Proceedings of the International Symposium
on

Genetics and breeding of durum wheat

Edited by:
E. Porceddu, A.B. Damania, C.O. Qualset

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Proceedings of the International Symposium
on

Genetics and Breeding of Durum Wheat

Dedicated to the memory of
Gian Tommaso Scarascia Mugnozza

CIHEAM

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Genetics and Breeding of Durum Wheat

Scientific Editors: E. Porceddu, A.B. Damania, C.O. Qualset

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Foreword

Durum Wheat is a main staple food crop in the Mediterranean area and in some marginal areas where it is critical to food security and income generation for resources limited farmers. However, options and interest for its utilization are increasing and expanding beyond the traditional cultivation area, to explore new environments and new positions on the cropping systems The scientific community recognizes that factors challenging durum wheat production and its global sustainability remain unresolved and require continued mobilization.

Traditional diseases, pests and environmental stresses continue to heavily limit crop production and to downgrade the commercial and utilization value of its harvested grain. Climate change will worsen these constraints and it is also pushing durum wheat cultivation toward higher latitude areas, where it will experience unfamiliar pests, diseases, weeds and different soil types. The range of products made from durum wheat is widening and their consumption increasing in regions where it is not cultivated and/or its products were not part of traditional consumption, pushing us to reconsider the key characteristics needed to obtain suitable processing quality. At the same time, advances in science are providing better research options and breeding strategies that can be used in developing varieties able to provide a sustainable production under these scenarios of more constraining environments and changing consumption trends. Advances in science allow also a more active exploitation of species' genetic resources and those in the wild and cultivated relatives.

In this context, the Accademia Nazionale delle Scienze detta dei XL - in collaboration with the Italian Consiglio Nazionale delle Ricerche (CNR), the International Center for Agricultural Research in the Dry Areas (ICARDA), the Centro Internacional de Mejoramento de Maíz y Trigo (CIMMYT), the Food and Agriculture Organization of the United Nations (FAO), the Centre International de Hautes Etudes Agronomiques Méditerranéennes (CIHEAM), and the Italian Agenzia nazionale per le nuove tecnologie, l'energia e lo sviluppo economico sostenibile (ENEA), and thesupport of the Italian Consiglio per la Ricerca e la Sperimentazione in agricoltura (CRA), the Società Italiana di Genetica Agraria (SIGA), Syngenta, Barilla, Societa Italiana Sementi, Divella, Perten Instruments,

Rummo and Wintersteiger - organized the International Symposium on "Genetics and Breeding of Durum Wheat", whose proceedings are presented in this volume.

Dedicated to the late President of the Accademia Nazionale delle Scienze, Gian Tommaso Scarascia Mugnozza, the meeting provided opportunity for the international durum wheat scientific community to gather and share results of research activities and progress made in the area of durum wheat genetics and breeding and discuss ways to address local, regional and international challenges that jeopardize the sustainability of durum wheat production. The symposium was noteworthy as a legacy to Professor Scarascia Mugnozza because he organized the first symposium, Genetics and Breeding of Durum Wheat, in 1973 held in Bari, Italy. There was great scientific interest in that symposium just as there was in the 2013 symposium, forty years later.

The Scientific Committee with the help of the Organizing Committee developed a technical program around seven themes: Origin and evolution of Durum Wheat, Genetic resources and durum wheat germplasm enhancement, Strategies and Tools in Durum Wheat Genetics and Breeding, Genetics and Breeding for Durum Wheat Yield and Sustainability, Genetics and Breeding for Durum Wheat Diseases and Pest Resistance, Genetics and Breeding for Nutritional and Technological Quality, Perspectives in Structural and Functional Genomics.Each theme was

developed through plenary sessions. More than 250 subject matter specialists, representing 44 countries, presented papers and/or posters in the symposium.

We hope that the Proceedings of the ISGBDW would serve a useful purpose for all those concerned with Durum Wheat cultivation and utilization.

Enrico Porceddu
National Academy of Sciences, Rome, Italy
Symposium Convenor

Preface

This volume is based on the presentations made by scientists at the International Symposium on Genetics and Breeding of Durum Wheat, held in Rome, Italy, on May 27-30, 2013. Though belonging to different disciplines, the participating scientists had a common focus: how their disciplines could contribute to improving Durum Wheat food production and quality, by using the most advanced techniques.

The text is organized into seven sections.

- Section I, “Origin and evolution of Durum Wheat”, deals with the origin, structure of current collections and with the changes correlated with the process of domestication and evolution of modern durum by comparison with landraces and wild tetraploids.
- Section II, ‘Genetic resources and durum wheat germplasm enhancement” reports the situation of durum wheat landraces, obsolete wheat, and wild relative accessions in the world’s gene-banks, the effects of climate change and its possible impact on wild relatives. Papers also provide good examples of success in transferring useful genes from wild species to cultivated material, along with the difficulties in making these transfers.
- Session III “Strategies and Tools in Durum Wheat Genetics and Breeding” presents ideas on how to overcome biotic and abiotic stresses and on how to use the tertiary gene-pool, including wild species, and the role of tools for evaluation of a myriad of characters.
- Session IV “Genetics and Breeding for Durum Wheat Yield and Sustainability” deals with adaptation and sustainability and the question of whether durum wheat is well-equipped to handle the coming climate change.
- Session V “ Genetics and Breeding for Durum Wheat Diseases and Pest Resistance” presents the situation of Durum Wheat genetic resistance to various diseases and pests, their influence on yield and the harvested grain quality and indicates strategies for reducing their incidence on crop harvest.
- Session VI “Genetics and Breeding for Nutritional and Technological Quality” recapitulates achievements and molecular methods to identify genes for good quality and stresses the need of having end products fine-tuned with consumers’ preferences and points out that yield improvement must not be achieved at the expense of quality.
- Session VII “Perspectives in Structural and Functional Genomics” underlines the progress made, tools currently available and the potential use of wild genetic resources in durum wheat improvement.
- The book is opened by messages of authorities, welcome addresses, and, a short presentation of the main events that characterised the life of Gian Tommaso Scarascia Mugnozza, to whom the Symposium was dedicated;it ends with some closing remarks, and the list of participants.

Whereas most papers have been edited for English language and style, some were substantially revised. For the papers requiring minor editing, the authors did not see these corrections. Every possible effort was made to ensure that each paper reflected the contributors’ ideas as accurately as possible. However, we solicit contributors’ indulgence for any mistakes and omissions that remain.

Publication of the proceedings would not have been possible without the cooperation and active involvement of the Accademia Nazionale delle Science, detta dei XL, Secretariat and of the editorial staff of the Mediterranean Agronomy Institute , Bari (IAMB), CIHEAM. Our warmest thanks to them. We take this opportunity to extend our warm gratitude to all the Invited Speakers, the Chairs of different sessions, the Scientific and the Organising Committee, the Partners, the

Sponsors and the Supporting Institutions.

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Opening session

Welcome addresses

Emilia Chiancone

Accademia Nazionale delle Scienze, detta dei XL, Rome, Italy

It is my very great pleasure to welcome you all to this International Symposium on the 'Genetics and Breeding of Durum Wheat', and to do so on behalf of the Accademia Nazionale delle Scienze detta dei XL, whose late president Gian Tommaso Scarascia Mugnozza organized the first Durum Wheat meeting in Bari 40 years ago.

First of all, I would like to thank Prof. Luigi Nicolais, the President of the Italian National Research Council (CNR), and the CNR Agri-Food and Biosciences Department for the generous support to this Symposium. Prof. Nicolais is outside Rome and asked me to express to all of you his welcome and his wishes for a most successful meeting.

The Minister for Agriculture, Food and Forestry Politics, Mrs. Nunzia De Girolamo, sent the following message: *'Unfortunately, due to Institutional commitments, I am not in the position of accepting your invitation to participate in the opening session of the International Symposium'.*

However, I want to underline the importance of your work and the precious contribution that research can give and has to give to agriculture.

I am deeply convinced that every study aiming at increasing knowledge and favoring scientific progress is indispensable and therefore has to be encouraged.

The agro-food world has to face many demanding challenges in the near future. The sharing of results, experiences and ideas represents the best way to deal with them.

I wish you a successful meeting.

The very fact that this Symposium is held here at the CNR, the major research institution in our country, allows me to put into perspective this Symposium and the CNR interest in durum wheat.

In the seventies, durum wheat improvement was one of the aims of the so-called Targeted Projects that led to the breeding and commercialization of several new varieties during the 1st generation of such Projects and continued during the 2nd and 3rd generation with increasing attention towards basic research.

Durum wheat was also one of the species studied at the CNR's Germplasm Institute directed at the time by Prof. Enrico Porceddu. Among the activities of this Institute it is worth recalling the many missions - carried out also with the support of FAO - to retrive and collect durum wheat in Algeria, Tunisia, Libya, and Greece, and similar missions in Ethiopia, one of the centers of species diversification. The material collected was multiplied and conserved at the Germplasm Institute, but was also distributed, when asked for, to a number of Institutions; it was shared with ICARDA and sent for long term conservation to the perma-frost facilities in the Svalbard Global Seed Vault.

The Germplasm Institute represented the Italian institution where modern and systematic studies on the technological and nutritional qualities of durum wheat grain started. These studies allowed the Germplasm Institute, now part of the Institute of Plant Genetics, to establish collaborations with many counties from the USA to Australia and have been pursued since by various Italian research Institutions.

Nowadays durum wheat represents the most wide-spread culture in Italy, the main durum wheat European producer, such that the number of exported tons of pasta exceeds by far the number of imported ones, not to mention the number of people involved in durum wheat transformation, in the production of processing machines, from milling to the final product.

Durum wheat provides an excellent opportunity of collaboration with industrialized and emerging countries. As examples can be taken on the one hand Australia, involved in the production of durum wheat for Asian countries, and on the other the Mediterranean countries and those of Central Asia, as they use durum wheat traditionally to prepare a variety of food products. Further, the collaboration with international institutions, like ICARDA and CIMMYT, that pursue actively research in durum wheat genetics and genetic improvement in cooperation with numerous institutes all around the world.

Last, but not least, I thank heartily all the Institutions that rendered this Symposium possible with their support: ICARDA, CYMMIT, ENEA, FAO, CIHEAM, CRA, SIGA, Zètema and the various Companies which cover the whole durum wheat production cycle from the seeds, namely SYNGENTA and SIS, to processing WINTERSTEIGER and PERTEN, and to the end product, BARILLA, DIVELLA and RUMMO.

Special thanks are due to the Academy staff, Dr. Giulia Trimani and Francesca Gitto in particular who take care of the Symposium Secretariat.

Thank you for your attention; I wish you all an interesting and enjoyable Symposium. I am sure it will have a particularly good flavor!

Opening address

Maarten van Ginkel

ICARDA

I am very pleased to be able to say a few words on behalf of ICARDA and CIMMYT, as requested by the chair, at this opening ceremony.

The CGIAR has recently reformed itself, to be able to work even better together with all our partners along the impact pathway, with the aim to improve rural livelihoods and enhance global food security. ICARDA and CIMMYT now work even more stronger together under this new CGIAR Research Program (CRP) called CRP/WHEAT. In addition to these two international Centers, WHEAT includes many dozens of partners, including NARS, Advanced Research Institutes, NGOs, farmers organizations, policy-makers, development agencies, private enterprise, processing industries and consumer organizations. Many of you participating in this Symposium are actively included. We expect that this closer cooperation among all of us will lead to new synergies and scientific breakthroughs, and even better outputs in term of more stable, higher yielding, durably biotic stress resistant, abiotic stress tolerant and better quality durum wheat germplasm and varieties.

As we, as global research-for-development practitioners, aim to improve rural agricultural resilience practiced by the resource-poor to stresses and open-up new opportunities for sustainable intensification, we also need to look beyond individual crops and their agronomy and policy environment. We need to study the entire integrated agro-ecosystem that includes not just one but several crops, vegetables, livestock, fish, trees, water, soil, market linkages, policies and institutions. This will mean that also in regard to new durum wheat varieties we need to study and determine how they fit best into dynamic integrated agro-ecosystems and add synergistically to the whole. As scientists we often tend to take reductionist approaches, but for farmers complexity is their reality.

I am pleased to see that the agenda includes significant emphasis on the use of durum wheat landraces and wild relatives to increase novel and diverse buffering capacities against multiple stresses and to be responsive to improved conditions. As we have seen in bread wheat, the use of Aegilops tauschii to develop so-called “synthetic” hexaploids and then through top-crosses synthetic derivatives, these can express multiple new traits or traits at higher levels of expression. Several such synthetic wheat derived varieties have been released in the past 20 years and are grown by farmers. In durum wheat this enriching of its genetic base is also being studied, but much more emphasis should be given to move these materials out of the breeders’ fields and into farmers’ fields.

Finally, I would like to stress that we should continue to exchange our newly developed germplasm among ourselves and with others. We all “stood on the shoulders of giants”, such as Dr. Norman E. Borlaug, Dr. Sanjaya Rajaram and Prof. Gian Tommaso Scarascia-Mugnozza, and need to continue along their path of free germplasm exchange as a joint research community. Teaming-up with other organizations to further enhance jointly shared germplasm can also open new doors of donors and funders to support such joint research. I wish you all a very productive and enjoyable Symposium.

Durum wheat in the Mediterranean

Cosimo Lacirignola

Secretary General of CIHEAM

CIHEAM is very pleased that its historical *Options méditerranéennes* collection will host these valuable contributions, developed at an international seminar on durum wheat genetics and cultivation dedicated to Gian Tommaso Scarascia Mugnozza in Rome 27–30th May 2013. Scarascia Mugnozza and his brother Carlo had a lasting influence on CIHEAM and played an important role in agricultural development in the Mediterranean. These contributions were provided by eminent scientists whose research focuses on this key Mediterranean product.

Wheat was actually the favourite cereal of ancient Mediterranean civilizations, and has since then remained a key element in the diet of this region, where bread is a vital and sacred product of intertwined religious practices and cultural dimensions.

Wheat constitutes a major area of scientific investigation, since it relates both to food security and to the evolution of consumption patterns. Continual increases in wheat yields have been imposed to keep pace with constantly rising demand. Productivity has also had to improve, as it has become increasingly difficult to expand agricultural lands for Mediterranean crops, due to the scarcity of soils and lack of water. Irrigation is used for cereal crops, but it is mostly rain water that allows wheat to grow in the Mediterranean region; it is therefore as necessary to consider rainfall as it is to carry out genetic research. Wheat production is constantly growing, but its diversity is gradually declining, although the Mediterranean has so many varieties! Wheat has multiple uses; durum wheat, in particular, is used for pasta and semolina, two products that have become socially and economically strategic for the Mediterranean countries.

Moreover, there are some present-day geopolitical concerns to take into account when considering durum wheat dynamics. I take the liberty of making these comments because this volume of *Options méditerranéennes* does not focus on these issues which complement scientific analysis on the genetics and use of wheat production.

- Firstly, it must be stressed that durum wheat represents only a very small fraction of world wheat production. It accounts for around 40 million tons (Mt) of the 700 Mt produced globally, which is just over 5%.
- Secondly, it must be specified that all of the world's durum wheat output is consumed by human beings, two-thirds of whom are in the Mediterranean area. Durum wheat is a cereal intended only for human consumption, and this should be remembered.
- The European Union (EU) produces about 20% of the world's durum wheat, i.e. 8 Mt. Half of all the durum wheat produced within the EU is grown in Italy. Then come France, Greece and Spain to complete European output on the Northern shores of the Mediterranean. Add to this the approximately 10 Mt of durum wheat produced in Southern and Eastern Mediterranean countries (Turkey, Morocco, Algeria, Tunisia and Syria), and we arrive at more or less 18 Mt of durum wheat produced in the Mediterranean region, i.e. nearly half of world production. In other words, one out of two tons of the world's durum wheat comes from the Mediterranean region.
- There are, however, large differences in durum wheat yields in the Mediterranean. France normally produces yields of over 5 tons per hectare (t/ha), and Italy has an increasingly

stable output of 3 to 3.3 t/ha. However, inter-annual variations are important in the other countries; yields in Spain and Greece may range between 1.5 and 3 t/ha, and this is also the case in Turkey. Yields in Morocco range between 1 and 2 t/ha, depending on the crop year.

– Canada produces only 10% of the world's durum wheat, but accounts for two-thirds of world exports. Together with US and Mexican outputs, 90% of the durum wheat traded in the world comes from North America. There is virtually no export of durum wheat from Mediterranean countries, where output satisfies the domestic demand of societies with high consumptions of pasta and semolina.

From a geo-economic point of view, these different situations may cause tensions on the markets. Agro-industries may certainly rely on harvests in Italy, Spain, France or North Africa if they are based in those countries, but most look to North America, which dictates the rate of international trade. When durum wheat production dips in Southern Europe and in the Maghreb, the food industry has even higher hopes of good harvests in Canada, the USA and Mexico.

Is it necessary to add that there is no futures market for durum wheat, unlike soft wheat, and that this does not afford industry operators any protection from inter-annual harvest fluctuations? Is it necessary to state that French, Italian and Spanish laws do not allow the use of soft wheat for pasta, to prevent producing sticky pasta and altering product taste? These considerations should be seen in the southern European context, where many cereal farmers have abandoned durum wheat in favour of more profitable cereals like maize in France or rice in Italy. The agricultural land under durum wheat in Italy has now shrunk to some of the lowest levels since 1945. However, the limited nature of the durum wheat market should not obscure its great sensitivity, and output is increasingly insufficient for consumption. In seven years between 2004/2005 and 2013/2014, i.e. in the last decade, world demand for durum wheat has exceeded global output.

Durum wheat has been a part of the history of the Mediterranean and its daily food patterns for centuries. Like all cereals, consumption of this valuable product depends on geographical and meteorological factors, but also increasingly on geopolitical parameters, given the importance of the North American powers in the international durum wheat trade. Although 50% of world output is still produced around the shores of the Mediterranean, the importance of the markets and their chronic tensions are a strategic area, where regional cooperation should play an active role.

In this respect, an essential step forward has been the 2014 establishment of the Mediterranean agricultural market information network (MED-Amin), coordinated by CIHEAM as a multilateral initiative of its 13 member States. By focusing on cereals (wheat, barley, rice and maize), the MED-Amin network intends to pursue several objectives with the aim of contributing to better food security in the region[1]. This is an example of a regional process showing the links of confidence between the Mediterranean countries, whose future will involve increasing levels of interdependence regarding adaptation to climatic constraints, in addition to agricultural research and trade.

[1] For more information on the activities of the MED-Amin network visit the following website : https://med-amin.ciheam.org/en/

Gian Tommaso Scarascia Mugnozza

Rome 1925 – 2011

Enrico Porceddu

Accademia Nazionale delle Scienze, detta dei XL, Rome, Italy

Madam President, prof. Giuseppe Scarascia Mugnozza, colleagues, ladies and gentleman.

Research institutions are evolving organisations which welcome newcomers and, as time passes by, acknowledge the contribution and achievements of their staff to advancement of science.

However, the celebration takes a different extent and shape when the person to be acknowledged is a founding father. His/her human values as well as scientific achievements must be remembered.

I will take this opportunity to take you on a brief journey across the life and work of a scientist who has left an ever-lasting mark in agricultural research in Italy and has played a leading role in the international arena. Rest assured it will not be a hagiography!

Gian Tommaso Scarascia Mugnozza (GTSM) was a loyal and just person, a very generous and caring friend, a man with a clear vision and able to stand his ground in defence of his beliefs and to deliver on his promises. Prudent and reserved in his behaviour, he never relented in passionately promoting new scientific enterprises.

He knew how to listen, how to advise and encourage through reasoning. He guided and inspired the cultural progress of many collaborators and his insights contributed to promote or encourage the engagement of research groups in agricultural genetics and plant breeding, as well as the setting up and/or the development of research programmes in agriculture. Today those many of those who had the good fortune of having him as a mentor are currently working at four different universities in Italy, in addition to retirees.

I myself am among the fortunate ones, since he was my mentor ever since 1970. Later when I became a colleague of his, I was able to enjoy his friendship and build a profound and mutual understanding . It would not be true to say we never had divergent opinions. They were very few and we were able to solve them by pondering the issue and analysing it carefully. It was a stimulating and enriching experience.

Allow me now to single out a few significant events that, in my opinion, are exemplary of his personal and professional life.

His days on earth came to an end on February 28, 2011 at age of 86 in Rome, where he was born on May 27, 1925 – exactly 88 years before this symposium was opened. It is a chance coincidence, since the date was not chosen on purpose, nevertheless it may be a good omen for agricultural research in general and for Durum Wheat research, in particular.

GTSM got a degree in Agricultural Sciences at the University of Bari, with a final dissertation on the diversity of grape vines from Apulia, the region his family originates from. His professional life started by working on tobacco mutagenesis and the results he got were outstanding so much so that he was asked to join the Italian delegation to the 1955 Geneva conference on “Atoms for Peace”. The issues on the agenda inspired him to promote the setting up of a Laboratory for nuclear energy applications in agriculture, which represented the original scientific nucleus of what has become the Casaccia nuclear centre (CNEN now ENEA). In cooperation with a group of scientists, some of whom are present here in this room, GTSM launched a research program on durum wheat.

Thousands of morphological and physiological mutants of several species were isolated and characterised before they became useful material for basic research. The topographic method used in the analysis of Durum Wheat (DW) mutants allowed for the calculation of the number of initial cells involved in spike development and the description of the crucial timing in spike organisation. Thanks to the sophisticated equipment available at the research centre, the group was able to measure the DNA content in DW meristems and to demonstrate that cell cycle is blocked both in pre (G1) and post DNA synthesis (G2). They also demonstrated the possibility of inducing mutations in only one of the two chromatids of the G2 cell chromosomes, thus giving rise to an heterozygote. Additional contribution to cell physiology derived from information on DNA synthesis, on mitotic activity of embryo cells, on packaging of storage compounds in seeds at different stages of development and with different water content.

Mutant lines and segregating material from crosses were tested at different locations in Italy and included in the “FAO/IAEA/CNEN Near East Uniform Regional Trials of Radio Induced DW Mutant Lines”, run for five years at different locations in Mediterranean countries, to Pakistan and India. Such trials are a treasure trove of information for potential future research work and in particular for genotype environment interaction studies. It has become evident the advantage of testing materials in a wide range of possible environments, for one season or a few seasons in identifying promising entries, which would later be tested over a number of years serving local interests.

Trials were a tremendous success in international cooperation coupled with local interests. Some of those lines were released as commercial varieties, which brought about a radical change in the DW scenario in Italy and in other countries, where they were included in crosses schemes.

At the end of 1968 GTSM returned in Bari as Chair Professor of Agricultural genetics, adding higher education to his scientific interests. Subsequently he set up the agricultural genetics institute, before being elected Dean of the faculty of Agriculture.

During those difficult years, marred by students’ protests, he set out to modernise university programs and courses in agriculture, working to craft an agreement between several Italian universities, avoiding useless duplicates and allowing for specialisation.

GTSM lent an interested ear to the young and promptly responded to their interests as in the case of natural resources, which are able to renew themselves and provide goods for an ever increasing human population through technology.

At that time he also paid special attention to Plant Genetic Resources (PGR). Following discussions held at the 1967 FAO technical meeting on Genetic Resources, he asked the Italian National Research Council (CNR) to set up the germplasm institute which he would have been glad to host at Bari University facilities. The institute became operational in 1970, when I moved to Bari, but its activities were soon expanded to include other Mediterranean countries, with the support of the FAO GR Unit. More than 11,000 seed samples were collected and characterised during the ‘70s. The results of these analysed and crucial aspects of the GR management were

presented as key note speeches at four subsequent wheat genetic symposia from 1973 to 1988, promoting the concept of core collections.

GTSM never ceased to be very active at the international level. In 1972 he attended the Beltsville meeting, where the Consultative Group on International Agricultural Research (CGIAR) system and its International Research Centres (IARCs) were first established.

Later he became a trustee of the newly created International Center for Agricultural Research in the Dry Areas (ICARDA), promoting the setting up of a GR conservation unit and related facilities, and eventually he was Technical Advisory Committee (TAC) member for two terms. All through those years he continued to press forward to promote research in the field of the Mediterranean agriculture and particularly in DW, the region's main crop. In fact he was also the founder of the FAO Network on DW for the FAO European Office and coordinated the network activities among the cooperating research institutions, besides seeing to a large cooperative research programme with Bolivia, for the Italian Ministry of Foreign Affairs.

In 1980 GTSM moved to Viterbo, where he was first Dean of the Faculty of Agriculture and then first Rector of the newly established University of Tuscia. For the third time he had to start from scratch. When he retired in 1998, the University had five faculties, fully equipped and staffed by motivated scientists. Needless to say a group of those scientists were still involved in DW research, with activities ranging from cytogenetics and disease resistance to genetic resources and grain quality aspects. Three of those scientists presented their achievements at this symposium and others are in the poster list.

During this period he was also elected by his colleagues President of the Italian University Rectors Conference, an organisation which consults with the Minister of University and Research as to the drawing up of university and research policies.

Research however was never side lined by his managerial responsibilities. Although rarely present in experimental fields and labs, he was able to follow research activities, discuss research results with his co-operators, attend national and international scientific conferences and symposia, present key note speeches and contribute with significant papers.

He developed two programs for Italy's CNR, while he was chairing the national advisory committee for agricultural sciences , which are further proof to his unrelenting interest in research. "Increasing the Productivity of the Agricultural Resources (IPRA)" and "Advanced Research for Innovations in the Agricultural System (RAISA)" were two targeted research programmes for the agricultural system which run during the '80s and the '90s, respectively, with a budget of 60 and 110 million euros, allocated to the 200 research units participating in the five year-activity. It is worth mentioning that on top of the numerous patents the programmes led to, 60% of their scientific results were published in internationally refereed journals, with a peak of 80% as to the results regarding plant science. Such a level of success is unparalleled to dates.

In recognition of his activities he was elected member of several academies. In 1984 he became a member of the National Academy of Sciences, the promoter of this Symposium, and was elected President of the Academy in 1989, a position he kept until the end. At the Academy, we use to pay homage to his outstanding contribution by providing the academy with facilities where to make available to the general public the rich library and archives containing volumes and documents since the academy was set up in 1782. But I would like to take this opportunity today to stress the wide scope of his activities and the themes discussed during Academy meetings comparing knowledge and new ideas, in line with the academy historical role. Mention should also be made to the continuous updating of working and information methods and tools. In fact GTSM was well aware of the challenge of promoting consistent and mutual formation and networked with academicians to press for a continuous pro-active participation in different initiatives, irrespectively of their specific cultural background.

In conclusion, during his life adventure as a scientists and mentor and in light of the wide diversity of scientific programmes he was involved in, some of which I have mentioned earlier, GTSM has played a major role in advising people, designing programmes and setting up institutions. Scientific rigor and latitude of approach, matched with a stimulating combination of fundamental aspects and practical results, have contributed to international cooperation and competition geared to quality and original, non-repetitive scientific production and encouraged people committed to research.

His last document, a volume of more than 400 pages, was dedicated to the history of agricultural research in Italy during the past 150 years. It illustrates stimulating future scenarios for agricultural research in Italy, of which we had a chance to talk during our last trip to Matera in Southern Italy, to attend the annual congress of the Italian Society of Agricultural genetics. I can tell you that, although he was ailing for some bone infection, GTSM through the journey and congress kept a lively and future looking conversation, commenting on past experiences and hypothesising possible future actions. It was only when we had our last telephone conversation on February 21, when he asked whether I would have been available to replace him on an advisory committee, he showed he was accepting his health condition.

The Academy of sciences chose to commemorate his human and scientific achievements with a ceremony which was held at the Senate library and attended by several distinguished academicians, mentees, former colleagues and politicians.

It is to his memory that the Academy of sciences wishes to dedicate this International Symposium on Genetics and Breeding of Durum Wheat, 40 years after he organised the first symposium on the same topic. Today in Rome we are discussing results, achievements and research hypothesis for which GTSM has played a vital role.

Thank you Madam President for allowing me to briefly portray the relevance and scope of GTSM's life achievements.

Symposium remarks

Enrico Porceddu

Accademia Nazionale delle Scienze, detta dei XL, Rome, Italy

Global durum wheat acreage and production only amounts to 5 to7% of the total wheat production, the other 93 to 95% being bread wheat (also known as common wheat). Thus, wheat can sometimes be considered a minor crop. However, it is the main crop and staple food in specific regions, such as the Mediterranean, where it was domesticated 10 to12 thousand years ago and where as much as 75% of the world's durum wheat production is harvested. Syria, Turkey, and Italy are the largest durum producers, followed by Morocco, Algeria, Spain, France and Tunisia.

In the Mediterranean area, it is used to make various end-products, such as pasta, flat bread, couscous, frekeh, and bulghur, which are presently consumed all over the world.

J. Blondel defined the Mediterranean basin as a "continent with solid borders." Borders mainly made by hills and mountains, which allowed limited contacts and communications (except by sea) thus, creating a wealth of diversity in space and time of both environmental and human societies. The succession of civilisations that existed at different places in this region over millennia has had a great impact on ecosystems and specifically on agro-ecosystems, with interactions between their components and human societies, determining a sort of coevolution, in which durum wheat played a major role and created a wealth of diversity. This symposium was designed to provide answers to some important theoretical and practical questions, such as: how did this material evolve; how much of that variation is still present and available in nature and/or in storage facilities; how much of the available variation is utilised directly and/or indirectly in crosses; and what difficulties are experienced in the utilisation of this material in Durum wheat breeding.

Mediterranean climate is characterised by erratic events which constrain durum wheat production.

Environmental constraints such as drought and temperature extremes cause stresses; unpredictability of seasonal precipitations is also important and the occurrence of moisture stress in certain plant development stages, such as booting and pollination may be extremely harmful. Hot winds and heat during grain filling period are not rare and damaging to grain yield and quality. Climate change is exacerbating some of these conditions. While mild winters cause leaf rust, yellow rust also causes problems in some areas; insects and root diseases, once a minor problem, are becoming serious threats to production in other areas, with Hessian fly being a major constraint in some of them. The array of traditional end-products is widening to include the exploitation of starch and protein in industrial non-food production.

Sessions at the Symposium were planned to update knowledge and present the state-of-theart techniques in breeding to overcome, mitigate, or tolerate these constraints. Two sessions were specifically devoted to explore alternative strategies and the wealth of variation and take advantage of progress in genomic analysis. Landraces and wild relatives are being utilised in crosses to produce new gene combinations in lines that are tested under different environments, but new strategies are needed to facilitate the production of those new combinations and to identify germplasm that combines tolerance, productivity, stability and resistance to biotic stresses.

Application of genomic tools is leading to major milestones in understanding the structure and function of the common wheat genome, and it promises to help identify the genes underlying genetic traits, their nature of dominance and epistatic interactions, and their fine tuning to specific

cell types, and in certain plant developmental stages. As a result of advances in biotechnology, germplasm from the ‘secondary’ gene pool is also available for utilization in crop improvement.

Extensive information on the above is now available, however, it is often scattered in various data bases and journal papers. The invited speakers were asked to bring some coherent pictures on the above-mentioned aspects and elaborate on new prospects.

I thank you for your attendance and your interest in durum wheat, and wish you a very productive Symposium.

Session 1

Origin and evolution of durum wheat

Durum wheat evolution-- a genomic analysis

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Abstract. Durum wheat appears in the archaeological record, very sporadically, ~7000 years before present, but becomes the dominant tetraploid wheat in the Levant and in the Mediterranean basin ~2500 years ago. Here, we discuss the archeological insights on durum wheat evolution and we focus on the analysis of the genomic changes that are correlated with the process of domestication and evolution of modern durum by comparing four genetic groups: wild emmer, domestic emmer, durum landraces and modern durum varieties. Changes in gene expression and copy number variation of genes and transposons were analyzed in the genetic groups. Genes were clustered based on their pattern of change during Durum evolution, e.g. gradual increase, or decrease, or increase at the onset of domestication and plateauing later on. There were not many genes that changed >2 fold in copy number. However, interestingly, the copy number of transposons increased with domestication, possibly reflecting the genomic plasticity that was required for adaptation under cultivation. Extensive changes in gene expression were seen in developing grains. For example, there was an enrichment for certain functions: genes involved in vesicle trafficking in the endosperm showed a gradual increase in expression during durum evolution and genes related to germination and germination inhibition increased in expression in the embryo, in the more recent stages of durum evolution. The approach described here enables better understanding of the genetic events that shaped modern wheat and identifies genes that can be used for crop improvement.

Keywords. Durum wheat – Evolution – Genomics domestication.

L'évolution du blé dur -- une analyse génomique

Résumé. *Le blé dur a fait son apparition dans les archives archéologiques, très sporadiquement, il y a environ 7000 ans, mais il est devenu le blé tétraploïde prépondérant au Levant et dans le bassin méditerranéen il y a environ 2500 ans. Nous allons parcourir ici les connaissances archéologiques sur l'évolution du blé dur et focaliser l'attention sur l'analyse des changements génomiques liés au processus de domestication et d'évolution du blé dur moderne, en comparant quatre groupes génétiques : amidonnier sauvage, amidonnier domestique, variétés locales et variétés modernes de blé dur. Les changements de l'expression génique et la variation du nombre de copies de gènes et de transposons ont été analysés dans les groupes génétiques. Les gènes ont été regroupés en fonction de leur profil de changement au cours de l'évolution du blé dur, par exemple, augmentation ou bien réduction progressive, ou augmentation au début de la domestication et plafonnement successif. On n'a pas trouvé beaucoup de gènes montrant une variation du nombre de copies >2 fois. Cependant, il est intéressant de noter que le nombre de copies de transposons a augmenté au fur et à mesure de la domestication, ce qui indiquerait une certaine plasticité génomique nécessaire pour l'adaptation au système de culture. Des changements importants dans l'expression des gènes ont été observés au niveau des grains en développement. Par exemple, le renforcement de certaines fonctions : l'expression des gènes impliqués dans le transport vésiculaire de l'endosperme a augmenté progressivement au fil de l'évolution du blé dur et l'expression des gènes liés à la germination et à l'inhibition de la germination a augmenté au niveau de l'embryon dans les stades les plus récents de l'évolution. L'approche décrite permet de mieux appréhender les événements génétiques qui ont façonné le blé moderne et d'identifier les gènes utilisables en vue de l'amélioration de la culture.*

Mots-clés. *Blé dur – Évolution – Génomique de la domestication.*

I – Introduction

Durum wheat, Triticum turgidum ssp. durum, is a tetraploid species whose genome is genetically very close to that of its progenitor, wild emmer wheat, Triticum turgidum ssp. dicoccoides (2n=4X=28, Genome BBAA) (Feldman 2001). The F1 hybrid between these two species is fully fertile and cytological analysis show that in most accessions of emmer wheat, chromosomes show full pairing with durum wheat. The first step in durum evolution was the domestication of wild emmer wheat through the loss of fragility of the spike, namely of its disarticulation into spikelets, the basic dispersal units (Feldman 2001; Salamini *et al.* 2002). This step was probably a gradual process as suggested from both genetic and the archeological evidence. Indeed, 2-3 major loci and several modifiers, with additive effects are involved in the control of fragility (Chen *et al.* 1998; Levy and Feldman 1989a; Millet *et al.* 2013; Nalam *et al.* 2006; Watanabe *et al.* 2002). It might thus have taken time for the appearance and fixation of the mutations and the full loss of fragility. In addition, the archeological record shows that in ancient sites where agriculture was practiced, a mixture of fragile and non-fragile types were found, and it took 3-4 thousand years until the non-fragile spikes became prominent in farming units in emmer wheat (Kislev 1984) and in einkorn wheat (Tanno and Willcox 2006). One interpretation of this observation is that the first mutants in the fragility locus were still partially fragile until a second and additional modifier mutations appeared, and/or that wild and domesticated spikes were grown in parallel. The loss of fragility gave rise to the first known domesticated wheat, Triticum turgidum ssp. dicoccum, or emmer wheat, which is grown to this day, albeit on a small scale (De Vita *et al.* 2006). Its spike is not fragile, however, like its wild progenitor, it has only 2 kernels per spikelet which are tightly wrapped in stiff glumes and it is not free threshing. How did durum wheat evolve from *dicoccum*? Did it get its naked kernels directly, through a mutation in the genes that control glume stiffness (the Q factor and the *Tenacious Glumes* (*TG*) locus) or, was there another intermediate step? The appearance of naked kernels was the second most important step in durum domestication after non-brittle spikes. The archeological record shows a very sporadic appearance of durum-like wheat ~ 7000 years BP (before the present) in the near east and is only ~ 2500 yrs ago that durum becomes a major crop in the Mediterranean basin (Feldman 2001). The question 'why *durum* was not cultivated before the Helenistic period?' remains puzzling. Perhaps it was susceptible to some disease. On the other hand, *Triticum turgidum* ssp. *parvicoccum,* a tetraploid wheat "fossil" species was relatively abundant in the archeological record starting already 9000 yrs BP, but disappeared ~ 2000 yrs BP. It had a compact spike and was free-threshing, suggesting that it probably already contained the Q and *tg* mutations prior to durum (Feldman and Kisslev 2007). This raises the possibility that *durum* received these mutations from *parvicoccum*, rather than evolving them independently from emmer wheat (Fig. 1). Durum may thus have derived from hybridization between *parvicoccum* and *dicoccum* receiving the free-threshing trait from *parvicoccum* and the large grains from *dicoccum*. The large grain of durum was probably preferred to the small grains of *parvicoccum* that lead to the prominence of durum as a tetraploid wheat and to the extinction of *parvicoccum.* While the origin of the free-threshing trait of durum, whether directly from *dicoccum* or via *parvicoccum*, remains uncertain, the molecular evidence suggests that there was a bottleneck in the formation of durum and it became isolated from its Near-Eastern emmer wheat center of origin (Oliveira *et al.* 2012; Ozkan *et al.* 2011).

In addition to the above-mentioned classical domestication traits that were selected in the process of durum evolution, other domestication "syndrome" traits were selected that were advantageous to the farmer, such as plant erectness versus the wild grassy types, increased number of seeds per spikelet, and reduced seed dormancy (Feldman 2001). It is likely that many other traits were selected, including many QTLs, which are not easily visible to the eye, such as resistance to abiotic and biotic stresses, physiological parameters that contribute to yield (Peleg *et al***.** 2009), as well as quality parameters (Levy and Feldman 1989c) and in recent decades, following the green revolution, adaptation to the new cultivation conditions including chemical fertilizers and mechanical harvest.

So far, with the notable exception of the Q locus, domestication genes identified in wheat have been characterized only through mapping. Two major genes that control spike fragility are *Brittle Rachis 2* and *Brittle Rachis 3* located on the short arms of chromosomes 3A and 3B, respectively (Nalam *et al.* 2006); in addition, another locus for spike brittleness was mapped to chromosome 2A (Peleg *et al.* 2011; Peng *et al.* 2003). The differences between studies mapping spike fragility suggest that there is diversity among wild accessions in the number and location of loci involved. Tenacious glumes, and Soft glumes are 2 independent loci that affect glume tenacity and spike threshability (Sood *et al.* 2009). Similarly, many domestication-related QTLs were mapped (Gegas *et al.* 2010; Peleg *et al.* 2009; Peng *et al.* 2003), but the underlying genes were not identified at the molecular level. The Q locus, located on chromosome 5A is the only one that was so far characterized at the molecular level. It is one of the most significant domestication loci as it controls spike compactness, glume tenacity and fragility. It encodes for the APETALA2-like transcription factor (Simons *et al.* 2006) and while the 5A homeoallele has the most significant contribution, other homeoalleles were also shown to be involved in the domestication traits (Zhang *et al.* 2011).

Among the traits that were affected by domestication are the storage proteins, in particular the high molecular weight (HMV) glutenins whose variability and amounts are higher in wild than in domesticated tetraploid wheat (*Laido et al.* 2013; Levy and Feldman 1988; 1989b). Recently, a *NAC* genes from emmer wheat that contributes to high protein percent, a trait that affects both the nutritive value and the processing of wheat and was lost during domestication, has been isolated (Uauy *et al.* 2006).

The identification of additional loci that control domestication-related traits will be facilitated by the new arsenal of genomic tools in wheat. Despite the complexity of the wheat genome, due to its polyploidy and to the large amount of transposons in its genome, there has been remarkable progress in the amount of datasets and tools for wheat genomics. To reduce complexity, several studies have chosen the strategy to sequence BAC libraries of single flow-sorted chromosomes. This lead to a high-resolution map of chromosome 3B, the largest wheat chromosome (1Gb) (Paux *et al.* 2008) and more recently to a high density map of chromosome 1BL (Philippe *et al.* 2013) and of group 7 (Berkman *et al.* 2013). New mapping tools are available including a large number of SNPs spread across the genome that have been developed for sequence-based mapping for bread wheat (Saintenac *et al.* 2013) and more specifically for *durum* (van Poecke *et al.* 2013). In fact, SNP mapping in a broad collection of wheat landraces and modern varieties has indicated the genomic regions that underwent selection (selective sweep) during post-domestication wheat breeding (Cavanagh *et al.* 2013). Whole genome sequences are also available for the A (Ling *et al.* 2013) and D (Jia *et al.* 2013) genomes, however a good assembly of contigs is still missing. Recently, a major advance in *durum* transcriptome analysis was the development of tools for the discrimination of homeologues from the A and B genomes from expression sequence data such as RNA-Seq (Krasileva *et al.* 2013). Data sets from small RNAs are also becoming available (Kenan-Eichler *et al.* 2011; Yao and Sun 2012).

In order to identify the global genomic changes that occurred during wheat domestication, we performed a genomic analysis, using a microarray to measure gene copy number and expression patterns in ~ 40,000 genes of tetraploid wheat and ~ 400 transposable elements (TEs). The wheat lines represent a gradient of domestication including a collection of wild emmer wheat; of domesticated emmer wheat (*dicoccum*); of *durum* landraces; and of modern *durum* cultivars. Genes were sorted according to patterns of evolution, showing different modes of increase or decrease during *durum* wheat evolution.

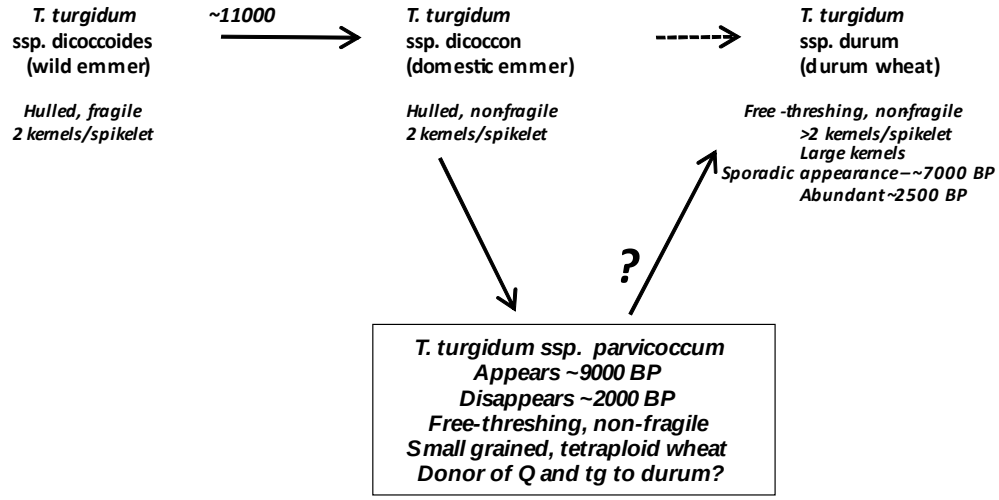

Figure 1. Evolution of durum wheat: The durum wheat wild progenitor, wild emmer wheat, *Triticum turgidum ssp. dicoccoides* was domesticated ~ 11,000 BP, giving rise to domestic emmer wheat, *Triticum turgidum ssp. dicoccon* through selection for non-brittle rachis. Naked kernels tetraploid wheat appears ~ 9000 BP in the near east. Its seeds and spike morphology are different from durum: the grains are small and it has relatively compact spike and short glumes. This sub-species, named *Triticum turgidum ssp. parvicoccum* disappears ~ 2000 BP. It might have a mutation in the Q factor and the *Tenacious Glume* genes, however, this cannot be confirmed as the species exists only in the archaeological record. The *parvicoccum* wheat might have contributed to the formation of durum wheat, providing it with the free-threshing trait.

Alternatively durum might have originated directly from emmer wheat through independent mutations in the *Q* and *Tg* genes.

II – Material and methods

1. Plant material

Thirty-six wheat lines that correspond to the various stages of domestication of the tetraploid level were grown and analyzed at the transcriptome and phenotypic levels. These wheat types consist of (a) the wild tetraploid varieties *T. turgidum* ssp. *dicoccoides* (11 lines), domesticated varieties including (b) primitive tetraploid wheat *T. turgidum* ssp. *dicoccum* (6 lines), (c) traditional tetraploid lines of *T. turgidum* ssp. *durum* (landraces) that were collected from traditional farmers, mostly from middle-eastern villages (7 lines), and (d) modern high yielding tetraploid macaroni wheat, *T. turgidum* ssp. *durum* varieties (5 lines). The plants were grown under the same conditions, in a net-house with 3 replicas per line, each replica being grown in a separate block. All plants were grown in 3-liter pots during the winter. The lines analyzed were of a broad range of eco-geographical origins (Table 1) in order to cover as much as possible of the variation typical of the subgroup analyzed.

Table 1. Tetraploid wheat lines used in this study (lab number and origin).

Wild emmer *Triticum turgidum* ssp. *dicoccoides*	Domestic emmer *Triticum turgidum* ssp. *dicoccum*	Durum Landraces *Triticum turgidum ssp. durum*	Durum modern *Triticum turgidum* ssp. *durum*
TTD12 (Ammiad,) Galilee, Israel	TTC1 (cv. Farrum, Italy)	TTR25 (cv. Ma'ari, Beit Sira Judean Mt., Israel)	TTR2 (cv. Hazera 163 Nursit, Israel)
TTD24, (Bet-Meir Judean Mts. Israel)	TTC4 (cv. Nigro-ajar)	TTR6 (cv. Beladi, Hevron Israel)	TTR19 (cv. Cappelli, Italy)
TTD28 (Northern Samaria, Israel)	TTC2 (cv. Khapli, India)	TTR265 (Mehola, Jordan Valley, Israel)	TTR1 (cv. camara, Portugal)
TTD31 (Lebanon)	TTC8 (cv. Submajus, India)	TTR333 (Turkey)	TTR298 (cv. Westbred 881, USA)
TTD32 (Turkey)	TTC6 (origin unknown)	TTR86 (Israel)	TTR16 (cv. Langdon, North Dakota, USA)
TTD35 (Turkey)	TTC7 (cv. macro antherium)	TTR60 (Israel)	
TTD 37 (Iran)		TTR42 (Israel)	
TTD48 (Shahabad-Ilam Iran)			
TTD49 (Rosh Pinna-Zefat Rd. Easten Galilee, Israel)			
TTD64 (Diyarbakir, Turkey)			
TTD150 (Northern Iraq)			

2. RNA extraction and quality control

RNA was extracted from each replica from the developing seed (embryo or endosperm) (14 days after anthesis). Embryos were manually dissected from ~6 developing seed in each spike and the endosperm "milky" liquid was collected separately for each seed.

All tissue samples were immediately frozen in liquid nitrogen, and total RNA was extracted from 1.0 g of each pool tissue type using the Trizol® Plus RNA Purification Kit (Invitrogen, Carlsbad, CA) with an on-column DNase treatment.

Total RNA integrity was assessed using RNA 6000 Nano Lab Chip on the 2100 Bioanalyzer (Agilent, Palo Alto, CA) following the manufacturer's protocol. Total RNA purity was assessed by the NanoDrop® ND-1000 UV-Vis Spectrophotometer (Nanodrop technologies, Rockland, USA). We considered RNA to be of good quality based on the 260/280 values (Nanodrop), rRNA 28S/18S ratios and RNA integrity number (RIN) (Bioanalyzer).

3. Labeling and microarray hybridization

For each block, an equal amount of RNA was pooled for each genetic group, namely we had 4 RNA samples, one for each of the Wild, Primitive (*dicoccum*), Land race durum, and modern durum lines. Microarray experiments were performed with 2 biological replicas (one series of 4 samples for each block).

The samples were labeled using Agilent Quick Amp Kit (Part number: 5190-0442). 500ng of total RNA was reverse transcribed using oligo-dT primer tagged to T7 promoter sequence. cDNA thus obtained was converted to double stranded cDNA in the same reaction. Further the cDNA was converted to cRNA in the in-vitro transcription step using T7 RNA polymerase enzyme and Cy3 dye was added into the reaction mix. During cRNA synthesis Cy3 dye was incorporated into the newly synthesized strands. cRNA obtained was cleaned up using Qiagen RNeasy columns(Qiagen, Cat No: 74106). Concentration and amount of dye incorporated were determined using Nanodrop. Samples that pass the QC for specific activity were taken for hybridization. 600 ng of labeled cRNA were hybridized on the custom Microarray Wheat 8x60K designed by Genotypic Technology Private Limited (AMADID: 037650) using the Gene Expression Hybridization kit (Part Number 5190-0404; Agilent) in Sure hybridization Chambers (Agilent) at 65º C for 16 hours. Hybridized slides were washed using Agilent Gene Expression wash buffers (Part No: 5188-5327). The hybridized, washed microarray slides were then scanned on a G2505C scanner (Agilent Technologies).

For copy number variation (Comparative genome hybridization - CGH) and gene (as well as transposons) expression profiling, we used a custom designed Agilent microarray chips of ~160,00 probes for the CGH (four for each EST, from which we choose the best probe in terms of quality for further analysis), and 60,000 probes for the gene expression analyses. The transposon fraction was assembled using data from the TREP database, which contains a collection of repetitive DNA sequences from different *Triticeae* species. The 10th version of this database, which was used here, contains a list of 477 sequences composed of DNA transposons, retrotransposons and other, non-classified repetitive sequences (http://wheat.pw.usda.gov/ITMI/Repeats/). Four Oligos were selected for each TE type from conserved regions that are representative of the TE family.

4. Microarray Feature Extraction and Data Analysis

Data extraction from Images was done using Feature Extraction software of Agilent V-10.7.3.1. Feature extracted data was analyzed using GeneSpring GX Version 11 software from Agilent. Normalization of the data was done in GeneSpring GX using the 75th percentile shift. Percentile shift normalization is a global normalization, where the locations of all the spot intensities in an array are adjusted. This normalization takes each column in an experiment independently, and computes the nth percentile of the expression values for this array, across all spots (where n has a range from 0-100 and n=75 is the median). Fold change expression values in test samples were obtained with respect to the specific control samples. Significant genes up and down regulated within the group of samples were identified. Statistical t-test was calculated based on volcano plot. For differential expression and clustering we used the EXPANDER and Cluster Identification via Connectivity Kernels (CLICK) algorithms (Sharan *et al***.** 2003).

5. Calculation of copy number variation (CNV)

We first calculate the median signal (gMedianSignal) and background (gBGMedian) signal for each array from the raw data files along with probe names. We then averaged the background and subtracted gMedianSignal by gMedianSignal – gBGMedianSignal and then convert to log base 2 for all arrays. In each sample the log transformed intensity values for each probe is subtracted by the calculated 75th percentile value of the respective array and expression values are obtained like so:

R= (P/100)* (N+1)

Where: R = Rank; P = Percentile; N = Number of Entities (Rows).

III – Results and Discussion

1. Changes in gene expression of the developing kernel during durum evolution

To examine the genes that show the most significant change in gene expression in embryo or endosperm of developing kernels, during durum wheat evolution, we divided all the probes on the chip by their expression patterns in the different genetic groups (Wild, Primitive, Landrace and Modern) using the CLICK clustering solution (Sharan *et al***.** 2003) and we selected genes which showed > 3 fold change. We chose to analyze significant clusters that have > 150 probes and used a stringent averaged homogeneity Pearson correlation coefficient r-value > 0.7. In Figure 2 we present such gene clusters for embryonic tissues dissected from two-week old seedlings.

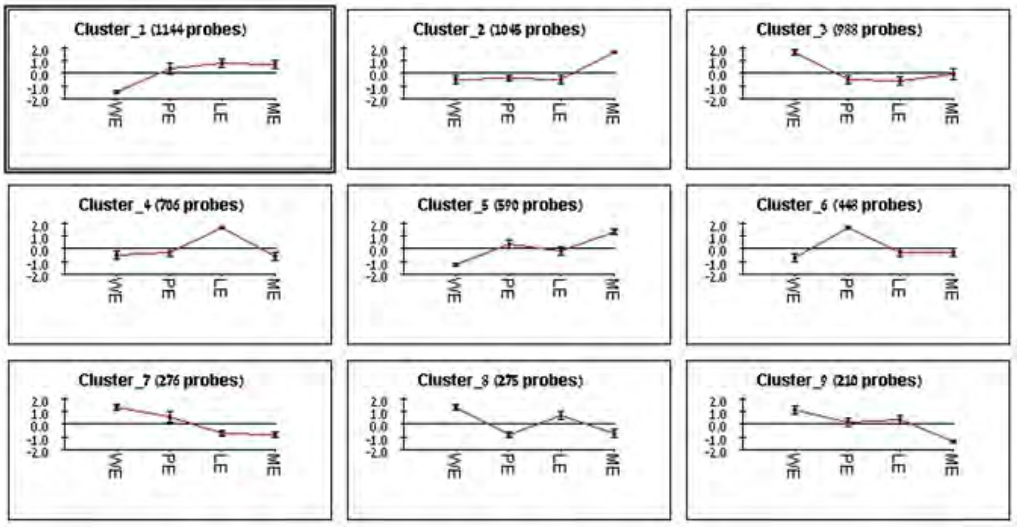

Figure 2. Clustering of probes corresponding to patterns of altered gene expression in the embryo during durum domestication. The X axis corresponds to the different stages of domestication, starting from the wild progenitor (WE= *T. turgidum* ssp. *dicoccoides*), the primitive non-fragile, non-free threshing tetraploid wheat (PE= *T. turgidum* ssp. *dicoccum* the *T. turgidum* ssp. *durum* landraces (LE) and modern durum cultivars (ME). The Y axis corresponds to the log value of fold change.

Within the embryonic tissue dataset, clusters 1, 2, 3, 5, 7 and 9 showed expression profiles in consistent with durum domestication and breeding. Cluster1 showed a gradual increase in expression during evolution with most changes occurring between the wild and primitive stage, namely at the onset of domestication. Cluster 2 showed most changes that occurred during recent amelioration (between landraces and modern varieties). These clusters were examined using BLASTx, Blast2GO (Conesa and Gotz 2008) and Ontologizer (Bauer *et al***.** 2008) suites for GO enrichment. In this way, the biological processes could be examined on a larger scale for each cluster. Interestingly, we found that for gene expression in embryonic tissue, Cluster 2 has

a significant number of probes (p-value 0.001) associated with alpha-amylase inhibitor (Figure 2 and Table 2). This is interesting in light of its role in the regulation of seed dormancy, a trait that was counter-selected in domesticated wheat (Feldman 2001).

Alpha-amylase is an enzyme which aids in the breakdown of starch into maltose by hydrolyzing bonds between glucose molecules (Tanaka and Akazawa 1970). Regulation of alpha-amylase would allow for control over the availability of sugars in the embryo needed for germination (Garcia-Maya *et al***.** 1990). As uniform germination would be a trait that would have been selected for during domestication, this becomes highly relevant. Other functions that were enriched in Cluster 2 are annotated as 'extracellular regions'. In this case the role for selection for enhanced expression in such genes is less clear but the analysis provides leads on potentially interesting genes.

Table 2. Biological processes, corresponding to Cluster2 pattern of evolution that were significantly enriched within embryonic tissues.

GO ID	Ontology Description	Total genes in GO category and annotated genes= 20772)	Genes in GO category (% total in Cluster2 and % total genes in cluster=539	Fold enrichment (p value)
GO:0019012	virion	30	8	10
		(0.14%)	(1.4%)	(0.005)
GO:0005576	extracellular	640	32	1.92
	region	(3.08%)	(5.93%)	(0.002)
GO:0045735	nutrient reservoir	243	75	11.89
	activity	(1.17%)	(13.91%)	(0)
GO:0030234	enzyme regulator	398	32	4.99
	activity	(1.19%)	(5.94%)	(0.00004)
GO:0015066	alpha-amylase	39	11	10.74
	inhibitor activity	(0.19%)	(2.04%)	(0.001)
GO:0009405	pathogenesis	34	6	6.94
		(0.16%)	(1.11%)	(0.018)

2. Changes in endosperm tissue gene expression during durum evolution

GO enrichment was also performed on clusters of genes consistent with patterns of changes relevant to durum evolution for endosperm tissue (Figure 3). Enrichment for cytoplasmic vesicle in cluster 5, (Table 3), a group of genes that showed gradual increase during durum evolution, is of interest since it might point to selection made for better starch and protein highways in the endosperm during domestication. Plant seeds accumulate starch in starch granules, providing sugars to the germinating embryo, and storage proteins which are a source of amino acids for use during germination are deposited into protein bodies (Takahashi *et al.* 2005). Both starch granules and protein bodies require vesicles for their packaging. In addition unique precursor-accumulating vesicles are known to mediate a transport pathway for insoluble aggregates of storage proteins directly to protein storage vacuoles (Hara-Nishimura *et al***.** 1998). Better starch and protein trafficking in the developing seed could increase the ability to act as an efficient sink, which might be beneficial for both quality and yield.

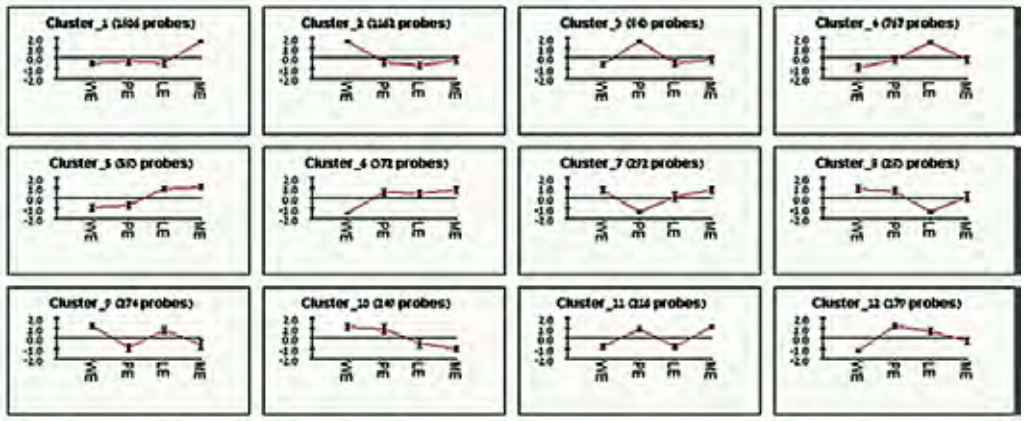

Figure 3. Clustering of probes in the endosperm, corresponding to patterns of altered gene expression during durum domestication. The X axis corresponds to the different stages of domestication, starting from the wild progenitor (WE= *T. turgidum* ssp. *dicoccoides*), the primitive non-fragile, non-free threshing tetraploid wheat (PE= *T. turgidum* ssp. *dicoccum* the *T. turgidum* ssp. *durum* landraces (LE) and modern durum cultivars (ME). The Y axis corresponds to the log value of fold change.

Within the embryonic tissue dataset, clusters 1, 2, 3, 5, 7 and 9 showed expression profiles in consistent with durum domestication and breeding. Cluster1 showed a gradual increase in expression during evolution with most changes occurring between the wild and primitive stage, namely at the onset of domestication. Cluster 2 showed most changes that occurred during recent amelioration (between landraces and modern varieties). These clusters were examined using BLASTx, Blast2GO (Conesa and Gotz 2008) and Ontologizer (Bauer *et al*., 2008) suites for GO enrichment. In this way, the biological processes could be examined on a larger scale for each cluster. Interestingly, we found that for gene expression in embryonic tissue, Cluster 2 has a significant number of probes (p-value 0.001) associated with alpha-amylase inhibitor (Figure 2 and Table 2). This is interesting in light of its role in the regulation of seed dormancy, a trait that was counter-selected in domesticated wheat (Feldman, 2001).

Alpha-amylase is an enzyme which aids in the breakdown of starch into maltose by hydrolyzing bonds between glucose molecules (Tanaka and Akazawa 1970). Regulation of alpha-amylase would allow for control over the availability of sugars in the embryo needed for germination (Garcia-Maya *et al*. 1990). As uniform germination would be a trait that would have been selected for during domestication, this becomes highly relevant. Other functions that were enriched in Cluster 2 are annotated as 'extracellular regions'. In this case the role for selection for enhanced expression in such genes is less clear but the analysis provides leads on potentially interesting genes.

3. Changes in copy number variation during durum evolution

The microarray was hybridized with genomic DNA to determine changes in copy number of genes or of TEs. With a few exceptions listed in the pie chart (Figure 5), there were no major changes in gene copy number during the various stages of durum evolution. Nevertheless, a cluster of 396 probes that exhibited > 2 fold change increase in copy number was detected between the wild emmer wheat and the modern durum cultivars. The pattern of copy number accumulation for these probes suggests a gradual accumulation of gene copies over the course of domestication (Figure 4).

Table 3. Biological processes, corresponding to Cluster5 pattern of evolution that were significantly enriched within endosperm tissues.

GO ID	Ontology Description	Total genes in GO category and (%total annotated genes =20772)	Genes in GO category in Cluster5 and (% total genes in cluster=240)	Fold enrichment and (p-value)
GO:0016023	cytoplasmic membrane bounded vesicle	4012 (19.3%)	99 (41.2%)	2.13 (0.0000)
GO:0065009	regulation of molecular function	555 (2.67%)	18 (7.5%)	2.81 (0.002)
GO:0050790	regulation of catalytic activity	549 (2.64%)	18 (7.5%)	2.84 (0.002)
GO:0044092	negative regulation of molecular function	338 (1.62%)	17 (7.08%)	4.37 (0.0005)
GO:0043086	negative regulation of catalytic activity	337 (1.62%)	17 7.08%)	4.37 (0.0005)
GO:0030234	enzyme regulator activity	398 (1.92%)	18 (7.5%)	3.91 (0.0005)
GO:0004857	enzyme inhibitor activity	270 (1.3%)	17 (7.08%)	5.45 (0.0002)
GO:0016787	hydrolase activity	3517 (16.91%)	64 (26.67%)	1.58 (0.0003)
GO:0052689	carboxylic ester hydrolase activity	197 (0.95%)	14 (5.83%)	6.14 (0.0006)
GO:0030599	pectinesterase activity	71 (0.34%)	13 (5.42%)	15.94 (0.0003)

These genes cover all levels of plant cell functions and maintenance. There was no obvious enrichment for any particular function in the cluster of genes whose pattern of CNV is as shown in Figure 5, namely, a gradual increase. It seems that if there was selection for copy number increase of specific characteristics it was done through the modulation of broad cellular mechanisms complexes.

Among these genes we found 13 genes related to ubiquitin and E3 ligase members, which are part of the autophagy mechanism. Particularly interesting is the *Opaque-2* transcription factor which appeared in both the copy number variation data set and the differential gene expression data set. In Maize, the *Opaque-2* has been shown to be involved in the regulation of expression of major storage proteins and other important genes involved in seed development. It is a major regulator in the balancing of starch and protein in maize seeds (Zhang *et al.* 2012). As it is regulated in a phosphorylation/dephosphorylation manner it is likely to be closely involved with kinases and phosphorylases (Guo *et al***.** 2012) which also appear in our results.

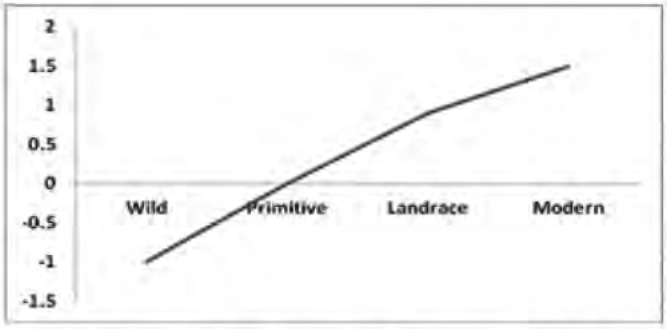

Figure 4. Cluster of 396 probes that increase > 2 fold in copy number during Durum wheat domestication. Samples of wild emmer wheat (Wild); domesticated emmer wheat (Primitive); of durum landraces (Landrace); and of modern durum cultivars (Modern) were tested using CGH on a custom designed microarray. Probes were clustered according to patterns of copy number variation (calculated as follows: the background was averaged and subtracted gMedianSignal by gMedianSignal – gBGMedianSignal and then converted to log base 2) This cluster shows a gradual increase in gene copy number over the course of tetraploid wheat domestication.

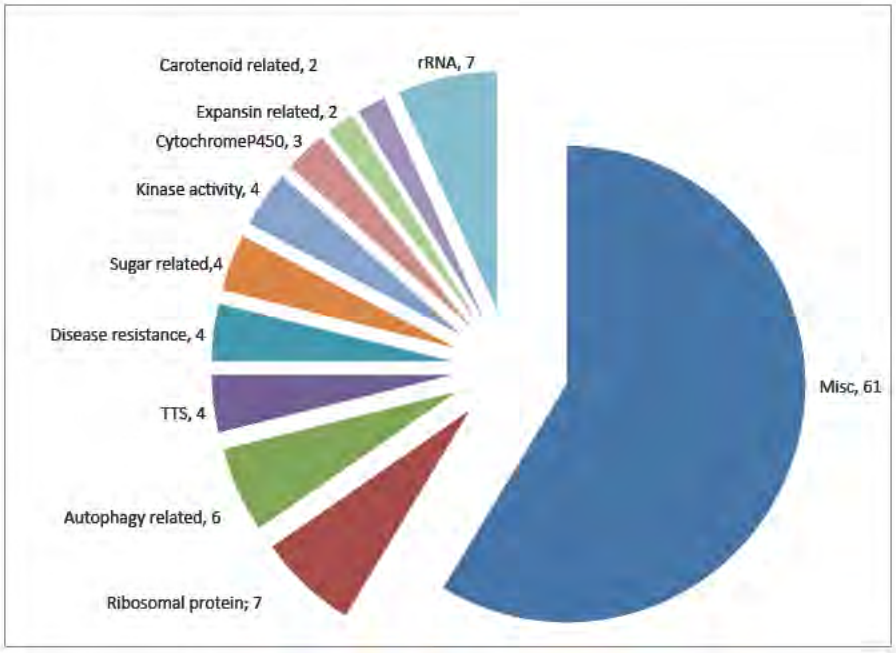

Figure 5. Representation of the functional annotation of genes found in the copy number module. The relative percent is given for each category.

When examining copy number variation on its own, we observed an over-representation of carotenoid biosynthesis related genes (carotenoid cleavage dioxygenase 1; CCD1, Chlorophyll synthase, viviparous-14; *vp14* and a light-harvesting complex I protein; *Lhca1*) (p-value 0.020). These five genes showed an increase in copy number between wild and modern tetraploid

wheat lines. Previous work has shown a link between domestication of maize and wheat and the accumulation of high levels of β-carotene in various tissues normally devoid of carotenoids (Rodriguez-Concepcion and Stange 2013). There are several reasons why the carotenoid pathway may have been selected for during domestication. Carotenoids are known to contribute to the stress response via ROS quenching and as a precursor to the plant hormone abscisic acid (Bradbury *et al***.** 2012). They are also a supporting mechanism for chlorophyll biosynthesis and the photosynthetic pathway in general.

As with any broad data set, there are several classes/families of genes which one would expect to be present on the bases of statistics alone. Many classes of genes participate in diverse functional mechanisms through their many members/constituents. Therefore it was not surprising to see that within our dataset we identified 16 ribosomal genes, six Cytochrom P450 genes and several protein kinases with diverse functions (data not shown).

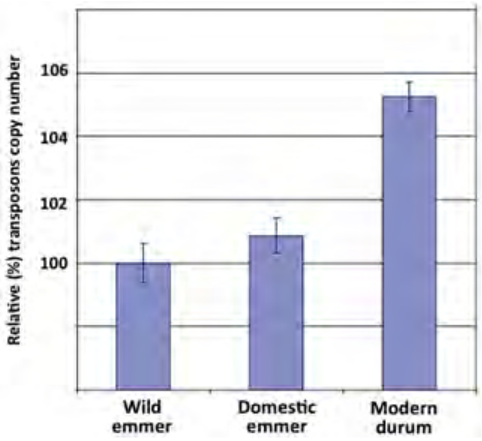

Figure 6. Relative change in transposable elements copy number during durum evolution. The copy number is expressed relatively to the wild emmer wheat. The results are for 477 sequences including DNA transposons and retrotransposons.

IV – Conclusions

While earlier studies on wheat domestication have mostly mapped the typical domestication syndrome genes (e.g. fragility and free-threshing traits), we have studied the genomic analysis of durum wheat evolution. We studied genome-wide changes in copy number and gene expression during the first stage of domestication, namely the transition from wild to domesticated emmer wheat, then the stages from *dicoccum* to *durum*, free-threshing landraces and modern varieties. For this purpose we have used a broad collection of lines (Table 1) from varied eco-geographical origins.

We have analyzed gene expression in developing kernels, in embryonic and endosperm tissues and we have classified genes according to different patterns of evolution. This analysis sheds light on genes whose expression was up regulated or down regulated at the various stages of evolution. For example, we could discover non-obvious targets of evolution, such as an enrichment for genes whose expression is related to vesicles and trafficking in the endosperm. It is tempting to speculate that the up-regulation we have observed was the result of human selection for types better adapted to agriculture conditions. There are many such examples, each of which requiring a deeper analysis to understand the functional significance of the observed changes.

Only a few genes showed some trends in copy number variation, but unlike for expression, these were few and overall rarely changed beyond 2 fold. Transposons seem to have increased in copy number. The increase was only of ~5% but considering that these correspond to a large fraction of the genome, this may have slightly affected genome size. More importantly, it is possible that the selection pressure of the new habitat of agriculture has served as a stress that activated copy number, or conversely, that only lines where transposons were active could provide the new mutations controlling the traits needed for evolution. Combining mapping data that should be soon available from whole genome sequences, together with a genomic analysis should point to new targets for further breeding of durum wheat.

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Biodiversity of tetraploid wheats: taxonomy, studying, increasing and preservation

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Abstract. Tetraploid wheats have played a critical role in human history. They were the first polyploids domesticated by man. *Triticum durum* Desf. was bred nearly 2000 years ago and the last 70 years, breeders have been working with only this one agricultural important tetraploid wheat species. Related wheat species having preserved higher polymorphism than that of cultivated ones could be an additional source of increasing biodiversity. Solving the problems of effective utilization and preservation of the biodiversity of wheat related species is poss ble in four basic trends: arranging a scrupulous preliminary comparative-genetic studying of related species and generic genepool, i.e., revising their biodiversity; aimed at usage of accessions with a preliminary established presence of gene(s) of interest for introgressive hybridisation or amphidiploidisation; obligatory cataloguing of accessions with introgression of genes or whole genomes in genebanks for their preservation; producing a new genus *Triticum* taxonomy including man-made species.

Keywords. Tetraplolid wheat – Taxonomy – Biodiversity – Preservation.

Biodiversité des blés tétraploïdes : taxonomie, étude, augmentation et préservation

Résumé. *Les blés tétraploïdes ont joué un rôle crucial dans l'histoire humaine. Ils ont été les premiers polyploïdes domestiqués par l'homme. Triticum durum Desf. a été sélectionné il y a environ 2000 ans et ces 70 dernières années, les obtenteurs ont travaillé seulement à cette espèce de blé tétraploïde importante du point de vue agricole. Les espèces de blé apparentées ayant conservé un polymorphisme plus élevé par rapport aux espèces cultivées pourraient constituer une source supplémentaire de biodiversité. Il est possible de résoudre les problèmes de l'utilisation efficace et de la conservation de la biodiversité des espèces de blé apparentées en suivant quatre approches : réaliser une étude préliminaire fine de génétique comparative sur les espèces apparentées et le pool génétique générique du blé, c'est-à-dire, reconsidérer leur biodiversité ; viser à utiliser des accessions chez lesquelles ont été identifiés des gènes d'intérêt pour une introgression ou une amphi-diploïdisation ; répertorier obligatoirement les accessions avec introgression de gènes ou de génomes entiers dans des banques de gènes pour leur conservation ; produire la taxonomie d'un nouveau genre Triticum incluant les espèces obtenues par l'homme.*

Mots-clés. *Blé tétraploïde – Taxonomie – Biodiversité – Conservation.*

I – Introduction

Searching for ways of increasing biodiversity and preservation is the key point in biology of the 21st century, whereas preservation of cultivated wheat species biodiversity is a strategic task of food security. Genus *Triticum* L. is includes di- (2*n*=14), tetra- (2*n*=28) and hexaploid (2*n*=42) species. Tetraploid wheats are represented by 2 wild and 12 cultivated species including into two evolutionary lines (sections) – Emmer and Timopheevii (Goncharov, 2011; Hammer *et al*., 2011). At present, only five of them, namely *Triticum durum* Desf., *T. turgidum* L., *T. dicoccum* (Schrank) Schuebl., *T. aethiopicum* Jakubz. and *T. turanicum* Udazch. are cultivated. Nowadays durum wheat is the primary wheat for pasta and semolina production and the second-most cultivated wheat after common (bread) wheat. Rivet, emmer and other tetraploid wheats practically disappeared from cultivation during the 20th century and its extinction was prevented only by inclusion of them accessions in germplasm bank collections. collections of cultivated plant are traditionally regarded as the material used mainly for breeding purposes. However, they can also be used in genetic or botanical investigations. Rearrangement of huge germplasm bank collections is the taxonomy task.

II – Taxonomy

Traditionally, the taxonomy methods are based on revealing the affinity among organisms, determining the homology of their traits and common origin. At present, there is a tendency of juxtaposition of classical taxonomy, which had historically developed on the basis of comparative morphology, against modern taxonomy based on genetic and molecular-genetic investigations (see review Goncharov, 2011).

Swaminathan and Rao (1961) showed that differences in taxonomically important traits of hexaploid wheats are controlled by four pairs of nonallelic genes. taxonomically important traits are absent in tetraploid wheats. Unfortunately, tetraploid species do not possess such genes. The only exceptions are *P1* and *P2* y *T.polonicum* and *T. ispahanicum* (Watanabe, 1994), *Ta – T. carthlicum* (Haque *et al*., 2011) and *Pp1 T. .aethiopicum* (Dobrovolskaya *et al.*, 2006; Khlestkina *et al.*, 2010).

Wheat taxonomy has a long history. The main goal of modern wheat taxonomy is to establish such a classification of wheat genera and species which would reflect both their phylogenetic relationships and genetic structure. Good and rigorous taxomony is necessary for effective conservation and increasing cultivated plant biodiversity by introgressive hybridization. This is complicated by the lack of consensus concerning the taxonomy of tetraploid wheats and by unresolved questions regarding the domestication and spread of naked wheats. These knowledge gaps hinder crop diversity conservation efforts and plant breeding program (Nachit *et al.* 2001).

The classification that I have proposed (Goncharov, 2002; Goncharov *et al.*, 2009) follows in the Körnicke–Flaksberger–Dorofeev tradition and includes 29 species in five sections (Table 1). I do not divide the genus into subgenera and have instead designed sections (except for section *Compositum* N.P. Gontsch. which includes most of the artificial man-made species) based on ploidy levels, cytoplasm types and genome compositions. Traits were evaluated in terms of their variation and genetic control at the three different ploidy levels. Only experimental comparative-genetic studies will permit identification of individual 'species-forming' genera, determination of their allelism, and further evaluation of the species recognized. A detailed classification would permit easy identification of the material being stored and reproduced in genebanks (Filatenko and Hammer 1997).

Poor classifications are not just less useful, they are positively harmful. In the absence of acceptable criteria for distinguishing individual taxa, genebank staff cannot be expected to monitor the purity of their accessions, and important accessions may be eliminated because their significance is not appreciated. Indeed, failure to provide formal taxonomic, and hence nomenclatural, recognition of distinct entities may lead to what Dr. Michael Windham has referred to as ''extinction by nomenclature.'' Clearly, a classification that requires expertise in cytogenetic and/or molecular genetics will not be practical for many of those who work with *Triticum*. What is needed is a classification system that takes account of phylogenetic, cytogenetic, and molecular information but is accompanied by detailed morphological descriptions, workable keys, and correct nomenclature (Morrison 1995, 2001; Goncharov 2002).

The two examples illustrate the primary disadvantage of Mac Key's (2005) approach to the classification of *Triticum* (Table 2). It overlooks and conceals many of the demonstrably distinct entities within the genus. This tends to result in the exclusion of these entities and the diversity they represent from research studies and may lead to the elimination of important accessions from the world's genetic resources. It can also lead to problems with the identification of existing genetic resources. Examination of 576 accessions identified as *T. turgidum* and 1,189 accessions identified as *T. aestivum* in the International Center for Agricultural Research in the Dry Areas (ICARDA) and Uzbek Institute of Plant Industry genebank, respectively, revealed that about 5 and 8% did not belong to the designated taxon (Table 2).

Table 1. *Triticum* L. classification ((Goncharov, 2002) with additions according to: Goncharov *et al*. (2009)).

Section	Group of species	Species	2*n*	Genomes
Monococcon Dum.	Hulled	*T. urartu* Thum. ex Gandil.	14	A^u
		T. boeoticum Boiss.	14	A^b
		T. monococcum L.	14	A^b
	Naked	*T. sinskajae* A. Filat. et Kurk.	14	A^b
Dicoccoides Flaksb.	Hulled	*T. dicoccoides* (Körn. ex Aschers et Graebn.) Schweinf.	28	BA^u
		T. dicoccum (Schrank) Schuebl. [a]	28	BA^u
		T. karamyschevii Nevski	28	BA^u
		T. ispahanicum Heslot	28	BA^u
	Naked	*T. turgidum* L.	28	BA^u
	tetraploids	*T. durum* Desf.	28	BA^u
		T. turanicum Jakubz.	28	BA^u
		T. polonicum L.	28	BA^u
		T. aethiopicum Jakubz.	28	BA^u
		T. carthlicum Nevski	28	BA^u
Triticum	Hulled	*T. macha* Dekapr. et Menabde	42	BA^uD
		T. spelta L.	42	BA^uD
		T. vavilovii (Thum.) Jakubz.	42	BA^uD
	Naked	*T. compactum* Host	42	BA^uD
	hexaploids	*T. aestivum* L.	42	BA^uD
		T. sphaerococcum Perciv.	42	BA^uD
Timopheevii A. Filat. et	Hulled	*T. araraticum* Jakubz.	28	GA^u
Dorof.		*T. timopheevii* (Zhuk.) Zhuk.	28	GA^u
		T. zhukovskyi Menabde et Erizjan	42	GA^uA^b
Compositum N.P.	Hulled	*T. palmovae* G. Ivanov	28	$DA^b(DA^u)$
Gontsch.		*T. dimococcum* Schieman et Staudt	42	BA^uA^b
		T. kiharae Dorof. et Migusch.	42	GA^uD
		T. soveticum Zhebrak	56	BA^uGA^u
		T. borisii Zhebrak	70	BA^uDGA^u
	Naked octoploid	*T. flaksbergeri* Navr.	56	GA^uBA^u

[a] *In botanical literature there is a rule to Latinize Greek word ending. The noun "dicoccon" from Greek "χοχχον" (grain) when forming adjectives becomes 'dicoccus, -a, -um' in Latin. So there is no reason to change T. dicoccum for T. dicoccon. Moreover, Schrank used name 'T. dicoccon' only 'for the time being' (for detail see review L.R. Morrison (1998)). Hence, his binominal proves to be only provisional name.*

Table 2. Investigations into the authenticity of a collection of ''tetraploid'' wheats (*T. turgidum*) from West Asia and North Africa (WANA) country genebank (ICARDA), and a collection of hexaploid wheats (*T. aestivum*) from Uzbek Institute of Plant Industry wheat collections.

Species	No. of studied accessions	No. of misidentified accessions	Percent of non-conformity
T. turgidum	576	44[a]	7,64
T. aestivum	1189	59[b]	4,96

[a] - *Number of hexaploids;*

[b] - *Number of accessions not corresponding to their passport botanical variety.*

III – Biodiversity

Genetic resources provide the basic input to all plant breeding programs. Nowadays the genetic diversity and the population structure of tetraploid wheats has received a lot of attention (Li *et al.*, 2006; Yifru *et al.*, 2006; Moragues *et al.*, 2007; Oliveira *et al.*, 2012; Leigh *et al.*, 2013; among others). The first step of reasonable biodiversity preservation is drawing up a phenotypic identification and inventory and the second is its genetic analysis. Development of a database describing phenotypic and genetic collections is crucial for their goal-oriented biodiversity preservation (Goncharov and Shumny, 2008). Phenotypic collections contain accessions showing contrasting or alternative characters. Genetic collections contain accessions showing characters whose genetic control is known. The probability for biodiversity preservation is higher for accessions of genetically identifiable pure lines than for those reproduced as small populations, i.e., "native" populations. However, the question remains open of how many plants should be included in genebanks populations for preservation of gene pools of collected native populations. In fact, varieties compete, when maintained as small populations, and some varieties disappear, others show sharply altered gene frequencies in the course of reproduction.

Distribution areas of related wheat species are continuously reducing. So collecting, replenishing, reproducing, studying and maintaining those species living, being a constant supply for breeding are important to preserve biodiversity resources and future food security. It is obviously not feasible to gather again Vavilov's or Kihara's wheat biodiversity collections of tetraploid wheats, even after following the routes of their expeditions. Nature has not spared the biodiversity existing in their times, and this emphasizes the significance of reasonable maintenance of the maximally possible biodiversity presently stored in genebanks. The questions of how to preserve and of what to undertake so that biodiversity would not be subjected to erosion are more timely as ever. Reduction in the natural areas of wild endangered wheat species, as well as in their polymorphism due to their reproduction in small populations: in genebanks, decrease the potential biodiversity of cultivated tetraploid wheat species. To knowledgeably preserve gene pools maintained as small size populations, accessions should be fuller genetically characterised. This would allow goal-oriented preservation of the natural gene pool of the accessions.

Polymorphism of cultivated wheat species is inconsiderable in many traits (Boggini and Pogna, 1989; Pecetti and Annicchiarico, 1998). The wild and con-cultivated tetraploid species (fig.1) are still a valuable source of useful agronomic traits for the continued improvement of cultivated wheat species. Wide hybridization of cultivated wheats with wild ones, coupled with cytogenetic manipulation of the hybrid material, has been instrumental in the genetic improvement of durum and common wheats. What are the prospects of searching for polymorphic traits in wild related species? Let us demonstrate the statement using two types of traits – adaptive and neutral.

1. Adaptive trait

Low adaptability of cultivated tetraploid wheat *T. durum* complicates its successful cultivation in many agricultural areas and field experiments. Duration of vegetation period is one of the basic traits among those determining plant wheat adaptability to environments (Vavilov, 1935). Its cultivar character is the most important parameter in *T. durum* breeding programs. Despite considerable achievements in studying earliness, it remains so far the factor that limits agricultural cultivation on these or that regions. Earliness of tetraploid wheats is a complicated trait controlled by genes with different interaction effects. Basic differences in its manifestation are determined by *Vrn* genes controlling growth habit (spring *vs.* winter) and *Ppd* genes controlling photoperiod sensitivity (Wilhelm *et al*., 2009). It is shown that *Vrn* genes control not only one of the cardinal ways of developmental switch to spring or winter growth habit but also determine maturity rate. By the way, different dominant *Vrn* genes condition basic distinctions in earliness in spring common wheat cultivars (Kato *et al.*, 1997).

Although the length of vegetation period in tetraploid wheats is controlled only by two not four dominant *Vrn* genes just like in common wheat, the expressiveness of character in studied cultivars of *T. durum* in Kazakhstan doesn't differ from the one in common wheat cultivars (fig.2).

2. Neutral trait

Neutral trait, i.e. the trait whose spreading in populations proceeds without the effect of natural and/or artificial selection - glucose-phosphate-isomerase (EC 5.3.1.9). Using a relatively 'neutral' trait allows us to estimate some formal-genetic parameters: level of polymorphism, degree of heterozygosity, relative genetic distance of those or that forms from each other, degree of isolation among close-related species, overlapping of close species genepools, parameters of reproduction systems (obligatory self-pollination and the presence of this or that degree of intraspecific cross-pollination).

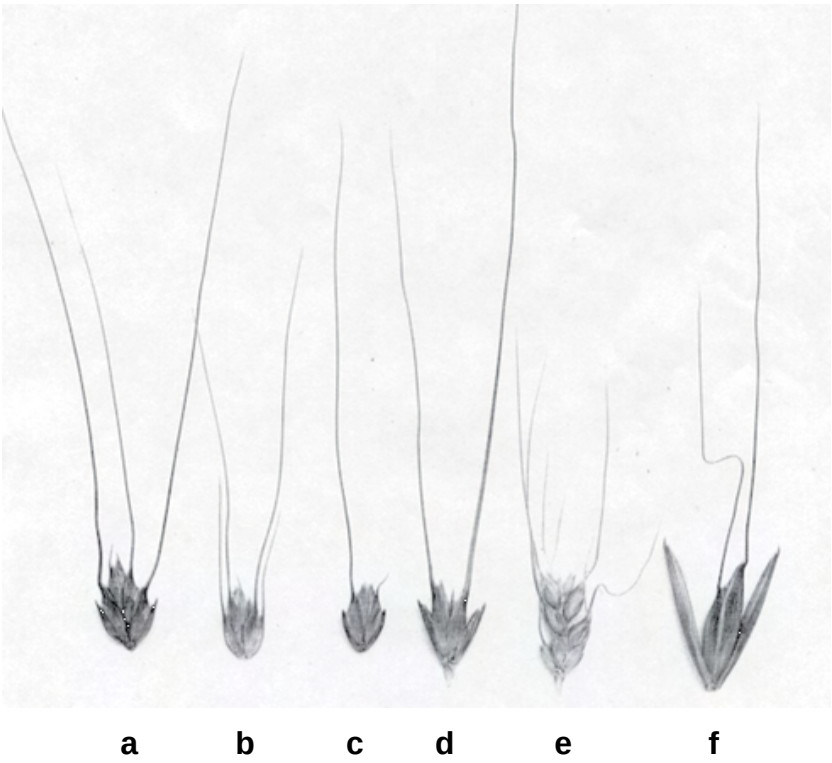

Figure1. The spikes of *T. durum* (a), *T. carthlicum* (b), *T. dicoccum* (c), *T. dicoccoides* (d), *T. turgidum* (e), *T. polonicum* (f).

Polymorphism on locus *Gpi-1*(glucose-phosphate-isomerase) was described in a genera *Triticum* and *Aegilops*. Its presence was shown in all donors of elementary genomes – *T.boeoticum*, *T.urartu*, *Ae.speltoides* and *Ae.aucheri*. However, it is worth noticing that frequencies of accessions with 'rare' variants are small. For example, analysis of 207 *T. urartu* accessions from Small Grain and VIR collections allowed to find out 9 of such variants with GPI mobility, different from the rest 199 studied (Table 3). It complicates their wide use for introgressive hybridization**.** No polymorphism was detected at locus *Gpi* in tetraploid species belonging to Dicoccoides section (Goncharov *et al.*, 1998).

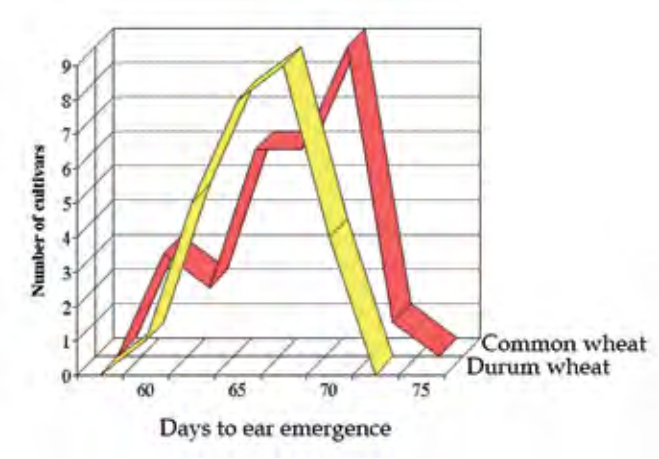

Figure 2. Distribution of Kazakhstan common and durum wheat cultivars according length of vegetation period (Almaty, field).

The obtained results are presented in Table 3. Some diploids of *Triticum* produce a monomorphic *GPI-1* band while others display composite and polymorphic patterns at locus *Gpi-1*. The heterozygotes present in the samples have most likely rresulted from cross-pollination.

Therefore, the task of related wheat species genepool preservation is simplest when solved first with an aimful collection, inventorisation and further their preservation in genebanks; second, by means of including their genepool in the genepool of cultivated species and making up gene storage, i.e. those of disease resistance; adaptivity; induces of grain quality, etc., also those controlling the morphological traits untypical for cultivated wheat species.

IV – Increasing biodiversity

Involving tetraploid species related to wheat and nowadays non-cultivated wheat tetraploids in interspecific hybridisation for introgression of genes and/or their alleles into cultivated species (especially *T. durum*) could be one of the ways to solve the problem of increasing genetic diversity source for durum wheat. These problems require an urgent solution for increasing *T. durum* biodiversity, hopefully, will enable us not to decrease grain presently and in the future. BA-genome species, except for part of *T. dicoccoides,* are easily crossed with each other producing fertile hybrids. Related tetraploid wheat species having preserved higher polymorphism than that of cultivated *T.durum* could be an additional source of increasing biodiversity. It is not complicated to obtain the hybrids between tetra- and hexa-, tetra- and diploid wheat species.

Table 3. Genetic distinctions on locus *Gpi-1* in wheat species having A^b genome.

Species	Genome	Number of found *Gpi-1* genotypes						Total
		ββ	βδ	δδ	εξ	2β	γγ	
T. boeoticum	A^bA^b	1^a	1^a	26				27
T. monococcum	A^bA^b	2		142				144
T. sinskajae	A^bA^b	1						1
T. urartu	A^uA^u	6			196		3	207
T. araraticum	GGA^uA^u		$3+19+2^b$		$6+14+2^b$			44^b
T. timopheevii	GGA^uA^u		10		4	11		25

a – polymorphic accessions are presented in different columns; b – heterozygotes of two types.

Searching for not only agronomic traits, but also marker-genes of these or other traits in wild related species of cultivated plants with their further introgression into genomes of improved cvs is an effective base to increase cultivated species biodiversity.

1) Characters on which a taxonomy of tetraploid wheats are based, namely:
   a. branched spike from *T.turgidum*;
   b. purple seed from *T.aethiopicum*;
   c. the presence of awns at the same time with flower and awn glume from *T.carthlicum*;
   d. elongated glume from *T.polonicum* and *T.isphahanicum*.

2) Characters appearing as a result of intraspecific hybridization in tetraploids:
   a. the semicopactoid (semiclub) spike;
   b. absence of nuclear organizer on chromosome 1B (lines Friebe 256/8/5 produced by Dr. Ponga from *durum* with *S. cereale* L).

3) *T. durum* mute collections.

4) Tetraploid wheat characters with the same genetic control as at hexaploid wheat (Table 4).

Table 4. List of tetraploid wheat genetic collection.

Phenotypes	Gene symbols	No. of genes and their chromosome localization	Accession with	
			Dominant genes	Recessive genes
Growth habit	*Vrn*	2 (5A, 5B)	BS1E, Bs2E	BWE
Hairy glume	*Hg*	1 (1AS)	Bs1E	Angara
Black glume	*Bg*	1 (1AS)	BS1E	Beloturka
Red grain	*R*	2 (3AL, 3BL)	tetraCS	K-43766
Awnedness	*B*	2 (5A, 6B)	Sharik, tetraCS	BWE
Hybrid dwarfness	*D2*	1 (2BL)	Loro	BWE
Hybrid necrosis	*Ne1, Ne2*	2 (5BL, 2BS)	Gaza, K-35116	BWE
Glaucousness (waxlessness)	*W*	1 (2BS)	Gaza, Nursit	Angara
- " -	*w*	1 (2bS)	-	BS1Ew
Hairy peduncle	*Hp*	1 (5A)	BS1Ehp	Angara
Hairy node	*Hn*	1 (5A)	tetraCS	TetraThatcher
Hairy leaf	*Hl*	2 (4A, 5A)	K-47759	tetraCS
Hairy leaf sheath	*Hs*	1	K-20403	Beloturka
Lack of ligules	*lg*	2	Mavroullos	Vroullos
Red coleoptiles	*Rc*	2 (7A, 7B)	K-29145	K-18999
Semicompactoid	*sc*	2	Angara	BWE, tetraThatcher
Chocolate color of glume		7BS	cv. Langdon mute	Beloturka
Purple pericarpe	*Pp3, Pp1*	2AL, 7BS	GAW 414	BWE
Branch sp ke	*bh*	2AS	branch line	BS1E
Tetraauricle	*ta*	5A	*T.carthlicum*	BS1E

BWE – Black Winter Emmer.

Availability of genetic collections of tetraploid wheats would allow us to:

- transfer genes from a wheat species at one ploidy level to another wheat species at a ploidy level different from it and *vice versa* with the expectation to increase the biodiversity of wheat species at any ploidy level;
- study the effect of ploidy level on the expression of wheat characters;
- study the effect of different kinds of wheat cytoplasms on gene expression;
- map characters that could not be introgressed to another ploidy level;
- investigate the effect of different cytoplasms on the traits expression;

– produce comparative gene mapping at different ploidy levels;
– obtain a model to study trait inheritance controlled polymerically - simplify models for studying the inheritance of characters under polygenic control.

We hope that maintenance and use of phenetic and genetic collections of di- and tetraploid wheat species are also a good strategy for biodiversity preservation.

V – Preservation

The two ways of preserving biodiversity are its to increase the long-term storage of the seeds.. The first way to do this was mentioned above.

The analysis of various methods of long-term storage of genetic resources was carried out. The conclusion was that the optimal method was cryopreservation method in a layer of permafrost in North-East Russia provided the following criteria has been made: 1) the maximal economic profitability and biological efficiency, 2) reliability and security from various natural and technogenic accidents, and 3) minimization of expenditures on labour. The project of creation of International cryobank for genetic resources with the use of «free and reliable natural cold» of permafrost is offered and directions of its activity are formulated (Kershengolts *et al*., 2012). So in addition to the one created in Norway at the Svalbard Global Seed (Qvenild, 2008), one more is being built in Yakut region of Russia in the permafrost. To destroy the layer of permafrost in Yakutsk the general thaw of Earth to 20^0 C is necessary.

VI – Conclusion

Existing germplasm collections are not being effectively used in agricultural science and breeding programs. The effective use of wheat biodiversity in breeding programs is dependent on a sound conservation strategy for sources of biodiversity, and on appropriate techniques of incorporation into modern cultivars. Studying the genetics of tetraploid wheat genome species donor showed the presence of polymorphism in them on very different traits. Therefore, at present both the task of collection, preservation, and study and the problem of introgression of part of related species genes into the genepool of cultivated tetraploid species having lost wide polymorphism during breeding and multi-centennial cultivation are topical.

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Global durum wheat diversity: structure and origin revealed by means of the gliadin markers

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Abstract. Genetic diversity for the alleles of gliadin-coding loci was studied within 563 durum wheat cultivars from 42 countries and in 98 Bulgarian durum wheat landraces. In total 116 alleles for 4 gliadin-coding loci were identified. The highest genetic diversity was revealed for durum wheat cultivars having origin from Middle East, Trans Caucasia, the Pyrenean Peninsula, and the Balkans countries. Ward's clustering analysis based on gliadin-calculated Euclidian distances among cultivars from different countries divided the collection on three separate groups. The groups significantly differed in the gliadin alleles frequencies. Two groups were proposed be formed by ancient genetic branches of durum wheat. A "Southern" branch included mostly durum wheats from the Mediterranean region, the Middle East, and Transcaucasia. A "Northern" branch included Russian and Ukrainian cultivars as well as cultivars bred on their basis in North America, China and some other countries. An additional group included durum wheat cultivars that had been bred in several past decades on the basis of the material of the International Agricultural Research Institutes, viz.,CIMMYT and ICARDA. This group displayed low genetic diversity.
The results made it possible to emphasize the factors forming present global durum wheat genetic diversity: climatic conditions and historical factors in the areas of cultivation on one hand and an international breeding trend on the other hand.

Keywords. Gliadin loci – Genetic diversity – Geno-geography – Durum wheat.

Diversité du blé dur à l'échelle mondiale : structure et origine révélées à l'aide des marqueurs de la gliadine

Résumé. *La diversité génétique des allèles des loci codant pour la gliadine a été étudiée chez 563 cultivars de blé dur provenant de 42 pays et 98 variétés locales bulgares de blé dur. Au total, 116 allèles pour 4 loci codant pour la gliadine ont été identifiés. Les cultivars de blé dur originaires du Moyen-Orient, de la Transcaucasie, de la péninsule des Pyrénées et des Balkans ont montré la diversité génétique la plus importante. L'analyse de regroupement selon la méthode de Ward, basée sur les distances euclidiennes calculées pour les gliadines au niveau des cultivars de différents pays, a permis de répartir la collection en trois groupes distincts. Les groupes différaient significativement pour la fréquence des allèles de la gliadine. On a avancé l'hypothèse que deux groupes étaient formés par les anciennes branches génétiques du blé dur. Une branche du "Sud" incluait la plupart des blés durs de la région méditerranéenne, du Moyen-Orient, et de la Transcaucasie. Une branche du "Nord" incluait des cultivaras russes et ukrainiens ainsi que des cultivars sélectionnés sur leur base en Amérique du Nord, en Chine et dans d'autres pays. Un groupe supplémentaire incluait des cultivars de blé dur qui ont été sélectionnés au cours de plusieurs décennies, en s'appuyant sur le matériel des Instituts internationaux de recherche agricole CIMMYT et ICARDA. Ce dernier groupe se caractérisait par une faible diversité génétique.*
Les résultats ont permis de faire ressortir les déterminants de la diversité génétique actuelle du blé dur à l'échelle mondiale : des conditions climatiques et des facteurs historiques dans les zones de culture, d'une part, et une tendance à la sélection internationale, d'autre part.

Mots-clés. *Loci de la gliadine – Diversité génétique – Géno-géographie – Blé dur.*

I – Introduction

The application of polymorphic DNA markers in biodiversity and phylogenetic studies in plants has become a routine research procedure now (Agarwal *et al*., 2008). However, in addition to

DNA based methods some polymorphic proteins could be successfully used. Such markers are probably less modern and advanced genetic tools than DNA, but also very informative and useful. For more than 30 years the Plant Genetics Department of the Vavilov Institute of General Genetics Russian Of the Academy Of Sciences has been studying polymorphism of gliadins – the wheat seed storage proteins. These proteins are very readily available and cheap to extract as markers, and allow the exploration of genetic differentiation among wheat cultivars as well as internal genetic structure of each cultivar involved in the analyses (Novoselskaya-Dragovich *et al.*, 2011). The genetics of gliadins is well studied and documented. Being separated by acid polyacrylamide gel (PAG) electrophoresis, gliadins form an electrophoretic spectrum containing about 40 distinct bands All these protein bands are coded by individual gliadin-coding genes in clusters and located on short arms of the chromosomes of the 1st and 6th homeological groups (Wrigley and Shepherd, 1973). Tetraploid durum wheat has 4 loci of gliadin-coding genes – located on the chromosomes 1A, 1B (loci *Gli-1*) and 6A, 6B (loci *Gli-2*) (Joppa *et al*., 1983). Each locus usually contains more than one gliadin-coding gene and controls more than one band in the typical electrophoretic spectrum. The genes gathered in one locus are closely linked genetically and are even often separated by retrotranspason elements (Gu *et al*., 2004). There is no recombination in the gliadin-coding locus. Consequently, the gliadin proteins controlled by such gene clusters are inherited together as a single Mendelian trait (Metakovsky *et al*., 1984). Such group of proteins were named as blocks of gliadin components and it is possible to discriminate all four blocks which form total electrophoretic spectrum of durum wheat gliadins (Kudryavtsev, 1994). Due to the polyallelism in gliadin-coding loci, allelic variants of blocks of gliadin components differ in mobility, staining intensity, and amount of their components (Metakovsky *et al*., 1984). That is the reason why different wheat cultivars display distinct gliadin spectra – almost each cultivar has its own, unique electrophoretic spectrum of gliadin.

In this study we applied gliadin markers to study a global collection of modern breeding cultivars of durum wheat.

II – Material and methods

To estimate the global diversity of durum wheat *Triticum durum* Desf., we examined 563 cultivars, which were developed mostly from the 1940s to the 1990s, and 28 landraces from 45 countries. Grains were obtained from the collection of the N.I. Vavilov All Russian Institute of Plant Industry and from our colleagues from different countries and organizations. Eight to one hundred grains were examined for each accession. Gliadin was extracted with 70% ethanol; polyacrylamide gel electrophoresis was carried out by a standard method (Metakovsky and Novoselskaya, 1991).

The allelic variants of gliadin component blocks were identified and designated according to available catalogs and an accepted system of allele designation (Kudryavtsev, 1994; Kudryavtsev *et al*, 1996; Melnikova *et al*., 2012). Genetic diversity for the loci of gliadin-coding genes was estimated according to Nei (Nei, 1973) as $H = 1 - \Sigma pi$ where H is Nei's index of genetic diversity (per locus) and pi is the allele frequency for the locus. Statistical analysis was performed using the Statistica (StatSoft) software package. To estimate the genetic similarity for groups of accessions from different regions and countries, we computed the Euclidean distances on the basis of allele frequencies and performed a clustering according to Ward (Ward, 1963).

III – Results and Discussion

In total 119 alleles for 4 gliadin-coding loci of durum wheat were identified. Most of these alleles have low occurrence and only 15 ones were relatively frequent (more than 5%). The most common alleles for four loci were: *c* (frequency 0.42), *g* (0.17), and *b* of the *Gli-A1*^{d} locus; *c* (0.49), *a* (0.30), and *b* (0.12) of the *Gli-B1*^{d} locus; *a* (0.21), *g* (0.21), *b* (0.18), and *o* (0.14) of the *Gli-A2*^{d}

locus; and *h* (0.45)and *a* (0.25) of the *Gli-B2*^{d} locus. At the same time, it was found that allele frequencies vary significantly in material from different countries. For example the allele *Gli-A1g* has frequency 0.17 in whole world collection. In Italian durum wheats it is rather rare allele with the frequency 0,07 but in Russian germplasm it is predominant allele having frequency 0,6 in old breeding varieties and 1.0 in modern ones (Melnikova and Kudryavtsev, 2009).

The genetic distances between cultivars from different countries were calculated using routine statistical procedure: On the basis of the allele frequencies we computed the Euclidean distances between the sets of national cultivars and performed clustering according to Ward. Three distinct clusters of durum wheat cultivars were isolated (fig.1).The first cluster join durum wheat accessions from Australia, Spain, Tunisia, Azerbaijan, Portugal, Turkey, the Republic of South Africa, Israel, Moldova, Bulgaria, India, Uzbekistan, Egypt, Cyprus, Syria, Bolivia, Peru, Armenia, Iraq, Iran, Hungary, Romania, and Georgia. The second cluster included the accessions of Austria, the United States, Canada, Kazakhstan, China, Russia, Ukraine, Germany, Poland and Yugoslavia. The third cluster included the accessions of Algeria, Morocco, Italy, Argentina, Chili, San Marino, Ethiopia, Greece, Jordan, Mexico, France, and Kenya.

The clusters (or groups) significantly differed in allele frequencies. In the case of the *Gli-A1* locus, the most common alleles were *b* (frequency 0.25) and *c* (0.25) in group I, *g* (0.45) in group II, and *c* (0.85) in group III. In the case of the *Gli-B1* locus, higher frequencies were observed for allele *c* (0.45) in group I, *a* (0.68) in group II, and *c* (0.75) in group III. In the case of the *Gli-A2* locus, the most common was allele *g* (0.31) in group I, allele *a* (0.45) in group II, and allele *b* (0.32) in group III. In the case of the *Gli-B2* locus, higher frequencies were characteristic of allele *h* in groups I and III (0.44 and 0.65, respectively) and allele *a* (0.58) in group II.

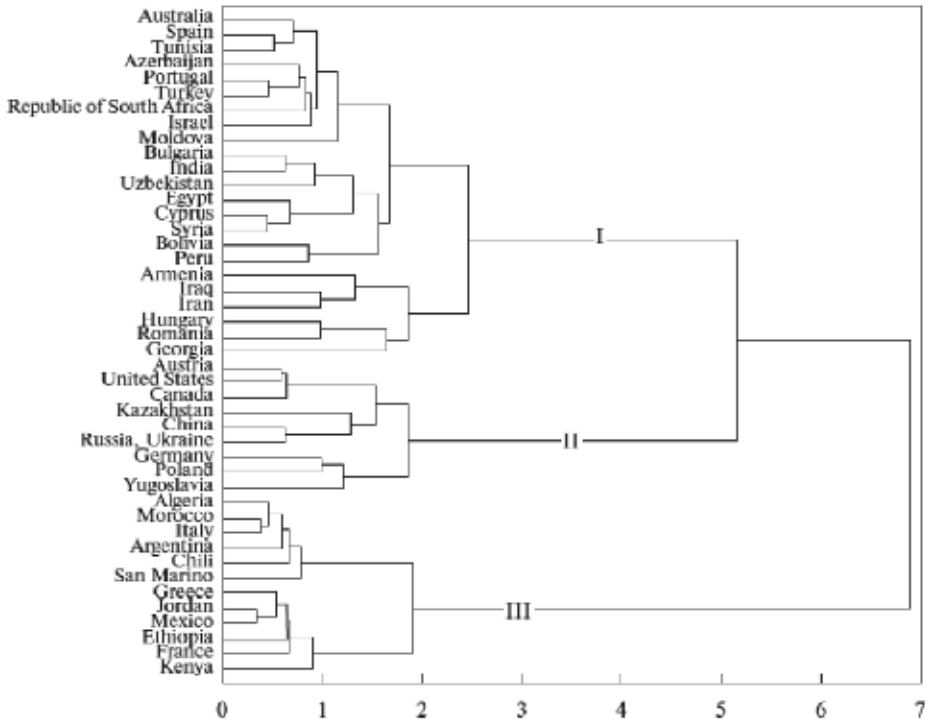

Figure 1. Clustering of the samples of durum wheat accessions from different countries by alleles of the gliadin-coding loci.

The Nei heterogeneity indexes (*H)* were calculated for each country and for each group (fig.2) On this diagram the countries are arranged according to the diversity level and shaded according to their group attribution. In general, genetic diversity was higher (0.64 on average) in the accessions of group I and lower (0.42 on average) in the accessions of group III. The genetic diversity indexes greatly varied in the cultivars of group II, averaging 0.5.

It is well known that the genetic diversity of cultivated plants usually is higher in the historical centers of origin or secondary diversification (Vavilov and Dorofeev, 1992). So looking on this graph we can suppose that there were at least two historical centers of durum wheat diversification: the first one is in the Mediterranean region, and the second one is in Russian and Ukrainian steppes. In a separate study, we demonstrated that this steppe ecotype of durum wheat could be brought from South Russia or Volga river region into the territory of ex-Yugoslavia by ethnic Bulgarians who migrated into Balkans after the Great Bulgaria disintegration about one thousand years ago and then evolved there independently (Melnikova *et al*., 2010). We know also that durum wheat of Canada, China and USA were bred not more than one hundred years ago based on Russian durum wheat germplasm. This is an explanation why the genetic diversity is wide in Russia, Ukraine and ex-Yugoslavia and relatively narrow in other countries of the second group. As to the third group of cultivars, it seems that we are dealing with the most advanced breeding cultivars which substituted completely the local durum wheat germplasm of these countries (if such landraces existed before). At least, this can be surely affirmed concerning Ethiopian and Italian durum wheats. We have studied old landraces of these countries and found cardinal genetic differences between new and old cultivars (un published results). Probably the genetic diversity in this group was formed also as a result of breeding activity of the International Centers like CYMMIT and ICARDA.

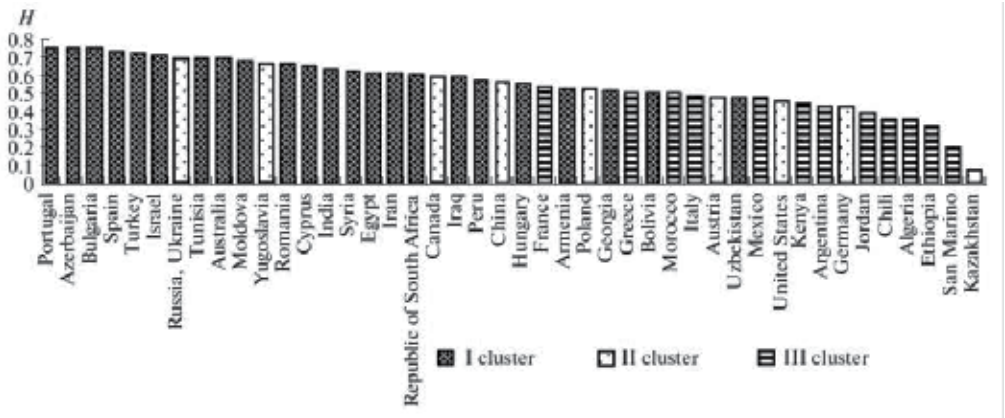

Figure 2. Genetic diversity *H* for the accessions from different countries. The clusters are indicated by column filling.

All these results and our observations bring up again the question of genetic erosion in modern durum wheats. In general, the genetic erosion has two aspects of manifestation. The first one is the loss of genetic heterogeneity and the second one is the loss of specific, local alleles due to their replacement by foreign ones. For the countries of the third cluster it is clear that here we deal with both aspects of the erosion - with complete change of local alleles on new ones and with the decreasing of the heterogeneity level. For the countries of first cluster it seems that genetic heterogeneity has not decreased during breeding process, however in this case we can observe certain signs of genetic erosion defined by the allele loss or exchange. The same situation is for Russian durum wheat (second group). The most clear erosion was shown earlier for *Gli-A1* locus. The Russian landraces displayed wide allele diversity for this locus, but now we have only one allele *g* (Melnikova and Kudryavtsev, 2009).

At least one important practical conclusion could be deduced from these results: Evidently durum wheat cultivars form two evolutionary old groups (the first and the second ones) that are adapted to different climatic conditions and represent two different ecotypes of the species. Therefore, the national agricultural research centers should consider this fact and develop breeding strategies based on use of proper donor genotypes which genetically belong to the group appropriate for local climatic conditions. As to the third group, cultivars it could be supposed that they belong to the same agroecological type as the first group of cultivars but differ from them with some technological characteristics. In this case we deal with clear trend of globalization in durum wheat breeding which is stimulated by practical needs but result in genetic erosion.

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Session 2

Genetics resources and durum wheat germplasm enhancement

Broadening the genetic bases of durum wheat

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Abstract. A large reservoir of genetic diversity is available in the *Triticum turgidum* sub-species. This diversity can be used in breeding programs but the method by which it could be introgressed in the elite durum pool is challenging once the objective is to cover the whole genome. *T. turgidum* accessions are usually classified in sub-species or taxa while evidence is accumulating that a more complex genetic structure can be revealed using no *a priori* approaches. Wild and cultivated emmers are the most diverse compartment and some cultivated emmers are strongly differentiated from naked wheats while other emmers are closer to the current durum. Recent and successive bottlenecks (in the XXth century) explain a large part of the structuration of the modern durum pool and the loss of molecular diversity. The use of *T. turgidum* germplasm in classical genealogical breeding may lead to some disappointments if a quick return is researched while some advances in breeding elite lines can be achieved with more recombination and selection. Evolutionary Pre-Breeding appears as a valuable alternative. Building and managing composite cross populations for a long period leads to in innovative genitors, with an enriched allelic diversity and reduced long range linkage disequilibrium.

Keywords. *Triticum turgidum* – Durum – Pre breeding – Structuration – NGS – Linkage disequilibrium.

Élargir les bases génétiques du blé dur

Résumé. *Les sous-espèces de Triticum turgidum représentent un réservoir important de diversité génétique. Cette diversité peut être exploitée dans des programmes de sélection, mais la technique d'introgression dans le pool du blé dur élite pose des problèmes si on vise à couvrir l'ensemble du génome. Les accessions de T. turgidum sont généralement classées dans des sous-espèces ou taxons alors qu'il semble désormais évident qu'une structure génétique plus complexe peut être révélée sans utiliser des approches a priori. L'amidonnier sauvage et l'amidonnier cultivé sont les plus diversifiés et certains amidonniers cultivés se différencient beaucoup des blés nus alors que d'autres sont plus proches du blé dur actuel. Des « goulots d'étranglement » récents et successifs (au cours du XXe siècle) expliquent en grande partie la structuration du pool du durum moderne et la perte de diversité moléculaire. L'utilisation des ressources génétiques de T. turgidum dans la sélection généalogique classique peut se solder par un échec si on s'attend à un résultat rapide alors que certains progrès en matière d'amélioration des lignées élites peuvent être obtenus en se focalisant sur la recombinaison et la sélection. La pré-sélection évolutive s'avère être une alternative intéressante. La construction et la gestion à long terme de populations croisées composites permet d'obtenir des géniteurs innovants, avec une plus grande diversité allélique et un déséquilibre de liaison plus limité.*

Mots-clés. *Triticum turgidum – Blé dur – Pré-sélection – Structuration – NGS – Déséquilibre de liaison.*

I – Introduction

Durum wheat belongs to the family of the AABB tetraploid wheats (*Triticum turgidum* spp.), where others cultivated forms such as emmer, polonicum and many others taxa co-exists (Bozzini, 1988, Nesbitt and Samuel, 1996). A lot of work has been carried out to describe and explain this current diversity (Özkan *et al*., 2002, 2011, Kilian et al 2009, Luo *et al*., 2007, Thuillet *et al*., 2005, Haudry *et al.,* 2007). All these domestic forms derive from a common wild ancestor, the wild emmer, *Triticum dicoccoides* (Kilian *et al*., 2009). Among the cultivated forms, emmer, *T. dicoccum* appears as the current remnant form of the first domesticated taxa developed by

the first farmers 10 000 years ago in the Middle East (Zaharieva *et al*., 2010). It presents hulled grains and has lost the seed dispersal habit of its wild ancestor. Grains have also increased in size and their shape have dramatically evolved from a triangular to a more round section with a reduction in the grain length (Zaharieva *et al*., 2010). Dicoccum spread from its center of domestication and spread westward and eastward giving rise to number of other different hulled taxa. A more recent transition (7000 years ago) has led to the fixation in new taxa of an innovating trait, the free threshing of the kernels (Feldman and Kislev, 2007). The free threshing wheat group, sometimes improperly designed as naked wheats is large and morphologically diversified and the current durum wheat is its most representative and farmed member (Bozzini 1988, Kilian *et al*., 2009).

Evolutionary factors such as spread and selection for the adaptation to new environments and varied farming practices, rise and fixation of new mutations, gene flows, seeds exchanges among and between farmers communities and lately modern breeding for adapting cultivars to the intensification and specialization of the crop production had a profound impact on cultivated plant growth, development and physiology (Alonso-Blanco *et al*., 2009) agronomic performance. Those evolutionary factors also deeply affect the level of genetic diversity between and among each group (Spilane and Gepts, 2001 for a review, Buckler and Thornsberry 2002, Luo *et al*., 2007, Thuillet *et al*., 2005, Haudry *et al*., 2007, Laidò *et al*., 2013 for *T. turgidum*).

The complex genetic landscape of old and modern *T. turgidum* wheats resulting from these 120 centuries of evolution has been partly elucidated. The major split appears between hulled wheats that constitutes diversified genetic pool and free threshing wheats, that tare comparatively very poor in allelic diversity (Thuillet *et al*., 2005, Haudry *et al*., 2007). Diversity is usually seen as an essential resource for breeding (Tanksley and Mc Couch, 1997, Acosta-Gallegos et al 2007, Cooper, Spillane and Hodgkin, 2001, Spillane and Gepts, 2001), especially for new and unpredictable environments., Exploiting this “lost” or “neglected” diversity of hulled wheats for breeding elite cultivars is thus very attractive (FAO, 1996). This diversity is classically screened to seek for new resistance, find adaptation to harsh environments or to detect capacities to uptake more resources (water, light and nutrients) from the environment (Tanskley and McCouch, 1997).

However, the exploitation of this diversity is very challenging since the gene pools spanning these 12000 years of evolution have been diverging for growth habits, adaptation to new cropping practices and to very different environments, from the harsh and competing condition in the wild to the highly controlled and fertile conditions of a modern field. In maize, it has been suggested that 4% of genes experienced a selective episode from the wild form to the crop (Wright et al 2005). In durum the transition from wild to domesticated also involved numerous QTLs (Peleg *et al*., 2011) and many physiological pathways have been differentially tuned (Papa, 2013). Mixing alleles selected under different conditions may therefore results in some physiological incompatibilities at the whole genome level, since many traits are constrained by contradictory trade-offs, e.g., the classical apparent and strong negative correlation between productivity and protein content (Bogard *et al*., 2010).

The knowledge of the genetic structure of diversity of the *T. turgidum* compartment taken as a whole, the creating of a pre-breeding germplasm gathering the diversity of the wild and the primary domesticated relatives, and the use of new technologies for its exploration and valorization is challenging. In this lecture note, we will first briefly expose recent work on the genetic structure and diversity of the *T. turgidum* gene pool without any prior on the different taxa. Then we will sum up some results obtained during a classical breeding program, lead by collaboration with French private companies, that included wild accessions, and old landraces. Eventually, we will show how the concept of evolutionary breeding (Suneson, 1956, 1969, Brown *et al*., 1990, Phillips and Wolfe, 2005, Wolfe *et al*., 2008) can be extended to the pre-breeding of durum wheat by creating and monitoring composite cross population to broaden the genetic basis of durum wheat.

Finally, we will detail how high-throughput sequencing technologies can be used to detect the allelic diversity introgressed in such composite populations. More generally, we are convinced that current breakthroughs in massive DNA sequencing and in massive genotyping relying on thousands of single nucleotide polymorphisms (SNPs) (Kilian and Graner, 2012) are preparing an avenue for the use of these so far neglected diversity in pre breeding activities (Hajjar and Hodgkin, 1997).

II – Sampling diversity : links between genetic structure of *T. turgidum* and erosion of diversity

Many previous studies on the structure of *T. turgidum* genetic diversity relied on *a priori* assignation of the samples to the different taxa, based on discriminant morphological traits. This *a priori* classification was used to study the differentiation between taxa and to compare their levels of genetic diversity. But as the taxa discriminant traits may be based on very few major genes, they may not reflect the shared ancestry or the divergence within and between groups (i.e., common morphological traits may have arisen through different history), and the classification may conceal a very different genetic structure. Moreover, recent or ancient crosses may have altered the initial genetic structure, by introgressing new traits in the different taxa. We applied here a clustering method identifying groups of genetically related individuals without any a priori on the origin of those individuals (DAPC, discriminant analysis of principal components, Jombart, Devillard, and Balloux 2010). We then projected the individual taxon information on the groups obtained by the classification procedure.

Our sample was made up of 492 individuals: 52 *T turgidum* sp. *dicoccoides (*DD), 52 *T. turgidum sp. dicoccum* (DC), 29 *T. turgidum sp. polonicum* (PO), 33 *T. turgidum sp. turgidum* (TU), 252 *T. turgidum sp. durum (*DR) covering traditional landraces and elite varieties mostly from the French catalog and 33 *T. turgidum sp. carthlicum* (CA) on which we firstly checked the ploidy level using flow cytometry. For these latter, we kept only 4X accessions since carthlicum accessions may count (2n=4X=28 chromosomes, 4X) or (2n=6X=42 chromosomes, 6X) (see Thuillet *et al*., 2005 for details). Fourteen microsatellites locus (table 1) were used to genotype the whole sample on a capillary sequencer. The ADEGENET R package was used for the discriminant analysis of the groups (Jombart, 2008).

Table 1. List and position of the 14 microsatellite locus used to genotype the 457 accessions.

Locus	Chromosome location	Locus	Chromosome location
Xgpw7577	1B	Xgwm601	4A
Xgwm312	2A	Xgwm495	4B
Xgwm257	2B	Xgwm234	5B
Xgwm374	2B	Xgwm193	6B
Xgwm413	2B	Xgpw2103	7A
Xgwm2	3A	Xgwm297	7B
Xgwm285	3B	Xgwm537	7B

Nine groups were detected using the procedures defined by Jombart *et al.*, (2010), and the distribution of the different a priori taxa among groups is plotted figure 1. Taxa have been sorted according to their relative level of Nei's diversity. This suggests an historical interpretation.

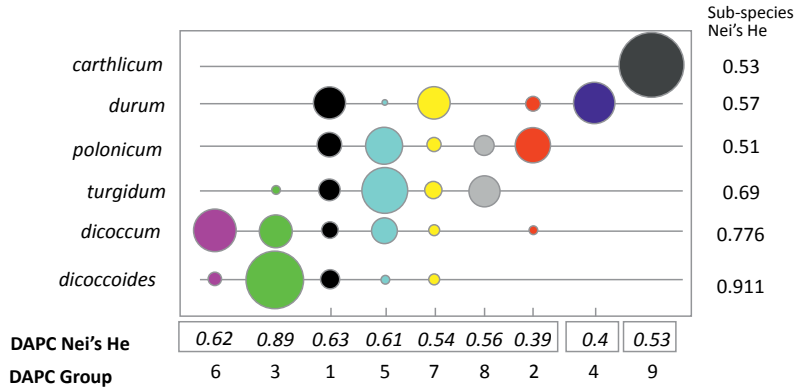

Figure 1. Distribution of the *a priori* taxon assignation of 492 *Triticum turgidum* spp. accessions within the DAPC groups obtained by a discriminant analysis of principal components obtained from 14 microsatellites locus (DAPC). The areas of the circle are proportional to the relative proportion within taxon. Nei's He are the Nei diversity index.

Dicoccoides is mostly present in DAPC group 3, very few accessions being attributed to other groups for this sub-species. It may be seen as the basal group of the turgidum species with the highest level of diversity (He=0.89). A significant fraction of cultivated emmer accessions also belongs to this DAPC group 3, they could be considered as the closest cultivated emmer to the wild emmer. The DAPC group 6 is mostly built on a portion of the sampled cultivated emmers and only a tiny portion of wild emmer accessions also belongs to this group. Note that some wild emmers in the DAPC group 6 could have been misclassified or be somewhat introgressed by domesticated emmers (Luo *et al*., 2007). No other sub-species contribute to this DAPC group 6 which appears then to be relatively disconnected of the rest of the cultivated turgidum sampled (graph not shown). This remarkable result suggests that this group did not participate to the emergence of the free-threshing wheat and remained isolated from the other cultivated sub-species. It has a relatively high level of diversity (He= 0.62). No obvious geographic localization could explain this structuration among cultivated emmers. Recent work support a polyphyletic origin of domesticated emmer from different sources of wild emmer (Civáň *et al*., 2013) and our results are somewhat congruent with this assumption.

The groups 1, 5, 7 and 8 have a complex composition which underlines the difficulty to resolve the taxonomy of *T. turgidum* in terms of history, using molecular markers. Namely, ssp. polonicum, turgidum and durum do not correspond to clear and distinguishable genetic entities. The group 1 appears relatively polymorphic (He=0.63) and spans all the sub species with the exception of carthlicum. This group may descent from the original domesticated genepool from which evolved the free threshing forms. Its complex structure is closed to that of the group 7 except that this latter has a reduced level of diversity.

The DAPC groups 2 and 4 are made almost exclusively with durum accessions and show a strong reduction of diversity (He=0.4). All carthlicum are grouped in a specific goup, DAPC group 9, with a medium level of diversity. These results are in strong agreement with the recent work of Laidò *et al* ; (2013). Our data does not permit to elucidate the origin of this group. *Triticum carthlicum* spikes resemble those of *Triticum aestivum* L. rather than those of free-threshing tetraploid wheats (Haque *et al*., 2011). The existence of 6X accessions in co-existence with 4X accessions suggests that this sub-species has had a specific evolutionary pattern and it may result from recurrent intercrossing between 4X and 6X specific gene pools in Georgia, Armenia, Azerbaijan, northern Iraq, and Iran where it is still cultivated (Metakovski *et al*., 1989).

In brief, like other authors in recent works (Civáň *et al*., 2013, Laidò *et al*., 2013), we found that *T. turgidum* subspecies diversity should not be based only on keys determining their sub species status. Like in molecular phylogeny, morphological resemblance or difference may be or may be not linked to a common or divergent evolutionary history. More works should be dedicated to a fine analysis of the origin of the different emmers, their potential and respective implication in the formation of the naked wheats. The understanding of origin and evolution of carthlicum also deserves deeper and appropriate sampling. Indeed these wheats are a very valuable source of traits for durum breeding. They have resistance to drought, frost, and resistance to ergot infection.

III – Impact of modern breeding on durum wheat structure and genetic diversity

Focusing on durum wheat, a more precise and recent pattern appears. We split the sample before and after 1950. After 1950, varieties were distributed by decades according to their registration in the French catalog. Their distribution between the different DAPC groups and their relative Nei's heterozygosity are plotted on figure 2. Landraces clearly belong to the DAPC group 1, the somewhat undifferentiated group described before. In 1950, two main groups 7 and 4 appear and a minor group (group 2) as well. These groups clearly experienced a strong reduction of diversity, the group 4 being the less diverse. A temporal evolution is also observed from 1950 to the post 1990's varieties. If the Nei's heterozygosity was around 0.56 in landraces and in the 1950's varieties, it regularly decreased and is now as low as 0.4, less than half of that found in dicoccoides (group 3). Modern breeding for the transition to short stature but probably also more recent effort for developing varieties with high quality standards (e.g., selection on the gliadin profile) led to a strong and continuous reduction in genetic diversity. Selection in interaction with genetic drift, probably at the whole genome level (selective sweep like in bread wheat (Cavanagh *et al*., 2013)) is likely responsible of this dramatic reduction of genetic diversity in modern cultivars. This confirms previous results (Thuillet *et al*., 2005) and more recent work on durum (Laidò *et al*., 2013). This continuing erosion of genetic diversity is alarming.

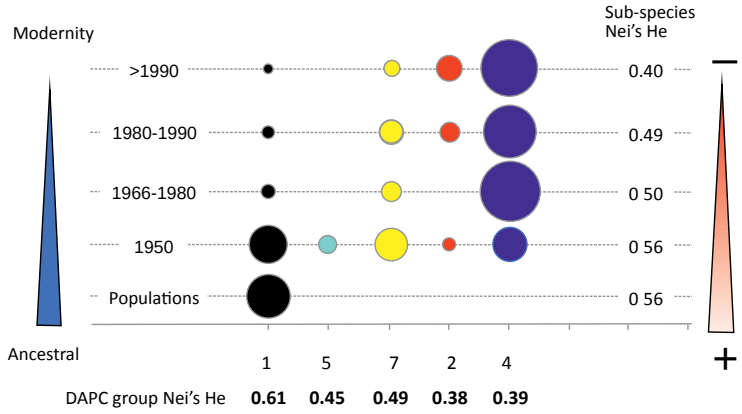

Figure 2. Distribution of the assignation of 252 *T. turgidum sp. durum* accessions within the DAPC groups obtained by a discriminant analysis of principal components obtained from 14 microsatellites locus (DAPC). These accessions are varieties sorted according to their period of release (see text for detail.). The areas of the circle are proportional to the relative proportion within time period. Nei's He are the Nei diversity index.

IV – Use of exotic diversity in pedigree breeding

Diversity *per se* may not be an interest even if the pledge for new alleles is attracting. Introgressing dicoccoides alleles in elite germplasm is usually realized after an identification of promising parents, a cross and successive backcrosses to eliminate undesirable chromosome fragments from the donor. This method of backcross has demonstrated a real efficiency to transfer monogenic traits, mostly resistance. In a larger view, broadening the genetic basis of a crop necessitates another, less targeted approach. Observing that the loss of diversity in modern durum wheat is really strong and assuming that wild and exotic germplasm can carry a number of valuable alleles for many traits, not easy to evaluate, or even not easy to identify at their sub species level, methods of non-targeted introgressions, guided by the idea of a broad and non-targeted introgression of new diversity have been proposed as a new pre-breeding challenge. With the help of a guilde of French durum wheat breeders (GIE Blé dur) we investigated the interests of the use of such germplasm.

During several years, more than 200 crosses have been realized between a core collection of tetraploid accessions and a set of elite genitors provided by the private partners. The core collection has been built from a 600 accessions sample by maximizing allelic richness on a set of 30 microsatellite locus used in diversity study (Thuillet *et al*., 2005). F2 seeds were distributed in a multi-site network of public (INRA) and French private partners (DESPREZ, SERASEM-RAGT, EURODUR-LIMAGRAIN, SYNGENTA, BENOIST, GAE), and a classical pedigree breeding method has been carried out to start the fixation of valuable lines that were generally used as genitors for backcrossing on durum elite lines. Several hundreds of thousands of individual F2 plants were evaluated by the partners. High throughput phenotyping for quality traits (protein content, yellow colour, semolina yield) were applied in the F4 selected families. Multi-site evaluation for frost and rust resistance were carried out on F5 families. The positive qualities of this material lied mostly in disease resistance (leaf rust, head blight, Mosaic virus (Wheat Spindle Streak Mosaic Virus, Soilborne Cereal Mosaic Virus) and a large morphological diversity. After the removal of the unfavorable undomesticated or primitive traits such as brittle rachis, hulled kernels, and tall tillers, the main caveats of this material were defaults in the kernel size and color in crosses involving *T. dicoccoides* in their genealogy, a lack of productivity and possibly an inefficient remobilization of nitrogen from leaves to the kernel during the senescence period. *T. polonicum* appeared as a very good source of kernel color and some accessions had good roots implantation. These primary and empirical observations justify new studies about the impact of domestication and recent breeding on the *durum* plant physiology, its N economy during its whole lifespan from uptake to remobilization. Domesticated and elite favorable alleles at some key locus, yet to be identified, may be important to explain a good end-use quality and productivity. Due to this lack of productivity, most of private breeders finally stopped this program since the agronomical level of the advanced inbred lines was not sufficient to register elite varieties for the current fertilization and treatment practices.

To keep up recombination and pre-breeding effort, INRA and Agri-Obtention went forward for more years with a policy of recombining several promising lines together as a priority instead of backcrossing recurrently on elite durum. The resulting most promising material increased in productivity, kept a good level of protein content and showed a high level of leaf rust resistance in untreated, and in treated as well, experiments (fig 3). Two lines are currently (in 2013) following the French registering procedure and we hope they will finally be registered in 2014-2015. Their advantage seems to lie in their good level of leaf rust resistance even under treated conditions that may itself come from the introgression of new major genes of resistance to this disease. In this case, this advantage might only last some years until new virulent strains of leaf rust become adapted to these new sources if the new lines finally succeed in being cultivated on a large area. This demonstrates that genetic advance for yield and quality relies on complex interactions between sanitary aspects, potential productivity in varying environments. These results clearly

indicate that pursuing efforts in long term recombination and selection could permit a valorization of exotic germplasm for durum wheat breeding. Productivity and quality traits can be improved, either via better exploitation of the resources and adaptation to harsh environment but also by using new patterns of genetic resistance to main disease. In the present case, investigations are necessary to identify the genetic basis of this enhanced leaf rust resistance in order to assess their sustainability. If finally registered, these lines will constitute the demonstration that the erosion of genetic diversity in the modern elite pool of durum wheat can be stopped and that new diversity will be available for all breeders. Whole genome investigation will rapidly permit to estimate in which chromosomic regions new alleles are brought by these new lines.

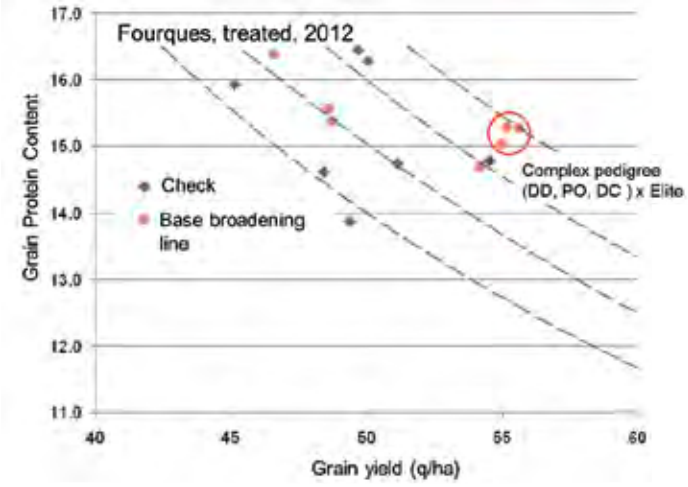

Figure 3. Comparison of agronomic performances of check lines (recent elite French durum cultivars) in blue versus lines derived from the pedigree base broadening program led by INRA Montpellier. Data are from Fourques in 2012 in a treated experiment. Dashed lines are the gradient of the grain yield x protein content product, i.e., the yield in protein/ha. The circled lines apply currently for a registration to the French durum wheat catalog.

V – Evolutionary Pre-breeding : presentation of the pre-breeding population of durum wheat

The term “pre-breeding” refers to the transfer of genes from related wild ancestors or from ancient varieties to breeding material (FAO, 1996). Pre-breeding activities span a very large set of methods, from interspecific crosses followed by recurrent back crosses to the management on the long term of composite cross populations. In this latter case, recombination and soft selection are used to introgress exotic material in an elite gene pool. Barley composite cross, started by Suneson (1956, 1969) and whose evolution was described by Allard and many others brought information about the very dynamic evolutionary processes at work in such long term monitored composite cross. More recent work on bread wheat confirmed that heterogeneous gene pools can adapt rapidly to different situations including climate gradients (Le Boulc’h *et al*., 1994, Goldringer *et al*., 2006, Rhoné *et al*., 2010), pathogen pressures (Paillard *et al*., 2000a, 2000b) maintaining their genetic diversity (Enjalbert *et al*., 1999). The lessons drawn from such experiments are that natural selection leads to adaptation to local condition (climate, disease) but also to competition between different architectural traits (Goldringer *et al*., 2001). Then, increasing the plant height in response to light competition may also drive to the fixation of bad alleles for productivity, as observed in all wheat populations in which semi-dwarf alleles disappeared (Le Boulc’h *et al*., 19994) and the harvest index evolved negatively.

Creating and managing composite cross (CC) in the long term could be a really interesting pre-breeding method but methodological work should be devoted to understanding the interplay between recombination, natural and human selection and genetic drift. Empirical and efficient rules for managing and improve such composites would permit to avoid undesirable evolution and create interesting new germplasm. Their role is to introgress massively interesting diversity at a whole genomic level. This needs that recombination and selection are finely tuned and like the CC cross of barley they have to be maintained for an indefinite number of generations and constitute an evolving reservoir of diversity (Henry *et al*., 1991). On durum wheat, our laboratory launched a pilot experiment; we used a durum wheat population in which segregates a nuclear male sterility gene donated by a former French INRA scientist, François Kaan. The male fertile allele Ms is dominant on the male sterile allele. Plants can be either hermaphrodites (Ms/Ms or ms/ms) or male sterile (ms/ms). A collection of flowering *T.dicoccoides*, *T*. *dicoccum*, *T*. *polonicum* accessions were crossed in 1997 on male sterile plants of this population. The resulting seeds were used to found a pre-breeding composite cross, the INRA Pre breeding durum wheat population (hereafter named IPBDWP).

Our aim was to combine recombination by promoting outcrossing and rapid fixation of favorable combinations by permitting selfing. The population is thus monitored under a mixed mating system thanks to the male sterility gene. This population is being reproduced as follows: every year, once the flowering starts and until harvest, the tallest tillers are eliminated to avoid a detrimental evolution of the IPBDWP due to competition of tall plants on short plants, male sterile spikes are identified by their wide glume opening at the blooming stage and marked by a red twist. These marked spikes are harvested and threshed in bulk separately from the selected fertile spikes. Hermaphrodite spikes are chosen visually at harvest for their shape, vigor and health status and then threshed in bulk. The new generation is composed then by 20% of seeds coming from the marked male ms/ms sterile spikes (outcrossing portion), 70% of the selected hermaphrodite spikes Ms/ms and Ms/Ms (selfing portion) and 10% coming from the best lines selected in the pedigree selection scheme presented above to bring new diversity and agronomic performance. The population introgressed a new diversity and is experiencing recombination at each generation, fixation of new combinations under the combined effect of anthropic selection for a return to agronomical conditions, natural selection for adaptation to the environment and of course random genetic drift. The restricted amount of outcrossing (20%) reduced also the selective pressure to adapt to allogamy which can be the major evolutionary force in such population of usually selfing crops (David and Pham, 1993). The project is now to verify the interest of such resources for breeding, either as a source of new alleles or gene combination or as a tool for deciphering the genetic basis of traits.

Recently interest in genome wide association studies (GWAS) pointed out the value of diversified panels to accurately detect chromosomal segments carrying valuable alleles for interesting agronomical traits (Maccaferri *et al*., 2010, 2012). As most of these panels are assembled from large and diverse collections, genetic structuration among accessions may lead to a high level of false positive associations. Even if several methods take into account this structuration, coping with it remains a challenge (Macafferi *et al*., 2005). The interest of evolving composite populations in the GWA approach is that the population can be seen as a reproductive unit and after several generations of partial outcrossing and effective recombination, a reduction of the genetic structuration and a consequent reduction of the statistical linkage between locus, especially those that are not closely physically linked is expected. Consequently the False Discovery Rate (FDR*), i.e.,* the ratio with spurious association between a polymorphism and a variation of a trait should decrease substantially in a composite cross compared to a panel made of lines from different geographical areas, from different periods or different breeding programs. The other interesting aspect of GWA in a diversified panels compared to biparental segregating population comes from a more robust estimation of allelic effects.

In the following, we investigated for the first time the genetic diversity content of a composite cross of durum IPBDWP and estimated the extent of linkage disequilibrium along chromosomal segments to determine whether such populations might be a good support for GWAS studies.

VI – First genomic investigations in the IPBDWP

Using New Generation Sequencing, information on thousands of candidate genes and candidate regions can be harnessed for thousands of individuals to sample genetic diversity within and between germplasm pools, to map Quantitative Trait Loci (QTLs), to identify individual genes, and to determine their functional diversity (Kilian and Graner, 2012). Here we applied for the first time in durum wheat such an approach on our population.

1. Data production and SNP detection

In 2009, 500 spikes were randomly harvested in IPBDWP and entered a 2 year fixation process. Hundred and six (106) of these lines were used to investigate the level of genetic diversity available in this composite. Seeds were germinated in growth chamber in standardized conditions and young coleoptiles were used to extract RNAs. CDNAs libraries were produced and tagged for each of these 106 genotypes. These 106 libraries were pooled, either by 24 samples or by 48 samples and sequenced on a HiSeq 2000 to produce 100 pb read pairs. Finally 813,110,268 cleaned reads were used to produce a *de novo* assembly using a bio-informatic pipeline (publication in prep). To separate homeologs between their A and B copies, we used an algorithm based on unbalanced expression ratio between the two copies implemented in the Homeosplitter software (Ranwez *et al*., 2013). The good split of copies were verified when possible by mapping reads sequences on *T. urartu and Ae. speltoides* transcriptomes produced and assembled by the same protocols. Finally, only good quality SNPs with no heterozygous excess were used in this preliminary study to evaluate the level of diversity and the extent of the linkage disequilibrium in IPBDWP.

Nucleotide diversity was estimated as proposed by Tajima (Tajima 1983). To estimate the decay of linkage disequilibrium, it was first necessary to obtain the position of the SNP on a reference genetic map. In this preliminary work, no segregation data were available for these SNPs in durum wheat and we used external and public data from bread wheat. Contigs containing SNPs were blasted against the sequence of the 9K SNP array defined and mapped on bread wheat polymorphism using several segregating populations (Cavanagh *et al*., 2013). To eliminate possible errors and to ensure appropriate genome localization, we kept only SNPs for which the genome localization was identified identically by mapping on bread wheat (Cavanagh *et al*., 2013) and properly assigned to a donor diploid species in our data (*T. urartu* for the A genome, *Ae. speltoides* for the S genome). Pairwise linkage disequilibrium was then computed and plotted against genetical distance between locus, distance estimated from the bread wheat data (Cavanagh *et al*., 2013).

2. Diversity and linkage

Finally, 13,911 SNPs on 5980 locus fulfilled the conditions to be kept for the study*, i.e*., no excess of heterozygous individuals. The nucleotide diversity π was computed for these 13,911 SNP and the obtained values vary between $\pi \sim 0.5$ to $0.9\ 10^{-3}$ per base pair. From previous evaluation on 21 genes (Haudry *et al*., 2007), estimations for the wild *dicoccoides* are $\pi \sim 2.5\ 10^{-3}$, $\pi \sim 1.3\ 10^{-3}$ for *dicoccum* and $\pi \sim 0.4\ 10^{-3}$ for all durum. It seems thus that if the population has effectively a good level of diversity compared to the whole durum sub-species it is still far from what it could have been if a large part of diversity from wild and cultivated emmer had been successfully introgressed in IPBDWP.

Out of the 5980 contigs, 553 blasted on the sequences of 9k bread wheat SNP arrays, giving a total of 1858 SNPs for studying the linkage disequilibrium decay. Discarding ambiguous SNPs, attributed to different genomes by the bread wheat mapping and by the proximity to one of the diploid ancestor, 1577 SNPs could be eventually used to evaluate the decay of the linkage disequilibrium in IPBDWP. Figure 4 illustrates this decay on the chromosome 1A, the other chromosomes showing very similar patterns.

As expected, the disequilibrium values between pairs of SNPs located in the same contig have the highest value, but their average value is far from the maximum value of 1, which would have mean complete linkage within genes and a low haplotype complexity. In this presentation paper, this apparent lack of linkage has not been fully investigated but it could mean that introgression of wild and exotic accessions has effectively enriched the haplotype diversity at very short genetic distance. Nevertheless, if some *de novo* assembled contigs are still chimeric between the A and B genome, low spurious r^2 values between some pairs of homeologous SNPs could decrease artificially the within contig linkage estimation. Between different contigs the decay of the linkage disequilibrium is decreasing very fast and is lower than the value found by Maccaferri *et al. (*2005) using microsatellites. A threshold value for r^2 around 0.1 is found after 70 cM very close to the value (dashed line) found for SNPs located on different chromosomes. Naturally, deeper investigations are needed to ascertain these linkage disequilibrium patterns but anyway this preliminary data suggest that evolving composite cross such as IPBWDWP could have very good and interesting properties for detecting markers closely linked to causal polymorphisms. They could constitute then very good alternative to association panels.

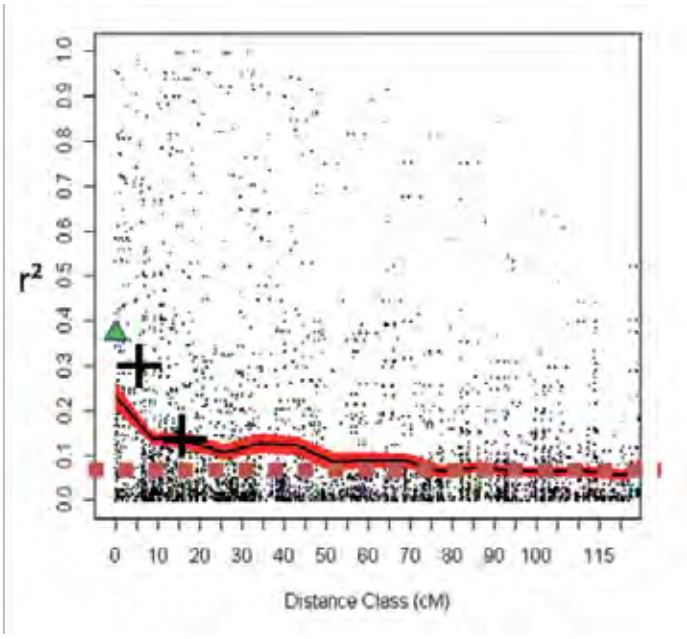

Figure 4. Evolution of linkage disequilibrium (r^2) between pair of SNPs located at different distances (cM) on the chromosome 1A in the INRA pre breeding Durum wheat population (IPBDWP). Mapping positions of the SNPs on the chromosome 1A were predicted by blasting contig sequence containing the SNPs on the sequence of the mapped markers of the 9K bread wheat micro array (Cavanagh *et al*., 2013). The green triangle is the average value of r^2 when the two SNP of a pair are located in the same contig. Black crosses are the values of r^2 estimated by Maccaferri *et al*. (2005) at similar distance classes in a durum wheat panel. In red, the within segmental average value of r^2 for 20 subsequent windows of equal genetic distance spanning the whole chromosome. The dotted brown line is the average value of pairwise r^2 when the two SNPs were assigned to different chromosomes.

VII –Conclusions & Perspectives

The modern elite pool of durum wheat has experienced several severe reduction of genetic diversity, and there is evidence that this genetic erosion is still continuing. The recent cultivars share a lot of common alleles and their deviation from the historical genetic background of the species seems accelerating at least until the end of the XXth century and for the French elite catalog. The same trend has been observed in the sample of durum recently investigated by Laido *et al*. (2013). If a lack of diversity in the elite pool is susceptible to impair future advances in the development of a sustainable durum wheat production, for which disease resistances, efficient nutrient uptake capacities, growth and flowering in harsher environmental conditions will be needed, the use of the genetic diversity of the whole *T. turgidum* species may be a key element of a germplasm development and integration strategy. But this will be a real challenge. Even though many studies demonstrated the worth of the genetic diversity helld in genebanks in the whole *Turgidum* subsp, especially in the wild and cultivated emmers, the use of this valuable diversity is not easy and may not be really successful if one expects the use of valuable alleles at some major genes, such as disease resistance.

In a collaborative program between INRA and GIE Blé dur, classical breeding led to some results by using intensive back crossing after the initial cross but the selected lines were not sufficiently productive in the first place to be registered as elite varieties in the French catalog by the Private Breeders. Nevertheless, some success was obtained by persevering in recurrently crossing advanced lines with introgressed backgrounds. Productivity eventually increased and some lines might become registered varieties in a close future, probably thanks to their good level of resistance to brown rust. This tolerance to rust probably provided a yield advantage to these advanced lines in an experiment where rust attack was important. This success should be confirmed on the long range since a quick overcome of the allelic of resistance is likely in the case of their commercial development.

As an alternative to this quick use of valuable germplasm, long term evolutionary pre-breeding programs may be of a great interest for creating new germplasm, integrating new alleles, promoting recombination and soft selection in populations of reasonable population effective size. In this paper, we reported the very first results on a composite cross population of a durum wheat population with a broaden genetic basis monitored for 12 years under a 20% outcrossing mating system. This current IPBDW population appears as an interesting resource for GWAS because of its reasonable level of genetic diversity, reduced long distance linkage disequilibrium and large phenotypic variation (data not shown). We are currently accumulating phenotypic data on a large number of traits (morphology, phenology, N status of leaves and grains) to verify if the sequencing effort yielded sufficient data to detect associations. RNAseq data obtained here will be directly be used as a genotyping method (Genotyping by sequencing, GBS) but a number of missing data arose since gene expression may greatly vary among individuals. The coverage of RNA seq for each individual, the standardization of the growing conditions before RNA extraction and the development of adapted bio informatics pipelines are key elements for the success of a RNA seq GBS approach. The detected SNPs here can also contribute to the assembly of a specific durum polymorphism database that can be used to develop a micro array chip within a durum wheat consortium.

Our 20% outcrossing mating regimes clearly reduced the long distance linkage disequilibrium in the population and also probably also reduced greatly the within population genetic structure that usually creates spurious association in GWA studies using panels assembled from different genetic sources. More methodological work is needed to set the most efficient value of the outcrossing rate in order to promote effective reduction of haplotype length, reduction of kinship structuration but also to promote a rapid fixation of valuable homozygous individuals in the population. If IPBWP appears as genetically diverse compared to a durum wheat panel, its nucleotide diversity is still much lower than the potential diversity available in the exotic parents

of the composite. Selection for plant height, removal of plants showing genetic incompatibility and other unidentified selective pressures for adaptation to climate and local pathogens may explain a strong loss of diversity by linkage drag and selective sweeps around the domesticated alleles at locus determining minimum agronomical values.

If the decay of linkage disequilibrium is rather steep in the population, low levels of linkage are still present at 50 – 70 cM. This suggests that effective recombination, led by the 20% outcrossing level, was not sufficient to break rapidly and efficiently mix the elite haplotypes with the introgressed ones. Furthermore, if many genes, and not only some major genes major responsible for dramatic and apparent changes in morphology and shape, (e.g., brittle rachis), have been involved in domestication and further improvement of durum quality and agronomic performance, it is likely at the whole genome level that valuable alleles in the exotic germplasm have good chance to be regularly associated with unfavorable alleles. In this case, a more appropriate method to enrich the allelic diversity of such pre-breeding populations would have been first to promote 100% outcrossing and recombination during the first generations before starting any conscious massal selection for a return to a "durum" like morphology compatible with modern agronomical practices. Such a strategy should reduce the number, strength and extent of the selective sweeps.

In conclusion we claim that new composite populations should be created by controlled crosses of male sterile plants with wild and cultivated emmers, traditional durum, polonicum and turgidum landraces and carthlicum as well sampled to cover the whole diversity of the genetic groups described in this paper (figure 1) or discovered elsewhere. From our first experience in IPBDW, outcrossing rate should be increased to promote effective and rapid recombination to avoid strong selective sweeps. The interplay between outcrossing and selection practices should be theoretically investigated. Our selection practices in IPBDW were probably too strong to eliminate wild traits such as dispersal or asynchronous growth habits, tallness and hulled kernels. Accepting that these traits co-exist for longer period along with the domesticated phenotypes could be a key for a good introgression of larger levels of diversity in valuable pre-breeding composites. This claims for theoretical approaches to deliver methodological recipes to create, monitor and use of evolutionary pre-breeding populations.

If our population is evolving in only one environment (Montpellier; Southern France), such prebreeding composites can be used to create a network of connected populations evolving in contrasted environments. Diversifying selection on a similar genetic background may help to detect chromosomal regions involved in different adaptations patterns (Beaumont & Nichols, 1996, Enjalbert *et al*., 1999) and are very well adapted to an international collaboration. IPBDW is available for distribution, lines and associated molecular data will soon be released.

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Positive effects on yield-contributing traits associated with *Thinopyrum ponticum* chromosome segments introgressed into durum wheat

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Abstract. As a possible means of coping with the many challenges in today's wheat breeding, widening of the crop genetic basis via exploitation of alien genetic variation from wild relatives is a promising and sustainable approach. Thanks to recent progress in chromosome engineering, through which alien chromosome segments can be transferred to wheat, it is now possible to target even complex traits such as those related to the yield. During the last three seasons, under Mediterranean rainfed conditions, three durum wheat-*Thinopyrum ponticum* near-isogenic recombinant lines with distal portions of their 7AL arm replaced by 23%, 28% and 40% of alien (= 7AgL) chromatin, respectively, containing the *Lr19+Sr25+Yp* genes, were included in field trials with, first, spaced plants (2 years) and then, plots (1 year) for evaluation of 7AgL-associated effects on yield-contr buting traits. Overall, the results revealed the involvement of defined 7AgL portions in the increase of traits such as flag leaf width and tiller number/plant (23-28% portion), grain number/m^2 and spike fertility index (28-40% portion), all traits contributing to the observed higher grain yield and biomass. Moreover, parameters measured in the plot trial (phenological phases duration, fertility at anthesis, chlorophyll content), suggested the presence in the 23-28% 7AgL region of loci significantly increasing booting-to-anthesis phase and chlorophyll content during grain filling. Conversely, the 28-40% interval was found to be associated with negative effects on biomass at anthesis and post-anthesis chlorophyll content, hence on grain filling.

Keywords. Chromosome engineering – *Triticum durum* – Alien gene transfer – Breeding – Yield QTL.

Effets positifs sur les caractères liés au rendement associés aux segments chromosomiques de Thinopyrum ponticum introgressés dans le blé dur

Résumé. *Afin de faire face aux nombreux défis que pose aujourd'hui la sélection du blé, une option possible est l'élargissement de la base génétique de la culture à travers l'exploitation de la variabilité génétique d'espèces sauvages apparentées, qui semble être une approche prometteuse et durable. Grâce aux récents progrès de l'ingénierie chromosomique permettant de transférer dans le blé des segments de chromosomes étrangers, il est maintenant possible de cibler aussi des caractères complexes tels que ceux liés au rendement. Au cours des trois dernières saisons, trois lignées recombinantes quasi-isogéniques de blé dur Thinopyrum ponticum, chez lesquelles les portions distales du bras 7AL ont été remplacées par 23%, 28% et 40%, respectivement, de chromatine étrangère (= 7AgL), contenant les gènes Lr19 + SR25 + Yp, ont été utilisées pour des essais sur le terrain, dans des conditions de culture en sec typiquement méditerranéennes. Dans un premier temps, on a mis en place un certain nombre de plantes espacées (2 ans), et ensuite, on a installé des parcelles (1 an) pour l'évaluation des effets associés au 7AgL sur les caractères liés au rendement. Dans l'ensemble, les résultats ont confirmé que les portions définies de 7AgL déterminent un renforcement de certains caractères tels la largeur de la feuille étendard et le nombre de talles par plante (portion 23-28%), le nombre de grains/m2 et l'indice de fertilité de l'épi (portion 28-40%), qui contribuent tous à l'augmentation observée du rendement en grain et de la biomasse. En outre, les paramètres mesurés au cours de l'essai en plein champ (durée des stades phénologiques, fertilité à l'anthèse, teneur en chlorophylle), ont permis de conclure à la présence dans la région 23-28% du 7AgL des locus qui augmentent significativement le stade gonflement-anthèse et la teneur en chlorophylle pendant le remplissage du grain. A l'inverse, il a été démontré que la portion 28-40% est associée à des effets négatifs sur la biomasse à l'anthèse et sur la teneur en chlorophylle post-anthèse, donc sur le remplissage des grains.*

Mots-clés. *Ingénierie chromosomique – Triticum durum – Transfert de gènes étrangers – Sélection – QTL de rendement.*

I – Introduction

Due to the current 'bottleneck' caused by the restricted crop genetic base, coupled with rising climatic and social challenges for wheat production increase of crop yield acquires an even more strategic importance among the goals of today's breeding programs. After more than 50 years of intensive efforts for agronomic and genetic improvement of this crucial crop for mankind, further increments in its yield are difficult to accomplish without the application of novel breeding strategies. For complex traits such as yield, with a typical multigenic control by several quantitative trait loci (QTL), a relatively low heritability and a significant interaction with the environment, a valid approach contemplates genetic dissection of the trait and effective genotyping and phenotyping of the available natural variation. The search for loci underlying yield-contributing traits can be extended to 'non-crop' species, including wild relatives, land races, and other non-adapted genetic materials, which display a wealth of potentially useful traits for crop improvement, along with undesirable ones. Indeed, the ability to transfer only the defined, target alien genes and get rid of unwanted ones is the key to harnessing alien genetic variation, making it an effective way to counter problems of crop genetic erosion.

The wheatgrass genus *Thinopyrum*, belonging to the wheat tertiary gene pool, represents a particularly large reservoir of desirable traits for improvement of cultivated *Triticum* species. The genus includes a large number of perennial diploid to decaploid species, used for more than half a century to enrich cultivated wheat germplasm with an array of genes for disease and pest resistance (e.g., Li and Wang, 2009), for tolerance to abiotic stresses (e.g., Colmer *et al.*, 2006, Li *et al.*, 2008), as well as for processing quality (Liu *et al.*, 2008), and even yield-related traits (Singh *et al*., 1998; Kuzmanović *et al.*, 2013). A *Thinopyrum* chromosome group turned out to be particularly rich in valuable genes for wheat improvement is the one sharing homoeology with wheat group 7 chromosomes, and perhaps the most extensively targeted is the one belonging to the decaploid *Th. ponticum* (tall wheatgrass, 2n =10x = 70), originally named 7Ag (Sears 1973) or 7el (Sharma and Knott, 1966; Knott *et al.,* 1977).

Thanks to the advances in 'chromosome engineering' approaches (Sears, 1972; Ceoloni and Jauhar, 2006) useful genes/QTL from the 7Ag chromosome were succesfully transferred into cultivated wheats since the mid 20th century. In particular, several major genes or QTL of proved or potential breeding value were found to be concentrated on its long arm. When introduced into wheat cultivars in the form of substitution and translocation lines, 7Ag chromosomes of different *Th. ponticum* accessions revealed the presence of genes controlling resistance to several wheat diseases, including rusts (e.g. *Lr19*, *Sr25*; e.g. Gennaro *et al*., 2009) and scab (or Fusarium head blight, FHB; see Forte *et al*., these Proceedings), as well as genes affecting grain pigment content (*Yp*) and even yield (for review see Ceoloni *et al.*, 2013). In general, there is a limited number of examples of wild genes used to improve yield in modern cultivars, due to the narrow or, by chance gained, knowledge of yield potential of wild germplasm.

The existence of loci associated with increase in yield in wheat-*Th. ponticum* genetic stocks was initially reported by CIMMYT, on the basis of results obtained by using near-isogenic lines (NILs) of the original T4 translocation (70% of 7AgL arm inserted into wheat 7DL) into various bread wheat backgrounds (Singh *et al*., 1998; Reynolds *et al*., 2001; Monneveaux *et al*., 2003). The effect of 7AgL translocation was found to consist of increased yield, biomass and grain number per ear (10-15%) in all backgrounds studied, and, though not consistently, to be particularly evident under non moisture stress. However, no precise information was available on the position along the large 7AgL segment of the loci underlying such traits. Interestingly, the largely syntenic and colinear 7AL region contains several QTL for yield-contributing traits in both bread and durum wheat (Kuzmanović *et al.*, 2013).

With the primary aim of transferring into durum wheat the *Th. ponticum Lr19+Yp+Sr25* linked genes (Ceoloni *et al.*, 2000, 2005; Gennaro *et al.*, 2003), distal portions of the same 7AgL segment

of line T4 were separately introduced into the 7AL arm of durum wheat recombinant lines (Ceoloni *et al*., 2005). Recent results from analysis of yield and yield-contributing traits on field-grown, spaced plants of three such durum wheat-*Th. ponticum* recombination lines, carrying 23%, 28% and 40% distal 7AgL chromatin on 7AL (Fig. 1), in combination with physical and genetic maps of recombinant 7AL-7AgL chromosomes, led to delineate functional sub-regions within the 40% distal 7AgL to which genes/QTL responsible for the conspicuous increase of flag leaf area, tiller number/plant, seed number/ear, grain yield/plant and above-ground biomass could be associated (Kuzmanović *et al*., 2013, and Fig. 1). In the first two seasons of agronomic analyses (2009 and 2010), in particular, total productive tiller number per plant was significantly increased in R112-4 (+25%) and R23-1 (+16%) recombinants, in the former recombinant being associated with significant increase in biomass per plant (28%). Of special interest showed to be the increase in R112-4 recombinant of the flag leaf width (11%), together with the increase in 2010 only in grain yield (36%) and seed number (27%) per plant of the same recombinant. Consequently, R112-4 ranked as the best line among the three tested. A stable increase in seed number per plant was also observed across the two years in R23-1 (22%), though accompanied by significant decrease in thousand kernel weight (-20%). In order to validate the expression of these and additional productivity traits in plot trials, a multi-year field experiment with the same 3 durum wheat-*Th. ponticum* recombinant lines was started in Viterbo, Central Italy, and here we report results of the first year analyses.

II – Material and methods

1. Plant materials and growth conditions

Materials employed in the field trial carried out in Viterbo in the 2011-2012 season were derivatives of the 3 durum wheat-*Th. ponticum* recombinant lines represented in Fig. 1. They had been subjected to several backcrosses (BC) to the recurrent cv. Simeto, so to produce near-isogenic recombinant lines (NIRLs). In particular, BC_5F_8, BC_5F_7 and BC_4F_7 progenies of R5-2-10, R112-4, and R23-1, respectively, were used. Genotypes were represented by homozygous carriers (= HOM+) and non-carriers (= HOM-) of the corresponding 7AgL segment. For each NIRL, HOM+ and HOM- variants were represented by 2 families originating from sister lines, replicated 3 times and randomized, to give a total of 36 plots (1.5 m x 1.5 m each). During the entire growth period, appropriate weed, disease and pest control measures were applied; plants were fertilized according to the standard procedure and grown under rainfed conditions.

2. Measurements of yield and yield-related traits and statistical analysis

During vegetative growth, at maturity and post-harvest stages, the following traits were measured: phenological phases – terminal spikelet (TS), booting (BS), heading (HD), anthesis (ANT), grain filling (GF), stem elongation (SE), booting to anthesis (BS-ANT); spike fertility traits (6 data points/plot) – spike dry weight at anthesis (SDW), biomass/shoot at anthesis (BST), fertile floret number/spike at anthesis (FF), spike length (SL), spike index (SI), No. spikelets/spike (SPNE), No. grains/spike (GNS), No. grains/spikelet (GNSP), grain yield/spike (GYS), spike fertility index (SFI); flag leaf traits and plant height (10 data points/plot) – flag leaf width (FLW), flag leaf length (FLL), flag leaf area (FLA), plant height (PH); chlorophyll content (SPAD) at watery ripe, early milk, medium milk and late milk stages of grain filling; productivity traits (25 tillers/plot) - grain yield/m^2 (GYM2), No. grains/m^2 (GNM2), No. spikes/m^2 (SNM2), biomass/m^2 (BM2), biomass/tiller (BTIL), grain yield/tiller (GYTIL), 1000 grain weight (TGW), harvest index (HI). Chlorophyll content was measured on the flag leaf by SPAD meter (Minolta, Japan). General linear model-ANOVA (GLM-ANOVA) was performed with SYSTAT12 (Systat Software Incorporated, San Jose, CA, USA) software package.

III – Results and discussion

The first plot trial with the durum wheat-*Th. ponticum* NIRLs has given encouraging results. With respect to the previous analyses on the same material (Kuzmanović *et al*., 2013), mainly focused on observations at maturity and post-harvest stages, the present work included as well analysis of traits at earlier developmental stages, such as biomass and spike fertility recorded at anthesis, and chlorophyll content, recorded from anthesis to ripening. Furthermore, the duration of phenological phases and their potential association with yield-related traits has been analysed. Several significant effects of given 7AgL segments on yield-related traits were confirmed, and new ones highlighted.

As emerged in previous analyses (Kuzmanović *et al*., 2013), the R112-4 recombinant confirmed to have significantly increased values for several yield-contributing traits due to the presence of its 28%-long 7AgL segment on 7AL. Firstly, compared to its control (HOM-), HOM+ plants of this NIRL showed to have significantly higher (+6%) FLW (Table 1), a trait positively correlated with TGW (not shown). These results support our hypothesis (Kuzmanović *et al*., 2013) of the location of a genetic determinant for FLW within the 7AgL segment present in R112-4 and absent from R5-2-10 (between 23% and 28% distal 7AgL chromatin on 7AL, see Fig. 1). Since this segment is common to the R23-1 7AgL portion, the lack of expression of this locus, as well as of others putatively assigned to the same region (see below), might be due to the presence of a Segregation distortion (*Sd*) gene(s) in the most proximal part of the R23-1 7AgL segment, negatively affecting a variety of plant traits (Ceoloni *et al*., 2013).

Secondly, R112-4 recombinant plants also confirmed to produce significantly higher number of spike-bearing tillers (SNM2), without any yield penalty (GYM2, Table 1). The observed 20% increase was highly remarkable, given the conventional sawing density of 350 seeds/m^2 adopted (in previous years, spaced plants showed a similar increase of 25%, see Kuzmanović *et al*., 2013). Since such a constant increase was unique to the R112-4 NIRL, a putative locus for tiller number appears to be located within 7AgL segment present in R112-4, absent from R5-2-10, and not expressed by R23-1 (see above; Fig. 1). Additionally, measurements performed with SPAD meter during grain filling revealed significantly higher chlorophyll content (+15%) at late milk stage in the R112-4 recombinant compared to its HOM- control and the other HOM+ genotypes (Table 1, Fig. 1). This indicates a potentially higher photosynthetic efficiency of R112-4, which contrasts with the significantly decreased chlorophyll content of R23-1 HOM+ plants throughout ripening (from watery to late milk stage). This, in turn, was probably largely responsible for the lower TGW observed in the latter recombinant (Table 1).

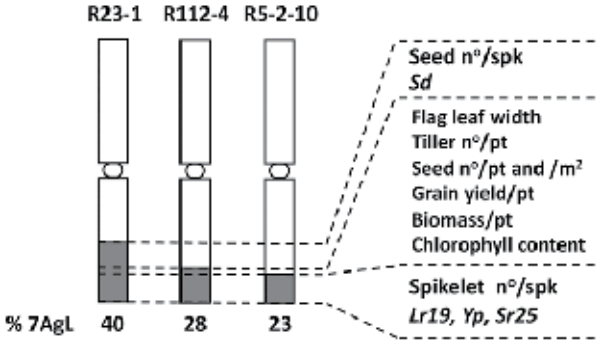

Figure 1. Recombinant 7AL-7AgL chromosomes representing the three durum wheat NIRLs used in the present study, with physical location within the 7AgL segments of main genes and newly identified loci for yield-contributing traits; spk: spike, pt: plant.

Table 1. Means and standard errors (SE) as from the ANOVA-GLM analyses for yield-contributing traits of the durum wheat 7AgL recombinant lines (HOM+) and their respective controls (HOM-) grown in Viterbo (Central Italy) in the 2011-2012 season

Trait	R5-2-10 HOM+		R5-2-10 HOM-		R112-4 HOM+		R112-4 HOM-		R23-1 HOM+		R23-1 HOM-	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
TS (N° days*)	125.2	0.71	125.2	0.71	126.0	0.71	124.5	0.71	125.2	0.71	125.7	0.71
BS (N° days)	149.7	0.49	149.7	0.49	150.0	0.49	149.2	0.49	151.7	0.49	151.8	0.49
HD (N° days)	152.3	0.75	152.2	0.75	152.8	0.75	151.8	0.75	155.7	0.75	155.5	0.75
ANT (N° days)	163.3	0.36	161.5	0.36	163.3	0.36	161.0	0.36	165.8	0.36	165.2	0.36
GF (N° days)	56.7	0.36	58.5	0.36	56.7	0.36	59.0	0.36	54.2	0.36	54.8	0.36
SE (N° days)	38.2	0.56	36.3	0.56	37.3	0.56	36.5	0.56	40.7	0.56	39.5	0.56
BO-ANT (N° days)	13.7	0.49	11.8	0.49	13.3	0.49	11.8	0.49	14.2	0.49	13.3	0.49
SDW (g)	0.6	0.02	0.6	0.02	0.6	0.02	0.6	0.02	0.5	0.02	0.6	0.02
BST (g)	4.0	0.10	4.3	0.10	3.9	0.10	4.0	0.10	3.6	0.10	4.1	0.10
FF	29.7	1.41	32.5	1.41	29.8	1.41	29.7	1.41	28.5	1.41	28.8	1.41
SL (cm)	6.0	0.11	6.1	0.11	5.9	0.11	5.8	0.11	6.7	0.11	6.7	0.11
SI	0.6	0.01	0.6	0.01	0.6	0.01	0.6	0.01	0.5	0.01	0.5	0.01
SPNE	16.9	0.35	16.8	0.35	16.5	0.35	16.4	0.35	18.3	0.35	18.0	0.35
GNS	39.1	1.43	39.1	1.43	40.5	1.43	38.7	1.43	50.7	1.43	46.2	1.43
GNSP	2.3	0.06	2.3	0.06	2.5	0.06	2.3	0.06	2.8	0.06	2.6	0.06
GYS (g)	2.8	0.09	2.7	0.09	2.7	0.09	2.7	0.09	2.3	0.09	2.9	0.09
SFI	68.5	3.42	67.1	3.42	71.3	3.42	68.2	3.42	112.5	3.42	81.3	3.42
FLW(cm)	1.6	0.02	1.6	0.02	1.7	0.02	1.6	0.02	1.5	0.02	1.5	0.02
FLL (cm)	12.8	0.48	12.8	0.48	12.5	0.48	12.8	0.48	12.5	0.48	13.6	0.48
FLA (cm^2)	21.0	0.99	20.8	0.99	21.3	0.99	20.7	0.99	1.0	0.99	20.9	0.99
PH (cm)	82.6	0.81	83.2	0.81	80.0	0.81	78.1	0.81	98.6	0.81	99.7	0.81
GYM2 (g)	408.9	20.52	397.6	20.52	440.7	20.52	428.2	20.52	415.0	20.52	423.2	20.52
GNM2	408.9	20.52	397.6	20.52	440.7	20.52	428.2	20.52	415.0	20.52	423.2	20.52
SNM2	239.8	10.42	241.6	10.42	315.5	10.42	263.8	10.42	299.4	10.42	272.2	10.42
BM2 (g)	974.3	46.62	929.8	46.62	1064.4	46.62	1026.4	46.62	1086.6	46.62	1081.2	46.62
BTIL (g)	6.3	0.20	6.0	0.20	6.0	0.20	6.2	0.20	5.6	0.20	6.5	0.20
GYTIL (g)	2.6	0.06	2.6	0.06	2.5	0.06	2.6	0.06	2.1	0.06	2.5	0.06
TGW (g)	72.0	0.57	71.8	0.57	67.7	0.57	71.0	0.57	48.3	0.57	64.2	0.57
HI	0.4	0.01	0.4	0.01	0.4	0.01	0.4	0.01	0.4	0.01	0.4	0.01
SPAD**	48.2	1.2	45.9	1.2	50.4	1.2	43.7	1.2	41.6	1.2	46.0	1.2

** from sawing date; ** chlorophyll content at late milk stage of grain filling*

On the other hand, R23-1 confirmed its ability to produce much higher seed number/spike, as seen from the 30% higher GNM2 accompanied by 38% higher SFI (Table 1). This increment was not exhibited by R5-2-10 nor by R112-4; moreover, no correlation was observed between No. grains/m^2 and No. spike/m^2 (not shown). All these observations suggest that the increase in seed number might be associated with a genetic factor independent of the locus controlling tiller number, and present on 7AgL chromatin exclusive to R23-1 (between 28% and 40% distal 7AgL; Fig. 1). In line with previous results (Kuzmanović *et al*., 2013), the higher seed number in R23-1 NIRL was not paralleled by an increase in yield, but, instead, accompanied by a much lower TGW (−25%, Table 1). This drawback, probably representing one of the side effects of the *Sd* gene(s), could be associated with the observed lower spike weight at anthesis and lower chlorophyll content of R23-1, as well as with its shorter grain filling period compared to other recombinants (Table 1), negatively correlated with TGW.

R5-2-10 and R112-4 recombinants showed to have slightly later anthesis date (ANT) compared to their respective controls (about 2 days), followed by, as expected, significantly shorter grain filling period (GF, Table 1). R23-1 HOM+ plants did not show significant alteration of the ANT or GF compared to HOM- plants; however, compared to the other HOM+ genotypes they had significantly longer ANT and shorter GF. Anthesis date was positively correlated with GNS and GNM2 (not shown), but it did not result in a significant increase in yield in any of the recombinants (Table 1). Duration of stem elongation phase (SE), known to be essential for spike growth and fertility, was significantly higher in R5-2-10 only, although all recombinants showed a tendency for longer SE compared to their HOM- controls (Table 1). On the other hand, the period comprised between booting and anthesis (BS-ANT), which appears to be the most important phase for nutrient transfer from stem into spike (e.g. Isidro *et al*., 2011), was significantly longer in R5-2-10 as well as in R112-4 NIRLs. This suggests that also the BS-ANT duration may contribute to the higher yield potential of R112-4.

Field trials, extended to a variety of locations, are being continued. So far, the 7AgL positive attributes expressed by the R112-4 recombinant appear the most readily exploitable in advanced breeding programs for yield improvement of durum wheat. Considering the additional beneficial genes present in the same alien segment (*Lr19+Yp+Sr25*), this represents a particularly demonstrative example of how a knowledgeable use of a suite of alien traits can result in effectively unlocking their great potential for breeding gains. On the other hand, the potentially enhancing yield traits associated with 7AgL chromatin unique to R23-1, primarily No. grains/spike and No. grains/m^2, might be potentially usable in bread wheat breeding, given the higher tolerance of the latter to sizable alien introgressions and to *Sd* gene effects as compared to durum wheat (Ceoloni *et al.*, 2013 and unpublished). To verify this, and to assess the effect of 7AgL portions smaller than the T4 translocation in a hexaploid background, the 3 durum wheat recombinants described here are being crossed and backcrossed with bread wheat cultivars to create hexaploid NIRLs to be used in future comparative trials.

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Searching for climate change related traits in plant genetic resources collections using Focused Identification of Germplasm Strategy (FIGS)

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Abstract. Prospects to assess and explore largely untapped plant genetic resources (PGR) collections to search for climate change related traits, such as drought and heat tolerance, as well as pest and disease resistance, are possible through new approaches such as the focused identification of germplasm strategy (FIGS). FIGS approach is based on the paradigm that any germplasm is likely to reflect the selection pressures of the environment under which it evolved. The approach uses trait and environmental data (climate data including phenology data) to develop *a priori* information based on the quantification of the trait-environment relationship. If a dependency between the trait and the environment is detected, the *a priori* information is then used to define subsets of accessions with a high probability of containing the sought after traits. The subsets of accessions are then used for *a posteriori* evaluation. Recent research comparing *a priori* and *a posteriori* information supports the assertion that FIGS can be used as an effective tool to search for traits of resistance to pests and diseases as well as traits to adapt to climate change. This paper presents and discusses some of the recent results where FIGS was used to develop subsets with high probability of finding desirable traits, such as resistance to stripe (yellow) rust, in durum wheat. It also addresses ways in which current FIGS based models could be further enhanced by working the ways in which the environmental data is presented to the models, thereby improving the detection of traits associated with climate change adaptation.

Keywords. Genetic resources – FIGS – Accessions – Pests – Diseases – Resistance – Climate change.

Recherche pour des caractères liés au changement climatique dans des collections de ressources phytogénétiques en utilisant la stratégie d'identification ciblée du matériel génétique (FIGS)

Résumé. *L'évaluation et l'utilisation des collections de ressources phytogénétiques largement inexploitées pour rechercher des caractères liés au changement climatique, comme la sécheresse et la tolérance à la chaleur, ainsi que la résistance aux organismes nuisibles et aux maladies, sont aujourd'hui possibles grâce à de nouvelles approches telles la stratégie d'identification ciblée du matériel génétique (FIGS). L'approche FIGS repose sur le paradigme que tout matériel génétique est susceptible de refléter les pressions de sélection de l'environnement dans lequel il a évolué. Cette stratégie utilise des caractères et des données environnementales (données climatiques, y compris les données phénologiques) pour développer une information a priori basée sur la quantification de la relation caractère-environnement. Si une dépendance entre le caractère et l'environnement est détectée, l'information a priori est alors utilisée pour définir des sous-ensembles d'accessions ayant une forte probabilité de porter les caractères cherchés. Les sous-ensembles d'accessions sont ensuite utilisés pour une évaluation a posteriori. Des recherches récentes comparant les informations a priori et a posteriori permettent d'affirmer que la FIGS peut être utilisée comme un outil efficace pour la recherche de caractères de résistance aux organismes nuisibles et aux maladies tout comme aux caractères d'adaptation au changement climatique. Dans cet article, on présente et on discute des résultats récents de l'application de la FIGS pour développer des sous-ensembles avec une haute probabilité de trouver les caractères cherchés, comme la résistance à la rouille jaune, chez le blé dur. On discute également les possibilités d'améliorer les modèles sur lesquels est basée actuellement la FIGS, en travaillant sur la façon dont les données environnementales sont intégrées dans les modèles, améliorant ainsi la détection des caractères associés à l'adaptation au changement climatique.*

Mots-clés. Ressources génétiques – FIGS – Accessions – Organismes nuisibles – Maladies – Résistance – Changement climatique.

I – Introduction

Prospects to assess and explore largely untapped plant genetic resources (PGR) collections for agronomically important traits, particularly those linked to climate change-adaptation, are possible through new approaches such as the Focused Identification of Germplasm Strategy (FIGS). Climate change, which is the result of greenhouse gas (GHG) emissions, is causing the atmosphere to heat up (Mendelsohn & Dinar 2009). Crops such as wheat are reported to be more vulnerable to heat stress than drought (Semenov & Shewry 2011). High temperatures during the reproductive phase can reduce the number of kernels per spike, which is an important component of yield (Semenov & Shewry 2011). Both heat and drought stresses are expected to increase in their frequency and intensity in dry areas (IPCC 2012) such as central North America, Northern Africa, Central Asia, West Asia and Western Australia. Although the global climate models (GCMs) differ substantially, they all tend to indicate significant temperature increases in these areas (Girvetz *et al*. 2009). Further, this increase in temperature as a result of GHG emissions is expected to increase depending on emissions scenarios and the extent of mitigation implemented measures to curb their effects (Howden *et al.* 2007, Mendelsohn and Dinar 2009).

Plant genetic resources have contributed enormously towards increased yield in crops (Hoisington 1999) and are a ready source of trait's variation (Qualset 1975). For example, a wheat landrace from Turkey that was conserved in a genebank in 1948 was later discovered, (in the 1980s) to carry genes that are resistant to a range of fungal diseases, and are still in use in current breeding programs (Atalan-Helicke 2012, FAO). However, searching for such traits can be a daunting and costly process given that PGR consists of large collections and populations maintained *in situ* or *on-farm* that are also more prone to yield climate change related traits but yet to sampled and collected. What is required therefore is an efficient method to select material from these genetic resources so that the probability of finding and locating the required variation is maximized while reducing the number of accessions evaluated and the onverall cost implications (Gollin *et al*. 2000). The FIGS approach represents one such method.

The FIGS approach is based on the paradigm that adaptive traits exhibited by germplasm are likely to reflect the selection pressures of the environment from which the germplasm was originally sampled (Mackay and Street, 2004). For example, if a plant population is exposed over a significant period of time to weather conditions that are favourable to consistently high pathogen populations then it is likely that a selection pressure will be imposed on the plant population for the emergence of resistance genes. Paillard *et al.* (2000) found this to be the case for the evolution of powdery mildew resistance in wheat and barley landraces. Thus if a dependency between a given trait and environmental parameters can be defined then the relationship can be used to predict the likelihood of finding a desired trait in a given environment (Mackay and Street 2004, Bari *et al.* 2012, Endresen *et al.* 2012). In this context information about the environmental origins of accessions are used to define trait specific subsets of germplasm with a higher probability of containing the sought-after traits.

This paper presents and discusses how FIGS has been applied to the search for resistance to stripe (yellow) rust in durum wheat. In previous FIGS studies predictive models were applied to historic climate data to search for traits of interest. In this study the models were tested with future climate change scenarios. However, adjustments may be required in the models for change climate scenarios as well as improvement by working ways in which environmental data is presented to the models to improve the search for traits to cope with climate change adaptation. The modelling process is considering separating the induced-shift climate change variation from the overall variation for better prediction.

II – Methodology

1. Data

This study was based on a field evaluation of durum wheat accessions for response to a naturally occurring yellow rust infection at ICARDA during the 2011/2012 season. The environmental data consisted of long-term climate monthly average data for the sites from which the accessions were originally sampled. The study also consists of projected climatic data extracted from three future climate scenarios based on the Canadian Climate Centre (CCC) global circulation model (Boer *et al*. 2000).

All the climate variables were extracted from a grid cell of 1 square km (Table 1) as monthly data (De Pauw 2008). Monthly data are coarse grained and thus more prone to be out of phase in relation to critical stages of crop development (Coops *et al*. 2001), which would be further amplified by climate change effects. Thus the study also used daily data which were derived from the monthly values using models proposed by Epstein (1991) (Hofstra *et al*. 2008).

To better capture the climate change induced-shifts the predictive models were applied to climatic conditions within the growing period. Thus data averages for stages in a crops development where compared to long term climatic averages expressed as monthly values alone. Thus in the modelling process the noise created by differences in phenology between sites and climate change induced-shifts would be eliminated facilitating higher resolutions to detect environment – trait linkages.

To estimate the crop development phases a day-degree accumulation model was used from an estimated onset date for each site. The onset date was estimated using a method which determines when neither moisture nor temperature would limit plant growth. The method is based on a modification of a model developed by the Food and Agriculture Organization of the United Nations (FAO 1978, De Pauw 1982).

Table 1. The environment variables used in the study.

Variable Type	Variable Name	Variable Description	Unit	Number
Climatic	*Tmin*	Monthly minimum temperature	°C	12
	tmax	Monthly maximum temperature	°C	12
	prec	Monthly precipitation	mm	12
	tmind	Daily minimum temperature	°C	365
	tmaxd	Daily maximum temperature	°C	365
	precd	Daily precipitation	mm	365
Phenology	*Onset*	Date of sowing	day	1

All variables were standardized to a mean of zero and a standard deviation of 1. After the transformation the data was standardized and a comparison made between the transformed and non-transformed data. This data pre-processing was systematically and automatically carried out through the different models.

2. Modelling

In previous models the predictions were limited to past climate data while here the modelling was also carried out on projected future scenarios. The stripe (yellow) rust disease evaluation scores of the growing season 2011/2012 were presented to the models to detect the trait by collection site environment dependency, if it exists. The models were then run on all the durum

collection data held at ICARDA using current climate set of data as well as 2 other sets of future climate data/maps reflecting two emission scenarios: *a* and *b* scenarios. The *b* scenario projects a doubling of CO2 relative to its preindustrial level (Franklin *et al*. 2013).

The modelling procedure was based on running two models using the current climate variables and then re-run the models using CC variables. The models used are SVM (Support Vector Machine) and RF (Random Forest). RF is a clustering algorithm developed to act like an ensemble classifier where the best splitters are randomly selected at each node among subset predictors ((Breiman 2001; Liaw and Wiener 2002). It is a procedure used in gene selection and classification of microarray data and genome-wide association studies for complex human diseases (Díaz-Uriarte and Alvarez de Andrés 2006; Lunetta *et al*. 2004). Support Vector Machines (SVM), on the other hand, maps input data to a more high-dimensional space that would lead to a better separation of data into respective classes by isolating those inputs which fall nearby the data boundaries (Cortes and Vapnik 1995; Principe *et al*. 2000). The mapping of input data to high-dimensional space is carried out through processes called kernel functions such as radial basis function (RBF) which is the kernel function used in this study for the SVM model. SVM models have been found to distinguish optimally between groups with minimum loss of information (Guo *et al*. 2004; Karatzoglou *et al*. 2006).

The predicted probabilities were then used to delimit areas where the conditions are conducive to occur. After defining the appropriate variograms, the maps were generated using kriging techniques (Cressie 1993). To create maps the R module was applied to irregularly spaced data (Figure 2) where the correlation between sites is (assumed) to be an exponential function of the distance.

III – Results

The results show the presence of relationship between the current or past climate data and the resistance to stripe rust. Both Receiver Operating Characteristics (ROC) values as well as Kappa values are all highly above acceptable values of 0.5 and 0.4 respectively. The ROC plots illustrate also that the curve for the two models were well above the diagonal line, which is expected when the model is different from random. The vertical trend towards the left-hand side is also an indication that the models classified the resistant accessions more correctly with fewer false positive errors (Fawcett 2006). The histograms (right side) illustrate further the extent of separation between the two trait states, resistant on one hand and tolerant on the other, with limited overlapping between the two states. The models were also able to correctly classify sites that yield either resistant or susceptible genotypes with a high correct classification when compared to the previous studies (Table 2). The accuracy of prediction as well as kappa increase reaching up to 0.83 and 0.70 respectively as we move from monthly data to aligned daily data based on onset data (Table 4).

Table 2. Accuracy and agreement parameters of daily two fit functions to generate daily data.

fit function	Stat	AUC	OR	SE	SU	CC	Kappa
Spline	mean	**0.80**	**0.32**	**0.68**	**0.92**	**0.87**	**0.61**
	upper CI	0.81	0.35	0.70	0.93	0.87	0.63
	lower CI	0.79	0.30	0.65	0.91	0.86	0.59
Loess	mean	**0.79**	**0.34**	**0.66**	**0.92**	**0.86**	**0.59**
	upper CI	0.80	0.37	0.68	0.92	0.86	0.60
	lower CI	0.78	0.32	0.63	0.91	0.85	0.57

Table 3. Accuracy and agreement parameters of daily data (spline fit function) and monthly data.

Data type		AUC	OR	SE	SU	CC	Kappa
daily data	**Mean**	**0.80**	**0.33**	**0.67**	**0.93**	**0.87**	**0.62**
	Upper CI	0.81	0.34	0.69	0.93	0.87	0.63
	Lower CI	0.79	0.31	0.66	0.92	0.87	0.61
monthly data	**Mean**	**0.79**	**0.31**	**0.69**	**0.90**	**0.85**	**0.58**
	Upper CI	0.80	0.33	0.71	0.90	0.86	0.60
	Lower CI	0.78	0.29	0.67	0.89	0.84	0.56

Table 4. Accuracy and agreement parameters of aligned data.

Data type		AUC	OR	SE	SU	CC	Kappa	
monthly	Max	0.81	0.28	0.72	0.90	0.86	0.61	
daily data	Max	0.82	0.30	0.70	0.93	0.88	0.64	
aligned daily data	Max	0.83	0.28	0.72	0.95	0.90	0.70	210 days

The presence of the existence of the dependency between climate data and the trait of resistance to stripe rust was used as a *priori* information for the prediction of stripe resistance in independent data. The results are shown in the maps for different CC scenarios. In terms of areas that might yield stem rust variation, Ethiopia was highest followed by India and Turkey. This is also similar to the results that have been reported on the regions that might yield resistance (Singh *et al*. 2006).

IV – Discussion

Recent findings on a study conducted to search for climate change traits such as traits of tolerance of drought where a comparison was made between *a priori* and *a posteriori* information supports the assertion that FIGS can be used as an effective tool to search for traits of adaptation to climate change (Khazaei *et al.* 2013). Similar comparison was also made recently for stripe rust resistance in durum wheat confirming also that FIGS is tool with potential to not only find the sought after traits but on a limited number of accessions (Bari *et al*. *in press*).

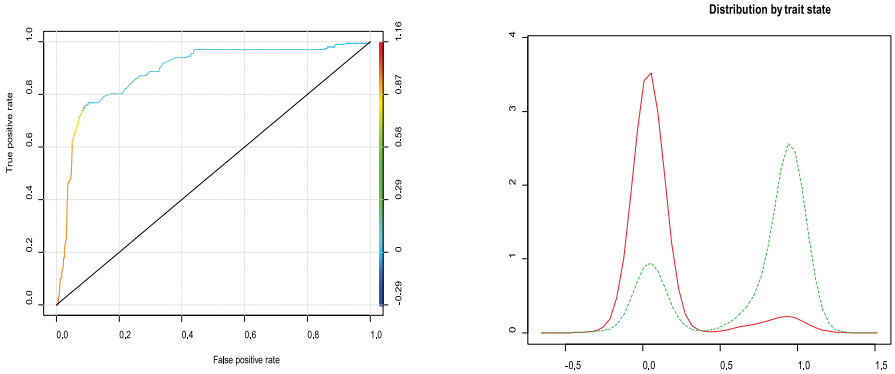

Figure 1. ROC plots for the RF and SVM models applied to the training set of accessions evaluated for Yr disease in 2011/2012 growing season at ICARDA (Left hand side). The ROC curve to the left of the diagonal plot is the true positive rate versus false positive rate. Density plots for prediction of resistance and susceptibility for the RF and SVM models (Right hand side).
[Green line indicates the probability density distribution for resistance and red line indicates susceptibility]

The FIGS conceptual framework was developed on the basis of past climate data with predictions were limited to geographical space. In the context however of CC where a shift is expected in the climate parameters bias in the prediction as a result of induced climate shift might be expected. Using future climate predictions may be more complex as this might also involve a shift in pest dynamics where mild winters and warmer weather may lead to other diseases outbreaks (Patterson et al. 1999) in areas different from where the accessions were originally sampled. The predictions showed that the range of some crop pests, such as the migratory grasshopper (*Melanoplus sanguinipes*), might be extended to areas beyond current agricultural land in North America (Olfert *et al.* 2011). This will also require a new modelling framework, or paradigm, in conjunction with the preliminary modelling framework being developed by Jenouvrier and Visser (2011). These two authors proposed a box-in-a-box modelling approach that couples population models to phenological change by linking these shifts to changes in population viability under various GHG emission scenarios. They expect the CC shift will be creating non-overlapping circumstances which in turn will lead to selection acting on phenology.

This study highlights the expected shift on the conditions of occurrence of diseases incidence of stripe rust based on past/current climate change. This shift could also be captured through phenology where the variation among collecting sites is a combination of both difference on crop phenology (growing season) and climate change. This will explored further by using both the auto-correlation and the variograms to better capture the dynamics of the distributions of traits.

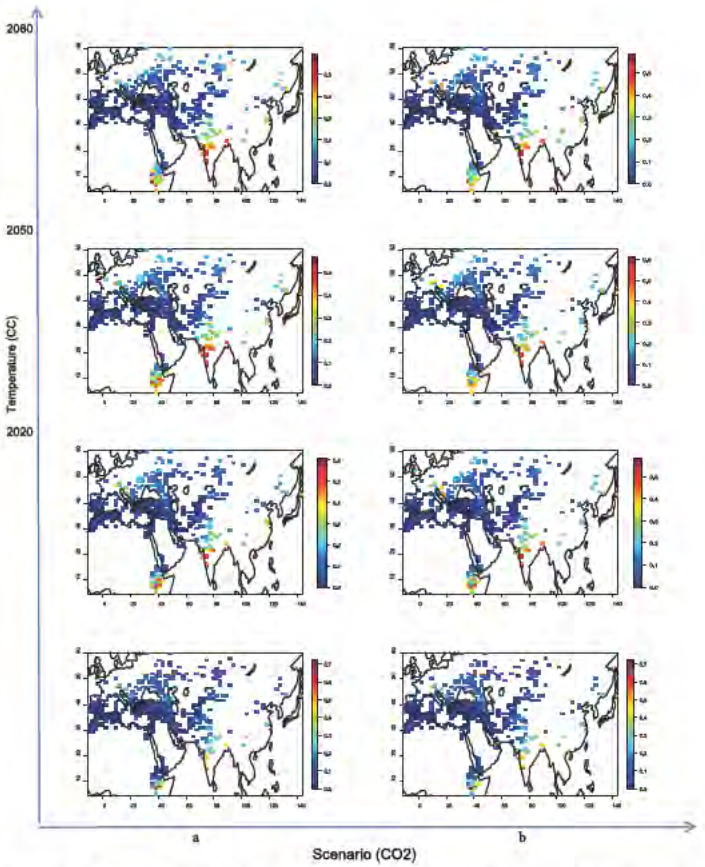

Figure 2. Maps for the different predicted probabilities of Yr occurrence for the temperatures (years) against the two CC scenarios (CO2).

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Intra-population variation for agronomic characteristics in the durum wheat landrace "SafraMa'an" (*Triticum turgidum* L. var. *durum*)

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Abstract. Two hundred and eighty six lines from tetraploid wheat (*Triticum turgidum* L. var. *durum*) landrace "SafraMa'an" were selected randomly during 1994-1995 growing season. The entire populations with three commercial check cultivars (Acsad 65, Hourani 27, and Amra) were evaluated at Maru Agriculture Research Station during 1995-1996 growing season for 16 characters including grain yield per plant. The objectives were to assess the magnitude of phenotypic variations for several traits in tetraploid wheat "SafraMa'an" and to evaluate the potential usefulness of some of the traits identified. Results showed wide range of phenotypic variation for most characters. Mono-morphism was common for juvenile growth habit, whereas the rest of the characters exhibited polymorphism in varying degrees. Considering all characters, the average diversity (H′) for "SafraMa'an" landrace was 0.65 ± 0.047. There were 10 lines superior to best check (Hourani 27) for grain yield per plant. Subsequently, the population lines were clustered into six distinct groups at a distance of about 0.55 based on their similarity for all traits. Acsad 65 and Amra were located in separate clusters whereas Hourani 27 cultivar was presented in cluster with most lines of "SafraMa'an". Thirteen lines from the population showed a bluish green cast or glaucousness characters. Glaucous lines have greater kernels per spike. In contrast, this character showed no significant association with grain yield per plant despite the greater grain yield per plant obtained for the glaucous lines. The results are important for the breeding and selection of this crop.

Keywords. Landrace – *Triticum turgidum* – Variation – Agronomic – Glaucous.

Variation intra-population pour les caractéristiques agronomiques de la variété locale de blé dur "SafraMa'an" (Triticum turgidum L. var. durum)

Résumé. *Deux cent quatre-vingt six lignées issues du blé tétraploïde (Triticum turgidum L. var. durum), variétés locales "SafraMa'an", ont été sélectionnées d'une manière aléatoire pendant la saison de végétation 1994-1995. Les populations entières avec trois cultivars commerciaux témoins (ACSAD 65, 27 Hourani, et Amra) ont été évaluées auprès de la Station de recherche agricole de Maru durant la saison de végétation 1995-1996 pour 16 caractères, incluant le rendement en grain par plante. Les objectifs étaient d'estimer l'ampleur des variations phénotypiques de plusieurs traits chez le blé tétraploïde "SafraMa'an" et d'évaluer l'utilité potentielle de certains des caractères identifiés. Les résultats ont montré une grande variabilité phénotypique pour la plupart des caractères. Le monomorphisme était commun pour le mode de croissance juvénile, tandis que le reste des caractères ont montré un degré variable de polymorphisme. Considérant tous les caractères, la diversité moyenne (H′) pour la variété locale "SafraMa'an" était de 0,65 ± 0,047. Il y avait 10 lignées supérieures par rapport au témoin le plus performant (Hourani 27) pour le rendement en grain par plante. Ensuite, les lignées de la population ont été réunies dans six groupes distincts, à une distance d'environ 0,55, sur la base de la similitude de tous les caractères. ACSAD 65 et Amra étaient situées dans des groupes séparés alors que le cultivar Hourani 27 était dans le groupe incluant la plupart des lignées de "SafraMa'an". Treize lignées de la population ont montré une dominante verte bleuâtre ou glauquescence. Les lignées glauquescentes avaient plus de grains par épi. En revanche, ce caractère n'a montré aucune association significative avec le rendement en grain par plante bien qu'on ait observé un rendement en grain par plante plus élevé pour les lignées glauquescentes. Les résultats sont importants pour l'amélioration et la sélection de cette culture.*

Mots-clés. *Variété locale – Triticum turgidum – Variation – Agronomique – Glauquescence.*

I – Introduction

Durum wheat is one of the most important of all crop plants cultivated to meet great demands for human food consumption in the Mediterranean basin, Europe and India (Abaye *et al*., 1997; Nachit *et al*., 1998). The world production of wheat increased by 9.5% during the period 2000-2004 to 2006-2010, while wheat harvested area increased by 2% during the same period. In Jordan, wheat production decreased by 44% while wheat harvested area decreased by 7%, during the same period (FAO, 2011). The major constraint affecting wheat production in Jordan is drought. Different methods could be used to increase cereal production, such as increasing area of production, effective cultural practices, and planting improved varieties (Cassman, 1999). In Jordan, as arable land is limited and most of the production area is under semi-arid conditions, developing high yielding varieties adapted to local conditions could be employed. Therefore, understanding the magnitude of existing variability, proper characterization of the most important physiological traits and their interrelationships with yield and yield components would be extremely helpful in the synthesis of most efficient and highly productive genotypes (Joshi *et al*., 1982). Cereal improvement depends on the continuous supply of new germplasm material to act as donor of various genes of agronomic importance. Landraces are possible source of this germplasm material.

Landraces are comprised of population mixtures that contain a great number of different hereditary types which, due to their genotypic diversity, are especially well adapted to the changes in the environmental conditions of their habitat. Compared to modern cultivars, they deliver only average but reliable yields (Kuckuck *et al.*, 1991; Tahir and Valkoun, 1994; Guarino, 1995). Landraces serve as good reservoir of genetic variability for germplasm collection programs (Welsh, 1981) and represent an important starting point for the successful development of improved varieties (cultivars) by exploiting genetic complexes governing adaptation or adaptability to the often very extreme environmental conditions of these countries (Kuckuck *et al.*, 1991). Gene pools from landraces can be used for further increasing durum wheat yields under rainfed conditions (Duwayri and Nachit, 1989). ICARDA′s cereal breeding efforts have concentrated on developing genotypes with high and stable grain and straw yields. Landraces and derived pure lines are being successfully used in crossing programs to transfer drought tolerance into otherwise adapted germplasm (ICARDA, 1989).Characterization of landraces is carried out by isolating single lines from the mixtures grown by farmers. Seeds of the best-adapted lines can then be multiplied and the lines released as cultivars in their own right. Arta is a typical success of this approach: a single-line selection from Syrian barley landrace *Arabi Abiad,* which currently out-yields any other line or cultivar in its target environment (ICARDA, 1996). Another way of utilizing the specific adaptation of landrace lines is to use them in breeding programs.

There are two main reasons (Tahir and Valkoun, 1994) for giving a special attention to landraces:(i) genetic erosion caused by the replacement of landraces by improved varieties, and (ii) landraces have good adaptation to the stressful and highly variable environments. In Jordan there are several landraces; one of them is “SafraMa’an” which belongs to tetraploid wheat *Triticum turgidum* L. var. *durum,* grown mainly in southern Jordan. There have been no previous studies on “SafraMa’an” landrace in Jordan. “SafraMa’an” has been used in plant breeding programs outside Jordan (Clarke *et al.*, 1994). The main objectives of this research were: (i) to assess the magnitude of phenotypic variation for several traits in the durum wheat landrace “SafraMa’an”, and (ii) to evaluate the potential usefulness of some of the traits identified.

II – Material and methods

Seeds of “SafraMa’an” landrace were obtained from the National Center for Agricultural Research and Extension (NCARE) in 1994. These seeds were collected from farmers’ fields at Al-Shoubak,

which is located in the southern part of Jordan in the 1993/1994 growing season. In the 1994/1995 growing season, seeds were space planted in Jubeiha and a random sample of 286 plants were selected, harvested and threshed as individual plants. The study was conducted in 1995/1996 growing season at Maru location (35*55* N latitude and 32*37* E longitude with an elevation of 500m). Detailed information on monthly rainfall and temperatures throughout the 1995/1996 growing season are shown in Table 1. A randomized complete block design (RCBD) with three replications was used. The experimental plot consisted of 1 row, 1 m long. Spacing between rows was 0.3 m and between seeds within row 10 cm. Three commercial durum wheat varieties Acsad 65, Hourani 27, and Amra were used as checks in this study.

Table 1. Distribution of rainfall and temperature regimes during 1995-1996 growing season in Maru agricultural research station.

Duration	Rainfall mm	Temperature C°
Oct, 1995	5.5	20.2
Nov, 1995	77.3	13.8
Dec, 1995	27.7	9.9
Jan, 1996	110.1	9
Feb, 1996	21.5	11.1
Mar, 1996	126.5	11.7
Apr, 1996	18.1	15.5
May, 1996	-	22.5

The following characters were measured in each plot: Early growth vigor (EGV) was recorded on Feb. 29, 1996, in the following three categories (1) weak, (2) intermediate, (3) healthy). Juvenile growth habit (JGH) was recorded on April 22, 1996, classifying plants as (1) erect, (2) semi-erect, and (3) prostrate). Glaucousness (GL) was recorded on April 17, 1996 (as one of the two categories (1) glaucous (2) non glaucous): Heading date (HD) was measured as Number of days from Jan 1 to date when 50% of the heads had emerged from the bootleaf; maturity date (MD) as Number of days from Jan 1 to date when 50% of the row showed physiological maturity - i.e the very first sign of the yellow color appearance on the flag leaf blade); grain filling period (GFP) was calculated as the difference between the (MD) and (HD)).

After physiological maturity (on May 28, 1996), five representative plants from center of each plot were taken and the following measurements recorded: Flag leaf area (FLA) (Calculated as flagleaf width (at the widest point) x flag leaf length (from tip to collar) x 0.65 at the time of physiological maturity); plant height (PH) (Height in centimeters from the soil surface to the tip of the spike (awn excluded) of the tallest culm); number of productive tillers (TN) (Total number of seed-bearing culm for each plant); number of spikelets per main spike (SS) (Total number of seed-bearing spikelets on the main head from each plant); spike length (SL) (Length in centimeters of the spike on the tallest culm): awn length (AWL) (Measured from the tip of the main spike to the end of the awn); spike density (SD) (Calculated as the ratio between the number of spikelets per spike over the spike length); number of kernels per spikelet (KS) (Calculated from each plant as kernels/ spike divided by spikelets/spike); thousands kernel weight (TKW); number of kernels per main spike (NKS) (Total number of kernels on the main spike from each plant); biological yield per plant (BY); number of heads per meter square (HM2); and Grain yield per plant (GYP).

Analysis of variance and t-test, were performed using SAS program (SAS, 1985). Estimates of phenotypic diversity index H′, Mean ($\overline{x}$) and standard deviation (S) were calculated for each quantitative trait. The two statistics were used to classify the trait into three groups: less than ($\overline{x}$ - S); between ($\overline{x}$ - S) and ($\overline{x}$ + S), greater than ($\overline{x}$ +S). Shannon´s information statistic ($h_{s.j.}$) (Tesfaye *et al*., 1991) was used to describe phenotypic diversity. The following formula was used for calculating $h_{s.j.}$ for the j^{th} trait with n categories:

$$h_{s.j} = -\sum_{i=1}^{n} P_i \log_2 p_i$$

where p_i is the relative frequency in the category of the j^{th} trait. Each value of $h_{s.j.}$ was divided by its maximum value ($\log_2{}^n$), which ensured that all scaled $h_{s.j.}$ values were in the range 0 to 1. The average diversity (H′) over k traits was estimated as:

$$H' = \sum_{i=1}^{k} h_{s.j} / k$$

The diversity indix (H′) was previously used for measurement and comparison of geographical patterns of phenotypic diversity in germplasm collections of wheat (Tesfaye *et al*., 1991). Cluster analyses were computed by using plant means for all quantitative traits; and plant means were clustered by the unweighted pair group method using arithmetic averages (UPGMA) as described in SAS (2002).

III – Results

1. Phenotypic variation

The results from analysis of variance for the investigated characteristics indicated the presence of a large variation observed for sixteen characters studied (Table 2). Line differences in most of the characters were significant at 0.1% level of probability. Hence a number of different stable lines could be derived from these populations to be utilized in breeding programs. Several lines from “SafraMa’an” landrace were better than the checks studied for several traits (Table 2). Comparisons between the local lines and the improved cultivars revealed that, in general, the former were taller, and had greater number of spikelets per spike, heavier thousands kernel weight and biological yield and, larger flag leaf area than the two checks, cultivars Acsad 65 and Amra. Also, the landraces gave greater grain yield and higher fertile tiller number per plant than Amra; the landraces were later both in heading and maturity time, and had larger awn length than the three check cultivars. The mean values of other characters compared to the three check cultivars are also presented in Table 2. There were ten lines superior to the best check (Hourani 27) for grain yield per plant and taller than other two checks Acsad 65 and Amra, whereas only 2 lines were taller than high yielding check (Hourani 27), one line was glaucous, seven lines had higher fertile tillers and eight lines had larger flag leaf area than the best check cultivar (Hourani 27). Among yield components, most of these ten lines were better than the checks in spike length, thousands kernel weight, and spikelet per spike. The grain yield and other characters of the ten superior plants and check varieties are presented in Table 3.

2. Variation among glacousness

Variation for glaucousness showed that only 4.5% of the in “SafraMa´an” lines were glaucous (Table 4). Glaucous lines gave a grain yield per plant non different from non-glaucous ones. The non-significant association of glaucousness with yield, detected in this study, could be probably due to favorable environmental condition during that specific growing season and to the small number of glaucous lines. Similar patterns have been reported for durum wheat by Clarke *et al.* (1991) and for barley by Baenziger *et al.* (1983).However other reports indicated that glaucous genotypes exhibited higher yield in wheat (Merah *et al*., 2000) and in wild rye (Jefferson,1994). Glaucous lines had greater tiller number, kernels per spike, spikelet fertility, spike density, number of head per meter square, short awn length, small flag leaf area, late in heading, and short in grain filling period than non glaucous lines. Grain yield and other characters of the 13 glaucous and check cultivars are presented in Table 5.

Table 2. Variation for 16 characters in 286 tetraploid "SafraMa'an" landrace compared with mean values of the standard check cultivars (ACSAD 65, HOURANI 27 AND AMRA).

Trait	Range	Mean ±SE	Std Dev	F. vlaues	LSD (P-0.05)	CV %	ACSAD 65	HOURANI 27	AMRA
PHt	78.60-118.40	105.15±0.38	6.34	28.14**	2.81	4.42	88.27	111.87	74.6
FN	5.00-11.60	7.96±0.08	1.31	39.88**	0.56	10.2	9.00	8.93	6.00
SL	6.47-10.29	9.19±0.03	0.48	16.38**	0.32	4.99	8.74	8.29	8.28
SS	22.2-27.06	24.49±0.05	0.91	7.24**	0.92	5.31	21.53	24.6	21.53
NKS	33.13-60.40	45.49±0.16	2.72	5.20**	3.29	10.14	52.53	47.80	58.20
KS	1.33-2.58	1.86±0.007	0.1	7.6**	0.13	9.17	2.44	1.925	2.70
TKW	22.78-42.46	33.13±0.23	3.82	5.91**	3.94	18.37	28.33	34.5	26.24
SD	2.36-3.68	2.67±0.008	0.13	14.17**	0.10	5.14	2.47	2.96	2.608
AWL	6.34-14.52	12.59±0.05	0.84	18.11**	0.15	6.10	11.48	10.31	10.15
FLA	31.47-46.99	39.52±0147	2.48	5.70**	2.69	10.19	28.39	37.05	31.86
GYP (g)	3.99-12.32	7.94±0.08	1.42	9.60**	1.19	22.3	8.84	9.74	5.83
GFP	26.33-36.33	29.09±0.07	1.20	17.76**	0.76	3.79	37.67	31.67	28.67
HD	94.33-112.00	107.02±0.08	1.46	27.21**	0.73	1.01	91.3	103.33	102.00
MD	130.67-140.0	136.12±0.05	0.89	32.22**	0.38	0.44	129.00	135.00	130.66
HM^2	167.7-382.23	269.63± 2.49	4289	84.04**	12.47	6.72	301.76	92.33	210
BY	20.77-57.54	37.35±0.41	6.97	27.81**	3.71	13.7	31.3	39.36	26.4

GYP: Grain yield / plant (g); PHt: Plant height (cm); TN: Fertile tillers / plant; FLA: Flag leaf area (cm^2); AWL: Awn length (cm); TKW: 1000 Kernel weight (g); NKS: Kernels / spike; SL: Spike length (cm); SS:Spikelets / spike; KS: Kernels / spikelet; SD: Spike density; GFP : Grain filling period (day); HD: Days to heading (day); MD: Days to maturity (day); HM^2: Head/ $meter^2$;BY : Biological yield (g).

Table 3. Grain yield per plants and other characters of the ten superior lines and the check cultivars.

Line	GYP	PHt	TN	FLA	AWL	TKW	NKS	SL	SS	KS	SD	GFP	HD	MD
185	12.32	109.80	10.80	43.13	13.16	38.02	45.40	9.60	22.47	2.02	2.62	29.34	107.67	137.00
191	11.42	110.46	10.80	39.21	12.12	34.67	45.67	8.84	23.46	1.95	2.64	27.67	108.67	136.34
113*	11.38	114.60	10.86	44.60	12.15	32.45	55.53	9.68	27.06	2.06	2.80	31.00	106.00	137.00
164	11.19	115.80	09.73	40.30	12.10	38.39	45.93	9.56	24.30	1.89	2.54	27.34	109.34	136.70
091	11.16	109.90	09.80	40.60	12.46	37.98	48.00	9.80	25.40	1.88	2.60	29.67	107.34	137.00
193	11.13	095.06	08.93	46.39	12.03	41.12	45.47	9.65	24.26	1.87	2.53	27.67	110.00	137.67
078	11.09	109.60	09.26	35.53	12.43	36.73	51.67	9.53	26.40	1.95	2.77	31.00	105.00	136.00
196	11.03	109.86	11.60	44.19	10.94	29.89	46.33	9.85	24.46	1.89	2.49	28.67	107.34	136.00
126	10.98	115.20	09.20	46.99	13.45	37.58	48.60	9.81	26.00	1.88	2.65	30.33	106.34	136.67
280	10.92	110.73	10.06	41.23	13.24	40.30	44.46	9.86	24.20	1.83	2.46	29.00	107.00	136.00
L286	07.94	105.15	07.96	39.53	12.59	33.14	45.49	9.19	24.49	1.86	2.67	29.09	107.03	136.12
L287	08.84	88.27	09.00	28.30	11.98	28.30	52.53	8.74	21.53	2.44	2.47	37.70	091.33	129.00
L288	09.74	111.87	08.93	37.05	10.31	34.50	47.80	8.29	24.60	1.93	2.96	31.70	103.30	135.00
L289	05.83	74.60	06.00	31.80	10.15	26.30	58.20	8.28	21.53	2.70	2.61	28.70	102.00	130.78

** Glaucous lines; A = Mean of 286 lines; L287 = line287 from Assad 65; L288 0 line from Hourani 27; L289 = line 389 from Amra GYP: Grain yield / plant (g); PHt: Plant height (cm); TN: Fertile tillers / plant; FLA: Flag leaf area (cm^2); AWL: Awn length (cm); TKW: 1000 Kernel weight (g); NKS: Kernels / spike; SL: Spike length (cm); SS:Spikelets / spike; KS: Kernels / spikelet; SD: Spike density; GFP : Grain filling period (day); HD: Days to heading (day); MD: Days to maturity (day); HM^2: Head/ $meter^2$; BY : Biological yield (g).*

Table 4.Variation among glaucous (g)/non-glaucous (ng) lines for 16 characters in 286 tetraploid "SafraMa'an" landrace.

Trait	g vsng	N	Range	Mean ± SE	Pr> |t|
Plant height (cm)	g	13	79 -114	102.46±2.56	0.12 ns
	ng	273	83-118	105.2±0.37	
Fertile tillers/plant	g	13	6.20 -10.93	8.47±0.0.53	0.18 ns
	ng	273	5.00 -12	7.94±0.08	
Spike length (cm)	g	13	6.47 -9.82	8.70±0.27	0.0001*
	ng	273	7.53 -10.29	9.21±0.03	
Spikelets / spike	g	13	22.80 -27.07	24.33±0.31	0.52 ns
	ng	273	22.20 -26.80	24.50±0.06	
Kernels / spike	g	13	41.66 - 60.40	48.26±1.58	0.0001*
	ng	273	33.13 - 53.8	45.36±0.15	
Kernels / spikelet	g	13	1.70 - 2.58	1.98±0.07	< 0.0001*
	ng	273	1.33 - 2.11	1.85±0.01	
1000 Kernel weight (g)	g	13	22.78-39.37	31.87±1.49	0.23 ns
	ng	273	26.28-42.46	33.20±0.23	
Spike density	g	13	2.53-3.68	2.84 ±0.09	< 0.0001*
	ng	273	2.36 - 3.08	2.66 ±0.01	
Awn length (cm)	g	13	6.34-14.50	11.64± 0.57	< 0.0001*
	ng	273	10.15-14.52	12.63±0.04	
Flag leaf area (cm^2)	g	13	34.63- 44.62	38.09±0.81	0.03*
	ng	273	32.15-46.99	39.59±0.15	
Grain yield / plant (g)	g	13	4.33 -11.37	8.10±0.48	0.67 ns
	ng	273	3.98 -12.32	7.93±0.09	
Grain filling period	g	13	26.33-31.00	29.02±0.70	2.870*
	ng	273	26.33-36.33	29.14±0.06	
Days to heading (day)	g	13	104.60-112.00	106.95±1.10	0.15 ns
	ng	273	94.33-110.33	107.01± 0.08	
Days to maturity (day)	g	13	133.34-139.00	135.37.±0.49	0.53 ns
	ng	273	130.66-140.00	136.17±0.05	
Head/ $meter^2$	g	13	208.89-367.70	292.73±1.54	0.15 ns
	ng	273	167.78-382.20	268.50±2.54	
Biological yield (g)	g	13	23.30-54.93	36.75 ±2.29	0.60 ns
	ng	273	20.77-57.54	36.36±0.42	

** Significant at 5%levelof probability.*

Table 5. Grain yield and other characters of the 13 glaucous lines and the check cultivars

Line*.	GYP	PHt	TN	FLA	AWL	TKW	NKS	SL	SS	KS	SD	GFP	HD	MD	HM^2	BY
113	11.38	114.60	10.86	44.60	12.15	32.45	55.53	9.68	27.06	2.06	2.80	31.00	106.00	137.00	313.33	54.94
258	10.19	090.13	09.54	37.26	11.90	38.98	47.50	8.55	23.74	2.00	2.78	28.30	108.00	136.30	322.23	35.10
279	09.29	108.13	10.87	36.42	10.37	31.51	41.66	8.80	24.54	1.69	2.78	28.34	107.66	136.00	366.70	42.66
270	08.79	104.60	06.94	42.11	12.98	37.97	44.53	9.82	24.80	1.81	2.67	28.00	108.34	136.34	235.60	36.54
111	08.34	099.13	09.54	34.63	11.29	30.47	45.86	8.69	25.80	1.78	3.03	29.00	109.67	138.67	324.40	36.56
264	08.17	110.87	10.94	38.54	06.34	22.78	52.20	6.48	23.80	2.19	3.68	27.34	109.00	136.34	367.80	43.93
269	08.12	101.60	08.20	36.79	12.39	33.64	42.80	8.85	23.60	1.82	2.67	29.34	106.67	136.00	278.90	33.27
271	07.91	108.06	07.27	36.11	12.78	31.78	45.60	9.37	24.24	1.83	2.66	29.00	107.67	136.67	254.50	37.59
268	07.79	099.60	07.00	40.19	12.11	39.73	48.67	8.88	23.54	2.07	2.65	27.00	109.34	136.34	236.70	29.44
201	07.10	105.80	09.40	39.74	14.51	26.59	60.40	6.91	23.44	2.58	3.39	26.34	109.67	136.00	313.34	41.14
170	07.08	078.60	06.10	35.19	12.00	31.90	54.07	8.86	22.80	2.37	2.58	36.33	094.34	130.67	201.13	26.24
153	06.85	104.10	06.27	36.83	13.24	33.37	46.07	9.21	24.26	1.89	2.64	29.67	106.60	135.67	218.90	32.09
137	04.33	105.73	07.20	35.94	09.30	23.00	42.60	9.04	24.06	1.77	2.67	27.67	108.00	135.67	242.30	23.30
L286	07.94	105.15	07.96	39.53	12.59	33.14	45.45	9.19	24.49	1.86	2.67	29.09	107.03	136.12	269.60	37.34
L287	08.84	088.27	09.00	28.30	11.98	28.30	52.53	8.74	21.53	2.44	2.47	37.70	091.33	129.00	301.10	31.30
L288	09.74	111.87	08.93	37.05	10.31	34.50	47.80	8.29	24.60	1.93	2.96	31.70	103.30	135.00	307.78	39.36
L289	05.83	074.60	06.00	31.80	10.15	26.30	58.20	8.28	21.53	2.70	2.61	28.70	102.00	130.78	210.00	29.40

A = Mean of 286 lines; L287 = line287 from Assad 65; L288 0 line from Hourani 27; L289 = line 389 from Amra

GYP: Grain yield / plant (g); PHt: Plant height (cm); TN: Fertile tillers / plant; FLA: Flag leaf area (cm^2); AWL:Awn length (cm); TKW: 1000 Kernel weight (g); NKS: Kernels / spike; SL: Spike length (cm); SS:Spikelets / spike; KS: Kernels / spikelet; SD: Spike density; GFP : Grain filling period (day); HD: Days to heading (day); MD: Days to maturity (day); HM^2: Head/ $meter^2$; BY : Biological yield (g)

3. Trait distribution

Frequencies of plants in desirable classes and the two additional classes are presented in tables 6 and 7. The desirable classes ranged from low of 4.6% for glaucousness to 100% for erect juvenile growth habit. Most lines (73%) had excellent early growth vigor and al (100%) had erect juvenile growth habit, two of the most important traits for drought tolerance. Plant height and tillering capacity of these lines indicated their adaptability to semiarid environments, where grain and straw yield are equally important (Jaradat, 1992b). Similarly, high frequency of lines with excellent agronomic score (27%) may suggest that "SafraMa′an" population have high genetic diversity. Frequencies in desirable classes of spike related traits reflect the high level of adaptability of this population to semiarid environment. The high frequency of long spike (50.7) and the low frequency (17.8) of high 1000 kernel weight (10.9) of high number of kernels/spike and dense (9.1) spikes demonstrate the selective pressure in this population. Frequency of this population with early heading, early maturity and long grain filling period are considered as indicators of increase tolerance to drought (Blum *et al.,* 1989; Jana *et al.,* 1990).

4. Estimates of Diversity Indices (H′)

Variation or polymorphism was common, with different degrees, for most traits, indicating a wide variability within population of "Safra Ma´an" landrace. Estimates of (H′) for individual traits are presented in Table 6 and 7. These estimates ranged from 0.0 (monomorphic) for Juvenile growth habit to 0.91 (highly polymorphic) for spike length, while most traits showed relatively high levels of polymorphism. Few of these traits (e.g. early growth vigor and glaucousness) displayed low (H′) estimates. However, a low (H′) estimate may reflect unequal frequencies of different class rather than the absence of the desirable class for a particular trait.

Average (H′) estimate for "SafraMa´an" landrace population, based on traits evaluated in this study, was 0.65 ± 0.047. However, when only drought-related traits were considered, as done by Blum *et al.* (1989), Jana *et al.*(1990; and Jaradat (1992a), (H′) estimate dropped to (0.61 ± 0.08). Similar pattern of reduction was obtained by Jaradat (1992a).

5. Cluster Analysis

Cluster analysis was performed with the quantitative data only according to Weltzien (1989). This analysis resulted in 6 clusters (Table 8). The means are presented for each quantitative trait for all clusters. Cluster 1 contains most lines of the population including Hourani. The landraces in this cluster were moderate in heading and maturity, shorter in grain filling period, taller than the mean, higher in grain yield per plant, lower number of tillers than the mean and larger flag leaf and taller awn length. Cluster 2 contains one line from "Safra Ma′an" population and Acsad 65, which is a check variety. Lines in this cluster, characterized by shorter, less number of tillers than those in the first cluster, showed longer grain filling period, earlier in heading and maturity, taller awn length and smaller flag leaf area than the first cluster. Cluster 3 had only one line characterized by low number of kernel, large thousand kernel weight. It was shorter than the mean, and has long awn length and large flag leaf area than the first two clusters. Cluster 4 contains only Amra, cultivated in Jordan, and characterized by low number of tillers and short plant, medium in filling period, heading and maturity dates. Amra yielded less than "Safra Ma′an" landrace population and was shorter in awn length and had smaller flag leaf area compared to "Safra Ma'an" landrace population. In cluster 5 there was only one line which was characterized by taller than the mean, longer awn length and larger flag leaf area than the mean of the landrace. Cluster 6, characterized by taller in height but shorter in awn length than the mean of "Safra Ma′an" landrace, had greater flag leaf area and grain yield per plant than mean of landrace.

Table 6. Frequency in three class's and diversity index (H′) estimates for 16 quantitative plant characters in "SafraMa'an" landrace population

Trait	N	Desirable Class	$C_1 \leq \overline{X} - S_d$	$\overline{X} - S_d < C_2 < \overline{X} + S_d$	$C_3 \geq \overline{X} + S_d$	H′
Plant height (cm)	286	Tall	14.70	72.00	13.30	0.72
Tillers/plant	286	High	13.60	68.90	17.50	0.76
Spike length (cm)	286	Tall	15.00	34.00	50.70	0.91
Spikelets/sp ke	286	High	16.40	66.40	17.10	0.79
Kernels/spike	286	High	10.20	78.90	10.90	0.61
1000 KW (g)	286	Heavy	15.70	66.40	17.80	0.79
Kernels /sp kelet	286	High	9.40	80.40	10.10	0.57
Spike density	286	Dense	13.30	77.60	9.10	0.67
Awn length (cm)	286	Tall	10.10	77.60	12.20	0.62
Flag leaf area (cm^2)	286	Large	15.70	68.20	16.10	0.77
Grain yield (g)	286	High	15.40	69.90	14.70	0.75
Grain filling period (day)	286	Long	12.50	71.90	15.40	0.71
Days to heading (day)	286	Early	10.80	78.70	10.50	0.60
Days to maturity (day)	286	Medium	9.10	74.50	16.40	0.66
Head per $meter^2$	286	High	14.00	68.90	17.10	0.76
Biological yield (g)	286	High	15.70	68.20	16.10	0.77

Table 7. Percentage of each category of the qualitative traits to the total number of cases of"Safra Ma'an" lanrace population

Trait	Desirable class	Category 1	Categery 2	category 3	H′
Early growth vigor	Exellent	4	23	73	0.63
Juvenile growth habit	Erect	100	0	0	0
Glaucousnes VS. non glaucousnes	Glaucous	4.6	95.4	0	0.27

Table 8. Quantitative plant characteristics in 289 lines, aggregated into 6 clusters

Cluster	Lines	PHt	TN	SL	SS	NKS	KS	TKW	SD	AWL	FLA	GYP	GFP	DH	DM	HM²
1	283	105.3	07.9	09.2	24.5	45.4	1.90	33.2	2.70	12.6	39.5	7.90	20.09	107.0	136.0	269.3
2	2	083.4	07.5	08.8	22.2	53.3	2.40	30.1	2.50	11.8	31.8	7.90	37.00	092.8	129.8	251.0
3	1	089.9	08.5	08.8	24.8	33.1	1.33	41.2	2.81	14.0	43.3	7.30	29.60	110.3	140.0	290.0
4	1	074.6	06.0	8.28	21.5	58.2	2.70	26.3	2.60	10.1	31.9	5.82	28.60	102.0	130.7	210.0
5	1	105.8	09.4	06.9	23.5	60.4	2.50	26.6	3.40	14.5	39.7	7.10	26.40	109.7	136.0	313.3
6	1	110.9	10.9	06.5	23.8	52.2	2.19	22.8	3.70	06.3	38.5	8.20	27.40	109.0	136.3	368.0
Mean		095.0	08.4	8.08	23.4	50.4	2.17	30.1	2.95	11.5	37.5	7.40	28.10	105.0	135.0	283.3

PHt: Plant height (cm); TN: Fertile tillers / plant; SL: Spike length (cm); SS: Spikelets / spike; NKS: Kernels / spike; KS: Kernels / spikelet; TKW: 1000 Kernel weight (g); SD: Spike density; AWL: Awn length (cm); FLA: Flag leaf area (cm²); GYP : Grain yield / plant (g); GFP : Grain filling period (day); HD: Days to heading (day); MD: Days to maturity (day); HM²: Head/ meter² .

able 9. Quantitative plant characteristics in 289 lines, aggregated into 11 clusters

Cluste	Line	PHt	TN	SL	SS	NKS	KS	TKW	SD	AWL	FLA	GYP	GFP	DH	DM	HM²
1	278	105.3	07.95	09.21	24.49	45.5	1.86	33.16	2.67	12.65	39.5	7.95	29.08	107.1	136.3	269.4
2	2	110.7	07.95	08.82	24.4	46.1.	1.89	34.1	2.77	11.48	38	8.45	30.5	105.5	136	275
3	1	105.7	07.2	09.05	24.6	42.6	1.78	23.0	2.66	9.3	35.94	4.33	22.7	108	135.6	242.3
4	1	114.0	10.87	09.68	27.1	55.53	2.06	32.6	2.81	12.15	44.62	11.38	31	106.0	137.0	358.9
5	1	89.9	8.47	08.84	24.8	33.13	1.33	41.28	2.81	14.05	43.1	7.31	29.67	110.3	140.0	290.0
6	1	105.8	9.4	06.92	23.5	60.4	2.57	26.6	3.39	14.51	39.74	7.1	26.33	109.6	136.0	313.3
7	1	110.9	10.94	6.48	23.8	52.2	2.19	22.8	3.68	6.34	38.54	8.16	27.33	109	136.3	367.8
8	1	78.6	6.07	8.87	22.8	54.1	2.38	31.9	2.58	12.1	35.2	7.9	36.33	94.3	130.7	201
9	1	92.4	6.0	7.54	23.2	43.2	1.86	33.7	3.08	13.2	40.1	5.52	29.7	108	137.7	205.6
10	1	88.2	9.0	8.74	21.5	52.5	2.44	28.3	2.47	11.9	28.3	8.84	37.7	91.3	129	301.1
11	1	74.6	6.0	8.28	21.5	58.2	2.70	26.3	2.61	10.15	31.8	5.83	28.7	102	130.8	210
Mean		095.0	08.4	8.08	23.4	50.4	2.17	30.1	2.95	11.5	37.5	7.40	28.10	105.0	135.0	283.3

PHt: Plant height (cm); TN: Fertile tillers / plant; SL: Spike length (cm); SS: Spikelets / spike; NKS: Kernels / spike; KS: Kernels / spikelet; TKW: 1000 Kernel weight (g); SD: Spike density; AWL: Awn length (cm); FLA: Flag leaf area (cm²); GYP : Grain yield / plant (g); GFP : Grain filling period (day); HD: Days to heading (day); MD: Days to maturity (day); HM²: Head/ meter² .

The above results indicate that “Safra Ma′an” landrace population is similar to Hourani 27, at a distance of 0.55, but different from the other cultivated checks Acsad 65 and Amra, at a distance of 0.48: Hourani 27 locate at separate cluster with one line from “Safra Ma′an” population and the population with 3 checks will separate into 11 clusters (Table 9). The run of cluster analysis over the complete data sets resulted in 82 clusters at a distance of 0.25; more the 50% of clusters consisted of one or two plants only. This was considered systematically unreasonable although it demonstrated the magnitude of polymorphism in this population. The presence of several clusters in this population at the 48% of the total Euclidean distance indicates the high variability within this population.

IV Discussion

The variation exhibited by the lines in 16 quantitative characters indicates that “Safra Ma’an” landrace population is a heterogeneous population, which includes a number of genotypes differing for quantitative characters of agronomic importance as well as for morphological and quality characters; thus, selection for several of these characters may be effective. Plant height is believed to be an important character for adaptation in non-irrigated areas under late season water stress condition (Okuyama *et al*., 2005) because one of the main effects of a dry spell during the growing season is a drastic reduction of stem elongation with a reduction of straw yield and the impossibility of combine harvesting the crop (Ceccarelli *et al.*, 1987). Therefore, it was interesting to find a large number of lines significantly taller than the tallest local cultivar Hourani. The finding ws expected since several studies have indicated the presence of variation within landrace populations in quantitative and qualitative traits (Poiarkova and Blum, 1983; Ceccarelli *et al.,* 1987; Ehdaie and Waines, 1989; Jaradat, 1992a; Jaradat, 1992b; Jaradat *et al*.,2004; Al-Nashash *et al*.,2007). Drought stress is probably the most important environmental factor affecting plant productivity. Because of the prevalence of drought, plants have various morphological and physiological characteristics that enable them to grow and reproduce in low rainfall environment. Studies with isogenic lines have shown that glaucousness out yielded non-glaucousness especially under stress condition. Glaucousness reduced residual transpiration (Clarke and Richards, 1988) and thus represents a desirable character for plant adaptation to drought. Thus, selection for glaucousness may be a goal in breeding programs. The greatest difference between the glaucous and non-glaucous lines for most characters must have resulted from better water use efficiency, as a result of low residual transpiration rates (Clarke and Richards, 1988). These results indicate that under dry land conditions the breeding programs should be directed toward the increase of glaucous lines in order to increase these characters. Frequencies in desirable classes of traits that are known to confer drought tolerance in wheat (Blum *et al.*, 1989; Jana *et al.,* 1990; Jaradat, 1992b) were relatively low, especially when compared with Jordan landraces. These results indicate a low pressure for selection compared to selection pressure obtained by Jaradat (1992b) in Jordan landraces. Estimates of diversity indices (H′) is relatively smaller than the one reported for Jordan wheat landraces (0.707 ± 0.05) which was based on 24 morphological traits (Jaradat, 1992a). Also, (H′) estimate for “Safra Ma′an” population is lower than that reported for Mediterranean region (0.792 ± 0.04) (Jana *et al.,* 1990), which was based on 27 traits most of which were include in this population. From this result, we can conclude that “Safra Ma´an” wheat landrace population could be an important source of genetic variability for selection procedure, the initial stage of wheat breeding.

V Conclusions

This study was conducted to assess the magnitude of phenotypic variation for several traits in tetraploid “SafraMa‘an” wheat landrace and to evaluate the potential usefulness of several traits after planting 286 lines from “Safra Ma‘an” landrace wheat and three check cultivars during 1995/1996 growing season at Maru Agriculture Research Station.

Polymorphism was common, in varying degrees, for most traits as indicated by a wide phenotypic variation within population of “Safra Ma‘an” landrace. Lines with glaucousness character were found in this population without being significantly different from non glaucous lines in grain yield per plant. Extensive variation is found in this landrace population and thus improvement in this wheat landrace may be possible.

The information generated in this study can be utilized in a breeding program in at least two different ways. First is the release of the highest yielding lines as pure line varieties, after testing their stability in different environments (locations and years). Second is the utilization of superior plants, for yield as well as for other characters, as parents in the crossing program to introduce additional desirable characters in an adapted genetic background.

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Exploiting landrace genetic diversity for germplasm enhancement in durum wheat breeding in Morocco

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Abstract. Traditional durum wheat farming communities have contributed for centuries to the evolution and enrichment of on-farm conservation of diverse wheat landraces, and to the development of farmer's seed exchange in order to ensure the continued evolution and diversification of these landraces especially under dry growing conditions. Landraces are genetically heterogeneous and have over many generations become adapted to the local environment and cultural conditions under which they are grown. However, during the last century, the introduction of high-yielding varieties, and the structural changes in wheat farming systems, led to the loss of genetic diversity of wheat landraces. In Morocco, landraces of durum wheat are still cultivated by farmers especially in marginal regions such as mountains and Saharan areas. Durum wheat landraces are highly appreciated for their adaptation to some abiotic stresses and mainly for their good grain and straw qualities. This paper summarizes some studies aiming to assess the amount of diversity of Moroccan landraces collected in two different agro-ecological areas of Morocco, and determine options for adding the value of these landraces. The evaluation of these landraces focused on agro morphological characters and specific quality parameters. The results showed a large genetic variability in this germplasm proving the possibility of using landraces as promising genepool in breeding program especially for improving grain quality. The results indicated also the possibility of improving on farm landraces productivity through "composite landraces" approach and low cost agricultural packages.

Keywords. Durum wheat landraces – Traits donors – Genetic diversity – Breeding – Adding value.

Exploiter la diversité génétique des variétés locales pour la valorisation du matériel génétique de blé dur au Maroc

Résumé. *La culture traditionnelle du blé dur par les communautés rurales a contribué au fil des siècles à l'évolution et à l'enrichissement de la conservation in situ de diverses variétés locales de blé, et au développement de l'échange de semences par les agriculteurs afin d'assurer l'évolution et la diversification continues de ces variétés locales, en particulier en conditions pluviales. Les variétés locales sont génétiquement hétérogènes et depuis de nombreuses générations, elles se sont adaptées à l'environnement local et aux conditions de culture. Cependant, au cours du siècle dernier, l'introduction de variétés à haut rendement, et les changements structurels dans les systèmes de culture du blé, ont conduit à la perte de la diversité génétique des variétés locales de blé. Au Maroc, les variétés locales de blé dur sont encore cultivées par les agriculteurs, en particulier dans les zones marginales telles que les montagnes et les régions sahariennes. Les races primitives de blé dur sont très appréciées pour leur adaptation aux stress abiotiques et principalement, pour la bonne qualité de leur grain et de leur paille. Dans cet article, on parcourt des études réalisées afin de mesurer la diversité des variétés locales marocaines collectées dans deux domaines agro-écologiques différents du pays et de déterminer les options possible pour accroître la valeur ajoutée de ces variétés locales. L'évaluation de ces races primitives est axée sur des caractères agro-morphologiques et des paramètres de qualité spécifiques. Les résultats ont fait ressortir une grande variabilité génétique de ce matériel indiquant ainsi la possibilité d'utiliser les variétés locales comme fond génétique prometteur dans des programmes de sélection, en particulier en vue d'améliorer la qualité du grain. Les résultats ont également confirmé qu'il est possible d'améliorer la productivité des variétés locales in situ par une approche "variétés locales composites" et des paquets agricoles à faible coût.*

Mots-clés. *Variétés locales de blé dur – Caractères des donneurs – Diversité génétique – Sélection – Valeur ajoutée.*

I – Introduction

The area planted with cereals in Morocco is about 5 million ha. Durum wheat (*Triticum turgidum* var. L. *durum*) is grown on over one million hectares. 45% of which are sown in the arid and semi-arid regions, 11% in high altitudes and 44% in more favorable areas (Nsarellah *et al*., 2011). The average durum wheat consumption is about 90kg/person/year. Morocco is ranked third in Mediterranean regions and first in North Africa and Middle East regions in term of durum wheat acreage. Because of the importance of this crop in Morocco, breeding program at Institute National de la Recherche Agronomique (INRA) has always provided the necessary efforts to release new varieties of durum wheat, that are productive, resistant to biotic and abiotic stresses, and of good quality. The official national catalogue lists over 35 durum wheat varieties. However, the agro-industries provide almost 99% of their supply from abroad. The release of durums with high quality standards is one of the priorities of breeding programs in the country in recent years. Landraces may provide new alleles for the improvement of commercially valuable traits.

Increasingly, the country is considered as centre of diversity for a number of cultivated crop plants and wild relatives. Indeed, Morocco constitutes one of the most important areas of diversity in the Mediterranean region. It is an important centre of diversity for global crops such as barley, faba bean, and wheat (Neal-Smith, 1955; Nègre, 1956 and Perrino *et al*., 1984). Morocco's crop diversity results from long-term adaptation to various local environmental conditions such as drought, cold and salinity (Sauvage, 1975; Graves, 1985). In many Moroccan traditional cropping systems, genetic diversity may be the only resource available to resource-poor farmers to cope with the environmental conditions and optimize their crop production. Moroccan durum wheat landraces represent an important source of valuable genetic resource (Sadiki *et al*., 2000). In fact, landraces of durum wheat are still cultivated especially by farmers in the mountains and arid regions of the country. They are highly appreciated by farmers for their adaptation to abiotic stresses and mainly for their good grain and straw qualities.

However, genetic diversity of the major crops including the durum germplasm has suffered an overall reduction over time as a consequence of their replacement by high-yielding varieties and urbanization (Zine el abidine *et al*., 1995). This genetic diversity is also facing the climate change threat. Durum wheat landraces have been largely replaced, in their centers of diversity by monocultures of pure genotypes. This genetic erosion resulted in significant loss of valuable genetic diversity of quality traits and resistance or tolerance to biotic and abiotic stresses. Also, durum wheat landraces from the mountainous areas of Morocco are known for their stem solidness which is an important trait for resistance to wheat stem sawfly (Damania, 1991).

Ex situ (gene bank) and *in situ* (on farm) conservation of these valuable genetic resources are ways to safeguard this genetic diversity from extinction for their present and future sustainable uses. Effective management and potential use of these genetic resources in breeding program require evaluation and description of the diversity in the gene pool, characterization of available accessions in order to detect the presence of variants of possible interest for breeding purposes.

II – Analysis of genetic diversity of Moroccan durum wheat landraces

The complexity of the population structure of wheat landraces may arise from a number of different homozygotes and the occurrence and frequency of heterozygotes in populations. The assessment of genetic diversity between and within wheat landraces is essential to utilize landraces as donors of traits in wheat breeding, and to identify priority areas for on-farm conservation.

Thirty nine landraces collected from oasis (Errachidia site) and nine from mountains (Taounate site) (Table 1) were characterized for the main agro-morphological traits according to descriptors suggested by Bioversity International; growth habit, plant height, spike characters (spike length and density, length and colour of awn, size and colour of grain, number of spikelets per spike and number of grains per spike.

Table 1. List of landraces collected from Errachidia and Taounate sites.

Site	Reference's landraces
Errachidia	CM98E1, CM98E2, CM98E3, CM98E4, CM98E5, CM98E6, CM98E7, CM98E8, CM98E9, CM98E10, CM98E11, CM98E12, CM98E13, CM98E14, CM98E15, CM98E16, CM98E17, CM98E18, CM98E19, CM98E20, CM98E21, CM98E22, CM98E23, CM98E24, CM98E25, CM98E26, CM98E27, CM98E28, CM98E29, CM98E30, CM98E31, CM98E32, CM98E33, CM98E34, CM98E35, CM98E36,CM98E37, CM98E38, CM98E39
Taounate	CM98T40, CM98T41, CM98T42, CM98T43, CM98T44, CM98T45, CM98T46, CM98T47, CM98T48

Variance analysis showed a high genetic diversity between and within all the landraces analysed in the two sites (Table2). Principal and factorial components analysis led to the classification of landraces into homogeneous groups characterized by specific traits (Figure 1 and Table 3).

Similar studies were done on Moroccan barley landraces and showed a high genetic variability between and within landraces (Rhrib and Taghouti 2001). Zarkti *et al*. 2010 measured genetic distance and diversity of seventeen Moroccan landraces through molecular markers analysis. The results revealed a high genetic diversity between the analyzed landraces; the hierarchical classification came up with five clusters related to earliness.

Table 2. Extent of genetic variability in Errachidia and Taounate landraces for the main agro-morphological traits.

Traits	Observed F Differences between landraces[1]	F test intra Errachidia accessions	F test intra Taounate accessions
Growth habit	3.19***	-	-
Height (cm)	4.25***	-	-
Awn length (cm)	6.56***	6.04***	4.19***
Awn color	105.33***	2.93***	13.35***
Spike length (cm)	13.42***	3.37***	2.17***
Spike density	8.62***	NS	4.13***
Number of sp kelets/spike	5.17***	2.06***	NS
Number of grains/sp kelet	4.65***	1.86***	2.10***
Thousand kernel Weight (g)	1.27NS	1.81***	2.21

*1 level of significance of F test: * significant at 0.05. ** significant at 0.01. *** Significant at 0.001, NS: Not significant.*

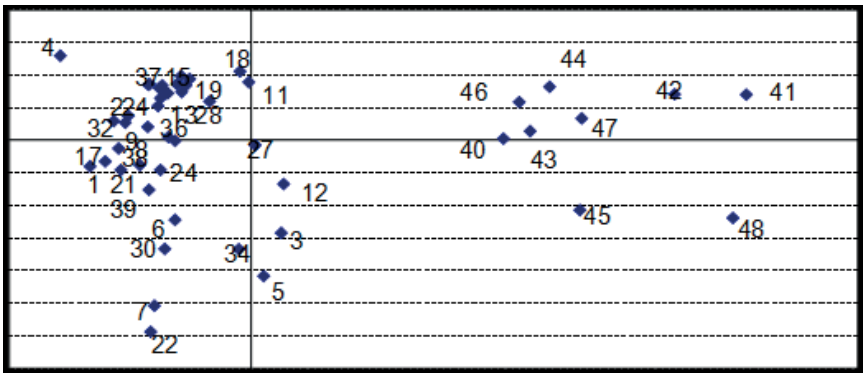

Figure 1. Representation of the Errachidia and Taounate Landraces in the graph formed by PCA1 and PCA2 axes.

Table 3. Groups of durum wheat landraces based on the multivariate analysis (FCA).

Groups	Traits	Traits level	Landrace origine
1	Grain color Grain size Spike density Number of grains/sp ke Thousand kernel Weight (g) Height (cm) Growth habit	Brown Intermediate Intermediate 25-43 22-44 107-137 Low	CM98T40, CM98T41, CM98T42, CM98T43, CM98T44, CM98T45, CM98T46, CM98T47, CM98T48
2	Number of grains/sp ke Number of sp kelets/spike Grain size Awn length (cm) Spike length (cm) Height (cm)	45-53 21-23 Large 18-19 5.9-6.9 137-153	CM98E1, CM98E8, CM98E14, CM98E16, CM98E19, CM98E20, CM98E23, CM98E25, CM98E28, CM98E29, CM98E31, CM98E32, CM98E33, CM98E37, CM98E38
3	Grain size Growth habit	Intermediate strong	CM98E 2, CM98E4, CM98E5, CM98E10, CM98E11
4	Grain size Awn color Awn length (cm) Growth habit	Large Black 16-18 medium	CM98E 3, CM98E 21, CM98E22, CM98E24, CM98E27, CM98E30, CM98E34, CM98E39
5	Spike length (cm) Awn length (cm) Number of sp kelets/spike Growth habit	5.9-6.9 17-21 18-22 strong	CM98E 15, CM98E25, CM98E7, CM98E33, CM98E35.

III – Adding value of durum wheat landraces

The local ecotypes are an important reservoir of genetic variability. Many authors were unanimous on the adaptation of landraces to their environment and confirmed that they outyielded the improved varieties in marginal areas and under low-input farming systems (Weltzein and Fishbek, 1990, Jaradat, 2011; Jaradat, 2013). Maintaining these landraces is therefore related to their utilization by farmers. The improvement of their productivity would surely contribute to their conservation.

On-farm conservation goal in Errachidia and Taounate sites is to encourage farmers to continue to maintain and manage durum wheat landraces. The primary method for achieving this goal is to Increase the value of durum wheat landraces on farm. Farmers on these two sites are still using traditional techniques in their parcels. The weeding is generally not practiced or if done manually

it is too late. Seed borne diseases especially smut, bunt, and fusariose lead to the reduction of germination and thus of the yield.

Fungi seed treatment against seed borne diseases and chemical weeding of the crop are technologies that can increase the landrace productivity and ensure that they have a better added value. Furthermore, composite landraces made up of promising lines of selected landraces could be another technique for durum wheat landraces valorisation.

1. Influence of fungi treatment of seeds and chemical weeding on grain yield of durum wheat landraces

Five to ten kilogramms of seed lots of five landraces collected from five farmers of Errachidia and Taounate sites were divided into four lots. These seed lots were sown in plot on farmer field under four treatments; 1) fungi treated seeds in weeded plot, 2) fungi treated seeds in non-weeded plot 3) non fungi treated seeds in weeded plot and 4) non fungi treated seeds in non-weeded plot (check).

The analysis of variance (ANOVA) showed the significant effect of the fungi seed treatments and weeding; combined weeding and fungi seed treatment effect on grain yield of landraces was depending on the sites (Table 4). The combined effect of fungi treatment of seeds and weeding was significant in Taounate site and not significant in Errachidia site. Whereas, the individual effect of fungi treatment and weeding was significant in Taounate site and allowed an increase of yield of 33% and 17% respectively. But in Errachidia site, the only significant effect was fungi seeds treatment that allowed a grain yield gain of 34%. Similar study was conducted on Moroccan landrace of barley in Taounate site and showed the positive effect of fungi seeds treatment and weeding on grain yield (Rhrib and Amri, 2002).

Consequently, some farmers of the two sites were provided with simple manual machines for seed treatment (Photo 1). Farmers were taught how to operate and manipulate these machines and encouraged to integrate fungi seed treatment before sowing.

Table 4. Effects of fungi treatments of seeds and weeding on grain yield of durum wheat landraces in Taounate and Errachidia sites.

Treatments	Taounate site			Errachidia site		
	Test F[1]	Probability	Mean (kg/ha)	Test F	Probability	Mean (kg/ha)
Fungi seeds treatment and weeding combined	5.28	0.0052**	227.10	1.88	0.1732 NS	502.50
Fungi seeds treatment	4.50	0.0429*	246.70	5.06	0.038*	575.00
weeding	10.74	0.0038**	216.70	0.29	0.59NS	485.00

*1 level of significance of F test: * Significant at 0.05. ** Significant at 0.01.*

2. Evaluation of yield potential of composites durum wheat landraces

Three types of genotypes have been used:

- A composite landrace composed of a mixture of the most productive lines selected from the Errachidia landraces,
- One landrace originating from Errachidia site,
- An improved durum wheat variety; Oum Rabiaa.

This trial was conducted in a plot of $10m^2$ each with two replications in Errachidia farmer's fields. The measures recorded were grain and straw yield. The results showed that the composite population out yielded the original landrace. The yield gained by the mixture was about 8%

compared to the original landrace. Both of them have out yielded the improved variety Oum Rabiaa (Figure 2).

This result revealed the possibility to increase the productivity of durum landraces by the development of new landraces composed of a mixture of promising lines. The study done by Tesemma, (1996) showed that composite landrace of durum wheat outyielded the most common improved variety in Ethiopia by 40% and the original landrace by 37%. Similar studies have been done on barley (Ceccarelli and Grando, 2000; Rhrib and Taghouti, 2002) and bread wheat (Moghaddan *et al*., 1997).

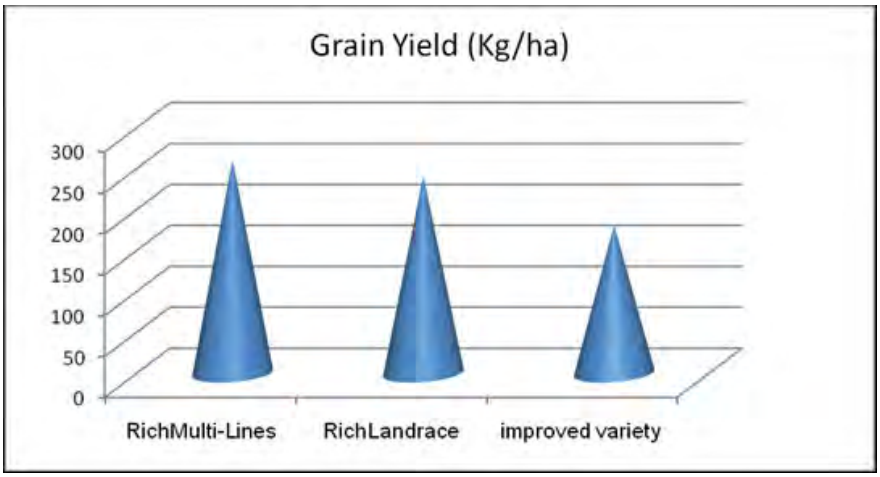

Figure 2. Grain yields of the three types of genotypes (multilines, landrace and improved variety) used on farm.

IV – Potential value of landraces in durum wheat breeding programs

Landraces could act as donors of important characteristics, such as drought and cold tolerance, and mainly grain quality. In general, they represent significantly broader genetic diversity than modern varieties and, therefore, they could contribute to extend the genetic base of modern cultivars. Moroccan durum wheat landraces hold large genetic variability and considerable number of alleles with the probability of having some of these alleles associated with stress tolerance and yield (Nachit *et al*., 2004; Pagnotta *et al*., 2004). In Mediterranean countries, durum wheat landraces were largely used in breeding programs and contributed to the development of improved varieties in dry areas (Nachit, 1992). The identification of quality parameters such as protein content, gluten strength, yellow pigment and their integration in the improved varieties is a priority in research on durum wheat (Nachit *et al*., 1995). Mineral content in modern wheat cultivars has significantly decreased, including copper, iron, magnesium, manganese, phosphorus, selenium, and zinc. High levels of these nutrients can be found in landraces and old low-yielding varieties (Jaradat, 2011).

1. Agronomic evaluation of durum wheat local lines

Eight hundred lines derived from thirty-five landraces from Rif Mountains and pre-saharan regions of Morocco were evaluated. The lines were sown at the INRA experimental station of Merchouch in Augmented Design: 2 lines of 2.5m / line. Two improved varieties Oum Rabia and Karim were

used as checks. The traits scored for each line are the days to heading, the days to maturity, plant height, grain and straw yield and the spike length, the awn length and the number of grains/spike.

The ANOVA showed significant differences between the local lines for the majority of characters. Overall, the local lines of durum wheat are later in maturity and have greater plant height than the improved varieties Oum Rabia and Karim. The average height of local lines landraces overstep the varieties by 26 cm. In addition, they remain less productive in grain yield and more productive in straw than the checks (Table 5). Nevertheless, the existence of variability in the material for days to heading, days to maturity, plant height, spike, and awn length and the number of grains per spike is very useful for the national durum wheat breeding program.

The factorial analysis applied on the Manhattan dissimilarity matrix allowed local lines distribution on two axes (Figure 3). Results showed high genetic variability in the material studied. The majority of local lines of durum wheat differed from improved varieties Karim and Oum Rabia. Similar work has been done on a global collection of durum wheat and showed that the Moroccan germplasm contains valuable breeding characters such as earliness, the character "Straw semi-dwarf" and high values of the number of grains per spike and thousand kernel weight (Pecetti *et al.* 1992).

Table 5. Mean values, minimum and maximum of durum wheat local lines and mean values of two checks for all characters.

Traits	Local lines			Checks	
	Min	Mean	Max	Oum.Rabia	Karim
Days to heading	86	108,62*	135	94.28	92.75
Days to maturity	137	165,27	196	153.4	154.5
Height	75	111,72	145	85.83	85.31
GrainYield (g)	9	519,74*	1146	666.3	679.31
StrawYield (g)	79	1145,4	2827	944.8	867.56
Awn Lenght (cm)	15	21,6***	28	19.8	19.56
Spike Lenght(cm)	5.61	7,88***	16.33	7.52	7.83
Number of grains/sp ke	33	61.05**	93.67	58.87	59.73

*Level of significance of test F dans l'ANOVA: * significatif à 0.05, ** significatif à 0.01, *** significatif à 0.001.*

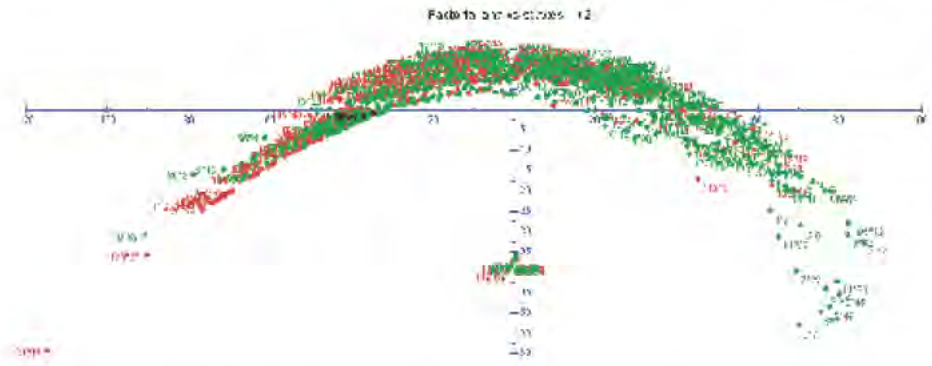

Figure 3. Dispersion of local lines on the axes of the factorial analysis. The arrow indicates the position of checks Karim and Oum rabia.

2. Evaluation of quality traits of local durum wheat lines

One hundred and fifty lines selected from durum landraces already evaluated on experimental station, and fifty advanced durum wheat lines have been evaluated for some physio-chemical quality parameters: Gluten strength was estimated by SDS sedimentation test according to the standard Moroccan method NM 08.1.217 (Anonymous), The yellow pigment content representing carotenoid content extracted by n-butanol saturated with water and expressed in micrograms of beta carotene per gram of dry matter (ppm) was determined according to the standard Moroccan method NM .1.216 08 (anonymous). The protein content expressed on the dry matter was determined by the Kjeldahl method, based on the standard method (AFNOR, 1991) (anonymous).

Uni-variate analysis showed highly significant differences between local and advanced lines for all quality parameters. Local lines showed a genetic diversity higher than in advanced lines mainly for SDS sedimentation index and yellow pigments rate (Table 6).

Table 6. Mean values ± standard error, minimum and maximum values for advanced and local durum wheat lines for the studied characters.

Quality traits	Mean ± standard error	Minimum	Maximum
Advanced lines			
SDS volumes (ml)	38.10 ± 1.79	17	70
Yellow pigments (ppm)	6.85 ± 0.26	2.53	9.32
Protein content (%)	12.80 ± 0.14	11.07	14.96
Local lines			
SDS volumes (ml)	71.65 ± 1.12	40	94
Yellow pigments (ppm)	8.62 ± 0.12	4.18	12.55
Protein content (%)	12.31 ± 0.11	10.41	15.88

Factorial analysis also showed a wide range for quality traits studied. The hierarchical clustering tree has grouped the local durum wheat lines into five distinct branches (B1, B2, B3, B4, and B5) (Table 7). Within each branch, groups and subgroups were identified. Lines within each group were characterized by well-defined quality criteria.

Genetic diversity highlighted in the local germplasm could be used in breeding programs to improve the technological quality of durum wheat varieties. Thus, quality improving attributes in these varieties will be based on the choice among these groups of durum wheat landraces lines those with high levels of these quality criteria and included them as parents in the breeding program. For example, Group 4 of the branch 2 contains lines with a high rate of yellow pigments.

Table 7. Groupment of local lines based on factorial analysis on quality parameters

Branch	Group	Sub Group	Lines of landraces	Mean values of traits		
				SDS (ml)	YP(ppm)	PC(%)
B1	G1	SG1	CM00E5(14), CM00E7(28,29), CM00E10(19,36), CM98T112(3).	62.8	8.07	12.55
		SG2	CM00E5 (13, 36, 42, 49), CM00E8(12).	62.2	8.58	13.28
		SG3	CM00E10(5, 18, 29, 48), CM00E5(5,12,23,29,34)	69.4	8.96	13.03
	G2	SG1	CM00E5(16), CM00E 12(3), CM00E 7(5,26,37), CM00E6(6), CM00E4(47).	71.8	7.45	12.39
		SG2	CM00E1(44), CM00E7(42), CM00E10(49).	73.0	8.71	14.97
		SG3	CM00E4(18), CM00E5(3,6,7,18,2,22,39) CM00E7(24, 49), CM00E10(30, 35, 46,47), CM00E11(48), CM98E105(8).	75.1	9.14	11.80

Branch	Group	Sub Group	Lines of landraces	Mean values of traits		
				SDS (ml)	YP(ppm)	PC(%)
B2	G1	SG1	CM98E10(3,4,48), CM00E12(4).	91.3	8.01	12.82
		SG2	CM00E5(1), CM00E7(38), CM00E10(42),	87.2	8.19	12.17
		SG3	CM00E12(8,11). CM00E5(41, 48), CM00E8(9), CM00E10(10,24,34)	89.3	9.85	11.45
	G2		CM00E5(38), CM00E7(48)	84.0	8.6	11.34
	G3	SG1	CM00E10(6), CM98E11(16).	79.0	6.67	11.74
		SG2	CM00E5(8), CM00E10(39), CM98E105(19).	80.3	10.06	11.74
		SG3	CM00E5(19,44), CM00E10(1,8,14,15,26,33).	79.3	8.70	11.85
	G4	SG1	CM00E5(9,30,47), CM00E7(25), CM98E10(2,7,11,16,20,32), CM98E18	82.4	9.21	12.20
		SG2	CM00E2(47), CM00E10(12).	82.0	11.73	11.54
		SG3	CM00E5(2,10,20,32,), CM00E7(23), CM00E10(17,31,43,47), CM98E19(5).	84.5	9.91	12.09
B3	G1	SG1	CM00E7(8,21).	71.0	6.35	12.37
		SG2	CM00E10(9).	83.0	12.52	10.41
		SG3	CM00E1(22), CM00E 7(9,22,30,47).	78.4	7.81	12.05
B4	G1	SG1	CM00E10(13,22,25,27), CM00E 11(6), Karim, Oum Rabiaa	53.6	8.52	12.27
		SG2	CM98T112(34), CM00E12(5).	57.5	6.17	12.10
	G2	SG1	CM00E2(15,40), CM00E6(31), CM00E10(40), CM00E12(10).	43.0	8.09	13.67
B5	G1	SG1	CM00E9(56), CM98T112(33).	66.0	6.45	11.62

V – Conclusions

Moroccan landraces analyzed displayed, as expected, a wide range of genetic diversity. This local germplasm forms an interesting source of favorable quality traits such as protein content, gluten strength and yellow pigments content useful to durum wheat breeders.

The persistent cultivation of durum wheat landraces in some Moroccan regions attests to their continued value to farmers, and to their competitive agronomic or nutritional advantage relative to modern varieties. Adding value of these landrace is the main motivating factor for their on- farm conservation. Fungi seed treatment against seed-born diseases and chemical weeding at the right time could improve the landraces productivity in a simple way.

Furthermore, Composite landraces made up of promising lines selected from landraces could be another way for durum wheat landraces valorization.

But, on-farm conservation of durum wheat genetic resources in Morocco could be more efficient provided that legislation changes are made that make it possible to market landraces as diversified genetic materials and encourage their consumption.

Moroccan durum wheat landraces have over many generations become adapted to the local environment and cultural conditions under which they are grown. Development of new varieties from landraces could be a viable strategy to improve yield and yield stability, especially under stress and future climate change conditions.

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Allelic variation for *GS* and *GOGAT* genes in a tetraploid wheat collection

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Abstract. Nitrogen is one of the major limiting nutrients in most plant species and is mostly assimilated as reduced form of ammonium. Ammonium is assimilated into amino acids through the synergic activity of two enzymes: glutamine synthetase (GS) and glutamate synthase (GOGAT). While Glutamine synthetase genes are a gene family whose enzymes are located in both cytoplasm (GS1, GSe and GSr) and plastids (GS2), glutamate synthase exists in two different isoform depending on the electron donor used as cofactor: NADH-dependent and Fd- dependent GOGAT, both active in plastids. GS catalyses the incorporation of ammonium into glutamate, producing glutamine. GOGAT catalyses the transfer of the amide group of glutamine to 2-oxoglutarate, resulting in the formation of two molecules of glutamate. This assimilation requires cofactors, reducing equivalents and other compounds generated during photosynthesis. Glutamine and glutamate serve as nitrogen donors for the biosynthesis of many other molecules, mainly for amino acid, directly involved in protein biosynthesis and ultimately in grain protein content. The aim of the present work was to assess the correlation between grain protein content and GS genes through identification of new allelic variations in a collection of durum wheat genotypes. For this purpose a collection of 240 tetraploid wheat genotypes (*Triticum turgidum* L.), was analyzed allowing the identification of 5 different haplotypes for the genes *GS2-A2* and *GS2-B2* of which the "a" allele of *GS2-A2* was found significantly correlated with grain protein content.

Keywords. GS – Wheat – Functional markers – Association mapping.

Variation allélique des gènes GS et GOGAT dans une collection de blé tétraploïde

Résumé. *L'azote est l'un des principaux nutriments limitant pour la plupart des espèces de plantes et est le plus souvent assimilé comme une forme réduite de l'ammonium. L'ammonium est assimilé aux acides aminés grâce à l'activité synergique de deux enzymes: la glutamine synthétase (GS) et la glutamate synthase (GOGAT). Alors que les gènes de la glutamine synthétase sont une famille de gènes dont les enzymes sont situées à la fois dans le cytoplasme (GS1, GSe et GSr) et les plastes (GS2), la glutamate synthase existe sous deux isoformes différents selon le donneur d'électrons utilisé comme cofacteur: GOGAT NADH dépendante et FD-dépendant, tous les deux actifs dans les plastes. GS catalyse l'incorporation de l'ammonium dans le glutamate, produisant la glutamine. GOGAT catalyse le transfert du groupe amide de la glutamine à 2-oxoglutarate, conduisant à la formation de deux molécules de glutamate. Cette assimilation nécessite des cofacteurs, des équivalents réducteurs et d'autres composés générés lors de la photosynthèse. La glutamine et le glutamate servent de donneurs d'azote pour la biosynthèse de nombreuses autres molécules, principalement pour les acides aminés, directement impliqués dans la biosynthèse des protéines et en fin de compte dans la teneur en protéines du grain. L'objectif de ce travail a été d'évaluer la corrélation entre la teneur en protéines du grain et les gènes GS grâce à l'identification de nouvelles variations alléliques dans une collection de génotypes de blé dur. A cet effet, une collection de 240 génotypes de blé tétraploïdes (Triticumturgidum L.) a été analysée permettant l'identification de 5 haplotypes différents pour les gènes GS2-A2 et GS2-B2 dont l'allèle "a" de GS2-A2 a été trouvé significativement corrélé avec la teneur en protéines du grain.*

Mots-clés. *GS – Blé – Marqueurs fonctionnels – Cartographie d'association.*

I – Introduction

Wheat, together with rice and maize, is one of the most important cereal crops grown worldwide and provides most of the proteins in human diet. As reviewed by Chatzav *et al*. (2010), the demand for cereal continues to grow as a consequence of a constantly increasing world population (for wheat, approx. 2% per year; Skovmand *et al*., 2001). So far, increasing yield has been one of the main objectives in plant breeding programs. But another important concern of wheat breeding programs is the nutritional value of staple food crops (Cakmak, 2008; Cakmak *et al*., 2010; Chatzav *et al*., 2010). Genetic diversity existing in crop plants is important for their eventual use in breeding programs for enhanced food production. As a result of the intense breeding techniques carried in the last decades, modern variety show a high uniformity with a reduction of genetic variation. Genetic diversity could be the result of geographical impact through evolution and hence traits could be considered as a function of variety (Benadeki, 1992). Choosing the appropriate parents is essential in crossing programs aimed to enhance the genetic recombination for potential yield increase (Islam, 2004). Among the most efficient tools for parental selection in wheat hybridization programs there are the estimation of genetic distance and the evaluation of the level and structure of genetic diversity (Khodadadi *et al*., 2011). In this context, landraces, wild forms *(Triticum* ssp.), and other related wild species can have crucial roles in breeding programs (Peleg *et al*., 2008).

Nitrogen uptake is an essential element in crop improvement, either directly for grain protein content or indirectly for photosynthetic production. One factor determining nutritional value in cereal is grain protein **c**ontent (GPC), also strictly related to the baking properties of common wheat (*Triticum aestivum* L. ssp*. aestivum*) as well as the pasta-making characteristics of durum wheat (*Triticum turgidum* L. ssp*. durum*) (Blanco *et al*., 2012). Domestication and the intense wheat management practices used in the last decades determined a serious erosion of genetic diversity resulting in genetic uniformity of modern varieties (Tanksley and McCouch, 1997; Ladinzinsky, 1998). GPC is a typical quantitative trait controlled by a complex genetic system and influenced by environmental factors and management practices, as well as nitrogen and water availability, temperature and light intensity. This character was found influenced by two major enzymes responsible for cyclic assimilation of ammonium into amino acids in the biochemical pathway of $NH4^+$ assimilation; i.e., glutamine synthetase: (GS) and glutamine-2-oxoglutarate amidotransferase: (GOGAT) (Nigro *et al*., 2013; Gadaleta *et al*., 2011; Gadaleta *et al*., 2014). These two enzymes are involved in assimilation and recycling of mineral N catalyzing ATP-dependent conversion of glutamine into glutamate using ammonia as substrate (Cren and Hirel, 1999; Ireland and Lea, 1999). Glutamine synthetase gene encodes for an enzyme responsible of the first step of ammonium assimilation and transformation into glutamine, essential compounds in aminoacid-biosynthetic pathway. On the bases of phylogenetic studies and mapping data in wheat, ten *GS* cDNA sequences were classified into four sub-families denominate *GS1* (a, b, and c), *GS2* (a, b, and c), *GSr* (1 and 2) and *GSe* (1 and 2) (Bernard *et al*. 2009). Genetic studies in rice (Obara *et al*. 2004) and maize (Hirel *et al.* 2001, 2007; Galais and Hirel 2004) demonstrated co-localisations of QTLs for GS protein or activity with QTLs relating to grain parameters at the mapped *GS* genes. The aim of the present work was to assess the genetic variation of GS2 genes in a collection of durum wheat genotypes.

II – Material and methods

A collection of 229 tetraploid wheat genotypes (*Triticum turgidum* L.), including old and modern cultivars of durum wheat (*T. turgidum* L. ssp*. durum*) and wild relatives was used for genetic studies. The collection, including 128 old and modern cultivars of durum wheat (*T. turgidum* L. var. *durum*) and 103 wild and domesticated tetraploid wheats, was grown in the experimental field of the University of Bari at Valenzano (Bari, Italy) in 2009 using a randomized complete block design with three replications and plots consisting of 1-m rows, 30 cm apart, with 50 germinating seeds per plot. Genomic DNA was isolated from fresh leaves using the method described by Sharp *et al*.

(1988) and subsequently purified by phenol-chloroform extraction. DNA quality and concentration was determined by spectrophotometer analysis at 260 and 280 nm (A260/A280 ratio = 1.6-1.8) and by agarose gel electrophoresis. Functional markers were designed by using Primer3 and OligoExplorer software for GS2 genes based on sequences reported by Gadaleta et.al. (2011).

DNA amplifications for fragment sequencing were carried out in 25 µl reaction mixtures, each containing 25 ng template DNA, 2 µM of each primer, 200 µM of each dNTP, 2.5 mM MgCl2, 1X PCR buffer (10 mM Tris-HCl, pH 8.3, 10 mM KCl) and 0.5 unit of Taq DNA-polymerase. The following PCR profile in a Bio-Rad DNA Thermal Cycler was used: initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 1 min, 55°C/65°C (depending on the tested primer combination) for 2 min, 72°C for 1 min with a final extension at 72°C for 15 min. PCR products were detected by agarose gel electrophoresis 1.5%. Single PCR fragments were directly purified with EuroGold Cycle Pure Kit and sequenced in both strands by BMR Genomics service. Sequence assembly was obtained with “Codone Code Aligner” and “Geneious” assembly programs. Gaps and uncertain sequence were resolved by primer walking. Regions of less coverage or ambiguous reads were rechecked with primers designed to cover those regions.

III – Results and discussion

Genetic diversity in wheat refers to both genetic and phenotypic variance. For example, plants could show different seed size, height, flowering time, flavor, but also they could be different for other characteristics such as resistance to biotic and abiotic stresses, as diseases and pests or heat/cold response, respectively. That means that variations exist in almost every trait, also complex ones such as nutritional quality and taste. But, when a trait of interest cannot be found in the modern crops due to their genetic uniformity, valuable alleles could be identified in in the wild ancestors of crop plants (Aaronsohn, 1910; Tanksley and McCouch, 1997). For this purpose, association mapping analysis was applied for studying nitrogen metabolism in wheat. In higher plants inorganic nitrogen, in the form of ammonia, is assimilated via the glutamate synthase cycle or GS-Gogat pathway.

In the present work candidate gene approach has been applied to the study of grain protein content in durum wheat, focusing the study on the glutamine synthetase genes as potential candidates for determining grain protein content (GPC) (Gadaleta *et al*., 2011). The aim of the present work was to assess the correlation between grain protein content and GS2 genes through a study of association mapping in a collection of durum wheat genotypes. For this purpose a collection of 240 tetraploid wheat genotypes (*Triticum turgidum* L.), including old and modern cultivars of durum wheat (*T. turgidum* L. ssp. *durum*) and wild relatives were evaluated for grain protein content in replicated trials and under different environmental conditions. The analysis of variance revealed highly significant differences at $P<0.001$ among genotypes. The mean GPC of the 234 tetraploid accessions was 46.2% with a range of 11.8% to 25.2%; the hereditability was of 0.64.Two functional markers were designed for the two homoeologous genes *GS2-A2* and *GS2-B2* genes and analyzed in the whole collection. Different aplotypes were identified for both genes. The analysis allowed the identification of 5 different haplotypes for both genes confirmed by sequences analysis of the obtained fragments. Functional markers were amplified in the wheat collection and electrophoretic pattern of GS-A2 gene is reported in figure 1. In particular, we considered two alleles for the gene *GS2-A2* (named “a” and “b”, corresponding to the presence/absence of a fragment of 480 bp physically mapped on 2A chromosome. The regression analysis carried out between the functional markers and grain protein content trait showed a positive significant correlation. The “a” allele of GS2-A2 was found significantly correlated with grain protein content, with a probability of $P>0.001$. The GS2-A2 gene co-localized with a major QTL for GPC identified by Gadaleta *et al*. (2011) and Blanco *et al*., (2012).

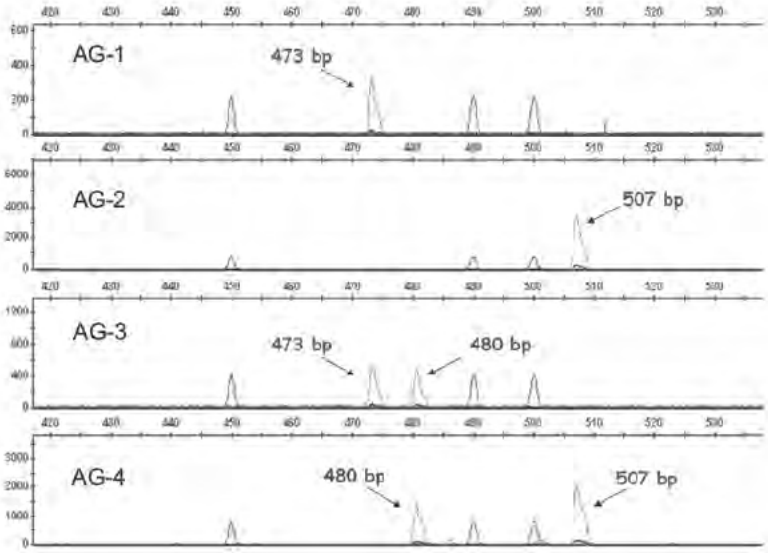

Figure 1. Electrophoretic pattern of four different haplotypes detected by using a *GS2-A2* functional marker.

IV – Conclusions

In higher plants, ammonium, whether resulting from nitrate assimilation or from secondary sources, is first incorporated into glutamine in a reaction catalysed by glutamine synthetase, and then glutamate synthase catalyses the combination of glutamine with 2:oxoglutarate to form two molecules of glutamate, one of which serves as substrate for GS, while the other one is available for transport, storage or further metabolism. These two reactions form a cycle referred to as the GS/GOGAT pathway.

We conclude that the results presented in the present study confirm the involvement of the GS2 enzymatic complex in the accumulation of grain protein in kernels and identified new alleles increasing this important character useful in assisted selection programs. Besides the present work open the way to further investigation using the forward and reverse genetic approaches that have been successfully used to validate the role of GS genes for grain production both in rice and maize (Fontaine *et al.* 2009).

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Evaluation of a hulled wheat (emmer and spelt) collections

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Abstract. A collection of 422 accessions of hulled wheat (219 emmer and 203 spelt) was studied to individuate the variability for some morphological and qualitative traits (heading date, plant height, thousand kernel weight, grain protein content, SDS sedimentation test). Results of this work highlighted a huge variability for the examined traits both for emmer and spelt accessions. Several accessions, possessing useful agronomical traits like earliness, short straw, large kernel, high protein content and SDS values, were identified for further evaluation in replicated trials.

Keywords. Hulled wheat – Emmer – Spelt – Variability – Germplasm collection – Marginal environments – Qualitative traits.

Évaluation de collections de blé mondé (amidonnier et épeautre)

Résumé. *Une collection de 422 accessions de blé mondé (219 d'amidonnier et 203 d'épeautre) a été étudiée pour identifier la variabilité de certains caractères morphologiques et qualitatifs (date d'épiaison, hauteur de la plante, poids de mille grains, teneur en protéines du grain, test de la vitesse de sédimentation SDS). Les résultats de ces travaux ont mis en évidence une grande variabilité des caractères examinés pour les accessions d'amidonnier et d'épeautre. Plusieurs accessions, possédant des caractères agronomiques utiles comme la précocité, la paille courte, un gros grain, une teneur en protéines et des valeurs du SDS élevées, ont été identifiées pour une future évaluation dans des répétitions.*

Mots-clés. *Blé mondé – Amidonnier – Épeautre – Variabilité – Collection de matériel génétique – Milieux marginaux – Caractères qualitatifs.*

I – Introduction

Triticum turgidum L. subsp. *dicoccon* Schrank (emmer) and *Triticum aestivum* subsp. *spelta* (spelt) are among the most ancient cereal crops (Nesbitt and Samuel, 1996). Over the centuries the cultivation of these hulled wheats was replaced by free-threshing and higher-yielding wheats. At present emmer and spelt are considered minor crops, cultivated in marginal areas of several European countries, including Italy (Perrino *et al.*, 1996). Their main value lies in their ability to give good yield in poor soils and tolerance to abiotic and biotic stresses. Hulled wheats should know a new development due to the nutritional value of the grain, the special taste of the products and their characters of resistance to pests and disease (Zaharieva *et al*., 2010). The increasing interest for ecologically grown products and for special diets based on health foods has led to a renewed interest in their cultivation, mainly for organic farming.

The growing attention for hulled wheats led scientists to start improvement programs to increase their adaptability, yield and qualitative characteristics as well as identify useful traits that could be transferred to durum and bread wheat (Pagnotta *et al*., 2009).

The aim of this work is to describe the variability for some morpho-physiological and qualitative traits of an emmer and spelt collection.

II – Material and methods

The collection, mainly from Institute of Plant Genetics of the Italian National Research Council (CNR-IGV), consists of 422 accessions (219 emmer and 203 spelt). Accessions were grown in single-row plots in 2011 at CRA-QCE experimental farm in central Italy (Rome-41°58'N 12°28'E alt 20 m asl) on deep soil having an outright clayey texture. Sowing date was February 9 and harvest date was July 15.

Some morpho-phisiological and qualitative traits were determined: heading date, plant height, thousand kernel weight (TKW), grain protein content, sodium dodecyl sulphate sedimentation (SDS).

The heading date, reported as number of days after April 1st, is the date in which 70% of the plants of the plot shows the spike emerged. Plant height (cm) is the average size of the plants of the plot from the ground level at the peak of the spike, excluding awns.TKW was determined by counting the number of kernels in a sample of at least 5 g. The sample was obtained by manual removing the hulls from spikelets.

Protein content (% d.m.) was performed by Dumas combustion method and Leco FP 428 instrument; SDS test (ml) was carried out following the ICC method 151, using a solution of SDS in lactic acid at 3% for tetraploid wheats and at 2% for hexaploid wheats.

III – Results and discussion

A summary of geographical origin of the accessions is reported in Table 1.

Table 1. Geographical origin of the accessions.

Geographical areas	Number of Accessions	
	Emmer	Spelt
Central East Africa	22	
Western Asia	20	
Eastern Europe	12	
Spain	11	72
Balkans	10	
Germany	1	6
Switzerland		11
Other areas	8	3
Unknown origin	14	7
Total	219	203

Most of the emmer accessions are from Central-East Africa (48 accessions) and Western Asia (44 accessions) while most of spelt accessions are from Spain (147 accessions).

A high level of variability was detected for most of the traits of emmer and spelt (Table 2 and Figure 1). Heading date of 219 emmer accessions ranged from May 8 to June 3 (38 days and 64 days after April 1st, respectively), with an average value of 52.7 da; 57% of the accessions showed a cycle length not lower than 53 da. Heading dates of 203 spelt accessions ranged from May 16 to June 10 (46 days and 71 da after April 1st, respectively) with an average value of 62.1 da, about ten days later than emmer. Almost 80% of the spelt accessions showed late cycle with heading dates ranging from 60 to 65 da.

Hulled wheats are generally tall plants. In the examined collection, values of emmer plant height were between 60 and 143 cm, with an average of 96.8 cm and half the accessions shorter than

100 cm. Average plant height of spelt accessions was 119.4 cm, taller than emmer one, ranging from 73 to 146 cm; more than 90% of the materials were taller than 100 cm.

Table 2. Means and range of variations for some traits measured for 219 emmer and 203 spelt accessions.

	Emmer				Spelt			
	Average	Min	Max	SD	Average	Min	Max	SD
Heading time	52.7	38	84	6.2	62.1	46	71	3.2
Plant height	96.8	60	143	15.7	119.4	73	146	12.6
TKW	36.1	21.1	51.3	6.6	39.8	23.2	56.6	5.7
Grain protein	17.3	13.2	22.8	1.7	18.5	13.6	23.5	1.4
SDS	32.1	14	80	13.5	56.3	15	90	11.5

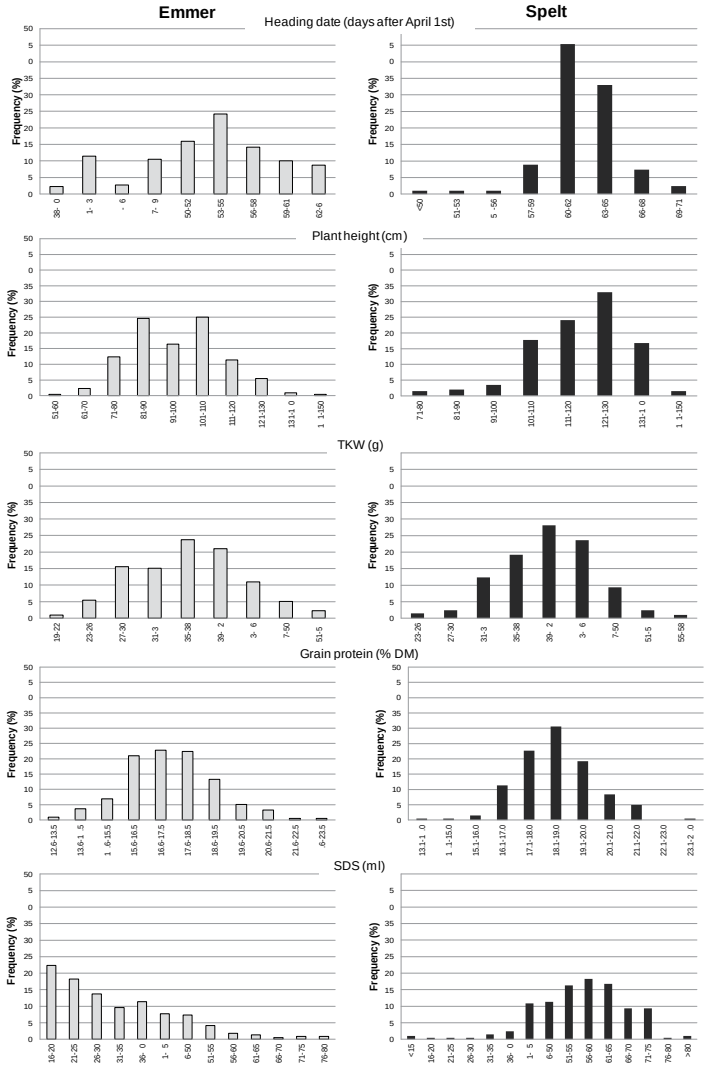

Figure 1. Distribution of frequency for heading date, plant height, TWK, grain protein and SDS measured on the Emmer (219 accessions) and Spelt (203 accessions) collection.

TKW is the character indicating seed dimension. TKW of 219 emmer accessions ranged from 21.1 to 51.3 g, with an average of 36.1 g; TKW of 203 spelt accessions was slightly higher than emmer, ranging from 23.2 g to 56.6 g with an average of 39.8 g. About one-half of spelt materials (99 accessions) achieved TKW value higher than 40 g, while such threshold was exceeded only by 58 emmer accessions (about a quarter).

Hulled wheats are characterized by high protein content that can reach 18-23% (Blanco et al., 1990; Perrino *et al*., Cubadda and Marconi, 1994). Analysis of grain protein content highlighted that all the accessions of the collection were characterised by a protein level higher than 13% (emmer accessions: range 13.2 - 22.8%; spelt accessions:13.6-23.5%), with 9 out to 10 materials having a protein content exceeding 15.5% for both species. Grain protein content was higher than 17% for 171 spelt accessions (84%) and 118 emmer accessions (54%).

SDS sedimentation volume is correlated with rheological parameters (Borghi *et al.,*1996) and gives a reliable evaluation of quantitative and qualitative aspects of protein, particularly of gluten.

A high variation was found for SDS in hulled wheats (Blanco *et al*., 1990; Perrino *et al*., 1993). In the examined collection, the range of SDS test values was similar for the two species (emmer: 14-80 ml; spelt: 15-90 ml) but more than 90% of the spelt accessions were characterised by high values, exceeding 40 ml (average value 56.3 ml); on the contrary emmer SDS values were lower: 75% of accessions was lower than 40 ml and 50% lower than 30 ml, with an average value of 32.1 ml.

IV – Conclusions

Results of this work highlighted a huge variability for the examined traits both for emmer and spelt accessions. Several accessions of emmer and spelt, possessing useful agronomical traits like earliness, short straw, large kernel, high protein content and SDS values, were identified for further evaluation in replicated trials. It is worth to highlight the large number of spelt accessions showing both high grain protein content and SDS sedimentation test values.

Further field trials are needed in order to identify the accessions suitable for different Italian environments or useful as source of genetic diversity for future wheat breeding programs.

The cultivation of these crops may highlight the links of products with territory and its history offering, at the same time, final products with high qualitative, organoleptic and nutritional traits.

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The strategies to serve and conserve Moroccan durum wheat genetic diversity before it is lost

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Abstract. Morocco is considered as a center of diversity of many species, including *Triticum* spp. This diversity is under the verge of extinction and hence urgent actions are needed to conserve it. Many actions are undertaken to reach that goal. Collecting missions were undertaken across Morocco and accessions are preserved at Gene Bank of Morocco. To widen this collection, recently two collecting missions were undertaken. One covered the Atlas Mountains, Ziz and Daraa valleys and carried out during 2009-10 cropping season, and the other covered the northern part of Morocco and carried out during 2010-11. The number of accessions collected were 16 in the South and 6 in the North. To assess the variation present in the Moroccan material, accessions from different provinces were tested along with improved cultivars were tested at different experimental station during 2009-11. The results indicated that the most prevalent diseases were yellow rust and to some extent leaf rust. Some of the old accessions are good sources of resistance to these diseases.
To promote on-farm conservation of landraces, on-farm field experiments using the same sub-core collection were carried out at different places. The result of these trials revealed that almost all improved cultivars were selected both by farmers and scientists along with some landraces. The magnitude of the positive correlation between agronomic scores attributed by farmers and scientists shed light on the importance to involve farmers in selection. The main traits of selection by the latter are grain yield, biomass, grain size and color, and earliness.

Keywords. Morocco – Durum wheat landraces – Genetic diversity – Participatory plant breeding Rusts.

Les stratégies pour soutenir et conserver la diversité génétique du blé dur marocain avant qu'elle ne soit perdue

Résumé. *Le Maroc est considéré comme un centre de diversité de nombreuses espèces, y compris Triticum spp. Cette diversité est menacée d'extinction et des mesures urgentes sont donc nécessaires pour la préserver. Plusieurs actions ont été entreprises pour atteindre cet objectif. Des missions de collecte ont été menées à travers le Maroc et les accessions sont conservées auprès de la Banque de Gènes du Maroc. Pour élargir cette collection, deux missions de collecte ont été effectuées dernièrement. La première, couvrant les montagnes de l'Atlas et les vallées du Ziz et Daraa, a été réalisée durant la saison de culture 2009-10 et l'autre, dans la partie nord du Maroc, a été effectuée en 2010-11. Le nombre d'accessions collectées étaient de 16 dans le Sud et 6 dans le Nord. Pour évaluer la variabilité du matériel marocain, les accessions provenant de différentes provinces ont été testées et comparées avec des cultivars améliorés, dans différentes stations expérimentales en 2009-11. Les résultats ont indiqué que les maladies prédominantes sont la rouille jaune et, dans une certaine mesure, la rouille des feuilles. Certaines des anciennes accessions constituent des sources utiles de résistance à ces maladies.*

Pour promouvoir la conservation à la ferme des variétés locales, des expériences au champ, avec la même sous-collection, ont été réalisées dans différents endroits. Le résultat de ces essais a révélé que presque tous les cultivars améliorés ont été sélectionnés à la fois par des agriculteurs et des chercheurs à l'instar de quelques variétés locales. L'ampleur de la corrélation positive entre les scores agronomiques attribués par les agriculteurs et les chercheurs a mis en évidence qu'il est important d'impliquer les agriculteurs dans la

sélection. Les principaux caractères retenus par ces derniers pour la sélection sont le rendement en grain, la biomasse, la taille et la couleur du grain et la précocité.

Mots-clés. *Maroc – Variétés locales de blé dur – Diversité génétique – Sélection végétale participative – Rouilles.*

I – Introduction

The flora of Morocco consists of more than 7000 plant species with more than 20% of endemism. Owing to its richness, in terms of the number of species and the high rate of endemism, Morocco harbors two internationally recognized biodiversity hotspots, the Atlas mountains and the Rif mountains among the 10 Mediterranean Basin Biodiversity hot spots. Morocco is also considered as a center of diversity of many cultivated species including *Triticum* spp. The local landraces of these crops offer an important gene pool as sources of adaptation and tolerance to many biotic and abiotic stresses. This important genetic material is continuously subjected to genetic erosion due to habitat degradation and the adoption of the improved varieties that are reducing significantly the acreage grown to landraces in many parts of Morocco. This loss of diversity is detrimental as farmers, mainly in marginal areas, rely on this diversity for their income and well-being. These landraces harbor large amounts of genetic variability and they need to be conserved before they are lost.

Two main strategies were adopted to alleviate the genetic erosion: Preservation through *ex-situ* conservation and encouraging their on-farm conservation (*in-situ*) through local use. On-farm conservation through local use is undoubtedly the best and long-lasting strategy to serve and conserve the biodiversity. To reach this goal and to promote the local use of landraces, on-farm field experiments using a core collection were carried out at Errachidia (2009-10), Rich and Taounat (2010-11) and using farmer's participatory breeding/variety selection approaches to involve farmers in the process of selection in order to pave the way for a successful re-adoption and local use of landraces. Moreover, two collecting missions were undertaken during two consecutive years to explore areas that have not been covered or less covered in the previous missions and to broaden the genetic base. A total of 549 accessions of durum wheat were collected during previous missions and are preserved at INRA-Genebank. Their repartition by province is listed in Table 1. The geographic localization of some of these accessions is exhibited in Figure 1.

Collecting missions should be an on-going process in order to broaden the genetic base and to long-term conserve the diversity. However, the collected landraces are to be used in breeding programs as donors of valuable traits and/or promoted for their re-adoption by farmers. In order to screen good sources of resistance to biotic stresses and to select the best performing accessions amongst the old wheat previously collected and preserved in gene-bank, core collections covering all provinces were sown at Annoceur experimental station during 2009-2010, and sub-core collections, tested on-farm, were sown at Meknès (Morocco) and Njoro (Kenya) experimental stations during 2010-2011 cropping season.

Table 1. Number of durum wheat accessions available at INRA Settat Genebank and those tested at Annoceur during 2009-10and at Meknès (Morocco) and Njoro (Kenya) during 2010-11.

	Available	Tested at	
	At Settat	Annoceur	Meknès and Njoro
Province	Genebank	(2010)	(2011)
AGADIR	25	7	3
Al HOCEIMA	21	6	4
AZILAL	18	17	8
BENI MELLAL	29	9	2
BOUARFA	6	2	1
EL JADIDA	8	2	2
ERRACHIDIA	29	13	2
FES	44	11	5
KHENIFRA	22	5	1
KHOURIBGA	3	1	0
MARRAKECH	57	15	7
MEKNES	26	8	2
MISSOUR	2	0	0
OUJDA	51	14	4
OURZAZAT	27	19	10
RABAT	49	7	3
TANGER	3	1	0
TAZA	50	12	4
TETOUAN	52	16	3
TIZNIT	27	8	1
Total	**549**	**173**	**62**

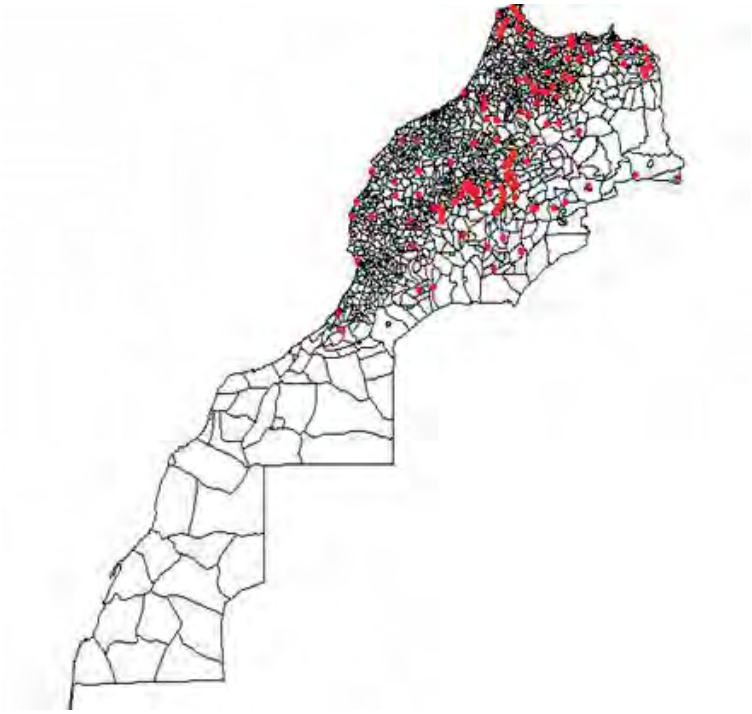

Figure 1. Sites of some previously collected Durum accessions available at INRA-Settat Genebank.

II – Methodology

1. Collecting missions

Defining target areas to be covered by the survey and landraces collections a very important issue to avoid redundancy and to fill the gap. That is, to conduct surveys in areas that were not covered previously. For that purpose, information regarding the covered areas (number of collected accessions of durum wheat by province) during previous collecting missions was documented. The available accessions are listed in the Table 1, from where we might detect the gap to be filled. In addition to areas that were not covered, those where the number of accessions is less than 20 (Table 1) were re-prospected to have a larger collection.

The best period to do the survey and collection is at maturity growth stage to collect individual spikes to be increased by single-spike descent and separately evaluated. Moreover, to gather as much information as possible, a questionnaire was prepared beforehand. For each collected sample, the data recorded were: name of the site and its coordinates (geo-referenced data), label of the collection, local name of the collected wheat, kind of collection (grains or spikes), origin of the seeds (on farm production, bought from neighbors or local market), seed treatment before sowing, since when this wheat is cultivated, the reasons behind the use of these old wheats (yield, biomass, grain color and size, biotic and/or abiotic resistance/tolerance, quality criteria (bread, couscous, or other uses).

Since landraces are becoming scarcer and scarcer and to collect as much as possible, we adopted many strategies during the missions of collection. Landraces were then collected from:

i. Farmers houses (grain of previous year);

ii. Spikes from fields of landraces (mainly durum);

iii. Spikes of old cultivars from fields of improved cultivars;

iv. Spikes from threshing areas;

v. Grains from local markets.

The itineraries of the collecting missions of wheat landraces undertaken during 2010 (south) and 2011 (north) are shown in Figure 2. The first mission covered the Atlas mountains and ZIZ and DARAA valleys, including the area between Errachidia, Rissani and Marzouga (not shown on the map), and the second one covered three provinces (Chefchaouen, Tetouan, and Taounate) with 6 rural counties (Zinat, BniLeit, Al Hamra, Derdara, Bab Taza, and Kissane).

Figure 2. Itinerary of the collecting mission of wheat landraces undertaken during May-June 2010 (left) and during July 2011 (right), within the frame of ITPGRFA (FAO) – INRA Morocco project.

2. On-farm field experiments and Farmers' participatory selection approach

The on-farm trials were carried out at Errachidia during 2009-2010 and at Rich and Taounat during 2010-11 cropping seasons. A core collection of 62 accessions was chosen on the basis of the amount of seeds available in the genebank and area of collection (Table 1) along with 18 commercial cultivars as local checks (Table 2). The repeated local checks were assigned according to an augmented design to control the experimental error. Each experimental plot was 3 rows of 1.5 m each and 30 cm apart. Plots were 60 cm apart from each other within blocks and 1 m from each block. The experiments were carried out by farmers using their cultivation methods.

Table 2. Durum wheat improved cultivars included in the experiment carried out at INRA-Annoceur Experimental station during 2009-2010 and at Meknes and Njoro during 2010-2011

Durum wheat cultivars			
Amjad	**Marjana**	Ourgh	Anouar*
Amria	Marouane	Tomouh	Tarek
Irden	Marzak	***Vitron*** **	Jawhar
Isly	Nassira	Yasmine	Sarif
Karim	Oum Rabia	Massa*	

** Tested only in Meknès and Njoro.*
*** Not tested in Meknès and Njoro,*

At physiological maturity, two simultaneous approaches were adopted: screening by scientists just before the arrival of farmers; and screening by farmers in groups of 3 to 5 farmers. Both scientists and farmers were requested to give an agronomic score ranging from 1 (the worst) to 5 (the best entry) and the reasons of selecting or discarding a given cultivar.

3. Ex-situ field experiment

To evaluate the accessions previously collected and taking into account the amount of seeds available, 173 accessions and covering all provinces (Table 1) were evaluated at Annoceur during 2009-2010 and 62 of them were tested at Meknès and Njoro during 2010-2011, in addition to 18 commercial cultivars (Table 2). Experimental plots were two rows of 1 m long each and 30 cm apart and 60 cm between plots.

The main objective of this experiment was to evaluate these accessions regarding their reaction to the prevalent biotic stresses namely yellow and leaf rusts in Morocco (Annoceur and Meknès) and stem rust in Kenya (Njoro). The severity and response ratings for the adult plant reaction under field conditions to rust diseases were based on the modified Cobb scale, where the severity was scored from 0 to 100% and the host infection response was rated as I = immune response, no sign of infection; R = resistant, very small uredinia with necrosis; MR = moderately resistant, small to moderate uredinia with necrosis; MS = moderately susceptible, small to moderate uredinia with chlorosis; and S = susceptible, large uredinia without necrosis or chlorosis.

The experiment at Njoro was carried out during the main-season (sowing date: June 29, 2011) andit was possible because of the cooperation with CIMMYT, ICARDA and KARI-Njoro, under the auspice of BGRI.

III – Results and discussion

1. Collecting missions

Despite the wide area covered during the collecting missions and the strategy adopted, the number of collected landraces is no more than 16. The magnitude of genetic erosion is even worse

in the north since the collected accessions were only 6. This fact means that genetic diversity of Moroccan wheat is on the verge of extinction. So, collecting should be an ongoing process to conserves this diversity *ex-situ*.

2. On-farm field experiments and farmers' participatory selection approach

To adopt a long-lasting strategy of preservation of local landraces of a given crop, the involvement of farmers in selection of the best ones and promoting the local use of these selected materials is a prerequisite. However, to be realistic, the local landraces will not be preserved unless they perform at least as well as the improved cultivars. Otherwise, there is no way to convince farmers to do so. This is the reason behind the incorporation of the improved cultivars in the experiment.

The analysis of the scoring notes attributed by farmers and scientists revealed that these scores are highly correlated (Figure 3) meaning that the involvement of farmers in breeding programs is reliable.

These on-farm trials revealed that all improved durum cultivars incorporated in the experiments were selected. However, the breakthrough and optimistic achievement of this experiment is that amongst the 62 durum landraces screened, 17, 16 and 27 were selected by farmers at Errachidia, Rich and Taounat respectively. That is, the improved cultivars might be considered as the main threat of diversity but also that there is a hope to serve and conserve it through the promotion of the old accessions that exhibited the same or even better agronomic performances and hence reducing the magnitude of the genetic erosion.

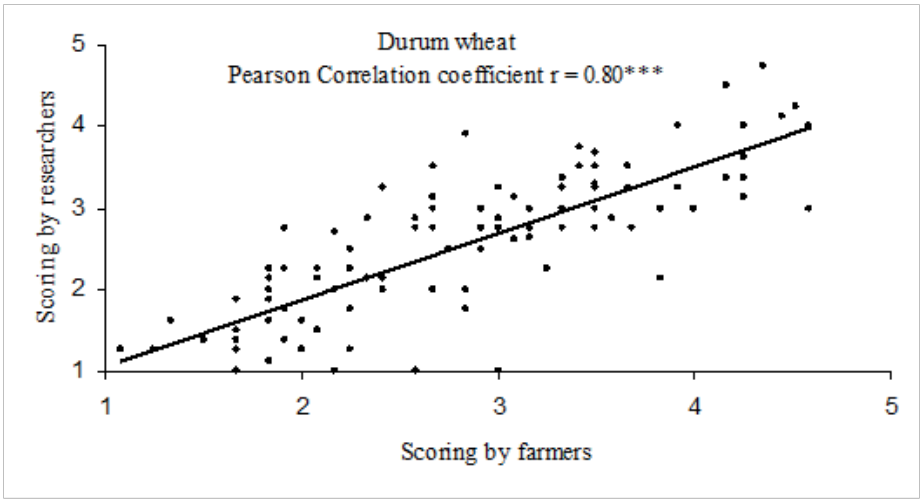

Figure 3. Magnitude of the correlation between scoring of wheat landraces and commercial cultivars by farmers and scientists.

3. *Ex-situ* field experiments

At Annoceur experimental station during 2009-2010, the severity of yellow rust on old durum ranged from 0 to 40% with 12% of them exhibited complete immunity. Leaf rust scores ranged from 0 to 15% with 44% of them were completely immune. The accessions that were relatively late compared to those at grain-fill stage exhibited a wide range of reactions towards yellow and leaf rusts, the most prevalent diseases. So even if these accessions were relatively late and consequently are subjected to the threat of extinction because they will be undoubtedly discarded

by farmers at least under the same conditions of Annoceur, some of them are good sources of resistance to yellow rust and/or leaf rust. The sound strategy to preserve them is through *ex-situ* conservation for future use in breeding programs. Regarding commercial cultivars, all of them had a high agronomic score and had a very good level of resistance against yellow rust where the most susceptible one had a severity of 5%, and a good level of resistance to leaf rust where the most susceptible one (Oum Rabia) had a severity of 20%.The agronomic score of old accessions ranged from 1 to 5 with some of the best performing one exhibited also a good level of resistance to both rusts.

A sub-subset of 62 landraces and 18 cultivars previously tested on-farm at Errachidia, Rich and Taounat were tested at Meknès and Njoro during 2010-2011. The result revealed that 22 accessions exhibited a very good level of resistance to stem rust where the relatively most susceptible one exhibited a severity of 20MS. Amongst them, 4 accessions were almost immune to stem rust (5R) but two of them (MGB 3060 and MGB 3065) were highly susceptible to yellow rust and the two others (MGB 3206 and MGB 3207) exhibited a very good level of resistance to yellow rust too (40R). These two latter accessions were not good from the agronomic performance point of view but are very promising lines as donors of resistance to both diseases (Table 3).

The severity of stem rust on improved durum cultivars ranged from 20MR to 70S, that is, none of them were immune to this disease and only four of them were relatively more resistant (20MR for ISLY and 20 MR-MR for AMJAD, NASSIRA and YASMINE). In contrast, yellow rust was not a real threat since the most susceptible one exhibited a severity of 30S (Table 4).So, using the best old landraces as donors of resistance to stem rust might further improve resistance to this threatening disease.

Table 3. List of durum wheat landraces resistant to stem rust at Njoro and their reaction towards yellow rust and their agronomic scores at Meknès during 2010-2011 cropping season.

MGB code (durum wheat)	Stem Rust (Njoro)	Yellow Rust (Njoro)	Yellow Rust (Meknès)	Agro Score (Meknès)
3207	5R	5R	40R	2
3206	5R	20S	40R	3
3060	5R	60S	80S	4
3065	5R	60S	80S	2
3054	10R	30S	60S	3
3055	10R	30S	80S	2
9393	15MR-MS	20RMR-90S	5 MR-MS	5
3085	15MR-MS	50SMS	60S	2
3082	15MR-MS	50SMS	80S	1
9399	15MR	10RMR	10MR-MS	5
3062	15MR	40S	80S	3
3108	15MR	50MS	80S	3
3059	15MR	60S	80S	3
3058	20MR	40S	80S	3
2812	20MR	60S	70S	2
3124	20MR	70SMS	70S	4
2997	20MR-MS	30S	50S	4
9404	20MR-MS	5RMR	10MR-MS	5
9302	20MR-MS	5S	0	5
3114	20MR-MS	20MS	60S	4
3071	20MR-MS	60S	70S	3
3064	20MS	60S	60S	2

Table 4. Reaction of improved durum wheat cultivars towards stem and yellow rusts at Njoro and towards Yellow rust and their agronomic scores at Meknès, during 2010-2011 cropping season.

Cultivars	Stem Rust (Njoro)	Yellow Rust (Njoro)	Yellow Rust (Meknès)	Agro Score (Meknès)
Isly	20MR	20S	Traces	4
Amjad	20MR-MS	5S	0	4
Nassira	20MR-MS	Traces	0	5
Yasmine	20MR-MS	30S	0	4
Sarif	30MR-MS	0	0	5+
Marzak	40MR-MS	Traces	Traces	4
Oum Rabia	40MR-MS	5S	0	4
Tomouh	40MR-MS	TR	5S-MS	3
Jawhar	40MR	0	Traces	4
Ourgh	40MS	5S	0	3
Amria	50S	20S	10 MR-MS	5
Massa	50S	0	0	5+
Anouar	60S	Traces	20R-MR	5+
Irden	60S	5S	40MR-MS	5+
Karim	60S	Traces	5MR	5
Tarek	60S	0	0	5
Marouane	70S	40MR-MS	40MR-MS	4

IV – Conclusion

Genetic diversity of Moroccan wheat is undoubtedly on the verge of extinction, bearing in mind that despite the wide areas covered during the collecting missions and the strategy adopted, the landraces were very scarce mainly in the northern part of Morocco.

The main reasons behind the increasing risk of extinction are:

- The adoption of improved cultivars mainly in irrigated areas of o*ases* and mountains;
- Shifting towards barley in some rainfed areas because of recurrent drought.

The best strategies to conserve such diversity are:

- Collecting missions should be an ongoing process to collect and conserve the diversity *ex-situ* for use in breeding programs;
- Involvement of farmers to select landraces that perform at least as well as commercial cultivars is for sure the best and long-lasting efficient strategy to serve and conserve the diversity, knowing that quite a few old landraces were selected by farmers and those landraces performed even better that commercial cultivars.

The evaluation of old accessions preserved in INRA Genebank revealed that quite a few of them exhibited a multiple disease resistance, including stem rust.

A deep and thorough evaluation and characterization of a large sample of the old accessions preserved in INRA Genebank is of paramount importance.

Evolution of durum wheat from Sicilian landraces to improved varieties

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Abstract. In the early twentieth century a large number of durum wheat landraces were grown in Sicily. They were characterized by high adaptability to Mediterranean environments and were composed of a mixture of different genotypes. In recent decades the genetic improvement has led to the constitution of modern durum wheat cultivars made up of individual pure lines, highly uniform and productive. Today, the old tetraploid landraces are a precious source of biodiversity and can be successfully used in breeding programs aimed to improving technological quality and resistance to biotic and abiotic stresses. Some of these landraces are still cultivated and used for the production of typical local breads. In the present work twelve Sicilian old landraces have been compared with sixteen durum wheat improved cultivars. For all genotypes morphological parameters were detected according to UPOV and IPGRI descriptors; biochemical characterization was performed by SDS – PAGE of low molecular weight and high molecular weight gluten subunits; molecular analyses were carried out using SSR with thirteen primer pairs. The joint analysis of the results allowed an assessment of genetic diversity between the two groups of genotypes.

Keywords. *Triticum turgidum* subsp. *durum* – Genetic development – Local populations – Cultivar – SSR.

Evolution des variétés locales siciliennes de blé dur pour améliorer les variétés

Résumé. *Au début du XXe siècle, bon nombre de variétés locales de blé dur étaient cultivées en Sicile. Elles étaient caractérisées par une grande capacité d'adaptation aux conditions de milieu méditerranéennes et étaient composées par un mélange de différents génotypes. Au cours des dernières décennies, l'amélioration génétique a conduit à l'obtention de cultivars de blé dur modernes, incluant des lignées pures individuelles, très homogènes et productives. Aujourd'hui, les anciennes variétés locales tétraploïdes sont une précieuse source de biodiversité et peuvent être exploitées avec succès dans les programmes de sélection visant à améliorer la qualité technologique et la résistance aux stress biotiques et abiotiques. Certaines de ces variétés locales sont encore cultivées et utilisées pour la production de pains typiques de la région. Dans le présent travail, douze anciennes variétés locales siciliennes ont été comparées avec seize cultivars améliorés de blé dur. Pour tous les génotypes, les paramètres morphologiques ont été détectés en fonction des descripteurs de l'UPOV et de l'IPGRI ; la caractérisation biochimique a été effectuée par SDS - PAGE des sous-unités gluténines de bas poids moléculaire et de haut poids moléculaire ; les analyses moléculaires ont été réalisées à l'aide de SSR avec treize paires d'amorces. L'analyse conjointe des résultats a permis d'évaluer la diversité génétique entre les deux groupes de génotypes.*

Mots-clés. *Triticum turgidum ssp. durum – Développement génétique – Populations locales – Ccultivar – SSR.*

I – Introduction

In the first half of the last century, many durum wheat landraces were grown in Sicily. The large number of these cultivars were grown due to their specific characteristics and their adaptability to Mediterranean environmental conditions (De Cillis, 1942). These ecotypes are characterized by tall plants, good quality characteristics of the grain and long biological cycle (Boggini *et al*., 1989). Because of their high adaptability and their particular qualitative characteristics some landraces are still cultivated in small areas of Sicily. In recent decades the genetic improvement has led to the composition of modern durum wheat cultivars made up of individual pure lines, highly uniform

and productive. Today, the old tetraploid landraces are a precious source of biodiversity that must be preserved and can be successfully used in breeding programs aimed to improve technological quality and resistance to biotic and abiotic stresses.

Many studies aimed to characterize and identify accessions in wheat species are based on morphological characterization according to UPOV and IPGRI descriptors (Zarkti *et al*., 2010). Morphological traits have been studied for the estimation of genetic diversity and as selection criteria for wheat breeding (Schut *et al*., 1997; Marti *et al*., 2007), but are influenced by environmental factors. Conversely, biochemical characterization and molecular analysis, based on DNA polymorphism, are independent of environmental conditions.

In this study, old Sicilian durum wheat landraces have been compared with modern improved cultivars, taking into account morphological traits, biochemical parameters and molecular markers.

II – Material and methods

A set of 28 accessions of durum wheat (*Triticum turgidum* L. ssp. *durum*) was studied: 12 old Sicilian landraces and 16 improved varieties as shown in table 1.

Table 1. Durum wheat accessions used in the experiment.

Improved varieties	Constitution Year	Sicilian landraces
Cappelli	1930	Biancuccia
Grifoni 235	1955	Bivona
Capeiti	1955	Castiglione
Trinakria	1970	Ciciredda
Appulo	1973	Cotrone
Creso	1974	Duro Lucano
Valnova	1975	Farro Lungo
Produra	1980	Gioia
Valforte	1980	Regina
Primadur	1984	Ruscia
Lira	1985	Sammartinara
Simeto	1988	Timilia
Colosseo	1995	
Bronte	1996	
Sant'Agata	2004	
Ciclope	2006	

1. Morphological characterization

An agronomical trial was carried out in location Libertinia (Catania), with cultural techniques typical of the area. The genotypes tested were grown in 10 m^2 plots, with 3 replications. The morphological characters measured were plant height, culm solid/hollow, ear shape, awn length, rachis length and caryopsis shape.

The data matrix was then standardized and elaborated with "R" software for the calculation of principal components (R Development Core Team, 2008).

A principal component analysis (PCA) of standardized data was applied to observe the main defining characteristics. A Genotype x Trait biplot (GT biplot) was created to recognize the genetic variability and the relationships among durum wheat genotypes. The first two PC (PC1 and PC2) were used to generate the GT biplot. For the assessment of the distance of similarity a dendrogram has been developed by the Ward's method was applied.

2. Biochemical characterization

The grain storage proteins were characterized by SDS-PAGE electrophoretic patterns of low molecular weight (LMW) and high molecular weight (HMW) glutenin subunits, according to the method described by Payne *et al*. (1980). As Sicilian populations are heterogeneous, 26 seeds were analysed for each landrace and the prevalent composition was detected, also recording different electrophoretic patterns. Three seeds of each improved cultivar were analysed.

3. Molecular characterization

DNA was extracted from fresh tissue of plants germinated in Petri dishes. Genomic DNA was isolated using 100 mg of fresh tissue by DNeasy Plant Mini Kit (Quiagen). All extracted samples were estimated by spectrophotometer readings and by electrophoresis. Thirteen primer pairs Xgwm localized on chromosomes of the A and B genomes were used (Table. 2). Amplification products derived from fluorescently labeled primers were resolved by capillary electrophoresis on the ABI Prism 3130 Genetic Analyzer (Applied Biosystems).

III – Results

1. Morphological evaluation

The distribution of the accessions in the dendrogram suggested a cut at a height of 8, resulted in three clusters. The clusters were then reported in a biplot of the first two principal components, PC1 and PC2, which explained more than 50% of the total variation (PC1=29%; PC2=24%).

The ear profile, awn length, rachis length and plant height had long vectors, suggesting that there was relatively large variation among accessions (Fig. 2).

In contrast, the fullness of the culm had short vectors, suggesting that there was little or no variation among accessions. The cosine of the angle between the vectors of two traits measures the similarity or the correlation between them relative to their variation among genotypes. An angle of zero indicates a correlation of +1, an angle <90° suggests a positive correlation, an angle of 90° indicates no correlation, implying independence, an angle >90° indicates negative correlation, and an angle of 180° represents a correlation of -1.

Thus, rachis length, culm fullness, awns length had acute (<90°) angles between them, indicating that their variations are similar. On the contrary, these morphological traits had obtuse (>90°) angles with ear profile and caryopsis shape, indicating that their variations are negatively correlated. In particular, awn length had a near-right angle with height or ear profile against caryopsis shape and rachis length, indicating that their variations are more or less independent of these traits.

The genotypes studied, grouped into 3 groups as represented in the biplot (Fig. 2) could be described for traits in common. So the biplot shows that cluster 1 is characterized by units in which the overriding factor is the shape the caryopsis. The accessions of this cluster are also distinguished by having short awns. It consists of quite old varieties except Biancuccia which is an ancient Sicilian population.

The second cluster is characterized by accessions discriminated for plant height that are also distinguished, in part, to the fullness of culm and for the length of rachis. This cluster consists exclusively of Sicilian populations.

The third cluster is characterized by accessions determined by the profile of ear more predominant than the other observed factors. Its accessions present small stature and it groups together almost entirely varieties except the landrace Timilia.

Table 2. List of primers, with their forward and reverse primers, repeat motif and annealing temperature (AT).

Locus	Forward primer	Reverse primer	Repeat	An. Temp.
Xgwm 6-4B	CGTATCACCTCCTAGCTAAACTAG	AGCCTTATCATGACCCTACCTT	(G A)40	55°
Xgwm 46-7B	GCACGTGAATGGATTGGAC	TGACCCAATAGTGGTGGTCA	(GA) 2GC (GA) 33	60°
Xgwm 67-5B	ACCACACAAACAAGGTAAGCG	CAACCCTCTTAATTTTGTTGGG	(CA) 10	60°
Xgwm 95-2A	GATCAAACACACACCCCTCC	AATGCAAAGTGA AAA ACCCG	(AC) 16	60°
Xgwm 107-4B	ATTAATACCTGAGGGAGGTGC	GGTCTCAGGAGCAAGAACAC	(CT) 21	60°
Xgwm 124-1B	GCCATGGCTATCACCCAG	ACTGTTCGGTGCAATTTGAG	(CT) 27 (GT) 18imp	60°
Xgwm 131-1B	AATCCCCACCGATTCTTCTC	AGTTCGTGGGTCTCTGATGG	(CT)22	60°
Xgwm 153-1B	GA TCTCGTCAC CCGGAATTC	TGGTAGAGAAGGACGGAGAG	(GA) 18	60°
Xgwm 155-3A	CAATCATTTCCCCCTCCC	AATCATTGGAAATCCATATGCC	(CT) 19	60°
Xgwm 408-5B	TCGATTTATTTGGGCCACTG	GTATAATTCGTTCACAGCACGC	(CA) > 22 (TA) (CA) 7 (TA) 9	55°
Xgwm 413-1B	TGCTTGTCT AGA TTGCTTGGG	GATC GTCTCGTCCTTGGCA	(GA) 18	60°
Xgwm 5l3-4B	ATCCGTAGCACCTACTGGTCA	GGTCTGTTCATGCCACATTG	(CA) 12	60°

2. Biochemical characterization

The results of protein characterization pointed out large biodiversity among Sicilian landraces. Regarding the HMW glutenin subunits encoded by the locus Glu-B1 in the long arm of chromosome 1B, six landraces had subunit “20” as prevalent composition, two had the subunit pair “13+16”, three had the subunit pair “6+8” and one the “7+8” (Table 3).

The landraces Bivona and Timilia showed polymorphism within the landrace populations, with prevalent composition “6+8” and presence of subunit “20”. All the tested landraces were “Null” for HMW subunits encoded by the locus Glu-A1 in the long arm of the chromosome 1A. Concerning the LMW glutenin subunits, all landraces presented the subunit type “2” in all seeds analysed.

The study of 16 improved varieties showed the HMW glutenin subunits “6+8” and “7+8” for the most of new cultivars. Four genotypes, the oldest varieties, showed the subunit “20”. Only one cultivar, Colosseo, had “13+16” subunit.

3. Microsatellite marker analysis

Thirteen Xgwm microsatellite markers localized on chromosomes of A and B genomes were used to test polymorphism between accessions of durum wheat (Table 2).

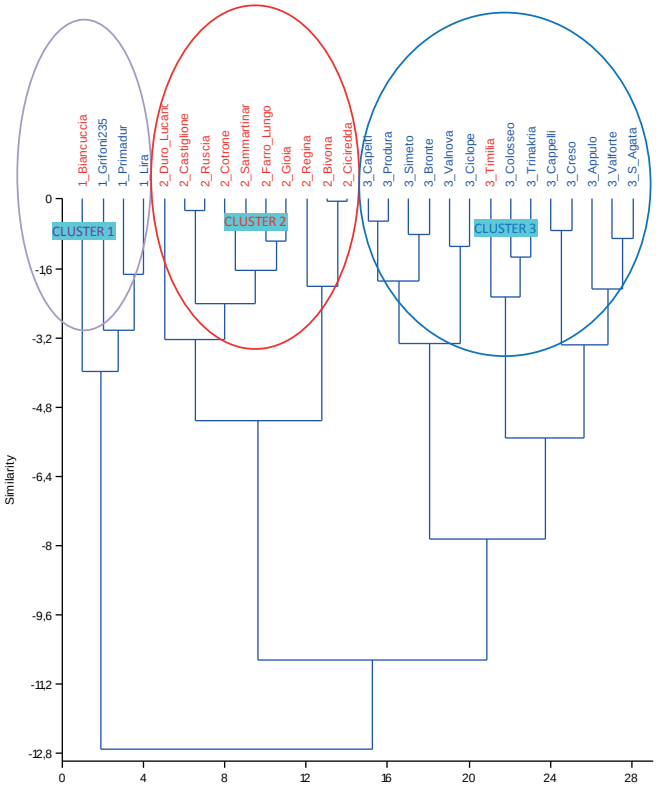

Figure 1. Dendrogram for the assessment of similarity distance of the durum wheat tested accessions (Ward’s method).

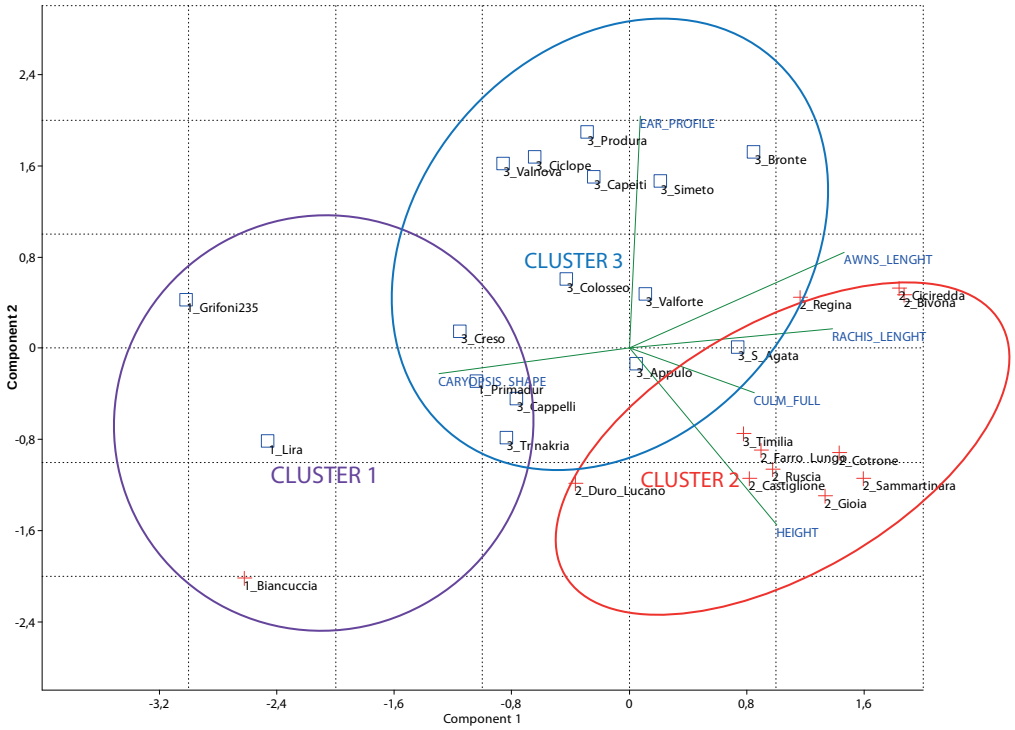

Figure 2. Biplot showing distance among 28 durum wheat accessions constructed on morphological traits.

Table 3. HMW glutenin subunits in Sicilian landraces and improved varieties.

Sicilian landraces		Improved varieties	
Biancuccia	20	Cappelli	20
Bivona	6+8/20	Griggoni 235	7+8
Castiglione	20	Cappelli	20
Ciciredda	13+16	Trinakria	20
Cotrone	20	Appulo	20
Duro Lucano	20	Creso	6+8
Farro Lungo	20	Valnova	7+8
Gioia	20	Produra	6+8
Regina	7+8	Valforte	7+8
Ruscia	6+8	Primadur	6+8
Sammartinara	13+16	Lira	6+8
Timilia	6+8/20	Simeto	7+8
		Colosseo	13+16
		Bronte	7+8
		Sant'Agata	7+8
		Ciclope	7+8

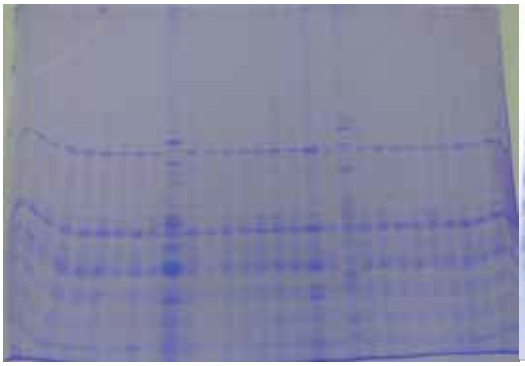

Figure 3. Sicilian landrace: Castiglione.

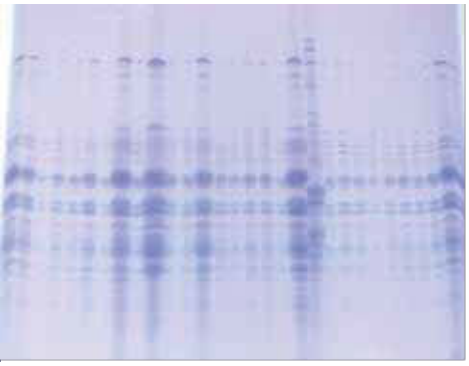

Figure 4. – Improved variety: Sant'Agata.

Preliminary results have shown amplification products between 120 and 270 bp. The most polymorphic primers were Xgwm 413-1B and Xgwm 513-4B. The Xgwm 413-1B produced fingerprinting patterns easily distinguishable for the cultivars Sant'Agata, Ciclope and Simeto, whereas Xgwm 153-1B was able to detect polymorphism intra and inter landraces. The results indicate that microsatellite loci of the B genome are highly variable.

V – Conclusions

Concerning morphological evaluation of old and new genotypes, the evolution of durum wheat varieties occurred primarily in morphological changes of plants, which led to the creation of more productive varieties. The plant height reduction and the compactness of the ear have allowed the plant to take advantage of a greater quantity of assimilated.

As regard protein composition, the results showed a progressive affirmation: the modern cultivars have the "6+8" and "7+8" HMW glutenin subunits that are suitable for the technological process.

The molecular characterization confirmed that DNA markers, which are not subject to environmental influences, are useful tools for genetic fingerprinting of old and new genotypes and to improve the efficiency in programs of gene recombination and selection.

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Genetic improvement of durum wheat establishment under fluctuating environmental conditions

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Abstract. Early sowing is critical for achieving high grain yields in wheat, especially under semi-arid environmental conditions where terminal drought limit the season. Fluctuating and sporadic precipitation early in the season could delay sowing or even terminate seedling growth. By applying deep sowing technique farmers have a tool to ensure adequate seed-zone moisture before germination leading to increasing seed establishment. This is often not feasible because of the extensive growing of semi-dwarf wheat containing the gibberellin insensitive genes *Reduce height* (*Rht*)-*B1b*/*Rht-D1b* which have shorter coleoptiles and low vigor. On the other hand, several alternative dwarfing genes with longer coleoptile have been reported. Our working hypothesis is that introduction of these alternative dwarfing genes into modern durum cultivars can improve emergence and seedling establishment under fluctuating environmental conditions. Emergence tests of durum wheat landraces from across the Mediterranean region, alongside with elite Israeli durum wheat cultivars, showed significant advantage of these pre- "Green Revolution" cultivars to emerge from soil-depth. While the durum Israeli cultivars germinated poorly from 10cm depth, several durum landraces show improved emergence rate, establishment and early vigor. Our results demonstrate the potential of using alternative dwarfing genes to improve wheat seedling survival under fluctuating environmental conditions and enhance yields.

Keywords. Dwarfing genes – Durum landraces – Coleoptile – Emergence – *Rht* – Early vigor – Drought resistance.

L'amélioration génétique de l'ancrage au sol du blé dur dans des conditions environnementales fluctuantes

Résumé. *Le semis précoce est essentiel pour obtenir chez le blé des rendements en grains élevés, en particulier dans des conditions environnementales semi-arides où la sécheresse terminale limite la saison. Les précipitations fluctuantes et sporadiques au début de la saison pourraient retarder le semis ou même mettre fin à la croissance des plantules. En appliquant la technique du semis profond, les agriculteurs ont un outil pour assurer une humidité appropriée de la zone autour des semences avant la germination, conduisant à l'augmentation de l'ancrage au sol des semences. Ceci n'est souvent pas possible en raison de la culture extensive du blé semi-nain contenant les gènes insensibles aux gibbérellines, Rht -B1b / Rht-D1b, qui a des coléoptiles courts et peu vigoureux. D'autre part, plusieurs autres gènes de nanisme induisant des coléoptiles plus longs ont été rapportés. Notre hypothèse de travail est que l'introduction de ces gènes de nanisme alternatifs dans les cultivars de blé dur moderne peut améliorer la levée et l'ancrage au sol des semis sous des conditions environnementales fluctuantes. Des essais sur la levée des variétés locales de blé dur de toute la région méditerranéenne, en plus des cultivars de blé dur israéliens d'élite, ont montré que ces cultivars pré - «révolution verte» ont une plus grande capacité à émerger du sol profond. Alors que les cultivars de blé dur israéliens présentent une capacité de germination plus faible à 10cm de profondeur, plusieurs variétés locales de blé dur s'avèrent être plus performantes en termes de levée, ancrage au sol et vigueur précoce. Nos résultats démontrent le potentiel de l'utilisation de gènes de nanisme alternatifs pour améliorer la survie des semis de blé dans des conditions environnementales fluctuantes et augmenter les rendements.*

Mots-clés. *Gènes de nanisme – Variétés locales de blé dur – Coléoptile – Levée – Rht – Vigueur précoce – Résistance à la sécheresse.*

I – Introduction

Durum wheat (*Triticum turgidum* ssp. *durum* (Desf.) MacKey.) is an important grain-crop, particularly in the Mediterranean basin. While in the past Israeli wheat fields were dominated by traditional durum wheat, nowadays only small portion of the fields is allocated for growing durum wheat and most fields replaced by bread wheat. The Mediterranean region is characterized by a long, hot, dry summer and a short, mild, wet winter (Loss and Siddique 1994). In recent years, global warming processes resulted in greater fluctuations in amounts and distribution of precipitation. In agreement with these environmental changes, farmers have shifted the sowing date from early November to mid-December. On the other hand, late sowing results in short growing season, as the terminal drought is common in early March. In cereals, yield is determined by five major components, including number of plants per unit area, number of spikes per plant, number of spikelets per ear, number of kernel-bearing florets per spikelet, and average grain weight. Drought has an effect on all the developmental stages of plant during the season and may cause a reduction in all yield components. Thus, more effective use of the entire growing season is essential for enhancing yield.

Early-season sowing into dry soil exposes the germinating seeds to risk of dehydration due to high precipitation fluctuation. Sporadic 30-50 mm of rainfall could suffice for emergence, but with no additional rainfall, drought stress will be imposed upon the young seedlings and in severe cases force re-sowing. This has become more important in the post- 'Green Revolution' cultivars containing the gibberellin-insensitive (GAI) dwarfing genes, ***R****educed* ***h****eight* (*Rht*)-*B1b* and *Rht-D1b*. These varieties have shorter coleoptiles and will not establish well if sown too deep (Allan, 1980). Several alternative dwarfing genes that are responsive to endogenous GA and exhibit no reduction in coleoptile length have been identified and characterized (Ellis *et al*., 2004). Lines containing these genes emerge more successfully when sown deep or when used in conservation farming systems (Rebetzke *et al*., 2012).

The objectives of the current study were to (*i*) characterize the field germination ability of modern Israeli cultivars sown in soil-depth (*ii*) test modern durum wheat cultivars and durum landraces for emergence ability, and (*iii*) develop the genetic infrastructure for future wheat breeding for improved tolerance to fluctuating environmental condition and enhance yields.

II – Material and methods

Field experiment: Two commercial modern Israeli wheat cultivars (Shaphir and Galil) were sown at two locations (Kfar Hanagid and Bet-Dagan, respectively) at two soil depths, 2 cm (control) and 10cm. Numbers of emerging seedlings were counted daily and after harvest, total biomass and grain yield were calculated.

Germination test: Eight modern wheat cultivars and 47 durum landrace wheat lines were tested in two soil depths (2 and 10 cm). Eight seeds were placed at the two soil depths in controlled conditions (dark, 15 ^{o}C). The numbers of emerging seedlings were recorded daily and coleoptile length was measured 10 days after sowing.

Field emergence assay: A subset of eight durum landrace wheat lines and one modern commercial durum cultivar (C-61, control) were sown in the field at 2 and 10cm soil depths. The numbers of emerging seedling, numbers of tillers, leaf size, plant height and grain yield were analyzed.

Gibberellin responsiveness assay: The response to GA was assessed by measuring coleoptile length of seedlings grown in hydroponic solution with GA_3 (10^{-5}M) or control.

III – Results and discussion

The 'Green Revolution' including the introduction of semi-dwarfing genes (*Rht-B1, Rht-D1*) led to impressive increases in wheat yields. The reduced culm length in these cultivars resulted from limited response to GA via DELLA proteins. As a consequence, most modern wheat cultivars have very short coleoptiles and show reduced emergence in deep sowing. Field experiments at two locations showed that deep sowing resulted in significant reduction in seedling stand (31.5 and 42% for Shaphir and Galil, respectively) as compared with the control treatment. As expected, this reduction in field stands was expressed later in lower grain yields (34 and 28.5%, respectively).

Controlled emergence tests from soil depth (2 and 10 cm) of 47 durum landraces, alongside with eight elite durum wheat cultivars showed significant differences: Most durum landraces (64%) emerge well from 10 cm soil-depth, with average of 6-9 days. On the contrary, the modern durum wheat cultivars were not able to emerge from soil-depth (with exception of cv. Afik which showed good emergence from 10cm soil depth). This higher emergence rate was associated with significantly longer coleoptile of the landraces lines as compared with the modern cultivars. For example, the landraces Abu Fashi and Gaza had a coleoptile length of 14.8 ± 0.6cm and 13.6 ± 0.7cm, respectively, while the modern cultivars Ayalon and C-9 were significantly shorter (8.3 ± 0.9cm and 9.4 ± 0.7cm, respectively). In agreement, responsiveness to exogenous GA test revealed positive significant effect in Abu Fashi seedlings while Ayalon and C-9 did not respond to the GA_3 treatment.

These greenhouse results were also supported by a field evaluation for emergence and development. Only slight or no significant reduction in emergence was observed for the local durum landraces were as commercial cultivar showed significant reduction in emergence rate when sown at 10cm. Furthermore, several landraces showed improved establishment and early-vigor which was manifested in minimal or no reduction in growth parameters (i.e. number of leaves, number of tillers and leaf width) when sown at soil-depth. Furthermore, most landraces presented enhanced early-vigor compare to the modern cultivars. For example, based on measurements of the last fully exposed leaf-width, the line Gaza showed significantly higher early-vigor compared to modern cultivars. In this line leaf width was not reduced in plots were seeds were sown at 10 cm. Interestingly other wheat lines which showed good emergence from soil-depth had relatively narrow leaf width (i.e., Abu Fashi). This might imply independent inheritance of the two traits.

In conclusion, our results demonstrate the breeding potential of replacing the GAI dwarfing genes with alternative dwarfing genes from pre "Green Revolution" germplasm, to improve wheat establishment under fluctuating water availability and enhance grain yields. Currently ongoing phenotypic and genotypic selection of crosses between modern Israeli cultivars and potential landraces lines with improved germination from soil-depth is underway.

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Variability of total antioxidant capacity among durum wheat genotypes

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Abstract. Durum wheat (*Triticum turgidum* L., ssp. *durum)* contains many health-promoting components involved in different biological activity, partly attr buted to potential chemo-preventive substances (phytochemicals), including antioxidants present in high amounts in vegetable crops and also in cereal grains. Recently, the determination of total antioxidant capacity (TAC) has gained a growing interest as a tool for exploring the putative role of antioxidant-rich products in the prevention of degenerative diseases and for the selection of varieties with potentially positive health benefits. The aim of the present study was to determine the influence of the genotype, year and environment on the TAC level in different cultivars of durum wheat during 2009, 2010 and 2011 crop years and in 3 environments. The results showed that year (Y), environment (E) and genotype (G), as well as their interactions, significantly influenced the TAC value in the durum wheat grains. Principal component analysis (PCA) identified genotypes with high and stable TAC values over the environments. Correspondence analysis and boxplots were also useful for assessing more stable cultivars over the years. Among different genotypes, the TAC values ranged between 36,55 to 55,83 mmolTEAC/kg (dry matter, DM).

Keywords. Durum wheat – TAC – Genotype – Environment.

Variabilité de la capacité antioxydante totale parmi les génotypes de blé dur

Résumé. *Le blé dur (Triticum turgidum L., ssp. durum) contient de nombreux composants bénéfiques pour la santé impliqués dans différentes activités biologiques, en partie attribués à des substances chimio-préventives potentielles (phytochimiques), incluant les antioxydants présents en grande quantité dans les cultures maraîchères et aussi dans les grains de céréales. Récemment, la détermination de la capacité antioxydante totale (CAT) a suscité un grand intérêt comme outil pour explorer le rôle potentiel des produits riches en antioxydants dans la prévention des maladies dégénératives et pour la sélection de variétés avec des qualités potentiellement bénéfiques pour la santé. Le but de cette étude était de déterminer l'influence du génotype, de l'année et de l'environnement sur le niveau de la CAT dans différents cultivars de blé dur durant les années de culture 2009, 2010 et 2011 et dans 3 environnements. Les résultats ont montré que l'année (Y), l'environnement (E) et le génotype (G), ainsi que leurs interactions, ont influencé de façon significative la valeur de la CAT dans les grains de blé dur. L'analyse en composantes principales (ACP) a identifié des génotypes avec des valeurs élevées et stables de CAT pour les environnements. L'analyse des correspondances et des boxplots a également été utile pour évaluer les cultivars les plus stables au fil des années. Parmi les différents génotypes, les valeurs de la CAT ont varié entre 36,55 à 55,83 mmolTEAC / kg (matière sèche, MS).*

Mots-clés. *Blé dur – CAT – Génotype – Environnement.*

I – Introduction

Epidemiological studies underlined the potential protective role of consumption of whole-grain cereals against several chronic diseases (Thompson 1994). These health benefits have been partly attributed to a wide variety of potential chemo-preventive substances, so-called phytochemicals, including antioxidant compounds present in high amounts in different vegetable crops and also in cereal grains (Frusciante *et al.* 2007, Serafini *et al.* 2002). The global action of all antioxidant compounds present in a raw material is generally expressed as total antioxidant

capacity (TAC). This parameter has gained a growing interest as a tool for exploring the putative role of antioxidant-rich products in the prevention of degenerative diseases, as well as for the selection of varieties/species rich in bioactive compounds with potentially positive health benefits. Wojdylo and Oszmainski (2007) measured in oat the free radical scavenging ability of methanolic and enzymatic extracts comparing the DPPH radical method (Yen and Chen, 1995) and the ABTS radical method (Re *et al.* 1999); other authors (Lavelli *et al.* 2009, Hidalgo *et al.* 2006) measured the total free radical scavenging capacity with DPPH radical of extracts from different wheat species using different extraction protocols and solvents. Several methods have been developed to measure the whole matrix antioxidant capacity and data are often variable and underestimated due to the different extraction methods (Frankel *et al.* 2008), considering that there is not a unique solvent suitable to solubilize all antioxidants present in a complex food matrix and generally the extraction procedures employ hydrophilic or lipophilic solvents and then measure the TAC separately. More recently a procedure for the measurement of the total antioxidant capacity was developed by Serpen *et al* (2008) without the extraction step, but directly on the solid food matrix. This method, was deeply evaluated by Gokmen *et al.* (2009) and defined as QUENCER (Quick, Easy, New, CHEap and Reproducible); it overcomes the difficulties of the extraction step, highlighting the synergistic effect of different antioxidants molecules, partially lost during antioxidants extraction or during the measurements on different extracts.

The aim of the present study, carried out in the AGER project “From seed to pasta” was to determine in a group of durum wheat cultivars the influence of genotype, year and environment on the total antioxidant capacity measured by a direct method.

II – Material and methods

Twenty durum wheat cultivars (Achille, Alemanno, Anco Marzio, Arnacoris, Biensur, Ciccio, Claudio, Creso, Duilio, Dylan, Iride, Minosse, Neolatino, Latinur, Liberdur, Severo, Saragolla, Simeto, Tirex, and Trionfo) were grown in Montelibretti (Rome) during 2009, 2010 and 2011 in a national network experimental trial; a set of 10 cultivars, Anco Marzio, Ciccio, Claudio, Creso, Duilio, Dylan, Iride, Latinur, Saragolla and Simeto, grown during the same period in other two locations (Jesi, Foggia) representing the different agroclimatic areas typical of durum wheat crop in Italy, were considered for evaluating the environment influence. Grain samples were ground with a laboratory mill and a sieving of 1 mm (Cyclotec, PBI), to obtain wholemeal employed for the TAC determination, applying the TEAC direct method described by Serpen *et al.* (2008). The TAC analytical procedure is based on an immersion of a pulverized solid matrix (sample) in a 50% ethanol solution containing 2,2’-azinobis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical. The final radical absorbance was set at 0.7 nm and the solution with the solid sample was incubated in an orbital shaker (190 rpm) at 25°C for 50 min. The absorbance measurements were performed at 734 nm. The antioxidant capacity was expressed as mmol of Trolox equivalent antioxidant capacity (TEAC) per kg of *2* sample by means of a Trolox dose-response curve. All samples were diluted with cellulose powder (1:9 w/w), inert toward the ABTS reagent, to obtain an absorbance in the valid range of the calibration curve. The TAC analysis was performed in triplicate.

The effects of year (Y), genotype (G) and environmental factors (E) and their interactions on TAC values have been studied using different statistical approaches. Analysis of variance (ANOVA) was performed by MSTATC program (Michigan State University, East Lansing, MI). Principal component analysis (PCA) and correspondence analysis were performed by MATLAB software (R2010a version, MathWorks Inc., USA) to study variation associated with genotype and environment for TAC values.

III – Results

1. Genotype influence

Mean TAC value for 20 cultivars grown in Montelibretti during three years was 43.64±3.03; the highest value was performed by Trionfo in 2009 (49.64 mmol TEAC/kg) and the lowest by Alemanno in 2010 (35.78 mmol TEAC/kg). All genotypes showed a different year-dependent behaviour (Fig. 1). The data show that genotypes influenced the TAC level, but a year-genotype interaction is always present, as also pointed out by ANOVA (Table 1).

Table 1. Analysis of variance: mean square and F value of year, environment and their interaction.

Sources	DF	Mean squares	F value
Replication (R)	2	3.103	1.54
Year (Y)	2	3.972	1.97
RxY	4	2.020	
Genotype (G)	19	35.340*	15.68
YxG	38	22.344*	9.92
Error	114	2.253	

**Significant at P<0.001.*

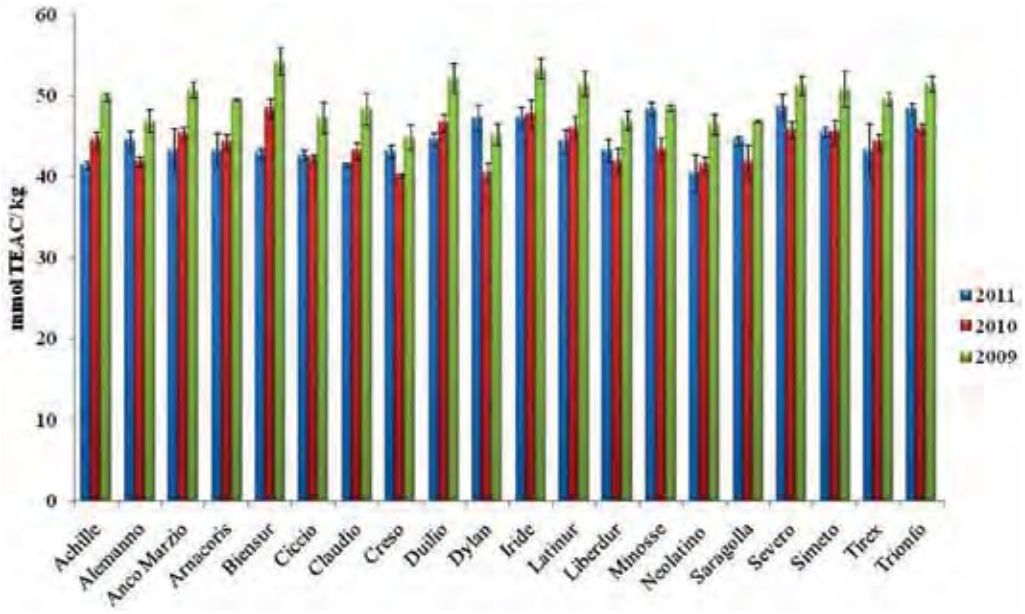

Figure 1. TAC of different *T. durum* cvs grown in Montelibretti during three crop years.

The whole data set was converted to a frequency table considering the cultivar as first variable and the TAC value as second variable, identifying for this last variable three arbitrary categories (low, medium, high) based on the mean values and standard deviations. Cultivars were graphically located near Low, Medium and High TAC values (Fig. 2). In the graphical representations of the frequency table the distances between the points representing the cultivars are a measure of the similarity of the cultivars-frequency profiles. Each trait-category point will lie close to the cultivars

for which the trait category is prominent. In particular, in terms of frequencies the cultivars Trionfo and Iride are the highest while Achille and Alemanno are the lowest (Fig. 2).

2. Environment and year influence

The behaviour of 10 cultivars during 3 years was quite different in 3 environments, with the exception of Montelibretti where TAC level was rather stable. Among the cultivars (Fig. 3), Ciccio and Duilio showed high TAC variability in the environments during the 3 years, while Iride was quite stable. The combined ANOVA on 10 cultivars grown in 3 environments during 3 years (Table 2) showed that TAC is mostly influenced by year (Y), followed by environment (E) and then by genotype (G), the interaction YxE was also significant.

Factor analysis (PCA) used to evaluate simultaneously all variables and their relationships (Fig. 4) identified that two factors explaining 80% of the total variance: The first factor (PC1) appears mainly linked to TAC and explained the 48.85% of the total variance, while the second (PC2) seems mainly associated to the cultivar stability and explained the 30.21% of the total variance. All environments were in similar positions on the positive side of PC. Jesi 2009 and 2010 and Montelibretti 2010 are located on the negative side for PC2, probably due to different agroclimatic conditions (i.e., high seasonal rainfall). Among the cultivars, Simeto had the highest TAC values, but was not stable across the environments, Iride presented a high TAC value and high stability; Creso and Claudio had lowest TAC values and seem less affected by environment (Fig. 4).

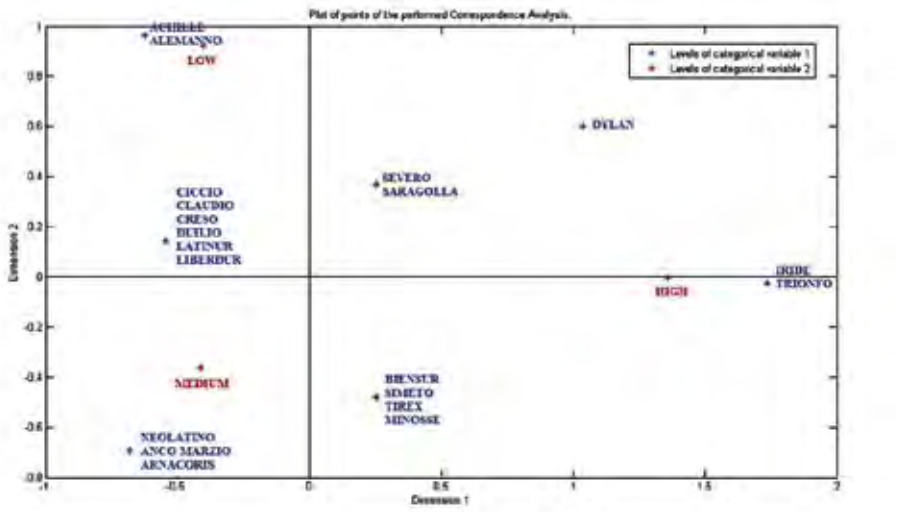

Figure 2. Correspondence analysis of the twenty cvs grown in Montelibretti (RM) in the three years.

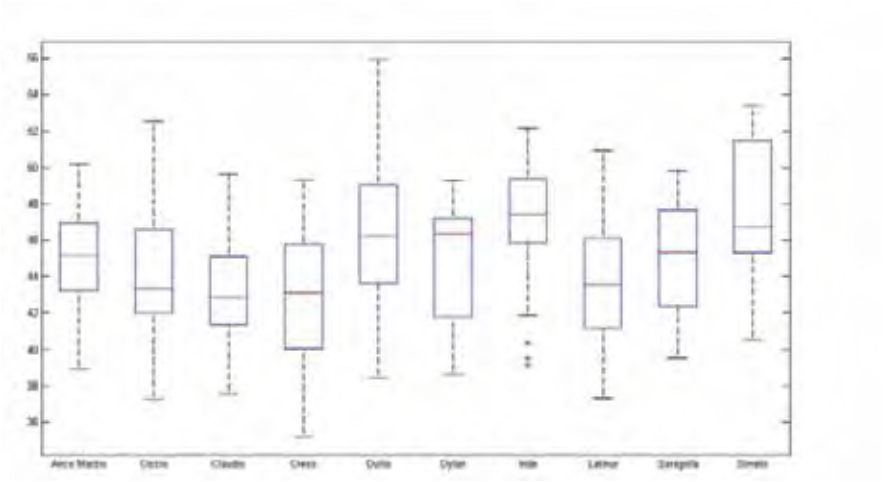

Figure 3. Boxplots of the cultivars behaviour in three environments and during 3 years.

Table 2. Analysis of variance of data from 10 cvs grown at three environments for three years.

Sources	DF	Mean squares	F value
Year (Y)	2	445,75*	111,24
Environment (E)	2	211,25*	52,72
Genotype (G)	9	62,54*	15,61
YxE	4	92,56*	23,10
YxG	18	22,29*	5,56
ExG	18	19,69*	4,91
YxExG	36	17,24*	4,30
Error	180	4,01	

**P significant P<0.001*

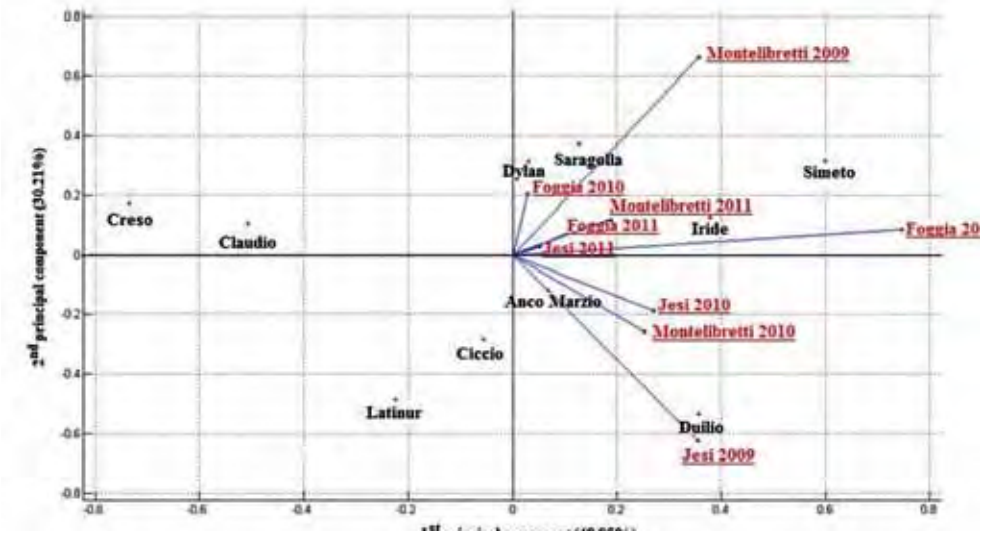

Figure 4. PCA analysis of the durum cvs grown in three environments and three years: year and environment effects.

IV – Conclusions

In this study we were able to classify some durum cultivars on the basis of total antioxidant capacity (TAC). It was possible to evaluate the influence of year, environment and genotype on the TAC, highlighting the year as the main factor affecting the antioxidant capacity followed by environment and genotype; moreover it was possible to evaluate the cultivar stability across years. The results suggested that it is possible, on the basis of TAC values, to choose the more suitable cultivars for use in breeding programs to select varieties naturally rich in antioxidants.

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Evolution of durum wheat breeding in Italy

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Abstract. The paper reports on the scientific effort for improving durum wheat production and quality made by Italian breeders and geneticists by exploiting the rich reservoir of genetic variation present in the Mediterranean area and in the germplasm introduced from distant geographical areas, as well as in attempting to understand the genetic control of agronomically and technologically important traits.

Keywords. Durum wheat – Breeding – Genetics, Italy.

Evolution de la sélection du blé dur en Italie

Résumé. *Dans cet article, nous allons parcourir les efforts scientifiques déployés par des sélectionneurs et des généticiens italiens pour améliorer la production et la qualité du blé dur en exploitant l'important réservoir de diversité génétique qu'abrite la région méditerranéenne et le matériel génétique provenant d'autres zones géographiques éloignées. Parallèlement, nous allons essayer de comprendre le contrôle génétique de certains caractères d'intérêt agronomique et technologique.*

Mots-clés. *Blé dur – Sélection – Génétique – Italie.*

I – Introduction

Durum wheat is the main crop in Italy in terms of land area being grown on about 1.3 million ha (Table 1), although it accounts only for 20% of the cereals production and 2% of the agricultural Gross Domestic Product (GDP). It is grown mainly in the southern part of the peninsula and in the islands, with Apulia and Sicily accounting for about 50% of the durum area. The European Union (EU-27) is the first producer of durum wheat in the world with more than 10.0 million tons of grain, with Italy playing a leading role: 50% of the durum area and production (Table 2). The European Union is also the most important consumer and from middle 1990s it has become a net importer of this cereal to offset the pasta export. This context accounts for the scientific effort to improve durum wheat production and quality made by Italian breeders, exploiting the rich reservoir of genetic variation present in the Mediterranean area as well as the germplasm introduced from distant geographical areas.

II – Breeding activities

Durum wheat breeding activities began at the outset of the XX century by exploiting the genetic variation present in landraces from southern Italy, North-Africa and West Asia. Todaro (1921) released Brottu, Lachesos, Sardaresu, Strampelli (1932) the varieties Azizia 17-45, Dauno, Duro di Puglia, Tripolino, and Senatore Cappelli, De Cillis (1942) bred Russello and Timilia, and Conti (1948) selected Azizia 301 and Azizia 302, Capinera, Ricco, Rossarda, Rossello, Sardo, Triminia. Overall a set of successful varieties were still present in southern Italy and islands during '70s. In fact, a FAO mission in 1971 was able to trace them still grown in Sicily (Porceddu and Bennet, 2071), and a numerical taxonomy analysis (Bogyo and Porceddu, 1075; Porceddu et al., 1981) confirmed the classification produced by De Cillis (1927). A special mention deserves Senatore Cappelli. Released in 1915, it represented a milestone in durum wheat breeding; its cultivation

covered more than 60% of the durum wheat area for several decades (Bozzini, 1989) and it is still grown in some areas for specific purposes or specific brand end-products, such as the monovarietal pasta manufactured in Apulia and Sardinia. Cappelli was selected from the North African landrace Jean Khetifah and well delineates the "North African" plant ideotype (waxy, tall, yielding but rather late ripening, good quality). For decades it has been crossed to "Syrian" types (no-waxy, shorter and early ripening but prone to lodging, low quality) and to other genetic material, including bread wheat, to endow the new releases with earliness, yield stability and quality across environments and years.

Several varieties, such as Garigliano (Strampelli, 1932), Capeiti, Patrizio (Casale, 1955), Grifoni, Appulo (Grifoni, 1964), Karel, Maristella (Barbieri and Deidda, 1968), and Trinakria (Ballatore, 1970) were released in the 1930-1970 period. They were characterized by good yielding ability, good quality, and adaptability to the southern Italy pedo-climatic conditions. An additional variety, ISA-1, selected in Apulia by Dionigi (1971), was characterized by earliness and thus able to escape to drought stress, although its yielding ability and quality did not meet the farmers' preference. Overall, these varieties moved yield from 1.0-1.2 to approximately 1.8-2.0 t/ha; however, despite this 80% increase, durum wheat yield was well below that of common wheat. Consequently, starting from '70s, yield increase became the main target of many breeding programmes, to be achieved by means of the introgression of useful traits from the hexaploid and wild wheat species or by mutagenesis programmes, fostering the breeding strategy adopted by breeders after world war II in selecting for reduced number of tillers per plant, increased number of spikes per unit of land, short stature plants, able to exploit higher fertiliser inputs and improved harvest index. Instrumental in this endeavour were the crosses between Italian genetic resources and short stature bread wheat, such as the Japanese red wheat Aka komugi, already used by Strampelli in bread wheat breeding. Thus by means of introgression from common wheat and selection for high spikelet fertility, Maliani (1968) obtained the cvs. Viscardo Montanari, Carlo Jucci, Giovanni Raineri.

An additional push in this direction was produced by D'Amato, Scarascia, Bozzini, Bagnara, Rossi and Mosconi, that, while studying the effects of radioactive mutagens on plants at the CNEN Casaccia Research Centre, were able to isolate some short statured lodging resistant, early ripening lines, four of which were released as finished varieties under the name of Castelporziano and Castelfusano, obtained from Cappelli, Casteldelmonte from Grifoni, and Castelnuovo from Garigliano (Scarascia Mugnozza, 1968; Bogyo *et al*., 1969; Bozzini and Girotto, 1971). Thanks to its erectoid gene, Castelporziano was able to stand in the most adverse lodging conditions. Stranger enough the molecular mechanism of the erectoid gene has yet to be clarified. Selection of segregating lines from a cross between one of those mutant lines (CpB144) and a dwarf CIMMYT line produced Creso, a high yielding variety, good quality, well adapted to different environmental conditions, almost filling the gap between durum and common wheat. In fact, in some environments Creso yield reached 10 t/ha and for a number of years it had more than 60% share in durum wheat land area (Bozzini *et al*., 1984; Bozzini, 1985). The attempt to reduce plant height by using CIMMYT lines carrying the Norin 10 dwarfing gene *Rht1* on chromosome 4A, in which a point mutation has produced a stop codon (the substitution of T with C converts the CGA codon for alanine in the TGA stop codon), was already in use at the Experimental Institute of Cereal Research by Vallega and Zitelli. They succeeded in selecting dwarf, disease resistant lines, which were later released as varieties having the prefix VAL- in their name (Zitelli and Vallega, 1968; Zitelli, 1973). The use of CIMMYT lines not only produced dwarf, lodging resistant plants, but also with a higher spikelet fertility and therefore a higher number of seeds per spike.

Table 1. Area (hectars) and production (metric tons) of durum wheat cultivated in individual regions of Italy during the 2011-2013 period.

Regions	2011		2012		2013		Averages	
	Ha	T	Ha	T	Ha	T	Ha	T
Piemonte	3,362	14,995	1,146	2,670	895	3,989	1,801	7,218
Valle d'Aosta	3	8	2	6	-	-	3	7
Lombardia	8,653	45,175	9,124	55,145	7,997	38,370	8,591	46,230
Veneto	8,110	45,225	7,676	50,492	3,859	24,734	6,548	39,995
Friuli-Ven. Giulia	763	3,269	741	3,180	-	-	752	3,225
Emilia-Romagna	41,993	252,438	47,388	287,928	43,388	267,077	44,256	269,148
Toscana	74,918	248,532	92,117	292,176	61,279	191,526	76,012	244,078
Umbria	18,005	100,995	18,000	105,487	17,355	99,525	17,786	102,002
Marche	120,380	479,819	132,350	606,711	123,604	491,855	125,444	525,561
Lazio	45,441	161,415	77,450	-	41,600	125,200	54,830	168,528
Abruzzo	29,860	111,015	34,083	130,161	32,240	133,846	32,061	124,570
Molise	50,766	153,819	61,500	172,200	59,600	166,880	57,288	164,300
Campania	55,239	144,134	55,317	188,212	59,609	189,622	56,721	173,317
Puglia	272,750	813,430	274,700	750,810	349,500	1,131,300	298,980	888,893
Basilicata	117,350	344,550	82,113	334,310	116,943	327,008	105,468	335,289
Calabria	23,537	58,861	31,037	80,195	31,537	91,814	28,703	76,286
Sicilia	295,690	818,314	301,641	872,287	287,331	800,690	294,887	818,235
Sardegna	32,154	62,490	34,036	82,084	34,514	74,935	33,568	73,169
North	62,884	361,109	66,077	399,420	56,139	334,169	61,700	364,899
Center	258,744	990,760	319,917	1,233,398	243,838	908,106	274,166	1,044,088
South	877,346	2,506,612	871,581	2,610,258	971,274	2,916,093	906,734	2,677,654
Italy	1,198,974	3,858,481	1,257,575	4,239,426	1,271,251	4,158,369	1,243,456	4,058,973

Source: ISTAT.

Table 2. World production of durum wheat (million tons) during the 2009-2013 period.

Country	2009	2010	2011	2012	2013	Average
EU-27	10.0	8.7	9.1	8.2	7.9	8.8
France	2.1	2.1	2.5	2.1	2.4	2.2
Greece	1.1	1.3	1.3	0.9	0.7	1.1
Italy	5.2	3.6	4.1	3.9	4.2	4.2
Spain	1.1	1.4	0.9	0.9	0.4	0.9
Kazakhstan	2.5	2.6	1.7	3.0	1.4	2.2
Canada	5.5	5.4	3.0	4.2	4.6	4.5
Mexico	2.0	2.2	2.2	2.2	2.1	2.1
USA	2.3	3.0	2.9	1.4	2.2	2.4
Argentina	0.2	0.2	0.3	0.2	0.2	0.2
Syria	1.2	1.8	1.6	1.7	1.5	1.6
Turkey	3.0	3.1	2.9	3.0	3.0	3.0
India	1.1	1.0	1.0	1.1	1.2	1.1
Algeria	0.9	2.9	2.2	2.5	3.0	2.3
Libya	0.1	0.1	0.1	0.1	0.1	0.1
Morocco	1.0	1.9	1.6	1.7	1.3	1.5
Tunisia	1.4	1.4	0.6	1.2	1.3	1.2
Australia	0.5	0.5	0.5	0.6	0.5	0.5
Others	7.2	6.2	5.3	5.7	5.1	5.9
WORLD TOTAL	38.9	41.0	35.0	36.8	35.4	37.4

Source: IGC – CWB.

The following years were characterized by intense breeding programmes, with CIMMYT materials included in almost every cross as partner of Italian germplasm. This allowed the release of a

number of high yielding cultivars by the Experimental Institute of Cereal Research (Foggia section) and by private seed companies, such as Società Produttori Sementi (Bologna), Società Italiana Sementi (Bologna), Eurogen (Enna), ISEA (Ancona) and COSEME (Foggia). The number of registered varieties, which was lower than 10 at the beginning of 1980s, grew up tremendously (6 until 1980, 19 in the decade 1981-1990, 62 in 1991-2000, and 145 in 2001-2013), thanks also to the EU financial support for durum wheat linked to the use of registered variety seed and to the introduction in Italy of the National Variety Register. In 2013, the National Seed Certifying Agency, ENSE, certified approximately 203,585 t of durum wheat seed (Table 3), with Iride having the highest share (12.4%), followed by Simeto and Saragolla with 11.4% and 9.3%, respectively; 76.1% of certified seed was interested by 20 cultivars, out of 136 registered varieties. Creso, 35 years old, was still grown on 1.3% of the durum wheat land devoted to certified seed.

Table 3. Certified seed (t) of the top twenty durum cultivars grown in Italy in 2013.

Cultivar	Certified seed		Cultivar	Certified seed	
	(t)	(%)		(t)	(%)
Iride	25,310	12.4	Anco Marzio	3,993	2.0
Simeto	23,241	11.4	Achille	3,936	1.9
Saragolla	18,987	9.3	Tirex	3,593	1.8
Core	12,175	6.0	San Carlo	2,507	1.7
Claudio	11,960	5.9	Pietrafitta	3,492	1.7
Quadrato	7,453	3.7	Dylan	3,483	1.7
Duilio	6,971	3.4	Svevo	2,675	1.3
Levante	5,968	2.9	Creso	2,646	1.3
Orobel	4,814	2.4	Miradoux	2,164	1.1
Rusticano	4,454	2.2	Other cvs	48,659	23.0
Arcangelo	4,104	2.0	TOTAL	203,585	100.0

An analysis of a Mediterranean collection of varieties by means of molecular markers, four per chromosome (Maccaferri *et al*., 2005), shed some light in the history of the last 50 years of breeding activity and strategic approaches followed by different breeding groups, and allowed to indicate some important points: 1) the main Italian durum cultivars, with the exception of Svevo, Neodur and Rusticano, have Cappelli in their pedigree, indicating that the ideotype preferred by Strampelli is somehow still valid, with the exception of plant height; 2) breeding has not added genes one by one but rather has restructured the entire genome; 3) breeding has preserved entire gene blocks for a long period, whereas more recently has produced a fine restructuring.

III – Selection targets

As far as the selection targets are concerned, lodging resistance has been already stressed as one of the most important targets, followed by earliness, limited number and simultaneously flowering fertile tillers, and number of fertile flowers per spikelet. Breeding progress in morpho-physiological, agronomical and qualitative traits of durum wheat cultivars released in Italy during the 20th century was recently investigated by De Vita *et al*. (2007) who showed that differences in agronomic traits are generally similar to differences observed in hexaploid wheat, with an annual genetic yield gain of 19.9 kg ha^{-1} $year^{-1}$. The genetic gain was most clearly associated with a higher kernels number m^{-2} indicating a larger grain-sink size and a higher number of spikes m^{-2}. The gradual reduction in plant height associated with an increased harvest index has represented the main breeding goal with an effect on the sink capacity and on the biomass partitioning.

1. Genetic resistance

About twenty pathogens and five insects can undermine wheat yield. The breeders prevalent attitude was to prefer low levels of long-lasting resistances, based on several genes, instead of high level of resistance controlled by single genes, in spite of the wide genetic variability present in cultivars and wild populations of the related species.

Stem rust (*Puccinia graminis* f. sp. *tritici*) has caused the heaviest losses in durum wheat. The first sources of resistance studied were a group of wheats from North Dakota (Yuma, Ld 390, Lakota and Wells), which were known to carry resistant factors derived from durum wheat, and some accessions of ssp. *dicoccum* (Zitelli, 1968), and were of great importance in controlling the pathogen's races existing in Italy (Bozzini, 1966; Zitelli, 1973). The sources of resistance to leaf rust, *Puccinia recondita*, were all found with poor agronomic performance. Using lines derived from Ld 390, Beladi 116, Tremez molle, Kyperunda and Gaza, Zitelli (1973) was able to transfer resistance to leaf rust into Italian varieties, thereby obtaining the Giorgio and Gerardo breeding lines used for further work by several durum breeders. The first source of resistance factor to powdery mildew (*Blumeria graminis*) was Yuma, whose resistance factors derived from ssp. *dicoccum* cvs Vernal and Khapli (Bozzini, 1966; Zitelli and Vallega, 1968; Zitelli, 1972). Later, a number of resistance genes were identified and mapped by using molecular markers, such as the novel resistance gene to mildew, *Pm36,* identified in one accession of ssp. *dicoccoides* and mapped on the chromosome bin 5BL6-0.29-0.76; the 244 bp allele of the EST-SSR marker BJ261635 can be used for marker-assisted selection during the wheat resistance breeding process for facilitating gene pyramiding. Later, many resistance genes for rust, mildew and *Fusarium* have been identified in related species and introduced in durum wheat (see recent review by Ceoloni *et al*., 2014). Interestingly, the Italian durum wheat cultivar Creso possess a high level of durable resistance to leaf rust (*Puccinia triticina* Eriks.) based on both hypersensitive and non-hypersensitive components. In order to investigate the genetic basis of this resistance, a segregating population composed of 123 recombinant inbred lines (RILs) derived from the cross Creso × Pedroso, was evaluated for disease severity in adult plants under field conditions (Marone *et al*., 2009). Besides some minor QTLs, one major QTL explaining both reduction of disease severity in the field and increased latency period was found on the long arm of chromosome 7B, and closely associated PCR-based and DArT markers were identified. Association mapping on a germplasm collection of 164 elite durum wheat accessions confirmed the presence of the *Lr14* resistance gene on 7BL in the cultivars Llareta and Creso (Maccaferri *et al*., 2010). *Lr14* can be considered as an important gene for resistance to leaf rust currently exploited by durum breeders in the Mediterranean areas.

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is one of the most important diseases of wheat worldwide, resulting in yield losses and mycotoxin contamination. Transgenic wheat plants expressing the bean PvPGIP2 (polygalacturonase-inhibiting proteins) in their flowers were found to have a significant reduction of symptoms when infected with *F. graminearum* (Ferrari *et al*., 2012) and suggest that polygalacturonases (PGs) likely play a role in of floral tissues infection, and that PGIPs incorporated into wheat may be important for increased resistance to FHB. Cereals contain xylanase inhibitor (XI) proteins which inhibit microbial xylanases and are considered part of the defense mechanisms to counteract microbial pathogens. A number of transgenic plants constitutively overexpressing TAXI-III, a member of the TAXI type XI that is induced by pathogen infection, were produced by Moscetti *et al*. (2013) and results showed that TAXI-III endows the transgenic wheat with new inhibition capacities which correlate with a significant delay of Fusarium head blight disease symptoms. These results provide clear evidence in planta that XI are involved in plant defense against fungal pathogens and show the potential to manipulate TAXI-III accumulation to improve wheat resistance against *F. graminearum*.

2. Drought stress

Drought is by far the most important factor affecting production and production stability; it has been therefore a classical topic in durum research. A number of morphological and physiological indices have been proposed for the selection of tolerant lines, such as stomata form, dimensions, and number per unit of leaf area, leaf water potential, osmotic adjustment ability, relative moisture content, accumulation of osmolites and abscissic acid (Tuberosa and Salvi, 2002; Cattivelli *et al*., 2008; Tuberosa, 2012). The best test, although empirical, is provided by the evaluation of lines in normal and stress conditions, and computing methods for establishing the different environmental conditions have been proposed. It is important to underline that phenotyping continues to represent the key step in understanding stress tolerance in the molecular era.

Gene cloning has opened new prospects in elucidating plant mechanisms elicited by stress and has led to the isolation of genes whose activity is controlled by stress events, although their precise function is still to be defined. Particularly interesting in this issue are results related to the expression of sequences and mRNA in plants of Capeiti and Creso, tested under different levels of water stress at the flowering stage (Cattivelli *et al*., 2002). A genomic map of major loci and QTLs affecting stress tolerance identified the crucial role of the group 5 chromosomes, where the highest concentration of QTLs and major loci controlling plant's adaptation to the environment (heading date, frost and salt tolerance) has been found. Extensive molecular studies have led to the cloning of many stress-related genes and responsive elements. The expression of some stress-related genes was shown to be linked to stress-tolerant QTLs, suggesting that these genes may represent the molecular basis of stress tolerance (Aprile *et al*., 2013).

Nine populations of durum wheat, from three environmentally contrasting regions of Ethiopia (Tigray, Gonder and Shewa), were analyzed by SSRs markers in order to verify the presence of LD and to detect loci with reduced variation, possibly as consequence of selective sweeps. Results indicated the existence of high linkage disequilibrium among loci and the presence of some selective sweeps in chromosome 4A sequences, close to loci (QTL) previously identified as related to drought tolerance (Pagnotta *et al*., 2007). Genes from the DREB family are involved in plant's responses to dehydration and possibly play a role in their ability to tolerate water stress. The isolation and characterization of a gene in durum wheat, namely TdDRF1, which belongs to the DREB gene family and produces three forms of transcripts through alternative splicing, has been reported (Latini *et al*., 2007). Recently, many transcription factors for tolerance to salt and drought stresses have been identified, and the multi-alignments of conserved domains in DREB1, WRKY1 transcription factors (TFs), and HKT-1 has allowed to identify functional single nucleotide polymorphisms (SNPs) (Mondini *et al*., 2012). All the discovered mutations were able to generate changes in amino acid sequences of the corresponding proteins. Most of the identified SNPs were found in salt and drought tolerant durum wheat genotypes. A different stress responsive strategy was found in two durum wheat cultivars characterized by different water use efficiency, subjected to drought, heat and a combination of both stresses (Rampino *et al*., 2012): the cv Ofanto (lower water use efficiency) activated a large set of well-known drought-related genes after drought treatment, while Cappelli (higher water use efficiency) showed the constitutive expression of several genes induced by drought in Ofanto and a modulation of a limited number of genes in response to stress.

A review of breeding progresses on drought stress tolerance (Cattivelli *et al*., 2008) pointed out that selection for high yield in stress-free conditions has indirectly improved yield in many water-limiting conditions. To reduce the gap between yield potential and actual yield in drought-prone environments, three main approaches can now be exploited: (i) plant physiology has provided new insights and developed new tools to understand the complex network of drought-related traits; (ii) molecular genetics has detected many QTLs affecting yield under drought or the expression of drought tolerance-related traits; (iii) molecular biology has provided genes useful either as candidate sequences to dissect QTLs or for a transgenic approach. The extent of information,

that breeders currently have, offers new tools for breeding, such as markers for QTLs and single genes for plant transformation. This strategy will lead to new cultivars with high yield potential and high yield stability, that in turn will result in superior performance in dry environments.

3. Grain and pasta quality

Durum wheat grain is today essentially used in pasta manufacturing in Italy and pasta quality has constantly been one of the most important breeding objectives. In southern Italy durum grain is also utilised in typical bread preparation, such as the well-known “Pane di Altamura”. De Cillis (1942) was the first to show that pasta produced by using vitreous grains, which possess higher protein content, has better cooking quality than that obtained from starchy grains, which possess lower protein content. The same conclusions were reached, years later, by many other scientists who also showed the existence of a consistent relationship between gluten properties and content and pasta cooking quality (Novaro *et al*., 1993; Mariani *et al*., 1995; Autran and Galterio. 1989; D’Egidio *et al*., 1990). These findings promoted a wide array of studies on grain protein composition, grain protein content and grain colour, to which Italian scientists participated actively using different approaches.

4. Grain protein composition

Pasta quality is strongly depending from gluten quantity and quality. Gluten is a proteic complex composed of gliadin and glutenin components confering viscoelasticity and plasticity to gluten mass, respectively. Since gluten was shown to be composed by polypeptides controlled by single co-dominant factors, specific attention has been deserved to the analyses of these genes (Dal Belin Beruffo *et al*., 1981, 1982; Porceddu *et al*., 1983; Lafiandra *et a*l., 1984, 1987, 1990). It was thus possible to demonstrate that: a) gliadins components are coded by genes *(Gli-1)* located on short arms of homoeologous chromosomes of groups 1 and 6 (Lafiandra *et al*., 1984); b) loci for low molecular weight (LMW) glutenin subunits (*Glu-3*) are located on short arms of group 1 chromosomes closely linked to *Gli-1* (Pogna *et al*.,1990); c) a tremendous amount of genetic variation exists for gliadin and glutenin subunits, both in cultivated wheats and wild relatives (Ciaffi *et al*., 1992) offering opportunity for selection. In fact a number of breeding lines has been selected for the presence of different gliadin and glutenin components. Related to these aspects, many studies have been carried out on the allelic variability of *Gli* and *Glu* loci and on the relationships with technological quality. Pogna *et al*. (1988), utilising a recombinant line containing ɣ-42 gliadin and LMW-2, firstly reported by Margiotta *et al*. (1987), were able to show the functional direct role of LMW subunits in gluten viscoelastic properties. Pogna *et al*. (1990) reported that genes different from those at *Glu* loci could be involved; in fact negative correlations between proteolitic activity and cooking quality was found (Dal Bellin Peruffo and Pallavicini, 1981; Petruzzelli *et al*., 1981). The total content of the glutenin components, the presence of specific HMW-GS and of specific gliadin subunits has been directly associated with the higher rheological characteristics of gluten. These proteins include the γ-45 gliadin and various ϖ and β gliadins coded by the Gli-*B1 l*ocus localized on the short arm of chromosome 1B and genetically associated to the *Glu-B3* locus coding for a group of LMW-GS, named LMW-2. Wheats with the γ-45 subunit have six different alleles at *Glu-B3,* and this explain the wide variability of grain quality (from average to high) of durum lines all presenting the γ-45 gliadin component. The γ-45 band represents, actually, a quality marker since *Gli-B1* is closely associated to the *Glu-B3* locus. Indeed, gluten quality essentially depends on specific glutenin subunits coded by *Glu-A3, Glu-B3* and *Glu-B2* loci. Protein bands, visualized after electrophoresis on poliacrylammide gel, are widely used as biochemical markers of grain quality in modern breeding programs.

These findings promoted additional studies, such as those on the analysis of a family of nine genes, located on group 4 chromosomes, coding for protein disulfide isomerase (PDI), which has a redox role and may affect protein folding and assembling (Ciaffi *et al*., 1999; Ciaffi *et al*., 2001);

PDI genes have been cloned and sequenced (Ciaffi *et al*., 2006) and transgenic plants have been produced in cooperation with CIMMYT (D'Aloisio *et al*., 2010; Paolacci *et al*., 2011; Ciaffi *et al*., 2013).

Moreover, Resmini and Pagani (1981) observed that differences in the semolina and spaghetti protein matrix could promote possible interactions not only among different protein types but also between proteins and carbohydrates in determining pasta quality. However, there were differences depending on whether semolina or dry pasta was used in the analyses. When semolina was used, the role of proteins was prevalent and that of other components was irrelevant. When pasta was used, the dominance of proteins decreased and the role of starch increased and was positive; amylopectin behaves in the same manner as starch, and starch change at the expenses of amylose is to be preferred (D'Egidio *et al*., 1979). Quite similar results were obtained with pentosans, which, as known, consist of highly branched linear xylanes. Medcalf *et al.* (1968) showed that water soluble pentosans from durum wheat are more branched than those from hard red spring wheat, and even small differences in the branching degree may greatly alter the degree and type of interactions of polysaccharides with proteins. The yield of water soluble pentosans from spaghetti is much higher than that from semolina, whereas the opposite is true for the water insoluble ones (Lintas and D'Apollonia, 1973) supporting the occurrence of differences in starch gelatinisation.

Manipulation of starch composition in cereals, and particularly in wheat, is receiving increasing attention due to recognition of its important role in food and nonfood applications. The amylose/amylopectin ratio influences the physicochemical properties of starches and nutritional value of derived end-products (Lafiandra *et al*., 2013). Identification of the key enzymes involved in the starch biosynthetic pathway has opened new avenues for altering the amylose and amylopectin ratio in durum and bread wheat. The granule bound starch synthases (GBSSI), or waxy proteins, are the enzymes responsible for amylose synthesis in storage tissues; amylopectin is produced by the concerted action of different enzymes, including starch synthases (SS), branching (SBE), and debranching enzymes (DBE). By altering the level of key enzymes involved in the regulation of starch synthesis, it is possible to generate novel starches with unique functional properties. In this respect, both low and high amylose starches are particularly interesting because they are associated with industrial and processing properties as well as with human health and nutrition (Lafiandra *et al*., 2010). The characterization of waxy genes that modify the relative amount of amylose and amylopectine was reported by Monari *et al.* (2005) and NIL have been produced and tested (Jonnala *et al*., 2010).

The remarkable innovations in pasta production processes, in particular drying technologies based firstly on low temperatures (40 - 50° C) and later raised to 60-70°C or even to 80°C, have allowed the production of pasta with an acceptable or good cooking strength by using poor quality raw materials (De Stefanis and Sgrulletta, 1990). High temperatures have been quickly adopted from pasta industry not only for baking quality improvement but also for the higher healthy conditions and the reduced times of drying. However, also with these new technologies, protein content remain a parameter of primary importance for the production of higher quality pasta (Novaro *et al*., 1993).

Durum wheat is traditionally used for the production of numerous types of pasta; however, significant amounts are also used for bread-making, particularly in southern Italy. The glutenin subunits 1Dx5 and 1Dy10, encoded by the *Glu-D1* locus on chromosome 1D in bread wheats, are positively correlated with higher dough strength. Transgenic plants for glutenin subunits have been obtained at Experimental Institute for Cereal Research (in cooperation with University of Bristol) (Terzi *et al*., 2005) and University of Bari (in cooperation with USDA, Albany) (Gadaleta *et al*., 2008). In order to study the effects of stable expression of the 1Dx5 and 1Dy10 glutenin subunits in different wheat genotypes, four durum cultivars commonly grown in the Mediterranean area (Svevo, Creso, Varano and Latino) were co-transformed, via particle bombardment of

cultured immature embryos, with the two wheat genes *Glu-D1-1d* and *Glu-D1-2b* (Gadaleta *et al*. 2008). Small-scale quality tests showed that accumulation of Dx5, Dy10 or both in transgenic durum seeds resulted in doughs with stronger mixing characteristics. Sissons *et al*. (2013) studied the effect on technological properties of pasta and bread made from durum wheat cv. Svevo and two isogenic genotypes carrying pairs of additional subunits 5+10 (S 5+10) or 2+12 (S 2+12), normally present in bread wheat. The dough properties of the S 5+10 line were markedly different from Svevo, having over-strong, stable dough, low wet gluten and elasticity; S 2+12 also displayed stronger dough. Pasta prepared from these genotypes showed lower cooked firmness. Bread loaf volume and loaf score decreased as more bakers flour was replaced by durum flour, but the decline varied with the genetic material and dosage. The greatest reduction in loaf volume occurred using S 5+10 and the least with S 2+12, which was similar to Svevo. Bake score was reduced with S 5+10 only. These work show that it is possible to manipulate the processing properties of pasta and durum-bread-wheat blends by altering the glutenin subunit composition.

5. Grain protein concentration

Protein content and other qualitative parameters of durum wheat grain are polygenic characters strongly influenced by environmental factors. Their evaluation and the obtainment of improved lines are expensive, laborious and time-consuming because of the low heritability and of the complex biological bases. Segregant off-springs and lines have to be evaluated in different environments in order to obtain reliable data and the identification of superior genotypes. Such evaluations sometimes require an elevated amount of grain. For these reasons, breeders are looking for alternative strategies, quicker and more reliable of conventional ones. The mapping of loci for quantitative characters allows the identification of associated molecular markers that can be used in assisted selection and, therefore, to perform a genotypic selection in alternative to the conventional phenotypic selection. In durum wheat a segregating population of recombinant inbred lines has been obtained from crossing the cv. Messapia and an accession of *dicoccoides* with high protein content; for this population a molecular map that now comprises 458 markers comprehensive of morphologic, biochemical, RFLP, AFLP and microsatellites markers has been produced (Blanco *et al*., 1998; Blanco et al, 2004). The analysis of the segregant population in eight different environments has allowed to map seven different QTLs for protein content on six chromosome arms and to clear the bases of the negative correlation between protein content and productivity observed in all cereals (Blanco *et al*., 2006). Such correlation is generally attributed to environmental factors, to nitrogen dilution in the kernels, to a higher amount of carbohydrates, to the higher energetic demands for protein synthesis with respect to carbohydrates, to genetic components. Six of the seven QTLs for the high protein content had pleiotropic effects or they were associated to QTLs for low productivity. These results are obviously important in assisted selection programs where the use of markers for the desired character should not have negative consequences on other correlated agronomic traits. In a recent study carried out on a RIL segregating population derived from crossing two commercial elite cultivars (Svevo e Ciccio), 10 independent genomic regions involved in the expression of GPC were detected, six of which were associated with QTL for one or more grain yield components (Blanco *et al*. 2012). QTL alleles with increased GPC effects were associated with QTL alleles with decreased effects on one or more yield component traits, or vice versa (i.e. the allelic effects were in opposite direction). Four QTL for GPC showed always significant effects, and these QTLs should represent genes that influence GPC independently from variation in the yield components. Such genes are of special interest in wheat breeding since they would allow an increase in GPC without a concomitant decrease in grain yield.

6. Grain colour

Yellow pigment concentration (YPC) in durum wheat is an important criterion for the assessment of semolina quality, particularly in determining the commercial and nutritional quality of end-

products. The pigment content has been taken into serious consideration only recently from Italian breeders. Yellow colour depends on several factors: carotenoid content of kernels, residual content of pigments after grain or semolina-bran conservation, the grinding rate, the oxidative degradation of enzymes, like lipoxigenases (LOX), and the conditions of pasta-making process. Genetic variability of YPC and carotenoid components was analysed in 102 wild and cultivated tetraploid wheat accessions (Di Gesù *et al*., 2009). Overall, modern cultivars showed significantly higher values of YPC compared to old cultivars and wild *dicoccum* and *dicoccoides* accessions. Lutein was the main component of carotenoids, followed by zeaxanthin and β-carotene; α-carotene and β-cryptoxanthin were minor components. Pigment concentration was negatively correlated with kernel weight and grain protein concentration; significant positive correlations were found between the yellow index *b** and YPC. The value of 4,2 ppm is considered the minimal amount in order to obtain pasta of acceptable colour. The majority of the recent French varieties and some of the Italian ones, like Grecale and Svevo exceed this value. The total carotenoid content is a polygenic character with high heritability. The isolation of BAC clones containing genes coding for three different enzymes of the carotenoid biosynthesis pathway: phytoene synthase (PSY), phytoene desaturase (PDS), and carotene desaturase (ZDS) was reported by Cenci *et al*. (2004). Primers were designed on the basis of wheat ESTs similar to the sequences of these three genes in other species, and used to screen a durum wheat BAC library (Cenci *et al*., 2003). PSY clones were localized on chromosomes 5A and 5B, PDS on chromosomes 4A and 4B, and ZDS on chromosomes 2A and 2B. Recently, 150 SSR and EST-SSR markers and 345 DArT markers, were used to construct the linkage map Latino x Primadur for subsequent carotenoid components QTL analysis (Blanco *et al*., 2011). Clusters of QTLs for total and/or one or more carotenoid compounds were detected on the same chromosome regions (2A, 3B, 5A and 7A) where QTLs for yellow pigment concentration and yellow index were identified. The molecular markers associated to major QTL would be useful for marker-assisted selection programs to facilitate high carotenoid concentration with high nutritional carotenoid compounds in wheat grain.

During pasta processing, oxidative degradation of carotenoid pigments occurs mainly due to lipoxygenase enzymes (LOX). In durum wheat, two *Lpx-1* genes have been identified on chromosome 4B, and evidences have been reported that the deletion of *Lpx-B1*.1 is associated with a strong reduction in LOX activity in semolina. The *Lpx-B1* gene family was characterized in a durum germplasm collection and showed that all of the genotypes have one of the three *Lpx-B1.1* alleles, associated with either *Lpx-B1.2* or *Lpx-B1.3*, and accounts for most of the total LOX activity in the mature grains (Verlotta *et al*., 2010; De Simone *et al*., 2010). Information on these *Lpx-B1* haplotypes provides significant improvement for prediction of LOX-1 activity levels in mature grains, and will therefore help in breeding programs aimed at selection of new durum genotypes with higher carotenoid contents in their end-products.

IV – Future perspectives

The selection of new and better genotypes of durum wheat can be obtained by several breeding strategies, all based, however, on the availability of genetic variability. The classical way consists in crossing parental lines selected for higher phenotypic traits (productivity, production stability, tolerance to drought, nutrients using efficiency, above all nitrogen, resistance to the main pathogens, etc.) and the subsequent evaluation of segregating off-springs through the pedigree method or other conventional methodology. With this approach, the possibility of selecting improved lines depends on the choice of the parental lines, especially on their genetic distance and, therefore, from the possibility to obtain a high number of recombinants. The wheat breeder must, therefore, select a combination of genes useful for a superior productive and qualitative performance of the new cultivar. However, to arrange desired genes in a single plant is not easy and it is a long-lasting and laborious process, in particular considering that, with the classic

methodologies of genetic improvement, the available genetic variability is that enclosed between individuals of the same or closely related species.

The molecular markers-assisted selection (MAS), as an approach for the identification of genotypes having certain QTLs, can be a valid instrument in order to accelerate the procedures and to reduce, therefore, the time necessary for the identification of superior genotypes (Collard and Mackill 2008). MAS uses molecular markers in linkage disequilibrium (LD) with the useful genes. However, agronomical important traits are complex and affected by many genes, each with small effect. Classical marker-assisted selection has been ineffective for such quantitative traits. The dramatic drop of the cost of DNA markers should accelerate the obtainment of crop varieties with improved yield and yield stability, quality, disease resistances and drought stress tolerance. The introduction of genomic selection (GS) is a new approach for improving quantitative traits in large crops breeding populations that uses whole genome molecular markers. Genomic prediction combines marker data with phenotypic and pedigree data to increase the prediction accuracy of breeding and genotypic values. Selection can be based on GS predictions, potentially leading to more rapid and lower cost gains from breeding (Bernardo and Yu, 2007; Goddard. and Hayes, 2007; Heffner *et al*., 2009).

The use of mutagenesis can be still used in order to increase the genetic variability, in particular for the identification of new useful characters. Thus, recently, at the Cereal Research Institute (Foggia) an experiment of chemical mutagenesis has been carried out and interesting mutants have been isolated, such as the “stay-green” that shows an extended photosynthetic activity, or other mutants characterized by elevated concentrations of K+ ions in the culm, resistance to salinity, elevated metabolic efficiency (Rascio *et al*., 2007). The reverse-genetics approaches are becoming appealing thanks to the improvement in high-throughput DNA screening techniques and the increasing number of available gene sequences. TILLING (Targeting Induced Local Lesions In Genomes; McCallum *et al*., 2000) is a significant and emerging reverse-genetics strategy that combines standard chemical mutagenesis with high-throughput techniques to screen and to identify induced point mutations in candidate genes. TILLING has been proposed as an innovative approach to generate and detect novel allelic variants starting from known genes of interest, as well as phenotypes suitable for breeding purposes. The seed company Società Produttori Sementi S.p.A. (PSB; Bologna, Italy) and the Department of Science and Technology for Agriculture, Forest, Nature and Energy (DAFNE) of Tuscia University (Viterbo, Italy) have developed a durum TILLING population of 2601 M_3 families from cv. Svevo using ethyl methanesulfonate as a chemical mutagen (Bovina *et al*., 2013). Despite the polyploid nature of the wheat genome, a preliminarily phenotypic screening of the entire M_3 population in a field-grown experiment showed a high frequency of morphological alterations (~22%). Furthermore, a reverse-genetics experiment was performed on DNA collected from M_2 leaves for the homoeologous genes *SBEIIa-A* and *SBEIIa-B* involved in starch metabolism and one non-sense mutation for both genes was identified.

In alternative to crossing, the transgene technology can be a powerful tool for widening the genetic variability and for the production of new lines. Currently, recombinant DNA technology supplies advanced instruments for the identification and the isolation of genes encoding specific characteristics in a determined organism and to transfer copies of these genes in a completely different organism. Recombinant DNA technology has been applied also in durum wheat for the production of new lines without using the classic methodologies of crossing and selection. The collaboration between the Cereal Research Institute (Foggia) and the Department of Agricultural Sciences of the University of Bristol (UK), has allowed to optimize the technique of transformation in the durum wheat (species well-known to be recalcitrant to transformation) and to obtain plants of the cultivar Ofanto transformed for glutenin subunits (Terzi *et al*., 2005). Similar results have been obtained through a collaboration between the section of Genetics and Plant Breeding of the DiSSPA (University of Bari) and the USDA of Albany (USA) that has allowed to obtain plants of three different durum cultivars (Creso, Svevo, Varano) transformed for the 5+10 HMW-

GS, normally present on the D genome of common wheat (Gadaleta *et al*., 2008). Moreover, studies are performed with the objective to dissipate doubts and perplexity about GMO in agriculture, above all those linked to the use of particular genetic markers. It is well known that the development of transgenic plants requires the use of selectable marker genes, as the efficiency of plant transformation is less than optimal for many important species, especially for monocots such as durum wheat. Many concerns have been expressed about the persistence of currently used marker genes in plants used for field cultivation. To sustain further progress in this area, alternative efficient selection methods are desirable. The 'selection efficiency' of a commonly used negative selection method that employs the *bar* gene to confer resistance to the herbicide bialaphos was compared to a positive selection employing the phosphomannose isomerase (*pmi*) gene as the selectable gene and mannose as the selective agent (Gadaleta *et al*., 2006). The selection efficiency was higher when *pmi* was used as the selectable marker gene (90.1%) than when bar was used (26.4%). Thus, an efficient selection method for durum transformation was established that obviates the use of herbicide résistance genes. At the same time, the "gene clean' technique has been setting up to combine biolistic transformation by minimal gene cassettes with genetic segregation to make marker-free transgenic wheat plants with new traits (Gadaleta *et al*., 2008).

Useful instruments in cultivated plants are the "BAC libraries" that allow cloning of large DNA fragments and generally are used for the construction of genomic libraries of whole genomes. BAC libraries are preferred to YAC libraries for their simpler realization and higher stability. In cultivated plants, these BAC libraries are useful for positional gene cloning, genomic structural analyses, comparative analyses of genomes of related species, saturation maps with molecular markers and microsatellites extracted from specific regions. For durum wheat is now available a BAC library realized in the LANGDON cultivar, composed of 516.096 clones singularly maintained in 1344 plates with 384 clones (Cenci *et al*., 2003). This BAC library has a 5X genome coverage and supplies a high probability to identify and to isolate the desired gene. A first use has been that to isolate clones containing gene sequences coding for the phytoene sinthase, phytoene desaturase and carotene desaturase, enzymes involved in the carotenoid biosynthesis (Cenci *et al*., 2004).

Genome sequencing and associated bioinformatics resources are now a popular research tool in wheat for accelerating the analysis of genome structure and function because it leverages similar work from other crops and plants. Despite wheat is one of the world's most important crops, progress in wheat genomics has been slow due to its large and complex genome. Several studies, coordinated by the International Wheat Genome Sequencing Consortium (http://www.wheatgenome.org) are in progress with the aim of obtaining and characterizing the wheat genome. One way to reduce the genome complexity is to purify single chromosomes using flow cytometry and to perform the analysis at the sub-genomic level (Doležel *et al*., 2007). The massively parallel 454 pyrosequencing was recently used to obtain a 2x coverage of wheat chromosome 5A (Vitulo *et al*. (2011) and the resulting sequence assembly was used to identify TEs, genes and miRNAs, as well as to infer a virtual gene order based on the synteny with other grass genomes. Repetitive elements account for more than 75% of the genome, while the coding fraction represents 1.08% and 1.3% of the short and long arm, respectively, projecting the number of genes of the whole chromosome to approximately 5,000. A particularly challenging task is the anchoring of BAC contigs to a genetic map, for which the availability of high density linkage maps are crucial. In wheat, the limitations of the large genome size and lack of polymorphism can be overcome by targeted mapping, made possible by the isolation of more than 400 deletion lines for the 21 chromosomes of the wheat cultivar Chinese Spring. The deletion mapping strategy has allowed to provide deletion maps for wheat the 5A and 5B chromosomes and a genetic map of 5A enriched with popular microsatellite markers, which could be compared with other existing maps and useful for mapping major genes and QTLs (Gadaleta *et al*., 2012).

Using next-generation sequencing technologies it is possible to resequence entire plant genomes or sample entire transcriptomes more efficiently and economically. Rather than sequencing individual genomes, it is possible the sequencing of hundreds of related genomes to sample genetic diversity within and between germplasm pools. Next-generation sequencing (NGS) technologies can be applied in some important areas such as the large-scale development of molecular markers for linkage mapping, association mapping, wide crosses and alien introgression, epigenetic modifications and population genetics to advance crop genetics and breeding (see review by Varshney *et al*. 2009). The application of complexity reduction of polymorphic sequences (CRoPS ®) technology for the discovery of SNP markers in durum wheat has been reported by Trebbi *et al*. (2011). A next-generation sequencing experiment was carried out on reduced representation libraries obtained from four durum cultivars and SNP validation was carried out on a panel of 12 cultivars. A total of 2,659 SNPs were identified on 1,206 consensus sequences. Of these SNPs, 157 were mapped in one of two mapping populations (Meridiano × Claudio and Colosseo × Lloyd) and integrated into a common genetic map. The validated CRoPS-derived SNPs showed valuable features for genomics and breeding applications such as a uniform distribution across the wheat genome, a prevailing single-locus codominant nature and a high polymorphism.

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Session 3

Strategies and tools in durum wheat genetics and breeding

Developing improved durum wheat germplasm by altering the cytoplasmic genomes

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Abstract. In eukaryotic organisms, nuclear and cytoplasmic genomes interact to drive cellular functions. These genomes have co-evolved to form specific nuclear-cytoplasmic interactions that are essential to the origin, success, and evolution of diploid and polyploid species. Hundreds of genetic diseases in humans and phenotypic variations in plants are known to be the result of alterations affecting nuclear-mitochondrial (NM) communication. The genetic bottleneck in the nuclear genome of modern polyploid wheat species is mirrored by the homogeneity of cytoplasmic genomes in durum and bread wheat cultivars. This lack of variation is illustrated by our data indicating that the mitochondrial genome of durum wheat is almost identical to that of published bread wheat genome. The data by our group and others clearly illustrate that genes affecting NM interactions are directly or indirectly related to hybrid compatibility. Therefore, their manipulation and use would permit wider usage of alien germplasm and more efficient introgression. Thus, we have embarked on a series of studies to: 1) isolate, characterize and manipulate genes involved in NM interaction; 2) better understand the influence of cytoplasmic genome by analyzing the vast collection of wheat alloplasmic lines; and 3) determine the extent of mitochondrial genome variability in *Triticeae* and *Aegilops* species in order to generate more cytoplasmically variable, and agronomically adapted cultivars.
Utilizing traditional genetic mapping and radiation hybrid mapping, we located a gene in durum wheat (*T. turgidum* L. var. *durum*) involved in NM compat bility to a chromosome segment of a few hundred Kb in size. Isolation and characterization of this gene will provide us the ability to understand and manipulate regulatory mechanisms respons ble for a number of developmental processes in durum wheat, including embryo/seed development and plant vigor. In parallel, we have demonstrated that variation in the cytoplasmic genome can influence plant-pathogen response such as the interaction with *Pyrenophora tritici-repentis* (tan spot) and *Puccinia triticina* (leaf rust). Sequencing the mitochondrial genome of an alloplasmic wheat line indicated a great amount of sequence and structural changes in the genome, and at a much higher frequency than is observed in evolutionarily distant species. Additionally, our data indicated paternal leakage, heteroplasmy and stoichiometric changes in the mitochondrial genomes. These results have important implications in terms of the potential to manipulate plant mitochondrial genomes and select for changes that are critical to plant development and adaptation**.**

Since plants cannot escape from adverse environmental conditions, adaptation is paramount to species survival. Cytoplasmic genomes play a critical role in adaptation, and poss bly speciation. Therefore, manipulation of mitochondrial genomes and creation of new cytoplasmic variability may provide a further mechanism for stress tolerance.

Keywords. Cytoplasmic variability – Alloplasmic – Stress tolerance – Mitochondria – Breeding.

Amélioration génétique du blé dur à travers la modification des génomes cytoplasmiques

Résumé. *Dans les organismes eucaryotes, les génomes nucléaires et cytoplasmiques interagissent pour diriger les fonctions cellulaires. Ces génomes ont co-évolué pour produire des interactions nucléaires-cytoplasmiques spécifiques qui sont essentielles pour l'origine, le succès, et l'évolution des espèces diploïdes et polyploïdes. Des centaines de maladies génétiques chez l'homme et des variations phénotypiques chez les plantes sont connues pour être le résultat des altérations de la communication nucléaire-mitochondriale (NM). Le goulot d'étranglement génétique au niveau du génome nucléaire des espèces de blé polyploïdes modernes est reflété par l'homogénéité des génomes cytoplasmiques chez les cultivars de blé dur et de blé tendre. Cette absence de variabilité est illustrée par nos données indiquant que le génome mitochondrial du*

blé dur est presque identique à celui du blé tendre publié. Les données de notre groupe et d'autres groupes montrent clairement que les gènes intervenant dans les interactions NM sont directement ou indirectement liés à la compatibilité de l'hybride. Par conséquent, leur manipulation et leur utilisation permettrait d'exploiter davantage le matériel génétique étranger et de mieux réussir l'introgression. Ainsi, nous avons entrepris une série d'études pour : 1) isoler, caractériser et manipuler les gènes impliqués dans l'interaction NM ; 2) mieux comprendre l'influence du génome cytoplasmique à travers l'analyse de la vaste collection de lignées de blé alloplasmiques ; et 3) déterminer l'importance de la variabilité du génome mitochondrial des espèces Triticeae et Aegilops afin d'obtenir des cultivars plus variables du point de vue cytoplasmique et adaptés sur le plan agronomique.

En utilisant la cartographie génétique traditionnelle et la cartographie des hybrides d'irradiation, nous avons localisé un gène dans le blé dur (T. turgidum L. var. durum) impliqué dans la compatibilité NM sur un segment chromosomique de quelques centaines de Kb. L'isolement et la caractérisation de ce gène nous permettra de comprendre et de manipuler des mécanismes de régulation responsables d'un certain nombre de processus de développement du le blé dur, y compris le développement embryon/semence et la vigueur de la plante. Parallèlement, nous avons démontré que la variation du génome cytoplasmique peut influencer la réponse plante-pathogène comme dans le cas de l'interaction avec Pyrenophora tritici-repentis ('helminthosporiose) et Puccinia triticina (rouille des feuilles). Le séquençage du génome mitochondrial d'une lignée de blé alloplasmique a mis en évidence de nombreux changements des séquences et des structures du génome, et ce, avec une fréquence beaucoup plus élevée par rapport aux espèces distantes du point de vue évolutif. En outre, nos données indiquent une perte de génome paternel, des changements de l'hétéroplasmie et des changements stœchiométriques dans les génomes mitochondriaux. Ces résultats sont importants dans la mesure où ils offrent un élan potentiel à la manipulation des génomes mitochondriaux des plantes et à la sélection des changements qui sont essentiels pour le développement et l'adaptation des plantes.

Comme les plantes ne peuvent pas échapper aux conditions de milieu défavorables, l'adaptation est primordiale pour la survie des espèces. Les génomes cytoplasmiques jouent un rôle fondamental dans l'adaptation et, probablement, la spéciation. Par conséquent, la manipulation des génomes mitochondriaux et la création d'une nouvelle variabilité cytoplasmique peuvent fournir un mécanisme supplémentaire pour la tolérance au stress.

Mots-clés. *Variabilité cytoplasmique – Alloplasmique – Tolérance au stress – Mitochondries – Sélection.*

I – Introduction

Wheat belongs to the Triticeae tribe of grasses, a group comprising some 300 species (Matsuoka, 2011). Wheat is widely adapted, grown on more land than any other agricultural plant, and - with rice and maize - vies yearly for the greatest tonnage of worldwide production (Shewry, 2009). Wheat also is one of the oldest crops, as established from abundant archaeological, religious (e.g., Biblical stories), and historical evidence indicating its importance to human civilization (Fuller, 2007). Wheat cultivation occurred around 10,000 years ago when human beings started to shift from hunting and gathering to self-production (Shewry, 2009). The early species cultivated by man were mostly diploid einkorn (AA) and tetraploid emmer (AABB) wheat (Dubcovsky and Dvorak, 2007). The *Triticum-Aegilops* species diverged from each other around 3 million years ago (Chalupska *et al*., 2008; Dvorak and Akhunov, 2005) (Fig. 1). This divergence followed changes in chromosome number as a result of two episodes of allopolyploidization leading to the formation of hexaploid cultivated bread wheat (Fig. 1). The first hybridization leading to the formation of cultivated tetraploid wheat (i.e., durum or pasta wheat) occurred ~0.5 million years ago and the second event leading to the formation of hexaploid wheat happened ~8,000 years ago (Fig. 1) (Chalupska *et al*., 2008; Dvorak and Akhunov, 2005). These events created genetic bottlenecks, which excluded potentially valuable alleles from the polyploid forms (Dubcovsky and Dvorak, 2007).

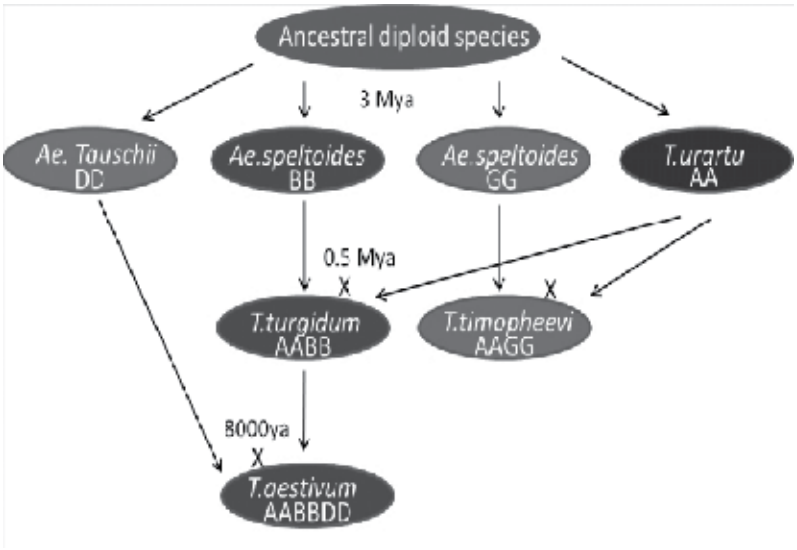

Figure 1. Evolutionary relationship of *Aegilops* and *Triticum* species leading to the formation of tetraploid pasta and hexaploid bread wheat. The wheat B genome donor is extinct, and is believed to be best represented by the S genome of *Ae. speltoides*. The cytoplasmic component of the genome is identified by ellipses filled with different shades of gray while the genomes are identified by their designation (e.g. DD for *T. tauschii*). MYA= million years ago.

It seems less than 15% of the *Ae. tauschii* and 30% of wild emmer wheat genetic variability is present in the D and A+B genomes of hexaploid wheat, respectively. However, the genetic diversity present in cultivated emmer wheat is 58% of the wild emmer wheat (Dubcovsky and Dvorak, 2007). The genetic bottleneck in the nuclear genome of modern polyploid wheat species is mirrored by the homogeneity of cytoplasmic genomes in durum and bread wheat cultivars. The hybridization event leading to the formation of tetraploid wheat was a rare event. The cytoplasmic genome, derived from an extinct species related to *Ae. speltoides*, coming from the female parent went through a similar reduction in variation (Fig. 1). Early in wheat research, Kihara (Kihara, 1954) showed that in crosses of polyploids with diploids, viable seed is more likely when the former is used as a female parent. Therefore, as the nuclear genome recovered some of its variation through introgressions from wild species, the cytoplasmic genome remained homogeneous due to uni-directional interspecific cross incompatibilities (Maan and Endo, 1991). This bottleneck was reiterated when the tetraploid wheat hybridized with Ae. tauschii forming the hexaploid wheat (Fig. 1). Lack of cyptoplamic variation is illustrated by a study that compared the mitochondrial genomes of 29 tetraploid and hexaploid wheat accessions with 21 microsatellite loci indicating that they are all the same (Ishii *et al*., 2006). Recently, we sequenced mitochondrial genomes of several Triticum species using 454 GS FLX technology. The *T. turgidum* mitochondrial genome is 451,925 bp in size and is almost identical in size to that of T. aestivum genome (452,528 bp) (Ogihara *et al*., 2005). The two genomes showed only 40 single nucleotide polymorphisms (SNPs) as compared with 605 SNPs between *T. aestivum* and *T. tauschii*.

In eukaryotic organisms, nuclear and cytoplasmic (mitochondria and plastids/chloroplast) genomes interact to drive cellular functions and biomass production. These genomes have co-evolved to form specific nuclear-cytoplasmic interactions that are essential to the origin, success, and evolution of diploid and polyploid species (Woodson and Chory, 2008). Plastids are known for their contribution to photosynthesis and storage of biomolecules such as carbophydrates, aminoacids and hormones. Therefore, the appropriate function of plastids in maintaining plant development and physiological process depends on the efficiency of the communication between

nucleus and plastids in the cell (Jung and Chory, 2010). Mitochondria are also essential in the cell by providing the cellular energy through production of ATP needed for daily functions. Coordination of gene expression between the nuclear and mitochondrial genomes is critically important for all eukaryotic cells (Woodson and Chory, 2008). Plant mitochondria are not only vital for cell respiration, but are also involved in many important physiological functions such as oxidative stress (Mittler, 2002), alternative oxidase pathway (McDonald, 2008), programmed cell death (Vianello *et al.*, 2007) and cytoplasmic male sterility (Hanson, 1991). Therefore, the lack of variability of the cytoplasmic genome in polyploid wheat has significantly reduced our ability to develop valuable germplasm for crop improvement.

Although synthetic wheat production has been utilized to increase the spectrum of nuclear genome variability in wheat (Dubcovsky and Dvorak, 2007) wheat breeders have yet to utilize cytoplasmic variability. Alloplasmic lines (lines with alien cytoplasm) are created by replacing the nucleus of one species, through substitution backcrossing, with that of another species (Tsunewaki *et al.*, 1996). Thus, in an alloplasmic line, a new combination of nucleus and cytoplasm is created. A large collection of alloplasmic lines has been created in wheat (Tsunewaki *et al*., 1996; Tsunewaki *et al*., 2002). All alloplasmic lines are derived by using cytoplasmic donors as the female parent and a wheat or a bridging species as the nuclear donor parent followed in certain situations by embryo rescue of the resulting hybrid (Tsunewaki *et al*., 1996). In order to better understand the role of cytoplasmic genomes in wheat development and to increase their variability in modern cultivars, we have embarked on a series of studies to: 1) isolate, characterize and manipulate genes involved in Nuclear mitochondrial (NM) interaction; 2) better understand the influence of cytoplasmic genome by analyzing the vast collection of wheat alloplasmic lines; and 3) determine the extent of mitochondrial genome variability in *Triticeae* and *Aegilops* species in order to generate cytoplasmically more variable, and agronomically more adapted cultivars.

II – Genes involve in nuclear cytoplasmic (NC) compatibility

Over evolutionary time, many mitochondrial genes have been transferred to the nuclear genome, making proper NM interaction essential for cell function (Woodson and Chory, 2008). Changes in nuclear or mitochondrial genomes may interrupt intracellular communication, resulting in nuclear cytoplasmic (NC) incompatibility. The results of NC may include cytoplasmic male sterility (CMS), stunted growth, or seed abortion (Chase, 2007; Mlchalak de Jimenez *et al.*, 2013. Key genes involved in NC compatibility are of critical importance for alloplasmic wheat production in the breeding programs (Maan, 1992a). According to the *scs* hypothesis, each diploid genome has at least one copy of the gene in the nucleus that facilitates NC compatibility (Maan, 1992b). These genes were named species-cytoplasm specific (*scs*) genes by Maan (Maan, 1975) or later as nuclear-cytoplasm compatibility (*Ncc*) genes (Asakura *et al*., 1997). *Ncc* and *scs* genes were found to be located on chromosome 1 group of *T. timopheevii* and *Ae. tauschii* which are also present in the D genome of wheat (Anderson and Maan, 1995; Asakura *et al*., 2000; Maan *et al*., 1999).

Maan (1992b) observed that tetraploid wheat is more sensitive to cytoplasm substitution than hexaploid wheat . The nucleus of hexaploid wheat (*T. aestivum*) was fully compatible with the cytoplasm of *Ae. longissima* (S1S1; 2n=2x=14) or *Ae. tauschii* but not the nucleus of tetraploid wheat (*T. turgidum*). However, male sterile alloplasmic lines of durum wheat in *Ae. longissima* or *Ae. tauschii* cytoplasm could be viable by transferring the whole or part of chromosome 1A from *T.timopheevi* or chromosome 1D from *T. aestivum* (Asakura *et al*., 1997; Asakura *et al*., 2000; Hossain *et al*., 2004b; Maan, 1992b). The *scs* genes originating from *T. timopheevii* Chromosome 1A and *T. aestivum* chromosome 1D designated as scs^{ti} and scs^{ae}, respectively in the durum wheat background. The positions of these genes were mapped in the alloplasmic lines of durum wheat having *Ae. longissima* cytoplasm or simply (lo) durum line using genetic mapping (Simons

et al., 2003) and radiation hybrid mapping (Hossain et al., 2004a). The two mapping strategies have been further implemented to identify potential candidate genes. Recently, by using the radiation hybrid mapping and designing gene based markers with the help of synteny between wheat, rice and *Brachypodium* the location of *scs* locus could be narrowed to a 1.1 Mb segment (MIchalak de Jimenez *et al*., 2013). The genetic mapping population also increased to 5,932 lines facilitated the fine mapping of the region on 1A for *scs*^{ti} (Ghavami *et al.*, 2010). Our results show that *scs*^{ti} and *scs*^{ae} are homoeoalleles (Fig. 2).

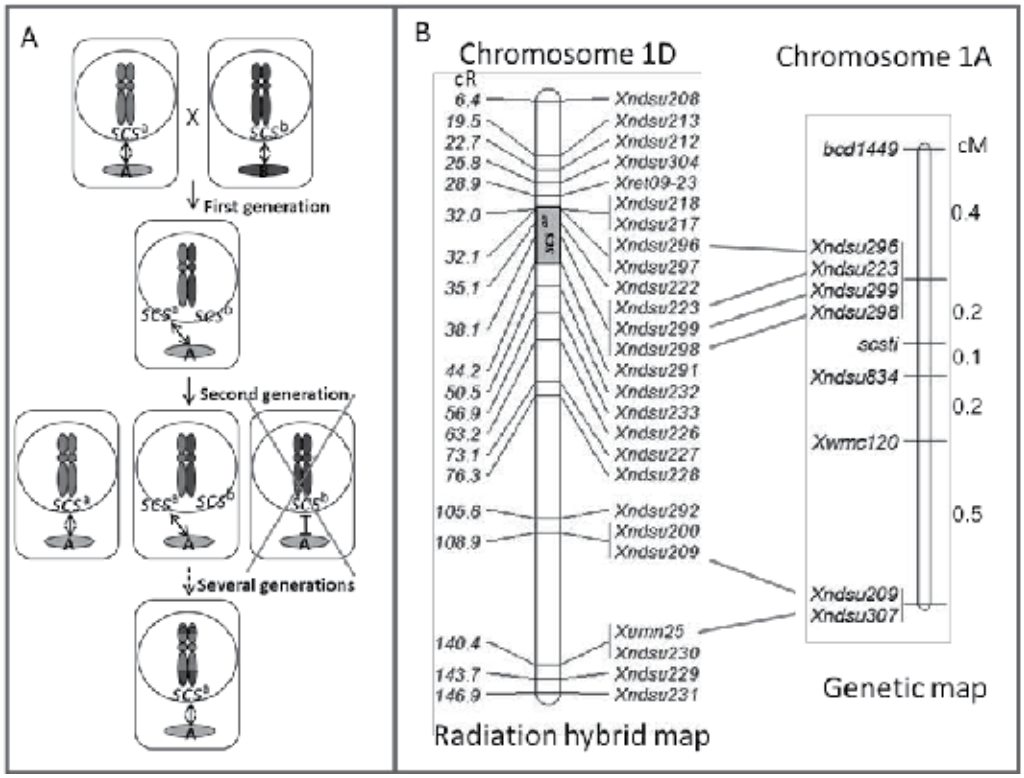

Figure 2. The hypothetical action of the *scs* gene during self- pollinated plant evolution (A) and the location of the *scs* homoeoalleles on chromome 1A and 1D mapped in an alloplasmic line of *T. turgidum* with *Ae. longissima* cytoplasm. The *scs* gene facilitates nuclear-cytoplasmic compatibility and during the evolution needs to be preserved. The *scs*^{ae} which can restore the compatibility between the *T. turgidum* nucleus and the *Ae. longissima* cytoplasm, was mapped to the long arm of chromosome 1D via radiation hybrid mapping. The genetic map also revealed the location of the *scs*^{ti} (derived from *T. timopheevii*) to almost the same location on chromosome 1A (B). For more information regarding the *scs* gene and marker sequences, see De Jimenez *et al*. (2013).

The homoeologous relationship between *Ncc-tmp1A* (from *T. timopheevii* 1A chromosome) and *Ncc-tmp1G* (from *T. timopheevii* 1G chromosome) has been confirmed before (Asakura *et al.*, 2000). It is very likely that common wheat carries three different *scs* gene homoeoalleles present in A, B and D genomes; however, only the *scs* on chromosome 1B is responsible for the NC compatibility, since both the cytoplasm and the B genome originated from *Ae. speltoides* (Fig. 1). The other *scs* gene homoeoalleles on chromosomes 1A and 1D of *T. aestivum* ensure NC compatibility when combining a nucleus of common wheat with different species carrying other plasmon types. Consequently, *T. aestivum* is compatible with *Ae. tauschii* as well as *T. urartu* (Tsunewaki, 1980). The presence of the three different *scs* gene homoeoalleles in wheat is

explained by the fact that *T. aestivum* is more compatible in regards to cytoplasm substitution than durum wheat, which has only two different *scs* gene homoeoalleles. This hypothesis could also explain why it is not possible to produce alloplasmic durum lines with the *Ae. tauschii* cytoplasm, whereas this was not an issue with nuclear genome of common wheat. Cloning of the *scs* gene could facilitate development of further alloplasmic line with durum wheat nucleus with additional species in *Triticum-Aegilops* tribe that are thus far have failed.

III – Analyzing the mitochondrial genomes from the bread wheat ancestors

In most plant species including wheat, mitochondrial DNA (mtDNA) is transmitted to the progeny maternally. However, minor paternal leakage has been observed in some cases, especially in alloplasmic lines of wheat (Tsukamoto *et al*., 2000). Although size of the wheat mitochondrial genome (mt genome) is less than 0.5 Mb (Ogihara *et al.*, 2005), sequencing mitochondrial genome is difficult due to the presence of multiple copies of mt genome in the cell having different rearrangements due to recombination (Burger *et al*., 2003). Most of the previous works on mt genome variation among *Triticeae-Agilops* species were based on restriction fragment length polymorphism (RFLP) analysis (Skuza *et al*., 2007; Wang *et al*., 2000) or PCR based markers (Wang *et al*., 1997).

The full-length sequence of wheat (*T. aestivum* cv. Chinese Spring) mt genome became available in 2005 (Ogihara *et al*., 2005). Since then, additions to the mt genome are limited to a single other wheat cultivar (*T. aestivum* cv. Chinese Yumai;) (Cui *et al*., 2009) and an alloplasmic line of wheat with *Ae. kotschyi* (Liu *et al*., 2011) cytoplasm. The sequence of the Chinese Yumai cultivar was almost identical to the Chinese Spring with a few single nucleotide polymorphisms (SNPs) in non-coding regions (Cui *et al*., 2009). The size of the alloplasmic mtDNA originating from *Ae. kotschi* was larger than *T. aestivum* (647 kb compared with 452 kb) and there were differences in gene structure and significant changes in non-coding regions of the genome (Liu *et al.*, 2011). The mt genome sequence and structure of wheat ancestors have not been reported. Recently, we sequenced the mt genome of *T. turgidum* var. durum and *Ae. tauschii* using the 454 GS FLX sequencing technology to gain insight into the variation and evolutionary changes that have occured in *Triticum-Aegilops* species (unpublished data). All genes previously described in *T. aestivum* mt genome (Ogihara *et al*., 2005) were present in both species. However, major gene differences in atp6, nad6, nad9, rps19-p, cob were found between *Ae. tauschii* and the other two *Triticum* species. Only five SNPs were identified in the gene space, and 40 SNPs in total between the two *Triticum* species. When mt genome of *Ae. tauschii* was compared with *T. aestivum*, 27 SNPs were found in gene space and 679 SNPs in total. Comparison of gene order showed multiple rearrangements between diploid *Ae. tauschii* and tetraploid and hexaploid wheat (Fig. 3). An alloplasmic line of durum wheat carrying the cytoplasm of *Ae. longissima* [(lo) durum line] was also sequenced and compared to the sequence of its parents. The mt genome of the alloplasmic line was significantly different from its maternal parent *Ae. longissima* , indicating accelerated evolutionary changes as a possible result of nuclear genome substitution. Accelerated evolution in mt genome of alloplasmic lines from other species (Allen *et al.*, 2007; Bentolila and Stefanov, 2012) emphasizes the importance of alloplasmic lines for enhancing the variation of cytoplasmic genomes that exist in the nature. The amount of changes observed in mitochondrial gene structure of *Ae. tauschii* as compared with *T. turgidum* may explain why the production of this alloplasmic condition failed in durum wheat as it likely interrupts the NM interaction.

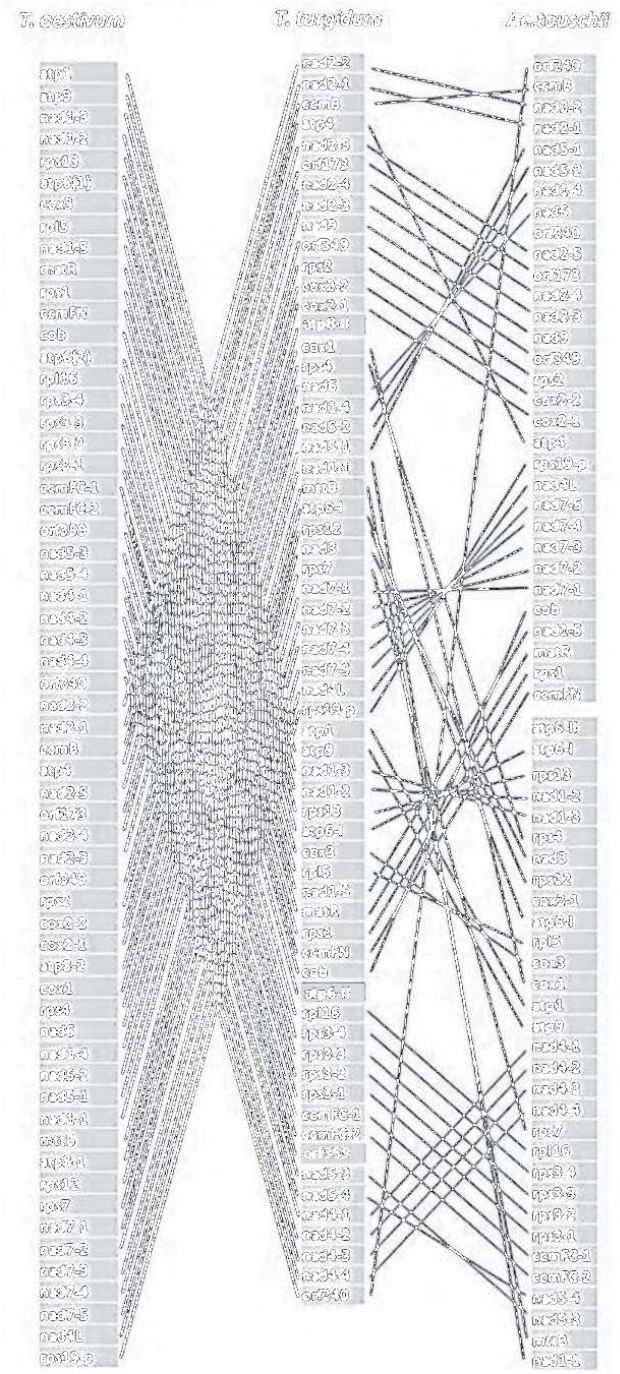

Figure 3. The arrangement of genes in the mitochondrial genome of *T. aestivum*, *T. turgidum* and *Ae. tauschii* indicating various rearrangements. The increased amount of rearrangements observed between *T. turgidum* and *Ae.tauschii* as compared with *T. aestivum* is not surprising considering the evolutionary distance.

IV The influence of cytoplasmic genome on wheat cultivar performance

Effect of cytoplasm on several morphological traits were studied in alloplasmic lines of wheat. (Tsunewaki *et al*., 2002) could classify the forty-six plasmons from *Triticum-Aegilops* species into 17 distinct groups based on their effects on 21 wheat characters. The classification based on phenotypic effects was in agreement with the plasmon genotyping, based on RFLP analysis (Wang *et al*., 2000). Therefore, it can be concluded that the diversity in cytoplasmic genomes is mirrored by diversity in the phenotype of the plants. Plasmon changes affected the number of selfed seed (male fertility), in contrast to female sterility, which was not affected by cytoplasm exchange. Wang's paper illustrates the significant effect on all studied characters, indicating the indispensable role that the mitochondria and chloroplast genomes have in plant development.

Alloplasmic condition in wheat has also proven valuable in improving plant responses to biotic and abiotic stresses (Hou *et al*., 2000; Klimov *et al*., 2005; Liu *et al.*, 2002). A number of these alloplasmic lines also show improved vigor and higher yield relative to parental controls (Tsunewaki *et al*., 2002). We analyzed differential responses of various alloplasmic lines to wheat foliar pathogens *Puccinia triticina* (*Pt*, leaf rust) and *Pyrenophora tritici-repentis* (*Ptr*, tan spot), both major disease problems worldwide. In this study some alloplasmic lines of tetraploid durum wheat (*T. turgidum*) 56-1, and hexaploid wheat (*T. aestivum*) cultivars 'Chris' and 'Selkirk' were tested for disease response to *Ptr* isolates BR15 and Pti2 (Table 1). The experiment was conducted with multiple replications under conditions that promote disease growth (unpublished data). The alloplasmic lines of the same *T. aestivum* cultivar also showed different responses to leaf rust. The *T. dicoccoides* cytoplasm confers resistance to tan spot, making it a candidate source for cytoplasmic substitution in both hexaploid and tetraploid wheat.

Table1. Responses of alloplasmic lines of *T. aestivum* cv. Selkirk, *T. aestivum* cv. Chris and *T. turgidum* var. durum line 56-1 to two different isolates of *Pyrenophora tritici-repentis* as compared with their euplasmic donors.

Cytoplasm	Nucleous	BR15 isolate	Pti2 isolate
Original (euplasmic)	Se kirk	Moderately susceptible	Resistant
Ae. cylindrica	Se kirk	Susceptible	NSD
Ae. mutica	Se kirk	Resistant	NSD
T. dicoccoides	Se kirk	Resistant	NSD
Ae. bicornis	Se kirk	Resistant	NSD
Original (euplasmic)	Chris	Moderately susceptible	Moderately susceptible
Ae. crassa	Chris	Susceptible	NSD
Ae. variabilis	Chris	Resistant	Resistant
Ae. heldreichii	Chris	Resistant	Resistant
Ae. squarrosa	Chris	Resistant	NSD
Original (euplasmic)	56-1	Susceptible	Moderately susceptible
Ae. longissima	56-1	NSD	Resistant
Ae. sharonensis	56-1	Resistant	NSD
Ae. variabilis	56-1	Resistant	Suscept ble
T. dicoccoides	56-1	Resistant	Resistant

NSD=Not significantly different

The importance of cytoplasm effect on resistance to fungal diseases is not new, and is well established (Mullaney, 1981; Voluevich and Buloychik, 1992). Wu *et. al.* (1998b) found *Ae. ventricosa* cytoplasm substitution in wheat cultivars delivers strong and stable resistance to alloplasmic wheat cultivars against wheat scab. Durum wheat breeding programs lack good wheat scab resistance sources (Buerstmayr *et al*., 2009).

Cytoplasmic substitution can be an alternative approach for enhancing durum wheat germplasm in this regard.

Many alloplasmic lines exhibit prolonged plant life span and delayed flowering (Tsunewaki *et al.*, 2002). However, there are some NC combinations that combine earliness with large ears (Wu *et al*., 1998a). This raises the possibility of using alloplasmic lines to improve yield. We examined a number of alloplasmic lines of hexaploid wheat cultivars 'Selkirk' and 'Chris' with *Ae. mutica* and *Ae. cylindrica* cytoplasm along with their euplasmic lines and also their hybrid progeny (Selkirk×Chris and Chris×Selkirk) for dry matter weight (unpublished data). Maternal cytoplasm (MC), nuclear-maternal cytoplasm (N×MC) interaction and maternal cytoplasm-paternal cytoplasm (MC×PC) each show significant effect on dry matter weight. Therefore, not only cytoplasm itself is important but also the proper combination with the nucleus makes a significant difference in improving certain characters (Table 2).

Table 2. Analysis of variance for organelle effect on dry mater weight in alloplasmic lines of wheat that carry cytoplasms of *Ae. mutica* and *Ae. cylindrica.*

Effects	DF	Mean Square	F Value	Pr > F
Replication (Rep)	9	206.45274	0.65	0.5926
Nucleus N	2	878.80567	2.79	0.0883
N×Rep	18	315.40490	1.27	0.2549
Maternal cytoplasm (MC)	1	13058.89861	120.21	<.0001
N×MC	2	1188.82826	10.94	0.0003
N×MC×Rep	27	108.62990	0.44	0.9877
Paternal cytoplasm (PC)	1	83.56247	0.34	0.5652
N×PC	2	9.84394	0.04	0.9612
MC×PC	1	4680.47174	18.81	<.0001
N×MC×PC	2	5991.62809	24.08	<.0001

V – Conclusions

Different studies conducted during the last fifty years have shown that the mitochondrial genome in wheat can be changed by nuclear genome substitution, creating new variations that can be exploited for germplasm enhancement and crop improvement. The advantage of these lines as a source for biotic and abiotic stress tolerance is that their integration into a cultivar improvement program is relatively simple for it merely requires their use as female in a backcrossing scheme. This method eliminates the need for making large, bi/multi-parental populations and recurrent selection in the breeding program. The most difficult aspect of this strategy is to establish an array of alloplasmic lines in improved backgrounds of the modern durum and bread wheat cultivars. Once established, the alloplasmic line of interest can be used in a recurrent backcrossing scheme to develop additional cultivars (Wu *et al*., 1998). Xiaoshan2134 is the only alloplasmic wheat cultivar released in China, and had 20% increase in yield over the control checks in testing over 1991 to 1996 (Wu *et al*., 1998a). In conclusion, using alloplasmic wheat may be an efficient alternative approach in plant breeding that justifies more attention.

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The progeny from the [(*T. turgidum* X *Dasypyrum villosum*) amphiploid X *Triticum aestivum*] hybridization is an effective source of new durum wheat inbred lines

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Abstract. The potential of recombination and chromosome elimination in progenies from homoploid hybridization between an amphiploid $A^dA^dB^dB^dVV$ and *Triticum aestivum* $A^aA^aB^aB^aDD$ leading to novel durum wheat inbred lines, remains largely unknown. Here, we report the results of homoploid hybridization performed among three $A^dA^dB^dB^dVV$ amphiploids ('M × V', 'C × V', 'Cr × V_B' obtained, after chromosome doubling of the hybrids from crossing *Dasypyrum villosum* (*Dv)* to the *T. turgidum* ssp *durum* cvs 'Modoc', 'Capeiti'and 'Creso', respectively), and five $A^aA^aB^aB^aDD$ bread wheat varieties ('Agadir', 'Chinese Spring' ('CS'), 'Provinciale', 'Sagittario', and 'Salgemma') and one inbred line ('41-3').
The average floret fertility upon controlled hybridization was 0.55 (1 caryopsis with F_1 embryo every 2 pollinated florets). After selfing, the F_1 plants produced caryopses with F_2 embryo in 40% of the florets. The chromosome number of the F_2 seedlings ranged from 2n=28 to 2n=42. The expected proportion of viable F_2 seedlings with 2n=28 was 3.72×10^{-9} while the observed proportion (0.065) was about 7 orders of magnitude higher. The observed frequency of the F_2 seedlings with chromosome number 2n>42 was below 0.03, which was in line with the expectation that F_2 zygotes with 2n>42 had a very low viability. The expected proportion of such zygotes was 0.57 determining an expected F_1 floret fertility of 43%, amazingly close to the observed average floret fertility of the F_1 plants. Seven new durum wheat inbred lines have been derived from the 'M × V' × 'CS' hybridization, two of them ('5-04' and '13-04') showing satisfactory agronomic and grain quality traits.

Keywords. Intergeneric hybridization – Breeding methods – Grain yield – Grain quality – Amphiploids – *Dasypyrum villosum* –*Triticum turgidum* L. ssp *durum.*

La descendance de l'hybridation [(T. turgidum X Dasypyrum villosum) amphiploïde X Triticum aestivum] est une source efficace de nouvelles lignées consanguines de blé dur

Résumé. *Le potentiel de recombinaison et d'élimination des chromosomes chez les descendants d'une hybridation homoploïde entre un amphiploïde AdAdBdBdVV et Triticum aestivum AaAaBaBaDD, conduisant à de nouvelles lignées de blé dur consanguines, reste encore largement inconnu. Nous allons illustrer les résultats d'une hybridation homoploïde réalisée avec trois amphiploïdes AdAdBdBdVV ('M x V', 'C × V', 'Cr × VB' issus du doublement des chromosomes des hybrides du croisement Dasypyrum villosum (Dv) et T. turgidum ssp durum cvs Modoc, 'Capeiti' et Creso', respectivement), et cinq variétés de blé tendre AaAaBaBaDD ('Agadir', 'Chinese Spring' ('CS'), 'Provinciale', 'Sagittario', et 'Salgemma') et une lignée consanguine ('41-3').*

Le fertilité moyenne des épillets lors de l'hybridation contrôlée était de 0,55 (1 caryopse avec un embryon F1 toutes les 2 épillets pollinisés). Après autofécondation, les plantes F1 ont produit des caryopses avec des embryons F2 dans 40% des épillets. Le nombre de chromosomes des semis F2 variait de 2n = 28 à 2n = 42. La proportion attendue de semis F2 viables avec 2n = 28 était de 3,72 × 10-9 tandis que la proportion observée (0,065) était environ sept fois plus élevée. La fréquence observée des semis F2 avec un nombre de chromosomes 2n> 42 était inférieure à 0,03, confirmant l'hypothèse avancée selon laquelle les zygotes F2 à 2n> 42 avaient une très faible viabilité. La proportion attendue de ces zygotes est de 0,57 et elle détermine une fertilité attendue des épillets F1 de 43%, étonnamment proche de la fertilité moyenne des

épillets observée chez les plantes F1. Sept nouvelles lignées consanguines de blé dur ont été obtenues par l'hybridation 'M x V' × 'CS' et, deux d'entre elles («5-04» et '13 -04 ') ont montré des caractères agronomiques et de qualité du grain satisfaisants.

Mots-clés. *Hybridation intergénérique – Méthodes de sélection – Rendement en grain – Qualité du grain – Amphiploïdes – Dasypyrum villosum – Triticum turgidum L. ssp durum.*

I – Introduction

In most regions where agriculture began, primary crops such as wheat, were domesticated only once or very few times (Blumler 1998) starting from local wild gene-pool. In the Fertile Crescent area, the A genomes of the diploid species *T*. *urartu* (Dvorak *et al*. 1993) in combination with a species that belonged to the lineage of the current wild wheat species, *Aegilops speltoides* (SS genome), initiated the evolution of the tetraploid AABB and AAGG genome species less than 0.5 million years ago (Matsuoka, 2011). Harlan and Zohary (1966) suggested that a large-seeded race of wild emmer wheat (*T. dicoccoides*), from the vicinity of the Upper Jordan Valley, was the likely progenitor of cultivated emmer.

Population genetic studies based on molecular data, indicated that also the northern populations of the Fertile Crescent area had an important role in the domestication of emmer wheat, although evidence for the site of domestication remains inconclusive (Matsuoka, 2011). The non-brittle rachis mutant phenotype had a role in the domestication of the hulled emmer wheat as well as the genotypic change from *qqTgTg* to *QQtgtg* which was essential for the emergence of the free-threshing phenotype in tetraploid wheats. Free-threshing durums derived from domesticated hulled emmer wheats migrated northeastward in association with the spread of agriculture across and beyond the Fertile Crescent region. Kihara (1944), McFadden and Sears (1946), and Kihara and Lilienfeld (1949) evidenced as the spontaneous hybridization between individuals of the populations of the hulled tetraploid wheat with those of the sympatric populations of the wild diploid species *T. tauschii*, the donor of the D genome, gave rise to the hexaploid wheat *T. aestivum*. However, Dvorak *et al* (2012) proposed that the tetraploid parent of hexaploid wheat was not hulled emmer but the free-threshing form of tetraploid wheat. In Armenia and the south west coastal area of the Caspian Sea and a corridor between the two areas, the "strangulata" genepool of *T. tauschii* hybridized with the free-threshing tetraploid and fertile hexaploid amphiploids were produced by self-pollination of the triploid hybrids (Dvorak *et al* 1998a) due to high production of unreduced gametes (Kihara *et al.* 1950). Once a single free-threshing amphiploid was established, alleles contributed by subsequent intercrossing with hulled/spelt hexaploids from wild hulled tetraploids and *T. tauschii* hybridization, were particularly disadvantaged in the fields of free-threshing wheat and were lost because their adhering glumes would tend to eliminate them during threshing (Dvorak *et al.* 1998b).

This brief history of wheat domestication indicated that: (a) because the majority of accessions of ancestral hulled emmer wheat species were not involved in free-threshing speciation, many of their unique genes may not be present in the released *Td* varieties (Reif *et al.* 2005; Warburton *et al,* 2006); (b) free-threshing speciation caused a genetic bottleneck for adaptive traits that hinder resilience of the current wheat germplasm to pressures from global warming; (c) the differences in ploidy levels between *Td* and *Ta*, may have caused divergence in gene expression and gene evolution, especially for quantitative trait loci (QTL) in the AB genomes of tetraploids and hexaploid wheat species (Zhang *et al*, 2012); (d) under cultivation, the AB genomes of the restricted gene-pool of free-threshing tetraploid and hexaploid wheats evolved independently but followed a common domestication path: new alleles were generated by mutation and novel allele combinations formed through recombination which were selected by early farmers, resulting in landraces adapted to specific local climatic conditions.

Since the beginning of the domestication of the free-threshing durum and bread wheat in the eastern Mediterranean region (Feldman and Kislev 2007, Luo *et al*. 2007), the crop varieties were obtained from shuffling and selecting the genes inherited from the restricted number of free-threshing landraces that moved along the farmers while agriculture gradually diffused. During the last century the traditional landraces were continually replaced by modern wheat elite cultivars with a dual result of erosion of wheat genetic resources (van de Wouw *et al.* 2009) and a further reduction of genetic diversity in the cultivated gene pools. This exposed wheat farmers to the risk of yield reduction due to epidemics and vulnerability to environmental changes and the effect of global warming.

Different approaches are being pursued to introgress new genes in the cultivated durum wheat gene-pool to enlarge the genetic diversity necessary for further adaptations and yield increase.

One approach is the hybridization of the tetraploid durum wheat [*Triticum turgidum* L. spp*. durum* (Desf.) Husn. (=*Td*); chromosome constitution $A^dA^dB^dB^d$; 2n=4x=28] with the hexaploid bread wheats [*T. aestivum* L.) (=*Ta*); chromosome constitution $A^aA^aB^aB^aDD$; 2n=6x=42]. In this case, it is expected that (i) the genetic enhancement of *Td* occur by recombining the shared, but evolutionary divergent, $A^aA^dB^aB^d$ tetraploid chromosome complement, (ii) transfer the desirable D genome loci into durum (Boggini *et al.*, 2000), and (iii) the loss of the majority of the D chromatin. Kihara (1982) observed that the pentaploid F_1 plants from *Ta* × *Td*, contained 35 chromosomes consisting of 14 bivalents and seven univalents. In successive generations, plants divided into an 'increasing group', which included plants that returned to the hexaploid state and a 'declining group', which lost all D genome chromosomes, resulting in a tetraploid state. Recombination events after durum × bread wheat hybridization and their impacts on the selection and performance of new durums are well documented (Gilbert 2000, Wang *et al.* 2005, Lanning *et al.* 2008).

Another approach point to unlock the genetic variation concealed in the AB genome of bread wheat was coupled to recombination with the V genome of *Dasypyrum villosum* (*Dv*). This methodology may provide the necessary novel allele combinations for durum wheat trait enhancement and is based on the use of *T. turgidum* ssp *durum* × *Dasypyrum villosum (Dv)* amphiploid (genomes $A^dA^dB^dB^dVV$) instead of a *Td* parent, in the cross to *Ta* (De Pace *et al.* 2011a). The additional expectation from this method was the genetic enhancement of *Td* by the potential transfer of desirable V genome loci into *Td* genome complement. Genes from the V chromosomes have already been demonstrated to contribute to the improvement of grain yield and grain quality performance when introgressed in the wheat genomes (De Pace *et al.* 2011b).

The main objective of this study was the production of a set of progenies from the homoploid 'A^dB^dV-amphiploid' × '*Ta'* hybridization in order to assess (i) the average floret fertility of the parental amphiploid upon controlled hybridization with *Ta* pollen, (ii) the average fertility of the florets of the F_1 plants and the chromosome number of the F_2 seedlings, (iii) the expected and observed proportion of viable F_2 seedlings with 2n=28, (iv) the proportion of plants of the 'A^dB^dV-aphiploid' × '*Ta'* progeny that 'declined' to the durum chromosome number, and (v) the field performance of the derived new durums containing the $A^aA^dB^aB^d$ genomes and putative introgressed D or V genome loci.

II – Material and methods

1. Material

Three $A^dA^dB^dB^dVV$ amphiploids, 'M × V', 'C × V', and 'Cr × V_B' were obtained after crossing *Dv* to the *T. turgidum* ssp *durum* cvs 'Modoc', 'Capeiti' and 'Creso', respectively, followed by chromosome doubling after colchicine treatment of the seedlings from the F_1 embryos cultured *in vitro* ('M × V' amphiploid; Jan *et al.* 1986) or as a consequence of the union of unreduced gametes on the

untreated F_1 plants from normal and rare caryopses developed in the spike of the durum wheat female parent after the cross pollination with *Dv* pollen ('C × V' and 'Cr × V_B' amphiploids; De Pace et al. 2003). A multi-hybridization experiment was conducted in the last ten years among those amphiploids and *Triticum aestivum* $A^aA^aB^aB^aDD$ wheat varieties 'Agadir', 'Chinese Spring' ('CS'), 'Provinciale', 'Sagittario', and 'Salgemma', and the inbred line '41-3'. A total of 9 $A^aA^dB^aB^dDV$ F_1 progenies were produced (Table 1 A). Seven new durum wheat lines ('1-04', '5-04', '13-04', '1/07a', '1/07b', '2/07', and '3/07'), were selected and tested in the field.

Table 1. Cross-combinations among *T. aestivum* entries and three $A^dA^dB^dB^dVV$ amphiploids, and floret fertility expressed as proportion of the emasculated florets that produced caryopses with hybrid embryo.

Female parent		$A^aA^aB^aB^aVDD$ *T. aestivum* (Male parent)					
		'Agadir'	**'CS'**	**'Provinciale'**	**'Salgemma'**	**'Sagittario'**	**'41-3'**
(A) Cross-combination							
$A^dA^dB^dB^dVV$ amphiploid	'M × V'		X			X	
	'C × V'	X					x
	'Cr × V_B'		X	X	X	X	
(B) Proportion of the emasculated florets that produced caryopses with hybrid embryo							
$A^dA^dB^dB^dVV$ amphiploid	'M × V'		0.49			0.38	
	'C × V'	0.11					0.04
	'Cr × V_B'		0.55	0.24	0.45	0.03	

2. Methods

A. Root-tip preparation for chromosome counting

Seminal roots were treated with α-bromonaphtalene for 6 hours, fixed in ethanol-glacial acetic acid 3:1 (v / v) and stored at 4°C before enzyme treatment. The root-tips were washed with a citrate buffer (sodium citrate 6 mM and citric acid 4 mM, pH 4.6), for 20 minutes at room temperature under stirring. The root-tips were then treated with a solution of pectinase 6% and cellulase 10% in citrate buffer for 60 to 90 at 37°C, and squashed under a coverslip in a drop of 60% acetic acid. The coverslip was removed by the dry ice method and the preparations were dried overnight at 37°C. The chromosomes were fixed with paraformaldehyde 4%, washed with 2xSSC and 4xSSC/ Tween 20, and stained with a 2% solution of DAPI (4,6-diamidino-2-phenylindole) in McIlvaine buffer pH 7.0.

B. Estimate of the expected chromosome number of the F_2 embryos formed upon self-fertilization of the $A^aA^dB^aB^dDV$ F_1 plant

Considering that during meiosis occurring in florets of the $A^aA^dB^aB^dDV$ F_1 plant, the homologous chromosomes of the A and B genomes pair regularly during prophase I, only the 7 D and 7 V univalents are expected to migrate randomly at one or the other pole during anaphase I. The binomial expectation for the frequency of gamete types can be determined using the formula:

$$P_k = \frac{n!}{k!(n-k)!} p^k q^{(n-k)}$$

where k is the number of D and V univalents that migrate to the same pole at anaphase I, ranging from 0 to 14; n = 14 is the total number of univalents; p=1/2 is the probability that a given univalent is pulled to one pole and q=1/2 is the probability that the same univalent is pulled towards the other pole. The expected probability (P_z) to find each type of F_2 zygote resulting from the random union of one of the possible female gametes (set with probability $P_{k(f)}$) and one of the possible male gametes ($P_{k(m)}$), is $P_z = P_{k(f)} \times P_{k(m)}$ (see Table 2).

Table 2. The expected probability (P_z) of each type of F_2 zygotes resulting from the random union of one of the possible female gametes (P_k(f)) and one of the possible male gametes (P_k(m)).

k (number of D and V unit alleles)	Binomial coeff.	Chrom. Number in gametes	Male →	n = 14+0	n = 14+1	n = 14+2	n = 14+3	n = 14+4	n = 14+5	n = 14+6	n = 14+7	n = 14+8	n = 14+9	n = 14+10	n = 14+11	n = 14+12	n = 14+13	n = 14+14
		Female ↓	P_k	0.00000	0.00080	0.00555	0.02222	0.06110	0.12219	0.18329	0.20947	0.18329	0.12219	0.06110	0.02222	0.00555	0.00080	0.00000
0	1	n = 14+0	0.00000	0.0000000037	0.00000035	0.00000034	0.000001	0.000004	0.000007	0.000011	0.000013	0.000011	0.000007	0.000004	0.000001	0.000000	0.000000	0.000000
1	14	n – 14+1	0.00085	0.0000005715	0.0000007	0.0000045	0.000019	0.000052	0.000104	0.000157	0.000175	0.000157	0.000104	0.000052	0.000019	0.000005	0.000001	0.000000
2	91	n 14+2	0.00555	0.0000033900	0.0000475	0.0000305	0.000123	0.000339	0.000679	0.001010	0.001163	0.001010	0.000679	0.000339	0.000123	0.000031	0.000005	0.000000
3	364	n = 14+3	0.02222	0.0000135501	0.0001838	0.0012340	0.004941	0.001357	0.002715	0.004072	0.004654	0.004072	0.002715	0.001357	0.000494	0.000123	0.000019	0.000001
4	1001	n 14+4	0.06110	0.0000372902	0.0005221	0.0033934	0.013587	0.003733	0.007469	0.011198	0.012798	0.011198	0.007469	0.003733	0.001357	0.000339	0.000052	0.000004
5	2002	n = 14+5	0.12219	0.0000746803	0.0010441	0.0067858	0.027156	0.007466	0.014931	0.022396	0.025596	0.022396	0.014931	0.007466	0.002715	0.000679	0.000104	0.000007
6	3003	n = 14+6	0.18329	0.0001119705	0.0015662	0.0101787	0.040712	0.011198	0.022396	0.033594	0.038394	0.033594	0.022396	0.011198	0.004072	0.001018	0.000157	0.000011
7	3432	n 14+7	0.20947	0.0001279520	0.0017899	0.0116345	0.046528	0.012798	0.025596	0.038394	0.043879	0.038394	0.025596	0.012798	0.004654	0.001163	0.000179	0.000013
8	3003	n 14+8	0.18329	0.0001119705	0.0015662	0.0101787	0.040712	0.011198	0.022396	0.033594	0.038394	0.033594	0.022396	0.011198	0.004072	0.001018	0.000157	0.000011
9	2002	n = 14+9	0.12219	0.0000746803	0.0010441	0.0067858	0.027156	0.007466	0.014931	0.022396	0.025596	0.022396	0.014931	0.007466	0.002715	0.000679	0.000104	0.000007
10	1001	n 14+10	0.06110	0.0000372902	0.0005221	0.0033934	0.013587	0.003733	0.007469	0.011198	0.012798	0.011198	0.007469	0.003733	0.001357	0.000339	0.000052	0.000004
11	364	n = 14+11	0.02222	0.0000135501	0.0001838	0.0012340	0.004941	0.001357	0.002715	0.004072	0.004654	0.004072	0.002715	0.001357	0.000494	0.000123	0.000019	0.000001
12	91	n – 14+12	0.00555	0.0000033900	0.0000475	0.0003085	0.001235	0.000339	0.000679	0.001010	0.001163	0.001010	0.000679	0.000339	0.000123	0.000031	0.000005	0.000000
13	14	n = 14+13	0.00085	0.0000005715	0.0000073	0.0000475	0.000019	0.000052	0.000104	0.000157	0.000179	0.000157	0.000104	0.000052	0.000019	0.000005	0.000001	0.000000
14	1	n = 14+14	0.00000	0.0000000373	0.0000005	0.0000034	0.000001	0.000004	0.000007	0.000011	0.000013	0.000011	0.000007	0.000004	0.000001	0.000000	0.000000	0.000000
			1.0000															

Expected P_z for F_2 zygotes with 2n > 42	0.57	Expected P_z for F_2 zygotes with 2n = 28	3.7E-09
Expected P_z for F_2 zygotes with 2n =< 42	0.43	Observed P_z for F_2 zygotes with 2n = 28	0.065
		Odds in favour of 2n = 28 zygotes	1.7E+07

C. Field performance of the new durum wheat lines

The three new durum wheat lines '1-04', '5-04', and '13-04' were compared in 1 × 1 m plots arranged in a randomized block field design replicated twice at the experimental field of University of Tuscia (Viterbo) and CRA-SCV (S. Angelo Lodigiano, Lodi) in 2006 and 2007. The 'Modoc', 'M × V', and 'CS' parents and the durum wheat cultivar 'Creso' and line '4.5.1' were used as controls. Plants were evaluated for heading time (days from Jan. 1st), culm length (cm), response to air-born inoculum of *Blumeria graminis* f.sp. *tritici* (the causal agent of powdery mildew) and *Puccinia triticina* (leaf rust) (symptoms were scored as percentage of the leaf area covered by pustules). Grain quality traits (hardness, protein content, sodium-dodecyl-sulfate sedimentation volume, and specific sedimentation volume) were evaluated using the methodologies reported in Vaccino *et al.* (2010).

D. Technological quality analyses

The semolina required for the technological quality analyses of the seven new durum lines and controls grown at the Experimental farm of University of Tuscia, Viterbo, in 2011, was prepared using a Chopin CD2 laboratory mill and Chopin Semolina Purifier (Chopin Technologies, Villeneuve-la-Garenne, France). The yellow index (Minolta b*) of the durum wheat varieties and breeding lines was recorded using a Minolta CR-300 chroma meter (Minolta Camera Co. Ltd., Osaka, Japan).The wet gluten content and the gluten index of each durum wheat sample was determined on the basis of the ICC 158 standard method using a Perten Glutomatic 2200 instrument and a Perten 2015 Centrifuge (Perten Instruments AB, Hägersten, Sweden). Zeleny sedimentation volume was analysed by ICC 116/1 method. Crude protein content was determined by Kjeltec 1035 Analyzer (ICC105/2) from whole grain meal. Samples were analysed for total- (TOT-AX) and water-extractable- arabinoxylane (WE-AX) content with the pentosan method of Douglas (1981). The total amount of mixed-linkage β-glucan was determined using a Megazyme kit (ICC 168, AACC Method 32-23). Amylose and amylopectin content of starch were measured by the Megazyme method which is a modification of a Con A method developed by Yun and Matheson (1990).

E. Data analyses

Descriptive statistics, ANOVA, and Bonferroni's method for multiple comparison tests of the means, were performed using the GenStat 16 ed. (VSN International Ltd) software.

III – Results and discussion

The average floret fertility upon controlled hybridization was 0.55 and ranged from 0.03 ('Sagittario' × 'Cr × V_B') to 0.8 ('Chinese Spring' × 'M × V'). The average spikelet fertility of the F_1 plants was low for the 'Sagittario' × 'Cr x V_B' and '41-3' × 'C × V' hybrids, while it was the highest for the 'Salgemma' × 'Cr x V_B' and 'CS' × 'M × V' hybrids (Table 1B). The largest progenies (number of F_2 caryopses) were obtained from the F_1 'M × V' × 'CS' and 'C × V' × '41.3'.

Homologous pairing and recombination between the A and the B genome chromosomes of durum and bread wheat and the random assortment of the chromosomes of the D and V genomes occurred at first division of meiosis of the F_1 plants. This determined the formation of diads and gametes with constant AB chromosome number (7 $A^{a/d}$ plus 7 $B^{a/d}$) plus various inclusion (from 0 to 14) of the 14 D and V univalents, including the very rare configurations of the euploid parental genomes A^aB^a, A^dB^d, A^aB^d, A^dB^a, A^aB^aV, A^dB^dV, A^aB^dV, A^dB^aV, A^aB^aD, A^dB^dD, A^aB^dD, A^dB^aD, A^aB^aDV, A^dB^dDV, A^aB^dDV, and A^dB^aDV. The expected chromosome number (n) in the gametes ranged from 14 (7 A and 7 B chromosomes) to 28 (7A, 7B and 1 to 14 D and/or V-univalents (Table 2). Fifteen gamete types differing in chromosome number were expected, the variation being attributed to

the number of univalents (k) included in each of them. Their respective frequency was equal to their probability (P_k) of being set in the male or female germline. The expected absolute frequency of the F_2 zygotes formed by the random union of those gametes was estimated by the product (P_z) of the probability of the uniting gametes. The expected frequency of the F_2 embryos with 2n=28 was 3.7×10^{-9}. The chromosome number detected in the root-tip of a sample of 62 F_2 seedlings, ranged from 2n=28 to 2n=42 (Caceres *et al.* 2011). The proportion of the F_2 seedlings displaying 2n=28 ($A^{a/d}A^{a/d}B^{a/d}B^{a/d}$) was examined in the largest ('M × V' × 'CS') of the nine F_1 progenies, and 2n=28 was detected in 4 out of 62 F_2 seedlings, an absolute frequency (0.065) which is about 7 order of magnitude higher then the expected frequency (3.7×10^{-9}). Three additional F_2 seedlings with 2n=28 were found in a further sample of 41 F_2 seedlings from the same hybrid progeny.

The cumulative expected probability of F_2 zygotes with chromosome number 2n ≤ 42 was 0.43 and the cumulative expected probability of F_2 zygotes with 2n > 42 was 0.57 (Table 2). In the F_2 seedlings examined by Caceres *et al.* (2011), the proportion of the F_2 seedlings with chromosome number 2n > 42 was below 0.03, which fitted the expectation that the F_2 zygotes with 2n > 42 had a very low viability. Therefore when the probabilities in Table 2 are converted to frequencies, than 57% of the F_2 zygotes with 2n > 42 are expected to be unviable, causing an F_1 floret fertility of 43%. F_2 caryopses were formed in 609 of the 1522 florets examined in the spikes of the nine F_1 hybrids (Table 3) providing an observed F_1 floret fertility of 0.40, which meant that 60% of the F_1 florets did not set F_2 caryopses and 40% of the F_1 florets formed F_2 caryopses, a proportion of non-fertile vs fertile floret that was amazingly close to the expected proportion under the hypothesis that almost all the zygotes with 2n > 42 were unable to live or develop normally.

Table 3. Floret fertility in F_1 plants from some cross-combinations among *T. aestivum* entries and $A^dA^dB^dB^dVV$ amphiploids.

	'Cr × V_B'			'M × V'			'C × V'		
T. aestivum	**Florets No.**	**Caryo-pses No.**	**Floret fertility**	**Florets No.**	**Cary-opses No.**	**Floret fertility**	**Florets No.**	**Caryo-pses No.**	**Floret fertility**
'Provinciale'	68	16	0.24						
'Salgemma'	62	28	0.45						
'Sagittario'	62	2	0.03	129	39	0.30			
'CS'	54	11	0.20	513	226	0.44			
'Agadir'							72	41	0.57
'41-3'							562	246	0.44
Total	246	57	0.23	642	265	0.41	634	287	0.45

Overal floret fertility 0.40.

Table 4. Chromosome number assessed in F_3 seedling from F_2 plants obtained by crossing the amphiploid 'M x V' and *T. aestivum* cv 'CS' and displaying durum wheat spike and kernel morphology. The karyological events observed by Caceres et al. (2011) in the embryo from which the F_2 mother plant was rised, are also reported.

F_3 seedlings analyzed No.	Chromosome No.	Karyological event*
5	28	None
6	28	None
4	28	None
6	28	None
6	28	A-B Recombination
2	28	None
4	28	A-B Recombination
2	42	None

**Karyological event observed by GISH in the root-tips of the embryo from which the F_2 mother plant was risen.*

Table 5. Analysis of variance for six traits recorded on plants of 3 new durum wheat lines obtained from crossing the amphiploid 'M × V' and 'CS' ('1-04', '5-04', and '13-04') and 5 controls (the parental entries 'Modoc', 'CS', and 'MxV' amphiploid, and the durum wheats cv 'Creso' and inbred line '4.5.1'), grown for two years (2006 and 2007) according to a randomized block field design replicated twice at the Experimental farms of Univ. of Tuscia (Viterbo) and CRA-SCV (S. Angelo Lodigiano, Lodi).

Source of variation	d.f.	Heading time (days from 1st Jan)		Culm length (cm)		Protein content (% dry weight)		Grain Hardness		Sedimentation volume (mL)		Specific Sedim. volume (mL)	
		MS	F prob.	MS	F prob.	MS	F prob.	MS	F prob.	MS	F prob.	MS	F prob.
Line	7	101.4	<.001	2084.3	<.001	54.13	<.001	8598.1	<.001	228.7	<.001	2.2	0.009
Year	1	2334.4	<.001	501.6	0.003	153.28	<.001	682.9	<.001	280.6	<.001	8.4	0.001
Location	1	79.8	0.002	1686.5	<.001	0.01	0.944	425.7	<.001	33.0	0.093	4.6	0.013
Line x Year	7	16.0	0.062	39.6	0.583	1.06	0.755	18.0	0.511	21.3	0.10	0.6	0.558
Line x Loc	7	20.8	0.020	419.6	<.001	2.08	0.348	54.2	0.024	9.3	0.558	0.2	0.918
Year x Loc	1	51.6	0.012	78.0	0.215	6.79	0.059	23.1	0.288	17.1	0.222	2.9	0.046
Line x Year x Loc	7	7.0	0.483	50.8	0.421	2.49	0.238	53.1	0.026	16.1	0.216	0.4	0.80
Residual	32	7.3		48.7		1.78		19.8		11.0		0.7	
Total	63												

The chromosome counting in root tip from the F_3 embryos formed by self-fertilization of the F_2 plants with 2n=28 confirmed the 2n=28 chromosome number, except in one instance where the F_3 progeny was made by a mixture of 2n=28 and 2n=42 embryos (Table 4). In this case, an accidental kernel mixture during threshing of the F_2 spikes cannot be excluded.

The seven F_3 progenies with 2n=28 provided new durum wheat inbred lines that were tested in field trials to ascertain their agronomical and grain technological performance.

The new durum wheat lines '1-04', 5-04' and 13-04' were compared to the parental 'Modoc', 'M × V', and 'CS' entries and to the durums 'Creso' and '4.5.1' for two years and in two locations. It was ascertained that the interactions of the entries with the different yearly and location climatic conditions were not significant (Table 5). There were significant differences among the entries, but the new durum wheat lines were similar, or even better, than the 'Modoc' and 'Creso' durum wheat checks for several of the examined traits (Table 6). In each year and location the line '1-04' expressed high resistance to powdery mildew and leaf rust, while 'M × V' expressed immunity to powdery mildew (due to genes inherited from *Dv*), and 'CS' was resistant to leaf rust. The resistance to powdery mildew in '1-04' can be explained by the introgression of the *Dv* gene for resistance from 'M × V.

Table 6. Multiple comparison tests of the means, performed using Bonferroni's method, for six traits recorded on plants of 3 new durum lines ('1-04', '5-04', and '13-04') and 5 controls (the parental entries 'Modoc', 'Chinese Spring', and 'M × V' amphiploid, and the durum wheats cv 'Creso' and inbred line '4.5.1'). Means are overall years (2006 and 2007) and locations (Experimental farms of Univ. of Tuscia, Viterbo, and CRA-SCV S. Angelo Lodigiano, Lodi) of the trials.

Heading time (days from 1st Jan)		Culm length (cm)		Protein content (% dry weight)		Grain Hardness		Sedimentation Vol. (mL)		Specific Sedim. Vol. (mL)	
123	bc	105.7	b	16.3	b	105.3	b	37.5	cd	2.4	ab
116	a	77.0	a	14.0	a	106.7	b	33.5	bc	2.8	ab
122	bc	73.3	a	14.3	ab	104.4	b	39.0	cd	3.1	b
116	a	76.7	a	14.3	ab	103.9	b	28.8	ab	2.2	ab
118	ab	109.7	b	15.3	ab	32.2	a	42.3	d	3.1	ab
116	a	102.2	b	21.4	c	34.3	a	26.3	a	1.7	a
125	c	81.0	a	13.5	a	102.7	b	37.0	cd	3.2	b
120	ab	69.7	a	13.8	a	100.8	b	37.0	cd	2.9	ab

Significant differences were detected for seven traits related to grain quality recorded on plants of 7 new durum wheat lines, 2 parental checks, and four durum wheat checks (Table 7). The wet gluten content of the lines carrying alien genetic material ranged between 28.8% and 36.5% (Table 8). Two of the lines ('5-04' and '13-04') had significantly higher wet gluten content than 'Creso' and two further lines exceeded the value of 'Modoc'. The gluten structure – measured by gluten index – was excellent (>85) in most lines, one line had good and one further line ('1/07') had below average gluten strength. The yellow index values were very low in the whole experiment (14.0–18.9) in part due to the unfavorable climatic conditions during harvest, however, the yellow index of two lines was even higher than that of the 'Creso' variety. Falling number values were high, while the protein content and the Zeleny sedimentation volume of most of the lines were comparable with that of the durum wheat controls (Table 8).

Table 7. Analysis of variance for seven traits related to grain quality, recorded on plants of 7 new durum wheat lines, 2 parental controls, and four durum wheat controls.

Source of variation	d.f.	Wet gluten content (%)	Gluten index	Yellow Index	Amylose content	β-glucan	Arabinoxylan (Total)	Arabinoxylan (We)
Entry	12	34.4	718.4	22.85	5.5	1.4	116.4	7.0
Residual	13	0.2	5.5	0.09	1.3	0.1	3.6	0.2
Total	25							

Table 8.Multiple comparison tests of the means, performed using Bonferroni's method, for seven traits recorded on plants of 7 new durum wheat lines obtained from crossing the amphiploid 'M × V' x 'CS', the two parental *Triticum* cvs 'Modoc' and 'CS', and four additional *T. turgidum* ssp *durum* cultivars used as controls. The variables were: wet gluten content (WGC), gluten index (GI), yellow index(YI), amylose content(AC), β-glucan content (βGC), Arabinoxylan Tot. content (ATC) arabinoxylan We. content (AWe). Falling number (FN), Zeleny sedimentation test (ZST ml), and protein content (PC%) were evaluated in one sample only.

ENTRY	WGC (%)		Gi		YI		AC		β-GC		ATC		AWe		FN	ZST	PC
'M × V' × 'CS'	$\dot{X}^1$	MC*	$\dot{X}^1$	MC*	$\dot{X}^2$	MC*	$\dot{X}^3$	MC*	$\dot{X}^3$	MC*	$\dot{X}^4$	MC*	$\dot{X}^4$	MC*	$\dot{X}^5$	$\dot{X}^5$	$\dot{X}^5$
'1/07a'	**36.5**	f	42.0	A	**18.9**	f	25.7	ab	4.1	cd	21.2	bcd	**6.2**	de	510	16	**16.1**
'1/07b'	28.8	abc	85.4	C	14.8	bc	26.2	ab	3.2	ab	21.9	cde	**5.8**	Cd	377	17	15.1
'2/07'	30.9	cde	86.2	cd	15.8	de	26.8	b	3.2	A	18.4	B	**6.0**	Cd	371	28	14.7
'3/07'	29.4	bcd	94.2	cde	15.4	cd	27.0	B	3.7	abcd	20.1	bcd	**6.1**	De	390	20	14.0
'1-04'	35.4	f	74.9	B	**18.2**	f	26.5	Ab	4.2	Cd	20.3	bcd	4.6	Ab	676	28	15.8
'5-04'	**32.5**	e	**92.8**	cde	**16.0**	de	25.5	Ab	3.9	abcd	19.2	Bc	4.0	A	**433**	20	**16.1**
'13-04'	**31.9**	e	**86.5**	cd	**16.5**	e	23.8	A	3.6	abcd	21.6	bcd	**5.7**	Cd	**352**	25	**14.4**
Parental check																	
'Modoc'	28.4	ab	96.1	de	13.9	b	25.0	Ab	**4.1**	Cd	21.1	bcd	4.8	B	369	26	14.6
'CS'	42.3	g	45.0	A	7.4	a	23.6	A	**5.4**	E	15.0	A	4.9	B	503	21	16.0
Other durum wheat check																	
'Creso'	30.9	cde	100.0	E	16.2	de	26.9	B	**4.0**	bcd	28.9	G	6.8	Ef	538	23	15.6
'Duilio'	31.4	de	85.6	C	14.8	bc	25.6	Ab	**3.5**	abcd	22.7	De	5.3	Bc	376	27	14.9
'Simeto'	27.1	a	97.2	E	16.0	de	26.8	B	**3.5**	abc	25.1	Ef	7.4	F	572	27	14.6
'4.5.1'	28.3	ab	97.0	E	14.0	b	24.5	Ab	**4.3**	D	28.3	Fg	6.3	De	322	22	14.5
Grand mean	31.8		83.3		15.2		25.7		3.9		21.8		5.7		445	23	15.1

1 Mean of two replicates; 2: Mean of three replicates; 3: Mean of four replicates; 4: Mean of eight replicates; 5: No replicates
** Multiple comparisons test of significance of 91 pair of means using an experiment-wise error rate of 0.05 and a comparison-wise error rate of 0.0006*
Values which are significantly better or differently by chance from either 'Modoc' or the best durum check 'Creso', are highlighted in bold

In order to get some information about the health related properties of the studied genotypes, the amylose content of the starch, the β-glucan content of the seed and the quantity of the total- (TOT-AX) and water-extractable-arabinoxylan (WE-AX) were measured (Table 8). These components contribute to the total dietary fibre content of the wheat. As the results show, none of the lines had significantly higher amylose (23.8-27.0%), β-glucan (3.2-4.2 mg/g) or total-arabinoxylan content (18.4-21.9 mg/g) than the control 'Modoc' or 'Creso', but there was a significant difference in the water extractability of the arabinoxylan. Five of the seven 'M × V' × 'CS' lines had significantly higher WE-AX content (5.7-6.2 mg/g) than the Modoc (4.8 mg/g) control.

Compared to the 'CS' control, all the studied lines had significantly high TOT-AX content. Altogether we can say that health related properties of the new lines were improved through the increased level of the arabinoxylan.

IV – Conclusions

Our results showed large variability among hexaploid genotypes for their ability to produce viable progeny when crossed to 'A^dB^dV-amphiploid', and suggested that 'Chinese spring' wheat may be a good bridge variety for homoploid crosses.

Forty-three percent of the F_1 florets were fertile and 57% were sterile. This last percentage coincided with the proportion of the expected F_2 embryos with 2n > 42 suggesting an association between high chromosome number (>42) and reduced F_2 zygote viability.

The proportion of the F_2 seedlings displaying 2n=28 ($A^{a/d}A^{a/d}B^{a/d}B^{a/d}$) was about 7 order of magnitude higher than the expected frequency (3.7×10^{-9}), indicating that the progeny from the [(*T. turgidum* x *Dasypyrum villosum*) amphiploid x *Triticum aestivum*] hybridization is an effective source of new durum wheat inbred lines where each A and B chromosome is a chimera ($A^{a/d}$ and $B^{a/d}$) of the genes in the A^dB^d genomes of durum wheat and A^aB^a genomes of bread wheat as consequence of homologous pairing and recombination of the A and B chromosome in the 'A^dB^dV-amphiploid' x '*Ta*' F_1.

The agronomic performance of some of the new durums was similar or even better then either wheat parents and in one case ('1-04') there was evidence of the gene transfer for disease resistance from the V chromosome of the parental amphiploid.

The parameters measuring the value of the grain for technologically complex traits of two lines ('5-04' and '13-04') were superior to the parental durum 'Modoc' and very similar to that of 'Creso'.

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Integrated crop solution as new approach to combine genetics and other innovative inputs in wheat varieties development

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Abstract. Syngenta is, a leading company in agribusiness with operations world-wide. Syngenta implemented the concept of “integrated crop solution” years ago in order to give concrete answers to the growers, in terms of availability of complete, innovative, and easy to manage solutions in agricultural practises, to meet the market requirements in term of yield and food safety. For field crops such as wheat, the concept is carried out establishing a link between genetics, breeding, crop protection and agronomical know-how during different stages of wheat varieties development, focusing on the synergy of all combined technical inputs. One of the most interesting example is the development of durum wheat varieties with enhanced tolerance to *Fusarium* head blight, that was screened evaluating the combined synergic response with different fungicide applications on disease level symptoms and mycotoxins content. Other ongoing goals in cereal breeding focus on combining genetics, crop enhancer products, and growth regulators; these different approaches can achieve relevant results in terms of resistance to biotic and abiotic stress acting, for example, on root development and biochemical modification for an improved tolerance to soil and environmental adverse conditions in optimal balance with yield and quality requirements.

Keywords. Crop solution – Agr business – Disease tolerance – Fusarium spp. – Abiotic stress – Growth regulators – Seed care – Wheat varieties.

Solutions intégrées pour les cultures comme nouvelle approche pour combiner la génétique et d’autres moyens innovants dans le développement des variétés de blé

Résumé. *Syngenta est un leader dans le secteur de l’agro-industrie avec ses entreprises répandues dans le monde entier. Syngenta a mis au point le concept de « solution intégrée pour les cultures » il y a quelques années, visant à donner des réponses concrètes aux producteurs, à travers des solutions de pratiques agricoles complètes, innovantes et faciles à gérer, pour répondre aux exigences du marché en termes de rendement et de sécurité alimentaire. Pour les grandes cultures telles que le blé, cette démarche est réalisée en conjuguant génétique, sélection, protection des cultures et savoir-faire agronomique dans les différents stades de développement des variétés de blé, en mettant l’accent sur la synergie de tous les moyens techniques combinés. Un des exemples les plus intéressants est l’obtention de variétés de blé dur chez lesquelles a été améliorée la tolérance à la fusariose de l’épi, évaluée en considérant la réponse synergique combinée, dans le cas de différents traitements fongicides, sur le plan des symptômes de la maladie et de la teneur en mycotoxines. D’autres objectifs fixés pour la sélection des céréales concernent la combinaison multiple de gènes, les additifs des cultures et les régulateurs de croissance ; ces différentes approches peuvent produire des résultats pertinents en termes de résistance aux stress biotiques et abiotiques en intervenant, par exemple, sur le développement des racines et la modification biochimique en vue d’améliorer la tolérance aux sols et aux conditions environnementales défavorables dans un équilibre optimal avec les exigences de rendement et de qualité.*

Mots-clés. *Solution pour les cultures – Agro-industrie – Tolérance aux maladies – Fusarium spp., stress abiotique – Régulateurs de croissance – Protection des semences – Variétés de blé.*

I – Introduction

The concept of integrated crop solution when applied to varietal development of all arable and specialty crops, can be considered as an innovative approach to enlarge the possibility of the use of genetics to meet targets in terms of adaptation and performance.

Syngenta's worldwide expertise in many crops addresses the research and development efforts combining the know-how of its seeds and crop protection corporate departments in finding integrated and scalable solutions to maximize the plant potential of different crop species in balance with sustainability of the practices developed.

One the most relevant example is the diseases tolerance of varieties; the introgression of most complex resistance traits and the associated genetic loci (QTLs) coming from conventional breeding bring relevant benefits for the genetic material in terms of *Fusarium* head blight (FHB) resistance or tolerance. The higher number of QTLs introgressed the greater the resistance to FHB, but the stack of many QTLs also increases negative traits such as low protein quality, low yield, low gluten index, etc.

However, unfortunately, some disease reactions are difficult to reliably score, while others are highly sensitive to the environment; a variety with good resistance in one location may be unacceptably susceptible in another.

For these reasons, by combining tolerance traits, proper fungicide applications, and good agronomic practices in field, it is possible to achieve interesting practical results in terms of crop protection, crop safety, quality, and yield in a wide range of conditions where only the varieties features could not be sufficient to ensure the general crop performance.

Other crop solution in wheat can be identified with the growth regulator (GR) application to further improve the approaches of conventional breeding relating to the development of drought-resistant varieties. These aspects could be referred to the chemically-induced resistance in plants, that in its broadest sense, is well-known and has been studied for a long time both on mono- and di-cotyledoneae species. This induction is responsible for the expression or the overexpression of certain genes that can potentially increase the resistance to biotic factors but also for some abiotic factors such as drought, frost, etc.

Although high yield potential is a main target of most cereal breeding programs, this cannot be always considered compatible, for example, with high levels of drought resistance (DR). The observation and the studies during variety development concerning in-field GR application can rebalance the inverse relationship between yield potential, DR, water-use efficiency, and other relevant genetic traits linked to abiotic stress tolerances.

GRs are normally used at farm level in cereals to reduce the height and to strengthen the stem; thus leading to an increase in resistance to lodging; some of them, as trinexapac-ethyl (Moddus®), when applied at early stage, can improve root growth especially in non-optimal growing conditions with water shortage or reduced nutrient fertilizers, returning traits of "hardiness" in phenotypes potentially suited to high yields.

Is also known that DR can be positively influenced by the radical health care provided by the use of seed treatments and foliar fungicides applications as some triazoles, strobilurins and SDHI fungicides, that combine the effects of disease control with a physiological action on plants by improving dehydration avoidance and osmotic adjustment and preventing the effects of early senescence of leaves due to partial slowing of ethylene biosynthesis.

II – Wheat genetic disease resistance and FHB – mycotoxins management with integrated crop solutions

It's a common understanding that the durum species is more sensitive for certain kind of fungal diseases than other species of *Triticum* genus and all its types of active resistance mode of actions are considered less effective.

Due to the high sensitivity of this species to the diseases and the potential risk of mycotoxins pollution on grains, in certain agricultural settings according to climate, crop rotations, soil tillage, etc, FHB resistance/tolerance is one of the most important targets for durum breeding.

Moreover, as already mentioned, the introduction of specifics QTLs for diseases tolerance within genotypes may be associated with non-positive traits whose phenotypic expression would not lead to a high value of the varieties developed. To modulate all this kind of disadvantages, the combination of resistant varieties with other inputs as chemical control of disease showed successful results.

For this reason many efforts have been directed towards enhancing the effects resistance induction on FHB, not only based on Durum genetics improvement; fungicide applications with active ingredients with proven efficacy and selectivity, if applied at the right time in the field, can reduce with high significance the mycotoxins content induced by the pathogens, even in situations of higher risk, where only the genetic tolerance would not be able to provide an absolute absence of phytosanitary risk.

This risk is universally recognized and it depends in particular, on crop rotation and tillage used, as shown in figure 1.

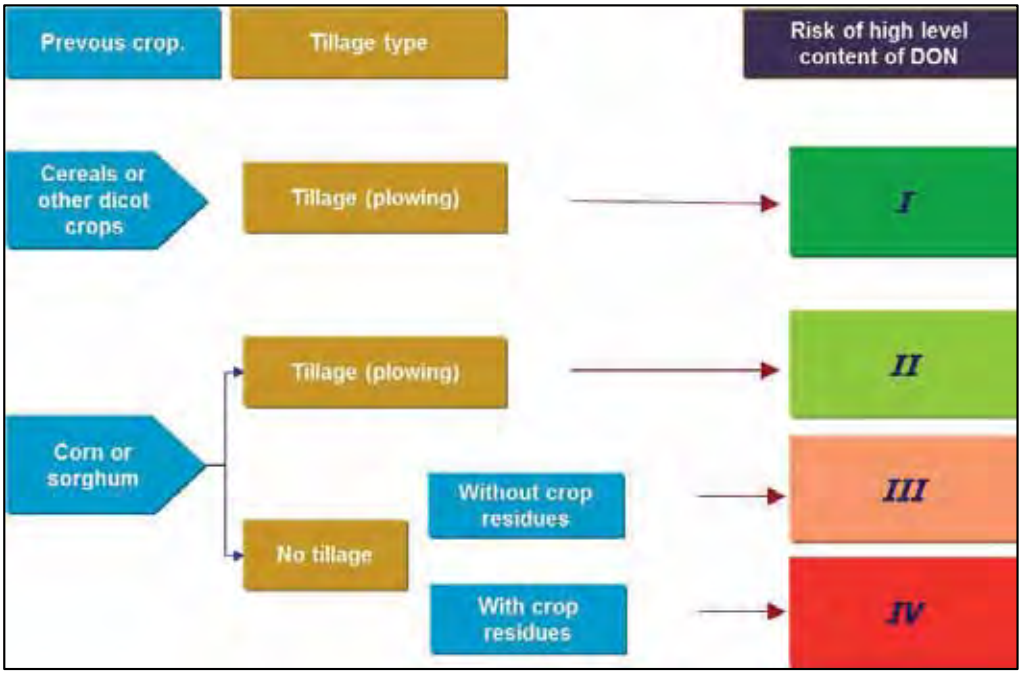

Figure 1. Definitions of the 4 levels of agronomic risk for DON levels on the wheat yield.
(source Arvalis –modified)

Theoretically, this experimental evidence is not only linked to a possible additive effect of the combination of tolerance-fungicidal efficacy, but also to an enhancement of FHB resistance mechanisms active within the plant, in particular of those of type 2 and 3 that indicate the resistance to the fungal growing inside the head and the resistance to colonization of grains.

Accordingly, with these indications, a good example of crop solution approach on FHB tolerance can be considered the seed dressing practice. Applications made directly on seeds with specific fungicides, can oppose the effect of the endogenous migration of *Fusarium graminearum* from seed to the ear in field. Several studies have shown that for the control of this pathogen that develops on culms in growth, the use of seed dressing can also significantly reduce the detectable level of deoxynivalenol (DON) on the ear, particularly in high-risk agronomic situations.

In situations of risk identified with levels 3 and 4 (Fig. 1), the use of a FHB-resistant variety is highly recommended but this would not necessarily entail absolute safety with respect to the DON content in terms of law.

The synergy between tolerance to FHB and seed dressing, as shown in figure 2, would bring a further lowering of the level of DON close to 20% and more in situations of higher risk, giving a wider safety range to the combined solution variety–seed care at field level.

As shown in figure 3, the contribution of the variety in common wheat for the management of the FHB problems is essential, because the variety considered sensitive, if referred to the resistant variety (Illico), in the field trials carried out, appears to have a 39 times higher potential for accumulation of DON. Moreover, also mid-sensitive varieties showed to have this 2-4 times greater potential compared to Illico. In extreme high-risk conditions this difference may further increase.

Still focusing about the levels of agronomic risk previously mentioned on the incidence of FHB foliar treatments with fungicides at the flowering stage are effective to minimize the risk of high incidences of disease.

In figure 4, we can highlight the fact that in a high-risk situation, this differential between the accumulation of a variety FHB sensitiveness with respect to a resistant one, can be up to a factor of 85. In this case the application of specific fungicides for the control of FHB further contributes to lower the content of mycotoxins to greater safety levels.

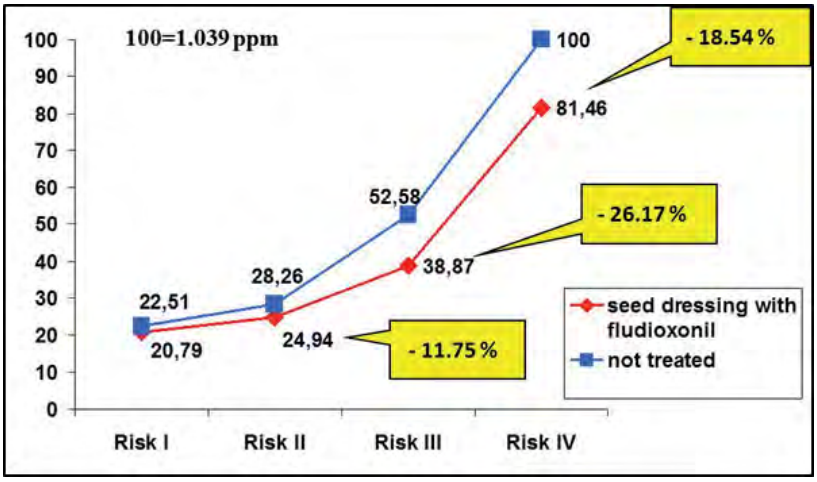

Figure 2. DON content on durum wheat yield (average of 13 trials carried out in France) in different agronomic risk level using fludioxonilvs untreated seed.
(source Syngenta internal)

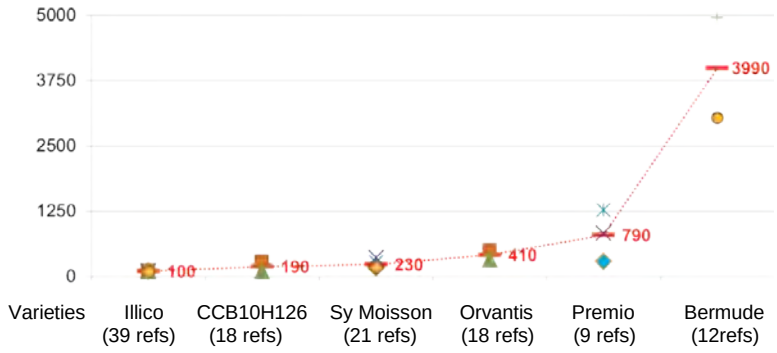

Figure 3. Winter wheat varieties evaluated in terms of DON content in 7 different locations in France during 2012. *Illico is the resistant reference, base 100 of DONon all trials; Bermude is the most sensitive , accumulation of 39.9 more DON than Illico***.**
(source Syngenta internal)

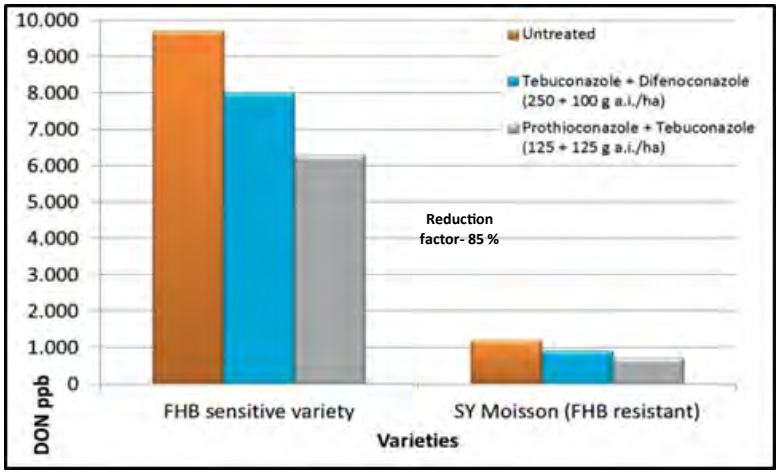

Figure 4. Two winter wheat trials with average DON content in extremely high-risk agronomic conditions (level IV) for FHB incidence. Interaction with fungicide application at flowering stage. Comparison between 2 varieties .
(source Syngenta internal)

In Durum wheat these differences tend to amplify and the management of an acceptable level of DON is very critical. Varieties with traits of good pest resistance become essential for those reasons, especially within certain agricultural conditions to get down the level of higher risk.

Figure 5 shows the high level of severity of attack by FHB and the significant difference between the expression of the symptoms on the ear with and without the artificial inoculation of the pathogen found on more sensitive varieties.

In these situations, the contribution of an integrated solution as seed dressing and foliar fungicide application can stabilize the results of the plant protection; even on more tolerant varieties the result is often greatly improved. Obviously, the integrated approach must also consider the agronomic management of the crop on the choice of the type of tillage and crop rotation, in order to bring the level of risk of contamination by DON and other mycotoxinsas as low as possible.

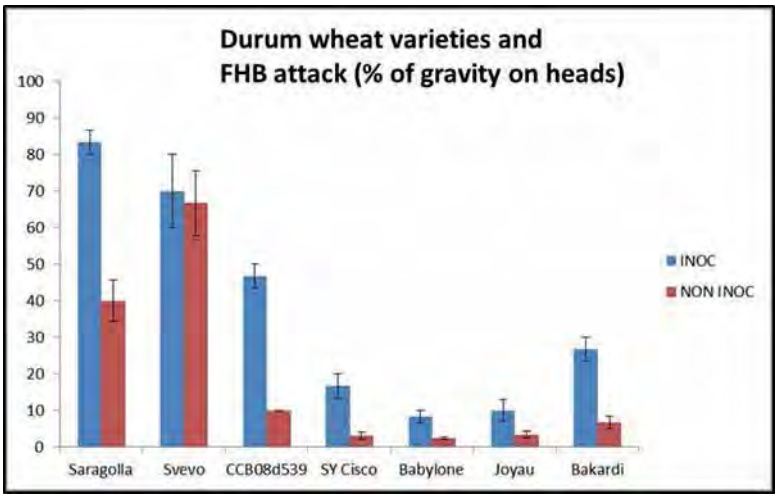

Figure 5. Different sensitivity to FHB of some durum varieties with and without artificial inoculation -2012 .
(source Turin University and Syngenta)

III Interactions with genetic drought resistance and application of grow regulators (GR) and fungicides in wheat

The application of GR on crops, as already mentioned, can potentially have different types of advantages for the cultivation of wheat varieties with traits of quality and productivity of high interest. In addition to the prevention of the lodging, which is the typical result expected from the use of these products in the field, some active ingredients showed the peculiarity of having a positive influence on the root system development.

Although the genetic trait of resistance to drought is characterized by the high complexity of phenotypic response, the practical experiments conducted on uses of GR as Trinexapac-ethyl, constantly appear in an improvement of the root development state, especially in the most critical situations for the absorption of water and nutrients. This possibility of self-regulation of the root system development as a function of water stress is important for the benefit that this kind of physiological adaptation can meet in terms of vegetative response.

As reported in figure 6, in many trials conducted over several years in various parts of the world with extensive cereal cultivations, the results of the use of Trinexapac-ethyl highlights, in general terms, a reduction of internodes width, especially of those lower down, and a clear increase in the root system development. Moreover, in most of the cases observed, an improvement of tillering and an increased level of filling of the grains is possible and this clearly helps a yield increase.

This results would lead to an interesting practical answer to the dilemma of the drought-resistant ideotype which is sometimes considered opposed to the expression of a high yield potential.

During the last few years interesting results were also acquired concerning drought resistance as a secondary effect obtained by the use of fungicides belonging to different chemical groups. In the specific field tests dedicated to examining these aspects, on plots treated with Isopyrazam (IZM) a significant improvement of some biochemical and physiological parameters was observed, with reference to the conditions of water availability on irrigated wheat varieties under test.

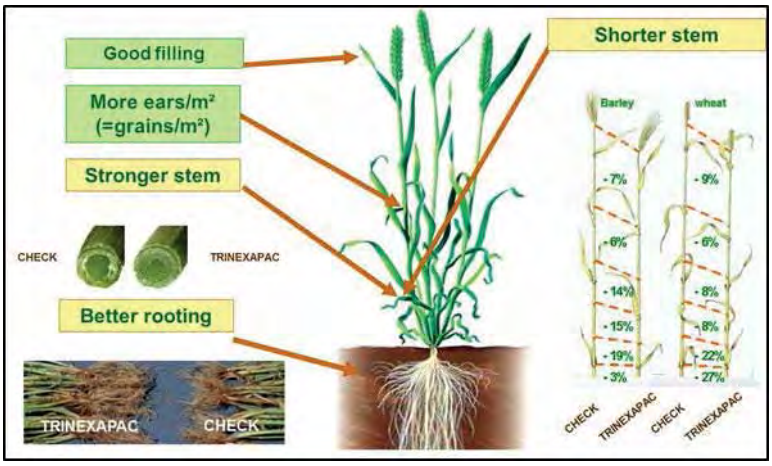

Figure 6. Anti-lodging and other effects of Trinexapac-ethyl applications in cereals. The effect on root development in drought conditions can improve the adaptation of varieties without relevant traits of water-use efficiency mechanisms.
(source Syngenta internal)

Entering into detail of this issue, Figure 7 shows the results of an experiment where the application of Isopyrazam (SHDI chemistry) in a standard protection program of the main fungal diseases such as powdery mildew and Septoria, showed an improvement in the size of the leaf area with a consequent increase in the efficiency of vegetative conditions where water availability was lower.

This positive physiological situation was determined measuring the relationship between electron transport rate (ETR) and the photosynthetic active radiation, both linked to photosynthetic efficiency.

As shown in Figure 8, the ETR is reduced under drought conditions. ETR was assessed on the flag leaves using chlorophyll fluorescence techniques; the difference between the ETR factor is particularly clear in the plots where the crop was maintained under conditions of water scarcity.

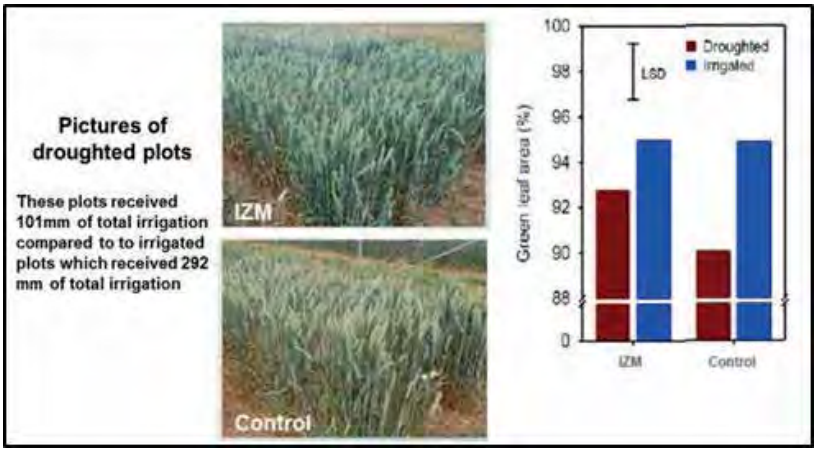

Figure 7. Effects on green leaf area in drought and irrigated conditions of Isopyrazam (SDHI fungicide) application in wheat for disease control purpose on Solstice variety.
(source Rothamstedand Syngenta)

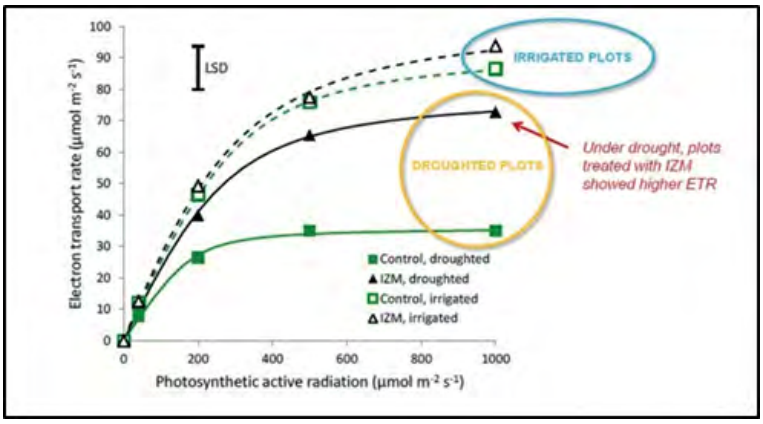

Figure 8. Effects of Isopyrazam (SDHI fungicide) application in wheat for disease control purpose on the photosynthetic efficiency in wheat (var. Solstice).
(source Rothamsted and Syngenta)

IV – Conclusions

As noted in several aspects, the possibilities that the implementation of integrated crop solutions might lead to interesting contributions in order to improve the activity of development of new wheat varieties of agronomic interest and for food downstream are manifold.

The combination of integrated crop solution and breeding with introgression of traits and their associated QTLs can lead to further benefits in durum wheat varieties development with high levels of quality, productivity, and food security, “correcting” any failure in order to achieve optimal characteristics and introducing more stable performances in different situations and crop conditions.

If verified during the early stages of varietal development, these integrated inputs, together with the use of correct agronomic practices, can lead to a speedup of the development process and to the possibility of a wider adaptation of varieties with benefits in terms of costs and enhanced responsiveness to the market needs.

One possibility offered by the integrated crop solution is to provide farmers for each variety developed, with a precise “user guide” to maximize the performance in different environments, optimizing all aspects of crop management in terms of costs and overall sustainability of cultivation practices. It is also important to consider the effectiveness and the role of integrated crop solutions to promote stronger connections among different expertise; through a close collaboration between breeders, agronomists and plant pathologists, exploration in cereals variety development may achieve important benefits in all situations but especially in critical ones where the growing demand for food, environmental deterioration, low sustainability levels, and climate changes threaten the entire social system of rural communities.

Acknowledgments

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Detection of molecular markers associated with yield and yield components in durum wheat (*Triticum turgidum* L. var. *durum*) under saline conditions

Markers for yield in durum wheat

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[3] International Centre for Agricultural Research in the Dry Areas (ICARDA), Morocco

Abstract. Durum wheat is one of the most important staple food crops grown mainly in the Mediterranean region where its productivity is drastically affected by salinity. The study objectives were to identify markers associated with grain yield and its related traits under saline conditions. A population of 114 F_8 recombinant inbred lines (RILs) was derived by single-seed descent from a cross between Belikh2 (salinity tolerant variety) and Omrabi5 (less salinity tolerant) was grown under non-saline and saline conditions in a glasshouse. Phenotypic data of the RILs and parental lines were measured for fifteen agronomic traits. Association of 48 SSR loci covering all 14 chromosomes with fifteen agronomic traits was analyzed with a mixed linear model. A total of 28 SSR loci were significantly associated with these traits. Under saline condition, 13 markers were associated with phenological traits while 19 markers were associated with yield and yield components. Marker alleles from Belikh2 were associated with a positive effect for the majority of markers associated with yield and yield components. Under saline condition, four markers (*Xwmc182*, *Xwmc388, Xwmc398*, and *Xbarc61*) were closely linked with grain yield, located on 3A, 3B, 4B, 5A, 6B, and 7A. These markers could be used for marker-assisted selection in durum wheat breeding under saline conditions.

Keywords. Keywords Association mapping – Durum wheat – Marker-assisted selection – Salinity tolerance – SSR.

Détection de marqueurs moléculaires associés au rendement et à ses composantes chez le blé dur (Triticum turgidum L. var dur.) sous conditions de stress salin. Marqueurs du rendement chez le blé dur

Résumé. *Le blé dur est une des cultures vivrières de base les plus importantes, cultivées principalement dans la région méditerranéenne où sa productivité est très affectée par la salinité. Cette étude avait pour objectif d'identifier des marqueurs associés au rendement en grains et à ses caractères corrélés dans des conditions de salinités. Une population de 114 lignées recombinantes (RIL) F8, issue de la descendance mono-graine d'un croisement entre Belikh2 (variété tolérante à la salinité) et Omrabi5 (moins tolérante à la salinité), a été cultivée en serre en conditions non salines et salines. Les données phénotypiques des RIL et des lignées parentales ont été mesurées pour quinze caractères agronomiques. L'association de 48 loci SSR, couvrant l'ensemble des 14 chromosomes, avec quinze caractères agronomiques a été analysée à l'aide d'un modèle linéaire mixte. Au total, 28 loci SSR étaient significativement associés à ces caractères. Dans des conditions de salinité, 13 marqueurs étaient associés à des caractères phénologiques alors que 19 marqueurs étaient associés au rendement et à ses composantes. Les allèles des marqueurs obtenus de Belikh2 étaient associés avec un effet positif pour la majorité des marqueurs associés au rendement et à ses composantes. Sous des conditions de salinité, quatre marqueurs (Xwmc182, Xwmc388, Xwmc398, et Xbarc61), situés sur les chromosomes 3A, 3B, 4B, 5A, 6B et 7A, étaient étroitement associés à la production de grain. Ces marqueurs pourraient être utilisés pour la sélection assistée par marqueurs dans l'amélioration du blé dur dans des conditions de salinité.*

Mots-clés. *Cartographie d'association – Blé dur – Sélection assistée par marqueurs – Tolérance à la salinité – SSR.*

I – Introduction

Salinity is one of the most serious abiotic stresses limiting crop production globally and has become more serious in recent years. It is estimated to affect nearly one-fifth of the world's irrigated land and causes ten million irrigated hectares to be abandoned each year (Flowers and Yeo, 1995). Although durum wheat cultivars are more salt sensitive than bread wheat and may yield less when grown in saline soils, the usual high price of durum wheat in the international market can bring a better return to farmers than bread wheat and other crops (Lindsay *et al*., 2004). Improving the salinity tolerance of durum wheat and increasing its productivity has been an important objective in wheat breeding programs. Salinity tolerance reflects the ability of a genotype to grow and yield well in a saline environment. It is generally measured as the relative biomass production or relative yield under saline and non-saline conditions (Munns, 2002).

Similar to other agronomical traits, breeding for salinity tolerance requires (a) economic justification, (b) genotypic variation, (c) a rapid and reliable selection method, and (d) understanding of genetic control. The first two criteria are satisfied, but the third and fourth criteria require further work. The current situation is that salinity tolerance is difficult to assess in the field due to spatial and temporal variation, although alternative screening methods have been developed (Munns and James, 2003), they are generally time-consuming, expensive (Lindsay *et al*., 2004) and require validation in the field. Salinity tolerance remains complex both physiologically and genetically (Koyama *et al*., 2001; Colmer *et al*., 2005; Munns and Tester, 2008; Genc *et al*., 2010). Pyramiding of salinity tolerance traits into breeding programs using association mapping and subsequent marker-assisted selection (MAS) have a great potential to accelerate the breeding process.

In the last decade, markers associated with salinity tolerance have been mapped in rice (Gong *et al*., 1999; Koyama *et al*., 2001; Lin *et al*., 2004), barley (Mano and Takeda, 1997; Xue *et al*., 2009) and soybean (Lee et al., 2004). In wheat, differences in salinity tolerance including physiological and agronomical response have been reported, but few researches have been done in genetic analysis. Lindsay et al. (2004) identified markers linked to salt tolerance at seedling stage in durum wheat. Although identification of the markers associated with salt tolerance in terms of yield at late growth stage is particularly important, few relevant studies have been done to date. Dura et al. (2013) identified markers linked to drought tolerance using recombinant inbred lines of durum wheat, derived from a cross between Omrabi5 and Belikh2 parents. Omrabi5 durum cultivar combines drought tolerance with yield and yield stability and Belikh2 was developed for saline areas (Dura *et al*., 2011). In the present study, the same mapping population was grown under non-saline and saline glasshouse conditions to (1) identify markers associated with salinity tolerance traits, (2) understand the relationships among these traits and (3) determine their genetic value for marker-assisted selection.

II – Material and methods

1. Plant material

The plant material used in this study was a population which originated from a cross between Omrabi5 with Belikh2. The population consisted of 114 F8 single seed descent recombinant inbred lines (RILs) developed in 2005 by the durum wheat breeding program at the International Center for Agricultural Research in the Dry Areas (ICARDA). Omrabi5 and Belikh2 are durum wheat cultivars developed for the Mediterranean conditions (Nachit, 1998). Omrabi5 was developed from a cross between the Middle East landrace Haurani and the improved cultivar Jori-C69, while Belikh2 (Cr/Stk) was bred at ICARDA for saline area. Omrabi5 was released in Jordan, Turkey, Algeria, Morocco, Iran and Iraq for commercial production; it combines drought tolerance with yield and yield stability, whereas Belikh2 was released in Lebanon and Syria.

2. Glasshouse experiment

The experiment was conducted in a glasshouse of the University of Jordan in 2007 using 114 RILs and the parental genotypes tested under two salinity levels with three replications. Plastic pots were filled with washed sandy soil, each containing 10 kg soil (dry wt. basis). The seeds were germinated in transplanting trays. After 10 days at two leaf stage, seedlings were transferred into each pot at a rate of three seedlings per pot. Seedlings were watered initially with tap water (0.2 mM NaCl), and then quarter strength Hoagland nutrient solution was introduced two days after transplanting and increased to full strength at three weeks after transplanting. The salinity concentration was increased gradually in aliquots of 10 mM NaCl every day until the required concentration of 100 mM NaCl was reached. Salinity treatments were begun 14 days after the start of the experiment.

The following traits were recorded on three plants of each pot. Days to heading (DH) was recorded as the number of days from emergence to the day when half of the spikes have appeared in 50% of the plants. Days to maturity (DM) was recorded as the number of days from emergence to the day when the peduncle was completely discoloured in 90% of the plants. Plant height (PH) was measured at harvest maturity from the ground level to the top of the spikes excluding awns. Peduncle length (PL) was measured from the node to the ligule of the flag leaf. Spike length (SL) was measured from the base to the top of the spike excluding the awns. Awns length (AL) was measured from the top of the spike to the top of awns. Number of tillers (NT) and number of fertile tillers (NFT) was counted. Main spike weight (WS), number of grains per plant (NG) and number of spikelets per spike (NSS) were counted. Thousand-grain weight (TGW) was measured by weighing grains taken from the plant and converted to the weight of 1000 grains. Biological yield (BY) was measured as the weight of aboveground dry matter (straw + grain). Grain yield was measured as the weight of grain harvested from the plant. Straw yield was calculated as the difference between biological yield and grain yield. The design used was a Complete Randomized Design (CRD) with three replications.

3. Molecular analysis

The following studies were conducted on plant materials grown in 2007 at ICARDA, Aleppo, Syria using ICARDA durum wheat MAS lab.

The DNA was extracted using SDS method from 3-5 gm leaf tissue of each RIL seedling eight-weeks after sowing according to the protocol developed at ICARDA durum wheat MAS lab (Nachit *et al*., 2001) and quantified by the spectrophotometer.

Wheat microsatellites *wmc* (wheat microsatellite) and *barc* (Beltsville agriculture research center) were used as described by Nachit *et al*. (2001). The parents were screened using 300 primer pairs of SSRs out of which 48 (15%) were polymorphic. The Polymerase Chain Reaction (PCR) amplification was carried out in Eppendorff thermal cycle, in a 7.5 µl reaction mixture. Each reaction contained 10 X Taq polymerase buffer, 200 µM of each dNTPs, 0.5 µM of each of the two primers, 1 U Taq polymerase, and 20 ng of genomic DNA as template. Amplifications were performed as follows: 94 °C for 5 min, 35 cycles of (94 °C 1 min, 63-56 °C 1 min, 72 °C 1 min), 72 °C for 5 min. PCR products were mixed with loading buffer, 5-10 µl of mixture was denaturated and loaded into wells in 0.4 mm thick 15% acrylamide gel resolved at constant power (30 w) in 1 X TBE running buffer for 15 min to one hour depending on size of the primer pairs of SSRs. Bands were visualized by silver-staining method as described by Nachit *et al*. (2001).

4. Statistical analysis and association mapping

The statistical analysis was performed using the MIXED procedure of the SAS statistical package (SAS, 1998). Pearson's correlations between phenotypic traits were calculated using SPSS 17.0 statistical software. Forty-eight SSR markers covering the whole durum AB genome were used.

Because of the low number of molecular markers probed in this study to utilize for genetic mapping, we have opted for association mapping between molecular markers and traits. We have a mixed linear model (MLM) within the program TASSEL version 2.0.1 (http://www.maizegenetics.net) where the marker was considered as a fixed-effects factor and the lines of the population considered as a random-effects factor (Kennedy *et al*., 1992). Significance of associations between loci and traits was based on an F-test, at a level α_c corresponding to α corrected for multiple testing. Corrected significance levels α_c were computed by 1000 permutations within a chromosome. The additive effects of the markers were estimated using Genstat (Version11).

III – Results

1. Phenotypic

A total of 114 lines and their parents (Omrabi5 and Belikh2) were investigated under salinity stress and normal conditions. The grand means and ranges of measured fifteen agronomic traits for the parent and RIL population are presented in Table 1. The two parents showed the great difference in all fifteen traits. The values of fifteen agronomic traits showed more reduction in Omrabi5 than in Belikh2 when the plants were exposed to salinity stress, which was consistent with the fact that Belikh2 is a well-known salt-tolerant genotype. On an average of all RIL, each value of 15 agronomic traits was obviously reduced under salinity stress relative to the control.

The phenotypic distributions of all examined traits for the RIL displayed a continuous normal pattern. Obviously, these traits were quantitatively inherited. In addition, transgressive segregation in both directions was observed for all traits (Table 1) under both the control and salinity stress.

Significant correlations ($P < 0.05$) were observed between GY and WS, TGW, NG, BY, and SY, irrespective of the control and salinity conditions. However, there was no significant correlation between GY and DH, PL, SL, AL, NT, NFT and NSS in both conditions. GY was positively correlated with PH, PL, SL, AW, NFT, TGW, NG, BY, and SY, and negatively correlated with DH, DM, NT, and NS under salinity stress (Tables 2 and 3).

2. Marker-trait association

A total of 28 SSR markers for 15 agronomic traits were located on all 14 chromosomes of durum wheat (Tables 4 and 5); being 15 and 13 markers under control and salinity stress, respectively. Only markers significant at the multiple testing-corrected significance levels for at least one trait are presented in Tables 4 and 5.

3. Phenological traits

For DH, one significant marker (Xwmc177) was detected both for the control and salt stress located on chromosome 2A accounted for 10.5, 31.0% of the total DH variation, respectively. There was one marker, Xwmc24 on chromosome 1A under the saline condition accounted for 1.6% of the phenotypic variation. All these markers had alleles from parent Belikh2 (Tables 4 and 5). One significant marker (Xwmc617 for DM was detected under the control and salinity stress conditions and mapped on chromosomes 4A, and 4B accounted for 2.6, 3.1% of the phenotypic variation, respectively. Three other markers (Xbarc61, Xbarc353, Xbarc1025) located on chromosomes 2A, 4B, and 7A were detected under the control condition accounted for 2.6, 2.3, and 3.4% of the DM variation, whereas another marker; Xwmc626 ($P < 0.01$) was found under salinity stress accounted for 5.1% of the total variation. All of these markers except; Xbarc61 had alleles from Belikh2 (Tables 4 and 5). Two significant markers; Xwmc177, and Xwmc617 influencing PH were detected under the two environments (control and salt stress).

Table 1. Mean performance, standard deviations, and ranges of traits under the control (S1) and salinity (S2) conditions for the parents and RILs.

Trait	Belikh 2S1 Mean ± SD	Omrabi 5S1 Mean ± SD	RILs SI Mean ± SD	Range	Belikh 2S2 Mean ± SD	Omrabi 5S2 Mean ± SD	RILs S2 Mean ± SD	Range
DH Days to Heading	111.7 ±3.9	93.5 ±1.4	114.5 ±13.7	92.0-160.0	83.8 ±1.6	81.2 ±2.6	102.7 ±13.4	82.0-150.0
DM Days to Maturity	152.0 ±8.3	114.2 ±1.7	157.8 ±24.6	115.0-201.5	119.5 ±3.1	101.8 ±2.6	122.9 ±16.1	102.0-145.5
PH Plant height (cm)	71.2 ±3.6	78.4 ±3.2	79.5 ±14.1	55.4-108.5	60.8 ±4.6	30.0 ±6.3	62.6 ±12.1	35.6-89.5
PL Peduncle length (cm)	22.5 ±2.7	32.3 ±4.1	17.9 ±7.3	1.2-36.9	9.4 ±2.0	1.8 ±0.4	9.5 ±5.4	0.0-26.8
SL Spike length (cm)	9.8 ±0.50	7.0 ±0.4	9.5 ±1.3	7.0-15.5	8.7 ±0.60	6.0 ±0.04	8.0 ±0.9	5.7-11.8
AL Awns length (cm)	14.5 ±0.92	12.0 ±0.69	13.9 ±1.9	9.6-21.5	11.0 ±0.72	7.2 ±0.7	10.9 ±1.7	7.0-15.5
NT Tillers plant^{-1} No	7.3 ±0.41	5.8 ±0.5	5.7 ±1.6	3.0-13.0	5.3 ±1.0	1.1 ±0.2	4.1 ±1.4	1.0-8.0
NFT Fertile til-lers plant^{-1}No.	7.3 ±0.42	5.7 ±0.5	5.1 ±1.8	2.0-13.0	4.3 ±0.76	2.0 ±0.6	3.8 ±1.0	2.0-8.0
WS Spike Weight (g)	2.6 ±0.61	2.8 ±0.4	2.4 ±0.5	1.4-4.2	2.4 ±0.41	1.4 ±0.5	2.1 ±0.5	1.2-3.5
NG Grains plant^{-1}No.	127.3 ±31.8	122.8 ±20.8	122.6 ±28.4	82.1-227.0	90.7 ±22.0	30.9 ±6.3	91.1 ±25.9	48.0-187.0
NSS Spikelets spike^{-1}No.	25.7 ±1.3	21.5 ±1.2	24.7 ±2.2	20.0-30.0	24.5 ±1.5	14.5 ±2.6	20.7 ±2.4	15.0-26.0
TGW 1000-grainweight (g)	56.9 ±8.1	47.4 ±4.8	59.0 ±6.7	54.8-67.7	52.1 ±10.4	12.1 ±4.8	41.2 ±14.7	15.8-79.6
BY Biological yield (g plant^{-1})	15.9 ±1.7	15.8 ±2.0	14.8 ±2.7	9.8-24.1	8.9 ±2.3	1.7 ±0.44	6.7 ±1.6	0.6-11.5
SY Straw yield (g plant^{-1})	8.4 ±1.4	7.8 ±2.0	8.4 ±2.3	4.4-15.1	7.1 ±1.8	1.5 ±0.46	5.5 ±1.6	0.5-10.2
GY Grain yield (g plant^{-1})	7.5 ±0.62	7.9 ±1.1	6.4 ±1.0	4.1-9.4	1.9 ±0.45	0.12 ±0.02	1.2 ±0.3	0.1-1.2

Table 2. Simple phenotypic correlation coefficients between days to heading (DH), days to maturity (DM), plant height (PH), peduncle length (PL), spike length (SL), awns length (AL), number of tillers (NT), number of fertile tillers (NFT), weight of spike (WS), number of spikelets per spike (NSS), 1000-grain weight (TGW), number of grains per plant (NG), biological yield (BY), straw yield (SY) and grain yield (GY) under the control conditions.

	DM	PH	PL	SL	AL	NT	NFT	NSS	TGY	GW	NG	BY	SY	GY
DH	.47	-15	-.28	.01	-.23	.27**	.15	.23	-.16	.29**	.23*	-.15	-.13	-.12
DM		-.02	-.20*	-.01	-.01	.78**	-.05	.10	-.06	-.20*	-.18*	-.17	-.17	-.007
PH			.53**	.01	.43**	-.15	-.28**	-.04	-.06	-.05	-.02	.05	.03	.07
PL				-.06	.55**	-.13	-.16	-.15	.17	.08	-.05	.08	.07	.09
SL					.08	.22*	.14	.13	.01	.07	.06	.13	.10	.18
AL						.02	-.15	-.10	.07	.03	-.03	.03	.02	.03
NT							.39**	-.01	-.15	-.12	-.03	-.09	-.08	-.05
NFT								.07	.14	.006	-.12	.16	.15	.08
WS								.01	.10	.18*	.10	.03	-.007	.22*
NSS									-.05	-.06	-.04	.03	.05	-.12
TGW										.59**	-.11	.20*	.17	.18*
NG												.08	.02	.31**
BY													.98**	.21*
SY														.02

**. Correlation is significant at the 0.05 level. **. Correlation is significant at the 0.01 level.*

Table 3. Simple phenotypic correlation coefficients between days to heading (DH), days to maturity (DM), plant height (PH), peduncle length (PL), spike length (SL), awns length (AL), number of tillers (NT), number of fertile tillers (NFT), weight of spike (WS), number of spikelets per spike (NSS), 1000-grain weight (TGW), number of grains per spike (NG), biological yield (BY), straw yield (SY) and grain yield (GY) under the saline conditions.

	DM	PH	PL	SL	AL	NT	NFT	WS	NSS	TGY	NG	BY	SY	GY
DH	.78 **	-.12	-.39 **	.23 *	-.24 **	.21 *	.28 **	-.12	.23 *	-.06	-.34 **	-.17	-.14	-.13
DM		-.14	-.42 **	.20 *	-.21 *	.09	.20 *	-.18 *	.319 **	-.032	-.28 **	-.14	-.09	-.20 *
PH			.58 **	-.15	.29 **	-.20 *	-.25 **	.04	.11	.17	-.07	.12	.06	.18*
PL				-.29 **	.36 **	-.10	-.21 **	.20 *	-.11	.10	.03	.031	.009	.06
SL					-.14	.18 *	.19	.17	.30 **	.01	.05	-.027	-.042	.025
AL						-.18 *	-.29 *	.27 **	-.14	.09	.15	.118	.068	.170
NT							.75 **	-.10	-.01	.03	-.09	.134	.115	.101
NFT								-.17	.05	.02	-.06	.24 **	.25 **	.08
WS									-.031	.21 *	.13	.14	.08	.19 *
NSS										-.04	-.07	.004	.03	-.06
TGY											-.11	.45 **	.36 **	.41 **
NG												.45 **	.41 **	.28 **
BY													.938 **	.57 **
SY														.25 **

These markers, located on chromosomes 2A, 4A, and 4B, accounted for 4.4-13.7% of the total phenotypic variation, with positive alleles coming from parent Belikh2. Under the control condition another marker; *Xbar61* ($P < 0.001$), located on chromosome 4B, accounted for 3.2% of PH variation. For salinity, marker; *Xwmc182* ($P < 0.019$), detected on 3B and 6B, accounted for 3.2% of the total variation, with positive alleles also coming from Belikh2 (Tables 4 and 5).

Three significant markers for PL were detected. Of them, one marker (Xwmc625) was mapped on chromosome 3B under both conditions accounted for 2.4% of the total phenotypic variation and its positive alleles came from Belikh2. Under salt stress, three markers were mapped on chromosomes 2B, 3B, 5B, 7B accounted for 2.4-5.4% of PL variation, and their positive alleles also came from Belikh2 (Tables 4 and 5). For SL three significant markers were found. Only one marker; Xwmc488 was mapped on chromosome 7A under the control condition accounted for 5.3% of the total phenotypic variation. Under salinity stress, two markers were mapped on chromosomes 3B, 4A, and 4B accounted for 2.4-8.6% of the SL variation. The alleles of these markers; which increased SL, came from Belikh2 (Tables 4 and 5). For AL only one significant marker; Xwmc625 ($P < 0.045$) was detected under the saline stress. This marker was located on chromosome 3B accounted for 3.4% of the total phenotypic variation and its positive alleles came from parent Belikh2 (Tables 4 and 5).

4. Yield components

For TN, three significant markers were detected. Under control condition, two markers; *Xwmc667*, and *Xbarc353,* located on chromosome 2A accounted for 3.5, 4.0% of the total phenotypic variation, respectively. For salinity, only one marker; *Xbarc100,* on 2B and 5A accounted for 6.4% of the total variation. The positive alleles also came from Belikh2 (Tables 4 and 5). Only two significant markers for FTN were detected. One off them, *Xwmc426,on 7B,* accounted for 6.6% of the total phenotypic variation. For salinity, another marker (*Xbarc100*) was mapped on 2B and 5A and accounted for 3.3. All of these markers had alleles from Belikh2 (Tables 4 and 5). Six markers were detected for WS. Of them, four were mapped on chromosomes 2A, 3B, 4B, 6B, 7A, and 7B under the control condition accounted for 1.8-3.9%. Under salinity stress, two markers (*Xwmc182*, *Xbarc70*) mapped on chromosomes 3B, 4B, and 6B, explaining 3.6 and 4.9% of the phenotypic variation, respectively. The positive alleles came from Belikh2 (Tables 4 and 5).

5. Yield components

For TN, three significant markers were detected. Under control condition, two markers; *Xwmc667*, and *Xbarc353* were located on chromosome 2A accounted for 3.5, 4.0% of the total phenotypic variation, respectively. For salinity, only one marker; *Xbarc100* detected on 2B, and 5A accounted for 6.4% of the total variation. The positive alleles also came from Belikh2 (Tables 4 and 5). However, two significant markers for FTN were found. Of them, one marker (*Xwmc426*) accounted for 6.6% of the total phenotypic variation and located on chromosome 7B under the control condition. For salinity, another marker (*Xbarc100*) was mapped on 2B and 5A and accounted for 3.3. All of these markers had alleles from Belikh2 (Tables 4 and 5). Six markers were detected for WS. Of them, four markers were mapped on chromosomes 2A, 3B, 4B, 6B, 7A, and 7B under the control condition accounted for 1.8-3.9%. Under salinity stress, two markers (*Xwmc182*, *Xbarc70*) were mapped on chromosomes 3B, 4B, and 6B, explaining 3.6 and 4.9% of the phenotypic variation, respectively. The positive alleles came from Belikh2 (Tables 4 and 5).

Only one marker (*Xwmc597*) was detected in both environments for NSS, being located on chromosomes 1B, 2B, 3B, 4A, and 6B. Under salinity condition, another marker was detected on chromosome 7B accounted for 7.0% of the NSS variation. All of these markers had alleles from Belikh2 (Tables 4 and 5). For TGW, only one significant marker (*Xbarc32*) was found in salinity environment accounted for 9.1% of the total phenotypic variation and located on chromosomes 5B, and 7B and its alleles came from Belikh2 (Tables 4 and 5). Under salinity condition, two

markers were located on chromosomes 1A and 3B accounted for 2.8, and 5.9% of the total variation respectively, and its positive alleles came from Belikh2 (Tables 4 and 5). Five genomic regions related to NG were detected. Of them, three markers were mapped on chromosomes 4A, 4B, 5A, 5B, and 7B under the control condition accounted for 2.8-5.9% of the total phenotypic variation, whereas other markers (*Xwmc398*, *Xbarc315*) were found under salinity stress, being mapped on chromosomes 4A, 6B, and 7B accounted for 2.6, and 5.2% of the phenotypic variation, respectively. The positive alleles are from Belikh2 (Tables 4 and 5).

Table 4. Comparison-wise ρ-values association of SSR loci for days to heading (DH), days to maturity (DM), plant height (PH), peduncle length (PL), spike length (SL), number of tillers (NT), number of fertile tillers (NFT), weight of spike (WS), number of spiklets per spike (NSS), number of grains per plant (NG), biological yield (BY) and straw yield (SY) under control condition.

Trait	Locus	df_Marker	F_Marker [a]	P_Marker	Marker effect [b]	Allele [c]
DH	WMC177@2Abp190	1	30.34	0.00**	10.5	Blk
DM	WMC617@4B4Abp200	2	8.87	0.00**	2.6	Blk
DM	BARC61@4Bbp150	2	4.44	0.013*	2.3	Mrb
DM	BARC353@2Abp205	1	4.00	0.047*	3.4	Blk
DM	BARC1025@7Abp125	1	8.05	0.005**	5.2	Blk
PH	WMC177@2Abp190	1	12.79	0.000**	4.4	Blk
PH	WMC617@4B4Abp200	2	3.88	0.023*	13.7	Mrb
PH	BARC61@4Bbp150	2	7.21	0.001**	3.2	Blk
PL	WMC625@3Bbp110	1	6.90	0.009**	2.4	Blk
SL	WMC488@7Abp120	1	4.72	0.032*	5.3	Blk
NT	BARC353@2Abp205	1	7.00	0.009**	4.0	Blk
NT	WMC667@2Abp110	1	4.24	0.041*	3.5	Mrb
NFT	WMC426@7Bbp210	2	4.65	0.011*	6.6	Blk
WS	WMC177@2Abp190	1	3.95	0.049*	3.9	Blk
WS	WMC603@7Abp95	2	3.31	0.040*	2.5	Mrb
WS	BARC1025@7Abp125	1	4.15	0.044*	1.8	Mrb
WS	WMC218@7Bbp110	1	3.93	0.049*	3.3	Blk
NSS	WMC597@1B2B3B4A6Bbp240	2	4.41	0.014*	2.7	Blk
NG	WMC617@4B4Abp200	2	4.14	0.018*	5.9	Blk
NG	BARC32@5B7Bbp135	2	3.94	0.022*	2.8	Mrb
NG	WMC475@5A7Bbp125	1	6.58	0.011*	4.2	Blk
BY	WMC617@4B4Abp200	2	4.22	0.017*	5.7	Blk
BY	BARC32@5B7Bbp135	2	3.24	0.042*	3.3	Blk
BY	WMC475@5A7Bbp125	1	4.24	0.041*	2.8	Blk
SY	WMC475@5A7Bbp125	1	5.24	0.024*	4.8	Blk

*a Only markers significant at the multiple testing-corrected significance level $\alpha_c = 0.05$ for at least one trait are shown. *, ** indicate significance at $\alpha_c = 0.05, 0.01$ respectively.*
b Positive and negative values indicate that MRBmrabi5 and Belikh2 alleles increased the phenotypic values, respectively.
c Mrb and Blk indicate Omrabi5 and Belikh2, respectively.

Table 5. Comparison-wise ρ-values association of SSR loci for days to heading (DH), days to maturity (DM), plant height (PH), peduncle length (PL), spike length (SL), awn length (AL), number of tillers (NT), number of fertile tillers (NFT), weight of spike (WS), number of spikelets per spike (NSS), thousand grain weight (TGW), number of grains per spike (NG), biological yield (BY), straw yield (SY) and grain yield (GY) under salinity condition.

Trait	Locus	Df	F[a]	P	E[b]	Allele[c]
DH	WMC177@2Abp190	1	13.5986	0.000**	31.0	Blk
DH	WMC24@1Abp125	1	6.2468	0.0139*	1.6	Blk
DM	WMC617@4B4Abp200	2	6.4589	0.0023**	3.1	Blk
DM	WMC626@7Abp180	1	6.7748	0.0105*	5.1	Blk
PH	WMC177@2Abp190	1	10.787	0.0014**	4.4	Blk
PH	WMC182@3B6Bbp160	1	5.6152	0.0195*	3.2	Blk
PH	WMC617@4B4Abp200	2	3.2302	0.0437*	13.7	Mrb
PL	BARC32@5B7Bbp135	2	3.4317	0.0359*	2.6	Mrb
PL	BARC114@2Bbp130	1	5.2301	0.0241*	5.4	Blk
PL	WMC625@3Bbp110	1	4.9527	0.0281*	2.4	Blk
SL	WMC617@4B4Abp200	2	3.8406	0.0247*	2.4	Blk
SL	BARC344@3Bbp240	1	4.8399	0.0299*	8.6	Blk
AL	WMC625@3Bbp110	1	4.0904	0.0456*	3.4	Blk
NT	BARC100@2B5Albp140	1	7.3021	0.008**	6.4	Blk
NFT	BARC100@2B5Albp140	1	6.0286	0.0157*	3.3	Blk
WS	WMC182@3B6Bbp160	2	3.4352	0.0357*	3.6	Blk
WS	BARC70@4Bbp240	1	4.0537	0.0466*	4.9	Blk
NSS	WMC662@7Bbp190	2	3.4547	0.0355*	5.2	Blk
NSS	WMC597@1B2B3B4A6Bbp240	2	3.1269	0.0478*	7.0	Blk
TGW	BARC32@5B7Bbp135	2	4.3779	0.0148*	9.1	Blk
NG	WMC398@6Bbp90	2	3.9572	0.0222*	2.8	Blk
NG	BARC315@4A7Bbp75	1	6.8057	0.0104*	5.9	Mrb
BY	WMC398@6Bbp90	2	4.0111	0.0211*	2.5	Blk
BY	BARC315@4A7Bbp75	1	6.0583	0.0154*	2.6	Blk
SY	BARC59@4Bbp185	2	3.425	0.0362*	2.2	Blk
SY	WMC475@5A7Bbp125	1	8.3393	0.0047**	4.8	Blk
GY	WMC182@3B6Bbp160	2	3.6303	0.0298*	2.7	Blk
GY	WMC388@3A5A7Abp150	1	7.6316	0.0067**	4.6	Blk
GY	WMC398@6Bbp90	2	3.257	0.0426*	2.3	Blk
GY	BARC61@4Bbp150	2	4.7463	0.0106*	6.1	Blk

6. Yield

Five significant markers for BY were identified. Under the control condition, three markers (*Xwmc617*; P < 0.017, *Xwmc475*; P < 0.042, *Xbarc32*; P < 0.043), being mapped on chromosomes 4A, 4B, 5A, 5B, and 7B accounted for 5.7, 2.8, and 3.3% of the total phenotypic variation, respectively, and their positive alleles are from Belikh2. Other two markers (*Xwmc388*, *Xwmc398*) were identified on chromosomes 4A, 6B, and 7B accounted for 2.5, and 2.6% of the BY variation under saline condition. The positive alleles also are from Bekih2 (Tables 4 and 5). Only one significant marker; *Xwmc475* for SY was identified in both environments accounted for 4.8% of the total SY variation. There was other one marker (*Xbarc59*) on chromosome 4B under the salinity accounted for 2.2% of the total variation. The positive alleles also are from Bekih2 (Tables

4 and 5). Four associated markers for GY were detected under salinity stress. Each of them, accounted for 2.3-6.1% of the total phenotypic variation, and the alleles from parent Belikh2 could increase GY, being *Xbarc61* and *Xwmc388* under salinity condition (Tables 4 and 5).

IV – Discussion

The two parents (Belikh2 and Omrabi5) differed significantly in the measured traits when they were exposed to non- and salinity stresses. Dura *et al*. (2011) found that germination percentage, seedling growth, vegetative growth, grain production, exclusion of Na+ and Cl-, and K+/Na+ ratio were higher in Belikh2 than Omrabi5 under saline conditions. The means of all RILs were close to the mid-parental values for all traits in both treatments (Table 1). Although phenotypic distribution of RILs was normal, transgressive segregation was also observed in both directions for all traits (Table 1). In the past decade, few genetic and molecular analyses were conducted and a small number of QTLs were mapped in durum wheat (Genc *et al*., 2010). Some reports showed that there was a large genotypic diversity for wheat in salinity tolerance (Koyama et al., 2001; Lindsay *et al*., 2004; Genc *et al*., 2010; Dura *et al*., 2011). Ma *et al*. (2004) found markers controlling salt tolerance at germination stage on homologous chromosomes 3, 4, 5 and 7 and at seedling stage on homologous chromosome 1 and 3 in bread wheat. Few research of such molecular analysis has been done under field condition. However, Quarrie *et al*. (2005) detected 7 markers controlling grain yield at mature stage under saline condition using field irrigated with saline water. In this study, a total of 28 markers for the examined fifteen traits were detected under the two treatments. It is suggested that different markers or alleles at the same locus are responsible for genetic variation under diverse environment conditions. The results were consistent with the study of Austin and Lee (1998) in which QTL was analyzed under stress and non-stress environments. The case was same for the markers controlling DH, DM, PH, PL, and NS (Tables 4 and 5).

The results suggested that these markers were stable and not greatly influenced by environments. Most of the detected markers locations were mapped on the same region of chromosomes 3B, 4A, and 7B which accounted for 3.1-6.8% of the total phenotypic traits (Tables 4 and 5). Moreover, these markers represented 73.7% of the total markers found under salt stress, and all their positive alleles came from Belikh2. The results indicated that this region of chromosomes 3B, 4A, and 7B and its homologous are important for salt tolerance in durum wheat. It may be assumed that there is a QTL cluster for salt tolerance in the region of chromosome 3B, 4A, and 7B and its homologous (Tables 4 and 5), and thus the region may be used as an important target for improving salt tolerance of durum wheat.

Under normal (non-stress) environment, one significant marker (Xbarc353) associated with DM and NT were mapped on chromosome 2A and its alleles with positive effect coming from Belikh2, supported by significantly positive correlation (r = 0.78**) between the two traits. Similar results were found for DH, DM, PH, NG, WS and BY in the markers; Xbarc1025, Xbarc61, Xwmc177, Xbarc1025, Xwmc617, and Xbarc32 on chromosomes 2A, 4A, 4B, 5A, 7A, and 7B (Tables 2 and 3). However, these markers were not found under salt stress. It may be assumed that the genes in these regions, controlling DH, DM, PH, NG, WS, and BY are expressed normally under the condition without salt stress, while their expression is greatly inhibited when the plants are exposed to salt stress. Moreover it was found that the markers detected under no salinity differed markedly from those detected under salt stress, and there were many markers which are co-located or tightly linked with these agronomic traits.

Salinity tolerance genes are located throughout the genome and are genotype dependent. In this study, it was found that for grain yield four markers were derived from Belikh2. However, some of the markers were also derived from sensitive parent of the population. This study confirms that salinity tolerance is a quantitative trait and that apparently sensitive parents may contain alleles for tolerance, which may not be found in the tolerant parent. It can be concluded that the sensitive

parent Omrabi5 may contain some tolerance alleles that when combined with alleles from tolerant parents can results in increased level of tolerance.

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Salinity tolerance genes are located throughout the genome and are genotype dependent. In this study, it was found that for grain yield four markers were derived from Belikh2. However, some of the markers were also derived from sensitive parent of the population. This study confirms that salinity tolerance is a quantitative trait and that apparently sensitive parents may contain alleles for tolerance, which may not be found in the tolerant parent. It can be concluded that the sensitive parent Omrabi5 may contain some tolerance alleles that when combined with alleles from tolerant parents can results in increased level of tolerance.

V – Conclusion

Molecular markers closely linked to genes of agronomic importance traits have been demonstrated to be useful tools for indirect selection in durum wheat breeding programs (Nachit *et al*., 1998). Further investigations for salinity tolerance will be required to establish the importance of the identified genomic regions in other backgrounds. In addition, field evaluation is required to establish the effectiveness of the salinity screening system in modeling water responses and in evaluating the stability of QTLs across environments (Mohan *et al*., 1997). Our results indicate the existence of genes, which are involved in the control of the phenotypic variation in quantitatively inherited traits related to salinity tolerance. Compared with conventional methods, QTLs and molecular markers provide breeders new alternatives for selection. Marker-assisted selection can accelerate breeding by reducing the time to develop new cultivars (Landjeva *et al*., 2007). Further research is needed on molecular markers and QTL mapping to screen potential genotypes for salinity tolerance in wheat.

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In vitro gynogenesis in some varieties of durum wheat (*Triticum durum*. L.)

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Abstract. To succeed in the production of doubled-haploid plants of durum wheat *(Triticum durum*. L), gynogenesis was studied in six durum wheat varieties Anwar (AN), Jawhar (JW), Yasmine (YS), Be bachir (BB), Sebou (SB) and Kyperonda (KP). The MS medium (Murashige and Skoog 1966) supplemented with 9% sucrose and 0.8% agar was used as basal medium. 2mg /l of three auxins [2,4-D (2,4-dichloro- phenoxy acetic acid), NAA (naphthalene acetic acid), and 2.4.5-T (trichlorophenoxy acetic acid)] were added to the induction medium. The embryogenic calluses were transferred onto regeneration medium R9 without growth regulators for two weeks then the same medium supplemented with 2 mg /l BAP (benzyl adenine purine) and 0.1mg /l NAA. The results showed that 2,4-D was the most reactive auxin for all varieties studied. The effect of genotype was significantly very marked in the presence of the three auxins tested. The best rate of embryogenic callus induction was 63% obtained from the Anwar variety. The presence of light (16h per day) favorably affected the kinetics of appearance of gynogenetic embryogenic callus. The first results appeared in the second week of culture whereas, in darkness, this period lasted up to nine weeks. The study also showed a genotypic effect on the regeneration phase with the best rate (27%) obtained with the variety Anwar.

Keywords. *Triticum durum* – Gynogenesis – Haploid methods – Genotypes – Green plants.

Gynogenèse in vitro dans certaines variétés de blé dur (Triticum durum. L.)

Résumé. *Pour réussir la production d' haploïdes doublés de blé dur (Triticum durum. L), on a étudié la gynogenèse dans six variétés de blé dur Anwar (AN), Jawhar (JW), Yasmine (YS), Belbachir (BB), Sebou (SB) et Kyperonda (KP). Le milieu MS (Murashige et Skoog, 1966), additionné de 9% de saccharose et 0,8% de gélose, a été utilisé comme milieu de base. 2 mg/l de trois auxines [2,4-D (2,4-dichlorophénoxyacétique), NAA (acide naphtalène acétique) et 2.4.5-T (acide trichlorophénoxyacétique)] ont été ajoutés au milieu d'induction. Les cals embryogènes ont été transférés sur un milieu de régénération R9 sans régulateurs de croissance pendant deux semaines, puis le même milieu a été additionné de 2 mg/l de BAP (benzyle-adénine-purine) et de 0,1 mg/l de NAA. Les résultats ont montré que le 2,4-D était l'auxine la plus réactive pour toutes les variétés étudiées. L'effet du génotype était significativement très marqué en présence des trois auxines testées. Le meilleur taux d'induction du cal embryogène était de 63% pour la variété Anwar. La présence de la lumière (16h par jour) avait une incidence favorable sur la cinétique d'apparition de cals embryogènes gynogénétiques. Les premiers résultats ont été obtenus dans la deuxième semaine de culture alors que, dans des conditions d'obscurité, cette période s'est étendue jusqu'à neuf semaines. L'étude a également montré un effet du génotype sur la phase de régénération avec le taux le plus élevé (27%) observé chez la variété Anwar.*

Mots-clés. *Triticum durum – Gynogenèse – Méthodes haploïdes – Génotypes – Plantes vertes.*

I – Introduction

The application of haplodiploidisation to cereals is a technique, at present, integrated into breeding programs and selection (Gallais, 2011). The cultures of anthers, microspores or pollen grains have led to spectacular results in many cereal crops: corn, wheat, rice and barley (Devaux, 1998, Cherkaoui *et al*., 2000, Bordes *et al*., 2006,).

However, few successes are noted in durum wheat. The species is considered recalcitrant to this technique because of the low induction rates of androgenic embryos and the abundance of albinism. The use of gynogenesis (culture of unfertilized ovaries) as a second way of production of pure green lines has been successful since the 1960s for barley (San Noeum 1967), and subsequently in other barley species (Wang and Kuang 1981, Zhou and Yang 1980), corn (Gbaguidi, 2010) rice (Zhou and Yang, 1980) bread wheat (Zhu and Wu, 1979, Devaux, 1998). In durum wheat, few research results are published and the successes achieved remain limited. Determining factors and culture conditions of *in vitro* culture of the female gametophyte would allow the improvement of the potential of durum wheat for the production of doubled haploid green plants. Several studies have shown the significant effect of culture conditions of unfertilized ovaries in some cereals (Mdarhri *et al*., 1998, Mdarhri, 2000, Chlyah *et al*., 2001, Mdarhri *et al*., 2005, Bordes, 2006, Gbaguidi, 2010). In this study, we try to determine the effect of the season, of three auxins and of the photoperiod on gynogenesis of six durum wheat varieties We have already shown the haploid nature of the green plants produced (Mdarhri *et al.*, 1998).

II – Material and methods

In late binucleate stage before anthesis, ears of six varieties of durum wheat (*Triticum durum* L.) Anwar (AN), Jawhar (JW), Yasmine (YS), Belbachir (BB), Sebou (SB) and Kyperonda (KP) were harvested and submitted to a pretreatment in the cold (4°C) during 10 days, then they are treated 3 minutes in 70% ethanol prior to disinfection. This involves, in sterile conditions, immersion in a solution of 2% Tween 20 for 2 min followed by a 20 min treatment in commercial bleach. Finally ears are rinsed three times with sterile distilled water at a rate of 3 min for every rinse. Disinfected ears are stripped of their husks and chaff.

Embryogenic calluses were initiated by culturing unfertilized ovaries on MS basal medium containing 2mg /l 2,4-D, NAA or 2.4.5-T. Cultures were incubated in a growth chamber at (25 ± 1) °C in the absence of light or under a photoperiod of 16h/8h (light /dark).

The embryogenic calluses formed were subcultured onto R9 regeneration medium without growth regulators for two weeks. Then they were transferred onto the same medium supplemented with 2 mg /l BAP and 0,1 mg /l NAA. The shoots obtained were transferred to a rooting medium: MS containing 1mg/l IAA and 1mg/l kinetin.

Results focused on the rate of induction of embryogenic calluses, the kinetics of their appearances and the rate of chlorophyll regeneration. The results of the various experiments were treated by analysis of variance and the significantly different averages were separated by Student's t-test and Newman Keuls at the probability level of 5%.

III – Results

1. Harvest season of spikes and ovaries

A preliminary study was made to determine the best time for harvesting spikes and excision of unfertilized ovaries. The results showed (Fig. 1) that the best rates were noted for the induction period which runs from early March to mid-April with an average rate of 3%. For this study all genotypes were grouped.

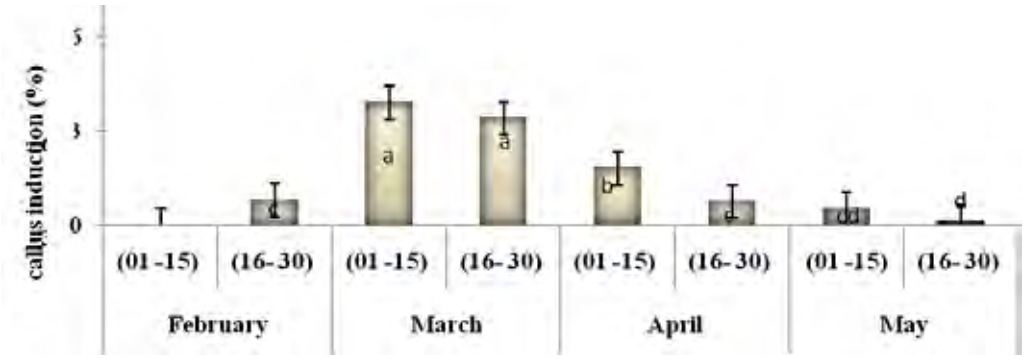
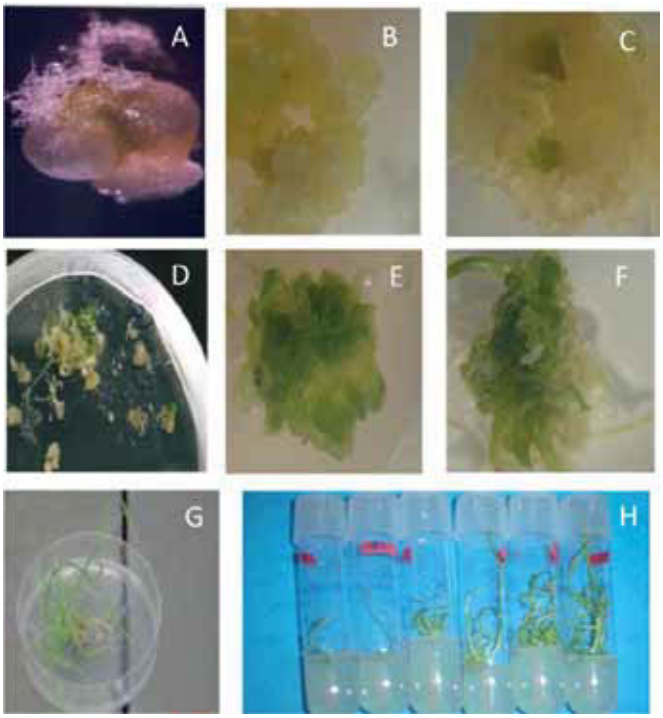


Figure 1. Callus formation as function of the date of culture.

2. Effect of growth regulators and genotype on the induction phase

For the six varieties studied in the presence of the three auxins tested, whitish friable calluses were induced. Transplanting to the regeneration medium resulted in the formation of tufts of green shoots. When these were transplanted to MS rooting medium, they formed roots (Fig. 2). However, genotypic differences significantly affected the rates of callus induction. The variety Anwar (An) has always given the best response allowing the production of 63.52% , 47.21 % and 29.34% in the presence of 2,4- D, T 2.4.5 or NAA respectively (Table 1).



Figue 2. Stages of haploid plant production in durum wheat from unfertilized ovary culture. A) Gynogenetic callus coming out at the base of the ovary. B) Gynogenetic callus transferred to the regeneration medium. C) Green shoots appear in the callus. D) Shoots develop into small plants. E) and F) Tufts of green plants. G) Rooted green plants. H) Multiplication and rooting of plants.

Table 1. Comparison of the effect of three auxins on the induction rate (%) of embryogenic callus from unfertilized ovary culture in six durum wheat varieties.

	Growth Regulator (2mg/l)		
Variety	**2,4-D**	**2,4,5 T**	**NAA**
AN	63.52a	47.21a	29.34a
JW	59.06b	38.64c	17.02c
YS	27.64f	27.87d	19.32bc
BB	45.87c	25.21d	21.41b
KY	38.74[e]	13.26[e]	7.61d
SB	40.39d	41.06b	28.07a
Mean	45.87	32.20	20.46
LSD	1.53	0.76	0.97

The mean values of the same column with the same letter are not significantly different at the 5% level (test of Student-Newman Keuls).

The study of the effect of three auxins on the induction rate showed that 2,4-D gave the best results. Figure 3 shows that the rate of callus induction decreased significantly in the presence of NAA. This variability was marked and highly significant for all varieties. Genotypes were classified according to their mean rates of callus formation after variance analysis (student –Neuman and Keuls test).

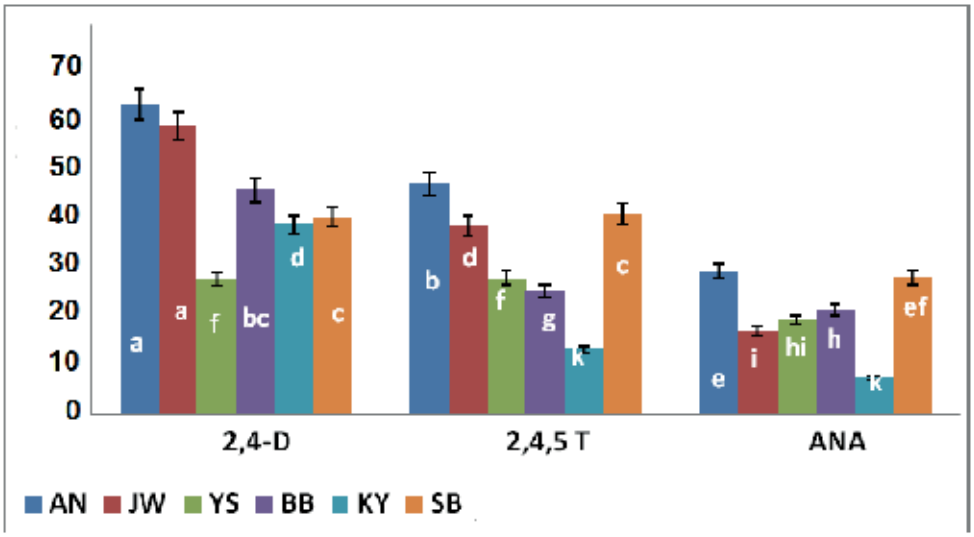


Figure 3. Effect of plant growth regulators on the rate of induction of gynogenetic callus for six varieties of durum wheat. Blocks of the same color with the same letter are not significantly different at the 5% level (test of Student-Newman Keuls).

3. Induction kinetics as a function of photoperiod

After monitoring the kinetics of appearance of embryogenic callus from cultures incubated in the dark and cultures submitted to a photoperiod of 16h per day we concluded the beneficial effect of alternating light and dark (Figure 4). In this last case, the initial responses were observed from the third week of culture with an optimum at the sixth week with an induction rate of 49.78%.

The percentage of induction fell gradually after the seventh week and ended in the 13th week. Cultures incubated in darkness started to produce callus only in the 11th week of culture, this rate increased up to the 17th week with the optimum at the 16th week at 27.89%.

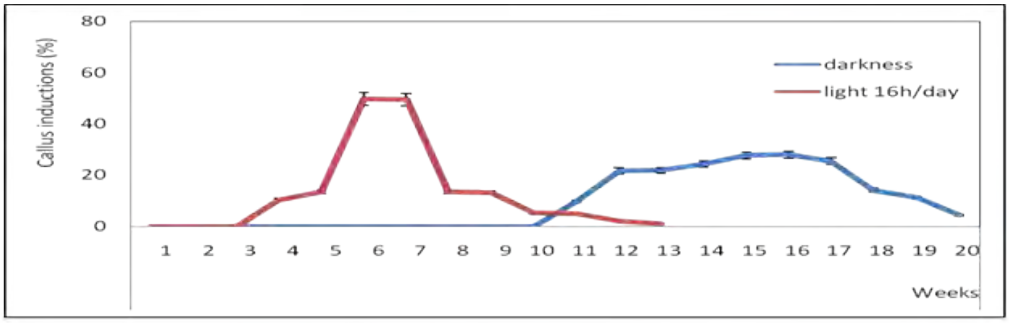


Figure 4. Kinetics of callus induction according to the light conditions.

4. Regeneration of haploid plants

Embryogenic callus obtained from the induction phase were transferred onto the R9 medium without growth regulators at first and then in the presence of BAP and NAA (2,01 mg /l) . The observations showed that all the regenerations were green in the form of tufts. The genotypic effect was striking (figure 5). Average rates of regeneration were between 15 % and about 4.4%, respectively for Anwar and Belbachir. No plants were regenerated from callus derived from the Yasmine and Kyperounda varieties.

The ANOVA showed significant differences among genotypes at a probability level of 5% by the Student- Newman and Keuls test.

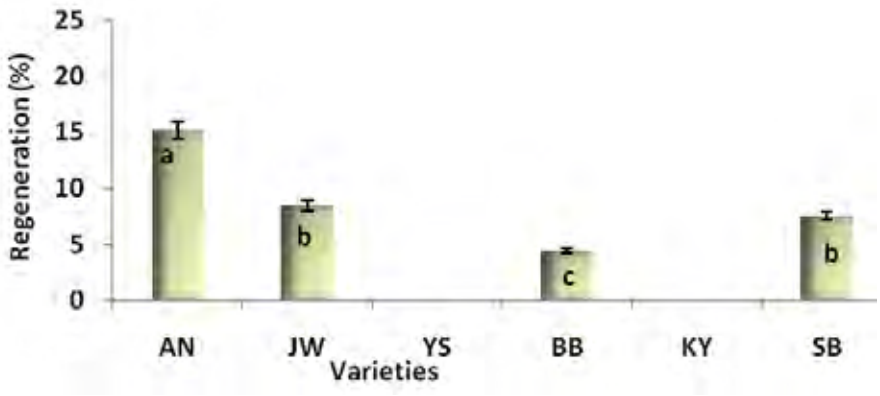


Figure 5. Regeneration of gynogenetic plants.

IV – Conclusion

Improvement of the technique of gynogenesis in durum wheat could bring about its integration into breeding and selection programs. This study showed the importance of culture conditions

and the variable performances of different genotypes. The Anouar variety gave best results both for callus induction and for green plant regeneration.

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Exploitation of SNP markers located on wheat 5A chromosome for the study of syntenic relationship with model species

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Abstract. Wheat is one of the most important world's crop species, however genomic studies are made difficult by the high complexity and width of its genome, which is very large and polyploid. For these reasons, sequence information from sequenced genomes of model species together with the study of the syntenic relationships among them represent a very important tool to elucidate the structure and the function of the complex wheat genome. In the present work, we analyzed the syntenic relationship existing between wheat, rice and *Brachypodium distachyon*, focusing in particular on SNP markers previously mapped on wheat 5A chromosome. The comparative genomic study described in the present work confirmed the great importance of sequence information associated with SNP markers combined with comparative genomics studies. In particular, the work evidenced a co-linearity and a good conservation of loci order between wheat and the two model reference species; such studies will be very useful for understanding the evolution of grass genomes, map based cloning of important genes, and for future wheat genome sequencing and the acceleration of genomic based improvement of these important crops.

Keywords. Wheat – SNP – Synteny – *B. distachyon* – Rice.

Exploitation des marqueurs SNP situés sur le chromosome 5A du blé pour l'étude des relations synténiques entre des espèces modèles

Résumé. *Le blé est une des cultures les plus importantes au monde et cependant, les études génomiques s'avèrent être difficiles à cause de la grande complexité et de la taille de son génome et de sa polyploïdie. Pour ces raisons, les informations de séquence obtenues à partir des génomes séquencés des espèces modèles et l'étude de leurs relations synténiques représentent un outil très important pour expliquer la structure et la fonction du génome du blé si complexe. Dans le présent travail, nous avons analysé la relation synténique entre le blé, le riz et Brachypodium distachyon, en nous concentrant, en particulier, sur les marqueurs SNP précédemment cartographiés sur le chromosome 5A du blé. L'étude génomique comparative illustrée dans ce travail a confirmé la grande importance des informations de séquence associées à des marqueurs SNP, combinées avec des études de génomique comparative. En particulier, les travaux ont mis en évidence une co-linéarité et une bonne conservation de l'ordre des loci entre le blé et les deux espèces modèles de référence ; ces études seront très utiles pour la compréhension de l'évolution des génomes des graminées, pour le clonage de gènes importants à l'aide de la cartographie, et pour le séquençage futur du génome du blé et l'accélération de l'amélioration basée sur la génomique de ces cultures importantes.*

Mots-clés. *Blé – SNP – Synténie – B. distachyon – Riz.*

I – Introduction

Despite the economic importance of wheat, genomic studies on this crop are very difficult due to the high complexity and size of its genome (16,937 Mb), its polyploid nature (2*n* = 4*x* = 28 for durum wheat or 2*n* = 6*x* = 42 for bread wheat), and the large extension (about 80%) of repetitive elements (Vitulo *et al*., 2011). Moreover, the extension of the coding portion of wheat genome, which is expected to account for about 90,000 genes, is only a very small percentage of the total,

and it is quite similar to other grass species such as rice (*Oryza sativa,* 430 Mb) or *Brachypodium distachyon* (300 Mb) with have much smaller genomes (Arumuganathan and Earle, 1991). For all these reasons, sequence information from sequenced genomes of model species, and the study of the syntenic relationships among them represent a very important tool to elucidate the structure of the complex wheat genome, and to infer information about gene order and function on the different chromosomes.

The syntenic relationships existing among *Poaceae* have been well described since many years (Moore *et al*., 1995). In particular, rice and *B. distachyon* have been recently recognized as model species for both comparative and functional genomics in grass species, due to the limited size and to the availability of complete sequences of their genomes. Several authors showed a good co-linearity among large chromosome segments of these two genomes with that of wheat (Sorrels *et a*l., 2003). In particular, regions of synteny to wheat chromosome 5A have been found in many grass species (Sorrels *et al*., 2003); moreover, studies by Moore *et al.* (2005) and Sorrels *et al*. (2003) reported that wheat homoeologous group 5 shows one of the most complex syntenic relationships with rice.

Wheat 5A chromosome accounts for a total size of 827 Mb, representing about 4.9% of wheat entire genome (Safar *et a*l., 2010), with predicted chromosome arms lengths of 295 Mb and 532 Mb for 5AS and 5AL, respectively (Safar *et al*., 2010). The importance of this chromosome is due to the fact that it carries lots of genes controlling important traits, such as *domestication* (*Q*) (Simons *et al*., 2006), resistance to abiotic stresses, frost tolerance (*Fr1*, *Fr2*), vernalization requirement (*Vrn)*, regulation of homoeologous chromosome pairing (*Ph1*), loci (QTLs) for yield and productivity, and several genes for pathogens resistance (e.g. Fusarium head blight).

Several studies have described a co-linearity between wheat 5A short arm and rice chromosomes R12, R11, R5 and R1 (Sarma *et al*., 2000; Qi end Gill, 2001). In particular, Linkiewicz *et al.* (2004) confirmed that the co-linearity between wheat chromosome 5A and rice chromosome 12 spans from 5A short arm to the proximal region of the long arm, suggesting the presence of similar centromere location. Wu *et al.* (1998) hypothesized that similarity between wheat 5AS and rice R11 could have been originated from the duplication of a larger segment of chromosome R12 in R11. On the other hand, 5A long arm shows syntenic regions with the rice chromosomes 9 and 3 (Vitulo *et al*., 2011). The 5A short arm is co-linear to *Brachypodioum* chromosome 4, whereas the long arm shows a close synteny with chromosomes 4 and 1 (Vitulo *et al*., 2011).

In the present study the SNP markers genetically and physically mapped on wheat 5A chromosome by Gadaleta *et al*. (2014, in press) were exploited to study the syntenic and functional relationships among wheat and the model genomes of rice and *Brachypodium distachyon*..

II – Material and Methods

The 90K iSelect array developed by Illumina CSPro® and described by Wang *et al*. (2014) was used to survey a set of 81,587 SNP markers across a recombinant inbred line (RIL) population developed by Gadaleta *et al*. (2012) by crossing the wheat cv. Chinese Spring (CS) and the line Chinese Spring-5A dicoccoides, which is a disomic substitution line carrying the 5A chromosome of CS replaced by the 5A chromosome from *Triticum turgidum* ssp. *dicoccoides*. The same SNP markers were also assayed on a set of aneuploid lines derived from Chinese Spring, including nulli-tetrasomic (NT), di-telosomic (DT) and 12 deletion bin lines, of which four dividing the short arm into 5 bins and 8 dividing the long arm into 9 bins. SNP markers data were integrated into a previous genetic linkage map of wheat 5A chromosome developed by Gadaleta *et al*. (2012), in order to produce a high-density and more saturated map. A sub-set of 50 SNP markers genetically and physically mapped on wheat 5A chromosome were employed to carry out a comparative study among wheat and the sequenced genomes of two model species, represented by *Brachypodium*

distachyon and *Oryza sativa*, with the objective to detect orthologous and syntenic relationships among the three genomes. The similarity search was carried out by launching each wheat SNP sequence against *B. distachyon* and *O. sativa* genomes by means of the BLAST tool at the site http://www.phytozome.it. The orthologous SNP sequences were also subjected to a BLASTX analysis against non-redundant protein database for assigning putative functions. For both analysis, an E value cut-off of 10^{-7} was used.

III – Results and Discussion

In the present work we analysed the orthology relationships among wheat, *Brachypodium distachyon* and *Oryza sativa* by focusing on wheat chromosome 5A, and in particular exploiting 50 of the SNP markers genetically and physically mapped on this chromosome by Gadaleta *et al*. (2014, in press). We choose this chromosome because different studies (Moore *et al*., 2005; Sorrels *et al*., 2003) reported that wheat homoeologous group 5 has one of the most complex syntenic relationships with rice and other grass species among all the chromosome groups. Out of 50 SNP sequences analysed, 45 of them identified a putative orthologous with an E value > 10^{-5}; in particular, 35 wheat SNP sequences showed similarity with both model species, 10 SNP sequences only with *B. distachyion* and five only with *O. sativa*. The majority of the orthologous wheat SNPs were found to be related to *Brachypodium* sequences located on chromosomes 4 and 1, while the remaining 2 showed similarity with sequences on chromosomes 2 and 3, respectively. Rice sequences with the highest level of homology to wheat SNPs were identified on chromosomes 3, 12, 9. In particular, 30 wheat.

SNPs matched with genomic sequences located on rice chromosomes 12 and/or 3, while seven wheat SNPs matched with rice chromosome 9, and three with rice chromosome 7. These results are in agreement with what reported by previous studies (Vitulo *et al*., 2011), as the wheat SNPs genetically and physically mapped on 5A chromosome short arm showed a close relationship to the rice chromosome 12, while the majority of SNP sequences located on 5A long arm showed a closer synteny with rice sequences on chromosomes 9 and 3.

By comparing the map position of the analyzed 50 wheat SNP markers with the genomic position of the syntenic sequences found on *Brachipodium distachyon* and rice chromosomes, we confirmed the good co-linearity existing among these three genomes. In fact, a good conservation of sequence order was observed among the wheat chromosome 5A, and the chromosomes 12, 9, 3 of rice and 1 and 4 of *Brachipodium*, as already reported by other several authors (Sidhu *et al*., 2008; Sorrels *et al*., 2009; Vitulo *et al*., 2011). Similar homologous relationships were also reported by Sorrels *et a*l. (2009), in fact a small number of SNPs found homologous sequences to the *Brachipodium* chromosomes 2 and 3 and to rice chromosomes 2 and 7. Comparative genomics studies and the elucidation of the syntenic relationships existing among species in the grass family, is of particular importance because the family comprises a number of economically important crops, and the genomic analysis using model species could offer a potentially useful strategy for the development of highly saturated genetic linkage maps and for gene discovery in cultivated wheat (Gadaleta *et al*., 2012).

In order to identify some of the functional relationships underlying the synteny among wheat, rice and *Brachypodium* genomes, we tried to attribute a putative function to all the 50 analysed SNPs (Fig.1).

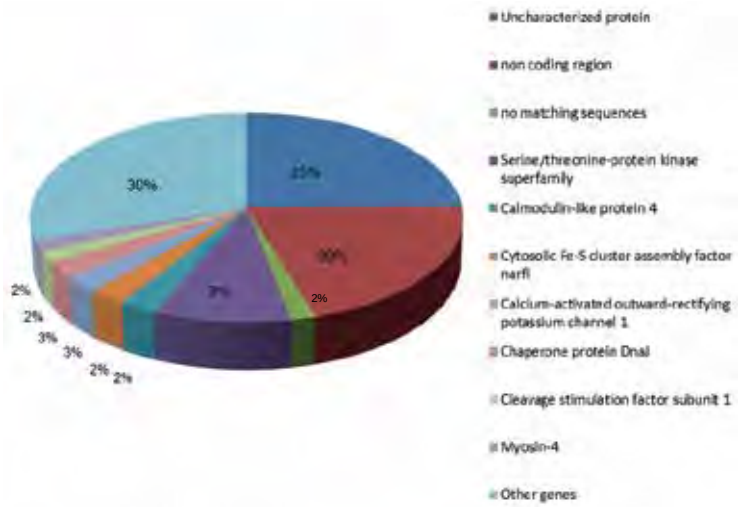

Figure 1. Graphic representation of putative function of 50 SNP sequences mapped on chromosome 5A by Gadaleta *et al*. (2014, in press).

Interestingly, we found that most of the wheat SNP sequences fell into coding regions, while only 20% matched with sequences belonging to non-coding regions of the two reference genomes. A very high percentage of the orthologous sequences identifying coding regions (25%) matched with predicted uncharacterized proteins, i.e., proteins of unknown function belonging to rice, whereas the other identified genes were found to be involved in several metabolic pathways. In particular, 30% of wheat SNP sequences matching with genes identified calmodulin-like proteins, while 9% were found to match with polypeptides of known function represented by a protein kinase superfamily. The remaining 16% represented other classes of genes. In conclusion, we can say that this work confirmed the great importance of sequence information associated with SNP markers combined with comparative genomics studies. Because of the syntenic relationships existing among grass species, knowledge from a model species could greatly facilitate the study of other important cereal crops. In particular in wheat, which is characterized by a very wide and complex genome, not yet completely sequenced, SNPs represent a powerful tool which could facilitate comparison with model species with the aim to infer information about the evolutionary mechanisms, order of genes and the putative function of genomic sequences.

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Efficient callus induction, plantlets regeneration and genetic transformation of durum wheat

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Abstract. In this study, we tested matured embryos as explants from eight Moroccan durum wheat varieties (Irden, Marzak, Kyperounda, Isly, Amria, Karim, Marouane, and Tomouh) and five induction media (M1 to M5) based on MS media (macro and oligo-elements) which differed with respect to concentrations of plant hormones (2,4-D and BA), vitamins, sucrose, maltose, L-asparagine, and solidifying agents. All tested media induced embryogenic callus for the varieties and regenerated plantlets. However, a significant effects of variety, medium and variety x medium interaction were observed for callus induction and regeneration. We used embryogenic callus derived from immature embryos for genetic transformation of *HVA1* gene of barley. In this study, we identified for the first time, favorable media for induction and regeneration from mature embryo explants of Moroccan durum wheat varieties. Also, we successfully transformed durum wheat with *HVA1* gene via particle bombardment. Since the transgenic plants developed in this study contained barley *HVA1* gene, further analysis for tolerance to water and salt stresses in subsequent generations needs to be undertaken.

Keywords. Durum wheat – Mature embryos – Plantlets regeneration – Somatic embryogenesis – Genetic transformation – HVA1 gene.

Induction efficace de cal, régénération de plantules et transformation génétique du blé dur

Résumé. *Dans cette étude, nous avons testé des explants d'embryons matures, provenant de huit variétés marocaines de blé dur (Irden, Marzak, Kyperounda, Isly, Amria, Karim, Marouane et Tomouh) et cinq milieux de culture d'induction (M1 à M5) à base de milieu MS (macro et micro-éléments) qui différaient par la concentration d'hormones (4,4-D et BA), vitamines, saccharose, maltose, L-asparagine et agents solidifiants. Tous les milieux testés ont induit un cal embryogène chez les variétés et les plantules régénérées. Cependant, un effet significatif de la variété, du milieu, et de l'interaction milieu x variété a été observé pour l'induction de cal et la régénération. Nous avons utilisé des cals embryogènes issus d'embryons immatures pour la transformation génétique avec le gène HVA1 d'orge. Dans cette étude, nous avons identifié pour la première fois, un milieu favorable pour l'induction et la régénération à partir d'explants d'embryons matures des variétés marocaines de blé dur. Nous avons aussi transformé avec succès le blé dur avec le gène HVA1 d'orge par bombardement de particules. Puisque les plantes transgéniques développées dans cette étude contenaient le gène HVA1 d'orge, des analyses supplémentaires devraient être menées pour évaluer la tolérance au stress hydrique et salin chez les générations suivantes.*

Mots-clés. *Blé dur – Embryon mature – Régénération de plantules – Embryogénèse somatique – Transformation génétique – Gène HVA1.*

I – Introduction

Durum wheat (*Triticum turgidum* L. subsp. *durum*) is the most important cereal crop in the Mediterranean basin. In Morocco, durum wheat is grown over an area ranging from 1 to 1.2 million hectares annually, and ranks the third after bread wheat and barley with respect to production (MAPM 2011). The country's wheat productivity has been affected by various biotic and abiotic stresses (Karrou 2003).

Immature embryos were the most efficient tissue source to regenerate plants *in vitro* (Jones 2005). However, it is usually difficult to obtain immature embryos throughout the year, and the

suitable stage for their culture is also strictly limited. The use of mature embryos from dry seeds has several advantages: mature embryos are easy to handle, available year round and in bulk quantities. For this purpose, mature embryos as a favourable source of explants are explored broadly in wheat tissue culture. Though the major hurdle with mature embryos as explants is their low frequency of plant regeneration (Ren *et al*. 2010). Although plant regeneration has been achieved previously from callus cultures derived from mature embryos of durum wheat (Neiverth *et al*. 2010), the regeneration efficiencies were inconsistent and also depended on genotype and medium composition (He *et al*. 1988). Moreover, studies on *in vitro* plantlet regeneration in durum wheat using mature embryos as explants derived from Moroccan varieties are lacking.

In Morocco, drought is a major environmental stress that limits cereal productivity and consequently Morocco is not self-sufficient in cereal production. Genetic transformation of crops is a powerful research tool for gene discovery and function to investigate genetically controlled traits and is fast becoming a key element in the process of varietal improvement. Development of a reliable genetic transformation protocol is necessary to facilitate genetic improvement of wheat for drought tolerance. The development of methodology for the delivery of genes from other species into intact plant tissues by particle bombardment has revolutionized the field of wheat transformation (Bahieldin *et al*., 2005; Matsumoto and Gonsalves, 2012). However, no reports are available regarding genetic transformation of Moroccan durum wheat cultivars.

The objective of this study was to define suitable media for callus induction and plant regeneration of Moroccan durum wheat varieties using mature embryos as explants. While doing so, we compared the effects of media, varieties and their interactions on callus induction and plant regeneration obtained from mature embryos as explants. Here we also report successful transformation of a Moroccan durum wheat variety by particle bombardment using immature embryos as explants source.

II – Materials and Methods

1. *In vitro* culture

Field grown seeds (matured caryopses) of durum wheat cultivars Irden, Marzak, Kyperounda, Isly, Amria, Karim, Marouane and Tomouh were used as the source for mature embryo culture. The seeds were procured from Experimental Research Station of INRA at Marchouch, Rabat, Morocco.

The seeds were then surface-sterilized (Tinak *et al*. 2013), Mature embryos were aseptically dissected away from the caryopses, and the remaining endosperm and radical were removed to prevent early germination. The embryos were placed in a Petri dish containing the induction medium based on M1 (Iraqi *et al*. 2005), M2 (Karim *et al*. 2005), M3 (Gadaleta *et al*. 2006), M4 (Pellegrineschi *et al*. 2002),or M5 (Przetakiewicz *et al*. 2003) (Table 1). The relative fresh weight growth rates (RFWGR) of callus were determined:

$$RFWGR = (FW_f - FW_i) / FW_i \times 100$$

where FW_f = final fresh weight and FW_i = initial fresh weight.

After five weeks, embryogenic calli from each replication were transferred to the regeneration medium (Iraqi *et al*. 2005). Percentage of plants regenerated was calculated as follows: (the number of plantlets regenerated / the number of callus transferred to the regeneration medium) x 100.

Table 1. Media composition.

	Medium tested				
Component	**M1**	**M2**	**M3**	**M4**	**M5**
Macroelements	MS	MS	MS	MS	MS
Oligoelements	MS	MS	MS	MS	MS
Vitamins	MS	MS	Thiamin	MS	B5
Fe-EDTA	MS	MS	MS	MS	MS
L-asparagine (mg/L)	150	-	150	-	-
Myo-Inositol (mg/L)	100	100	100	100	100
Sucrose (g/L)	20	20	-	30	20
Maltose (g/L)			40		
2,4-D (mg/L)	2	2.5	1	2.5	3
BA (mg/L)	-	2.5	-	-	-
pH	5.7- 5.8	5.7- 5.8	5.7- 5.8	5.7- 5.8	5.7- 5.8
Phytagel (g/L)	2.5	2.5	3.5	-	2.5
Bacto agar (g/L)	-	-	-	8	-

2. Genetic transformation

Immature embryos were excised out from immature seeds collected 12-16 days post-anthesis sterilized (Iraqi *et al*., 2005) and cultured on induction and maintenance medium (MS Asp; Iraqi *et al*., 2005). The embryos whose cells started rapid division were selected for subsequent transformation and subculturing. The plasmid used for bombardment pBY520 contained the linked selectable marker/herbicide resistance *bar* (phosphinothricin acetyl transferase) gene (driven by cauliflower mosaic virus 35S promoter and the nopaline synthase nos terminator) and the barley *HVA1* gene (driven by rice *Act1* promoter and terminated by the potato protease inhibitor *pin II*). After the bombardment, embryos were left in the same medium in the dark for 16 hours at 25 °C and subsequently transferred to the MS Asp medium without mannitol for a period of 4-5 days. The resistant calluses were transferred to the regeneration medium (Iraqi *et al*., 2005) supplemented with PPT. For root induction, the regenerated shoots were transferred in MS half-strength medium lacking hormones and supplemented with PPT. Herbicide resistance of the putative transgenic wheat plants was determined by painting leaves of plants at the fifth or sixth leaf stage with basta (0.3% w/v) with 7 days between applications to minimize escapes. Plants were scored as susceptible or resistant according to the degree of leaf desiccation after 7 days (Pellegrineschi *et al*., 2002).

III – Results and discussion

1. *In vitro* culture

Callus production was strongly influenced by the media and the variety used. A significant ($p < 0.001$) interaction between variety and medium was observed. RFWGR of callus calculated after 5 weeks of culture on different induction and maintenance media showed that the highest RFWGR was observed on M1 for varieties Irden (8914%), Marouane (7249%), Marzak (8969%) and Amria (9792%); and for the rest of varieties, M5 gave the highest RFWGR (Table 2). In all these varieties, except Irden, culturing on M3 medium resulted in lowest RFWGR of callus.

Table 2. Effect of medium and variety on relative fresh weight growth rate of callus (RFWGR) and plantlet regeneration in durum wheat.

	RFWGR (%)					Plantlet regeneration (%)				
Variety	**M1**	**M2**	**M3**	**M4**	**M5**	**M1**	**M2**	**M3**	**M4**	**M5**
Irden	8914	1773	2462	5576	6713	30.6	34.58	34	21.2	11.2
Marouane	7249	6832	4281	5458	5634	35.44	35.54	51.32	24.5	15.88
Kyperounda	5438	3520	3290	5558	6039	39.6	23.92	55	21	13.2
Isly	5712	3379	3956	3826	6153	37	47	49.2	23.9	20.22
Marzak	8969	4668	4396	6760	6861	24.6	17.54	52.8	32.42	31
Karim	6832	5118	4335	6788	8137	44.36	17.2	58.58	29.8	47.42
Amria	9792	6090	4930	6764	8500	27	39	36.4	9	20
Tomouh	2231	1975	2020	1594	2620	35.6	31.8	65	32	11.8

After 5 weeks, callus was transferred to the regeneration medium. After 8 weeks of culturing, the number of plantlets regenerated was recorded (Table 2). The induction and maintenance media used for callus induction had a significant effect on plantlet regeneration ($p < 0.001$). Even though M1 and M5 showed higher RFWGR for callus induction after 5 weeks of culture (Table 1), the plantlet regeneration rates were lower from those calluses, 34.27% and 21.34%, respectively (Table 1). On the other hand, M3 medium which induced least amount of callus, regenerated the highest percentage of plantlets (50.29%; Table 1), indicating M3 medium induces more embryogenic callus than other media.

M1 medium yielded the highest RFWGR for the varieties Irden, Marouane, Marzak and Amria, whereas M5 for the rest of the varieties (Table 2). In all these varieties, except 'Irden', culturing on M3 medium resulted in lowest RFWGR of callus. These results indicate that callus weight improved by increasing 2,4-D (auxin) to 2 mg/L (as in the case of M1) in agreement with the finding of Malik *et al*. (2003) with mature seeds of wheat in the subculture media ; or 3 mg/L (as in the case of M5), similar to the results obtained by Munazir *et al*. (2010) with mature seeds culture of wheat. The beneficial effect of 2 and 3 mg/L of 2,4-D on callus induction of wheat mature embryos was also found by Raziuddin *et al*., 2010. In contrast, Mendoza and Kaeppler (2002) showed in bread wheat cultivar Bobwhite that callus weight tended to decrease when concentration of 2,4-D was increased.

Regeneration of plantlets from mature embryos derived callus was also controlled by their genetic makeup (Bahman *et al*., 2012). In our study, the varieties Karim, Isly, and Tomouh produced higher plantlet regeneration, whereas Irden and Amria produced significantly lower plantlet regeneration. The other genotypes were in between. However, plantlet regeneration varied significantly depending on the varieties and the induction and maintenance media used. For the varieties Marouane, Kyperounda, Marak, Karim, and Tomouh, the favorable medium was M3, whereas, for Isly, Irden and Amria, both M2 and M3 were favorable (Table 2).

2. Genetic transformation

The untransformed callus became yellow, like that of the controls (Fig. 1c). The resistant callus was then transferred to regeneration media (Iraqi *et al*., 2005) supplemented with 3 mg/L of basta. Green and vigorously growing plants survived (Fig. 1d) were transferred to the rooting medium (half-strength MS) also with 3 mg/L of basta (Fig. 1e). The regenerated plantlets were transferred to pots in the greenhouse for acclimation (Fig. 1f). 14 plants, only from 'Irden' variety, survived on the 3 mg/L of basta-selection. Recognizing that the selection system could permit false positive plants, a second selection was done by painting leaves with 0.3% of basta to demonstrate the expression of the basta herbicide-resistance gene *bar* (Pellegrineschi *et al*., 1998). After 7 days, for all the putative transformants, leaves painted stayed green, whereas for the control they became yellow and died. These putative transformants were phenotypically normal and fertile (Fig. 1g).

In this study, we identified for the first time, favorable media for induction and regeneration from mature embryo explants of Moroccan durum wheat varieties. Also, we successfully transformed durum wheat with *HVA1* gene via particle bombardment. Since the transgenic plants developed in this study contained barley *HVA1* gene, further analysis for tolerance to water and salt stresses in subsequent generations needs to be undertaken.

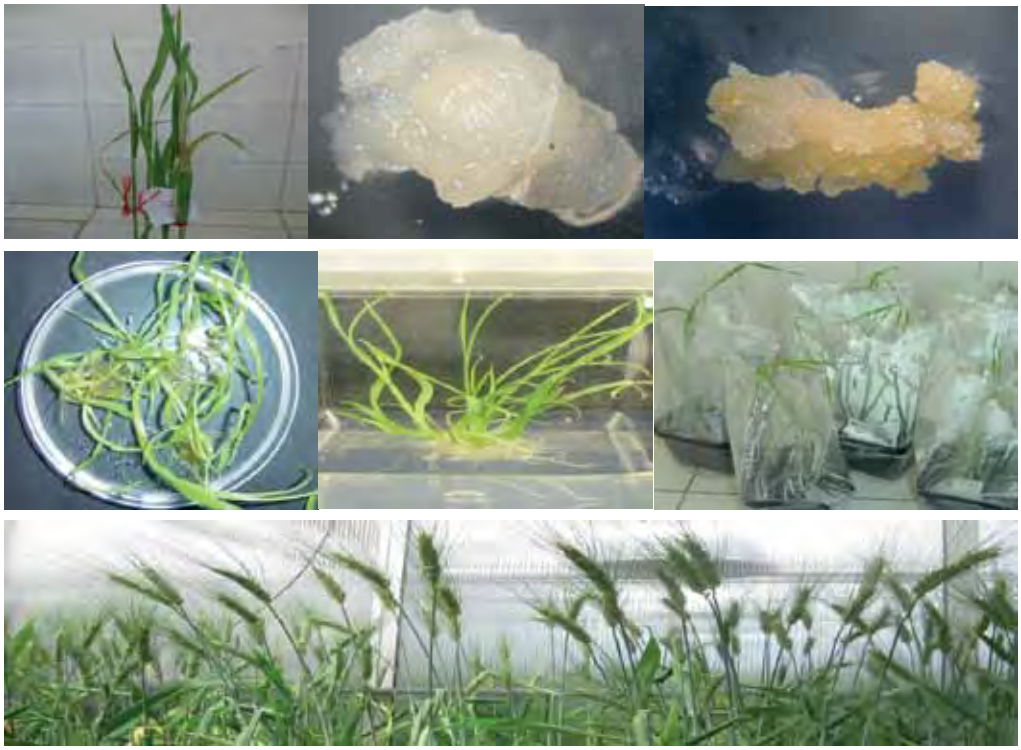

Figure 1. Genetic transformation of variety 'Irden' of durum wheat by particle bombardment using immature embryo-derived calli as the target tissue (Upper a, b, c; middle d, e, f; bottom g). The calli were bombarded with plasmid pBY520. a: basta leaf paint assay for transformant. b, c : callus tissue after selection with 3 mg/L of basta: untransformed calli (yellow) and transformed callus (white). d: putative transformed plantlets in regeneration medium with 3 mg/L of basta. e: putative transformed plantlets in rooting medium with 3 mg/L of basta. f: acclimatation of putative transformed plants. g: T1 progeny plants.

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Session 4

Genetics and breeding for durum wheat yield and sustainability

Durum wheat adaptation and sustainability: ensuring accurate phenotyping for improving drought tolerance and yield stability

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Abstract. In most of its area of cultivation, durum wheat is facing water shortage. Climate change is expected to produce more frequent drought events. As a consequence, durum wheat breeders are now considering drought tolerance as an essential breeding objective. However, phenotyping still represents a major bottleneck in selecting for abiotic tolerance traits. Efficient phenotyping implies accurate i) definition of target populations of environments based on the performance of known varieties, ii) choice and characterization of the managed stress environment, iii) stress monitoring and iv) "secondary" (drought tolerance related) traits measurement. Improving drought phenotyping in durum wheat should take advantage of the new technologies developed to refine target populations of environments definition, precisely describe managed stress environments and efficiently monitor drought stress. This will permit establishment of a precise typology of target populations of environments based on drought scenarios, better predict adaptation of the tested germplasm, and finally increase response to selection. The utilization of geographic information system (GIS) tools and more integrative drought tolerance related traits assessment methods should be encouraged. The development of research networks among different partners and establishment of phenotyping platforms in the main durum wheat cultivation areas could simulate sharing of knowledge and experience and quicker evaluation of germplasm in diverse environments and facilitate dissemination and germplasm products, thus ensuring larger impact of breeding efforts.

Keywords. Drought – Secondary traits – Target populations of environments – Managed stress environments – *Triticum durum* – Phenotyping.

Adaptation et durabilité du blé dur : assurer un phénotypage précis pour améliorer la tolérance à la sécheresse et la stabilité du rendement

Résumé. *Dans la zone de culture du blé, l'eau est généralement rare. D'après les prévisions, le changement climatique s'accompagnera de l'apparition plus fréquente des sécheresses. Par conséquent, les sélectionneurs du blé dur considèrent actuellement la tolérance à la sécheresse comme un objectif essentiel pour la sélection. Toutefois, le phénotypage représente encore un obstacle majeur dans la sélection pour les caractères de tolérance abiotique. Un phénotypage efficace implique i) la définition précise des populations cibles des environnements, basée sur la performance des variétés connues, ii) le choix et la caractérisation de l'environnement sous conditions de stress, iii) le suivi du stress et iv) la mesure des caractères « secondaires » (associés à la tolérance à la sécheresse). L'amélioration du phénotypage de la sécheresse chez le blé dur devrait tirer parti des nouvelles technologies développées pour affiner la définition des populations cibles des environnements, décrire précisément les environnements sous condition de stress, et suivre efficacement le stress de la sécheresse. Cela permettra d'établir une typologie précise des populations cibles des environnements basée sur des scénarios de sécheresse, de mieux prévoir l'adaptation du matériel génétique testé et enfin, d'augmenter la réponse à la sélection. L'utilisation d'un système d'information géographique (SIG) et l'intégration des méthodes d'évaluation des caractères liés à la tolérance à la sécheresse devraient être encouragées. Le développement de réseaux de recherche entre les différents partenaires et la création de plateformes de phénotypage dans les principales zones de culture du blé dur pourraient stimuler le partage de connaissances et d'expérience et favoriser une évaluation plus rapide du matériel génétique dans des environnements divers, facilitant ainsi la diffusion des ressources phytogénétiques et assurant une plus grande efficacité des efforts de sélection.*

Mots-clés. *Sécheresse – Caractères secondaires – Populations cibles des environnements – Gestion des environnements de stress – Triticum durum – Phénotypage.*

I – Introduction

Durum wheat is cultivated on 17 million ha worldwide and represent around 8% of the total wheat area and 6% of the wheat production (Belaid, 2000). It is mainly grown in West Africa (4.5 million ha) and North Africa (3.3 million ha). In Europe, where the crop covers around 2.5 million ha, durum wheat is cultivated in the southern part of the continent (Italy, France, Spain and Greece). In North America (2.9 million ha), it is mainly found in the Saskatchewan province in Canada, in North Dakota, Montana, Minnesota, South Dakota and California States in the USA, and in the States of Sonora, Baja California, Sinaloa, and Baja California Sur in Mexico. In South America, its cultivation is limited to the central part of Chile and the southern part of the Buenos-Aires Province in Argentina. Durum wheat cultivation is also significant in Australia (New South Wales and Queensland), Russia and India. In most of these regions, environmental stresses as well as pests and diseases drastically limit crop production and reduce the commercial and utilization value of the grain (Morancho, 2000). Climate change is expected to increase the effects of these constraints and to move durum wheat cultivation toward higher latitude areas where it will experience unfamiliar pests, diseases, weeds, and soil constraints.

The socio-economic impact of environmental stresses on yield and quality is of particular importance in the marginal areas of West Asia and North Africa (WANA). In this region durum wheat, an important component of cropping systems, is a main staple food crop that it is critical to food security, and income generation for resources-limited farmers. It is mainly grown under rainfed conditions (Nachit and Ouassou, 1988) and rainfall explains 75% of its yield variation (Blum and Pnuel, 1990). Finally, these regions are also predicted to face the most dramatic and negative changes in climate predicted for any part of the world, particularly more frequent droughts, increased evapo-transpiration, and changes in rainfall patterns (Thomas, 2008). Crop yields are expected to decrease by as much as 10–30% by the 2080 if no efforts are made to mitigate climate change effects (IPCC, 2001).

In many countries of the WANA region, farmers still traditionally grow durum landraces that are well adapted to severe moisture stress conditions but give a poor yield in more rainy years relative to modern cultivars. Those landraces still cover more than 20% of the area (Heisey *et al.*, 2002). Over several decades, breeders have attempted to produce wheat cultivars adapted to these semi-arid environments with limited success in earlier years. Breeding work for drought-prone environments was largely empirical, with grain yield being the primary trait for selection. Then, with the use of indirect selection, modern cultivars have been developed that yield the same as the traditional cultivars in dry years while showing a better response to more favourable conditions of moisture and nutrient supply (Osmanzai *et al.*, 1987). Due to their improved yield stability, these modern cultivars are increasingly grown in dry regions, with rates of adoption approaching those attained in irrigated and high rainfall areas (Heisey *et al.*, 2002).

Further progress in developing drought tolerant germplasm depends on the efficiency of breeding methodologies. Despite the huge amount of information provided by molecular biology in the past few decades, the application of these techniques in the development of improved germplasm has been quite disappointing, largely because the present phenotyping approaches and methods still limit our ability to capitalize on plant functional genomics and modern breeding technologies (Tuberosa, 2012). An improvement of approaches and tools and a more rigorous application of the proposed methods are required to accurately address complex traits and generate the high-quality quantitative data that are needed for genetic analysis and gene identification and transfer. This may allow information from molecular experiments to be more efficiently translated into plant performance in farmers' fields.

II – Phenotyping, the main bottleneck in breeding for abiotic stress tolerance

Plant phenotyping (from the Greek *phainein*, to show) is the comprehensive assessment of plant complex traits such as growth, development, tolerance, resistance, architecture, yield, and the basic measurement of individual quantitative parameters that form the basis for the more complex traits. Plant phenotyping has been performed by farmers, since humans started to select plants, to increase yield or enhance other desirable traits, and during the last century by breeders. It was at that time mostly based on experience and intuition. Over the last two decades, some progress has been done in the development of more reproducible measurements reducing the individual subjectivity factor of the phenotyping person. However, the basic attributes of a good phenotyping approach are not just the accuracy and precision of measurements, but also the relevancy of experimental conditions. Efficient phenotyping implies accurate i) definition of target population of environments, ii) characterization of the testing environment or managed stress environments (MSE), iii) stress monitoring and characterization and iv) measurement of secondary traits.

III – Identification of target populations of environments

Any variety is adapted to several environments. Fischer *et al.* (2003) refer to this group of environments as the target population of environments (TPE). Deploying different cultivars for different TPEs is the only way to reduce genotype by environment interactions. A TPE, also called yield stability target by Annicchiarico (2002), can be defined as the set of all environments in which an improved variety is expected to perform well. An important objective for breeders is to clearly define the TPE for which each variety is developed. The environments constituting a TPE must be sufficiently similar for one genotype to perform well in all of them.

There are several complementary ways to define the TPE. A first step is the definition of mega-environments, based on information about environmental constraints, mainly derived from breeder's experience (Rajaram *et al.*, 1995). The provided information can be refined through an analysis of the performance of known varieties and the genotype by environment interaction (Nachit *et al.*, 1992). More recently, new tools provided by spatial analysis can also help defining TPE and target genotypes (Hyman *et al.*, 2013).

1. Definition of mega-environments

The definition of mega-environments is mainly based on spatial information (mainly provided by breeder's experience) about environmental constraints (including water availability) at the ecosystem or sub-ecosystem level. A total of 12 wheat mega-environments have been defined by Rajaram *et al.* (1995) (Table 1). These mega-environments are broad, often non-contiguous or trans-continental areas with similar biotic or abiotic stresses and cropping systems (Braun *et al.*, 1996).

Durum wheat is mainly cultivated in Mediterranean-type climates (i.e. the Mediterranean Basin which represent 60% of the total area under Mediterranean climate, Central Chile, Western and Southern Coast of Australia and California). Durum wheat growth and yield are limited, in these environments, by low temperatures shortly after the crop establishment and water deficit often associated with high temperatures during the reproductive phase of the growth cycle. This situation corresponds in Rajaram's classification of mega-environments to ME4A (winter rain or Mediterranean-type drought mega-environment) which cover half of the total durum wheat cultivated area and in which durum wheat is more cultivated than bread wheat (Table 2). Durum wheat is also cultivated in ME1 (Nile Valley, Egypt and Yaqui Valley, Mexico), ME2A (Ethiopia), ME4B (southern Cone of Latin America), ME4C (India), ME11 (Russia) and ME12 (Turkey). The

high yields obtained in ME1 and ME11, compared to other MEs highlight the impact of water limitations on grain yield (Heisey *et al.*, 2002).

Table 1. Wheat mega-environments with their main features (according to Rajaram *et al.*, 1995).

ME[a]	Sub ME	Moisture regime	Temperature	Wheat type	Area (%)	Production *(Ml t)*
ME1		Irrigated	Temperate	Spring	36.1	83
ME2		High Rainfall (>500 mm)	Temperate	Spring	8.5	25
ME3		High Rainfall (>500 mm) Acid Soil	Temperate	Spring	1.9	3
ME4		Low Rainfall (<500 mm)	Temperate/hot	Spring	14.6	20
	ME4A	Winter rain or Mediter.-type drought				
	ME4B	Winter drought or Southern Cone-type rainfall				
	ME4C	Continuous or subcont.-type drought				
ME5		Tropical	Hot	Spring	7.1	12
	ME5A	Low-humidity tropics				
	ME5B	High humidity tropics				
ME6		Semi-arid	Temperate	Spring	6.2	13
	ME6 A	High rainfall				
	ME6 B	Semi-arid				
ME7		Irrigated	Cool	Facult.		
ME8		High Rainfall	Cool	Facult.	10.0	23
ME9		Semi-arid	Cool	Facult.		
ME10		Irrigated	Cold	Winter		
ME11		High Rainfall	Cold	Winter	15.0	30
ME12		Semi-arid	Cold	Winter		

Table 2. Wheat mega-environments in which durum wheat is significantly cultivated (from Heisey *et al.*, 2002).

Mega-environment	Area (million ha)	Percentage of the total durum wheat cultivated area	Percentage of the total wheat area	Average durum wheat yield (t ha^{-1})
ME1	0.6	7	1.6	4.15
ME2A	2.1	26	29.6	1.99
ME4A	4.0	50	67.8	1.19
ME4B	0.1	1	3.1	2.06
ME4C	0.1	1	1.5	0.97
ME11	0.1	1	2.8	4.80
ME12	1.1	14	19.3	1.45

Within the Mediterranean region, Nachit (1998) identified three main agro-ecological zones (continental areas with low winter temperatures, temperate areas with mild winters and high altitude areas with severe cold winters). Similarly, Eser (1998) defined three environments for durum wheat cultivation in Turkey, the spring zone, the central plateau and transitional zone (winter and facultative wheat), and the southeast (spring and facultative).

However, the mega-environments and agro-ecological zones do not always offer a sufficient level of resolution in the definition of TPEs. This is particularly true for the Mediterranean region where rainfall and temperatures markedly differ due to differences in topography, nearness of regions with temperate or arid climates and maritime and continental influences (Ryan *et al.*, 2006). Genotype by environment interaction analysis and spatial analysis are useful tools to refine the TPE definition.

2. Use of genotype by environment interaction analysis

A. Implementation of multi-local trials

An important objective, by implementing multi-local trials and analyzing genotype by environment interaction (GEI) is, besides describing the behaviour of genotypes across different environments, to define groups of locations that share the same best cultivar(s), ie, that show little or no crossover (Yan and Rajcan, 2002). As there is a large non-predictable component of GEI associated with year-to-year variation, particularly in the Mediterranean climate considered as the most variable of the world (Ward *et al.*, 1999) and characterized by a high fluctuation of precipitations (Keatinge *et al.*, 1986), it is sometimes difficult to define consistent patterns for the grouping on the basis of locations (Cooper *et al.*, 1999). Substantial datasets are consequently required to accurately estimate frequencies of environmental types based on variable water conditions.

If the TPE is too narrowly defined, few trials will be conducted within each TPE and least significant difference values will be very large, preventing accurate evaluations and reducing progress from selection. The TPE might include three to five evaluation sites. Evaluation of the GEI helps to decide on the number of TPEs for the breeding program. In rain-fed environments, GEI may be large and a high number of TPEs, each served by different varieties, may be optimal. Since each new TPE will need additional breeding and testing resources, there is however a practical limit to the number of TPEs used in a breeding program. Moreover, in some TPEs, the size of the target area can be insufficient to justify the resources required for a separate effort, and the breeders should rely on the spill-over of a variety from another TPE. A compromise should be consequently searched between precisely defining the TPE and achieving enough replication within it. The biplot analysis and the AMMI (additive main effects and multiplicative interaction) and GGE (genotype main effects and genotype × environment interaction effects) models are the most commonly used for clustering location and defining TPEs (Yan *et al.*, 2007). Table 3 provides a list of attempts to define TPEs for durum wheat in the Mediterranean region.

B. Analysis of historical data

Most breeding programs routinely collect data from multi-environment trials (METs). From the 1960s to the 1980s, the Centers of the Consultative Group on International Agricultural Research (CGIAR) produced great networks of testing sites all over the world, particularly for wheat (e.g., Peterson and Pfeiffer, 1989). Many of the results are archived, and the analysis of these historical sets of data can contribute defining TPEs, by allowing clustering of environments, based on the correlation of variety means across trials. This method of grouping environments in the TPE should only be used if data from trials containing 20 or more varieties are available over several years.

3. Use of spatial analysis

Several advances over the last few decades have improved the capacity of spatial analysis to contribute to phenotyping and GEI analysis (Hyman *et al.*, 2013). Advances in the development of computer hardware and software have permitted types of analysis that were impossible to carry out before and availability of climate data in digital formats has been key resource for spatial analysis in agriculture. These advances have led to the development of more precise agro-ecological zoning maps as the agro-climatic map developed for the Mediterranean region by UNESCO (1979) which includes 37 different zones (Ryan *et al.*, 2006). They also allowed sophisticated statistical analysis of GEI (Crossa *et al.*, 2004), improving our understanding of spatial and temporal aspects of the interactions (Loffler *et al.*, 2005).

The grouping of trial sites provided by the GEI analysis does not tell us ultimately where genotypes can perform well because the sites only represent a limited number of point locations. By using soil and climate information on the trial sites it is possible to classify these point locations into more or less homogenous environment types (DeLacy *et al.*, 1994; Roozeboom *et al.*, 2008).

Linking individual trial sites to larger regions for which they are representative is very useful for develop maps of TPEs and, ultimately, for introducing varieties into environments where they are expected to perform well (Gauch and Zobel, 1997). In the case of durum wheat, spatial analysis combined with GEI has been for example used by Annicchiarico *et al.* (2002) to define durum wheat TPEs in Algeria and recommend cultivars for specific locations.

Table 3. Examples of contribution to the definition of durum wheat TPEs through GEI analysis in the Mediterranean region (the clusters and sub-clusters defined as a results of the analysis can be considered as TPEs).

Region	Design	Type of analysis	Clusters	Reference
Mediterranean area	CIMMYT Elite Durum Wheat Yield Trial, 32 locations, 5 years	Pattern analysis	Two main clusters and six subclusters	Abdalla *et al.* (1996)
Algeria	24 genotypes, 18 locations, 2 years	Pattern analysis and AMMI	Two major clusters	Annicchiarico (2002)
Ethiopia, Bale Highlands	16 genotypes, 7 locations, 2 years	GGE	Two clusters: Selka, Gassera, Sinana, Sinja and Adaba, Robe, Agarfa.	Letta *et al.* (2008)
Iran	20 genotypes, 4 locations, 3 years	GGE	Two clusters: cold (Maragheh, Shirvan and Kermanshah) and warm (Ilam) environments	Mohammadi *et al.* (2009)
Italy	65 genotypes, 3 locations, 4 years	AMMI	3 clusters, one comprising locations from South Italy and Sicily	De Vita *et al.* (2010)
Iran	20 genotypes, 19 locations, 3 years	Pattern analysis and AMMI	Three clusters: cold (Maragheh, Shirvan), mild (Kermanshah) and warm (Ilam) environments	Mohammadi *et al.* (2011)
Morocco	23 genotypes, 6 sites, 4 years	AMMI	Two clusters: Deroua, Marchouch; and Tassaout, Jemaat –Shaim, Khemis-Zemamra, Sidi-El-Aydi, mainly based on temperatures	Nsarellah *et al.* (2011)
South Portugal	9 genotypes, 11 locations, 2 years	AMMI	A small cluster (Elva) and a larger cluster with the remaining ten environments	Rodrigues *et al.* (2011)
Algeria	12 genotypes, 5 locations, 1 year	AMMI and GGE	No clustering among the five locations (Harrouch, Khroub, Setif, Sidi Bel Abbes and Saïda)	Nouar *et al.* (2012)
Iran	20 genotypes, 5 locations, 3 years	GGE	Three clusters: (1) Moghan, Gorgan, (2) Gachsaran and (3) Ilam	Sabaghnia *et al.* (2012)

IV – Choice and characterization of managed stress environments

1. Choice of managed stress environments

The major concerns in germplasm evaluation are: i) the choice and further characterization of the sites where to test the genetic material and ii) the capacity of this evaluation to predict the performance of genotypes in the range of target environments under which the released varieties will be grown.

The choice of the specific experimental sites for drought tolerance phenotyping studies should take into account their representativeness with regard to economic and social factors, information on agriculture, cropping systems, and edaphic and climatic conditions (based on historical

weather data and soil features including hydrology, physical properties, soil moisture retention curves and chemical properties (Gomide *et al.*, 2011).

In the past, plant breeders in rainfed systems have been quite reluctant to select under drought stress and preferred to screen for traits such as height, maturity, plant type, pest tolerance, and grain quality under optimal conditions on research stations. They evaluated under the stress conditions of farmers' fields only at the advanced testing stage, when relatively few genotypes remained. The result was often a variety performing well under well-watered conditions but poorly under stress. Growing evidence indicates that varieties developed for improved yield under drought stress may respond to well-watered conditions if there is an early selection in both environments and if the choice of stressed environments effectively takes into account the previously described TPEs. Once the TPEs have been defined, a breeding strategy can then be developed for each one, based on the adaptation to the prevalent water supply and type of drought.

The choice and monitoring of the managed stress environments (MSE) directly determine the potential genetic gains in the TPE. Ideally, the MSE should mimic the TPE for water distribution, profiles, potential evapo-transpiration rates, and physical and chemical soil properties. Any deviations may result in significant GEI between TPEs and MSEs, and genetic gains achieved in the MSE may not be expressed in the TPE. Geographic information system (GIS) tools can help considerably in describing the relationships between TPEs and MSEs through establishing homology maps that show the degree of similarity between any set of stations or a continuous surface through spatial interpolation of climate data (Hyman *et al.*, 2013).

2. Characterization of managed stress environments

A. Documentation of climate and soil characteristics

For planning a drought phenotyping experiment, information is required on weather conditions (rainfall events and evapotranspiration levels) occurring during the experiment and those that can be expected during specific periods of the growing season, based on long-term climatic data. Actual environmental climatic characterization and recording are essential to quantify evapo-transpiration and crop water requirements, in order to control different water regime treatments and crop water stress levels. Their comparison with long-term average data is also important to know to which extent weather data of the year are representative of the climate of the location. The main atmospheric parameters which must be registered close to the vegetation surface are air temperature, global solar radiation, air relative humidity (RH), wind speed, air water vapor pressure deficit (VPD) and precipitation. Acquisition of weather data should be done by means of an automatic or a standard weather station.

The atmospheric evaporative demand (ET_o) is the main factor that drives the water consumption of the crop and its knowledge is essential to an accurate environment characterization. ET_o can be calculated according to FAO standards (Allen *et al.*, 1998) using the ET_o calculator, http://www.fao.org/nr/water/eto.html. (FAO, 2009). Procedures are incorporated to estimate missing climatic data from temperature data or from specific climatic conditions. Maximum and minimum air temperature data are the minimum dataset, but estimations become more precise if data on air humidity, radiation and wind speed are available.

Some tools have been developed to generate historical information, like the software package RAINBOW, http://www.iupware.be. (Raes *et al.*, 2006b) that estimates the magnitude of events by the mean of frequency analysis. Together with RAINBOW, New_LocClim, http://www.fao.org/nr/climpag/locclim/locclim_en.asp. (FAO, 2005) is a useful tool for choosing suitable experimental locations (i.e., targeting) and planning experiments. New_LocClim permits an estimate of average climatic conditions in locations where no observations are available, using climatic data of almost 30,000 meteorological stations worldwide from the FAO and after interpolation, create climatic

maps and graphs of annual cycles of the climate by month and extract numerical data in various formats for further processing.

Soil characterization of potential sites for drought is important as differences in soil depth and water holding capacity can affect the imposition of stress. Soil depth affects rooting volume and consequently nutrient and water availability. Compaction, aluminum toxicity and soil acidity will also reduce root depth. Soil texture is a major determinant of water holding capacity and water release characteristics (Gomide *et al.*, 2011).

As far as the aim is to develop varieties with adaptation to water constraints, it is important to know more about the patterns of water supply and the type of drought faced by the MSE. Water balance models are highly valuable tools to characterize environments based on predicted water availability. Physiologically based crop growth models or mechanistic models like STICS (Brisson *et al.*, 2003), CropSyst (Stöckle *et al.*, 2003) and DSSAT (Jones *et al.*, 2003) have been developed that give a good understanding of the exact influence of environmental characteristics and plant properties on crop development. However, they are sometimes difficult to apply in field situations, due to the relatively large amount of inputs required. Functional or engineering models like BUDGET, AQUASTAT and UPFLOW (Raes *et al.*, 2006b) are more problem-oriented, with more empirically derived functional relationships (Hoogenboom, 2003). BUDGET, http://www.iupware.be. (Raes *et al.*, 2006a) is suitable for assessing crop water stress under rain-fed conditions throughout the season, estimating yield response to water and designing irrigation schedules.

B. Spatial homogeneity

T Uniformity represents an essential criterion in the selection of suitable phenotyping sites and any fields with significant heterogeneity must be eliminated as a potential phenotyping site to avoid introducing unwanted experimental error. Without a homogenous phenotyping site, the value of data acquired, regardless of cost and time, is limited (Masuka *et al.*, 2012). Spatial variability affects the detection of treatment differences by inflating the estimated experimental error variance. Moreover, the effects of soil heterogeneity become more apparent under drought (Gomide *et al.*, 2011).

Spatial variability depends on the soil formation process and on complex interactions among natural environmental factors and human activities (Webster, 2000). As variability may be in the range of one meter or less (Solie *et al.*, 2001), the level of resolution of regional soil maps is not sufficient for the objectives of a precise experimental site. In addition, some important agronomic characteristics, such as soil compaction and soil water availability, are not usually displayed in regional soil maps. The past use and management of experimental fields are not always carefully registered and their effects generally not well identified. As a consequence of this, additional information on soil variability should be searched through soil analysis and mapping.

Direct assessment of soil variability within a field site for key soil physical and chemical properties can be made through destructive soil sampling at 30 cm depth intervals (to a depth of 90 or 120 cm soil depth). The location of soil samples could be positioned by GPS to allow the test results to be mapped to the exact location (Campos *et al.*, 2011). Soil samples should be analyzed at a minimum for texture, pH, macro and micro-nutrients. High-throughput techniques are now available for mapping variability within field sites based on penetrometers (Cairns *et al.*, 2011), soil electrical conductivity sensors (Cairns *et al.*, 2012), spectral reflectance (Rossel *et al.*, 2006; Dang *et al.*, 2011) and thermal imagery of plant canopies (Campos *et al.*, 2011).

Knowledge of soil variability can be used to ensure planting within areas of the least spatial variability to further reduce unwanted experimental error (Cairns *et al.*, 2009). This decision, together with the use of adapted trial designs (Federer and Crossa, 2011) is essential to reduce experimental error.

V – Stress monitoring

The ability to manage drought episodes (timing, frequency and intensity) of drought episodes and characterize (soil, plant measurements) is a key factor in mimicking the environmental conditions prevailing in the TPE and ensuring accurate drought phenotyping (Tuberosa, 2012).

1. Stress application and control

A. Out-of-season testing

An increasing number of breeding programs are conducting field trials in dry locations or "out-of-season", i.e., in seasons that are not the cropping season of the crop but are characterized by very low rainfall. Under such conditions the dynamics and intensity of drought episodes can be tightly controlled through the frequency and volume of irrigation treatments. Trials in dry sites also offer the advantage of a lower incidence of noise factors which can bias the evaluation. The option of field testing in dry areas or during dry seasons is however not always available or possible. The dry season should be sufficiently long to cover the whole growth cycle and photoperiod. Furthermore, conditions during the dry season are harsh for plants and generally do not reflect the environmental conditions plants will experience during a natural drought in the main (wet) season, temperatures and vapor pressure deficit (VPD) being generally higher (Jagadish *et al.*, 2011). These differences lead to genotype-by-season interactions and do not allow results obtained from the out-of-season experiments to be easily extrapolated to the growing season conditions.

B. Water application

Different traits will confer adaption to different types of drought stress, thus drought experiments should aim to impose a similar water stress (in terms of timing, frequency and intensity) as experienced in the TPE. For example, tolerance to drought stress before anthesis in wheat does not necessarily confer tolerance to drought stress after anthesis (Monneveux *et al.*, 2005). To ensure that drought is imposed at the correct phonological stage, irrigation should be withheld prior to this stage. A crop water balance should be used to determine the last date of irrigation to ensure plants experience drought stress at the target stage.

As there is generally a substantial variation in phenology across genotypes and drought stress is imposed at the same time across all genotypes within an experiment, genotypes with different phenologies are expected to face different stress duration. The presence of large differences in flowering time among genotypes bias the interpretation of the influence of drought-adaptive traits on yield. To overcome that difficulty, genotypes can be grouped into subsets of similar maturity and planted at different times to ensure phenological synchronization across genotypes at the crucial stage when drought stress is imposed. A preliminary study can be used to determine the phenology of genotypes prior to drought experiments. Another option is to use the information on phenology as a covariate adjustment. Finally, irrigation systems must be carefully chosen to ensure optimum control of the irrigation water. Drip irrigation is recommended to allow plot level control of irrigation.

C. Rainout shelters

Static or moveable rainout shelters represent another alternative of investigating the adaptive response of crops to a desired level of drought stress, avoiding the bias of unpredictable rainfall patterns. Major inconvenient to the use of rainout shelters are (in addition to the high construction and operating costs), the usually rather limited area protected by a shelter which, in turn, limits the number and size of experimental plots that can be tested.

D. Controlled environments

As the environment where selection and testing work are done is often variable in terms of rainfall, breeders are searching for more reliable phenotyping protocols that can accelerate progress. This can be made by controlling the environment and phenotyping in greenhouses or growth chambers, with increasingly sophisticated systems (eg, high-throughput screening based on robotized systems and advanced image analysis software). Greenhouse research increases the speed at which large numbers of plants can be phenotyped in a reproducible and precise manner. It also allows control of other environmental influences on phenotype expression that could confound data interpretation. Carefully controlled environments (such as pots, soil-filled pipes and hydroponics) are generally favored by molecular-oriented researchers because unwanted environmental variation can be minimized. However, by choosing to work in highly controlled environment, breeders should be aware that controlled conditions tend to be very different to those prevailing in the target population of environments (TPE) and may limit the application of results in germplasm development. In particular, irrigation in pots creates a situation that is very different from that occurring under field conditions (Passioura, 2005). Significant differences in transpiration response were noted by Wahbi and Sinclair (2005) between plants grown in a potting mixture and in field conditions, plants in pots being exposed to stress earlier in the drying cycle and with a more rapid depletion of moisture. An additional factor to be also considered is the more uniform pore distribution existing in potting mixtures, compared to natural soils, which can lead to hypoxia (Passioura, 2005). Finally, the temperature of the substrate used to fill pots or containers used in greenhouse experiments can be different from field soil temperature (Passioura, 2005).

2. Stress characterization

Drought covers different ranges of intensity and timing. These differences cause differential responses of the genotypes under consideration. Therefore, the intensity timing and timing of drought in the phenotyping experiment should be very well controlled and in areas where drought severity fluctuates widely, phenotyping should preferably be carried out under well-watered conditions and at different levels of drought stress (e.g., intermediate and severe). A sound interpretation of the results of an experiment conducted under conditions of water shortage requires an accurate characterization and monitoring of the water status of both soil and plant. In a review of molecular papers focusing on the effects of drought on gene expression or transgenes under drought stress, Jones (2007) highlighted that over half of the published papers had no measure of plant or soil water status. Measuring soil and plant water status also permits to optimize irrigation scheduling and crop management and allows the repetition of the experiment under comparable conditions. Soil or plant water status can be monitored by measuring the amount of water or its energy status (Kirkham, 2004).

At the plant level, emphasis has traditionally been devoted to water potential (Blum, 2009). The relative water content of the leaf also provides important information on the water status of the plant (Riga and Vartanian, 1999), offering the advantage of collecting a high number of samples in a short time. Both leaf water potential and relative water content provide an integrated measurement of the interaction among the factors involved in maintaining the flow of water through the plant. As components of leaf water relations change during the day as irradiance and temperatures vary, the change is small for about two hours at and after solar noon. Therefore, this is an appropriate time window for investigating leaf water relations in a large number of genotypes.

Different methods are available to measure the amount of water stored in the soil. The gravimetric method (i.e., weighing samples of soil columns before and after oven drying) provides an accurate but cumbersome measurement of soil moisture. Furthermore, the gravimetric method is destructive and requires dedicated plots distributed across the other experimental plots. Tools such as the neutron probe extensively used to estimate soil water status since the 1970's (Hignett and Evett, 2008) and the capacity probe (Nagy *et al.*, 2008) allow quicker and less labor-intensive

measurement. Several dielectric based soil water monitoring techniques have been developed, like the time-domain reflectometry (TDR), and the (single and multi-sensor) capacitance probe (CP) systems (Fares and Polyakov, 2006). These techniques greatly simplify the real-time determination of water content on a fine spatial and temporal scale. TDR techniques are of the most widely used thanks to their high precision, non-ionising radiation and low influence of soil salinity, bulk density and texture (Noborio, 2001). However, they generally not permit detailed measurement along the soil profile (Manieri *et al.*, 2007). Because of their relatively low cost and ease of operation, CP systems have met widespread acceptance as a means of closely monitoring soil moisture by collecting high-resolution soil-water content data in the rhizosphere. More recently, two dimensional geo-electrical tomography has been used for monitoring soil-water redistribution due to water uptake (Werban *et al.*, 2008). This technique permits to image and monitor diurnal soil-water redistribution. An additional option is provided by the use of a polymer-based tensiometer (POT) designed to measure matric potentials down to –1.6 MPa, thus allowing a better resolution of levels of local water stress and quantification of root water uptake in dry soils (van der Ploeg *et al.*, 2008). The choice of methodology used for monitoring soil water content will depend on many factors including the cost, intensity of drought, field variability, and accuracy and precision required.

3. Reducing noise factors

Experimental conditions on the MSE should ensure target stress to be imposed without interference from additional stresses, and with minimal environmental heterogeneity to reduce experimental error. The crop facing water deficit or heat stress simultaneously experiences a number of additional stress factors (e.g., micronutrient deficiency, soil compaction, salinity, nematodes, fungal pathogens) that exacerbate the effects of studies stresses. Typical case scenarios are those involving factors that cause mechanical damage to roots (e.g., nematodes, root-worms), impair root growth (e.g., soil acidity, boron toxicity, salinity) and reduce water availability to the crop (e.g., presence of weeds) and source capacity (e.g., foliar diseases, insect damage to the canopy). When one or more of these constraints affects the experimental plots, genetic variability among the tested germplasm for resistance to these stress agents inevitably biases an accurate evaluation of the effects of the drought or heat tolerance. Important and more subtle interactions may also occur when the effects of water deficit are evaluated in the presence of other abiotic stress factors (eg, high temperatures) that enhance leaf senescence and the role of specific adaptive mechanisms, such as the relocation of stem water soluble carbohydrate. This is typically the case for durum wheat experiencing combining drought and heat stress during grain filling in Mediterranean environments.

Efforts should be made to remove all other constraints except drought, or to implement additional trials where only this constraint is applied, in order to evaluate its specific impact (eg, trial under full irrigation in heat prone areas to isolate the specific effect of high temperatures). Soil surveys may allow the identification of selection sites or fields that avoid confounding factors. In some cases, these surveys allow identifying sites where the selection pressure for these stress factors permit the selection of genotypes targeted for regions where these stresses interact with drought. They could also identify the within-site distribution of e.g., nematodes (Nicol and Ortiz-Monasterio, 2004) or zinc deficiency (Ekiz *et al.*, 1998). These 'noise' factors can be partially overcome through adequate replication within and across environments.

Another solution to this problem, at least for traits other than grain yield and its components, which are best evaluated under field testing, is to collect phenotypic data from plants grown in controlled facilities (greenhouse, growth chamber, etc). This allows for an accurate control of the main environmental parameters (temperature, air humidity, light, etc…) but, as already mentioned, makes more difficult to mimic the real conditions of the target environment. Other major inconvenient is the limited volume of genetic material that can be evaluated and the high operating costs.

4. Accurate statistical designs and interpretations

It is recognized that an important part of the efficiency of modern breeding is due to the accurate phenotyping of large numbers of plots, made possible by more sophisticated and high-throughput experimental machinery (e.g., plot combines able to measure yield directly in the field), as well as the automation of tedious manual operations. The labeling of a large number of plots and samples, data collection and storage are now facilitated by the use of electronics (eg, bar-coding) and dedicated software (e.g., spreadsheets, databases, etc). The effectiveness of field experiments and the management and interpretation of phenotypic data can be enhanced through the utilization of the most appropriate experimental designs (Federer and Crossa, 2011), to allow for better control of within-replicate variability and reduce or remove spatial trends.

VI – Traits measurement

1. General Requirements

After having used yield under drought as an exclusive breeding objective, most breeders have progressively replaced this empirical approach by a more analytical one, the so-called "indirect selection" (Jackson *et al.*, 1996) based on the selection for "secondary traits" or plant characteristics other than grain yield that provide additional information about how the plant performs under a given environment (Lafitte *et al.*, 2003). For a secondary trait to be useful in breeding programs, it has to comply with several requirements (Edmeades *et al.*, 1997). A secondary trait should ideally be: (i) genetically associated with grain yield under drought; (ii) genetically variable; (iii) highly heritable; (iv) easy, inexpensive and fast to observe or measure; (v) non-destructive; (vi) stable over the measurement period; and (vii)not associated with yield loss under unstressed conditions. The heritability of indirect traits itself varies according to the genetic make-up of the materials under investigation, the conditions under which the materials are investigated and the accuracy and precision of the phenotypic data. The identification of secondary traits requires analyzing their association with yield on genetic pool with wide genetic basis, a condition not always met (Annichiarico *et al.*, 2005). The accuracy of secondary traits measurement is closely related to precision or repeatability, the degree to which further measurements show the same or similar results. For a number of traits measured with mechanical or electronic devices, accuracy and precision in measurements require calibration of the instrument prior to data collection. Finally, secondary traits can improve the selection response for stress conditions only if they avoid any confounding effects of stress timing on yield (eg, drought and flowering dates). The set of genotypes to be evaluated may be composed accordingly, grouping the genotypes by similar earliness or using irrigation methods (eg, drip irrigation) allowing precise water supply at the plot level.

Examining morpho-physiological traits in landraces from different origins can eventually help in the identification of traits of adaptation to specific environments and understanding of adaptation patterns. Ali Dib *et al.* (1992) compared the two durum landraces Haurani (from Middle-East) and Oued-Zenati (from Algeria) and found that the latter was characterized by later heading, taller stature, more developed root systems, larger and decumbent leaves, lower number of fertile tillers, longer awn, and heavier kernels, compared to the Middle-East landrace. They suggested that some of these characteristics could confer specific adaptation to stress conditions prevailing in the two regions, i.e., longer cold spells and intermittent drought in Algeria and severe terminal drought stress in the Middle-East. Moragues *et al.* (2006) reported that durum wheat landraces from South Mediterranean regions had larger plot stand at jointing, produced more biomass at anthesis (distributed mostly in the main stem) and were more efficient in the allocation of biomass to reproductive organs because their higher mean harvest index (HI). They suggested that these traits could have major importance in harsh Mediterranean environments.

Most of the traits currently mentioned in the literature associated with drought adaptation in durum wheat are shown in Table 4. Secondary traits can be classified according to their relationship to drought escape, pre-anthesis growth, access to water, water-use efficiency and photoprotection. In addition to these traits that may improve yield under drought, any other characteristic of socio-economic importance may obviously be considered. A good example is, for durum wheat, the case of straw production in cereal-livestock Mediterranean farming system which can be used to feed animals (Isaac and Hrimat, 1999). Traits that confer this characteristic like stem height (Annicchiarico and Pecetti, 2003) or tillering and should be consequently considered in the breeding process.

2. Traits related to drought escape

In low rainfall areas, earliness is considered as fundamental adaptive trait (Blum, 1988). In Mediterranean conditions characterized by drought developing increasingly throughout the late reproductive and grain-filling phases (ME4A mega-environment), earliness allows grain filling to take place under conditions of lower drought and high temperature stress (Loss and Siddique, 1994). Breeding for earliness of flowering is relatively simple, as major genes responsible for insensitivity to photoperiod and vernalization which allows anticipating heading are well known and relatively easily manipulated (Slafer, 1996). However, in most Mediterranean regions where cereal breeding has been carried out for decades, selection for earliness has already taken place (Siddique *et al*., 1989) and there may be only marginal scope for further raising yield due to selecting for even earlier flowering crops (Slafer *et al.*, 2005). Under optimal conditions, as grain yield is often positively correlated with crop duration, selection for shorter duration may impose a substantial yield penalty (Evans, 1993). In high altitude or continental areas, a compromise is requested between the need of escaping late frosts prior to anthesis on one hand and terminal drought and heat stress on the other (Annichiarico and Pecetti, 1998; Hafsi *et al.*, 2006).

3. Traits related to pre-anthesis growth

A. Controlled environments

Under drought prone environments, rapid ground cover through vigorous crop establishment is a highly desirable trait as it improves radiation interception by the crop at the early stages of growth (Ludlow and Muchow, 1990) and helps to shade the soil and suppress weeds that compete for water (Richards, 1987). In Mediterranean types of drought environment (ME4A) where 40% of available water may be lost by evaporation (Loss and Siddique, 1994), it also increases water use efficiency by reducing evaporation (Turner and Nicolas, 1987). Early vigor and associated larger root mass may also help to maintain a better water balance under early water stress (ME4B) if water is available deeper in the soil profile (Mian and Nafziger, 1994). Significant association has been found between biomass at the second leaf stage and final yield in durum wheat by Royo *et al.* (2000) and Aparicio *et al.* (2002). Ground cover can be estimated visually, recorded quantitatively by measuring plant dry weight, or assessed by digital image analysis (Regan *et al.*, 1992).

Large seed and embryo size favors early vigor. In durum wheat, seed size has been showed to be strongly associated with seedling development and seedling biomass by Aparicio *et al.* (2002). Similar associations were reported by Amin and Brinis (2013). Akinci *et al.* (2008) also reported an association of seed size and emergence rate. Rapid ground cover was found to be associated to thinner and wider leaves in bread wheat (Richards, 1996) but not in durum wheat (Araus *et al.*, 2002). In addition, a negative association between large leaves and frost tolerance has been reported in durum wheat (Pecetti *et al.*, 1993) suggesting that this trait could be a disadvantage in continental or high altitude areas.

Another seedling trait useful to improve crop establishment under drought conditions is coleoptile length. Genotypes with a long coleoptile allow sowings at greater soil depth. This trait is particularly useful when the crop grows exclusively on stored soil moisture (ME4C), to avoid extremely hot soil surface temperatures and rapid soil drying. The association between the presence of dwarfing gene *Rht1* and coleoptile length, stronger in durum wheat than in bread wheat because of dosage effect, makes the selection for long coleoptile quite difficult in durum wheat. A significant genetic variation was however observed for this trait in durum wheat by Alaei *et al.* (2010).

B. Tillering survival and recovery

An intermediate level of potential tillering is favorable in drought prone areas (Loss and Siddique, 1994). In durum wheat, a positive association has been reported in Morocco under early-season drought between high tiller survival rate and yield (El Hafid *et al.*, 1998). Garcia del Moral *et al.* (2003) also reported that the number of spikes per square meter predominantly influenced grain production in the warmer environments of Spain.

C. Total biomass

T Final grain yield is determined in durum wheat by total biomass production and the proportion of biomass allocated to grains (Van den Boogaard *et al.*, 1996). As a consequence biomass should be considered in breeding programs targeting drought prone environments. Significant correlation has been reported in durum wheat between grain yield and biomass at maturity (Waddington *et al.*, 1987) and anthesis (Villegas *et al.*, 2001; Royo *et al.*, 2005). Under Mediterranean climate (ME4A), the magnitude of the correlation is expected to increase with drought intensity association between biomass and grain yield, since canopy photosynthesis is inhibited by post-anthesis drought and final yield depends increasingly on the re-mobilization (Blum, 1998).

Measurement of total biomass is cumbersome and destructive. Samplings reduce the final area available for determining final grain yield on small plots (Whan *et al.*, 1991). The measurement of the spectra reflected by crop canopies has been largely proposed as a quick, cheap, reliable and noninvasive method for estimating plant above-ground biomass production in cereals (Aparicio *et al.*, 2002; Elliot and Regan, 1993; Smith *et al.,* 1993). Biomass can be estimated by measuring the spectra reflected by crop canopies in the visible (VIS, λ=400-700 nm) and near-infrared (NIR, λ =700-1300 nm) regions of the electromagnetic spectrum (the crop's ability to intercept radiation and photosynthesize (Ma *et al.*, 1996). Estimation is now feasible using spectro-radiometers to measure the spectra of light reflected by the canopy (Royo *et al.*, 2003). Spectral reflectance information from leaves or canopies is used to build vegetation indices which are simple operations (e.g., ratios and differences) between spectral reflectance data at given wavelengths. The normalized difference vegetation index (NDVI) and simple ratio (SR) have been reported as the best traits to assess biomass (Table 5), and stages 65 and 75 of the Zadoks scale the most accurate period for measurements (Aparicio *et al.*, 2002; Cabrera-Bosquet *et al.*, 2011). Vegetation indices have been used to estimate biomass (Aparicio *et al.*, 2002) and yield (Aparicio *et al.*, 2000) of durum wheat, but phenotypic correlation coefficients found are usually weak and largely dependent on the range of variation of the tested material (Royo *et al.*, 2003). Easy-to-handle spectro-radiometers such as the GreenSeeker are now available which gives the basic spectro-radiometric index of green biomass, NDVI. As the GreenSeeker includes its own radiation source, it may be used independently of atmospheric conditions. Spectro-radiometric measurements are been quite intensively used to evaluate biomass in durum wheat. Alternative techniques such as the use of an affordable conventional digital camera may provide information about the portion of the soil occupied by green biomass, the percentage of yellow leaves, or even yield components such as the number of spikes per unit land area (Casadesús *et al.*, 2007).

4. Traits related to remobilization and sink strength

A. Carbohydrates reserves

When drought stress occurs after anthesis, as it is frequently the case in Mediterranean drought environments (ME4A), photosynthesis is limited and yield depends greatly on the remobilization to the grain of pre-anthesis assimilates accumulated in leaves and stems (Álvaro *et al.*, 2008). Post-anthesis maximum water soluble carbohydrates (WSC) content has been consequently proposed as a selection criterion to stabilize grain yield under stressful environments (Edhaie *et al.*, 2006). In durum wheat an accumulation of WSC has been noted under water stress in vegetative tissues by Kameli and Lösel (1996).

Traits that may also contribute to remobilization during grain filling include long and thick stem internodes and peduncle, and solid stems. In studies where crosses were made between bread wheat lines contrasting in the solid-stem trait, the solid-stem progeny contained more soluble carbohydrate per unit of stem length (Ford *et al.*, 1979). In durum wheat, Kaya et al. (2002) and Bogale *et al*. (2011) reported a positive association between peduncle length and yield under drought.

The capacity of a genotype to support grain filling from mobilized stem and leaf reserves can be also assessed through application of chemical desiccants as potassium iodide which inhibit stem and leaves photosynthesis (Blum, 1988). Although chemical selection seems to have successfully used to screen for remobilization of pre-anthesis reserves (Blum *et al.*, 1991), the method is not currently used in breeding programs.

B. Spike fertility

Annicchiarico and Pecetti (1993), Simane *et al.* (1993), Kiliç and Yağbasanlar (2010) found that spike fertility was the component most highly correlated with yield in drought prone environments.

C. Grain filling duration

A significant positive association between grain filling rate and grain yield has been found in durum wheat (Gebeyehou *et al.*, 1982). It is generally accepted that grain filling duration is largely affected by environmental conditions, as its heritability is medium to low (Egli, 1998). .

5. Traits related to water status: *Root characteristics*

Root systems determine the potential volume of soil that can be explored for water and nutrients. Variation in root characteristics includes differences among wheat genotypes in the ability to establish a deep root system quickly (Siddique *et al.,* 1990), in root length density (Mian *et al.,* 1994), in root distribution (Ford *et al.,* 2006), in post-anthesis root growth (Ford *et al.,* 2006) and in the numbers of seminal roots (Robertson *et al.,* 1979) and total roots (Box and Johnson, 1987). Manschadi *et al.* (2006) found a relation between the angular orientation of wheat seminal roots, root and water uptake. reported an Associations have been postulated between drought tolerance and root length density in deeper soil layers (Manske and Vlek, 2002) and rooting depth (Lopes and Reynolds, 2010). Optimal root characteristics can vary in relation to the type of drought (Ali Dib *et al.*, 1992). Deep rooting appears more important when the crop depends on residual soil moisture (Mian *et al.,* 1994) whereas higher root density at intermediate soil depths (0.15–0.60 m) is more important in Mediterranean environments (Gregory *et al.,* 2009). In durum wheat, Motzo *et al.* (1993) reported an association between high root mass and tolerance to severe drought. However, extensive root systems also have higher respiration costs for plants. Root depth (Simane *et al.*, 1993) and root length density (El Hafid *et al.*, 1998) appears as better candidate traits for drought tolerance in durum wheat in Mediterranean conditions.

In the practice, root patterns have been poorly studied because root trait evaluation under field conditions is tedious and impractical for large populations. Nakhforoosh *et al.* (2012) reported some encouraging results concerning the use of electrical capacitance to screen for root length and root surface. In order to reduce the variability observed in field studies, root screening can also be made under controlled environments using rhizotrons, pots, hydroponics, or gel-filled containers. Some attempts have also been made to follow root growth in controlled and field conditions using Nuclear Magnetic Resonance but this technique is not yet available e for high throughput phenotyping. Table 6 provides a list of the main techniques that are available actually, with their mains advantages and limitations.

6. Traits related to drought escape

A. Stomata conductance

Traits that are indicative of the water status of a plant, especially when measured during periods of peak stress, are useful indicators of the plant's capacity to match evaporative demand by exploring and extracting soil water. Significant correlation between stomata conductance and yield has been reported in durum wheat by Monneveux *et al.* (2006). Viscous-flow porometers have been developed that allow a quick assessment of stomata conductance (Richards *et al.*, 2001). It is however difficult to accurately assess stomata conductance in a large number of plants while properly accounting for the fluctuation in the main environmental factors that affect stomata conductance during the day (wind, solar radiation, humidity, etc.).

A more integrative way of monitoring stomata conductance is based on the measurement of the natural oxygen isotope composition ($\delta^{18}O$) in leaf and grain materials (Barbour *et al.*, 2000). Measuring $\delta^{18}O$ in plant material allows for the collection of a large number of samples, and requires very little labor in the field. Significant association was found between leaf $\delta^{18}O$, stomata conductance and grain yield in bread wheat (Barbour *et al.*, 2000) and durum wheat (Cabrera-Bosquet *et al.*, 2011).

B. Abscisic acid

An increase in ABA concentration is a universal response observed in plants subjected to drought (Quarrie, 1991). ABA is a fundamental component of the mechanisms allowing the plant to match water demand with water supply and optimize growth and survival in response to environmental fluctuations. ABA has been shown to affect many of the traits that influence the water balance of the plant through both dehydration avoidance and dehydration tolerance (Thompson *et al.*, 2007). It also appears to pre-adapt plants to stress by reducing rates of cell division, reducing organ size, and increasing the rate of development. The analysis of the effects of ABA accumulation on other drought-related traits and yield showed some contradictory results (Tuberosa, 2012), thus limiting potential applications in breeding.

C. Canopy temperature depression

Among the traits relating to access to water, by far the easiest to measure in the field is canopy temperature depression (CTD) or difference in temperature between the canopy surface and the surrounding air, a quick and non-destructive method. Because a major role of transpiration is leaf cooling, canopy temperature and its reduction relative to ambient air temperature are an indication of how much transpiration cools the leaves under a demanding environmental load. Higher transpiration means colder leaves and higher stomata conductance, both aspects favoring net photosynthesis and crop duration. A relatively lower canopy temperature in drought-stressed crops also indicates a relatively greater capacity for taking up soil moisture or for maintaining a better plant water status. The addition of CTD as a selection criterion in wheat nursery improved

considerably the identification of the highest yielding materials (van Ginkel and Ogbonnaya, 2007).

CTD is useful mainly in hot and dry environments typical of countries with a Mediterranean climate. Although canopy temperature may seem very easy to measure, in practice there are methodological problems, particularly when there is variation in the air temperature with wind or cloudiness (Araus *et al.*, 2002; Royo *et a.l*, 2002). Screening by canopy temperature measurements under drought stress can be done only after full ground cover has been attained and before inflorescence emerges, at high vapour-pressure deficits and without the presence of wind or clouds (Royo et *al*., 2005).

In durum wheat, association was found between CTD and yield under stress by Royo *et al*. (2002) in Spain and further by Bahar *et al*. (2008) in Turkey, Guendouz *et al*. (2012) in Algeria, Karimizadeh and Mohammadi (2011), Moayedi *et al*. (2011) and Shefazadeh *et al*. (2012) in Iran.

D. Plant water status

Ability to maintain leaf hydration under drought stress is related to root growth, low residual transpiration and osmotic adjustment. Leaf rolling protects the leaf against excess of solar radiation which cannot be dissipated by transpiration, but is also an indicator of turgor loss (Nachit *et al.*, 1992). Positive association was found between leaf rolling and yield in durum wheat in Ethiopia (Bogale *et al.*, 2011). Low residual transpiration, the sum of cuticular transpiration and residual stomata transpiration (due to an incomplete closure of stomata) is expected to limit water loss under harsh drought conditions (Rawson and Clarke, 1988). Genotypes with low RT tend to have higher yield under drought conditions (Clarke and Romagosa, 1991). Lower residual transpiration was found by Febrero *et al.* (1991) in durum wheat landraces from the Middle-East, compared to landraces from North-Africa and improved cultivars.

Osmotic adjustment (OA) is the process by which plants accumulate solutes in their cells to minimize water loss and maintain cell function under drought conditions. OA has been identified as a mechanism to maintain grain yield under stressed conditions by allowing root growth and maintaining water and nutrient capture (Morgan and Condon, 1986), thereby mitigating some of the most detrimental effects of plant water deficit. A number of experiments have shown that wheat lines selected for high OA in response to the lowering of leaf water potential have higher grain yields in field experiments. However, OA is difficult to measure in large samples under field conditions. Moreover, filed conditions generate confounding effects related to genotypic differences in soil water exploration by roots. In durum wheat genetic variation in OA has been established under controlled conditions (Rekika *et al.*, 1998).

A positive relationship was noted by El Hafid et al. (1998) between relative water content (RWC) and grain yield in durum wheat. As RWC measurement is cumbersome, plant water status can be assessed directly by reflectance (Table 5), using the water index, WI = R900/R970 (Peñuelas *et al.*, 1993). WI has been used to detect variation in relative water content, leaf water potential and canopy temperature depression, but only when plant water stress is well developed. The ratio of WI to NDVI has also been proposed for estimating relative water content (Peñuelas *et al.*, 1997).

E. Carbon isotope discrimination

Carbon isotope discrimination ($\Delta^{13}C$) measures the ratio of stable carbon isotopes ($^{13}C/^{12}C$) in the plant dry matter compared to the ratio in the atmosphere (Condon *et al.*, 1990). Because of differences in leaf anatomy and mechanisms of carbon fixation between species with C_3 and C_4 photosynthetic pathway, studies on $\Delta^{13}C$ have wider implications for C_3 crops (Monneveux *et al.*, 2007). $\Delta^{13}C$ is generally negatively associated with water use efficiency over the period of dry mass accumulation (Condon *et al.*, 2004) and positively associated to stomata conductance (Condon *et al.*, 2002). In wheat, the relationship between $\Delta^{13}C$ and grain yield depends on the

environmental conditions, the phenology of the crop and the plant organ (e.g., leaf or grain) from which the samples are collected (Merah *et al.*, 2002). In durum wheat cultivated in Mediterranean environments, $\Delta^{13}C$ (particularly when measured in mature grains) is positively correlated with grain yield (Araus *et al.*, 1998; Hafsi *et al.*, 2001; Merah *et al.*, 2001; Monneveux *et al.*, 2005). One of the reasons for this positive relationship is that a genotype exhibiting higher $\Delta^{13}C$ has higher stomata conductance. The higher correlation generally observed under Mediterranean conditions with harvest index and grain yield, compared to those with biomass, suggest that higher $\Delta^{13}C$ values also indicate higher efficiency of carbon partitioning to the kernel (Merah *et al.*, 2001). High genetic variation and heritability was reported $\Delta^{13}C$ (Merah *et al.*, 2001). For all these characteristics, $\Delta^{13}C$ is an attractive breeding target for improving WUE and yield, while the high cost required for measuring each sample makes it an interesting candidate for marker assisted selection.

F. Ash content

Carbon isotope discrimination ($\Delta^{13}C$), despite being a very promising trait, is probably less widely accepted because of the cost of its determination. Several surrogate approaches have been proposed that are cheaper, faster and easier. The option most studied has been to use the mineral or ash content of leaves (Araus *et al.*, 1998; Merah *et al.*, 1999) or grains (Monneveux *et al.*, 2005; Misra *et al.*, 2006). A significant negative association was found in durum wheat between ash content and grain yield by Bogale and Tesfaye (2011) in Ethiopia. A promising option relies on the estimation of ash content through the near-infrared spectroscopy (NIRS) technique (Ferrio *et al.*, 2001) which has the additional advantage to be non-destructive.

7. Traits related to water use efficiency

Measurement of carbon isotope discrimination of grain or other tissues can be used to estimate the water-use efficiency (WUE) of the crop, since their signals are based on the integration of plant water status over a period of time (Condon *et al.*, 1993). However, these data must be interpreted with care. While in Australia, under conditions where wheat is grown on stored soil moisture, better performance of wheat cultivars indicated an advantage for high WUE genotypes (Rebetzke *et al.*, 2002), under Mediterranean drought conditions high yield is associated with lower WUE, reflected by high $\Delta^{13}C$ values (Monneveux *et al.*, 2005).

A. Spikes photosynthesis

Spikes photosynthesis contributes up to 40 percent of total carbon fixation under moisture stress (Evans *et al.*, 1972) and to 10-70 percent of final grain weight (Duffus et *al.*, 1985). Spikes have higher WUE than leaves due to the fact that they can refix respiratory carbon (Bort *et al.*, 1996). Moreover, they are able to maintain a better water status than leaves, through a higher OA and a more xeromorphic structure (Tambussi *et al.*, 2005). While gas exchange measurement of spikes is time consuming and difficult to standardize (Araus *et al.*, 1993), chlorophyll fluorescence should be considered as a more rapid means of screening for spike photosynthetic capacity under stress.

B. Awn length

In durum wheat, awns contribute substantially to spike photosynthesis and longer awns are a possible selection criterion (Villegas *et al.*, 2006).

C. Harvest index

Genes that increase partitioning of assimilates to the sink, resulting in a higher harvest index (HI), would be expected to improve yield under drought. They however often affect root development and access to soil water. As a consequence, a compromise should be found, depending on

environmental conditions (input level, occurrence of constraints) and particularly on drought stress intensity.

D. Senescence

Changes in leaf color can reflect a variation in partitioning of assimilates to the sink. Stress accelerates the senescence of leaves. Delayed senescence of leaves has been proposed as a secondary trait for performance under drought by Rharrabti *et al.* (2001). However, the relationship between delayed senescence and yield has been found by other authors to be unstable and highly dependent on drought intensity (Hafsi *et al.*, 2006; Guendouz and Maamari, 2011). According to Blum (1998), the stay-green trait may indicate the presence of drought avoidance mechanisms and contribute to yield per se if there is no water left in the soil profile by the end of the cycle to support leaf gas exchange, but may be detrimental if it indicates lack of ability to remobilize stem reserves. To check for delayed senescence of leaves, particularly flag leaves, portable chlorophyll meters such as the Minolta SPAD are extensively used, due to their speed and ease of use. Image analysis techniques are more precise but less time-effective (Hafsi *et al.*, 2000).

8. Traits relating to photo-protection

Decreased stomata conductance in response to drought leads to warmer leaf temperatures and insufficient CO_2 to dissipate incident radiation, both of which increase the accumulation of harmful oxygen radicals and photo-inhibitory damage. Photo-inhibition can be mitigated by some leaf adaptive traits such as glaucousness, pubescence, rolling, thickness or posture (Richards, 1996). These traits decrease the radiation load to the leaf surface. Benefits include a lower evapo-transpiration rate and reduced risk of irreversible photo-inhibition. However, they may also be associated with reduced radiation use efficiency, which would reduce yield under more favorable conditions. In durum wheat, glaucousness (waxy covering over the plant cuticle) was found to reduce water loss after stomata closure (Qariani *et al.*, 2000) and provide a yield advantage under drought stress (Merah *et al.*, 2000).

A. Photosyntetic pigments

In theory, chlorophyll content is a desirable characteristic as it indicates a low degree of photo-inhibition. However, in hot and high light intensity environments, a pale-green color, related to low chlorophyll content, could limit the energy load from strong sunlight, as suggested in barley (Tardy *et al.*, 1998) and the wild wheat *Aegilops geniculata* (Zaharieva *et al.*, 2001). No clear relationship with yield under drought was found in durum wheat (Royo *et al.*, 2000). Additionally to handheld devices for measurements of chlorophyll indices (e.g., SPAD meter), parameters of canopy reflectance via remote sensing approaches have been intensively investigated. Several reflectance methods have been proposed to estimate the concentration of chlorophyll and other pigments (Table 5). Chlorophyll concentration can be assessed by direct measurement at 675 nm (R_{675}) and 550 nm (R_{550}). R_{675} is very sensible to changes in chlorophyll concentration at relatively high concentrations. R_{550} can be used at low chlorophyll concentrations, but is less sensible (Lichtenthaler *et al.*, 1996).

The carotenoid to chlorophyll ratio can be used to estimate the intensity of stress faced by the plant (Young and Britton, 1990). It can be estimated using the pigment simple ratio (PSR) or the normalized pigment index (NDPI). As these indices are affected by variation in leaf surface and structure, Pañuelas et al. (1995a) developed a new index, structural independent pigment index (SIPI).

Violaxanthin, a xanthophyll carotenoid present in the photosynthetic apparatus of plants, is rapidly and reversibly de-epoxidized into zeaxanthin via the intermediate antheraxanthin under high-light stress (Horton *et al.*, 2005). This chemical transformation of violaxanthin, called the xanthophyll cycle, is required for the conversion of PSII from a state of efficient light harvesting to a state of

high thermal energy dissipation, which is usually measured as a nonphotochemical quenching (NPQ) of chlorophyll (Chl) fluorescence. NPQ protects PSII from photoinhibition, at least under short-term light stress (Niyogi *et al.*, 1998). Zeaxanthin synthesis in high light was also found to prevent photo-oxidative stress and lipid peroxidation (Havaux *et al.*, 2000). In a number of cases, accumulation of zeaxanthin was shown to increase tolerance to photo-oxidative stress (Havaux *et al.*, 2004). In durum wheat, an increase in zeaxanthin was noted under drought stress in the cultivar Adamello by Loggini *et al.* (1999). A reflectance based measurement of zeaxanthin has been proposed by Pañuelas *et al.* (1995b) using the photochemical index (PI). Relationship between the non-photochemical quenching and the photochemical Index across different stress intensities has been reported by Tambussi *et al.* (2000).

B. Chlorophyll fluorescence

Chlorophyll fluorescence can be used to estimate the activity of thermal energy dissipation in photosystem II and has been proposed to screen durum wheat accessions for drought tolerance (Flagella *et al.*, 1995; Flagella *et al.*, 1998; Royo *et al.* 2000). Under Mediterranean conditions, F_o, F_m and F*v* have been used successfully to detect differences across genotypes and showed high heritability (Araus *et al.*, 1998). F_v/F_m is only sensitive to very severe stress conditions and has a poor heritability. ΦPSII and $F_{v'}/F_{m'}$ as they are sensible to light intensity variation, are difficult to measure in field conditions. Fluorescence imaging should become a promising tool if portable systems are available as this technique accounts for spatial variation within the leaf and plot.

C. Antioxydants

The effects of photo-inhibition can be alleviated by antioxidants such as superoxide dismutase (SOD) and ascorbate peroxidise, which have been shown to increase in quantity in response to drought stress (Mittler and Zilinskas, 1994). Thermal dissipation through the xanthophyll cycle is another protective mechanism that can dissipate as much as 75 percent of absorbed light energy (Niyogi, 1999). In durum wheat, Zaefyzadeh *et al.* (2009) found higher SOD in drought tolerant landraces from Iran and Azerbaijan than in susceptible ones.

9. Application of secondary traits in breeding

The use of any trait and its further application in breeding should be first considered in relation to the type of stress (intensity, timing) faced by the crop in the TPE. As mentioned by Tardieu *et al.* (2011), most traits presumably associated with drought tolerance have a dual effect, positive in some conditions and limited or negative in others. A strong association reported between a given trait and yield in a specific environment may be weaker or disappear in others. A typical case in durum wheat is the association between grain yield and grain $\Delta^{13}C$, constantly positive under the typical post-anthesis drought of Mediterranean countries (ME4A), but highly dependent on the intensity of drought and particularly on the quantity of water stored in the soil in ME4B and ME4C (Monneveux *et al.*, 2005). Some other examples of traits effects changing according to the environment have been mentioned in this paper (earliness, chlorophyll concentration). Others have been reported by Tardieu *et al.* (2011).

While many traits have been studied for their use in breeding for drought resistance, there is a general consensus among breeders that only a few of them can be recommended for practical use in breeding programs at this time. The use of some traits in breeding is sometimes prevented by their low heritability but more often because of their lack of accuracy and precision. Some traits are difficult to assess on a large number of plants and their measurement is consequently affected by the fluctuation of environmental factors. In many cases, “instantaneous” measurements also face a problem of sampling (e.g., to which extent a measurement done on one leaf of few plants is representative of a plot and to which extent the time of measurement (hour of the day) affects the results of the measure. The development of techniques that are more time- and space-integrative

(spectrometry, thermal imaging) should solve most of these difficulties and the development of new equipment will facilitate measurements. Other traits cannot yet be recommended as part of an ongoing breeding program, because they are too expensive. However, some such as $\Delta^{13}C$ can be used for the selection of parents (Misra *et al.*, 2006; Xu *et al.*, 2007).

Vegetation indices have been defined to estimate different plant characteristics such as photosynthetic active biomass, pigment content and water status (Table 5). An extensive study conducted by Royo *et al.* (2002) on a collection of genotypes showed that Reflectance at 550 nm (R_{550}), water index (WI), photochemical reflectance index (PRI), structural independent pigment index (SIPI), normalized difference vegetation index (NDVI) and simple ratio (SR) explained jointly a 95.7% of yield variability when all the experiments were analyzed together, 92% being explained by R_{550}. When regression analyses were carried out separately for each experiment, spectral reflectance indices explained from 17.3% to 65.2% of total variation in yield, and the indices that best explained differences in yield were experiment-dependent. The same authors especially recommended the use of reflectance at 680 nm (R680), WI and SR as suitable estimators of durum wheat grain yield under Mediterranean conditions, when determined at milk-grain stage. Thermal imaging and color imaging techniques are expected to greatly facilitate large scale evaluations in the next future (Cabrera-Bosquet *et al.*, 2012).

Conventional cameras have been proposed as a selection tool for cereal breeding by Casadesus *et al.* (2007) and Mullan and Reynolds 2010). In breeding programs, photographic sampling can be cost-efficient because a large number of samples can be obtained with minimum effort. Calculations from those images can also be cost-effective since they are based on rather simple methods that can be automated for application to a large number of images.

VII –Traits measurement

Drought is expected to increasingly affect durum wheat in most regions where it is cultivated, with potential consequences on food security. Genomics approaches to improve drought tolerance will bring new opportunities over the next few years, but their impact in farmer's fields will mainly depend on the actual progress in our understanding of the physiology and genetic basis of drought-adaptive traits. The effective implementation in breeding programs of accurate and cost-effective phenotyping methods will be consequently essential to ensure research impact.

Efforts should focus on a more precise definition of TPEs, a better control of the stress monitoring in the MSEs and a more accurate assessment of drought tolerance related traits. Geographic information system tools, new equipment for the measurement of soil and plant water content, and more integrative drought tolerance related traits assessment methods can contribute largely in these efforts. But the success will also depend on a closer cooperation among partners. Collaborative efforts could include development of free-access long-term climatic data bases, multi-local and multi-institutional trials including common sets of cultivars, establishment of a well-documented database of durum wheat MSEs, registration of field data in common databases, web-sharing of experiences and organization of training courses. The development of networks among different partners and establishment of shared phenotyping platforms will allow quicker evaluation of germplasm in diversified environments, broader dissemination of germplasm products and larger impact of breeding efforts.

Table 4. Main secondary traits that can be used to improve drought tolerance in durum wheat, associated characteristics, measurement methods, references, ease of use and main target environment of application.

Secondary trait	Associated characteristics	Measurement method	References	Heritability	Ease of use	Target environment
Traits related to drought escape						
Earliness	Drought escape	scoring	Annichiarico and Pecetti (1998), Hafsi *et al.* (2006)	high	+++	ME4A, ME4C
Traits related to pre-anthesis growth						
Early ground cover	Decrease of evaporation, increase of radiation use	scoring, digital image analysis	Regan *et al.* (1992), Annicchiarico and Pecetti (1993)	moderate	+++	ME4A, early
Large seed size	Emergence, early ground cover	measurement	Aparicio *et al.* (2002a), Amin and Brinis (2013)	high	+++	ME4A
Long coleoptiles	Emergence from deep sowing	measurement	Giriyappanavar *et al.* (2010)	moderate	+++	ME4C
Number of sp kes (fertile tillering)	Tiller Survival and recovery	scoring	El Hafid *et al.* (1998) Annichiarico *et al.* (2002)	low	++	ME4A (early-season drought)
Pre-anthesis biomass		Measurement NDVI	Villegas *et al.* (2001), Royo *et al.* (2005)	low	++	ME4A
Traits related to remobilization and sink strength						
Stem water soluble carbohydrates	Storage of carbon products	biochemical analysis	Kameli and Lösel (1996)	moderate	+	ME4A
Peduncle length	Storage of carbon products	measurement	Kaya *et al.* (2002), Bogale *et al.* (2011)	moderate	+++	ME4A
Spike fertility	Sink strength		Gebeyehou *et al.* (1982)	moderate	+++	-
Grain filling duration	Grain filling, thousand kernel weight	measurement	Simane *et al.* (1993), Annicchiarico and Pecetti (1998)	low to moderate	+++	Drought around flowering
Traits relating to water status						
Root mass	Water uptake	see Table 5	Motzo *et al.* (1993)	low	+	Severe drought
Root depth	Water uptake	see Table 5	Simane *et al.* (1993)	low	+	ME4C
Root length density	Water uptake	see Table 5	El Hafid *et al.* (1998)	low	+	ME4A
Stomata conductance	Transpiration and CO_2 assimilation	gas exchange, porometry	Monneveux *et al.* (2006)	moderate	++	ME4A
^{18}Oxygen	Transpiration	mass spectrometry	Cabrera-Bosquet *et al.* (2011)	high	++	ME4A
Canopy temperature depression	Stomata conductance	infra-red thermometry	Royo *et al.* (2002)	moderate	+++	Hot and dry environments
Leaf rolling	Loss of turgor	score	Bogale *et al.* (2011)	high	+++	-

Residual transpiration	Cuticular and residual stomata transpiration	weighting	Febrero *et al.* (1991)	high	+++	Severe drought
Osmotic adjustment	Minimization water loss	measurement of water status parameters under controlled conditions	Rekika *et al.* (1998)	moderate	+	Moderate drought
Relative water content	Maintenance of cell function	Weighting reflectance (WI)	El Hafid *et al.* (1998)	moderate	+	-
^{13}Carbon	Stomata conductance	mass spectrometry	Araus *et al.* (1998), Merah *et al.* (2001)	high	++	Mainly for ME4A
Ash content	^{13}Carbon, transpiration	Combustion, near-Infrared spectrometry (NIRS)	Araus *et al.* (1998), Merah *et al.* (1999), Ferrio *et al.* (2001)	high	++	-
Traits relating to water-use efficiency						
Root xylem diameter	reduction in root conductance	measurement	Richards and Passioura 1989)	high	+	ME4C (Australia)
Spike photosynthesis	Contr bution to photosynthesis	gas-exchange measurements, Δ of water soluble fraction, fluorescence (?)	Araus *et al.* (1993)	moderate	+	ME4A
Awn length	Contr bution to photosynthesis	measurement	Villegas *et al.* (2006)	moderate	+++	ME4A
Senescence	drought avoidance, partitioning	SPAD	Hafsi *et al.* (2003), Hafsi *et al*. (2006), Guendouz and Maamari (2011)	moderate	++	-
Traits relating to photo-protection						
Glaucousness	radiation load to the leaf surface, water loss	scoring	Qariani *et al.* (2000), Merah *et al.* (2000)	high	+++	Severe drought
Chlorophyll fluorescence	activity of thermal energy dissipation in photosystem II	fluorimetry	Araus *et al.* (1998)	high	++	Severe drought
Carotenoid content						
Antioxidants (S.O.D., ascorbate peroxidise)		biochemical analysis	Zaefyzadeh *et al.* (2009)	moderate	++	-

Table. 5. Spectral vegetation indices (adapted from Araus *et al.*, 2001 and Mullan, 2012).

Measured trait and corresponding indices	Calculation	Reference
Photosynthetic size of canopy		
Simple ratio	$SR = R_{NIR}/R_{red}$	
Normalized difference vegetation index	$NDVI = (R_{NIR} – R_{red})/(R_{NIR} + R_{red})$	
Modified NDVI	$NDVI = (R_{701} – R_{520})/(R_{701} + R_{520})$	Carter (1998)
Soil adjusted vegetation index	$SAVI = [(R_{NIR} – R_{red})/(R_{NIR} + R_{red} + L)] (1 +L)$*	Huete (1988)
Transformed soil adjusted vegetation index	$TSAVI = a(R_{NIR} – R_{red} – b)/[R_{red} + a(R_{NIR} – b) + 0.08(1 + a^2)]$**	Baret and Guyot (1991)
Perpendicular vegetation index	$PVI = [(R_{redsoil} – R_{red\ vegetation})^2 + (R_{NIR\ vegetation} – R_{NIRsoil})^2]^{1/2}$	Richardson and Wiegand (1977)
Water status		
Water index	$WI = R_{900}/R_{970}$	Pañuelas *et al.* (1993)
Normalized water index - 1	$NWI\text{-}1 = (R_{970} – R_{900}) / (R_{970} + R_{900})$	Babar *et al*., 2006b
Normalized water index - 2	$NWI\text{-}2 = (R_{970} – R_{850}) / (R_{970} + R_{850})$	Babar *et al*., 2006b
Normalized water index - 3	$NWI\text{-}3 = (R_{970} – R_{920}) / (R_{970} + R_{920})$	Prasad *et al*., 2007
Normalized water index - 4	$NWI\text{-}4 = (R_{970} – R_{880}) / (R_{970} + R_{880})$	Prasad *et al*., 2007
Chlorophyll		
Simple chlorophyll index	R_{675}	Jacquemoud and Baret (1990)
Simple chlorophyll index	R_{550}	Jacquemoud and Baret (1990)
Ratio of reflectance	R_{750}/R_{550}	Lichtenthaler *et al.* (1996)
Ratio of reflectance	R_{750}/R_{700}	Lichtenthaler *et al.* (1996)
Green normalized difference vegetation index	$NDVI_{green} = [R_{NIR} – R_{540}/R_{570})]/ [R_{NIR} + R_{540}/R_{570})]$	Gitelson and Merzlyak (1997)
Wavelength of the red edge	λ_{re}	Filella *et al.* (1995)
Maximum amplitude in the first derivative of the reflectance spectra	dR_{re}	Filella *et al.* (1995)
Sum of amplitudes between 680 and 780 nm in the first derivative of the reflectance spectra	$\Sigma dR_{680\text{-}780}$	Filella *et al.* (1995)
Normalized difference red edge	NDRE = (R790 – R720) / (R790 + R720)	Barnes *et al.* (2000)
Modified spectral ratio (chlorophyll concentration)	MSR = (R750 – R445) / (R705 – R445)	Sims and Gamon (2003)
Chlorophyll degradation		
Normalized phaeophytinization index	$NPQI = (R_{415} – R_{435})/(R_{415} + R_{435})$	Pañuelas *et al.* (1995c)
Chlorophyll a		
Ratio analysis of reflectance spectra (Chla)	$RARSa = R_{675}/R_{700}$	Chapelle *et al.* (1992)

Ratio analysis of reflectance spectra (Chla)	RARSa* = R_{680}/R_{800}	Blackburn (1998)
Pigment specific simple ratio (Chla)	PSSRa = R_{800}/R_{675}	Blackburn (1998)
Chlorophyll b		
Ratio analysis of reflectance spectra (Chlb)	RARSb = $R_{675}/(R_{650} \times R_{700})$	Chapelle *et al.* (1992)
Pigment specific simple ratio (Chla)	PSSRb = R_{800}/R_{650}	Blackburn (1998)
Carotenoid		
Ratio analysis of reflectance spectra (car)	RARSc = R_{760}/R_{500}	Chapelle *et al.* (1992)
Carotenoid to chlorophyll ratio		
Pigment simple ratio (PSR)	PSR = R_{430}/R_{680}	Pañuelas *et al.* (1993)
Normalized difference pigment index (NDPI)	NDPI = $(R_{680} - R_{430})/(R_{680} + R_{430})$	Pañuelas *et al.* (1993)
Structural independent pigment index (SIPI)	SIPI = $(R_{800} - R_{435})/(R_{800} + R_{435})$	Pañuelas *et al.* (1995a)
Zeaxanthin		
Photochemical reflectance index (PRI)	PRI = $(R_{531} - R_{570})/(R_{531} + R_{570})$	Pañuelas *et al.* (1995b)

**L = 1 for low soil coverage, L = 0.25 for high soil coverage*

*** a is the slope and b the intercept of the linear equation* $R_{NIRsoil} = a\ R_{redsoil} + b$

Table 6. Main techniques available for assessing root characteristics (adapted from Herrera *et al.* (2012).

Method	Short description	Reference	Accu-racy	Time-effecti-veness	Cost effecti-veness	Through-put
Trench walls	The soil next to a plant is dug in such a way that the root systems become visible	-	++	---	+++	---
Mesh bags	The dynamics of root growth and root turnover can be studied by placing bags containing root-free soil in the field and removing them at regular intervals	-	-	--	++	--
Monoliths	A cubic section of soil that contains roots (monolith) dug out from the soil or obtained from a container in which the plant has been grown is washed to remove soil and separate roots.	McCully (1999)	+++	---	+++	---
Soil core	A soil core, small compared to the rooting volume is taken from the rhizosphere. The amount of roots can be estimated by breaking the soil core horizontally and counting the roots exposed on both faces of the breakage or by washing the samples and recovering the roots	Kumar *et al.* (1993); Yamaguchi (2002); Pierret *et al.* (2005),	++	---	+++	---
Two-dimensional (2D) rhizotrons	the plant is grown in a flat container with side walls made of a transparent material such as glass	-	+	-	-	+
Mini-rhizotrons	small-diameter transparent tubes inserted into the soil for the observation of root	Smit *et al.* (2000a)	-	-	-	+
Optical scanners	used to process samples obtained by soil coring or by burying them in the soil to study roots in a similar way as with 2D rhizotrons	Dannoura *et al.* (2008)	+	+	--	+
Electrical capacitance	based on measuring the electrical capacitance of an equivalent parallel resistance-capacitance circuit formed by the interface between soil water and the plant root surface	Chloupek *et al.* (2006) Amato *et al.* (2009)	--	+++	+	++
Ground-penetrating radars	Used to study the root biomass of trees, to be validated for cereals	Hruska *et al.* (1999)	?	++	--	++
Computed tomography methods	allow to image root growing and water uptake in the soil non-invasively	Tracy *et al.* (2010)	+++	++	---	+

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Adaptation of durum wheat to a changing environment

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Abstract. The expected climatic changes will lead to an environment characterized by elevated atmospheric CO_2, increased temperatures and increased risks for drought due to an elevated atmospheric evaporative demand. Therefore the traits involved in the adaptation to abiotic stresses, particularly drought and heat, as well the capability of the plants to adjust their metabolism to the increased CO_2 concentration will play a key role to sustain yield (and yield quality) in the durum wheat crop in the next years. This report summarizes a number of studies dedicated to the understanding the physiological and molecular basis of durum wheat adaptation to environmental stress. The overall findings highlight the existence of a significant degree of genetic variation that will allow the selection of new cultivars with a specific adaptation mechanism for the new or changed climatic conditions.

Keywords. Durum wheat – Stress tolerance – Molecular response – Stress related genes.

Adaptation du blé dur à un environnement changeant

Résumé. *Les changements climatiques attendus devraient conduire à un environnement caractérisé par une augmentation du CO_2 atmosphérique, la hausse des températures et des risques accrus de sécheresse en raison d'une demande évaporative atmosphérique élevée. Par conséquent, les caractères intervenant dans l'adaptation aux stress abiotiques, en particulier la sécheresse et la chaleur, ainsi que la capacité des plantes à ajuster leur métabolisme par rapport à la concentration accrue de CO_2, joueront un rôle déterminant pour maintenir le rendement (et la qualité du rendement) de la culture de blé dur dans les prochaines années. Dans ce travail, nous présentons les grandes lignes d'un certain nombre d'études centrées sur les bases physiologiques et moléculaires de l'adaptation du blé dur au stress environnemental. Les conclusions générales indiquent l'existence d'un degré significatif de variation génétique qui permettra la sélection de nouveaux cultivars, dotés d'un mécanisme d'adaptation spécifique aux conditions climatiques nouvelles ou modifiées.*

Mots-clés. *Blé dur – Tolérance au stress – Réponse moléculaire – Gènes liés au stress*

I Stress tolerance is a main component of yield stability

During the past century breeding activity has been characterized by the constant release of leading cultivars that in turn became progenitors of new cultivars, selected to perform well under intensive crop management and characterized by an increased yield potential (De Vita *et al*., 2007). Nevertheless, in recent years average grain yield has not increased at the pace registered from the 1950s to the 1990s. In many crops and certainly in durum wheat, an insufficient yield stability has been recognized as one of the main factors responsible for the gap between yield potential and actual yield, particularly in drought-prone environments. It is worthy to noticing that enhanced yield potential places a greater demand on field resources, thereby resulting in greater stress frequency unless the higher yield potential is associated to an increased stress tolerance. As a consequence, yield stability and stress tolerance are highly associated, and in many cases stress tolerance represents the main factor limiting yield stability (De Vita *et al.,* 2010).

A work describing the changes in adaptation and yield stability achieved over the last century in a historical collection of Italian durum wheat genotypes (landraces, old and new cultivars with different years of release and advanced breeding lines) has been carried out by De Vita *et al*. (2010). The breeding strategies adopted during the last decades have contributed to reduce the interaction of genotypes with environments selecting genotypes with better stability across a wide range of locations and years, as a consequence the modern genotypes outperformed the old ones in all tested environments with a strong yield capacity in highly fertile environments. This trend suggest that the traditional breeding has been able to select, indirectly, for abiotic stress tolerance; nevertheless a number of evidences highlights that many specific stress tolerance mechanisms that can be found in old cultivars/landraces have been lost during the selection.

II – Ofanto and Cappelli, a couple of varieties with contrasting drought response strategies

Senatore Cappelli (generally named Cappelli) is an old, low yielding, tall cultivar selected from a Tunisian landrace and released in 1915. When Cappelli was compared to a typical modern high yielding short cultivar (Ofanto, released in 1990), constitutive differences in Water Use Efficiency (WUE) and adaptive strategies were noticed. Integrated WUE, as recorded by grain isotopic discrimination, consistently showed a higher WUE of the variety Cappelli, associated with lower stomatal conductance over a wide range of relative soil water contents. The differences in WUE thus turned out to be constitutive (Rizza *et al*., 2012). These finding suggest that the durum wheat cultivars Ofanto and Cappelli can represent an ideal experimental system to investigate the water and heat stress responses in durum wheat.

III Ofanto and Cappelli show a largely different molecular response to high temperature and drought

When a transcriptomic analysis of the molecular response to drought, heat, and to a combination of both stresses was carried out in plants of Ofanto and Cappelli, two largely different responses were found. For instance, Ofanto activated a large set of well-known drought-related genes after drought treatment, while Cappelli showed the constitutive expression of several genes that in Ofanto are induced by drought and a minimal modulation of gene expression in response to stress. Assuming that the extent of gene modulation (number of genes modulated in response to stress) is a consequence of the stress signal perception, the same experimental conditions had a different impact both on stress signalling in Cappelli and Ofanto. Despite the lower Relative Water Content of Cappelli compared to Ofanto, the former cultivar showed minimal gene activation in response to drought. The lower stomata conductance and the constitutive expression of some drought-related genes might contribute to limit the effect of drought and the stress perception in Cappelli which, in turn, is reflected in a minimal drought-induced gene expression (Aprile *et al*., 2013).

Durum wheat often faces water scarcity and high temperatures, two events that usually occur simultaneously in the fields. The combination of drought and heat stress in plants is a unique stress sharing a marginal portion of the molecular responses activated by drought and heat stress alone. With respect to the response to the combination of heat and drought conditions, Ofanto and Cappelli are characterized by two opposite stress-responsive strategies. In Ofanto the combination of drought and heat stress led to an increased number of modulated genes, exceeding the simple cumulative effects of the two single stresses, whereas in Cappelli the same treatment triggered a number of differentially expressed genes, lower than those altered in response to heat stress alone (Aprile *et al*., 2013).

IV The RIL population of Ofanto x Cappelli offers a tool for the dissection of the genetic basis of trait associated to stress tolerance

Given the significant differences observed in terms of physiological and molecular mechanisms involved in the adaptation to abiotic stress condition, a RIL population was derived from the cross Ofanto x Cappelli and used to build a molecular marker map. A total of 618 molecular markers were assembled into 30 linkage groups that covered all of the durum wheat chromosomes except 1A (Marone *et al*., 2012a, 2012b).

This genetic map was used to dissect the genetic bases of leaf porosity (a stomatal-conductance-related trait) measured under field conditions as well as the loci controlling the expression of a gene differentially expressed in response to stress between the two parental lines. Six QTLs were detected for leaf porosity, among them, the one located on chromosome 3B appeared to be more stable across different environments (Paino *et al*., 2012). A gene of unknown function having the greatest expression difference in response to drought stress between the two cultivars was selected and used for expression QTL analysis, a single e-QTL with a strong effect (more than 90% of explained phenotypic variability) was mapped on chromosome 6B (Aprile *et al*., 2013). The fact that the e-QTL was coincident with the locus of the position of the gene strongly suggests that the main factor controlling its expression relies in the gene sequence itself.

The mapping of the QTLs controlling leaf porosity and of the e-QTL controlling the expression of a stress related gene, provides clear evidences that the genetic system based on Cappelli and Ofanto represents an useful tool for the genetic dissection of the molecular response to drought and heat stress in durum wheat.

V – Functional analysis of selected stress responsive genes

The analysis of gene expression often leads to a list of candidate genes that need to be further validated to understand their role in the stress response. Early works on gene expression have identified a number of genes with potential regulatory role in the response of durum wheat to could and drought stress (Mastangelo *et al*., 2005, De Leonardis *et al*., 2007, Aprile *et al*., 2009). One of them, *TdRF1* a gene encoding an E3 ubiquitin ligase, was then subjected to a functional characterization. Its E3 ligase activity was demonstrated and a network of proteins interacting with TdRF1 was described. The E3 enzymes are responsible of recruiting the proteins targeted by the ubiquitination process, which in turn drive the proteins to degradation through the 26S-proteasome. Furthermore, the functional characterization of TdRF1 has highlighted a small interactome represented by 4 interacting proteins, besides TdRF1, the following proteins were involved: the mitogen-activated protein kinase TdWNK5 was able to phosphorylate TdRF1 *in vitro*, the transcription factor WBLH1 was degraded in a TdRF1-dependent manner through the 26S proteasome *in vivo*, and the RING-finger protein WVIP2 was shown to have a strong E3 ligase activity. Furthermore, *TdRf1* and the genes coding for the TdRF1 interactors were all responsive to cold and/or drought stress, and a negative regulative function in dehydration tolerance was observed for the barley homolog of *WVIP2* (Guerra *et al*., 2012).

The involvement of E3 ubiquitin ligases in the response to drought and cold stress points out the role of post-translational modifications of proteins in the adaptation to environmental changes in durum wheat.

Besides the genes whose role in stress tolerance is postulated based on their expression profile, other sequences can be identified because involved in metabolic pathways modulated during stress response. An example is represented by the Phospholipases A2 gene family known to

mediate signalling cascades during plant growth and development, as well as biotic and abiotic stress responses. A specific study was undertaken to assess the involvement of specific PLA2s in durum wheat response to drought stress. Three sequences encoding putative PLA2s were found modulated by drought stress suggesting that PLA2 in durum wheat that have roles in orchestrating the plant response to drought (Verlotta *et al*., 2013).

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Durum wheat (*T. durum* Desf.) vs. bread wheat (*T. aestivum* L. em.Thell.) in South-East Anatolia, Turkey

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Abstract. This study aimed to compare grain yield, marketing price and net return per unit area of durum wheat vs. bread wheat in the South-East Anatolia.16 bread wheat + 9 durum wheat advanced lines were tested employing randomized complete block design with 3 replications in 2010-11 and 2011-12 cropping seasons in 2 locations (Ş.urfa and Adıyaman) in each year. Mean separations for grain yields of combined analysis of variance (ANOVA) indicated that bread wheat in average over yielded durum wheat with 7.14%. Rank stability analysis further indicated that bread wheat entries fell into high stable area more than durum wheat. Marketing prices of entries was estimated in Ş.Urfa commodity market. Durum wheat entries in average received higher marketing price offers than bread wheat with 4.97%. A visual characteristic of 1000 kernel weight affected marketing prices for both durum and bread wheat significantly (r= 0.699**). Average net return of bread wheat in average was higher than that of durum wheat with 2.6%. Additional premium (as much as 2 times of Std. deviation of marketing prices) given to highest income generating durum wheat entry did not change the profitability rank. It was concluded that higher yielding bread wheat entries generated higher net returns. Unless purchasers give adequate premium to durum wheat, farmer preference for durum wheat cannot be achieved under supplementary irrigation in SE. Anatolia.

Keywords. Grain yield – Marketing price – Visual quality – Stability – Net return.

Blé dur (Triticum durum Desf.) vs blé tendre (T. aestivum L. em.Thell.) dans le Sud-Est de l'Anatolie, Turquie

Résumé. *Cette étude visait à comparer le rendement en grain, le prix de vente et le rendement net par unité de surface du blé dur et du blé tendre dans le Sud-Est de l'Anatolie. Seize lignées avancées de blé tendre et neuf de blé dur ont été testées en utilisant un dispositif expérimental en blocs aléatoires complets avec 3 répétitions, au cours de la saison de culture 2010-11 et 2011-12 et chaque année, dans deux endroits différents (Ş.urfa et Adıyaman). L'écart moyen des rendements en grain de l'analyse combinée de la variance (ANOVA) a mis en évidence que le blé tendre, en moyenne, a un rendement de 7,14% plus élevé que celui du blé dur. En outre, l'analyse de la stabilité des rangs a montré que les données d'entrée du blé tendre se situent davantage dans la zone de plus grande stabilité par rapport au blé dur. Le prix de vente des produits a été estimé au marché de Ş.Urfa. En moyenne, pour le blé dur, les offres de prix de marché dépassaient de 4,97% celles du blé tendre. Une caractéristique visuelle du poids de 1000 grains influait sur le prix de vente du blé dur et du blé tendre de manière significative (r = 0,699 **). Le rendement net moyen du blé tendre était plus élevé en moyenne de 2,6% par rapport à celui du blé dur. Le supplément de prix (jusqu'à 2 fois l'écart-type des prix de vente) payé pour le blé dur générant un revenu plus élevé n'a pas changé le rang de la rentabilité. Il a été conclu que les entrées de blé tendre à rendement supérieur ont généré des rendements nets plus élevés. A moins que les acheteurs ne soient disposés à payer un supplément de prix pour le blé dur, les agriculteurs continueront à lui préférer le blé tendre dans le Sud-Est de l'Anatolie où ils ont recours à l'irrigation d'appoint pour cette culture.*

Mots-clés*. Rendement en grain – Prix de vente – Qualité visuelle – Stabilité – Rendement net.*

I – Introduction

Durum wheat (*T. durum* Desf) comprises approximately 8-10 % of World wheat production (Ozberk et.al., 2005a; Oztahtacı,2000; Sardana, 2000; Nachit,1998; Abeye *et al.*,1997). The average annual durum wheat production between 2003-04 and 2009-10 was as 33,620 mil. tons from a harvested area of 14-16 mil. ha (Anonymous, 2012). More than 85% of the World durum wheat production area is located in the Mediterranean basin. It occupies about 11. mil. ha in this region. Manufacturing and marketing of durum products are also concentrated in the region (Nachit *et al*., 1998). Turkey is one of major durum wheat producer with an average 3,057 mil. tons between 2003-04 and 2009-10 (Anonymous, 2012) with an average 1.658 mil.ha area during the same period (Ozberk *et al*., 2005c). Wheat origins from the area called 'Fertile Crescent'. Wild relatives of wheat are widespread in Turkey, especially in South East Anatolia (Karagoz and Ozberk, 2010).SE. Anatolia is known to be the durum wheat belt of the country (Ozberk *et al*., 2005c). This area is the most favourable for durum wheat production (Kun *et al*.,2005).There are no significant yield differences in favour of bread wheat in this area (Bagcı and Ekiz, 1993). Average grain yield under rain fed condition is about 2 ton ha^{-1} whereas, a 6 ton ha^{-1} grain yield can be achieved under supplementary irrigation (Ozberk *et al*., 2011). Twenty-five percent of the national durum wheat production is met by this region (Ozberk *et al*.,2011).

Production capacity of macaroni industry in Turkey exceeded 1,2 mil. ton $year^{-1}$in 2009 (Bozkurt, 2010). But the capacity use was 67% (Bayram, 2010). Thirty-five % of this capacity is located in one of SE Anatolian city of Gaziantep. Bulgur a second important durum product is also produced over one mil. ton $year^{-1}$(Bayram ,2010). Only 218,000 tons of bulgur is produced by 242 bulgur plants. The rest comes from homemade production (Bayram, 2010). Another SE Anatolia city of Ş. Urfa is one of leading bulgur producer with 28 running plants. Macaroni consumption is about 6 kg $year^{-1}$ per head (Koksel *et al*, 2010). Whereas, bulgur consumption is about 12 kg $year^{-1}$per head (Bayram, 2010). Macaroni export figures changes year by year with an average of 189,000 tons $year^{-1}$.Wheras, bulgur and semolina export figures reach an average of 115,600 tons $year^{-1}$ between 2007-2009 (Bayram, 2010).

Durum wheat varieties Fırat-93, Sarıcanak-98, Ege-88, Fuat bey-2000, Svevo, Zenit, Burgos are the major dominating varieties in acreage in the region. Last three possessing high yellow pigmentation characteristic were introduced by private companies (Ozberk *et al*., 2011). Ceyhan-99, Dariel, Meta-2002, Adana-99, Pehlivan, Sagitario are leading bread wheat cultivars in the region. Yield potentials of durum wheat cultivars grown in the region varies from 4,925 ton ha^{-1} to 5,809 ton ha^{-1}(Ozberk *et al*.,2011). In a regional bread wheat trials with 20 entries, yield potential of standards varied from 4,491 to 5,282 ton ha^{-1} (Ozberk *et al*, 2006). In variety release and registration trials carried out in 2009, recently developed bread wheat and durum wheat candidates with highest yielding standards were tested at same location side by side. Bread wheat standards at combined ANOVA mean separation for 4 locations (Nurkent, Adana-99, Sagitario, Pehlivan, Ziya Bey-98, Basri Bey-05, Ceyhan-99, Pamukova-97) performed varying from 4,439 ton ha^{-1} to 4,991 ton ha^{-1} . Average yield of standards was 4,723 ton ha^{-1}.Whereas; durum wheat standards (Fırat-93, Sarıcanak-98, Svevo, Solen-2002, Ege-88, Zenit, Fuat Bey-2000, Amanos-97) performed from 4,654 ton ha^{-1} to 5,476 ton ha^{-1}. Average grain yield of standards was 5,048 ton ha^{-1} (Anonymous, 2010).

Ş.Urfa commodity market is the third largest market in Turkey with over 500,000 tons of summer season marketing capacity (Ozberk *et al*., 2005 a). Although there are many other quality requirements for durum wheat in the international marketing, some physical characteristics such as 1000 kernel weights and hectolitre weights determine the marketing price (Ozberk *et al*., 2006). Moreover, if the grain belongs to a highly reputed variety it attracts even higher market price. Portable protein analysers have been introduced to the purchasers in Ş.Urfa commodity market since 2006. Purchasers also refer to protein content (%) in marketing price offers.

The criterion of farmers is productivity and his concept of quality is closely linked to the need to obtain high yield in order to maximize profit (Troccoli *et al*., 2000; Inglis, 1992). Similar results were achieved by Ozberk *et al*., 2011. In which high quality cultivars were not given adequate premiums and high yielding cultivars were found to be high income generating in durum wheat.

II Material and methods

25 wheat entries (16 bread wheat + 9 durum wheat) consist of recently developed advanced lines (Table 1) were tested employing randomized complete block design with 3 replications in 2 locations (Ş. Urfa, Adıyaman) in 2010-11 and 2011-12 cropping seasons. Except 2011-12 Adıyaman experiment, the rest were grown under supplementary irrigated conditions. Annual rainfall in Ş.Urfa was 351,4 mm in 2010-11 and 296,5.mm in 2011-12 season. These turned out to be 679,6. mm in 2010-11 and 812,3.mm in 2011-12 in Adıyaman.

Table 1. Names and pedigrees of entries

Pedigree/Cross No:	
1.MILAN/KAUZ//HD29/2*WEAVER/3/KAUZ	RSM(BW)124-2002T-54CJ-010T-010CJ-010T-0CJ
2.CHIBIA//PRL/CM65531/3FISICAL	INDIA-0CJ
3.WAXWING*2/KIRATATI	INDIA-0CJ
4.PRL/2*PASTOR//SERI	RSM(BW)043-2002T-52CJ-010T-010CJ-010T-0CJ
5.BLANCA FUERTE	USA-0CJ
6.04W44509	USA-0CJ
7.02W50274_1	USA-0CJ
8.06W31187	USA-1T-0CJ
9.06W31455	USA-4T-0CJ
10.06W31455	USA-5T-0CJ
11.06W31582	USA-2T-0CJ
12.BERKUT	CMSS96M05638T-040Y-26M-010SY-010M-010SY-4M-0Y-05T-03CJ-03T-0CJ
13.PRL/2*PASTOR	CGSS97Y00034M-099TOBP-027Y-099M-099Y-099M-25Y-0B-05T-03CJ-03T-0CJ
14.VAR1/4/MILAN/KAUZ//PASTOR/3/CROC1/AE.SQUARROSA(224)//OPATASI85-03-040T-040CJ-4T-03CJ-5T-0CJ	
15.VAR1/F4SR S-2013	SI88-03-040T-040CJ-8T-03CJ-4T-0CJ
16.CROC/AE.SQUARROSA(205)/BOURLOG95/3/2*MILAN	
17.JUPARE(2001)/3/SOMAT/TILO//LOTUS	SI57-04-11T-03CJ-6T-0CJ
18.JUPARE(2001)/3/SOOTY_9/RASCON_37//SITE/3*MUSK_4	
	SI63-04-9T-03CJ-2T-0CJ
19.RIO COLORADO/ICARDA 94-MK3	SI74-04-8T-03CJ-4T-0CJ
20. RIO COLORADO/ICARDA 94-MK3	SI74-04-8T-03CJ-6T-0CJ
21. RIO COLORADO/ICARDA 94-MK3	SI74-04-8T-03CJ-8T-0CJ
22.RIO COLORADO/4/YAZI1/AKAKI 4…..	SI80-03-040T-040CJ-2T-05CJ-4T-0CJ
23.RIO COLORADO/6/ CHEN1/TEZ/3/GUILT….	SI84-03-040T-040CJ-4T-05CJ-10T-0CJ
24.ICASYR 2	SYRIA-010CJ-7T-0CJ
25.ICASYR 2 (SYRIA)	SYRIA -03CJ-03T-0CJ

Field trials were sown in mid-November under cotton-wheat crop rotation system in Ş. Urfa and wheat-food legumes rotation in Adıyaman. Sowing rate was 500 grain m^{-2} and 60 kg ha^{-1} pure P_2O_5 and 140 kg ha^{-1}(split) nitrogen were applied. Plot size was 6 m and 6 rows (1.2m) at planting and 5 m and 6 rows at harvest. All other necessary agronomic measures were taken to obtain healthy data. Two irrigations were practiced in grain filling period and the amount of water delivered was not measured.

Individual and combined analysis of variance was performed. Statistical prerequisites were taken into consideration prior to combine ANOVA. Data obtained from field trial carried out under rain fed condition in Adıyaman in 2011-12 seasons was also included for combined ANOVA. Therefore the performances of all entries under both conditions were assessed. Duncan multiple range test was employed for mean separations. Yield stabilities of all entries were assessed through 'Rank Stability Analysis' (Huhn, 1990).

Grain samples of all entries obtained from 2011-12 Ş. Urfa field trials were joined and cleaned by dockage cleaner. Subsequently, HI (Anonymous, 1990) and 1000 kernel weights (Uluöz, 1965) were scored.

The grain samples (1kg) were presented to 6 randomly selected grain purchasers in Ş.Urfa commodity market in April, 2013. Relationship between hl and 1000 kernel weights vs. marketing prices was investigated through correlation analysis. Marketing price estimates were analysed by randomized complete block design with 6 replications (purchasers). Duncan multiple range test was employed for mean separation.

Production income (US$ ha^{-1}) was calculated by multiplying grain yield (ton ha^{-1}) x marketing price (US$ ton^{-1}) for each entry. Profitability estimates (average, min, and max.) of bread wheat vs. durum wheat were compared and promising entries were offered for release. SPSS statistical software was used for statistical analyses.

III – Results

Grain yield data obtained from the locations and two years were subjected to individual and combined analysis of variance (data not shown) results from all years and locations indicated that entries were found to be significant ($F_{Ş.Urfa\ ,2010-11}$ = 2.569**, $F_{Ş.Urfa,\ 2011-12}$ = 2.547**, $F_{Adıyaman,\ 2010-11}$ = 3.491**, $F_{Adıyaman,\ 2011-12}$ = 1.940*). Replications were also found to be significant for all experiments. Coefficients of variations (CV%) for Ş. Urfa- 2010-11, Ş. Urfa 2011-12, Adıyaman 2010-11 and Adıyaman 2011-12 were found to be 12.85%, 11.52%, 10.74% and 21.05% respectively.

Combined ANOVA was performed to test the presence of GxE interactions. Results revealed that locations (F= 665,97**), years(F= 246,59**), years x locations (F= 19.19**) and varieties x locations x years (F= 1,91**) were found to be significant. CV% was 16.26. Ş. Urfa location gave a 6.292 ton ha^{-1} grain yield whereas; Adıyaman gave 3.939 ton ha^{-1} in average.

Duncan multiple range test was performed for mean separations and the results showed that (Table 2) first 8 top ranking entries were 3, 7, 8, 6, 4, 3 and 9 giving 5.946, 5.788, 5.647, 5.571, 5.455, 5.421, 5.391 and 5.306 ton ha^{-1} respectively. Durum wheat entry no 19 took place at 9^{th} at rank giving 5.223 ton ha^{-1}. Rank stability analysis also indicated that bread wheat entries 2,6,7,8 and 13 were found to be stable for grain yield. Many of durum wheat entries fell into average rank and rank standard deviation area.

Marketing price data for grain samples of all entries for Ş. Urfa (2011-12) location were subjected to analysis of variance. Entries turned out to be significant (F= 45,12**). Purchasers (replications) were found to be non-significant (F= 2.16^{ns}).CV% was 3.25. Duncan mean separation test showed that (Table 2) durum wheat entries 17, 19, 25, 21,18, 20, 22, 23 and 24 took place at first 9 top ranking entries giving 459.76, 458.82, 457.44, 457.11, 456 05, 454 67, 454 23, 452 65 and

451 9 US $ ton^{-1} respectively. Bread wheat entry no 16 ranked at 10th place giving 441 06 US$ ton^{-1} marketing price.

Average Hl weight of bread wheat was 81 68 kg. Whereas; this was 81 84 kg for durum wheat. Average thousand kernel weights for bread wheat was 40 53 g. this was 45 79 g for that of durum wheat. The coefficient of correlations between hl weights vs. market price was not significant (r= 0.086 ^{ns}). Whereas, that for 1000 kernel weights vs. market price turned out to be highly significant (r= 0.699**).

Net returns (US$ ha^{-1}) (Table 2) showed that top five ranking entries were 13, 2, 7, 19 and 6 giving 2608.0, 2551.84, 2424.3, 2396.46 and 2382.23 US $ ha^{-1} respectively. Durum wheat entry no 19 was alone taking into top 5 ranking entries for net returns.

Table 2. Duncan's mean separations for combined grain yield, marketing prices and net returns

Entry No.	Grain yield/groups ton ha^{-1}		Marketing price/groups US$ ton^{-1}		Net return/groups US$ha^{-1}	Income rank
13	5.946	a	438.57	ef	2608,08	1
2	5.788	ab	440.84	e	2551,84	2
7	5.647	a-c	429.33	h	2424,3	3
8	5.571	a-d	427.0	hi	2378,85	7
6	5.455	a-d	436.69	ef	2382,23	5
4	5.421	a-d	439.01	ef	2379,96	6
3	5.391	a-d	434.86	fg	2344,59	8
9	5.306	b-d	434.86	fg	2307,41	11
19	5.223	b-e	458.82	ab	2396,46	4
18	5.138	c-f	456.05	a-d	2343,18	9
23	5.069	c-f	452.85	cd	2295,67	13
10	5.069	c-f	426.12	hi	2160,13	21
21	5.049	c-f	457.11	a-d	2308,22	10
5	5.020	c-f	431.15	gh	2164,37	20
12	5.017	c-f	434.42	fg	2179,87	16
17	4.996	d-f	459.76	a	2297,23	12
14	4.996	d-f	434.86	fg	2172,77	18
20	4.996	d-f	454.67	a-d	2271,66	14
15	4.957	d-f	439.12	ef	2176,93	17
16	4.950	d-f	441.06	ef	2183,51	15
1	4.947	d-f	438.07	ef	2167,39	19
25	4.639	e-g	457.44	a-c	2122,20	22
24	4.548	f-g	451.9	d	2055,60	23
11	4.537	f-g	423.79	i	1922,90	24
22	4.223	g	454.23	b-d	1918,44	25
CV%	16.25		CV%	3,25		

IV – Discussion

The entries under this study were very competitive for grain yields as the yield performance of varieties grown in the region (Ozberk *et al*., 2011; Ozberk *et al*., 2006). Significant effects of replications in the individual ANOVA's for grain yield can be attributed to the heterogeneity of experimental fields and differences in irrigation water given to plots. Although adequate rainfall for Adıyaman was received the distribution of rainfall was not homogeneous and lack of grain filling period. This resulted in lower average yield inevitably. Except Adıyaman 2011-12 experiments, all other CV's (%) were quite reliable. Combined ANOVA indicated the presence of GXE interactions. The presence of GXE interactions were further investigated through 'Rank Stability Analysis' and the entries with lower ranking and lower standard deviations were determined. Grand mean of

durum wheat entries was 4,876 ton ha^{-1} whereas; that of bread wheat entries was 5251 ton ha^{-1}. There was a 7.14% yield differences in favour of bread wheat (Table 3).There was also 12.16% yield gap in favour of bread with between highest yielding bread wheat vs. durum wheat entries. This turned out to be 6.9% for the lowest yielding bread wheat vs. durum wheat entries. In the field trials carried out by 'Variety Release and Registration Institute in 2009 found similar grain yield advantages in favour of bread wheat with 6.88%.

Hl weights of both bread and durum wheat were almost same. But average 1000 kernel weights of durum wheat was higher than that of bread wheat with 5.26 g (12.93%). Highly significant correlation coefficient between 1000 kernel weights vs. marketing price as indicated earlier (Ozberk *et al*., 2011; 2006; 2005a; 2005b) visual characteristics of grains in commodity market are main criteria for high market price offers.

Grand mean of marketing price for durum wheat entries was 455,83 US$ ton^{-1}.Whereas;that of bread what was 434.25 US $ ton^{-1}.marketing price advantage for durum wheat was 4,97%. When the highest market price receiving entries compared, there was a 4.24% advantage in favour of durum wheat. This was 6.63% for the lowest marketing price receiving durum vs. bread wheat entries.

Table 3. grain yield, marketing price and net return comparisons for durum wheat vs. bread wheat.

Comparisons	D W	BW	A d v a n t a g e		
	ton ha^{-1}	ton ha^{-1}	BW%	DW%	BW%
Grain yield					
Grand mean	4,876	5,251	7,14		
Highest mean	5,223	5,946	12,16		
Lowest mean	4,223	4,537	6,9		
Marketing price					
Grand mean	455,83	434,25		4,97	
Highest mean	459,76	441,06		4,24	
Lowest mean	451,90	423,79		6,63	
Net income					
Grand mean	2223,18	2281,57			2,6
Highest mean	2396,46	2080,8			8,83
Lowest mean	1918,44	1922,90			0,23

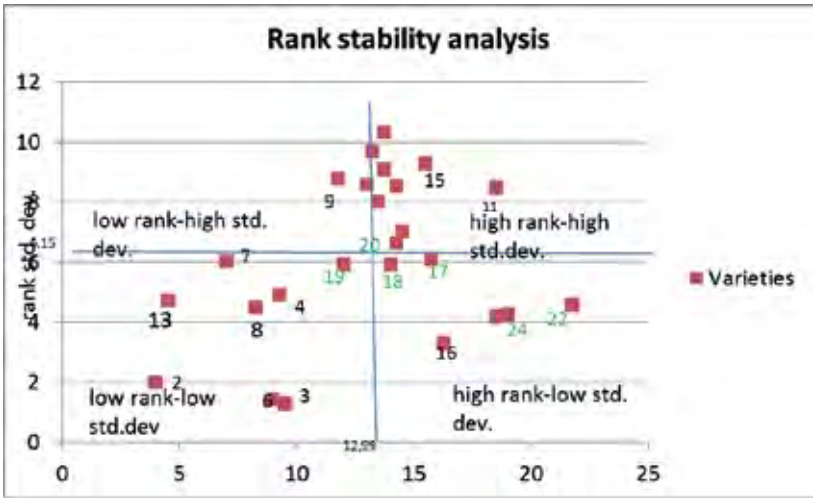

Figure 1. Rank stability analysis of bread wheat and durum wheat entries.

The criterion for farmers in variety preference is productivity and his concept of quality is closely linked to maximize profit (Ozberk *et al*., 2011; Ozberk *et al*.,2006; Troccoli *et al*., 2000; Inglis, 1992).

Grand mean of net return for durum wheat entries was 2238,18 US$ha^{-1} whereas; that of bread wheat was 2281,57 US$ ha^{-1}There was 2,6% net return advantage in favor of bread wheat. This was 8,83% for highest net return generating bread wheat vs. durum wheat entries. Net return advantage of bread wheat was 0,23% for the lowest net return generating bread wheat vs. durum wheat.

In a simulation study additional premium (as much as 2 times of std. deviation of marketing price) given to high income generating durum wheat entry (19) did not change income rank.

By this time of the year in Ş. Urfa commodity market, marketing price differences between durum wheat vs. bread wheat was 5-7% (in favour of durum wheat). This is normally 15-20% throughout the year. It means that durum wheat can be a rival for bread wheat for net return. But durum wheat still needs to have additional premium support for sustainable and profitable production in the SE Anatolia.

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Durum wheat breeding for high yield potential in Egypt

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Abstract. ICARDA-ARC Wheat improvement program (ICARC-WIP) is a joint project between ICARDA and Agricultural Research Center (ARC) in Egypt. The project, which commenced in 2009, aimes to breed for high yielding potential and rust resistance in durum wheat. Nineteen durum wheat genotypes were selected from advanced yield trials in addition to five checks from the growing season 2010-2011 and were planted across five environments at north, middle and south of Egypt as elite durum wheat yield trail (EDWYT) in growing season 2011-2012. Four durum wheat genotypes were selected based on multi location testing by ICARC-WIP and are candidates for inclusion in the preliminary yield trail at the national program in Egypt in the growing season 2012-2013. The pedigree of the 4 durum wheat promising lines are ICAMO-R-TA04-61/Mrb3, Quarmal/Gbch-2//Terbol97-4, Bushen-4/2*Green-18//M ki-1/3/Icasyr-1//Saadi 1989/Chan, and Mrf1/Stj2//Gdr2/Mgnl1. Data were collected on yield and yield components. The previous lines had high yielding ability compared to the grand mean (8786.51 kg/ha) and were resistant to rusts and lodging. The grain yield for these lines were 9061.585, 10422.68, 10805.61 and 9980.483 kg/ha respectively.

Keywords. Durum wheat – Breeding – Yield potential – Egypt.

L'amélioration du blé dur pour un rendement potentiel élevé en Egypte

Résumé. *Le programme ICARDA-ARC pour l'amélioration du blé (ICARC-WIP) est un projet conjoint entre l'ICARDA et le Centre de recherche agricole (ARC) en Egypte. Le projet, qui a débuté en 2009, a pour objectif de réaliser une sélection pour un potentiel de rendement élevé et pour la résistance à la rouille du blé dur. Dix-neuf génotypes de blé dur ont été sélectionnés sur la base du test de rendement avancé, en plus de outre cinq témoins, dans la saison de croissance 2010-2011 et ont été plantés dans cinq différents endroits dans le nord, le centre et le sud de l'Egypte pour effectuer des tests de rendement sur le blé dur d'élite (EDWYT) dans la saison de croissance 2011-2012. Quatre génotypes de blé dur ont été sélectionnés sur la base de tests multi-sites par ICARC-WIP et sont candidats pour être inclus dans le test de rendement préliminaire du programme national égyptien pour la saison de croissance 2012-2013. Les pédigrées des 4 lignées de blé dur prometteuses sont ICAMO-R-TA04-61/Mrb3, Quarmal/GBCH-2//Terbol97-4, Bushen-4/2*Green-18//Miki-1/3/Icasyr-1//Saadi 1989/Chan, et Mrf1/Stj2//Gdr2/Mgnl1. Des données ont été collectées sur le rendement et ses composantes. Les lignées précédentes avaient une capacité de rendement élevée par rapport à la moyenne générale (8786,51 kg/ha) et étaient résistantes à la rouille et à la verse. Le rendement en grain de ces lignées était de 9061.585, 10,422,68, 10805,61 et 9980,483 kg/ha, respectivement.*

Mots-clés. *Blé dur – Sélection – Potentiel de rendement – Egypte.*

I – Introduction

Durum wheat represents 8-10% of the wheat grown and produced worldwide (FAOSTAT, 2006). The production is concentrated in relatively small geographical areas where it often plays a major role in the food security and in the livelihood and nutrition of urban communities (Ammar *et al.,* 2008). About 50% of total area is in the developing countries. In these countries, durum wheat occupies approximately 11 million hectares of which 80% is found in the Mediterranean region of West Asia and North Africa (WANA) (Nachit, 1992). The importance of durum wheat is attributed to multiple usage for human consumption in bread-making, macaroni industry, and it is high in

protein and gluten contents (Rachon *et al*., 2002). In Egypt durum wheat growing areas are concentrated in Middle and Upper Egypt and used in bread and macaroni industry. Since that time many durum wheat cultivars have been identified (Bani Swif 1, Bani Swif 3, Bani Swif 4, Bani Swif 5, Bani Swif 6 and Sougage 3).

By 2020, wheat production must increase 40% to meet the global demand - mainly through elevating yield. “Increasing the intensity of production in those ecosystems that lend themselves to sustainable intensification, while decreasing intensity of production in the more fragile ecosystems” may be the only way for agricultural to keep pace with population (Borlaug and Dowswell, 1997). Hence, future crop improvement has to emphasize grain yield potential (GYP), yield stability, and user preference in concerted interdisciplinary approaches. Issues of environmental sustainability must be an integral part of the research agenda (Pfeiffer *et al*., 2000).

The need to accelerate genetic progress in the yield potential of crops is widely acknowledged (Royal Society, 2009; Philips, 2010), Breeding for high yield potential is very important for many reasons, among them are: 1) increased demand for food; 2) the multiple challenges associated with climate change; 3) declining crop productivity due to attrition of natural resources (Matthew *et al*., 2011). Cox *et al.* (1988) found that 0.6% annual gain in hard red wheat yield in highly productive environments as compared with only 0.4% in stress environments between 1919 and 1987. To achieve environmental sustainability, durum wheat breeding at ICARDA aims to protect high genetic yield potential. ICARDA, Agricultural Research Center (ARC), Wheat Improvement Program (ICARC-WIP) is a joint project between ICARDA and ARC in Egypt conducted at Sids station. Sids station is specific for high yield potential, and the project aimed to 1) breeding for high yield potential, 2) breeding for rust resistant, and 3) capacity building. The ICARC-WIP project supplies CWANA region with different materials..

II – Material and methods

Eight durum wheat experiments including 192 genotypes derived from ICARDA material and acquired by ICARC-WIP were planted in the advanced durum yield trial (ADYT) as augmented design in growing season 2010-2011 at Sids Station 23m asl South of Cairo. Nineteen durum wheat lines were selected from ADYT based on grain yield, rusts reaction (yellow, leaf and steam rust) and resistant to lodging. The selected lines in addition to five checks were planted as elite durum yield trail (EDWT) designed by alpha lattice with three replication in growing season 2011-2012 across six environments representing North, Middle and Upper Egypt. Four promising durum wheat lines were selected from EDYT based on multi locations testing, rust resistant and lodging resistant. These lines are now under evaluation at the national wheat program in the growing season 2012-2013. Table (1) shows the name and pedigree of the EDYT genotypes. Regarding the planting method, it was on flat terrain and all recommendation package were applied for each location.

III Results

Fig. 1. shows variation between durum wheat genotypes under study for grain yield affected by multi location testing across five environments representing North, Middle and Upper Egypt. The highest values observed by genotypes 5, 6, 7 (local check), 14, 19, 21 and 24 which gave 10439.69, 10422.68, 10969.27, 10805.61, 10706.1, 10433.26, 10666.92 kg/ha yield respectively, but some of them were susceptible to rusts.

Regarding yield components Fig. 2, 3 and 4 show the mean values of number of spikes/m^2, number of kernels/spike, and 1000-kernel weight respectively.

Table 1. Name and pedigree for 24 durum wheat genotypes under study.

Sn.	Name/pedigree
1	MORL-F38//Bcrch1/Kund1149/3/Bicrederaa1/Miki
2	Omrabi-5 (check)
3	Azeghar-2/4/Stj3/3/Gdfl/T.dicds-SY20013//Bcr
4	ICAMOR-TA04-61/Mrb3
5	Magh72/Rufo//Alg86/Ru/3/Altar 84/Ald/4/../5/Msbl-1/Quarmal
6	Quarmal/Gbch-2//Terbol97-4
7	Bani Swif-5 (check)
8	Marsyr-3//Mrf-2/T.Dids SY 20123
9	Ouasloukos-1/5/Azn1/4/BEZAIZ-SHF//SD-19539/Waha/3/Gdr
10	CM829/Cando cross-H25
11	Korifla (check)
12	CM829/Cando cross-H25
13	Ouasloukos-1/5/Azn1/4/BEZAIZ-SHF//SD-19539/Waha/3/Gdr
14	Mrf1/Stj2//Gdr2/Mgnl1
15	Marsyr-3//Lgt3/Bcrch1
16	Waha (check)
17	Geromtel-1/Icasyr-1
18	Mrf1/Stj2/3/1718/BT24//Karim
19	ICAMOR-TA04-68/6/21563/AA//Fg/3/D68-10-2A-2A-1A/4/Vitron/5/Bcr
20	Miki-2 (check)
21	ICAMOR-TA04-73/Ammar-8
22	Bushen-4/2*Green-18//Miki-1/3/Icasyr-1//Saadi 1989/Chan
23	Geromtel-1/Icasyr-1
24	Atlast1/961081//Icasyr-1

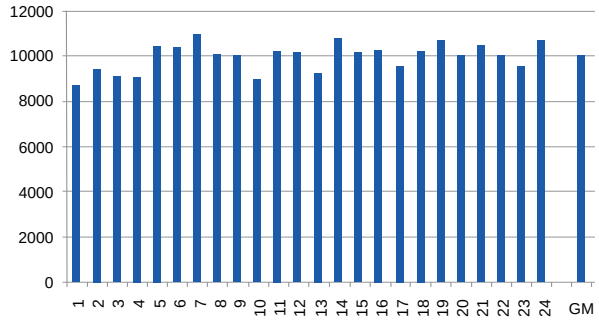

Figure 1. Grain yield performance (kg/ha) over all Egypt compared to checks and grand mean.

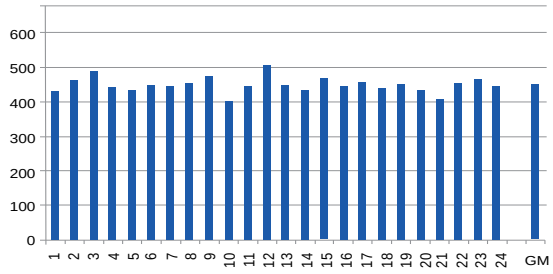

Figure 2. No. of spikes /m² for each genotype at Sids station.

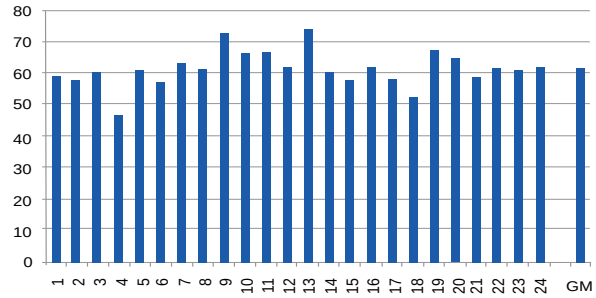

Figure 3. No. of kernels/spike for each genotype at Sids station.

The genotypes 3, 9 and 12 had the highest values for number of spikes/m^2 (488, 477 and 510 respectively); the highest values of kernels /spike were 58, 61, 67, 67, 74, 67 and 65 observed in genotypes 7, 9, 10, 11, 13, 19 and 20 respectively; genotypes 2, 3, 5, 12, 17, 18, 22 and 24 had the highest values for 1000-kernel weight with 53.4, 56.9, 52.2, 52.5, 52.7, 58, 52.5 and 56.9 gms respectively.

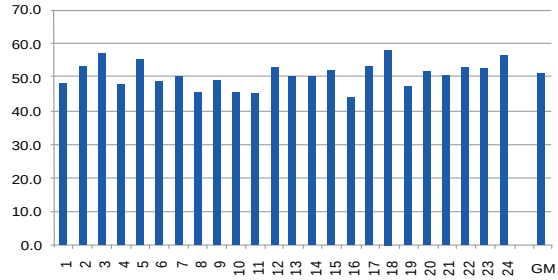

Figure 4. 1000-kernel weight for each genotype at Sids Station.

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Molecular responses to drought and heat stress in durum wheat

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Abstract. Water scarcity and high temperature stress and their combination are the most important stresses experienced by durum wheat in the field. The responses induced by drought and heat stress at transcriptional level have been described, but much less is known about plant response to simultaneous drought and heat stress, although this is the most common event in field conditions. Several data indicate that in plants the molecular response to combined heat and drought stress activates networks which are different from that activated by the single heat or drought stress.

Keywords. Heat stress – Drought – Combined stress – Transcriptome – Microarray.

Réponses moléculaires à la sécheresse et au stress thermique chez le blé dur

Résumé. *Le déficit hydrique et le stress thermique et leur combinaison sont les contraintes les plus importantes auxquelles est exposé le blé dur au champ. Les réponses induites par la sécheresse et le stress thermique au niveau transcriptionnel ont été décrites, mais on n'a pas beaucoup d'informations sur la réponse des plantes au stress dû la sécheresse combinée à la chaleur, bien que celle-ci soit la condition la plus fréquente au champ. Plusieurs données indiquent que chez les plantes, la réponse moléculaire au stress combiné chaleur-sécheresse active des réseaux différents par rapport à ceux activés par l'une des deux contraintes seulement.*

Mots-clés. *Stress thermique – Sécheresse – Stress combiné – Transcriptome – Micropuces.*

I – Introduction

Wheat is one of the oldest crops and helped humans to develop their social communities, evolving itself (domestication processes) from the primitive form (emmer wheat) into the presently cultivated species (Peña 2002). Selection and human habits have led mainly to the cultivation of two wheat species: *Triticum aestivum* L. (hexaploid bread wheat) and *T. turgidum* L. var. *durum* (tetraploid durum wheat). Durum wheat (*Triticum turgidum* L. ssp. *durum*) is a typical Mediterranean crop and is widely used to mill semolina for pasta production. Durum wheat is used also to make regional food (such as couscous). Although the main form of utilization of durum wheat is still represented by pasta, an increasing proportion of production is used for a wide range of baked goods and breads characterized by different flavors and shapes.

About 90 to 95 percent of the wheat produced in the world is common wheat, the rest is mostly durum wheat. Durum wheat adapts to all diverse climatic conditions and is cultivated all around the world. Even if Canada is the country with highest durum wheat production, the Mediterranean countries (Italy, Turkey, Syria, Spain, Algeria, Morocco, Tunisia and France) cover the 80% of world production.

In the Mediterranean region where durum wheat is grown under rainfed conditions, drought and heat are common abiotic stress factors. Heat and drought stress strongly affect grain quality enhancing or decreasing protein content, and often limit yield potential (Farooq *et al*., 2012).

In this review, we present the molecular responses of durum wheat to drought and elevated temperatures.

II – Impact of drought and heat stress

Drought and heat stresses strongly affect the cell physiology of plants because they interfere in photosynthesis and respiration, the two main cell processes. Chlorophyll and fluorescence parameters measured in the flag leaves of durum wheat genotypes, under control and heat-stress conditions in the grain-filling phase was measured by Dias *et al.* (2011), concluding that the chlorophyll reduction and the decrease in Fv/Fm probably resulted in an increasing energy dissipation (i.e., thermal energy) mediated by photoprotective mechanisms. Different studies indicate that loss of chlorophyll during grain-filling is associated with reduced yield (Reynolds *et al.* 1994).

Comparing their data to *T. aestivum*, Dias *et al*. (2010) concluded that the photosynthetic performance of durum wheat is better than bread wheat. Chlorophyll reduction was observed also by Akhkha *et al*. (2011) during drought stress.

A recent study (Li *et al*., 2013) reported the effects of drought and heat stress on yield and quality parameters of durum wheat grains, showing that protein content and SDS sedimentation volume increased under these stress conditions. Other quality parameters related to gluten-strength, were also significantly increased or decreased (Flagella *et al.*, 2010). Moreover drought and heat stress reduce grain yield but enhance flour yellowness and these differences are not equal among cultivars, suggesting that accurate comparative experiments should be done.

III Response mechanisms to drought and heat stress

The molecular mechanisms involved in drought and heat stress responses were well described in studies on *T. aestivum* (Qin *et al.*, 2008; Szucs *et al.*, 2010; Ge *et al*., 2012; Bowne *et al*., 2012; Ford *et al*., 2011), but less was done on *T. durum* wheat, both because *T. aestivum* is the most cultivated among wheat species and because *T aestivum* data are considered informative for all wheat species.

Aprile *et al*. 2009 have carried out a comparative transcriptomic study on drought stress in durum cultivar Creso and bread wheat cultivar Chinese Spring describing a series of molecular mechanisms activated by durum wheat in response to water deprivation.

1. ABA

Abscisic acid content is often associated to plant stress response; in particular as a result of increased water stress. Under intense water stress, the concentration of ABA in plants increases, which triggers a number of processes starting from decrease in turgor pressure, decline in cellular expansion, then stomatal closure to reduce water loss in leaves (Thompson *et al*., 1997). In durum wheat the levels of ABA in response to water stress were studied by Mahid *et al*. (2011) and Akhkha *et al*. (2011) and confirm the findings of other experiment in plants: ABA content is correlated to drought stress level.

The key enzyme in ABA biosynthesis is NCED (9-cis-epoxycarotenoid dioxygenase) and mRNA level, protein level, and ABA content are closely correlated in dehydrated leaves and roots,

indicating a regulatory role of NCED in ABA biosynthesis (Qin and Zeevart 1999). In Creso durum wheat the expression level of the main genes involved in ABA pathway were investigated under drought stress and control conditions (Aprile *et al*., 2009), highlighting not only the *NCED* gene up-regulation, but a general up-regulation of the ABA pathway (Figure 1).

2. Osmolite accumulation

Proline accumulation in higher plants is a characteristic physiological response to osmotic stress. Proline is considered to play an important role in defense mechanisms of stressed cells and can work in protection against oxidative stress (Szekely, 2004).

In durum wheat the proline accumulation was also proposed as drought stress indicator (Dib *et al*., 1994) demonstrating the high correlation between proline content and drought stress level. In plants, proline can be synthesized starting from either glutamate or ornithine (Delauney *et al*., 1993; Kavi Kishor *et al*., 1995) and catalyzed, respectively, by P5CS and ornithine-d-aminotransferase. However, only *P5CS* gene was found differentially expressed in durum wheat in response to drought, whereas both genes are induced in bread wheat (Aprile *et al*., 2009). Metabolic data reported that proline tended to accumulate early, at the onset of the stress, while glycine betaine accumulation was observed during prolonged stress (Carillo *et al*., 2009). Glycine betaine pathway was found activated by drought stress in durum wheat also by Aprile *et al.*, 2009.

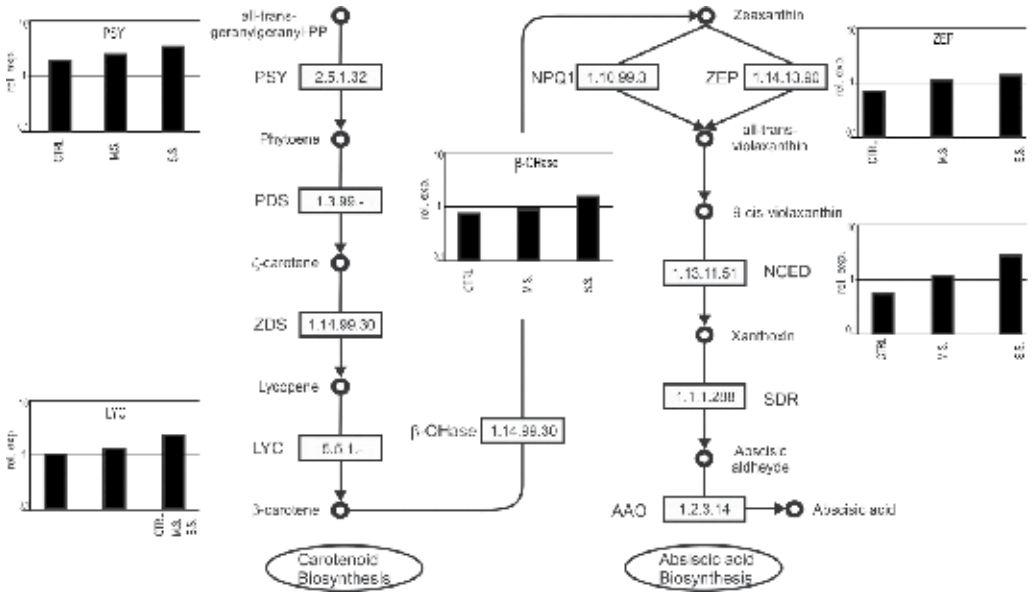

Figure 1. Brief overview of the ABA pathway (inferred by Aprile *et al*., 2009). On the left side the b-carotene biosynthesis steps. On the right the ABA-dedicated enzymatic reactions. Several probe sets related to ABA synthesis enzymes (PSY, LYC- b, β -OHase, NCED) were up-regulated by drought stress. Their expression levels based on array data are showed in the corresponding histograms. 2.5.1.32 = Phytoene synthase (PSY); 1.14.99.-= Phytoene desaturase (PDS); 1.14.99.30 = z-carotene desaturase (ZDS); 1.14.-.-= Lycopene β-cyclase (LYC-b); 1.14.13.- = β -carotene hydroxylase (β -OHase); 1.10.99.3 = Violaxanthin de-epoxidase (NPQ1); 1.14.13.90 = Zeaxanthin epoxidase (ZEP); 1.13.11.51 = 9-cis-epoxycarotenoid dioxygenase (NCED); 1.1.1.288 = xanthoxin dehydrogenase (SDR); 1.2.3.14 = Abscisic aldehyde oxidase (AAO).

3. Heat shock protein

The heat shock proteins (HSPs) were extensively studied among plant species. In durum wheat the HSP26 and HSP70 are activated by heat stress (Laino *et al*., 2010; Rampino *et al*., 2012). Moreover, differences in HSP transcripts accumulation were observed among durum wheat cultivars, and the HSP mRNA levels are related to the acquisition of thermotolerance (Rampino *et al*., 2009). *HSP101* gene was found differentially expressed also after water deprivation as well as the heat transcription factor HSF-C1 /Aprile *et al*., 2009).

4. Transcription factors

Transcription factors (TFs) are key molecular regulators that control genes and gene clusters (Nakashima *et al*., 2009). Many families of transcription factors have been demonstrated to play a role in stress responses in plants. Among them, the bZIP, WRKY, AP2, NAC and C2H2 zinc finger families comprise a high proportion of abiotic stress-responsive members (Rahaie *et al*., 2013).

The DREB proteins, known also as the C-repeat (CRT) binding factors (CBFs), regulate expression of drought/cold stress-related genes, while the ERFs are known to be involved in biotic and abiotic stress responses and both families of proteins contain the Apetala2 (AP2) domain. In durum wheat both DREB and ERF proteins regulate expression of the Cor410b gene, coding a dehydrin, a typical drought stress gene (Eini *et al*., 2013).

Two elements of the NAC family were studied by Baloglu *et al*. (2012), revealing that expression profiles *TaNAC69-1* and *TtNAMB-2* under drought, salt, cold, and heat stress conditions are strongly modulated. bZIP, MYB and WRKY drought-sensitive transcription factors were also found in durum wheat (Aprile *et al*., 2009).

The integration of information about durum wheat physiology and molecular mechanism with conventional or molecular assisted breeding will help to develop new durum wheat varieties with higher performances also if contrasted by drought and heat stress events.

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Durum wheat and local chains: A new strategy to strengthen locally selected genotypes

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Abstract. Wheat is the most important crop in many areas of the Mediterranean Region. In Sardinia (Western Mediterranean, Italy), this crop has been grown for a very long time. As a consequence, the Island is rich in traditional breads and pasta completely different from those that can be found in other parts of Italy and the Mediterranean. Hence, a specific regional breeding programme has been developed in Sardinia to meet the different needs of farmers, millers, end-product processors and consumers as well as to strengthen the peculiarities of the Sardinian durum wheat chain. This programme aims to release specific cultivars showing strong genotype × environment interaction (GE) to Sardinian growing conditions. At the moment, two Sardinian varieties have been released in the last years: Karalis and Ampsicora. These varieties have been grown and tested in different areas of the island together with other varieties used as a check and the main agronomic and quality traits have been analysed. Finally, these varieties have been used either in purity or in blends, to make Sardinian traditional breads and pasta. The success of this programme enabled the development of a local durum wheat chain identified by a quality brand called *Semenadura.*

Keywords. Durum wheat breeding – Local chain – Bread-making – Pasta-making.

Le blé dur et les chaînes locales : une nouvelle stratégie pour renforcer les génotypes sélectionnés localement

Résumé. *Le blé est la culture la plus importante dans de nombreuses zones de la région méditerranéenne. En Sardaigne (Méditerranée occidentale, Italie), cette plante est cultivée depuis très longtemps. Par conséquent, l'île a une richesse de pains et de pâtes traditionnels, très différents de ceux qui peuvent être trouvés ailleurs en Italie et en Méditerranée. Par conséquent, un programme spécifique d'amélioration régionale a été élaboré en Sardaigne pour répondre aux différents besoins des agriculteurs, des meuniers, des transformateurs de produits finis et des consommateurs et pour mettre en valeur la chaîne du blé dur sarde. L'objectif de ce programme est d'obtenir des cultivars spécifiques montrant une forte interaction génotype × environnement (GE) dans les conditions de culture sardes. Deux variétés sardes ont été obtenues au cours des dernières années : Karalis et Ampsicora. Ces variétés ont été cultivées et testées dans différentes zones de l'île avec d'autres variétés utilisées comme témoins et leurs principales caractéristiques agronomiques et leur qualité ont été analysées. Enfin, ces variétés ont été utilisées soit à l'état pur soit mélangées pour préparer des pains et des pâtes traditionnels sardes. Le succès de ce programme a permis le développement d'une chaîne locale du blé dur identifiée par une marque de qualité appelée Semenadura.*

Mots-clés. *Amélioration du blé dur – Chaîne locale – Fabrication du pain – Fabrication des pâtes.*

I – Introduction

Durum wheat is the most widespread crop in many areas of the Mediterranean Region. In Sardinia (Italy), this species is extensively grown in spite of a substantial reduction in recent years (Fig. 1). This downward trend is due to several reasons, not least the difficulty of finding durum wheat varieties suited to the special pedo-climatic conditions of Sardinia. To tackle this downward trend, the regional agencies, Agris and Laore, have developed an on-going project aiming to enhance a durum wheat local chain to meet the needs of farmers, millers, end-product processors, and consumers as well as to exploit the peculiarities of Sardinian durum wheat products. In fact, this

Island is rich in traditional breads and pasta that are completely different from those that can be found either in Italy or in the Mediterranean region (Figs. 2 and 3).

II – Material and methods

This project aims to find durum wheat cultivars showing strong genotype × environment interaction (GE) to Sardinian growing conditions. To achieve this goal a two-stage approach was developed: (1) choosing a reduced number of top-yielding varieties with good quality, in the short term; (2) releasing selected genotypes specifically targeted onto the local agro-ecological conditions of Sardinia, in the medium-long term. In particular, two varieties from the Agris breeding programme were released, Karalis and Ampsicora, and used in this project. The chosen genotypes were tested in experimental and extension fields all over Sardinia. After the harvest, the grain was milled and used either in purity or in blend to make Sardinian breads and pasta.

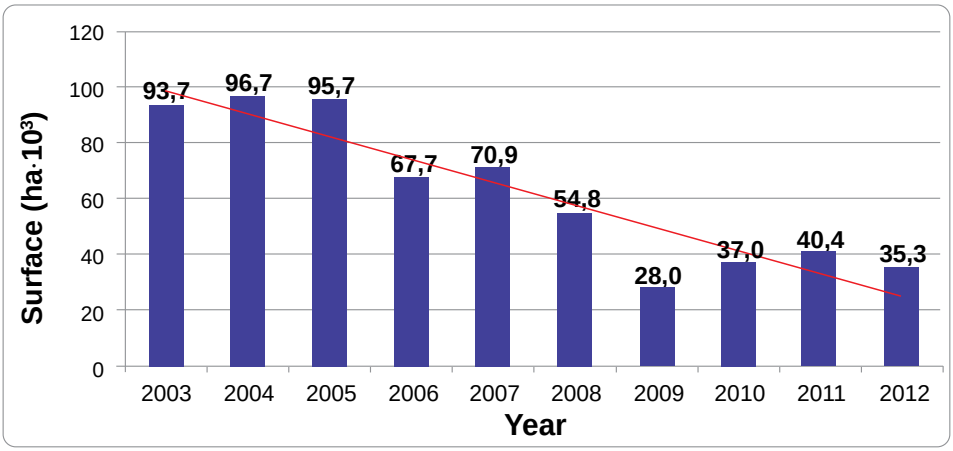

Figure 1. Trend of durum wheat surface in Sardinia (2003-12).

Figure 2. Typical Sardinian pasta: *culurgiones* (left) *and malloreddus* (right).

Figure 3. Typical Sardinian breads: *carasau* (left) *and civraxiu* (right).

III – Results and discussion

Figure 4 shows the effect of using well adapted genotypes to specific agro-ecological conditions: reducing the number and using well adapted or locally selected genotypes resulted in increasing yields across the main durum growing areas of Sardinia. Moreover, the reduction of cultivated varieties allowed a more rational storage process on the basis of protein content and gluten strength. Thus, the offer of Sardinian durum wheat is currently improving its quality standard in order to meet the needs of local millers as well as pasta- and bread-makers.

Among the cultivars currently grown in Sardinia, Karalis showed the greatest yield stability as well as excellent grain and technological quality and has been the most widely grown variety in Sardinia over the last years. Likewise, Ampsicora is rapidly spreading over the main durum growing areas of the Island.

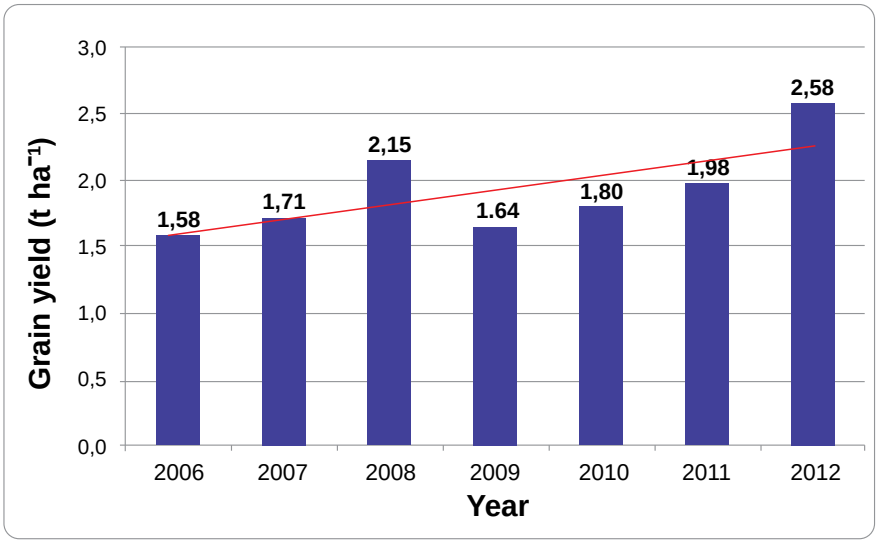

Figure 4. Trend of durum wheat yields in Sardinia (2006-12).

Quality data shown in Table 1 confirm the good quality standard of durum wheat grown in extension fields in the period 2008 to 2010. These results are remarkable given the great year-to-year variability of climatic conditions with a special focus on water supply, as well as the great soil variability due to the ancient geological origin of the island.

Table 1. Yields and data concerning grain and technological quality from extension fields in Sardinia (2008-10).

Area	Year	Protein %	Dry gluten, %	Test weight Kg/hl	Grain t/ha
Sardinia North	2008	13.5	9.2	77.2	3.14
	2009	12.3	8.6	85.1	1.97
	2010	12.1	8.1	81.0	2.55
	Mean	12.6	8.7	81.1	2.55
Sardinia South	2008	13.0	10.1	77.1	2.26
	2009	12.5	9.4	80.4	2.62
	2010	11.8	7.5	75.6	2.29
	Mean	12.4	9.0	78.0	2.36
Sardinia	Mean	12.5	8.8	79.6	2.46

IV – Conclusions

The success of this project is proven by the increasing share of local durum wheat milled and processed "on spot". Furthermore, the increasing interest of demonstrative and promotional activities resulted in the design of a quality brand addressing to this specific durum local chain. This brand is called "SEMENADURA" (*sowing* in the Sardinian language) and it will guarantee provenance and quality of typical agro-food products made of Sardinian wheat (Fig. 5).

In the future, the project will emphasize breeding activities to release high-yielding, top-quality genotypes well adapted to the Sardinian agro-ecological conditions and agronomic management to preserve the long-term soil fertility by means of conservation tillage and rotation with legumes as well as sustainability of agricultural systems (Fig. 6).

Figure 5. Two examples of the quality brand "Semenadura".

Figure 6. Cropping systems trials.

The n-alkylresorcinols in durum wheat: genotypic and environmental variability

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Abstract. The polyphenols commonly present in some cereal grains (*Triticum* species, rye and barley, mainly) include the 5-n-alkylresorcinols, alternatively called a kylresorcinols (ARs). The major AR in cereal grain contains the saturated chain with an odd number of carbon atoms in the range 17-25. These polyketides exh bit a wide spectrum of biological activities (antimicrobial, interaction with proteins, biological membranes, and enzymatic activities) which may be associated with their amphiphilic structure. Content and composition of ARs are affected by several factors such as plant species, cultivar, organ, physiological stage and environment.

The aim of the present study was to evaluate AR variability (content and homologue composition) by GC-MS of fifteen cultivars of durum wheat grown in three Italian locations (Jesi, Montelibretti and Foggia) during 2009 and 2010.

The environment (E) and the genotype (G), as well as their interaction (GxE), appeared to significantly influence the AR content in the durum wheat grains. On average, the analysed genotypes showed a variability range from 173.9 to 415.2 µg/g (dry matter, DM) and revealed similar composition of AR homologues with a high proportion of higher chain length C21:0, C23:0 and C25:0. Finally, in the present study the potential antifungal activity of ARs extracted from durum wheat against four different *Fusarium* species was also described.

Keywords. Durum wheat – Alkylresorcinols – Antifungal activity – Functional food .

Les n-alkylrésorcinols chez le blé dur : variabilité génotypique et environnementale

Résumé. *Les polyphénols présents couramment dans les grains de certaines céréales (l'espèce Triticum, le seigle et l'orge, principalement) incluent les 5-n-alkylrésorcinols, connus aussi comme alkylrésorcinols (ARs). Le principal AR dans le grain de céréale contient la chaîne saturée avec un nombre impair d'atomes de carbone allant de 17 à 25. Ces polykétides ont plusieurs fonctions biologiques (activité antimicrobienne, interaction avec les protéines, membranes biologiques et activités enzymatiques) qui peuvent être associées à leur structure amphiphile. Le contenu et la composition des ARs sont influencés par de nombreux facteurs tels que l'espèce végétale, le cultivar, l'organe, le stade physiologique et l'environnement.*

Le but de cette étude était d'évaluer la variabilité des AR (teneur et composition homologue) par GC-MS de quinze variétés de blé dur cultivées dans trois différents endroits en Italie (Jesi, Montelibretti et Foggia) en 2009 et 2010.

L'environnement (E) et le génotype (G), ainsi que leur interaction (GxE), semblent influencer de manière significative la teneur en AR dans les grains de blé dur. En moyenne, les génotypes analysés ont montré une variabilité allant de 173,9 à 415,2 mg/g (matière sèche, MS) et ont révélé une composition similaire des homologues d'AR avec une forte proportion de la longueur de chaîne supérieure C21: 0, C23: 0 et C25: 0. Enfin, l'activité antifongique potentielle des ARs extraits de blé dur contre quatre espèces différentes de Fusarium a également été explorée.

Mots-clés. *Blé dur – Alkylrésorcinols – Activité antifongique – Aliments fonctionnels.*

I – Introduction

Wheat is a major crop and one of the most important staple foods of the human diet. In recent years, research has shown that whole grain consumption was associated with significant health benefits in the management of important chronic diseases (Jacobs *et al*., 2004; Riccioni *et al*., 2012; de Munter *et al*., 2007; Aune *et al*., 2011). Current consumer demand for healthier foods has lead to an increased focus on the characterization of health-beneficial compounds and their contents in whole grain. Among health promoting phytochemicals residing in whole grain, phenolic lipids have gained interdisciplinary interest in many scientific research areas as they have antioxidant properties and biological activity in prevention of cardiovascular diseases and cancer. Phenolic lipids are synthesized both during normal development and in response to stress conditions and their content is affected by several factors such as plant species, cultivar, organ, physiological stage and environment (soil, agronomy and climate) (Carbone *et al*., 2011; Yu *et al*., 2004). The polyphenols commonly present in the cereal grains include the 5-n-alkylresorcinols, alternatively called alkylresorcinols (ARs) which belong to an extensive family of bioactive compounds, widely distributed in plants, fungi and bacteria (Kozubek and Tyman, 1999; Ross *et al*., 2003).

5-n-alkylresorcinols are characterized by two hydroxyl groups at positions C1 and C3 of the aromatic ring, with an odd number of saturated alkyl chains of different lengths (C15:0, C17:0, C19:0, C21:0, C23:0, C25:0) at the C5 position of the benzene ring. The amphiphilic nature of their structure could be responsible for their ability to interact with biological membranes and related with their biological properties (antimicrobial, cytotoxic, antioxidant, antitumor activity etc.) (Kozubek and Tyman, 1999; Ross *et al*., 2003, Linko *et al*., 2005). Among cereals, wheat, rye and triticale contain high levels of these compounds which occur only in an intermediate layer of the caryopsis, including the hyaline layer, inner pericarp, and testa and are not detected in other parts of the grain (Landberg *et al*., 2008). Due to their periferal location and biosynthesis specifically during seedling stage, alkylresorcinols and their derivatives are thought to serve important roles as phytoanticipins and allelochemicals, although direct evidence is still somewhat lacking (Suzuki and Yamaguchi, 1998; Zarnowski *et al*., 1999).

Moreover, limited information is available on the effect of genotype and growth conditions on the concentration and composition of AR in durum wheat. In a recent paper (Bellato *et al*., 2013) the authors have shown data on the phytochemical profile of the durum wheat grains also, considering the AR variability. In the present study additional information on the effects of genotype (G), environmental factors (E) and their interaction (G×E) on AR accumulation was provided. As regard the potential of these compounds to inhibit fungal growth, in this work the antifungal activity of 5-(n)-alkylresorcinol extract, from durum wheat whole grain, against four species of *Fusarium* (*F. graminearum* Schwabe, *F. culmorum* (W.G.Smith) Sacc, *F. avenaceum* (Fr.) Sacc. and *F. poae* (Peck) Wollenw.) was described.

II – Material and methods

Fifteen Italian commercial varieties of durum wheat (*T. turgidum*, L. ssp. *durum*) were grown in two different geographical areas, Jesi and Foggia located in central-north and southern Italy, respectively, in two successive years (2008–09 and 2009–10) with two replications. The environmental conditions for the selected growing areas were reported previously (Ciccoritti *et al.*, 2011). Moreover, during 2009-10, the Montelibretti area was also included. From sowing time to harvest, temperature and rainfall data were considered normal for this area of central Italy. The samples, immediately after harvest, were milled using a laboratory cyclone mill (Cyclotec 1093, Foss, Italy) to pass a 0.5 mm screen, to produce wholemeal flour.

To maximize the extraction, 1g of milled samples of each variety was extracted with 40 ml of acetone for 24h (Ross et al. 2001) and total AR content and relative homologue composition in

the extracts were determined by GC-MS analysis according to Landberg *et al*. (2009). All the analytical details are provided in Ciccoritti *et al*. (2013).

To test the antifungal activity by minimizing co-extraction of interfering substances, AR extract was prepared from durum wheat whole grains by using cyclohexane solvent (ratio 1:5 w:v) (Nocente *et al*., 2012). The extract was then filtered through Whatman paper, dried and finally redissolved in 1 ml of the solvent.

Then the fungistatic activity against *F. graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae* was tested. One 4-mm diameter plug of the isolates growing on PDA was placed in the center of the 60 mm PDA plates previously prepared by spreading 500 µl of the extract. After 4 to 6 da from inoculation the diameter of colonies was measured and compared to control plates and the percentage of growth inhibition was calculated.

The combinations of years and locations were treated as five environments. Analysis of variance was performed with the MSTATC program (Michigan State University, East Lansing, MI) using a factorial model (mod.9) with G, E (locality and year) and G x E interaction. Genotype means were separated using Duncan's multiple range test ($p < 0.05$) by combining the results across environments. Principal component analysis (PCA), performed with MATLAB software (R2010a version, MathWorks Inc., USA), was used to study the variation associated with the genotype and the environment.

III – Results

1. Variability of AR in relation to genotype and environment factors

On average, the analysed durum wheat genotypes showed a variability range from 173.9 to 415.2 µg/g (dry matter, DM). Data for the 15 durum wheat common cultivars grown in the selected five environments were analyzed by ANOVA (Table 1).

Table 1. Mean value, standard deviation (SD) and mean squares of genotype (G), environment (E) and interaction (G x E) for 5-n-alkylresorcinol (AR)

Source of variation	Degrees of freedom	Mean square
Replication	1	172.83
Genotype (G)	14	6194.93***
Error	14	205.5
Environment (E)	4	24680.30***
G × E	56	2418.08***
Error	74	299.31
*P<0.001*** Mean value (%DM) ± SD=*	*264.0 ± 32.9*	

Results showed that both G and E had highly significant effects on AR content ($p < 0.001$). In addition, the contribution of G × E interaction to the total variability was lower than that of the genotype or environment alone. Anderson *et al*. (2010) also reported a strong influence of genotype as well as of environment on the AR content in bread wheat. Our data showed that E was the main factor contributing to the total variation in the parameters that were measured.

Box plots were used to compare the variation in the AR content within and between environments (Fig. 1).

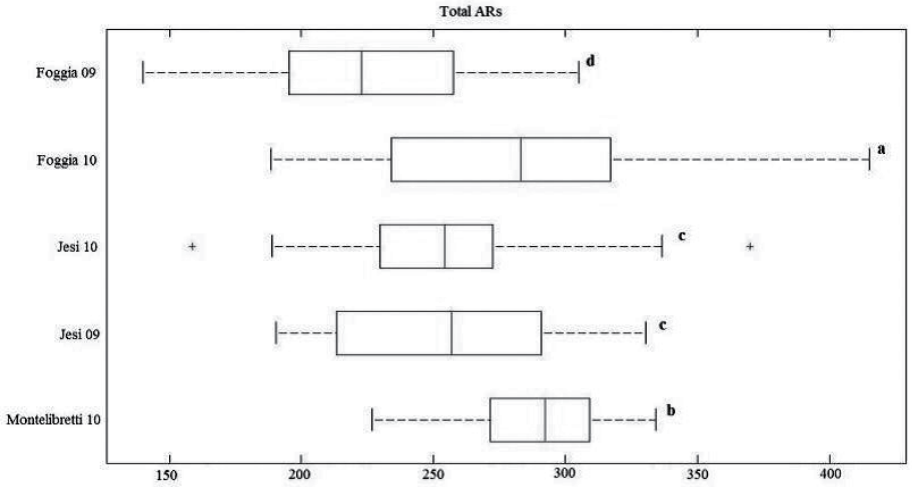

Figure 1. Dataset box plots: evaluation of E variability on AR content (µg/g DM). Different letters indicate that means are significantly different from each other (P<0.05).

The AR content showed a significantly higher mean value in Foggia 2010 (302.7 µg/g DM) with a range between 198.0 and 415.2 µg/g DM. On average, the lowest AR content (241.7 µg/g DM) was recorded in Foggia in 2009 and the highest in Foggia in 2010, which is the environment where the lowest amount of precipitation occurred. These results are in agreement with Anderson and co-workers (2010) who have observed a great variability in the AR content of bread wheat between years and locations, with highest contents in an environment characterized by hot dry conditions during grain filling.

Box plots of the 15 common varieties grown in all of the environments, as well as the variation in the measurement within a single variety are shown in Fig. 2.

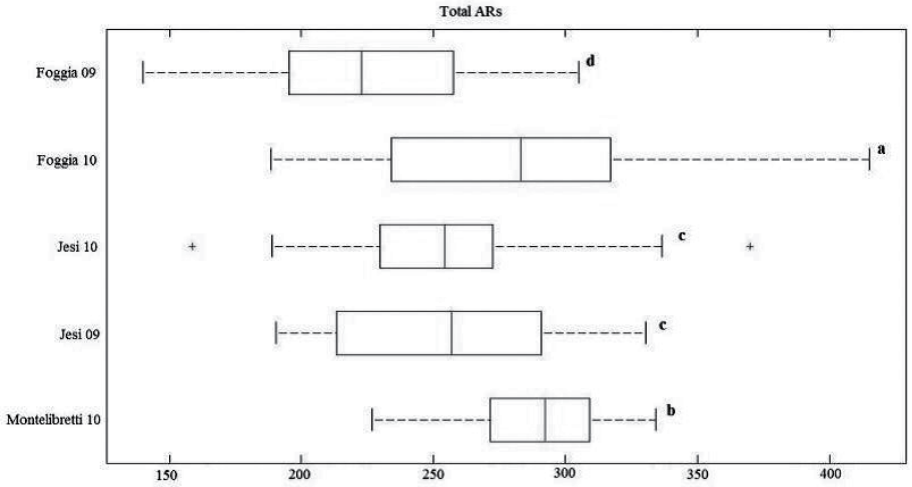

Figure 2. Dataset box plots: evaluation of G variability on AR content (µg/g DM). Different letters indicate that means are significantly different from each other (P<0.05).

The genotypes significantly differed in their AR content and the mean values ranged from 210.0 (Normanno) to 324.1 µg/g DM (Iride). The box plots show limited variation in some of the genotypes (Minosse, Normanno, Tirex, Ciccio, and Simeto) underlying the significant contribution of genotypic characteristics to the AR accumulation. The characterization of AR extracts by using GC-MS technique showed no significant differences of homologue composition in the analyzed durum wheat cultivars, which presented high proportion of homologues with high chain length C21:0, C23:0 and C25:0. This is in agreement with previous findings that the variations in AR composition are mainly due to *Triticum* species (Ciccoritti *et al*., 2013). The effect of the environment on AR content can be clearly seen by PCA of the varieties and environments (Fig. 3). The environments are in similar positions on the positive side of PC1 (explained variance = 66%); in the biplot Foggia 2010 and Montelibretti 2010 (the latter appears very close to the axes) are located on the positive side for PC2 (explained variance = 30%). The varieties with positive scores for PC1 and PC2 generally had AR content slightly above the average. Among them, only Iride (positive value for PC1) showed higher and more stable contents of AR across environments. Four varieties with positive scores for PC1 showed very strong interaction with the environment and had the highest contents of AR in Foggia 2010.

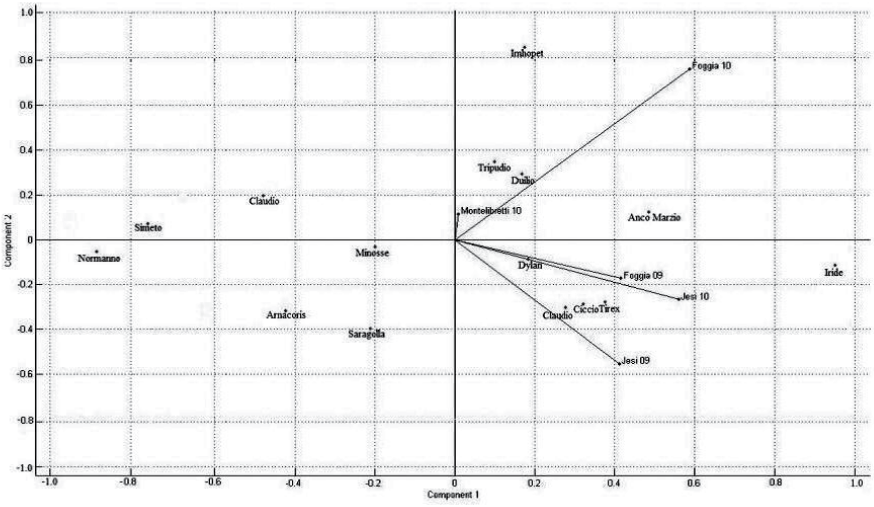

Figure 3. Genotype × Environment biplot from PCA for AR content of 15 durum wheat varieties grown in five environments.

2. Antifungal activity of ARs

Following the indications of previous study (Nocente *et al*., 2012), to test the antifungal activity of AR extract from durum wheat grain, cyclohexane solvent was used. Gas chromatography (GC-MS) analysis confirmed that cyclohexane AR extract contained a higher proportion of C21:0 homologue, which represented 59.9% of ARs, and lower percentages of the other homologues. Moreover in agreement with Zarnosky *et al*. (2004) the GC-MS analysis showed low amount of ballast substances co-extracted with AR (*data not shown*).

The fungistatic activity of cyclohexane extract on mycelial growth of *F. graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae* was tested (Fig.5).

The analysis of variance performed on the colony diameter, measured after 4 and 6 days from inoculation, showed statistically significant differences on the effect of ARs against the four pathogens compared with the respective control. In particular, after six days it was observed a growth inhibition of 47% for *F. graminearum*, 41% for *F. culmorum*, 55% for *F. avenaceum* and 40% for *F. poae.*

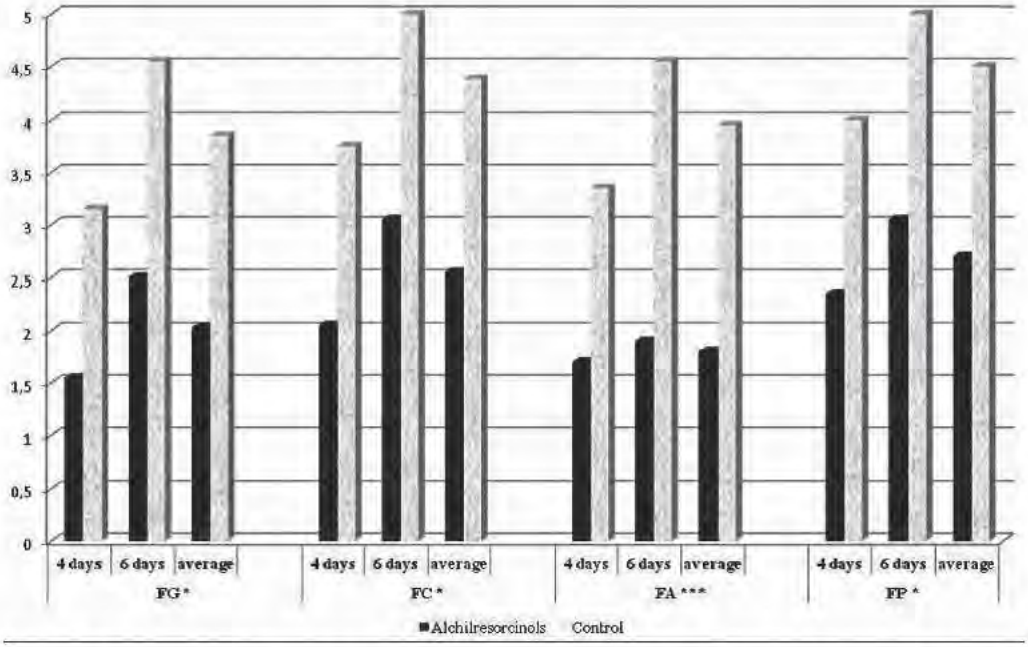

Figure 5. Effects of cyclohexane extract, containing ~500 µg of AR, on mycelial growth of *Fusarium* spp. (FG= *F. graminearum*; FC= *F. culmorum*; FA= *F. avenaceum*; FP= *F. poae*). Colony diameter (cm) after 4 and 6 days of incubation (average values of triplicate experiments are given) *** P=0.001 *P=0.05.

IV – Discussion and conclusion

The results of this study will provide useful information to durum wheat scientists regarding the relative importance of genotype and environment on the accumulation in the grains of 5-n-alkylresorcinols. Principal component analysis identified genotypes that were richer in ARs and more stable across environments. The findings in the present paper indicate that there is a wide variation in the AR content; significant E and G effects were found ($p < 0.001$). In addition the contribution of G x E to the total variance appears much lower than that due to the G and E effects, and E accounted for the highest proportion of the variation. These data clearly show that the effects of E and G×E on the levels of AR should be taken into account during the breeding programs aimed at improving the health benefits of wheat. The beneficial antifungal and 5 antibacterial activities have led to the general assumption that alkylresorcinols play a defensive role during plant growth, even though they occur as a minor components. To test antifungal properties of 5-n-alkylresorcinols various organic solvents were used following the objective to extract alkylresorcinols by minimizing co-extraction of interfering substances which might reduce the inhibitory effects of 5-n alkylresorcinols on pathogen fungi (Zarnowski and Suzuki, 2004; Nocente *et al.*, 2012). Among the suggested organic solvents, in the present investigation

cyclohexane was chosen as extractant of 5-n-alkylresorcinols from intact kernels. In these analytical conditions, our data demonstrated antifungal activity of AR extracts from durum wheat grain against the tested *Fusarium* fungi; in particular, high growth-inhibiting activity of the extract was evidenced against *F. avenaceum*, this fungus appeared more sensitive towards AR than *F.graminearum*, *F. culmorum* and *F. poae*. The results suggest that the ARs can be considered the major components responsible for the fungistatic properties of cyclohexane extract from durum wheat grain. However, it cannot be excluded that the minor components in the extract may also contribute to the antifungal activity.

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Application of the international crop information system for retrieval and usage of pedigree and phenotypic data for use in durum research and breeding

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Abstract. The International Crop Information System (ICIS) links pedigrees to phenotypic and genotypic data that is made easily accessible. The Genealogy Management System (GMS) interfaces 'Central' public databases that can have various levels of access, and 'Local' private databases within institutions. This facilitates the global sharing of non-sensitive pedigrees, selection histories and other descriptors in the Central database while interfacing with Local databases which contain sensitive data. Canadian public-sector durum wheat researchers have set up databases containing extensive phenotypic data (agronomic, disease and end-use functionality) from cultivar registration and pre-registration trials conducted over the past 25 years. Pedigree information on the lines is uploaded to the globally-available Central database so that pedigrees of Canadian lines can be traced back to ancestors and easily visualized for presentation purposes. The power of this system was demonstrated by tracing the sources of the low grain cadmium concentration allele by selective phenotyping of the lineages of diverse durum cultivars, and management of data for association mapping studies for discovery of QTL for biotic stresses.

Keywords. *Triticum turgidum* L. var *durum* – Data management – Pedigree – Grain cadmium.

Application du système international d'information sur les cultures pour la récupération et l'utilisation des données de pédigrées et phénotypiques pour une utilisation dans la recherche du blé dur et la sélection

Résumé. *Le système international d'information sur les cultures (ICIS) fait le lien entre les pédigrées et les données phénotypiques et génotypiques qui sont rendues facilement accessibles. Le système de gestion des données généalogiques (GMS) fait l'interface entre des bases de données publiques « centrales », qui peuvent avoir différents niveaux d'accès, et des bases de données privées «locales», au sein des institutions. Cela facilite le partage mondial des pédigrées non-sensibles, des histoires de sélection et d'autres descripteurs présents dans la base de données centrale, tout en permettant l'interfaçage avec des bases de données locales qui contiennent des informations sensibles. Au Canada, les chercheurs du secteur public qui travaillent sur le blé ont mis en place des bases de données réunissant de nombreuses données phénotypiques (aspects agronomiques, maladies et fonctionnalité à l'utilisation finale) provenant des essais pour l'homologation et la pré-homologation des cultivars, réalisés ces 25 dernières années. Les informations sur le pédigrée des lignées sont chargées dans la base de données centrale, disponible à l'échelle mondiale, pour pouvoir remonter aux ancêtres et visualiser les pédigrées des lignées canadiennes à des fins de présentation. La puissance de ce système a été démontrée en traçant les sources des allèles de la faible concentration de cadmium dans le grain à travers un phénotypage sélectif des lignées de divers cultivars de blé dur, et la gestion des données pour des études de cartographie d'association visant la détermination de QTL pour des stress biotiques.*

Mots-clés. *Triticum turgidum L. var durum – Gestion des données – Pédigrée – Cadmium du grain.*

I – Introduction

The International Crop Information System (ICIS) links pedigrees to phenotypic and genotypic data for easy querying and use. The Genealogy Management System (GMS) permits various levels of access when interfacing 'Central' public databases (e.g., IWIS, International Wheat Information System), and 'Local' private databases within institutions. This facilitates the global sharing of, for example, non-sensitive pedigrees and selection histories in the Central database while interfacing with Local databases which may contain sensitive data (6.5M). This system has the advantages of accommodating input of different synonyms depending on the source of information, and allowing determination of the wheat pedigrees by many experts from around the world.

ICIS is a very useful repository of valuable data that were used, in addition to the primary function as a breeding tool, for estimation of genetic gain (Clarke *et al.,* 2010) and association genetics studies (Pozniak *et al*., 2012). In this report, we look at usage of ICIS pedigree and molecular databases to track sources of the low grain cadmium concentration allele in diverse durum lines, and the phenotypic database to manage data for association mapping studies.

Cadmium is a heavy metal potentially toxic to humans (McLaughlin *et al.,* 1999) that occurs naturally in trace quantities in almost all soils. North American durum wheat (*Triticum turgidum* L. var *durum*) was reported to accumulate higher Cd levels in grain than hexaploid wheat (*T. aestivum* L.) (Zook *et al*.,1970). Breeding for low grain cadmium concentration began in Canada in the early 1990s (Clarke *et al.,* 2010) due to observed genetic variation in durum wheat cadmium concentration (Penner *et al.,* 1995), which was simply inherited (Clarke *et al*., 1997). This led to the commercialization of the now widely-grown low cadmium cultivar Strongfield (Clarke *et al.,* 2005). However, the ancestral source of the low cadmium trait is not known.

Resistance to biotic stresses is a long-standing focus of Canadian durum breeding. The wheat stem sawfly (*Cephus cinctus* Norton) is a major insect pest of the Canadian durum growing area. Changes in North American races of leaf rust (*Puccinia triticina* Eriks.) and stripe rust (*Puccinia striiformis* f. sp. *Tritici*) are a concern due to widespread virulence on Canadian durum germplasm. Discovery of QTL for resistance to these biotic stresses is a high priority using bi-parental populations and association mapping panels, with data management facilitated by ICIS.

II – Material and methods

Canadian public-sector durum wheat researchers maintain databases containing extensive phenotypic data (agronomic, disease and end-use functionality) from cultivar registration and pre-registration trials conducted over the past 25 years. Pedigree information on the lines is uploaded to the globally-available Central database so that pedigrees of Canadian lines can be traced back to ancestors and easily visualized for research purposes at (https://www. integratedbreeding.net/crop-information/wheat).

The origin of the low cadmium allele was traced in the cultivar Biodur (Valdur//Wascana/Durtal), which originates from Germany. It was used as a donor of the low cadmium trait and stem solidness in Canadian durum breeding programs. Solid stems reduce damage by the wheat stem sawfly. The pedigree of Biodur in ICIS was updated or corrected where necessary following cross-checks with the European Wheat Database of the European Cooperative Programme for Plant Genetic Resources (http://genbank.vurv.cz/ewdb/), research publications and communication with durum wheat breeders. The Draw Tree option in ICIS was used to generate seven generations of pedigrees of Biodur to track the origin of the allele. As many key ancestors as possible were obtained for testing cadmium genotype. Multiple accessions of each ancestor were tested where possible to detect heterogeneity. Seed not already in our possession was obtained from the

USDA Small Grains Collection, Plant Gene Resources Canada, or directly from durum breeding colleagues. Lines and accessions were tested with the marker *XBF474090* (Weibe *et al*., 2010) to detect presence of the low cadmium allele.

Other capabilities of ICIS are demonstrated, such as calculation of coefficient of parentage among solid-stem lines in an association mapping panel and production of publication quality tables of germplasm lists with pedigrees and origins.

III – Results and discussion

Biodur, by its pedigree, could derive the low cadmium allele from hexaploid wheat, Durtal having come from the cross of a semidwarf hexaploid with the durum Sentry. Durtal, however, turned out to have high cadmium phenotype (Fig. 1). Valdur was therefore the donor of the low allele, possibly obtained from M'Rari via D117. However, Sterpe 131066, which we could not obtain, could be a co-donor. Biodur is the donor of the solid stem trait and co-donor of low cadmium in two new durum cultivars CDC Fortitude (Pozniak *et al*., unpublished) and AAC Raymore (Singh *et al,.* unpublished).

In other tested lineages (not shown), we were able to demonstrate that the low cadmium allele was in some cases obtained from hexaploid wheat parents. Hexaploid wheats were the sources of Rht-B1b dwarfing genes in durum, so the low cadmium allele in many CIMMYT durum lineages may derive from those crosses. We are conducting further testing to confirm our preliminary observations. This demonstrated the utility of ICIS to generate the pedigrees of low cadmium durum cultivars. Combined with molecular information available in ICIS, these data were a powerful tool to determine the likely source of the allele conferring low grain cadmium concentration.

We made extensive use of ICIS for retrieval of phenotypic data from Canadian durum registration trials for association mapping studies (Pozniak *et al*., 2012) using DArT markers. The phenotypic information comprising agronomic, end-use quality and disease resistance data for 14 years of the registration trial were retrieved. The data consisted of individual replicated plot data for agronomic and disease traits, and within location or among location composite data for end-use quality. The 'SETGEN' feature of ICIS was used to create a publication-ready list of the lines in the study, together with pedigrees and origin of the material (Table 1). The same dataset is being used to evaluate the recently-available genotypic data from the Infinium 90K iSelect array.

ICIS was also used for management of data and pedigrees for an association mapping panel used for study of linkage disequilibrium (Somers *et al*., 2007) and identification of QTL associated with semolina yellow pigment (Reimer *et al*., 2008). The same panel of lines is currently being used for association mapping of stripe rust resistance with the Infinium 90K iSelect array and for validation of markers for stem solidness. Phenotyping of the panel for stem solidness identified several solid stem accessions from Italy. The coefficient of parentage (COP) was calculated among these lines using ICIS (Table 2), and with the recently released Canadian cultivars CDC Fortitude and AAC Raymore, and their ancestor Biodur. The COP showed that Biodur is not closely related to the Italian lines. In contrast, Biodur showed a larger COP with all three Canadian lines because it is the source of solidness, and Biodur also has a Canadian ancestor (Wascana). The COP of the Italian cultivars Fortore, Lesina and Mongibello was greater than 0.3 as would be expected given their similar pedigrees.

In summary, ICIS is a useful tool in our durum breeding and research activities. Use of ICIS offers time efficiencies over maintenance of phenotypic, molecular and pedigree data in spreadsheets or text files. Local curation of our database enables correction of errors as they are found by users, thus providing users with access to the most up to date version of all data sets. Use of ICIS for data management also ensures access to properly annotated datasets by future users,

overcoming the all too common situation where data are lost when a researcher moves to a new job or retires.

Acknowledgments

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Durum wheat and climate change: simulation models as a tool to support decisions in targeting genotypes and crop breeding

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Abstract. The CERES-Wheat crop model, included in DSSAT v. 4.0, was used to simulate the grain yields of three Italian durum varieties--Creso, Duilio and Simeto-- grown in two sites, Benatzu (high-fertility soil) and Ussana (low-fertility soil), in Southern Sardinia (Italy). The model was cal brated and validated using long-term weather and agronomic data-sets over the period 1973 to 2004. To assess the responses of durum wheat varieties to increasing temperatures and decreasing rainfall, 48 scenarios were used: 6 scenarios with increasing maximum air temperature from 1 to 6 °C incremented by 1 °C steps; 6 with decreasing rainfall from 5 to 30% of the annual measured amount reduced by 5% steps; 36 combining increasing temperature and decreasing rainfall scenarios. The simulated impact of increasing temperatures and decreasing rainfall scenarios on the grain yields of Creso, Duilio and Simeto were evaluated at both sites and resulted in grain yield reduction for all varieties and sites. The late variety Creso proved to be the most sensitive to the effects of the simulated scenarios. In contrast, the early genotypes Duilio and Simeto showed the lowest grain yield reduction. Compared to results from real experiments in different pedoclimatic conditions, CERES-Wheat model responses effectively express reality. Hence, CERES-Wheat can be reliably used to evaluate plant responses to projected climate change conditions and used successfully to support mitigation strategies such as choice and selection of adapted genotypes to tackle the negative impact of climate change.

Keywords. Durum wheat – Climate change – Simulation models.

Blé dur et changement climatique : les modèles de simulation comme outil d'aide aux décisions de sélection des génotypes et à l'amélioration des cultures

Résumé. *Le modèle de culture CERES-Blé, inclus dans DSSAT version 4.0, a été utilisé pour simuler les rendements en grain de trois variétés de blé dur italien - Creso, Duilio et Simeto-- cultivées dans deux sites, Benatzu (sol à haute fertilité) et Ussana (sol à faible fertilité), dans le sud de la Sardaigne (Italie). Le modèle a été étalonné et validé en utilisant des séries de données météorologiques et agronomiques de long terme couvrant la période 1973-2004. Pour évaluer les réponses des variétés de blé dur à la hausse des températures et à la baisse des précipitations, 48 scénarios ont été élaborés : 6 scénarios avec augmentation de la température maximale de l'air de 1 à 6°C incrémentée par paliers de 1°C ; 6 avec la diminution de la pluviométrie de 5 à 30% de la quantité mesurée annuelle réduite par paliers de 5% ; 36 scénarios combinant température croissante et précipitations décroissantes. L'impact simulé des scénarios envisageant une hausse des températures et une diminution des précipitations sur les rendements en grains de Creso, Duilio et Simeto a été évalué sur les deux sites, révélant une réduction du rendement en grain pour toutes les variétés et les sites. La variété tardive Creso s'est avérée être la plus sensible aux effets des scénarios simulés. En revanche, les génotypes précoces Duilio et Simeto ont montré la plus faible réduction du rendement en grain. Par rapport aux résultats des expériences réelles dans différentes conditions pédoclimatiques, les réponses du modèle CERES-Blé traduisent bien la réalité. Par conséquent, CERES-blé peut être utilisé de manière fiable pour évaluer les réponses des plantes aux conditions du changement climatique prévu et appliqué avec succès pour soutenir les stratégies d'atténuation telles le choix et la sélection de génotypes adaptés pour lutter contre l'impact négatif du changement climatique.*

Mots-clés. *Blé dur – Changement climatique – Modèles de simulation.*

I – Introduction

Crop simulation models are useful tools to determine the potential impact of climate change on production and to define adaptation strategies in order to analyse the most appropriate actions to mitigate the potential negative effects as well as to propose guidelines for plant breeding and agricultural policies. In this study the CERES-Wheat crop model included in DSSAT v. 4.0 (Godwin *et al.*, 1990; Jones *et al.*, 2003) was applied to assess the simulated impact of increasing temperatures and decreasing rainfall on grain production of three durum wheat varieties grown at two different sites in Southern Sardinia (Italy). In addition, the simulated responses of the three varieties to increasingly harsh growing conditions was evaluated.

II – Material and methods

The Italian varieties Creso, Duilio and Simeto were used to test the model performances. The CERES-Wheat model had been calibrated and validated in the test area for the same varieties in a previous study (Dettori *et al.* 2011). Two sites were considered: Benatzu (clay soil with high-yielding potential) and Ussana (sandy-clay soil with low-yielding potential). Experimental data of both sites came from the Italian Durum Wheat Variety Trials. The following study periods were considered: 1974-2004 and 1975-2004 for Creso at Benatzu and Ussana, respectively; 1985-2004 and 1986-2004 for Duilio at Benatzu and Ussana, respectively; 1989-2004 for Simeto both at Benatzu and Ussana. To assess the simulated effects of increasing temperatures and decreasing rainfall on the grain yields of the three durum wheat varieties, 48 scenarios were used to represent paths of possible future climate: 6 scenarios with increasing maximum air temperature from 1 to 6 °C incremented by 1 °C steps; 6 scenarios with decreasing rainfall from 5 to 30% of the annual measured amount reduced by 5% steps; 36 scenarios obtained by combining increasing temperature and decreasing rainfall. The simulated impacts of increasing temperatures and decreasing rainfall on grain yields of the three varieties at Benatzu site were compared over the study period 1990 to 2004, when field trial tests were conducted simultaneously for the three varieties.

III – Results and discussion

Figure 1 shows the predicted effects of simulated scenarios on the three varieties in comparison with the observed values. The increasing negative impact on grain yields when passing from the mildest (temperature increase: +1 °C; rainfall reduction: 5%) to the worst scenario (temperature increase: +6 °C; rainfall reduction: 30%) is clear, especially in the low fertility soil of Ussana. Figure 2 shows the simulated reduction of grain yield over the period 1990-2004 at Benatzu for the same climate change scenarios T1_R5 and T6_R30. Creso (late genotype) seems to be the most prone to the negative impact of climate change when compared to the early varieties Duilio and Simeto. These preliminary results confirm the plausibility of the CERES-Wheat model in evaluating the impact of climate change on wheat production and its possible use to support decisions in targeting genotypes and crop breeding.

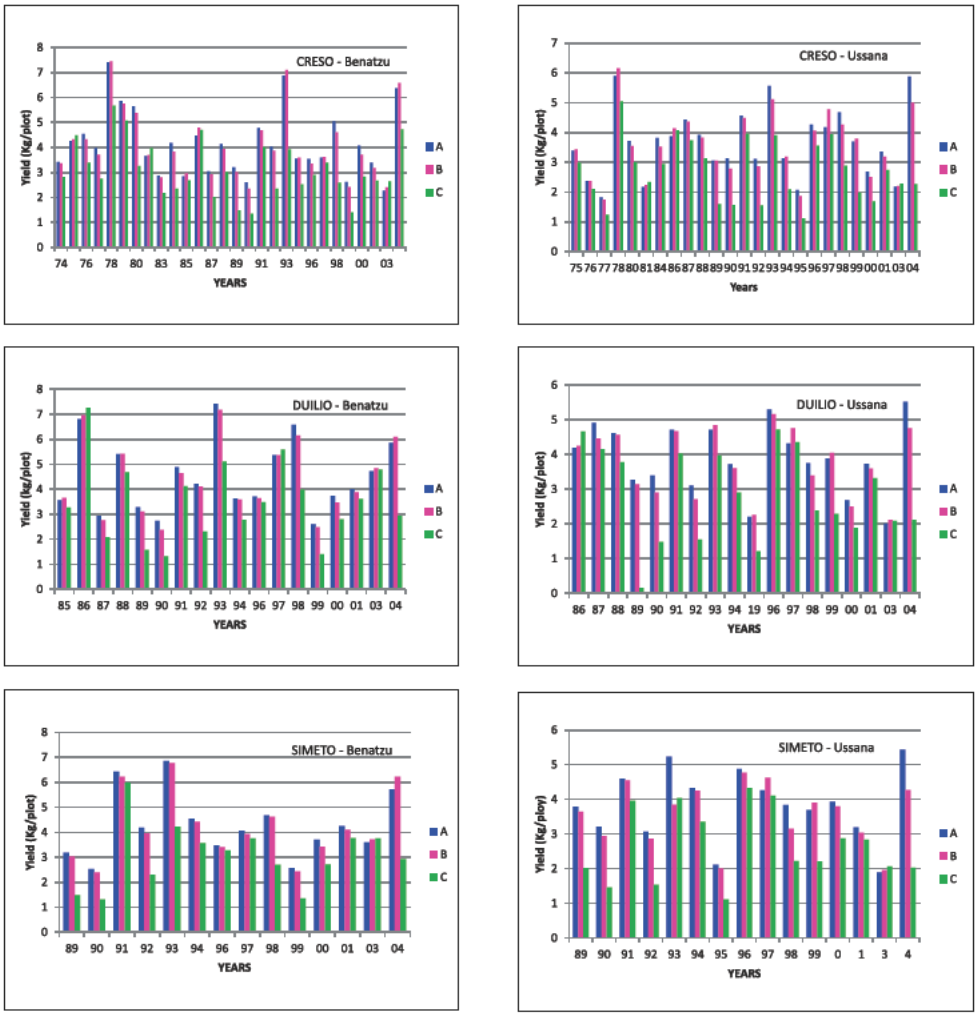

Figure 1. Effects of climate change scenarios on grain yield of durum wheat varieties Creso, Duilio, and Simeto, at Benatzu and Ussana sites. Observed yield data (A), and simulation results from scenarios T1_R5 (temperature increase: +1 °C); rainfall reduction: 5% (B), and T6_R30 (temperature increase: +6 °C; rainfall reduction: 30%) (C).

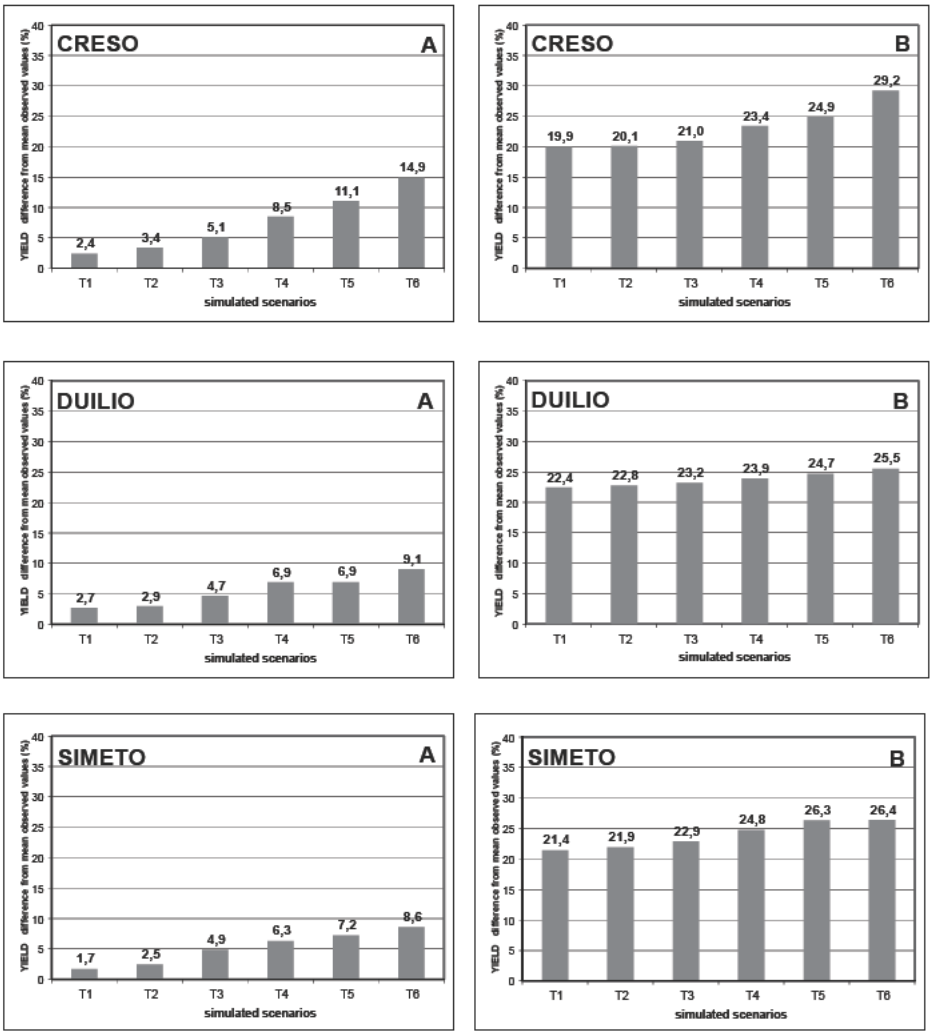

Figure 2. Percentage decline of grain yield over the period 1990-2004 at the experimental site of Benatzu for climate change scenarios characterized by increasing temperature (from +1 °C to +6 °C) and decreasing rainfall by 5% (A) and 30% (B).

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The salt tolerance candidate genes family in wheat and its relationship to the phylogenetic complexity of cereals

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Abstract. Salt is a major abiotic stress affecting crop plants worldwide. In Morocco, the problem is worsening with climate change. More than 700.000 hectares are affected by salt, therefore a large number of south Moroccan farms is affected in some way by soil and groundwater salinity. So to understand the mechanisms underlying the response of cereal species to salinity in natural systems, our study focused on reverse genetics for the characterization of genes identified as l kely to increase tolerance of wheat to salinity. Salt tolerance comes from genes that limit the rate of salt uptake by the plant from the soil and the transport of salt throughout the plant, adjust the ionic and osmotic balance of cells in roots and shoots, and regulate leaf development and the onset of senescence. Salt-tolerant candidate genes families in wheat and other cereals such as HKT, NHX, SOS, and HAK were identified and downloaded from the NCBI and wheat genomics of abiotic stress (WGAS) databases. Comparative studies and analyses, such as search for domains, multiple sequence alignments and phylogenetic tree constructions as well as primers design were carried out using bioinformatics tools. Sixty candidate genes for salt-tolerance were identified. Several protein domains have been characterized: (i) the protein family of HKT genes contains a TrKH (cation transport protein) domain, (ii) the protein family of NHX genes contains a Na+/H+ exchanger domain, (iii) *a Ktrans (K+ potassium transporter) domain for the protein family of HAK genes,* (iv) *a Na+/H+* exchanger domain and a cNMP (cyclic nucleotide binding domain) for SOS1 genes, (v) a serine/threonine protein kinase domain for SOS2 genes, and (vi) an EF-hand (calcium binding protein) domain for SOS3 genes. Multiple sequence alignments of HKT genes in wheat revealed a high frequency of glycine and serine amino acids conserved in the consensus sequence. According to the phylogenetic tree analysis, HKT genes were grouped into two subfamilies, and this division is associated with a substitution of a glycine/serine residue intended to be in first loop pores of the protein. All members of the subfamily 1 have a serine at this position, whereas members of subfamily 2 (except OsHKT1) have a glycine. The RT-PCR primers associated with the candidate genes for the studied trait were designed as markers for selection to assist the cereal breeding program.

Keywords. Bioinformatics – Salt-tolerant genes – RT-PCR primers – *Triticum .*

Famille des gènes candidats pour la tolérance à la salinité chez le blé et sa relation à la complexité phylogénétique des céréales

Résumé. *La salinisation est un stress abiotique majeur pour les plantes cultivées dans le monde entier. Au Maroc, le problème est aggravé par les effets du changement climatique. Plus de 700 000 hectares sont affectés par la salinité et donc, bon nombre d'exploitations agricoles dans le sud du Maroc sont touchées à un degré différent par la salinité du sol et des eaux souterraines. Pour comprendre les mécanismes régissant la réponse à la salinité des espèces céréalières dans les systèmes naturels, notre étude a été axée sur la génétique inverse pour la caractérisation des gènes identifiés comme susceptibles d'augmenter la tolérance du blé à la salinité. La tolérance au sel provient des gènes qui limitent la vitesse d'absorption du sel par la plante dans le sol et le transport de sel tout au long de la plante, contrôlent l'équilibre ionique et osmotique des cellules des racines et des pousses, et règlent le développement de la feuille et le début de la sénescence. Les familles de gènes candidats pour la tolérance à la salinité chez le blé et chez d'autres céréales telles que HKT, NHX, SOS, et HAK ont été identifiées et téléchargées à partir des bases de données NCBI et des données génomiques des stress abiotiques du blé (WGAS). Les études et les analyses comparatives, telles que la recherche des domaines, des alignements de séquences multiples et les constructions d'arbres phylogénétiques ainsi que la conception des amorces ont été réalisées à l'aide des outils de la bioinformatique. Soixante gènes candidats pour la tolérance à la salinité ont été identifiés. Plusieurs domaines protéiques*

ont été caractérisés : (i) la famille de protéines des gènes HKT contient un domaine TrKH (protéine de transport des cations), (ii) la famille de protéines des gènes NHX contient un domaine échangeur de Na+/H+, (iii) un domaine Ktrans (transporteur de potassium K+) pour la famille des protéines des gènes HAK, (iv) un domaine échangeur Na+/H+ et un cNMP (domaine de liaison à un nucléotide cyclique) pour les gènes SOS1, (v) un domaine sérine/thréonine protéine kinase pour les gènes SOS2, et (vi) un domaine EF-hand (protéine de liaison du calcium) pour les gènes SOS3. Des alignements de séquences multiples des gènes HKT de blé ont révélé une fréquence élevée des acides aminés de la glycine et de la sérine conservés dans la séquence consensus. Selon l'analyse de l'arbre phylogénétique, les gènes HKT ont été regroupés en deux sous-familles, et cette division est associée à une substitution d'un résidu glycine/sérine destiné à se situer dans les pores de la première boucle de la protéine. Tous les membres de la sous-famille 1 ont une serine dans cette position, alors que les membres de la sous-famille 2 (sauf OsHKT1) ont une glycine. Les amorces de RT-PCR associées aux gènes candidats pour le caractère étudié ont été conçues comme des marqueurs de sélection pour faciliter le programme d'amélioration des céréales.

Mots clés. *Bioinformatique – Gènes de tolérances à la salinité – Amorces RT-PCR – Triticum.*

I – Introduction

Abiotic stresses are a serious problem to crop production under dry land conditions in arid and semi-arid regions of the world. The area is still increasing as a result of irrigation or land-clearing (FAOSTAT, 2012). These abiotic stresses include high and low temperatures, water deficit, sodicity, alkalinity, acidity, ion deficiencies and toxicities and salinity (Javid *et al.,* 2011). The major salinity problem in Morocco is in dry lands (more than 700.000 hectares), the overuse of surface and ground water, coupled with agricultural intensification, generates soil salinity and sodicity problems (Bannari *et al.*, 2008). Whole plant tolerance to soil salinity involves numerous processes in many different tissues and cell types. For many cereals, sensitivity to salinity is due to the accumulation of sodium (Na+) to toxic concentrations in the leaves (Bryt, 2008). Recent advancements in biotechnology have led to the development of more efficient selection tools to substitute phenotype-based selection systems (Ashraf and Foolad, 2012). The mechanism of the molecular response of higher plants against water stress has been analyzed by studying a number of genes in *Arabidopsis thaliana* responding to drought, high-salinity and cold stress at the transcriptional level (Seki *et al*., 2002) (Fig.1).

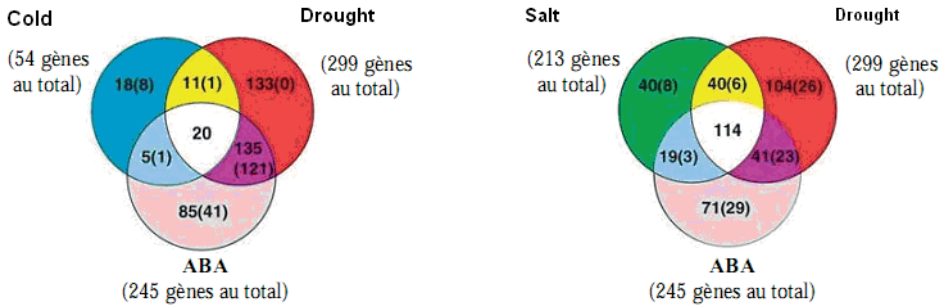

Figure 1. Venn diagrams describing the genes regulated during abiotic stress in Arabidopsis thaliana. ABA, abscisic acid. (Seki *et al*., 2002).

With the genomes of various plants having been sequenced, the total complement of potential proteins involved in Na+, K+ and Cl- transport can be surmised. Fig. 2 gives an overview of the main classes of monovalent ion transporters, often derived from large gene families (Mian *et al*. 2009).

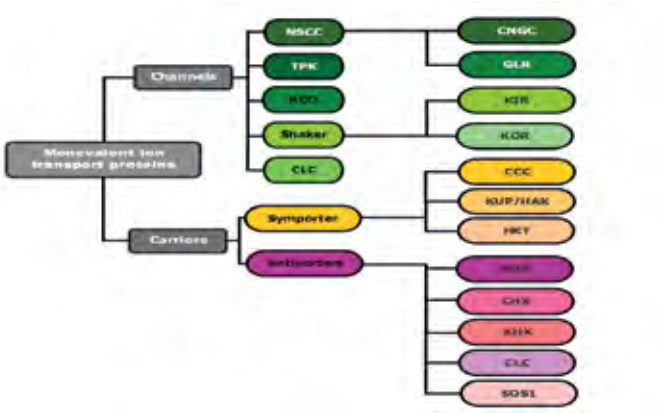

Figure 2. Overview of gene families involved in Na+, K+ and Cl- homeostasis in rice during salt stress. Abbreviations: CCC, cation chloride co-transporter; CHX, cation/H+ exchanger; CLC, voltage gated Cl- channel; CNGC, cyclic nucleotide gated channel; GLR, glutamate like receptor; HKT, high affinity K+ transporter; KHX, K+/H+ exchanger; KIR, Shaker type K+ inward rectifier; KOR, Shaker type K+ outward rectifier; KUP/HAK, K+ uptake permease; NHX, Na+/H+ exchanger; NSCC, non-selective cation channel; TPK, two-pore K+ channel (Mian *et al*. 2009).

Improving crop plants genetically for salt tolerance represents an important part of basic plant biology (Zhu, 2000). Breeding for salt tolerance can be thought of as selecting plants that withstand salt stress most effectively (Shannon and Qualset, 1984). Whole plant tolerance to soil salinity involves numerous processes in many different tissues and cell types. For many cereals, sensitivity to salinity is due to the accumulation of sodium (Na+) to toxic concentrations in the leaves. Recent advancements in biotechnology have led to the development of more efficient selection tools to substitute phenotype-based selection systems (Ashraf and Foolad, 2012). The marker-assisted selection is a process of indirect selection in which the character in question has a high heritability, since not influenced by environmental factors. There is increased efficiency of plant breeding, reducing the number of progenies and the number of generations for the stabilization of the genotypes. The selection can be performed in early generations (Eduardo, 2011). The procedures require integrating multidisciplinarity involving researchers with backgrounds in classical plant breeding, chemistry, biochemistry, plant physiology, statistics, computer science and bioinformatics. Biotechnology and Bioinformatics carries benefits for plant researchers: it can aid in plant breeding and genetic engineering, and allow plant scientists to produce salt tolerant for the future (Mochida and Shinozaki, 2010). Hence, a detailed understanding of the basic mechanisms involved in the plant salt tolerance is an important prerequisite to improve the performance of crop plant in saline soils (Binzel and Reuveni, 1994; Eduardo, 2011).

Furthermore, promotion of comparative genomics among model and applied plants allows us to grasp the biological properties of each species and to accelerate gene discovery and functional analyses of genes (Mochida and Shinozaki, 2010). The objective of this study, for better understanding of the mechanisms that can contribute in salt tolerance of cereals, is to identify candidate genes involved in this mechanism and to develop In Silico some markers associated to those genes using bioinformatics tools.

II – Methodology

In this study, candidate genes involved in the tolerance to salt mechanism have been searched, identified and used. Twenty-three genes of the HKT family, twenty-four genes of the NHX family,

five genes of SOS family, and eight genes of the HAK family, which are homologous genes, were downloaded. Table 4 summaries the approach and tools used in this study.

1. Bioinformatics tools

Several bioinformatics tools were downloaded from the Internet and used to meet our objective in the context of our study but we opted for a few that applies to the described methodology. We, however, mostly used CLC Main Workbench because it includes several features that make certain operations effective on biological data (DNA, RNA, proteins, etc.), and accepts different file formats (GB: GenBank, GFF: Generic Feature Format, Fasta, etc.) which is limited to others. In addition, it helps to have direct access to some external databases such as NCBI (National Center for Biotechnology Information), UniProt (Universal Protein Resource), and Pfam (Protein families' database). It has a good GUI (Graphical User Interface). The list of databases and bioinformatics tools used in this study is shown in Table 1.

Table 1. List of databases and bioinformatics tools used.

Bioinformatics tools and databases	Functions and approach
NCBI (http://www.ncbi.nlm.nih.gov/)	Protein and nucleotide sequence download.
AMIGO (http://amigo.geneontology.org/cgi-bin/amigo/go.cgi)	Identification of genes' functions.
Pfam (http://pfam.janelia.org/)	Search for protein domains.
CLC MAIN WORKBENCH (http://www.clcbio.com)	Protein sequence analyses.
GeneFisher2 (http://bibiserv.techfak.uni-bielefeld.de/genefisher2/submission.html)	Primers design.
Oligo Calc: Oligo nucleotide Properties Calculator (http://www.basic.northwestern.edu/biotools/oligocalc.html)	Validation of the primers designed.

2. Collection of candidate genes

The availability of research platforms, such as the web tools of the National Center for Biotechnology Information (NCBI) has transformed the time-consuming task of identifying candidate genes from genetic studies to an interactive process where data from a variety of sources are obtained to select likely genes for follow-up (Sadasivam *et al.,* 2009; Sanchez *et al.,* 2011). Salt-tolerant candidate genes were known from scientific journals. Basic Local Alignment Search Tool (BLAST) is a sequence similarity search program that can be used via a web interface or as a stand-alone tool to compare a user's query to a database of sequences. Several variants of BLAST compare all combinations of nucleotide or protein queries with nucleotide or protein databases (Johnson *et al*., 2008). The nucleotide and protein sequences of salt-tolerant candidate genes were collected and saved in a Fasta file format which was later used to perform multiple sequence alignments.

The genes identified were downloaded and are detailed in the results and discussion section, with the names of candidate genes, their protein and nucleotide sequence accessions, descriptions, dates of modification and lengths in amino acids (aa) and base pairs (bp).

3. Search for candidate genes' functions

AmiGO is a web application that allows users to query, browse and visualize ontologies and related gene product annotation data (Carbon *et al*., 2009). AmiGO can be used online at the Gene Ontology (GO) website to access the data provided by the GO Consortium; it can also be downloaded and installed to browse local ontologies and annotations. AmiGO is free open source software developed and maintained by the GO Consortium. Every page in AmiGO offers a simple search box through which users can query the GO database for GO terms or gene products. AmiGO returns search results ordered by how closely the result matches the original

query; results can also be sorted by other parameters, such as accession in term searches, or gene symbol when querying for gene products..

4. Search for protein domains

Pfam is a database of protein families, where families are sets of protein regions that share a significant degree of sequence similarity, thereby suggesting homology. Similarity is detected using the HMMER3 (http://hmmer.janelia.org/) suite of programs (Finn *et al.,* 2010). This database helped to further confirm candidate gene.

5. Multiple Sequence Alignment (MSA)

CLC Main Workbench is developed for Windows, MacOSX and Linux. The software for either platform can be obtained from http://www.clcbio.com. CLC Main Workbench 5.7.1 software was installed on a Microsoft windows operating system. CLC Main Workbench was chosen amongst other bioinformatics tools such as Geneious, Bioedit, ClustalW etc., because it offers more advantages especially in the graphical presentation of sequences from multiple sequence alignments.

6. Construction of phylogenetic trees

Phylogenetic relationship was determined based on multiple sequence alignments by using the CLC Main Workbench 5.7.1.

7. Primers design

GeneFisher2 is a recent reimplementation of the original GeneFisher application which recreates the overall functionality of its predecessor while enhancing usability and user experience (Hagemeier, 2006). This new version accesses basically the same underlying tools, now turned into components, via a web application, and conducts the whole application from a Web GUI by means of AJAX (Asynchronous Java-Script and XML) technology. GeneFisher accepts single or multiple DNA and protein sequences as input. As primers are calculated for a single DNA sequence, multiple input sequences are aligned using alignment programs such as ClustalW or DCA. From the alignment, a consensus sequence is derived and used as input for the primer calculation step. GeneFisher selects PCR primers with certain criteria such as: melting temperature Tm, GC content, primer length, 3' clamp GC content and degeneration, hairpin loop structure detection, primer-primer dimers detection, primer degeneration, amplified region length, and primer uniqueness (Hagemeier, 2006). In this study, Primers were designed from the coding portion of the nucleotide sequences (mRNA) downloaded from the NCBI database.

The choice of good primer is then validated by the Oligonucleotide Properties Calculator application software which is used to calculate all the parameters as well as check for complementarily of the primers designed (Chavali *et al*., 2006). Generally, in this study, the primer length must be within the range of 18-30 nucleotides, and its composition in GC must be between 40-60%. The primers should neither form self-dimers nor cross dimers or hairpin structures.

III – Results

1. Salt-tolerant candidate genes

In this study, salt-tolerant candidate genes were searched, identified and downloaded (twenty-three HKT genes, twenty-four NHX genes, five SOS genes, eight HAK genes) (Annex 1).

2. Overview of candidate genes' functions

HKT and HAK gene families are transporters of potassium (K+) while NHX gene families are exchangers of Na+/H+. However, SOS gene families are antiporters of Na+/ H+.

3. Search for protein domains

The search for protein domain using pfam has shown that HKT genes belong to a family of proteins known as TrkH: Cation transporting proteins, which consists of various cation transport proteins (Trk) and the subunit of ATP synthase, sodium or ATPase translocation. These proteins are involved in the active absorption of sodium by using ATP in the process of absorption. Trk/ HKT transporters are reminiscent of K+ channels in that they possess in a single polypeptide chain and four domains resembling P-loops. These P-loop-like domains are weakly conserved to K+ channel P-loops (Platten *et al*. 2006).

However for NHX genes, the protein families are exchangers of sodium (Na+) and hydrogen (H+) ions. This family is also called antiporters of Na+/H+, which act as transporters that play a major role in maintaining the pH of actively metabolizing cells. The molecular mechanisms of antiporters are not clear (Rodriguez-Rosales *et al*., 2009).

As for SOS1 genes, they present a protein family of exchangers of sodium and hydrogen ions, as well as cyclic nucleotide-binding domains.

Regarding the SOS3 gene, it encodes an EF-hand type calcium-binding protein with similarities to animal neuronal calcium sensors and the yeast calcineurin B subunit. As regards SOS2 gene, it encodes a serine/threonine type of protein kinase. The SOS2 gene physically interacts with SOS3, and is activated by the latter. The SOS3-SOS2 kinase complex represents a regulatory pathway that specifically controls the homeostasis of Na+ and K+ and tolerance of salt in plants. The product of this pathway is the upregulation of SOS1 expressed under NaCl stress (Shi *et al*., 2000). In addition, HAK genes belong to a protein family of potassium carriers (Ktrans: K+ potassium transport) in different species.

4. Multiple Sequence Alignments

Multiple sequence alignments (MSA) were carried out using protein sequences of wheat and related species such as barley (*Hordeum vulgare*), maize (*Zea mays*), rice (*Oryza sativa*), poplar (*Populus trichocarpa*), as well as selaginella (*Selaginella moellendorffii*), tomato (*Solanum lycopersicum* L.) and the model species thale cress (*Arabidopsis thaliana*), for a comparative study of these candidate genes across species.

Conserved regions, significant similitude were also observed in NHX, HAK and SOS candidate genes, respectively. MSA of HKT genes revealed conservation region which can be explained by the similarity in amino acid residues of their protein sequences. There is a high frequency of glycine and serine amino acids conserved in the consensus sequence, which is specific to the HKTs.

5. Phylogenetic tree construction

Phylogenetic trees of publicly available full-length HKT coding sequences or HKT amino acid sequences show that the gene family splits into two major branches (Fig. 2). To establish the relationship between the different salt tolerance genes in cereals , only one representative of each of the gene groups (preferentially the homologous with cut-of of 95%) was included in this analysis. In this study, we used UPGMA (Unweighted Pair Group Method with Arithmetic Mean) as our analysis method. HKT genes are homologs because they have a common ancestor. The

ancestor also known as root on this tree is *Selaginella moellendorffii* (SmHKT2). According to the phylogenetic tree, these genes were grouped into two subfamilies (subfamily 1 and subfamily 2), and this division is associated with a substitution of a glycine/serine intended to be in the first loop pores of the protein. All members of the subfamily 1 have a serine at this position, whereas members of subfamily 2 (except OsHKT1) have a glycine (Fig. 3). Functional analyses of TaHKT1, AtHKT1, and rice suggest that this particular amino acid could play a central role in determining the Na+ selection by the carrier.

The analysis of the four family of genes from cereals (Fig.4) reveals a diversification clustering in the same family of gene between species.

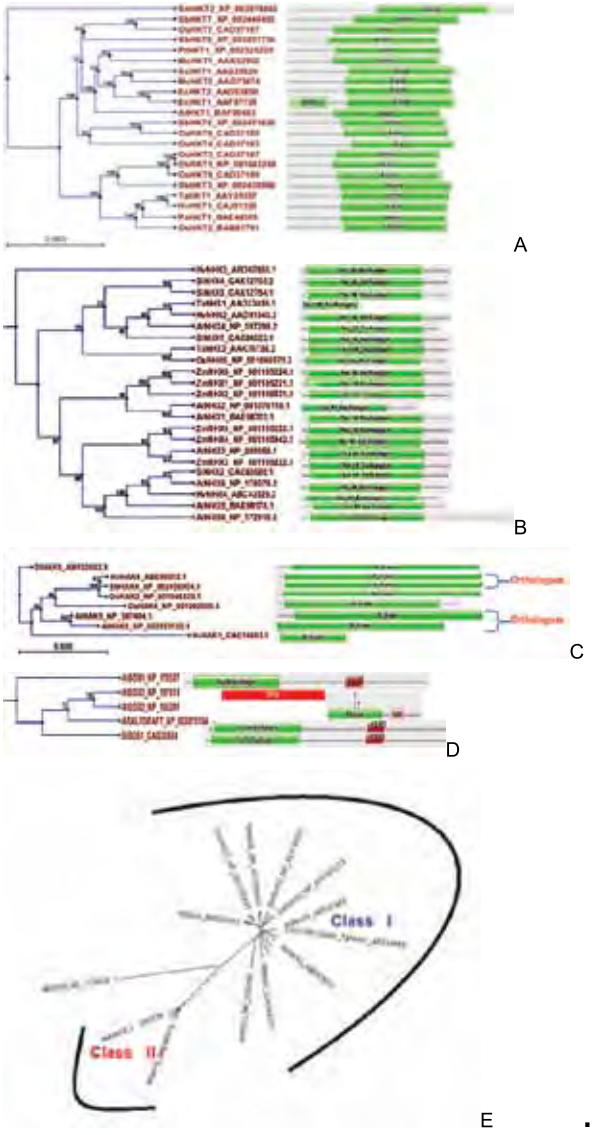

Figure 3. Phylogenetic relationships between salt tolerance genes: In the left (A) HKT genes, (B) HAK genes, (C) SOS genes and (D) NHX genes. Protein domains for HKT, HAK, NHX and SOS genes, done by CLC Main Workbench 5.7.1 using the UPGMA method with a bootstrap of 1000 (E).

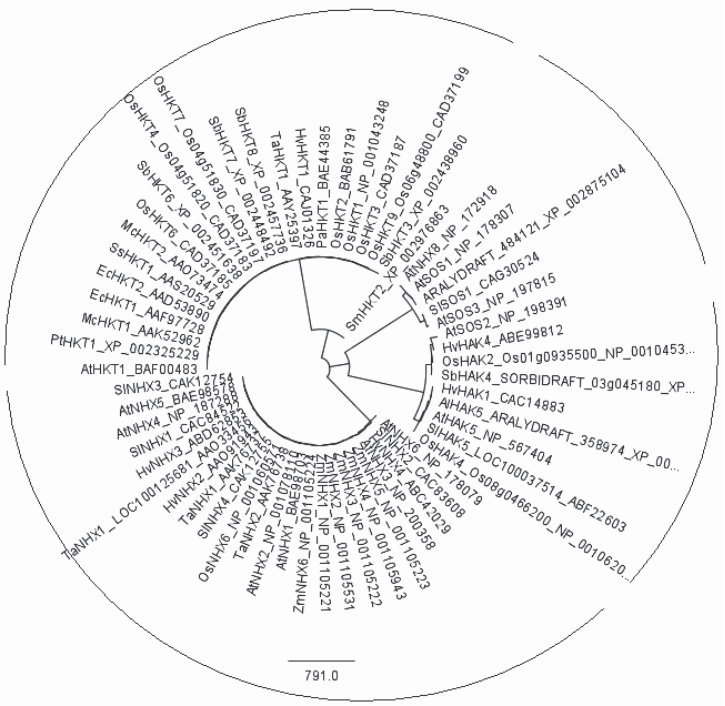

Figure 4. Phylogenetic relationships between salt tolerance genes (HKT, NHX, HAK, and SOS).

6. Primers design

The primers, designed by GeneFisher2 and validated by OligoCalc, are listed in Annex 2.

IV – Conclusion and perspectives

The search for salt-tolerant candidate genes among species via bioinformatics tools identified and characterized twenty-three HKT genes, twenty-four NHX genes, five SOS genes and eight HAK genes. Analyses such as search for genes' functions, search for protein domains, multiple sequence alignments, phylogenetic tree construction, carried out on these genes, showed that HKT and HAK genes play a role in the transport of potassium, NHX genes as exchangers of Na+/H+, and the SOS1 genes as antiporters of Na+/H+. Protein domains revealed that HKT genes belong to a protein family of TrKH involved in the active absorption of sodium by using ATP in the process of absorption while NHX family of genes are exchangers Na+/H+ that play a role in maintaining the pH of actively metabolizing cells.

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ANNEX 1. List of salt-tolerant candidate genes: HKT, NHX, SOS, and HAK genes with their protein sequence accessions, descriptions, modification dates, lengths in amino acids, nucleotide sequence accessions, and lengths in base pairs, respectively.

Gene	Protein accessions	Description	Date	Length aa	Nucleotide Accession	Length (bp)
HKT						
AtHKT1	BAF00483	Sodium transporter [*Arabidopsis thaliana*].	27/07/2006	506	AK228564	1695
PtHKT1	XP_002325229	Sodium transporter hkt1-like protein [*Populus trichocarpa*].	04/12/2009	506	XM_002325193	1772
TaHKT1	AAY25397	High-affinity potassium uptake transporter [*Triticum aestivum*].	07 /05/2005	533	DQ009003	1981
McHKT1	AAK52962	Putative potassium sodium transporter [*Mesembryanthemum crystallinum*].	14 /05/2001	505	AF367366	1904
OsHKT1	NP_001043248	Os01g0532600 [*Oryza sativa Japonica* Group].	08/06/2010	509	NM_001049783	1587
EcHKT1	AAF97728	Potassium-sodium symporter HKT1 [*Eucalyptus camaldulensis*].	13/11/2000	550	AF176035	1992
HvHKT1	CAJ01326	High-affinity sodium transporter [*Hordeum vulgare* subsp. *vulgare*].	17/11/2005	531	AM000056	1925
PaHKT1	BAE44385	High-affinity potassium transporter [*Phragmites australis*].	11/03/2008	530	AB234304	1828
SsHKT1	AAS20529	Suaeda salsa HKT1 (hkt1) mRNA, complete cds.	12/03/2008	550	AY530754	2033
SmHKT2	XP_002976863	Sodium transporter [*Selaginella moellendorffii*].	13/08/2010	745	XM_002976817	2238
McHKT2	AA073474	Mesembryanthemum crystallinum high affinity potassium transporter 2 (HKT2) mRNA, complete cds.	01/04/2003	543	AY231175	2230
EcHKT2	AAD53890	Potassium-sodium symporter HKT2 [*Eucalyptus camaldulensis*].	13/11/2004	549	AF176036	1915
OsHKT2	BAB61791	Potassium-sodium symporter [*Oryza sativa* Indica Group].	14/02/2008	530	AB061313	1781
OsHKT3	CAD37187	Putative sodium transporter [*Oryza sativa* Japonica Group].	02/07/2003	509	AJ491820	1587
SbHKT3	XP_002438960	Hypothetical protein SORBIDRAFT_10g029000 [*Sorghum bicolor*].	13/07/2009	545	XM_002438915	1638
OsHKT4 (Os04g51820)	CAD37183	Putative sodium transporter [*Oryza sativa* Japonica Group]	02/07/2003	552	AJ491816	1669
OsHKT5	----------	Oryza sativa Japonica Group hkt5 pseudogene, exons 1-3.	14/11/2006	----	AJ506745	3203
OsHKT6 (Os02g07830)	CAD37185	Putative sodium transporter [*Oryza sativa* Japonica Group]	02/07/2003	531	AJ491818	1679
SbHKT6	XP_002451638	Hypothetical protein SORBIDRAFT_04g005010 [*Sorghum bicolor*].	13/07/2009	532	XM_002451593	1899
OsHKT7 (Os04g51830)	CAD37197	Putative sodium transporter [*Oryza sativa* Japonica Group].	14/11/2006	500	AJ491853	5400
SbHKT7	XP_002448492	Hypothetical protein SORBIDRAFT_06g027900 [*Sorghum bicolor*].	13/07/2009	563	XM_002448447	1692
SbHKT8	XP_002457736	Hypothetical protein SORBIDRAFT_03g012590 [*Sorghum bicolor*].	13/07/2009	498	XM_002457691	1497
OsHKT9 (Os06g48800)	CAD37199	Putative sodium transporter [*Oryza sativa* Japonica Group].	02/07/2003	509	AJ491855	1557

Gene	Protein accessions	Description	Date	Length aa	Nucleotide Accession	Length (bp)
NHX						
AtNHX1	BAE98703	Na+/H+ exchanger [*Arabidopsis thaliana*].	27/07/06	538	AK226586	2346
ZmNHX1	NP_001105221	Na+/H+ antiporter NHX1 [*Zea mays*].	14/12/2007	540	NM_001111751	1623
TaNHX1	AAK76737	Na+/H+ antiporter [*Triticum aestivum*].	28/07/2001	546	AY040245	2017
TaNHX1_ LOC100125681	AAO33456	NHX1 [*Triticum aestivum*].	02/02/2003	204	AF472486	612
SlNHX1	CAC84522	Na+/H+ antiporter, isoform 1 [*Solanum lycopersicum*].	19/06/03	534	AJ306630	2122
AtNHX2	NP_001078110	NHX2 (SODIUM HYDROGENEXCHANGER 2); sodium ion transmembrane transporter/ sodium: hydrogen antiporter [Arabidopsis thaliana].	21/08/2009	421	NM_001084641	1486
ZmNHX2	NP_001105531	Na+/H+ antiporter NHX2 [*Zea mays*].	14/12/2007	540	NM_001112061	1623
SlNHX2	CAC83608	Na+/H+ antiporter, isoform 2 [*Solanum lycopersicum*].	19/06/03	531	AJ306631	2267
TaNHX2	AAK76738	Na+/H+ antiporter [*Triticum aestivum*].	27/02/2003	538	AY040246	2422
HvNHX2	AAO91943	Vacuolar Na+/H+ antiporter [*Hordeum vulgare*]	29/01/07	546	AY247791	1941
ZmNHX3	NP_001105222	Na+/H+ antiporter NHX3 [*Zea mays*].	14/12/2007	539	NM_001111752	1620
SlNHX3	CAK12754	(Sodium/potassium)/proton exchanger 3 [*Solanum lycopersicum*].	09/05/2006	537	AM261866	1614
HvNHX3	ABD62853	Na+/H+ antiporter [*Hordeum vulgare*].	17/04/2006	541	DQ372061	1794
AtNHX3	NP_200358	ATNHX3; sodium ion transmembrane transporter/ sodium:hydrgen antiporter [*Arabidopsis thaliana*].	21/08/2009	529	NM_124929	1861
AtNHX4	NP_187288	NHX4 (SODIUM HYDROGENEXCHANGER 4); sodium iontransmembrane transporter/ sodium:hydrogen antiporter [*Arabidopsis thaliana*].	21/08/2009	503	NM_111512	2207
HvNHX4	ABC42029	Na+,K+/H+ exchanger [*Hordeum vulgare*].	14/06/06	510	DQ314285	2117
SlNHX4	CAK12755	(Sodium/potassium)/proton exchanger 4 [*Solanum lycopersicum*].	19/04/2007	536	AM261867	1611
ZmNHX4	NP_001105943	Na+/H+ antiporter NHX4 [*Zea mays*].	14/12/2007	538	NM_001112473	1617
AtNHX5	BAE98578	Na+/H+ exchanger 5 [*Arabidopsis thaliana*].	27/07/06	521	AK226435	1786
ZmNHX5	NP_001105223	Na+/H+ antiporter NHX5 [*Zea mays*].	14/12/2007	545	NM_001111753	1638
ZmNHX6	NP_001105224	Na+/H+ antiporter NHX6 [*Zea mays*].	14/12/2007	541	NM_001111754	1626
AtNHX6	NP_178079	Sodium proton exchanger, putative (NHX6) [*Arabidopsis thaliana*].	21/08/2009	535	NM_106609	1943
OsNHX6	NP_001060571	Os07g0666900 [*Oryza sativa* Japonica Group].	08/06/10	536	NM_001067106	2227
AtNHX8	NP_172918	ATNHX8; lithium:hydrogen antiporter/ sodium ion transmembrane transporter/ sodium:hydrogen antiporter [*Arabidopsis thaliana*].	21/08/2009	756	NM_101333	2471
SOS						

Gene	Protein accessions	Description	Date	Length aa	Nucleotide Accession	Length (bp)
SlSOS1	CAG30524	Putative plasmalemma Na+/H+ antiporter [Solanum lycopersicum].	26/05/2005	1151	AJ717346	3823
AtSOS1	NP_178307	SOS1 (SALT OVERLY SENSITIVE); sodium:hydrogen antiporter	21/08/2009	1146	NM_126259	3682
AtSOS2	NP_198391	SOS2 (SALT OVERLY SENSITIVE 2); kinase/ protein kinase [*Arabidopsis thaliana*].	21/08/2009	446	NM_122932	1757
AtSOS3	NP_197815	SOS3 (SALT OVERLY SENSITIVE 3); calcium ion binding / calcium-dependent protein serine/threonine phosphatase [*Arabidopsis thaliana*].	21/08/2009	222	NM_122333	759
ARALYDRAFT_484121	XP_002875104	Hypothetical proteinARALYDRAFT_484121 [*Arabidopsis lyrata* subsp. *lyrata*].	11/06/2010	1135	XM_002875058	3537
HAK						
HvHAK1	CAC14883	Putative potassium transporter [*Hordeum vulgare*].	14 /11/2006	255	AJ297888	766
OsHAK2 (Os01g0935500)	NP_001045320	Putative HAK2 [*Oryza sativa* Japonica Group].	16/02/2008	783	NM001051855	2939
LOC100037514	ABF22603	HAK5 [*Solanum lycopersicum*].	23/01/2007	786	DQ489721	2361
HvHAK4	ABE99812	Potassium transporter HAK4 [*Hordeum vulgare*].	19/11/2009	785	DQ465924	2808
SbHAK4 (SORBIDRAFT_ 03g045180)	XP_002456904	Hypothetical protein SORBIDRAFT_03g045180 [*Sorghum bicolor*].	13/07/2009	783	XM_002456859	2765
OsHAK4 (Os08g0466200)	NP_001062000	Os08g0468200 [*Oryza sativa* Japonica Group].	08/06/2010	916	NM_001068535	2579
AtHAK5	NP_567404	HAK5 (HIGH AFFINITY K+ TRANSPORTER 5); potassium ion transmembrane transporter/ potassium: sodium symporter [*Arabidopsis thaliana*].	21/08/2009	785	NM_117416	2623
AlHAK5 (ARALYDRAFT_358974)	XP_002863132	Hypothetical protein ARALYDRAFT_358974 [*Arabidopsis lyrata* subsp. *lyrata*].	11/06/2010	645	XM_002863086	1938

Annex 2. List of primers designed from candidate genes of salt tolerance.

Gene	Nucleotide accession	Forward Primer	Reverse Primer	%GC (F /R)	°CTm (F/R)	Length (bp) (F/R)	Product size
HKT							
AtHKT1	AK228564	CCATCACCGTCTCTTCCA	TTAGGAGCCAGATGAGA	56/47	54.9 /48.7	18 /17	500
PtHKT1	XM_002325193	ACTTTCCAGGGCTAGAGA	GAGACTGTGATAGCGAGA	50/50	52.7/51.8	18/18	500
TaHKT1	DQ009003	GTCGCTGAAACCAAGCA	TAGTGAGAAGAGCACGA	53 /47	53.8/49.7	17/17	501
McHKT1	AF367366	CATAGGAAGAGGGAGCAA	CACCACAAACTGAACCA	50/47	51.8/50.3	18/17	500
OsHKT1	NM_001049783	CGCAGTAGGTTTCAGTCA	CCAGTGGACAACCCAA	50 /56	53.3/50.5	18/16	501
EcHKT1	AF176035	CCTGATTCTCATCCCTCA	CAGGTAGGAAAGCTGTGA	50/50	51.5/52.5	18/18	500
HvHKT1	AM000056	GTACAGGGTTGAAGAGGA	GGCCATCAAACACGGAA	50/53	51.7/53.4	18/17	500
PaHKT1	AB234304	GCACCACCAAACAGAGA	TTGCTCCACTAGGATCCA	53/50	52.6/53.2	17/18	500
SsHKT1	AY530754	CCATTTTTCGGCCTCGAA	CCTAGCATCCATATCGA	50/47	54.7/47.7	18/17	500
SmHKT2	XM_002976817	CTGTGGCTTTTCCCCAA	CCACGAAGTAGATCGAGA	53 /50	52.7/51.8	17/18	500
McHKT2	AY231175	ACTACCACAACCACCACA	GAACCCGAAAACGGTCA	50/53	54.4/53.1	18/17	500
EcHKT2	AF176036	CCTCTGTCTCCTTGGCTA	GGTCGACACAGTGGTGA	56/59	53.8/54.4	18/17	499
OsHKT2	AB061313	CACCCATTCTGGATCCAA	TGTACCAGAGAACCAGCA	50/50	52.8/53.8	18/18	500
OsHKT3	AJ491820	TGCTACTCATTGGCCAGA	TTGAAGCCGAGTGGTGA	50/53	54.3/53.8	18/17	501
SbHKT3	XM_002438915	GGAGAGAAGCTCACCAA	TGAGGAGAACAAGGAGCA	53/50	51.1/53.8	17 /18	500
OsHKT4	AJ491816	TTGGGAGAAAGCTCAGCA	GTACCTGGTTTTGCGACA	50/50	54.9/54.3	18/18	501
OsHKT5	AJ506745	ACTTCTGTGTCCACAGCA	ATCATTTACCGGGGTGA	50/47	54.8/50.2	18/17	500
OsHKT6)	AJ491818	TCTGAACTCCGCATGGAA	AGCTCCACTCCAAAGAGA	50/50	54.7/53.5	18/18	500
SbHKT6	XM_002451593	CTGAACTCCACTTGGAA	GAGCTCCATTCTAGAGA	47/47	48.9/47.0	17/17	501
OsHKT7	AJ491853	CCATTTCCCAGTTCGTGA	TTCCTAAACCAGGCTGCA	50/50	53.6/54.8	18/18	501
SbHKT7	XM_002448447	GGGGAGAAGCTGTCCAA	TGCCTCCTTTCATGCTGA	59/50	53.9/54.9	17/18	500
SbHKT8)	XM_002457691	TCCACTTCACCTTCACCA	AATGGAGAGCCTGAGGAA	50/50	54.0/53.5	18/18	501
OsHKT9)	AJ491855	CAAGAGGAGCTGCCACA	GGAGGTTAGCCCTGCAA	59/59	54.8/54.4	17/17	501

Gene	Nucleotide accession	Forward Primer	Reverse Primer	%GC (F /R)	°CTm (F/R)	Length (bp) (F/R)	Product size
NHX							
AtNHX1	AK226586	GCAGTGAGCTCAATCCTA	GTCGCATGAAGGAGTCA	50/53	52.6/52.1	18/17	501
ZmNHX1	NM_001111751	AGCCAAGATGAGACACCA	GACTTACCTGGGGTGTCA	50/56	54.2/54.3	18/18	501
TaNHX1	AY040245	ACCGTGTTCTTCTGTGGA	GTCTTGGTGGGATGAGGA	50 /56	54.5/54.4	18/18	500
TaNHX1	AF472486	AAGCAATTCCTCCGCAA	GCCACTCAGATCCAGCA	47/59	52.4/54.4	17/17	500
SlNHX1	AJ306630	GGGGTGGTAAATGATGCA	CTTCCAACCAGAACCAGA	50/50	53.5/52.6	18/18	501
AtNHX2	NM_001084641	AGAAGCATCAGAGCGAGA	TCATGGCTGCTTCTCTCA	50/50	54.1/54.3	18/18	500
ZmNHX2	NM_001112061	TGATGTTGGTCCACTCGA	GAAAGACAGGGTGGCAA	50/53	54.2/52.4	18/17	501
SlNHX2	AJ306631	CCTTGGTGGAGTTACGTA	CGACACAAATCGCTGTGA	50/50	52.2/54.8	18/18	500
TaNHX2	AY040246	ATGACCACCAAGGGGAA	GACGTATGCACTAAGCA	53/47	52.7/49.8	17/17	500
HvNHX2	AY247791	TCATTCTGCTCTGCACCA	GCACTAAGCAATCCAGCA	50/50	54.9/54.3	18/18	500
ZmNHX3	NM_001111752	TCTATCTACTGCCGCCAA	CAGGACCATAATGGCCAA	50/50	53.8/53.2	18/18	501
SlNHX3	AM261866	AAGAGTCACCACCAAGCA	AAGTGGCACGATGAAGGA	50/50	54.7/54.9	18/18	500
HvNHX3	DQ372061	TTCCAGGGTCACAACCAA	AAGGACTTTGGGCTGGA	50/53	54.6/53.1	18/17	500
AtNHX3	NM_124929	GGGGTTGAGTGCTAGAGA	CTCCGCAATAAAGGACA	56/47	54.1/49.6	18/17	501
AtNHX4	NM_111512	CCTGGTCAGTCGATTGGA	GCGGAACACTATTCTCA	56 /47	54.9/49.3	18/17	499
HvNHX4	DQ314285	GCAGAGTTTGAGCACGA	CCACAGAGGCAAGACGA	53/59	53.2/54.4	17/17	500
SlNHX4	AM261867	GGATACCGAGAAGTGGAA	TGGTGCGGTAAGTAGCA	50/53	51.9/53.5	18/17	501
ZmNHX4	NM_001112473	GTAGTGAACGATGCCACA	TCTGCCTAGCATGACCA	50/53	53.7/52.7	18/17	500
AtNHX5	AK226435	CAGGTTAAGCAGCAGCAA	CTCCAAAGACCAAAGCA	50/47	54.6/50.0	18/17	501
ZmNHX5	NM_001111753	AGGAGAACAGATGGCTCA	CGAAGACTTGGAGTGCA	50/53	53.5/52.4	18/17	500
ZmNHX6	NM_001111754	CAATATTGGAGCCCTCGA	AATGAGAGGGTCGCGAA	50/53	52.7/53.6	18/17	500
AtNHX6)	NM_106609	TAAATCCGTCGAGGCGTA	CAGGATCAGTGGCTGAGA	50/56	54.3/54.5	18/18	500
OsNHX6	NM_001067106	GAGTTTGCCAGTGACAGA	CGCCAGTAGTAGTGGACA	50/56	53.5/54.6	18/18	501
ATNHX8	NM_101333	CGTACAAATCACCGGAGA	CCAAGCTCCTTTAGCAA	50 /47	53.1/49.7	18/17	501

Gene	Nucleotide accession	Forward Primer	Reverse Primer	%GC (F /R)	°CTm (F/R)	Length (bp) (F/R)	Product size
SOS							
SlSOS1	AJ717346	GAAAGCGAGGAAGAAGGA	TGTAAGGCTTTCCCACCA	50/50	52.9/54.4	18/18	500
AtSOS1	NM_126259	TGCTCTTGGATCTCTCGA	CGCCAGACCAATGCCTA	50/59	53.4/54.9	18/17	501
AtSOS2	NM_122932	GGTTACGATGGTTCAGCA	CAGCCTCAATGTTAGCA	50/47	53.6/49.9	18/17	501
AtSOS3	NM_122333	CCACCGGGATATGAGGA	GAGCGATGGATTCAAGGA	59/50	52.7/53.1	17/18	499
ARALYD	XM_002875058	ATGCTTGATGAGGGCAGA	TTCAAACCGGTCTGGACA	50/50	54.6/54.8	18/18	501
HAK							
HvHAK1	AJ297888	TGAGCTTGGCTTTCCAGA	AGGACCACTTGTGTCTGA	50/50	54.8/53.7	18/18	500
OsHAK2	NM_001051855	GGTGGATCTATGGACAGA	TCATGAAAGGCAGGGAGA	50/50	51.1/53.8	18/18	500
LOC1000	DQ489721	TCAGACAGGGTTTGGAGA	TTGGCCACTGTAAGCTGA	50/50	53.3/54.8	18/18	501
HvHAK4	DQ465924	GGTGGATCTATGGGCAGA	TCATGAAAGGCAGGGAGA	56/50	54.2/53.8	18/18	500
SbHAK4	XM_002456859	GCAGTTGTTGGTAGCCAA	CAAGGCTTGGACCCAGA	50/59	54.5/54.2	18/17	501
OsHAK4	NM_001068535	ATGTTGAAGCCGGACAGA	GAGTCCATCAATCGCTGA	50/50	54.9/53.1	18/18	501
AtHAK5	NM_117416	GGTCACTTCAGTGTTCGA	CTATGGAGCCAAAGACGA	50/50	53.2/52.7	18/18	500
AlHAK5	XM_002863086	GGAAGCCATGTTTGCTGA	AGAACGTAGCGATCCACA	50/50	54.5/54.5	18/18	501

Screening durum wheat for heat tolerance

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Abstract. In the Australian wheat belt, periods of moderate to extreme high temperatures at the end of the season frequently reduce grain yield and also impact on end-use quality. With global warming, extreme heat events are expected to become more frequent in the southern Australian wheat belt, making heat stress an increasing concern. Clearly, varieties with improved heat tolerance would be highly desirable. The extent of genetic variation for heat tolerance in Australian durum wheat germplasm is largely unknown and anecdotal evidence suggests that Australian durum wheat varieties may be uniformly heat susceptible. We obtained durum genotypes from various sources and plants were grown at an irrigated field site in NSW in 2011. A late-sown trial was used to expose plants to heat stress during flowering and seed set, and a second trial was sown at the normal time to provide a control. Both trials were flood irrigated to minimize water stress. Observed effects of heat on yield, single grain weight and quality will be presented and a list of potential heat tolerant lines suggested.

Keywords. Heat stress – Durum – Screening.

Sélection du blé dur pour la tolérance à la chaleur

Résumé. *Dans la ceinture de blé d'Australie, les périodes de températures modérées à extrêmement élevées à la fin de la saison réduisent souvent le rendement en grain et ont également un impact sur la qualité d'utilisation finale. Avec le réchauffement climatique, les vagues de chaleur extrêmes devraient être plus fréquentes dans la ceinture de blé de l'Australie du Sud, faisant du stress thermique une préoccupation croissante. De toute évidence, il serait très souhaitable d'obtenir des variétés plus tolérantes à la chaleur. La mesure de la variation génétique de la tolérance à la chaleur du matériel génétique de blé dur australien est largement inconnue et des preuves anecdotiques suggèrent que les variétés de blé dur australien peuvent être uniformément sensibles à la chaleur. Nous avons obtenu des génotypes de blé dur provenant de diverses sources et les plantes ont été cultivées sur un site irrigué dans le NSW en 2011. Dans un premier traitement, un semis tardif a été effectué pour exposer les plantes au stress thermique lors de la floraison et de la grenaison alors que pour un second traitement, utilisé comme témoin, le semis a été réalisé dans la période normale. Dans les deux traitements, on a utilisé l'irrigation par inondation pour minimiser le stress hydrique. Les effets de la chaleur sur le rendement, le poids d'un grain unique et la qualité seront illustrés et une liste de lignées potentiellement tolérantes à la chaleur sera présentée.*

Mots-clés. *Stress thermique – Blé dur – Sélection.*

I – Introduction

In Australian wheat growing environments, heat stress days (>30 °C) begin occurring at the flowering stage and become more frequent and severe during grain fill (Wardlaw and Wrigley, 1994). Yield losses due to heat stress have been estimated at 10 to 15% in Australia and the USA (Wardlaw and Wrigley, 1994) and can be attributed to reductions in both grain number and size. Heat stress can also impact quality traits that relate either to harvest value (e.g. increased % screenings) or processing (e.g. dough mixing characteristics). In this study, we surveyed the

extent and variability of tolerance to the yield and quality effects of heat stress in a collection of tetraploid wheat (*Triticum turgidum* L. *subsp. durum*) lines, including landraces and Australian durum varieties and breeding lines. Two years of field trials were performed in central NSW, in which relative performance under late sown (heat stressed) vs. normal sown (control) conditions were used as a measure of heat tolerance.

II – Material and methods

1. Germplasm

Heat tolerance was assessed in 34 tetraploid genotypes, including 10 varieties, 17 breeding lines (BL codes) and 7 Ethiopian landraces (AUS accessions). Except for AtilC2000 and Kronos, all of the named varieties were Australian. The breeding lines were provided by Durum Breeding Australia. The same trials also included 252 hexaploid (*Triticum aestivum*) varieties and landraces but their performance will be described in detail elsewhere

2. Field trials

Field trials were conducted in Leeton in 2011 and in Wagga Wagga in 2012. At each location, duplicate trials were sown close to the optimal time (1st and 8th June, respectively) and late (1st and 8thAugust, respectively). Flood irrigation was applied to late- and normal-sown trials to minimize drought stress. Gypsum blocks were used to monitor soil moisture levels, and irrigation scheduled as needed. Genotypes had two (occasionally one) 7.5 m^2 replicate plots per trial, arranged randomly. Propiconazole (Throttle) was applied at a rate of 250-500 ml/ha as needed to control stripe rust infection. Weather data for the trial periods were obtained from the Bureau of Meteorology web site, for the weather stations located closest to the field sites (Yanco Agricultural Institute 074037 and Wagga Wagga AMO 072150).

3. Analysis of yield, physical grain and processing quality traits

Each plot was assessed for days to anthesis, grain yield and % screenings (2 mm sieve; corrected for visually assessed % of fragmented grains), and the overs fraction analysed for 1000-grain weight, hectolitre weight and grain number. Additional analyses were performed on the Leeton-2011 grain. These included % grain protein (using NIR; adjusted to 11% moisture basis), single kernel hardness index (SKHI; measured using SKCS 4100 device), % semolina milling yield (Buhler MLU202) and semolina yellowness (b*; Minolta chromameter). Semolina dough was also analysed in a 10g mixograph to determine time to peak resistance (mix time) and resistance breakdown (RBD; based on reduction in envelope width at 8 minutes vs. at peak resistance). A standard semolina sample (Jandaroi) was analysed at intervals to allow for temporal and operator effects. Gluten index was determined using a Glutomatic (Perten Instruments). Five genotypes had insufficient grain for milling from one or both sowing dates (either as single or pooled sample) and were therefore not analysed for semolina and mixograph traits.

III – Results and discussion

1. Flowering time and physical grain traits

Warmer conditions experienced by the late-sown plots accelerated development relative to the normal sown plots (Table 1), but the 60 day delay in sowing still set back anthesis date appreciably (relative to normal-sown plots), by an average of 26 and 18 days, at Leeton and Wagga Wagga, respectively. Consequently, more hot (>30 °C) days were experienced in the

critical developmental period during the first 30 days of grain filling by the late sown plots vs. normal sown plots: 15.1 vs. 2.6 days at Leeton, and 7.7 vs. 3.9days at Wagga Wagga.

Late sown plots yielded less total grain than the normal sown plots, for all genotypes/trials. Per cent screenings was increased by the late sowing and reductions in single grain weight and number of grains/ha accounted for roughly equal proportions of the yield loss in the overs fraction. Hectolitre weight was also reduced.

Flowering times well correlated between the normal and late sowings(r=0.96 and 0.79 at Leeton and Wagga Wagga, respectively). Genotypes that took longer to flower tended to suffer greater losses with late sowing, for yield (Figure 1), and for the other physical grain traits (not shown), as these genotypes experienced more days of heat stress late in development. Hexaploid wheat genotypes of similar flowering times were less affected by late sowing (e.g., for yield, Figure 1), consistent with anecdotal reports that durum wheat in Australia is generally more heat sensitive than bread wheat.

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Genotypes that were least affected by late sowing were regarded as potentially possessing heat tolerance. After accounting for the effects of flowering time, elite tetraploids that showed the most consistent tolerance to the effects of heat on yield across the two trials were Jandaroi, Saintly and BL15,while the lines with best tolerance to grain size related effects were Jandaroi, Caparoi, BL3 andBL5.These and the best performing exotic lines could be used in genetic analysis to identify chromosome regions controlling heat tolerance, with the ultimate aim of providing molecular markers for heat tolerance to breeders.

Table 1. Traits measured in tetraploid accessions in the heat tolerance trials. t-test probability refers to the comparison of means for June vs. August sowing at each location.

Trait	Leeton 2011				Wagga Wagga 2012			
	June sown	Aug sown	Aug/ June	t-test prob.	June sown	Aug sown	Aug/ June	t-test prob.
Days to anthesis.	114	80	0.70	<0.001	122	80	0.66	<0.001
Grain yield (t/ha)	6.57	3.95	0.60	<0.001	4.84	3.14	0.65	<0.001
1000-g wt. (g)	48.2	36.7	0.76	<0.001	45.1	37.2	0.82	<0.001
% screenings	0.55	4.73	8.60	<0.001	0.67	1.85	2.76	<0.01
Grain no./ha ($\times 10^6$)	135	103	0.76	<0.001	107	83	0.78	<0.001
Hectolitre wt. (kg)	80.6	76.1	0.94	<0.001	81.2	76.6	0.94	<0.001
% grain protein	12.7	14.6	1.15	<0.001	-	-	-	-
SKHI	84.0	89.2	1.06	<0.001	-	-	-	-
% milling yield	70.9	70.2	0.99	<0.001	-	-	-	-
b*	23.6	26.1	1.11	n.s.	-	-	-	-
Mix time (min)	3.88	3.60	0.93	n.s.	-	-	-	-
RBD	56.4	45.3	0.80	n.s.	-	-	-	-
Gluten index (%)	72.7	72.9	1.00	n.s.	-	-	-	-

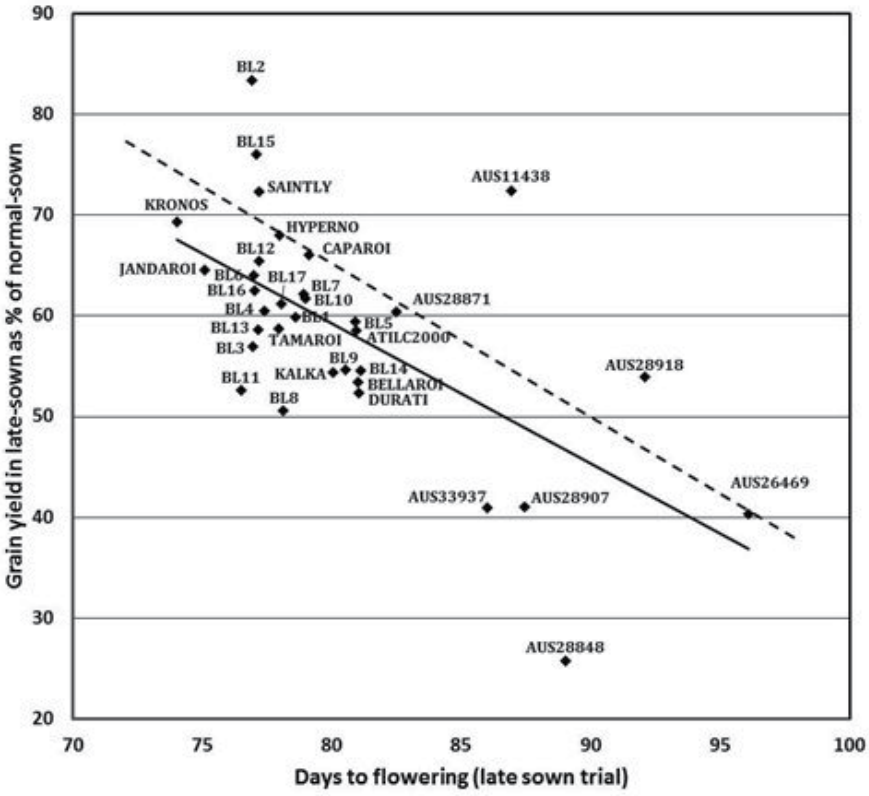

Figure 1. Relative grain yield under heat stress versus flowering time (Leeton 2011). The solid line shows the linear regression for the tetraploid accessions, and the dotted line shows the linear regression for the hexaploids (for which data points are not shown).

2. Processing quality traits

Late sowing reduced semolina milling yields, probably due to a reduced endosperm: germ ratio expected in the smaller grains (Table 1). The heat-affected grains contained elevated concentrations of protein. This is a typically observed effect of heat stress (e.g., Blumenthal *et al*. 1995) and presumably derives from a reduction in starch quantities caused by the particular heat-sensitivity of the starch biosynthetic machinery in the developing wheat grain (Zahedi *et al*. 2003). Grain from late sown plots also had harder grain (higher SKHI) which may relate to the elevated protein concentration, since interactions between starch granules and the protein matrix is regarded as a determinant of hardness (Pauly *et a*l. 2013). Heat stress did not significantly alter yellowness (b*).

On average, late sowing did not significantly alter mix time or resistance breakdown (Table 1). These mixograph traits are indicators of processing quality and can be responsive to heat. They are correlated with the proportion of glutenin that is highly polymerized, but their behaviour is largely independent of the concentration of protein *per se* (Blumenthal *et al*. 1995). Gluten index, another indicator of glutenin polymerization, was also not significantly affected (Table 1). The presence and direction of heat effects on dough physical and biochemical characteristics can depend on the timing and severity of heat stress as well as the wheat genotype (Blumenthal *et*

al. 1995; Corbellini *et al.* 1998; Stone and Nicolas 1996). The absence of a sowing time effect on dough traits could therefore be due to insufficient levels of heat stress (timing and number of days over 30 °C) and/or the presence of a high proportion of genotypes that were resistant to the quality effects of heat stress.

Ongoing quality testing of grain from these trials, including pasta making and biochemical analyses, may shed further light on the effects of heat stress on quality in durum wheat, including its biochemical basis.

Acknowledgments

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Durum wheat cultivation and breeding in the Altai Russian region

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Abstract. Altai territory is the third – fourth largest durum producer in Russia as a whole. Durum wheat appeared on the territory at the middle of 19th century. From the first steps durum appeared to be good yielder under varying sowing area: 3 000 ha after World War II till 400 000 ha at the end of stagnation time. Breeding work was initiated in 1929 and since 1970 it is incessant. Peculiar S berian climate calls for specially adapted varieties. Since 1970 8 varieties of durum wheat have been released. Breeding progress in grain productivity made up 44%. At present major challenges are: (i) high and stable yield through good adaptation to abiotic (moisture deficit, heat stress) and biotic (loose smut, Septoria tritici blotch, common root rot, black point, ergot, sawfly, cereal leaf beetle and others) stresses; (ii) improvement of quality parameters – protein content, gluten content and quality, kernel vitreousness, semolina color, cooking strength, firmness of pasta; (iii) resistance to lodging (traditional type with strong culm); (iv) ease of threshing and some other. For the development of new genetic diversity inter- (*Triticum aestivum, T.dicoccum, T. turgidum, T.timopheevii, T. persicum, T.monococcum, T.bioticum, T.turanicum* in descending order) and intraspecies hybridization is used.

Keywords. *Durum wheat – Cultivation – Breeding – Yield – Selection.*

Culture du blé dur et sélection dans la région russe de l’Altaï

Résumé. *Le territoire d’Altaï est le troisième-quatrième plus grand producteur de blé dur dans toute la Russie. Le blé dur est apparu sur le territoire au milieu du 19ème siècle. Dès le début, le blé dur a fait preuve de son rendement élevé dans diverses zones de culture, passant de 3 000 ha après la Seconde Guerre mondiale à 400 000 ha à la fin de la période de stagnation. Le travail de sélection a été lancé en 1929 et depuis 1970, il est devenu incessant. Le climat sibérien particulier demande des variétés adaptées spécifiquement. Depuis 1970, huit variétés de blé dur ont été obtenues. Les progrès de la sélection pour la productivité des grains a permis une augmentation de 44%. À l’heure actuelle, les principaux défis sont les suivants : (i) un rendement élevé et stable grâce à une bonne adaptation aux stresses abiotiques (déficit d’humidité, stress thermique) et biotiques (charbon nu, septoriose du blé due à Septoria tritici, pourriture commune des racines, maladie du point noir, ergot, cèphe du blé, criocère des céréales et autres) ; (ii) l’amélioration des paramètres de qualité - la teneur en protéines, la teneur et la qualité du gluten, la vitrosité du grain, la couleur de la semoule, la force de cuisson, la fermeté des pâtes ; (iii) la résistance à la verse (type traditionnel avec une forte chaume) ; (iv) la facilité de battage et autres. Pour le développement d’une nouvelle diversité génétique inter- (Triticum aestivum, T.dicoccum, T. turgidum, T.timopheevii, T. persicum, T.monococcum, T.boeoticum, T.turanicum par ordre décroissant) et intra-spécifique, l’hybridation est utilisée.*

Mots-clés. *Blé dur – Culture – Amélioration génétique – Rendement – Sélection.*

I – Introduction

The south of Western Siberia, including Altai territory, is a marginal zone for durum wheat production. Altai territory is situated in latitude 50-55° North and at longitude 77-87° East. Total West to East length is about 600 km, North to South - about 400 km. Climate of the territory is acutely continental with cold winter and hot summer. Average temperature of January (the coldest) is -16 -20°C, of July (the hottest) is 18-20°C. Average year temperature is 0,5 – 2,1°C. Day duration in summer time is 13-17 hours. Period without air frost is 105-140 days. Durum

growing period averaged 89 days with variation 76 – 100 and is timed to May - August. There are several agricultural zones differing in rain fall, no-frost period, sum of temperatures and other characteristics (Table 1).

Altai territory is a large agricultural province of Russian Federation. Agricultural land occupies 10,6 million ha, arable land is about 5,5-5,9 million ha, that is the largest arable land among Russian administrative units. Each year 5,4 – 5,5 million ha is sown mostly with spring crops: 3,8 million ha with cereals and legumes, 0,62 million ha with industrial crops, including 0,5 million ha of sunflower. Fodder crops take up more than 1 million ha.

Durum wheat appeared on Altai first half of XIX century. The crop was brought to Siberia with migrants from European part of Russia. Durum was a good yielder from the first steps in the province. Durum area varies in a large scale (Figure 1). The highest results were obtained at the end of stagnation time. For 1986-1990 yearly state deliveries of Altai made up 155 thousand t of high-quality grain – 30% of all Soviet Union deliveries and 85% of Western Siberia. In 1989 state durum deliveries made up 324 thousand ton. At present Altai is the third-fourth largest durum producer in Russia after Orenburg and Chelyabinsk region.

Average durum yield of commercial crop is about 1,5 t/ha that is similar to bread wheat. In dry years durum yields less, in other cases – better. Realized yield potential of the crop in production area at present is 5,1 t/ha. In the beginning of XX century advanced farmers – participants of the movement for high yields managed to receive yields 6,1 -7,8 t/ha of durum wheat. They applied a lot of manure and elite seeds were selected by hands. Average yield under experiments is much higher – about 3 t/ha with variation 0,9 – 5,1 t/ha (Figure 2).

Table 1. Climatic characteristics of agricultural zones of Altai territory.

Zones	Arable land, ha x 10^6	Precipitation for growing period, mm	Share of years with hard water deficit,%	Duration of air drought in May-June, days	Sum of temperature >+10°
I West-Kulunda steppe	1,0	140	80	10-17	2300
II East-Kulunda steppe	1,1	170	70	8-15	2250
III Rubtsovsk-Aleisk steppe	1,2	200	60	7-11	2200
IV Ob' forest-steppe	1,2	250	40	3-9	2100
V Foothills of Altai	0,8	300	10	2-3	2000
VI Biysk-Choumysh forest-steppe	0,8	280	20	3-5	2000
VII Foothills of Salair	0,7	310	10	1-3	1900

Source: Agroclimatic resources of Altai territory (1971).

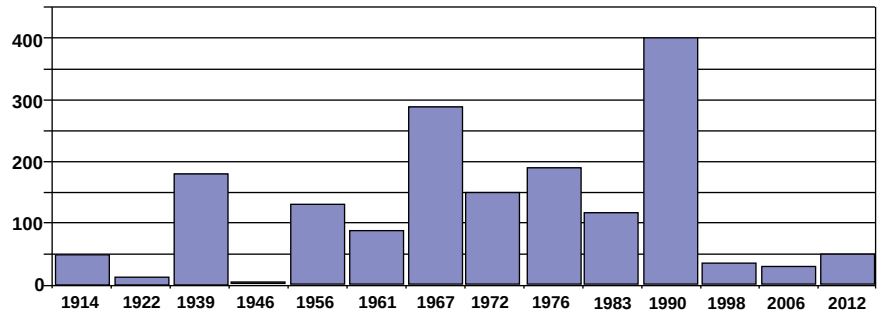

Figure 1. Durum area in Altai territory, 1000 ha.

Major durum processing plant is the Pospelikha pasta plant – the part of the "Altan" holding company. Yearly durum grain demand of the plant is 30 000 t. Other Altai macaroni producers are dealing mostly with high-quality bread wheat and from time to time with durum. Altai durum is also bought by Chelyabinsk, Moscow, Saint-Petersburg and some other plants.

Durum wheat breeding was started on Altai in 1929 with collecting samples over the territory and intra-varietal selection (Yanchenko *et al*., 2001). The work was interrupted and since 1970 it is incessant. Basic variety was Kharkovskaya 46. Since its release 9 varieties have been developed: Altaika, Hordeiforme 53, Altaiskaya niva, Zarnitsa Altaya, Altaiskiy yantar, Aleiskaya, Salyut Altaya, Pamyatie Yanchenko and Solnechnaya 573. The last variety is now in the State Variety Testing trials. All other varieties have been released. At present Altaiskiy yantar, Aleiskaya, Salyut Altaya and Pamyatie Yanchenko are recommended for commercial use. For the period since 1970 grain productivity was essentially increased (Figure 3).

Figure 2. Durum productivity in cooperative yield trial, 1985 – 2012.

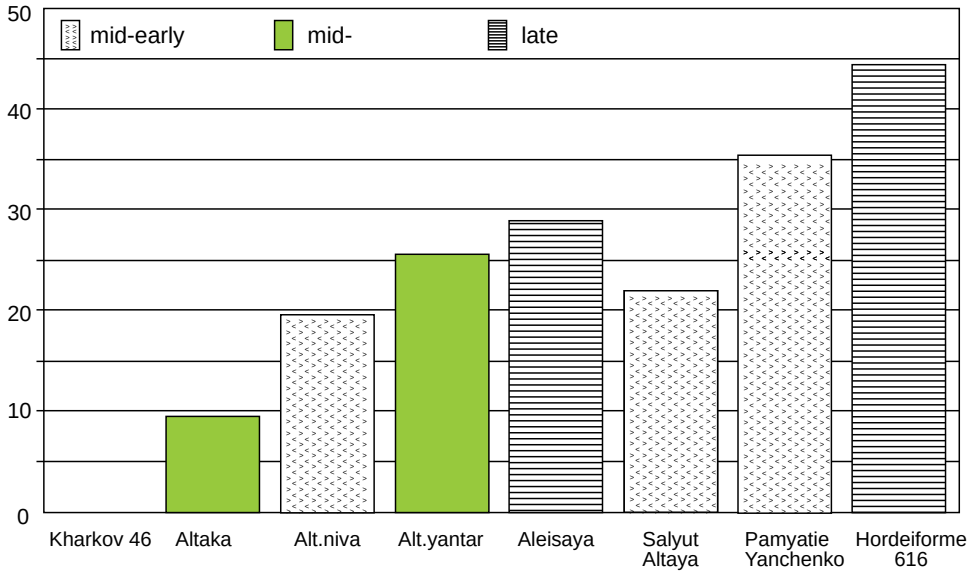

Figure 3. Breeding progress in durum grain productivity on Altai (% higher than Kharkovskaya 46).

Considerable rise in grain yield was reached with the development of Altaiskaya niva, which has *Triticum dicoccum* in its pedigree. Compared to the previous variety Altaika, Altaiskaya niva yields better, especially in low-yielding environments. At level from 1,5 to 3,0 t/ha surplus of Altaiskaya niva to Altaika made up 20 – 60%. The surplus is higher at the lower the yield level of the environment. For the period 1985 – 2012 grain productivity of Altaika makes up 2,92 t/ha and Altaiskaya niva – 3,34 t/ha.

On the next stage, the responsiveness to water and resources supply was improved with the development of the variety Altaisky yantar. The variety has high number of plants before harvest and increased number of kernels per spikelet. Further perfection of varieties developed was realized through slight multiple shifts in many features and structural elements.

II – Current activities

Nowadays the aim of durum wheat breeding is the development of a system of complementary varieties for different agroclimatic zones of the south of Western Siberia (Working Program of Breeding Center…, 2011). Most varieties bred on Altai are of mid-early type. Two varieties are mid-ripening and one of mid-late type. There is strong necessity in developing mid-ripening and mid-late genotypes. Predominant type in all zones is mid-ripening with 88 (variation over years 74 – 99) days to ripeness. For steppe zones mid-late varieties are of acute interest and steppe zone is more than half of sowing area in Altai.

Diversity of agrozones as well as farms with different economic and financial levels determines the relevance of varieties with specific adaptedness. Unpredictable freaks of nature and wide variations of environmental elements in time and space cause the development of varieties with relatively wide adaptation.

A special and vital topic is the quality of grain and end-products.The quality of varieties released is within demands of State Standards, but much effort is given to improving level and stability of vitreousness, gluten content and its quality, semolina and macaroni color, cooking firmness, and some others.

For the time being major challenges in breeding spring durum wheat for the south of Western Siberia are:

- high and stable yield through good adaptation to abiotic (moisture deficit, heat stress) and biotic (loose smut, *Septoria tritici* blotch, common root rot, black point, ergot, sawfly, cereal leaf beetle and others) stresses,
- improvement of quality parameters – protein content, gluten content and quality, kernel vitreousness, semolina color, cooking strength, firmness of pasta,
- resistance to lodging (traditional type with strong culm),
- ease of threshing.

In breeding durum wheat bulk-method is mostly used. To widen adaptability under abiotic stresses inter-species hybridization with *Triticum aestivum* and *Triticum dicoccum* as well as crosses of ecologically distant genotypes remains an important strategy for our breeding program. More than 100 double and complex crosses are made yearly. For selecting ecologically adapted lines different preceding crops and sowing dates are used.

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Proteomic analyses of the effect of nitrogen assimilation in wheat cultivars under different fertilization regimes

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Abstract. Nitrogen nutrition is one of the major factors that limits growth and production of crop plants. It affects many processes, such as development, architecture, flowering, senescence and photosynthesis. Although the improvement in technologies for protein study and the widening of gene sequences have made poss ble the study of the plant proteomes, only limited information on proteome changes occurring in response to nitrogen amount are available up to now. In this work, two-dimensional gel electrophoresis (2-DE) has been used to investigate the protein changes induced by different nitrogen sources of wheat plants.

Keywords. Nitrogen assimilation – Proteomics – Wheat – Fertilization regimes.

Analyses protéomiques de l'effet de l'assimilation de l'azote chez les cultivars de blé sous différents régimes de fertilisation

Résumé. *La fertilisation azotée est l'un des principaux facteurs qui limitent la croissance et la production des plantes cultivées. Elle intervient dans de nombreux processus tels que le développement, l'architecture, la floraison, la sénescence et la photosynthèse. Bien que l'amélioration des technologies pour l'étude des protéines et l'élargissement des séquences de gènes ait permis d'étudier les protéomes de plantes, peu d'informations sont disponibles à présent sur les modifications du protéome induites par la quantité d'azote apportée. Dans ce travail, l'électrophorèse bidimensionnelle sur gel (2-DE) a été utilisée pour étudier les changements des protéines induits de par différentes sources d'azote chez les plantes de blé.*

Mots-clés. *Assimilation de l'azote – Protéomique – Blé – Régimes de fertilisation.*

I – Introduction

Wheat (*Triticum aestivum* and *T. turgidum*) is one of the three most important cereal crops worldwide. Its cultivation presents a dominant position in the European agriculture due to its adaptability and large consumption in all the Mediterranean countries. A number of projects worldwide have been focusing on understanding the uptake, assimilation and utilization of nitrogen to improve the efficiency of nitrogen recovery in the grain. Whilst the physical processes of nitrogen and sulphur remobilization have been studied in detail, the genetic control of these processes and their contribution to agronomic productivity are less well understood. The peculiarity of the flag leaf allows for an efficient translocation of assimilates until the very late stages of leaf senescence, and the relative contribution of the flag leaves to the final grain nitrogen level is essential.

Although generally low, soil nitrogen availability can fluctuate greatly in both space and time due to factors such as precipitation, temperature, soil type and pH. Therefore, the preferred form in which N is taken up depends on plant adaptation. Nitrate uptake occurs at the root level and two nitrate transport systems have been shown to coexist in plants and to act co-ordinately to take up nitrate from the soil solution and distribute it within the whole plant (Tsay *et al*., 2007).

The analysis of the protein profile of plant tissue is an optimal method for quantifying changes in protein abundance caused by cropping systems. Proteomics is the study of the expression genes that have physiological effects on the plant. By identifying these proteins we can then link the protein back to the gene. In this way, candidate genes for agronomic traits can be identified, leading to the development of functional molecular markers for accelerating and assisting crop breeding practices (Varshney *et al*., 2005).

Transcriptomics has previously been used to directly identify genes involved in N metabolism and storage protein synthesis which are differentially expressed in response to organic and conventional fertilisers (Lu *et al*., 2005).

The main advantage of using a proteomics approach allows the observation of post-transcriptional changes to gene products that would not be identified in the transcriptome, such as protein degradation involved in important plant physiological processes, including N remobilization.

The objectives of the study presented here were to compare the effect of contrasting components of organic and conventional cropping systems on a) agronomic/physiological traits, b) the wheat flag leaf proteome, c) the association between the flag proteome and agronomic/physiological traits. This is a first step towards identifying functional molecular marker for subsequent marker-assisted breeding of wheat.

II – Material and methods

In the present research, we analysed two durum wheat cultivars (Creso and Dylan) under different fertilization regimes (Table 1) normally used in organic and conventional agriculture. A 2-dimensional electrophoresis gel coupled with mass-spectrometry approach was used, according to Vita *et al*. (2013), on wheat leaf samples and significant differences in expressed proteins were detected. To confirm these data, analyses related to transcript levels on nitrogen transporter genes through qPCR were performed. Primer pairs were designed using conserved sequences in related species (e.g. *Brachypodium distachyon*, *Triticum aestivum*).

Table 1. Nitrogen fertilization (Kg/ha) applied.

N treatment	Pre-sowing(P)	Emergence (E)	Coverage ©
Control	0	0	0
Synthesis	40	40	40
Leather	40	40	40
Protein hydrolysate 1	0	60	60
Protein hydrolysate 2	0	60	60
Rhizovit	0	60	60

III – Results

1. Effect of crop management on protein expression in flag leaves

About 72 2-DE gels (Fig. 1) have been analyzed by using Progenesis Samespot (software version 3.2.3). Six gels were realized for each experimental condition. Bioinformatics analyses revealed that contrasting fertilization regimes resulted in significant differential expression of 30 protein spots of interest, which distinguish cultivars among them and between treatments (some examples in Fig. 2), selected for the protein identification through mass-spectrometry analysis. The selection of spots was made on the basis of fold change (>1,3) and ANOVA values (p value <0,05).

2. Creation of subgroups for analysis

To compare the protein profile of different cultivars and treatments, 4 subgroups of analysis were created.

1. Subgroup Creso-Dylan,
2. Subgroup Creso (6 treatment),
3. Subgroup Dylan (6 treatment),
4. Each treatment (Creso vs. Dylan).

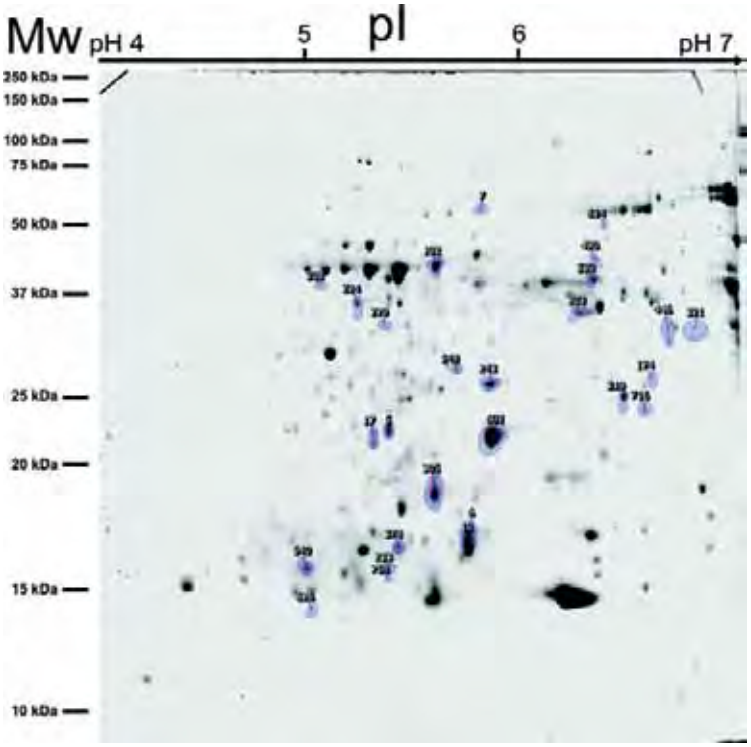

Figure 1. Reference Gel.

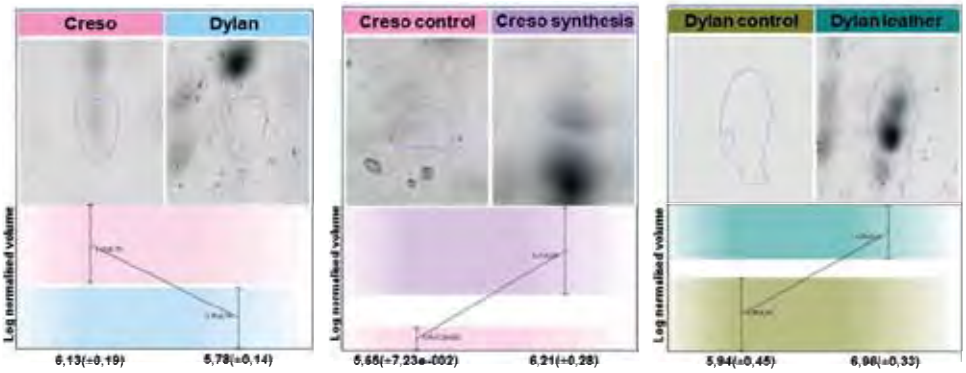

Figure 2. Examples of differential expressed spots: 1. Subgroup Creso – Dylan, 2. Subgroup Creso, 3. Subgroup Dylan.

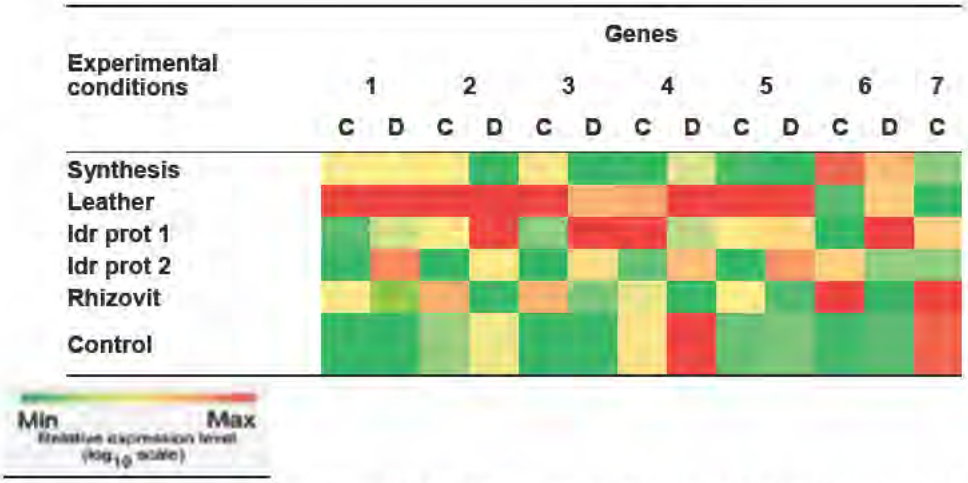

Figure 3. Pathway of nitrogen uptake for primer selection qPCR of the genes related. C=Creso; D=Dylan. 1. Nitrate transporter 2 (NRT 2); 2. Ferredoxin nitrite reductase (E.C. 1.7.7.1); 3. Low affinity nitrate transporter (NRT 1.2); 4. Glutamine synthetase isoform a (GS2a) (E.C. 6.3.1.2); 5. Nitrate transporter 2.6 (NRT 2.6); 6. Asparagine synthetase (AS) (E.C. 6.3.5.4); 7. Aspartate aminotransferase (AspAT) (E.C. 2.6.1.1).

IV – Conclusion and future perspectives

It clearly ensues from RT_PCR analysis, that treatment with leather induces both high and low affinity nitrate transporters and moreover this treatment, in general, induces the entire pathway of nitrogen uptake in both cultivars (Creso and Dylan). *Arabidopsis thaliana* NRT 1.2 (NTL1) represents one of the most highly expressed nitrate genes in shoots (Okamoto *et al.*, 2003).

Otherwise, the use of Rhizovit as nitrogen source seems to be responsible for the high expression levels of asparagine synthetase (AS) and aspartate aminotransferase (AspAT) in Creso cultivar. Rhizovit stimulates plant nitrogen uptake because it contains a microbial activator which stimulates the activity of microorganisms that transform nitrogen into a form easily taken up by plants. Works are in progress to identify those proteins of the nitrogen uptake pathway more influenced by treatments.

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QTL mapping of morphological traits associated with drought adaptation in a Iranian mapping population of durum wheat

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Abstract. Drought stress represents one of the most important abiotic constraints to durum wheat production. The identification of genotypes carrying drought-related resistance traits and the mapping of the corresponding genomic regions are fundamental requirements to sustain the selection of new cultivars more adapted to semiarid conditions. Toward these aims a new genetic linkage map was constructed based on a cross between the local durum wheat local variety Zardak and the landrace 249 collected in the semiarid Kermanshah region (Iran). One hundred thirty single-seed descent derived F_6 recombinant inbred lines (RILs) were evaluated in a drought stress (rainfed) and supplemental irrigation conditions to investigate QTLs of drought tolerance and its associated traits. A primary skeletal molecular map constructed using 71 SSR markers has been developed and it is currently under implementation with AFLP markers. The linkage analysis defined 15 linkage groups. Several QTLs were found for morphological (peduncle length, awn length and flag leaf length) and physiological traits (relative water loss and exited leaf water retention) under rainfed and supplemental irrigation conditions.

Keywords. Quantitative trait loci (QTLs) – Durum wheat – Simple sequence repeat (SSR) markers – Drought stress.

Cartographie de QTL des caractères morphologiques liés à l'adaptation à la sécheresse dans une population de cartographie iranienne de blé dur

Résumé. *La sécheresse représente l'une des plus importantes contraintes abiotiques pour la production de blé dur. L'identification des génotypes portant des caractères de résistance à la sécheresse et la cartographie des régions génomiques correspondantes sont des exigences fondamentales pour soutenir la sélection de nouveaux cultivars plus adaptés aux conditions semi-arides. Face à ces objectifs, une nouvelle carte de liaison génétique a été élaborée sur la base d'un croisement entre la variété locale de blé dur Zardak et la variété primitive 249, collectées dans la région semi-aride de Kermanshah (Iran). On a évalué cent trente lignées pures recombinantes (RIL) F6, en filiation monograine, sous stress hydrique (régime pluvial) et on a exploré les conditions d'irrigation d'appoint pour identifier des QTL de tolérance à la sécheresse et ses caractères associés. Une carte moléculaire squelettique préliminaire a été élaborée en utilisant 71 marqueurs SSR est actuellement, on est en passe de la compléter par des marqueurs AFLP. L'analyse de liaison a défini 15 groupes de liaison. Plusieurs QTL ont été trouvés pour des caractères morphologiques (longueur du pédoncule, longueur des arêtes et longueur de la feuille étendard) et physiologiques (perte d'eau relative et rétention de l'eau provenant de la feuille) sous régime pluvial et d'irrigation d'appoint.*

Mots-clés. *Loci des caractères quantitatifs (QTL) – Blé dur – Marqueurs de répétition de séquence simple (SSR) – Sécheresse.*

I – Introduction

Durum wheat (*Triticum turgidum* L.), possessing the A- and B genomes, is an important cereal crop used mainly for different food products such as pasta, couscous, and burghul (Kubaláková *et al.,* 2005). Durum wheat is traditionally grown in semiarid environments where drought stress is

the main constraint. Iran, with about 220 mm of average annual rainfall is a generally dry country with the exception of some northern provinces located in the vicinity of the Caspian Sea (Nouri-Ganbalani *et al.,* 2009). Landraces from Iran should therefore carry a number of adaptive traits allow them to growth under limited water availability, the identification of the genetic bases of such adaptation mechanisms might contribute to improve drought tolerance in modern durum wheat cultivars. Drought tolerance is a complex trait controlled by many genetic factors characterized by strong interactions with the environment (Reynolds *et al.,* 2006).

Genetic linkage maps are powerful tools for many studies, such as gene tagging, genome characterization, QTL analysis and evolutionary studies (Chu *et al.,* 2010). Construction of a genetic map plays a vital role in linkage analysis of agronomic traits and can be used to detect QTL for both abiotic and biotic stresses and therefore facilitate marker-assisted selection (MAS) (Peleg *et al.,* 2008). Identification of QTLs influencing grain yield and related traits in dry environments is needed to expand our knowledge on the adaptation of durum wheat under limited conditions and support the selection of more stress-tolerant cultivars, thus improving yield and yield stability in marginal regions. The application of molecular markers, such as amplified fragment length polymorphisms (AFLP), simple sequence repeats (SSR) and random amplified polymorphism DNA (RAPD), has provided effective approaches to dissect complicated quantitative traits into component loci to study their relative effects on a specific trait (Langridge *et al.,* 2001; Doerge, 2002.

The aims of this study were 1) to construct a genetic linkage map of Zardak × 249 (local variety and landrace from Kermanshah province, Iran), 2) to evaluate the performance of the RILs population under rain-fed and irrigated conditions and 3) to determine the chromosomal locations of the QTLs controlling yield and yield-related traits under drought conditions.

II – Material and methods

1. Materials and methods

One hundred-thirty F_6 recombinant inbred lines (RILs) deriving from the cross between the durum wheat genotypes 'Zardak' and '249' (local variety and landrace from Kermanshah province, Iran, respectively) were used in this study. Field experiments were carried out at the Research Station of Faculty of Agricultural, Razi University, Kermanshah, Iran (latitude 34° 21′, longitude 47° 9′, altitude 1319 m) in 2009-2010 cropping season. Young leaves from F_6 seedling were cut as tissue samples for DNA extraction. Total genomic DNA was isolated according to the protocol described by Murray and Thompson (1980).

2. Phenotyping

The experiment was laid out in one-replicate within RCBD augmented design. To study the environmental effects on the expression of the traits, the RILs were sown at two locations namely, rain-fed and irrigated (supplemental irrigation) conditions. Fourteen days after anthesis, a supplemental irrigation was applied to one of the two field trials, whilst other field was carried out under rain-fed conditions. Morphological characters, including peduncle length (PED), awn length (AL) and flag leaf length (FL) were measured in rain-fed and irrigated conditions. Physiological traits including RWL (relative water loss) and ELWR (excised leaf water retention) were evaluated under both conditions. For RWL (%)) five young fully expanded flag leaves were sampled from each plot at anthesis stage. The leaf samples were weighed (FW), wilted for 4 h at 35°C, reweighed (W4h), and oven-dried for 24 h at 72°C to obtain dry weight (DW). RWL % = [(FW - W4h)/(FW - DW)] × 100 and ELWR % = [1- ((FW- W4h)/FW)] × 100, according to method of Farshadfar *et al. (*2002).

3. Molecular characterization

A total of 382 molecular markers, in particular 360 simple sequence repeats (SSRs) and 22 RAPDs were tested to detect polymorphism between parents. The forward primer of each pair of SSR markers was synthesized with the universal M13 tail at the 5′ end. The M13 tail was labeled either with carboxyfluorescein (FAM) or hexachlorofluorescein (HEX) fluorescent tags. Multiplexes of SSR fragments, different for color and size, were separated using an ABI 3130xl Genetic Analyzer sequencer (Applied Biosystems) and the GeneScan ROX 500 was used as size standard. Visualizations and sizing of the SSR fragments were performed using the GeneMapper software version 4.0 (Applied Biosystems). The RAPDs were analyzed on 1.2% agarose gel and stained with ethidium bromide.

4. Linkage analysis and map construction and QTL analysis

The genetic linkage map was constructed with JoinMap v. 4 (J.W. van Ooijen, 2006) and the Kosambi mapping function was used to calculate map distance (Kosambi, 1944). Markers were placed with a LOD threshold of 3.0 and a maximum REC frequency= 0.40. The association between phenotype and marker genotype was investigated using different mapping procedures, Simple Interval Mapping (SIM, Lander and Botstein, 1989) and Multiple QTL Model (MQM, Jansen and Stam, 1994) implemented in the MapQTL 6.0 software (Van Ooijen, 2009). Simple interval mapping (SIM) was used to identify the markers most significantly associated with variation. To enhance the power of QTL detection, the analyses were repeated using these markers identified by SIM as co-factors in a multiple QTL model (MQM).

III – Results

1. Linkage analysis

The preliminary genetic linkage map consists of 71 loci on 15 linkage groups including 62 SSR loci, 7 EST-SSR markers and one RAPD locus (Figure 1). These loci were mapped on chromosomes 1A, 1B, 2A, 2B, 3A, 4B, 5B, 6B, 7A and 7B. Total map coverage is 913.5 cM, excluding chromosome 3B, 4A, 5A and 6A which contained unlinked markers. This map provides an average distance of 12.86 cM between loci.

2. QTL analysis with simple interval mapping

Peduncle length (PED). Six QTLs were detected for PED under rain-fed conditions located on chromosomes 1B, 3A, 5B and 7A (Table 1). Under irrigated conditions, two QTLs were identified on chromosomes 5B and 6B (Table 1). One overlapping peak for PED under rain-fed and irrigated conditions was detected on chromosome 5B.

Table 1. Genetic characterization of QTL linked to PED under rainfed and irrigated conditions.

Environment	Chromosome	Locus	LOD	Explained variance (%)	Donor	Additive
Rainfed	1B	Xgwm18	2.52	8.5	Zardak	1.04
	1B	Xbarc181	2.27	7.7	Zardak	1.11
	3A	XDuPw227	2.29	7.8	Zardak	1.11
	3A	Xwmc264	2.16	7.4	249	-1.05
	5B	Xgwm234	4.66	9.80	Zardak	1.41
	7A	XDuPw254	2.08	7.1	249	-0.92
Irrigated	5B	Xgmw234	2.00	6.8	Zardak	0.85
	6B	BF483631	3.26	10.9	249	-1.03

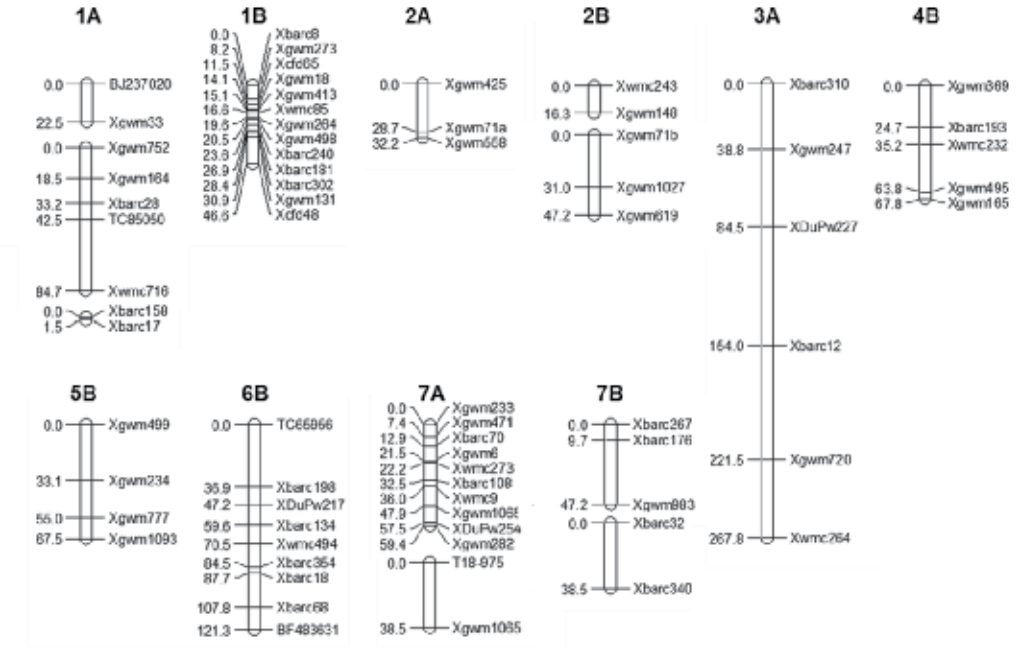

Figure 1. Linkage groups for the Zardak/249 F6 population.

Awn length (AL). In total, 6 QTLs were mapped for AL under rain-fed conditions, while eight QTLs were identified for the same trait under irrigated conditions (Table 2). Five QTLs located on chromosomes 6B and 7A were common under both conditions.

Flag leaf length (FL). Nine QTLs were detected on chromosomes1A, 4B, 6B, and 7A for flag leaf length under rained conditions (Table 3). Under irrigated conditions, eight QTLs significantly associated with FL were identified on chromosomes 6B and 7A (Table 3), all of them, except Xbarc108 located on 7A, were coincident with the loci detected in the rain-fed field trial. In the study of Dodig *et al* (2012) the variation in flag leaf width was found to be related with major yield QTL on 7A chromosome, expressed mainly under stressed conditions.

Relative water loss (RWL). Only two QTLs for RWL under rainfed conditions were found on chromosome 4B and 7B (Table 4). These QTLs were found to be environment responsive, most influential or exclusively detected under the dry treatment. No other QTLs were detected under irrigated conditions.

Excised leaf water retention (ELWR). A single QTL for ELWR was located on chromosome 7B under rain-fed conditions (Table 5). This QTL was found to be environment responsive, most influential or exclusively detected under the dry treatment. No other QTLs were detected under irrigated conditions.

Table 2. Genetic characterization of QTL linked to AL under rainfed and irrigated conditions.

Environment	Chromosome	Locus	LOD	Explained variance (%)	Donor	Additive
Rainfed	5B	Xgwm234	2.34	8.0	Zardak	0.47
	6B	Xbarc68	5.34	17.2	249	-0.65
	6B	BF483631	2.66	9.0	249	-0.47
	7A	Xgwm471	2.74	9.3	249	-0.49
	7A	Xgwm6	3.29	11	249	-0.52
	7A	Xbarc108	2.51	8.5	249	-0.49
Irrigated	4B	Xgwm165	2.28	7.8	249	-0.53
	6B	Xbarc68	2.41	8.2	249	-0.42
	6B	BF483631	4.0	13.2	249	-0.62
	7A	Xgwm471	2.04	7.0	249	-0.40
	7A	Xbarc70	2.41	8.2	249	-0.43
	7A	Xgwm6	3.41	11.4	249	-0.58
	7A	Xbarc108	3.24	10.8	249	-0.59
	7A	Xwmc9	2.68	9.1	249	-0.46

Table 3. Genetic characterization of QTL linked to FL under rainfed and irrigated conditions.

Environment	Chromosome	Locus	LOD	Expl.: Var %	Donor	Additive
Rainfed	1A	BJ237020	3.92	13.0	249	-0.022
	4B	Xgwm369	2.92	9.8	249	-0.020
	6B	Xbarc354	3.32	11.1	249	-0.026
	6B	Xbarc68	6.45	20.4	249	-0.028
	6B	BF483631	3.17	10.6	249	-0.019
	7A	Xgwm233	3.53	10.6	249	-0.019
	7A	Xgwm471	4.28	14.1	249	-0.024
	7A	Xbarc70	3.38	11.3	249	-0.022
	7A	Xgwm6	3.52	11.7	249	-0.022
Irrigated	6B	Xbarc354	2.24	7.6	249	-0.020
	6B	Xbarc68	2.44	8.3	249	-0.0177
	6B	BF483631	2.47	8.4	249	-0.0177
	7A	Xgwm233	2.43	8.2	249	-0.0179
	7A	Xgwm471	3.26	10.9	249	-0.0216
	7A	Xbarc70	3.76	12.5	249	-0.0222
	7A	Xgwm6	4.56	14.9	249	-0.0250
	7A	Xbarc108	2.29	7.8	249	-0.0167

Table 4. Genetic characterization of QTL linked to RWL under rainfed conditions.

Environment	Chromosome	Locus	LOD	Expl. var. (%)	Donor	Additive
Rainfed	4B	Xwmc232	2.18	7.4	Zardak	0.027
	7B	Xgwm983	2.21	7.5	Zardak	0.024

Table 5. Genetic characterization of QTL linked to ELWR under rainfed conditions.

Environment	Chromosome	Locus	LOD	Explained variance (%)	Donor	Additive
Rainfed	7B	Xgwm983	2.18	7.4	249	-1.54

IV – Discussion

Most periods of drought stress encountered in the major grain-growing areas worldwide are transient, unpredictable, and imprecisely measured. These features result in the difficulties in breeding efforts for drought tolerance. Moreover, plant drought tolerance is a complex trait controlled by many genes and a large "genotype × environment" interaction. For AL, FL and PED we detected some consistent QTLs in two environmental conditions, suggesting the presence of genes related to stability of these traits under drought stress conditions. The magnitudes and signs of the additive effects reflect no genotype-environment interaction observed in these traits, as for the allele coming from a parent resulted in the same behavior in two environments. Although stable QTLs for these traits were identified, a more enriched molecular map as well as a more deep phenotypic characterization are essential to validate the QTL.

Table 6. MQM analysis for the traits under rainfed and irrigated conditions for RILs population of durum wheat.

Environment	Traits	Chromo-	Locus	LOD	Expl./ var. (%)	Donor	Additive effects
Rainfed	PED	5B	Xgwm234	5.13	16.5	Zardak	1.484
	AL	6B	Xbarc68	5.3	16.5	249	-0.640
	FL	7A	Xgwm233	3.18	10.6	249	-0.019
Irrigated	PED	6B	BF483631	3.26	10.9	249	-1.033
	FL	7A	Xgwm6	4	13.2	249	-0.020

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The history of wheat breeding in Algeria

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Abstract. Research on wheat in Algeria started several centuries ago. In the pre-colonization period (before 1830) it was just a botanical curiosity and from then acquired the status of real plant breeding using most of the modes to increase genetic variability in new varieties (i.e. hybridization, selection, testing, release), to develop better varieties for a rapidly increasing population. For a long time researchers thought that bread wheat did not exist in North Africa before the arrival of the Arabs. In 1930, during the French occupation of Algeria, only durum wheat was cultivated in the plain areas. Bread wheat was considered a separate crop and was only found as undesired mixtures in durum fields. From the end of the XVIIIth and early XIXth century, only durum wheat was given importance by the farmers and was largely cultivated. There are archeological, historical and phylogenetic arguments that support the presence of durum wheat in Algeria.
After the colonists and first botanical studies era to the post independency period, several germplasm enhancement programs were launched aimed at good adaptation and high yield potential. Collaboration with International Centers, from the late 1960's (FAO, European CC and CIMMYT) was very strong and interactive and achieved all planned objectives. In 1980 ICARDA participated in contributing to Algeria's field crops research and development.
In light of achieving the main government goal to ensure food security by 2015 by producing 10 million tons of wheat a new bilateral (INRAA/ITGC) research approach (NWIP) was launched with the collaboration of ICARDA. Regional and multidisciplinary approaches have been adopted and several promising cultivars were developed through a participatory approach as a final step. This material helped in evolution of good yields during this last decade.

Keywords. Durum wheat – History – Germplasm enhancement – Yield – Production.

L'histoire de l'amélioration du blé en Algérie

Résumé. *La recherche sur le blé en Algérie a commencé il y a plusieurs siècles. Dans la période de pré-colonisation (avant 1830), il s'agissait juste d'une curiosité botanique et c'est successivement qu'on a commencé à réaliser une véritable sélection, en utilisant la plupart des moyens connus pour augmenter la variabilité génétique des nouvelles variétés (hybridation, sélection, tests, diffusion des obtentions végétales). Et ce, afin d'obtenir des variétés meilleures pour satisfaire aux besoins d'une population qui s'accroît de plus en plus rapidement. Pendant longtemps, les chercheurs avaient pensé que le blé tendre n'existait pas en Afrique du Nord avant l'arrivée des Arabes. En 1930, pendant l'occupation française de l'Algérie, seul le blé dur était cultivé dans les zones de plaine. Le blé tendre était considéré comme une culture distincte, qui poussait spontanément dans des champs de blé dur. Entre la fin du XVIIIe et le début du XIXe siècle, les agriculteurs ne s'intéressaient qu'au blé dur qui était donc largement cultivé. De nombreuses données archéologiques, historiques et phylogénétiques révèlent la présence du blé dur en Algérie.*

Après la période coloniale et la première ère des études botaniques jusqu'à la période post-indépendance, plusieurs programmes d'amélioration génétique ont été entrepris pour améliorer l'adaptation et accroître le potentiel de rendement. La collaboration avec les centres internationaux, depuis la fin des années 1960 (FAO, CC européenne et CIMMYT), a été très forte et interactive et a atteint tous les objectifs prévus. En 1980, l'ICARDA a participé en contribuant à la recherche et au développement des grandes cultures en Algérie.

En vue d'atteindre l'objectif principal du gouvernement pour garantir la sécurité alimentaire à l'horizon 2015, en produisant 10 millions de tonnes de blé, une nouvelle approche de recherche (NWIP) bilatérale (INRAA/ITGC) a été lancée avec la collaboration de l'ICARDA. Des approches régionales et multidisciplinaires ont été adoptées et un certain nombre de cultivars prometteurs ont été mis au point grâce à l'utilisation d'une approche participative dans la phase finale. Ce matériel a contribué à faire augmenter le rendement au cours de cette dernière décennie.

Mots-clés. *Blé dur – Histoire – Amélioration du matériel génétique – Rendement, production.*

I – Introduction

Algeria is the largest country in Africa covering more than two million square kilometers. Most agricultural activities in Algeria are situated in the north of the country due to the large non-arable desert areas stretching towards the south. The dominant crops are annual in nature and involve mainly field crops such as, cereals, forages, food legumes, and potatoes. With 238 million ha of arable land, Algeria has only 3.4% of potentially arable land, of which less than 20% is actually cultivated (about 8.6 million ha). Of the remaining area 4.3 million ha is used for forestry, 34.3 million ha for rangelands, and 191 million ha is unproductive land.

Cereals are the predominant crops grown by Algerian farmers covering annually 3 to 3.5 million ha which is nearly 40% of Algeria's total agricultural land. Irrigated cereals cover about 245,000 ha. However, the country depends on imports for 45% of its food consumption. Although agriculture does not contribute significantly to the country's Gross Domestic Product (8.1%), it is an important sector as it contributes to food security for its 35 million people, of which 14% are employed in agriculture. Therefore, since independence in 1962, Algeria has continued to give high priority to agricultural research and development both for social and economic reasons.

Wheat research has been conducted in Algeria since several centuries. It was just a botanical curiosity before 1830, but got the status of real plant breeding efforts using hybridization, selection, testing, release etc… to develop better varieties. For a long time researchers thought that bread wheat had not existed in North Africa until the arrival of the Arabs. However, Porteres (in Laumont and Erroux, 1961) indicated that due to its better resistance to drought and its use in semolina, durum wheat replaced actually existing bread wheat cultivation.

In 1930, during the French occupation of Algeria, only durum wheat (Laumont *et al*., 1961) was cultivated in the plains. Bread wheat (Trabut in Laumont and Erroux (1961), Boeuf (1925), Ducellier (1921), Miège (1922) was only found as undesired mixtures in durum fields (Ducellier (1930), Trabut in Laumont *et al*., 1961. R. Desfontaines, who explored the regencies of Tunis and Algiers during the period 1773-1786, mentioned only durum wheat in his remarkable book on Flora Atlantica. Schousboe in Ducellier 1921, who studied the flora of Morocco during the same period (1771-1793), also reported only the production of durum wheat.

It is important to notice that the Arab invasion in the 7th and 8th centuries did not affect the whole of the country, as the countryside mostly remained settled by the autochthonous Berber people.

II – Evidences

1. Archeological arguments

The numerous ruins of settlements built and occupied by Romans did not reveal any durum wheat grains. According to samples found at Timgad and Djmila ruins, Ducellier advanced the conclusion that durum wheat had not been cultivated in Algeria until the Arab invasion.

2. Historical arguments

We know that during the Roman occupation era North Africa furnished to the empire's capital, Rome significant quantities of grains representing taxes taken from local communities. This is why North Africa was called "Rome's granary.

3. Phylo-genetical arguments

The historical documents allow us to determine the species that formed the basis of present-day wheats. We consider most often that these wheats were *Triticum diccocum, T. spelta,* and *T. turgidum* or *T. vulgare*.

In fact, the large species diversification in the North African region made Vavilov himself consider the region to be a secondary center of origin of *T. durum*, the primary center being Abissinia.

The numerous botanical varieties (more than 20), each comprising of a multiple number of types could be explained by spontaneous hybridization according to Ducellier (1921, 1930). In addition, it seems possible that even more diversity may have arisen from natural mutations, which would have required more time to stabilize and could have resulted in the novel types found in the early 19th Century. One such product was an inter-generic cross of *Aegilops ovata* x *T. durum* found near the city of Guelma, and studied by Laumont and Errox (1961).

Historical arguments reviewed by Jasny (1944) using old texts and work by predecessors, established a sound argument in favor of the existence of wheat in North Africa before the Arab invasion. Jasny (*l. c.*) concluded that during classical antiquity, durum wheat was the most cultivated crop in the Mediterranean region. It is also interesting to note that Loret (1892) cited that durum wheat existed in Egypt as far back as during the Pharaohs' era. In summary, it seems clear that durum wheat has a long history in North Africa.

III – Variations in the durum wheat group

Because we ignore the authors from antiquity and their allusions to durum wheat, we had to wait according to Kornicke (1885) till 1566 to find a first description of durum wheat made by Dodoens (Dodonaeus in Latin). In 1913, Schulz refers to Dodonaeus in his publication "*historia frumentorum, leguminum, palustrium, and aquatilium herbarum*". But it is only in 1798 that durum wheat was recognized as a specific species by Desfontaines in "Flora Atlantica". Among the main characteristic traits indicated by Desfontaines, there are the solid stem, pubescent lemma, and the vitreous and elongated kernels. Desfontaines addresses Kornicke's classification in his book "The Wheat Plant" (1921).

The pubescent trait (*glumis pubescentibus spica villosa*) was described by Orlov as occurring in 11 Algerian varieties, with 13 others being glabrous. Orlov's classification retained pubescence/glabrousness among the primary traits for species identification (Orlov, 1923).

In other words, the durum species from the *Triticum* genus includes numerous cultivated forms not only in North Africa but also in other Mediterranean areas (e.g. Bulgaria, Greece, Italy, Portugal, Spain, and Turkey.

Within all durum wheats cultivated all over the world, Flaksberger's (1935) classification distinguished two sub-species: subsp. *abessinicum* var. and subsp. *expansum* var. These two sub-species are subdivided in three groups, including several races or "proles" characterized by ecological and morphological considerations, as in this following list:

Subsp. *abessinicum*

1. *Proles tenerum*
2. *Proles expansoïdes*
3. *Proles tenero-expansoïdes*

Subsp. *expansum*

1 Group *commune*

a. Series *prolum mediterranea*

i. Proles jordanica
ii. Proles syriaca
iii. Proles sardinicum
iv. Proles intermedium

b. Series prolum europaea

i. Proles densiusculum
ii. Proles taxiusculum
iii. Proles cyprium
iv. Proles asiaticum
v. Proles endemicum
vi. Proles villosum
vii. Proles melitense

2. Group *duro-oblungum*

a. Proles orientale
b. Proles falacatum

3. Group *orientale*

a. Proles duro-compactum
b. Proles maroccanum
c. Proles horanicum
d. Proles aegyptiacum

Algerian wheat belongs to the sub species: *expansum commune*.

IV – Cultivated durum wheat in Algeria and general traits

The diversity among Algerian durum wheat is large. Orlov indicated the presence of 22 out of 34 known botanical varieties. Within these botanical varieties we can also distinguish more “races” according to morphological and physiological traits. This explains why Algeria has to be considered as a secondary center of diversity of durum wheat (Boeuf, 1932). This extensive polymorphism in the durum wheat was also observed by the native farmers, who found several mixtures in their fields and separated them under such names as “Kahla”, “Hamra”, “Adjini”, “Mahmoudi”, etc.

A study of durum wheat in Algeria therefore must comprise:

1. Inventory and recognition of botanical varieties according to Orlov,
2. Recognition of the diverse cultivated types (descriptions, nomenclature used and regional names)

This description recalls different traits, such as the primary ones indicated by Orlov in 1923 (pubescence or glabrousness of spikes or awns, and grain color), and several other traits indicated by Orlov, Boeuf and Miège that require in-depth observations using a microscope.

Spike density of the different durum wheat types: It is important to point out that Abyssinian wheats are very dense. *T.durum* Desf. *aristantum duro compactum* Flaksb with short stature and

small grains had not been inventoried in Algeria by Orlov, who could only find forms belonging to *T. durum* Desf. *aristantum commune* Flaksb.

Lemma and palea traits: These traits are also used to discriminate botanical groups from agricultural varieties. The awns that extend from the palea are parallel (e.g., Hedba 3), or diverging and deviating on one side of the spike (e.g. Adjini 9-19, Mekki 14.970).

Vegetative traits: Vegetative traits such as plant (erect or prostrate), type of leaves, grain and leaf glaucescens, leaf color, plant height and straw structure contribute also to the determination of the different varieties.

V – Wheat introduction into Algeria

The first colonists (French farmers that came to settle in Algeria) brought with them seeds of the varieties they used to grow in their country of origin (Tuzelle from south-west France, Mahon from the Baleares islands). These old introduced wheat were called wheat of the country or white wheat because of their adaptation to the environment. The needs of modern flour mills focused on strong wheat in terms of mixing and dough properties. The national research center that has the duties to perform breeding oriented then its activities towards developing new lines through intercrossing, while continuing the introduction of new varieties and advanced lines from abroad. This research work continued by INRA, France until Algeria's independence in 1962. The Algerian Center for Agronomic, Scientific and Economic Research (CARASE) or INRA, Algeria took over the selection work at the different stations (1963 -1969) where the existing collections were maintained. In the mean-time testing of different varieties and/or populations was increased (INRAA-CNRA, 1970).

Since 1969, as stakeholders became increasingly aware of the importance of cereal production to national food security, the Ministry of Agriculture (MOA) started to evaluate new improved and high-yielding germplasm introduced mainly from the International Maize and Wheat Improvement Center (CIMMYT).

Overall cereal production intensification could not be pursued without a concerted and harmonized plan of action through the entire intensification chain to be transferred to farmers. Thus, in August of 1971 the "Projet Céréales" (Cereals Project) was created with the purpose of integrating the different elements within a technological package. This project that involved FAO (Projet Algérie/37) and the CCCE (Central Cash of European Community), was the real beginning of a new research-for-development process targeting cereal production intensification and dryland field crop diversification.

Three years later (1974) this cereal project evolved into the creation of the Field Crops Development Institute (IDGC) that took up this major responsibility and ever since has been in charge of organizing the development of cereals, forages and food legumes within the country.

We may recall that in the early 1970s cereal production was increased through new varieties obtained using natural populations or selections from within these populations, such as:

- Durum wheat: Bidi17, Oued Zenati368, Hedba3, and Mohamed Ben Bachir,
- Bread wheat: Mahon Demias and Florence Aurore,
- Barley: Saïda183 and Tichedrett.

During this same period, science-based genetic improvement began, but was confined mainly to varietal development through either mass selection within local populations or through line derivation from crosses among local populations. The IDGC's germplasm enhancement program targeted good adaptation of new varieties to diverse conditions (sufficiently long vegetative growth

cycle to avoid frost during flowering in spring, while avoiding damage due to the hot sirocco winds in early summer), high fertility/productivity, disease and pest resistance, good end-use quality (French type breads) with good baking quality. The wheat types that were cultivated could be classified into four groups.

Wheats cultivated before the arrival of botanists and French researchers, old introduced wheats, newly introduced wheats, and new wheats derived from cross-hybridization (Benbelka cem 1993).

IDGC proceeded by the conservation of botanical collections, the conservation and utilization of local landraces, and plant selection in early generations and the introduction of new genotypes from abroad for adaptation trials and use in crosses.

The collaboration with CIMMYT was very strong and interactive from the very start, and starting from 1980, the International Center for Agricultural Research in the Dry Areas (ICARDA) participated in contributing to Algeria's field crops research development.

VI – ITGC and INRAA Research approaches

To ensure food security by 2015, the Algerian government set the official goal of producing 10 million tons of wheat annually in the early 2000. The main approach was genetic improvement to create more new varieties with all desirable attributes. The available genetic resources collections were characterized. Plant physiological studies were undertaken to understand their response to abiotic environmental stresses. An inventory of the major diseases and insects affecting wheat was assembled and an integrated disease and pest management program was started. Biochemical, mixing and baking studies were considered to establish the end-use qualities of all improved genotypes.

Almost all genetic material existing at INRAA (Institute National de la Recherche Agronomique d'Algérie), i.e. before the creation of the Institute Technique des Grandes Cultures (ITGC) from the IDGC, originated from improved local populations, which had been established through pure-line selection, aiming for homogeneity and stability of the resulting lines. Such selections had been carried out on the cultivated populations of Bidi17, Hedba3, Oued Zenati368, Mohamed Ben Bachir, and others. The other varieties developed by INRAA in its National Center for Agronomic Research (CNRA) were created through cross-hybridization and selection of outstanding lines from such crosses. The initial crosses included Zenati/Bouteille, *T. polonicum*/Zenati Bouteille, Florence Aurore/Mahon, and Pusa/Mentana, yielding stable lines that were kept pure through maintenance selection.

By the time the Cereals Project was completed, the IDGC had made significant advances in breeding efficiency. The most utilized breeding/selection methodology was achieved by experienced breeders in segregating populations following hybridization, initially using the pedigree selection method, and later the modified bulk method. The back-cross method was only occasionally used. Among other approaches to creating genetic variability, the earlier ITGC tried also a mutation breeding program, but it achieved no lasting success.

VII –Varietal achievements

With the introduction of semi-dwarf varieties from CIMMYT during 1968 to 1971, and by a more intensive varietal development program, numerous new cultivars have been developed and cultivated on a large acreage by farmers. They replaced for a majority the old traditional varieties by getting much higher yields even under the local dry land conditions, expressing a good level of resistance to diseases (mainly to all rusts). Peak yields of 5.2t/ha were obtained in breeder seed plots with the bread wheat variety Hidhab = HD1220/*3Kal//Nac, and around 4.5t/ha with

the durum varieties Hoggar and Sahel. The number of newly registered and cultivated varieties increased from six in 1975/76 to 33 in 1991, 49 in 2010 and 61 in 2012. New durum wheat and bread wheat varieties accounted for about 75% of the total number of these newly released varieties (Table 1).

Table 1. List of new varieties released since 1975 in Algeria.

Durum Wheat		Bread Wheat	
Chougrane	Boussellem	Hodna	Aïn Abid
Rahouia80	Simeto	Zidene	Orion
Guemgoum Rkhem	Ofanto	Nesser	Almirante
Sebaou	Gur/Dur.	Mimouni	ElWiffak
Ar bs	Eider	Soummam	Hamam-1
Righa	Carioca	Chelif	Anapo
Sahel	Cirta	Anza	Tiddis
Chen'S'	Orjaune	Tessalah	Massine
Bibans	Poggio	Rhummel	Boumerzoug
Khroub 76	Wahbi	Sidi Okba	Akhamokh
Z bans	Beni Mestina	Ziad	Yacine
Tassili	Sigus	Isser	
Hoggar	Aïn Lehma	Arz	
Kebir03	Ammar 6	Hidhab	
Belikh2	Setifis		
OumRabi	Megress		
Sham3	Tejdid		

Since 2005, the wheat improvement programs of the ITGC and INRAA merged to a single and unique National Wheat Improvement Program (PNAB), and further strengthened through collaboration with ICARDA.

Most of latest breeding efforts in Algeria are now concentrating on maximizing yield potential under more favorable rain fed production conditions, (investments should go to an irrigated wheat system). Efforts are on intensifying higher potential areas in the north of Algeria (rainfall >400mm), in addition to breeding for tolerance to major biotic and abiotic stresses (Benbelkacem 1996). In the low rainfall areas, the priority is given to tolerance to drought and resistance to biotic and abiotic stresses, such as cold and frost. More than 50% of the improved genetic material is derived from new selections or crosses in the national wheat improvement breeding program involving interdisciplinary inputs.

All deliverables (i.e. varietal development) were to involve a participatory approach with farmers to better target and promote new products. During this latest period (2005-2012) germplasm development has progressed very well and several new varieties were released (Table 1). These varieties improved consequently farmers' wheat production.

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Avenues for increasing salt tolerance of Tunisian durum wheat cultivars

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Abstract. Salt-tolerant durum wheat cultivars offer great potential to grow them in marginal lands. To develop high-yielding, salt-tolerant cultivars for the various salt-affected areas of Tunisia we adopt two approaches. The first approach is the study of genetic variability for salt tolerance within Tunisian varieties using agro-physiological traits. We studied numerous agro-physiological traits but only few of them change very significantly and contribute to the salt tolerance mechanism. This allowed us to choose reliable screening traits and criteria useful for germplasm breeding programmes. The second approach is the introgression of *Nax* genes into elite Tunisian varieties by marker-assisted backcrossing (MAB). These *Nax* genes reduce leaf [Na^+] and increase durum wheat grain yield on saline soils.

Keywords. Durum wheat – Salt tolerance – Marginal land – Nax genes – Agro-physiological traits.

Pistes pour augmenter la tolérance à la salinité des cultivars de blé dur tunisien

Résumé. *Les cultivars de blé dur tolérants au sel offrent un grand potentiel pour la culture sur des terres marginales. Afin de développer des cultivars à haut rendement, tolérants au sel pour les différentes régions de la Tunisie affectées par la salinité, nous avons adopté deux approches. La première approche est l'étude de la variabilité génétique pour la tolérance au sel chez des variétés tunisiennes sur la base des caractères agro-physiologiques. Nous avons étudié de nombreux caractères agro-physiologiques, mais nous avons observé que quelques-uns seulement varient de façon très significative et contribuent au mécanisme de la tolérance au sel. Cela nous a permis de choisir des caractères de sélection fiables et des critères utiles pour les programmes d'amélioration du matériel génétique. La seconde approche est l'introgression des gènes Nax dans les variétés élites tunisiennes par rétrocroisement assisté par marqueurs (MAB). Ces gènes Nax réduisent le [Na+] de la feuille et augmentent le rendement en grain du blé dur sur des sols salins.*

Mots-clés. *Blé dur – Tolérance au sel – Terre marginale – Gènes Nax – Caractères agro-physiologiques.*

I – Introduction

More than 3/4 of the Tunisian's arable land is located in arid or semi-arid areas. A significant proportion of these lands are affected by either drought and/or salinity. These areas are generally cultivated with cereals and results in lower yield when grown in salt-affected soils. Comparing to other cereals, the high price of durum wheat enhances farmers to cultivate more durum wheat than bread wheat or other cereals. Increase in salt tolerance of durum wheat is needed to sustain agriculture in these areas. Salt tolerance of wheat is known to change with growth stage. There are three avenues by which to introduce salt tolerance into durum wheat: traditional breeding techniques using physiologically-based phenotyping, marker-assisted selection, and through transformation of genes known to improve Na^+ exclusion or tissue tolerance (Lindsay *et al*. 2004). Compared with conventional techniques that score and rank salt tolerance genotypes based on single trait, some success has already been realized by using multiple agronomic traits simultaneously at different growth stages (Zeng *et al*., 2002). Identifying the multiple traits associated with salt tolerance during different growth stages is important for evaluating wheat genotypes and improving their salt tolerance (El-Hendawy *et al*., 2005). Salt tolerance in wheat and many other species is associated with the ability to exclude Na^+ so that high Na^+

concentrations do not occur in leaves, particularly in the leaf blade (Munns, 2005). Durum wheat (*Triticum turgidum* L. *subsp. durum* [Desf.]) is particularly sensitive to salinity and has higher rates of Na+ accumulation and poor K^+/Na^+ discrimination and is less salt tolerant than bread wheat (Munns et al., 2006). A durum wheat Line 149, resulted from a cross between an old durum cultivar (Marrocos) and a *Triticum monococcum* accession was selected as having exceptionally low rates of Na^+ accumulation in leaves (Munns *et al*., 2000). The low Na^+ phenotype was found to be controlled by two dominant interacting genes of major effect (Munns *et al*., 2003). These genes, named *Nax1* and *Nax2*, enhance removal of Na+ from the xylem, leading to low Na^+ concentrations in leaves (James *et al*. 2006). *Nax1* was mapped as a QTL to the long arm of chromosome 2A, tightly linked to flanking molecular markers, gwm312 and wmc 170 (Megan *et al* 2004). *Nax2* was located in the terminal 14% of chromosome 5AL, using telomeric deletion lines (Byrt *et al*. 2007). A tightly linked marker 'cslinkNax2' is used for selection of lines containing *Nax2.* These tightly linked markers can be used to introgress the *Nax* genes into elite varieties to improve their salt tolerance by means of marker-assisted selection. The objectives of this study were to identify the relative importance of morpho-physiological and molecular traits associated with salt tolerance, to screen Tunisian durum wheat genotypes and to develop salt-tolerant cultivars either by conventional or molecular approaches.

II – Material and methods

1. Phenotyping

Six principal Tunisian varieties of durum wheat were used in this study (Karim, Khiar, Maali, Nasr, Razzek and Salim). These varieties were grown under semi-controlled conditions during the 2011/2012 growing season in pots (4 plants/pot) filled by a loamy sand soil collected from the soil surface (0–15 cm) at the Ariana experimental station of INRAT. The soil was air-dried, ground, passed through a 5-mm mesh screen, and thoroughly mixed. The experiment was conducted in triplicate with a completely randomised design. In our previous studies (Chaabane *et al*, 2011) it was shown that 10g/l NaCl significantly affects the majority of agro-physiological characters in durum wheat. Similarly, El-Hendawy *et al*. (2011) reported that variations in salt tolerance indexes among spring wheat (*Triticum aestivum* L.) genotypes were reduced at high salinity (150mM NaCl). This suggests that the selection criteria can be considered appropriate for screening wheat genotypes only when they are measured under high salinity. Therefore, two treatments were used, a saline treatment (150 mM NaCl) and a control (no NaCl). The salinity treatment was initiated at three-leaf stage. Agro-physiological measurements were conducted at different growth stages (60, 80, 100, 110, and 120 days after sowing and final harvest). Chlorophyll (Chl) content of the flag leaves was measured at 60, 80, 100, 110, and 120 days after sowing (DAS). Three different measurements were performed at the base, the middle and apex of the leaf using a portable Minolta SPAD 502 Meter. In this protocol the rate of Chl was estimated per unit SPAD. The height of the main shoot of each plant was measured with a ruler at 50, 60, 70, 80 and 90 DAS. Tiller number was recorded at 120 DAS. Heading date and flowering dates were also recorded. After harvesting, shoots were oven-dried at 70°C for 48 h to determine the dry weight (DW). The number of spikes/plant, the number of spikelets/spike, the grain number, the grain weight/spike and the 1000-grain weight were also determined at final harvest (150 DAS). The ratio of harvested grain to total shoot dry matter known as harvest index was calculated. The data were also converted to salt tolerance index (STI) to allow comparisons among genotypes for salt sensitivity. A STI was defined as the observation at salinity divided by the average of the controls (El-Hendawy *et al*. 2005). Basing on STI the genotypes were grouped according to a one-way ANOVA, followed by Newman–Keuls' post hoc tests. Analyses of variance (ANOVA) (Tables 3, 4) were performed using Statistica 5.0 v. '98 Edition.

2. Genotyping

A. DNA extraction

Total DNA was extracted from young leaves of a single plant per genotype. The extraction buffer (pH 8) was composed of 20 mM EDTA, 100 mM Tris-HCl (pH 8.0), 1.44 mM NaCl, 3% CTAB (w/v), 1% β-mercaptoethanol (v/v). All reagents were from Sigma-Aldrich (St. Louis, USA). DNA was purified by a treatment with RNase (10 mg/ml, Fermentas) at a final concentration of 10µg/ml followed by a phenolic extraction: A treatment by equal volume Sigma-phenol:chloroform:Isoamyl alcohol 25:24:1, followed by a treatment by equal volume of Sigma-chloroform:isoamyl alcohol 24:1. DNA concentration was quantified by gel electrophoresis. The average DNA yield was 15 µg DNA/g of tissue.

B. Molecular analysis

PCR reactions were carried out in a 25-µl reaction volume containing 1 U of taq polymerase, 50-100 of template DNA, 0.25 µM of each primer, 0.2 mM of each dNTP, 2mM of MgCl2 and 1X PCR reaction buffer. Amplifications were performed in a DNA thermocycler (Biometra Thermocycler, Goettingen, Germany) programmed for one cycle of 95°C for 3 min and 35 consecutive cycles of [1 min denaturing at 94°C, 1 min annealing at 55°C and 2 min extension at 72°C] followed by 10 min at 72°C. Amplified PCR products were separated by electrophoresis using a 2% agarose 1X TBE gel, stained with 0.5 mg/ml ethidium bromide and visualized under UV light and photographed by a gel documentation system (GDS). A 100-bp DNA ladder (Promega, Ariana, Tunisia) was used as the molecular size standard.

III – Results and discussion

1. Phenotyping

Salinity affected all of the considered traits at different growth stages. At the vegetative growth stages, tiller number, plant height, spikes per plant, spike-bearing tillers, shoot dry weight (DW), Chlorophyll content at 60, 80, 100, 110, and 120 DAS and heading date were significantly affected by salinity. At harvest, the shoot DW, the number of spikes per plant and the total grain yield were significantly affected by salinity.

At vegetative growth stages the heading date, the mean tiller number (Fig.1) and the plant height (Fig.2) were the most affected traits by salinity. Comparing to the control, the heading date of all varieties was earlier in the salinity treatments. The heading date at salinity treatment was one day (Karim) to five days (Khiar) before the control treatment. These results are in accordance with those obtained by Royo et al. (2003) who reported that salinity induced a heading date 6 days earlier for the different varieties in the most saline treatment. At salinity treatment, the plant high was reduced by 16,2% as compared with the control treatment. The tiller number for all varieties at salinity treatment was reduced by 43% as compared with the control treatment. These results confirm our previous results (Chaabane *et al*. 2011, 2012) and they are in accordance with those obtained by several authors: El-Hendawy *et al*. (2005) reported that tiller number was significantly more affected by salinity than leaf number and leaf area at the vegetative stage; Eugene *et al*. (1994) reported that salinity stress strongly influenced the distribution of spike-bearing tillers; Nicolas *et al*. (1994) found that salt stress during tiller emergence can inhibit their formation and can cause their abortion at later stages; Jones et al. (1977) reported that breeding genotypes with fewer, but less vulnerable tillers could substantially increase yields on salt-affected soils. The salt tolerance indexes of tiller number (Table 1) ranged from 0.46 (Khiar) to 0.74 (Maali). Therefore, for tiller number, Khiar was the most affected genotype by salinity and Maali was the least affected. Tiller number at salinity was decreased by 54% for Khiar and 26% for Maali, as

compared with the control. The average chlorophyll content of the flag leaf measured in Unit SPAD had a decreasing trend with time after the 60th DAS. Compared to the control treatment the average chlorophyll content at salinity treatment of the six varieties was increased by 4.78%, 4.6% and 8,0% respectively at 60, 80 and 100 DAS. At 110 and 120 DAS the average chlorophyll content of the 8 varieties was decreased by 37,8% and 54.0%. This reveals that senescence processes were promoted by salinity.

The most affected traits at harvest were the number of spikes per plant, the shoot dry weight (Fig.3) and the grain yield (Fig. 4). As compared with the control treatment, the number of spikes per plant was reduced by 37,3%, the shoot dry weight was reduced by 29,7% and the grain yield was reduced by 30.8%. However, some yield components (spikelets/spike, grains/spike) were much less affected by salinity. The number of spikelets per spike was reduced by 0.03% and the number of grains per spike was reduced by 1.4% as compared with the control treatment.

The salt tolerance indexes of all traits varied among varieties. The salt tolerance indexes (Table 1) for Chl (day 60), Chl (day 80), tiller number, spikes per plant, 1000-grain weight, and grain yield were significantly affected by salinity. These significantly affected traits can be used to compare the behaviour of the different analysed varieties in salt conditions. Maali and Salim were the less affected varieties (Table 1) for three traits (tiller number, spikes per plant, 1000-grain weight, and grain yield). Kerim and Khiar were the most affected varieties for the majority of traits.

Pearson's correlations were computed between salt tolerance indexes of different traits. Salt tolerance index of grain yield showed a very highly significant ($P<0.001$) positive correlation with salt tolerance index of shoot dry weight ($r=0.80$) and with harvest index ($r=0.67$). The STI of grain yield showed also a high correlation ($P<0.05$) with tiller number ($r=0.43$), spikelets per spike ($r=0.56$) and flowering date ($r=0.49$). These correlation studies showed that the grain yield sensibility to salt stress is highly correlated with the sensibility of shoot dry weight, tiller numbers, flowering date, spikelets per spike and harvest index. These traits are sensitive traits that affects final yield under salinity conditions. The significantly affected traits identified at early stages are not always correlated with that of harvest. Salt tolerance at early growth stages does not always correlate with that at ensuing growth stages (Zeng *et al*. 2002; El-Hendawy *et al*. 2011).

Table 1. Salt tolerance indexes.

Variety	Chl. Day 60	Chl. Day 80	Tiller No.	Spikes/ plant	1000 GW	Grain yield
Karim	1,00 (a)	1,00 (a)	0,51 (ab)	0,55 (a)	0,86 (a)	0,64 (a)
Khiar	1,04 (ab)	1,06 (ab)	0,46 (a)	0,54 (a)	0,91 (ab)	0,68 (ab)
Maali	1,06 (ab)	0,99 (a)	0,74 (b)	0,71 (b)	1,19 (b)	0,72 (b)
Nasr	1,00 (a)	1,15 (b)	0,54 (ab)	0,65 (ab)	0,99 (ab)	0,71 (ab)
Razzek	1,10 (b)	1,00 (a)	0,59 (ab)	0,65 (ab)	0,87 (a)	0,67 (ab)
Salim	1,07 (ab)	1,07 (ab)	0,72 (b)	0,71 (b)	0,92 (ab)	0.74 (b)

(a), (ab),(b): Newman–Keuls comparison tests.

The STIs of previously reported sensitive traits are themselves correlated with STI of other significantly affected traits. Thus, these traits indirectly affect final yield at salinity conditions; therefore salinity effects on traits at early stages may affect directly or indirectly yield.

Finally, screening for salt tolerance should be done by studying and combining the maximum values of significantly salt affected agro-physiological traits evaluated at different growth stages and those of which STI are correlated with STI of final yield. All these traits could be used as simple, non-destructive criteria to target wheat genotypes in breeding programs for genetic improvement of the analysed varieties.

2. Genotyping

Introgression of *Nax* genes into durum wheat reduce leaf Na+ and increase yield on saline soil. In young plants grown in 150 mM NaCl, *Nax1* reduced the leaf Na^+ concentration by 3-fold, *Nax2* by 2-fold and both *Nax1* and *Nax2* together by 4-fold (James *et al.*, 2012). Field trials on saline soils demonstrate that the presence *Nax2* locus significantly reduces leaf [Na^+] and increases durum wheat grain yield by 25% compared to near-isogenic lines without this locus (Munns *et al.*, 2012) indicating that this material is suitable for breeding commercial durum wheat with improved yield on saline soils. The introgression of *Nax* genes in elite Tunisian durum wheat varieties can be made by marker-assisted backcrossing. The desired outcome is elite Tunisian durum wheat varieties containing *Nax* genes and more tolerant to salt stress. Before starting the introgression of these genes, molecular markers (gwm312, wmc170, Cslink Nax2) linked to *Nax* genes were tested on six Tunisian durum wheat elite varieties. For *Nax1* we analyzed the gwm312 and wmc170 (Fig. 5) molecular markers profiles. For *Nax2* we analyzed the CslinkNax2 molecular marker profile (data not shown). This will allow us to know which varieties will be possible to follow the transfer of *Nax* genes using these flanking molecular markers. This will be possible when the varieties do not have the marker allele. For example, it would be possible to transfer *Nax1* by using microsatellite marker gwm312 in the varieties Karim and Salim. As shown in Figure 5 these varieties do not have the allele (199 bp) indicating the presence of the *Nax1* gene.

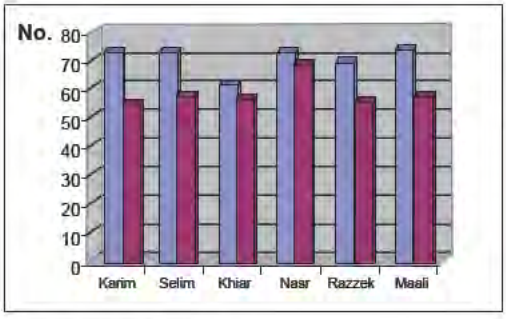

Figure 1. Tiller number

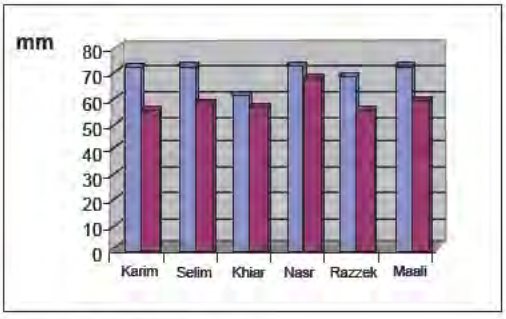

Figure 2. Plant heght

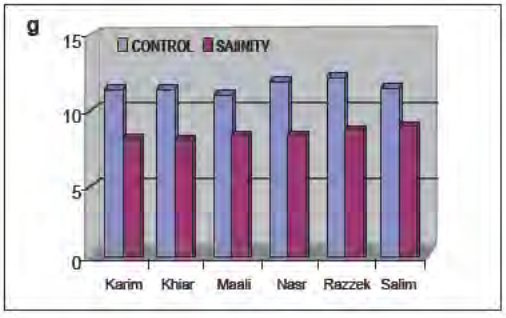

Figure 3. Shoot fry weight

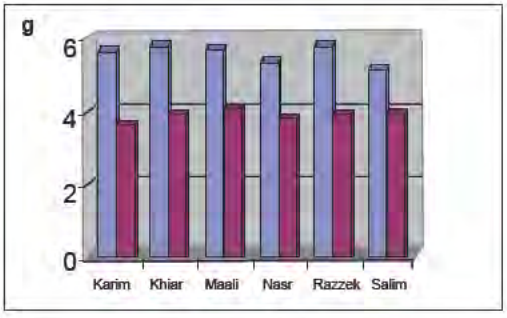

Figure 4. Total grain yield

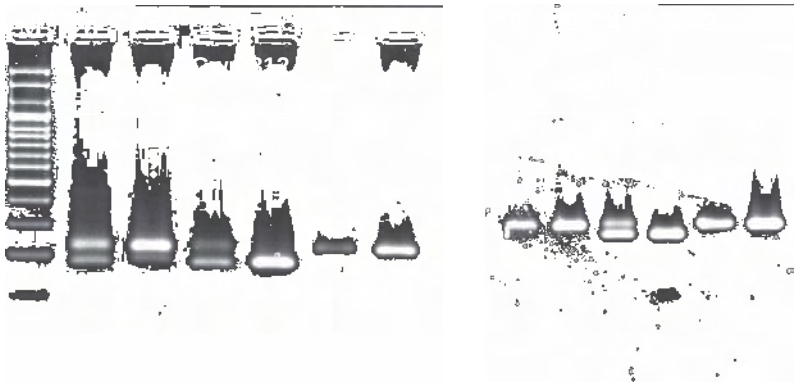

Figure 5. Agarose (2%) gel electrophoresis of PCR products obtained using gwm312 and wmc170 primers. M:100 bp, 1:Karim, 2: Salim, 3: Khiar, 4:Nasr, 5: Razzak 6, Maâli.

IV – Conclusions

Salinity affected all of the considered traits. At all vegetative growth stages, tiller number, plant height, spikes per plant, spike-bearing tillers, shoot dry weight (DW), chlorophyll content (60, 80, 100, 110, and 120 DAS), and heading date were significantly affected by salinity. At harvest, the shoot DW, the number of spikes per plant, and the total grain yield were significantly affected by salinity. The different measured traits showed differential response to salt stress among the wheat cultivars. At vegetative growth stages the heading date, the plant height and the mean tiller number were the most affected traits by salinity. At harvest the most affected traits were the number of spikes per plant, the grain yield and the shoot dry weight. The correlation between the different STI showed that the grain yield sensitivity to salt stress is highly correlated with the sensitivity of shoot dry weight, tiller numbers, flowering date, spiklets per spike, and harvest index. The significantly affected traits identified at early stages were not always correlated with that of harvest. Therefore, screening for salt tolerance should be done by studying and combining the maximum values of significantly salt affected agro-physiological traits evaluated at different growth stages and those of which STI are correlated with STI of final yield. All these traits could be used as simple, non-destructive criteria to target wheat genotypes in breeding programs for genetic improvement of the analysed varieties. Finally, the present study showed that both conventional and molecular approaches are useful for improving salt tolerance of Tunisian durum wheat.

Acknowledgments

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Durum wheat cultivation and use in the USA with special reference to California

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Abstract. Durum wheat (*Triticum durum* Desf.), one of the first crops to be domesticated, originally came to us from the Levant Region of the Near East and the Ethiopian Highlands about 10,000 years ago. Man has depended upon the wheat plant for himself and his beasts of burden for thousands of years. It is now cultivated and traded worldwide. A global wheat failure would be a disaster that few nations could survive. Wheat cultivation was introduced into Mexico in 1521 by early colonizers, but it did not appear in the territory that would later become Canada and the United States until 1602 with the arrival of the first explorers, settlers, and adventurers. After introduction, the main wheat production today comes from Montana, North Dakota, and Kansas among others in the U.S. and mainly the provinces of Manitoba and Saskatchewan in Canada. In the state of California, the San Joaquin Valley and the Imperial Valley are the two growing areas. According to FAO (2010), world production of both durum and bread wheat was 651 million tons, making it the third most-produced cereal after maize (844 million tons) and rice (672 million tons). U.S. durum wheat production in 2012 was 1.5 million tons, but due to demand and upward moving prices the 2012-2013 projected yields is expected to be 2.5 million tons. However, since the U.S. also exports half of durum wheat production and because pizzas and pasta are very popular, significant imports of durum wheat is also needed to fulfill the total demand. Durum wheat in general commands higher prices in the world market than bread wheat. There are many other uses of durum wheat besides pasta and pizzas. For example durum wheat is extensively used in biscuit-making. However, it is the yellow endosperm of durum wheat that gives pasta its familiar color. The ground endosperm of durum wheat is called semolina. There are several major pasta-makers both domestic and foreign. Italian pasta-makers like Barilla and De Cecco are famous in the U.S. due to their aggressive advertising campaigns. A world without pasta seems inconceivable.

Keywords. California – Climate change – Desert durum® – Durum wheat history – Pasta products – United States – “Wheat Kings”.

Culture et utilisation du blé dur aux Etats-Unis et plus particulièrement en Californie

Résumé. *Le blé dur (Triticum durum Desf.), une des premières cultures domestiquées, nous est parvenu initialement de la région du Levant, au Proche-Orient, et des hauts plateaux éthiopiens, il ya environ 10 000 ans. Pendant des milliers d’années, l’homme a été dépendant des plantes de blé pour ses propres besoins et ceux des bêtes de somme. Le blé est aujourd’hui cultivé et commercialisé dans le monde entier. Un déficit important de blé à l’échelle mondiale serait un désastre auquel peu de nations pourraient survivre. La culture du blé a été introduite au Mexique en 1521 par les premiers colonisateurs, mais elle n’a fait son apparition sur les territoires qui deviendront plus tard le Canada et les États-Unis qu’en 1602, avec l’arrivée des premiers explorateurs, colons et aventuriers. Depuis son introduction, la production de blé est assurée principalement par le Montana, le Dakota du Nord, et le Kansas, entre autres, aux États-Unis, et par les provinces du Manitoba et de la Saskatchewan au Canada. Dans l’État de Californie, la vallée de San Joaquin et la Vallée Impériale sont les deux zones de culture. Selon la FAO (2010), la production mondiale de blé dur et de blé tendre était de 651 millions de tonnes, ce qui en fait la troisième céréale la plus produite après le maïs (844 millions de tonnes) et le riz (672 millions de tonnes). La production de blé dur des États-Unis en 2012 était de 1,5 millions de tonnes, mais en raison de la demande et de la tendance à la hausse des prix, le rendement prévu pour 2012-2013 devrait s’élever à 2,5 millions de tonnes. Cependant, comme les États-Unis exportent aussi la moitié de leur production de blé dur et les pizzas et les pâtes sont très populaires, des importations importantes de blé dur sont également nécessaires pour satisfaire la demande totale. En général, le blé dur obtient des prix plus élevés sur le marché mondial que le blé tendre. A part les pâtes et les pizzas, on peut faire du blé une utilisation variée. Par exemple, le blé dur est largement utilisé dans la biscuiterie. Toutefois, c’est l’endosperme jaune du blé dur qui donne aux pâtes leur couleur connue. L’endosperme broyé de blé dur est appelé semoule. Il y a plusieurs grands fabricants de pâtes nationaux et étrangers. Les producteurs*

de pâtes italiens comme Barilla et De Cecco sont célèbres aux Etats-Unis en raison de leurs campagnes publicitaires agressives. Un monde sans pâtes semble inconcevable.

Mots-clés. *Californie – Changement climatique – Blé dur des zones désertiques (durum wheat®) – Histoire du blé dur – Pâtes alimentaires – Etats-Unis – « Rois du blé ».*

I – Introduction

Through the archeological evidence left by nomadic humans in west Asia, researchers have learned that humans adapted from hunting animals to also gathering seeds for food. Periods of glaciers no doubt inspired this move by reducing available game. The early gatherers were also the first millers and selected grains that could be most easily released from their glumes or husks and prepared. People parched, simmered, and ground these grains and prepared flat cakes. Thus, using grains as food changed the way early ancestors lived their daily lives, in addition to providing basic sustenance. The evolution of agriculture and cultivating seeds for harvest (which occurred about 9,000 to 10,000 years ago) changed not only the available food supply but how people moved about. Human beings' ability to process (mill), store, cultivate, and trade grain marked the beginnings of civilization.

Durum wheat (*Triticum durum* Desf.), is a type of wheat that is high in protein, gluten, and generally very firm and strong. Its kernels are usually large and amber colored. This wheat is most commonly used for making pasta rather than for baking because of its denseness and cooking quality. Pasta made from this type of wheat is typically yellow in color because of the wheat's yellow endosperm. The endosperm of wheat is found in the kernel and is usually full of niacin, iron, starch, and protein. De Vita (2009) lists over 300 shapes, sizes, and types of pasta. In fact, the earliest cookbook from the Middle-East, and the first to mention pasta, was written in the 10th century AD during the reign of the Abbasids whose capital was Baghdad (Verde and Verde Barr, 2013).

Durum wheat or 'macaroni wheat', as it is commonly called, is the only tetraploid species of wheat of commercial importance that is widely cultivated today. It was developed by artificial selection of the domesticated emmer wheat strains formerly grown in Central Europe and the Near East around 7000 B.C.E., which developed a naked, free-threshing form. Durum in Latin means "hard", and the species is the hardest of all wheats. Its high protein content, as well as its strength, make durum good for special uses, the most well-known being pasta which in Italy is exclusively made from the flour of durum wheat. Durum wheat is also used extensively in flat-bread making. However, it is unusual in that, despite very high protein content, it is low in desirable gluten needed to form a glutinous web necessary for loaf bread to rise. As a result, although 100 % durum wheat breads do exist, such as pagnotte di Enna from Sicily, as well as others, in most instances bread doughs contain only a portion of durum wheat and are supplemented substantially with commercial white flours, oftentimes those higher in gluten necessary to offset the poor gluten contribution of durum flour. When durum flour is used as the sole flour in bread (such as focaccia bread), substantial additions of isolated wheat gluten are necessary to effect rising. Without it, 100 % durum wheat breads are often heavy, with very close grain, and will split easily when risen for baking. When we were at the CNR's Istituto del Germoplasma, we used to often partake of the famous focaccia Pugliese.

Milling durum wheat turns it into ground semolina, which is made into many types of pasta. Semolina is a grainy substance that may be off-white or yellowish in colour. The next step after grinding the wheat is usually mixing it with water to form dough. Semolina dough is often very stiff, which generally makes it easier to use for melding into various pasta shapes. Dies and metal discs are commonly used for creating the many different shapes.

II – Historical background

Even in Europe the daily bread (as is now in the countries of the Near-East), a prerequisite to stability and satisfaction of the less privileged class. Poor harvests, dearth and famine put the social order and peace in peril. Ultimately the King was responsible. In fact, in 1898 bread riots broke out in Milan, Italy, and were so violent that it cost the lives of 24 of the protestors and ultimately the King Umberto I, who was assassinated in 1900. The *Battaglia del Grano*, unleashed by Mussolini (Il Duce) from 1923 to 1932 successfully doubled wheat production and cemented his popularity and support among the Italians. It was not until very recently (in 2006) that the price control on bread was abolished in Italy (Bjornstad, 2012).

Wheat cultivation was introduced into Mexico in 1521, but it did not appear in the territory that would become Canada and the United States until 1602. Wheat was introduced to North America by explorers, traders, settlers, and soldiers in the sixteenth and seventeenth centuries. Kansas is the biggest wheat producing state in the U.S. Large-scale mechanized farming and continued planting of wheat without regard to crop rotation exhausted the soil of large areas. High-yield wheat, one of the grains resulting from the Green Revolution, requires optimal growth conditions, e.g., adequate irrigation and high concentrations of fertilizer.

Discovering wheats suitable for new areas was a reoccurring struggle. In the more temperate 'Middle Colonies', the cultivars transplanted from Western Europe fared better. However, the challenges were particularly acute when pioneers moved wheat cultivation westward onto the northern Prairies, Great Plains, and Pacific Coast. All these regions eventually became major wheat suppliers, but only after farmers learned to overcome climatic conditions far different from those prevailing to the East and in Western Europe. The initial attempts to grow traditional wheat cultivars imported from Europe frequently failed.

The California Gold Rush (1848-1855) resulted in rapid expansion of the urban population. Wheat was a natural crop that was adapted to California conditions which included very fertile and flat valleys that required little clearing to become extremely good arable land. Wheat exports were used to procure other foods for the fast growing population. Exports to Britain and Europe increased because of the California wheat varieties' excellent milling quality, high gluten content, and ability to absorb large quantities of water and produce a large and heavy loaf of bread. San Francisco became the hub of flour milling in California. Before the advent of the railroads, wheat for export was moved through such ports as Oakland and Stockton. The grain fleet sailing through San Francisco Bay constituted a major part of commerce in the region from 1870 to 1900 with over 300 vessels departing each year. This circa 1900 photo shows wheat harvesting activity on the Wiseman family ranch, located within the Sacramento Valley. In 1899, Yolo (2.5 million), Sacramento (1.1 million), Colusa (3.2 million), Sutter (1.2 million) and San Joaquin (4.1 million) counties produced some 12 million bushels of wheat. Combine harvesters pulled by 27 mules and horses were put to work the vast fields of bread and durum wheats in California. One such animal-drawn combine harvester is stored in a barn near the city of Woodland in Yolo county (Fig. 1).

III – American "Wheat Kings"

Not even the shrewdest grain trader from the East (New York), including "Wheat King" Arthur W. Cutten on the cover of TIME magazine (TIME, 1928) (Fig. 2), could have foreseen the tremendous increase in wheat production in California, especially from the Sonoma and Napa Valleys. In fact Santa Clara county alone could produce enough wheat to meet the requirement of the entire state of California (Rothstein, 1987). However, after 1928 the prices of wheat declined in the global market and at the same time farmers were turning to orchards and growing plums, almonds, pistachios, and rice in the delta regions (Drynan, 1986). The arrival of refrigeration in the 1920s

further led to the decline of wheat production in California, and led to the bankruptcy of many a "wheat king", including Cutten, considered to be one of the great bulls of Wall Street, who lost $60 million almost overnight due to speculation of wheat prices and thereafter died of a heart attack. The Sonoma and Napa Valleys began to plant wine grapes and the flat Jan Joaquin and Imperial Valleys began to plant fruits and nuts. The urbanization of California after WWI created a significant local demand for perishable farm products. The latter coupled with demand for alfalfa cattle-feed for the fast growing dairy industry and the use of bees for pollination on a grand scale meant that California had literally, a 'land of milk and honey' (Rothstein, 1987)

Figure 1. A combine harvester pulled by 27 horses in the great plains in U.S.

Figure 2. "Wheat King" Arthur W. Cutten (1870-1936) on cover of TIME magazine. The Bull and the Bull's Uncle.

In the United States (U.S.) today, North Dakota is the state where the majority of durum wheat is produced. Roughly 73% of durum that is used in the U.S. comes from this state, although Montana, Minnesota, and South Dakota also produce durum wheat. Countries outside the U.S. States normally import durum wheat grown in these areas because it is typically very strong. However, in recent years California grown durums have also gained popularity abroad. The majority of the wheat planted in the U.S. is fall-seeded winter wheat, and only about 6% is durum.

In 2013 an estimated 690,000 acres were planted to wheat out of which only 10% were durum (J. Cooper, pers comm.). A downward shift in durum was observed due to lower prices and problem of water in the Imperial Valley. The top varieties in Southern California were Desert King, Orita,

and Havasu, whereas Volante, Westmore, and Platinum were the top three durum varieties in the San Joaquin Valley. Worldwide nearly 90% of wheat planted is bread wheat and only 10% is planted to other wheats including durum wheat. However, 80% of the worldwide 10% of durum wheat is planted in the West Asia and North Africa region. Pasta is increasingly becoming very popular globally and especially in the U.S. which means that acreage devoted to durum wheat plantings will increase (Fig. 3).

Figure 3. A hoarding along a highway promoting pasta product near Los Angeles.

The pedigree and development of the well-known bread wheat, Marquis, by David Fife and later by C.E. Saunders in Ontario, Canada is a well-known fact and I will not go in to it here. However, the first introductions of durum wheat were made by a certain group of Mennonites who emigrated to the U.S. from southern Russia and settled in the middle Great Plains which included Kansas. They brought with them wheat selections from Turkmenistan which were found to be well-adapted to the fields in the vicinities of towns of Newton and Halstead. At the time the new durum variety attracted only some local attention, but it was not until a USDA cerealist named M.A. Carleton who picked it up because of its tolerance to drought and good yields under adverse climatic and soil conditions. In fact, he even went to Russia and brought back several varieties like Turkey, Kharkof, Crimean, Beloglina, etc. which he introduced to the U.S. The work of USDA through Carleton was instrumental in establishing wheat industry. Up to this time durum wheat was also planted by several farmers in the U.S., but had failed to achieve commercial success. Once again it was the Russians settled in North Dakota who brought with them Arnautka and began to market it commercially in 1898. Two years later, in 1900, Carleton introduced the durum variety Kubanka in the middle Great Plains and also brought in some seeds of Arnautka. However, after decades of cultivation Kubanka is still considered as the most widely adapted durum wheat in the U.S. In fact, Kubanka is the standard durum variety against which new durum varieties are judged for over 100 years.

Other wheat breeders, such as H.L. Bolley and N.E. Hansen of the North and South Dakota Experimental Stations, were responsible for further introductions of durum wheat. Bolley introduced Pentad and Monad material, both rust resistant durums, from which a variety Kota was selected. Pentad, a red durum, was the first variety to be widely grown in the U.S. Its popularity increased rapidly and by 1911 its acreage had increased to a wide area. This commercial success was no so much for semolina or macaroni manufacture since the grain quality was not up to par, but it was a great success in the feed grain business.

After the re-discovery of Mendel's work by de Vries, Correns, and Tschermak in Europe and Spillman in the U.S., new methods of breeding wheat were developed. Selection work at the Minnesota Agricultural Experiment Station resulted in a variety called Minnesota Durum (shortened to "Mindum"). It had become very popular in the mid to late 1930s. It was well received by the wheat traders and was as popular as Marquis at that time. Mindum's positive traits were high yields, high straw production, and a bright amber color that was unanimously accepted by the pasta makers.

IV – Pasta products

Commercially produced dry pasta, or *pasta secca*, is made almost exclusively from durum semolina. Certain home made fresh pastas (*pasta fresca*), such as orecchiette, cavatelli, and malloreddus, also utilize durum wheat, while others, such as *tagliatelle*, utilize only soft wheat, often "00," or a combination of soft and hard wheats. The availability of commercially prepared pasta is relatively recent. Before that, all pasta used to be made at home and each Italian housewife had her own secret recipe which she proudly guarded (Fig. 4).

Husked but unground, or coarsely ground, it is used for *semoules* in the couscous of North Africa, and other parts of the Arab world. It is also used for Levantine dishes such as *tabbula*, *kishk*, *kibba*, *bitfun* and the *bulghur* for pilafs. In Arab cuisine, it forms the basis of many soups, gruels, stuffings, puddings and pastries. When ground as fine as flour, it is used all over the Middle East, for baking flat round breads, and in Europe and elsewhere, it can be used for pizza, *torte*, etc. It is not, however, good for cakes, which are made from soft wheat to prevent toughness. The use of wheat to produce pasta was described as early as the 10th century by Ibn Wahshīya of Cairo. The Arabs called the product *itrīya*, from which Italian sources derived the term *tria* (or *aletria* in the case of Spanish sources) during the 15th century. Another type of pasta, *al-fidawsh* (called "dry pasta"), was popular in al-Andalus. From there it was transmitted to Christian Spain, and it frequently appears in Hispano-Muslim cookbooks. From *al-fidawsh* was derived the Spanish word for noodles, *fideos*, and the Italian *capellini*.

In the American Great Plains, durum wheat is used almost exclusively for making pasta products such as spaghetti and macaroni. Most of the durum grown today is amber durum, the grains of which are amber-colored and larger than those of other types of wheat. Durum has a yellow endosperm, which gives pasta its color. When durum is milled, the endosperm is ground into a granular product called semolina. Semolina made from durum is used for premium pastas and breads. There is also a red durum, used mostly for livestock feed. The cultivation of durum generates greater yield than other wheats in areas of low precipitation (300–500 mm). Good yields can be obtained by irrigation, but this is rarely done. In the first half of the 20th century, the crop was widely grown in Russia. Durum is one of the most important food crops in West Asia. Although the variety of the wheat there is diverse, it is not extensively grown there, and thus must be imported. West amber durum produced in Canada is used mostly as semolina/pasta, but some is also exported to Italy for bread production. In the Middle East and North Africa, local bread-making accounts for half the consumption of durum. Some flour is even imported. On the other hand, many countries in Europe produce durum in commercially significant quantities.

Figure 4. Italian women making pasta in the home kitchen in the 19th Century.

Pasta fresca, as its name implies, is fresh home-made pasta. Soft and pliable, *pasta fresca* is meant to be cooked promptly. While, strictly speaking, it can be made with durum wheat flour, sometimes the flour is enriched with eggs and becomes easier to work, especially by hand. *Pasta secca*, on the other hand, is the kind of pasta commonly found on grocery-store shelves. This form of pasta can be made only with durum flour, because durum's unique properties permit its nearly indefinite preservation. Writing in the 14th century, the Mamluk civil servant Al-Umari cited a government report that claimed that the durum wheat of North Africa "could be stored for 80 years in silos," and, in the 11th century, Andalusian geographer Al-Bakri boasted that one of the characteristics of Toledo is that "its wheat never changes or goes bad over the years."

Table 1. Durum Wheat Area (x 1000 ha) and Production (x 1000 tons) in different continents.

Region	Area	Production
Western Europe	2,490	5,730
North America	2,960	5,756
South America	102	196
Middle East	4,462	6,950
North Africa	3,290	3,214
Others	3,756	3,540
World	17,060	25,360

V – Desert Durum®

Desert Durum" is a collection of wheat varieties that were developed by and under the ownership of the Arizona Grain Research & Promotion Council and the California Wheat Commission. These wheats are produced in the deserts and dry lowlands of both states under irrigation. These are regions of high temperature (above 32C in May and June) and low rainfall (annual precipitation of less than 200 mm). The wheat is typically planted from November through February, and harvested in May or June. This gives Desert Durum an advantage because they enter the international and domestic market from 1 to 3 months before the spring durum crops from other parts of North America. The Desert Durum varieties are "Desert King", "Duraking", "Havasu", "Kronos", "Maestrale", "Ocotillo", "Orita", "RSI 59", "Saragolla", "Sky", "WB-Mead", and "Westmore". These wheats have been thoroughly evaluated for various agronomic and quality characters: Protein and Moisture Content, 100-KW, Kernel Size, Milling and Semolina characteristics, and Pasta-Making quality including color and firmness. A detailed chart of all this data is available.

There is considerable export demand surfacing for U.S. durum in the last 2-3 years and domestic demand is still fairly sluggish. But there are encouraging signs that domestic pasta makers will buy more Desert Durums in the future. In fact some of these varieties have already started to move to the elevators.

Looking to USDA report the planting estimate for the U.S. desert durum region in 2012 was 90,000 acres in Arizona – up 13 percent from last year, and California was up 17 percent at 140,000 acres for a combined planting of 230,000 desert durum acres. Estimating yield at about 100 bushels/acre, the potential yield is around 23 million bushels – up from the area's more traditional level of 16-18 million in recent years, but lower than the all-time high of more than 30 million bushels.

VI – The future of durum production and climate change

I don't think there's any question" that climate change is already affecting durum wheat production in North Dakota, says Roger Johnson, a former durum farmer who was the state's agriculture commissioner from 1996 to 2009. Johnson points out that Dakota Growers Pasta Co., one of the nation's leading pasta producers, built a combined durum mill and pasta-making plant in Carrington, a town in eastern North Dakota, in 1993. At the time, the decision made economic sense. But as the durum zone has shifted west, transport costs have increased, putting the Carrington plant at a competitive disadvantage. "Looking at the cost of logistics, it has certainly had a negative impact," says Ed Irion, the plant's general manager.

Extreme and volatile weather patterns are especially threatening to durum, which is more finicky than conventional wheat varieties. If too much rain falls at the wrong time, durum's quality can be ruined. Too little rain is not good either. Because durum is trickier to grow, farmers require a price premium over what conventional wheat earns. Already, farmers complain, grain companies have been shrinking these premiums to boost their own profit margins. As climate change intensifies

and durum gets even harder to grow, how high will the price premium have to rise to entice farmers to take the risk? Opland, a durum farmer, wonders whether he will plant durum at all next year.

Moving west also puts durum in direct competition with the richest business enterprise in human history, an industry that has very different plans for the prairies of North Dakota.

The local water supply and its quality are also threatened. Fracking pumps millions of gallons of fresh water underground at high pressure to force oil and gas deposits to the surface. This water is extracted from an aquifer beneath North Dakota, “and we have no right to do that to future generations who’ll need that water,” say the natives. Then the contaminated water is brought back to the surface and disposed of in huge storage ponds, risking spills that can pollute creeks and soil for generations to come.

Perhaps most worrisome for the future of pasta, is ecological fall out of the Bakken oil boom in North Dakota that is gobbling up prime farmland. By an accident of geology, the Bakken oil deposits lie beneath the very area to which climate change has shifted durum production, an area that in recent years also has accounted for most of the durum exports. The U.S. and Canada, are the two leading exporters of durum, with most of their production coming from western North Dakota, eastern Montana, and the southern half of Saskatchewan province. Lay a map of the durum production zone onto a map of the Bakken oil deposits, and the two line-up almost exactly.

Driving west from Minot (a city in North Dakota) one afternoon, Opland passed a new housing development and a freshly completed La Quinta Inn—one of 18 hotels recently built to accommodate oil-boom workers. “That housing development covers 160 acres, and the hotels even more,” Opland laments. “That land won’t come back to farming, not in our lifetimes.” The Bakken oil reserves are large enough to last at least 100 years at full production capacity.

The end of pasta production will not come overnight. If it comes, and it can still be avoided, if humans act swiftly enough. It will come in fits and starts, as harvests falter one year but not the next, and it will be expressed more in shockingly high prices for pasta than in an absolute disappearance of spaghetti and macaroni from grocery-store shelves (Hertsgaard, 2012).

But this need not happen if the U.S. finally gets serious about climate change. That means, among other things, shifting to climate-smart agriculture. If we want to continue enjoying pasta and many other foods we currently take for granted, we need more farmers to emulate the sustainable practices of Glen Bauer and Fred Kirschemann. We also need, desperately, to limit global warming, because even the most skillful adaptation measures cannot cope with 7˚F of global temperature rise. That means the federal government must stop ignoring the mounting climate crisis and take swift aggressive action to slash greenhouse gas emissions.

The televised horrors of Hurricane Sandy may help break the climate silence that still afflicts many Americans. “Mother Nature is better at bringing people to Jesus than any politician is,” notes Jay Fuhrer, the extension agent. But a fear of offending friends and neighbors still inhibits many. “The first thing we always talk about here is the weather, because it affects our lives so much,” says Donny Nelson. “But global warming, people just don’t get into it.”

VII –Conclusion

Traditionally durum wheat has received much less attention of the breeders then bread wheat. But since around the mid-1970s all that has changed and in recent years the prices of durums has always been a little higher than bread wheat, thus giving a good incentive for farmers to grow more durums. The future for durum wheat in the U.S. is excellent, both in consumption as well as production. In fact, California has even begun exporting durum wheat to Italy. But climate change

and oil drilling in the lands that are best for durum production threaten to take over and thereby putting the U.S. durum wheat exports in to jeopardy.

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Yield and nitrogen use efficiency as influenced by rates of nitrogen fertilizers of some Tunisian durum wheat cultivars

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Abstract. Nitrogen use efficiency of durum wheat grain (*Triticum turgidum* ssp. *durum*), as valuable indicator for rational N fertilization supply, was investigated in a field experiment in the subhumid area of Tunisia with four durum wheat genotypes: two improved genotypes and two landraces, grown under three mineral N fertilization treatments (0,75 and 150 kg N ha^{-1}) during two cropping seasons (2008/09, 2009/10). Total N content in grain, yield, N uptake by grain, and soil N ($N\text{-}NH_4$, $N\text{-}NO_3$) status were examined to indicate relations between N trial treatments and growing years. Analysis of variance indicated that significant interaction was noted for nitrogen x years and genotypes. Over total investigated period, above-mentioned factors significantly differ per nitrogen levels and years. The highest total N content in durum wheat grain was recorded in N150 treatment during 2009/10, but the highest yield was reached in 2008/09 growing season under 150 kg N ha^{-1}. Drought and elevated temperatures prevailing during 2009/10 cropping season were associated with lower yielding ability of most genotypes along with higher NUE per fertilization treatment, compared to 2008/09. NUE values varied from 7.85% in 2008/09 up to 24% in 2009/10 for the 150 kg N ha^{-1} treatment. During investigated growing years NUE was increased with increasing nitrogen fertilization levels.

Keywords. Nitrogen use efficiency – Fertilizer rates – Grain yield – Durum wheat.

Production et efficacité d'utilisation de l'azote influencées par le taux d'engrais azotés de certains cultivars de blé dur tunisien

Résumé. *L'efficacité d'utilisation de l'azote (EUA) des grains de blé dur (Triticum turgidum ssp. durum), comme indicateur significatif pour un apport raisonné de cet élément, a été étudiée à travers un essai en plein champ dans la zone subhumide de la Tunisie avec quatre génotypes de blé dur : deux génotypes améliorés et deux variétés locales, cultivés sous trois traitements de fertilisation azotée minérale (0,75 et 150 kg N ha^{-1}) au cours de deux saisons de culture (2008/09, 2009/10). La teneur en N total dans le grain, le rendement, l'absorption de N par le grain et la réserve azotée du sol ($N\text{-}NH_4$, $N\text{-}NO_3$) ont été examinés pour déterminer les relations entre les traitements azotés et les années de croissance. L'analyse de la variance a révélé une interaction significative entre l'azote et les années et les génotypes. Pour toute la période examinée, les facteurs évoqués diffèrent de manière significative en fonction des niveaux d'azote et des années. La teneur en N total la plus élevée dans le grain de blé dur a été enregistrée dans le traitement N150 pendant 2009/10, mais le rendement le plus important a été observé dans la saison de croissance 2008/09 avec 150 kg N ha^{-1}. La sécheresse et les températures élevées qui ont caractérisé la saison de culture 2009/10 ont été associées à une plus faible capacité de rendement de la plupart des génotypes avec une EUA plus élevée par traitement de fertilisation, par rapport à 2008/09. Les valeurs d'EUA variaient de 7,85% en 2008/09 à 24% en 2009/10 pour le traitement 150 kg N ha^{-1}. Au cours des années de croissance étudiées, on a observé un accroissement de l'EUA suite à l'augmentation des niveaux de fertilisation azotée.*

Mots-clés. *Efficacité d'utilisation de l'azote – Doses d'engrais – Rendement en grain – Blé dur.*

I – Introduction

Durum wheat is the main staple food for Mediterranean populations (Arregui and Quemada, 2008) and the major cultivated cereal in Tunisia (Latiri *et al*., 2010). N fertilizer use is one of

the most important agronomic practices in cereals, particularly when crop rotations are lacking (Crews and Peoples, 2004).

Nitrogen use efficiency (NUE) is perceived as a valuable indicator for rational N supply of mineral nitrogen fertilizer in durum wheat which depends on nitrogen status in soil and plant.

In crop production, nitrogen application is a common practice to improve yield and grain quality. In cereals, nitrogen is applied at sowing. However, applied rates of nitrogen are usually split in two applications: at sowing and prior to anthesis to increase protein concentration (Austin *et al.*, 1977; Palta *et al.*, 1994; Fangmeier *et al.*, 1999). High nitrogen use efficiency (NUE) is a required trait in cereals, particularly when nitrogen application is made in advanced vegetative growth stage (at flowering) as compared to early application (Wuest and Cassman, 1992; Raun and Johnson, 1999; Cassman and Walters, 2002). The late application of nitrogen could alter protein composition and nitrogen accumulation in the spike (Johansson and Svensson, 2004).

Estimated NUE is within 33% in developed countries, whereas it is at lower rate in developing countries (Raun and Johnson, 1999). Field experiments indicated that no more than 45-70% of the applied N fertilizers are recovered under average growing conditions (King *et al*., 2001; Noulas *et al*., 2004). NUE is a complex trait and it is associated with N uptake efficiency (UPE) and N utilization efficiency (UTE). Moll *et al.* (1982) and Ortiz-Monasterio *et al.* (1997) noted that UPE reflects the efficiency of the crop in obtaining N from the soil, while UTE reflects the efficiency of nitrogen use of a plant in the translocation process contributing to grain yield.

Nitrogen use efficiency is also driven by genotype effects (Le Gouis *et al*., 2000) and influenced by growing conditions (Bertic *et al*., 2007). The NUE genotypic variation of durum wheat (Giambalvo *et al*., 2010) was attributed to high N uptake and/or high N use efficiency (Dawson *et al*., 2008). Selecting for high NUE genotypes would lead to the reduction of N applications and then a low environmental contamination risk (Giambalvo *et al*., 2010).

With regard to management practices, the choice of plant genotype is particularly important. In fact, several studies have shown that many crop species have genetic variability for NUE (Fageria *et al*., 2009) and that the use of the best-adapted genotype can contribute to improved efficiency in how cereal crops acquire and use soil N or fertilizer N. Foulkes et al. (1998) found that modern wheat genotypes were less efficient at recovering soil N than older genotypes, which suggests that old genotypes may be the best choice for low input and organic growing systems. In contrast, other researchers (Le Gouis *et al*., 2000; Brancourt-Hulmel *et al*., 2003; Guarda *et al*., 2004) have found that NUpE and NUE have increased with the introduction of improved genotypes, and that modern genotypes give the best result seven under limited N availability. Sylvester-Bradley and Kindred (2009) state that wheat breeding has greatly increased grain yield associated with an increase in optimum N rate. Besides, the integration of agro-physiology and molecular N pathway traits to screen wheat genotypes seem to be useful to optimize yields and NUE (Vinod, 2007). The objective of this study was to determine how different mineral nitrogen rates affect total N content in grain, yield, N uptake by grain, and grain N use efficiency during two growth cycles.

II – Material and methods

1. Description of the field experiment site

The field experiment was conducted at the experimental station of the School of Higher Education in Agriculture of Mateur (latitude: 37.04 m, longitude: 9.66 m, altitude: 51 m), located in the subhumid area of Tunisia, during two growing seasons 2008/2009 and 2009/2010. Moderate annual rainfall across the years and distribution during the cropping season were the main characteristics of this site. The area received an annual rainfall of 253 mm during the cropping season (November-June, 2009) which was lower than the mean annual rainfall of the next year

2010. Mean maximum and minimum temperatures recorded at the station during the season (November-June, 2010) were 24 and 10.76°C, respectively (Table1).

Table 1. Agro-meteorological parameters of the experimental site.

Parameter	Year	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	Mai	Jun.	Season
Mean air temperature, °C										
	2009	18.3	11	9.5	9.7	11.3	15.6	17.5	19	63.1
	2010	16.5	13	10.8	12.3	13.9	14.1	24.0	24	128.6
Rainfall, mm										
	2009	58.0	55.6	141.8	99.4	80.4	190.5	2.8	1.6	252.9
	2010	68.7	53	80.5	71.5	90.4	35.7	4.8	1	405.6

2. Experimental design and treatments

The experiment was laid out in a randomized complete block design (RCBD) with three replications. A control with no N fertilizer and three nitrogen levels were applied (75 and 150 kg N ha^{1}). Nitrogen was applied as ammonium nitrate (33.5% N) divided into three applications at different growth stages: early tillering Zadoks 13 (30%), elongation Z16 (40%), and 2nd node Z32 (30%). Four *Triticum turgidum* ssp. *durum*) cultivars were included in this study with two landraces (Bidi AP4 and Azizi AC2) and two high-yielding cultivars (Khiar and Om Rabia). Date of sowing was on November and December 26th, 2008 and 2009, respectively, at a rate of 300 seeds m^{-2}. Plots were 3 m long with six rows, spaced 20 cm apart. At harvest, a 0.5 m^2 portion at the center of each plot was sampled.

3. Soil sampling and analysis

The soil of the experiments has a silt clay loam texture with a low content of organic matter (3%). The pH (H_2O) of the field was 6.7 in 2009 and 8.5 in 2010. The relevant soil characteristics at the study site are presented in Table 2. In the two years, soil samples were taken on all plots prior to sowing and after harvesting, at a depth of 90 cm. All samples were analyzed for nitrate and ammonium content according to the Devarda"s Alloy reagent method (Sims *et al*., 1995) and ammonium was measured using the distillation–titration proceeding method (Rhine *et al*., 1998).

Table 2. Soil properties at three depths in the experimental field prior to sowing.

Soil property %	0-10 cm	10-30 cm	≥ 30 cm
Clay	22.5	21.6	18.5
Silt	57.3	57.3	52
Sand	17.3	18.3	16.1
Limestone	20.1	20.9	19.9
Mineral calcite	10.1	9.8	10.1
Organic matter	1.90	2.08	1.83
Total N	0.20	0.21	0.21
pH	8.3	8.4	8.5

4. Straw nitrogen and grain protein analysis

Straw and grain N concentration was determined using the Kjeldahl procedure and N concentration was determined using the method outlined by Cataldo *et al*. (1974). At maturity, samples of non-grain above-ground plant parts (stems, leaves and chaff) were obtained from the central unit areas of 0.5 m^2. Plant samples were oven-dried at 80 °C and the dry weight measured. The samples were ground by a rotor mill and a sample of 200 mg was used for the digestion with sulfuric acid (H_2SO_4).

5. Data measurements

Grain Yield (kg ha^{-1}): Obtained from the harvested central unit areas of 0.5 m^2. Samples were cleaned following harvesting and weighed using an electronic balance.

Nitrogen uptake (kg N ha^{-1}): Nitrogen contained in the grain was calculated as grain yield*grain protein/5.7.

Total Nitrogen uptake (kg N ha^{-1}): Total N uptake is the sum of N_{straw} and N_{grain}, whereas N accumulated in the crop residue at harvest (kg N. ha^{-1}) was calculated as the total biomass - grain yield * $\%N_{straw}$.

Crop N supply: This measure includes the sum of soil NO_3-N at sowing, mineralized N and N fertilizer (Moll *et al*., 1982). Mineralized N was estimated as the difference between pre-sowing and post-harvest plant and soil NO_3 -N in a check plot.

Nitrogen use efficiency (NUE; kg.kg^{-1}): Total N in the straw and grain samples was used to compute N use efficiency according to an expanded model of Moll *et al.* (1982) and Ortiz-Monasterio *et al.* (1997). The following N-efficiency parameters were calculated for each: N use efficiency (NUE; kg kg^{-1}), defined as grain production per unit of N in the soil.

6. Statistical analyses

Genotypes, N level and their interactions were assessed using a SAS GLM procedure (SAS Institute Inc., 1999) for all traits. The treatment means were compared by Duncan's multiple range test ($\alpha = 0.05$).

III – Results and discussion

1. Effect of N application on grain yield

The mean squares calculated for both cropping seasons (Table 3) indicated that variation for grain yield was significantly ($p \leq 0.01$) affected by the interactions of both N rate × year, N rate × cultivar and year × cultivar. The mean values for the cultivars across all N rates and years showed the lowest grain yield of the improved genotypes (Om Rabiaa and Khiar) without nitrogen supply compared with the local genotypes (Bidi and Azizi) (Table 4). The mean grain yield obtained for Khiar at 0 kg N ha^{-1} was lower than the mean yield of Bidi at the same N application rate (Table 4). Grain yield differed significantly among the two studied years. The grain yields of the landraces were significantly higher than that of the improved cultivars in 2009 and in 2010 (Table 5). This indicates differences in the genetic background of the four cultivars for yield potential. Cultivar x N application rate indicated progressive increases in grain yields ranging from 2025 kg.ha^{-1} to 6253 kg.ha^{-1} of the cultivars with increased application of nitrogen. Maximum yield increase of 208% and 179% for Khiar and Om Rabia were obtained at the highest level of N application compared to the control. There were large yield reductions for all cultivars grown without nitrogen application as compared to those fertilized plots. Huggins *et al*. (2010) reported that, in an optimal yield environment, higher levels of N fertilizer would reduce grain yield responses.

In general, grain yields in the cropping season 2009-2010 were lower than those in 2008-2009. This was attributed to the drought and the high temperatures during 2010. It is apparent, that elevated temperatures and drought prevailed during the vegetative growth stages during both cropping seasons. Johnson and Raun (2003) noted that temporal yield variability was greatly affected by differences in temperature and cumulative precipitation.

Table 3. Mean squares of grain yield (GY), nitrogen uptake (NUp), total nitrogen content (TNc), crop N supply (CNS) and nitrogen use efficiency (NUE) of four wheat cultivars grown under three Nitrogen levels and two years in subhumid conditions of northern Tunisia.

Sources of variation	DF	GY kg ha^{-1}	NUE kg kg^{-1}	NUp kg N ha^{-1}	TNc kg N. ha^{-1}	CNS kg N. ha^{-1}
Years (Y)	1	27280880**	3872**	94	70	254643**
Blocks in Year	4	320719	3	453	955*	649
Cultivars (C)	3	327545	13	608	631	928
Nitrogen (N)	2	70952346**	89**	34453**	38974**	38564**
N x C	6	1857441**	13	682*	847*	702
YxC	3	1649772**	15	969*	926*	928
YxN	2	3997202**	26	3330**	3966**	31184**
YxNxC	6	602299	7	473^{s}	392^{s}	702
Error	44	348842	9	235	290	634

, significant differences at α = 0.05 and α = 0.01 respectively.*

Table 4. Two-year mean durum wheat grain yields (kg.ha^{-1}) under three nitrogen levels.

Cultivars	0 N	75 N	150 N	Mean
Azizi	2305G	4434DE	5174CD	3971A
Bidi	2496G	4588DE	5358BC	4147A
Khiar	2024G	3395F	6253A	3891A
Om Rabia	2112G	3498F	5908AB	3839A
Mean	2235C	3979B	5673A	

LSD (0.05) = 396.8 and 370.6 for comparison of cultivar means and treatment means, respectively.
Different letters indicate significant differences between cultivars (within-row) and treatments (within-column) at α = 0.05.

Table 5. Means of NUE components of durum wheat cultivars of two years (2009 and 2010). Data represent means of three nitrogen levels.

NUE component	Cultivars	2009	2010	Mean	LSD
GrainYield (kg.ha^{-1})	Azizi	4361B	3581C	3971A	† : 396.8
	Bidi	5133A	3163C	4148A	‡ 370.6
	Khiar	4238B	3544C	3891A	
	Om Rabia	4580AB	3099C	3840A	
N uptake (kg.ha^{-1})	Azizi	74.3B	85.3A	84.8AB	† 10.29
	Bidi	88.2A	76.8AB	88.7A	‡ 13.93
	Khiar	64.6B	73.9AB	74.9B	
	Om Rabia	88.4A	70.1B	85.5AB	
Total N uptake (kg.ha^{-1})	Azizi	78.8ABC	90.8AB	84.8BA	
	Bidi	93.7A	83.5ABC	88.6A	† 11.45
	Khiar	71.0C	79.0ABC	74.9B	‡ 20.23
	Om Rabia	94.4A	76.5BC	85.5BA	

† For comparison of cultivar means; ‡ For comparison of year means.
Different letters indicate significant differences between genotypes (within-row) and treatments (within-column) at α =0.05.

2. Effect of N-application on grain N-uptake and total N-uptake

Nitrogen uptake in grain and total nitrogen uptake had a significant response to nitrogen and years and different genotypes and years (Table 3). Grain N-uptake increased significantly with N supply during the two years. The highest grain N-uptake was noted for Om Rabia (88.42 kg ha^{-1}). Om Rabia accumulated the maximum N content in grain (88.42 kg ha^{-1}) which may be associated with maximum yield. Fageria *et al*. (2003) and Shinano *et al.* (1995) reported that in cereals, N accumulation is associated with dry matter production and shoot yield and grain representing

the total biomass. Nitrogen uptake in the straw increased significantly with N (data not shown). An application of 150 kg N ha^{-1} caused the highest content of N uptake (104.55 kg ha^{-1}) in 2009 and in 2010 (129.46 kg ha^{-1}) (Table 6). Fageria *et al.* (2009) argued that this response could be associated with maximum yield of shoot yield.

Total N uptake differed significantly among years (Table 3). For the two years study as a whole, total mean N uptake of111.23 kg.ha^{-1} and 138.90 kg.ha^{-1} for both cropping seasons and suggesting that N uptake was proportional to yield: 5859 and 5487 kg N. ha^{-1} in 2009 and 2010, respectively. However, in their study of bread wheat, Limaux *et al.* (1999) reported a significant effect on partitioning of added N between soil and plant.

Differences between species in N uptake were noticeable at heading and maturity. Barley took up 70-73% of the total N before heading, whereas wheat and oat averaged 64% (Peltonen-Sainio *et al*., 2007b). Our results for durum wheat are comparable to those of earlier studies (Bulman and Smith, 1994; Delogu *et al*., 1998). These results indicated that wheat had much lower N uptake up to heading than the 90 to 100% reported by Clarke *et al.* (1990) and Heitholt et al. (1990). According to our results, wheat had up to 69% higher heading N uptake than barley, possibly because they require much longer growing period under northern growing conditions than barley (Peltonen-Sainio *et al*., 2007b). These results did not support those of earlier studies which suggested that higher N uptake of wheat would contribute to improved NUE in wheat (Van Sanford and MacKown, 1986; May *et al*., 1991; Le Gouis *et al*., 2000).

Table 6. Means of NUE components of durum wheat cultivars of two years. Data represent means at three nitrogen levels.

NUE component	N rate	Year				
	kg ha^{-1}	2009	2010	Mean	LSD1	LSD2
Grain yield	0	2898C	1572D	2235C	370.6	343.6
(kg.ha^{-1})	75	4977B	2981C	3979B		
	150	5859A	5488A	5674A		
N uptake	0	49.6E	33.2F	41.4C	13.93	8.91
(kg.ha^{-1})	75	82.4C	66.9D	74.6B		
	150	104.6B	129.5A	117.0A		
Total N uptake	0	53.7E	35.5F	44.6C	20.23	9.91
(kg.ha^{-1})	75	88.4C	72.9D	80.7B	† :	
	150	111.2B	138.9A	125.1A		
Crop N supply	0	35.2D	79.3C	57.2	6.67	14.65
(kg.ha^{-1})	75	39.2D	164.0B	101.6		
	150	43.3D	231.2A	137.2		

LSD1 and LSD2 for comparison of year means and treatment means, respectively.
Different letters indicate significant differences between cultivars (within-row) and treatments (within-column) at α = 0.05.

3. Effect of N application on N use efficiency under different N treatments

NUE refers to the total nitrogen available to the plant either from the soil or from the fertilizers. It has been shown that under suboptimal yields, NUE increases with the increase of the total available N (Raun and Johnson, 1999). NUE was significantly affected by N fertilizer rates and years (Table 3).The NUE has been increased under nitrogen application (Figure1). Maximum NUE (15.67%) was observed at 150 kg N/ha; while the lowest efficiency (11.87%) was noted in the control. In addition, no significant difference was noted between genotypes for the NUE of durum wheat genotypes cultivated in subhumid growing conditions of northern Tunisia. These results would imply that sufficient available nitrogen for cereal exists in the soil of the experimental station or it has been leached. In fact, the soil total N content of the experimental field was very low (Table 2). The quite high yield levels obtained at N control could be attributed to the high averages

ofl organic content at maximum root growth depth (Table 2) which could constitute an additional N source from soil organic mineralization process driven by rainfall and high temperature during March and April (Cabrera *et al.*, 2005). Mineral nitrogen will be then available till near anthesis growth stage for a greater resulting grain yield (Woolfolk *et al.*, 2002; Kara, 2010) and quality (Ottman *et al.*, 2000). This confirms the major contribution of high levels of residual soil N to grain yield, due to the accumulation of N fertilizer over time, as indicated earlier. Results showed that with increasing N application, NUE increased (Figure1a). NUE was significantly affected also by years (Figure1b). However, greater value of NUE was noted during 2010 cropping season which was three times more than the mean value for 2009 (Figure1b). This increase is larger than that reported by Sowers *et al.* (1994), and was similar for the results obtained for bread wheat by Lopez-Bellido and Lopez-Bellido (2001) and Lopez-Bellido *et al.* (2005), under similar Mediterranean conditions.

Zhang *et al.* (2010) reported the same results. When higher nitrogen is supplied, NUE will decrease and was attributed to an inconsistent increase between grain yield and N supply. In these conditions, plants are unable to assimilate enough nitrogen and N losses raised (Dawson *et al.*, 2008). In our experiment, all measured nitrogen efficiency components increased under maximum N supply. This indicates that the maximum N rate of 150 kg $N.ha^{-1}$ is unlikely to be sufficient for the tested genotypes to reach their optimum genetic yield. Besides, the optimum N and NUE are closely related to the water availability (Latiri, 2000; Latiri *et al.*, 2010). In our experimental conditions, available water for plants was not a limiting factor during the cropping season 2009/2010 with 406 mm.

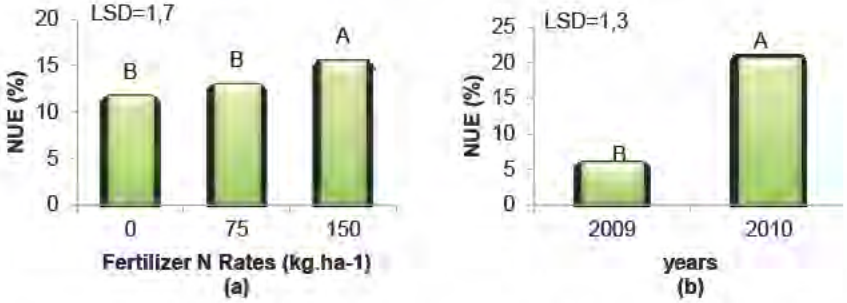

Figure 1. Variation of mean nitrogen use efficiency (NUE) of four durum wheat cultivars among N fertilization rates and cropping periods.

IV – Conclusions

The described differences in N dynamics and N use efficiency shown in this work emphasizes the need for characterization of durum wheat germplasm for NUE for plant breeding programs and more accurate agricultural management practices. The NUE characterization inputs will be adapted for crop management practices and thereby will reduce environmental impacts of N losses.

Based on these results, selecting for better NUE in breeding programs would be still challenging, due to the complexity of NUE. N uptake would be the way to address the better N recovery from the soil decreasing the potential for leaching and volatilization of N fertilizers. During the grain filling, N relocation to grains constitutes the main part of N dynamics which together with increased biomass production would increase grain yield and final NUE. Durum wheat total grain N content, yield, and grain NUE are conditioned by soil type and climate factors such as rainfall and temperature during vegetation stages. The results showed that improved genotypes require

higher levels of nitrogen to fully express their genetic potential. Nitrogen fertilization level of 150 kg N. ha^{-1} leads to the best average use efficiency (Giambalvo *et al*., 2010). To improve durum wheat NUE, more information is required on seasonal soil changes and its impact on crop utilization.

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Durum wheat grain yield and quality under elevated CO_2 : first results of a free air carbon dioxide enrichment (FACE) experiment

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Abstract. Free-air CO_2 enrichment (FACE) experiments the study of the effects of elevated [CO_2] on plants and ecosystems grown under natural conditions without enclosure. Here the results of the first harvest 2011/2012 on the aboveground biomass production, grain yield and grain quality of 12 durum wheat (*Triticum turgidum* var. *durum*) genotypes grown under FACE conditions. These genotypes are representative of the durum wheat breeding history in Italy. The free-air system, installed in the experimental farm of the Genomics Research Centre of CRA in Fiorenzuola d'Arda, allows the study of the effect of increased atmospheric CO_2 mixing ratios, expected for the mid of the 21st century on crop yield and quality. The results showed an increase in biomass and grain yield and a decrease in grain crude nitrogen content due to elevated CO_2. Moreover, high genetic variability was observed for all of these traits within the genotypes.

Keywords. Free Air Carbon Dioxide Enrichment (FACE) – Genetic diversity – Grain yield – Grain quality.

Rendement et qualité des grains de blé dur sous CO_2 élevé : les premiers résultats d'une expérience d'enrichissement en dioxyde de carbone à l'air libre (FACE)

Résumé. *L'enrichissement en CO_2 à l'air libre (FACE) permet d'étudier expérimentalement les effets de la hausse du [CO_2] dans l'atmosphère sur les plantes et les écosystèmes cultivés en plein champ et à l'air libre. Nous allons illustrer les résultats de la première récolte 2011/2012 sur la production de biomasse aérienne, le rendement en grain et la qualité du grain de 12 génotypes de blé dur (Triticum turgidum var. durum) cultivés dans des conditions FACE. Ces génotypes sont représentatifs de l'histoire de la sélection du blé dur en Italie. Le système à l'air libre, installé dans la ferme expérimentale du Centre de recherche en génomique du CRA à Fiorenzuola d'Arda, permet d'évaluer l'effet de l'augmentation des rapports de mélange du CO_2 dans l'atmosphère, annoncée pour le milieu du siècle, sur le rendement et la qualité des cultures. Les résultats ont montré une augmentation de la biomasse et du rendement en grains et une diminution de la teneur en azote brut du grain liées à la concentration accrue de CO_2. En outre, une forte variabilité génétique a été observée pour l'ensemble de ces caractères entre les génotypes.*

Mots-clés. *Enrichissement en dioxyde de carbone à l'air libre (FACE) – Diversité génétique – Rendement en grain – Qualité du grain.*

I – Introduction

Continued growth of the world population, motorization and industrialization is resulting in an increased emission of greenhouse gases, especially CO_2, from combustion of fossil fuels, industrial processes, and deforestation. Based on the reports by the IPCC (Intergovernmental Panel on Climate Change, 2007), atmospheric CO_2 concentration is continuously rising (Balouchi *et al*., 2009). The measurements at the Mauna Loa station (Hawaii) show an increase from below 320 ppm at the start of the measurements in 1958 to 393 ppm in 2011. Further increases are expected during the 21st century due to continued use of fossil fuels, leading to estimated concentrations around 550 ppm for the mid-century. The rising atmospheric CO_2 levels are a

cause of the ongoing anthropogenic climate warming and thus a matter of concern with respect to global change.

CO_2 is the main source of organic carbon of living beings. Plant photosynthesis fixes and reduces CO_2, incorporating the carbon into biomolecules. The direct causes of the instantaneous increase in C3 photosynthesis with elevation of CO_2 are two properties of the primary carboxylase of C3 photosynthesis: ribulose-1,5-bisphosphatecarboxylase/oxygenase (RuBisCo). The enzyme catalyzes the carboxylation of ribulose-1,5-bisphosphate (RubP) with CO_2 to yield two molecules of 3-phosphoglyceric acid (3PGA). First, RuBisCo is not saturated at present levels of atmospheric CO_2, and so elevated CO_2 increases the velocity of carboxylation and net photosynthesis. In addition, CO_2 is a competitive inhibitor of the oxygenation reaction, which leads to photorespiratory release of CO_2 (Long *et al*., 2006; Ainsworth and Rogers, 2007) and so elevated CO_2 reduces the rate of oxygenations. Thus, the historical rise in atmospheric CO_2 as well as the expected further increases during the coming decades have the potential to lead to increased carbon assimilation by C3 photosynthesis. Many studies on the effects of elevated CO_2 on C3 plant photosynthesis and growth have demonstrated a stimulation of photosynthetic production and, subsequently, growth, although not always as high as expected on the base of the enzyme kinetic properties of RuBisCo. The photosynthetic capacity is often reduced after long-term exposure to elevated CO_2, a phenomenon known as down-regulation (Arp, 2006).

Change in biomass in response to CO_2 enrichment has been reported to vary with species and a persistent increase of biomass production during growth in elevated CO_2 was observed. Besides carbon fixation, also the plant tissue chemistry of nitrogen is greatly affected by atmospheric CO_2 enrichment. In particular, the most commonly reported effect is a decrease in the dry mass concentration of N (Nm). It was reported that the mean value of Nm decreased by 14% in above-ground tissues and 9% in roots, reaching a 12.9% decrease for leaves in free-air carbon dioxide enrichment (FACE) experiments and 14% for seeds (Taub and Wang, 2008).For wheat, barley and rice, the reduction in grain protein concentration was 10-15% of the value at ambient CO_2 (Taub *et al*., 2008). This effect, known as growth dilution, leads to reduced concentration of protein with increased yield. Such decrease in Nm can have important implications for plant physiological processes and for food chains, as well as on the performance of insect herbivores and can affect herbivore population dynamics.

Many research efforts to understand how plants and ecosystems will respond to rising atmospheric CO_2 have been undertaken. The primary effects on plants of elevated CO_2 have been well documented and include reduction in stomata conductance (gs) and transpiration, improved water-use efficiency (WUE), higher rates of photosynthesis (A), and increased light-use efficiency. The majority of these conclusions have come from studies of individual species grown in closed controlled environments. While the conclusions from these experiments form the basis for the knowledge of plant physiological responses to elevated CO_2, there are serious limitations to using enclosure systems when studying the effects of elevatedCO_2 on plants. Chambers also are limited in size and may have limited capacity to allow investigators to follow trees and crops to maturity within an experimental facility. Large-scale free-air CO_2 enrichment (FACE) experiments allow the exposure of plants to elevated CO_2 under natural and fully open-air conditions. FACE technology uses no confinement structures, rather an array of vertical or horizontal vent pipes to release jets of CO_2-enriched air or pure CO_2 gas at the periphery of vegetation plots. FACE relies on natural wind and diffusion to disperse the CO_2 across the experimental area. More recent field studies have employed a FACE technique in which pure CO_2 gas is released as high-velocity jets from emission tubes (through numerous small perforations) positioned horizontally at the periphery of a FACE octagon (Miglietta *et al.,* 2001). FACE design allows good temporal and spatial control of CO_2 concentrations throughout crop canopies and forest plantations (Long and Ainsworth, 2005).

There is now a pressing need to understand more about long-term adaptation and genetic changes in future CO_2 concentrations, particularly for adaptive traits that are relevant to plant productivity and ecological characteristics that determine survival, fitness, yield and interaction with pathogens. Genetic variability in this response needs to be characterized to identify the most promising genotypes for breeding of new varieties that optimally exploit elevated levels of atmospheric CO_2.

A FACE experiment was conceived to study the effects of elevated CO_2 on growth, yield and grain quality of 12 durum wheat (*Triticum turgidum* var. *durum*) genotypes that were products of the durum wheat breeding history in Italy. Genetic variability and GxE interactions under ambient/elevated CO_2 are been observed for plant development and growth, canopy-related traits, yield, yield components, quality traits and metabolite composition. Furthermore, eco-physiological analyses are carried out to describe the physiological mechanisms modified in response to elevated CO_2 and flag leaf samples are collected for transcriptomic (RNAseq) and metabolomic studies, and grain for quality. The analyses of the samples collected during the first growing season (2012) have been partially completed and the presentation illustrates the first main findings and summarizes the expected impact of increased atmospheric CO_2 on yield response and quality in durum wheat.

II – Material and Methods

Twelve durum wheat genotypes (*Triticum turgidum* var. *durum*) were grown within the FACE facility of the Genomics Research Centre of the Consiglio per la Ricerca e sperimentazione in Agricoltura (CRA-GPG) at Fiorenzuola d'Arda (44.927°N, 9.893°E) applying a split-plot design with FACE and control octagons distributed at random within the experimental field (4 FACE, 4 controls). The single FACE and control systems contained two blocks (northern and southern side) with plots (1.32 x 2.2 m) for the 12 genotypes as sub-plots. The genotypes include modern high-yielding varieties (Simeto, Ciccio, Claudio, Anco Marzio, Saragolla), varieties with high protein content (Svevo, Aureo), varieties with a prominent role in Italian durum wheat breeding (Cappelli, Creso, Ofanto) and two lines of the Ofanto x Cappelli mapping population (RIL11 and RIL28). Sowing at optimal sowing time (October 19th 2011) was assured by a pre-harrowing irrigation due to dry soil conditions. The CO_2 mixing ratio for the FACE treatment target was fixed at 570 ppm representing a value within the upper range of scenarios for the mid Century atmospheric mixing ratio. Carbon dioxide sensors are located in the centre of the octagons and of the anemometers on top of the control units. The readings of the CO_2 concentration, wind speed and velocity are used to calculate the level of the fumigation and the sectors in which CO_2 is released. The readings of CO_2 sensors as well as the variables describing the fumigation are transferred to a central server within the institute *via* fibre glass cables. FACE treatment was started on November 16th, 2011 and stopped when leaves were senescent at June 14th, 2012. The experiment was performed according to standard local agronomic practice and with the objective to avoid major pests and diseases. The plots were fertilised with application of an N:P:K fertiliser at pre-seeding and two top-dressings with ammonium nitrate for a total of 149 kg N ha^{-1}. At final harvest (July 2nd, 2012) 1.5 linear meters per plot were harvested for determination of yield components. Subsequently, the whole plots were harvested manually and aboveground biomass and grain yield were determined. Grain nitrogen content determined with the Kjeldahl method, and grain crude protein content calculated as 5.7*N.

Figure 1. Two of the durum wheat FACE octagons in April 2012 with the CO_2 tank in the background.

III – Results and Discussion

The traits related to growth showed a more vigorous development under elevated CO_2 (Badeck *et al*., 2012). Flag leaf light-saturated photosynthesis was higher under elevated CO_2 when measured at growth conditions and stomata conductance substantially reduced leading to an increased instantaneous water use efficiency (+30 to 60%) at the leaf level (Badeck *et al.,* in press). The increased leaf level water use efficiency (WUE) was not fully compensated by the higher transpiring surface area, as evidenced by measurements of soil water content in the uppermost 6 cm of soil (data not shown), that showed a slightly higher water content in the elevated CO_2 treatment.

A good growing season led to high yield in the ambient as well as the elevated CO_2 treatments. The average grain dry mass yield was 7.91 t DM ha^{-1} under ambient CO_2 and the FACE treatment increased the average yield significantly ($p<0.01$) to 8.91 t DM ha^{-1}. Total aboveground dry biomass at harvest was 18.5 t DM ha^{-1} under ambient CO_2 and increased ($p<0.01$) to 21.6 t ha^{-1} under FACE. There is a considerable and statistically significant genetic variability of yield as well as of the CO_2 response of yield. Grain dry matter yield was increased between 4.4% (var. Ciccio) and 20.4% (RIL28) (Fig.2). The average increase in grain yield of durum wheat by 12.6% due to elevated CO_2 was similar to the medium effect of 14.4 % reported by Ainsworth and Long (2005) for bread wheat based on a meta-analysis of five FACE experiments. The variability among the durum wheat cultivars (+4.4 to 20.4%) filled a substantial part of the confidence range found for the effect in bread wheat (-1.6 to +33.1%). The increase in grain yield of durum wheat was mainly due to increased numbers of tillers, while the number of grains per spike and the thousand kernel weight (TKW) changed only marginally (Fig.3).

Averaged across all genotypes, the crude grain protein content decreased by 7.0% for plants grown in elevated CO_2 relative to the controls with a substantial variation between genotypes (-2.2% for Ofanto to -10.8% for Aureo).

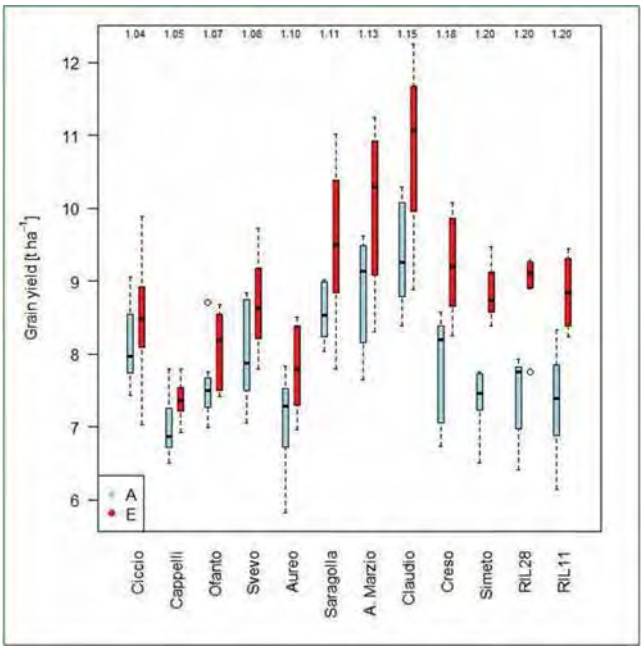

Figure 2. Graph describing the grain yields of 12 genotypes of durum wheat grown in the FACE octagons (E enrichment red bars) and in atmospheric CO_2 concentration (A ambient blue bars).

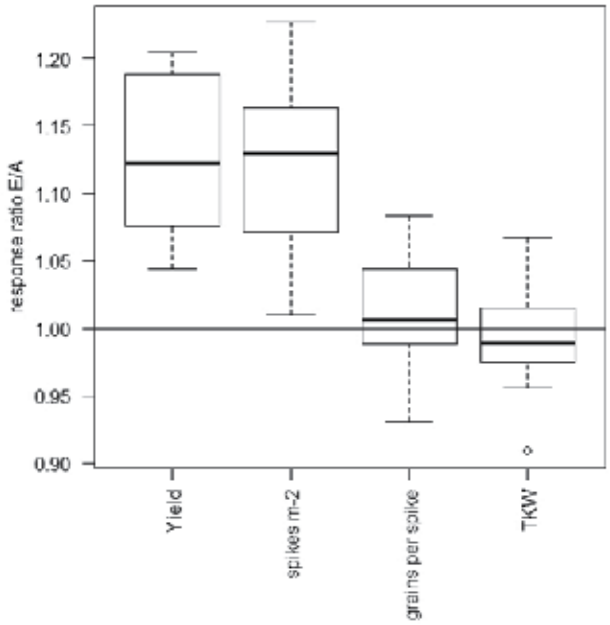

Figure 3. Effect of elevated CO_2 on grain yield and yield components. The box plots show the distribution of genotype means.

IV – Conclusions

Atmospheric CO_2 content elevated to 570 ppm lead to a stimulation of grain yield in durum wheat that is comparable to results obtained on bread wheat, whereas crude grain protein content decreased indicating potential losses in grain quality. Substantial between genotype variability in the yield and quality response to elevated CO_2 hints to genetic variability that can be exploited for selection of varieties best suited for the mid-century atmospheric CO_2 content.

Acknowledgments

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Session 5

Genetics and breeding for durum wheat disease and pest resistance

Genetic resources for stem rust resistance in cultivated and wild tetraploid wheats

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Abstract. New emerging races of *Puccinia graminis* f. sp. *tritici* are a serious threat to durum and bread wheat production worldwide because of their virulence on many cultivars and rapid spread. Our research objective was to indentify new sources of stem rust resistance in cultivated and wild tetraploid wheats that could be utilized in durum breeding. We characterized 3500 durum (*T. turgidum* ssp. *durum*) and 360 emmer wheat (*T. turgidum* ssp. *dicoccum*) accessions for stem rust resistance in multiple field and seedling evaluations. Search for resistance through seedling evaluation was also conducted in 1770 accessions of wild and cultivated *T. turgidum* ssp. and *Aegilops* ssp. Accessions exhibiting a high level of stem rust resistance to TTKSK (or Ug99 race) and other races were observed in all the species evaluated. Studies on the inheritance of TTKSK resistance revealed that resistance was conferred mostly by one and two genes. Our studies concluded that wild and cultivated tetraploids are a rich source of resistance to race TTKSK, and may contribute with novel stem rust resistance genes.

Keywords. Genetic resources – Resistance genes – *Puccinia graminis* f. sp. *tritici* – Ug99.

Ressources génétiques de la résistance à la rouille noire dans les blés tétraploïdes cultivés et sauvages

Résumé. *De nouvelles races émergentes de Puccinia graminis f. sp. tritici posent une grave menace pour la production de blé dur et de blé tendre dans le monde entier en raison de leur virulence sur de nombreux cultivars et de leur propagation rapide. L'objectif de cette recherche est d'identifier de nouvelles sources de résistance à la rouille de la tige des blés tétraploïdes cultivés et sauvages qui pourraient être utilisées pour l'amélioration du blé dur. Nous avons caractérisé 3500 accessions de blé dur (T. turgidum ssp. durum) et 360 d'amidonnier (T. turgidum ssp. dicoccum) pour la résistance à la rouille noire par de nombreuses évaluations sur le terrain et au niveau des semis. La recherche de la résistance à travers l'évaluation des semis a également été menée sur 1770 accessions de T. turgidum ssp. et Aegilops ssp sauvages et cultivées. Des accessions présentant un niveau élevé de résistance à la rouille noire TTKSK (ou race Ug99) et à d'autres races ont été observées dans toutes les espèces étudiées. Les études sur l'héritage de la résistance TTKSK ont révélé que la résistance est principalement conférée par un ou deux gènes. Nos études ont permis de conclure que les tétraploïdes sauvages et cultivés sont une riche source de résistance à la race TTKSK, et que leurs gènes pourraient être utilisés pour améliorer la résistance à la rouille de la tige.*

Mots-clés. *Ressources génétiques – Gènes de résistance – Puccinia graminis f. sp. tritici – Ug99.*

I – Introduction

Stem or black rust, caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn. (Pgt), is one of the most destructive diseases of durum wheat [*Triticum turgidum* L. ssp. *durum* (Desf.) Huns.] and bread wheat (*T. aestivum* L.) worldwide. The recent emergence of TTKS (or Ug99 race) group in Eastern Africa with broad virulence to wheat cultivars worldwide (Jin *et al.*, 2007) represents a new threat to wheat production at a global scale. Since first reported in 1999 (Pretorius *et al.*, 2000), TTKSK and its variants have been found throughout eastern and southern Africa (Jin *et al.*, 2008; Singh *et al.* 2011; Viser *et al.* 2011; Wanyera *et al.* 2006; Wolday *et al.*, 2011), and Iran (Nazari *et al.*, 2009). In addition to TTKSK, Pgt races found in Ethiopia that are more adapted to durum and have combined virulence to important durum resistance genes, such

as *Sr13* and *Sr9e*, increase the vulnerability of durum to stem rust. The limited availability of resistance to TTKSK in adapted germplasm (Jin *et al.* 2007) coupled with its rapid evolution and spread urgently requires the identification and introgression of effective resistance genes from all genepools of wheat.

Tetraploid wheats with AABB genome (*Triticum turgidum* L.) comprise eight cultivated and wild subspecies. The only wild form is wild emmer [*T. turgidum* ssp. *dicoccoides* (Körn. ex Aschers. & Graebn.) Thell.], the progenitor of cultivated tetraploid and hexaploid wheat (Zohary, 1970). In addition to durum wheat, there are four other free-threshing cultivated subspecies of *T. turgidum* including Persian wheat [*T. turgidum* L. subsp. *carthlicum* (Nevski) Á. Löve and D. Löve], Polish wheat [*T. turgidum* L. subsp. *polonicum* (L.) Thell.], Oriental wheat [*T. turgidum* L. subsp. *turanicum* (Jakubz.) Á. Löve and D. Löve], and Poulard wheat (*T. turgidum* L. subsp. *turgidum*) (van Slageren, 1994). These tetraploid wheats are ancient cereal crop species derived from cultivated emmer wheat [*T. turgidum* L. subsp. *dicoccum* (Schrank) Thell.] (Feldman *et al.*, 1995). Tetraploid wheats have contributed with important genes for stem rust resistance, such as *Sr2, Sr9d, Sr9e, Sr12, Sr13, Sr14*, and *Sr17* (Heermann and Stoa, 1956; McFadden, 1930; McIntosh *et al.*, 1995), but have not been extensively characterized for resistance to the new races on the TTKSK group.

Aegilops is the most closely related genus to *Triticum* (Gill and Friebe, 2002) and comprises 23 species that include diploid, tetraploid, and hexaploid genomes (van Slageren, 1994). *Aegilops* species are known to be rich sources of resistance to various pathogens and pests, including stem rust (Alam and Gustafson 1988; Anikster *et al.* 2005; Gill *et al.* 1985; Pasquini 1980), and many resistance genes have been transferred into wheat. However, limited information for resistance to race TTKSK is available except for *Ae. tauschii* (Rouse *et al.* 2011).

The objective of this study is to identify and characterize new sources of stem rust resistance against race TTKSK and other races with broad virulence in *T. turgidum* L. ssp. and *Aegilops* ssp., and to investigate the genetic bases of stem rust resistance.

II – Material and methods

1. Germplasm

A total of 5359 accessions of seven subspecies of *T. turgidum* deposited at the USDA-ARS National Small Grain Collection (NSGC) (Aberdeen, ID) were evaluated in this study. The collection includes: 3500 accessions of durum wheat, 359 of cultivated emmer, 880 of wild emmer, 77 of Persian wheat, 63 of Polish wheat, 66 of Oriental wheat, and 414 of Poulard wheat. We also evaluated 1220 *Aegilops* accessions including 260 of *Ae. biuncialis,* 151 of *Ae. cylindrica*, 182 of *Ae. geniculata*, 202 of *Ae. neglecta*, 73 of *Ae. peregrina*, and 233 of *Ae. triuncialis*. Thirteen accessions (4 durum, 4 emmer, 2 wild emmer, 1 Persian wheat, 1 Polish wheat, and 1 Poulard wheat) were selected for inheritance and allelism studies based on their reaction to races TTKSK, TRTTF, and TTTTF. Crosses were developed to investigate the number of genes conferring resistance to race TTKSK. F_1 plants were grown and selfed to produce F_2 populations. Individual F_2 plants were then selfed to produce $F_{2:3}$ families.

2. Disease assessment

Adult evaluation. All the durum and emmer entries were evaluated for resistance in field tests in the stem rust nursery at Debre Zeit, Ethiopia. Accessions rated as resistant with 30% or less stem rust severity and moderately or lower susceptible infection response in the Debre Zeit field nursery were further evaluated in the Debre Zeit and St. Paul nurseries in two growing seasons. In St. Paul, the nursery was inoculated with a composite of six US races (TPMKC, RKQQC,

RCRSC, QTHJC, QFCSC, and MCCFC). The Debre Zeit nursery was artificially inoculated with race TTKSK and a bulk of Ethiopian isolates collected from durum lines at a ratio of 50/50. Details about the management of nurseries at St. Paul and Debre Zeit, and inoculation and disease assessment procedures, were described by Olivera *et al.* (2012a). Plants were evaluated for their infection responses (pustule type and size) (Roelfs *et al.*, 1992), and disease severity following the modified Cobb scale (Peterson *et al.*, 1948). Infection responses R and RMR were considered as indicative of resistance, and infection responses MR, MRMS, and MS with 30% or less stem rust severity were considered intermediate.

Seedling evaluation. The entire *T. turgidum* ssp. collection of the NSGC was evaluated for reaction to three Pgt races with broad virulence and different geographic origin: TTKSK (Kenya), TRTTF (Yemen), and TTTTF (United States). Accessions exhibiting resistance to race TTKSK were further characterized against race JRCQC (Ethiopia), a race with virulence combination to *Sr9e* and *Sr13* that are important for stem rust resistance in durum, and to six representative US races (TMPKC, RKQQC, RCRSC, QTHJC, QCCLC, and MCCFC). All the *Aegilops* accessions were evaluated only against races TTKSK, TRTTF and TTTTF. The race designation is based on the letter code nomenclature system (Roelfs and Martens, 1988; Roelfs *et al.*, 1993), modified to further delineate races in the TTKS group (Jin *et al.*, 2008). Information about the stem rust isolates used in the disease phenotyping tests is summarized in Table 1. Five seedlings per accession were inoculated on the fully expanded primary leaves 8 to 9 days after planting. Experimental procedures in inoculation and disease assessment were done as described by Jin *et al.* (2007). Wheat cultivar McNair 701 (Cltr 15288) was used as the susceptible control. All the assessments were done with one replicate and were repeated once.

3. Inheritance studies

To determine the genetic control of resistance to wheat stem rust at the seedling stage, crosses between resistant and susceptible accessions were evaluated. F_1 plants were evaluated for the response to races TTKSK to assess gene action. F_2 and $F_{2:3}$ progenies were evaluated against race TTKSK to determine the inheritance of resistance based on phenotypic ratios. Twenty plants from each $F_{2:3}$ family were tested. According to Hanson (1958), this $F_{2:3}$ family size has a 99% probability of distinguishing between segregating and non-segregating families for monogenic inheritance.

Table 1. Isolate designation, origin, and virulence phenotype of *P. graminis* f. sp. *tritici* races used to evaluate resistance in *T. turgidum* ssp and *Aegilops* spp.

Race[1]	Isolate	Origin	Virulence / avirulence formula
TTKSK	04KEN156/04	Kenya	*Sr5 6 7b 8a 9a 9b 9d 9e 9g 10 11 17 21 30 31 38 McN / Sr24 36 Tmp*
TRTTF	06YEM34-1	Yemen	*Sr5 6 7b 9a 9b 9d 9e 9g 10 11 17 21 30 36 38 McN Tmp / Sr8a 24 31*
JRCQC	09ETH08-3	Ethiopia	*Sr6 9e 9g 10 11 17 21 McN Tmp / Sr5 7b 8a 9a 9b 9d 24 30 31 36 38*
TTTTF	02MN84A-1-2	USA	*Sr5 6 7b 8a 9a 9b 9d 9e 9g 10 11 17 21 30 36 38 McN Tmp / Sr24 31*
TPMKC	74MN1409	USA	*Sr5 7b 8a 9d 9e 9g 10 11 17 21 36 McN Tmp / Sr6 9a 9b 24 30 31 38*
RKQQC	99KS76A-1	USA	*Sr5 6 7b 8a 9a 9b 9d 9g 21 36 McN / 9e 10 11 17 24 30 31 38 Tmp*
RCRSC	77ND82A	USA	*Sr5 7b 9a 9b 9d 9g 10 17 21 36 McN / 6 8a 9e 11 24 30 31 38 Tmp*
QTHJC	75ND717C	USA	*Sr5 6 8a 9b 9d 9g 10 11 17 21 McN / 7b 9a 9e 24 30 31 36 38 Tmp*
QFCSC	06ND76C	USA	*Sr 5 8a 9a 9d 9g 10 17 21 McN / 6 7b 9b 9e 11 24 20 31 36 38 Tmp*
MCCFC	59KS19	USA	*Sr5 7b 9g 10 17 McN Tmp / 6 8a 9a 9b 9d 9e 11 21 24 30 31 36 38*

[1]Race designation based on the letter code nomenclature system (Roelfs and Martens, 1988; Roelfs et al., 1993), modified to further delineate races in the TTKS group (Yin et al., 2008).

III – Results

1. Resistance in durum and emmer wheat

Resistance to wheat stem rust at the adult stage was observed in durum in the Debre Zeit nursery, as 914 (26.1%) entries exhibited a resistant to moderately resistant response (Table 2). These entries were further evaluated in Debre Zeit and St. Paul nurseries in two additional growing seasons. Two hundred eighty (8.0%) entries exhibited a resistant to moderately resistant response in all the field evaluations (Table 2). The highest frequencies of resistance were observed in entries from Africa (Ethiopia and Egypt) and North America (Mexico and USA). From these 280 field-resistant entries, 123 exhibited resistant reactions to all the Pgt races used in the seedling evaluation (Table 2). These accessions likely possess useful resistance genes and could be used in durum improvement for stem rust resistance. Ten entries were susceptible in all seedling evaluations (Table 2). This result may indicate the presence of genes for adult plant resistance (APR) in these accessions.

A high frequency of resistance at the adult stage was observed in emmer wheat, as 164 (50.3%) accessions exhibited a resistant to moderately resistant response in the first evaluation at the Debre Zeit nursery (Table 2). However, only 39 (10.9%) accessions remain resistant to moderately resistant in all the field evaluations at Debre Zeit and St. Paul nurseries (Table 2). The highest frequencies of resistance in emmer were from Ethiopia and the Middle East. Twenty-eight of these resistant accessions in field evaluations exhibited a resistant reaction to all Pgt races at the seedling stage. Selection of resistance based on seedling tests can be effective, as resistance detected at the seedling stage remains effective at the adult stage. Only four accessions that were susceptible to races TTKSK, TRTTF, and TTTTF in seedling evaluations remained resistant to moderately resistant across the two evaluations performed at the adult stage (Table 2).

Table 2. Number and percentage of durum (*Triticum turgidum* ssp. *durum*) and emmer (*T. turgidum* ssp. *dicoccum)* entries resistant (R) to moderately resistant (MR) to wheat stem rust in field evaluations, and resistant (R) and susceptible (S) in seedling evaluations.

	Durum		Emmer	
	No.	%	No.	%
Total number of entries	3500	100.0	359	100.0
R to MR[1] in Debre Zeit nursery	330	9.4	51	14.2
R to MR in St. Paul nursery	425	12.1	85	23.7
R to MR in all field evaluations	280	8.0	39	10.8
R against all Pgt races at seedling stage[2]	123	3.5	28	7.8
S against all Pgt races at seedling stage	10	0.3	4	1.1

[1] Accessions characterized as resistant to moderately resistant with a maximum 30% stem rust severity and maximum moderately susceptible infection response.
[2] Entries evaluated against races TTKSK, TRTTF, TTTTF, JRCQC, TPMKC, RKQQC, RCRSC, QTHJC, QFCSC, and MCCFC.

2. Resistance in wild emmer, Persian, Polish, Oriental, and Pollard wheat

Seedling resistance was observed in these five *turgidum* ssp., as 250 (17.9%), 319 (25.2%), and 304 (21.5%) accessions exhibited a resistant reaction to race TTKSK, TRTTF, and TTTTF, respectively (Table 3). The highest frequency of TTKSK resistance was observed in Persian wheat (44.6%), whereas low frequencies were observed in Pollard (12.3%) and Oriental wheat (9.4%). The percentage of resistance to the three Pgt races was similarly high in wild emmer and Polish wheat. However, in Persian, Oriental, and Pollard wheat, the percentage of resistance varied markedly depending on the pathogen race (Table 3). One hundred and one (6.7%) accessions

were resistant to all the races evaluated (Table 3). The characteristic infection types (IT) of wild emmer, Polish, Oriental, and Pollard wheat resistant accessions to the three races evaluated ranged from $2^{=}$ to 2^{+} (Table 3). However, most of the Persian wheat resistant accessions exhibited intermediate types (IT ;32 and 3-2;). Thirteen wild emmer and 62 cultivated tetraploid accessions were resistant against all Pgt races evaluated (data not shown).

3. Resistance in *Aegilops* ssp.

A high frequency of resistance was observed in this group of *Aegilops* spp. as 896 (73.2%), 767 (62.7%) and 849 (69.3%) accessions exhibited low infection types to races TTKSK, TRTTF, and TTTTF, respectively (Table 4). Five hundred nine (41.6%) accessions were resistant to the three races. With the exception of *Ae. biuncialis,* all species exhibited a frequency of resistant accessions to race TTKSK over 80% (Table 2). We observed a high degree of association for resistance to the three Pgt races in *Ae. geniculata* and *Ae. neglecta*; over 75% of the accessions were resistant against races TTKSK, TRTTF, and TTTTF. However, race specificity was apparent in accessions of the remaining species. In particular, *Ae. cylindrica* had only one accession that was resistant to race TRTTF.

Table 3. Number and percentage of *T. turgidum* ssp. *carthlicum,* ssp. *polonicum,* ssp. *turanicum,* and ssp. *turgidum* accessions exhibiting resistant, susceptible, and heterogeneous[1] reaction to *P. graminis* f. sp. *tritici* races TTKSK, TRTTF, and TTTTF.

	TTKSK		TRTTF		TTTTF	
	No.	%	No.	%	No.	%
T. turgidum ssp. *dicoccoides*	152	17.2	178	25.1	123	14.4
T. turgidum ssp. *carthlicum*	33	44.6	12	16.4	19	27.1
T. turgidum ssp. *polonicum*	15	24.6	14	22.6	13	21.7
T. turgidum ssp. *turanicum*	6	9.4	1	1.6	0	0.0
T. turgidum ssp. *turgidum*	44	12.3	114	31.8	146	40.7
TOTAL	250	17.9	319	25.2	304	21.5

[1] Accessions that contained both resistant and susceptible plants.

4. Inheritance of stem rust resistance

The infection type displayed by the F_1 plants from the crosses between resistant and susceptible accessions, and the segregation ratios observed in the resulting F_2 and $F_{2:3}$ progenies indicate that resistance to race TTKSK at the seedling stage in *T. turgidum* ssp. is controlled mostly by single gene (Table 5). Two resistant genes effective against TTKSK was observe only in two wild emmer and one durum accessions. Genes with complete dominance, partial dominance, and recessive effects were observed in the selected resistant parents (Table 5).

Table 4. Number and percentage of *Aegilops* accessions exhibiting resistant reaction to *P. graminis* f. sp. *tritici* races TTKSK, TRTTF, and TTTTF at the seedling stage.

Species	Genome	Access.	TTKSK		TRTTF		TTTTF		3 races	
		No.	No.	%	No.	%	No.	$	No.	%
Ae. cylindrical	DDCC	151	133	88.1	1	0.7	102	67.5	1	0.7
Ae. geniculata	MMUU	183	145	79.2	159	86.9	156	85.2	136	74.7
Ae. triuncialis	UUCC	353	290	82.2	198	56.1	315	89.2	166	47.0
Ae. biuncialis	UUMM	262	75	28.6	179	68.3	82	31.3	34	13.0
Ae. neglecta	UUMM	202	189	93.6	183	90.6	170	84.2	158	78.2
Ae. peregrina	SSUU	73	64	87.7	47	64.4	24	32.9	14	19.2
Total		1224	896	73.2	767	62.7	849	69.3	509	41.6

IV – Discussion

The TTKS race group of *Puccinia graminis* f. sp. *tritici*, and races found in Ethiopia that are more adapted to durum (Olivera *et al.*, 2012b), pose serious challenges to durum production at a global scale. The limited number of stem rust resistance genes effective against these new emerging Pgt races requires the search of additional stem rust genes that are effective against them. Results from this study demonstrate that cultivated and wild tetraploid wheats (*T. turgidum* ssp.) are a good reservoir of genes and could be used in durum improvement for stem rust resistance. Two hundred eighty (8.0%) durum accessions exhibited a resistant to moderately resistant in all field evaluations (Debre Zeit, Ethiopia and St. Paul, MN). These frequencies of resistance are much lower to the ones reported for North American (Pozniak *et al.*, 2008), and CIMMYT and Egypt (Singh *et al.*, 2011) durum lines at the field stem rust nursery in Njoro, Kenya (TTKSK and TTKSKT inoculum). These results confirmed that Pgt races present at the Debre Zeit (Ethiopia) nursery are more adapted to durum wheat and overcome the TTKSK resistance present in many of the durum lines. The highest frequency of durum resistant accessions is from cultivars and breeding lines from USA and Mexico, and landraces and old cultivars from Ethiopia and Egypt. Ethiopian durum cultivars and landraces have been previously reported as a good source of stem rust resistance (Admassu *et al.*, 2012; Beteselassie *et al.*, 2007; Bonman *et al.*, 2007; Denbel and Badebo, 2012). Ethiopia is a center of diversity of tetraploid wheat (Harlan, 1969) and the majority of the durum varieties are landraces (Tessema *et al.*, 1993) that have been co-evolving for centuries with local pathogen populations. Efforts should be made in incorporating these effective resistance genes into modern durum cultivars. Stem rust resistance in North American durum cultivars largely relies on *Sr13* and *Sr9e* (Klindworth *et al.*, 2007). However, the occurrence of a high frequency of resistant accessions originated from North American breeding programs indicates that additional effective genes are present in these germplasms.

Table 5. Segregation of $F_{2:3}$ families of various crosses of *T. turgidum* ssp. to race TTKSK of *Puccinia graminis* f. sp. *tritici*.

	Resistant	$F_{2:3}$ families[1]			Ratio tested		Genes	
Species	line	HR	Seg.	HS	(HR:Seg:HS)	*p*	Number	Effect
Durum	PI 428549	33	63	39	1:2:1	0.567	1	D
Durum	PI 298547	53	69	16	7:8:1	0.027	2	D
Durum	PI 479959	45	102	43	1:2:1	0.585	1	D
Durum	PI 519559	25	56	17	1:2:1	0.191	1	D
Emmer	PI 101971	31	48	28	1:2:1	0.522	1	PD
Emmer	PI 217640	37	80	33	1:2:1	0.644	1	PD
Emmer	PI 298582	22	45	19	1:2:1	0.821	1	R
Emmer	PI 319869	22	38	19	1:2:1	0.843	1	R
Wild emmer	PI 466946	46	74	9	7:8:1	0.179	2	PD
Wild emmer	PI 466960	42	69	16	7:8:1	0.003	2	PD
Persian	PI 387696	20	33	17	1:2:1	0.784	1	D
Polish	PI 384339	22	40	12	1:2:1	0.203	1	PD
Poulard	PI 384339	32	83	30	1:2:1	0.213	1	R

[1]HR=homozygous resistant; Seg=segregating; HS=homozygous susceptible; D=Dominant; PD=Partially Dominant; R=Recessive.

Cultivated emmer accessions highly resistant in field and seedling evaluations have been observed in this study, and can contribute genes for stem rust resistance in durum. Most of the field resistant emmer accessions remain resistant against all Pgt races evaluated at the seedling stage. Selection of resistance based on seedling tests can be effective, as resistance detected at

the seedling stage remains effective at the adult stage. Emmer wheat has contributed race specific stem rust resistance genes; *Sr13* and *Sr14* from Khapli (Heermann and Stoa, 1956), *Sr9e* from Vernal, and *Sr9d* and *Sr17* from Yaroslav (McIntosh *et al.*, 1995). The infection types observed in the 28 emmer accessions resistant to all the Pgt races evaluated indicate that resistance genes present in these accessions were likely different from the abovementioned genes. Emmer is also the donor of *Sr2* (McFadden, 1930), the most important APR gene for stem rust resistance in wheat. A selected group of emmer and durum accessions are being investigated for the presence of *Sr2* or additional APR genes based on stem rust evaluations in seedling and adult plant stages as well as available markers (Mago *et al.*, 2011).

Wild emmer, Persian, Polish, Oriental, and Poulard wheats may provide additional diversity of resistance to race TTKSK and other Pgt races from different origins and broad virulence spectra. Resistance to stem rust in these wild and cultivated tetraploids has been previously reported (Anikster *et al.*, 2005; McVey, 1991; Nevo *et al.*, 1991). The results from our study revealed that these subspecies exhibited different frequencies of resistance to race TTKSK. The highest frequency of resistance was observed in Persian wheat (45%), followed by Polish wheat (25%). These frequencies are comparable to the one reported by Olivera *et al.* (2012a) in cultivated emmer wheat (32.2%). These frequencies of resistance in cultivated and wild tetraploid wheats are lower than that reported by McVey (1991) using North American races, suggesting that race TTKSK overcame many of the genes present in these subspecies. According to infection type patterns, wild emmer exhibited the highest level of diversity for stem rust resistance (data not shown), and a high level of race specificity as a limited number of accessions were resistant to all races evaluated.

In the six tetraploid *Aegilops* species we evaluated, a high frequency of resistance against races with broad virulence spectrum was observed in all species. High frequencies of resistance against Ug99 have been also reported in diploid *Aegilops* species (Olivera *et al.*, 2007; Rouse *et al.*, 2011), confirming that this genus, closely related to *Triticum*, can contribute with potential new genes for stem rust resistance. We have selected resistant and susceptible parents from all evaluated species to produce biparental crosses in order to study the genetics of TTKSK resistance in these species. Gene introgression from these tetraploid *Aegilops* ssp. will likely not be a straightforward process, and cytogenetic manipulation will be required.

One of our major research objectives is to develop several mapping populations for all the *T. turgidum* subspecies level to determine the inheritance of TTKSK resistance and then mapping the resistance genes identified. Our results indicate that resistance to race TTKSK at the seedling stage is mostly conferred by single genes with both dominant and recessive action (Tables 5). The simple inheritance of TTKSK resistance in these species should simplify the transfer of resistance to durum and bread wheat.

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Pyramiding of resistance genes *Sr36 and Sr2* in durum wheat background (HI 8498) through marker assisted selection for resistance to stem rust race 117- group pathotypes

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Abstract. Stem rust (*Puccinia graminis* f. sp. *tritici)* has historically been one of the major constraints in realizing stabilized durum wheat yields in central India. Pyramiding of genes into a single genotype has been one of the preferred strategies in wheat rusts management. Currently, HI 8498 (Raj 6070/Raj 911) is the most popular durum wheat cultivar in central India. However, it is susceptible to a number of Indian pathotypes of stem rust race 117-group. Hence, a planned breeding programme was taken up to develop derivatives of HI 8498 with improved resistance by pyramiding stem rust resistance genes *Sr36* and *Sr2* through marker assisted selection. These were targeted as besides being broad-spectrum resistance genes, they have had their origin in tetraploid wheats, *Sr36* in *Triticum timopheevii* and *Sr2* in *T. turgidum* var. *durum*. While, durum wheat genotype IWP 5070 was chosen as donor parental line for *Sr2* gene, based on the presence of 'pseudo-black chaff (*pbc*)', a tightly linked phenotypic marker; Australian bread wheat variety 'Songlen' was used as donor for *Sr36*, since none of the 75 durum wheat genotypes tested was found positive for this gene using tightly linked co-dominant microsatellite marker *Xstm773-2*. Moreover, Songlen is known to carry *Sr2* gene as well. The markers being used for foreground selection in the HI 8498 derivatives are '*pbc*' and CAPS marker *csSr2* for the gene *Sr2* and SSR marker *Xstm773-2* for the gene *Sr36.* The stem rust resistance in the 'HI 8498' derivatives (BC_3F_1) carrying *Sr2* and *Sr36* individually has improved significantly (terminal disease severity 0 to 5S), compared to the background cultivar HI 8498 (30S – 40S). Furthermore, a total of 770 representative SSR markers distributed throughout the wheat genome were screened for polymorphism between parental genotypes HI 8498, Songlen and IWP 5070. Of these, 165 markers showed polymorphism and are being used as effective markers in Marker Assisted Background Selection (MABS) to identify 99 % of recurrent parent genome (RPG) i.e., HI 8498 in BC_3F_1 generation of both the populations involving *Sr36* and *Sr2* genes to facilitate their pyramiding in common recipient parent background.

Keywords. Durum wheat – Stem rust resistance – MAS – Gene pyramiding.

Cumul des gènes de résistance SR36 et Sr2 chez le génotype de blé dur (HI 8498) par la sélection assistée par marqueurs pour la résistance à la rouille noire de la race du groupe pathotypes 117

Résumé. *La rouille noire (Puccinia graminisf. sp. tritici) a toujours été l'un des principaux obstacles à la réalisation de rendements stables pour le blé dur dans le centre de l'Inde. Le cumul de gènes dans un seul génotype a été l'une des stratégies privilégiées dans la gestion des rouilles du blé. Actuellement, HI 8498 (Raj 6070/Raj 911) est le cultivar de blé dur le plus populaire dans le centre de l'Inde. Cependant, il est sensible à un certain nombre de pathotypes indiens de la rouille noire de la race du groupe 117. Ainsi, un programme de sélection planifié a été proposé pour développer des dérivés de HI 8498 avec une meilleure résistance à la rouille noire par le cumul des gènes de résistance SR36 et Sr2 à travers la sélection assistée par marqueurs. Ces gènes ont été ciblés comme étant des gènes de résistance à large spectre, ils tirent leurs origines de blés tétraploïdes, T. timopheevii pour SR36 et T. turgidum var. durum pour Sr2. Alors que le génotype de blé dur IWP 5070 a été choisi comme lignée parentale donneuse pour le gène SR2, basée sur la présence de « pseudo-black chaff (pbc) », un marqueur phénotypique étroitement lié; la variété australienne de blé tendre Songlen a été utilisé comme donneur pour SR36, puisque aucun des 75 génotypes de blé dur testées a été trouvé positif pour ce gène en utilisant le marqueur microsatellite co-dominant Xstm773-2 étroitement lié. En outre, Songlen est connu pour porter aussi le gène Sr2. Les marqueurs utilisés pour la sélection de premier plan dans les dérivés de HI 8498 sont 'pbc' et le marqueur CAPS csSr2 pour le gène*

Sr2 et le marqueur SSR Xstm773-2 pour le gène SR36. La résistance à la rouille noire chez les dérivés de HI 8498 (BC3F1) portant Sr2 et SR36 individuellement a amélioré de manière significative (sévérité terminale de la maladie de 0 à 5S), par rapport au cultivar HI 8498 (30S - 40S). En outre, un total de 770 marqueurs SSR représentatifs répartis sur tout le génome du blé ont été analysés pour le polymorphisme entre les génotypes parentaux HI 8498, Songlen et IWP 5070. Parmi ceux-ci, 165 marqueurs ont montré un polymorphisme et sont utilisés comme marqueurs efficaces pour la sélection du fond génétique assistée par marqueurs (MABS) pour identifier les 99% du génome du parent récurrent (RPG), à savoir, HI 8498 dans la génération BC3F1 des deux populations impliquant les gènes SR36 et Sr2 pour faciliter leur cumul dans le fond génétique du parent destinataire commun.

Mots-clés. *Blé dur – Résistance à la rouille noir – MAS – Cumul de gènes.*

I – Introduction

Durum wheat (*Triticum turgidum var. durum*) is the second most important wheat species globally as well as nationally, after bread wheat with a share of around 5% in the total wheat production of >90 million tons in India. Durum wheat cultivation offers many advantages like saving irrigation water due to its high water-use efficiency, field tolerance to loose smut and Karnal bunt diseases, employment generation through durum based fast food industry and provide better nutrition as it is rich in protein, β-carotene and essential micronutrients like iron and zinc. In fact, durum wheat was the predominant wheat species grown in central India, particularly in the Malwa plateau in Madhya Pradesh, parts of Gujarat, southern Rajasthan and Bundelkhand region of Uttar Pradesh. However, area under durum wheat cultivation continuously declined due to limited yield potential and susceptibility to rust diseases of old varieties, and by the seventies, durum wheat got almost out of cultivation. Hence, durum improvement programme was intensified at IARI – Regional Station, Indore.

A large number of rust resistance donors, particularly among the exotic collections, were identified and utilized in crop improvement. Three improved durum wheat varieties HI 8381 (Malav-shri), HI 8498 (Malavshakti) and HD 4672 (Malav Ratna) were released by the station, which immensely contributed to the revival or resurrection of durum wheat in Central India. While Malavshri and Malavshakti were released for irrigated timely sown conditions, and Malav Ratna was for rainfed and limited irrigation. HI 8498 (Malavshakti) is currently the most popular durum wheat cultivar in Central India under irrigated, timely sown (November sowing) conditions. Combining high yield with earliness, disease resistance, and excellent grain quality, it has proved to be a truly 'landmark' variety in the history of wheat crop improvement in central India. It showed resistance to stem rust pathotypes *40A* and *40-1* which exhibit high degree of virulence to bread wheat varieties. However, it showed susceptibility to several pathotypes of stem rust race *117* group like *117-6* (*37G19*), *117A* (*38G2*), *117-1* (*166G2*), and *117A-1* (*38G18*).

Breeding for durable resistance by pyramiding multiple resistance genes, both major and minor ones, is an effective strategy in managing plant diseases (Gupta *et al,* 1999, Singh *et al,* 2001). Therefore, a planned breeding programme was initiated to pyramid the genes *Sr2* and *Sr36* into HI 8498 background for improving resistance to the aforesaid pathotypes of stem rust race *117* group. Wheat stem rust resistance gene ***Sr36*** (syn. *SrTt-1*), derived from *Triticum timopheevii* (Allard and Shands, 1954), shows effectiveness to many stem rust pathotypes including *117-6* which is highly virulent to durum wheat (Mishra *et al* 2009). The gene ***Sr2*** was originally transferred from Yaroslav emmer wheat into hexaploid wheat (Mc-Fadden, 1930), which has been utilized in breeding for around 60 years as a source of durable and broad-spectrum adult-plant resistance. *Sr2* confers partial resistance only in the homozygous state because of its recessive inheritance due to which traditional breeding with *Sr2* is difficult to carry out. *Sr2* is closely linked to pseudo-black chaff ('pbc'), controlled by partially dominant gene which produces the characteristic stem and head melanism in wheat, but its levels of expression vary with genetic backgrounds and environments (Mc-Fadden, 1939, Kota *et al.,* 2006).

Both *Sr2* and *Sr36* are widely effective against the Indian stem rust populations including the race *117*-group pathotypes. Hence, transferring these genes into HI 8498 background could broaden and diversify its resistance base. However, pyramiding genes in a single line through classical breeding methods can be time consuming or even impossible, especially when more than one gene confers resistance against known races of *P. graminis* f.sp. *tritici*; as it becomes difficult to identify genotypes carrying combinations of more than one gene (Tsilo *et al.,* 2008). Hence, phenotypic and molecular marker assisted selection was resorted to for pyramiding the 'target' genes in common background.

II – Material and Methods

1. Material

Recipient parent HI 8498, a high yielding durum wheat variety with good adaptability was released for growing under timely sown conditions. Australian bread wheat cultivar 'Songlen' which was documented to carry *Sr36,* and IWP 5070, a durum wheat genetic stock carrying *Sr2* were used as donors for these resistance genes. Stem rust pathotype *117-6* was used for tracking resistance derived from the donors. Crosses between Songlen/HI 8498 and HI 8498/IWP 5070 were performed during *rabi* 2009-10. During the same time, parental polymorphism survey was done between recipient parent HI 8498 and the two donor parents Songlen and IWP5070 using 730 markers covering all the chromosomes (Sourdille *et al., 2004*). The F_1 seed obtained were sown in *rabi* 2010-11 at IARI –RS farm, Indore to produce BC_1F_1. All the plants in BC_1F_1 were screened for the presence of resistance genes *Sr36* and *Sr2* with the help of *Xstm773-2* and CAPS marker *csSr2* along with pseudo-black-chaff trait, respectively. The positive plants based on molecular analysis results and 'resistance phenotype' was utilized to produce BC_2F_1 populations. Markers which were polymorphic between HI 8498 and Songlen, and HI 8498 and IWP 5070 were used for background selection in backcross (BC_2F_1 and BC_3F_1) populations generated from the respective crosses to identify individuals with maximum genome of HI 8498. The foreground positive plants of both the populations with maximum recovery of recurrent parent HI 8498 were utilized for pyramiding of genes into the background of HI 8498. At all stages, the pathological screening in the field was done by syringe inoculating each plant with freshly collected uredospores of pathotype *117-6*.

2. TPCR analysis

For molecular analysis, DNA was extracted directly from two leaf stage of young plants with CTAB method to get pure form of genomic DNA for PCR analysis. Two pairs of primers were used for PCR analysis. Primers *Xstm773-2*F 5' AATCGTCCACATTGGCTTCT 3' and *Xstm773-2*R 5'-CGCAACAAAATCATGCACTA 3'were designed based on the published sequence (Tsilo *et al., 2008*), and the amplified fragment co-segregating with the *Sr36* gene was used for foreground selection of *Sr36* gene. The PCR conditions for *Xstm773-2* were *:* denaturing step: 95°C, 4 min, amplification step (40 cycles): 94°C, 30 sec; 60°C, 30 sec; 72°C, 30 sec, extension step: 72°C, 7 min (PCR amplified products were resolved on 3.5% metaphor gel for SSR marker). Similarly, Primers *csSr2*F 5'CAAGGGTTGCTAGGATTGGAAAAC 3' and *csSr2* R 5'AGATAACTCTTAT GATCTTACATTTTTCTG 3' were designed according to sequence information (McNeil *et al.,* 2008), and were used for detection of *Sr2* gene. The amplification products derived from the former primer pair are co-dominant, whereas those from the latter pair are dominant and require further digestion of amplified PCR product through *Bsp*HI restriction enzyme. The PCR conditions for *csSr-2* marker were : denaturing step : 95°C, 2 min, amplification step (30 cycles): 94°C, 30 sec; 60°C, 40 sec; 72°C, 50 sec, extension step: 72°C, 5 min. For CAPS analysis after completion of PCR, an additional 5 µl of mix consisting of 2.5 ml of 10x NEB buffer 4 and 0.5 µl of *Bsp*HI (10 U/ µl; NEB) was added and the tubes were incubated at 37°C for 1 h. After completion of restriction digestion the product was separated on a 2.5 % (w/v) agarose gel (R. Mago *et al.,* 2011).

III – Results and Discussion

Among the parents, amplification was noticed with ***Xstm773-2*** marker only with different amplicon sizes i.e., 155 bp – Resistant and 190 bp – Susceptible utilizing *Sr36*-Near isogenic line (NIL) as positive control. It was observed that *Sr36*-NIL and Songlen had the band 155 bp i.e., resistant (presence of *Sr36*) (Fig. 1), while other varieties were having the band 190 bp i.e., susceptible (absence of *Sr36*).

With the help of CAPS marker *csSr-2* (Fig. 2) with *Bsp*HI restriction enzyme, we had validated the presence of *Sr-2* gene linked to "pbc" phenotype in IWP 5070 viz., the presence of the band 172 bp, 112 bp and 53 bp i.e., resistant (presence of *Sr2 gene*) along with the pseudo-black chaff *(pbc*) phenotype in the field. Similar band pattern with CAPS marker *csSr-2* with *Bsp*HI restriction enzyme digestion was observed in IWP 5070 and Songlen as showed in Hope by R. Mago *et al.,* 2011, whereas, in HI8498, null type allele (lack of amplification) was observed as in Chinese Spring. Therefore, this CAPS marker behaved like a dominant marker.

Figure 1. Band pattern of plants with *Sr36* with *Xstm773-2* marker.

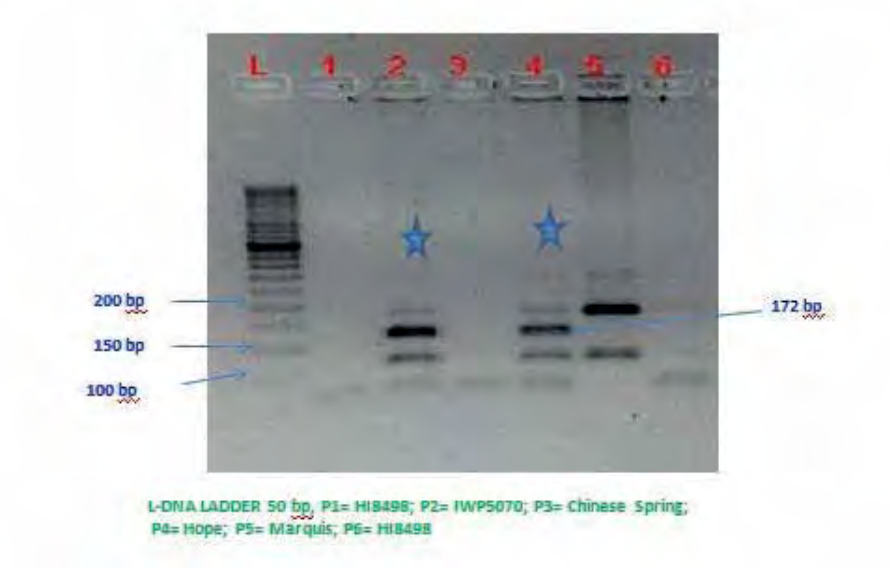


Figure 2. Band pattern of plants with *Sr2* with CAPS marker *csSr-2.*

1. Screening of durum wheat germplasm for presence of *Sr36* gene

Presence of the gene *Sr36* is not documented in any of the known durum genotypes. Hence, 75 durum wheat genotypes representing a cross section of Indian durum wheat germplasm were analyzed using closely linked molecular marker *Xstm773-2,* but none of the durum genotypes tested was found to carry the gene (Singh *et al., 2013*).

2. Parental polymorphism of parents

Marker assisted background selection is used for recurrent parent genome selection, which requires more number of polymorphic markers equally distributed in the genome of parents. So for this purpose, we have screened 730 SSR primers distributed in all the chromosomes of wheat, out of which 177 SSR primers were found to be polymorphic, and these will be used for background selection of parent HI 8498 (Fig. 3 and 4). 151 SSR primers were found polymorphic between Songlen and HI 8498, while, 77 primers between IWP 5070 and HI 8498. Due to differences at species and ploidy level, a large number of polymorphism was observed between Songlen and HI 8498, compared to IWP 5070 and HI 8498.

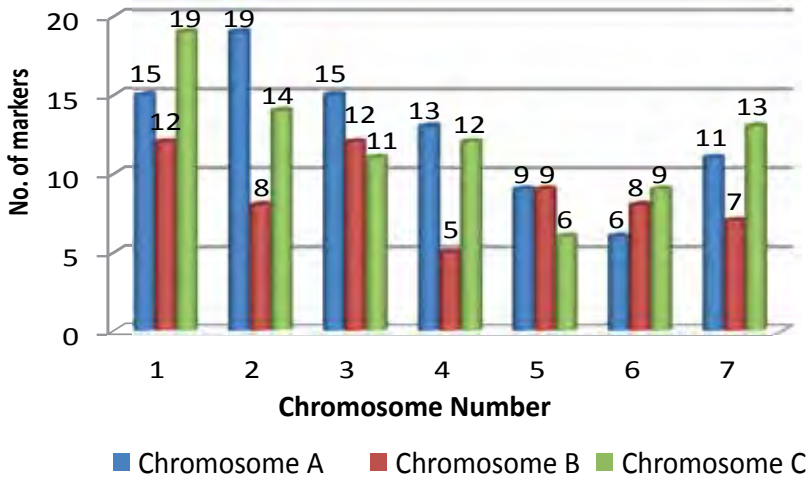
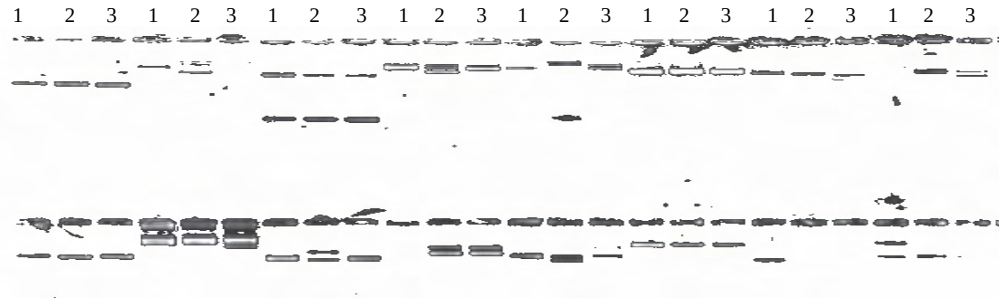


Figure 3. Chromosmal distribution of polymorphic SSR primers.

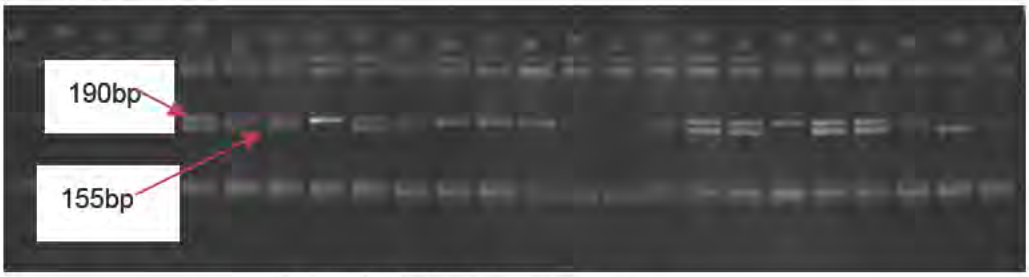


1= HI8498; 2 = Songlen; 3 = IWP5070 for 16 different markers

Figure 4. Parental polymorphism with different SSR markers.

3. Screening of backcross population for foreground selection of *Sr36* gene (*Xstm773-2* marker)

In BC_1 population, out of 261 plants, 90 plants showed the presence of the band 155 bp i.e., resistant (presence of *Sr36*), whereas, other plants had band 190 bp *i.e*, susceptible (absence of *Sr36*). Plants positive for *Sr36* showed good stem rust resistance in the field along with good expression and were used to develop the BC_2 population by crossing with the recurrent parent HI 8498. In BC_2 population, out of 267 plants, 48.7 *per cent* of the plants (130 plants) showed the presence of the band 155 bp, with good stem rust resistance; whereas, other plants (51.3%) had band 190 bp i.e., susceptible (absence of *Sr36*). In BC_3 population, out of 92 plants, 27 *per cent* of the plants (24 plants) showed the presence of the band 155 bp i.e., resistant (presence of *Sr36*) (Fig 5) with good stem rust resistance and durum wheat 'HI 8498' plant type; whereas, the remaining plants (73%) had band 190 bp i.e., susceptible (absence of *Sr36*). The stem rust resistance in the 'HI 8498' derivatives (backcross populations) carrying *Sr36* individually has improved significantly (terminal disease severity 0 to 5S), compared to the background cultivar HI 8498 (30S – 40S).



Marker-Xstm773-2; BC_3 population

Figure 5. Band pattern of plants in the BC_3 population for *Sr36*.

4. Recurrent parent genome recovery % (RPG) among backcross populations through Marker Assisted Background Selection

Sr36 **:** Based on the information gained through parental polymorphism, polymorphic markers were utilized for background selection to select plants with high recurrent parent genome recovery (RPG) i.e., band pattern for markers CFA2158 and *Xgdm*219 is shown as example in Fig 6 and RPG recovery of BC_3F_1 population through GGT2 program was shown in Fig 7. In BC_3F_1, RPG ranged from 87.5 to 100 percent in the foreground positive plants, out of which 15 plants showed > 95% with homozygous bands as recipient parent, HI 8498 (Table 1). The rust reaction of these positive plants are in the range of 0 to 5S. Of these 15 plants, 3 plants showed 100 % RPG and similar plant type of HI 8498, which were selected for pyramiding of the gene *Sr36*.

Figure 6. Band pattern of plants in the BC_3 population for *Sr2*.

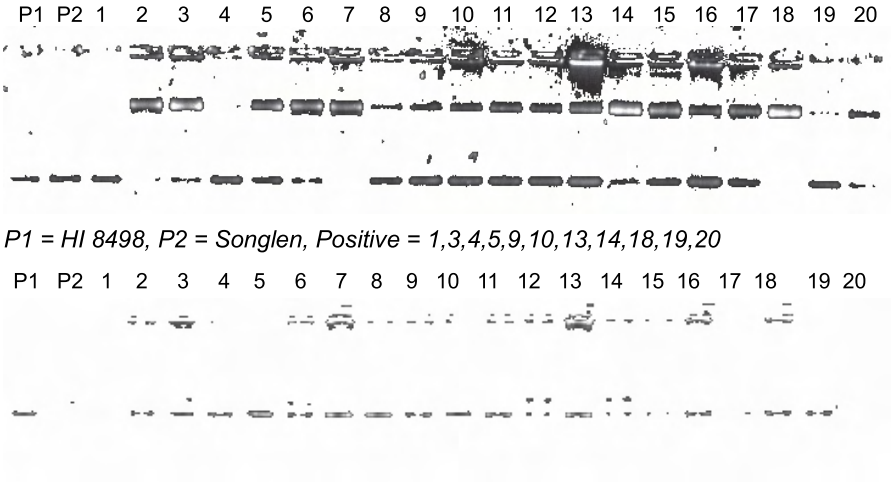


Figure 7. Background selection (MABS) of foreground positive plants for *Sr36.*

Table 1. RPG of foreground positive plants of HI 8498 along with *Sr36.*

S. No.	Plant ID No.	Percentage recovery
1	WF1	95.8
2	WF3	95.8
3	WF4	95.8
4	WF6	95.8
5	WF16	97.5
6	WF17	100.0
7	WF18	100.0
8	WF33	97.5
9	WF37	97.5
10	WF42	100.0
11	WF46	97.8
12	WF50	98.9
13	WF58	95.8
14	WF60	97.5
15	WF61	95.8

Sr2: Based on the information gained through parental polymorphism, polymorphic markers were utilized for background selection to select plants with high recurrent parent genome recovery (RPG) *i.e*, band pattern for markers CFD238 and CFD54 is shown as example in Fig 8 and RPG recovery in BC_3F_1 population through GGT2 program was shown in Fig 9. In BC_3F_1, RPG ranged from 82 to 100 percent in the foreground positive plants, out of which 14 plants showed > 95% with homozygous bands as recipient parent, HI 8498 (Table 2). The rust reactions of these positive plants are in the range of 5R to 10S. Of these 14 plants, 4 plants showed 100% RPG and similar plant type of HI 8498, which were selected for pyramiding of the gene *Sr2*.

Figure 8. Graphical representation of RPG of HI 8498 along with *Sr36.*

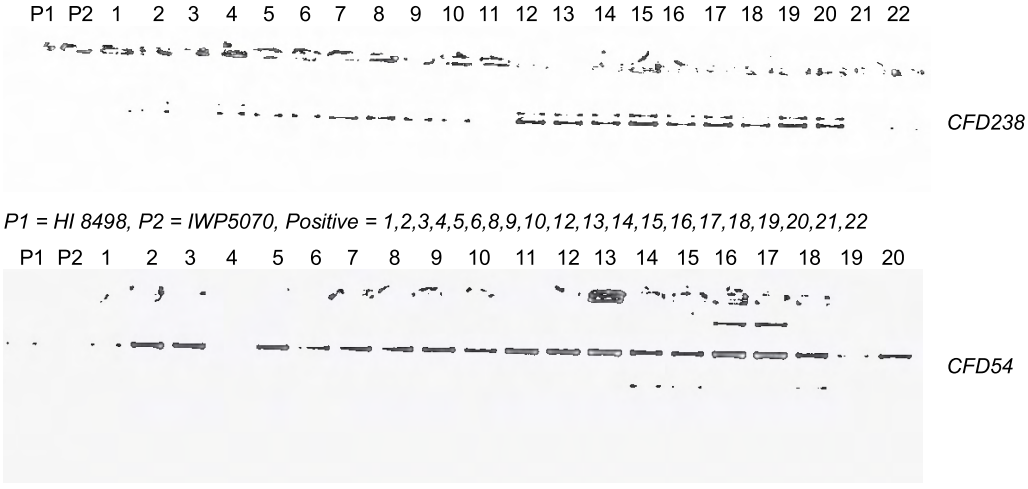


Figure 9. Background selection (MABS) of foreground positive plants for *Sr2.*

Table 2. RPG of foreground positive plants of HI 8498 along with *Sr2*.

S. No.	Plant ID No.	Percentage recovery
1	WE 29	95.4
2	WE 32	100.0
3	WE 42	95.4
4	WE53	100.0
5	WE76	95.4
6	WE 117	97.2
7	WE127	100.0
8	WE 130	95.4
9	WE131	97.2
10	WE 143	97.2
11	WE 145	97.2
12	WE 151	95.4
13	WE 161	95.4
14	WE 163	100.0

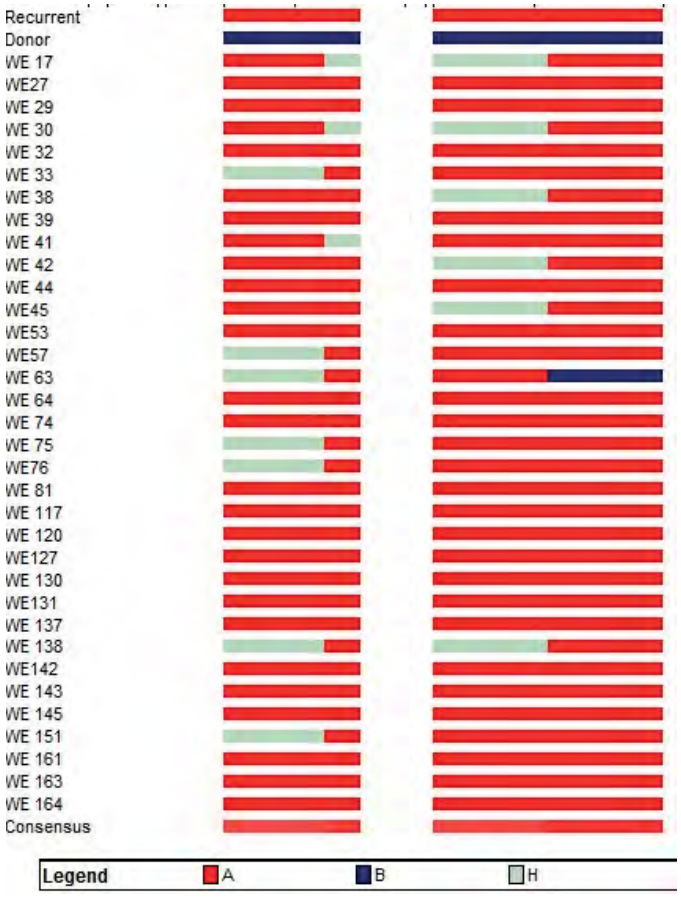


Figure 10. Graphical representation of RPG of HI 8498 along with *Sr2*.

5. Pyramiding of *Sr36* and *Sr2* in HI 8498 background

Foreground positive plants with maximum recurrent parent genome were selected in BC_3F_1 population derived from Songlen and IWP 5070 with HI 8498 background. Crosses were made among high RPG plants with resistance genes *Sr36* and *Sr2*, looking mostly like HI 8498 in field with good resistance to stem rust race *117-6*. DNA markers *Xstm773-2* and *csSr2* were utilized for individual selection of both the genes in a single plant which really helped us to discriminate the plants for the presence of both the genes in a single cultivar, which would have been impossible in the phenotypic screening because *Sr36*-resistance could mask the *Sr2* resistance which is expressed in adult plant stage. Positive homozygous plants for both the genes will be selected for multiplication and further utilization. Efforts are in progress to select homozygous plants in F_2 of the pyramided population with resistance genes *Sr36* and *Sr2*, looking mostly like HI 8498 in field with good resistance to stem rust race *117-6* using both PCR analysis and phenotyping. It was observed that the positive plants selected through MAS functioned normally as recipient parent HI 8498. Positive homozygous plants of this population involving *Sr36* and *Sr2* genes will help to facilitate their pyramiding in common recipient parent HI 8498 background, its multiplication and further utilization.

Acknowledgments

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Breeding durum wheat for crown rot tolerance

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Abstract. Our approach involves a multi-pronged strategy to identify, characterise and use the available variation for crown rot (CR) tolerance in material from pre-breeding projects, advanced breeding lines, germplasm lines and released varieties, to incorporate it into commercially suitable backgrounds. Our preliminary results have shown the presence of useful genetic variation within durum germplasm for resistance (reduced expression of disease symptoms) and tolerance to CR (reduced loss of yield potential in the presence of crown rot), both of which are part of our strategy. Compared with resistance to CR, we consider tolerance to CR a more worthwhile trait to target because it represents all the processes in the plants leading to better yield performance under high disease pressure. Our approach for developing CR tolerance includes establishment of trial sites in CR prone areas in western NSW and also evaluation of advanced lines for tolerance to CR in inoculated yield trials. A permanent CR disease nursery to screen material for resistance to CR is being established. Molecular markers will be used to provide additional data. Whilst progress is expected to be gradual, these strategies should generate high quality data to conduct effective selection for CR tolerance.

Keywords. Durum wheat – Crown rot – Resistance – Tolerance – Breeding.

Amélioration du blé dur pour la tolérance à la pourriture du collet

Résumé. *Dans le présent travail, nous allons illustrer une approche multiforme utilisée en vue d'identifier, caractériser et exploiter la variabilité disponible pour la tolérance à la pourriture du collet dans le matériel issu des projets de pré-sélection, des lignées de sélection avancées, des lignées de matériel génétique et des variétés homologuées, pour l'incorporer dans des génotypes adaptés aux besoins du marché. Nos résultats préliminaires ont indiqué la présence d'une variabilité génétique dans le matériel de blé dur utile pour la résistance (réduction de l'expression des symptômes de la maladie) et la tolérance à la pourriture du collet (moindre réduction du potentiel de rendement en présence de la pourriture du collet), qui sont toutes les deux intégrées dans notre stratégie. Par rapport à la résistance à la pourriture du collet, la tolérance est un caractère plus intéressant à cibler parce qu'elle renferme l'ensemble des processus déterminant une meilleure performance des plantes, en termes de rendement, dans des conditions de pression élevée de la maladie. Notre approche pour le développement de la tolérance comprend l'établissement de sites d'essai dans les zones exposées au risque de pourriture dans l'ouest de la Nouvelle-Galles du Sud (NSW) et aussi l'évaluation des lignées avancées pour la tolérance dans des essais de rendement sous l'effet de l'inoculation. Actuellement, nous travaillons à la mise en place d'une pépinière où sera maintenue en permanence la pourriture du collet pour sélectionner le matériel résistant. Les marqueurs moléculaires seront utilisés pour fournir des données supplémentaires. Bien que nous prévoyions des résultats progressifs, ces stratégies devraient générer des données de haute qualité pour procéder à une sélection efficace pour la tolérance à la pourriture du collet.*

Mots-clés. *Blé dur – Pourriture du collet – Résistance – Tolérance – Sélection.*

I – Introduction

Crown rot (CR) of Wheat is caused by the fungal pathogen *Fusarium pseudograminearum*. It is the most important disease of durum wheat in Northern NSW and Queensland and is a significant factor limiting expansion of the durum industry. With the wide adoption of minimum tillage based production systems, CR disease pressure is expected to increase in future seasons. Also, the expected increase in frequency of droughts due to climate change will add to the increasing risk

of CR because of the observed link between drought conditions and higherexpression of CR disease. It is therefore important to develop genetic resistance and tolerance to the disease. The best source of resistance to date has been a bread wheat line, 2-49 (Gala/Gluyas Early) but it is agronomically poor. Sunco, an Australian commercial bread wheat cultivar, has useful adult plant resistance to CR but it is susceptible in seedling stages (Martin *et al*., 2013). Variation for CR resistance/tolerance within durum germplasm has not been studied in detail to date. This study describes our initial examination of genetic variation for CR resistance and tolerance in durum lines, and, development of a breeding approach based on these results.

II – Material and methods

A set of durum lines containing released varieties and advanced breeding lines, including bread wheat check varieties, was evaluated for CR tolerance in a yield trial at Tamworth Agricultural Institute in the 2010 season containing inoculated (2g CR inoculum/m row) and uninoculated treatments as described by Dodman and Wildermuth (1987). The trial was designed as a randomised complete block with 3 replicates on a red-brown vertosol with light-medium clay content, pH 6.4 (1:5 CaCl2). 50kg/ha of N as urea and 50kg/ha of Granulock 12Z were applied at sowing.

Disease severity was visually assessed at harvest on 25 random plants from each plot as the extent of basal browning. Each plant was assessed for total number of tillers (a), number of tillers with any browning of the first internode (b), and the height of browning on a 0-3 scale designated as “c” (0 = no browning, 0.5 = partial browning of the first internode, 1 = complete browning of the first internode, 1.5 = complete browning of the first internode plus partial browning of the 2nd internode etc.). CR severity was calculated using this data as ((b/a X 100)/3)X c as described by Martin *et al*. (2013). The above set of lines was also put through a glasshouse CR pot test (Raju and Turner, 2008) at The University of Sydney, Cobbitty, to obtain additional CR resistance data (based on a 0-4 scale incorporating basal browning and whiteheads/deadheads). This test involved placing a 5mm plug of the fungal mycelium from a 5 day old culture near the base of the seedlings and covering with unprocessed wheat bran. Fungus growth on wheat bran around the seedlings was visible in 48-72h after inoculation and crown rot symptoms (leaf and stem browning) were seen within 7 days after inoculation. The plants were allowed to grow normally until maturity. At maturity disease severity was assessed on a 1-4 scale (0 = No lesions, 1 = First internode partially lesioned, 2 = First internode full lesioned and second internode fully or partially lesioned, 3 = More than two internodes lesioned, 4 = Dead head (white head or no head) due to crown rot.

III – Results and discussion

All entries, including 2-49 (resistant bread wheat check), showed reduced yield in inoculated plots relative to the untreated checks (Figure 1) although 2010 season was not conducive to CR disease development. As expected, 2-49 was the lowest yielding line in the trial in both inoculated and un-inoculated categories. Yield loss due to CR was highest in EGA Bellaroi and lowest in BO4-17, a CIMMYT durum line. Lines 241000, 241046 (both NSWDPI) and Hyperno (released SA variety) also showed relatively low levels of yield loss from CR infection. Caparoi showed significantly better tolerance to CR relative to EGA Bellaroi and this is consistent with the observation that Caparoi performs well under both dry and wet conditions. Five lines including 241046 (NSWDPI), BO4-17 (CIMMYT) and three from University of Adelaide node of DBA (WID052, Yawa and WID091) produced high yields in inoculated treatments (Table 1).

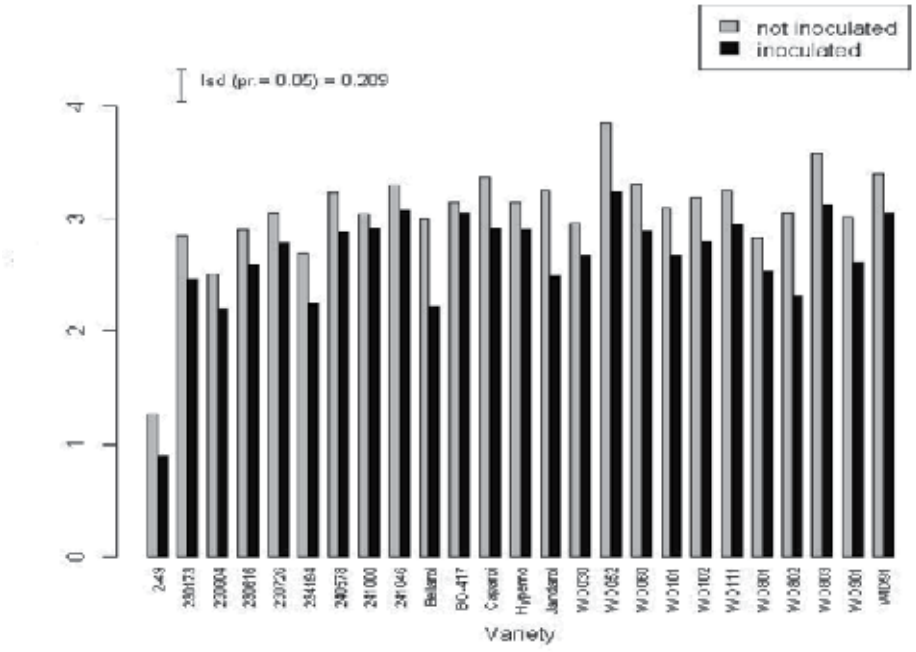

Figure 1. Performance (Yield /ha) of durum lines in presence of CR relative to un-inoculated checks in 2010 in Tamworth.

The apparent lack of agreement between yield loss and disease severity assessments, for example, BO4-17 showing the lowest loss of yield but high levels of disease severity could be explained on the basis that the measures based on the extent of disease symptoms do not fully represent the impact of the disease on yield performance of the genotype. For this reason tolerance to CR would be a better trait to target although it is difficult to screen genotypes in large numbers for this trait.

Table 1. CR tolerance and resistance data for selected durum lines.

Lines	Yield (added CR kg/ha)	Yield loss (%)	Field CR severity (%)	Pot test CR severity
WID052	3250	15.5	39.6	3.8
Yawa	3123	12.9	39.2	3.6
BO4-17	3059	2.8	44.7	3.2
WID91	3050	10.1	40.8	3.2
241046	2998	9.0	37.7	4.0
Caparoi	2914	13.6	42.7	3.7
EGA Bellaroi	2231	25.4	45.5	3.7
LSD (0.05)	289		4.7	0.7

Correlation between CR severity data from the two tests was low (0.26), most likely as a result of lower disease pressure in the field trial due to lack of moisture stress. However, both tests detected significant variation among durum lines. On the basis of these results we conclude there is useful genetic variation for CR tolerance in durum wheat which can be characterised using resistance and tolerance criteria.

IV – Breeding approach

We are working to characterise the material generated by GRDC-funded durum CR pre-breeding project (NSWDPI/USQ) for CR resistance and agronomic traits. Best selections from this material and other durum germplasm lines that have shown CR tolerance in our work will be crossed to advanced durum lines to incorporate the trait into high yielding and high grain quality backgrounds. In early stages (up to S1), evaluation would be based on performance in disease nurseries, marker information and/or glasshouse tests. For lines in intermediate stages (S2/S3), evaluation will be in CR prone trial sites and disease nursery. Advanced (S4) lines will be assessed in inoculated trials to provide CR tolerance data.

Acknowledgments

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Diverse sources of resistance to Indian pathotypes of stem rust and leaf rust in durum wheat

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Abstract. Stem rust (*Puccinia graminis* f. sp. *tritici*) and leaf rust (*P. triticina*) are among the most serious biotic stresses for durum wheat cultivation in India. Constant broadening of resistance base is necessary for maintaining effective and longer lasting rust resistance in view of the continued evolution of rust pathogens. Hence, Mendelian inheritance and extent of diversity for resistance were studied among five rust resistant durum wheat genetic stocks of diverse origin viz., 'B 662', 'ED 2398-A', 'HG 110', 'IWP 5019' and 'Line 1172'. Of these, B 662, IWP 5019, and Line 1172 were derived from inter-specific hybridization involving crosses of different durum wheat genotypes with *Triticum aestivum*, *T. turgidum* var. *dicoccum*, and *T. militinae* lines, respectively. HG 110 was developed from an intra-specific (durum wheat / durum wheat) cross, while ED 2398-A was an Ethiopian durum wheat land race. The test pathotypes included *40A* (*62G29*) and *117-6* (*37G19*) of stem rust, the former being the most predominant in nature and the latter being the most virulent one on Indian durum wheat; and the prevalent and durum-specific leaf rust pathotypes, *12-2* (*1R5*) and *104-2* (*21R55*). While seedling tests were conducted with the pathotypes *40A*, *12-2* and *104-2*, adult-plant studies were made with the pathotype *117-6*. Analysis of the F_2 populations and F_3 families derived from the crosses of the aforesaid resistant stocks with three suscept ble durum wheat varieties- 'Motia', 'Malvi Local' and 'Sarangpur Local' showed that resistance was governed by one or two genes. In all, four genes for resistance to the pathotype *40A*, and eight genes each for resistance to *117-6* and *12-2* were identified among the five resistant stocks studied; while three genes for resistance to *104-2* were identified among B 662, ED 2398 A and IWP 5019, based on the allelic tests. Though the identity of these genes is not known, the ones for stem rust resistance are different from *Sr2*, *Sr7b*, *Sr9e* and *Sr11*, and those for leaf rust resistance are different from *Lr23*, the documented stem rust and leaf rust resistance genes commonly postulated among Indian durum wheat genotypes. Thus, the reported genetic stocks should contr bute to enrich the gene pool in durum wheat improvement as diverse sources of resistance to stem rust and leaf rust.

Keywords. Durum wheat – Stem rust resistance – Leaf rust resistance – Genetic diversity – Indian rust pathotypes.

Diverses sources de résistance aux pathotypes indiens de la rouille noire et de la rouille brune du blé dur

Résumé. *La rouille noire (Puccinia graminis f. sp. Tritici) et la rouille brune (P. triticina) sont parmi les plus graves contraintes biotiques pour la culture du blé dur en Inde. Un élargissement constant de la base de la résistance est nécessaire pour assurer une résistance efficace et plus durable aux rouilles vu l'évolution continue de ses agents. Par conséquent, l'héritage mendélien et l'étendue de la diversité de la résistance ont été étudiés parmi cinq stocks génétiques de blé dur de diverse origine résistant aux rouilles, à savoir 'B 662', 'ED 2398-A', 'HG 110', 'IWP 5019' et 'Lignée 1172'. Parmi ceux-ci, B 662, IWP 5019, et Lignée 1172 ont été obtenus par l'hybridation inter-spécifique impliquant des croisements de différents génotypes de blé dur avec Triticum aestivum, T. turgidum var. dicoccum, et les lignées T. militinae, respectivement. HG 110 a été développé à partir d'un croisement intra-spécifique (blé dur/blé dur) tandis que ED 2398-A était un blé dur de race primitive éthiopienne. Les pathotypes testés comprenaient le 40A (62G29) et le 117-6 (37G19) de la rouille noire, le premier étant prédominant dans la nature et le dernier plus virulent sur le blé dur indien ainsi que les pathotypes de la rouille brune plus répandus et spécifiques du blé dur, 12-2 (1R5) et 104-2 (21R55). Alors que les tests de semis ont été réalisés avec les pathotypes 40A, 12-2 et 104-2, les études sur les plantes adultes ont été réalisées avec le pathotype 117-6. L'analyse des populations F_2 et des familles F_3 issues des croisements de ces stocks résistants avec trois variétés de blé dur sensibles 'Motia', 'Malvi local' et 'Sarangpur local' a montré que la résistance est contrôlée par un ou deux gènes. Au total, quatre gènes de*

résistance au pathotype 40A, et huit gènes de résistance pour chaque race, 117-6 et 12-2, ont été identifiés parmi les cinq stocks résistants étudiés ; en plus, trois gènes de résistance à 104-2 ont été identifiés parmi B 662, ED 2398 A et IWP 5019, sur la base des tests alléliques. Bien que leur identité ne soit pas encore connue, les gènes pour la résistance à la rouille noire sont différents de Sr2, Sr7b, Sr9e et Sr11, et ceux pour la résistance à la rouille brune sont différents de Lr23, les gènes de la résistance à la rouille noire et à la rouille brune documentés parmi les génotypes de blé dur indiens. Ainsi, les stocks génétiques explorés devraient contribuer à enrichir le patrimoine génétique pour l'amélioration du blé dur comme sources différentes de résistance à la rouille noire et à la rouille brune.

Mots-clés. *Blé dur – Résistance à la rouille noire – Résistance à la rouille brune – Diversité génétique – Pathotypes de rouille indiens.*

I – Introduction

India produces >90 million tons of wheat from an area of >25 million hectares, to which the contribution of durum wheat is about 5%. However, durum wheat has a special niche in Indian wheat economy for at least two reasons. Indian durum wheat is typically purchased by the private trade at a price premium, mainly for processing of high value products. In addition, durum wheat is preferred over bread wheat for several local food preparations. In India, durum wheat is mainly grown in the central and peninsular parts of India including the states of Madhya Pradesh, Rajasthan, Gujarat, Uttar Pradesh (Bundelkhand region), Maharashtra, and Karnataka, where stem rust and leaf rust are the major disease problems affecting the wheat crop. Relatively little work has been done on the inheritance of rust resistance in durum wheat, compared to bread wheat. Hence, studies were conducted using appropriate rust pathotypes to determine the mode of inheritance and extent of diversity for resistance to stem and leaf rusts among five durum wheat genetic stocks *viz*. 'B 662', 'ED 2398-A', 'HG 110', 'IWP 5019' and 'Line 1172', which have been showing high levels of resistance to both the rusts in the disease screening nurseries under heavy inoculum pressure since 1997. Results of these studies are presented in this communication.

II – Material and Methods

1. Host material

Five rust resistant durum wheat genetic stocks viz., 'B 662', 'ED 2398-A', 'HG 110', 'IWP 5019' and 'Line 1172' of diverse origins were selected for the present studies (Table 1). Three durum wheat land races viz., Motia, Malvi Local, and Sarangpur Local were used as 'susceptible' parental lines in crosses with the above listed 'resistant' stocks.

2. Rust pathotypes chosen for the studies

Stem rust pathotypes 40A (62G29) and 117-6 (37G19) since the former is currently the most prevalent one (Anonymous, 2012), while the latter is highly virulent to Indian durum wheat germplasm (Mishra *et al*., 2001a; Mishra *et al*., 2009) among the stem rust pathotypes occurring in India.

Leaf rust pathotypes 12-2 (1R5) and 104-2 (21R55) since the former is durum-specific (Mishra *et al.*, 2001a; Mishra *et al*., 2009), while the latter is widely prevalent (Anonymous, 2012), and durum-virulent (Mishra *et al*., 2001a; Mishra *et al*., 2009) among the Indian leaf rust pathotypes.

The avirulence / virulence characteristics of these pathotypes on the stem rust and leaf rust differentials being currently used in India, based on seedling tests, are as follows (Nayar *et al*., 2001):

40A (62G29): P Sr13, Sr30, Sr37, Einkorn (Sr21), Khapli (Sr7a, Sr13, Sr14) / p Sr8b, Sr9b, Sr9e, Sr11, Sr28, Marquis (Sr7b+), Kota (Sr28+), Reliance (Sr5+), Charter (Sr11+),

117-6 (37G19): P Sr8b, Sr9b, Sr28, Sr30, Sr37, Kota (Sr28+), Reliance (Sr5+), Khapli (Sr7a, Sr13, Sr14) / p Sr9e, Sr11, Sr13, Marquis (Sr7b+), Einkorn (Sr21), Charter (Sr11+),

12-2 (1R5): P Lr10, Lr13, Lr15, Lr17, Lr18, Lr19, Lr24, Webster (Lr2a), Thew (Lr20), Malakoff (Lr1), Benno (Lr26), HP 1633 (Lr9+) / p Lr14a, Loros (Lr2c), Democrat (Lr3),

104-2 (21R55): P Lr10, Lr13, Lr15, Lr19, Lr24, Webster (Lr2a), Thew (Lr20), HP 1633 (Lr9+) / p Lr14a, Lr17, Lr18, Loros (Lr2c), Democrat (Lr3), Malakoff (Lr1), Benno (Lr26).

Table 1. List and characteristics of host material.

Genetic stock	Parentage / Source	Important phenotypic traits
ED 2398 A	Ethiopian local durum wheat variety from the germplasm collection of IARI-RS, Indore	Tall in height (>110 cm) Late in heading (~90 days) Long ears with glabrous glumes Purple pigmentation on stem and auricle
HG 110	Sarangpur Local/HI 8185 (Sarangpur Local - local durum variety, HI 8185 – an advanced generation durum line developed at Indore	Medium Tall (<110 cm) Medium early in heading (~70 days) Long ears with pubescent glumes Purple pigmentation on auricle
B 662	PBW 34*2/Chuanmai #18 (PBW 34 – a released durum cultivar, Chuanmai # 18 – a Chinese accession of *Triticum aestivum* carrying *Rht8* gene for dwarfism)	Triple dwarf (Height <50 cm) Medium late in heading (~80 days) Long ears with pubescent glumes
IWP 5019	HD 4519*2/NP 200 (HD 4519 – an advanced generation durum line NP 200 – a released cultivar *of T. dicoccum*	Double dwarf (Height 80-85 cm) Very early heading (>70 days) Short ears with Glabrous glumes Grains with high protein content and high SDS value
Line 1172	MACS 9*2/*T. militinae* (MACS 9 – a released durum wheat cultivar, *T. militinae* – a free-threshing mutant of *T. timopheevii*)	Tall in height (>110 cm) Medium late in heading (~80 days) Glabrous glumes Grains very bold

B 662, IWP 5019 and Line 1172 were developed at IARI, New Delhi through interspecific hybridization.

3. Methods

Studies were carried out in the glasshouse, and in the field at the Indian Agricultural Research Institute (IARI), Regional Station, Indore, India during the regular wheat crop season (November-April). The 'susceptible' varieties 'Motia', 'Malvi Local', and 'Sarangpur Local' were used as female parental lines in crosses with each of the five 'resistant' stocks, 'B 662', 'ED 2398-A', 'HG 110', 'IWP 5019' and 'Line 1172'. Also, the resistant stocks were crossed among themselves in all possible combinations without reciprocals. A few F_1 seeds were saved for tests, while others were grown to obtain F_2 seeds from individual F_1 plants. The F_3 families were constituted from harvest of the individual F_2 plants. The parents, F_1s, F_2 populations and F_3 families were tested in the seedling stage with stem rust pathotype *40A*, and leaf rust pathotypes *12-2* and *104-2* in a glasshouse at 22°C ± 2°C using standard glasshouse procedures (Roelfs *et al*., 1992; Nayar *et al*., 1997); and in the adult-plant stage with the stem rust pathotype *117-6* in an isolated nursery following recommended crop cultivation practices.

In the adult-plant tests involving the stem rust pathotype *117-6*, seeds of the parental lines, the F_1s and the F_2 populations were dibbled with 10 cm spacing between seed-to-seed in 1.5 m long rows, planted 30 cm apart. The F_3 families derived from the F_2 plants of 'susceptible parent x resistant parent' crosses as well as the ones derived from the F_2 plants classified as "susceptible" (putative susceptible segregants) among 'resistant parent x resistant parent' crosses were tested to confirm the F_2 observations. Around 50 seeds derived from each of the F_2 plants was hand-drilled in 2.5 m long rows planted 30 cm apart. The parental lines were replicated twice along with the F_1s, F_2 populations, and F_3 families of each of the crosses. Rust spreader rows consisting of mixtures of highly susceptible wheat varieties were planted after every 20 test rows, and all around the experimental plot as well. Beginning 50-60 days after sowing, the disease spreader rows were inoculated with aqueous suspension of the uredospores of the stem rust pathotype *117-6*, freshly collected from the actively sporulating pots maintained in isolation in the glasshouse. Both hypodermic syringes and sprays were used to inoculate the disease spreader rows to ensure timely establishment of stem rust in the field. The spore suspension was sprayed on to the test rows as well, but no syringe inoculations were made in order to simulate natural stem rust epidemics. Stem rust scores were recorded combining the disease severity as per the modified Cobb's scale (Peterson *et al*., 1948), and the host response (Roelfs *et al*., 1992).

The F_2 plants were grouped in to 'resistant' and 'susceptible' classes on the basis of their infection types in seedlings or their response combined with severity in case of adult plants, and were counted to determine the F_2 ratios. The F_3 families derived from the F_2 plants were classified as homozygous resistant (R), segregating (Seg), or homozygous susceptible (S), based on the presence of exclusively resistant plants, both resistant and susceptible plants, and exclusively susceptible plants, respectively. The chi-squared test was used to test the goodness-of-fit of the observed F_2 and F_3 ratios to the expected ones on the basis of Mendelian segregation.

III – Results

1. Adult-plant resistance to stem rust pathotype 117-6

Among the five resistant durum genotypes studied, only B 662 showed seedling resistance to the stem rust pathotype *117-6,* while all the five expressed adult-plant resistance to this pathotype (Table 2). Hence, genetics of adult-plant resistance was studied using the stem rust pathotype *117-6*. The F_1s from all of the 'susceptible parent' / 'resistant parent' (S / R) crosses were resistant except the one from 'Sarangpur Local' / 'B 662' cross, indicating the dominant mode of inheritance of adult-plant resistance to stem rust pathotype *117-6* in the five resistant genotypes studied (Table 3). Analysis of F_2 and F_3 ratios involving 'S / R' crosses showed the presence of a single dominant resistance gene in B 662 and IWP 5019, while two independent dominant genes were operative for resistance in each of the three remaining resistant genotypes (Table 3). Sarangpur Local', one of the three susceptible parental lines used in the study, showed the presence of a suppressor gene against the resistance gene in B 662 (Table 3). Allelic tests involving 'resistant parent' / 'resistant parent' (R / R) crosses showed that all of these genes were different from each other (Table 3). Thus, a total of eight diverse dominant genes were identified for adult-plant resistance among the five resistant genotypes studied.

2. Seedling resistance to stem rust pathotype *40A*

All the five resistant genotypes and the F_1s from all of the S / R crosses were resistant except the one from 'Sarangpur Local' / 'B 662' cross, indicating the dominant mode of inheritance of seedling resistance to stem rust pathotype *40A* in the five resistant genotypes studied (Table 2). Study of the F_2 and F_3 populations derived from 'S / R' crosses showed the presence of a single dominant resistance gene in ED 2398-A, HG 110, and IWP 5019, while one dominant +

one recessive gene occurred each in B 662 and Line 1172 (Table 4). However, in the 'Sarangpur Local' / 'B 662' cross, the F_2 ratio fitted to 7R : 9S, indicating modification to the two recessive genes. Analysis of the F_2 ratios involving 'R / R' crosses revealed that while the dominant genes in B 662 and ED 2398-A were unique, the dominant gene was common among HG 110, IWP 5019 and Line 1172. Likewise, the recessive gene was common between B 662 and Line 1172. (Table 4). Thus, a total of four diverse genes including three dominant genes and one recessive were identified for seedling resistance to stem rust pathotype *40A* among the five durum wheat genotypes studied.

3. Seedling resistance to leaf rust pathotype *12-2*

All the five resistant genotypes and the F_1s from all of the S / R crosses were resistant, indicating the dominant inheritance of seedling resistance to leaf rust pathotype *12-2* in the five resistant genotypes studied (Table 2). Analysis of the F_2 and F_3 ratios involving 'S / R' crosses showed the presence of a single dominant resistance gene in HG 110 and Line 1172, while two independent dominant genes each conditioned resistance to this pathotype in B 662, ED 2398-A and IWP 5019 (Table 5). Allelic tests involving 'R x R' crosses showed that all of these genes were different from each other (Table 5). Thus, a total of eight diverse dominant genes were identified for seedling resistance to the leaf rust pathotype *12-2* among the five resistant genotypes studied.

Table 2. Seedling and adult-plant responses of the parental lines and the F_1s to the rust pathotypes used in the study.

Material	Seedling Infection Types				Adult-plant response to117-6
	40A	12-2	104-2	117-6	
Parental line					
B 662	;1$^-$	0;	;2N	;1	5RMR
ED 2398-A	;1N	;1	;1	23$^+$	TR
IWP 5019	;1	;1N	;2	34	10S
HG 110	;1	X$^+$	34	34	5S
Line 1172	;1	X	34	34	TS
Motia	34	34	34	34	80S
Malvi Local	34	34	34	34	80S
Sarangpur Local	34	34	34	34	60MSS
F_1S					
Motia / B 662	;1	;1	;2$^+$N	NT	10MR
Malvi Local / B 662	;1	;1	;2$^+$N	NT	10MR-TS
Sarangpur Local / B 662	34	;1	;2$^+$N	NT	60MSS
Motia / ED 2398-A	;2$^-$N	;1$^+$	;1$^+$	NT	TMR
Malvi Local / ED 2398-A	;2$^-$N	;1$^+$	;1$^+$	NT	5MR
Sarangpur Local / ED 2398-A	;2$^-$N	;1$^+$	;1$^+$	NT	TMR
Motia / IWP 5019	;1$^+$	;2$^-$N	;3$^-$	NT	20S
Malvi Local / IWP 5019	;1$^+$	;2$^-$N	;3	NT	20S
Sarangpur Local / IWP 5019	;1$^+$	;2$^-$N	;3	NT	20S
Motia / HG 110	;1$^+$	X^{++}	NT	NT	10S
Malvi Local / HG 110	;1$^+$	X^{++}	NT	NT	10S
Sarangpur Local / HG 110	;1$^+$	X^{++}	NT	NT	10S
Motia / Line 1172	;1$^+$	X$^+$	NT	NT	5S
Malvi Local / Line 1172	;1$^+$	X$^+$	NT	NT	5S
Sarangpur Local / Line 1172	;1$^+$	X$^+$	NT	NT	5S

NT – Not Tested.

4. Seedling resistance to leaf rust pathotype *104-2*

Three of the five resistant genotypes studied viz., B 662, IWP 5019, and ED 2398-A as well as the F_1s from their crosses with the susceptible parental lines were resistant, indicating the dominant inheritance of seedling resistance to leaf rust pathotype *12-2* in these three genotypes (Table 2). Seedling tests involving the F_2 and F_3 populations derived from 'S x R' crosses showed the presence of a single dominant resistance gene each in B 662 and IWP 5019, while two independent dominant genes controlled resistance to this pathotype in ED 2398-A (Table 6). Allelic tests involving 'R x R' crosses showed that while the gene in B 662 was unique, one of the genes in ED 2398-A was common with that of IWP 5019, as no susceptible segregant was observed in the 'ED 2398-A' x 'IWP 5019' cross (Table 6). Thus, at least three diverse dominant genes were identified for seedling resistance to leaf rust pathotype 104-2 among the aforesaid three resistant genotypes studied. The other two genotypes, HG 110 and Line 1172, being seedling susceptible to this pathotype, were not included in the study.

IV – Discussion

With the possible exception of the dominant gene in B 662, the genes for adult-plant resistance to the stem rust pathotype *117-6* identified are different from those detected in seedlings using stem rust pathotype *40A*, since only B 662 showed resistance to *117-6* in seedlings, while others including ED 2398-A, HG 110, IWP 5019, and Line 1172 were susceptible. Thus, a total of 11 stem rust resistance genes including 10 dominant and one recessive were identified in the present study, since the dominant gene for resistance to the pathotype *40A* among HG 110, IWP 5019 and Line 1172 was common, and the recessive gene between B 662 and Line 1172 was common for resistance to the same pathotype. In an earlier study, a single dominant gene was found to control seedling resistance in B 662 to the stem rust pathotype *117-6* (Mishra *et al*., 2005), and it could be the same gene that has been identified in the present study for the adult-plant resistance to this pathotype in B 662. Though the identity of the genes identified in the present study is not known, they are different from the stem rust resistance genes *Sr2*, *Sr7b*, *Sr9e* and *Sr11*, which have commonly been postulated in Indian durum wheat germplasm (Nayar *et al*., 2001), since *Sr2* is expressed only in adult-plants and is recessively inherited, while the other three genes are ineffective against the stem rust pathotypes *40A* and *117-6*. Presence of a suppressor gene was observed in Sarangpur Local for the dominant gene in B 662 for adult-plant resistance to stem rust pathotype *117-6*. Suppressor genes for adult-plant resistance to stem rust, and for adult-plant as well as seedling resistance to leaf rust in durum wheat have been reported earlier also (Mishra *et al*., 1989a; Mishra *et al*., 1989b).

Presence of unique genes for leaf rust resistance in HG 110 and Line 1172, since both were susceptible to the pathotype *104-2*, and presuming that the genes in B 662, ED 2398-A and IWP 5019 were common for resistance to the leaf rust pathotypes *12-2* and *104-2*, it can be inferred that at least eight diverse dominant genes were identified for leaf rust resistance among the five resistant durum genotypes studied. These genes are different from the leaf rust resistance gene *Lr23*, which has been commonly postulated in Indian durum wheat germplasm (Nayar *et al*., 2001), as *Lr23* is ineffective against both the leaf rust pathotypes *12-2* and *104-2*.

The aforesaid five durum wheat genetic stocks were reported as new sources of rust resistance in 2001 (Mishra *et al*., 2001b), based on their continued expression of resistance since 1997. It may be noted that subsequently they have been observed to maintain their resistance status till date for stem and leaf rusts in the disease screening nurseries under heavy inoculums pressure, not only at Indore, but at other hot-spot locations in the country as well. With the establishment of genetic diversity among them for resistance to both the rusts through the present study, these genotypes can contribute to broaden the rust resistance base in durum wheat leading to prolonged durability of rust resistance in future durum varieties.

Table 3. Segregation for adult-plant resistance to stem rust pathotype 117-6 in F_2 plants / F_3 families derived from 'susceptible parent' / 'resistant parent' (S / R) and 'resistant parent' / 'resistant parent' (R / R) crosses *R: Resistant. (S: Susceptible, Seg: Segregating for resistance).*

Cross	Number of F_2 plants					Number of F_3 families					
	R	S	Total	χ^2	P	R	Seg	S	Total	χ^2	P
S / R crosses											
Motia' / 'B 662'	72	30	102	1.06 (3:1)	>0.30	25	59	15	99	5.66 (1:2:1)	>0.05
Malvi Local' / 'B 662'	85	35	120	1.11 (3:1)	>0.20	20	39	17	76	0.29 (1:2:1)	>0.50
Sarangpur Local' / 'B 662'	22	59	81	3.76 (3:13)	>0.05	5	46	30	81	1.59 (1:8:7)	>0.30
Motia' / 'ED 2398 A'	82	4	86	0.38 (15:1)	>0.50	36	34	9	79	4.25 (7:8:1)	>0.10
Malvi Local' / 'ED 2398 A'	68	7	75	1.21 (15:1)	>0.20	26	36	6	68	1.31 (7:8:1)	>0.50
Sarangpur Local' / 'ED 2398 A'	111	13	124	3.79 (15:1)	>0.05	45	51	4	100	0.87 (7:8:1)	>0.50
Motia' / 'HG 110'	97	5	102	0.32 (15:1)	>0.50	44	49	5	98	0.24 (7:8:1)	>0.80
Malvi Local' / 'HG 110'	108	12	120	2.88 (15:1)	>0.05	45	56	8	109	0.40 (7:8:1)	>0.80
Sarangpur Local' / 'HG 110'	106	11	117	1.98 (15:1)	>0.10	44	67	6	117	2.48 (7:8:1)	>0.20
Motia' / 'IWP 5019'	86	27	113	0.07 (3:1)	>0.70	25	53	22	100	0.54 (1:2:1)	>0.70
Malvi Local' / 'IWP 5019'	89	33	122	0.27 (3:1)	>0.50	28	62	29	119	0.23 (1:2:1)	>0.80
Sarangpur Local' / 'IWP 5019'	103	41	144	0.93 (3:1)	>0.30	29	69	33	131	0.62 (1:2:1)	>0.70
Motia' / 'Line 1172'	149	16	165	3.35 (15:1)	>0.05	70	80	14	164	1.46 (7:8:1)	>0.30
Malvi Local' / 'Line 1172'	60	3	63	0.24 (15:1)	>0.50	23	33	3	59	0.85 (7:8:1)	>0.50
Sarangpur Local' / 'Line 1172'	94	6	100	0.01 (15:1)	>0.90	32	49	5	86	1.71 (7:8:1)	>0.30
R / R crosses											
B 662' / 'ED 2398-A'	459	08	467	0.07 (63:1)	>0.70	Susceptible F_2 plants were progeny tested to confirm the segregating F_2 ratios in the R / R crosses					
B 662' / 'HG 110'	829	19	848	2.53 (63:1)	>0.10						
B 662' / 'IWP 5019'	1104	89	1193	2.96 (15:1)	>0.05						
B 662' / 'Line 1172'	789	12	801	0.02 (63:1)	>0.80						
ED 2398-A' / 'HG 110'	1284	06	1290	0.18 (255:1)	>0.50						
ED 2398-A' / 'IWP 5019'	845	12	857	0.15 (63:1)	>0.50						
ED 2398-A' / 'Line 1172'	617	02	619	0.07 (255:1)	>0.70						
HG 110' / 'IWP 5019'	839	17	856	1.00 (63:1)	>0.30						
HG 110' / 'Line 1172'	1398	07	1405	0.41 (255:1)	>0.50						
IWP 5019' / 'Line 1172'	1397	29	1426	2.04 (63:1)	>0.10						

Table 4. Segregation for seedling resistance to stem rust pathotype 40A in F_2 plants and F_3 families derived from 'susceptible parent' / 'resistant parent' (S / R) and 'resistant parent' x 'resistant parent' (R / R) crosses.

Crosses	F_2 plants No..					F_3 famIlies No					
	R	S	Tot	χ^2	P	R	SegR	S	TotS	χ^2	P
S / R Crosses											
Motia' / 'B 662'	69	19	88	0.47 (13:3)	>0.30	30	33	5	68	0.16 (7:8:1)	>0.90
Malvi Local' / 'B 662'	64	17	81	0.27 (13:3)	>0.50	28	34	7	69	1.85 (7:8:1)	>0.30
Sarangpur Local' / 'B 662'	38	40	78	0.78 (7:9)	>0.30	27	31	8	66	4.07 (7:8:1)	>0.10
Motia' / 'ED 2398 A'	60	19	79	0.04 (3:1)	>0.80	11	19	8	38	0.47 (1:2:1)	>0.70
Malvi Local' / 'ED 2398 A'	68	22	90	0.01 (3:1)	>0.90	15	31	13	59	0.29 (1:2:1)	>0.80
Sarangpur Local' / 'ED 2398 A'	59	26	85	1.41 (3:1)	>0.20	12	29	16	57	0.58 (1:2:1)	>0.70
Motia' / 'HG 110'	61	18	79	0.20 (3:1)	>0.50	17	40	21	78	0.46 (1:2:1)	>0.70
Malvi Local' / 'HG 110'	61	24	85	0.47 (3:1)	>0.30	15	32	18	65	0.29 (1:2:1)	>0.80
Sarangpur Local' / 'HG 110'	63	23	86	0.13 (3:1)	>0.70	13	33	16	62	0.55 (1:2:1)	>0.70
Motia' / 'IWP 5019'	52	22	74	0.88 (3:1)	>0.30	10	25	13	48	0.45 (1:2:1)	>0.70
Malvi Local' / 'IWP 5019'	53	24	77	1.56 (3:1)	>0.20	14	31	16	61	0.15 (1:2:1)	>0.90
Sarangpur Local' / 'IWP 5019'	71	20	91	0.44 (3:1)	>0.50	19	37	21	77	0.22 (1:2:1)	>0.80
Motia' / 'Line 1172'	61	15	76	0.05 (13:3)	>0.80	26	33	5	64	0.42 (7:8:1)	>0.70
Malvi Local' / 'Line 1172'	40	11	51	0.27 (13:3)	>0.50	21	24	4	49	0.31 (7:8:1)	>0.80
Sarangpur Local' / 'Line 1172'	64	22	86	2.63 (13:3)	>0.10	30	35	7	72	1.49 (7:8:1)	>0.30
R / R crosses											
B 662' / 'ED 2398-A'	124	09	133	1.29 (61:3)	>0.20						
B 662' / 'HG 110'	130	11	141	3.06 (61:3)	>0.05						
B 662' / 'IWP 5019	137	13	150	5.32 (61:3)	>0.02						
B 662' / 'Line 1172'	144	00	144	5.24 (247:9)	<0.05						
ED 2398-A' / 'HG 110'	134	15	149	3.71 (15:1)	>0.05						
ED 2398-A' / 'IWP 5019	140	09	149	0.01 (15:1)	>0.90						
ED 2398-A' / 'Line 1172'	139	07	146	0.004 (61:3)	>0.90						
HG 110' / 'IWP 5019'	145	00	145	9.66 (15:1)	<0.01						
HG 110' / 'Line 1172'	139	00	139	6.84 (61:3)	<0.01						
IWP 5019' / 'Line 1172	159	00	159	7.82 (61:3)	<0.01						

R: Resistant, S: Susceptible, Seg: Segregating for resistance.

Table 5. Segregation for seedling resistance to leaf rust pathotype 12-2 in F_2 plants / F_3 families derived from 'susceptible parent' / 'resistant parent' (S / R) and 'resistant parent' / 'resistant parent' (R / R) crosses.

Cross	F2 plants No.					F3 families No.					
	R	S	Tot.	χ^2	P	R	Seg	S	Tot	χ^2	P
S / R crosses											
'Motia' / 'B 662'	**93**	**4**	**97**	**0.75 (15:1)**	**>0.30**	**33**	**45**	**5**	**83**	**0.60 (7:8:1)**	**>0.70**
'Malvi Local' / 'B 662'	**156**	**9**	**165**	**0.18 (15:1)**	**>0.50**	**26**	**39**	**7**	**72**	**2.56 (7:8:1)**	**<0.20**
'Sarangpur Local' / 'B 662'	**147**	16	163	3.53 (15:1)	>0.05	39	45	8	92	0.94 (7:8:1)	>0.50
'Motia' / 'ED 2398 A'	158	12	170	0.19 (15:1)	>0.80	35	39	5	79	0.01 (7:8:1)	>0.99
'Malvi Local' / 'ED 2398 A'	173	13	186	0.17 (15:1)	>0.80	33	43	4	80	0.54 (7:8:1)	>0.70
'Sarangpur Local' / 'ED 2398 A'	175	13	188	0.14 (15:1)	>0.90	31	39	3	57	0.73 (7:8:1)	>0.50
'Motia' / 'HG 110'	165	59	224	0.21 (3:1)	>0.80	22	47	18	87	0.93 (1:2:1)	>0.50
'Malvi Local' / 'HG 110'	172	47	219	1.47 (3:1)	>0.30	23	52	16	91	2.94 (1:2:1)	>0.20
'Sarangpur Local' / 'HG 110'	189	54	243	1.00 (3:1)	>0.30	17	41	12	70	2.90 (1:2:1)	>0.20
'Motia' / 'IWP 5019'	99	11	110	2.65 (15:1)	>0.10	34	32	7	73	1.99 (7:8:1)	>0.30
'Malvi Local' / 'IWP 5019'	138	8	146	0.15 (15:1)	>0.90	41	51	5	97	0.36 (7:8:1)	>0.80
'Sarangpur Local' / 'IWP 5019'	138	4	142	2.86 (15:1)	>0.05	31	39	6	76	0.51 (7:8:1)	>0.70
'Motia' / 'Line 1172'	120	32	152	1.27 (3:1)	>0.20	18	43	19	80	0.47 (1:2:1)	>0.70
'Malvi Local' / 'Line 1172'	102	32	134	0.09 (3:1)	>0.70	14	41	12	67	3.48 (1:2:1)	>0.10
'Sarangpur Local' / 'Line 1172'	190	73	263	1.07 (3:1)	>0.20	20	47	16	83	1.84 (1:2:1)	>0.30
R / R crosses											
'B 662' / 'ED 2398-A'	768	3	771	0.00 (255:1)	>0.99	The F3 families from the R /R crosses not tested					
'B 662' / 'HG 110'	656	8	664	0.55 (63:1)	>0.30						
'B 662' / 'IWP 5019'	449	3	452	0.88 (255:1)	>0.30						
'B 662' / 'Line 1172'	352	6	358	0.03 (63:1)	>0.80						
'ED 2398-A' / 'HG 110'	385	3	388	1.57 (63:1)	>0.20						
'ED 2398-A' / 'IWP 5019'	611	3	614	0.15 (255:1)	>0.90						
'ED 2398-A' / 'Line 1172'	379	4	383	0.67 (63:1)	>0.70						
'HG 110' / 'IWP 5019'	344	3	347	1.10 (63:1)	>0.20						
'HG 110' / 'Line 1172'	652	49	701	0.66 (15:1)	>0.30						
'IWP 5019' / 'Line 1172'	593	14	607	2.19 (63:1)	>0.10						

R: Resistant, S: Susceptible, Seg: Segregating for resistance.

Table 6. Segregation for seedling resistance to leaf rust pathotype 104-2 in F_2 plants / F_3 families derived from 'susceptible parent' / 'resistant parent' (S / R) and 'resistant parent' / 'resistant parent' (R / R) crosses.

Cross	F_2 plants No.					F_3 famllles No.					
	R	S	Tot	χ^2	P	R	Seg	S	Tot	χ^2	P
S / R crosses											
Motia' / 'B 662'	48	11	59	1.27 (3:1)	>0.20	15	30	10	83	1.37 (1:2:1)	>0.50
Malvi Local' / 'B 662'	96	29	125	0.22 (3:1)	>0.50	16	41	21	78	0.85 (1:2:1)	<0.50
Sarangpur Local' / 'B 662'	121	45	166	0.39 (3:1)	>0.50	17	46	22	85	1.17 (1:2:1)	>0.50
Motia' / 'ED 2398 A'	77	4	81	0.24 (15:1)	>0.50	33	39	3	75	0.67 (7:8:1)	>0.70
Malvi Local' / 'ED 2398 A'	103	10	113	1.30 (15:1)	>0.20	39	57	8	104	1.76 (7:8:1)	>0.30
Sarangpur Local' / 'ED 2398 A'	78	5	83	0.01 (15:1)	>0.90	41	37	7	85	1.64 (7:8:1)	>0.30
Motia' / 'IWP 5019'	71	29	100	0.85 (3:1)	>0.30	16	52	21	89	3.09 (1:2:1)	>0.20
Malvi Local' / 'IWP 5019'	48	17	65	0.05 (3:1)	>0.80	12	32	15	59	0.72 (1:2:1)	>0.50
Sarangpur Local' / 'IWP 5019'	59	18	77	0.11 (3:1)	>0.70	16	36	13	65	1.03 (1:2:1)	>0.50
R / R crosses											
B 662' / 'ED 2398-A'	320	8	328	.65 (63:1)	>0.10	The F_3 families from R /R crosses not tested					
B 662' / 'IWP 5019'	285	21	306	0.20 (15:1)	>0.50						
ED 2398-A' / 'IWP 5019'	257	0	257	4.02 (63:1)	<0.05						

R: Resistant, S: Susceptible, Seg: Segregating for resistance.

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Genetic basis of resistance to leaf rust in tetraploid wheats

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Abstract. Leaf rust, caused by *Puccinia triticina* Eriks., is one of the major constraints to durum wheat production. It is globally distributed with different race structures that continuously evolve and form novel virulent races. Growing resistant cultivars represent the most effective way of controlling rust diseases in wheat. In this paper we report a summary about the leaf rust genes (*Lr*), the quantitative trait loci (QTLs) and significant regions detected in tetraploid wheats.

Keywords. *Puccinia triticina* – Tetraploid wheats – Genetic resistance – Mapping.

Base génétique de la résistance à la rouille brune chez les blés tétraploïdes

Résumé. *La rouille brune, causée par Puccinia triticina Eriks., est l'un des principaux obstacles à la production de blé dur. Ce pathogène est distribué à l'échelle mondiale et présente des structures de races différentes qui évoluent continuellement et forment de nouvelles races virulentes. Cultiver des variétés résistantes représente le moyen le plus efficace de lutte contre les maladies de la rouille du blé. Dans cet article, nous allons parcourir les gènes de la rouille brune (Lr), les loci des caractères quantitatifs (QTLs) et les régions significatives détectées dans les blés tétraploïdes.*

Mots-clés. *Puccinia triticina – Blés tétraploïdes – Résistance génétique – Cartographie.*

Leaf rust, caused by *Puccinia recondita* Rob. ex. Desm. f.sp. tritici Eriks. & E. Henn. (syn. *P. triticina*), is an important disease in durum wheat that causes significant reduction in grain yield and quality in most wheat growing areas (Samborsky, 1985). The level of damage inflicted by leaf rust varies with the growth stage of plants at the initial infection and environmental conditions (Kolmer *et al.*, 2007). The use of resistant cultivars is the most effective way to control this disease and the constant search for novel resistance genes is essential to cope with the dynamic and rapidly evolving pathogen populations (Kolmer, 2005; Bolton *et al.*,2008). Sources for the identification of new resistance genes frequently include the wild relatives of crop plants and germplasm from the center of diversity of the cultivated species, and wild emmer (*T. turgidum* ssp. *dicoccoides*) has represented an useful source of genes for resistance to pathogens, including leaf rust, in wheat (Marais *et al.*, 2005; Dyck 1994; Xie *et al.*, 2012). Similarly, also *T. turgidum* ssp. *dicoccum*, one of the earliest domesticated wheat derived from wild emmer (Kilian *et al.*, 2009) acted as a donor of genes for resistance to leaf rust (Piarulli *et al.*, 2012; Liu *et al.*, 2005; McIntosh *et al.*, 1995).

Knowledge of genetic nature of the resistance to infective diseases, the genes at the basis of this character, as well as their inheritance and interaction, is essential for breeding for resistance.

Usually, the identification of genes/quantitative trait loci (QTLs) for resistance to fungal pathogens has been carried out through linkage mapping, but association mapping also revealed to be a useful tool for finding significant associations between molecular markers and the resistance to

leaf rust in durum wheat. As shown in Table 1, 15 different genes and 4 QTLs have been identified in durum wheat using linkage mapping, and different types of closely linked molecular markers have been found. Leaf rust resistance (*Lr)* genes were detected along all chromosomes except for 2A, 3A, 4A, 4B and 5A, while the QTLs regions were identified on chromosomes 1B, 2B and 7B.

More recently, the use of association mapping with durum wheat germplasm collections has been introduced to discover new useful allelic variants through genome-wide scan. Association mapping has several advantages over biparental mapping, including increased mapping resolution and reduced research time by utilizing historic recombination rather than developing new mapping populations, and the ability to detect a greater number of alleles at a particular locus (Yu and Buckler, 2006). A high number of molecular markers associated to the resistant phenotype were recently identified through association mapping in a collection of 164 elite cultivars of durum wheat analyzed with 25 different *P.triticina* isolates (Maccaferri *et al*. 2010), as reported in Table 1.

Information on genetic loci for resistance to leaf rust, closely linked molecular markers and the genotype source of the resistance (as reported in Table 1) is an important prerequisite for marker assisted selection (MAS) programs. With respect to traditional breeding, the use of genetic markers for MAS can greatly shorten the duration of a breeding program, increase the selection efficiency, and limit the phenotypic assessment, which is often laborious and time-consuming. Many efforts have been made internationally to incorporate modern selection technologies into breeding programs. An example of this is the WHEAT CAP project (http:// maswheat.ucdavis.edu/), which is aimed at preparing MAS protocols to incorporate valuable genes for many traits of interest into the best wheat breeding lines (Borrelli *et al.* 2009). For instance, more than 160 leaf (*Lr*), stem (*Sr*) and stripe (*Yr*) rust resistance genes have been found and characterized in common hexaploid wheat, tetraploid durum wheat, and many diploid wild wheat species (Todorovska *et al.* 2009). Nevertheless, the knowledge of the gene sequences linked to the resistance is still lacking, even if it is of great importance, as this allows the design of perfect molecular markers that are not subject to the risk of recombination between the marker and the R gene. Three genes for leaf-rust resistance that confer race-specific resistance have been isolated in bread wheat: *Lr1* and *Lr10*, which originated from common wheat, and *Lr21*, which originated from *Triticum tauschii* (Cloutier *et al.* 2007; Feuillet *et al.* 2003; Huang *et al.* 2003). With the rapid progress of “omics” technologies, great efforts should be aimed at the isolation and cloning of genes and QTL for resistance to leaf rust also in durum wheat, to understand the genetic and molecular mechanisms of resistance and to use this information for the release of cultivars characterized by high and durable resistance.

Table 1. Leaf rust resistance genes (*Lr*), QTLs (*QLr*) and significant regions detected using linkage mapping (LM) and association mapping (AM, marker-wise significant of P ≤ 0.01) approaches on durum wheat. Information on durum wheat genotypes, chromosome location (Chr.) and closely linked markers are also provided.

	Donor genotypes	Chr.	Closely linked markers	Method	References
Lr3a	Storlom	6BL	AFLP: Xmwg798; cDNA marker:TaR16; UBC849540	LM	Herrera-Foessel et al., 2007; Danna CH et al., 2002; Khan RR et al., 2005
Lr10	Altar, Russello	1A	Xsfr1, Xsfrp1	LM	Schachermayr et al., 1997
Lr14a	Lloreta INIA, Somateria	7B	Xwmc273, Xgwm344	LM	Herrera-Foessel et al., 2008a
Lr19	UC1112, UC1113, Ammar9 and Azeghar2	7A	Xwg420, Xmwg2062, SSR-Gb	LM	Zhang et al., 2005; Kassem et al 2011

	Donor genotypes	Chr.	Closely linked markers	Method	References
Lr23	Altar84; W-7974	2BS	Xksu904	LM	Nelson et al., 1997;Faris et al., 1999
Lr26	Cando2/Veery; KS91WGRC14	1BL	IB-267, iag95	LM	Mago et al., 2002; Friebe et al., 1993
Lr27	Benimichi C2004, Jupare C2001	3B	XksuG53	LM	Huerta-Espino J et al., 2009; Nelson et al., 1997
Lr31	Benimichi C2004, Jupare C2001	3B	XksuG10	LM	Huerta-Espino J et al., 2009; Nelson et al., 1997
Lr47	-	7AS	PS10	LM	Dubcovsky et al., 1998
Lr50	TA870, TA 145, TA874, TA 870, TA895 (*T. armeniacum*)	2B	Xgwm382,Xgdm87	LM	Brown-Guerdira et al., 2003
Lr53	98M71 and 479 (*T. dicoccoides*)	6BS	PSR167	LM	Marais et al., 2003,2005;
Lr61	Guayacan 2, Guayacan INIA	6BS	AFLP: P81/M70269/P87/ M75131; SSR: Xwmc487	LM	Herrera-Foessel et al., 2008b
Lr64	8404 (*T. dicoccoides*)	6AL	Xbarc104, Xgwm427	LM	Kolmer JA 2008 Personal communication
LrWo	Wollaroi AUS99174	5BS	Xgwm234; wPT-1420	LM	Singh et al. 2010
QLr	Sachem	7BL	Xgwm146	LM	Singh et al., 2013
QLr	Sachem	1BL	wPt-3579	LM	Singh et al., 2013
QLr	Strongfield	2B	wPt-3632	LM	Singh et al., 2013
*QLr. ubo-*7B.2	Creso/Colosseo	7BL	Xgwm344.2 and DaRT 378059	LM	Marone et al., 2009; Maccaferri et al., 2008
-	164 elite durum wheat accessions	1A	Xgpw2276, Xwmc24, Xwmc469, Xcfa2129	AM	Maccaferri et al., 2010
-	164 elite durum wheat accessions	1B	Xwmc500.1, Xgwm124, Xbarc188, Xwmc44, Xbarc80, Xgwm140, Xcfd251.1	AM	Maccaferri et al., 2010
-	164 elite durum wheat accessions	2A	Xbarc212, Ppd-A1, Xcfa2201, Xgwm1198.2,Xgwm1198.3, Xwmc552.1	AM	Maccaferri et al., 2010
-	164 elite durum wheat accessions	2B	Xbarc2318, Xwmc770, Xgwm410.1, Xgwm148, Xbarc183.1, Xbarc40, Xbarc101.1, Xwmc175, Xgwm846.2	AM	Maccaferri et al., 2010
-	164 elite durum wheat accessions	3A	Xwmc388.2, Xwmc264, Xcfa2193	AM	Maccaferri et al., 2010
-	164 elite durum wheat accessions	3B	Xgwm685, Xbarc84, Xgwm299	AM	Maccaferri et al., 2010
-	164 elite durum wheat accessions	4A	Xgwm894, Xbarc155, Xwmc313	AM	Maccaferri et al., 2010

	Donor genotypes	Chr.	Closely linked markers	Method	References
-	164 elite durum wheat accessions	4B	Xbarc193, Xwmc524, Xgwm856, Xgwm6	AM	Maccaferri et al., 2010
-	164 elite durum wheat accessions	5A	Xwmc489.1, Xbarc303, Xwmc705, Xwmc805, Xgwm1570, Xgwm410.2	AM	Maccaferri et al., 2010
-	164 elite durum wheat accessions	5B	Xgwm335, Xcfa2121, Xwmc640.1	AM	Maccaferri et al., 2010
-	164 elite durum wheat accessions	6A	Xgwm1009, Xksum98	AM	Maccaferri et al., 2010
-	164 elite durum wheat accessions	6B	Xwmc486, Xgwm1682	AM	Maccaferri et al., 2010
-	164 elite durum wheat accessions	7A	Xgwm233, Xgwm1187, Xwmc488	AM	Maccaferri et al., 2010
-	164 elite durum wheat accessions	7B	Xwmc323, Xgwm1184, Xgwm333, Xwmc396	AM	Maccaferri et al., 2010

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Durum wheat improvement against fungal pathogens by using protein inhibitors of cell wall degrading enzymes

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Abstract. We report the use of three glycosidase inhibitors, the bean polygalacturonase inhibiting protein 2 (PvPGIP2), the kiwi pectin methyl esterase inhibitor (AcPMEI), and the *Triticum aestivum* xylanase inhibitor III (TAXI-III), to control leaf blotch and Fusarium Head Blight (FHB) symptoms caused by the fungal pathogens *Bipolaris sorokiniana* and *Fusarium graminearum*. We produced transgenic durum wheat lines by particle bombardment using these inhibitors singly or in combination. We pyramided these transgenes also by classical crossing of transgenic lines carrying a single transgene. Phyto-pathological tests performed in controlled conditions showed that the expression of these inhibitors has the potential to engineer a broad-spectrum disease resistance in wheat.

Keywords. Disease resistance – Cell wall degrading enzymes – Glycosidase inh bitors – Fungal pathogens – *Triticum durum.*

Amélioration du blé dur contre les pathogènes fongiques à l'aide de protéines inhibitrices des enzymes dégradant la paroi cellulaire

Résumé. *Nous allons nous intéresser à l'utilisation de trois inhibiteurs de glycosidases, la protéine 2 inhibitrice de la polygalacturonase des légumineuses (PvPGIP2), la protéine inhibitrice de la pectine méthylestérase (AcPMEI) du kiwi, et la protéine inhibitrice de xylanases III de Triticum aestivum (TAXI-III), pour contrôler les symptômes de la tache foliaire et de la fusariose de l'épi (FHB) causés par les pathogènes fongiques Bipolaris sorokiniana et Fusarium graminearum. Nous avons produit des lignées de blé dur transgéniques par bombardement de particules en utilisant ces inhibiteurs seuls ou en combinaison. Nous avons aussi pyramidé ces transgènes par croisement classique des lignées transgéniques portant un transgène unique. Les tests phyto-pathologiques réalisés en conditions contrôlées ont montré que l'expression de ces inhibiteurs permet d'élaborer une résistance aux maladies à large spectre chez le blé.*

Mots-clés. *Résistance aux maladies – Enzymes dégradant la paroi cellulaire – Inhibiteurs de glycosidases – Pathogènes fongiques – Triticum durum.*

I – Introduction

Broad-spectrum and durable resistance to diseases is one of the most attracting perspective in breeding projects aimed at increasing crop resistance. Since most microbial pathogens need to surmount the plant cell wall to penetrate the host tissue, the reinforcement of this complex compartment should increase the capacity of the host plant to resist the attack of different pathogens. We pursued this goal by enhancing the host ability to abolish or limit the activity of Cell Wall Degrading Enzymes (CWDEs) secreted by pathogens during the penetration and colonization of the host tissue (Ten Have *et al*., 2002).

Plants counteract CWDEs by expressing protein inhibitors which contrast the activity of these degradative enzymes (Juge *et al*. 2006). These inhibitors include polygalacturonase inhibiting

protein (PGIP), xylanase inhibitor (XI), pectin lyase inhibiting protein (PNLIP), xyloglucan-specific endoglucanase inhibitor protein (XEGIP) and pectin methyl esterase inhibitor (PMEI).

We concentrated our efforts on the containment of the activity of two different CWDEs: the polygalacturonases (PGs) and the xylanases.

PGs are among the first CWDEs secreted by fungal pathogens during infection and in some patho-systems they are virulence factors (Ten Have *et al*., 2002). PGs depolymerize the cell wall pectin, a minor component of wheat cell wall, and are inhibited by PGIPs (De Lorenzo *et al*., 2001). PG activity is also negatively affected by a high degree of pectin methyl esterification (Bonnin *et al*., 2002). The level of pectin methyl esterification is controlled by the activity of plant pectin methyl-esterases (PMEs), which remove the methyl groups, and by its protein inhibitor PMEI (Wolf *et al*., 2009). Thus, PMEI may indirectly affect negatively the activity of PGs by maintaining a high degree of pectin methyl esterification.

Xylanases are key enzymes in the degradation of xylans, a main component of wheat cell wall (Vogel, 2008). These enzymes have been shown to be virulence factors for the fungal pathogen *Botrytis cinerea* (Brito *et al*., 2006). The activity of microbial xylanases is controlled *in vitro* by XIs (Dornez *et al*., 2010).

II – Observations

By using a transgenic approach we showed that the constitutive expression of PGIP or PMEI endows durum wheat with new capacities to control the activity of fungal PGs, possibly through a direct interaction or indirectly by modifying the level and pattern of methyl esterification of cell wall pectin (Janni *et al*., 2008; Volpi *et al*., 2011). Similarly, transgenic durum wheat plants over-expressing constitutively TAXI-III, a member of the TAXI-type XIs, showed new abilities to control fungal xylanases in all tissues, including those that normally do not accumulate this inhibitor (Moscetti *et al*., 2013).By phytopathogenic tests we demonstrated that the over-expression of PGIP, PMEI or TAXI-IIIis effective in limiting wheat diseases caused by the fungal pathogens *Fusarium graminearum* and *Bipolaris sorokiniana*(Janni *et al*., 2008; Volpi et al., 2011; Ferrari *et al*., 2012; Moscetti *et al*., 2013). The extent of symptom reduction obtained with the over-expression of each glycosidase inhibitor varies between 25-30% for FHB caused by *F. graminearum* and about 50% for leaf blotch caused by *B. sorokiniana* (Janni *et al*., 2008; Volpi *et al*., 2011; Ferrari *et al*., 2012; Moscetti *et al*., 2013). This level of protection is similar to that observed in transgenic dicot plants expressing PGIP or PMEI (Powell *et al*. 2000; Ferrari *et al*., 2003; Agüero *et al*., 2005; Manfredini *et al*., 2006; Joubert *et al*., 2006; Ferrari *et al*., 2012; Borras-Hidalgo *et al*., 2012; Hwang *et al.*, 2010; Perez-Donoso *et al*., 2010), although the level of pectin content in wheat is much lower than in dicots (Vogel, 2008).

For wheat transgenic plants expressing PGIP, we showed also that the reduction of disease symptoms is associated with a reduced accumulation of mycotoxins and a significant reduced loss of starch accumulated in the grains compared to control plants (D'Ovidio *et al*., 2012).

Plants over-expressing constitutively TAXI-III were very useful to demonstrate, for the first time, that XIs are indeed involved in plant defence; however, its constitutive over-expression caused transgene silencing at high frequency (Moscetti *et al*., 2013), indicating that for practical application a different strategy should be considered, including the expression of XI in specific tissues or regulated by induced promoter.

Finally, the pyramiding of these three glycosidase inhibitors through co-bombardment or crossing resulted in transgene silencing at high frequencies which prevented test their combined effect on disease symptom development (Kalunke *et al*., 2013). Probably the presence of a high number of transgene copies driven by the same constitutive promoter such as *Ubiquitin1*(*Ubi-1)*, may

have triggered homology-dependent gene silencing (HDGS) (Meyer and Saedler 1996). To these unwanted results the constitutive expression of TAXI-III, normally expressed in the endosperm tissue, could have also contributed.

III – Conclusions

In conclusion, these results indicated that the host cell wall polysaccharides, irrespective of their amount and type, play a key role as functional barrier against different pathogens and that the increased accumulation of glycosidase inhibitors can contribute to maintain the integrity of the cell wall and improve wheat resistance against fungal pathogens.

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Pyramiding resistance genes to Fusarium head blight and rusts from *Thinopyrum ponticum* into durum wheat

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Abstract. Taking advantage of climate changes, unfamiliar pests and diseases are challenging wheat crop species. This is the case for Fusarium Head Blight (FHB), which has recently become a threat in unusual environments, including those where durum wheat is traditionally cultivated. Since currently available durum wheats are largely suscept ble to FHB, new varieties are needed capable of maintaining yield capacity and grain quality under the disease pressure. A sustainable approach to achieve this aim is represented by transfer of resistance genes/QTL from related Triticeae species by means of "chromosome engineering". We resorted to this cytogenetic strategy, efficiently complemented with advanced characterization and selection systems, to transfer into durum a gene/QTL for FHB resistance (provisional designation *Fhb-7el$_2$*) located on the 7el$_2$L arm of the wild *Thinopyrum ponticum*. A bread wheat 7DS.7el$_2$L translocation line was employed as donor of the trait in crosses with previously developed durum wheat 7AS.7AL-7el$_1$L recombinant genotypes, carrying additional resistance genes (*Lr19*+*Sr25*) deriving from a different *Th. ponticum* accession. Given the nearly complete homology between the 7el$_1$L and 7el$_2$L arms, and in spite of some pairing reduction in the pentaploid F1's, pyramiding into durum of target genes/QTL from the two *Th. ponticum* accessions was successfully achieved. The selected multiple recombinant lines exhibited up to 80% reduction of suscept bility following *Fusarium* inoculation. The present proof of the *Fhb-7el$_2$* efficacy also in durum wheat opens the way for its straightforward breeding exploitation.

Keywords. Chromosome engineering – Wheat-alien transfer – *Triticum durum* – FHB – Scab – Lr19 + Sr25 genes.

Pyramidage des gènes de résistance à la fusariose de l'épi et à la rouille de Thinopyrum ponticum dans le blé dur

Résumé. *A la suite des changements climatiques, des ravageurs et des maladies auparavant inconnus chez le blé ont fait leur apparition sur cette culture. Tel est le cas de la fusariose de l'épi (FHB), qui représente une nouvelle menace pour certains environnements, y compris ceux où le blé dur est traditionnellement cultivé. Puisque les blés durs disponibles aujourd'hui sont très sensibles à la FHB, il est nécessaire d'obtenir de nouvelles variétés capables de maintenir le potentiel de rendement et la qualité du grain sous pression de maladie. Une approche durable pour atteindre cet objectif est le transfert de gènes de résistance/QTL à partir d'espèces apparentées à Triticeae par le biais de « l'ingénierie chromosomique ». Nous avons eu recours à cette stratégie cytogénétique, complétée efficacement par des systèmes de caractérisation et de sélection avancés, pour transférer chez le blé dur un gène/QTL pour la résistance à la FHB (désignation provisoire Fhb-7el2), situé sur le bras 7el2L de l'espèce sauvage Thinopyrum ponticum. Une lignée de translocation de blé tendre 7DS.7el2L a été utilisée comme donneur de ce caractère dans les croisements avec les génotypes recombinants de blé dur 7AS.7AL-7el1L développés précédemment, portant des gènes de résistance supplémentaires (Lr19 + SR25) issus d'une accession différente de T. ponticum. Compte tenu de l'homologie presque complète entre les bras 7el1L et 7el2L, et malgré une certaine réduction d'appariement dans les pentaploïdes F1, le pyramidage dans le blé dur des gènes cibles/QTL des deux accessions de Th. ponticum a été réalisé avec succès. Les lignées recombinantes multiples sélectionnées affichaient jusqu'à 80% de réduction de la vulnérabilité après l'inoculation de Fusarium. Cette preuve de l'efficacité du Fhb-7el2 aussi chez le blé dur ouvre la voie à son exploitation directe dans la sélection.*

Mots-clés. *Ingénierie chromosomique – Transfert de gènes étrangers chez le blé – Triticum durum – FHB – Fusariose – Gènes Lr19 + SR25.*

I – Introduction

In recent years, climatic changes have favoured the spread of previously uncommon fungal diseases, including Fusarium Head Blight (FHB), in several wheat growing areas, resulting in damage to wheat production and quality (Chakraborty and Newton, 2011). An efficient and sustainable strategy to counter the spread of the pathogen is development of resistant/tolerant varieties able to respond to the current and future demand for high-yielding and low-impact crops. Given the scarcity of resistance sources in cultivated *Triticum* species and even among their close relatives (Buerstmayr *et al*., 2009, 2012, 2013), we have looked outside the primary gene pool and targeted perennial wheatgrass species belonging to the *Thinopyrum* genus. *Thinopyrum* possesses a considerable array of genes for disease and pest resistance as well as for tolerance to environmental stresses, and even yield-related traits (Kuzmanović *et al*., 2013), some of which have been exploited in wheat breeding (reviewed in Ceoloni *et al*., 2013). *Thinopyrum* species are also valuable donors of effective resistance to FHB (Cai *et al*., 2005). Both the diploid *Th. elongatum* (2n = 14) and the decaploid *Th. ponticum* (2n = 70) were shown to harbour a major gene/QTL for FHB resistance on the long arm of a homoeologous group 7 chromosome, namely on 7EL and on $7el_2L$ respectively. While the $7el_2L$ gene/QTL has been mapped toward the distal end of the arm in close association with *XBE445653* and *Xcfa2240* marker loci (Shen and Ohm, 2007; Zhang *et al*., 2011), position of the 7EL locus(i) along the arm has not been determined so far (Shen *et al*., 2004; Shen and Ohm, 2006). The $7el_2L$ arm also carries the effective, but still unmapped, stem rust resistance gene *Sr43* (Kibirige-Sebunya and Knott, 1983; Xu *et al*. 2009), whereas it lacks any major leaf rust resistance gene (Kim *et al*., 1993).

On the other hand, on the $7el_1L$ arm (Sharma and Knott, 1966; Dvorak and Knott, 1977), also called 7AgL (Sears, 1973), originating from a different *Th. ponticum* accession, the leaf and stem rust resistance genes *Lr19* and *Sr25* are distally located (Ceoloni *et al*., 2005, 2013; Gennaro *et al*., 2009), in close linkage with a *Yp* gene contributing to yellow endosperm pigmentation (similarly present on $7el_2L$, see Kibirige-Sebunya and Knott, 1983). Both *Lr19* and *Sr25* are highly valuable resistance sources effective against a large majority of races of the corresponding fungal pathogen that has spread worldwide (Singh *et al*., 2008; Gennaro *et al*., 2009; Jain *et al*., 2009). Notably, they display their full efficacy in areas where durum wheat is the main cereal crop (such as central Italy) and rust diseases represent a constant challenge (leaf rust e.g., Gennaro *et al*., 2007) or tend to re-emerge (stem rust, see Nocente *et al*., 2011).

As $7el_1L$ proved to be fully homologous to $7el_2L$ (Forte *et al*., 2011; Zhang *et al*., 2011) and closely homoeologous to 7EL (Dvorak, 1975; Forte *et al*., 2011), pyramiding of the different *Thinopyrum* genes was considered a feasible target. Chromosome engineering strategies have been undertaken for the recombination-based pyramiding of resistance genes/QTL from both of the above-mentioned *Thinopyrum* species into bread and durum wheat recombinant lines. While the work involving the *Th. elongatum*-derived FHB resistance is underway, we present here the results of successful pyramiding of FHB resistance from *Th. ponticum* $7el_2$ chromosome into durum wheat lines already carrying the $7el_1L$-derived *Lr19* and *Sr25* rust resistance genes.

II – Material and methods

The KS24 bread wheat 7DS.$7el_2L$ centric translocation line (Kibirige-Sebunya and Knott, 1983; Shen and Ohm, 2007; Fig. 1) was used as FHB resistance donor (type II resistance, i.e., inhibition of disease spreading after infection) in crosses with durum wheat recombinant lines, named R5-2-10, R112-4 and R23-1 (Fig. 1). The latter genotypes have 23%, 28% and 40%, of $7el_1L$ replacing corresponding portions of their 7AL arms, respectively (Ceoloni *et al*., 2005). Meiotic metaphase I chromosomes of pentaploid F1 plants were subjected to Genomic In Situ Hybridization (GISH) to assess the frequency of $7el_1L$/$7el_2L$ pairing. F1's were backcrossed to normal durum cultivars

to recover the 2n = 28 chromosome number in the target genotypes. Selection for the desired loci was aided by use of polymorphic SSR, EST and STS markers in the regions of interest (Fig. 1; see also Ceoloni *et al*., 2013). Further characterization was carried out by GISH on somatic chromosomes of the selected genotypes. Selected plants carrying $7el_2L$ markers linked to the FHB resistance locus (here provisionally designated *Fhb-7el*$_2$) were subjected to infection with *Fusarium graminearum*. A pair of central spikelets of each ear (one ear/plant) was inoculated by spore injection, and the disease spreading followed at 7, 14 and 21 days post-inoculation (dpi). The KS24 line, previously proved to be highly resistant also toward Italian *Fusarium* pathotypes, was included in the infection test in addition to several susceptible controls including the 7AL-$7el_1L$ recombinant lines R5-2-10 and R112-4, as awell as various durum wheat cultivars.

III – Results and discussion

GISH analyses on meiotic metaphase I cells confirmed the considerable pairing affinity between the largely homologous chromosomes $7el_1$ and $7el_2$. However, in contrast to their virtually complete pairing observed in bread wheat F1's from the cross of the KS24 line with the T4 translocation line (70% of 7DL replaced by $7el_1L$) (Shen and Ohm, 2007; Forte *et al*., 2011), $7el_1L/7el_2L$ pairing in KS24 x R5-2-10/R112-4/R23-1 pentaploid F1's, always detected in the form of a 7AL.7AS/7AS.7AL-$7el_1L/7el_2L$.7DS trivalent configuration, dropped to less than 40% frequency. This can probably be attributed to the fact that the homologous $7el_1L$ and $7el_2L$ portions lye on otherwise homoeologous chromosomes of the durum wheat parent (7A) and of the bread wheat parent (7D), the former having its complete 7A also present in the same cell.

In line with the observed pairing frequency, around 18% $7el_1L$-$7el_2L$ recombinants were identified in the progeny from the cross of (KS24 x R5-2-10/R112-4/R23-1) F_1 plants x durum cv. Ariosto, analysed with suitable molecular markers (Fig. 1). GISH applied to somatic chromosomes of the putative recombinant types revealed that only a minority of them had the desired combination of $7el_1$ and $7el_2$ target loci on wheat 7AL arm, the remaining ones showing $7el_1L/7el_2L$ recombined chromatin onto the 7DL arm. Of two 7AL recombinants, R85, like R23-1, has 40% of distal 7elL (Fig. 1), while R129, like R5-2-10, has 23% distal 7elL (Fig. 1). Molecular markers revealed that both R85 and R129 recombinants carry *Lr19* ($7el_1L$), as well as the $7el_2L$ allele for the most distal CFA2240 marker, to which the FHB resistance QTL seems to be more tightly associated (Zhang *et al*., 2011). However, the longer $7el_2L$ segment of R129 also includes $7el_2L$ alleles for the more proximal *XBE445653* and *XBF145935* EST marker loci, besides that for the *Yp* gene-linked *XSTSPsy1* locus (Fig. 1).

Based on selection by molecular markers homozygous plants, both carriers and non-carriers of the distal *Thinopyrum* segment, were isolated in F_2 progeny of R85 crossed with normal durum wheat, and these were subjected to infection with *Fusarium* ssp. to assess their resistance/susceptibility against Italian pathotypes. A pair of central spikelets of each ear (one ear/plant) was inoculated by spore injection and the disease spreading followed at 7, 14 and 21 days post-inoculation. As susceptible controls, plants of the R5-2-10 and R112-4 recombinant lines and durum wheat varieties Simeto and Duilio were also included in the experiment.

The phenotypic assay confirmed the tight association of the *Fhb-7el*$_2$ QTL with the CFA2240 marker (the *XBE445653* marker locus has a $7el_1L$ allele in R85), and for the first time it showed its efficacy, previously reported only in bread wheat (Shen and Ohm, 2007), to be fully displayed in durum wheat as well. In fact, the selected R85 homozygous plants showed a significant reduction of susceptibility to FHB, ranging between 60 and 80%.

In F_2 progeny of R85 recombinant heterozygous for a normal 7A, some deviation from normal transmission was observed, likely attributable to the known presence of a Segregation distortion (*Sd*) gene in its most proximal portion, i.e. comprised between its $7el_1L$-7AL breakpoint (= R23-1)

and that of line R112-4 (see Fig. 1). In order to eliminate drawbacks associated with presence of the *Sd* gene (Ceoloni *et al*., 2013), R85 was crossed with R112-4 and R5-2-10 recombinants. This allowed isolation of secondary recombinant types, named R193 and R216, with the same $7el_1L/7el_2L$ content of target loci as R85, but with overall shorter 7elL segments (Fig. 1), hence undergoing normal transmission (not shown). Homozygous plants of such recombinants, as well as of R129, are currently isolated and will be subjected to *Fusarium* infection to corroborate previous evidence on R85. Resistance to leaf rust conferred by *Lr19* was also validated in these materials, both in seedlings and adult plants, while presence of *Sr25* remains to be ascertained.

In conclusion, the recombinant durum wheat genotypes identified in this work represent novel and highly valuable material to be introduced in durum wheat breeding programs aimed at enhancing and widening the spectrum of resistance to a variety of relevant diseases, both traditional and newly emerged that are greatly challenging the crop.

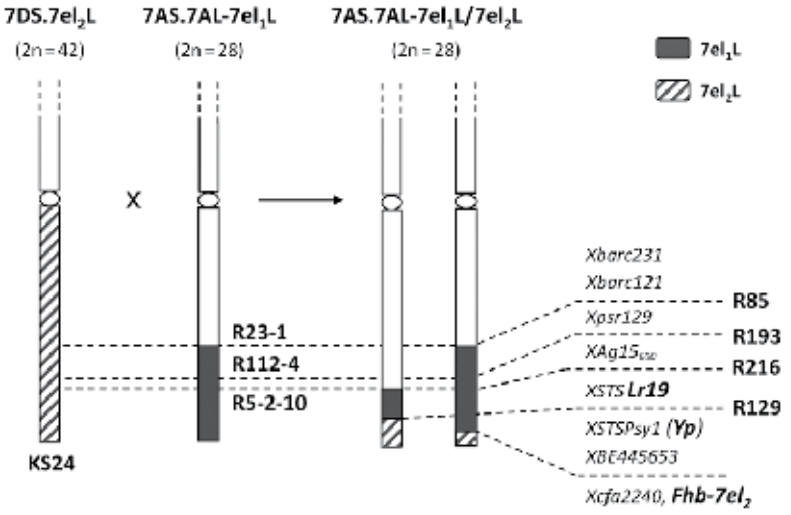

Figure 1. Pyramiding genes/QTL from *Th. ponticum* $7el_1L$ and $7el_2L$ chromosome arms: parental lines and their durum wheat recombinant products carrying different amounts of total 7elL chromatin and combinations of target and marker loci.

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Characterization of sources of resistance to leaf rust in durum wheat germplasm

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Abstract. A nursery with 184 entries including French, European, North African and CIMMYT/ICARDA lines, was phenotyped for its resistance in field trials inoculated with wheat leaf rust, in 4 locations in France and 2 locations in Mexico, in 2009 and 2010. Moreover, the 184 entries were phenotyped for their resistance to 9 pathotypes in the glasshouse.

Genes *Lr27+31* and *Lr3* were effective in France, but given their breakdown in Mexico, they are unl kely to be durable sources of resistance in France. Genes *Lr61*, *LrCamayo*, *Lr19* and *Lr47* were efficient both in Mexico and in France, and could represent valuable sources of resistance. Some lines displayed a high level of resistance in all locations, l kely due to an unknown major gene. Four French entries, as well as several slow rusting lines from CIMMYT, displayed a good level of partial resistance in all environments tested.

Association mapping, using 1300 DArT markers and 34 variables from the phenotyping studies, revealed two QTLs and one locus corresponding to a major gene: i) on chromosome 2B, a QTL was tagged by wPt-1064, wPt-6477 and wPt-0408 ii) on chromosome 6B, a QTL was tagged by wPt-8059, wPt-7065 iii) on chromosome 7B, a major gene was tagged by wPt-0465, wPt-3700 and wPt-9515, which corresponded to *Lr14a.* This gene is not effective in France, whereas it is still efficient in Mexico.

Keywords. *Puccinia triticina* – Resistance phenotyping – QTL – Association mapping – DArT markers.

Caractérisation des sources de résistance à la rouille brune chez le matériel génétique de blé dur

Résumé. *En 2009 et 2010, 184 accessions de pépinière, incluant des lignées françaises, européennes, nord-africaines et du CIMMYT/ICARDA, ont été phénotypées pour leur résistance en réalisant des essais d'inoculation de la rouille brune du blé au plein champ, sur 4 sites en France et 2 sites au Mexique. De plus, les 184 accessions ont été phénotypées pour leur résistance à 9 pathotypes en serre.*

Vu que les gènes Lr27 + 31 et Lr3 étaient efficaces en France mais déjà contournés au Mexique, il est fort improbable qu'ils constituent une source durable de résistance en France. Les gènes LR61, LrCamayo, Lr19 et Lr47 étaient efficaces au Mexique et en France, et ils pourraient donc représenter des sources de résistance importantes. Certaines lignées ont affiché un niveau élevé de résistance dans tous les endroits, probablement en raison de la présence d'un gène majeur encore inconnu. Quatre accessions françaises, ainsi que plusieurs lignées « slow-rusting » du CIMMYT ont montré un niveau de résistance partielle intéressant dans tous les environnements testés.

La cartographie d'association, réalisée à l'aide de 1300 marqueurs DArT et 34 variables issues des études de phénotypage, a révélé deux QTL et un locus correspondant à un gène majeur : i) sur le chromosome 2B, un QTL a été marqué par wPT-1064, wPT-6477 et wPT-0408 ii) sur le chromosome 6B, un QTL a été marqué par wPT-8059, wPT-7065 iii) sur le chromosome 7B, un gène majeur a été marqué par wPT-0465, wPT-3700 et wPT-9515, qui correspond à Lr14a. Ce gène n'est pas efficace en France, alors qu'il est encore efficace au Mexique.

Mots-clés. *Puccinia triticina – Phénotypage de la résistance – QTL – Cartographie d'association – Marqueurs de DArT.*

I – Introduction

Strong leaf rust epidemics, caused by *Puccinia triticina*, regularly occur in the durum wheat growing areas in France and Mexico. Yield losses up to 80% were registered on susceptible cultivars in south-eastern France in 2007, and considerable economic impact was reported in Mexico after the 2001 epidemics (Huerta-Espino *et al.* 2011). Although resistance to this disease has been a concern to breeders globally, the resistance level has to be improved when objectives have been set to curb fungicide use for both environmental and economic reasons. Moreover, most of the resistance sources used in the French germplasm broke down following the evolution of *P. triticina* populations in 2001, and again in 2007 (Goyeau *et al*., 2012).Thus resistance sources should be diversified to respond to a fast changing pathogen population. The objective of this work was to evaluate a collection of selected genotypes with global relevance to wheat leaf rust resistance for i) their seedling reaction against a collection of French and Mexican pathotypes, and ii) their field reaction at adult stage. In addition, the phenotypic data generated was used in combination with DArT genotyping in an association mapping exercise to detect major genomic areas influencing leaf rust reaction in the panel of genotypes.

II – Material and methods

A set of lines and cultivars was selected, including i) breeding lines and cultivars displaying some resistance to wheat leaf rust, ii) lines from CIMMYT/ICARDA germplasm with efficient major genes, or a combination of minor resistance genes, and iii) susceptible germplasm as a control.

1. Phenotyping

Phenotyping was performrd in a greenhouse. Evaluation of the material was conducted by inoculating the set of lines with well-characterized pathotypes individually in separate experiments. In France. The five pathotypes identified up to now in the French wheat leaf rust population (Goyeau *et al.* 2012) were used. In Mexico, pathotypes 61/61 (virulent on *Lr61*), BBG/BP, CBG/BP, BBG/BN (Huerta-Espino *et al*., 2011) were used. Plants were inoculated at the seedling stage by spraying spores suspended in Soltrol® oil, then incubated in a dew chamber at 15-20°C for 24 h, placed in the greenhouse for the next 10 days and assessed for their infection types according to the Stakman *et al*. (1962) scale. In the field, nurseries were sown in France in 4 locations (Lectoure, Montbartier, Castelnaudary and Grisolles) and in Mexico in two.

In France, a mixture of two pathotypes was used, so as to combine the virulences for *Lr14a*, *Lr23* and *Lr72*. In Mexico, a mixture of pathotypes BBG/BP (virulent on *Lr3*) and BBG/BN was used. In France, in each location, the maximum percentage of diseased leaf was assessed independently by two to three people, using the modified Cobb scale (Peterson *et al*., 1948). In Mexico, in each location four to five disease assessments were made, allowing calculation of the area under the disease progress curve (AUDPC).

2. Genotyping

Association mapping was performed for 182 lines or cultivars, using 1300 DArT markers. Analyses were conducted independently by four different collaborators, to compare results obtained with different statistical softwares. Each collaborator used a mixed linear model as described by Yu *et al*. (2006) to calculate the marker-trait association analysis. Mixed linear model can reduce both type I and type II errors as this model simultaneously takes into account population structure and kinship. Significance of associations between loci and traits was described as p-value and the QTL effects level was evaluated by R^2 of the peak marker. All the variables issued from phenotyping were analysed independently, except for one collaborator who grouped highly correlated variables.

III – Results

1. Phenotyping

Cultivars and lines were grouped according to their profiles of infection types against the pathotypes at the seedling stage in the greenhouse. When including information provided by CIMMYT about major *Lr* genes and minor resistance genes identified in the lines, resistance groups could be defined, postulated to differ for the genetic basis of their wheat leaf rust resistance, from information with the French (Table 1) and the Mexican (Table 2) pathotypes. Field Epidemic development was good in the two Mexican locations in 2009 and 2010; in France, it was satisfactory in 2009 in three out of four locations, and in four locations in 2010. In France, a high level of resistance, due to efficient major genes, was achieved in 18 lines from CIMMYT, carrying one of the genes *Lr3*, *Lr19*, *Lr47*, *Lr61* and *LrCamayo*, as well as in Anco Marzo (*Lr27+31*), and in three cultivars (Byblos, Saragolla, and Gaza) postulated to carry unidentified major genes. Quantitative resistance was also expressed: a moderate final disease level (35-60%) was displayed by 39 lines, and 9 cultivars (Acalou, Altar, Arnacoris, Brennur, Lemur, Liberdur, Nautilur, Sachem, and Virgilio); a low level of quantitative resistance, with a final disease level of 60-70%, was displayed by 15 lines and one cultivar (Poulit). Overall, glasshouse and field phenotyping yielded 34 variables (Table 3).

Table 1. Resistance profiles of the lines and cultivars, combining information from i) infection types from the seedling tests in the greenhouse using 5 French pathotypes and ii) presence of known *Lr* genes or minor genes based on information from CIMMYT. Infection types after Stakman *et al*., (1962).

RESISTANCE FROUP	Pathotype (see Goyeau *et al*., 2012)					
	no vir.	vir 23, Altar	vir 14a	vir14a, 23	vir Altar, 23, (Gaza)	Number of lines.
No effective major gene	3+	3+	3+	3+	3+	40
Lr14a only	X++	X	3+	3+	X++	38
CIMMYT lines with minor genes	Y++	3+	Y++	X++3	3+	13
Lr23	12	3+	X-	X++3	3+	4
Lr72	1	3+	;	X++3	3+	19
Lr14a + unidentified major gene	;	X++	X++	X++3	X--	10
Lr14a + *Lr72*	0;	X-	;--	X++3	X++	28
Unidentified major gene	X+	Y+	X++	X+	X+	7
Lr14a + *Lr72* + unidentified major gene	;--	;1+	;	X++	X++	2
Unidentified major gene (Gaza), *Lr61* (Guayacan Inia)	;	;	;12	;12	X++	4
Lr72+ unidentified major gene	;-	X--	;1	X-	X-	4
Saragolla	;12	;12	;12	;1+	;1	1
Lr_{Camayo}	;	;12+	;	;	;12	3
Lr3/*Lr19*/*Lr47* or unidentified major gene	;-	;	;-	;	;-	10
Byblos	X	;-/X++	;--/X++	;--/X++	0;	1
					TOTAL	184

Table 2. Resistance profiles of the lines and cultivars, combining information from i) infection types from the seedling tests in the greenhouse using 4 Mexican pathotypes and ii) presence of known *Lr* genes or minor genes based on information from CIMMYT. Infection types after Stakman *et al*., (1962).

	A	B	C	D	Lines No.
Lr72 but *Lr14a* positive	x	3+	3+	3+	2
Lr61	3+	;1=	;1=	;1=	2
Lr27+31	;1	33+	33+	1++	3
Lr3	0;	0;	33+	0;	6
Undecided/lost/ inconclusive	-	-	-	-	13
Lr72	x	3+	3+	3+	22
Uncharacterised Seedling Resistance	;1=	x	x	x	26
No detectable seedling resistance	33+	33+	33+	33+	29
Lr14a (based on the marker)*	1=	x=	;1=	x=	81

A = Race BBG/BP vir Lr10,23,61
B =Race CBG/BP vir Lr10,11,23,27+31,72
C = Race BBG/BP virLr3,10,11,23,27+31,72
D = Race BBG/BN vir Lr10,11 23 72
**could be with or without Lr72 or any other gene*

Table 3. Phenotyping variables included in the association mapping analyses.

Name	location	year	Variable
V1, V2, V3	Castelnaudary	2009	Final % of diseased flag leaf, assessed by 3 people
V4, V5, V6	Castelnaudary	2010	Final % of diseased flag leaf, assessed by 3 people
V7, V8, V9	Montbartier	2009	Final % of diseased flag leaf, assessed by 3 people
V11, V10, V12	Montbartier	2010	Final % of diseased flag leaf, assessed by 3 people
V13, V14	Lectoure	2009	Final % of diseased flag leaf, assessed by 2 people
V15, V16	Lectoure	2010	Final % of diseased flag leaf, assessed by 2 people
V17	Grisolles	2010	Final % of diseased flag leaf
V18, V19	Obregon	2009	Final % of diseased flag leaf, RAUDPC
V20, V21	Obregon	2010	Final % of diseased flag leaf, RAUDPC
V22, V23	Batan	2009	Final % of diseased flag leaf, RAUDPC
V24, V25	Batan	2010	Final % of diseased flag leaf, RAUDPC
V26, V27, V28, V29, V30	GH France	2009 - 10	Infection types to 5 pathotypes
V31, V32, V33, V34	GH Mexico	2009 - 10	Infection types to 4 pathotypes

GH = Greehouse.

2. Genotyping

Independent analyses by four collaborators yielded similar results. The very few markers identified as significant by only one collaborator were dropped, so as to keep markers significant for at least two collaborators and two variables. A first analysis detected 37 DArT markers, corresponding to at least 3 chromosomal regions (2B, 6B, and 7B). On the chromosome 2B, markers wPt-1064, wPt-6477, and wPt-0408 were significant, with a low effect, and for four variables only (final disease scoring for one location one year in France, and two French pathotypes in the glasshouse). On the chromosome 6B, markers wPt-8059 and wPt-7065 were significant, with a low effect, and for nine variables only (two French field locations in 2009 and one in 2010). On the chromosome 7B, markers wPt-0465, andwPt-9515 were significant in the field in Mexico in 2009 and 2010; marker wPt-3700 was significant in the field in France and in Mexico, in 2009 and 2010. These three latter markers were also significant in the greenhouse with the four Mexican pathotypes, and with two French pathotypes. The corresponding QTL has a strong effect, particularly in Mexico (45% of the phenotypic variance). Comparison of mapping with DArT markers used in the present study and SSR markers performed at CIMMYT established that this QTL corresponded to gene *Lr14a*.

Haplotype 011 for markers wPt-0465, wPt-3700, and wPt-9515, respectively, was associated to an increased resistance level in Mexico, whereas it was associated to an increased susceptibility in France. A second analysis was performed, dropping lines with haplotype 011 to check whether gene *Lr14a* could mask the expression of other QTLs. For the 80 lines left, 23 DArT markers were significant; however, most of these markers were not mapped.

IV – Discussion and perspectives

The present study brought information on the effectiveness and the diversity of sources of resistance to wheat leaf rust in durum germplasm. Combined greenhouse and field phenotyping of lines and cultivars allowed detection of useful efficient major genes. However, breeding cultivars with single major genes should be avoided, as they have frequently proven to be quickly overcome, as for *Lr3* (race CBG/BP) and *Lr27+31* (race BBG/BP) in Mexico (Huerta-Espino *et al*., 2011) and *Lr14a* in France (Goyeau *et al*., 2010). A number of lines, carrying minor resistance genes, displayed an interesting level of quantitative resistance in the field. Phenotyping also brought valuable information about the diversification level of the resistance sources investigated, yielding a classification in different groups of resistance. However, genotyping is necessary to determine whether the genetic basis is indeed diversified, and to identify markers useful for marker-assisted selection. Association mapping revealed three chromosomal regions (2B, 6B, and 7B) involved in the resistance, as well as other interesting markers, which should be further investigated using a map with a higher density of markers. The bimodal distribution of French lines when dropping lines carrying *Lr14a*, suggested another major gene in this germplasm, for which we did not have close DArT markers. Moreover, our analysis revealed an increased susceptibility of lines carrying *Lr14a* in French field trials which raises the question of a deleterious effect of this gene on the resistance level. Another hypothesis could be that, given its efficiency in Mexico, and its efficiency in France before 2000, lines and cultivars with *Lr14a* could not be evaluated for their quantitative resistance, and may lack any QTL, when lines without *Lr14a* could have been selected for their good level of quantitative resistance.

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Qualitative and quantitative resistance against powdery mildew in wheat

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Abstract. Bread and durum wheats are among the most important cultivated crop plants worldwide. Powdery mildew caused by *Blumeria graminis* f. sp. *tritici* is one of the most destructive foliar diseases of wheat, affecting yield and end-use quality, especially in areas with a cool or maritime climate. Breeding for resistance using diversified disease resistance genes is the most promising approach to prevent outbreaks of powdery mildew. To date, more than 60 genes/alleles have been identified and mapped on the wheat chromosomes, and many of these genes have been extensively used in breeding. Very few have been cloned, but most of them have been tagged with molecular markers, especially microsatellites, useful for marker-assisted selection, allowing selection for resistance in the absence of the pathogen. The details about most of the resistance genes mapped on the wheat genome, the source of resistance and molecular markers tightly associated to them have been reviewed.

Keywords. Wheat – Resistance to powdery mildew – Pm genes – Marker-assisted selection.

Résistance qualitative et quantitative contre l'oïdium du blé

Résumé. *Les blés tendre et dur sont parmi les principales espèces végétales cultivées dans le monde entier. L'oïdium causé par Blumeria graminis f. sp. tritici est l'une des maladies foliaires du blé les plus destructrices, affectant le rendement et la qualité d'utilisation finale, notamment dans les régions à climat froid ou océanique. La sélection pour la résistance utilisant différents gènes de résistance aux maladies est l'approche la plus prometteuse pour prévenir l'apparition de l'oïdium. À ce jour, plus de 60 gènes/allèles ont été identifiés et cartographiés sur les chromosomes du blé, et beaucoup d'entre eux ont été largement utilisés dans la sélection. Un petit nombre de ces gènes ont été clonés, mais la plupart d'entre eux ont été marqués avec des marqueurs moléculaires, en particulier des microsatellites, utiles pour la sélection assistée par marqueurs, permettant la sélection pour la résistance en l'absence de l'agent pathogène. Dans ce travail, nous allons focaliser l'attention sur la plupart des gènes de résistance cartographiés sur le génome du blé, la source de résistance et les marqueurs moléculaires qui leur sont étroitement liés.*

Mots-clés. *Blé – Résistance à l'oïdium – Gènes Pm – Sélection assistée par marqueurs.*

I – Introduction

Bread and durum wheat are among the most important cultivated crops worldwide in terms of cultivated area and food source. Powdery mildew of wheat, caused by the biotrophic pathogen *Blumeria graminis* f.sp *tritici*, is one of the most devastating foliar diseases in temperate climates and usually leads to yield losses ranging from 5 to 34% and affects end-use quality (Conner *et al*., 2003). The disease is favoured by intensive cultivation methods associated with modern agriculture such as the use of semi-dwarf and high-yielding cultivars in combination with high levels of nitrogen fertilization. Growing resistant cultivars is the most economical and environmentally sound method to decrease the use of fungicides and to reduce crop losses due to this disease. This approach, however, requires comprehensive exploration of potential genetic resources and an in-depth understanding of their resistance mechanisms.

II – Scientific evidence

Two types of powdery mildew resistance exist in wheat: qualitatively and quantitatively inherited resistances. Qualitative resistance, also called “monogenic” or “vertical” or “race-specific”, is controlled by major race-specific genes that are generally effective only against some isolates of powdery mildew, providing a complete protection of the crop. The resistance (R) gene-mediated resistance belongs to the category of “gene-for-gene” interaction (Bennett *et a*l., 1984; Hsam and Zeller, 2002). Unfortunately, qualitative resistance is usually of short durability due to frequent changes in the pathogen population (Hsam and Zeller, 2002). Consequently, new resistance genes are continuously needed to replace the defeated ones. To date, more than 60 powdery mildew resistance genes/alleles have been reported in common and durum wheat (Alam *et al*., 2011) and some of these genes have been cloned, supported by the genome sequence information of wheat species with lower ploidy levels. In particular, *Pm3b* from hexaploid wheat is a member of the coiled-coil nucleotide binding site leucine-rich repeat (NBS-LRR) class of disease resistance genes (Yahiaoui *et al*., 2004). A putative serine/threonine protein kinase gene (*Stpk-V*) was also characterized conferring the durable resistance in the *Pm21* locus, located on the chromosome 6V of *Dasypyrum villosum* [syn. *Haynaldia villosa*] and transferred to wheat as a 6VS·6AL translocation (Cao *et al*., 2011).

The second type of powdery mildew resistance is represented by adult plant resistance (APR), also called “slow-mildewing” or “partial resistance” (Alam *et al*., 2011). It can be identified in cultivars with defeated race-specific genes or lacking known resistance genes and allows the plants to be infected with the pathogen, but significantly retards the development of disease in adult plants (Hautea *et al*., 1987). Even if it has been shown to be more durable, the quantitative nature of partial resistance to powdery mildew makes it more complicated to handle in a breeding program compared to race-specific resistance. Examples with good levels of partial resistance include the winter wheat cultivar Knox (Shaner, 1973) and the derived cultivar Massey (Liu *et al*., 2001), which have provided effective resistance against powdery mildew in the southeastern United States for half a century. Breeding for resistance has been greatly enhanced by the use of molecular markers. Many reports about high-density linkage maps used to map *Pm* genes and quantitative trait loci (QTL) governing this trait are available in literature (i.e Zhang *et al*., 2008; Lan *et al*., 2010; Muranty *et al*., 2010). Very often the partial resistance is controlled by a number of genes, but this is not always the case. An example of monogenic partial resistance is the gene *Mlo*. Homologs of barley gene *Mlo* were found in syntenic positions in the three genomes of hexaploid wheat (Elliot *et a*l., 2002; Salmeron *et al*., 2000; Niu and He, 2009; Konishi *et al*., 2010). The gene *Mlo*, isolated by positional cloning, consists of an integral membrane protein with seven transmembrane helices and two casein kinase II motifs (Büschges *et al*., 1997). Chromosomal positions of the main mapped powdery mildew resistance loci are reported in Table 1. The powdery mildew resistance genes are not equally distributed in the genome, but often form clusters of genes. Particularly reach of genes of resistance to powdery mildew are the chromosomes 7A and 2B (Table 1). The D genome seems to be the one with the lowest number of mapped genes, except for the chromosome 5D. As reported in Table 1, some genes were transferred from wild relatives, such as *T. turgidum* var. *dicoccoides* and var. *dicoccum*, *T. timopheevii*, *T. monococcum*, *T. tauschii*, *Ae. speltoides*, or from more distant species, like *Secale cereale*. It is well established that the genetic diversity of crop plants has been eroded with respect to their wild relatives as a result of the genetic bottleneck associated with the domestication process and subsequent modern breeding processes (Ladizinsky, 1998). This genetic erosion had far-reaching agronomic consequences limiting our ability to protect crop plants from biotic and abiotic stress factors and to meet future global challenges (e.g., Harlan, 1972; Zamir, 2001). Using crosses between domesticated and wild species of inbreeding plants, alleles that were “left behind” during domestication may be reintroduced into the domesticated gene-pool. Nevertheless, other genes have been identified in *T. aestivum*, and this permits to hypothesize that cultivated wheats can be even explored to identified new alleles.

Table 1. Chromosomal location, source and reference of the most mapped *Pm* genes.

Gene	Chromosome	Source	Reference
Pm3g	1A	*T.aestivum*	Bougot *et al*., 2002
Pm3e	1A	*T. aestivum*	Mohler *et al*., 2011
Mlar	1A	*T. aestivum*	Sourdille *et al*., 1999
Pm3a	1A	*NA*	Chen *et al*., 2009
Pm24	1DS	*T. aestivum*	Huang *et al*., 2000
Pm24b	1DS	*T. aestivum*	Xue *et al*., 2012
Pm4d	2A	*T. monococcum*	Schmolke *et al*., 2012
Pm23 (Pm4c)	2AL	*T. aestivum*	Hao *et al*., 2008
Pm4b	2AL	*T. dicoccum*	Mingeot *et al*., 2002
PmHNK54	2AL	*Secale cereale*	Xu *et al*., 2011
MlIW70	2B	*T. dicoccoides*	Liu *et al*., 2011
MlZec1	2BL	*T. dicoccoides*	Mohler *et al*., 2005
PmJM22	2BL	*T. aestivum*	Yin *et al*., 2009
PmPS5B (Pm33)	2BL	*T. carthlicum*	Zhu *et al*., 2005
Pm6	2BL	*T. carthlicum*	Zhu *et al*., 2005
MlAB10	2BL	*T. dicoccoides*	Maxwell *et al*., 2010
Pm42	2BS	*T. dicoccoides*	Hua *et al*., 2009
Ml5323	2BS	*T. dicoccum*	Piarulli *et al*., 2012
Pm43	2DL	*Th. intermedium*	He *et al*., 2009
Pm41	3BL	*T. dicoccoides*	Li *et al*., 2009
Pm2026	5A	*T. monococcum*	Xu *et al*., 2008
Pm36	5BL	*T. dicoccoides*	Blanco *et al*., 2008
Ml3D232	5BL	*T. dicoccoides*	Zhang *et al*., 2010
Pm16	5BS	*T. dicoccoides*	Chen et al., 2005
PmD57-5D	5D	*T. aestivum*	Ma *et al*., 2011
Pm46	5DS	*T. aestivum*	Gao *et al*., 2012
Pm34	5DL	*Ae. Tauschii*	Miranda *et al*., 2006
Pm35	5DL	*Ae. Tauschii*	Miranda *et al*., 2007
PmY201	5DL	*Aegilops tauschii*	Sun *et al*., 2006
PmY212	5DL	*Aegilops tauschii*	Sun *et al*., 2006
MlRE	6AL	*T. dicoccum*	Chantret *et al*., 2000
Pm12	6B	*Ae. spelotides*	Song *et al*., 2007
Pm27	6B	*T. timopheevii*	Jarve *et al*., 2000
PmG3M	6B	*T. dicoccoides*	Xie *et al*., 2011
PmD57 (Pm45)	6DS	*T. aestivum*	Ma *et al*., 2011
MlAG12	7A	*T. timopheevii*	Maxwell *et al*., 2009
Pm37	7A	*T. timopheevii*	Perugini *et al*., 2008
PmNCAG11	7A	*T. timopheevii*	Srnic´ *et al*., 2005
PmNCA4	7A	*T.monococcum*	Srnic´ *et al*., 2005
Mlm80	7A	*T. monococcum*	Yao *et al*., 2007
Mlm2033	7A	*T. monococcum*	Yao *et al*., 2007
PmG16	7AL	*T. dicoccoides*	Ben-David *et al*., 2010
MlIW72	7AL	*T. dicoccoides*	Ji *et al*., 2008
NCA6Pm	7AL	*T. monococcum*	Miranda *et al*., 2007
Pm1a	7AL	*T. aestivum*	Neu *et al*., 2002
PmU	7AL	*T. urartu*	Qiu *et al*., 2005
Pm22(Pm1e)	7AL	*T. aestivum*	Singrun *et al*., 2003
MlRD30	7AL	*T. aestivum*	Singrun *et al*., 2004
PmTm4	7BL	*Secale cereale* L.	Hu *et al*., 2008
Pm5e	7BL	*T. aestivum*	Huang *et al*., 2003
Pm5d	7BL	*T. aestivum*	Nematollahi *et al*., 2008
mlxbd	7BL	*T. aestivum*	Xue *et al*., 2009
Pm40	7BS	*Elytrigia intermedium*	Luo *et al*., 2009
Lr34/Yr18/Pm	7D	*T.aestivum*	Spielmeyer *et al*., 2005

NA: Not Available.

Molecular markers have been largely used for mapping to specific chromosomes or chromosome regions a number of these genes (Zhang *et al*., 2010). Currently, SSRs are the markers of choice for mapping in wheat and numerous microsatellites have been found to be associated to *Pm* resistance genes, such as *Ml3D232* on chromosome 5BL that is flanked by *Xgwm415* and *Xwmc75* (Zhang *et al*., 2010) or *Pm37* on chromosome 7AL for which two markers *Xgwm332* and *Xwmc790* have been found tightly linked to the gene (Perugini *et al*., 2008). Molecular markers have also been used to map quantitative trait loci (QTL) for partial resistance to powdery mildew in several wheat cultivars, including the Swiss winter wheat Forno (Keller *et al*., 1999), the French winter wheats RE714 (Chantret *et* al., 2000, 2001; Mingeot *et al*., 2002) and RE9001 (Bougot *et al*., 2006), the North American winter wheats Massey (Liu *et al*., 2001) and USG3209 (Tucker *et al*., 2007) and the Japanese cultivar Fukuho-komugi (Liang *et al*., 2006).

Molecular markers tightly associated to resistance QTL/genes have a great potential for utility in plant improvement and for breeders to adopt marker-assisted selection (MAS). As an example, in publicly financed wheat breeding programs in the USA, Australia and Canada, about 50 genes are used in MAS for resistance to the main wheat diseases, which include powdery mildew, rusts, cereal cyst nematode, and viruses, and similar numbers of resistance genes are available in barley (Marone *et al*., 2013). In particular on the MAS wheat website (ttp://maswheat.ucdavis.edu) in which MAS protocols to incorporate valuable genes for many traits of interest into the best wheat breeding lines are described, a MAS protocol is available for the gene *Pm34*, derived from *Ae. tauschii* and carried by the North Carolina germplasm line NC97BGTD7, and for *Pm35*, present in germplasm line NC96BGTD3, with the closely linked SSR *Xcfd26*. The knowledge of the gene sequences linked to the resistance is very important, as this allows the design of perfect molecular markers that are not subject to the risk of recombination between the marker and the R gene. A functional marker has been developed by Qin *et al*., (2012) for the gene *Pm6*, localized on chromosome 2B, which has been introduced from the tetraploid wheat *T. timopheevii* into the hexaploid common wheat. The sequence of the barley RFLP probe BCD135 found to be closely linked with the powdery mildew resistance gene *Pm6,* corresponded to a putative receptor-like protein kinase gene (*HvRPK*) in barley, a protein implicated in diverse signaling pathways such as the disease response.

III – Conclusions

A great number of resistance genes to powdery mildew have been identified and mapped in bread and durum wheat. Most of them are race-specific and therefore characterized by a short durability. To prolong and enhance the effectiveness of race-specific resistance, gene pyramiding, multi-lines, and cultivar mixtures have been proposed and used in wheat breeding programs. The availability of molecular markers, co-dominant and PCR-based, facilitates the wheat breeders in marker assisted selection (MAS). Near-complete resistance in a wheat cultivar is expected to be obtained by pyramiding the major and minor resistance genes to reach a more complete level of resistance.

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Additional genetic factors of resistance to stem rust, leaf rust and powdery mildew from *Dasypyrum villosum*

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Abstract. The gene diversity for rust and powdery mildew disease resistance is very narrow in durum wheat varieties. The chromosome 6V#4 from *D. villosum* contains genes for broad-spectrum resistance to diseases caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*) (stem rust), *Puccinia triticina* Eriks. (*Pt*) (leaf rust), *Puccinia striiformis* f. sp. *tritici* Er ks. (*Pst*) (stripe rust), and *Blumeria graminis* f. sp. *tritici (Bgt)* (powdery mildew). Progenies from the cross of a durum wheat F_7 line (derived from 'Cappelli' × 'Peleo') with CS-DA6V#4 (a disomic addition line of chromosome 6V#4 to the *T. aestivum* 'Chinese Spring' genomic background), were backcrossed to durum wheat lines in order to selected plants for resistance to airborne *Bgt* inoculum in the greenhouse as a marker for the presence of chromosme 6V#4. The chromosome number of the progenies of two of those plants, '467-68.1' and '491-50.2', ranged from 28 to 36 with an average of 2n=31, and the presence of 6V#4 was revealed by GISH. The seedlings of the two progenies were tested for response to different races (isolates) of *Pgt* and *Pt* under controlled experiments at CAR-HAS in Hungary, and to *Pgt* and *Bgt* under controlled experiments at CRA-QCE in Italy. All the seedlings from the '467-68.1' and '491-50.2' progenies, were resistant to *Pt* and *Bgt*, and the '467-68.1' progeny displayed resistance to *Pgt.* The NAU/Xibao15$_{902}$ molecular marker linked to *Pm21,* a putative locus in 6V#4 with a gene determining resistance to *Bgt*, was detected in all the seedlings of the two progenies. Plants with chromosome number ranging from 28 to 30 are now field tested and are being prepared for the final round of backcross to the '4.5.1' durum wheat recurrent parent.

Keywords. Gene for resistance – Plant disease – *Triticum turgidum* L. var *durum* – Interspecific hybridization – Gene transfer.

Autres facteurs génétiques de résistance à la rouille noire, la rouille brune et à l'oïdium de Dasypyrum villosum

Résumé. *La diversité génétique pour la résistance aux maladies de la rouille et de l'oïdium est très limitée dans les variétés de blé dur. Le chromosome 6V#4 de D. villosum contient des gènes de résistance à large spectre pour les maladies causées par Puccinia graminis f. sp. tritici (Pgt) (rouille noire), Puccinia triticina Eriks. (Pt) (rouille brune), Puccinia striiformis f. sp. tritici Eriks. (Pst) (rouille jaune), et Blumeria graminis f. sp. tritici (Bgt) (oïdium). Les descendants du croisement d'une lignée F7 de blé dur (issue de «Cappelli 'x' Peleo ') avec CS-DA6V#4 (une lignée d'addition disomique du chromosome 6V#4 au génome de T. aestivum 'Chinese Spring'), ont été rétrocroisés avec des lignées de blé dur pour sélectionner des plantes pour la résistance à l'inoculum aérien de Bgt en serre, en tant que marqueur pour la présence du chromosome 6V#4. Le nombre de chromosomes des descendants de deux de ces plantes, «467-68,1» et «491- 50,2", varie de 28 à 36 avec une moyenne de 2n=31, et la présence de 6V#4 a été révélée par GISH. Les semis des deux descendants ont été testés pour leurs réponses à différentes races (isolats) de Pgt et Pt en conditions expérimentales contrôlées au CAR-HAS en Hongrie, et à Pgt et Bgt en conditions expérimentales contrôlées au CRA-QCE en Italie. Tous les semis des descendants de «467-68,1» et «491-50,2 étaient résistants à Pt et Bgt, et le descendant 467-68,1' affichait une résistance à Pgt. Le marqueur moléculaire NAU/Xibao15902 lié à Pm21, un locus putatif de 6V#4 avec un gène déterminant la résistance à Bgt, a été détecté dans tous les semis des deux descendants. Les plantes avec un nombre de chromosomes compris entre 28 et 30 sont maintenant testées sur le terrain et soumises à la préparation pour la phase finale de rétrocroisement avec le parent récurrent de blé dur "4.5.1".*

Mots-clés. *Gène de résistance – Maladies des plantes – Triticum turgidum L. var durum – Hybridation interspécifique – Transfert de gène.*

I – Introduction

A strong global demand for durum wheat grains is expected until the year 2020. The management issues that are yet to be resolved to consistently sustain production till that time, include those related to phytopathological concerns and climate-related environmental stresses. Rusts and powdery mildew cause major production losses in bread as well as durum wheat.

There is a need for greater genetic diversity for durum wheat improvement in order to face the recent increase in occurrence of virulent and highly aggressive rust strains on all continents (including Europe) (Solh *et al*., 2012; Hodson *et al.,* 2012).

Genes for rust and powdery mildew resistance are numerous in bread wheat but few have been found in durum wheat. Many of the most effective genes have been transferred from wild wheat relatives and species from the secondary genepool, as deduced from the following review.

1. Stem rust

Wheat stem rust is caused by the fungus *Puccinia graminis* f. sp. *tritici* Eriks. & E. Henn (*Pg*t). Wheat production is threatened by the spread of a new dangerous race designated as Ug99. Currently, approximately 30 major genes conferring resistance to *Pg*t races from the seedling stage are known, plus five slow-rusting or resistance genes at adult plant stage, are being studied (Pumphrey, 2012). Thirty-eight near-isogenic lines of bread wheat carrying 21 single designated *Sr* genes for resistance to stem rust were produced and tested with nine races of stem rust by Knott (1990). To date, molecular markers have been identified for several stem rust resistance genes (*Sr 2, 6, 9a, 13, 24, 25, 26, 31, 36, 38, 39, 40*) to deploy them in new elite cultivars (Simons *et al*., 2011) and diagnostic DNA markers are being developed for other *Sr* genes (Pumphrey, 2012).

Some of those genes have been introgressed in durum wheat, and others are being transferred.

Sr9d is present in the Stakman *et al.* (1962) durum differentials Mindum, Arnautka and Spelmar; Many North American durums appear to carry *Sr9e.*

Sr13 is the only studied gene found in durum wheat with moderate resistance and effectiveness against the TTKSK race, one of the three races (the other two being TTKST and TTTSK) within the TTKS lineage originally designated Ug99. *Sr13* localized in the distal region of the long arm of chromosome 6A of several *Triticum turgidum* ssp. *durum* cultivars (McIntosh, 1972; Pumphrey 2012), was mapped within a 1.2–2.8 cM interval (depending on the mapping population) between EST markers CD926040 and BE471213 (Simons *et al*., 2011). The Ethiopian land race ST464 (PI 191365) and the domesticated emmer wheat (*T. turgidum* ssp. *dicoccon* L.) 'Khapli' (CItr 4013) have been the two major sources of *Sr13* in durum (Knott 1962; Klindworth *et al*. 2007) and nowadays *Sr13* is contained in a number of Ug99-complex resistant durum (i.e.,'Kronos', 'Kofa', 'Medora' and 'Sceptre'), in the Canadian durum wheat 'Stewart 63' (together with *Sr7* and *Sr11*) (Knott 1963; Kuznestova, 1980), and cultivated emmer varieties, although its moderate resistance to TTKS races makes it a good candidate for gene pyramiding with other stem rust resistance genes. The *Sr13* resistance gene was transferred, together with *Sr9e*, from 'ST464' to other durum wheat varieties such as 'Leeds' (Luig, 1983).

Sr14 is located very close to the centromere on chromosome 1BL (McIntosh, 1980). *Sr14,* similarly to *Sr13,* was an effective gene for resistance to *Pg*t and was transferred from dicoccum wheat which is called 'Khapli' in India to the hexaploid cv. Steinwedel, resulting in cv. Khapstein (PI 210125) (McIntosh, 1972). However its response to *Pg*t is reduced under high temperature and high light conditions (Knott, 1962; Luig, 1983; Gousseau *et al.,* 1985).

Several effective *Sr* resistance genes had been transferred to wheat from relative species. *Sr21* and *Sr22* were transferred from *T. monococcum*. *Sr24* was originally transferred in 'Agent' bread

wheat from *Thinopyrum ponticum* and is present in a translocation involving wheat chromosome 3D and one *T. ponticum* chromosome; *Sr24* is effective against most stem rust races worldwide (Smith *et al*., 1968; Yu *et al*., 2010). The initial TTKSK race was not virulent on the *Sr24* gene but the new variant of TTKS (TTKST) identified in Kenya (Jin *et al*., 2008; Jin *et al*., 2009) was virulent on *Sr24*. *Sr25* is present in 'Agatha' which also has a translocation involving wheat chromosome 7D and an *Agropyron* chromosome; *Sr26* is in a wheat-*Agropyron* translocation derived from 'Agrus" and involving wheat chromosome 6A; and *Sr27* has been found in a wheat-rye (*Secale cereale* L.) translocation line 73.214.3-1 from the University of Sydney. The lines carrying those genes were resistant to all nine *Pgt* races tested by Knott (1990).

Sr31 is a gene located in the short arm of chromosome 1R from 'Petkus' rye and introgressed into hexaploid wheat as a 1RS·1BL translocation, and *Pgt* race TTKSK was the first stem rust race reported to be virulent on this gene (Zhang *et al.,* 2010).

Sr33 gene was discovered from *Ae. tauschii*, the diploid progenitor of the D genome in hexaploid wheat and was introgressed into common wheat (*Triticum aestivum*, genomes AABBDD) (Kerber and Dyck, 1978). It is tightly linked to *Gli-D1* on chromosome arm 1DS (5.6 to 7.6% recombination) and less tightly to the centromere (29.6% rec.) and to *Glu-D1* (39.5% to 40.9 % rec.) (Jones *et al.,* 1991). The *Sr33* gene encodes a coiled-coil, nucleotide-binding, leucine-rich repeat protein and is orthologous to the barley (*Hordeum vulgare*) *Mla* mildew resistance genes that confer resistance to *Blumeria graminis* f. sp. *hordei*. It has been recently cloned (Periyannan *et al*., 2013) and when used for genetic transformation experiments of the 'Fielder' wheat cultivar, which is susceptible to the Australian *Pgt* race 98-1,2,3,5,6, the resulting transgenic lines expressed the *Pgt* resistant phenotype. When introgressed alone into hexaploid wheat, *Sr33* provides a valuable, intermediate level of resistance to diverse *Pgt* races, including the race TTKSK (Rouse *et al.,* 2011) but, preferably, *Sr33* should be deployed together with genes like *Sr2* to maintain its resistance.

Sr35, originally transferred from *Triticum monococcum* to hexaploid wheat (McIntosh *et al*., 1984), is effective against TTKSK (Jin *et al*., 2007) displaying a strong hypersensitive reaction to that race. Monogenic lines carrying *Sr35* exhibited resistant to moderately resistant infection responses with relatively low disease severity in field nurseries in Kenya in 2005 and 2006 (Jin *et al*., 2007). *Sr35* was first assigned to the long arm of chromosome 3A (McIntosh *et al*., 1984) and later mapped 41.5 cM from the centromere and 1cM from the red grain color gene *R2*. *Sr35* shows also hypersensitive reaction to TRTTF race groups when introgressed into hexaploid wheat but is susceptible to some *Pgt* races and, therefore, should not be deployed alone. The *Sr35* gene has recently been cloned and it was demonstrated (Saintenac *et al*., 2013) that is a coiled-coil, nucleotide-binding, leucine-rich repeat gene lacking in the A-genome diploid donor and in polyploid wheat. The identification and cloning of *Sr33 and Sr35* opens the door to transgenic approaches to control the devastating races of *Pgt* in both durum and bread wheat cultivars.

Sr36 is an additional wild-relative-derived stem rust resistance gene frequently used by wheat breeders (Olson *et al*., 2010a). *Sr36* was transferred from *Triticum timopheevii* (Allard and Shands, 1954) and is present in several commercial wheat varieties (Olson *et al*., 2010a; Yu *et al*., 2010). The initial TTKSK race was not virulent on that gene. Unfortunately, the new variant of TTKS (TTTSK) identified in Kenya (Jin *et al*., 2007; Jin *et al*., 2009) was virulent on plants carrying *Sr36* gene.

Sr44 maps on the short arm of the *Th. intermedium* 7J#1S short chromosome arm. Liu *et al*. (2013) produced a line with a homozygous compensating wheat-*Th. intermedium* T7DL•7J#1S Robertsonian translocation which carries *Sr44* on the 7J#1S fragment. *Sr44* confers resistance the Ug99 race complex including races TTKSK, TTSKT, and TTTSK.

Sr52 was transferred into wheat from *Dasypyrum villosum*. A set of whole arm Robertsonian translocations involving chromosomes 6A of wheat and 6V#3 of *D. villosum* was produced

through centric breakage-fusion (Qi *et al*., 2011). *Sr52* was mapped to the long chromosome arm 6V#3L of *D. villosum*, and when it was transferred to wheat it translocated with chromosome arm 6AL. *Sr52* shows a temperature-sensitive resistance pattern to stem rust race Ug99 (TTKSK): it is most effective at 16°C, partially effective at 24°C and ineffective at 28°C. *Sr15* becomes also less effective at higher temperatures (Roelfs, 1988). The variation of resistance related to the temperature could hinder field deployment, since the fungal pathogen is more active at warmer temperatures.

Significant stem rust resistance quantitative trait locus (QTL) were detected on chromosome 4B of the durum wheat cv Sachem (Singh *et al*., 2013).

2. Leaf rust

Leaf rust caused by *Puccinia triticina* Eriks. (*Pt*) is an important disease that causes significant wheat production losses worldwide. At present over 50 genes controlling wheat leaf resistance are known (McIntosh *et al.* 1995) and only two of them, *Lr14a* and *Lr23,* originated from tetraploid wheat (Herrera-Foessel *et al.*, 2005). Survey studies based on tests of seedlings with different rust isolates and molecular genotyping have shown the presence of *Lr1*, *Lr3*, *Lr10*, *Lr14a*, *Lr16*, *Lr17a*, *Lr19*, *Lr23*, *Lr25*, *Lr33*, *Lr61* and *Lr64* in the elite durum wheat germplasm (Terracciano *et al*., 2013).

Race-specific genes for leaf rust resistance frequently undergo “boom-and-bust” cycles. Examples of this are given by genes *LrAlt* in ‘Altar 84’ released in 1984 which was broken down in 2001 by race BBG/BN; and genes *LrAlt, 27+31 in* ‘Jupare’ released in 2001 which broke down in 2007 by race BBG/BP (Singh, 2012). The novel virulent leaf rust race BBG/BN and its variant BBG/BP overcame the resistance of widely adapted durum cultivars in North-western Mexico which had been effective and stable for more than 25 years (Huerta-Espino *et al.,* 2009 a, b).

Lr14a is a dominant leaf rust resistance gene originally transferred from emmer wheat ‘Yaroslav’ to the hexaploid wheat lines Hope and H-44 by McFadden (1930). It has been found in the Chilean durum cv. ‘Llareta INIA’ and in CIMMYT-derived durum ‘Somateria’. The *Lr14a*-resistance gene was also present in the durum wheat cv. ‘Creso’ and its derivative cv. ‘Colosseo’ is one of the best characterized leaf-rust resistance sources deployed in durum wheat breeding. It was mapped to chromosome arm 7BL through bulked segregant analysis using the amplified fragment length polymorphism (AFLP) technique. Several simple sequence repeat (SSR) markers, including *Xgwm344-7B* and *Xgwm146-7B*, were associated with the *Lr14a* resistance gene in both common and durum wheat (Herrera-Foessel *et al*., 2008) in the distal portion of the chromosome arm 7BL, a gene-dense region (Terracciano *et al*., 2013). Gene *Lr14a* is linked to stem rust and powdery mildew resistance genes *Sr17* and *Pm5*, respectively. However, the original ‘Yaroslav’ accession that carried the relevant genes (i.e., *Sr17, Lr14a*, and *Pm5*) and the slow-rusting stem rust resistance gene *Sr2* (chromosome 3B) has been lost (McIntosh *et al.,* 1995.).

Lr19 was a highly effective gene against five different *Pt* pathotypes (TKF/H, SKF/G, PHT/B, THT/F, and KHP/C) and was identified in ‘Dur’ and ‘Valdur’ varieties (Shynbolat and Arakeyat, 2010).

Lr23 was shown to be an effective resistance gene against the five mentioned *Pt* pathotypes avirulent on *Lr19* and was found in the durum wheat varieties ‘Albatross’, ‘Cocorit71’, ‘VZ-187’ and ‘Nauryz6’ (Shynbolat and Arakeyat, 2010). *Lr23* was transferred to common wheat from durum wheat cv. ‘Gaza’ and cytogenetically mapped to chromosome 2BS (McIntosh and Dyck, 1975).

The wild emmer wheat *T. turgidum* ssp. *dicoccoides* was the source of many genes for resistance to *Pt* such as *Lr53,* located in chromosome 6BS (Marais *et al*., 2005) and another genes expressing the same infection types as *Lr33* (Dyck, 1994).

Evidence have been provided that resistance to *Pt* in the F_2 and F_3 progenies of 'Atil C2000' (susceptible durum parent) × 'Hualita' (resistant durum parent) was due to complementary leaf rust resistance genes (Herrera-Foessel *et al*., 2005). Previously identified and designated complementary leaf rust resistance genes were *Lr27* and *Lr31* in bread wheat (Singh and McIntosh, 1984 a, b) which were located on chromosomes 3BS and 4BL, respectively (Singh and McIntosh, 1984b). Gene *Lr31* is either completely linked or the same as *Lr12* (Singh *et al.,* 1999).

The French durum wheat cultivar Sachem was resistant, while Strongfield, the predominant cultivar grown on the Canadian prairies, was moderately susceptible to stripe rust, BBG/BN leaf rust race and Ug99 stem rust races. A major leaf rust QTL was identified on chromosome 7B at *Xgwm146* in Sachem. In the same region on 7B, a stripe rust QTL was identified in Strongfield. A significant leaf rust QTL was detected on chromosome 2B where a *Yr* gene derived from Sachem conferred resistance (Singh *et al*., 2013).

Adult-plant resistance genes *Lr13* and *Lr34* singly and together have provided the most durable resistance to leaf rust in bread wheat throughout the world (Kolmer, 1996). *Lr34* has been found in Strampelli varieties 'Ardito' and 'Mentana' (Salvi *et al*., 2013) and in 'Chinese Spring' bread wheat in which the *Lr12* gene is also present (Dyck, 1991). Previous studies have located the codominant gene *Lr34* on the short arm of chromosome 7D. This location hindered the transfer of *Lr34* in durum wheat to support durable resistance. *Lr34* is linked to *Yr18* and co-segregate with other traits such as leaf tip necrosis (*Ltn1*), *Pm38* for powdery mildew resistance and *Bdv1* for tolerance to Barley yellow dwarf virus (Kolmer *et al,* 2008). *Lr34* has been cloned (Krattinger *et al.* 2009) and when deployed with other adult plant resistance genes, near-immunity can be achieved (Singh and Trethowan, 2007).

It would be extremely useful if an *Lr34*-like gene associated to other multiple disease resistance could be found in diploid relatives, because it will provide breeders with diverse genes for pyramiding and increase the durability of resistance in durum wheat.

3. Stripe rust

Stripe rust (or yellow rust) of wheat, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), has become more severe in eastern United States, Australia, and elsewhere since 2000. Markell and Milus (2008) observed that isolates collected before 2000 had diverse virulence phenotypes, were usually virulent only on a few of the differential lines, and were always avirulent on resistance genes *Yr8* and *Yr9*. On the other hand, isolates collected since 2000 had similar virulence phenotypes, were usually virulent on approximately 12 of the differential lines, and were always virulent on differentials carrying *Yr8* and *Yr9*. Those results indicated that isolates causing severe epidemics in the United States since 2000 did not arise by mutation from the existing population and were most likely from an exotic introduction adapted to warmer temperatures (Milus *et al.,* 2009).

About 52 permanently named and more than 40 temporarily designated genes or quantitative trait loci (QTL) for stripe rust resistance have been reported (Chen *et al.*, 2002; Chen 2005; Ren et al. 2012). Among the permanently named resistance genes, *Yr11*, *Yr12*, *Yr13*, *Yr14*, *Yr16*, *Yr18*, *Yr29*, *Yr30*, *Yr34*, *Yr36*, *Yr39*, *Yr46*, *Yr48* and *Yr52* confer adult plant or high-temperature adult plant (HTAP) resistance, which is expressed when plants grow old and weather becomes warm, whereas the others confer all-stage resistance (Park *et al*., 1992; Xu *et al.*, 2013). Of the permanently named *Yr* genes, 14 were transferred from common wheat relatives, such as *T. aestivum* ssp. *spelta* var. *album*, *T. dicoccoides*, *T. tauschii*, *T. turgidum*, T*. turgidum* var. *durum*, *T. ventricosum*, *Aegilops* (*Ae*.) *comosa*, *Ae. geniculata*, *Ae*. *kotschyi*, *Ae*. *neglecta*, *Ae*. *sharonensis*, *Dasypyrum villosum*, and *Secale cereale* (Chen 2005; Xu *et al*., 2013). At least one gene for resistance to *Pst* was located on the short arm of chromosome 6V of *D. villosum* in the

6VS/6AL-translocation line from cv. Yangmai-5 (obtained by Chen PD, CAAS, China); this gene was named *Yr26* (Yildirim *et al*., 2000).

Resistance genes *Yr7*, *Yr15*, *Yr24*/*Yr26* and *Yr36* originated from tetraploid wheat accessions (Xu *et al*., 2013). *Yr36* was first discovered in wild emmer wheat (*T. turgidum* ssp. *dicoccoides* accession FA15-3. In controlled environments, plants with *Yr36* are resistant at relatively high temperatures (25° to 35°C) but susceptible at lower temperatures (e.g., 15°C) (Fu *et al*., 2009). The *Yr36* gene has been cloned but it has not yet been transferred in modern durum and bread wheat varieties (Fu *et al*., 2009). The durum wheat PI 480148 from Ethiopia possessed the gene *Yr53,* was resistant to *Pst* races under controlled greenhouse conditions at the seedling stage, and was resistant also at multiple USA locations subjected to natural infection of *Pst* for several years (Xu *et al*., 2013). The gene was mapped to the long arm of chromosome 2B and is flanked by the SSR marker *Xwmc441* (5.6 cM) and RGAP marker *XLRRrev/NLRRrev350* (2.7 cM). Xu *et al*, 2013, found that the gene is different from *Yr5*, which is also located on 2BL, 21 cM away from the centromere (Law, 1976). The *Yr5* gene confers resistance to all *Pst* races identified so far in the United States.

4. Powdery mildew

Wheat powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is one of the most severe diseases of wheat worldwide. Up to now, 41 loci (*Pm1 to Pm45*, *Pm18=Pm1c*, *Pm22=Pm1e*, *Pm23=Pm4c*, *Pm31=Pm21*) with more than 60 genes/alleles for resistance to *Bgt* isolates have been identified and located on various chromosomes in bread wheat and its relatives (Alam *et al*., 2011). Thirteen *Pm* genes were found in tetraploid wheats but only one gene, *Pm3h*, might have originated from a cultivated *T. durum* Ethiopian line (Hsam and Zeller, 2002). Several genes were identified and transferred from other domesticated as well as wild relatives, such as *T. timopheevii* (Zhuk.), *T. monococcum* (L.), *T. tauschii* (Schmalh), *Aegilops speltoides* (Tausch), *Thinopyrum intermedium (Pm43), Secale cereale* (L.) and *Dasypyrum villosum*. In this last species, a putative serine/ threonine protein kinase gene (*Stpk-V*) in the *Pm21* locus (Cao *et al*. 2011) was characterized as conferring durable resistance and was located on the short arm of chromosome 6V (Chen et al., 1995). Pm21 provide a broad-spectrum resistance to *Bgt* which cannot easily be overcome by newly developed *Bgt* races and is correlated with durability of resistance; *Pm21* was transferred to wheat as a 6VS · 6AL translocation (Cao *et al*., 2011).

The above information indicate that durum wheat has a narrow genetic basis for rust and powdery mildew resistance, and only few well characterized disease resistance genes are known in that species, which have been prevalently transferred from Ethiopian accessions or its wild relative *T. dicoccoides*. Transfer of disease resistance genes from wheat relatives to bread wheat occurred directly neglecting the role of durum wheat as a bridge species especially in the transfer of disease resistance genes from diploid wheat relatives. Most designated *Sr*, *Lr*, *Yr*, and *Pm* genes which are effective in the wheat genetic background have been transferred from wild relatives. Some of those genes provide broad-spectrum resistance such as the stem rust resistance *Sr33* from *Ae. tauschii*, the leaf rust resistance gene *Lr34* from Chinese bread wheat landraces, the stripe rust resistance gene Yr36 from T. turgidum ssp. dicoccoides, and the powdery mildew resistance gene *Pm21* in *D. villosum.*

Those genes are scattered in different chromosomes of diverse varieties and are difficult to pyramid in one wheat variety. However, the above review indicated that chromosome 6V from the diploid wild species *D. villosum* of the secondary gene-pool of wheat (De Pace *et al*., 2011), contains genes at the *Sr52* locus for resistance to *Pg*-Ug99 races, and at the *Yr26* and *Pm21* loci for resistance to *Pst* an *Bgt* races, respectively. Other observations indicated that 6V contain stronger genes then *Lr34* for resistance to *Pt* (Bizzarri *et al*., 2009). Therefore, 6V is a rich source of genes for broad-spectrum resistance to *Pg*, *Pt*, *Pst*, and *Bgt*, which can simultaneously be transferred to wheat in one round of hybridization. This has been achieved, and the 6V#4 chromosome has

been added to the 'Chinese Spring' ('CS') genomic background as disomic addition (IBL CS×V63, 2n=44) or as disomic 6V#4(6B) substitution (IBL CS×V32, 2n=42). Those IBLs have repeatedly expressed adult plant resistance to *Pgt*, *Pt*, *Pst*, and *Bgt* under controlled greenhouse conditions and at two locations subjected to natural infection for several years, while 'CS', used as control, expressed susceptibility. Therefore, 6V#4 is a good candidate for simultaneously transferring multiple genes for rusts and powdery mildew resistance to durum wheat. Here we report the first attempts in such endeavor.

II – Material and methods

1. Plant material

The lines used in this study included: (a) the durum wheat line '4.5.1'; (b) the durum wheat cvs 'Cappelli' (used as control for the infection experiments in the greenhouse) and 'Creso' (used as control for the PCR experiments); (c) the introgression breeding lines (IBL) obtained after crossing *T. aestivum* cv 'Chinese Spring' ('CS') to *Dasypyrum villosum*, followed by backcrossing to 'CS' and several generations of selfing; the IBLs contained chromosome 6V#4 in 'CS' genomic background under the configuration of a disomic additon CS-DA6V#4 in line 'CSxV63' and as a disomic substitution CS-DS6V#4(6B) in line 'CSxV32'; and (d) two progenies from the plants '467' (progeny 68.1) and '491' (progeny 50.2) whose pedigree is depicted in Fig. 1. After the initial cross between a durum wheat F_7 line (derived from crossing the durum wheat cvs 'Cappelli' × 'Peleo') and 'CSxV63', the hybrid progenies were composed by the plants labeled '481', '488', and '494'. Those hybrid plants were crossed to '4.5.1' (selected from the progeny of 'Peleo' × 'Trinakria') and the resulting F_3 progenies were backcrossed to '4.5.1' to produce the progeny from which the plant '491' was selected. The hybrid plants were also crossed to the line '498' (from 'Cappelli × Peleo'), and the resulting F_3 progeny was crossed to '4.5.1' obtaining the progeny from which the plant '467' was selected. Plants '467' and '491' were selected for their resistance to air-born *Bgt* inoculum in greenhouse (Fig. 2). Caryopses of the '467-68.1' and '491-50.2' progenies were germinated and the root-tips were used for chromosome counting; the seedlings were tested for response to different races (isolates) of *Pgt* and *Pt* under controlled experiments at CAR-HAS, Martonvásár, Hungary, and to two isolates of *Pgt* and one isolate of *Bgt* under controlled experiments at CRA-QCE, Rome, Italy.

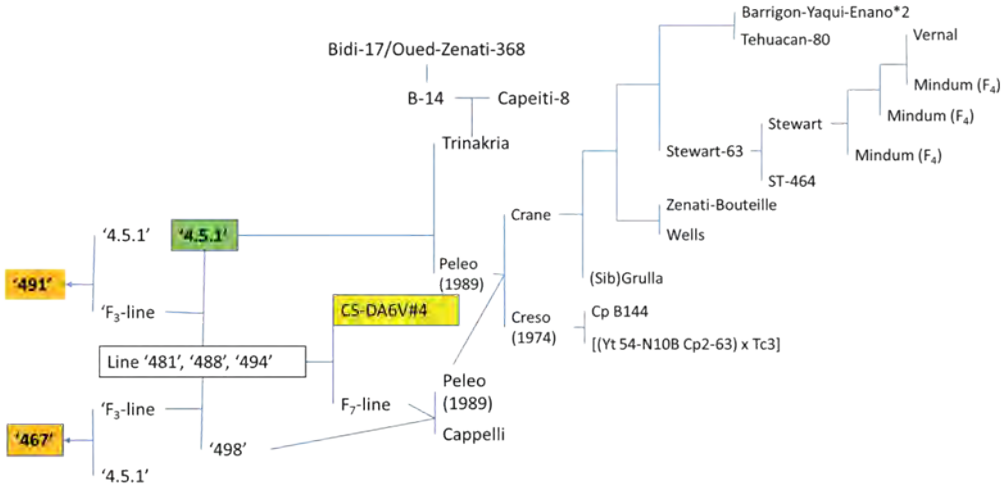

Figure 1. Pedigree of the '467' and '491' plants resistant to *Bgt* infection in the greenhouse.

Figure 2. *Bgt*-resistant plant '491' (right) and the *Bgt*-susceptible parental durum wheat line '4.5.1' (left), grown side-by-side: clear qualitative differences in their response to the natural mildew population in the greenhouse are displayed.

A. Chromosome counting and Genomic *in situ hybridization (GISH).*

For chromosome counting, the root apical meristems of seedlings from the '467-68.1' and '491-50.2' progenies were pretreated with a 0.05% aqueous solution of colchicine (Sigma) for 4 h at room temperature, fixed in ethanol-acetic acid 3:1 (v/v), and Feulgen-stained after hydrolysis in 1N HCl at 60°C for 8 min. The apices were treated with a 5% aqueous solution of pectinase (Sigma) for 30 min at 37°C and squashed under a coverslip in a drop of 60% acetic acid. The coverslips were removed by the solid CO_2 method. After air-drying, the slides were subjected to three 10-min washes in SO_2 water prior to dehydration and mounting in DPX (BDH).

B. Controlled infection at Martonvásár using Pgt *and* Pt *isolates*

The plants were inoculated in the seedling stage with a mixture of *Pt* or *Pgt* uredospores collected from varieties with various genetic backgrounds and multiplied in the greenhouse. The *Pt* pathogen population used was avirulent on the 'Thatcher'-based near-isogenic lines (NILs) with *Lr9*, *Lr19* or *Lr29* and the severity was less than 10% on the NILs carrying *Lr24*, *Lr25* or *Lr28* resistance genes in the adult plant stage. The pathotypes in the *Pgt* population were avirulent on the *Sr36* 'LMPG'-based NIL, and the severity was 20% with moderately susceptible response for NILs with *Sr9d* and *Sr31* genes. Seedlings were inoculated with uredospore suspension of *Pt* or *Pgt* by brush at GS11 and the symptoms were evaluated according to the 0-4 scale (0 = immune, 4 = susceptible; Stakman *et al*., 1962) ten days after inoculation.

C. Controlled infection CRA-QCE Rome using Pgt and Bgt races.

The material was tested at 10-day-old seedling stage in the greenhouse using the *Bgt* isolate O2 and the *Pgt* isolates 16716-2 and 16713-5-2, identified within the Italian pathogen populations of the respective pathogens. These isolates, collected from experimental nurseries located

in Central Italy, were chosen because of their virulence characteristics with respect to known resistance genes. The *Bgt* isolate O2 was virulent to many known mildew resistance genes, including *Pm1*, *Pm2*, *Pm3c*, *Pm4a*, *Pm4b*, *Pm5*, Pm6 and *Mli*, but it was avirulent to *Pm3a*, *Pm3b* and *Pm3d*. The two *Pgt* isolates showed low infection types (ITs 0; to 2) on differential lines with resistance genes *Sr17, Sr24, Sr26, Sr27, Sr31, Sr35*, *Sr38* and high infection types (ITs 3 to 4) on lines with *Sr5*, *Sr6*, *Sr7b*, *Sr8a, Sr8b, Sr9a, Sr9b*, *Sr9e, Sr9d, Sr9g, Sr10, Sr15, Sr22, Sr36, Sr38* and *SrTmp.* Seedlings, with the first leaf fully expanded, were inoculated and incubated at 100% relative humidity for 24h at 20°C in the dark and then placed on greenhouse benches covered with clear plastic chambers, at 22 ± 2°C with a photoperiod of 14 h.

For what concerns powdery mildew infection types at the seedling stage were recorded 10-12 days after inoculations, following the 0-4 infection type (IT) scoring system, in which ITs from 0 (no micelia) to 2 (small micelia spots) were considered the expression of resistance and ITs from 3 to 4 (dense and large micelia spots) were considered as host susceptibility (Pasquini and Delogu, 2003). Regarding stem rust, infection types (ITs) on the basis of a 0-4 scale according to Stakman *et al*. (1962) were assessed 12 and 15 days post inoculation. Also in this case infection types from 0 to 2 were considered as a low response, indicating a resistant or moderately resistant host. Infection types from 3 to 4 were considered as a high response, indicating a moderately susceptible or susceptible host.

D. DNA extraction and Marker Analysis

Seedlings from the controlled infection experiment carried-out at CRA-QCE, Rome, were sprayed with fungicide after scoring the response to *Bgt*, and moved to the glasshouse of University of Tuscia in Viterbo for growing until the grain ripening stage. The tips from newly emerged leaves were used for DNA extraction applying the DNeasy Plant Mini kit (Qiagen) according to the manufacturer instructions.

Polymerase chain reaction (PCR) amplification using the NAU/Xibao15$_{902}$ foreward and reverse primers flanking the coding sequence of *Pm21* gene located in the short arm of chromosome 6V#4 (Cao *et al*., 2006) took place in 25-µL volume, running in a GeneAmp PCR System 9700 (Applied Biosystems) thermocycler. The PCR mixture consisted of 1x PCR buffer, 0.2 mM of each dNTPs, 5 pmol of each primer, 1 unit of *Taq* DNA polymerase, and an amount of 20 ng of DNA template. Reagents were obtained from Applied Biosystems (Foster City, CA). Temperature profiles consisted of an initial DNA denaturation at 94° C for 3 min, and then 32 amplification cycles according to the following programme: 94° C for 30 s, 55° C for 30 s, and 72° C for 2 min. A final 8-min extension at 72° C was also employed.

The amplification products were separated on 1.5% (w/V) agarose gel in TBE buffer (1×), stained with ethidium bromide; the gels were visualized under UV light and pictured using the Kodak Gel Logic 100 Imaging System.

III – Results and discussion

The average chromosome number in the progenies ‘467-68.1’ and ‘491-50.2’ was 2n=31, and the highest proportion of metaphase plates contained 2n=32 chromosomes (Table 1). The homologous pair of 6V#4 was present among the 31 chromosomes of ‘467’-68.1, together to 14 A, 14 B, and 3 D chromosomes (Fig. 3). The NAU/Xibao15$_{902}$ molecular marker linked to the *Pm21* locus in 6V#4 which carry the putative gene determining resistance to *Bgt,* was detected in all the seedlings of the ‘467-68.1’ and ‘491-50.2’ progenies and in ‘CSxV63 (Fig. 4) but was absent from the ‘4.5.1’ and ‘Creso’ durums.

The parental lines ‘CSxV63’ and ‘4.5.1’ when tested at Martonvásár with *Pg*t isolates during the seedling stage, expressed infection types that denoted host susceptibility. When tested at CRA-

QCE-Rome, a similar response was observed for '4.5.1' but the 'CSxV63' line was resistant. This result might be explained by assuming different effects of the pathogen-genotype x host-genotype interaction exerted by the *Pg*t isolates used in Rome experiments compared to the *Pg*t isolates used in the Martonvásár experiments. The resistance to *Pgt* and *Pt* expressed at the seedling stage by 'CSxV63' is an unexpected observation, because in previous infection experiments, the genes for resistance to leaf rust were fully expressed at adult stage rather than at the seedling stage in the 'CSxV63' parental line (Bizzarri *et al*., 2009).

Table 1. Chromosome number counted in metaphase plates prepared from root-tips of seedlings of the progenies ' 491-50.2' and '467-68.1'. The progenies were obtained from the plants '467' and '491' whose pedigree is drawn in Fig. 1.

Chromosome	Metaphase plates (%)	
No.	491-50.2 progeny	467-68.1 progeny
26	9.4	3.2
27	0	4.3
28	3.1	10.6
29	6.3	1.7
30	14.6	21.3
31	6.3	8.5
32	18.8	28.7
33	15.6	3.2
34	15.6	8.6
35	9.3	0
Average	31.5	30.5

All the seedlings from the '467-68.1' progeny were consistently resistant to the *Pg*t and *Pt*, isolates used in the controlled infection experiments (Table 2). The seedlings of the '491-50.2' progeny expressed susceptibility symptoms when infected with *Pg*t isolates at Martonvásár (no data were available from the experiment in Rome due to poor seedling growth), but displayed resistance when infected with *Pt* isolates (Table 2). It is not known whether the rust resistance genes in 6V#4 interact with other genes in the chromosomes of the '467-68.1' line to produce improved resistance. However, the two lines had both an average chromosome number of 31, and the extra chromosomes over the euploid 2n=28 number, might be different between the two lines, providing opportunities for differential interaction. In other instances, it has been found that rust genes such as *Lr34* can interact with other genes to give enhanced levels of resistance (Dyck and Samborski, 1982; Dyck, 1991).

Table 2. Tested materials at the seedling stage for response to isolates of stem rust (*Pgt*), leaf rust (*Pt*) and powdery mildew (*Bgt*) in controlled infection experiments carried-out at CAR-HAS, Martonvásár (Hungary) and CRA-QCE, Rome (Italy).

	Response[(1)] to			
Tested entry	*Pgt*	*Pgt*	*Pt*	*Bg*
	Martonvasar	Rome	Martonvasar	Rome
467-68.1	0/N	1-	X	1=
391-50.2	3	°	2	0
CSxV63	3	0	4	0 to 1
CSx32	4	3+	X	0 1
4.5.1	3	3-	3	3-
Cappelli	4	4	3	3

(1) Infection types 0, N, X, 1, 2 indicate a resistant host response; Infection types 3-, 3, 3+ and 4 represent susceptible reactions.

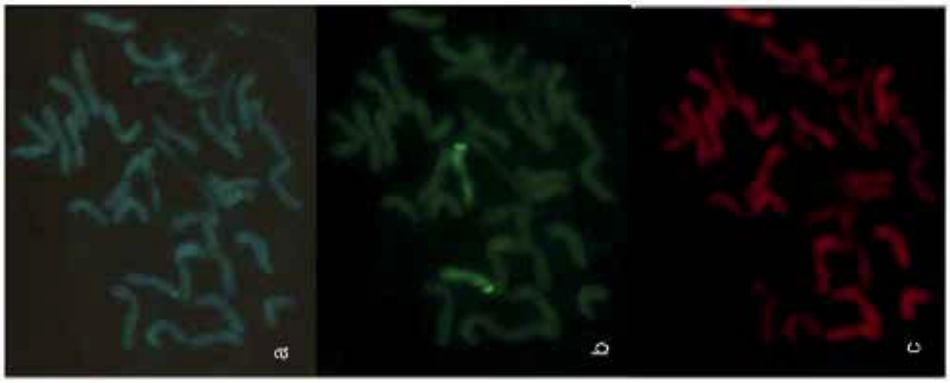

Figure 3. Metaphase plate in a root-tip meristem cell of the line '467-68.1' containing 33 chromosomes (14 'A', 14 'B', 3 'D', and 2 '6V'). (a) DAPI staining; (b) GISH using labeled DNA of *D. villosum* (FITC) and *Ae. speltoides* (wheat B genome) blocking DNA; (c) GISH using labeled DNA of *Triticum urartu* (A genome; Cy3) and *Ae. speltoides* blocking DNA. The 6V chromosome pair can be seen in b, and 14 chromosomes of wheat A genome can be seen in c. ×1,500.

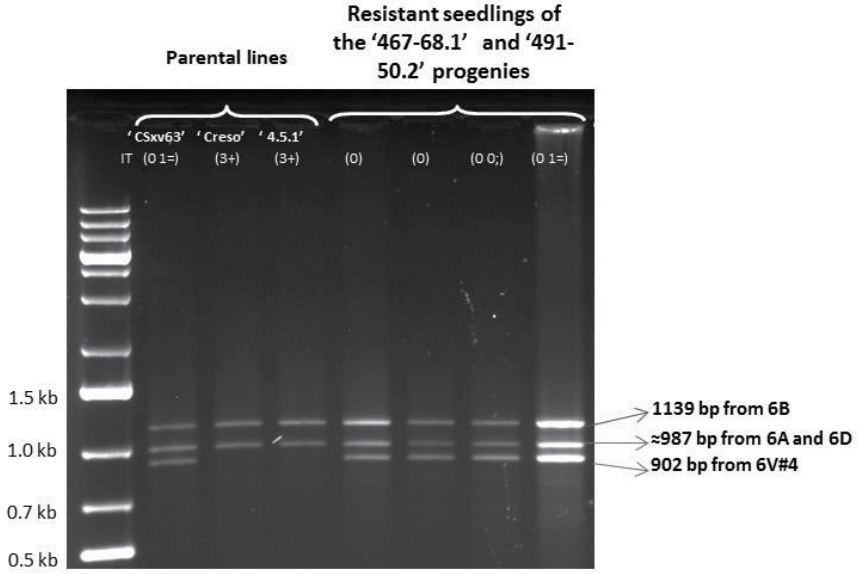

Figure 4. Amplicon of 902 bp obtained from the PCR using NAU/Xibao15 primers flanking the locus *Pm21* on the short-arm of 6V#4 containing a gene encoding a serine/ threonine protein kinase gene (*Stpk-V*) conferring broad-spectrum resistance to powdery mildew caused by Bgt. The primers amplify also an orthologous amplicon of 1.139 kbp from 6B, and another orthologous amplicon of about 0.987 kbp from 6A and 6D chromosomes. The 902 bp amplicon was absent in the pattern of the '4.5.1' parental line and in the 'Creso' durum wheat, but was present in 'CSxV63' parental line, and was detected in all the plants of the '467-68.1' and '491-50.2' progenies expressing infection type (IT) denoting host resistance to *Bgt*.

All the seedlings from the '467-68.1' progeny were consistently resistant to the *Pg*t and *Pt*, isolates used in the controlled infection experiments (Table 2). The seedlings of the '491-50.2' progeny

expressed susceptibility symptoms when infected with *Pg*t isolates at Martonvásár (no data were available from the experiment in Rome due to poor seedling growth), but displayed resistance when infected with *Pt* isolates (Table 2). It is not known whether the rust resistance genes in 6V#4 interact with other genes in the chromosomes of the '467-68.1' line to produce improved resistance. However, the two lines had both an average chromosome number of 31, and the extra chromosomes over the euploid 2n=28 number, might be different between the two lines, providing opportunities for differential interaction. In other instances, it has been found that rust genes such as *Lr34* can interact with other genes to give enhanced levels of resistance (Dyck and Samborski, 1982; Dyck, 1991).

Infection with *Pt* isolates demonstrated that both parental lines were susceptible at the seedling stage while the 'CSxV32' control line carrying also 6V#4 and both '467-68.1' and '491-50.2' progenies, displayed a resistant infection type. Since the 'CSxV63' and 'CSxV32' IBLs contain the same 6V#4 but in a different genomic background (6B is missing in 'CSxV32), the different reaction of the two IBLs to *Pt* infection at Martonvásár ('CSxV32' is more resistant than 'CSxV63) might reflect the possibility that the resistance genes to *Pt* in 6V#4 interact with genes in 6B of the 'CSxV63' line resulting in higher susceptibility rating. Such possibility of interactions in the tested lines needs further investigation.

All the entries with chromosome 6V#4 (the progenies '467-68.1' and '491-50.2', 'CSxV63' and 'CSxV32') were highly resistant to *Bgt*, while the durum wheat entries '4.5.1' and 'Cappelli' were susceptible, confirming that 6V#4 carry the allele for resistance to *Bgt* at the *Pm21* locus.

IV – Conclusions

All the seedlings from the '467-68.1' progenies were consistently resistant to virulent strain of the *Pg*t, *Pt*, and *Bgt* pathogens because they inherited, from the 'CSxV63' parental line, the chromosome 6V#4 with the genes for resistance to races of these pathogens. The resistance to *Pg*t and *Pt* expressed at the seedling stage was an unexpected observation, because in other experiments it was shown that the rust resistance genes were expressed at the adult stage. Selected plants from the '467-68.1' progeny with chromosome number ranging from 28 to 30 and expressing resistance to rusts and powdery mildew under controlled experiments, are the best candidates for: (a) scoring their response to airborne inoculum of *Pg*t, *Pt*, *Pst*, and *Bgt* at the adult stage and (b) completing the transfer of chromosome 6V#4 in the euploid 2n=28 durum wheat genome by a final round of backcross to the '4.5.1' durum wheat recurrent parent.

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Session 6

Genetics and breeding for nutritional and technological quality

Improvement of technological and nutritional quality in durum wheat: achievements and perspectives

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Abstract. Durum wheat represents one of the main food source for numerous countries of the Mediterranean area. It is mainly used for pasta production, but also in an array of other regional foods such as flat bread, cous cous, burghoul, etc. Breeding activities for quality in durum wheat have been mainly targeted towards the production of high-yielding cultivars with superior pasta-making characteristics. The role played by the gluten proteins in influencing processing characteristics of semolina, and in particular by the low-molecular weight glutenin subunits, has been elucidated. This has resulted in the development of breeding strategies for modifying protein composition in a predictable way and releasing durum wheat cultivars capable of satisfying processors and consumers requirements. More recently, the strong evidences between diet and health are leading to focus breeding activities on nutritional aspects and enhancement of wheat kernel components of health value. Among these, starch composition represents an important target due its role in influencing both technological and nutritional aspects of wheat end-products. In particular, high amylose starch can play an important role on human health preventing the onset of important diseases. In this paper, the manipulation of proteins and starch with the final goal to tailor novel durum wheat cultivars with improved technological and nutritional characteristics will be presented.

Keywords. Quality – Glutenin subunits – Starch – RNA interference – TILLING.

Amélioration de la qualité technologique et nutritionnelle du blé dur : réalisations et perspectives

Résumé. *Le blé dur représente l'une des principales sources de nourriture pour de nombreux pays de la région méditerranéenne. Il est essentiellement utilisé pour la production de pâtes, mais aussi pour l'élaboration de plusieurs autres produits régionaux tels que le pain plat, le couscous, le burghoul, etc. Les activités de sélection pour la qualité du blé dur ont été principalement orientées vers la production de cultivars à haut rendement avec des caractéristiques supérieures pour la fabrication des pâtes alimentaires. L'influence des protéines de gluten sur les caractéristiques de traitement de la semoule a été élucidée, en examinant en particulier le rôle joué par les sous-unités gluténines de faible poids moléculaire. Des stratégies d'amélioration ont donc été élaborées pour modifier la composition des protéines d'une manière prévisible et obtenir des cultivars de blé dur capables de satisfaire les exigences des transformateurs et des consommateurs. Plus récemment, les indications évidentes du rapport étroit entre alimentation et santé conduisent à concentrer les activités de sélection sur les aspects nutritionnels et l'amélioration des composants du grain de blé bénéfiques pour la santé. Parmi ceux-ci, la composition de l'amidon représente une cible importante dans la mesure où elle intervient dans les propriétés technologiques et nutritionnelles des produits finis du blé. En particulier, la haute teneur en amylose de l'amidon peut jouer un rôle important dans la santé humaine pour prévenir l'apparition de maladies redoutables. Dans cet article, nous allons présenter la manipulation des protéines et de l'amidon pour la production de nouveaux cultivars de blé dur avec des caractéristiques technologiques et nutritionnelles améliorées.*

Mots-clés. *Qualité – Sous-unités gluténines – Amidon – Interférence ARN – TILLING.*

I – Manipulation of gluten composition

Protein content and gluten strength play the major role in determining pasta making quality. In particular, durum wheat cultivars with strong gluten and good viscoelastic properties have been shown to be essential to produce pasta with superior cooking characteristics. Studies of the past decades have firmly established the role of the low molecular weight glutenin subunits (LMW-GS) encoded by genes present at the *Glu-B3* locus and tightly associated at the *Gli-B1* locus. The two major allelic variants of the LMW-GS identified at these loci: LMW-1/γ-42 and LMW-2/γ-45, have been found to be associated to poor and good pasta-making characteristics, respectively (Payne *et al.* 1984; Pogna *et al.* 1990).

Durum wheat is prevalently used to produce pasta but, in some parts of the world, is also used to make bread, though, in some cases, of inferior quality compared to bread wheat. This has promoted different research efforts aimed at transferring high molecular weight glutenin subunits (HMW-GS) present in common wheat, associated at the chromosome 1D, that have been shown to be important in determining bread-making quality in bread wheat. In particular, loci encoding subunits 5+10 and 2+12, associated to the *Glu-D1* locus in bread wheat, have been introduced into several durum wheat cultivars by different authors using either chromosome engineering or wheat genetic transformation (Lukaszewski, 2003; Gadaleta *et al.*, 2008; Gennaro *et al.*, 2012; Sissons *et al.*, 2014).

Alveographic measurements of the durum wheat lines containing either the pair 5+10 or the 2+12 are strongly influenced by the two different types of HMW-GS (Fig.1). A more equilibrated ratio between tenacity (P) and extensibility (L) along with an increase in dough strength (W) have been found associated with the lines possessing the HMW-GS 2+12. On the contrary the lines with the HMW-GS 5+10 have shown a strong increase in the tenacity.

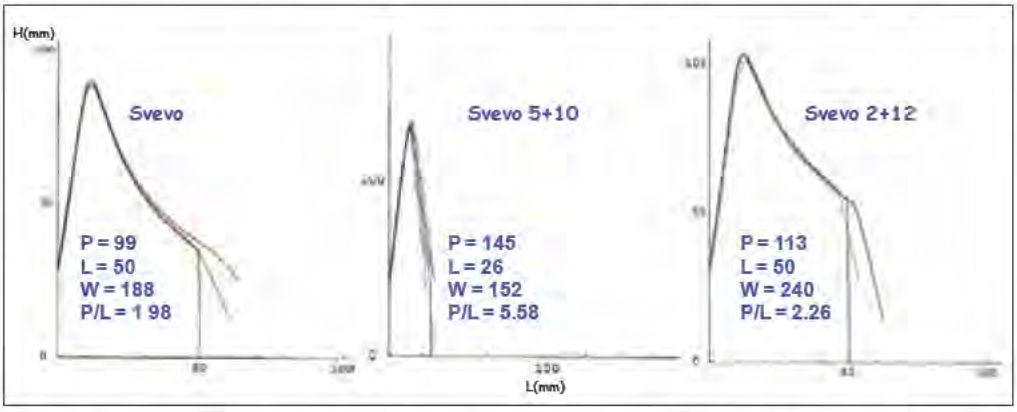

Figure 1. Alveographic measurements of semolina obtained by the durum wheat cultivar Svevo and derived lines possessing HMW-GS 5+10 or 2+12.

II – Manipulation of starch composition

Starch is composed from two glucan polymers (amylose and amylopectin), differing for the degree of polymerization and ramification. Biosynthesis of amylose and amylopectin is realized through the involvement of different types of enzymes (Starch synthases (SS), branching and debranching enzymes (SBE and DBE), through two different pathways having ADP-glucose as a common substrate.

Amylose/amylopectin ratio (1:3 in normal wheats) is one of the most important parameters that affect the chemical-physical properties of the starch; modulating the activity of key enzymes involved in starch biosynthesis, both low and high amylose starch have been produced in durum wheat (Lafiandra *et al.*, 2010; Sestili *et al.*, 2010a,b; Hogg *et al.*, 2013; Bovina *et al.*, 2013; Botticella *et al.*, unpublished).

Recent studies have demonstrated the existence of positive correlation between amylose content in flour and resistant starch (RS) in food products. RS has been shown to escape digestion to glucose in the stomach and plays a prebiotic role in the large bowel. In fact its fermentation produces small molecules, known as short chain fatty acids (SCFAs), representing important metabolites capable to promote optimal function of the viscera (Topping and Clifton, 2001). RS has a role similar to dietary fibre, protecting against diet-related diseases as colon cancer, type II diabetes, obesity and osteoporosis (Nugent, 2005). In addition, semolina containing high amylose amount improves the quality of pasta, increasing the firmness and reducing the stickiness and water absorption during the cooking (Soh *et al.*, 2006).

Different strategies have been applied to obtain high amylose in durum and bread wheat: silencing of genes involved in amylopectin biosynthesis (Starch synthase IIa or Starch granule protein 1 and Starch branching enzymes IIa), through use of natural mutant or TILLING (natural or induced mutants) and biotechnology tools (RNA interference silencing).

1. Natural mutants: Creation of a Starch granule protein 1 (Sgp-1) *null* collection in durum wheat

Single mutations for *Sgp-A1* (Chousen 30) and *-B1* (Kanto 79) genes, previously identified by Yamamori *et al.* (2000), were introgressed in the durum wheat cv Svevo with the aim to produce a complete Sgp-1 *null* line. An extensive SDS-PAGE electrophoresis of starch granule proteins was used to select the progeny of interest. Backcrosses between the parental cultivar and Sgp-1 *null* genotype (Chousen 30/Svevo/Svevo)//(Kanto 79/Svevo/Svevo) produced 144 Sgp-1 sister lines, that were characterized either for qualitative or quantitative traits. A set of fourteen sister lines, showing good or poor agronomic traits, was chosen and grown in two different years. These lines highlighted an increase in amylose content (AC) ranging between 36-45 %, but this result was also associated to a drastic loss in grain yield and starch content. Similar, but not identical effects have been previously reported in bread wheat and barley (Yamamori *et al.*, 2000 and 2006; Morell *et al.*, 2003). In barley, the lesion of the *Sgp-1* gene produced a strong increase in AC, (~70% of total starch), while in bread wheat the increase was more modest (~36%).

2. Transgenic approach: RNA interference silencing of Starch Branching enzyme IIa (SBEIIa)

Regina *et al*. (2006) used the RNAi technology to knock out the starch branching enzyme genes (*SBEIIa)* and increase the amylose content in bread wheat. Suppression of the activity of SBEIIa enzymes resulted in lines with amylose content above 80%. The same approach has been used by Sestili *et al*. (2010) in durum wheat, using two different cultivars (Svevo and Ofanto). Although two different methods were used for the genetic transformation, biolistic for cv Svevo and Agrobacterium for cv Ofanto, similar effects were observed on amylose content, granule morphology and starch composition in RNAi seeds. Amylose content was significantly increased in all the transgenic RNAi lines, but it varied between 31 and 75%. This result was probably due to the efficiency of gene suppression depending on transgene copy number and its position on genomic DNA. The value of resistant starch was also strongly increased in transgenic starch and resulted notably higher than in durum wheat cultivar Svevo (≈12% of total starch in transgenic line MJ16-112; 0.4% in Svevo). Regarding to starch granule phenotype, it was markedly affected in SBEIIa-silenced lines compared to the reference cultivars. In particular type-A granules were

smaller and deflated, whereas type-B granules lost their normal spherical shape and became more extended, similarly to that observed by Regina *et al.* (2006) in bread wheat.

With the aim to investigate the effects of biolistic and *Agrobacterium*-mediated transformation methods, a comparative proteomic approach has been undertaken to compare the proteome (starch granule and metabolic proteins) of mature and immature kernels of untransformed and transgenic durum wheat (Sestili *et al.* 2013). This study highlighted subtle differences, most of them considered as "predictable unintended effects" due to the silencing of *SBEIIa* genes. In conclusion, the comparison of the proteome of the transgenic lines obtained by two different transformation methods has shown only some small differences, that might depend on the different varietal responses.

3 Mutagenesis: a TILLING approach to suppress SBEIIa gene activity

T Mutagenesis represents a very effective strategy to generate novel genetic variation and its widespread use has resulted in the release of over 3000 crop cultivars with improved quality characteristics. Combination of the power of mutagenesis and a high throughput screening, based on PCR, to identify induced mutations in a target gene has resulted in the development of a powerful non transgenic technology, termed TILLING (Targeting Induced Local Lesions IN Genomes). Recently the TILLING strategy has also been applied in durum wheat and used to modify starch composition (Slade *et al*., 2005, 2012; Hazard *et al*., 2012; Bovina *et al*., 2014).

In particular, Bovina *et al*. (2014) have developed a mutagenized population by treating seeds of the durum wheat cultivar Svevo with Ethyl-Methan-Sulfonate (EMS). The M_1 generation was advanced by Single Seed Descent (SSD) to obtain M_3 seeds, whereas genomic DNA was extracted from each of the M_2 individual lines obtained. The entire M_3 population, consisting of 2601 families, was field-sown for both seed multiplication and phenotypical evaluation. Field phenotypic screening showed a high frequency of morpho-physiological alterations (ca. 22%). After harvesting, the M_4 seeds were also characterized for quality characteristics as yellow pigment, protein content and Sodium-dodecyl-sulfate (SDS) sedimentation test. Alterations of seed morphology, as kernel size/shape or colour were also identified.

In order to develop high-amylose durum wheat genotypes starting from knock-out mutants for the *SBEIIa* homeologus genes (*SbeIIa-A* chr 2AS; *SbeIIa-B*, chr. 2BS), the genomic DNA, isolated from the M_2 linesof the mutagenized population of Svevo, was screened by a TILLING approach. High Resolution Melting was applied to identify functional SNPs in the two homoeologous genes encoding SBEIIa-A and -B enzymes, using the strategy and primer pairs described by Botticella *et al.* (2011).

TILLING analysis permitted to identify 45 novel allelic variants: 39 for the gene *SBEIIa-A* and 6 for *SBEIIa-B*. Sequencing analysis confirmed that the mutations were G to A or C to T transitions as expected from alkylation by EMS. Note of worth a non-sense mutation for each homoeoalleles was identified. These single *null* mutants for SBEIIa-A and -B were crossed to obtain a complete *null* genotype. The screening of F_2 plants is in progress.

III – Conclusions

Technological and nutritional improvement of wheat is more feasible thanks to the possibility to integrate classical and novel approaches in both research and breeding. Increasing components present in the wheat kernel capable to exert beneficial effects on the onset of chronic diseases will open the possibility to develop novel end products with important added value and social benefits.

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Durum wheat production chain: research, quality and future challenges

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Abstract. Barilla ranks as one of today's top Italian food groups, leading in the global pasta business, the pasta sauces business in continental Europe, the bakery products business in Italy, and the crispbread business in Scandinavia. Being the first user of durum wheat worldwide for its pasta production, Barilla focused its research activity, since more than 20 years ago, on durum wheat breeding to meet its needs in terms of quality for pasta manufacturing. Moreover, the limited availability of durum wheat at the global level, compared to the other major cereals, led Barilla to adopt an integrated approach to manage its production chain from the field to the finish product.
Recently, Barilla has carried out a study on the environmental impacts of pasta conducted with the life cycle assessment methodology, through the publication of the Environmental Product Declaration. The study showed that, through durum wheat production chain, the cropping system is responsible for more than 80% of the ecological footprint, approximately 60% of the carbon footprint and almost for the entirety of the water footprint.
As a consequence, Barilla launched a specific project, the "Barilla Sustainable Farming" with the aim to increase the widespread use of sustainable cropping systems. The project has been focusing on identifying potential improvements of the most diffused cropping systems for the cultivation of durum wheat in Italy, maintaining high levels of quality and plant health conditions.

Keywords. Durum Wheat – Pasta – Breeding – Integrated chain – Sustainability.

Chaîne de production du blé dur : recherche, qualité et défis de demain

***Résumé**. Barilla est classé parmi les principaux groupes alimentaires italiens d'aujourd'hui, leader dans le secteur mondial des pâtes, dans le secteur des sauces pour pâtes en Europe continentale, dans le secteur des produits de boulangerie en Italie, et le secteur des biscottes en Scandinavie. Etant le premier utilisateur de blé dur dans le monde entier pour sa production de pâtes, Barilla a concentré son activité de recherche, depuis plus de 20 ans, sur l'amélioration du blé dur pour satisfaire ses besoins en termes de qualité pour la fabrication de pâtes. En outre, la disponibilité limitée de blé dur au niveau mondial, par rapport aux autres principales céréales, a conduit Barilla à adopter une approche intégrée pour la gestion de sa chaîne de production du champ au produit fini.*

Récemment, Barilla a réalisé une étude sur les impacts environnementaux des pâtes basée sur la méthodologie d'évaluation du cycle de vie, pour la publication de la Déclaration Environnementale de Produit. L'étude a montré que, tout au long de la chaîne de production du blé dur, le système de culture est responsable de plus de 80% de l'empreinte écologique, d'environ 60% de l'empreinte carbone et presque pour la totalité de l'empreinte eau.

Par conséquent, Barilla a lancé un projet spécifique, "L'agriculture durable Barilla" dans le but de promouvoir l'utilisation généralisée des systèmes de culture durables. Le projet a mis l'accent sur l'identification des améliorations potentielles des systèmes de culture les plus répandus pour le blé dur en Italie, en maintenant des niveaux élevés de qualité et de conditions phytosanitaires.

***Mots-clés.** Blé dur – Pâtes – Sélection – Chaîne intégrée – Développement durable.*

I – Introduction

The major use of durum wheat is for pasta production, particularly in the European and North American countries, whereas in other areas, such as the Middle East and North Africa, it is also used to make couscous, and for baking various types of bread (Troccoli *et al*., 2000).

In Italy, Pasta can be made with durum wheat semolina and water only, according to the Italian Law No. 580, 1967. Therefore, being the only ingredient used beside water, the semolina quality has a significant impact on the finished product quality. A high-quality pasta begins with good quality grain. The protein content and the gluten quality are the most important variables in determining the pasta cooking quality (D'Egidio. *et al.*, 1990; Novaro *et al.*, 1993). A good quality cooked pasta maintains a good texture, being resistant to surface starch leaching and stickiness, and retains a firm structure of "*al dente"* consistency. The Bright Yellow color of semolina is an important factor in high quality pasta manufacturing. This color is the result of the natural carotenoid pigments present in the kernel (Cubadda *et al.*, 1988).

II – Barilla "Integrated supply Chain" model

The production chain is a complicated network of interconnected businesses and activities related to the production and sourcing of raw materials, their processing towards finished products and distribution.

The continuous improvement of the sustainability of Barilla's strategic supply chains is implemented through projects and initiatives, developed together with partners along the supply chain.

Concerning durum wheat, Barilla operates by integrating the various stages of the production chain to create an "Integrated Supply Chain" model. Unlike the conventional supply chain concept where players follow each other in a top-down flow, Barilla's supply chain model has a circular structure in which players that operate at different stages are involved in a shared project that is focused on the same objective. Barilla's durum wheat breeding program, in collaboration with the breeding Company Produttori Sementi Bologna (PSB) represents the first step of this model: new and dedicated durum wheat varieties are developed to meet the production requirements and Barilla's quality standards (e.g., Svevo, Normanno and Aureo). The innovation embedded into new high quality varieties is transferred to farmers through the seeds, provided under cultivation agreements with Barilla, which uses the durum wheat produced for its pasta production closing the integrated supply chain.

Through a mutual collaboration among the production chain players, Barilla aims to manufacture safer, superior and more sustainable products.

III – Sustainability of the Barilla integrated supply chain

In order to assess the environmental impact of its production chain, Barilla carried out a Life Cycle Assessment analysis (LCA), using Carbon Footprint, Water Footprint and Ecological Footprint as indicators.

Barilla carried out this study at first on durum wheat pasta to evaluate the footprints of durum wheat cultivation and milling, pasta production, transport, packaging production and cooking for consumption. Results of this study have been published on durum wheat semolina dry pasta Environmental Product Declaration (EPD) (Ref. International EPD Consortium and EPD of durum wheat semolina dried pasta Barilla) (Fig.1).

The study underlined that the cultivation stage of durum wheat is the most significant in terms of emissions together with pasta cooking. The manufacturing of the packaging and transport contribute the least to greenhouse gas emissions (less than 5% each). The major impacts associated with farming activities are due to the use of nitrogen fertilizers and mechanical operations, particularly for working the land. More information on Barilla's activities, related to sustainability, is reported in the Sustainability report of the Company.

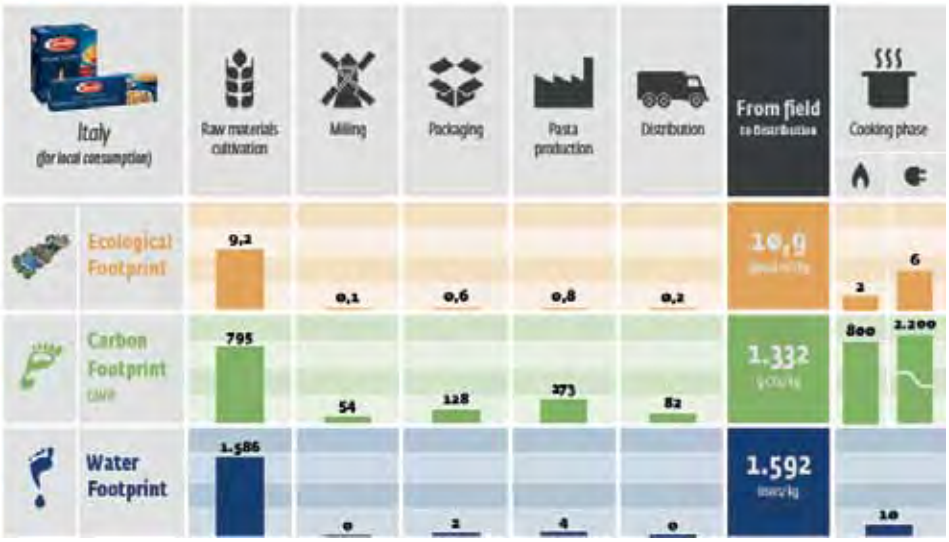

Figure 1. Life Cycle Assessment results of durum wheat pasta

IV – Barilla Sustainable Farming

Since it has been widely demonstrated that farming generates the most of pasta environmental impact, Barilla undertook a specific project using LCA methodology to analyze different cropping systems for durum wheat production. Carbon, water and ecological footprints were integrated with specific economic and agronomic indicators, in order to provide guidance on the "sustainability" - including the "feasibility" - of cropping systems that can represent alternatives for the cultivation of durum wheat in Italy, maintaining and improving quality and food safety standards of the products.

The system boundaries includes important elements, such as crop rotation, tillage activities, crop yields, fertilizers, herbicides and pesticides use, including relative emission to air, and water.

The durum wheat cultivation was analyzed by identifying different cropping systems currently followed in the three main geographical Italian areas: Northern Italy, Central Italy, and Southern Italy. The standard cropping system is a four-year rotation in which the cultivation of different crops, other than durum wheat, are involved.

The results of this study were published in the *Handbook for sustainable cultivation of quality durum wheat in Italy*, which was distributed to farmers. This document is intended as tool to disseminate knowledge and practical suggestions. It contains several guidelines concerning issues of crop rotation, soil tillage, nitrogen fertilization, sowing, and weed and pest management (*Barilla's Handbook*).

According to the *Handbook*, selecting appropriate *crop rotation* is a key issue for the sustainability of a farming system. When cultivating durum wheat it is best to avoid cereals as the preceding crop because the cultural residues of such crops are a favourable habitat for the fungi that propagate mycotoxins (i.e., deoxynivalenol**,** DON). Cultivation of a legume crop is recommended whenever possible insofar as it is able to fix the atmospheric nitrogen in the soil and, therefore, allow reduction of additional fertilizers required for crop growth in the following year.

Tillage is another important aspect both for the environmental and economic impacts which are mainly linked to diesel fuel use. The hilly central regions of Italy have a tradition of plowing yearly, which increases risk of erosion and costs due to fuel. Plowing, however, is not necessary for all

cases, and minimal or no tillage could be a valid solution to reduce environmental impacts and increase profitability. In areas with high risk of mycotoxins, such as Northern Italy, this solution cannot be implemented because plowing helps reduce mycotoxins.

The use of *fertilizers* is another key issue given the high impacts on both the production and use phase. Nitrogen fertilization causes the emissions of the greenhouse gas N_2O, and so certain factors must be contemplated, such as the timing of treatments, the quantity of nitrogen distributed in fields, and excessive and often unnecessary use of fertilizer applications during pre-sowing.

Seeding can indirectly influence the indicators considered because seeding time, rate and variety can affect the yield, which is the parameter by which the impacts of one hectare are divided by and hence "diluted".

This same reason also makes it important to undertake prompt and effective *management of weeds and pests* for sustainable crop production.

In 2011 – 2012, Barilla launched a specific project called "Barilla Sustainable Farming" with the aim to increase the widespread use of sustainable cropping systems. Thirteen farms (4 in Lombardia, 1 in Toscana, 6 in Marche and 2 in Puglia Regions) are involved in the project activities.

Farmers were asked to make a comparison of two different durum wheat crop management systems:

1. Usual and traditional crop management (own choices and strategies); and

2. Crop management implementing the Barilla's Handbook.

The results were very positive. By choosing implementing the handbook's recommendations, Farmers obtained an increase in yield (up to 20%), a reduction in carbon footprint (up to 36%), and in direct production costs (up to 31%).

Currently, in the 2012 – 2013 campaign, a second year of trials involves 25 farms and the results will be coming out soon with the next harvest. The objective for Barilla is to buy in the coming years, an ever increasing amount of durum wheat grown according to the identified sustainable farming techniques. The project is going be extended to other countries and to other strategic raw materials such as soft wheat, rye, and tomatoes. Furthermore, a prototype "*Decision Support System*" via web (www.granoduro.net) has been developed by Horta s.r.l (spin-off company of the University of Piacenza, http://www.horta-srl.com.) to provide wider assistance to Farmers. Also, the prototype can help to further reduce costs and environmental impacts.

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Quality in durum wheat: comparison between landraces and high yielding varieties

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Abstract. Eight durum wheat genotypes: four landraces and four high-yielding varieties were studied in two regions (sub-humid and semi-arid) of Tunisia, using four fertilizer treatments and during two cropping seasons. Three quality parameters were evaluated: thousand-kernel weight (TKW), yellow berry (YB) and protein content (P). Significant Genotype x Environment x Fertilizers interaction ($p < 0.05$) was noted for the quality related traits P and YB. TKW was dependent on Environment x Fertilizers and Environment x Genotype interactions ($p<0.05$). Landraces showed higher values of P (18.32 %) and TKW (44.49 g) than high yielding varieties (15.81%, 40.23 g) respectively. Conversely, landraces showed lower YB rates (4.14%) than high-yielding varieties (7.79%). A pronounced expression of these traits was noted in semi-arid region. N-fertilizer appeared to increase the protein content and reduce TKW. Landraces were associated with reduced YB levels in semi-arid and sub-humid areas, for potassium and nitrogen fertilizers. Nitrogen fertilizer reduced YB in semi-arid region from 11.48 % to 3.5 % in high yielding varieties. In sub-humid region YB rates were similar for nitrogen and potassium combination ($p>0.05$%) ranging from 6.77% to 8.92%. These results support that landraces are adapted to semi-arid area with low fertilizer input while high yielding varieties appeared to improve fertilizer use efficiency for quality traits.

Keywords. Durum wheat quality – Landraces - High-yielding varieties – Environmental interactions.

Qualité chez le blé dur : comparaison entre variétés locales et variétés à haut rendement

Résumé. *Huit génotypes de blé dur, quatre variétés locales et quatre variétés à haut rendement, ont été étudiés dans deux régions (sub-humide et semi-aride) de la Tunisie, en utilisant quatre traitements de fertilisation et pendant deux saisons de culture. Trois paramètres de qualité ont été évalués : le poids de mille grains (PMG), l'indice de jaune (YB) et la teneur en protéines (P). Une interaction significative du Génotype x Environnement x Engrais ($p <0,05$) a été notée pour les caractères liés à la qualité P et YB. PMG était dépendant des interactions Environnement x Engrais et Environnement x Génotype ($p <0,05$). Les variétés locales ont montré des valeurs plus élevées de P (18,32%) et PMG (44,49 g) que les variétés à haut rendement (15,81%, 40.23 g), respectivement. En revanche, les variétés locales ont montré des taux plus faibles de YB (4,14%) que les variétés à haut rendement (7,79%). Une expression prononcée de ces caractères a été observée dans la région semi-aride. L'engrais azoté augmentait la teneur en protéines et réduisait le PMG. Les variétés locales ont été associées à des niveaux réduits de YB dans les zones semi-aride et sub-humide, pour les engrais potassiques et azotés. Dans la région semi-aride, les engrais azotés réduisaient le YB de 11,48% à 3,5% chez les variétés à haut rendement. Dans la région sub-humide, les taux de YB étaient similaires pour la combinaison azote et potassium ($p> 0,05$%), allant de 6,77% à 8,92%. Ces résultats confirment que les variétés locales sont adaptées à la zone semi-aride avec un faible apport d'engrais alors que les variétés à haut rendement améliorent l'efficacité d'utilisation des engrais pour les caractères de qualité.*

Mots-clés. *Qualité du blé dur – Variétés locales – Variétés à haut rendement – L'interaction avec l'environnement.*

I – Introduction

Durum wheat quality is considered as a multidisciplinary concept. Quality aspects are defined according to the user's category. Protein content, test weight and grain moisture are important for commercial transactions; however, grain yield and stability are interesting aspects for the farmers.

For milling and pasta industries, semolina yielding ability, gluten and protein content are the most important traits considered for durum wheat quality (Troccoli *et al*., 2000). In addition, many researchers consider that storage proteins are responsible for the variation in physicochemical and technological durum wheat properties (Martinez *et al*., 2005; Simeone *et al*., 2001; Lerner *et al*., 2004). Strong relationships were detected between the high molecular weight glutenin subunits allelic composition and bread making quality (Ammar *et al*., 2000), spaghettis cooking quality (Aalami *et al*., 2007) and gluten strength (Sisson *et al*., 2005 and Saperstein *et al*., 2007). According to previous researches the durum wheat grain quality parameters are influenced by the environmental, genotypic factors and the potential interactions among them (Rharrabti *et al*., 2003b; Lerner *et al*., 2004; Edwards *et al*., 2007). Protein content is mainly controlled by fertilizer and environments (Mariani *et al*., 1995). Kernel weight and the kernel virtreousness parameters are strongly dependent on the water availability conditions during the grain filling period (Li *et al*., 2013; Jia *et al*., 1996).

The objectives of this study were to:

(i) compare the potential responses of protein content, yellow berry and thousand kernel weight of selected high yielding durum wheat cultivars and local landraces in different growing conditions,

(ii) characterize their high molecular weight-glutenin subunits,

(iii) evaluate the class of genotype, environments and fertilizer treatments effects on the assessed quality traits.

II – Material and methods

Two field trials were grown in sub-humid (BouSalem) and semi-arid (Kef) regions in Tunisia. Each trial included four durum wheat landraces (Chili, Biskri, Mahmoudi and Inrat 69) and four high yielding durum wheat cultivars (Karim, Razzak, Om Rabiaa and Khiar), which were grown using four fertilizer treatments N0K0 (no applied N or K fertilizer), N1K0 (applied N only), N0K1 (applied K only) and N1K1 (applied both N and K fertilizers). Trials were conducted during two successive growing seasons, viz., 2007 and 2008.

The quality parameters measured were: protein content, thousand kernel weight and yellow berry. Grain protein content (P) was determined using NIRS system (near infrared spectroscopy using a Perten-Inframatic-8600) (Sgruletta and Destefanis, 1997). Thousand kernel weight (TKW) was determined using the Numigral Chopin apparatus. Yellow berry (YB) rate was determined by inspecting 50 kernels sliced using thel farinotom apparatus by Pohl.

Glutenins extraction was made as described by Rhazi *et al*. (2009) on the eight durum cultivars. Glutenins were examined by microchip capillary electrophoresis-sodium dodecyl sulfate (microchip CE) platform, LabChip 90. The identification of HMW-GS at the Glu-B1 locus was carried out according to Rhazi *et al*. (2009).

Analysis of variance was carried out using proc anova of SAS with the option LSD to compare means.

III Results and discussion

1. Quality parameters variability with environments, genotypes and fertilizer treatments

The analysis of the variance showed that all three main effects environment (E), genotypic pools (G) and fertilizer treatment (F) were significant for the quality parameters protein content, thousand

kernel weight and yellow berry (Table 1). Environmental effect was very large in magnitude and appeared to be the principal effect for thousand kernel weight. Yellow berry appeared to be controlled mainly by genotype. Second order interaction (E x F x G) was significant only for yellow berry and protein content. E x G and E x F interactions were significant for the three measured parameters, whereas they were smaller in magnitude as compared to individual main effects (Table 1). This is in accordance with previous studies which showed that individual environmental and genotypic effects were more important than their interaction on the quantitative quality parameters (Li *et al*., 2013; Mariani *et al*., 1995).

2. Comparison between landraces and high yielding varieties

Landraces showed superiority over the high yielding varieties for the assessed quality traits for all regions-fertilizers' combinations. Overall cultivars means of protein content, thousand kernel weight and yellow berry comparison (Table 3) showed that protein content ranged from 17.76% to 18.70% for landraces (a mean 18.32 %), while it varied within the range of 15.29% to 16.20% for high yielding varieties (a mean 15.81%). Greater values of thousand kernel weight were observed for landraces, ranging from 43.29g to 45.48g (a mean 44.49g) than for high yielding varieties, ranging from 37.30g to 42.01g (a mean 40.23g). Yellow berry rates were lower for landraces, a mean 4.13% was scored ranging from 3.48% to 5.30% ($p>0.05$); whereas it varied significantly ($p<0.05$) from 6.45% to 9.22% for high yielding varieties (with a mean 7.79%). These results confirmed the genotypic effect observed on all three quality traits (Table 1) and suggest that landraces are characterized by a greater semolina yielding ability (Rharrabti *et al*., 2003a).

Table 1. Mean squares of thousand kernel weight (TKW), yellow berry (YB) and protein content (P) assessed on the two classes of genotypes (G) during two growing seasons using four fertilizer treatments (F) in both environments (E) sub-humid and semi-arid regions.

Source of variation	D.f.	TKW (g)	YB (%)	P (%)
E	1	8288.28**	3204.66**	1814.15**
r(E)	4	125.89	254.37	10.49
G	1	5224.12**	3854.78 **	1812.14**
F	3	1889.19**	1633.08 **	489.13**
E x G	1	150.07 *	504.42**	37.41**
G x F	3	43.74 ^{ns}	62.56^{ns}	2.41^{ns}
E x F	3	159.62**	280.44**	10.87**
E x F x G	3	79.25 ^{ns}	500.53**	26.46*
Error	1132	31.79	58.81	2.48

,, Significant at 5% and 1% , respectively.*

Comparison of cultivars responses to the assessed quality traits according to Environment x Fertilizers interaction (Table 2) showed that protein content was higher in the semi-arid area than in the sub-humi. This may be due to the water shortage that affected dry matter accumulation during grain filling period (Debaeke *et al*., 1996). In addition, protein content increased significantly with the application of nitrogen-fertilizers in both regions and for all genotypes confirming prior results (Lerner *et al*., 2006; Malik *et al*., 2012; Abad *et al*., 2005). In contrast, thousand kernel weight showed lower values in semi-arid area and decreased significantly with nitrogen-fertilizers application. These results would support the dilution effect of protein content which occurs mainly in favorable growing conditions (Jia *et al*., 1996). Yellow berry, the lowest value was observed in semi-arid area. Yellow berry showed similar means ($p>0.05$) for the two groups of genotype (11.53%) using N0K0 fertilizers treatments. Although comparable means for yellow berry was obtained for high yielding cultivars grown either in the sub-humid or semi-arid areas, a significant decrease of this trait was noted for landraces grown in semi-arid area (3.37%). The use of

potassium and nitrogen fertilizers alone or combined showed similar (p>0.05) yellow berry in the SH for each group of cultivars. Greater reduction of yellow berry was observed for landraces (4.64%) than for high yielding cultivars (8.01%) when no fertilizer application was used. These results suggested that landraces grown under optimal nitrogen application are characterized by a greater semolina yielding ability (Rharrabati *et al*., 2003a). However, in the SA region, yellow berry decreased from 11.48 to 8.88% within high yielding cultivars gene pool. This was attributed to a greater use efficiency of nitrogen than potassium fertilizers. Whereas, lower yellow berry rates (3.37%) were noted for landraces grown in the same areas and under similar fertilizers applications.

Table 2. Means variation of Thousand kernel weight (TKW), yellow berry (YB) and protein content (P), with second order interaction Environment (sub-humid (SH) and semi-arid (SA))x Genotype (Landraces and high yielding cultivars (High.y.cvs)) x Fertilizers (N0K0, N0K1, N1K0 and N1K1).

		TKW (g)		YB (%)		P (%)	
		SH	SA	SH	SA	SH	SA
Landraces							
	N0K0	50.40 a	43.48 cd	11.91 a	3.37 ef	14.79 h	18.80 b
	N0K1	48.98 a	43.97 bc	4.36 de	1.35 f	16.44 e	18.61 bc
	N1K0	45.78 b	40.62 e	4.10 e	1.42 f	18.16 cd	20.70 a
	N1K1	44.97 bc	37.72 f	5.48 de	1.08 f	18.16 cd	20.92 a
High.y.cvs							
	N0K0	43.72 cd	40.96 e	11.16 ab	11.48 a	13.78 i	15.32 g
	N0K1	43.95 bc	40.95 e	6.77 cd	8.88 bc	13.96 i	15.97 ef
	N1K0	40.64 e	35.53 g	8.36 c	3.29 ef	15.87 f	17.88 d
	N1K1	41.90 de	34.19 g	8.92 bc	3.51 ef	15.35 g	18.39 bcd

Means with the same letters do not differ significantly (P ≤0.05%) (LSD-test).

3. Quality evaluation in relation to high molecular weight glutenin subunits (HMW-GS) compositions

The results of HMW-GS at the Glu-B1 identification showed that three different compositions were found: 20x-20y, 6-8 and 7-8 (Table 3). The 6-8 subunits composition was detected in the cultivar Biskri and was associated with greater values of TKW (44.07g) and protein content (18.38%) and the lowest rate of yellow berry (3.78%).The subunits 20x-20y were observed in landraces Chili, Mahmoudi and Inrat 69 and the high yielding cultivar Om rabiaa. This composition was associated with intermediate values of TKW (with a mean of 43.96g); and protein content (17.77%) and yellow berry (5.04%). The 7-8 subunits composition was found in the high yielding cultivars Karim, Razzak and Khiar. It showed the lowest quality profile with scored means of TKW (39.64 g), P (15.68%) and the highest yellow berry rate (7.91%). ANOVA results in Table 4 showed that two different groups of varieties. The first group composed by varieties carrying the 20x-20y and varieties carrying the 6-8 subunits, and were associated with similar values of P, TKW and YB (p>0.05); the second group was composed by varieties carrying the 7-8 subunits only.

This classification could explain partly the consumers' preference for using derived end products from landraces instead of Tunisian high yielding cultivated varieties (Zaibet *et al*., 2007), especially since Aalami *et al*. (2007) found that varieties with 7-8 subunits showed a poor cooking quality for spaghetti. Therefore the relationships between HMW-GS and quality parameters could depend also from the LMW-GS compositions (Raciti *et al*. 2003). Previous studies showed that differences between 6-8 and 7-8 may be due to the composition of LMW-GS at Glu-A3 and Glu-B3 (Lerner *et al*., 2004; Vazquez *et al*., 1996).

Table 3. Overall means of thousand kernel weight (TKW), yellow berry (YB) and protein content (P) and High molecular weight glutenin subunits identification of the eight durum wheat genotypes (assessed in both sub-humid and semi-arid regions).

		HMW-GS	TKW (g)	YB (%)	P (%)
Landraces					
	Chili	20x-20y	45.48 a	3.98 de	18.45 a
	Biskri	6-8	44.07 ab	3.78 e	18.38 a
	Mahmoudi	20x-20y	45.08 a	3.48 e	18.70 a
	Inrat69	20x-20y	43.29 bc	5.30 cde	17.76 b
High yielding varieties					
	Karim	7-8	40.19 e	6.45 bcd	16.03 cd
	Omrabiaa	20x-20y	42.01 cd	7.43 abc	16.20 c
	Razzak	7-8	41.43 de	9.22 a	15.73 de
	Khiar	7-8	37.30 f	8.08 ab	15.29 e

Means with the same letters do not differ significantly (P ≤0,05%) (LSD-test).

Table 4. Means of thousand kernel weight (TKW), yellow berry (YB) and protein content (P) by HMW-GS composition.

HMW-GS	TKW (g)	YB (%)	P (%)
20x-20y	43.96 a	5.04 b	17.77 a
6-8	44.07 a	3.78 b	18.38 a
7-8	39.64 b	7.91 a	15.68 b

Means with the same letters do not differ significantly (P ≤0,05%) (LSD-test).

IV – Conclusions

The effect of fertilizers use on the protein content appeared to be conditioned by the combined effect of environments (semi-arid or sub-humid region) and by the genotypes. These results would assume that landraces outperform high yielding cultivars in protein content when grown under semi-arid and sub-humid conditions using combined potassium and nitrogen fertilizations.

Even though, the semi-arid region valorized better the expression of all quality parameters for both classes of genotypes than the sub-humid region.

Good management of nitrogen and potassium fertilizers use could improve the quality of high yielding cultivars in the sub-humid region.

In spite of their different HMW-GS composition (20x-20y) and (6-8), landraces showed similar values of the assessed quality traits and superiority over high yielding cultivars which carried the 7-8 HMW-GS subunits.

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Breeding and quality of soft-textured durum wheat

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Abstract. Puroindolines A (PIN-A) and B (PIN-B) encoded by genes *Pina-D1* and *Pinb-D1* on chromosome 5DS are the principal determinant factors of kernel hardness in common wheat, and exert a strong impact on several quality traits such as yield and granularity of flour, starch damage and water absorption, rheological and baking properties. Effects of grain texture on pastamaking and breadmaking quality were evaluated in soft-textured durum wheat lines (SDLs) as compared with their hard durum sister lines (HDLs). SDLs accumulated puroindolines on their starch granules and showed SKCS values significantly lower than those of their hard-textured counterparts lacking Pin-A and Pin-B. The average flour extraction rate of SDL was about 23% higher than that of HDL. Increasing kernel softness significantly affected rheological parameters, whereas spaghetti cooking quality was unaffected by kernel hardness. Loaf volume exhibited a 10% increase associated with kernel softening. In order to reduce plant height, soft durum lines with the lowest SKCS indexes were further crossed with durum wheat cv. Simeto and 17 F_6 progeny lines were evaluated in terms of stability for their short height, soft texture and gluten quality. Modulation of kernel texture in durum wheat was obtained as well by transgenic approach by inserting vromindoline genes from oats. Finally soft textured durum wheat were used in crosses with *Triticum aestivum* with the aim to obtain extra-soft common wheats that may supply breeders with a broader range of kernel texture.

Keywords. Kernel texture – Durum wheat breeding – Pasta-making quality – Bread-making quality.

Amélioration et qualité du blé dur à texture tendre

Résumé. *Les puroindolines A (PIN-A) et B (PIN-B) codées par les gènes Pina-D1 et Pinb-D1 sur le chromosome 5DS sont les principaux facteurs déterminants de la dureté du grain chez le blé commun, et exercent un fort impact sur plusieurs caractères de qualité tels que le rendement et la granularité de la farine, la dégradation de l'amidon et l'absorption d'eau, les propriétés rhéologiques et de cuisson. Les effets de la texture du grain sur la qualité de la production de pâtes et de la panification ont été évalués dans les lignées de blé dur Soft (SDL) par rapport à leurs lignées sœurs Hard (HDL). Les SDL ont accumulé les puroindolines sur leurs granules d'amidon et ont montré des valeurs SKCS significativement inférieures à celles de leurs homologues Hard sans Pin-A et Pin-B. Le taux d'extraction moyen de farine des SDL était d'environ 23% plus élevé que celui des HDL. L'augmentation de la tendreté du grain a affecté de façon significative les paramètres rhéologiques, alors que la qualité de cuisson des spaghettis n'a pas été affectée par la dureté du grain. Le volume du pain a présenté une augmentation de 10% associée au ramollissement de la graine. Afin de réduire la hauteur des plantes, les lignées de blé dur tendre avec les plus faibles indices de SKCS ont ensuite été croisées avec des blés durs cv. Simeto et 17 lignées de descendance F6 ont été évaluées en termes de stabilité pour leur faible hauteur, la tendreté et la qualité du gluten. La modulation de la texture du grain de blé dur a ainsi été obtenue par une approche transgénique, en insérant des gènes de la vromindoline de l'avoine. Enfin, le blé dur à grain tendre a été utilisé dans des croisements avec Triticum aestivum dans le but d'obtenir des blés communs extra-tendres qui peuvent fournir aux sélectionneurs une gamme plus large de texture des grains.*

Mots-clés. *Texture des grains – Amélioration du blé dur – Qualité de la production des pâtes – Qualité de la panification.*

I – Introduction

Kernel hardness is a main determinant of end product quality because of its strong effects on milling conditions, granularity of flour and starch granule integrity. In particular, common wheat

(*Triticum aestivum* L.) cultivars can be divided into three endosperm-texture classes based on their average SKCS (Single Kernel Characterization System) values, i.e. soft, medium hard and hard. On the contrary, all durum wheat (*T. turgidum* ssp *durum*) cultivars are characterized by an extra-hard kernel texture with SKCS index >90. This extremely hard texture is mainly due to the absence of PIN-A and PIN-B, two basic, tryptophan- and cysteine-rich polypeptides encoded by two closely linked genes named Pina-D1 and Pinb-D1, located in the distal part of the short arm of chromosome 5D (Mattern *et al*., 1973; Gautier *et al*., 1994) and consequently absent in AB-genome durum wheat. Extra-hard durum wheat grain is mainly ground to make semolina for the production of pasta and cous-cous, and in Mediterranean countries it is also used for breads of all types (Quaglia, 1988; Palumbo *et al*., 2000). Breeding programs have focused on selecting durum wheat genotypes with superior pastamaking quality because of its primary commercial importance, and selection for baking quality has been applied to a minor extent (Boggini and Pogna, 1989; Peña *et al*., 1994; Boggini *et al*., 1995; Liu *et al*., 1996; Palumbo *et al.*, 2000). To make a durum bread, semolina is reground to reduce its particle size and provide sufficient starch damage to assure appropriate gassing power during the fermentation process (Quaglia, 1988). Because of the extreme hardness of durum wheat grain, semolina regrinding can result in excessive starch damage, which alters alveogram and farinogram shapes, and exerts detrimental effects on baking performance (Dexter *et al*., 1994).

In order to insert puroindoline genes into durum wheat, Gazza *et al*. (2003) used durum wheat line "Cappelli M" lacking the Ph1 locus (Giorgi, 1978) as the female parent in a cross with the 5D(5B) substitution line of durum wheat cv. Langdon carrying wild-type alleles Pina-D1a and Pinb-D1a. The resulting soft-textured plants devoid of chromosome 5D were used as the male parent in crosses with commercial durum wheat cv. Colosseo (Gazza *et al*., 2008) and three F6 plants emizygous at the Pina-D1/Pinb-D1 locus from these crosses were self-pollinated for three generations to develop six F9 lines, i.e. three Soft Durum Lines (SDL) homozygous for wild-type alleles Pina-D1a and Pinb-D1a, and three Hard Durum Lines (HDL), lacking the Pina-D1 and Pinb-D1 genes.

Here, soft-textured and hard-textured durum wheat lines are compared for their milling properties, rheological characteristics, pastamaking and breadmaking quality. In addition, in order to reduce plant height a selected SDL line was crossed with durum wheat cv. Simeto and 17 F6 progeny lines were evaluated in terms of stability for their short height, soft texture and gluten quality. Modulation of kernel texture was also obtained in transgenic durum wheat cv. Svevo containing vromindolines, two puroindoline-like proteins bound to starch granules, and likely responsible of the extra-soft texture of oat kernels. Production of extra-soft common wheat lines deriving from a cross between SDLs and common wheat is discussed as well.

II – Material and methods

DNA was extracted from leaves by the CTAB method. Puroindoline genes were amplified by PCR as described by Gautier *et al*. (1994). SSR (Simple Sequence Repeat) sequences on chromosome 5D were used for microsatellite marker characterization (Somers *et al*., 2004; Song *et al*., 2005).

Starch-bound proteins were extracted with 50mM NaCl and 50% (v/v) propan-2-ol from 50 mg of air-dried starch granules as described previously (Corona *et al*., 2001). A-PAGE at pH 3.1 of starch-bound proteins was carried out as described by Corona *et al*. (2001). Reduced endosperm proteins were fractionated by SDS-PAGE as described previously (Pogna *et al*., 1990).

Kernel hardness was performed on 300 kernels-sample by the Perten SKCS 4100 (Springfield, IL, USA) following the manufacturer's operating procedure. The instrument was set in a range of hardness values between -40 and +120. Samples (3Kg) from soft-textured and hard-textured lines were milled with (i) the MCK Buhler experimental mill for durum wheat, (ii) the MLU 202 Buhler experimental mill for common wheat or (iii) the Bona 4RB (Bona, Italy) experimental mill for common wheat.

The milled samples were analyzed with the Chopin Alveograph (Chopin, Villeneuve La Garenne, France) according to the manufacturer's instructions as modified by D'Egidio *et al*. (1990). In addition, the flour samples obtained with the MLU 202 Buhler experimental mill were analyzed with the Brabender (South Hackensack, NJ) farinograph. Flour and semolina obtained from each soft-textured or hard-textured line with the MCK Buhler experimental mill for durum wheat were combined and mixed with tap water to reach a dough water content of 24.5% (for SDLs) or 30% (for HDLs). The dough was processed into spaghetti (1.7 mm in diameter) using a laboratory press. After drying at 50°C for 20 h, spaghetti (100 g) were cooked and evaluated for firmness, stickiness and bulkiness by a trained panel of three experts as described by D'Egidio *et al.* (1990).

Bread was baked according to the AACC Method 10-10B with minor modifications (Cattaneo and Borghi, 1979), using flour samples obtained with the milling for common wheat. Loaf volume was determined by rapeseed displacement.

All data are the means of at least duplicate determinations. Data were statistically evaluated by Student's *t* test or analysis of variance.

III – Results and Discussion

PCR amplifications of genomic DNA with primer pairs specific for seven microsatellites located on 5D chromosome suggested that SDLs contain only a small 5DS fragment, inferior to 14.4 cM in size, likely translocated to homoeologous chromosome 5BS. Upon A-PAGE fractionation, soft textured durum wheat lines were found to accumulate PIN-A and PIN-B on the surface of their starch granules in amounts comparable to those observed in soft-textured common wheat cultivars. Accumulation of puroindolines reduced SDLs mean SKCS indexes to 19.9 - 23.6, which are typical of soft-textured common wheat cultivars whereas hard-textured durum wheat lines HDL were similar to durum wheat varieties in lacking both puroindolines. According to SDS-PAGE fractionation, all durum wheat lines produced in the present study exhibited LMW-2 glutenin subunits, which are associated with superior gluten strength (Pogna *et al*., 1990), and inherited HMW glutenin subunit pair 6+8 from Langdon 5D(5B) substitution line.

SDLs revealed that grain hardness has a strong influence on several quality-related traits at the tetraploid level as well. In particular, the average flour extraction rate of SDLs was approximately 24% higher than that of HDLs, and even greater (about 60%) after milling with the MCK Buhler mill for durum wheat. Grain softness strongly decreased farinograph water absorption and consequently resulted in inferior dough tenacity (P), strength (W) and P/L ratio of SDLs with respect to HDLs. Moreover, the lower starch damage accounts for the higher farinograph dough stability and mixing tolerance of SDL milling products, which likely derives from their lower water absorption. It is noteworthy that the substantial variation in water absorption and rheological properties associated with the contrasting kernel textures of the durum wheat lines did not significantly affect firmness, stickiness and bulkiness of spaghetti. In addition, HDLs and SDLs did not differ significantly for their pasta-making quality as determined by the global quality score, and were comparable with high-quality durum wheat cultivars grown in Italy. On the other hand, soft-textured lines showed a small, but significant, increase of the bread loaf volume (approximately 10%) compared with their hard-textured counterparts. These results suggest that modulation of kernel hardness in durum wheat does not impair its pasta-making potential, and may improve its baking performance.

As compared with HDLs, the soft texture of SDLs resulted in significant lower yellow and brown indexes in both flour and semolina fractions obtained with the MCK Buhler mill. This suggest that color was strongly related to the particle size of the milling fractions, yellowness b* and brownness (100 - L*) being consistently and significantly lower in the finer flour and semolina of SDLs.

The high plant height (>120 cm) of SDLs and HDLs resulted in partial lodging at harvesting (until 60%). In order to breed shorter cultivars, an SDL line was crossed with durum wheat cv. Simeto. Segregation of allele *Pina-D1a* coding for wild-type PIN-A was followed by PCR amplification on single F_2 plants, whereas segregation of texture in F_3 kernels produced by each F_2 plant was determined by SKCS. Amongst the F_6 resulting progeny, ten soft-textured individuals, <85 cm in height, were selected (Table 1). Moreover, three genotypes (12-81, 12-88 and 12-91) were found to contain HMW glutenin subunit pair 7+8 (Fig.1), which are associated to gluten strength.

Table 1. Mean values of plant height, SKCS indexes and seed weight of 17F_6 individuals of the cross between SDL1 and durum wheat cv Simeto.

Genotype	Plant height ± SD (cm)	SKCS ± SD	Seed weight±SD (mg)
12.76	98.0±13.3	13.6±13.7	58.7±11.2
12.77	96.1±11.2	14.5±13.4	61.7±8.4
12.78	81.1±5.6	0.2±13.6	58.8±10.5
12.79	78.7±7.6	0.1±15.5	57.8±8.9
12.80	77.1±6.4	4.6±15.4	59.7±10.7
12.81	86.0±6.3	10.7±15.2	51.2±12.2
12.82	78.3±6.9	2.6±15.1	60.5±10.0
12.83	78.9±5.1	-2.1±14.4	58.8±11.2
12.84	102.7±6.1	6.6±12.3	56.0±9.1
12.85	75.5±9.2	-9.2±14.0	59.3±8.9
12.86	73.9±5.3	-10.1±12.9	62.1±9.45
12.87	74.8±6.2	39.2±30.4	49.8±12.2
12.88	74.5±5.3	35.4±34.0	58.5±9.3
12.89	95.5±7.7	3.3±13.0	47.9±10.2
12.90	92.2±6.1	2.0±13.8	51.6±8.9
12.91	68.5±4.5	6.5±14.5	55.4±12.3
12.92	70.7±5.0	-5.3±15.3	59.7±12.5

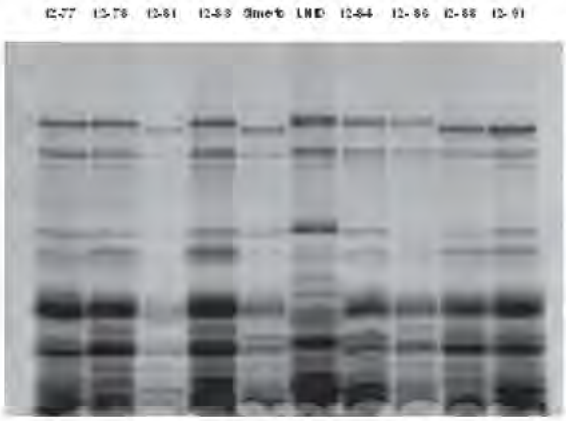

Figure1. SDS-PAGE fractionation of storage proteins of eight F_6 lines obtained from the cross SDL x Simeto. HMW glutenin subunits pair 7+8 is indicated.

Modulation of kernel texture was obtained in transgenic durum wheat containing vromindolines from oats as well. Two plasmids containing either *Vin-2* or *Vin-3* genes were used to co-transform durum wheat cv. Svevo by the biolistic method. *Vin-2* and *Vin-3* from oats (*Avena sativa*) code for vromindolines 2 and 3 (VIN-2 and VIN-3), respectively, two starch-bound, puroindoline-like

proteins sharing a tryptophan-rich domain of four tryptophan residues. A total of 24 T_1 plants and 50 T_2 plants expressing both *Vin-2* and *Vin-3* genes were grown in growth chambers, and characterized for their genetic structure and kernel texture.

When grown under chamber conditions, the 34 T_1 progeny of two transgenic T_0 individuals with expression of both *Vin-2 and Vin-3* were indistinguishable from control cv. Svevo for their morpho-physiological traits and fertility. Transgenic plants produced T_2 kernels significantly softer (mean SKCS = 37.1±12.5) than their sister plants without vromindoline transgenes (mean SKCS = 82.2±12.9, Table 2). *As expected, heterozygous T_1 plants* generated seeds with contrasting hardness characteristics, with SKCS values ranging from 0.4 to 115.7. These results suggest that vromindolines VIN-2 and VIN-3 are able to modulate grain texture in durum wheat as well.

Soft-textured F_6 lines of durum wheat containing PIN-A and PIN-B were crossed with common wheat cvs Eridano and Bilancia. The hard-textured cv. Eridano contains null allele *Pina-D1b* and wild-type allele *Pinb-D1a*, whereas the soft-textured cv. Bilancia has wild type alleles *Pina-D1a/ Pinb-D1a*. Amongst the F_2 progeny of cv. Eridano, individuals with three doses of puroindolines, *i.e*. one of PIN-A and two of PIN-B, were selected by PCR amplification with primers specific to *Pina-D1b* or *Pina-D1a* (Fig.2A), and found to have a mean SKCS value of 45 (Fig.2B). Moreover, the F_2 progeny of cv. Bilancia showed a mean SKCS values of 22, with extra-soft individuals exhibiting an SKCS index as low as -3.

Table 2. SKCS values of kernels produced by 34 T_1 plants from two T_0 plants of durum wheat cv. Svevo expressing *Vin-2* and *Vin-3.*

Genotype of T_1 plants	No. of T_1 plants	No. of T_2 kernels	Mean SKCS value ± SD	SKCS Min	Range Max
Homozygous	9	210	37.1 ± 12.5**	-11.3	72.2
Heterozygous	20	666	58.3 ± 23.4	0.4	115.7
Null	5	152	82.2 ± 12.9	49.3	117.6
Control cv. Svevo	5	300	88.8 ± 15.2 ns	41.1	110.2

*** t-value significant at P < 0.01 with respect to the null T_2 progeny; ns, t-value not significant with respect to the null T_2 progeny.*

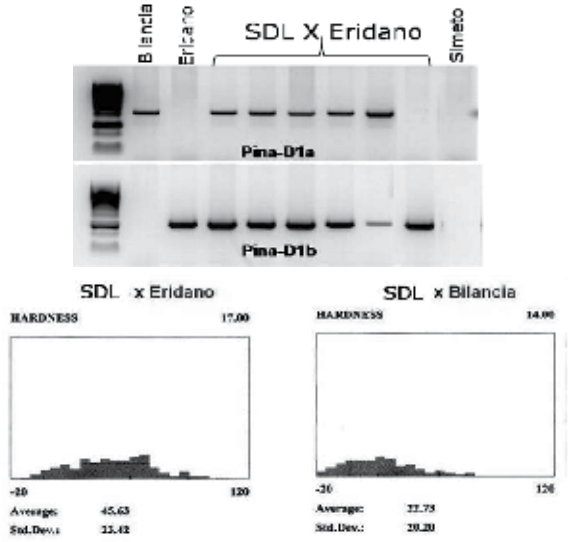

Figure 2. Results of PCR amplification of F2 SDL lines using allele PinaD1a or Pina D1b specific primer.

IV – Conclusions

Durum wheat lines homozygous for a <14.4 cM terminal fragment of chromosome 5D containing the Pina-D1a/Pinb-D1a alleles showed SKCS values typical of soft-textured kernels. Softening effect resulted in a about 24% higher flour extraction rates compared with hard-textured lines. Spaghetti cooking quality was unaffected by kernel hardness, whereas loaf volume exhibited a 10% increase associate with kernel softening. Availability of soft-textured durum wheat genotypes may have important practical and useful implications for breeding multiple-purpose durum wheat (pasta, bread, biscuits and other oven products), and for technological operations of industrial interest.

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Molecular characterization of candidate genes involved in nitrogen metabolism and relationship with the grain protein content of wheat

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Abstract. Wheat is one of the most important cereal crops grown worldwide and provides most of the proteins in human diet. Grain protein content (GPC) determines the nutritional value and the baking properties of common wheat (*Triticum aestivum* L. *ssp. aestivum*) as well as the pasta-making technology characteristics of durum wheat (*Triticum turgidum* L. *ssp. durum*). GPC is a typical quantitative trait controlled by a complex genetic system and influenced by environmental factors and management practices, as well as nitrogen and water availability, temperature and light intensity. In higher plants inorganic nitrogen, in the form of ammonia, is assimilated via the glutamate synthase cycle or GS-GOGAT pathway. This assimilation requires cofactors, reducing equivalents and carbon skeletons generated during photosynthesis. We focused on the Glutamine synthetase and Glutamate synthase, as potential candidates for determining grain protein content. While Glutamine synthetase genes are a family whose enzymes are located in both cytoplasm and plastids, glutamate synthase exists in two different isoform depending on the electron donor used as cofactor, NADH-dependent and Fd- dependent GOGAT, both active in plastids. In the present manuscript has been reported an overview on the candidate gene involved found in the control of grain protein content.

Keywords. Wheat – Glutamine synthetase (GS) – Glutamate synthase (GOGAT) – Sequencing – Real Time PCR.

Caractérisation moléculaire des gènes candidats impliqués dans le métabolisme de l'azote et relation avec la teneur en protéines du grain de blé

Résumé. *Le blé est l'une des cultures céréalières les plus importantes dans le monde entier et il fournit la plupart des protéines de l'alimentation humaine. La teneur en protéines des grains (GPC) détermine la valeur nutritionnelle et les propriétés de cuisson du blé commun (Triticum aestivum L. ssp. aestivum) ainsi que les caractéristiques de la production de pâtes du blé dur (Triticum turgidum L. ssp. durum). La GPC est un caractère quantitatif typique contrôlé par un système génétique complexe et influencé par des facteurs environnementaux et les pratiques de gestion, ainsi que par la disponibilité de l'azote et de l'eau, la température et l'intensité lumineuse. Chez les plantes supérieures, l'azote inorganique, sous forme d'ammoniac, est assimilé par l'intermédiaire du cycle de la glutamate synthase ou la voie GS-GOGAT. Cette assimilation nécessite des cofacteurs, des équivalents réducteurs et des squelettes de carbone, générés lors de la photosynthèse. Nous nous sommes concentrés sur la glutamine synthétase et la glutamate synthase, comme des candidats potentiels pour déterminer la teneur en protéines du grain. Alors que les gènes de la glutamine synthétase sont une famille dont les enzymes sont situés à la fois dans le cytoplasme et les plastes, la glutamate synthase existe en deux isoformes différents selon le donneur d'électrons utilisé comme cofacteur, NADH dépendant et GOGAT Fd- dépendant, tous les deux actifs dans les plastes. Dans le présent travail, nous allons présenter une vue d'ensemble des gènes candidats impliqués dans le contrôle de la teneur en protéines du grain.*

Mots-clés. *Blé – Glutamine synthétase (GS) – Glutamate synthase (GOGAT) – Séquençage – PCR en temps réel.*

I – Introduction

The nutritional quality of cereals is an important component of the population diet as the cereals represent the largest component of world food supplies. In the years ahead wheat, perhaps more than other cereals, can be expected to assume greater importance as a source of protein for much of the world's increasing population. In durum wheat, seed storage proteins are important not only from the nutritional standpoint, but they have even greater significance for pasta-making quality. Grain protein concentration, protein quality, are same of the major quality attributes affecting pasta-making technology characteristics and resistance to overcooking (Autran *et al*., 1996). The accumulation of protein in kernels is related to the nitrogen availability. Nitrogen uptake is an essential element in crop improvement, either directly for grain protein content or indirectly for photosynthetic production. Thus, nitrogen utilization is fundamental to crop productivity, and over the past 50 years nitrogen (N) fertilizers have been extensively used to increase both grain yield (GY) and grain protein content (GPC) in cereals and wheat - helping to support a vastly increased world population. However, this requires that growers must optimize the use of N fertilizers to avoid pollution, while maintaining reasonable profit margins. Such crops would make better use of nitrogen fertilizer supplies giving higher yields with improved protein contents. Therefore, selecting new crop varieties exhibiting improved nitrogen use efficiency (NUE; the yield of grain per unit of available N in the soil), and adapting agricultural practices to reduce the use of N fertilizers both represent challenges for both breeders and farmers (Hirel, 2007).

Whether N is derived from soil reserves, from N fertilizer, or from N_2 fixation, it is incorporated into the organic form via the assimilation of ammonia. However, the primary assimilation of ammonia from external inorganic N is only part of the process. N is also released from organic combination as ammonia and re-assimilated many times during the movement of N around the plant, from seed reserve, through transport to vegetative organs, to eventual re-deposition in a new crop of seeds. There is also a major release and re-assimilation of N during the process of photorespiration in C3 plants. The process of ammonia assimilation is thus of crucial importance to crop growth and productivity.

II – Detection of QTL for grain protein content

GPC is a typical quantitative trait controlled by a complex genetic system and influenced by environmental factors and management practices (nitrogen and water availability, temperature and light intensity). Heritability estimates for GPC ranged from 0.41 (Kramer 1979) to 0.70 (Suprayogi *et al*., 2009), depending upon the genetic material, environment and the method of computation. The extensive review by Konzak (1977) and more recent investigations by (Levy and Feldman 1989; Stein *et al*., 1992; Snape *et al*., 1995; Sourdille *et al*., 1996; Joppa *et al*., 1997; Prasad *et al*., 1999; Khan *et al*., 2000; Perretant *et al*., 2000; Dholakia *et al*., 2001; Zanetti *et al*., 2001; Campbell *et al*., 2001; Börner *et al*., 2002; Blanco *et al*. 2002, 2006; Olmos *et al*. 2003; Groos *et al*., 2003; Prasad *et al*., 2003; Gonzales-Hernandez *et al*., 2004; Turner *et al*., 2004; Huang *et al*., 2006; Nelson *et al*., 2006; Zhang *et al*., 2008; Mann *et al*., 2009; Raman *et al*., 2009; Suprayogi *et al*., 2009; Sun *et al*., 2010) have indicated that factors influencing protein concentration in cultivated and wild wheats are located on all chromosomes. The lack of sufficient genetic variation for useful traits within the cultivated wheats has limited the ability of plant breeders to improve grain yield and grain quality.

Recently Blanco *et a*l. (2012) in their study evaluated grain yield components and GPC in five field trials with twelve replicates and in a RIL population derived by the cross of two durum wheat cultivars Ciccio x Svevo. Ten independent genomic regions involved in the expression of GPC were identified, six of which were associated with QTLs for one or more grain yield components. QTL alleles with increased GPC effects were associated with QTL alleles with decreased effects

on one or more yield component traits. Four QTLs for GPC showing always significant effects should represent genes that influence GPC independently from variation in the yield components. We compared the genomic regions involved in the quantitative expression of GPC found in the Svevo x Ciccio RIL population with the map position of QTLs found in different genetic materials. A major QTL on chromosome 2A was further investigated. The influence of group-2 chromosomes on GPC control was firstly reported by Joppa and Cantrell (1990) using durum wheat - var. *dicoccoides* chromosome substitution lines then confirmed by Blanco *et al*. (2006) in the durum backcross line 3BIL-85 (Latino x *dicoccoides*) and by Suprayogi *et al*. (2009) in the Canadian durum line DT695.

III – Candidate genes approach

The candidate gene approach has been applied in plant genetics in the past decade for the characterization and cloning of Mendelian and quantitative trait loci (QTLs) as a complementary strategy to map-based cloning and insertional mutagenesis. Candidate genes analysis is based on the hypothesis that known-function genes (the candidate genes, CGs) could correspond to loci controlling traits of interest (Gebhardt *et al*., 2007). CGs refer either to cloned genes presumed to affect a given trait ('functional CGs') or to genes suggested by their close proximity on linkage maps to loci controlling the trait ('positional CGs'). In plant genetics the most common way to identify a CG is to look for map co-segregation between CGs and loci affecting the trait. Statistical association analyses between molecular polymorphisms of the CG and variation in the trait of interest can let to affirm the involvement of the CG in a specific metabolic pathway. To select the most promising candidates from a large number of functional candidate genes, gene sequences should be tested for linkage to QTL for the trait of interest by molecular mapping, thereby identifying positional candidates (genes co-localizing with a QTL) (Pajerowska *et al*., 2005).

In the present work this approach has been applied to the study of grain protein content in durum wheat. Several studies have attested the key-role of the glutamine synthetase enzyme (GS) in plant nitrogen metabolism (Bernard *et al*., 2009) and GOGAT (glutamine-2-oxoglutarate amidotransferase). Glutamine synthetase gene encodes for an enzyme responsible of the first step of ammonium assimilation and transformation into glutamine and glutamate, essential compounds in aminoacid-biosynthetic pathway. GS exists in multiple enzyme forms with the chloroplastic isozyme encoded by one gene (GS2) and the cytosolic encoded by 3–5 genes depending on the species. Studies have shown that both GS isozymes are regulated in a developmental manner in leaves and have different metabolic roles (Tobin *et al*. 1985; Kamachi *et al*., 1991; Finnemann and Schjoerring 2000; Habash *et al*., 2001). Cytosolic GS has multiple metabolic functions, such as assimilating ammonia into glutamine for transport and distribution throughout the plant; immunolocalisation studies in tobacco (Brugie're *et al*., 1999), pine (Canovas *et al*., 2007), potato (Pereira *et al*., 1995) and rice (Sakurai *et al*., 1996; Tabuchi *et al*., 2005) have shown predominant vascular location in different organs. Whilst studies on GS regulation in several species have shown some common regulatory mechanisms, also highlighted differences particularly in gene expression, protein and enzyme activity levels (McNally *et al*., 1983). Few studies are available about genomic variation of these genes, therefore, it is important to study the role of each GS gene in a variety of plant species.

On the bases of phylogenetic studies and mapping data in wheat, ten *GS* cDNA sequences were classified into four sub-families denominate *GS1* (a, b, and c), *GS2* (a, b, and c), *GSr* (1 and 2) and *GSe* (1 and 2) (Bernard *et al*., 2008). Bernard *et al*. (2008) reported that QTLs for flag leaf total GS activity were positively co-localised with others for grain and stem nitrogen, but smaller correlations were established with loci for grain yield components; they identified QTLs for GS activity co-localised to a *GS2* gene mapped on chromosome 2A and to the *GSr* gene on 4A. Genetic studies in rice (Yamaya *et al*., 2002; Obara *et al*., 2004) and maize (Hirel *et al*., 2001,

2007; Galais and Hirel, 2004) demonstrated co-localisations of QTLs for GS protein or activity with QTLs relating to grain parameters at the mapped *GS* genes. Bernard *et al*. (2008) auspicated to integrate the biochemical and genetic approaches to further establish allelic differences in GS isozymes and to uncover new regulatory loci modulating GS activity in diverse genetic material or mapping populations. The GOGAT enzyme catalyzes the reductive transfer of the amide group of glutamine to 2-oxoglutarate to form two glutamate molecules (Krapp *et al*., 2005). Together with GS, it maintains the flow of N from NH_4^+ into glutamine and glutamate, which are then used for several other aminotransferase reactions during the synthesis of amino acids (Ireland and Lea, 1999). Kinetic and inhibitory studies have suggested that GOGAT is the rate-limiting step in amino acid production (Chen and Cullimore, 1989; Baron *et al*., 1994). In rice, two different GOGAT enzymes have been identified based on the electron donor: a ferredoxin (Fd)-dependent GOGAT and a NADH-dependent GOGAT. In rice, NADH-GOGAT is active in developing organs, such as unexpanded non-green leaves and developing grains (Yamaya *et al*., 2002). NADH-GOGAT has been proposed to be involved in the use of remobilized nitrogen, because it is located in the specific cell types which are important for solute transport from the phloem and xylem elements (Hayakawa *et al*., 1994).

In the present work we focused the attention on *GS* genes and GOGAT with the objectives to isolate and characterize the complete genomic sequences of these genes in the A and B genomes of two elite durum wheat cultivars differing for grain protein content and to assess the association with QTLs for grain protein content.

IV – GS-GOGAT candidate genes

The isolation of the complete glutamine synthetase gene sequences and the localization on the two homeologous chromosome 2A and 2B in the durum wheat cvs. Ciccio and Svevo characterized by different grain protein content has been reported by Gadaleta *et al*. (2011). *GS2-A2* located on 2A chromosome was found comprised of 13 exons separated by 12 introns The *GS2-B2* has the same intron/exon structure, but the two cultivars differ for the insertion of a 33 bp sequence located in the second intron of the cv. Svevo. The complete cytosolic glutamine synthetase gene sequences of the durum wheat cvs “Ciccio” and “Svevo” was also reported by Gadaleta *et al*. (2014). *GSe-A4* was found located on 4A chromosome and was comprised of 12 exons separated by 11 introns, while the *GSe-B4* gene on 4B chromosome was comprised of 11 exons separated by 10 introns (Gadaleta *et al*., 2014).

Specific primer were designed in the polymorphic regions and in order to genetically map the genes in a RIL population, obtained by crossing the two durum wheat cultivars Svevo and Ciccio. Mapping data localized *GS2* and *GSe* genes on chromosomes 2A, 2B, (*GS2*) and 4A, 4B (*GSe*) where four significant QTLs for GPC where found by Blanco *et al*. (2012).

The high sequence homology was found for plant cytosolic and plastidic *GS* as also reported by Bernard *et al*. (2008) suggesting that they are derived from a common ancestor, and providing molecular evidence supporting the mechanism of chloroplast evolution (Weeden, 1981). This model proposed that genes for plastid isozymes evolved by duplication of nuclear genes and subsequent specialization of each locus.

The genomic sequences of the two homoeologous A, and B *NADH-GOGAT* genes were obtained in the same durum wheat cultivars by Nigro *et al*. (2013). Analysis of the gene sequences indicates that all wheat *NADH-GOGAT* genes are composed of 22 exons and 21. The two hexaploid wheat homoeologous genes show the same exon/intron number and size except intron 13 which shows differences in both length and sequence for all of three homoeologues. A comparative analysis of sequences has been conducted among di- and mono-cotyledous plants and shows both regions of high conservation and of divergence.

qRT-PCR performed with the two durum wheat cvs Svevo and Ciccio (characterized by an high and low protein content, respectively) was conducted for the three genes (*GS2*, *GSe* and *GOGAT*).

Total RNA extracted from plants grown in field conditions and reverse-transcribed for qRT-PCR analyses. To test if the homoeologous genes show differential expression patterns, qRT-PCR were performed using specific primers designed to preferentially amplify the A and B sequences for the six genes in leaves (at seedling stage) at three different phenotypic stages (first leaf, flowering and grain filling).

Different expression levels of the two *NADH-GOGAT-3A* and *NADH-GOGAT-3B* genes was observed, transcript levels in the first leaf and grain filling stages showed similar expression levels, while a significantly higher value of transcripts was observed during flowering ($P<0.01$) (Nigro *et al*., 2013). A similar trend was observed for both the homoeologous genes and in the two cultivars (Fig.1).

The physical chromosome position of the *NADH-GOGAT-3B* gene co-localize with Meta QTLs for high protein content reported by Quraishi *et al*. (2011). They showed, a NUE QTL conserved at the same orthologous loci as the *GOGAT* gene on wheat chromosome 3B, rice chromosome 1, sorghum chromosome 3 and maize chromosomes 3 and 8, despite 50–70 million years of separate evolution associated with considerable sequence shuffling. For these reasons *NADH-GOGAT* is one of the potential candidate genes involved in the control of the complex character trait GPC.

The transcription level of the *GSe* and GS2 was also investigated. A significant different expression was observed for both genes between the two cvs. Higher values of expression were observed for *GSe-A4* during the flowering time, while the higher value of *GSe-B4* expression was observed during the maturation, indicating that the homoeologous alleles play non overlapping roles in the different phenological phases and that alleles encoded by "Svevo" are more expressed than the "Ciccio" ones, probably due to differences in the promoter region or to a different gene regulation between the two cvs Ciccio and Svevo (data reported in Gadaleta *et al*., 2014).

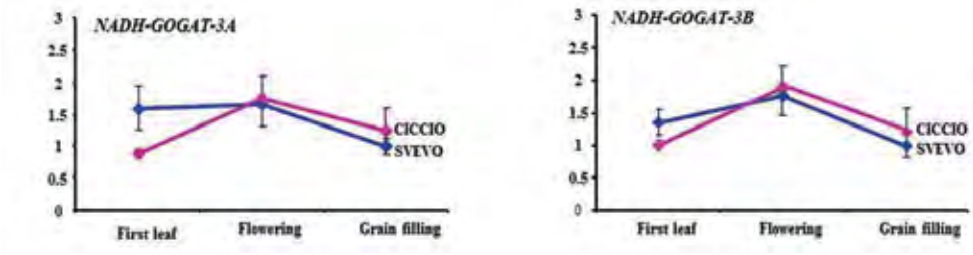

Figure 1. Comparison of the expression level of NADH-GOGAT-3A and NADH-GOGAT-3B genes in three different phenological phases (first leaf, flowering, grain filling) of cv Ciccio and Svevo.

A different trend was observed for *GS2-B2* whose transcript level increased during the three different phenological phases with a major value during maturation. ANOVA showed highly significant differences ($P<0.001$) between the two cultivars also for GS2-B2 gene during flowering and maturation (Fig.2). In conclusion we can say that in the present work candidate gene approach was efficiently applied for the study of grain protein content ion wheat.

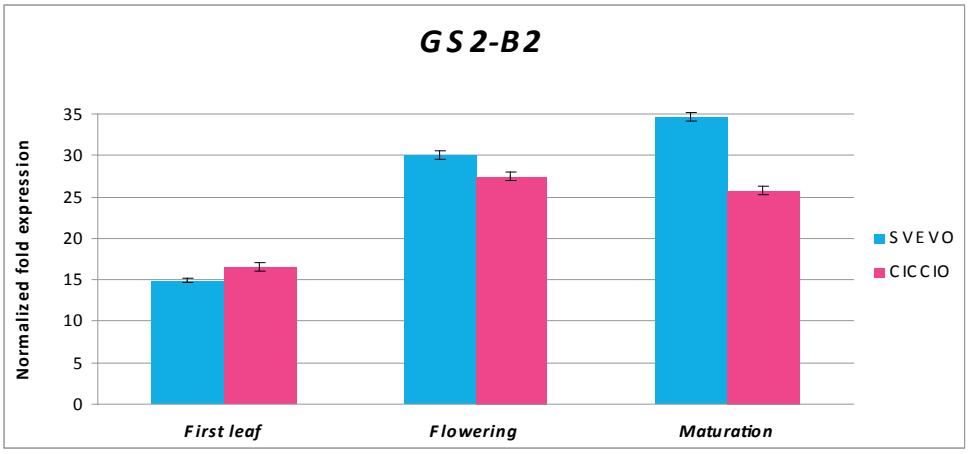

Figure 2. qRT-PCR conducted for GS2-B2 gene with specific probes in three different phenological phases (first leaf, flowering, grain filling) of cv Ciccio and Svevo.

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Mediterranean durum wheat landraces as a source of variability for quality improvement

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Abstract. A collection of 154 durum wheat landraces from 20 Mediterranean countries and 18 modern varieties was used to examine the existing variability for the main quality traits and to identify potential quality-enhancing genotypes for use in breeding programs. Field experiments were conducted during 3 years under rainfed conditions in north-eastern Spain. Based on yield and quality attr butes, landraces were clustered according to their region of origin in eastern Mediterranean, western Mediterranean, and North-Ba kan Peninsula. Landraces from the eastern Mediterranean countries had the highest global quality and the widest variability for quality traits, but were characterized by relatively small grains. Landraces from the western Mediterranean countries had the heavier grains, while landraces from the North-Balkan Peninsula had low quality and small quality variability. Modern varieties showed the highest global quality, but they had the lowest grain protein content and phenotypic variability. The assessment of the allelic composition at five glutenin loci (*Glu-A1*, *Glu-B1*, *Glu-A3*, *Glu-B3*, and *Glu-B2*) allowed identifying 114 alleles/banding patterns in the collection. Three rare banding patterns, found on a few number of landraces, affected significantly gluten strength. Landraces with improved quality traits, particularly gluten strength and grain weight were identified as potential donors for quality improvement.

Keywords. Protein content – Gluten strength – Kernel weight – HMW-GS – LMW-GS.

Variétés locales de blé dur de la Méditerranée comme source de variabilité pour l'amélioration de la qualité

Résumé. *Une collection de 154 variétés locales de blé dur provenant de 20 pays méditerranéens et 18 variétés modernes a été utilisée pour examiner la variabilité existante pour les principaux caractères de qualité et pour identifier des potentiels génotypes améliorateurs de qualité à intégrer dans les programmes de sélection. Des expériences de terrain ont été menées en sec pendant 3 ans dans le nord-est de l'Espagne. Sur la base du rendement et des attributs de qualité, les variétés locales ont été regroupées en fonction de leur région d'origine dans l'est de la Méditerranée, dans l'ouest de la Méditerranée et dans le nord de la péninsule balkanique. Les cultivars traditionnels provenant des pays de la Méditerranée orientale se caractérisent par la qualité globale la plus élevée et la plus large variabilité des caractères de qualité, mais ils présentent des grains petits. Les cultivars traditionnels provenant des pays de la Méditerranée occidentale ont les grains les plus lourds, tandis que les variétés locales du nord de la péninsule balkanique ont une faible qualité et une faible variabilité de la qualité. Les variétés modernes ont affiché la qualité globale la plus élevée, mais elles ont la plus faible teneur en protéines du grain et la plus faible variabilité phénotypique. L'évaluation de la composition allélique dans cinq loci de la gluténine (Glu-A1, Glu-B1, Glu-A3, Glu-B3 et Glu-B2) a permis d'identifier 114 allèles/profils de bandes dans la collection. Trois profils de bandes rares, observés sur un petit nombre de variétés locales, influent de manière significative sur la force du gluten. Les variétés locales avec des caractères de qualité améliorés, en particulier la force du gluten et le poids du grain, ont été identifiées comme donneurs potentiels pour l'amélioration de la qualité.*

Mots-clés. *Teneur en protéines – Force du gluten – Poids du grain – HMW-GS, LMW-GS.*

I – Introduction

The Durum wheat (*Triticum turgidum* L. var. *durum*) is a major crop in the Mediterranean Basin, a region close to the Fertile Crescent, where wheat was domesticated about 10,000 years BP. During the migration process East ward into the Mediterranean Basin, both natural and human

selection resulted in the establishment of landraces adapted to a diversity of agro-ecological zones and to local consumer preferences. From the early 1970s landraces were displaced by improved semi-dwarf cultivars which resulted in a decrease of diversity and the near-extinction of on-farm native genetic variability (Skovmand *et al*., 2005). Landraces, with their broad diversity within the species, are highly valuable sources for widening the genetic variability for numerous traits when made available to breeding programs. In particular, durum wheat landraces and old varieties from the Mediterranean region seem to retain a high level of polymorphism and large genetic diversity for grain and end-product quality (Aguiriano *et al*., 2008; Moragues *et al*., 2006).

The overall quality of durum wheat grain may be evaluated through the quality index (QI) established in 2003 by an EU Commission Regulation. The QI is based on protein content, gluten strength, yellow color index and test weight (or thousand kernel weight), and is expressed as a percentage with reference to specific check varieties. Gluten strength, one of the main factors influencing grain quality, strongly depends on the composition of storage proteins, among which glutenins are the most influential. The high molecular weight-glutenin subunits (HMW-GS) are encoded by the complex at the *Glu-1* loci (*Glu-A1* and *Glu-B1*), whereas the low molecular weight-glutenin subunits (LMW-GS) are encoded by genes at *Glu-A3*, *Glu-B3* and *Glu-B2* (Shewry *et al*., 1992; Vázquez *et al*., 1996).

This study was conducted with the aim of evaluating and characterizing the grain quality of a collection of Mediterranean landraces and a set of representative modern varieties −with special emphasis on banding patterns related to allelic variability for HMW-GS and LMW-GS−in order to detect the presence of variants of potential interest for breeding purposes. The geographic structure existing in the region was also assessed on the basis of yield and quality traits.

II – Material and methods

A collection 154 durum landraces from 20 Mediterranean countries and a set of 18 representative modern cultivars (see Nazco *et al*., 2012 for a complete description of genotypes), was tested during three years in Lleida (north-eastern Spain) in non-replicated experiments with three replicated checks. Plots were mechanically harvested, yield was expressed at 12% moisture level, and a sample of grain for each plot was used for quality determinations. Protein content (%) was determined by a near infrared spectroscope and gluten strength was assessed by the SDS (sodium dodecyl sulphate) sedimentation test (ml). Yellow color index was determined by means of a reflectance colorimeter. These three quality traits plus thousand kernel weight (TKW, g) were used to calculate the EU quality index (QI) relative to cv. 'Simeto', 'Gallareta' and 'Vitron' that were used as reference checks. The quotient between gluten strength and protein content was the sedimentation index (SI, ml/protein unit); test weight (TW, kg/hl) was determined by the Dickey-John equipment. The best linear unbiased predictors (BLUPs) were estimated for yield and quality data by Restricted Maximum Likelihood (REML). High- and low molecular weight glutenin subunit compositions at *Glu-A1*, *Glu-B1*, *Glu-A3*, *Glu-B3* and *Glu-B2* were assessed by electrophoretic analysis (1D SDS-PAGE).

III – Results and discussion

1. Variability and geographic structure

A large genotypic effect was observed for QI and gluten strength, but a high environmental influence was obtained for protein content as reported by Rharrabti *et al.* (2001, 2003). Landraces showed much larger variability than modern cultivars for the quality traits evaluated (Fig. 1), with

the widest variability recorded in landraces from the eastern Mediterranean Basin (Nazco *et al.,* 2012).

The first two PC axes of the Principal Component Analysis (PCA), conducted with yield and quality data, grouped the germplasm under study in four clusters, corresponding to (1) modern cultivars, (2) landraces from the Eastern Mediterranean Basin, (3) landraces from the western Mediterranean Basin, and (4) landraces from the north-Balkan countries (see Nazco *et al*., 2012). Landraces from the eastern Mediterranean Basin had the best overall quality among the set of landraces, on the basis of their high gluten strength and yellow index. Landraces from the north Balkan countries had high grain weight and low overall quality and gluten strength, while landraces from the western Mediterranean countries had intermediate properties between both groups (Fig. 2). Modern varieties showed the best average grain yield, gluten strength, yellow index, SI and overall quality. However, their protein content was lower than that of landraces (Fig. 2) as reported by previous studies (De Vita *et al*., 2007; Royo *et al*., 2007). Nevertheless, landraces were identified that could be used in breeding programs with the least negative effect on overall quality.

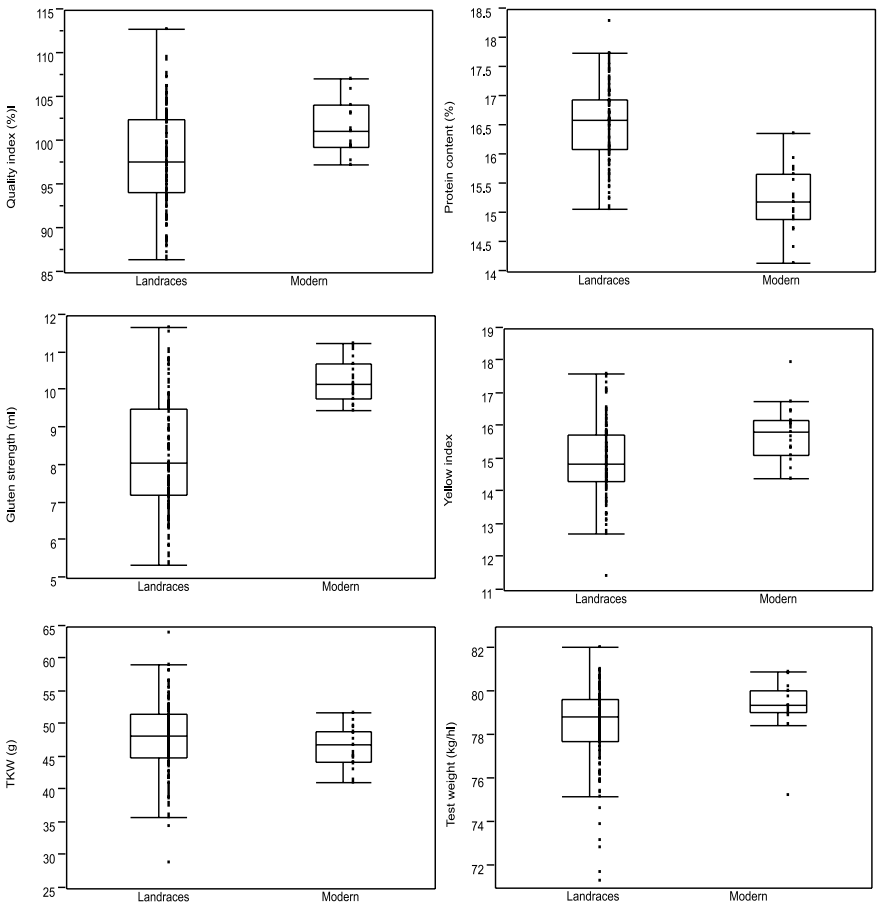

Figure 1. Box plots showing the variability existing within landraces and modern cultivars for grain quality traits. Data are 3-years adjusted means of samples from field experiments conducted in north-eastern Spain.

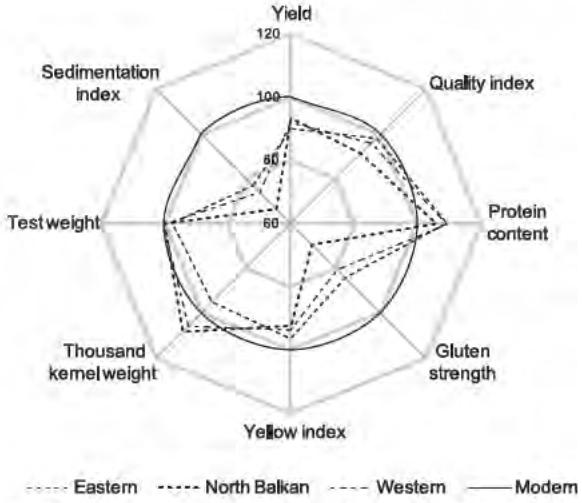

Figure 2. Comparison of relative yield and quality traits of landraces from three Mediterranean regions in relation to modern cultivars.

2. HMW-GS and LMW-GS allelic composition

A total number of 114 banding patterns, potentially *Glu-1*/*Glu-3* allele-specific were identified in the collection (see details in Nazco *et al.*, 2013). From them, 85% were present exclusively in landraces and at low frequencies (<0.05). All the patterns present in modern cultivars were also present in the landraces. The null allele was the most frequent at *Glu-A1*, as previously reported in other durum collections (Branlard *et al.*, 1989). It was detected in 62% of the landraces and in all modern varieties. However, none of the 5 alleles found at this locus had a significant effect on gluten strength. For the locus *Glu-B1*, the most frequent banding patterns were 7+8 in modern cultivars (55.5%) and band 20 (*Glu-B1e*) in the landraces (36.8%). All modern cultivars had SDS-values ≥ 9 ml, and therefore none of the HMW-GS allele/banding patterns had a significant effect on their gluten strength, as determined by SDS-sedimentation test. Of the 20 banding patterns distinguished in the landraces at *Glu-B1*, the banding pattern 20 had a small but positive and significant effect on the gluten strength of the landraces, increasing the average SDS-sedimentation value of the landraces carrying it by 6.2% (Fig. 3). Larger genetic variability was found at *Glu-B1* in this study than in a previous one (Moragues *et.al.*, 2006) also conducted with Mediterranean landraces.

For the LMW-GS, 15 patterns were identified at *Glu-A3* in the landraces and only 3 of them in modern cultivars (Nazco *et al.*, 2013). Alleles *Glu-A3a* (band 6) and *Glu-A3d* (bands 6+11) had a positive and significant effect on the gluten strength (Fig. 3). The most variable locus was *Glu-B3*, with72 banding pattern/putative alleles identified. Among them, the most frequent banding pattern in modern varieties (77.8%) was 2+4+15+19 (*Glu-B3a*), which within the set of landraces had an enhancing effect of SDS-value (Fig.3). This result was in agreement with previous studies relating good quality to the presence of *Glu-B3a* (Carrillo *et al.*, 2000; Nieto-Taladriz *et al.*, 1997).

It is important to note that the model LMW-1, associated with very week gluten properties, was absent in this collection, indicating that the effects of the alleles on gluten strength measured herein are quite significant in their magnitude (detected even in the presence in all genotypes of the strength-enhancing LMW-2 models).

Three among the rare banding patterns found in the landraces at *Glu-B3* had a significant effect on gluten strength, but only for two of them differences between gluten strength groups could

be associated with their frequency. They were banding pattern 2+4+14+15+18 (present in the Egyptian landrace PI-366109) and 2+4+15+18+19 (Fig.3), present in two landraces: 'Trigo Glutinoso' from France and 'Lobeiro de Grao Escuro' from Portugal (Nazco *et al*., 2013).The two alleles reported at *Glu-B2* (*Glu-B2b* or null and *Glu-B2a* or band 12) were found in both sets of germplasm at high frequencies and with a significant effect on the gluten strength of the landraces. However, while band 12 had a positive effect on gluten strength, the effect of the null allele was detrimental (Fig 3).

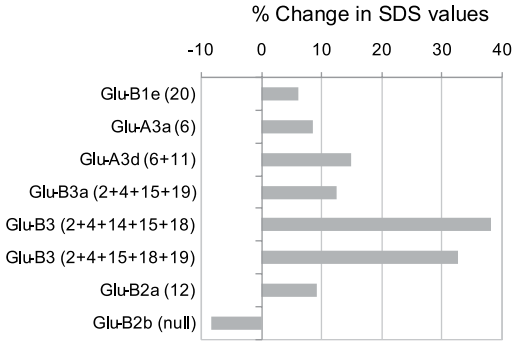

Figure 3. Alleles/banding patterns with significant effect (*P*<0.05) on the SDS-sedimentation volume of the landraces carrying them. Prepared from data shown in Nazco *et al*., 2013.

3. Landraces useful to enhance grain quality in breeding programs

At country level the greatest QI was recorded in Cypriot landraces, which also showed the best gluten strength and SI values (Nazco *et al.*, 2012). For individual genotypes, the best QI (113% relative to the average of the three check cultivars) was recorded in the Egyptian landrace identified in the USDA Germplasm Bank as PI-366109. This landrace and also 'Lobeiro de Grao Oscuro' from Portugal were among the 10th percentile for QI, protein content and gluten strength simultaneously.

The Spanish landrace 'Raspinegro de Alcalá' was among the best for QI, gluten strength, yellow index and SI simultaneously. The Israeli landrace 'Hati' was among the best entries for yellow index and TW simultaneously. The Spanish landrace 'Enano de Andújar' had the heaviest grains with a TKW surpassing a 23.5% that of Simeto, the modern cultivar with the heaviest grains. Among the modern set, the Italian cv. 'Svevo' and the U.S. desert durum cv.'Ocotillo' reached the greatest overall quality standards. Many new/un-reported banding patterns were identified and some apparently influencing the expression of gluten strength. These will need to be transferred to modern backgrounds and subsequently evaluated for their potential strength-enhancing effects or their capacity to produce gluten qualities that may be useful in the production of specific types of products.

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Purple grain colour genes in wheat

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Abstract. Purple colour of wheat grain is caused by accumulation of anthocyanins in the pericarp. Potential health benefits and adaptability may be the reason for a renewed interest in wheat with high anthocyanin content. Genetic bases underlying purple grain character as well as some practical aspects are reviewed in this paper. Two complementary dominant genes control purple pericarp coloration in both durum (*Triticum durum* Desf., *2n* = *4x* = 28) and bread (*T. aestivum* L., *2n* = *6x* = 42) wheat. In durum wheat, these genes are mapped to be on chromosomes 2A (*Pp3*) and 7B (*Pp-B1*), whereas in bread wheat they are located on chromosomes 2A (*Pp3*) and 7D (*Pp-D1*). Functional alleles of at least one of the two complementary genes determining purple pericarp exist in *Ae. speltoides*, *Ae. tauschii* and *T. timopheevii* – the species, in which purple-grained plants have never been descr bed. The wheat *Pp-1* and *Pp3* genes regulate transcription of the anthocyanin biosynthesis structural genes and may encode transcription regulatory factors that belong to the MYB- and MYC-l ke superfamilies, respectively. Their orthologues were cloned in maize, rice and barley and can be used for homology-based cloning of wheat *Pp-1* and *Pp3*. Usefulness of microsatellite markers closely linked to the *Pp* genes for marker-assisted selection has been demonstrated.

Keywords. Purple grain – Durum wheat – Bread wheat – Gene – Mapping – Orthologous genes – Marker-assisted selection.

Les gènes de la couleur violette du grain chez le blé

Résumé. *La couleur violette du grain de blé est déterminée par l'accumulation des anthocyanes dans le péricarpe. Les bienfaits potentiels pour la santé et l'adaptabilité peuvent être la raison d'un regain d'intérêt pour le blé ayant une teneur en anthocyanes élevée. Dans ce travail, nous allons examiner les bases génétiques du caractère grain violet ainsi que certains aspects pratiques. Deux gènes complémentaires dominants contrôlent la coloration violette du péricarpe chez le blé dur (Triticum durum Desf., 2n = 4x = 28) et chez le blé tendre (T. aestivum L., 2n = 6x = 42). Chez le blé dur, ces gènes sont cartographiés sur les chromosomes 2A (Pp3) et 7B (Pp-B1), alors que chez le blé tendre, ils sont situés sur les chromosomes 2A (Pp3) et 7D (Pp-D1). Les allèles fonctionnels d'au moins un des deux gènes complémentaires déterminant le péricarpe violet sont présents sur Ae. speltoides, Ae. tauschii et T. timopheevii - les espèces, pour lesquelles les plantes à grain violet n'ont jamais été décrites. Les gènes du blé Pp-1 et Pp3 régulent la transcription des gènes de structure de la biosynthèse des anthocyanines et peuvent coder pour des facteurs de transcription régulateurs qui appartiennent à la superfamille de type MYB et MYC, respectivement. Leurs orthologues ont été clonés chez le maïs, le riz et l'orge et peuvent être utilisés pour le clonage basé sur l'homologie du Pp-1 et PP3 du blé. L'utilité des marqueurs microsatellites étroitement liés aux gènes Pp pour la sélection assistée par marqueurs a été démontrée.*

Mots-clés. *Grain violet – Blé dur – Blé tendre – Gènes – Cartographie – Gènes orthologues – Sélection assistée par marqueurs.*

I – Introduction

Wheat grain may have white, red, blue or purple colour. Red colour is caused by proanthocyanidins in the seed coat, blue colour is due to production of anthocyanins in the aleurone layer, whereas purple colour is caused by accumulation of anthocyanins in the pericarp. Anthocyanins are flavonoid pigments well-known for their free radical scavenging capacity (Kähkönen and Heinonen, 2003). Besides antioxidant activity, other properties of anthocyanins have been described, such as estrogenic activity, enzyme inhibition, anti-inflammatory activity, capillary permeability and

fragility decrease, membrane strengthening, protection from DNA cleavage, *etc.* (Lila, 2004). The potential nutritional and health benefits may not be the only reason for a renewed interest to wheat with high anthocyanin content. Comparative analysis of wheat near-isogenic lines differing by anthocyanin content in the coleoptile and pericarp (Tereshchenko *et al*., 2013) showed higher drought tolerance of intensely colored seedlings (Tereshchenko *et al*., 2012a). Furthermore, the purple-grained NILs had better viability after artificial ageing compared to the recurrent parent lacking anthocyanins (Gordeeva and Khlestkina, 2013a). This is in agreement with finding of Debeaujon *et al*. (2000) in Arabidopsis: mutants affected in testa pigmentation showed a reduced germination capacity after long storage.

II – Inheritance of the purple grain character and mapping *Pp* genes using molecular markers

Investigation of the genetic basis of the purple pericarp trait has been performed in both durum (*Triticum durum* Desf., *2n* = *4x* = 28) and bread (*T. aestivum* L., *2n* = *6x* = 42) wheat. Sharman (1958) described dominant monogenic inheritance of purple pericarp trait in tetraploid wheat. Bolton (1970) reported dominant digenic inheritance with possible complementary effect in hexaploid wheat. Two dominant complementary genes for purple grain were localized by Piech and Evans (1979) on chromosomes 3A and 7B of bread wheat. Arbuzova *et al.* (1998) localized one of the two complementary *Pp* genes on chromosome 7B (*Pp1*) and suggested another two loci on chromosomes 6A (*Pp2*) or 2A (*Pp3*) of bread wheat. However, certain number of *Pp* genes and their precise chromosome location remained unclear until the molecular marker era.

In durum wheat cross 'TRI 15744' (purple-grained) × 'TRI 2719' (white-grained), the segregation ratio for grain colour was consistent with 9:7, suggesting the presence of two dominant complementary *Pp* genes in the purple-grained accession 'TRI 15744' (Khlestkina *et al.*, 2010a). One of these genes, *Pp1*, forms a cluster with dominant genes *Pc*, *Plb*, *Pls* (Khlestkina *et al.*, 2010a) and *Rc* (Tereshchenko *et al.*, 2012b) determining anthocyanin coloration of culm, leaf blades, leaf sheaths and coleoptile, respectively. This cluster has been mapped to be located on the short arm of chromosome 7B between the microsatellite loci *Xgwm0951* and *Xgwm0573* (Khlestkina *et al.*, 2010a). The complementary *Pp* gene has been mapped to the long arm of chromosome 2A between the loci *Xgwm0328* and *Xgwm0817* close to the centromere. This position is highly comparable with localization of the *Pp3* gene in bread wheat (Dobrovolskaya *et al.*, 2006), thus the *Pp* gene of durum wheat mapped to chromosome 2A has been designated *Pp3*. This gene is closely linked to the dominant *Pg* gene determining purple glume in durum wheat. Unlike purple pericarp, purple glume is a monogenically inherited trait (Khlestkina *et al.*, 2010a). Dobrovolskaya *et al.* (2006) has shown the bread wheat *Pp2* gene (suggested on chromosome 6A by Arbuzova *et al*., 1998) to be an allelic variant of *Pp3* on chromosome 2A.

In bread wheat, the gene complementary to *Pp3*, inherited from either 'Purple' or 'Purple Feed', has been mapped to the short arm of chromosome 7D (Tereshchenko *et al.*, 2012b). This gene forms a cluster with *Pan* (Laikova *et al.*, 2005), *Pc*, *Plb*, *Pls* and *Rc* genes (Tereshchenko *et al.*, 2012b), determining anthocyanin coloration of anthers, culm, leaf blades, leaf sheaths and coleoptile, respectively. This cluster has been mapped between the microsatellite loci *Xgwm0044* and *Xgwm0111* on chromosome 7DS. The bread wheat *Pp* gene on 7DS is homoeoallelic to the durum *Pp1* gene on 7BS, therefore these loci have been designated *Pp-D1* (on 7DS of bread wheat) and *Pp-B1* (on 7BS of durum wheat), respectively (Tereshchenko *et al.*, 2012b). Localization of one of the complementary *Pp* genes in the D-genome of bread wheat doesn't support the former view that the purple pericarp trait of bread wheat has been transferred from purple-grained Ethiopian tetraploid wheats (Copp, 1965; Bolton, 1970). At least one of the two complementary genes in bread wheat was inherited from *Aegilops tauschii* (Tereshchenko *et al.*, 2012b).

Bread wheat 'Novosibirskaya 67' carries the dominant *Pp* gene allelic to the *Pp-D1* locus of 'Purple' or 'Purple Feed', but has a recessive allele at the *Pp3* locus, therefore anthocyanins are not synthesized in the pericarp of this cultivar (Dobrovolskaya *et al.*, 2006). However, introgression of tetraploid timopheevii wheat chromosome segment (2G) into 'Novosibirskaya 67' chromosome 2B (Leonova *et al.*, 2002) causes production of anthocyanin pigment in pericarp (Gordeeva *et al*., unpublished). Furthermore, bread wheat introgression line 'PC' having *Pp-S1* gene inherited from *Aegilops speltoides* and introgressions of timopheevii wheat to chromosomes 2A and 2B has purple pericarp too (Tereshchenko *et al.*, 2012c). Thus, in tetraploid timopheevii wheat, the gene either allelic or homoeoallelic to *Pp3* may exist. Overall, functional alleles of at least one of the two complementary genes determining purple pericarp still exist in *Ae. speltoides*, *Ae. tauschii* and *T. timopheevii* – the species, in which purple-grained stocks have never been described (Tereshchenko *et al.*, 2012b, 2012c).

III – Orthologues and future prospects for homology-based cloning

In order to understand a nature of the two complementary factors encoded by the wheat *Pp-1* and *Pp3* loci, we compared map positions of these genes (Dobrovolskaya *et al.*, 2006; Khlestkina *et al.*, 2010a; Tereshchenko *et al.*, 2012b) with locations of well-studied rice and maize purple seed colour genes (Table 1; Fig. 1).

The most probable orthologues of the wheat *Pp3* locus are rice *Pb*/*Ra* and maize *Lc*/*R* genes determining pericarp colour and encoding MYC-like regulatory factor of anthocyanin biosynthesis (Dooner and Kermicle, 1976; Ludwig *et al.*, 1989; Hu *et al.*, 1996; Wang and Shu, 2007). The *Pb*/*Ra* gene is located on rice chromosome 4, which has a colinearity with Triticeae chromosome 2 (Stein *et al.*, 2007). The *Lc*/*R* gene has been mapped to maize chromosome 10, which has colinearity with rice chromosome 4 (Ahn and Tanksley, 1993). Recently, Cockram *et al.* (2010) isolated a candidate gene for the barley *ant2* (*anthocyaninless 2*) locus. *Ant2* is located on chromosome 2H (Lundqvist *et al.*, 1996), and its candidate gene encodes a transcriptional factor, which also belongs to the bHLH (MYC) family (Cockram *et al.*, 2010). In rye, the gene determining pericarp colour was localized in chromosomes 2R (de Vries and Sybenga, 1984) which has colinearity with *Triticum* chromosome 2 (Devos *et al*., 1993).

A putative orthologue of the wheat *Pp-1* gene is the maize *C1* gene (*colorless 1*) encoding MYB-like regulatory factors affecting transcription of the structural anthocyanin biosynthesis genes (Cone *et al*., 1986, 1993). Li *et al*. (1999) used the *C1* sequence as a probe to locate homologous sequences in wheat. They identified loci (designated *Mpc1*) in those regions of wheat homoeologous group 7 chromosomes (Li *et al*., 1999), that correspond to map positions of the genes *Pp-B1* and *Pp-D1* (Tereshchenko *et al.*, 2012b). In rice, the gene *Pa* (*purple apiculus*) has been mapped on chromosome 6, which has colinearity with Triticeae chromosome 7 (Stein *et al.*, 2007). In barley, the *ant1* (*anthocyaninless 1*) locus mapped to the short arm of chromosome 7H (Lundqvist *et al*., 1996) can be an orthologue of wheat *Pp-B1* and *Pp-D1* (Table 1).

Thus, the wheat *Pp-1* and *Pp3* genes may encode transcription regulatory factors that belong to the MYB- and MYC-like superfamilies, respectively. Tereshchenko *etal*. (2013) used wheat near-isogenic lines differing by allelic state of the *Pp-1* and *Pp3* genes and compared transcriptional activity of the anthocyanin biosynthesis structural genes (*Chs* encoding chalcone synthase, *Chi* – chalcone-flavanone isomerase, *F3h* – flavanone 3-hydroxylase, *Dfr* – dihydroflavonol-4-reductase, and *Ans* – anthocyanidin synthase) in the pericarp. It was shown that dominant *Pp-D1* and *Pp3* alleles activate *F3h* expression in pericarp and induced more intensive transcription of the other structural genes (*Chs*, *Chi*, *Dfr*, and *Ans*). This is in accordance with a suggestion that the *Pp-1* and *Pp3* genes are transcriptional regulators in the anthocyanin biosynthesis network.

Homology-based cloning of these genes can be performed using known sequences of the maize, rice and barley genes encoding the MYB- and MYC-like anthocyanin biosynthesis regulatory factors.

Table 1. Regulatory genes of the anthocyanin biosynthesis in barley, rice and maize, having orthologous map positions compared to wheat *Pp* genes, determining purple pericarp colour.

	MYB-like regulatory factor			MYC-like regulatory factor		
	gene designation	chromosome location	reference	gene designation	chromosome location	reference
Bread- wheat	*Pp-D1*	7D	Tereshchenko *et al*., 2012b	*Pp3*	2A	Dobrovolskaya *et al*., 2006
Durum- wheat	*Pp-B1*	7B	Khlestkina *et al*., 2010	*Pp3*	2A	Khlestkina *et al*., 2010
barley	*Ant1*	7H	Lundqvist *et al*., 1996	*Ant2*	2H	Lundqvist *et al*., 1996
rice	*Pa*	6	Liu *et al*., 2012	*Pb*/*Ra*	4	Wang and Shu, 2007
maize	*C1*	9	Cone *et al*., 1993	*Lc*/*R*	10	Dooner and Kermicle, 1976

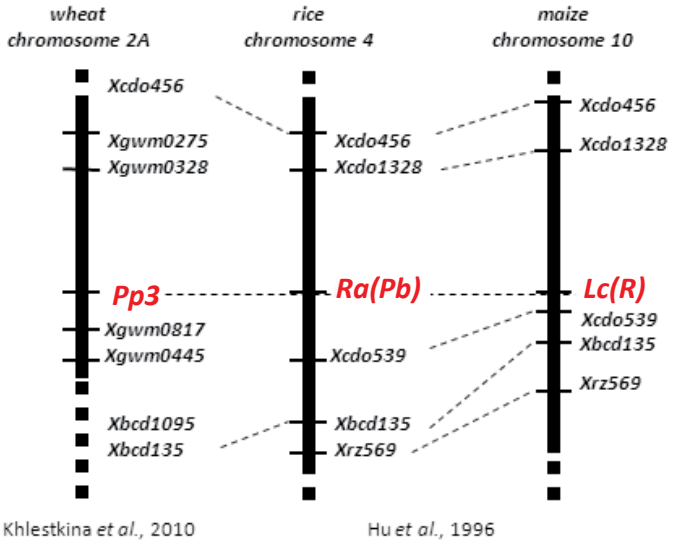

Figure 1. Comparative map positions of durum wheat *Pp3* gene determining purple pericarp colour and genes for purple seed in rice and maize.

IV – Marker-assisted selection in basic and applied research

In order to better understand the mechanisms of anthocyanin biosynthesis regulation in wheat pericarp and to determine particular role of each of the two complementary *Pp* genes, isogenic lines with different combinations of the *Pp* alleles were needed. To obtain a set of such lines we performed marker-assisted backcrossing of the near-isogenic line 'i:S29Pp1Pp2^{PF}' of bread wheat 'Saratovskaya 29' (Gordeeva and Khlestkina, 2013b). This line carries two introgressions from bread wheat 'Purple Feed' at the 'Saratovskaya 29' background (Arbuzova *et al*., 1998). The introgressions extend from microsatellite locus *Xgwm0558* on chromosome 2AS to *Xgwm0294* on 2AL and from *Xgwm0044* to *Xgwm0111* on 7DS (Tereshchenko *et al*., 2012b). These two

donor's segments carry dominant alleles of the genes *Pp3* (on chromosome 2A) and *Pp-D1* (on chromosome 7D), thus conferring intensive purple pericarp colour in 'i:S29Pp1Pp2^{PF}'. Donor's segment on chromosome 7D also carries dominant alleles at the *Rc-D1*, *Pc-D1*, *Pan-D1*, *Pls-D1*, *Plb-D1* loci, determining intensive anthocyanin pigmentation of the coleoptile, culm, anthers, leaf sheath and leaf blade, respectively (Tereshchenko *et al.*, 2012b). 'Saratovskaya 29' has no anthocyanin pigment in the pericarp, but has light pigmentation on the coleoptile, culm, leaf sheath and leaf blade, controlled by the dominant genes *Rc-A1*, *Pc-A1*, *Pls-A1* and *Plb-A1*, respectively (Khlestkina *et al.*, 2010b). We crossed 'i:S29Pp1Pp2^{PF}' with 'Saratovskaya 29' and used combined foreground and background marker-assisted selection approach to get homozygous F_2 lines with different combinations of *Pp* alleles (Gordeeva and Khlestkina, 2013b).

To choose the line with dominant *Pp-D1* and recessive *Pp3*, foreground (using chromosome 7D markers) and background (2A) selection was performed. In the selected line the recipient's chromosome 2A has been completely recovered, whereas chromosome 7D still carries the donor's segment. This line has lost anthocyanin pigment in the pericarp but retained intensive coloration of other organs (Gordeeva and Khlestkina, 2013b). Overall, wheat *Pp-1* gene is tightly linked with genes determining anthocyanin pigmentation of coleoptile, stem, leaves and anthers. This relationship was observed in durum wheat for *Pp-B1* (Khlestkina *et al.*, 2010a), in bread wheat for *Pp-D1* (Tereshchenko *et al.*, 2012b). In addition to material described in Khlestkina *et al.* (2010a) and Tereshchenko *et al.* (2012b), we studied coleoptile coloration of purple-grained wheats 'Konini', 'ANK-28A' and 'ANK-28B' and observed intensive anthocyanin pigmentation in each of them. However, this relationship can be a specific feature of wheat. For just a few of the purple-grained barley accessions maintained in IPK-Genbank have colored coleoptile.

To choose the line with dominant *Pp3* and recessive *Pp-D1*, foreground (using chromosome 2A markers) and background (7D) selection was carried out. In the selected line the recipient's chromosome 7D has been completely recovered, whereas chromosome 2A still carries the donor's segment. This line has light anthocyanin pigmentation of coleoptile, culm, leaf sheath and leaf blade similar to the recurrent parent 'Saratovskaya 29'. However, pericarp pigmentation is light purple, that is distinct from both parents, 'i:S29Pp1Pp2^{PF}' with intense anthocyanin pigmentation and 'Saratovskaya 29' without anthocyanins in pericarp (Gordeeva and Khlestkina, 2013b). This finding suggests interaction between dominant *Pp3* inherited from 'i:S29Pp1Pp2^{PF}' with another *Pp* gene, most likely *Pp-1* (*Pp-A1*) within a cluster of anthocyanin regulatory genes on chromosome 7A (Khlestkina *et al.*, 2010b). Further marker-assisted split of complementary *Pp* genes (*Pp3* and *Pp-A1*) into different near-isogenic lines is in progress.

The microsatellite markers successfully used for marker-assisted backcrossing of 'Saratovskaya 29' NILs, can be recommended for marker-assisted breeding of bread and durum wheat for purple grain colour. We propose that breeding material having red coleoptile is needed addition of *Pp3* dominant allele only in order to obtain purple-grained phenotype, whereas plants with green coleoptiles require introduction of both complementary genes *Pp3* and *Pp-1*.

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Biochemical and molecular approaches for the technological quality assessment of durum wheat varieties

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Abstract. Durum wheat (*Triticum turgidum ssp. durum*), one of the most important cultivated species in North-Africa, is mainly used for pasta and, to a lesser extent, as flat-bread and couscous for human consumption. A lot of breeding programs concentrate mainly on the dough quality. Quality value is determined by protein content, glutenin, and gliadin allelic composition, the different ratios between those subunits and with less importance the expression of other loci. The aims of our study are to determine some genetic tools of selection, from a choice of high quality wheat genotypes, and propose a breeding improvement program. Among thirty-seven varieties, we assessed the dough quality based on some technological parameters (such as, grain protein content, SDS-sedimentation volume, mixogram) and analysed the variability of gluten subunits (HMW and LMW glutenins and gliadins). Based on the results, we have characterized and selected varieties with better gluten strength and some landraces that displayed the best grain protein contents. The relationships between technological parameters and protein electrophoretic patterns showed that the presence of some *Glu-B1* subunits correlated, with a positive influence, on SDSS parameters value. In contrast other subunits of the locus *Glu-A1* have a negative influence on gluten strength.

Keywords. *Triticum turgidum* var. *durum* – Prolamins – Gluten quality.

Approches biochimiques et moléculaires pour l'évaluation de la qualité technologique des variétés de blé dur

Résumé. *Le blé dur (Triticum turgidum ssp. durum), l'une des espèces cultivées les plus importantes de l'Afrique du Nord, est principalement utilisé pour la fabrication des pâtes et, dans une moindre mesure, du pain plat et du couscous destinés à la consommation humaine. Bon nombre de programmes d'amélioration se concentrent essentiellement sur la qualité de la pâte. La valeur de qualité est déterminée par la teneur en protéines, en gluténine et la composition allélique de la gliadine, les différents rapports entre ces sous-unités et à un degré moindre, l'expression d'autres loci. Cette étude a pour objectif de déterminer des outils génétiques de sélection, à partir d'un choix de génotypes de blé de haute qualité, et de proposer un programme d'amélioration génétique. Parmi trente-sept variétés, nous avons évalué la qualité de la pâte sur la base de certains paramètres technologiques (tels que la teneur en protéines du grain, le volume de sédimentation en milieu SDS, le mixogramme) et analysé la variabilité des sous-unités du gluten (gluténines et gliadines de faible et de haut poids moléculaire). Les résultats obtenus nous ont permis de caractériser et sélectionner des variétés ayant une meilleure force du gluten et des variétés locales qui présentaient les meilleures teneurs en protéines du grain. Les relations entre les paramètres technologiques et les profils électrophorétiques des protéines ont montré que la présence de certaines sous-unités Glu-B1 est corrélée positivement à la valeur des paramètres SDSS. A l'inverse, d'autres sous-unités du locus Glu-A1 ont une influence négative sur la force du gluten.*

Mots-clés. *Triticum turgidum var. durum – Prolamines – Qualité du gluten.*

I – Introduction

Wheat endosperm proteins, namely prolamins are subdivided into gliadins and glutenins, according to their polymerisation properties (Miguel *et al.,* 2011). Durum wheat (*Triticum turgidum* var. *durum*), the preferred raw material for the production of pasta worldwide, is usually cultivated under rainfed conditions in the Mediterranean Basin, which often imposes a number of environmental stresses on the crop. Terminal drought stress during the grain-filling period, usually results in yield reductions, but in most cases results in high grain quality (Nazco *et al*., 2013). Several studies have shown the existence of variability for quality traits in durum wheat landraces (Moragues *et al*., 2006; Aguiriano *et al*., 2008; Nazco *et al*., 2013). Our capacity to gain profit from this diversity depends on the identification of accessions containing genes and alleles demonstrated to be useful in breeding programmes. Differences in dough properties and baking quality are largely determined by the superimposed effects of protein content, the size distribution of the polymeric glutenin, the allelic compositions of the HMW-GS and the LMW-GS, and on the relative amounts of the different glutenin subunits (Ruiz and Carrillo, 1995; Vazquez *et al.*, 1996; Patil *et al.*, 2006). Previous studies applied the γ-gliadins 45 and 42 as markers for good and poor quality of gluten quality, respectively (Damidaux *et al.*, 1978; du Cros *et al.*, 1982). This is due to the genetic linkage with LMW-GS (Payne *et al.*, 1980, 1983, 1984). In fact, pasta cooking quality and gluten strength were initially related to the negative and positive effects of the low Mr glutenin subunit patterns LMW-1 and LMW-2, respectively (du Cros, 1987; Pogna *et al.*, 1990).The HMW glutenin appears to have less critical effects than the LMW glutenin on the gluten strength of durum wheat (Vazquez *et al.*, 1996; Brites and Carillo, 2001). Nevertheless, this has not been clearly established due to limited genetic variability at the *Glu-1* loci present in modern durum wheat cultivars used in published studies (Sisson *et al.*, 2005). It has been suggested that the identification of genes influencing dough quality, other than those controlling the gluten fraction, might be useful way of recognizing others factors (Law *et al.*, 2005).The aims of this study are to analyse the variability of HMW-GS and B-LMW-GS and determinate the effect of *Glu-B1* subunits of HMW on gluten strength.

II – Material and methods

Thirty-seven durum wheat (*Triticum durum Desf*.) varieties (30 landraces and 7 modern cultivars) were included in this study.The varieties were sown in a randomised complete-block, with two repetitions, design under rainfed condition in two different localities. Proteins were extracted from crushed endosperm following a sequential procedure (Singh *et al.*, 1991). Electrophoresis of reduced and alkylated proteins (high and low molecular weight glutenin subunits) was performed on sodium dodecyl sulphate polyacrylamide gels (SDS-PAGE) according to Payne *et al.* (1980). Gliadins were fractionated by A-PAGE (Lafiandra and Kasarda, 1985). B-LMW glutenin alleles were designated according to Nieto-Taladriz *et al.* (1997) nomenclature. Protein content at 14% moisture basis, was estimated by a near-infrared reflectance analysis (NIR) using a Technicon Infralyzer 300. Gluten strength was estimated by SDS-sedimentation (SDSS) test according to Dick and Quick (1983). Rheological properties were determined by Mixograph-10g whole wheat meal (Finney and Shogren, 1972). The mixograph parameters measured were: mixing development time (MT), maximum peak height (MH), height at 3 min after the peak of the curve (H3), and resistance to breakdown (BDR) (difference in percentage between MH andH3). Values represent means of four repetitions (two repetitions in each locality).

III – Results and discussion

Durum wheat samples analysed in this study displayed a wide range in dough strength and gluten properties. In order to analyse the variability of *Glu-A1* and *Glu-B1* (HMW-GS), *Glu-A3, Glu-B3*

and *Glu-B2* (B-LMW-GS), and *Gli-B1* (γ-gliadin) loci, we were able to calculate a genetic diversity with the D index, according to the following formula:

$$Dj = 1 - \Sigma p^2_{ij}$$

Where p is the frequency of the i^{th} allele at j^{th} locus.

Considerable diversity was found in landraces from Spain and with a lesser degree within Tunisian landraces. The modern varieties had a poor genetic diversity index (Table 1). Diversity in gluten loci varied from D= 0,29 to D= 0,66. A high diversity was also recorded in *Glu-B1 and Glu-A3* loci, and a minor diversity in *Gli-B1.* Among the 37 genotypes, four were γ-gliadin 42 type, 31were γ-gliadin 45 type and two are rare alleles, Null and γ-44. The well-established tendency for γ-gliadin 42 types to be consistently weak is clearly evident. The γ-gliadin 45 types exhibited a wide range of strength from weak to very strong. Previous studies applied the γ-gliadins 45 and 42 as marker for good and poor quality of gluten quality, respectively (Damidaux *et al.*, 1978; du Cros *et al.*, 1982). ANOVA analysis for quality parameters has indicated that significant effect was recorded for B-LMW glutenin loci variation, while γ-45 pattern is present, accounting for 17.8%, 39.8% and 19.0% of the variation for SDSS, MT and BDR, respectively. A second ANOVA analysis of quality parameters showed a significant effect of all allelic prolamins variation. This last model explains 60.3%, 61.3% and 50.4% of the variance of SDSS, MT and BDR parameters respectively. When comparing these values with the previous model, which only took account of the variation of the B-LMW glutenin loci, we can deduce the importance of HMW glutenin loci *via* the increasing and improvement of the qualitative parameters. The gliadin influence is lower in this study, because most varieties possess γ-45. While the allelic variance (*F* values) of *Glu-A1, Glu-B1*, *Glu-A3*, *Glu-B3* and *Glu-B2* loci and the variation of quality parameters measured, demonstrate a high significant effect of *Glu-B1* (HMW-GS) on gluten strength (SDSS) and Mixograph test. Moreover, interaction between this locus (*Glu-B1)* and *Glu-A3* is recorded in SDSS volume and Mixing time. The second interaction between *Glu-B1* and *Glu-B3 allelic variation* marked a significant influence only on SDS-sedimentation volume (Table 2).

Table 1. Genetic diversity indices.

	Total Varieties	Landraces (Spain)	Landraces (Tunisia)	Modern Varieties
D (*Glu-A1*)	0,48	0,66	0,22	-
D (*Glu-B1*)	0,66	0,55	0,53	0,57
D (*Glu-A3*)	0,60	0,70	-	0,45
D (*Glu-B3*)	0,45	0,45	0,47	0,25
D (*Glu-B2*)	0,25	0,50	0,47	0,25
D (*Gli-B1*)	0,29	0,30	0,22	0,25
D-index	0,45	0,42	0,31	0,29

Table 2. The Analysis of variance (F values) of HMW and B-LMW loci and Quality parameters.

Source	Protein	SDSS (mm)	Mixing time (s)	Breakdown (%)
Glu-A1	1.40	0.75	0.36	6.10**
Glu-B1	0.93	6.41**	3.77*	3.55*
Glu-A3	1.05	9.02**	18.63**	1.81
Glu-B3	0.70	0.57	1.09	0.38
Glu-B2	0.34	2.43	6.04*	0.37
Glu-B1 x *Glu-A3*	0.66	6.35*	6.18*	0.03
Glu-B1 x *Glu-B3*	0.03	4.29*	1.17	2.32

Four different *Glu-B1* HMW-GS patterns were identified: *6+8, 7+8, 20x+20y* and *13+16* respectively allele *d, b, e* and *f* according to Payne and Lawrence (1983). The most prevalent HMW-GS pattern was *6+8* (17 genotypes), followed by *20x+20y* (12 genotypes) and with less frequency *7+8* and *13+16*. Glutenins of high molecular weight (HMW) in hexaploid wheat bakers explain 60% of the variation of gluten strength (Carrillo *et al.*, 1990). Nevertheless, in durum wheat, the influence of different HMW glutenins is fewer, having greater influence of LMW glutenin variation. In this study we considered that HMW glutenin have a considerable influence on gluten characteristics. We analyzed the influence of *Glu-B1* subunits variation on quality parameters in the presence of 4 different alleles of *Glu-A3:6 (a), 5 (b)* and *Null (h)* (according to nomenclature of Nieto-Taladriz *et al.*, 1997) and 5* previously described by Aguiriano *et al.* (2008).

Based on the values of SDSS and MT, illustrated in figures 1 and 2, it is noteworthy that the varieties harbouring the two pairs (*7+8*) and (*6+8*) within the *Glu-B1* subunit locus, display higher and better quality (gluten strength) than the ones harbouring (*20x+20y*), independently of the *Glu-A3 locus*. Whereas, varieties with (13+16) subunits have a poor gluten strength.

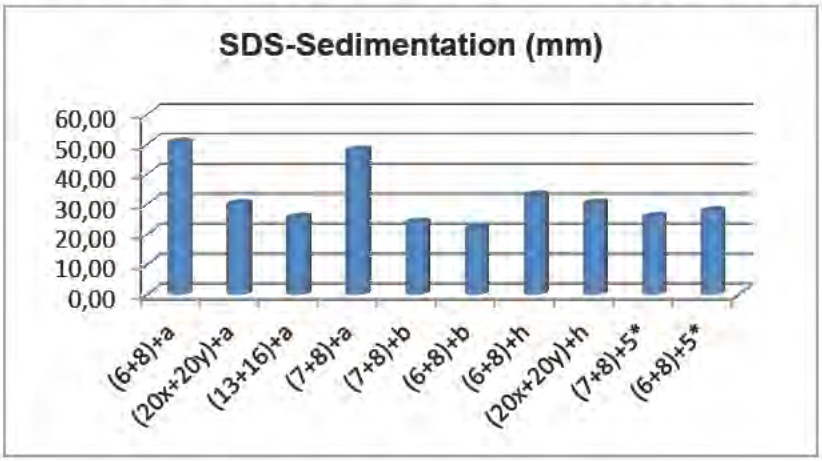

Figure 1. Influence of *Glu-B1* subunits variation in the presence of one *Glu-A3* (*a, b, h* and *5) on SDS-sedimentation volume.**

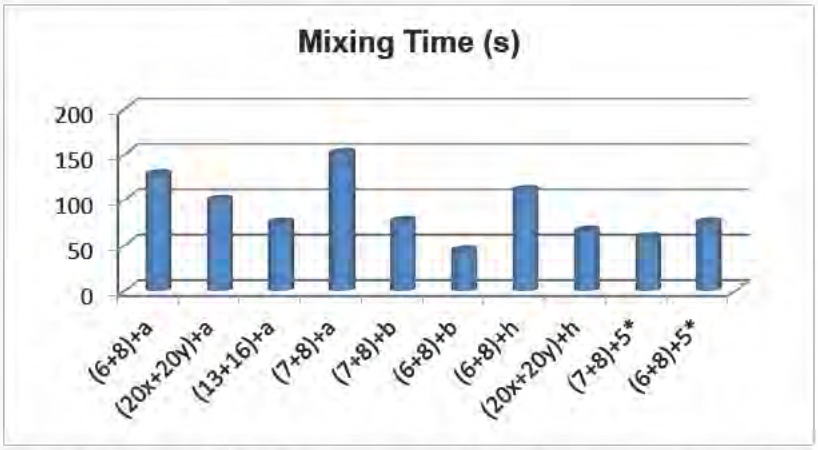

Figure 2. Influence of *Glu-B1* subunits variation in the presence of one *Glu-A3* (*a, b, h* and *5) on Mixing time (s).**

The allele 7+8 is linked to the highest Mixing Time of Mixograph values and SDS-Sedimentation volume in the presence of *Glu-A3a*. In the figure 4, we recorded the influence of *Glu-B1* subunit variation on SDS-sedimentation in the presence of 3 different patterns of *Glu-B3* subunits: *a* (*2+4+15+19*), *b* (*8+9+13+16*) according to Nieto-Taladriz *et al.*(1997) and 2+4+14+18 described by Rodriguez-Quijano *et al.* (2010) in one Ethiopian accession of Khorasan wheat and by Nazco *et al.* (2013) in two durum wheat landraces.

Varieties with (*7+8*) and (*6+8*), in the presence of different *Glu-B3* alleles, still have a high gluten strength; while varieties with (*20x+20y)* and (*13+16)* have always a lower SDS-sedimentation volume. Varieties displaying *Glu-B3a* and *Glu-B1b* (*7+8*) have a highest volume of SDS-sedimentation. Varieties with *Glu-A3a* and *Glu-B3a* possess a good gluten index (Sisson *et al.*, 2005). In the current study *Glu-A3a* and *Glu-B3a* (LMW-GS) with (7+8) in*Glu-B1* is a preferred combination giving high gluten strength. We also concluded that weak gluten is obtained in the presence of *b* and *5** alleles, despite the presence of *6+8* and *7+8* pair subunits of *Glu-B1.* Even glutenin subunits pair *6+8* in *Glu-B1* locus are considered linked to poor quality of bread wheat bakers; in durum wheat, both 6+8 and *7+8* exert a positive influence on semolina quality, especially when we compared these subunits with the effect of *20x+20y* and *13+16* subunits pair within the same locus (kovacs*et al.*, 1993; Martinez *et al.*,2005).

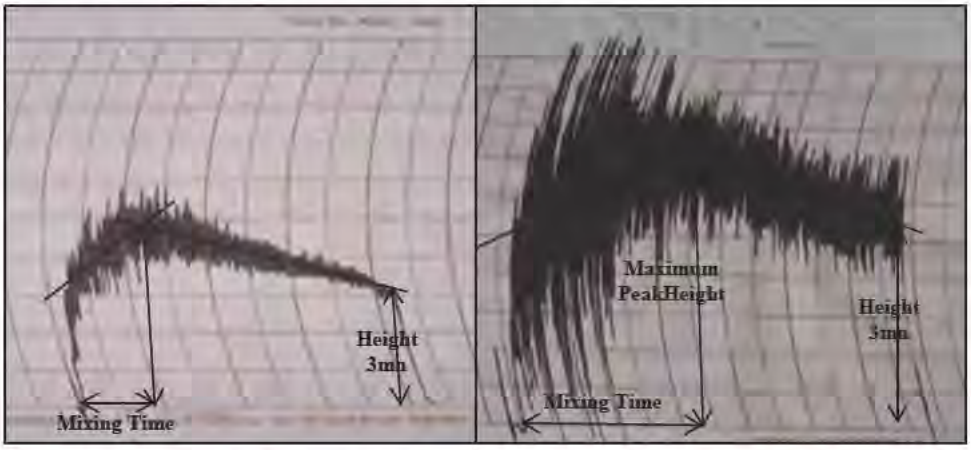

Figure 3. Mixographes of two contrasting varieties displaying respectively (13+16) subunits (a) and (7+8) subunits (b) in *Glu-B1*.

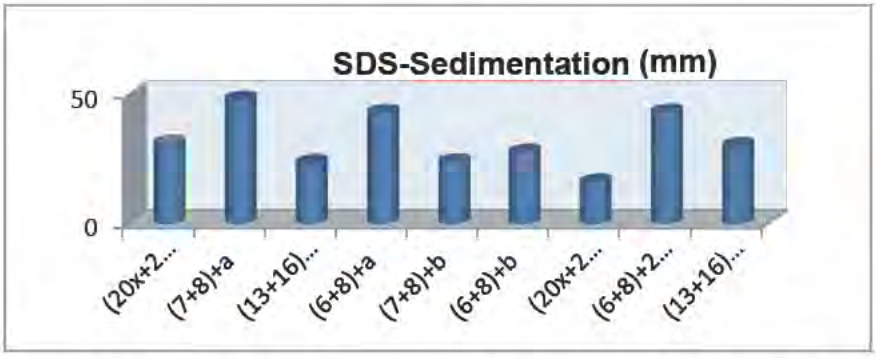

Figure 4. Influence of *Glu-B1* subunits variation in the presence of one *Glu-B3* subunit (*a*, *b*and the combination:*2+4+14+18*) on mixing time (s).

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Grain quality of durum wheat varieties

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Abstract. Fourteen commercial varieties of durum wheat bred in Kazakhstan and Siberia along with Barilla Italy varieties from Kostanay, Akmola, North Kazakhstan, Aktobe and Karaganda regions were analyzed for test weight, ash, protein content, gluten quality, carotene and amylose content, vitreousness, ratios of gliadin and glutenin. The regions differed in mean values of the traits. The region of Aktobe was characterized by high protein content (by maximizing gliadin fraction) with the best gluten quality, with the maximum ratio of gliadin/glutenin and ash content. In Kostanay region vitreouss grain was formed with a relatively high carotene content and in Karaganda region the highest amylose content. Ranking of varieties based on integrated assessment of 11 traits in 4 regions resulted in the following order: Kargala 29 (1.983) >Zhemchuzhina Sibirii (2.002) >Altaiskaya Niva (2.158) >Kustanaiskaya 12 (2.270) >Damsinskaya yantarnaya(2.308).

Keywords. Durum wheat – Test weight – Ash – Protein and gluten quality – Carotene – Amylose – Vitreousness.

Qualité des grains des variétés de blé dur

Résumé. *Quatorze variétés commerciales de blé dur sélectionnées au Kazakhstan et en Sibérie, avec des variétés Barilla d'Italie provenant des régions de Kostanay, Akmola, Nord Kazakhstan, Aktobe et Karaganda, ont été analysées pour évaluer le poids spécifique, les cendres, la teneur en protéines, la qualité du gluten, la teneur en carotène et en amylose, la vitrosité, le rapport gliadine/gluténine. Les régions diffèrent par les valeurs moyennes des caractères. La région d'Aktobe est caractérisée par une teneur en protéines importante (maximisation de la fraction gliadine), la meilleure qualité du gluten, le rapport gliadine/gluténine et la teneur en cendres les plus élevés. Pour la région Kostanay, la vitrosité du grain était associée à une teneur relativement élevée en carotène alors que pour la région de Karaganda, on a observé la teneur en amylose la plus importante. A travers une évaluation intégrée de 11 caractères dans 4 régions, il a été possible de classer les variétés selon l'ordre suivant : Kargala 29 (1.983) > Jemtchoujina Sibirii (2.002) > Altaiskaya Niva (2.158) > Kustanaiskaya 12 (2.270) > Damsinskaya Yantarnaya (2.308).*

Mots-clés. *Blé dur – Poids spécifique – Cendres – Qualité des protéines et du gluten – Carotène – Amylose – Vitrosité.*

I – Introduction

Durum wheat is an important cereal crop for different end-use (pasta, spaghetti, noodles, couscous, and bread-baking). The success of the breeding is defined by germplasm collection, effective breeding approaches and methods of grain identification for high quality. The climate conditions of spring sowing area in Kazakhstan favors high protein grain formation which also depends on the cultivars. The aim of this work was evaluation of grain quality of durum wheat cultivars from 5 regions under Kazakhstan-Siberian Network trials (KASIB 4-5, 2003-2004) and 14 commercially released cultivars from CIMMYT trials on quality characteristics of grain, gluten, semolina, and flour to reveal pasta and bread-making potential.

II – Material and methods

The material for investigations included 15 varieties of durum wheat grown in 5 regions (Akmola, Kostanay, Petropavlovsk, Aktobe, and Karaganda) under KASIB trials and 14 commercially

released varieties from CIMMYT trials. The check varieties (Altaiskaya Niva, Damsinskaya yantarnaya, Zhemchuzhina Sibirii, Kargala 29 and Kostanayskaya 12) were used for investigation of grain quality stability in different regions. The content of protein, gliadin and glutelin was determined according to Kjeldahl using NIR-calibrations (Peruanskyi *et al*., 1996). The test weight, vitreousness, gluten quality and quantity, carotenoid and ash content, total macaroni values were determined according to the State Standards. Sedimentation was determined in 2% acetic acid and hardness index by SKCS 4100. The physical properties were evaluated by farinograph (Brabender) and alveograph (Shopen) methods. The whiteness of flour was determined in accordance with the Russian Federation Standards 26361-84.

III – Results

The five check varieties were characterized by the highest variability of amylose content (5.1-16.3%) (Table 1). High variability in different regions was found for ash content. Variety Kargala 29 was superior for test weight and amylose content; variety Kostanayskaya 12 had highest protein content and gliadin/glutenin ratios; Altaiskaya Niva and Kostanayskaya 12 had the highest vitreousness and grain hardness index; Zhemchuzhina Sibiri had highest carotenoid content. Quality parameters of these varieties varied depended on cultivation region. Test weight of Kargala 29 ranged from 815 to 835 g/l.

Table 1. The grain quality variability of 5 spring durum wheat cvs in different growth conditions.

Indicator	Zhemchuzhina Sibirii	Altayskaya Niva	Damsinskaya yantarnaya	Kustanayskaya 12	Kargala 29
Nature mass, g/l	780-817	769-820	774-814	758-809	815-835
Vitreousness, %	58-64	62-69	57-80	54-82	54-65
Hardness index	107-112	98-118	96-108	99-121	103-111
Ash content, % DM	1,49-2,17	1,49-2,17	1,45-2,12	1,42-2,33	1,34-2,10
Protein, % DM)	15,3-19,0	15,4-17,6	16,0-18,5	16,4-20,8	15,4-18,0
Gliadin, % protein	30,9-37,1	31,6-36,9	30,8-35,4	31,1-37,9	30,1-36,1
Glutenin, % protein	21,4-23,2	20,9-22,8	20,1-22,4	21,0-22,4	21,3-23,1
Gliadin + glutenin, %	53,1-59,5	53,1-59,1	51,5-56,8	52,3-59,4	53,2-58,7
Gluten quality score	3,1-4,2	3,7-4,6	4,3-5,2	3,3-4,0	2,6-3,4
Carotenoids, % yellow	25,7-27,6	18,5-20,4	18,9-20,4	19,1-22,1	19,8-20,6
Amylose, 5 DM	5,76-16,26	5,13-14,65	5,76-15,87	6,91-12,13	6,91-14,65

The ash content ranged from 1,34 to 2,12%. Kostanayskaya 12 was distinguished by a number of traits: vitreousness (54-82%), protein content (16,4-20,8%), gliadin content (31,1-37,9%) and the gliadin+glutenin (52,3-59,4%), amylose content ranged from 5,1-16,3%. Ranking of spring durum wheat by grain quality depended on cultivation region. Variety Altaiskaya Niva was superior for five traits in Akmola region: vitreousness, hardness, gliadin content, gliadin/glutenin ratio and amylose content (Fig. 1).

In Kostanay region variety Kostanayskaya 12 had high protein content, vitreousness, hardness, gliadin content and the gliadin/glutenin ratio. Variety Kargala 29 had high quality indicators: test weight, gliadin and gliadin + glutenin content in the 1th Karaganda region. Variety Zhemchuzhina Sibiri ranked first in Aktobe region for carotene content, hardness index, and gliadin content. Among the studied durum wheat varieties Kargala 29 showed stability for amylose and glutenin content in all environments and variety Zhemchuzhina Sibiri had stability for high carotene and glutenin content. Regions differ for quality values for the same set of varieties. Thus, Aktobe region was characterized by high-protein grain with maximum gliadin fraction content and with the best gluten quality and ash content. In Kostanay region high vitreousness grain with a high

carotene content was formed and in Karaganda region grain was mostly characterized by high amylose content (Table 2).

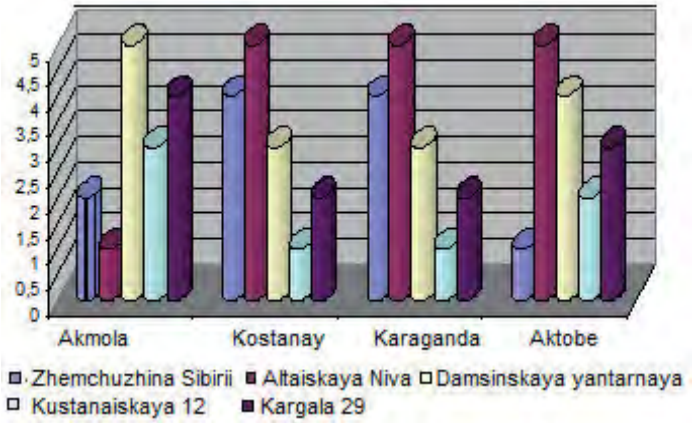

Figure 1. Ranking of spring durum wheat varieties in terms of grain quality, depending on the growing region.

Table 2. Average value of spring durum wheat grain quality by region.

Indicator	Akmola	Kostanay	Aktyubinsk	Karaganda
Test weight, g/l	820	828	792	803
Vitreousness, %	64	73,5	65	57
Hardness (SKCS 4100)	102	97	102	99
Ash content, % DM	1,52	1,71	2,00	1,45
Protein, % DM	15,9	14,9	18,5	17,5
Gliadin, % protein	31,2	30,5	33,5	31,8
Glutenin, % protein	22,4	22,4	21,9	21,1
Gliadin/glutenin	1,4	1,4	1,6	1,5
Gluten by ball	3,8	3,7	3,8	3,9
Carotenoids content, % DM	20,3	21,5	21,3	20,4
Amylose content,% DM	12,9	11,7	8,5	12,4
Gliadin + glutenin	53,8	53,5	58,7	52,9

KASIB trial is a comparison of the best varieties and lines from Russia and Kazakhstan with the aim to select the most high-quality genotypes for each of the limiting characteristics and their combination. Thus, for protein content varieties Damsinskaya yantarnaya, Kargala 30, Kostanayskaya 12 were superior in 2-3 regions out of five; variety Kargala 28 in 4 regions and varieties Asangali, Kargala 29 and Kostanayskaya 12 in 2 out of 5 regions. For gluten quality variety Collectivnaya 2 was the best in all 5 regions and variety Damsinskaya yantarnaya was best in 2 out of 5 regions. For amylose content variety Zhemchuzhina Sibiri and for sedimentation index varieties Collectivnaya 2 and Kargala 28 were superior in two out of three regions.

Comparison of varieties on all indicators of quality achieved by integrated assessment is demonstrated in Table 3. The most consistently balanced for all traits was variety Collectivnaya 2 (1th rank in North, Karaganda and 2th rank in Barnaul) and variety Gordeiforme 417 (rank 1 in Karabalyk, Omsk and rank 3 in Karaganda). Varieties Gordeiforme 417, Gordeiforme 415, Collectivnaya 2, Kargala 28 and Zhemchuzhina Sibiri were the best in all regions.

Analysis of the gliadin component composition revealed a close similarity in quality of cvs: Kargala 29 and Kargala 30 (rank 12, 14); Gordeiforme 91-144-4 and Kostanayskaya 12 (rank 13,

15); Gordeiforme 415 and Kargala 28 (rank 2 and 4). The best pasta evaluation and pasta color was observed for Zhemchuzhina Sibiri and Gordeiforme 91-144-1 and then varieties Asangali, Gordeiforme 415, Gordeforme 417, Kargala 28 and Kargala 30. The flour produced from durum was studied for whiteness. The samples different from the second type to first, mostly for varieties Kargala 29 (50,2); Asangali (49,3); Gordeiforme 415 (49,1); Collectivnaya 2. According to the mixing values (farinograph), varieties Gordeiforme 415, Kargala 28, Kargala 30, and TS-15 had good quality (>60%). The maximum values of gluten quality were observed fro varieties Damsinskaya yantarnaya, Damsinskaya 90, Kargala 34; for flour whiteness – Asangali, Kargala 34, Orenburgskaya 10, Nauryz 6, Kievlyanka. Maximum value of sedimentation was found for Damsinskaya yantarnaya, Damsinskaya 90 and Kargala 34; of amylose content for Kargala 34; strength of flour for Kargala 34 (W=210) and Damsinskaya 90 (W=202). Daminskaya yantarnaya (W=192); valormetric value (farinograph) for Kargala 34 was 60-55f, Damsinskaya yantarnaya 70-51f (Tohtabakieva and Abugalieva, 2006). Analysis of flour and dough physical properties and baking to evaluate selected varieties as having potential for bread-making (30-60 u.f.) as shown for Moroccan wheat (Benjnah *et a*l., 1999; Hareland *et al*. 1999).

Table 3. Integral assessment of the KASIB durum wheat varieties by grain quality (11 index).

Variety	2004		2005			Total Average		Rank
	B*	O	K1	K2	A	Rank	Rank	
Hiton	6	11	-	-	-	17	8,5	11
Collectivnaya 2	2	13	1	10	1	27	5,4	3
Zhemchuzhina siberii	8	5	4	6	10	33	6,6	5
Gordeiphorme 91-144-4	7	3	14	15	9	48	9,6	13
Altayskaya Niva	13	6	8	3	5	35	7,0	6
Gordeiphorme 415	9	4	5	5	2	25	5,0	2
Gordeiphorme 417	11	1	3	1	-	16	4,0	1
Damsinskaya yantarnaya	14	9	10	2	4	39	7,8	8
L 173/93-1	5	8	9	11	-	33	8,2	9
Kostanayskaya 12	12	12	7	13	6	50	10.0	14
Asangali	10	7	6	12	7	42	8,4	10
Kargala 28	4	2	-	9	11	26	6,5	4
Kargala 29	3	10	11	8	12	44	8,8	12
Kargala 30	1	14	13	14	8	50	10	14
TS-15	-	-	12	7	3	22	7,3	7

**Locations: B = Barnaul; O 0 Omsk; K1 = Karaganda; K2 = Karabalik; A = Aktobe.*

Analysis of the gliadin component composition revealed a close similarity in quality of cvs: Kargala 29 and Kargala 30 (rank 12, 14); Gordeiforme 91-144-4 and Kostanayskaya 12 (rank 13, 15); Gordeiforme 415 and Kargala 28 (rank 2 and 4). The best pasta evaluation and pasta color was observed for Zhemchuzhina Sibiri and Gordeiforme 91-144-1 and then varieties Asangali, Gordeiforme 415, Gordeforme 417, Kargala 28 and Kargala 30. The flour produced from durum was studied for whiteness. The samples different from the second type to first, mostly for varieties Kargala 29 (50,2); Asangali (49,3); Gordeiforme 415 (49,1); Collectivnaya 2. According to the mixing values (farinograph), varieties Gordeiforme 415, Kargala 28, Kargala 30, and TS-15 had good quality (>60%). The maximum values of gluten quality were observed fro varieties Damsinskaya yantarnaya, Damsinskaya 90, Kargala 34; for flour whiteness – Asangali, Kargala 34, Orenburgskaya 10, Nauryz 6, Kievlyanka. Maximum value of sedimentation was found for Damsinskaya yantarnaya, Damsinskaya 90 and Kargala 34; of amylose content for Kargala 34; strength of flour for Kargala 34 (W=210) and Damsinskaya 90 (W=202). Daminskaya yantarnaya (W=192); valormetric value (farinograph) for Kargala 34 was 60-55f, Damsinskaya yantarnaya 70-51f (Tohtabakieva and Abugalieva, 2006). Analysis of flour and dough physical properties and baking to evaluate selected varieties as having potential for bread-making (30-60 u.f.) as shown for Moroccan wheat (Benjnah *et al*., 1999; Hareland *et al*. 1999).

IV – Conclusions

The studied durum wheat varieties ranged for the most high-quality pasta traits 1) released varieties Kostanayskaya 12, Damsinskaya Yantarnaya, Zhemchuzhina sibiri, Altayka, Sid-88, and Damsinskaya 90, 2) varieties being officially tested - Damsinskaya Yantarnaya, Asangali, Kargala 34, Damsinskaya 90, Zhemchuzhina Sibiri, Toma; 3) germplasm from KASIB trial: Gordeiforme 417, Gordeiforme 415, Collectivnaya 2, Kargala 28, and Zhemchuzhina Sibiri. Cultivars Kargala 29 and Zhemchuzhina Sibiri were characterized for stability of grain quality (gluten quality and carotene content) formation in different conditions. Bread-making potential was revealed for the following durum wheat cultivars: Damsinskaya yantarnaya, Asangali, Kargala 34, Nauryz 7 and Nauryz 8. Varieties in Aktobe region excelled in formation of high-protein grain (due to maximum gliadin fraction) with the best gluten quality and ash content.

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Phenolic compounds and antioxidant activity in tetraploid wheat

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Abstract. Phenolic compounds have been widely studied for their health value, as they exert a protective role against chronic degenerative diseases, mainly due to their antioxidant properties. Cereals are at the basis of the food pyramid and, even if they are not one of the main sources of phenolic compounds, they can effectively contribute to the dietary uptake of these secondary metabolites. After an overview of functions and mechanisms of bioavailability, the extraction methods and varietal variability of phenolic compounds in tetraploid wheat are reviewed, in comparison with bread wheat. The quantitative distr bution of the various fractions and classes of phenolic compounds in the caryopsis are discussed, with special attention to ferulic acid. The state of the art about the production of phenolic extracts from bran is reviewed, pointing out the most recent technologies adopted to recover the insoluble-bound phenolic fraction.

Keywords. Phenolic compounds – Tetraploid wheat – Milling by-products – Functional ingredients – Antioxidant activity.

Composés phénoliques et activité anti-oxydante chez le blé tétraploïde

Résumé. *Les composés phénoliques ont été largement étudiés pour leurs vertus sanitaires, car ils exercent un rôle protecteur contre les maladies dégénératives chroniques, principalement en raison de leurs propriétés anti-oxydantes. Les céréales sont à la base de la pyramide alimentaire et, même si elles ne sont pas l'une des principales sources de composés phénoliques, elles peuvent contribuer efficacement à l'apport alimentaire de ces métabolites secondaires. Après avoir donné un aperçu des fonctions et des mécanismes de la biodisponibilité, nous passerons en revue les méthodes d'extraction et la variabilité variétale des composés phénoliques chez le blé tétraploïde, en faisant une comparaison avec le blé tendre. La distribution quantitative des différentes fractions et classes de composés phénoliques dans le caryopse sera aussi discutée, en focalisant l'attention sur l'acide férulique. Enfin, l'état des lieux sur la production d'extraits phénoliques du son sera présenté, en examinant les technologies les plus récentes adoptées pour récupérer la fraction d'acide phénolique insoluble lié.*

Mots-clés. *Composés phénoliques – Blé tétraploïde – Sous-produits de mouture – Ingrédients fonctionnels – Activité anti-oxydante.*

I – Chemistry of phenolic compounds

Phenolic compounds constitute one of the most numerous and widely distributed groups of secondary metabolites in the plant kingdom, all characterized by the presence of at least an aromatic ring bearing one or more hydroxyl substituents. They can be divided into at least 10 different classes depending on their basic chemical structure (Table 1). The most abundant are phenolic acids, lignans, stilbenes, flavonoids and tannins (Bravo, 1998); phenolic acids and flavonoids represent 30 and 60%, respectively, of total phenolic compounds in the Mediterranean diet (King and Young, 1999).

Phenolic compounds range from low molecular weight compounds to highly polymerized compounds, such as melanins, suberin, tannins, and lignins. Flavonoids are derivatives of benzo-γ-pyrone; on the basis of the oxidation state of heterocycles, and aromatic rings position, are classified into: anthocyanidins, flavonols, flavans, flavanones, flavones, isoflavones and hydrolysable tannins.

The predominant phenolic compounds in cereals are phenolic acids. They are derivatives of either benzoic acid or cinnamic acid. The latter group includes ferulic acid (4-hydroxy-3-methoxycinnamic acid) that is the most abundant phenolic acid of wheat at all stages of development. Its concentration increases steadily during grain development, prior to a 50% decrease during grain ripening (Mc Keehen *et al.*, 1999). This acid arises from the methabolism of phenylalanine and tyrosine (Graf, 1992) and is ubiquitously present in plant cell walls (Bravo, 1998). The presence of the CH=CH–COOH group in its structure is considered to be the key for significantly higher antioxidative efficiency than that of hydroxybenzoic acids (White and Xing, 1997). The *trans* isomer of ferulic acid is predominant, as it represents 90% of the total phenolic acids of wheat (Lempereur *et al.*, 1997).

Table 1. Basic chemical structure of the main phenolic compounds.

Class	Basic carbonious skeleton
Hydroxybenzoic acids	C_6–C_1
Hydroxycinnamic acids	C_6–C_3
Stilbenes, anthraquinones	C_6–C_2–C_6
Flavonoids	C_6–C_3–C_6
Lignans	$(C_6$–$C_3)_2$
Melanins	$(C_6)_n$
Lignins	$(C_6$–$C_3)_n$
Condensed tannins (proanthocyanidins or flavolans)	$(C_6$–C_3–$C_6)_n$

II – Functions and distribution of phenolic compounds in plants

With regard to the subcellular distribution of phenolic compounds, sites of biosynthesis and accumulation are different, due to the reactivity of these compounds against protoplasmic constituents, that could render them toxic for the cell. This toxicity can be prevented by conjugation with monosaccharides and cellular compartmentalization in the synthesis and transport processes (Wink, 2010). The synthesized products, in glycosylated form, are seized in specific regions of the endoplasmic reticulum to form membranous vesicles. Subsequently, these vesicles can move to the vacuole, where different classes of phenolic compounds are stored. Alternatively, they can head to the plasma membrane for secretion within the cell wall, thus contributing to the process of lignification (Wink, 2010). Phenolic compounds, in fact, confer mechanical stability to cells, by forming polymeric constituents of support structures, such as lignin and other constituents of the cell wall (Renger and Steinhart, 2000).

The structural variety of phenolic compounds reflects in a large array of functions and explains their extensive diffusion. Due to their strong antioxidant activity, they protect plants from UV radiation and oxidative stress, and have phytoalexin functions (Hammerschmidt, 1999). In addition, phenolic compounds have antibiotic, antifungal, and antiviral properties (Dixon, 2001). The lignificating ability and antioxidant properties of ferulic and other phenolic acids constitute a physical and chemical barrier to insect attacks. In wheat, they play a role in midge resistance (Abdel-Aal *et al.*, 2001) and contribute to *Fusarium* resistance (Mc Keehen *et al*., 1999).

III – Health value of phenolic compounds

Phenolic compounds have been extensively studied for their health value, as they exert a protective role against chronic degenerative diseases, mainly due to their antioxidants properties. In fact, phenolic compounds are scavengers of free radicals, primarily responsible for the oxidative damage caused to DNA, lipids and proteins (Graf, 1992). In particular, flavonoids and phenolic acids, including ferulic acid, protect low density lipoproteins (LDL) from oxidation by reactive

oxygen species (ROS), associated with the initial steps of the atherosclerosis process (Yu *et al*., 2005).

Moreover, phenolic compounds play a preventive role in the various stages of carcinogenesis, with different mechanisms: (i) by scavenging the carcinogenic agents (especially free radicals); (ii) by altering the production of key proteins and stopping the cell cycle; (iii) by inducing apoptosis of tumor cells; (iv) by expounding an angiogenic action (Thomasset *et al.*, 2007; Ramos, 2008). Finally, phenolic compounds exert anti-inflammatory, anti-hypertensive, anti-microbial and photoprotective activities (Bravo, 1998).

IV – Bioavailability of phenolic compounds

Phenolic compounds may be classified as soluble or insoluble in the most common solvents. The bonds with other molecules influence the physical and chemical characteristics of these compounds, including their solubility, and determine their cell location, functions, absorption, and metabolism. In particular, water-soluble phenolic compounds include (i) free aglycones; (ii) glycosides obtained by conjugation with one or more mono- or di-saccharides; (iii) esters of organic acids (Bravo, 1998). Phenolic acids may form both ester and ether linkages owing to their bifunctional nature through reactions involving their carboxylic and hydroxyl groups, respectively. This allows phenolic acids to form cross-links with cell wall macromolecules. The insoluble fraction originates from bonds between phenolic compounds and cell wall polymeric constituents. Ferulic acid, for example, contributes to the formation of insoluble fiber by cross-linking arabinoxylans (Renger and Steinhart, 2000). Phenolic compounds can bind also lyposoluble molecules such as phytosterols, terpene alcohols or triterpenes, commonly associated with the cell membrane (Miller and Engel, 2006). While lyposoluble and insoluble-bound phenolic compounds mainly play a structural role in the cell wall, water-soluble phenolic compounds have generally antioxidant and antimicrobial functions (Smith and Hartley, 1983).

To be absorbed, glycosides must be hydrolyzed by glycosidase in the gastrointestinal tract; this enzyme can be endogenous, or produced by colonic microflora (Kim *et al.*, 1998). The product of this enzymatic reaction are hydrophilic aglycones that can be absorbed in the small intestine by diffusion throughout biological membranes (Bravo, 1998). Phenolic compounds present in foods in insoluble-bound form, especially high molecular weight polymers, such as condensed tannins, are not bioavailable and have antinutritional properties: these molecules complex and precipitate proteins and divalent cations, interfering with their digestion and absorption (Bravo, 1998).

V – Extraction and quantification of wheat phenolic compounds

Phenolic compounds are usually extracted from wheat grains, preliminarily milled, by procedures that involve the use of polar solvents. The most commonly used are methanol, ethanol, and acetone (Table 2). After the addition of solvent, the supernatant (corresponding to the soluble fraction composed of free and conjugated phenolic compounds), and the solid residue (insoluble-bound forms) are separated. The residue undergoes subsequent treatments, usually alkaline or acidic hydrolysis, to release the bound fraction of phenolic compounds (Adom *et al.*, 2003; Kim *et al*., 2006; Arrantz and Saura Calixto, 2010). The subsequent determination of the total amount of phenolic compounds in the recovered fractions is performed by VIS spectrophotometry after Folin-Ciocalteu reaction, while reversed-phase HPLC/MS is usually applied for identifying and quantifying the individual compounds. The elution proceeds by increasing the concentration of either acetonitrile or methanol in the mobile phase, under acidic conditions (Lempereur *et al.*, 1997; Kim *et al.*, 2006; Arrantz and Saura Calixto, 2010; Heimler *et al.*, 2010).

Table 2. Comparison of phenolic compound (PC) contents in wholemeal of durum and bread wheat.

Number of cultivars	Extracting conditions	Extracted fraction	Content of PC Min - max	Reference
Triticum turgidum L. ssp. *durum (Desf.) Husnot*				
5	Alkaline hydrolysis and subsequent ether extraction	Esterified ferulic acid	0.69–2.44 mg/g d.m. as ferulic acid	Lempereur *et al.*, 1997
9	Ethanol/water/formic acid (70:29.5:0.5)	Free and soluble-conjugated pPC	0.70-1.20 mg/g f.w. as gallic acid	Heimler *et al.*, 2010
30	Methanol-water (80:20 *v/v*) acidified with 1% HCl	Free and soluble-conjugated PC	0.78–0.95 mg/g d.w. as ferulic acid	Menga *et al.*, 2010
Triticum aestivum L. ssp. a*estivum*				
3	Ethanol	Free and soluble-conjugated PC	0.49–0.93 mg/g d.w. as gallic acid	Yu *et al.*, 2002
25	Methanol/water (80:20 *v/v*) acidified with 1% HCl	Free and soluble conjugated PC	0.78–1.07 mg/g d.w. as ferulic acid	Menga *et al.*, 2010
17	Ethanol/water/formic acid (70:29.5:0.5)	Free and soluble-conjugated PC	0.65-1.12 mg/g f.w. as gallic acid	Heimler *et al.*, 2010
11	Ethanol	Free PC	119.6-201.2 µmol gallic acid/100 g of grain	Adom *et al.*, 2003
11	Alkaline hydrolysis of extraction residue of free and soluble-conjugated fraction and subsequent extraction with hexane and ethyl acetate	Insoluble-bound PC	508-700 µmol gallic acid/100 g	Adom *et al.*, 2003
Commercial sample	Methanol/acetone (M/A)	Free and soluble-conjugated PC	1.12 mg/g f.w. (direct sum of single HPLC identified compounds)	Arrantz and Saura-Calixto, 2010
Commercial sample	Acidic hydrolysis of residue of extraction of free and soluble-conjugated fraction and subsequent M/A extraction	Insoluble-bound PC	2.62 mg/g f.w. (direct sum of single HPLC identified compounds)	Arrantz and Saura-Calixto, 2010
Commercial sample	Alkaline hydrolysis of extraction residue of free and soluble-conjugated fraction and subsequent M/A extraction	Insoluble-bound PC	0.002 mg/g f.w. (direct sum of single HPLC identified compounds)	Arrantz and Saura-Calixto, 2010

The primary phenolic compounds detected in wheat caryopsis are phenolic acids and derivatives (Mateo Anson et al., 2008). More than 70% of them are insoluble-bound forms (Adom *et al.*, 2003; Kim *et al.*, 2006). Among phenolic acids, the most abundant in both soft (Klepacka and Fornal, 2006) and durum wheat (Lempereur *et al.*, 1997) is ferulic acid, followed by *p*-coumaric, sinapic and caffeic acid (Lempereur *et al.*, 1997). Bound ferulic acid, esterified to arabinose units of cell-wall arabinoxylans, accounts for 97% of total ferulic acid content of wholemeal flour (Adom *et al.*, 2003).

Most of wheat phenolic compounds are concentrated in the outer layers of the kernel, as they play a key role in plant defense against pests and diseases (Abdel-Aal *et al.*, 2001). Hence, the removal of these fractions, during flour refining processes, causes a relevant decrease of these functional ingredients. In particular, phenolic compounds are concentrated in the aleurone layer (Lempereur *et al.*, 1997; Mateo Anson *et al.*, 2008). Ferulic acid occurs in high concentrations in aleurone, pericarp and embryo cell walls; only in trace amounts in the starchy endosperm. Lempereur *et al.* (1997) detected high concentrations of ferulic acid esterified to cell-wall arabinoxylans in the aleurone layer of 5 durum wheat cultivars (69% of total ferulic acid), while the remnant was found in germ and in seedcoat (26.6% of total ferulic acid). Only 1.4% of total ferulic acid was detected

in the starchy endosperm. Adom *et al.* (2005) observed that the content of phenolic compounds in wheat bran and germ is significantly higher than in the endosperm.

Table 2 shows a comparison between phenolic contents of durum and bread wheat. It can be observed that the majority of studies regarded bread wheat. Wide varietal variability was observed by various authors. Significant differences among wheat cultivars may depend on both intrinsic factors, related to genotype, and extrinsic ones, such as agronomic conditions. However, differences may be imputable to technical issues related to analytical protocols of phenolics extraction, and different instrumental specificity and sensitivity of the quantifying methods.

No reports regard the analysis of phenolic compounds in wild accessions of tetraploid grains. Table 3 shows the results of quantitative determination of soluble phenolic compounds (free and soluble-conjugated with mono- or di-saccharides) in a set of various tetraploid grains comprising *Triticum turgidum* L. ssp. *turanicum* (Jakubz.) Á.Löve & D.Löve, *Triticum turgidum* L. ssp. *turgidum*, *Triticum turgidum* L. ssp. *carthlicum* (Nevski in Kom.) Á.Löve & D.Löve and *Triticum turgidum* L. ssp. *dicoccum* (Schrank ex Schübler) Thell. On the whole, relevant levels of phenolic compounds were observed, ranging from 1.74 to 2.69 mg/g as ferulic acid. These values seem higher than those reported for cultivated varieties of durum and bread wheat, listed in Table 2.

Table 3. Soluble phenolic compounds (PC) (free and soluble-conjugated components of the total phenolic compounds) extracted with acetone:water 50:50 (v/v) from wholemeal of different tetraploid wheat accessions.

Type of wheat	Samples No.	Content of PC (mg/g as ferulic acid (min-max)
Triticum turgidum L. ssp. *turanicum*	5	1.74-2.18
Triticum turgidum L. ssp. *turgidum*	4	2.08-2.40
Triticum turgidum L. ssp. *carthlicum*	3	2.01-2.36
Triticum turgidum L. ssp. *dicoccum*	5	2.48-2.69

VI – Effect of polyphenol oxidase on tetraploid wheat phenolic compounds

Phenolic compounds act as terminators of free radicals and chelators of metal ions that catalyze lipid peroxidation. They exert the antioxidant activity by donation of a hydrogen atom to radicals (Bravo, 1998). Moreover, the phenoxy radical intermediates are resonance stabilized; therefore, a new chain reaction is not easily inititated. Oxidation of phenolic compounds lead to quinones, that are characterized by brown colour (Nicolas *et al.*, 1993).

Many vegetable foods contain polyphenoloxidases (PPO) (E.C. 1.14.18.1), a class of enzymes catalizing the oxidation of phenolics to quinones in presence of oxygen. In bread wheat, PPO causes discoloration of oriental noodles, at an extent related to the enzymatic activity (Fuerst *et al.*, 2006). The same enzyme is responsible for dough browning also in tetraploid wheat (Feillet *et al.*, 2000; Taranto *et al.*, 2012). The assessment of PPO activity in a set of 113 wild tetraploid wheat accessions and durum cultivars evidenced significantly lower levels of enzyme activity in the latter (Pasqualone *et al.*, 2004; Taranto *et al.*, 2012).

VII –Phenolic content of bran: from waste to source of antioxidants

Table 4 reports the content of phenolic compounds of bran. It is well established that the majority of bran phenolic acids occur in insoluble-bound form (Kim *et al.*, 2006). To release them, increasing the extraction rates, either chemical (Adom *et al.,* 2003; Kim *et al.*, 2006; Arranz and Saura Calixto, 2010) or enzymatic hydrolysis (Bartolome *et al*., 1999) have been proposed. The reported results

about the efficiency of chemical hydrolysis in alkaline or acidic conditions are controversial. Kim *et al.* (2006) found the alkaline conditions as more efficient than acidic hydrolysis. Also Adom *et al.* (2003) preferred an alkaline hydrolysis.

Table 4. Content of phenolic compounds (PC) extracted from commercial wheat bran in various conditions.

Samples No.	Extracting conditions	Extracted fraction	Content of PC (min-max)	Reference
1	Methanol/acetone (M/A)	Free and soluble-conjugated PC	1.62 mg/g f.w. (direct sum of single HPLC identified compounds)	Arrantz and Saura-Calixto, 2010
1	Acidic hydrolysis of extraction residue of free and soluble-conjugated fraction and subsequent M/A extraction	Insoluble-bound PCs	15.89 mg/g f.w. (direct sum of single HPLC identified compounds)	Arrantz and Saura-Calixto, 2010
1	Alkaline hydrolysis of extraction residue of free and soluble-conju-gated fraction and subsequent M/A extraction	Insoluble-bound PC	3.72 mg/g f.w. (direct sum of single HPLC identified compounds)	Arrantz and Saura-Calixto, 2010
4	Methanol/water (80:20 v/v)	Free and soluble-conjugated PC	0.18–0.34 mg/g f.w. as gallic acid	Kim *et al.*, 2006
4	Alkaline hydrolysis of extraction residue of free and soluble-conjugated fraction and subsequent ethyl ether extraction	Insoluble-bound PC	2.14-2.33 mg/g f.w. as gallic acid	Kim *et al.*, 2006
4	Acidic hydrolysis of extraction residue of free and soluble-conjugated fraction and subsequent ethyl ether extraction	Insoluble-bound PC	0.65-1.07 mg/g f.w. as gallic acid	Kim *et al.*, 2006
1	Ultrasound-assisted extraction with 64% ethanol	Free and soluble-conjugated PC plus an aliquote of bound phenolics mobilised by ultrasounds	3.12 mg/g f.w. as gallic acid	Wang *et al.*, 2008
51	Aqueous ethanol 80% (v/v)	Free and soluble-conjugated PC	0.85-1.75 mg/g f.w. as gallic acid	Verma *et al.*, 2008
51	Alkaline hydrolysis of extraction residue of free and soluble-conju-gated fraction and subsequent ethyl ether and ethyl acetate extraction	Insoluble-bound PC	2.31-5.38 mg/g f.w. as gallic acid	Verma *et al.*, 2008

On the contrary, Arranz and Saura Calixto (2010), by performing HPLC analyses of methanol-acetone extracts, as well as of alkali and sulphuric acid hydrolysates of bran, found higher amounts of phenolic compounds in acidic (15.89 mg/g f.w.) than in alkaline hydrolysates (3.72 mg/g f.w.).

To enhance the efficiency of the release of phenolic compounds from bran it has also been proposed an ultrasound-assisted extraction, by using ethanol as solvent, with good results (Wang *et al.*, 2008). The effects of acoustic cavitations produced in the solvent by the passage of ultrasonic waves exert a mechanical effect, allowing greater penetration of solvent into the

sample matrix and increasing the contact surface area between solid and liquid phase; as a result the solute quickly diffuses from solid phase to the solvent (Wang *et al.*, 2008).

Many studies evidenced the antioxidant properties of bran, mainly attributable to its phenolic content (Zhou and Yu, 2004). Bran has been reported to be able to inhibit lipid oxidation catalyzed by either iron or peroxyl radicals (Baublis *et al.*, 2000). Bran extracts exert LDL protective effects in biologic systems (Yu *et al.*, 2005), and reduce lipid peroxidation of liposomes (Zielińsky and Kozłowska, 2000). Durum wheat bran has been the object of selections aimed to identify fractions with different functional and nutritional properties. It has been observed that the antioxidant activity is higher in the most internal bran fractions and increases in fractions having thinner granulometry (Esposito *et al.*, 2005). Durum bran extracts were observed to inhibit seed oil oxidation (Onyeneho and Hettiarachchy, 1992).

In the last decades, a large number of studies focussed their attention towards the employ of natural antioxidants to substitute synthetic molecules, and various agri-industry by-products have been proposed as source to extract antioxidant compounds (Moure *et al*., 2001; Fernàndez-Bolanos *et al.*, 2002). In this framework, the use of durum wheat bran to produce edible phenolic extracts has been proposed, with the final aim of enriching fresh pasta. The use of an edible solvent, such as aqueous ethanol, coupled to an alkaline hydrolysis by addition of either NaOH or KOH, with subsequent neutralization, has been experimented with good yields (Delvecchio *et al.*, 2012; Delvecchio and Pasqualone, 2012).

VIII – Conclusions

The varietal variability observed in the levels of wheat phenolic compounds suggest the possibility of identifying cultivars and wild accessions with higher levels of these secondary metabolites. Moreover, the outer layers of the caryopsis are particularly rich in phenolic compounds. These materials could be the starting point to prepare phenolic extracts useful in the formulation of wheat-based functional foods with enhanced antioxidant activity. Novel value-added utilisations of wheat milling by-products would enhance their marketing potential, and benefit the agricultural economy.

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Characterization of Phytoene synthase 2 (*Psy2*) genes in wheat

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Abstract. Phytoene synthase (*Psy*) is a key enzyme responsible in plant metabolism of carotenoids. *Psy* genes are important for their contr bution to flour color and nutritional aspects in human diet since they are precursor of vitamin A. In the grass family, PSY are nuclear enzymes encoded by a small gene family consisting of three genes: *Psy1*, *Psy2*, and *Psy3* localized on group 7 and 5 chromosomes.
The goal of our study was to characterize *Psy2* gene sequences and to verify its assessment with quantitative trait loci (QTL) involved in carotenoid expression in durum wheat. In the work we described the isolation of the *Psy2* sequences on A and B genomes in durum wheat cvs. Latino and Primadur characterized by a different carotenoid content. *Psy-A2* (2,593 bp) and *Psy-B2* (2,646 bp) were comprised of 6 exons separated by 5 introns. Alignment with *Brachypodium* and rice genomes confirmed the intron/exon structure. The study localized *Psy2* genes on chromosomes 5B and revealed the absence of linkage with QTLs for carotenoid content.

Keywords. Durum wheat – Phytoene synthase gene – Carotenoid.

Caractérisation des gènes de la phytoène synthase 2 (PSY2) du blé

Résumé. *La phytoène synthase (Psy) est une enzyme clé responsable du métabolisme des caroténoïdes chez les plantes. Les gènes Psy sont importants pour leur contribution à la couleur de la farine et aux aspects nutritionnels dans l'alimentation humaine, car ils sont précurseurs de la vitamine A. Dans la famille des graminées, les PSY sont des enzymes nucléaires codées par une petite famille de gènes composée de trois membres : Psy1, Psy2, et Psy3, localisés sur les groupes chromosomiques 7 et 5.*

Le but de notre étude était de caractériser les séquences du gène Psy2 et de vérifier son évaluation avec des locus de caractères quantitatifs (QTL) impliqués dans l'expression des caroténoïdes chez le blé dur. Dans ce travail, nous avons décrit l'isolement des séquences Psy2 dans les génomes A et B du blé dur cvs. Latino et Primadur, caractérisés par une différente teneur en caroténoïdes. Les Psy-A2 (2593 pb) et Psy-B2 (2646 pb) sont constitués de 6 exons séparés par 5 introns. L'alignement avec les génomes de Brachypodium et du riz a confirmé la structure intron/exon. L'étude a localisé les gènes Psy2 sur le chromosome 5B et a révélé l'absence d'association avec les QTL pour la teneur en caroténoïdes.

Mots-clés. *Blé dur – Gène de la phytoène synthase – Caroténoïdes.*

I – Introduction

Yellow pigment content (YPC) represents one of the major criteria in the assessment of durum wheat semolina quality. It is important in determining the commercial and nutritional quality of end-products such as pasta. Semolina colour is influenced by several factors, including the carotenoid pigments accumulation in grain (Panfili *et al*., 2004), the oxidative degradation processes and the transformation events of end-products (Borrelli *et al*., 1999).

The carotenoid biosynthesis involves several enzymatic steps, among which the step catalyzed by phytoene synthase (PSY) is assumed to be the rate-limiting one (Hirschberg, 2001). Phytoene synthase encodes for an enzyme responsible of the first step of C40 phytoene compound formation condensing two geranylgeranyl diphosphate molecules (GGDP).

In the grass species, *Psy* genes are classified into three paralogous sub-families: *Psy1*, *Psy2*, and *Psy3* (Li *et al*., 2008). The three *Psy* genes were characterized in rice (Gallagher *et al*., 2004; Welsh *et al*., 2008), maize (Li *et al*., 2008), sorghum (Fernandez *et al*., 2008) and recently in wheat (Pozniack *et al*., 2007; Dibari *et al*., 2012).

In wheat the YPC is under complex genetic control and several QTLs have been located on chromosomes 3A (Parker *et al*., 1998), 3B (Patil *et al*., 2008), 5A (Hessler *et al*., 2002), 7A and 7B (Crawford *et al*., 2011, 2013). However, the group 7 chromosomes appeared to contain genes critical for the carotenoid expression (Zhang *et al*., 2008; Blanco *et al*., 2011). Indeed *Psy1* locus was located on the long arm of group 7 chromosomes where a major QTL for YP was detected (Pozniak *et al*., 2007; Zhang and Dubcovsky, 2008; Blanco *et al*., 2011). The role and function of *Psy1* have been largely investigated since it was correlated with accumulation of endosperm carotenoids.

Partial sequences of *Psy2* from several durum wheat varieties are available, but there are no information about its function. Cenci *et al*. (2004) and Pozniak *et al*. (2007) located this locus on the short arm of group 5 chromosomes with no clear association to carotenoid content. On the same chromosome group (arm 5L), *Psy3* have been recently mapped and characterized. Dibari *et al*. (2012) showed their expression in roots during stress conditions (drought and salt stress) underlining the *Psy3* roles in the downstream carotenoid and abscisic acid (ABA) accumulation.

In the present work, we focused our attention on *Psy2* gene with the objectives: (a) to isolate and characterize the complete genomic sequences of this gene in the A and B genomes of wheat; (b) to develop and map functional markers for *Psy2*; (c) to assess the linkage between *Psy2* gene and QTLs for carotenoid pigment content.

II – Material and methods

A set of 121 F_2:F_3 families derived from crossing two durum wheat cultivars, Latino and Primadur (characterized by low and high values of carotenoid content), were used for *Psy2* mapping. Nulli-tetrasomic, di-telosomic, and deletions lines (NTs) of *Triticum aestivum* cv. Chinese Spring (Sears 1954; Sears and Sears 1978; Endo and Gill 1996) were used for the physical location of *Psy2* amplicons on chromosome bins. Genomic DNA was isolated from young leaves using the protocol published by Dvorak *et al*. (1998).

The identification of *Psy2* genes in *Brachypodium* (Bradi4g01100) and *Oryza sativa* (Os12g43130) genomes was carried out searching in BLASTn (http://blast.ncbi.nlm.nih.gov/ Blast.cgi) and Phytozome database (http://www.phytozome.net/). The physical mapping and the functional annotation in genomes of closed related species to wheat genome were obtained using the COGE website (http://genomevolution.org/CoGe/ CoGeBlast.pl) (Lyons et al., 2008). An expectation value (E) of e^{-10} was used as significant threshold. All detected sequences were aligned and analysed using ClustalW tools (http://www.ebi.ac.uk).

To isolate the phytoene synthase genes in wheat, we used partial cDNA sequences from durum wheat (DQ642445, DQ642446, DQ642441 and DQ642442) identifying *Psy-A2* and *Psy-B2*, respectively (Pozniak *et al*., 2007). The reconstruction of the complete gene sequences in wheat ran out with the cereals databases (http://www.cerealsdb.uk.net/).

A set of primer pairs was designed using Primer3 (http://frodo.wi.mit.edu/primer3/) to cover the entire gene sequence in the genome A and B of the hexaploid cv. Chinese Spring. Primer design was focused mostly on the nucleotide regions characterized by polymorphisms between the homoeologous wheat genes. The primer sequences used for the physical mapping are: for (5'-CCTCTCTGACACGGCGTCA-3') and rev (5'-AGGTCATATACCTCGATTTCCAA-3') primers for A genome, for (5'-TTGGAAATCGAGGTATATGACCT-3') and rev (5'-ACTGGACGAACTGG

CACAG-3') primers for B genome. Single PCR fragments were directly purified with EuroGold Cycle Pure Kit and sequenced following the manufacturer's instructions (http://www.bmr-genomics.it/).

In order to investigate the role of *Psy2* genes in cv. Chinese Spring and to have a preliminary correlation between YPC and gene transcript, an analysis *in silico* was carried out against a wheat 61K microarray platform (Dash *et al*., 2012). The Plant Expression Database (PLEXdb) Blast (E value <-10) allowed to identify the corresponding probe set on wheat GeneChip.

III – Results and Discussion

Psy genes play a key role in the biosynthesis of carotenoids components, important for their impact on flour color and on human diet for nutritional aspects. The syntenic relationships within monocots (rice, maize, *Brachypodium*, sorghum and *Triticeae*) are recently reassessed and allow the development of tools to identify precisely chromosome-to-chromosome orthologous relationships (Colasuonno *et al*., 2013).

The analysis, based on BLASTn and Phytozome databases, allowed the isolation of the *Psy2* gene in *Brachypodium* and rice genomes. In particular, in *Brachypodium* genome the *Psy2* gene, located on chromosome 4 (locus name: Bradi4g01100) (Fig.1a), consisted of 2,560 bp length, mRNA sequence of 1,185 bp length and protein of 394 amino acids. In rice, the *Psy2* gene was mapped on chromosome 12 (Os12g43130) and had a genomic sequence of 2,791 bp, a mRNA of 1,197 nucleotides and a protein of 398 amino acids. In both species, the gene had 5 introns and 6 exons structure. The syntenic analysis allowed the isolation of a partial wheat gene sequences. The 5' UTR region of *Psy2* genes showed high GC content regions. The complete gene sequences were obtained using primer pairs derived from the contig reconstruction sequences by Cereals db website.

The *Psy-A2* and *Psy-B2* genomic structures and sequences were obtained for the cv. Chinese Spring and confirmed in the two durum wheat cultivars (Latino and Primadur). The *Psy-A2* genomic sequence was 2,592 bp long with 50.2% GC content. The predicted gene sequence counted out a region 811 bp long of 5′ untranslated sequence and 1,049 bp long of 3′ untranslated sequence. The mRNA had a 1,193 bp length translating for 397 amino acids. The *Psy-B2* genomic sequence had a length of 2,645 bp (50.5% GC content) and a mRNA region of 1,193 bp. The 5′ and 3' untranslated sequences was of 778 bp and 747 bp, respectively. The predicted protein length consisted of 397 amino acids. In both genes, the intron borders matched the canonical plant intron borders (GT..AG) for all 5 introns. Consensus exon/introns boundaries were predicted using Softberry program (Fig.1). The two homoeologous wheat genes shared the same number of introns/exons with no differences in the open reading frame (ORF), an identity of 93% and 98% between genomic and mRNA sequences. In comparison with model species, *Psy-A2* gene shared a sequence identity of 88% with *Brachypodium* and rice, whereas *Psy-B2* gene presented a sequence identity of 92% with *Brachypodium* and of 95% with rice (Fig. 1).

The *Psy2* genes were physically mapped on wheat group 5 chromosomes using NT lines. They were localized on chromosome bin 5AS3-0.75-0.98 and 5BS6-0.18-1.00, respectively. The Latino × Primadur linkage map reported by Blanco *et al*. (2011) and Colasuonno *et al*. (2013) was used for the *Psy2* genetic mapping. The *Psy-A2* and *Psy-B2* functional markers designed on the basis of *Psy2* sequences produced polymorphic fragments only for the B genome. In details *Psy-B2* marker produced polymorphic fragments of 491 bp in the cv. Latino and 483 and 519 bp in the cv. Primadur. The segregation analysis in the Latino × Primadur population integrated the *Psy-B2* marker into a linkage group of 112 cM including 37 markers (15 SSRs, 3 EST-SSRs and 19 DArTs).

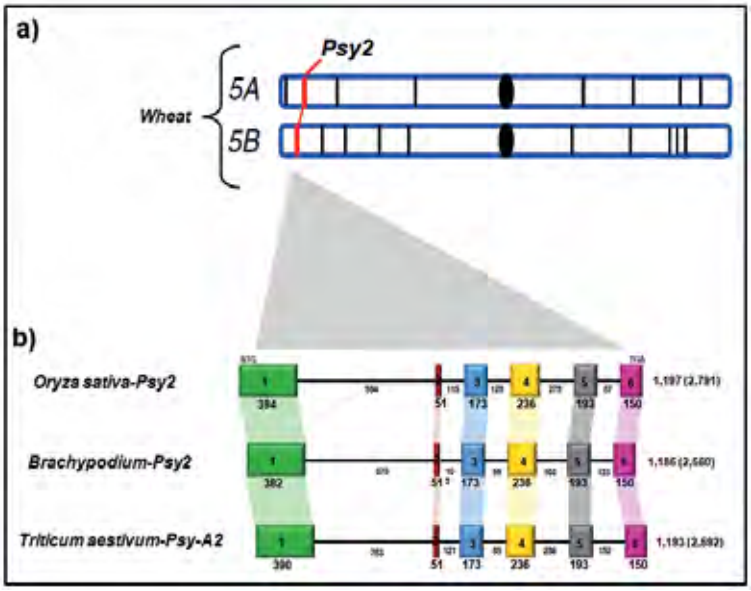

Figure 1. *Psy2* gene characterization. a) Schematic representation of physical position of *Psy2* on wheat chromosomes was showed. The physical mapping was referred to cv. *Chinese Spring*. b) Comparison of *Psy2* gene structure between *Oryza sativa*, *Brachypodium* and *Triticum aestivum* was presented basing on coloured boxes highlighting conserved exons. Introns and exons sizes were reported as well as the total gene (in brackets). cDNA and genomic sequence lengths were reported next to colour boxes.

The segregant population Latino × Primadur was evaluated for carotenoid content in four field trials in southern Italy as reported by Blanco *et al*. (2011). The ICIM (Inclusive Composite Interval Mapping) analysis detected the QTL on chromosome arm 5BS, flanked by *Xwmc73* and *wPt-3661*, closest to *Xgwm274* marker. The analysis revealed a different localization of the detected QTL-5B and *Psy-B2* gene, indicating the absence of the gene association to carotenoid content.

To further investigate the role of *Psy2* genes during plant development, a comparative matching analysis was done using the wheat 61k microarray platform at Plant Expression Database (PLEXdb). The BLAST search identified the probe Ta.18880.1.S1_at that had a high sequence identity with *Psy2*. The probe expression showed a constant pattern during wheat development suggesting its involvement in several biological mechanisms rather than in the endosperm development phase as the *Psy1*. This could justify an alternative *Psy2* role as detected in rice by Welsh *et al*. (2008). Indeed they detected PSY2 transcript levels increments in response to illumination during greening.

The present analysis identifies suitable *Psy2* markers and assesses the absence of linkage between the gene and QTLs for YPC, as confirmed by the 35 cM of distance between them in the linkage group map. This suggests a different role of *Psy2* in carotenoid expression, compared the other PSY gene family members (PSY1 and PSY3) involved directly in the control of the agronomic trait and resistance of stress, respectively.

Acknowledgments

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Evaluation of *Triticum durum* Desf. germplasm for the improvement of local products

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Abstract. The evaluation of '*ex situ*' germplasm collections allows us to use and conserve them more efficiently. A collection of 107 durum wheat (*Triticum durum* Desf.) accessions collected in Southern Italy from 1947 to 2009 was characterized using six morphological traits and 30 SSR markers. On the basis of the plant height, these accessions were classified into Pop 1 (plant height ≤70 cm), Pop 2 (70 cm <plant height <100 cm) and Pop 3 (plant height ≥100 cm). Significant differences were observed for morphological traits in these populations confirming the effects of the introduction of varieties with dwarfing genes. The 30 SSR markers identified 115 alleles, with an average of 3.83. The estimates of N_a, N_e, I and H_e for each population suggested a decrease in genetic diversity after the introduction of dwarfing genes in durum wheat germplasm. The level and the distribution of genetic diversity in the materials analysed in this study could be used as genetic criteria to protect these accessions in *ex situ* collections, to further investigate their characteristics and to use them both for breeding systems and for the improvement of typical products.

Keywords. *Triticum durum*, genetic resources, *ex-situ* conservation, morphological traits, SSR.

Evaluation des ressources génétiques de Triticum durum Desf. pour l'amélioration des produits locaux

Résumé. *L'évaluation des collections de ressources génétiques ex situ nous permet de les utiliser et de les conserver de manière plus efficace. Une collection de 107 accessions de blé dur (Triticum durum Desf.), collectées dans le sud de l'Italie de 1947 à 2009, a été caractérisée en s'appuyant sur six caractères morphologiques et 30 marqueurs SSR. Sur la base de la hauteur des plantes, ces accessions ont été classées en Pop 1 (hauteur de la plante ≤70 cm), Pop 2 (70 cm <hauteur de la plante <100 cm) et Pop 3 (hauteur de la plante ≥100 cm). Des différences significatives ont été observées pour les caractères morphologiques de ces populations confirmant les effets de l'introduction de variétés portant des gènes de nanisme. Les 30 marqueurs SSR ont identifié 115 allèles, avec une moyenne de 3,83. Les résultats obtenus pour Na, Ne, I et He dans chaque population ont suggéré une diminution de la diversité génétique après l'introduction des gènes de nanisme dans le matériel génétique de blé dur. Le niveau et la distribution de la diversité génétique dans le matériel analysé dans cette étude pourraient être utilisés comme critères génétiques pour protéger ces accessions dans des collections ex situ, pour mieux étudier leurs caractéristiques et les utiliser à la fois dans des systèmes d'amélioration génétique et pour l'amélioration des produits typiques.*

Mots-clés. *Triticum durum – Ressources génétiques – Conservation ex situ – Caractères morphologiques – SSR.*

I – Introduction

Durum wheat (*Triticum durum* Desf., 2n=4x=28; AABB genomes) is a tetraploid wheat species which is mainly used for human consumption. Over time in Italy plant breeding programs have introduced a number of varieties with always higher and more stable yield and improved grain quality that have continuously replaced the varieties previously locally grown. Genetic erosion of the available durum wheat germplasm has been prevented and a large number of accessions has been collected and preserved 'ex situ' for future breeding needs (Hagenblad *et al*., 2012). So far, only a small fraction of the huge worldwide collections of durum wheat have been characterized

and used. The analysis of genetic variation is a powerful tool to study germplasm resources and takes advantage by the development of a large number of molecular markers (Ganeva *et al*., 2010). Simple sequence repeats markers (SSR) or microsatellites, have been largely used to monitor the changes in genetic diversity in wheat germplasm (Landjeva *et al*., 2006; Mir *et al*., 2012).

In this study 30 SSR polymorphic markers are used (i) to assess the amount and the distribution of genetic diversity in a 'ex situ' collection of 107 accessions of durum wheat collected in the past 60 years in Southern Italy, and (ii) to evidence the genetic structure of the germplasm collected before and after the introduction of dwarf-gene varieties.

II – Materials and methods

1. Plant materials and morphological characterization

A germplasm collection of 107 durum wheat accessions that were collected in Southern Italy from 1947 to 2009 was used in the present study. Seed samples were kindly provided by the IPK genebank (Institute of Plant Genetics and Crop Plant Research), Gatersleben, Germany. Data were collected on morphological traits during the entire growing season in a field trial carried out at Azienda Agricola Sperimentale Dimostrativa (A.A.S.D.) Pantano in Pantano di Pignola, Potenza (Southern Italy) according to a randomized block design. Heading date, plant height, spike length, number of spikelet per spike, number of seeds per spike and weight of 1000 seeds were considered.

2. Genomic DNA extraction, PCR and SSR genotyping

For each accession genomic DNA was extracted from leaf tissues at the tillering stage using the automatic extractor ABI prism™ 6100 Nucleic Acid prep Station and the Trans-Prep protocol. Thirty SSR markers were selected on the base of their chromosome locations, T_{an} and degree of polymorphism. SSRs designation, chromosome location, primer sequences, T_{an} and the expected product size of the amplified loci were reported by Röder *et al*., (1998).

The PCR reaction was performed in a final volume of 50 µl composed of 10x PCR Buffer, 10 mM dNTP, 25mM $MgCl_2$, 10 µM forward primer, 10 µM reverse primer, 5 U AmpliTaq Gold DNA polymerase, 20 ng of genomic DNA and nuclease-free water. Amplification was performed by GeneAmp® PCR System 9700 as follows: 10 min at 95 °C; 1 min at 94 °C, 1 min at T_{an}, 1 min at 72 °C (35 cycles); and a final extension stage of 10 min at 72 °C. Following the PCRs, 3 µl of loading buffer 6x were added to 20 µl of each sample, then amplification products were analysed by electrophoresis in 2% agarose gels and visualised by ethidium bromide staining.

3. Statistical analysis

The germplasm collection was divided into three populations on the basis of the plant height: Pop 1 (plant height ≤70 cm), Pop 2 (70 cm <plant height <100 cm) and Pop 3 (plant height≥ 100 cm). Data were analysed by ANOVA using SAS 9.2 (TS2M3, SAS Institute Inc, NC, USA, 2002-2008) software package and means were compared by Duncan's multiple range test. Values were considered significant at $P < 0.005$.

For each SSR locus, the number of alleles detected, the gene diversity or unbiased expected heterozygosity (H_e; Nei 1978), and the polymorphic information content (PIC; Botstein *et al.*, 1980) were calculated using the program Power Marker 3.25 (Liu and Muse, 2005).

The genetic diversity in each population of durum wheat accessions was assessed using GenAlEx 6.5 (Peakall and Smouse, 2006, 2012). Number of alleles (N_a), number of effective alleles (N_e), Shannon's diversity index (I) and gene diversity (H_e: Nei 1978) were computed.

III – Results and discussion

Table 1 summarises the mean values for morphological traits in each population of durum wheat accessions. The accessions of Pop 1, collected after the introduction of dwarf-gene varieties, are characterized by a lower significant value for heading date and spike length than Pop 2 and Pop 3; vice-versa for the other morphological traits Pop 1 showed higher significant values. These results are in accordance with the effects of the introduction of dwarf varieties with higher-yielding potential due to an increased harvest index and better lodging tolerance, especially under high fertilizer and water inputs. In these varieties the translocation of assimilates to the ear allowed a higher number of seeds per spike despite the lower number of spikelet per spike.

All SSR markers used in the present study showed polymorphic fragments among all the 107 durum wheat accessions analysed; in total SSRs revealed 115 alleles. The number of alleles per locus varied among markers, ranging from two (Xgwm165-4A, Xgwm169, Xgwm357, Xgwm408 and Xgwm415) to seven (Xgwm6), with an average of 3.83 (Table 2). As a measure of the discriminatory power of each microsatellite locus, the average PIC value was 0.47, ranging from 0.09 for Xgwm374 to 0.79 for Xgwm6 (Table 2). The average PIC value suggested that SSR employed resulted adequate and efficient, considering that a PIC value > 0.5 accounts for a highly informative marker, 0.5 > PIC > 0.25 for an informative marker, and PIC ≤ 0.25 for a slightly informative marker (Botstein *et al.*, 1980).

Table 1. Means of six morphological traits collected in 107 accessions of *Triticum durum* from Southern Italy.

Morphological Trait	Pop 1 (h≤70 cm) n=20	Pop 2 (70cm<h<100cm) n=63	Pop 3 (h≥100 cm) n=24
Heading date (d)	173.76 c	183.94 b	186.42 a
Plant height (cm)	58.63 c	89.60 b	110.74 a
Spike length (cm)	6.16 c	7.25 b	8.63 a
Number of spikelet per spike (n)	19.13 c	21.62 b	22.86 a
Number of seeds per spike (n)	50.98 a	46.81 b	46.92 b
Weight of 1000 seeds (g)	51.26 b	61.30 a	57.66 a

Duncan's multiple range test, P < 0.001.

The overall genetic diversity (H_e) was 0.529, indicating that the durum wheat accessions used in this study displayed a substantial level of genetic diversity. This value was lower compared with those reported in other studies in wheat using SSRs (Landjeva *et al.*, 2006; Mir *et al.*, 2012). These differences can be explained by considering the genetic background of genotypes studied, the number of markers used and the techniques applied to detect polymorphism.

In order to reveal the genetic structure of the germplasm collected before and after the introduction of dwarf-gene varieties, we estimated various standard statistics for each population of accessions of durum wheat (Table 3). Pop 1 included accessions with a lower value for the plant height and collected after the introduction of dwarf-gene varieties, showed lower genetic diversity for all statistics measured (Wilcoxon signed-rank test, $P < 0.001$) compared to Pop 2 and Pop 3. This reduction in genetic diversity levels is in accordance with the results of previous studies on durum wheat (Roussell *et al.*, 2004; Reif *et al.*, 2005) and might be explained by the introduction of high-

yielding dwarf varieties based on a limited number of key parents and that rapidly dominated the wheat germplasm base.

Table 2. Chromosome location, total number of alleles, gene diversity and PIC values of the 30 SSR markers used to study genetic diversity in the germplasm collection of durum wheat from Southern Italy.

Locus	Chr. Loc.	All. No.	Gene div.	PIC	Locus	Chr. Loc.	All. No.	Gene div.	PIC
TAGLUT	1AS	3	0.595	0.529	Xgwm164	1AS	3	0.459	0.362
Xgwm357	1AL	2	0.213	0.191	TAGLGAP	1BS	3	0.612	0.543
Xgwm268	1BL	5	0.726	0.680	Xgwm95	2AS	3	0.585	0.496
Xgwm448	2AS	6	0.756	0.720	Xgwm526	2BL	4	0.627	0.553
Xgwm374	2BS	3	0.090	0.087	Xgwm155	3AL	3	0.369	0.333
Xgwm369	3AS	5	0.619	0.544	Xgwm493	3BS	4	0.562	0.471
Xgwm389	3BS	4	0.460	0.424	Xgwm165-4A	4AS	2	0.498	0.374
Xgwm610	4AL	4	0.572	0.489	Xgwm6	4BL	7	0.813	0.788
Xgwm495	4BL	3	0.549	0.448	Xgwm165-4B	4BL	4	0.713	0.663
Xgwm415	5AS	2	0.254	0.222	Xgwm304	5AS	5	0.650	0.605
Xgwm234	5BS	3	0.204	0.191	Xgwm408	5BL	2	0.463	0.356
Xgwm169	6AL	2	0.292	0.249	Xgwm570	6AL	4	0.393	0.369
Xgwm518	6BS	5	0.752	0.714	Xgwm219	6BL	4	0.639	0.573
Xgwm282	7AL	6	0.632	0.562	Xgwm332	7AL	4	0.431	0.350
Xgwm46	7BS	4	0.582	0.493	Xgwm611	7BL	6	0.761	0.723
Mean		3.83	0.529	0.470					

Table 3. Summary statistics of diversity for 30 SSRs detected in 107 durum wheat accessions subdivided by plant height in Pop1, Pop 2 and Pop3.

Pop	n	N_a	N_e	I	H_e
1 (h≤70 cm)	20	2.667 a	1.932 a	0.713 a	0.439 a
2 (70cm<h<100cm)	63	3.767 b	2.298 b	0.908 bc	0.507 b
3 (h≥100 cm)	24	3.400 c	2.528 c	0.952 c	0.551 c
All	107	3.278	2.253	0.858	0.499

Wilcoxon signed-rank test, P < 0.001.

The loss of genetic diversity may indicate an erosion of alleles valuable for plant improvement and future demands of producers and consumers. Currently some of the limiting factors in the use of 'ex situ' collections are linked to the missing or incomplete characterization of collections. The level and the distribution of genetic diversity in the materials analysed in this study could be used as genetic criteria to protect these accessions in 'ex situ' collections, to further investigate their characteristics and to use them both for breeding systems and for the improvement of typical products.

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Identification of molecular markers associated with yield and quality traits for Argentinean durum wheat breeding programs

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Abstract. Developing cultivars with high grain yield and optimal quality traits for pasta production is the main goal of durum wheat breeding programs worldwide. This work summarize the main results of our research group using a RIL mapping population (UC1113 x Kofa) evaluated in six environments from Argentina to detect QTLs and linked markers for yield and yield components, phenology, gluten strength, protein and color. QTLs affecting yield and yield components were mapped on chromosomes 3BS (*gwm493*), 2BS (*cfa2201*), 3AL (*ksm28-wmc428*) and 4AL. The first two QTL also affect heading date and/or flowering time. QTLs affecting flour yellow color (Fb*) were located on 4AL (*wmc219*), 6AL (*wmc553*) and 7BL (*cfa2040-barc1073*). The QTLs on 6AL and 7BL plus a 7BS QTL (*barc72*) also affect yellow pigment content (Ypc). Another important QTL for Ypc and Fb* was linked to *Psy-B1* gene. The deletion of the *Lpx-B1.1* from Kofa resulted in a significant decrease of lipoxigenase activity and in an improving in pasta color. For gluten strength, the most important and stable QTL was located on 1BL (Glu-B1) and two additional regions were located on 6AL (*wmc553*) y 6BL (*gwm219*). Two QTLs located on 3BS (*barc147-gwm493*) and 7BL (*cfa2040-barc1073*) were found as affecting protein content. The flanking markers of the QTLs detected in this work could be efficient tools to select superior genotypes to improve the Argentinean durum wheats.

Keywords. QTL – Yield – Quality – MAS – Durum wheat.

Identification de marqueurs moléculaires associés aux caractères liés au rendement et à la qualité dans les programmes d'amélioration du blé dur argentin

Résumé. *Le développement de cultivars à haut rendement en grain et avec des caractères de qualité optimaux pour la production de pâtes est l'objectif prioritaire des programmes de sélection du blé dur dans le monde entier. Ce travail résume les principaux résultats obtenus par notre groupe de recherche à l'aide d'une population de cartographie RIL (UC1113 x Kofa), évaluée dans six différents environnements en Argentine, pour détecter des QTLs et des marqueurs liés au rendement et à ses composantes, la phénologie, la force du gluten, les protéines et la couleur. Les QTLs affectant le rendement et ses composantes ont été cartographiés sur les chromosomes 3BS (gwm493), 2BS (cfa2201), 3AL (ksm28-wmc428) et 4AL. Les deux premiers QTL affectent également la date d'épiaison et/ou la date de floraison. Les QTLs affectant la couleur jaune de la farine (Fb*) sont situés sur 4AL (wmc219), 6AL (wmc553) et 7BL (cfa2040-barc1073). Les QTL sur 6AL et 7BL plus un QTL 7BS (barc72) affectent également la teneur en pigment jaune (Ypc). Un autre QTL important pour Ypc et Fb* est lié au gène Psy-B1. La délétion de la Lpx-B1.1 de Kofa a entraîné une diminution significative de l'activité lipoxygénase et une amélioration de la couleur des pâtes. Pour la force du gluten, le QTL le plus important et stable est situé sur 1BL (Glu-B1) et deux autres régions sont situées sur 6AL (wmc553) et 6BL (gwm219). On a observé que deux QTL situés sur 3BS (barc147-gwm493) et 7BL (cfa2040-barc1073) affectent la teneur en protéines. Les marqueurs flanquant les QTL détectés dans cette étude pourraient être utilisés efficacement pour sélectionner des génotypes supérieurs afin d'améliorer les blés durs argentins.*

Mots-clés. *QTL – Rendement – Qualité – MAS – Blé dur.*

I – Introduction

Durum wheat (*Triticum turgidum* L. ssp. *durum*) is mainly used to produce pasta because its grains are the only ones, among the cereals, able to produce semolina. The aptitude of semolina for pasta production is conferred by the particular characteristics of its endosperm storage proteins

that comprise the gluten matrix. A strong gluten and high protein content are conductive to the production of dough with excellent rheological properties for pasta making. Another important quality trait for durum wheat is the yellow color in milling products, due mainly to high carotenoid pigment content and low lipoxygenase activity. Developing cultivars with high grain yield and optimal quality traits for pasta production has been the main goal of durum wheat breeding programs worldwide. Since 2004 our group works in mapping quantitative trait loci (QTLs) associated with these traits in order to implement MAS in Argentinean breeding programmes (Carrera *et al*., 2007; Picca *et al*., 2008; Garbus *et al*., 2009; Conti *et al*., 2011; Roncallo *et al*., 2012). The starting point of our work in this field was done using a RIL population resulting from a cross of foreign germplasm. Genetic dissection using QTL analysis on local environments allowed to find genomic regions expressed in our durum wheat area. The present work resumed the main results of our research group using this bi-parental mapping population (UC1113 x Kofa) in order to detect QTL and linked markers for grain yield and yield components, phenology, gluten strength, protein and color. Field evaluations were performed in different locations of the main durum wheat area of the Province of Buenos Aires, Argentina.

II – Material and methods

1. Plant material

The mapping population consisted of 93 F9 recombinant inbred lines (RILs) obtained by single seed descent from the cross between the line UC1113 and the variety Kofa (Carrera *et al*., 2007).

2. Field trials

The 93 RILs and the parental lines were sown during two consecutive years (2006 and 2007) in three locations from Argentina (Cabildo [CA], Barrow [BW] and Balcarce [BC]). Field trials were sown following a randomized complete block design with three replications using experimental plots of 3 m^2 in size and 150/ m^2. Each year x location combination was considered an environment in the statistics analysis. Agronomical management of fertilization with nitrogen and phosphorus was performed in two applications, at presowing or sowing and tillering, according to local practices and doses for each experimental field. All traits were determined in the three replications of each genotype in each environment.

3. Quality traits

Whole-wheat flour was obtained by milling grains with an UDY experimental cyclonic mill (UDY Corporation) with a 1 mm sieve.

Gluten: Sedimentation volume test (Sv) was determined according to the technique described by Dick and Quick (1983) using 1 g of whole wheat flour.

Protein: Grain protein content (Gpc) was evaluated and expressed in percentage using Near-Infrared Transmission (NIRT) (Infratec 1226, Tecator, Suecia) according to IRAM procedure 15.879 and based upon 12% moisture content.

Color: Lipoxygenase activity (LOX) and yellow pigment content (Ypc) were determined. LOX extraction and substrate preparation were performed as described by Carrera *et al*. (2007) based on McDonald (1979) and Surrey (1964). Yellow pigments were analyzed using the protocol of Fares *et al*. (1991) as described in Roncallo *et al*. (2012). Flour yellow color (Fb) was measured with a MINOLTA chromameter (CIE L*a*b*).

4. Industrial Quality

Test weight (Tw) was obtained using a Schopper balance.

5. Yield and yield components

Grain yield (Yld) from each entire plot was obtained by weighing the harvested clean grains using a harvest machine (Kg/ha). Thousand grain weight (Tgw) was recorded by weighing two samples of 100 grains from each plot. Yield components were obtained from ten plants randomly collected from the central row of each plot after harvest maturity, expressed as mean value. The value per plant was calculated as an average of all ears by plant. Harvest index (Hi) was obtained as the ratio between the total weight of grains per plant and the weight of the plant. Spikelet number/ear (Sne) was obtained as the average number of total spikelets/ear, counting the number of spikelets in all the ears/plant. Grain weight/ear (Gwe) was determined by weighing the grains from each ear of the plant. Grain number/ear (Gne) was calculated as the product of the weight of grains/spike (Gwe) and the average weight of one grain which was obtained from the thousand grain weight. Spike fertility (Sf) was calculated as the ratio number of fertile spikelets/ear (Fse) and number of total spikelets/ear (Sne). Grain number per fertile spikelets (Gnfs) was calculated by dividing the grain number per ear with fertile spikelets per ear from each individual plant. Grain number per total spikelets (Gnts) obtained as the product of grain yield per ear and the individual grain weight obtained from thousand grain weight.

6. Morphological and phenological traits

Plant height (Ph) was measured as the distance from the edge of separation of the stem from the root to the tip of the spike (cm). Peduncle length (Pd) was measured as the distance from the last internode to the base of the spike (cm.). Heading date (Hd) was determined as the number of days between emergence and heading (Zadoks stage 55). Flowering time (Flt) was calculated as the number of days between emergence and flowering (Zadoks stage 65) (Zadoks *et al*., 1974).

7. QTL mapping

Genetic map: A total of 269 markers, including 23 SNP markers, were arranged on 14 linkage groups covering a total length of 2,140 cM, in this population (Zhang *et al*., 2008).

QTLs for lipoxygenases were mapped using a map constructed with 83 RILs based on the markers of Zhang *et al*. (2008) enriched with 44 AFLP, 9 RAPD, two isozymes and one storage protein. The genetic map was constructed using the software GQMOL (Schuster and Cruz, 2004) as was described in Picca *et al*. (2008).

Mapping method: QTL mapping was performed by the CIM method using the Windows QTL-Cartographer software v.2.5 (Wang *et al*., 2004) as was described in Roncallo *et al*. (2012).

III – Results

The main QTLs mapped using the UC1113 x Kofa mapping population are summarized in Table 1. QTL analysis showed several pleiotropic regions affecting correlated traits. Most of the positive alleles for quality were provided by Kofa whereas the positive alleles for yield came from UC1113.

The main QTLs affecting yield and yield components were located on chromosome arm 3BS, closest linked to the SSR marker *gwm493*. Kofa had the positive allele for this QTL that explained a maximum R^2 of 38% for yield in BC 2006 and also affected several yield components (Table 1).

Table 1. Main QTLs affecting yield, yield components and quality traits mapped in a durum wheat RILs population in six environments from Argentina.

QTL -Chr. arm	Closest marker	Positive allele	LOD score	Additive effect	R^2 (%)	Peak position (cM)	Environment	Individual envir. No.	Pleiotropic effect (positive allele)
QYld.cerz-1BL1	*BE443797_436*	UC1113	5.1	-122.65	13.4	47.1	Mean	1	Fb (K), Ypc (K), Gwe (U), Fse(U)
QSv.cerz-1BL2	*Glu-b1*	Kofa	17.6	5.19	46.2	81.8	Mean	6	
QYld.cerz-2BS	*cfa2201*	UC1113	7.1	-150.45	23.0	18.8	CA 2006	2	Hd(K),FltK),Sne(K), Gpc(U)
QYld.cerz-3AL	*Ksm28-wmc:428*	UC1113	6.9	-136.65	18.0	66.4	Mean	3	Gne (U), Hi(U)
QYld.cerz-3BS	*gwm493*	Kofa	8.1	149.43	20.7	13.0	Mean	2	Tw(K),Gne(K),Gwe(K), Ph(K),
QYld.cerz-4AL1	*dupw4-barc170*	Kofa	4.9	119.09	14.3	44.2	Ca 2006	1	PdL(K), Hd(KU), Flt(U), Gpc(U) Fb(U), Gpc(U), Sv(U), Hi(K), Gnfs(K)
QPh.cerz-4AL2	*wmc258-wmc718*	UC1113	5.9	-1.96	13.3	58.8	Mean	4	PdL(U), Gwe(U), Bpp(U)
QFb.cerz- 4AL3	*wmc219*	Kofa	4.0	0.24	10.6	126.2	Mean	3	
QLpx.cerz-4BS	*Lpx-B1.1*	Kofa	18.98	-	68.4	13.2	UC Davis	2 (2003-2004)	
QTgw.cerz-5BL	*BE495277_339*	UC1113	4.1	-0.69	13.2	73.3	Mean	2	Tw(U), Ypc(U), Gpc(U)
QYpc.cerz-6AL	*wmc553*	Kofa	10.5	0.43	29.9	65.4	Mean	5	Fb(K), Sv(K), Sne(K), Hd(K)
QTgw.cerz-6BL1	*BE604119_469 or wmc105*	Kofa	4.2	0.73	15.9	64.9	Mean	2	
QSv.cerz-6BL2	*gwm219*	Kofa	4.7	2.35	9.6	117.5	Mean	4	
QSv.cerz-7AS	*barc70*	Kofa	7.3	3.01	15.2	16.0	Mean	2	Hi(U), Sf(U)
QYpc.cerz-7BS	*barc72*	Kofa	4.1	0.27	9.5	59.2	BW 2007	2	Fb(K)
QGpc.cerz-7BL	*barc1073*	Kofa	4.3	0.21	15.6	185.6	Mean	3	Ypc(K), Fb(K), Sne(K)
QYpc.cerz-7BL2	*Psy-B1*	Kofa	2.9	0.24	7.9	195.5	Mean	1	Fb(K)

Yld= Yield, Sv= Sedimentation volume, Ph= Plant height,Fb= Flour b value, Lpx= Lipoxygenase activity, Tgw= Thousand grain weight, Ypc= Yellow pigment content, Gpc=Grain protein content,Gwe= Grain weight per ear,Fse=Fertile sp kelets per ear, Hd=Heading date,Flt= Flowering time, Sne=Sp kelets number per ear, Gne=Grain number per ear, Hi= Harvest index, Tw= test weight, PdL= Peduncle length, Gnfs= Grain number per fertile spikelets, Bpp= Total biomass per plant, Sf= spike fertility. Alleles: K= Kofa, U= UC1113. CA= Cabildo (Buenos Aires), BW= Tres Arroyos (Buenos Aires).

This QTL had a pleiotropic effect on heading date and/or flowering time, where a delay in spike maturity was produced by the UC1113 allele. Another QTL for yield was located on chromosome arm 2BS linked to *cfa2201*, having a more important effect on heading date and spikelet number per ear than over yield (data not shown. The most stable QTL for yield/yield components was mapped on the chromosome arm 3AL flanked by the markers *ksm28* and *wmc428*. Two linked QTL were mapped on the 4AL chromosome arm, with opposite alleles. The first one (*QYld.cerz-4AL.1*) affects yield/yield components and quality, whereas the second one (*QPh.cerz-4AL.2*) mainly have effect on plant height and peduncle length having a pleiotropic effect on some yield components.

The main QTLs affecting flour yellow color (fb*) were located on chromosome arms 4AL, 6AL and 7BL, linked to the markers *wmc219*, *wmc553* and to the interval *cfa2040-barc1073*, respectively. The QTLs on 6AL and 7BL also affect yellow pigment content, plus a 7BS QTL linked to the marker. *barc72*. These QTLs, in addition to the *Psy-B1* gene, located on 7BL proximal to *cfa2040-barc1073* interval, could be a useful target for breeding programs aimed at improving semolina color. Selection against high lipoxygenase activity using the deletion of the *Lpx-B1.1* from Kofa, would result in significant improvement in pasta color.

For gluten strength the most important and stable QTL affecting Sv was located on 1BL (*Glu-B1*). Other regions affecting this trait were located on 6AL (three environments and mean with LOD=2.9) and 6BL linked to the SSR markers *wmc553* and *gwm219*, respectively. Kofa had the positive allele on 6AL QTL simultaneously increasing Sv, Ypc and Fb. A QTL for Sne was mapped on 6AL. Sne and Sv were mapped with a well defined peak at 6-10 cM apart from Ypc QTL, but showing overlapped confidence intervals.

Two QTLs located on 3BS and 7BL were found as affecting protein content, flanked by the SSR markers *barc147-gwm493* and *cfa2040-barc1073*, respectively. The 3BS QTL correspond to the same mapped for yield, with opposite effects on protein and yield, whereas the 7BL QTL have pleiotropic effect increasing both Gpc and Ypc.

The 3BS QTL for yield and yield components overlaps with QTLs for plant height, peduncle length, heading time and flowering time. However, the peak positions for the two last traits were located at 9-20 cM apart of the first ones, with the Kofa allele being responsible for earliness (Fig.1). QTLs for plant height and peduncle length were consistently detected in the six environments, whereas Hd/Flt QTLs were less consistent in the results, although data for only 3 environments were collected in Hd/Flt.

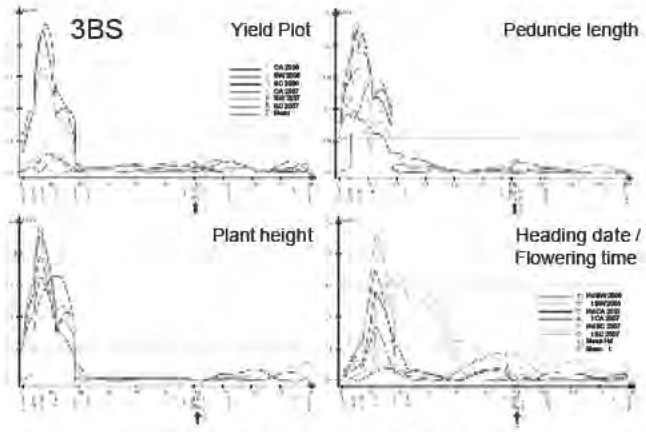

Figure 1. Graphical output of QTL analysis on 3B chromosome for yield (A), plant height (B), peduncle length (C) and heading date / flowering time (D). Red arrow indicate the approximate location of centromere.

IV – Discussion

Height reduction (−55%) caused by the putative *Rht5* gene located on chromosome 3BS was reported in bread wheat (Rebetzke *et al*. 2012), associated with delayed flowering, lesser number of grains by spike and yield. The UC1113 allele for the 3BS QTL (*gwm493-cfd79*) showed a similar effect in our analysis for these traits. The QTL mapped on 2BS was located near on *Ppd-B1* gene based on the consensus map of Sommers *et al*. (2004) and Mohler *et al*. (2004).

The markers presented here are in process to validation using an association mapping population consisting on 167 entries. The validated advantageous alleles will be used for MAS in public and private breeding programs in Argentina.

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Durum wheat breeding lines with new HMW glutenin subunit combinations selected for bread-making quality

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Abstract. A breeding program was carried out jointly by CIMMYT (Mexico) and CRA-ACM (Italy) in order to improve the bread-making quality of durum wheat (*Triticum turgidum* subsp. *durum*). The research, objective of the research was to expand the genetic diversity for glutenin subunits in durum wheat, and resulted in the development of breeding stocks with new HMW-GS subunits. Ninety-six breeding lines were tested at Sonora (Mexico) in irrigated experimental trials during the 2009-10 cropping season. The same genotypes were grown in Sicily (Italy) in the 2011-12 season under rainfed conditions. Most of the lines showed HMW-GS combinations that included subunits typically found in bread wheat (1 or 2*; 7+17 or 17+18, 5+10 at the Glu-A1, Glu-B1 and Glu-D1 loci, respectively). Main bio-agronomic parameters were evaluated and genotype adaptability in different environments was studied. Gluten quality-related analysis and experimental bread-making test were carried out in order to study the aptitude of semolina with different HMW-GS combinations to bread-making quality. Results showed that the highest bread volumes were obtained from genotypes characterized by the glutenin combination 7+8/5+10 and 7+17/5+10.

Keywords. *Triticum turgidum* subsp. *durum* – Protein composition interspecific crosses – Baking quality.

Lignées de sélection du blé dur avec de nouvelles combinaisons de sous-unités gluténines de haut poids moléculaire (HMW-GS) sélectionnées pour la qualité de panification du pain

Résumé. *Un programme de sélection génétique a été réalisé conjointement par le CIMMYT (Mexique) et le CRA-ACM (Italie) afin d'améliorer la qualité de panification du blé dur (Triticum turgidum subsp. durum). Cette recherche avait pour objectif d'accroître la diversité génétique pour les sous-unités gluténines chez le blé dur, et elle a permis de développer des stocks de reproducteurs avec de nouvelles sous-unités HMW-GS. Quatre-vingt-seize lignées ont été testées à Sonora (Mexique) dans des essais expérimentaux effectués avec irrigation au cours de la saison de culture 2009-10. Les mêmes génotypes ont été cultivés en sec, en Sicile (Italie), durant la saison 2011-12. La plupart des lignées ont montré des combinaisons HMW-GS qui comprenaient des sous-unités normalement présentes dans le blé tendre (1 ou 2*, 7+17 ou 17+18, 5+10 au locus Glu-A1, Glu-B1 et Glu-D1, respectivement). Les principaux paramètres bio-agronomiques ont été évalués et l'adaptabilité des génotypes aux différents environnements a été étudiée. Des analyses liées à la qualité du gluten et un essai expérimental de panification ont été réalisés afin d'étudier l'aptitude de la semoule avec différentes combinaisons HMW-GS à la qualité panifiable. Les résultats ont montré que les volumes de pain les plus élevés sont obtenus à partir de génotypes caractérisés par la combinaison de gluténines 7+8/5+10 et 7+17/5+10.*

Mots-clés. *Triticum turgidum subsp. durum – Composition en protéines – Croisements interspécifiques – Qualité de cuisson.*

I – Introduction

Genetic improvement of durum wheat has always been directed to the enhancement of technological parameters to produce high quality pasta. On the other hand, in many countries a large part of durum wheat production is used for of bread-making and other baked products (Palumbo *et al*., 2005; Spina *et al*., 2011a). Worldwide, 76% of durum wheat is used for the production of pasta, while the remaining 24% is used for the production of bread and other baked goods, as well as couscous, bulgur, etc.

In Italy, although most of the durum wheat harvest is devoted to pasta preparation (90.8%), part of durum wheat production is still used for manufacturing local, typical bread, and other baked food forms. UNIPI data for 2009 indicated that 6.6% is used in baking, 2.3% is exported as semolina and 0.3% for other domestic uses.

The bread-making aptitude is influenced especially by gluten extensibility (Ammar *et al*., 2000; Peña *et al*., 1994; Liu *et al*., 1996), which depends on high molecular weight glutenin components (HMW-GS) (Boggini and Pogna, 1989), many of which are present in genotypes derived from interspecific hybridization with *Triticum aestivum*. The presence of genes for those components allows the selection Durum Wheat genotypes characterized by good bread-making quality, irrespective of their origin (Boggini *et al*., 1995; Spina *et al*., 2009).

CRA, other institutions in Italy and CIMMYT in Mexico are leading a breeding program to improve bread-making quality of durum wheat (Palumbo *et al*., 2000; Boggini *et al*., 2003; Spina *et al*., 2011b). Durum wheat lines possessing glutenin subunits suitable for bread-making quality were crossed with durum and bread wheat cultivars and lines with good rheological and baking quality were selected from the segregating material. Most of these lines showed HMW-GS combinations that included subunits typically found in bread wheat (1 or 2*; 7+17 or 17+18, 5+10 at the Glu-A1, Glu-B1 and Glu-D1 loci, respectively).

II – Material and methods

Ninety-six F_6BC_5breeding lines were tested for agronomic traits at Sonora, Yaqui Valley (Mexico), which is 40 m above sea level during the 2009-10 cropping season, in comparison to 4 tester varieties, whereas 99 F_7BC_5 and F_8BC_5breeding lines (the 96 plus three additional lines) were tested at Libertinia, Sicily (Italy) (180 m above sea level) during the 2011-12 cropping season. Most of the lines, exhibited the HMW 1 and 2* in the A genome; 7+17, 17+18 in the B genome and 5+10 from the D genome, in addition to 6+8 and 7+8 HMW-GS, typical of durum wheat.

A square lattice experimental design, with 10 incomplete blocks and two repetitions was adopted at Sonora; the four rows plots had a 3.36 m^2 size. Full irrigation was applied. The trial carried out at Libertinia differed from the previous one for the plot size (1.88 m^2) and the rainfed conditions. The following bio-morphological parameters were recorded: date of ear emergence and flowering, awns color, and plant vigor. Yield, test weight, and thousand seeds weight were recorded on the harvested material. Electrophoretic analysis of storage proteins was performed on 5 seeds from 5 different spikes from each. Protein content was evaluated by NIR System unit (Foss Tecator) and ISIscan software. Additional parameters were recorded on whole grain semolina: yellow index (by Konica Minolta colorimeter CR-410 - Minolta method); sedimentation volume in SDS (using a stirrer apparatus for SDS test) and sedimentation index (as ratio between the sedimentation volume and protein content, as proposed by Peña *et al*., 1990). Bread-making test was performed, according to the AACC method 10-10. On the grain obtained from trials performed in Italy the following parameters were determined: protein content, gluten content and yellow index (using NIT apparatus - Foss Tecator). Stati.stical processing of agronomic trials was performed by means the software Genstat (Blues Spatial Analysis Results).The data concerning yield and quality parameters were processed using Statistics software version 6.

III – Results and discussion

Average values for Yield data from Sonora (7.44 t/ha) were higher than those from Libertinia (5.90 t/ha) (Fig. 1). The same trend was observed for grain parameters, such as test weight (TW) (83.2 *vs* 79.8 kg/hl), and thousand seeds weight (TSW) (47 *vs* 28.8 g).

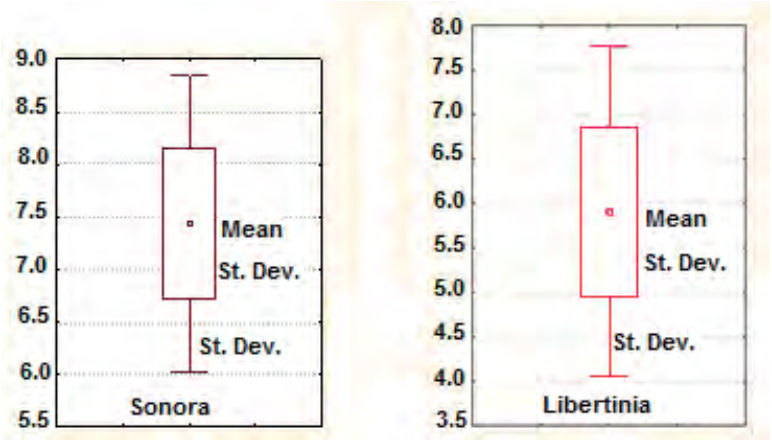

Figure 1. Yield results (Txha^{-1} of the trials carried out in 2009-10 at Sonora (left) and in 2011-12 at Libertinia (right).

A total of 14 different combinations of HMW-GS were detected in protein electrophoresis analysis (Table 1). D genome HMW subunits were present in both 1A and in 1B chromosomes, e.g. subunits 7+17, 5+10 and 17+18 were present on chromosome 1B, in addition to 6+8 and 7+8 typical of durum wheat germplasm). As far as LMW-GS was concerned, all lines were previously selected for the presence of type 2 subunits, and did not show any difference among them.

Table 1. High molecular weight glutenin subunit (HMW-GS) combinations and their frequency in the genotypes analyzed.

Lines with the same HMW-GS No.	HMW-GS	
	1A	1B
	glu-A1	glu-B1
16	Null	7+8
14	Null	7+17
10	Null	6+8
8	Null	17+18
8	6+8	5+10
8	7+17	5+10
6	2*	17+18
6	7+8	5+10
6	17+18	5+10
4	1	7+8
4	2*	7+17
2	1	6+8
2	1	7+17
2	2*	7+8

Protein content was significantly higher in the Libertinia harvest (16.1%, range 15.2 - 17.0%) than that from Sonora (12.0%, range 10.0 - 14.0%) (Fig. 2). The sedimentation volume of whole grain semolina was on average more than 12 cm^3 in the Sonora material, confirming the good quality of the gluten of most genotypes. Gluten content had an average value of 11.8% and a range of variation between 10.8 and 12.5%.

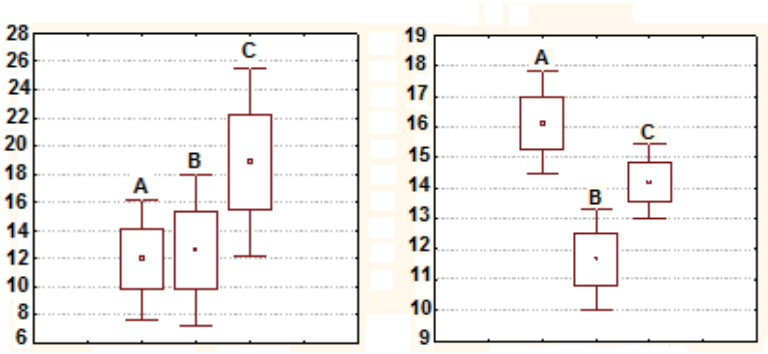

Figure 2. Grain protein (A), sedimentation volume (B) and grain color (C) in the analyzed material grown at Sonora (left) and at Libertinia (right).

Yellow index exhibited significant variability (from 15 to 22 b*) and satisfactory average value (19 b *), in the material from Sonora, whereas the material from Libertinia had showed lower average value (14.2 b*) and narrow range of variability.

The results of experimental baking test carried out on the 2010 trial are showed in table 2. The selected lines showed 557.1 cm^3 average value of bread volume, higher than that of durum wheat testers. The bread wheat testers, as expected, reached the highest volumes (> 800 cm^3).

Bread volume was significantly correlated with the sedimentation index (r = - 308 **).

Table 2. Results of baking test.

Bread-making parameters	Lines			Durum wheat Testers			Common wheat testers		
	Mean	Range	St. Dev.	Mean	Range	St. Dev.	Mean	Range	St. Dev.
Volume (cm^3)	557.1	445-785	63.9	504.4	475-650	81.3	807.1	740-900	56.6
Porosity (1-5)*	3.8	2-5	0.9	2.9	1-5	1.4	1,4	1-2	0.53
Crumb color (b**)	30.7	24.5-42.6	2.1	27.2	22.1-31,9	4.2	15.1	12.6-17.3	1.8

Concerning the relation between the glutenin composition and bread volume, the genotypes with the protein combinations 7+8/5+10, 7+17/5+10, 17+18/5+10 and 2*/17+18 showed higher bread volume (about 600 cm^3), indicating good bread-making quality. The 7+8/5+10, 7+17/5+10 HMW-GS combinations showed the best bread volume (616.7 and 616.3 cm^3, respectively).

The results of the study indicated that the introgression and expression of genes from bread wheat into durum wheat can be an efficient strategy for improving the bread-making quality of tetraploid wheat where such need exists for cultural, traditional, or local preferences.

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Importance of durum wheat breeding in terms of bulgur in Southeastern Anatolian Region of Turkey

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Abstract. Durum wheat is grown in specific regions of the world, with Turkey being a major producer with 3.5 million tons/year of grain. It has basically been used for the production of bulghur and pasta. Although Turkey is self-sufficient in durum wheat production, the quality falls short of the finest varieties grown in other parts of the world and Turkey has to import better quality varieties to close this quality gap.
In Turkey 66.5% of the durum wheat, termed as "macaroni wheat", is used for making bulghur and the rest for pasta products. Generally, bulghur is used to make "pillaf" as it is cheaper and healthier than rice. The importance of bulghur in human diet is being appreciated and this trend has progressed to Europe and even as far as America. During 2001 about 1 million tons of bulghur was produced by about 500 manufacturing plants throughout the country. In order to meet the demands of industry and consumer preferences, greater importance has been given to durum wheat breeding in Turkey in recent years.

Keywords. Durum wheat breeding – Quality – Bulghur – Southeastern Anatolia – Turkey.

Importance de l'amélioration du blé dur pour la production de boulgour dans le sud-est de la région anatolienne de la Turquie

Résumé. *Le blé dur est cultivé dans des régions spécifiques du monde dont la Turquie qui est un important producteur avec 3,5 millions de tonnes/an de grain. Il est essentiellement utilisé pour la production de boulgour et de pâtes. Bien que la Turquie soit autosuffisante pour la production de blé dur, la qualité est inférieure par rapport aux meilleures variétés cultivées dans d'autres parties du monde et ce pays doit donc importer des variétés de meilleures qualités pour combler cet écart.*

En Turquie, 66,5% du blé dur, qualifié de "blé macaroni", est utilisé pour la fabrication de boulgour et le reste est destiné à la production de pâtes. Généralement, le boulgour est utilisé pour faire le "pillaf", car ce produit est moins cher et plus sain que le riz. L'importance du boulgour dans l'alimentation humaine est appréciée et cette tendance s'est répandue en Europe et a gagné du terrain même en Amérique. En 2001, environ 1 million de tonnes de boulgour ont été produites par environ 500 usines de production disséminées à travers tout le pays. Afin de répondre aux demandes de l'industrie et aux préférences des consommateurs, une plus grande importance a été accordée à l'amélioration du blé dur en Turquie au cours des dernières années.

Mots-clés. *Amélioration du blé dur – Qualité – Boulgour – Sud-est de l'Anatolie –Turquie.*

I – Introduction

The Mediterranean region, including Turkey, has been known for durum wheat for centuries. The South, West and Thrace are most suitable regions both agronomically and quality-wise for the cultivation of durum wheat in Turkey (Doğan, 2004). Southeastern Anatolia Region has a huge agricultural potential with the presence of extensive land and favorable climate conditions for durum wheat (Genç *et al*., 1993). Therefore, this region has potential for producing more efficient and high quality bulghur products than other regions. In our country, more bulghur, noddles and flat-bread are produced from durum wheat than any other wheat, unlike other countries in the world (Özberk *et al*., 2003).

Grain based industry has a vital place in Turkey's food economy sector. Due to the country's rapidly growing population and constant increase in the demand for grain products, grain processing industry has a remarkable potential for growth. Durum wheat quality is one of the most important factors that determine the quality and consumer acceptance of the end-product (Millma, 2010).

Wheat quality is very important for both farmers and industrialists. Although the area planted to durum wheat in Turkey has not changed much in the last 40 years, increase in demand has been largely met with increase in production via utilization of certificated seed and high-yielding varieties, coupled with application of irrigation. However, this has resulted in some quality problems which has required import of high-quality durum wheat from other sources to about 3.5 million tons. In spite of the fact that durum wheat quality is improving, there is still much scope for improvement. This situation and unstable developments in the region and the growing of wheat imports in recent years has meant that serious measures should be taken to improve durum production and quality in Turkey (UHK, 2011).

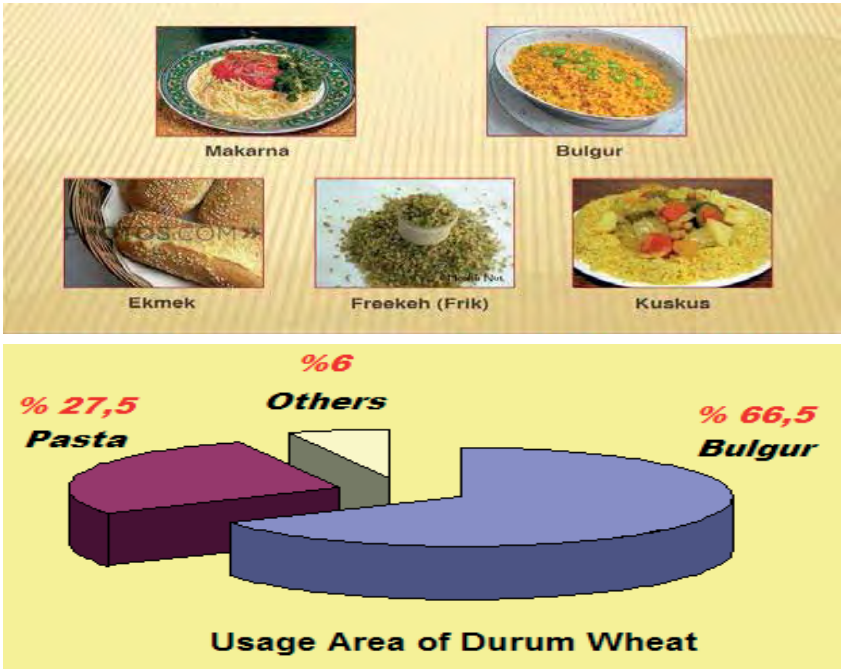

Figure 1. Durum wheat products and their relevance.

Southeastern Anatolia Region is an important center for durum wheat and its products (bulghur, pasta), however, the production of durum wheat at the desired quality level has not been reached. The farmers need high quality durum wheat to sow so that the same does not need to be imported as at present (TMSD, 2008).

II – Improvement, evaluation, interpretation

As mentioned above, durum wheat is used for making bulghur and pasta in Turkey (Kılıç et al., 2007; Anonymous, 2006). The production figures are as follows: (i) pasta 346,000 tons (27.5%); (ii) bulghur 839,000 tons (66.5%); (iii) other 75,000 tons (6.0%) (Zencirci and Aktan, 1998; TUIK, 1991).

Figure 2. Bulgur preparation at home.

Bulghur possesses high nutritional value. Compared to pasta and rice, in the same consumption-class, bulghur is cheaper and more nutritious. Especially, compared to rice with regards to B vitamin group, bulghur is very rich and therefore it is proposed as a substitute for rice in some countries, where there is high consumption of rice. Also it is a food item with durable and easy preparation, as far as the production technology (Özkaya, 1997; Bayram, 2000).

A study conducted in the USA in 1992, bulghur scored 69 points in the food table followed by wheat, rice, pasta and oats. In the same study bulghur took third place after barley and oats in terms of fiber and left behind pasta and wheat (Dönmez *et al*., 2004; Bayram, 2005).

Turkey is a country which imports rice and exports bulghur. Bulghur takes part among semi-finished food products which is rare in the food industry (Bayram *et al*., 1996). Today, the number of bulghur processing plants are nearly 500 and about 1 million ton of bulghur are produced annually in Turkey. This production was greater 2.5 times than macaroni (pasta) production. This amount of production increases when productions of urban homes and villagers are added (Bayram, 2005). Average consumption of bulghur is 12 kg per person on a country average. This value is around 23 kg in the East region and 7 kg in the the western regions. Amount of production and the economic value of bulghur have been increasing year after year. This increase in production, together with the recognition of bulghur as a superior food source, vitamins-wise, has been leading to an increase in bulghur exports (Bayram *et al*., 2002).

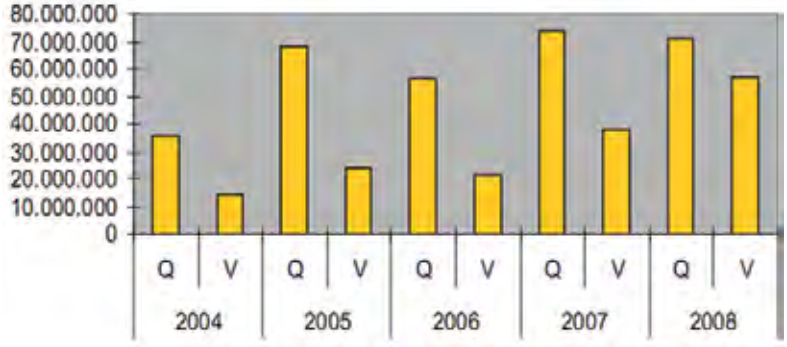

Figure 3. Amount (Q = Kg) and value (V = US $) of bulgur exports from Turkey with respect to year (IGEME, 2009).

In recent years, bulghur has been produced outside Turkey as well. For example, the total number of bulghur plants have jumped from almost nil to around twenty in USA and Canada alone. The annual bulghur production is 250,000 tons/year in United States. Bulghur is usually known and

consumed by Arabic, Greek, Armenian, and Turkish speaking peoples in the EU countries. Turkey is the largest provider of bulghur according to official records to these countries. Along with Turkey, there are bulghur manufacturers in France, Greece, and Sweden. Annual production of the manufacturers in Europe are around 2.000-3.000 tons/year, but sale value is low due to the problems of quality and acceptability. Factories have been also established in Arab and European countries in the last five years (Bayram and Öner, 2004). While bulghur was produced in limited quantities for those who migrated from Middle East to the United States in the past, The U.S. has begun to produce a wide range of products for export including the Middle East to other countries (Baysal, 1996).

Wheat type in bulghur production is one of the important factors affecting the quality. The bright yellow color and high protein is preferred (Tekeli, 1964; Elgün and Ertugay, 1992). However, the desired level of bulghur quality is always not possible. There are several reasons for this and one of the most important is that appropriate raw materials cannot be always be selected (Megep, 2008). To obtain a product of high quality at every stage of production it becomes necessary to select the most appropriate genotypes. Bulghur quality is under the influence of the variety and environment, too as with other types of wheat (Aydın *et al*., 1993).

Table 1. Export destination of bulgur from Turkey (Q = 000 Kg; V = 000US $).

	2006		2007		2008	
Country	**Q**	**V**	**Q**	**V**	**Q**	**V**
Iraq	16,152	5,582	21,587	8679	21,443	15,045
Liberia	7,988	2276	17,119	7394	18,493	10,268
Germany	5,871	3110	9,711	6090	8,379	10,091
Sierra Leone	5,283	1528	7,480	3112	6,408	3,583
Saudi Arabia	4,583	1627	913	452	1,992	1,574
France	585	274	1,224	920	1,393	1,621
The Netherlands	1,084	559	1,891	1297	1,262	1,557
Israel	3,042	1062	2,367	1063	1,776	1,403
UK	1,032	529	1,230	840	1,035	1,168
Mauritania	1,491	445	1,067	428	1,692	1,058
Sweden	849	485	1,012	1161	806	860
Australia	902	429	730	466	789	802
USA	646	310	740	470	688	770
Rep. N Cyprus	891	376	1,186	722	778	745
Belgium	607	304	834	558	550	641
Jordan	547	206	276	108	649	576
Russian Fed.	364	205	421	344	501	573
UAE	605	231	827	488	683	562
Austria	245	127	301	237	375	464
Denmark	303	147	321	218	311	380
Kuwait	679	207	539	292	416	378
Canada	214	104	515	185	374	378
Switzerland	218	112	243	176	308	352
Greece	283	110	270	182	274	268
Azerbaijan	105	50	221	271	271	250
Norway	129	74	90	70	138	173
Syria	0	0	6	3	193	134
Egypt	46	17	36	18	111	108
Kazakstan	89	48	115	86	99	104
Total (others included)	54,779	21,465	72,624	37,609	70,070	56,977

The wheat improvement concept of the future will be in the direction of satisfying consumer demands on the final product. In recent years, advances have been observed in technology and

the consumers research for new/better products. In addition, changes in the European Union processing laws brings about some regulations in Turkey too. Therefore, there is a need for development of platforms for relevant scientists and other persons from each sector to discuss these progress and regulations together. It is imperative that wheat producers, wheat sellers, such as mercantile exchanges and the Turkish Grain Board, research institutes, seed growing sector, universities, industrialists, and exporters should engage in more in dialogue together. Moreover, functional foods, nutrition, and food security are the topics that should also be discussed. Southeastern Anatolia Region falls within the gene center of wheat. Especially Şanlıurfa and its surroundings are known as our country's durum wheat belt. In this respect, the region is of capital importance in terms of durum wheat processing industry (Millma, 2010).

III – Conclusions

Nowadays, in order to increase the production of durum wheat and to have the desired high quality, it becomes necessary to concentrate on breeding for the quality of durum wheat varieties in addition to high-yield. In this way, it is hoped that the currently decreasing durum wheat production will rise again in Turkey, and the foreign-dependence of agricultural industry who are manufacturing this product will be reduce (Sözen and Yağdı, 2005).

Quality of raw materials should be improved in order to produce products that the consumers desire in the coming years (Millma, 2010). Contributions could be made to human health by: 1) increasing the bulghur consumption of Turkey on par with rice, 2) subject to genetic screening durum wheat cultivars and lines in order to identify genotypes with high values in terms of bulghur quality needed by the industrial sector, and 3) to focus on breeding programs to be executed for the high-quality bulghur varieties in Southeastern Anatolia Region.

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Breeding for improved technological quality in winter durum wheat

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Abstract. Under Hungarian conditions durum wheat must be capable of surviving several days of temperatures as low as –20°C without snow cover. Improving cold tolerance and technological quality simultaneously is no easy matter. The varieties used as sources of cold tolerance did not have satisfactory gluten strength and yellow pigment content, so high quality facultative durum wheat varieties were also included in the crossing programme. A period of ten years was required after gluten index measurements were commenced before over 70% of the new breeding lines had a gluten index significantly better than that of Parus, but since 2009 this proportion has been stable at over 80%. The gluten index of the majority of new winter durum wheat lines developed since the introduction of selection for gluten index is similar to that of high quality spring durum wheat genotypes. The other valuable technological quality trait of durum wheat is high yellow pigment content. The yellow index values of the advanced lines fluctuated over a wide range (17.7–32.2) in different years. Before selection was begun the average yellow index of these lines was 99.2% compared with that of the check variety Parus, while this value had risen to above 120% by 2006. The yellow index of the best lines exceeded that of Parus by 17.5–70.8% in different years.

Keywords. *Triticum turgidum* ssp. *durum*, – Gluten strength – Gluten index – Semolina colour – Yellow index.

Sélection pour l'amélioration de la qualité technologique du blé dur d'hiver

Résumé. *Dans les conditions de culture hongroises, le blé dur doit être capable de survivre plusieurs jours à des températures qui descendent même à -20°C sans couverture neigeuse. Améliorer à la fois la tolérance au froid et la qualité technologique n'est pas chose aisée. Les variétés utilisées comme sources de tolérance au froid n'ont pas une force du gluten et une teneur en pigment jaune satisfaisantes, donc des variétés de blé dur facultatives de haute qualité ont également été incluses dans le programme de croisement. Une période de dix ans a été nécessaire, après avoir commencé à mesurer l'indice de gluten, pour que plus de 70% des nouvelles lignées de sélection atteignent un indice de gluten significativement meilleur par rapport à celui de Parus, mais depuis 2009 cette proportion est restée stable à plus de 80%. L'indice de gluten de la majorité des nouvelles lignées de blé dur d'hiver développées depuis l'introduction de la sélection pour l'indice de gluten, est similaire à celui des génotypes de blé dur de printemps de haute qualité. L'autre caractère important de la qualité technologique du blé dur est la teneur en pigment jaune élevée. Les valeurs de l'indice de jaune des lignées avancées ont fluctué dans une large fourchette (de 17,7 à 32,2) sur différentes années. Avant d'entamer la sélection, l'indice de jaune moyen de ces lignes était 99,2% par rapport à celui de la variété Parus, alors que cette valeur a augmenté jusqu'à plus de 120% en 2006. L'indice de jaune des meilleures lignées a dépassé celui de Parus de 17.5-70,8% sur différentes années.*

Mots-clés. *Triticum turgidum ssp. durum – Force du gluten – Indice de gluten – Couleur de la semoule – Indice de jaune.*

I – Introduction

Winter durum wheat is mainly cultivated in the Eastern European countries. The most important producers of this crop are the former Soviet States (mainly Russia and Ukraine) but winter durum is also sown in other Eastern European countries (Bulgaria, Romania, Hungary, Slovakia, Serbia, Croatia, Macedonia) and in the Central Plateau of Turkey (Palamarchuk, 2005). Even breeding teams from the USA (Hall *et al*. 2011) and Canada (Tamburic-Ilincic *et al*., 2012) have recently released winter durum wheat varieties. The total production area of this crop is estimated at 1.2-

1.5 million hectares (Palamarchuk, 2005; EUROSTAT, 2013), which is about 8-10% of the spring durum wheat area. In Hungary it is cultivated on 8.5–14.9 thousand hectares (Hungarian Central Statistical Office, 2013).

Durum wheat generally has lower winter hardiness than bread wheat. However, in Hungary – due to the relatively short season for spring crops, often combined with drought stress during the spring period –winter durum is superior to the spring type. Under Hungarian conditions durum wheat often has to survive several days of temperatures as low as –20°C without snow cover, so the development of cold-tolerant varieties is a priority in breeding programmes.

The testing of the first winter durum wheat lines began in Martonvásár in 1982. These originated from the Odessa (Ukraine) breeding programme and had excellent cold tolerance and yielding ability, but their technological quality was poorer than that of spring durum wheat varieties. The genotypes first used as sources of cold tolerance had high protein content, but did not have satisfactory gluten strength and yellow pigment content. Later high quality facultative durum wheat varieties were also included in the crossing programme. The main objective of the Martonvásár durum wheat programme is to combine the high level of cold tolerance (also tested in phytotron chambers; Tischner *et al*., 1997) with strong gluten structure (measured by the gluten index test) and bright yellow colour (yellow index).

Gluten structure, including the strength of the matrix, is an important component of pasta quality (Cunin *et al.*, 1995). Gluten strength can be determined using several techniques. The laboratory equipment widely used for rheological measurements on bread wheat can also be used to analyse durum wheat samples (Sissons *et al.*, 2012). The determination of the gluten index (GI), based on the methodology elaborated by Perten (1990), is also a useful technique in durum wheat breeding programmes. A modified version of this method was first used for the analysis of durum wheat wholemeal and semolina samples by Cubbada *et al*. (1992). It is possible to perform the measurements even on wholemeal, and only 20 g samples are required for the analysis, so the method is particularly suitable in breeding programmes for analysing samples from early generations. The gluten index is a highly heritable, stable technological quality trait, mainly dependent on the genotype (Ames *et al*., 1999). In experiments performed by Clarke *et al*. (2000) the heritability of this trait in 120 progenies from three crossing combinations ranged from 0.84 to 0.93. Yellow pigment content is one of the most important technological quality parameters of semolina, the ground durum wheat product used in pasta-making (Irvine, 1971). The pigment, consisting mainly of lutein and esters of this compound (Lepage and Sims, 1968), has little or no influence on pasta-manufacturing and cooking properties (Dexter *et al*. 1981), but it leads to a considerable improvement in the aesthetic value, storability and marketability of pasta.

Yellow pigment content is a genetically determined trait (Braaten *et al.* 1962). Data in the literature suggest it is influenced chiefly by additive gene effects. The high heritability index of this trait also indicates that it is oligogenically inherited and determined by only a few alleles. Quantitative trait loci controlling the yellow pigment content (Elouafi *et al*., 2004; Patil *et al*., 2008; Zhang *et al*., 2008; Blanco *et al.*, 2011) and variation in the genes coding for key enzymes in carotenoid biosynthesis pathway (Cenci *et al*., 2004; Pozniak *et al*., 2007; Zhang and Dubcovsky, 2008) have been studied extensively.

Traditionally, total carotenoid content has been measured with a spectrophotometer (ICC Standard No. 152 and AACC International Method 14-60.01), but the yellow colour of the semolina can also be determined indirectly using a Minolta chromameter. In recent years this rapid method, which requires only small samples and no chemicals, has gained ground throughout the world (Borrelli *et al*. 1999). The Minolta b* index, known in the literature as the yellow index, is of most significance for the technological quality of durum wheat. This index is in very close correlation with the quantity of yellow pigment in the semolina. In experiments carried out by different research groups (Wehrle *et al.*,1997; Borrelli *et al.*,1999; Humphries *et al.*, 2004; Digesù *et al.*, 2009) the correlation coefficient between the two traits was found to be 0.88–0.95.

The present paper reports on the results achieved in improving the gluten strength and yellow index of winter durum wheat in the Martonvasar breeding programme.

II – Material and methods

The gluten index and yellow index of all the winter durum wheat varieties released from Martonvásár were tested in two replications in three consecutive years with contrasting weather conditions (2010 – extremely wet, 550.0 mm precipitation during the vegetation period; 2011 – dry, but high soil water content; 2012 extremely hot and dry, 202.5 mm precipitation).

Another analysis was made on data from experiments on advanced breeding lines to determine the effect of introducing tests on the gluten index and yellow index during the period 2000–2012. The number of lines included in the analysis ranged from 15 (2000) to 31 (2008–2012). Due to the nature of the experiment, the lines included in the analysis differed from year to year, so the data were compared with those of a check variety (Parus – a Ukrainian variety released in 1983; Palamarchuk, 2005), which was sown every year. The analyses were carried out in two replications.

Both experiments were set up on the same field in Martonvásár (47°18' N/18°49' E). According to the laboratory analysis of samples taken from the ploughed layer (1–20 cm) of the chernozem soil with forest residues, the soil of the selected area was sufficiently homogeneous. The topsoil, which contained no lime or damaging salts, had a weakly acidic pH and a loamy texture. Based on the humus content, it had moderate N supplies, while the AL-soluble values showed P contents close to the borderline between the good and very good categories (200 mg/kg) and good K supplies. With regard to microelements, the soil had a low Zn content, but good supplies of Cu.

Sowing was performed with HEGE-80 or HEGE-90 seed drills (Hans-Ulrich Hege GmbH und Co., Waldenburg, Germany) and in all the experiments the plant density was that recommended in Hungary (500 seed/m²). The crop was protected against weeds and insect pests throughout the growing season, but no fungicide was applied. The plots were harvested at full maturity with a plot combine (Wintersteiger AG, Reid, Austria).

The gluten index of each winter durum wheat sample was determined from semolina on the basis of the ICC158 standard (ICC, 1995) using a Perten Glutomatic 2200 instrument and a Perten 2015 Centrifuge (Perten Instruments AB, Hägersten, Sweden). The yellow index of the winter durum wheat varieties and breeding lines was recorded using a Minolta CR-300 chromameter (Minolta Camera Co. Ltd., Osaka, Japan).

The semolina required for the analyses was prepared using a Brabender Junior laboratory mill (Brabender GmbH & Co. KG, Duisburg, Germany), converted as suggested by Vasiljevic *et al.* (1977). The removal of the bran and separation according to particle size were carried out on a Retsch sieve series (Retsch GmbH, Haan, Germany). The 160–315 µm fraction was further cleaned with a Chopin Semolina Purifier (Chopin Technologies, Villeneuve-la-Garenne, France). From 2010 onwards the durum wheat samples were ground using a Chopin CD2 laboratory mill, but the semolina was cleaned using the instrument described above, so the particle size of samples prepared before and after 2010 was the same.

III – Results

The gluten and yellow index values of the Martonvasar winter durum wheat varieties are presented in Table 1. The first varieties from the breeding programme were released in 1996. Weak gluten was characteristic primarily of older genotypes, because gluten strength was not included among the selection criteria when these varieties were developed. The main goal at that time was to

improve adaptability, especially cold tolerance, in addition to which efforts had to be made to raise the yield potential to an acceptable level, since winter durum had to compete with winter wheat in their production zones (Dorofeev, 1987). Only when these aims had been achieved was it possible to turn attention to improvements in technological quality traits. This process is clearly illustrated by the gluten index values of winter durum wheat varieties bred in Martonvásár (Table 1).

Table 1. Gluten and yellow index of Martonvásár winter durum wheat varieties, 2010-2012.

Variety	Release Year	Gluten index				Yellow index			
		2010	2011	2012	Mean	2010	2011	2012	Mean
Odmadur 1*	1996	38.40	32.42	7.96	26.26	19.69	19.76	21.19	20.21
Odmadur 2*	1996	26.56	8.52	3.69	12.92	18.57	17.52	19.75	18.61
Martondur 1	1996	78.61	43.36	52.06	58.01	16.65	15.20	17.59	16.48
Martondur 2	1996	28.02	44.33	4.70	25.68	19.58	18.19	19.84	19.20
Martondur 3	1999	29.61	48.61	41.89	40.04	19.34	18.77	19.89	19.33
Mv Maxidur	2001	78.99	86.81	77.69	81.16	19.51	19.09	21.47	20.02
Mv Makaróni	2001	45.50	21.43	25.25	30.73	22.48	22.93	23.49	22.97
Mv Gyémánt	2004	72.69	56.53	4.94	44.72	22.56	18.26	20.14	20.32
Mv Hundur	2011	65.10	72.15	69.78	69.01	26.84	25.21	25.01	25.69
Mv Pennedur	2011	91.32	94.36	75.14	86.94	24.47	23.83	23.20	23.83
$LSD_{5\%}$		3.86	7.05	5.51	3.30	0.38	0.86	0.65	0.42

*Note: * Varieties bred jointly with the Plant Breeding and Genetics Institute Odessa, Ukraine.*

The Martonvásár durum wheat breeding programme was initiated in 1982, but right up to the mid-1990s selection was only made for kernel type and vitreousness. The use of instruments for analysis began in 1996, with measurements on the wet gluten content and the gluten and yellow indexes. The first four years of analysis were sufficient to demonstrate that there was great genetic variability both for gluten index and yellow index in the breeding material and that these technological parameters had little dependence on the year, thus making it possible to carry out efficient selection for stronger gluten and for brighter yellow semolina colour.

Improvements in the gluten and yellow indexes of the breeding lines had to be achieved while maintaining or improving the values of other traits (cold tolerance, protein content). The results achieved between 2000 and 2012 are presented in Figure 1. The gluten index of the lines referred to in the figure was significantly higher ($p = 0.05$) than that of Parus. In addition to the relative values, the figure also demonstrates the ratio of varieties in the given year with a gluten index classified in the 'good to very good' ($65 > GI \geq 85$) or 'excellent' ($GI > 85$) categories given by Cubadda *et al.* (1992). A period of ten years was required after gluten index measurements were commenced before over 70% of the new breeding lines had a gluten index significantly better than that of Parus, but since 2009 this proportion has been stable at over 80%. The lines examined included a high proportion of genotypes with good-to-excellent gluten strength, so this trait no longer represents a bottleneck in the selection of advanced lines.

The mean yellow index values recorded for advanced breeding lines over the last thirteen years also confirm that the technological quality of winter durum wheat genotypes can be substantially improved by selection. The lowest value recorded in the laboratory was 17.70 (in 2001) and the highest 32.16 (in 2004). The yellow index values exhibited a wide range during the 13 years of investigations. The yellow index of the majority of the genotypes was close to average, but in each year it was possible to identify and select for winter durum advanced lines with an exceptionally high yellow pigment content, close to, or even higher than that of spring varieties.

As the result of selection there has been a substantial increase in the yellow index of winter durum wheat lines awaiting state registration. The data for the lines are compared with those of Parus, a Ukrainian winter durum variety with favourable agronomic traits, excellent winter hardiness

and moderate yellow pigment content (Table 2). This variety has been sown as a control ever since the breeding programme was commenced. When selection was begun in 1996 the average yellow index of the lines compared with the control variety was 99.2%, while since 2006 this value is continuously above 120.0% (the largest difference was observed in 2009, when it was 142.8%).

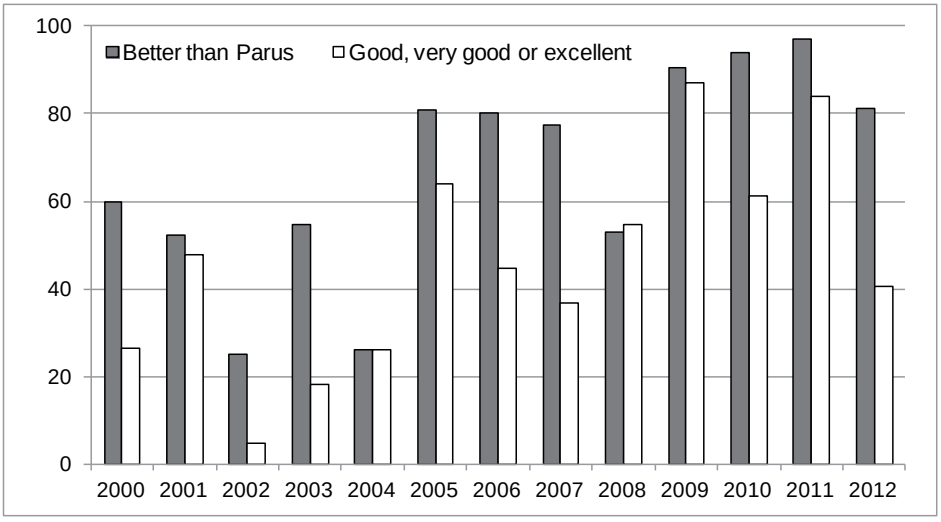

Figure 1. Ratio of Martonvásár winter durum wheat lines with a gluten index significantly better (p=0.05) than that of Parus, and with good-to-excellent gluten strength (gluten index>65). Martonvásár, 2000–2012.

Table 2. Yellow index of Parus and of Martonvásár winter durum wheat breeding lines, 2000–2012.

Year	No. of lines	Parus	Mv breeding lines			No. of lines		
			Min.	Max	Mean	> Parus	Medium quality*	High quality*
2000	15	20.64	20.06	27.04	23.09	11	9	6
2001	23	21.12	17.70	24.83	22.34	15	14	8
2002	20	24.06	22.94	28.84	25.15	12	6	14
2003	19	20.82	21.48	27.55	24.05	19	9	10
2004	22	23.67	25.32	32.16	27.21	22	0	22
2005	24	19.80	19.34	27.76	23.54	22	11	13
2006	28	20.65	20.61	31.24	26.41	27	3	25
2007	29	21.23	21.26	29.94	25.96	28	4	25
2008	31	20.68	22.10	29.08	25.59	31	2	29
2009	31	15.77	19.68	26.94	22.52	31	20	11
2010	31	18.56	20.55	26.84	23.80	31	13	18
2011	31	17.72	19.88	25.21	22.58	31	21	10
2012	31	17.73	19.14	25.08	22.80	31	21	10

** Classification according to Landi (1995). Medium quality: yellow index is between 19.0 and 23.5; High quality: yellow index is above 23.5.*

During the period 2000–2012 the yellow index of the best lines exceeded that of the check variety by 17.5 to 70.8%. Selection also led to a substantial reduction in the ratio of lines with a yellow index smaller than that of Parus. In the first year after the purchase of the Minolta CR-300 instrument (1996) this ratio was still 60%, while in 2003, after several years of selection, it had dropped to 0%. Since then, the majority of the lines (91.7–100.0%) have a yellow index significantly higher than that of Parus.

The gluten and yellow indexes are highly heritable traits in winter durum wheat, with little dependence on the environment. Both gluten index and yellow index measurements require small sample size, so these methods can be successfully used in winter durum breeding programmes for the improvement of gluten strength and yellow pigmentation. Using selection based on these parameters, the technological quality of winter durum wheat lines can be improved sufficiently to make them competitive with high quality spring varieties.

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Session 7

Perspectives in structural and functional genomics

A new and “open access” chromosome approach to complex genomes: flow sorting of FISH labeled chromosome in suspension

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Abstract. A number of leading crop species are either polyploid and/or hold a large genome as a result of the accumulation of highly repetitive sequences. These features hamper the assembly of complex genomes in spite of the fast development of new sequencing technologies (Next Generation Sequencing) and the availability of powerful bio-informatic tools. The chromosome approach, by enabling genome dissection into single chromosomes or chromosome arms via flow sorting, can contribute to reduce genome complexity. In all plants, this approach is restricted to species, or special cytogenetic stocks, as in the case of wheat, containing chromosome types that differ in size from the standard complement. We have developed an overall robust and easy method called FISHIS (Fluorescence *In Situ* Hybridization in Suspension) which overcomes the constrain of chromosome size and DNA content differences, allowing chromosome flow sorting on the base of fluorescent hybridization labeling of chromosomes in suspension. FISHIS relies on readily available synthetic, fluorescently labeled repetitive sequences and on a kaline DNA denaturation. We show that the method can discriminate between and hence isolate the A genome from the B *genome* of durum wheat (*Triticum turgidum* subsp. d*urum*), a number of the chromosomes of the same species and o*f T. monococcum*, as well as the whole complement of the diploid wheat relative *Dasypyrum villosum* (L.) Candargy.

Keywords. Fluorescence In Situ Hybridization In Suspension-FISHIS – Flow molecular cytogenetic approach, chromosome flow sorting – Wheats – *Dasypyrum villosum – Haynaldia villosa. T. monococcum.*

Une nouvelle approche chromosomique aux génomes complexes en « libre accès » : tri par flux des chromosomes marqués FISH en suspension

Résumé. *Un certain nombre d'espèces cultivées parmi les plus importantes sont soit polyploïdes et/ou ont un grand génome en raison de l'accumulation de séquences hautement répétitives. Ces caractéristiques ralentissent l'assemblage de génomes complexes, en dépit du développement rapide des nouvelles technologies de séquençage (Next Generation Sequencing) et la disponibilité d'outils bio-informatiques puissants. L'approche chromosomique, en permettant la dissection du génome en simples chromosomes ou bras chromosomiques via le tri par flux, peut contribuer à réduire la complexité du génome. Dans toutes les plantes, cette approche est limitée à des espèces ou à des stocks cytogénétiques particuliers, comme dans le cas du blé, contenant des types de chromosomes qui diffèrent de par la taille du complément standard. Nous avons développé une méthode globale robuste et facile appelée FISHIS (Hybridation In Situ Fluorescente en Suspension) qui surmonte la contrainte de la taille des chromosomes et des différences de contenus d'ADN, permettant de trier par flux les chromosomes sur la base du marquage par hybridation fluorescente des chromosomes en suspension. La méthode FISHIS s'appuie sur des séquences répétitives synthétiques facilement disponibles, marquées par fluorescence et sur la dénaturation alcaline de l'ADN. Nous allons montrer que la méthode permet de discriminer entre, et donc, d'isoler le génome A du génome B du blé dur (Triticum turgidum subsp. durum), un nombre de chromosomes de la même espèce et de T. monococcum, ainsi que le complément entier du blé diploïde apparenté Dasypyrumrapport villosum (L.) Candargy.*

Mots-clés. *Hybridation In Situ Fluorescente en Suspension – FISHIS – Approche du flux cytogénétique moléculaire – Tri par flux des chromosomes – Blés – Dasypyrum villosum – Haynaldia villosa – T. monococcum.*

I – Introduction

In plant genomics new avenues have been disclosed by the rapid development of next generation sequencing (NGS) technologies and new bioinformatics tools (Metzker 2010, Trengen and Salzberg 2012, Edwards *et al* 2013). However, the assembly of huge genomes is still challenging because of the polyploidy origin and the high content in repetitive sequences of many plant species. The "chromosome approach" (Dolezel *et a*l. 2007) consisting in isolation of individual chromosome or chromosomes arms by Flow Cytometry (FC) analysis and sorting, offers a clue to reduce genome complexity. FC relies on the passage of chromosome suspension through the focus of intense light source. Optical parameters, such as light scattering and fluorescence, related to chromosomes morphology and DNA content respectively, are detected by the instrument and finally display as an histogram or as a two dimensional representation (dot plot) of the distribution of the different chromosomes. Critical for chromosome discrimination and flow sorting is the occurrence of a sufficient difference (at least 10%) in DNA content among individual chromosomes, a condition quite uncommon in plants. In humans, where differences exist in base-pair composition of individual chromosomes, two different fluorescent dyes, that binds preferably to: AT (Hoechst 33258) or GC (Chromomycin A3) rich regions have been used to discriminate similar sized chromosomes. In plants all attempts to base the chromosome sorting (following the animal model) on variation in their A-T and C-G content have failed because of the uniformity across plant genomes with respect to base pair composition, probably due to the high amount of repetitive sequences scattered all over the genomes. The fluorescent labeling in suspension of such abundant repetitive sequences could represent an alternative approach to the flow discrimination of similar sized chromosomes. We developed a reliable, fast and cost effective method for Fluorescence *In Situ* Hybridization. In Suspension (FISHIS) of plant chromosomes using Simple Sequence Repeats (SSR) as probe, which allows, for the first time in plants, chromosome sorting based both on total DNA content (size) and on a FISHIS specific chromosome labeling pattern. The discriminatory power of FISHIS combined with the high throughput of flow cytogenetic analysis and sorting gives rise to the new "flow molecular cytogenetics" approach which has allowed to purify, separately, the A and the B genomes of durum wheat and several of its individual chromosomes. Besides we have isolated chromosome $6A^m$ from *T. monococcum* and all seven chromosomes of the wild diploid species *Dasypyrum villosum* (L.) Candargy. The development of this method offers many analytical and preparative opportunities for the extension of current genomic technologies to large and complex genome species.

II – Material and Methods

1. Plant material

Grain of the diploids *Triticum monococccum* and *Dasypirum villosum* were provided by S. Pogna (CRA Agricultural Research Council, Italy,) and C. De Pace (University of Tuscia, Viterbo, Italy), respectively. Seeds of durum wheat (*Triticum durum*) cv Creso were obtained from P. Gentili (ENEA, Rome, Italy).

2. Cell cycle synchronization and preparation of chromosome suspensions

The procedure for cell cycle synchronization and for preparation of chromosome suspensions were according to methods of Dolezel *et al.* (1999) and Giorgi *et al.* (2013).

3. DNA probes

The following probes were synthesized and labeled by Eurofins MWG Operon (Ebersberg, Germany): $(GAA)_7$, $(AG)_{12}$, 5'-FITC-$(GAA)_7$-3'-FITC, 5'-Cy3-$(AG)_{12}$, 5'-Cy3-$(AAT)_7$ and 5'-Cy3-

$(AAC)_5$. The HPLC desalted oligos were resuspended at 1µg/µl in 10mM Tris, 1mM EDTA. The 18S-5.8S-26S rDNA clone pTa71 probe (Gerlach and Bedbrook, 1979) was labeled by nick translation using standard kits (Nick Translation Mix, Roche) following the manufacturer's instructions.

4. ISFISH

A. Alkaline denaturation of DNA

The extent of DNA denaturation was determined experimentally by the addition of NaOH to a 150µl aliquot of suspended chromosomes ($2x10^6$ chromosomes/ml LB01) labeled with 36µM acridine orange (AO), followed by flow cytometric analysis . AO stains single-stranded DNA red and double-stranded DNA green. Flow data were collected in the form of dot plots by plotting the ratio ssDNA/dsDNA fluorescence against dsDNA fluorescence for 10^4 chromosomes per sample (for details see Giorgi *et al.* 2013). The optimum denaturation treatment was set at pH13 for 20min, followed by a return to pH8.0 by the addition of 1M TrisHCl pH 7.4 and maintaining the suspension on ice for 1min.

B. Fluorescent labeling

The oligonucleotides probes were dissolved in 300mM sodium chloride, 0.3mM trisodium citrate (2XSSC) at a concentration of 160ng/ml and directly added to the chromosome suspension immediately after neutralization. The pTa71 probe after dilution in 2XSSC at a concentration of about 300-400ng/ml was hot denaturated for 5' at 95°C and then added to the chromosome suspension. In both cases the labeling reaction was carried out for 1h at room temperature, without any washing or centrifugation steps. After hybridization, the samples were diluted 1:1 with LB01 buffer counter-stained with 7µM DAPI and analyzed by flow cytometry (300µl final volume). For chromosome identification by fluorescence microscopy (Fig. 3), 4µl chromosome suspension was mounted in 30% v/v LB01, 70% v/v Vectashield (Vector Labs, Burlingame, CA) containing 7µM DAPI.

C. Flow cytometry and chromosome sorting

The Chromosome analyses and sorting were performed with a dual laser FACS Vantage SE flow cytometer (BD Bioscience, San Jose, CA) (for details see Giorgi *et al* 2013).

D. T Fluorescence microscopy

FISHIS labeled chromosomes and nuclei, before and after flow cytometric analysis and chromosome sorting, were visualized through a Nikon Eclipse TE2000-S epifluorescence microscope equipped with a Hg100 lamp and filter sets appropriate for FITC, DAPI and Cy3 fluorescence. The single images from each filter set were captured and digitized using a cooled NIKON DXM1200 color camera (Nikon Instruments Europe, B.V. Amstelveen, The Netherlands). Fluorescence images were superimposed after contrast and background optimization using ImageJ v1.45 (rsbweb.nih.gov/ij/index.html).

III – Results and Discussion

The first set of experiments was performed in durum wheat with the aim to define the best conditions for controlled DNA denaturation and labeling of chromosome in suspensions. The unwinding of the DNA double helix, induced by alkaline pH treatment of varied duration, was assessed via the flow cytometric analysis of the metachromatic shift, from green to red fluorescence, of a

chromosome suspensions stained by Acridine Orange (AO). The optimal combination proved to be a 20min treatment at pH13 (Giorgi *et al.* 2013).

Different concentrations of probe were evaluated first using a $(GAA)_7$ microsatellite labeled with either FITC or Cy3, which has been shown to be highly effective in generating FISH karyotypes of wheat. (Kubalakova *et a*l. 2005, Cuadrado *et al* 2008). An intense and well defined hybridization pattern was achieved in chromosome suspension (Figure 1) using 160ng/ml $(GAA)_7$-FITC probe and the distribution of $(GAA)_7$-FITC sites identified in the durum wheat complement (genomes A and B) was highly reproducible and consistent with that observed in standard FISH on slide (Pedersen and Langridge 1997, Kubaláková *et al*. 2005). Moreover multi-color labeling was achieved by using different probe combinations (Fig. 1).

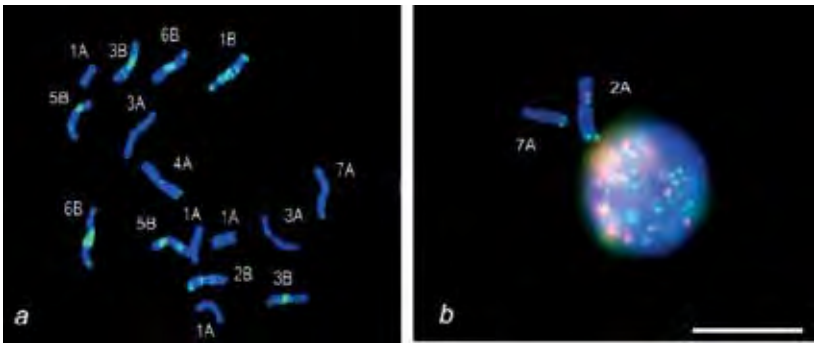

Figure 1. Single and double target FISHIS of durum wheat cv Creso chromosome suspensions. a) Chromosome suspensions hybridized with (GAA)7-FITC; b) chromosomes and a nucleus after (GAA)7-FITC and (AAC)5-Cy3 dual labeling. Bar=10µm (from Giorgi *et al.* 2013).

FISHIS proved to be effective in chromosome labeling of several *Triticeae* species (table1). In particular chromosome suspensions of *T. durum, T. monococcum* and *D. villosum* have been used for the flow cytometric analysis and chromosome sorting after FISHIS labeling. In durum wheat the standard flow karyotype, based on DNA DAPI staining, show three main peaks only one containing a single type chromosome, the 3B (Kubalakova *et al*. 2005; Giorgi *et al.* 2013). After $(GAA)_7$–FITC labeling, up to ten chromosome clusters were resolved in the dot blot by the FC analysis (Fig. 2). The unequal distribution of the GAA SSR among the A and B genome of pasta wheat, with the A genome chromosomes less intensely labeled compare to those in the B genome, allowed for the ready separation of the two whole chromosome set (Fig. 2).

Moreover several chromosome type as 1A, 6A and 2B could be isolated at high level of purity, respectively >90%, >93%, > 93%. In the wild species *D. villosum* (2n=14 ; genome VV), a far relative of cultivated wheats, the $(GAA)_7$ distribution on the V genome has been recently investigated (Grosso *et al*. 2012) by standard FISH on slide, which revealed the GAA chromosome specific hybridization pattern and its high discriminatory power. Such observation suggest a possible use of $(GAA)_7$ for the flow molecular cytogenetic analysis of *D. villosum.* Its standard flow karyotype comprised four peaks, only one represented by a single chromosome type, the 6V (Grosso *et al.* 2012). After $(GAA)_7$–FITC labeling of *D. villosum* chromosome suspension a dot plot karyotype was generated in which all seven chromosomes could be individually identified and isolated at a level of purity > than 85% (Figure 3).

Other oligonucleotides, besides $(GAA)_7$ have been used in single or double target FISHIS experiment and proved to generate a labeling pattern able to discriminate and hence to allow flow sorting of different chromosomes. The (AG)12 microsatellite showed hybridization signals on chromosomes 3B, 4B, 5B and 6B of both pasta wheat allowing the flow sorting of chromosome 5B

(double strong band) and 3B to a purity level above 90 (Giorgi *et al.* 2013). In *T. monococcum* the $(ACC)_5$ *and the* $(GAA)_7$ oligonucleotides markedly label the chromosome 6V, (Megyeri *et al* 2012). Combining, by FISHIS, the two different oligonucleotides labeled with the same fluorochrome (Cy3) it was possible to increase the specific fluorescence emission of chromosome 6A and to flow sort it at high level of purity (Figure 4).

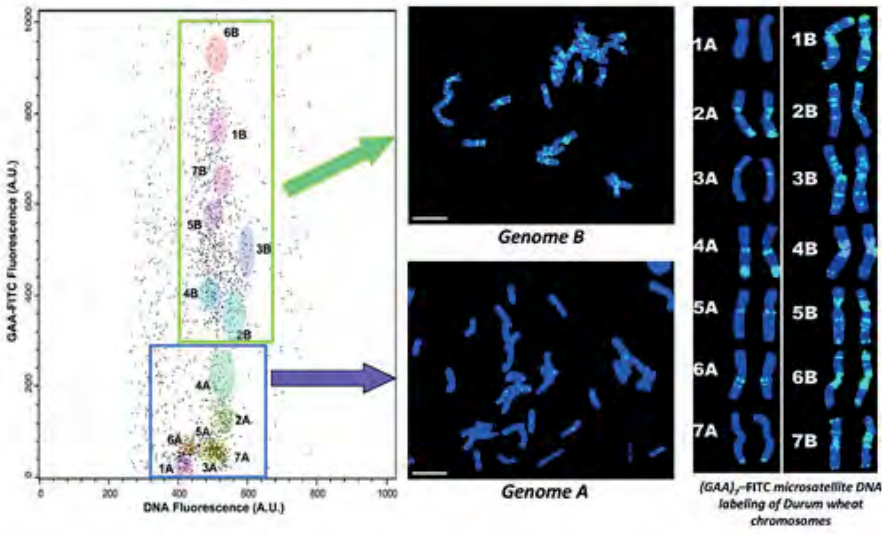

Figure 2. Biparametric dot plot analysis of durum wheat cv Creso chromosomes. The fluorescence intensity emissions from chromosomes stained with DAPI (DNA content) and FISHIS labeled with GAA)7-FITC are joint together into a bi-parametric dot plot where each dot represents a single particle (blue: DNA stained by DAPI; green: (GAA)7-FITC labeling). On the right is shown the different distribution of the GAA SSR among the A and B genomes of durum wheat. It allowed for the ready separation of the two whole chromosome set and of a number of single chromosome type (colored regions in figure) at high purity.

Different kind of repetitive sequences, other than oligonucleotides, were also used as FISHIS probes, i.e., the ribosomal DNA sequence from *T. aestivum* named pTa71. Such probe is known to label the 1B and the 6B chromosome of *T. aestivum* and allowed the flow sorting of both chromosomes together.

In plants, the reduction of the genome into its chromosomal components represents an effective means of acquiring the full genome sequence of polyploidy large genome such as that of bread wheat (Safàr *et al.* 2004). So far, the standard wheat DNA flow karyotype has delivered the purification of only a single entire chromosome, namely 3B, while the only way to isolate its whole chromosome complement is based on the exploitation of ditelosomic stocks in which one chromosome pair is replaced by its corresponding arms. A major drawback in this reliance on aneuploid stocks is that they have not been, and are unlikely ever to be developed for all but a small number of higher plant species. Moreover, such stocks are often developed using model varieties of low agronomic value. Here, we describe FISHIS a robust, rapid and low cost labeling method which combined with FC gives origin to the new" Flow Molecular Cytogenetic Approach" FMCA. Such approach allowed chromosome sorting to be independent from the use of aneuploid stocks, thereby potentially opening the access to the genome of all wild or cultivated species of interest. Besides, the sorted chromosomes can be used in a number of applications ranging from physical mapping to genomic studies using the NGS technologies, as recently reviewed by Dolezel *et al.* 2012.

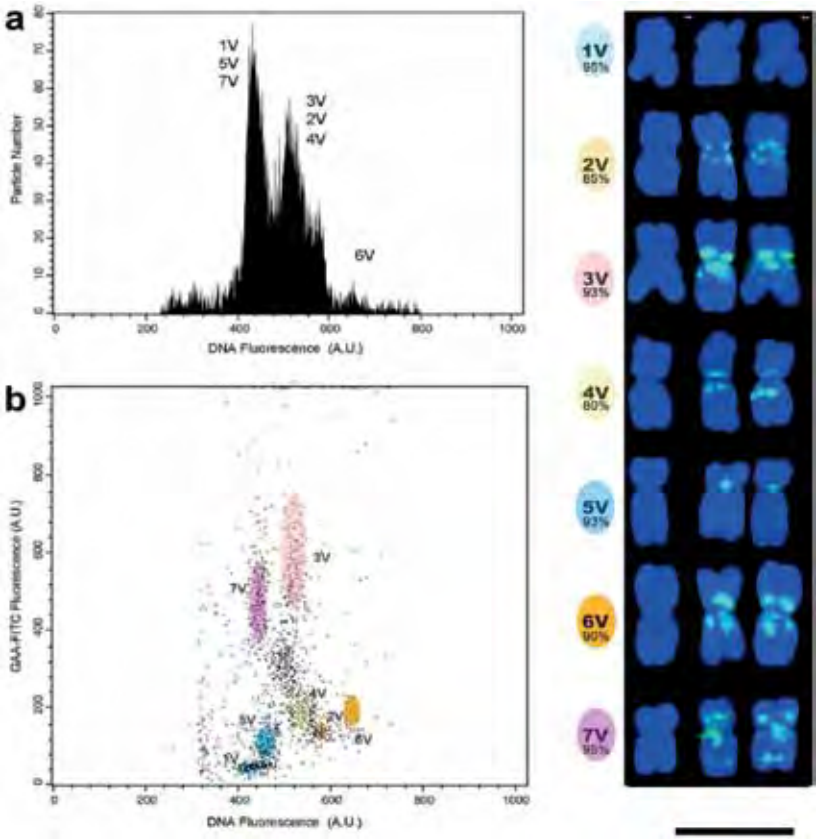

Figure 3. Flow karyotyping (a) and chromosome sorting of the whole complement of *Dasypyrum villosum* after (GAA)7-FITC labeling (b)). On the right: (GAA)7 distribution on *Dasypirum villosum* chromosomes (from Giorgi *et al.* 2013).

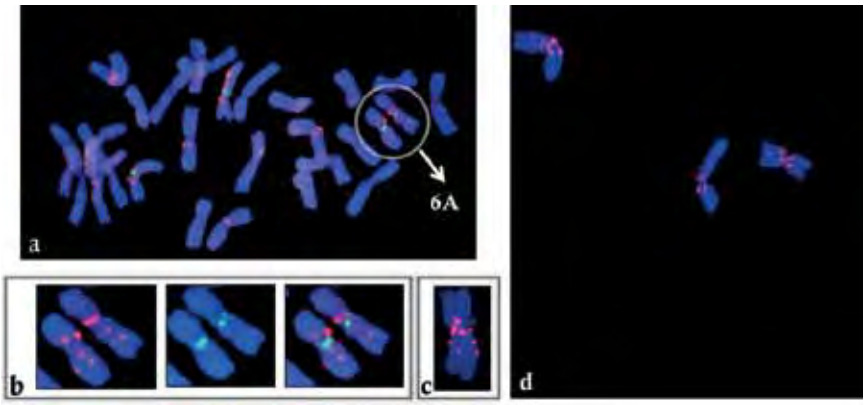

Figure 4. *T. monococcum* 6A chromosome sorting. a) Double target FISH with $(GAA)_7$-FITC (green) and $(ACC)_5$ Cy3 (red) SSR on T. monococcum chromosome spread. Chromosome 6A: single and double target hybridization with $(GAA)_7$ and $ACC)_5$SSR labeled with different (b) and with the same fluorochrome (c). Flow sorted chromosome 6A after $(GAA)_7$Cy3 and $(ACC)_5$ Cy3 FISHIS labeling (d).

IV – Conclusion

In conclusion FISHIS, by the innovative FMCA approach, extend the possible applications of FC to potentially all species of interest once a high quality chromosome suspension and the proper probes are available.

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Molecular analysis of a novel DNA transposon in *Triticeae*

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Abstract. A novel non-autonomous transposon element was identified in durum wheat and in various *Aegilops speltoides* accessions from the Fertile Crescent. It shows the standard transposon signals, such as a terminal inverted repeat (TIR-18bp), target site duplications (TSD-2bp-TC), many internal inverted repeats and a variable number of tandem repeats and does not code for a transposase enzyme. Interestingly, it is located inside the *Dehydration Responsive Factor 1* (*TdDRF1*) gene which codifies for transcription factors involved in the early response to drought by an alternative splicing mechanism. The transposon encompasses the gene sequence, from intron 1 to intron 3, including two translated regions, the exon 2 and the exon 3. Due to its peculiar position inside the CDS, a possible involvement in the molecular evolution of the gene was hypothesized. The transposon sequence and signals in all available relevant sequences from the same tr be, such as *Triticum durum, T. urartu, A. tauschii* were analysed and compared with the aim of drawing its phylogenetic story.

Keywords. Transposable Elements – DRF1 gene – Molecular evolution – Exonization.

Analyse moléculaire d'un nouveau transposon d'ADN chez les Triticées

Résumé. *Un nouvel élément transposon non autonome a été identifié chez le blé dur et chez diverses accessions d'Aegilops speltoides du Croissant Fertile. Il montre les signaux de transposons standards tels qu'une répétition terminale inversée (TIR-18BP), des duplications du site cible (TSD-2pb-TC), de nombreuses répétitions inversées internes et un nombre variable de répétitions en tandem et il ne code pas pour une enzyme transposase. Il est intéressant de noter que cet élément est situé à l'intérieur du gène Dehydration Responsive Factor 1 (TdDRF1) qui code pour les facteurs de transcription impliqués dans la réponse précoce à la sécheresse par un mécanisme d'épissage alternatif. Le transposon comprend la séquence du gène, de l'intron 1 à l'intron 3, incluant deux régions traduites, l'exon 2 et l'exon 3. En raison de sa position particulière à l'intérieur du CDS, une possible implication dans l'évolution moléculaire du gène a été avancée. La séquence du transposon et les signaux dans toutes les séquences pertinentes disponibles de la même tribu, comme Triticum durum, T. urartu, A. tauschii ont été analysés et comparés en vue de tracer son histoire phylogénétique.*

Mots-clés. *Eléments transposables – Gène DRF1 – Evolution moléculaire – Exonisation.*

I – Introduction

Transposable Elements (TEs) are genetic elements capable of transposing to different chromosomal locations and represent a large portion of the DNA in many species of animals and plants including agriculturally important crops such as corn and wheat (SanMiguel *et al*., 1996). Transposons are classified into two classes according to their mechanism of transposition: Class I – retrotransposons and Class II – DNA transposon. In particular, the DNA transposons are excised from one to another place with simple cut and paste mechanism. Based on the coding ability of transposase, the DNA transposons are categorized as autonomous and non-autonomous. Initially, it was supposed that TEs were simply 'junk' DNA, but subsequently it was demonstrated that they play an important role in evolution and speciation through mechanisms such as exonization and intronization (Fedoroff, 2000; Sorek, 2007; Sela *et al*., 2010; Chenais *et al*., 2012).

We identified a novel non-autonomous transposon element located inside the *Dehydration Responsive Factor 1* (*DRF1*) gene, a *DREB2*-related gene correlated to drought stress response in wheat. The gene consists of four exons and three introns and its expression is modulated by an alternative splicing mechanism (Latini *et al.,* 2007). Homologous genes sharing the same structure were isolated and analysed in wheat wild relatives, *Triticum urartu, Aegilops speltoides and Aegilops tauschii* and also in other *Poaceae* family members, mining sequences databases. The transposon inside *DRF1* gene was analysed for investigating its role in the gene evolution.

II – Material and methods

1. Transposon Mining

Genomic sequences of *DREB2*-related genes were accessed from GenBank (National Center for Biotechnology Information, NCBI; http:www.ncbi.nlm.nih.gov), Phytozome v9.1 (http://www.Phytozome.org) and TAIR (http://www.arabidopsis.org) databases, between January and June 2013. *AsDRF1* transposon sequence and *TdDRF1* gene sequence were used as BLAST search queries (version 2.2, http:blast.ncbi.nlm.nih.gov/Blast.cgi?).

2. Data analysis

Recovered sequences of DREB2-related genes were analysed by CLUSTAL W (Thompson *et al.,* 1994) and functionally aligned by BioEdit (http://www.mbio.ncsu.edu/bioedit/bioedit.html). Molecular evolutionary genetics analyses were carried out by MEGA 5 (Tamura *et al.,* 2011).

III – Results and discussion

1. The *DRF1* transposon and a hypothetical mechanism for transposon insertion

The analysis of the *DRF1* gene sequence in durum and its ancestors revealed some inverted repeats, target site duplications and the presence of many internal reverse and direct short tandem and long tandem repeats, all signals of transposable elements. In particular, it was observed a terminal inverted repeat (TIR-32bp) anchoring another internal 100% identity TIR-18bp, plus target site duplications (TSD-2bp-TC). Thus, a new transposon was identified and added to Repbase (Karthikeyan *et al.,* 2009). Because no sequence coding for a transposase enzyme was found, it represents a non-autonomous element. This transposon encompasses the *DRF1* gene sequence, from intron 1 to intron 3 and includes two translated regions, the exon 2 and the exon 3, suggesting a transposition event followed by exonization.

The overall structure of the transposon and the alignment of TIRs and TSDs in wheat and its ancestors are shown in Figure 1.

The transposon structure strongly supports that it could have played a vital role in the gene evolution. Actually, *DREB2* gene, firstly isolated in *Arabidopsis thaliana,* do not exhibit such a structure.

Based on the similarity between the 32bp and the 18bp TIRs, a double-step event for transposition can be hypothesized in this group of sequences. The 32bp TIRs suggest an older event of insertion of a non-autonomous transposon about 255bp in an ancestral gene sequence. It is possible to hypothesize that later, a transposase, carrying about 1190bp transposon containing 18bpTIRs and 4bp STRs, due to the high similarity of TIRs, inserted it in the target site. The final assembled gene structure is schematically shown in Figure 2.

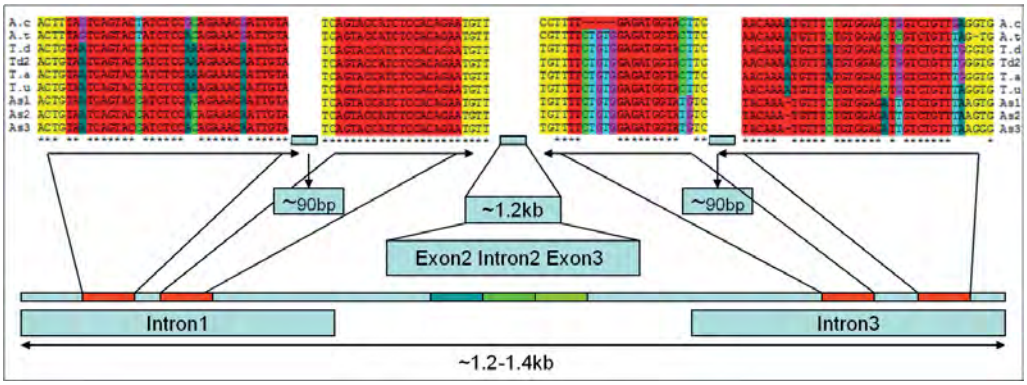

Figure 1. Structure and alignment of 18bp TIRs and 32bp TIRs in *DRF1* genes from different *Poaceae* members. TIRs are shown in red colour, TSDs are shown in yellow colour. A.c: acc. GU017675; A.t: acc. EU197052; T.d: acc. EU197052; Td2: acc. JN571425; T.a: sequence from Chr. AL at URGI (http://urgi.versailles.inra.fr); T.u: lab sequence acc. 57_7; As1: acc. FJ843102; As2: acc. FJ858188; As3: acc. FJ858187.

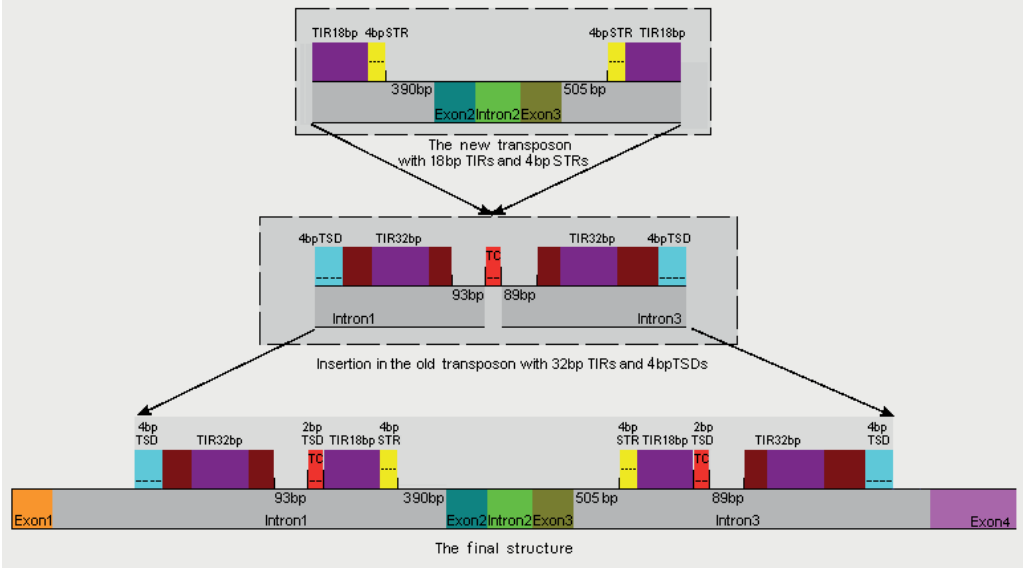

Figure 2. Hypothetical mechanism of the insertion of transposon inside *DRF1* gene sequence (the scheme is based on acc. FJ843102).

3. Analysis of *DRF1* transposon in orthologous sequences

Available databases were searched for retrieving sequences of orthologous DREB2-related genes. Just few genomic DNA sequences were available, being the great majority mRNA sequences. Sequences were functionally analysed and compared and it appears that only sequences from *Poaceae* family showed the same *DRF1* gene structure. In this subset, the sequences of transposon were isolated and aligned by ClustalW. A phylogenetic tree was built from the alignment (see Figure 3).

It is worth of noting that the obtained phylogenetic tree, able to clusterize correctly the species, exactly reflects the plant taxonomy.

Evolutionary analysis was carried out using 844 positions after eliminating gaps and missing data. The probability of transition resulted higher than transversion, thus suggesting a strong selection pressure to promote diversification.

Concerning TIRs, they appear to be well conserved in wheat and its wild relatives, being better conserved at 5', because, as known, the 3' TIRs are more susceptible to mutate. The core element, constituted by exon 2-intron 2-exon 3, is largely conserved in all analysed sequences, probably because it corresponds to the part of transposon which acquired a functional role in the gene. Concerning more distant species, just the 18bp TIRs can be recognized, thus the double transposition event cannot be hypothesized, and only the later insertion can be observed.

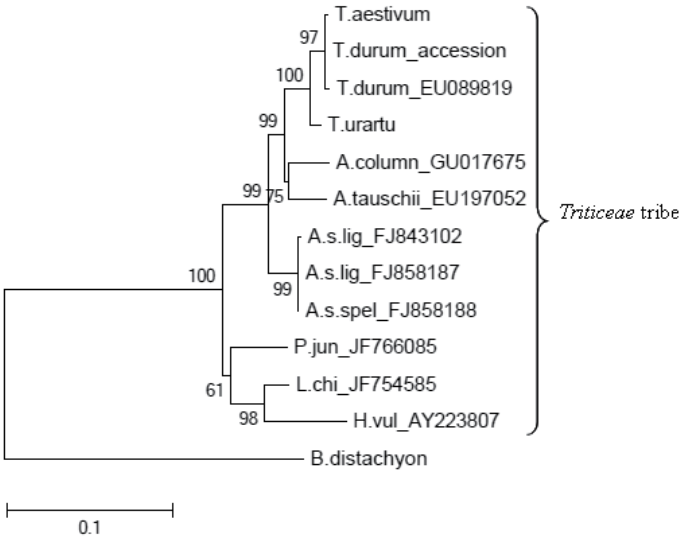

Figure 3. Phylogenetic tree from the alignment of the orthologous sequences of *DRF1* TE from T*riticeae* tribe and *B. distachyon* (Maximum likelihood method, Kimura 2 parameter model, 1000 bootstrap).

4. Looking for the ancestral gene sequence

Looking at the possible mechanism of transposon insertion, we speculated a double-step transposition inside an ancestral sequence. Thus, to mimics this ancestral sequence, we manually removed the whole transposon region encompassed between the 32 bp TIRs, including both external TSDs, and added two nucleotides, TC sequence.

This virtual ancestral sequence, built from *T. durum,* was used as template for a BLAST search in NCBI and PHYTOZOME databases. Interesting results were found in *Arabidopsis*. The *DREB2A* gene in *Arabidopsis thaliana* is located in Chromosome 5 (NCBI ID: AB016570 and consists of one intron and two exons and does not follow the GT-AG rule splice site. Furthermore, the final gene product consists of just the second exon translation. Beside the expected high homology between the AP2 domain of *Arabidopsis* and exon 4 of *DRF1* gene, an interesting similarity was found between 5'UTRs, 3'UTRs and Intron1 of *Arabidopsis* and the corresponding 5'UTRs, 3'UTRs and the virtual ancestral sequences (data not shown). The average score is about 55%

and the observed transition to transversion frequency is 2.44, suggesting that silent substitutions are predominant.

The overall results suggest that the virtual sequence of *DRF1* gene retains a relationship with intron 1 of *DREB2,* despite of the gene evolution. Thus, it is reasonable to assume that both them share a common ancestor and evolved separately after divergence between monocotyledons and eudicotyledons, in Magnoliophyta.

IV – Conclusions

The transposon located inside the *DRF1* gene was studied inside *Triticeae* tribe and in *Brachipodium distachyon*. Our results reflect the taxonomic relationships and accordingly cluster the sequences.

However, we were not able to find reliable relics of a possible ancestor of the gene in the current analysed sequences, even if *Arabidopsis* shares interesting features. More work is necessary to better understand the recovery traces of the past through the evolution.

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E3 ubiquitin ligases regulating plant stress responses: an overview

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Abstract. Post-translational modifications are emerging as a key regulatory component of many important cellular mechanisms. Among these ubiquitination, a multistep reaction, sequentially involving three enzymes named E1, E2 and E3, labels proteins with ubiquitin and led to 26S-mediated degradation. Protein ubiquitination plays a key role in a wide variety of cellular processes such as hormone signaling, DNA repair, biotic and abiotic stress response, cell cycle regulation to name few. In *Arabidopsis* more than one thousands of genes code for E3 ubiquitin ligase enzymes that specifically recognise target proteins. This suggests that a large amount of targets might be regulated by ubiquitination.

Keywords. Abiotic stress – Ubiquitination – E3 ligase – Protein protein interaction – Wheat.

Les E3 ubiquitines ligases régulant les réponses au stress de la plante : un aperçu

Résumé. *Les modifications post-traductionnelles sont en train de devenir un élément clé de régulation de nombreux mécanismes cellulaires importants. Parmi ces ubiquitinations, une réaction en plusieurs étapes, impliquant successivement trois enzymes nommées E1, E2 et E3, étiquette les protéines avec l'ubiquitine et conduit à la dégradation médiée par le 26S. L'ubiquitination de la protéine joue un rôle clé dans bon nombre de différents processus cellulaires tels que la signalisation hormonale, la réparation de l'ADN, la réponse au stress biotique et abiotique, la régulation du cycle cellulaire, pour n'en citer que quelques-uns. Chez Arabidopsis, plus d'un millier de gènes codent pour des enzymes E3 ubiquitines ligases qui reconnaissent spécifiquement les protéines cibles. Cela nous incite à avancer qu'une grande quantité de cibles pourrait être régulée par l'ubiquitination.*

Mots-clés. *Stress abiotique – Ubiquitination – Ligase E3 – Interaction protéine-protéine – Blé.*

I – Introduction

Abiotic and biotic stresses result in major constrains in growth and therefore productivity in crops. Understanding how plants respond to such stresses is of key importance to ensure and improve agricultural yield.

Post-translational modifications (PTMs) of proteins are deeply involved in the regulation of cellular processes in all organisms and also in adaptation to environmental changes including biotic and abiotic stress responses in plants. Ubiquitination, the attachment of ubiquitin to a protein substrate, has emerged as a key PTM involved in all aspects of plant physiology (Vierstra, 2009). Ubiquitin, discovered 30 years ago, is a 76 amino acid protein highly conserved in all eukaryotes, which is covalently attached to a target substrate. Ubiquitination was firstly described in the labeling of proteins for degradation through the 26S-proteasome (Hershko and Ciechanover, 1998). Protein ubiquitination is mediated by the sequential action of three enzymes, namely ubiquitin activating enzymes (E1), ubiquitin conjugating enzymes (E2) and E3 ubiquitin ligases (Vierstra 2009). Ubiquitinated proteins can undergo of several fates that include changes in their activity, re-localization within the cell or proteasome-mediated proteolysis. Ubiquitination in plants has been shown to regulate several processes including hormonal responses (Liu and Stone, 2011), light response (Roberts *et al*., 2011), control of the circadian rhythm (Cui *et al*., 2013), flowering

process (Pineiro and Jarillo, 2013), pathogen resistance (Trujillo *et al*., 2008), tolerance to abiotic stress (Guerra *et al*., 2012; Cho *et al*. 2008), sugar response (Huang *et al*., 2010), intracellular trafficking and vacuole biogenesis (Isono *et al*., 2010) among others. The functional diversity of the ubiquitin/26S proteasome system (UPS) pathway is reflected by the high number of proteins involved in the UPS occupying approximately 6% of the total proteins encoded by the Arabidopsis genome with about 1600 genes.

This review will present the current status on knowledge about the role of ubiquitination pathway in relation to plant stress response with special attention to the role of E3 ubiquitin ligases.

II – E3 ubiquitin ligases and stress response

As described above, the E3 enzymes are responsible of recruiting the target proteins, conferring specificity to the selection of the entire ubiquitination process. There are different types of E3 ubiquitin ligases, including monomeric ones such as RING, U-Box or HECT proteins, and multisubunit E3s such as cullin-RING ligases (CRLs).

RING E3s have been found to regulate specific molecular responses by targeting critical elements. In Arabidopsis the RING ubiquitin ligase DEHYDRATION RESPONSIVE ELEMENT BINDING PROTEIN2A (DREB2A)-Interacting Protein1 (DRIP1) acts as negative regulator of drought response mediating the ubiquitination and the degradation of DREB2A (Qin *et al*., 2008). Moreover the well characterized RING-finger protein high expression of osmotically responsive gene (HOS1) is induced during cold exposure to exert a negative control of the stress response through the ubiquitination of the key transcription factor Inducer of CRT/DRE-binding factor Expression1 (ICE1; Dong *et al*., 2006). SDIR1 a H2-type zinc finger-protein is a positive regulator of ABA signaling, acting upstream of the main transcriptional regulators of the ABA molecular response (Zhang *et al*., 2007). Indeed, the ectopic expression of SDIR1 gene greatly enhances ABA-induced stomatal closure resulting in increased drought tolerance. In cross-complementation experiments, the ABA-insensitive phenotype of the sdir1-1 mutant can be rescued by several transcription factor genes acting in the ABA pathway (ABI5, ABF3 and ABF4).

The Arabidopsis RING finger E3 ligase RHA2a is also a positive regulator of abscisic acid signaling during seed germination and seedling development. Moreover, RHA2a negatively regulates seed germination on salt medium (Bu *et al*., 2009). In recent work (Lee *et al*., 2009), another drought stress-induced RING finger protein, Rma1H1 for RING membrane-anchor 1 homolog 1, was isolated in *Capsicum annuum* and characterized through cross transformation in *A. thaliana*. Expression analysis on Rma1H1 showed a clear induction during drought stress with a major amount of transcript in leaf tissue. Rma1H1 is also induced by cold stress, mechanical wounding, high salinity and ethylene but not by ABA. Moreover Rma1H1 confer drought tolerance when overexpressed in Arabidopsis plants (Lee *et al*., 2009). In addition, there are several examples of E3 ligases with a pivotal role in the regulation of stress response, as well as in processes related to growth and development, thus ensuring the connections among different pathways. For instance, Delayed Seed Germination1 (OsDSG1) participates both in stress response and seed germination (Park *et al*., 2010). BTH-induced RING finger protein1 (OsBIRF1) is a rice (Oryza sativa) RING protein with pleiotropic effects on growth and defense response against multiple abiotic and biotic stresses (Liu *et al*., 2008).

Often E3 ligases represents a connecting point between different signaling pathways. The already mentioned RING ligase HOS1 is responsible for the 26S-mediated degradation of two transcription factors, ICE1, the master regulator of cold response, and CONSTANS, the central component of the flowering pathways (Dong *et al*., 2006; Lazaro *et al*., 2012). The RING-finger E3 ligase TdRF1 (*Triticum durum* RING-finger protein 1) represents an interesting example (Guerra *et al*., 2012). TdRF1 is induced upon exposure to low temperatures and dehydration.

TdRF1 was shown to be phosphorylated by the kinase TdWNK5 (With No Lysine [K]5) a member of the Arabidopsis WNK family of MAP kinases involved in flowering time and circadian clock regulation (Wang *et al*., 2008). Moreover TdRF1 interacts with another E3 ligase, WVIP2 (Wheat Viviparus1 Interacting Protein2) showing a strong up-regulation upon cold treatment and sharing high amino acid similarity with the wild oat VIP2 (Jones *et al*., 2000). Finally TdRF1 was shown to degrade *in vivo* the transcription factor WBLH1 (Wheat Bel1-Type Homeodo-main1), a previously described protein belonging to KNOX (Knotted1-like homeobox) gene family (Mizumoto *et al*., 2011). Finally the over-expression of TdRF1 increases tolerance of barley cells to dehydration, suggesting it as a positive regulator of plant response to drought and freezing conditions. In a recent work AtPUB22 U-box ligase, an Arabidopsis E3, was shown to negatively regulate immunity response and drought stress in *A. thaliana* (Trujillo *et al*., 2008, Cho *et al*., 2008). Indeed AtPUB22 shows a clear induction in response to several abiotic stresses such as cold, drought and salinity stresses but not ABA treatment. Finally PUB22 was demonstrated to interact and ubiquitinate RPN12a, a subunit of the 19S regulatory particle (RP) in the 26S proteasome (Cho *et al*., 2008). In a recent work the group of Dr. Trujillo discovered another protein target of AtPUB22, the exocytic machinery component Exo70B2 (Stegmann *et al*., 2012). The authors demonstrated that AtPUB22 undergoes proteasomal degradation by autocatalytic activity and by contrast it is stabilized upon pathogen elicitors treatment (Stegmann *et al*., 2012). Finally the authors propose the following action mechanism: in standard conditions AtPUB22 regulates itself by autoubiquitination, flg22 perception by FLS2 stabilize AtPUB22 allowing it to interact and ubiquitinate Exo70B2 finally leading to an attenuation of PAMP-induced signaling. Interestingly PUB22 was shown to coordinately co-operate with other PUBs in both abiotic and biotic stress responses indicating a certain degree of redundancy.

III – Conclusions

The emerging picture indicates the E3 enzyme as the hub point connecting regulatory proteins of different cellular processes, e.g., the response to abiotic and biotic stress and various aspects of plant development. Different E3 ligases can play a role in the same process by targeting the same substrates or they can regulate each other's besides other targets. All these scenarios create cross-talking between different signaling pathways or cellular processes. Otherwise a single E3 can regulate several aspects of plant life cicle simply by mediating degradation of different target proteins in a different temporal window. Thus plants can exploit ubiquitination to coordinate the functioning of different processes according to environmental and cellular conditions.

Finally an E3 is able to regulate all these processes mediating degradation of other proteins, thus identification of E3 targets is a crucial step to completely unravel ubiquitination role in regulating plant life cycle.

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Closing session

Closing remarks

Enrico Porceddu

Accademia delle Scienze, detta dei XL

Ladies and gentlemen, fellow scientists, colleagues past and present.

As convenor of the International Symposium on Genetics and Breeding of Durum Wheat, it is now my great honour to make some closing remarks based upon the last four days of most interesting and high level of research presented here in Rome at the Symposium dedicated to the late President of the Academy of Sciences of Italy, Professor Gian Tommaso Scarascia-Mugnozza.

The Symposium has come to an end, but what a great congregation of scientists, researchers and students it has been! In all, 52 oral papers and 130 posters were presented from around the world and a wide variety of institutions. It has been my great pleasure to have seen young scientists make some extremely good and informative presentations, as well as highly informative presentations from veterans of durum wheat research. It has truly been a great tribute to the memory of the late Professor G.T. Scarascia-Mugnozza.

To summarize briefly, let me start by recalling that the meeting was opened by the incumbent President of the Academy of Sciences, followed by representatives of the co-organising institutions, and other international organisations. The short presentation on the Symposium structure was followed by seven sessions, which covered almost all the aspects of durum improvement and research.

Session I on the Origin and Evolution of Durum Wheat set the tone for the meeting. The 12,000 year-old history of durum cultivation was presented and discussed. The great amount of diversity in durum germplasm both in cultivated landraces and wild progenitors was presented. The fact that some of the diversity has been lost and is still being lost due to several reasons was discussed. The meeting also noted the effects that the great upheaval in parts of the West Asia and North Africa region are having on breeding efforts, research, as well as potential loss of genetic resources of durum wheat.

Session II covered the Genetic Resources and Durum Wheat Germplasm Enhancement. There were only three or four persons present who participated in the first Symposium held at the University of Bari in 1973. After some recollection of that meeting, speakers discussed the situation of durum wheat landraces, obsolete wheat, and wild relative accessions in the world's gene-banks. It was noted that almost all the collections were duplicated and hence were safe, including the 'black box' collections deposited in the Svalbard seed vault. Effects of climate change and its impact on genetic resources was also noted. The question as to whether collecting activities should continue produced a general consensus that most of the landraces have been pretty much collected, and that also they did not exist anymore in many parts of the world having been replaced by modern varieties. Additional efforts should be devoted in protecting the remaining stands of wild progenitors and members of the secondary gene-pool, allowing them to continue to evolve especially under increasing global warming. As temperature rises, germplasm from the Irano-Afghanistan area was noted for its heat and salinity tolerance, a very valuable resource in the coming years. Good examples of success in transferring useful genes from wild species to cultivated material were provided, along with the difficulties in making these transfers. The need to augment this line of research was emphasized.

Session III was devoted to the Strategies and Tools in Durum Wheat Genetics and Breeding. Several researchers gave their ideas and presented their work on breeding strategies to overcome biotic and abiotic stresses operating on durum wheat. It was noted that durum, being a tetraploid, has a lower chromosome number and hence is relatively less hardy when it came to tolerating stresses than bread wheat. The use of the tertiary gene-pool, including wild species, such as *Dasypyrum villosum* was also presented. An important point was made - that breeders should look at the holistic picture when releasing varieties and not just yield. Local preferences, traditions, and tastes must also be taken in to consideration.

This led very nicely into the IVth session on the Genetics and Breeding for Durum Wheat Yield and Sustainability. Papers included adaptation and sustainability using various tools for evaluation of a myriad of characters. The question of whether durum wheat is well-equipped to handle the coming climate change which may result in higher temperatures and lower availability of moisture was also thoroughly discussed.

Session V dealt with the Genetics and Breeding for Durum Wheat Diseases and Pest Resistance. Various diseases and pests were analysed along with their influence on yield and grain quality of the harvested grain. Strategies for reducing the incidence of some diseases were mentioned, for instance the problem of the stem borer could be reduced by selection of solid-stem lines, originating from specific areas of the Mediterranean countries. A point was made that the disease "Tan Spot" had not been adequately covered in the session.

The Session VI dealt with the Genetics and Breeding for Nutritional and Technological Quality, in which past achievements and various molecular methods to identify genes for good quality were discussed. However, consumers' preferences may vary according to the various end products obtained from durum wheat, and thus products must be fine-tuned and targeted according to specific areas and demands of the consumers. Differences in preferences in nutritional quality and colour remain between industrialised and developing countries. A comparison between quality aspects of modern varieties and traditional landraces was made, and the point that yield improvement must not be achieved at the expense of quality was raised.

And finally the last Session VII covered the Perspectives in Structural and Functional Genomics. The session underlined the progress made in different species and the potential use of wild emmer genetic resources in durum wheat improvement. The possibility of sorting different chromosomes and the utilization of various genes that occur on them was also deliberated upon. A strong case for the potential of using wild emmer for improving quality, stress tolerance, etc. was made. Laboratories around the world already working on this line of research were mentioned.

A number of points emerging from presentations and discussions at the end of each session were offered to the participants of the meeting. Comments and points arose during this general, final discussion, and permitted me to rephrase them collectively as follows:

1. Usefulness of landraces and wild species, as source of genes for adaptability, and disease and pest resistance geared to sustainability was recognised along with difficulties and time required in their utilisation in practical breeding programmes. The need to set up pre-breeding programmes, which exploit advanced technologies in incorporation more than one gene at the same time, thus speeding up research programmes, was underlined.

2. The need to listen to the end-users of the new varieties, the farmers, and their requirements for material adapted to their environment and particularly to diverse soils and climate vagaries was stressed. The need for a global approach in plant breeding, where different factors receive proper attention emerged as a definite priority.

3. The need to take into account the views of consumers of end products and their preferences was also mentioned, since the processed product may be different in

various places, and thus may require grain with different technological and processing characteristics. Also, consumer preferences in industrialised countries may be different from those in the developing world as far as nutritional aspects and other quality parameters are concerned.

4. Concerted action between countries and among institutions working on durum wheat in the same country is urgently needed, since local problems can very rapidly become regional or global, as shown by the Ug99 epidemic. The meeting noted that cooperation among institutions, which used to be strong competitors in the past, is now increasing and progress is being made that promises good results. Advancement in science will help in achieving rapid progress, but the use of modern tools require expertise and support that may not be available at individual institutions and/or single countries. Even wider and stronger cooperation should be encouraged, also facilitating the set-up of international and regional networks.

5. Monitoring progress in durum improvement is absolutely necessary and scientists from different countries offered to organize durum wheat symposia at five years intervals, possibly in between two International Wheat Genetics Symposia, with the next meeting taking place in three years' time. The possibility of setting up a small International Organising Committee for these symposia will be explored.

Before closing, I would like to express my grateful thanks to the members of the International Scientific Committee, and the Local Organizing Committees, the co-organisers, ICARDA CIMMYT, FAO, CNR, ENEA and CIHEAM, the main sponsors, Syngenta, Barilla, SIS, Divella, Perten, Rummo, Wintersteiger, and the supporting institutions, CRA, SIGA and Zetema. We need to thank them for without their unwavering support this Symposium could not have been held. I would also like to thank the session chairs, speakers, discussants, and all the participants, who gathered here from far corners of the world and from a wide variety of backgrounds to make this Symposium a great success.

The Symposium Secretariat, who has been given certain extra work during the last months, and the CNR staff responsible for security in these excellent facilities deserve our special commendations and thanks for their tireless work.

Let us resolve to meet once again in a three years-time to contemplate the progress made in durum wheat research and production, and all the other topics that are linked to these issues.

Thank you very much to you all!

Now, I have the privilege of declaring this International Symposium on Genetics and Breeding of Durum Wheat closed!

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