Assessment in Syria of apple rootstocks resistance to woolly apple aphid (*Eriosoma lanigerum Hausm*)

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Activity background and context

Apple rootstocks are different in their susceptibility to woolly apple aphid (WAA) *Eriosoma lanigerum* (Homoptera: Aphididae), WAA is an invasive pest that cause severe damage, it can feed on both roots and vegetative parts of apple trees, however root infestation can cause the death of the tree in extreme cases, that the root infestation cannot be chemically controlled (Klimstra and Rock, 1985). Researchers found the efficient solution to this case is through using rootstocks which have the genetic resistance to this pest, that they are usually considered as field immune to the pest and they are used to prevent infestation of the belowground parts (Bus *et al*., 2008). There are three defined apple resources which have the resistance genes to WAA "Northern spy" cultivar is one of these resistant resources, it has the gene *Er1*, it was used in the past as an apple rootstock, then introduced into apple rootstock breeding program as a parent in East Malling institute in cooperating with John Innes institute.

While the rootstock "Robusta 5" which belongs to *M.* x *robusta* species, was identified as a resource of the gene *Er2* (King *et al.,* 1991; Alston *et al.,* 2000). Likewise, the gene *Er3* which also responsible to the resistance to WAA was identified in "Aotea 1" from *M. sieboldii* species (Bus *et al.,* 2008). These available resources are very important in apple breeding program, that it can be used as parents to produce resistance rootstocks. However these three genes are different in their resistant mechanism, which led to the strategy of pyramid the resistant genes to insure better resistant to WAA (Sandanayaka *et al.,* 2003). This can be achieved through the integration between field evaluation for WAA resistant seedlings, and linked resistant genes using molecular markers which is defined as marker assisted selection (MAS), to speed up apple breeding program for WAA resistant genes. However, the locus of *Er1, Er2* and *Er3* were determined on apple genetic map, that the *Er1*and *Er2* are the essential genes used in the selection of apple rootstocks resistant to WAA (Bus *et al.,* 2008).

Our research aimed to evaluate and identify some of apple rootstock genotypes have the genetic resistance to WAA for rootstock breeding program in Syria depending on phenotypic and genetic evaluation.

Methodological approach

The present investigation was carried out at the agricultural scientific research center –GCSAR- in Sweida province, which located at 1525m altitude in the south of Syria.

Plant material

One year old seedlings from a hybridization between the apple rootstock MM106 (semi vigor rootstock, has the resistance gene *Er1* to WAA from its parent "Northern Spy") and the local apple cultivar Sk(Skarji) which has many desirable agronomic traits and tolerant to environment stress. Thus by this hybridization we aimed to get new rootstocks have the desirable traits from the two parents particularly resistance to WAA.

Phenotypic for resistance to WAA

Seedlings were planted in lines, the planting distance was 25 cm between plants and 70 cm between lines, all the agricultural processes (irrigation, fertilization and weeding)were achieved, the infestation was done in late June 2010 by placing shoot pieces with heavily infested WAA colonies in each seedling, the infestation was repeated twice in interval two weeks, the seedlings were not subjected to chemical control all the season.

WAA infestation was assessed 4 months after inoculating at the first season, and at the end of second season using 6- point scales according to (Bus *et al.,* 2008):

- 0: No infestation
- 1: Light infestation consisting of several small, separate colonies
- 2: Medium infestation and galling with some colonies starting to coalesce
- 3: Many colonies coalescing and up to 2 shoots completely infested and galled
- 4: Heavy infestation and galling on 2-5 shoots
- 5: Heavy infestation and galling on more than 5 shoots

The percentage of infested seedlings in each scale within each genotype was calculated. For genetic evaluation seedlings classified as 0 or 1 to be resistant and those scoring 2-5 to be susceptible.

Genetic evaluation

DNA extraction

DNA extraction was achieved using CTAB protocol according to Porebski *et al.,* (1997), by collecting leaves from the resistant plants and some of susceptible ones in addition to the parents.

PCR amplification

PCR amplification was achieved using 8 markers (table 1) linked to the resistant genes for woolly apple aphid (*Er1, Er2* and *Er3*), which developed according to linkage map (Bus *et al*., 2008).

The reaction was performed with volume (10 µl) consisted of: 1 µl 10 X buffer + 1 µl dNTPs + 1 µl forward primer + 1 µl reverse primer + 3 µl DNA + 0.1 µl Taq + 2.9 µl dH_2O. The cycling profile for the markers NZsc_G327, NZsc_O05, NZsc_E01 and NZsc_A01 consisted of an initial denaturation step of 3 min at 94 c, followed by 35 cycles of 30 s at 94C, 30 s at 55 C and 1min at 72C, the amplification process was finished with 5 min at 72C. For the markers NZms_EB145764, NZms_EB106753, NZsn_O05 and NZsc_C20 were used touchdown PCR consisted of an initial denaturation step of 5 min at 94 c, followed by 10 cycles of 30 s at 94C, 30 s at 70 C and 45 s at 72C, the temperature was reduced 1C every cycle, followed by 20 cycles of 30 s at 94C, 30 s at 60 C and 45 s at 72 C, the amplification process was finished with 10 min at 72 C.

Table 1
Shows the markers linked to the resistant genes to woolly apple aphid, the sequence of forward and reverse primers, and the product size (bp)

Marker name	Marker type	Original RAPD/EST	WAA gene	Forward primer	Reverse primer	Product size (bp)
NZsc_C20	SCAR	OPC20	*Er1*	TCTCTAACT CAATAACTC CCAAGAC	ACTTCGCC ACCATTAT CACTCCTG A	2,000
NZsc_GS3 27	SCAR	GS327	*Er1*	GCCAAGCT TCAATGTC GGAGTAGA T	CAAGCTTC CCCTAAGG CTATTGCC A	1,600
NZsc_O05	SCAR	OPO05	*Er1*	CCCAGTCA CTAACATAA TTGGCACA	CCCAGTCA CTGGCAAG AGAAATTA C	1,700
NZsn_O05	SNP	OPO05	*Er1* *Er3*	AACGTCAT GTCAATAT	CCCAGTCA CTGGCAAG AGAAATTA C	880
NZsc_E01	SCAR	OPE01	*Er3*	CCCAAGGT CCGAACAC AAATGAGA G	CCCAAGGT CCAAAACT ATCCCGAA G	1,350
NZSc_A01	SCAR	OPA01	*Er3*	CAGGCCCT TCAGCAAA GAGGTGTC T	CAGGCCCT TCACTACT AATAAGAA C	1,250
NZms_EB1 06753	SSR	EB106753	*Er1* *Er3*	TCTGAGGC TCCCAAGT CC	TAGGAGCA GAAGAGGT GACG	175
NZms_EB1 45764	SSR	EB145764	*Er2*	TTCCAGCG ATCCAAAAC AAT	GCTCAGGA ACACCTCG TTCT	198

Visualization of the PCR products

The PCR products were detected by electrophoresis on 1% agarose gel in 1X TBE buffer, stained with ethidium bromide and visualized by UV light and photographed using gel doc. NZms_EB106753 and NZms_EB145764 markers detected by running PCR products on a 8% polyacrylamide gel in 1X TBE buffer.

Results and discussion

Breeders of apples rootstocks have focused on extending the range of rootstock attributes and the benefits they confer to scions propagated upon them. woolly apple aphid (*Eriosoma lanigerum*) is the considerable pest in all apple rootstock breeding programs to develop apple rootstocks which offer resistance to this pest depending on phenotypic and genetic evaluation.

Phenotypic evaluation

The results of seedlings infestation with WAA showed differences between the two seasons of assessment. At the first season all seedlings were presented in scale 0 and 1 (100% resistant). At the second season the percentage of resistant and susceptible seedlings were changed that the percentage of resistant seedlings became (40.6 %), and the susceptible seedlings grouped in the scale 3. These results were in agreement with Fazio and Beers (2010), that the resistant rootstocks did not change, while the infestation increased within the susceptible ones in the second season. The resistant seedlings due to the main role of the rootstock MM106 as a parent takes its resistance property from Northern spy cultivar which has the resistant gene *Er*1 for WAA (Webster *et al.*, 2000).

Table 2
The percentage of infested seedlings for each scale among studied seedlings during the two seasons

Season of assessment	Percentage of infestation %					
	0	1	2	3	4	5
2010	81.2	18.8	0	0	0	0
2011	28.1	12.5	0	59.4	0	0

Genetic evaluation

At the end of the second season the susceptible seedlings were excluded from the apple rootstock breeding program and the resistant seedlings were genetically evaluated to insure the presence of considered resistant genes for WAA. The results showed that the marker NZsn_O05 linked to *Er*1 and *Er*3 genes was the most efficient marker, it gave allele has the predictable size 880 bp according to Bus *et al*., (2008), in 6 seedlings (4 in the scale 0 and 2 in the scale 1), in addition to the rootstock MM106 (Figure 1) which used as control for the gene *Er*1.However, this marker could not distinguish all the resistance seedlings which lead to advance genetic researches and use new techniques to detect WAA resistant genes, this result was in agreement with Bus *et al.,* (2008),they found that this marker discriminated 70 plants from 77 ones showed the resistance property.

The marker NZSc_E01 linked to the gene *Er*3 gave three polymorphic alleles, one of them was 1350 bp as the same of predictable size by Bus *et al.,* (2008) which was noticeable in the most studied seedlings (resistant and susceptible ones), while the remaining seedlings have two other alleles size 700 bp was existed in 3 resistant seedlings while the 500 bp was found in two seedlings (one resistant and the other susceptible).

The markers NZsc_C20, NZsc_O05 and NZsc_GS327 linked to the resistant gene *Er*1 did not give any PCR products. On the other hand, the markers NZms_EB145764 (linked to the gene *Er*2), NZms_EB106753 (linked to the genes *Er*1 and *Er*3) and NZSc_A01 (linked to the gene *Er*3) gave same alleles in both resistant and susceptible seedlings (monomorphic alleles) so they were not able to distinguish between resistant and susceptible seedlings. Although, these markers gave the same expected size as mentioned by Bus *et al.,* (2008) except NZSc_A01. This is possibly that these markers were may not tightly linked to the resistant genes.

Concluding remarks

The studied markers could not discriminate between all resistant seedlings and susceptible ones except the marker NZsn_O05. Therefore, it is necessary to develop new linked markers to WAA resistant genes depending on studied plant material, through using available techniques such SNPs and SSR. In addition, breeding programs should depend on the strategy of pyramiding the resistant genes to give durable resistance to WAA.

Bibliography / More information

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