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Effect of different rates and application times of nitrogen fertilizers in a rice cropping system

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Abstract

The use of agrochemicals in European ricefields leads to a progressive pollution of surrounding waters and lost of soil quality due to their negative effects on soil micro - organisms, particularly on N_2-fixing cyanobacteria. The necessity of preserving natural resources makes essential to improve rice cropping system in terms of stability, sustainability and limited emissions to the environment. Therefore, the improvement of soil utilization by preserving and developing their natural ability of biofertilization due to the presence of N_2- fixing organisms, the reduction of the use of N fertilizers and the improvement of N-efficiency are of some importance.

In this way :

- (i) the effect of fertilization rates (0-150 kg/ha) and application times (basal or split) of N fertilizers and inoculation with N_2-fixing cyanobacteria on biological nitrogen fixation, rice crop performance and N utilization, and
- (ii) the N status of the rice plant at different stages of the growing season to establish a critical N leaf level for near-maximum grain yield was studied under controlled experimental conditions in Valencia, Spain, during six crop seasons.

The results showed that the paddy field ecosystem is favourable for growth of N_2-fixing cyanobacteria. Nitrogen fixation varied with time during the cultivation cycle and showed a negative correlation with the amount of N fertilizers used. Grain yield increased with increasing amounts of N fertilizers up to 70 kg N/ha, on soil with 4.8% organic matter content. At this reduced basal dressing of N, the apparent N-recovery was 0.32 kg N taken up per kg N applied and agronomic efficiency was 20.8 kg grain dry mass per kg N applied. On soil with 2.57% organic matter content, grain yield increased with increasing amounts of N fertilizers up to 100 - 125 kg N/ha. At this higher basal dressing of N, values of 0.26 and 16.3 $kg.kg^{-1}$ were obtained for the apparent N-recovery and agronomic efficiency, respectively. Critical values for leaf N content have been determined at mid-tillering, end-tillering and at panicle initiation. These values have been related to SPAD chlorophyll meter's readings.

The cyanobacterial inoculation had no significant effect on rice yield and did not quantitatively affect the occurrence of N_2-fixing cyanobacteria but produced a change of its qualitative composition.

Keywords

- Cyanobacteria, nitrogenase activity, cyanobacteria inoculation, basal and split N application, N-recovery, N-efficiency, N-status of the rice plant, .
- Spain

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Introduction

Direct seeding under irrigation is the main intensive form of rice cultivation in the world. However, intensive culture may involve environmental problems when nutrients added are lost from agricultural fields. In European ricefields, the high input of N fertilizers may cause water pollution and a progressive loss of soil quality due to their harmful effect on soil micro-organisms, particularly on N_2-fixing cyanobacteria (Roger & Kulasooriya 1980, Fernández Valiente et al 1997).

Most of the rice-growing area in Valencia, Spain, is located inside the Albufera Natural Park, with a consequent increasing awareness towards environmental quality. Rice farmers in Valencia traditionally incorporate high levels of chemical N fertilizers which may contaminate the groundwater (Ballesteros et al 1988) and be responsible for the eutrophication of the drainage channels (Forés 1992) which discharge into the Albufera lagoon, an hypereutrophic lake (García et al 1984), and subsequently into the Mediterranean.

The necessity of preserving natural resources makes essential to improve rice cropping system in terms of stability, sustainability and limited emissions to the environment. Moreover, the Common Agricultural Policy by European Union aims at supporting farming practices that reduce pollution. Therefore, the improvement of soil utilization, by preserving and developing their natural ability of biofertilization due to the presence of N_2-fixing organisms, the reduction of the use of N fertilizers and the improvement of N - efficiency are of some importance.

Ecological studies have indicated that the physicochemical characteristics of the paddy field ecosystem in Valencia provide favourable conditions for cyanobacterial growth (Quesada et al 1995 a, b). In fact, both their presence and ability to fix dinitrogen have been shown in the ricefields (Quesada & Fernández Valiente 1996). The effects of N fertilizers on N_2 - fixing cyanobacteria and biological N fixation are not well understood. N fertilizers have been shown to decrease cyanobacterial growth (Roger & Kulasooriya 1980). On the other hand, one report has demonstrated a beneficial effect of N fertilizers on soil algae (Mahapatra et al 1971). Since De (1939) attributed the natural fertility of the tropical paddy fields to these organisms, many trials have been conducted to increase rice yield and to maximize nitrogen fixation by cyanobacterial inoculation (algalization) of the soils. The effect of inoculation is erratic and unpredictable (Roger 1991). Nevertheless, this practice has been reported to have a beneficial effect on grain yield in China, Egypt, India, Japan, Russia and Philippines. There are also reports indicating failure of algalization (Roger & Kulasooriya 1980). However, published studies dealing with algalization in Spain are lacking.

In order to minimize N application, N management practices, based on N status of plant samples collected in different stages of the rice crop, should be favoured. Moreover, the efficiency of N fertilizer, under various environmental conditions, should be determined to optimize the use of N fertilizer for crop production. In this way, there are no reports comparing the relative N utilization of different cropping conditions in the rice area of Valencia.

In this paper we present the results of an agronomic study conducted in Valencia (Spain), under controlled experimental conditions, to investigate :
(i) the effect of different rates (0-150 kg/ha) and application times (basal or split) of N fertilizers and algal inoculation on soil cyanobacteria, rice crop performance and N utilization, and
(ii) the N status of a rice crop at different stages of a growing season, to establish a critical N leaf level for near-maximum grain yield.

Material and methods

Experiment I

This experiment was conducted during three consecutive crop seasons (1990-1992) to investigate the effect of different N rates on soil cyanobacteria, nitrogenase activity and N utilization (dry matter yield, N uptake, apparent N recovery and N use efficiency).

Soil and irrigation water

The soil of experimental fields have a silty clay texture, with an organic matter content 4.8%, available phosphorus content 37 mg/kg (Olsen 1954) and available potash 283 mg/kg by extraction with CH_3-COO NH_4.

Rice was grown in flooded soil. The irrigation water had an average NO_3^--N and NH_4^+-N content of 0.1 and 0.2 mg/l respectively and an average electrical conductivity of 1230µS/cm.

Experimental design

Rice (Oryza sativa) was given N at rates of 0, 17.5, 35, 70 and 140 kg/ha. Experimental plots of 100 m^2 (5 x 20 m) were laid out in randomized complete block design with four replications. Plots and treatments remained the same throughout the three crop seasons.

Observed data

The N_2-fixing cyanobacteria population in the top 0.5 cm of the soil was estimated at 4-5 weekly intervals throughout the growth cycle. A total of 7 sampling periods were done during two seasons. Soil samples for algal counts were collected with a cylinder 28 mm in diameter and 50 cm long, which was inserted into the soil with a rubber hammer, avoiding undue disturbance. Afterwards the core was extracted, water was removed with a syringe and soil was pushed up with a Teflon pestle, gathering the top 0.5 cm of the cored soil. Cores of the same plot were mixed and reduced to a unique plot sample, put in plastic bags and kept at 4ºC until processing : plating soil suspension dilutions onto agarized N-free medium (Mateo et al. 1986). The plates, 4 dilutions for each plot sample, were incubated for 4 weeks at 30±2ºC with continuous illumination (25 µE/m^2.s).The mean values of the 4 plates were considered for the statistical analysis.

Nitrogenase activity was studied, to assess N_2 fixation. The in situ acetylene reducing activity (ARA) method, as described by Quesada et al. (1989) was used. Assays were performed in 4 sampling periods, May, June, July and September, for 3 consecutive years. At each sampling period 3 assay chambers were placed in each plot, and the mean values of the 3 chambers were considered for the statistical analysis.

At maturity, 10 stalks from each plot were hand-harvested for both grain and stalk N determinations. Total N was determined using an elemental analyzer (Perking Elmer 2400 CHN) with a thermal conductivity detector.

At harvest, three 0.25 m^2 sample areas/plot were hand-harvested and harvest index was derived from the grain/straw ratio of the three sample areas. Individual whole plots were combine-harvested and grain yields were recorded. Moisture content was determined in order to express the yield at 14% moisture content. Total N uptake (kg/ha) was calculated as total dry matter yield x N concentrations. Apparent N recovery (%) was calculated by the difference method [(kg N at N_x - kg N at N_0)/ applied N at N_x) x 100]. Nitrogen use efficiency (kg dry matter produced per kg N applied) was calculated as : (yield at Nx - yield at N_0)/ applied N at N_x.

Experiment II

This experiment was conducted during 1994 to investigate the effect of split N application on biological nitrogen fixation. The experimental fields, irrigation water and nitrogenase activity determination method were the same as in Expt. I.

Experimental design

The field experiment layout was a randomized complete block design with four replications. Plot sizes were 8 by 10 m. Treatments consisted of one total N rate (75 kg N/ha) applied under four application schedules :

(1) complete basal application before flooding;
(2) split application, two-thirds of the N basally at sowing before flooding and the remaining one-third as topdressing at mid-tillering stage (MT) (45 days after sowing);
(3) split application, as 2) but at sowing and panicle differentiation stage (PD) (65 days after sowing);
(4) total N rate was topdressed, two-thirds at MT and the remaining one-third at PD.

Experiment III

This experiment was conducted during three crop seasons (1991-1993) to investigate the effect of inoculation with N_2-fixing cyanobacteria on soil cyanobacteria, nitrogenase activity and N utilization. The experimental fields, irrigation water and soil samples collection, nitrogenase activity and grain and stalk N determinations methodologies were the same as those in Expt.I. Cyanobacterial flora was evaluated by epifluorescence microscopy (González 1996).

Experimental design

The field experiment layout was a randomized complete block design with three replicated plots (3 x 2 m) per treatment. Treatments were 1) 0 kg/ha N, 2) 0 kg/ha N in presence of algal inoculum, 3) 35 kg/ha N, 4) 35 kg/ha N in presence of algal inoculum, and 5) 140 kg/ha N.

After 2 harrowings and flooding, Senia rice was transplanted with 28 days old seedlings at four plants/hill and hill spacing of 15 by 15 cm. Inoculation was made at one month after transplanting using dry inocula. The dry inoculum was a mixture of Nostoc punctiformi (Strain 205 from the collection of Universidad Autónoma de Madrid), Anabaena variabilis (Strain UAM 203), Calothrix marchica (UAM 214) and Gloeotrichia sp., previously isolated from rice fields of Valencia and grown in liquid culture under controlled conditions. The inoculum, previously dried and mixed with soil as support material, was broadcasted over the soil in the drained field at 1.4 kg/ha in 1991, 10.5 kg/ha in 1992 and 18.3 kg/ha in 1993. During the two last years Calothrix marchica was the dominant genus in the mixture.

Agronomic practices

Plots were kept flooded throughout the experimental period except during the drainage period, 15 days after transplanting, to control water weeds. Before reflooding, the plots were inoculated. Insecticide triclorfon (Dipterex) was applied at 1.8 kg ai/ha after inoculating. At maturity (end of September), individual whole plots were hand-harvested. Five stalks were separated from each plot for N determinations. Grain and straw yields were recorded for the whole plot area. Moisture content was determined in order to express the yield at 14% moisture content.

Experiment IV

This experiment was conducted in a different experimental field during two consecutive crop seasons (1995-1996) to investigate :

(i) the effect of different rates and times (basal or split) of nitrogen fertilizers on N utilization (dry matter yield, N uptake, apparent N recovery and N use efficiency) and,
(ii) the N status of the rice plant at different stages of the growing season to establish a critical N leaf level for near-maximum yield.

Soil and irrigation water

The soil of experimental fields have a loamy clay texture, with an organic matter content 2.57%, available phosphorus content 42 mg/kg (Olsen 1954) and available potash 250 mg/kg by extraction with CH_3-$COONH_4$.

Rice was grown in flooded soil. The irrigation water, from Júcar river, had an average NO_3--N and NH_4^+-N content of 0.16 and 0.28 mg/l respectively.

Experimental design

Split-plot design experiments were set out with four replications. Main plots were arranged in a randomized complete block and consisted of six basal N rates (0, 50, 75, 100, 125 and 150 kg N/ha. Subplots consisted of three N topdressing patterns : non topdressed, topdressed with 50 kg N/ha at MT growth stage (about 45-50 days after sowing), and topdressed with 50 kg N/ha at PD stage (70-75 days after sowing). Water flow was halted for 2 days following the N topdressing at PD stage. Plots and treatments remained the same throughout the two crop seasons. Main plot sizes were 30.0 by 15.0 m : subplot sizes were 5.0 by 15.0 m.

Observed and calculated data

During the sampling period, leaf chlorophyll contents were determined, using a portable chlorophyll meter (Minolta model SPAD 502), at MT, end-tillering (ET) (60-65 days after sowing), and PD stages. These readings are in SPAD units which are values defined by Minolta to indicate the relative amount of chlorophyll contained in plant leaves. At the three stages, the SPAD value was measured on the most recently matured leaf (Y leaf) of each plant at the point three-fourths of the way from base to leaf tip. Meter readings were taken on representative plants at 2 m intervals along a transect through the plots.

Following measurements on SPAD-502, the fresh leaf blades were cut from every measured leaf in each plot. Leaf blade samples were oven-dried at 70º C until constant weights were obtained. Dried samples were then ground in a Cyclotec sample mill to pass a 0.5 mm screen. Samples were analyzed for total N concentration by the Kjeldahl method. The SPAD measurements and leaf blade collections were carried out prior to N-topdressing at MT and PD stages.

At maturity, the stalk sample collection, N determination and grain yield, harvest index, N uptake, apparent N recovery and N use efficiency calculation procedures were equal to that in Expt. I.

Common agronomic practices to all experiments

The N fertilizer was applied in all cases as ammonium sulphate. Experimental plots were laterally isolated in the field by using plastic sheets pushed into the soil. Basal N rates and P fertilizer (100 kg/ha P_2O_5 as superphosphate) were applied as a single broadcast application c 3 cm deep, bellow the soil surface of the bed 3 - 7 days before flooding, depending on year and field conditions.

Every year, around mid May, all plots in Experiments I, II and IV were hand-sown with rice (Oryza sativa), cv Senia (medium grain) in Expt. I and cv Leda (medium grain) in Expts II and IV, at 200 kg/ha seed, pre-soaked in tap water.

Molinate (S-ethyl hexahydro-1H-azepine-1-carbothiolate) at the rate of 4.5 kg (ai)/ha at c 15 days after sowing or c 20-30 days prior transplanting, depending on Experiment, was applied for Echinochloa weed control. Water flow was halted for 3 days following the molinate application. The later Echinochloa infestations were controlled with 3.5 kg (ai)/ha of propanil [N-(3,4-dichloropropionanilide)].

About 25-35 days after sowing, depending on year and field conditions, stand establishment was made uniform in each plot by transplanting 4 week-old rice seedlings in hills of 1-2 plants each at 10-15 cm spacing.

Plots were drained once in the 6-7th week after sowing or 2-3th week after transplanting for a 1-2 week period. Then, bentazon + MCPA at the rates of 1.6 and 0.24 kg (ai)/ha respectively, or bensulfuronmethyl (Expt. IV) at the rate of 0.06 kg (ai)/ha were applied for aquatic and sedge weeds control. The plots were reflooded until they were drained for harvest 2-3 weeks before harvesting.

Results and discussion

Experiment I

Abundance of filamentous N_2-fixing cyanobacteria

In agreement with other reports (Roger & Kulasooriya 1980; Quesada et al. 1989) a significant negative correlation (r=-0.53, P<0.05, n=35) was observed between the abundance of N_2-fixing cyanobacteria and the amount of N fertilizer (Fig. 1). Mean values ranged from 1.16±0.2x105 at a rate of 140 kg N/ha to 2.20±0.7x105 colony forming units (CFU)/cm^2 without mineral N application.

Biological nitrogen fixation

Figure 2 shows the effect of N-fertilizers on acetylene reducing activity (ARA). During the growth cycle (May, June, July and September), nitrogenase activity decreased linearly (P<0.05) as the amount of N fertilizer increased. The decrease was more evident in June than in May, July or September (Fig. 2) as evidenced the statistical analysis (month x N rate interaction). A highly significant negative correlation (r=-0.90, P<0.05, n=60) was found between (ARA) and N-fertilizer rates. Similar relationships were found by Trolldenier (1987) and Prosperi et al. (1992).

Nitrogenase activity varied with time during the growth cycle (Fig. 2). The pattern of variation was similar to that reported for other rice fields (Roger & Kulasooriya 1980). Very low values were observed during the first 15 days after flooding and sowing. The underwater light intensity was very high (up to 1500 µE/m^2.s at midday) and would have had an inhibitory effect on N fixation, as reported by Leganés & Fernández Valiente (1991). Maximum values of nitrogenase activity were found at mid-tillering stage in June, about 40 days after sowing, when the plant cover was sufficiently dense to protect cyanobacteria from a high light intensity. From then, the nitrogenase activity decreased, as the underwater light intensity decreased, due to the increase in the plant canopy.

Values of ARA (Fig. 2) in unfertilized plots ranged from 56±62 µmol ethylene/m^2.h, at the start of the cultivation cycle, to 649±282 µmol ethylene/m^2.h at MT stage. In plots fertilized with 140 kg N/ha, values of ARA ranged from 23±18 to 295±120 µmol ethylene/m^2.h.

Grain yield and N utilization for Senia rice

Grain yield increased linearly (P<0.01) with the rate of N (Table 1). Nevertheless, as reported by Sendra et al. (1993), there were no significant differences between plots fertilized with 70 and 140 kg N/ha. Furthermore, the harvest index (Table 1) decreased with increments in N rates. Thus, at high N fertilizer rates, nitrogen taken up by the rice plant is more closely related to the formation of vegetative structures which are not desirable.

The total amount/ha of N removed by plants was directly related to the N-fertilizer rate. Nevertheless, despite that relationship, there were no significant differences between plots fertilized with 70 and 140 kg N/ha.

In plots with 140 kg N/ha, at least 25% of the N applied was not used by plants, and therefore it could have contributed to the pollution of surrounding waters. In other instances, and in agreement with Trolldenier (1987), the difference between removed and applied N was positive and higher at lower N input rates. This difference can be explained, as suggested by Trolldenier (1987) and Roger et al. (1993), by the utilization of dinitrogen fixation as an alternative source of N, since soil N content did not decrease after three crop seasons and the estimated amount of N supplied by irrigation water (< 10 kg N/ha per year) was not enough to explain such a difference. In fact, a significant positive correlation (r=0.85, P<0.05) between ARA and the difference between removed and applied N was found, suggesting that N_2 fixation contributes to the maintenance of grain yield and fertility in paddy fields at moderate or zero fertilization.

As reported by Trolldenier (1987) and as shown by orthogonal polynomial contrast, N-use efficiency and apparent N recovery (Table 1) increased linearly (P<0.01) as rate of N decreased, suggesting that it could be due to the higher N_2 fixation and lower N losses. Therefore, fertilization with 140 kg N/ha did not significantly increase grain yield and, furthermore, involve a higher fertilizer cost and likely a greater risk of environmental pollution than that with 70 kg N/ha.

Experiment II

Effect of the split N application on biological nitrogen fixation

The results (Fig. 3) are in agreement to those obtained in Expt. I. Differences among treatments were only significant (P<0,05) in June. The highest ARA value (831± 128 µmol ethylene/m^2.h) was obtained in the treatment where the entire N application rate was delayed after the maximum ARA occurred in June ($N_{0,50,25}$ treatment). On the contrary, the lowest ARA value (314± 89 µmol ethylene/m^2.h) was found in plots where the total N rate, 75 kg/ha, was applied early in May as a single basal application ($N_{75,0,0}$ treatment). After second N application in July, the total amount/ha of N applied was the same regardless of the treatment and in consequence there were no differences in ARA between treatments in July and September. Therefore, according to Goyal (1985), IRRI (1986) and Yanni (1991), split N applications with most of N rate applied late in July seemed to improve biological N fixation.

Experiment III

Effect of algalization on occurrence of N_2-fixing cyanobacteria and ARA

There were no significant differences in total and N_2-fixing cyanobacteria abundance between inoculated plots and controls (Fig. 4). Thus, inoculation did not quantitatively affect the occurrence of N_2-fixing cyanobacteria but produced a change on its qualitative composition, favouring the inoculated strains. In inoculated plots, at both 0 and 35 kg N/ha, the fluorescence units (FU) of inoculated heterocystous cyanobacteria were more abundant (18.67% of the total FU) than in plots non inoculated (10.68% of the total FU). But, in uninoculated plots the density of total N_2-fixing cyanobacteria was the same than that attained in inoculated plots. Accordingly, nitrogen fixation was not affected by algalization (Fig. 4). In accordance with Roger et al (1987), these results suggest that more attention should be given to agricultural practices, enhancing the growth of indigenous strains, than to algalization improvement.

Effect of algalization on grain yield and N utilization

The results of grain yield, harvest index, apparent N recovery and efficiency N use are shown in Table 2. There were no significant differences in all traits between treatments. According to Reddy et al (1986) and Watanabe (1973) but in contrast to other reports (Roger, 1991), the algal inoculation had no significant effect on rice yield in both the presence and the absence of N fertilizer (0 and 35 kg N/ha). Furthermore, the N fertilizer recovery and N use efficiency were not improved by algalization, in plots where nitrogen fertilizer was applied to the field (Table 2).

Experiment IV

Effect of N fertilization rates and timing on agronomic performance, grain yield and N utilization for Leda rice.

The effects of basal N rate or additional 50 kg N/ha application, at both the MT and the PD stages, on days to 50% heading were not significant (Table 3). In contrast and in agreement with Sendra et al (1993), days to maturity, plant height and lodging increased linearly as the rate of N increased. At all basal N rates, the additional topdressing N, at both stages, increased the above three traits (Table 3). Increased height with N applied at MT was higher than when was added at PD stage. Days to maturity, according to Moletti et al (1992), and lodging (Melgar et al 1994) from split N application were lower than the values achieved with total N rate applied under a entire basal preflooding application (Table 4). Lodging was also lower when the second part of two-split fertilizer applications was supplied at PD than at MT (Table 4).

In contrast to Expt I, and as a consequence of its less organic matter content, rough-rice grain-yield response to fertilizer N rate was significant up to 100 kg N/ha added as single basal application. Though differences with 100 kg N/ha were not significant, the greatest grain yield (7.41 t/ha) was obtained with 125 kg N/ha (Table 3). In correspondence with Expt. I, the harvest index decreased with increments in N rates. Grain yield increased with an additional application of 50 kg N/ha and was greater when applied at PD than at MT stage (Table 3). The increase in yield due to N topdressing depended on the basal N rate and, thus, on the N and chlorophyll status of the plant at any stage (Fig. 5).

According to Moletti et al (1992) and Hefner & Tracy (1991), at the highest N rate of 150 kg N/ha, the split-applying N in two applications with two-thirds applied before sowing and the last third during PD, resulted in higher grain yield than all N applied presowing or split applied at presowing and MT (Table 4). No general advantage of split N application vs all presowing N was observed for grain yield at the N rate for the maximum grain yield (Melgar et al 1994). This observation suggests, as reported by Senanayake et al (1996), that agronomic trials seeking to improve rice yields by optimizing N timing frequently show little effect largely because of compensation between yield components; rather it is total N supply that should be improved.

Regardless of N rate, split-applying N with the last application at PD resulted in higher harvest index value than either all N applied presowing or split-applied with the last application at MT (Table 4).

The N removed by plants, the apparent N recovery and the efficiency of N use were related to N rates. The relationships had a similar trend for the rice cultivars and soil characteristics of the experiments I and IV : Senia cv (Expt. I) and Leda cv (Expt. IV) on soils with 4.8 and 2.57% respectively. Furthermore, the absolute values for all traits at any N rate were similar in both experiments. Thus, apparent N recovery (Table 3) ranged from 29.4% at the rate of 150 kg N/ha to 35.8% at the rate of 50 kg N/ha. Values of efficiency of N use (Table 3) ranged from 12.7 kg grain/kg N, in plots fertilized with 150 kg N/ha, to 24.1 kg grain/kg N at the rate of 50 kg N/ha. This similarity in N response in both experiments suggests that the growing rice cultivars, with equal growth habit, and the soil characteristics, not very different and with an organic matter content greater than 2% in both experiments, have less effect than the constraints in the irrigation system. In fact, the ancient irrigation network that force the water to flow down from a field to other successive ones, the irrigation ordinances that through the Irrigation Council fix the dates of water delivery and draining, the splitting up of irrigated holdings and the dispersion of each individual rice grower acreage leads to a lack of control in water management. Hence, the farmer may have practical difficulties to irrigate after the incorporation of the fertilizer application. This, in accordance with Stutterheim et al (1994) and Barbier & Mouret (1992), is reflected in the low values of the apparent N recovery and agronomic efficiency and indicate that N-losses are substantial. Accordingly, the additional application of 50 kg N/ha decreased the apparent N recovery and agronomic efficiency (Table 3). This was probably due to the increase of the total applied N, consequence of that second application, and to the greater N losses attributed to the topdressed treatment (Bilal et al, 1979).

In consequence and according to Stutterheim et al (1994), nitrogen split-supply had no effect on apparent N recovery and agronomic efficiency (Table 4). This lack of effect can be explained because of the high organic matter content of the experimental plots. In this sense, Patnaik (1965) and Racho & De Datta (1968), demonstrated that soils rich in organic matter show a large N-supplying capacity due to immobilization-mineralization biogeochemical processes. In the final stages of the growing season, the remineralized N, coming from either the organic matter or the basal N fertilizer application, is placed in anaerobic soil layers and shows a lesser risk for losses than the inorganic N coming from the second split application at either mid-tillering or panicle differentiation stage (Savant & De Datta, 1982).

N status of the rice plant at different stages of the growing season

Leaf N (Schnier et al, 1990) and chlorophyll contents decreased quadratic or linearly respectively from mid-tillering until maturity in all N rates (Fig 5). The decrease had a similar trend among N rates. At any stage of the growth cycle, N and chlorophyll contents increased linearly as the amount of basal N application increased. Thus, leaf N contents in unfertilized plots ranged from 3.63% ± 0.060 to 2.29±0.017 at MT and PD stages respectively. In plots fertilized with 150 kg N/ha values of leaf N concentration ranged from 4.73% ± 0.061 at MT, to 3.23% ± 0.040 at PD. These values are in the range of those reported for other rice areas in Spain (Forés & Comin, 1989; Aguilar & Grau, 1996). Similarly, SPAD values in unfertilized plots ranged from 34.6±0.50 at MT, to 27.9±0.17 at PD. In plots fertilized with 150 kg N/ha SPAD values ranged from 40.9±0.50 at MT, to 36.8±0.41 at PD. This similar trend is evidenced by the fact that there was a good relationship between leaf N concentration on a dry-weight basis (N_{dw}) and SPAD values. Consistent with other reports (Takebe & Yoneyama 1989; Shaobing et al 1993), the relationship between SPAD and N_{dw} differed significantly depending on growth stage. The regression equations were (Fig. 6):

At MT: SPAD = 16.69+5.07(N_{dw}); r=0.73**, n=144
At ET: SPAD = 10.77+8.10(N_{dw}); r=0.83**, n=144
At PD: SPAD = 9.66+8.29(N_{dw}); r=0.68**, n=144

Based on pooled data from all stages : SPAD =22.1+4.06(N_{dw}); r=0.72**, n=432

The additional N applications, at MT and PD stages, temporarily increased the foliar N concentration (Schnier et al, 1990) and SPAD value. That increase involved an increase in yield depending on the basal N rate and, in consequence, on the N and chlorophyll status of the plant at any stage (Fig. 5). In other words, the leaf N concentration and SPAD value can determine the need for N topdressing at specific growth stages (Turner & Jund 1991; Chubachi et al 1986; Miyashita et al 1986; Takebe & Yoneyama 1989; Takebe et al 1990).

There was no significant correlation between SPAD value and the rice yield increase from N topdressing application at the PD stage. This is consistent with observations of Turner & Jund (1991) who found that the failure of the SPAD reading to predict the need for topdress N may be attributed to the applications of herbicides which may have altered leaf chlorophyll stability or light adsorption capability. In this experiment, herbicides were applied in the 2nd week of July to control late infestations of Echinochloa sp. In contrast, during the MT stage, a significant ($P<0.05$) though not very good relationship between SPAD value and the need for topdress N at MT was evident. The regression equations were :

Based on pooled data from two years :

y (yield increase due to topdress N)=9,969.94-254.4(SPAD);r^2=0.44,n=48
In 1995 : y=14,540.91-365.3(SPAD); r^2=0.52

In 1996, the range in leaf SPAD values narrowed to between 33 and 39 and, according to Turner & Jund (1991), may have contributed to a poor relationship.

The r^2 value of 0.44 indicated that 44% of the rice yield response to topdress N could be explained by SPAD readings. This r^2 value is not so negligible considering that during the years these data were obtained : rice yield varied by 500 kg/ha between years; LSD for rice yield was as high as 1,000 kg/ha; and the maximum yield increase due to N topdressing was 2,000 kg/ha.

Foliar critical values and adequate ranges (Fig. 5) for both, the N concentration and the SPAD reading, were determined by the method of Mikkelsen & Evatt (1973), Miller (1983) and Aguilar & Grau (1996). The upper limit of the range indicates the values above which no N topdressing is needed. The probability of obtaining a yield response from the addition of nitrogen depends upon whether the N concentration or SPAD reading is above or below the critical range. The values, for both the N foliar concentration and the SPAD reading, above which benefits from additional amounts of N are not likely to occur were : 5.36, 3.76 and 3.19% at MT, ET and PD respectively for leaf N concentration and 43.86, 41.19 and 36.10 SPAD units at the same stages of the growth cycle for SPAD reading. However, if the leaf N concentration falls bellow the critical value, the probability of a response from fertilization is very high. The critical values at MT, ET and PD stages were : 4.00, 2.83 and 2.61% respectively for leaf N concentration and 37.2, 33.7 and 31.5 SPAD units for SPAD reading.

Conclusions

The abundance of N_2-fixing cyanobacteria and biological nitrogen fixation, estimated by acetylene reducing activity (ARA), decreased linearly as the amount of N fertilizer increased. Nitrogen fixation contributes to the maintenance of grain yield and fertility in paddy fields at moderate or zero fertilization. Moreover, split N application with most of N rate applied late in July seemed to improve biological nitrogen fixation.

Since rice yield and biological nitrogen fixation was not affected by algalization, more attention should be given to agricultural practices, enhancing the growth of indigenous strains of cyanobacteria, than to algalization efforts.

To prevent yield decline, the amount of N applied as basal dressing must not exceed 70 and 125 kg N/ha on soils with 4.8 and 2.57% organic matter content, respectively. Agronomic efficiency values of 20.8 and 16.3 $kg.kg^{-1}$ were obtained at those basal N rates, respectively. These low values indicate that N-losses are substantial.

Split N application did not improve the agronomic efficiency and was not efficient for yield production but reduced days to maturity and lodging and increased the harvest index value.

The values above which no N topdressing is needed, for both the N foliar concentration and SPAD reading, were :

At MT: 5.36% N content, 43.86 SPAD reading
At ET: 3.76% " , 41.19 " "
At PD: 3.19% " , 36.10 " "

Not a clear correlation between SPAD value and the rice yield increase from N topdressing application at the PD and MT stages, was found.

Table 1.- Effect of N rate on grain yield and on N responses of rice cv. Senia*

N applied (Kg N/ha)	Grain yield (t/ha)	Harvest$ index	N removal (kg/ha)& By rice plants	N removal (kg/ha)& Minus N applied	Apparent N recovery& (%)	Efficiency N use (kg grain/N)
0	5.95	0.62	77.2	77.2	-	-
17.5	6.60	0.61	90.1	72.6	75	48.7
35	6.73	0.60	90.5	55.5	38	25.2
70	7.37	0.61	99.4	29.4	32	20.8
140	7.59	0.60	105.6	-34.4	20	11.1
SE N rate (DF)	0.158(12)	0.004(12)	3.42 (4)	3.42 (4)	17.6 (3)	9.55 (9)

* Randomized complete block design with four replications. Means of 3 years.
$ Means of 2 years
& Randomized complete block design with two replications. Means of 3 years.

Table 2.- Effect of N fertilizer and algalization on grain yield, harvest index and N utilization of transplanted rice cv. Senia

Treatment	Grain yield& (t/ha)	Harvest index&	Apparent N recovery$ (%)	Efficiency N use$ (kg grain/N)
0 kg N/ha	6.76 a	0.603 a	-	-
35 «	7.41 a	0.607 a	27.36 a	19.37 a
140 «	8.51 b	0.590 a	20.86 b	11.30 b
0 « + Inoc	6.78 a	0.610 a	-	-
35 « + Inoc	7.14 a	0.597 a	25.36 a	20.69 a

& Means of 3 years
$ Means of 2 years
Within columns, means followed by the same letter are not significantly different at P=0.05.

Table 3.- Effect of N rate on agronomic performance, grain yield and on N responses of rice cv. Leda*

Basal N fertilizer (Kg N/ha)	Growing cycle (days) 50% heading	Maturity	Plant height (cm)	Lodging$ (%)	Grain yield (t/ha)	Harvest index	Apparent N recovery& (%)	Efficiency N use (kg grain/N)
0	83.42	131.92	85.9	0.11	4.75	0.581	-	-
50	83.46	133.67	95.5	3.99	6.28	0.575	35.8	24.1
75	83.37	135.33	99.4	20.85	6.87	0.572	36.9	23.2
100	83.50	136.12	103.0	35.36	7.20	0.557	28.6	19.9
125	83.58	138.67	102.9	58.41	7.41	0.546	26.0	16.3
150	83.71	138.75	105.7	80.43	7.00	0.529	29.4	12.7
SE N rate (DF)	0.097(15)	0.317(15)	0.84(15)	0.090(15)	0.174(15)	0.0065(15)	4.09 (4)	1.8 (4)
Additional 50 kg N/ha at								
MT	83.58 a	136.33 a	101.70 a	33.71 a	6.79 b	0.5444 c	31.4 a	15.8 b
PD	83.48 a	135.94 b	98.60 b	27.17 a	7.10 a	0.5763 a	32.4 a	18.1 b
No addit. N	83.46 a	134.96 c	95.85 c	15.15 b	6.58 b	0.5590 b	32.9 a	23.9 a
SE addit. (DF)	0.091(36)	0.132(36)	0.390(36)	0.054(36)	0.088(36)	0.00250(36)	3.05 (10)	1.03 (30)

*Split-split-plot design with four replications. Years as sub-subplot. Means of 2 years.
$ Analysis of variance conducted on data transformed to square root of (x+0.5). The table gives backtransformed values.
& Split-split-plot design with two replications. Means of 2 years.
Within columns, means followed by the same letter are not significantly different at P = 0.05. MT = midtillering; PD = panicle differentiation

Table 4.- Effect of N application timing on agronomic performance, grain yield and on N responses of rice cv. Leda*

N timing (Kg N/ha)	Growing cycle (days) 50% heading	Maturity	Plant height (cm)	Lodging$ (%)	Grain yield (t/ha)	Harvest index	Apparent N recovery& (%)	Efficiency N use (kg grain/N)
100 B	83.37 abc	135.00 ef	101.1 cde	16.61 def	7.20 abc	0.559 de	44.8 a	24.5 ab
50 B + 50 MT	83.87 ab	134.25 f	99.1 e	13.74 ef	6.76 cd	0.566 bcd	30.7 a	20.1 bcd
50 B + 50 PD	83.25 bc	134.50 f	94.9 fg	0.69 g	6.92 bc	0.597 a	33.8 a	21.8 bc
125 B	83.37 abc	138.25 b	100.3 de	34.71 c	7.41 ab	0.552 de	26.0 a	21.3 bcd
75 B + 50 MT	83.62 abc	135.75 de	101.1 cde	29.19 cd	7.34 abc	0.555 de	28.9 a	20.8 bcd
75 B + 50 PD	83.37 abc	135.75 de	100.6 cde	25.32 cde	7.33 abc	0.590 a	28.0 a	20.6 bcd
150 B	84.00 a	138.50 ab	103.3 bc	73.46 a	7.00 bc	0.530 fg	29.4 a	15.0 de
100 B + 50 MT	83.62 abc	136.37 cd	105.8 b	57.11 ab	7.02 bc	0.527 fg	35.5 a	15.2 cde
100 B + 50 PD	83.50 abc	137.00 c	102.1 cd	38.43 bc	7.77 a	0.584 ab	30.4 a	20.2 bcd
SE (DF)	0.222(36)	0.325(36)	0.95(36)	0.326(36)	0.216(36)	0.0063(36)	6.83 (10)	2.30 (30)

*Split-split-plot design with four replications. Years as sub-subplot. Means of 2 years.
$ Analysis of variance conducted on data transformed to square root of (x+0.5). The table gives backtransformed values.
& Split-split-plot design with two replications. Means of 2 years.
Within columns, means followed by the same letter are not significantly different at P = 0.05. B = basal preflooding application; MT = midtillering application; PD = panicle differentiation application.

Fig 1

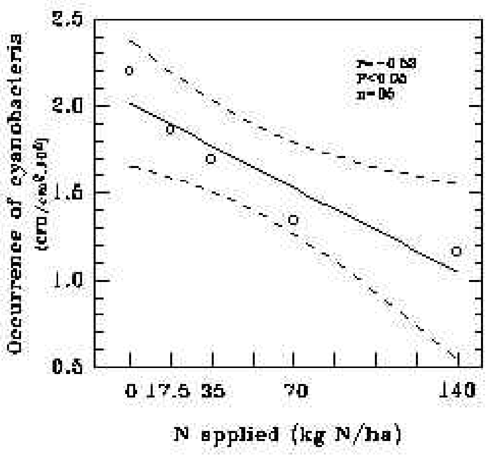

Fig. 1.- Effect of N rate on the occurrence of blue-green algae in ricefields. Data are the mean of two crop seasons. CFU= colony forming units.

Fig 2

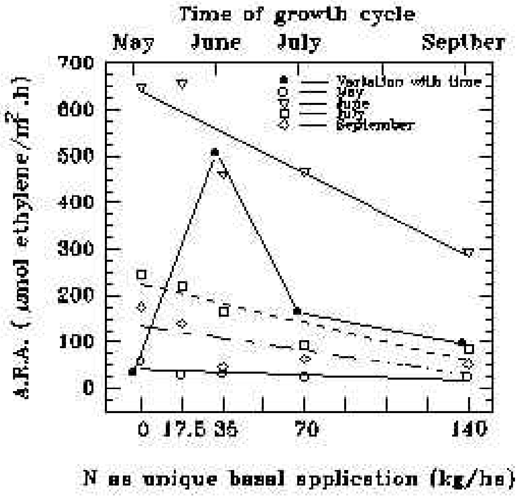

Fig. 2.– Effect of N rate on A.R.A. and variation with time during the growth cycle of rice. Data are mean values of three years. ARA= acetylene reducing activity.

Fig 3

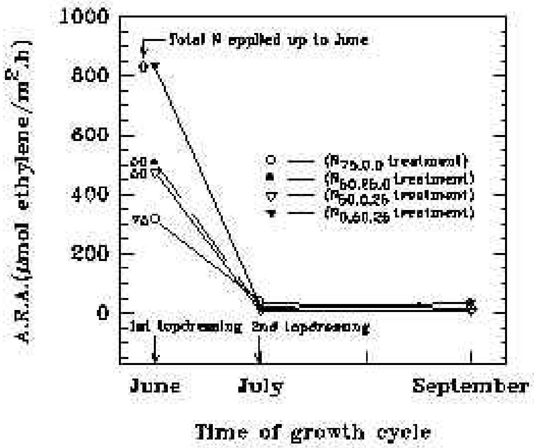

Fig. 3.– Effect of the split N application on A.R.A. during the growth cycle of rice. ARA= acetylene reducing activity.

Fig 4

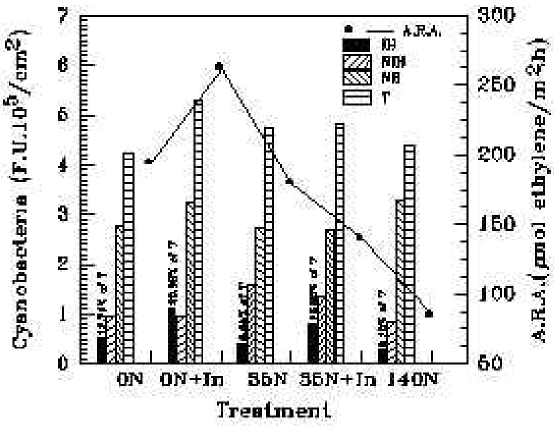

Fig. 4.– Effect of N fertilizer and algalization on the number of cyanobacteria and A.R.A. Data are mean values of two years; ARA=acetylene reducing activity F.U.=fluorescence units; IH= inoculated heterocystous cyanobacteria; NIH= non inoculated heterocystous cyanobacteria; NH= non heterocystous cyanobacteria; T= total cyanobacteria; 0N+in = 0 kg N/ha + inoculation.

Fig 5

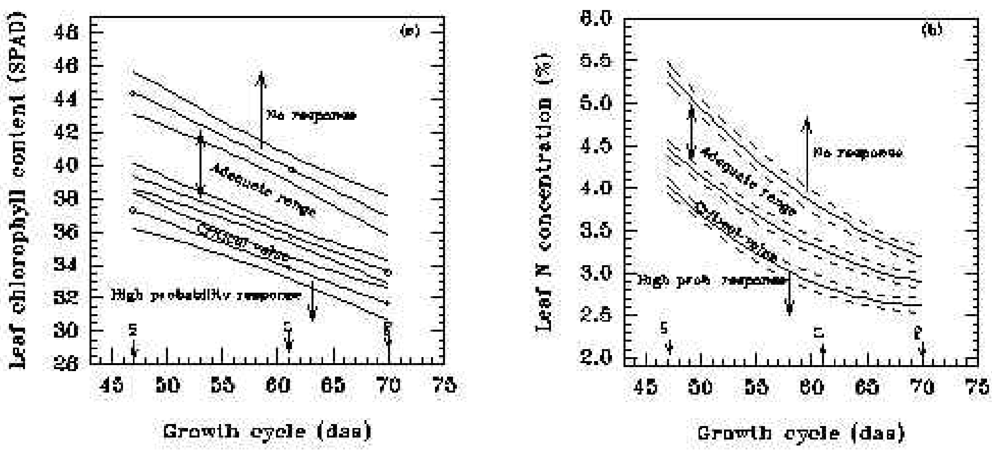

Fig. 5.– Grain yield response to N topdressing as affected by (a) rice leaf chlorophyll content (SPAD value) or (b) rice leaf N concentration at midtillering (MT), end tillering (ET), and panicle differentiation (PD). Data of two year. Adequate range limits calculated by the method of Mikkelsen & Evatt (1973), Miller (1983) and Aguilar & Grau (1996). The upper range limit for SPAD value was estimated from the N content throughout the regression equations for leaf chlorophyll content (SPAD) on the N concentration, at any growth stage; das = days after sowing.

Fig 6

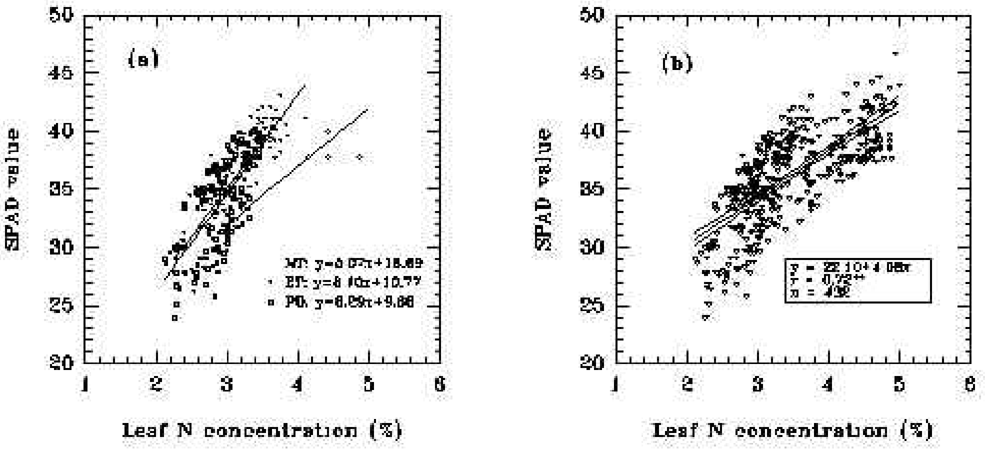

Fig. 6.- Linear regression of chlorophyll meter readings (SPAD values) on (a) leaf N concentration of Leda rice at midtillering (MT), end tillering (ET), and panicle differentiation (PD), or (b) on leaf N concentration for pooled data from the three developmental stages.

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