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Nitrogen Metabolism and Yield Response to Increases in Nitrogen-Phosphorus Fertilization: Improvement in Greenhouse Cultivation of Eggplant (*Solanum melongena* Cv. Bonica)

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ABSTRACT

The effect that application of nitrogen-phosphorus (NP) rates exerts on some parameters of nitrogen metabolism and on yield in aubergine plants (eggplant, *Solanum melongena* cv. Bonica) was studied. All plants were grown under controlled conditions in an experimental greenhouse. The treatments consisted of the combination of three rates of N in the form of KNO_3 (N_1: 15 g m^{-2}, N_2: 22.5 g m^{-2} and N_3: 30 g m^{-2}) together with two rates of P in the form of H_3PO_4 (P_1: 24 g m^{-2} and P_2: 36 g m^{-2}), for a total of six treatments. Results indicate that regardless of the P rate applied, an increase in the N fertilizer increased NO_3- assimilation as well as total and commercial yield. Use of the P_2 rate favored all of these processes in comparison with the results obtained with the P_1 rate, in addition to notably reducing the foliar levels of NO_3- and noncommercial yield.

Keywords: *Solanum melongena L.; NP fertilization; nitrogen assimilation; yield*

INTRODUCTION

Plant growth is dependent on an adequate nitrogen (N) supply in order to form amino acids, proteins, nucleic acids, and other cellular constituents. For most plants, inorganic N is obtained from the soil in the form of nitrate (NO_3^--N). It has long been recognized that the rate-limiting step for N assimilation, the reduction of NO_3^--N to nitrite (NO_2^--N) catalyzed by nitrate reductase (NR), is highly regulated (Solomonson and Barber, 1990; Huber et al., 1996; Sivasankar and Oaks, 1996). Nitrate availability, growth regulators, light, products of NO_3^--N assimilation and other physiological and environmental parameters are all factors in regulation of NO_3^--N assimilation (Campbell, 1988; Lillo, 1994; Kaiser and Huber, 1994; Crawford, 1995; Sivasankar and Oaks, 1996).

Appropriate levels of NO_3^--N derived from proper N fertilization increases the amount and activity of NR; this leads to a corresponding increase in the potential for NO_3^--N reduction and confers a greater capacity for general amino-acid synthesis, protein synthesis, or total N assimilation (Vincentz et al., 1993; Migge and Becker, 1996; Barneix and Causin, 1996). In addition, it has been shown that plant growth and yield rates are often dependent on N supply (Mattson et al., 1991). Nevertheless, excessive N fertilization can be harmful, and NO_3^--N accumulation is a problem in most crops (Steingröver et al., 1982; Blom-Zandstra and Lampe, 1985; Van der Boon et al., 1990; Quillere et al., 1994). Leaf tissues accumulate NO_3^--N when the rate of NO_3^--N delivery exceeds the NO_3^--N assimilation capacity (Mackown et al., 1990; Quillere et al., 1994). In addition, nitrogen fertilizers contribute to groundwater and surface-water pollution through leaching and soil erosion (Sisson et al., 1991).

The role of P in N metabolism has been studied in detail, although almost always from the standpoint of the effect of deficiencies in this element. Nitrate assimilation is altered when plants

are deprived of P. The most commonly described effects are: (i) reduction in NO_3^--N absorption through the roots (Rufty et al., 1990; Rufty et al., 1991; Pilbeam et al., 1993), possibly due to decreased availability of radicular ATP and a limitation in synthesis of the membrane transport system for NO_3^--N (Rufty et al., 1993); (ii) decrease in the translocation of NO_3^--N from the roots to the aerial part (Rufty et al., 1990; Pilbeam et al., 1993), related to a fall in water pressure via the root and xylem (Rufty et al., 1993); and (iii) increases in amino acid levels (Rufty et al., 1990; Rufty et al., 1993). These factors lead to a decreased activity of NR and therefore, in the assimilation of NO_3^--N (Pilbeam et al., 1993).

In the present work, we analyzed the effect of N(NO_3^--N) and P (H_3PO_4) fertilization on some parameters of N metabolism and on yield of eggplant, the latter being the final indicator of plant growth. Our aim was to determine the most appropriate rates of these elements for eggplant grown under greenhouse conditions.

MATERIALS AND METHODS

Crop Design. *Solanum melongena* cv. Bonica were seeded in cell flats (cell size 3 × 3 × 10 cm) filled with peat-lite mixture, placed on benches under the greenhouse conditions described below, for a period of 8 weeks; then seedlings were transplanted and grown under controlled conditions in an experimental greenhouse at Centro de Investigación y Desarrollo Hortícola, El Ejido, Almería, Spain. The experiment was conducted from 1993 to 1995. The climate is semiarid and the lands are intensively used for agriculture. The soil used was loamy-sand with the following characteristics: sand (37.3%), silt (48.6%), and clay (10.1%), $CaCO_3$ equivalent (26.82%), $CaCO_3$ active (14.35%), total N (3.5 g kg^{-1}), total organic C (36.1 g kg^{-1}), PO_4^{-3} (890 mg kg^{-1}), K^+ (5.34 g kg^{-1}), pH (H_2O, 8.45; KCl; 8.01), electrical conductivity (EC) 4.63 dS m^{-1}). The relative humidity was 60-80% and the temperature range 24 (4 °C with extremes 15 and 30 °C in the greenhouse. The experimental design was a factorial arrangement in a randomized complete block with six treatments.

Container-grown eggplants were transplanted into two rows 100 cm apart and trickle irrigated. Each treatment was replicated four times in four individual plots of 4 m × 2 m wide (24 plots). Each plot contained eight treated plants. The irrigation water had the following properties: pH, 8.05; EC 2.03 dS m^{-1}; Cl^- 483.90 mg L^{-1}; Na^+ 305.76 mg L^{-1}; K^+ 10.16 mg L^{-1}; $HCO_3$278.15 mg L^{-1}.

The different treatments consisted of applying increasing rates of both N and P in the following manner: N in the form of KNO_3 (N_1: 15 g m^{-2}, N_2: 22.5 g m^{-2}, and N_3: 30 g m^{-2}) and P in the form of H_3PO_4 (P_1: 24 g m^{-2} and P_2: 36 g m^{-2}). Calcium (11 g m^{-2}) and magnesium (3 g m^{-2}) were supplied as sulfates. Each nutrient was applied gradually at the specified rate with water irrigation over the entire growth period of the plants. Fertilization-irrigation was complemented with the following micronutrients: Fe: 0.5 mg L^{-1}; B: 0.1 mg L^{-1}; Mn: 0.1 mg L^{-1}; Zn: 0.075 mg L^{-1}; Cu: 0.075 mg L^{-1} and Mo: 0.05 mg L^{-1}. The pH values of the solution oscillated between 5 and 6; Fe was applied as FeEDDHA, B as H_3BO_3, and the remaining micronutrients as sulfates.

Plant Sampling. Leaf samples were taken only from plants with fully expanded leaves of the same size (> 25 cm). Leaves were picked about one-third of the plant height from the plant apex during flowering (95 days after transplanting until 170 days) every 2 weeks (Lo´pez-Cantarero et al., 1995). Leaves were rinsed three times in distilled water after being disinfected with nonionic detergent at 1% (Wolf, 1982), then blotted on filter paper. At each sampling, fresh leaf matter was used for the NRA assay, amino acids and proteins; then a subsample was dried in a forced air oven at 70 °C for 24 h, ground in a wiley mill, and then placed in plastic bags for further analyses (NO_3^- and organic N).

Plant Analysis. *NO_3 Determination.* NO_3^--N was analyzed from an aqueous extraction of 0.2 g of dried and ground leaf material in 10 mL of Millipore-filtered water; 100 μL aliquot was taken for NO_3^--N determination and added to 10% (w/v) salicylic acid in sulfuric acid at 96%, and the NO_3^--

N concentration was measured by spectrophotometry as performed by Cataldo et al. (1975). The results were expressed as mg g^{-1} dry weight (dw).

Detection of in Vivo NR Activity. The basic method was an adaptation of the in vivo NR assay by Jaworski (1971) and Maurin˜o et al. (1986). Leaves were cut into 5 mm sections and the sample (0.5 g) was placed in 10 mL incubation buffer (100 mM potassium phosphate buffer, pH 7.5) and 1% (v/v) propanol. The sample was infiltrated and the intracellular spaces of the tissues were flushed with buffer using a vacuum (0.8 bar). After 10 min, the vacuum was released and the samples were reevacuated. The samples were incubated at 30 °C in darkness for 1 h and placed in a boiling water bath to stop the NR activity. The resulting NO_2^- was measured by the method of Snell and Snell (1949), and the NR activity was expressed as μmol NO_2^- g^{-1} fresh weight (fw) h^{-1}.

Amino Acids and Soluble Proteins Determination. Fresh leaf samples (0.5 g) were crushed with cold phosphate buffer (50 mM KH_2PO_4, pH 7) and centrifuged at 12 000*g* for 15 min. The resulting supernatant was used for the determination of total amino acids by the ninhydrin method as described by Yemm and Cocking (1955); total free amino acids were expressed as mg glycine g^{-1} fw. Soluble proteins were measured by Bradford G-250 reagent (Bradford, 1976) and expressed as mg bovine serum albumin g^{-1} fw.

Organic N Determination. A 0.1 g dw subsample was digested with sulfuric acid with H_2O_2 (Wolf, 1982). After dilution with deionized water, a 1 mL aliquot of the digest was added to the reaction medium containing buffer (5% potassium sodium tartrate, 100 μM sodium phosphate, and 5.4% [w/v] sodium hydroxide), 15/0.03% (w/v) sodium salicylate/sodium nitroprusside, and 5.35% (v/v) sodium hypochlorite. Samples were incubated at 37 °C for 15 min and organic N was measured by spectrophotometry according to Baethgen and Alley (1989). The results were expressed as mg g^{-1} dw.

Yield. Plant yield was expressed as the mean of fruit weight. Collected eggplants were weighed on each plant at harvest. Commercial yield represents fruits with acceptable color and caliber, while those without acceptable features represent noncommercial or residual yield. Total yield (kg $plant^{-1}$) is the sum of both types of yield.

Statistical Analysis. The data were submitted to three statistical treatments in order to determine: (i) differences between N rates at each P level; (ii) differences between P rates at each N level; and (iii) differences between all fertilizer treatments. Standard analysis of variance (ANOVA) techniques were used to assess the treatment means. Where were no climatic effects on yield, so ANOVAs were performed on pooled data from both years. Treatment means were compared using the least significant difference at the 0.05 probability level. The mean of separation according the Duncan's Multiple Range test is represented with letters in the tables and figures. When appropriate, a simple regression analysis was performed between parameters: $p < 0.05$) *, $p < 0.01$) **, ns) not significant.

RESULTS AND DISCUSSION

Response to the N Rate. When the P rate was P_1, most of the N-metabolism parameters studied, and yield, were influenced directly by the N rate applied. One of the major and limiting stages of NO_3^- assimilation is NR activity (Huber et al., 1996; Sivasankar and Oaks, 1996). Figure 1 presents the values obtained in the *in vivo* assay of NR activity, presenting the maximal activity when the N_3 rate was used with a 12% increase with respect to the minimal activity found with N_1. On the other hand, the NO_3^- levels (Table 1) showed a behavior contrary to the NR activity (Figure 1), given that the maximal foliar levels were reached with N_1 (8.43 mg g^{-1} dw), while the minimum resulted from N_3 (7.29 mg g^{-1} dw). One of the principal factors regulating both the increase in *de novo* NR synthesis and its activity is the presence of NO_3^- (Campbell, 1988; Kaiser and Huber, 1994; Crawford, 1995; Sivasankar and Oaks, 1996). In view of this fact, an increase in N fertilization (as caused by the treatments N_2P_1 and N_3P_1) would raise the foliar levels of NO_3^-. This should stimulate NR activity (Figure 1) and explain the

decreased foliar NO_3^- content in these treatments (Table 1) due to its reduction. The relationship between the two parameters in our experiment proved negative (r^2)-0.754**). With respect to the nitrogenous compounds of high and low molecular weight, such as amino acids and proteins, these are the principal products of NO_3assimilation (Barneix and Causin, 1996). When the P rate was P_1, we found no effect of the different N rates on the foliar amino acid levels (Table 1). On the contrary, the protein levels (Table 1) reflected the greatest reduction of NO_3^-, since the N_2P_1 and N_3P_1 treatments registered the maximal concentrations. Organic nitrogen was another parameter that strengthened with intensified NO_3^- reduction (Vincentz et al., 1993). Compared to the amino acids and proteins, the organic-nitrogen concentration (Table 1) augmented proportionally to the increase in NR activity (Figure 1), given that the maximal concentration was found when the nitrogen rate was N_3 (47.84 mg g^{-1} dw). The relationship between the NR activity and the organic nitrogen concentration was positive (r^2) 0.827**), and there was a close relationship between the NO_3^- reduction and the organic-nitrogen content.

Nitrogen fertilization plays an important role in the growth and development of most plants (Silberbush and Lips, 1991; Mattson et al., 1991). Yield (g $plant^{-1}$) was clearly influenced by the nitrogen rate applied, since the maximum values in total as well as commercial and noncommercial yield were recorded for the N_3 treatment (Table 2). Increased N fertilization raised both the noncommercial and commercial yields (Table 2).

When the P rate was P_2, the trend described above varied for some parameters, indicating a possible influence of P. The NR activity (Figure 1) was again influenced by N fertilization, since as the N rate increased, so did enzymatic activities; N_3 registered a 16% increase with respect to the minimal activity values of N_1. In relation to NO_3^- (Table 1), in contrast to the previous case (P_1), no decrease was recorded in the foliar level in proportion to increased NR activity, given that N_1P_2 and N_3P_2 gave the minimal level (6.86 mg g^{-1}dw). In this case, these parameters showed no relationship (r^2=0.271 ns), indicating that a higher P application could influence foliar NO_3^- levels.

Amino acids and proteins (Table 1) showed a trend similar to that observed when the P fertilization wasP1, given that no clear effect was found of the N rate on these levels, as differences between treatments were minimal. Finally, organic N (Table 1), as was true when the P rate was P1, proved strongly influenced by N, the maximal concentration appearing at N_3(48.82 mg g^{-1}dw) and the minimum at N_1(45.39 mg g^{-1}dw). The relationship between the NR activity and the organic-nitrogen concentration was again positive (r^2=0.799**), indicating the lack of interference of P in NO_3^- assimilation

Yields were directly dependent on the N rate applied (Table 2), given that N_3 gave the maximum values (18.67 and 17.57 kg $plant^{-1}$, respectively). It is striking that noncommercial yield differed from the rest in that the minimal value was recorded for the N_3 rate (1.10 kg $plant^{-1}$), showing a trend completely opposite that shown when the P rate was P_1, and thus, increased P fertilization appears to diminish noncommercial yield.

In this sense, we conclude that regardless of the rate of P applied, the N fertilization exerts direct influence on NO_3^- assimilation with increases in NR activity, on the foliar concentration of organic N (Figure 1; Table 1), and on both total and commercial yield (Table 2). The data suggest that increased fertilization did not result in N intoxication as a result of (i) the low foliar NO_3^- levels found at N_3, indicating that levels of this ion that would saturate or exceed assimilation capacity were not reached (Mackown et al., 1990); and (ii) the low amino-acid levels (Table 1), indicating that N concentrations were not great enough to raise amino acid levels and thereby inhibit NO_3^- reduction (Barneix and Causin, 1996; Padgett and Leonard, 1996; Sivasankar and Oaks, 1996). Finally, P_2 played a part in the foliar NO_3^- content and noncommercial yield, decreasing the levels of both (Tables 1 and 2).

Response to P Rate. Regardless of the N level applied, the use of P_2 increased the NR activity (Figure 2) in comparison with the activity shown by this enzyme when the P rate was P_1, this increase in all cases exceeded 10%. The foliar NO_3^- content (Table 3) also showed an influence by the P_2

fertilization, since regardless of the N level used, the application of P_2 decreased NO_3^- levels. It is possible that the larger P application reversed the processes described in the Introduction in relation to P deprivation as well as the effect of this element on N metabolism, thereby increasing absorption, translocation toward the aerial part, and NO_3^- assimilation (Rufty et al., 1993; Pilbeam et al., 1993). This would explain the increased NR activity (Figure 2) and the decrease in the NO_3^- level (Table 3).

With respect to the amino acids and proteins (Table 3), the P rate had no appreciable influence, since either there was no statistical significance in the case of the amino acids or the behavior was variable in the case of the proteins. On the contrary, the organic-N concentration (Table 3) increased with P_2, due possibly to the strengthening effect of the P rate on NO_3^- assimilation. Finally, in relation to yield, P_2 showed a negative effect on total and commercial yield when the N rate was N_1, decreasing the values of both (Table 4), while for the N_2 and N_3 rates, an increase in P raised total and commercial yield (Table 4). The P_2 rate caused noncommercial yield to fall sharply and significantly regardless of the N level (Table 4).

Response to NP Fertilization. This aspect of our study was undertaken to compare statistically all of the fertilization treatments, in order to identify the most adequate combination or combinations. The values of NR activity (Figure 3) show that the minimum activity was given by N_1P_1, indicating that the combination of low rates of N (N_1 and N_2) and P (P_1) did not increase NR activity. On the contrary, the application of N_3P_2 produced greater NR activity (Figure 3), with an increase of 28% over the minimum. With respect to the NO_3^- values (Table 5), the treatments N_1P_2, N_2P_2, and N_3P_2 gave the lowest levels, with maximal decreases of 19% with respect to the maximum presented in the treatment N_1P_1.

Amino acids and proteins are the main products of NO_3^- assimilation (Barneix and Causin, 1996), but these did not show a clear effect of the treatments, and thus are not useful parameters for selecting the most adequate fertilization treatments. On the other hand, the organic N did present a positive relationship with NO_3^- reduction, since the maximal concentration was presented in the combination N_3P_2 and the minimum in N_1P_1 (Table 5).

Finally, the total and commercial yield (Table 6) was maximal in N_3P_2, with increases of 13 and 14%, respectively in comparison with the minimal yield obtained in the N_1P_2 treatment. Meanwhile, noncommercial yield registered its minimal value in N_3P_2, with a decline of 44% from the maximal value of treatment N_3P_1.

Taking into account the results of these experiments, we conclude that the most adequate fertilizer combination for eggplant was N_3P_2. This treatment gave the greatest NO_3^- assimilation (minimal NO_3^- levels; maximal NR activity, and maximal organic-N concentration), largest total and commercial yield and least noncommercial yield. Finally, P_2 is noteworthy for strengthening all of the parameters and processes mentioned above, and thus, it appears that an increased P application before high N rates can reduce noncommercial in favor of commercial yield, and can avoid or reduce both NO_3^- pollution as well as the negative effects of NO_3accumulation in different plant organs.

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Table 1. Accumulation of Various N Forms due toApplication of Different N Rates[a]

treatments		mg g^{-1} fw		mg g^{-1} dw	
		amino acids	proteins	organic N	nitrate
P_1	N_1	1.37 a	25.8 b	41.1 c	8.43 a
	N_2	1.37 a	27.4 a	43.4 b	7.80 b
	N_3	1.38 a	27.3 a	47.8 a	7.29 c
P_2	N_1	1.36 a	26.3 b	45.4 c	6.86 a
	N_2	1.35 a	27.4 a	46.6 b	7.01 a
	N_3	1.34 a	26.1 b	48.9 a	6.86 a

[a]Values followed by the same letter within a column and phosphorus treatment were not measurably different ($p<0.05$).

Table 2. Effect of N Rate on Yield of Eggplant[a]

treatments		yield (kg $plant^{-1)}$		
		total	commercial	noncommercial
P_1	N_1	17.7 b	15.9 c	1.83 b
	N_2	17.9 b	16.1 b	1.86 b
	N_3	18.3 a	16.4 a	1.97 a
P_2	N_1	16.3 c	15.2 c	1.16 a
	N_2	17.9 b	16.7 b	1.16 a
	N_3	18.7 a	17.6 a	1.10 b

[a]Values followed by the same letter within a column and phosphorus treatment were not measurably different ($p<0.05$).

Table 3. Accumulation of N Compounds due to Application of the Different P Rates[a]

treatments		mg g^{-1} fw		mg g^{-1} dw	
		amino acids	proteins	organic N	nitrate
N_1	P_1	1.37 a	25.8 b	41.1 b	8.43 a
	P_2	1.36 a	26.3 a	45.4 a	6.86 b
N_2	P_1	1.37 a	27.4 a	43.4 b	7.80 a
	P_2	1.35 a	27.4 a	46.6 a	7.01 b
N_3	P_1	1.38 a	27.3 a	47.8 b	7.29 a
	P_2	1.34 a	26.1 b	48.9 a	6.86 b

[a]Values followed by the same letter within a column and nitrogen treatment were not measurably different ($p<0.05$).

Table 4. Effect of P Rate on Yield of Eggplant[a]

treatments		yield (kg plant^{-1})		
		total	commercial	noncommercial
N_1	P_1	17.7 b	15.9 a	1.83 a
	P_2	16.3 b	15.2 b	1.16 b
N_2	P_1	17.9 a	16.1 b	1.86 a
	P_2	17.9 a	16.7 a	1.16 b
N_3	P_1	18.3 a	16.4 b	1.97 a
	P_2	18.7 a	17.6 a	1.10 b

[a]Values followed by the same letter within a column and nitrogen treatment were not measurably different ($p<0.05$).

Table 5. Accumulation of N Compounds due toApplication of the Different NP Rates[a]

	mg g^{-1} fw		mg g^{-1} dw	
treatments	amino acids	proteins	organic N	nitrate
N_1P_1	1.37 a	25.8 c	41.1 e	8.43 a
N_2P_1	1.37 a	27.4 a	43.4 d	7.80 b
N_3P_1	1.38 a	27.3 ab	47.8 b	7.29 bc
N_1P_2	1.36 a	26.3 b	45.4 cd	6.86 c
N_2P_2	1.35 a	27.4 a	46.6 c	7.01 c
N_3P_2	1.34 a	26.1 b	48.9 a	6.86 c

[a]Values followed by the same letter within a column were not measurably different ($p<0.05$).

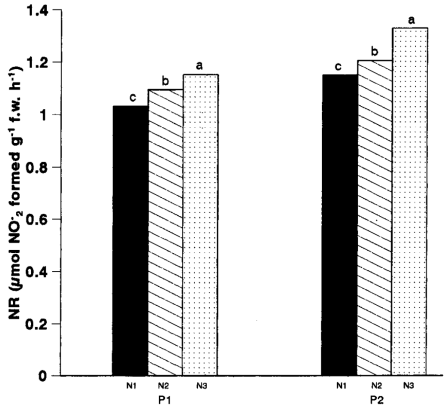

Figure 1. Effect of the different N rates (N_1 solid bar, N_2 striped bar, N_3 dotted bar) on the in vivo nitrate-reductase activity. For each P rate, different letters indicate a significant effect ($p<0.05$) according to Duncan's test

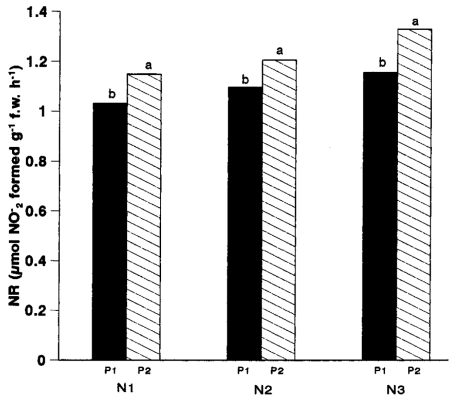

Figure 2. Effect of the different P rates (P_1 solid bar, P_2 striped bar) on the in vivo nitrate-reductase activity. For each N rate, different letters indicate a significant effect ($p<0.05$) according to Duncan's test.

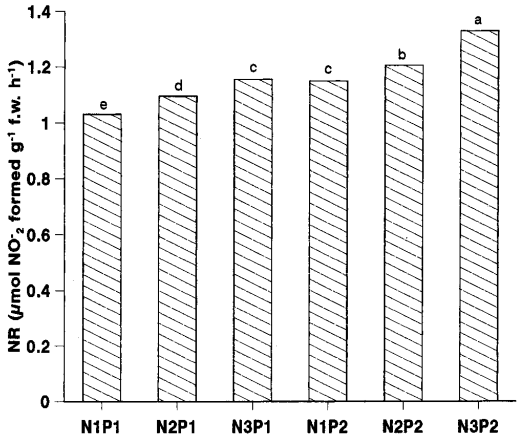

Figure 3. Effect of NP rates on the in vivo nitrate-reductaseactivity. Different letters indicate a significant effect ($p<0.05$) according to Duncan's test.