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

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The effect that application of nitrogen-phosphorus (NP) rates exerts on some parameters of phosphorus metabolism in 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<sub>3</sub> (N<sub>1</sub> = 15 g m<sup>-2</sup>; N<sub>2</sub> = 22.5 g m<sup>-2</sup>; and N<sub>3</sub> = 30 g m<sup>-2</sup>) together with two rates of P in the form of H<sub>3</sub>PO<sub>4</sub> (P<sub>1</sub> = 24 g m<sup>-2</sup> and P<sub>2</sub> = 36 g m<sup>-2</sup>), for a total of six treatments. The results obtained show a positive effect of NP fertilization on the nutritional status of P in the plants, clearly reflected in the response of bioindicators of the P (acid phosphatase activity and carbohydrates). The plants treated with N<sub>3</sub>P<sub>2</sub> registered greater integration or assimilation of inorganic P to organic P, a fact that may be related to the maximum total and commercial yield in these plants and minimum noncommercial yield.

Keywords: Solanum melongena L.; NP fertilization; phosphorus metabolism; bioindicators

## INTRODUCTION

Phosphorus (P), an essential element for higher plants and required in substantial concentrations in plant tissues, is particularly critical during vegetative growth (Jeschke et al., 1996). The principal effects of P deficiency include a reduction of foliar expansion (Fredeen et al., 1989), decrease in the number of leaves (Lynch et al., 1991), and loss in photosynthetic efficiency (Lauer et al., 1989). In terms of dry-matter yield, the roots are much less affected by P deficiency than are the leaves (Heuwinkel et al., 1992). Studies on the nutritional status of P indicate that the P concentration in the leaves has a powerful impact on the regulation of phosphate flux (Adalsteinsson et al., 1994).

The acquisition of nutrients of low mobility such as P is strongly influenced by the morphological and physiological properties of the roots (Adalsteinsson et al., 1994). Various physiological responses have been demonstrated within roots, such as the release of organic acids, especially citrate (Hoffland et al., 1989a), the release of phosphatase and, therefore, the increase of its activity in the rhizosphere (Barret-Lennard et al., 1993), and the acidification of the rhizosphere by the net release of H<sup>+</sup>, which takes place principally when plants are supplemented with NO<sub>3</sub><sup>-</sup>-N (Hoffland et al., 1989b; le Bot et al., 1990). All of the physiological responses enhanced the uptake of P from the soil.

In the present work, we analyzed the effect of N  $(NO_3^{-}-N)$  and P  $(H_3PO_4)$  fertilization on the nutritional status of P and investigated the response of the acid phosphatase activity (APA) and the foliar levels of carbohydrates as the principal bioindicators of the P. On the other hand, because nitrogen fertilizers  $(NO_3^{-}-N)$  are relatively expensive and can contribute to ground

and surface pollution through leaching and soil erosion (Sisson et al., 1991) and because of the low effectiveness in some cases of phosphorus fertilizers (Xiong and Zhou, 1995), the present work was also aimed at establishing the ideal NP dosage under our experimental conditions, with the ultimate objective of promoting efficient and nonpolluting cultivation of greenhouse eggplant.

## MATERIALS AND METHODS

Crop Design. Solanum melongena cv. Bonica were seeded in cell flats (cell size 3  $\times$  3  $\times$  10 cm) filled with peat-lite mixture, which were placed on benches under the greenhouse conditions described below for a period of 8 weeks; the seedlings were then 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<sub>3</sub> equivalent (26.82%); CaCO<sub>3</sub> active (14.35%); total N (3.5 g  $kg^{-1}$ ; total organic C (36.1 g  $kg^{-1}$ );  $PO_4^{-3}$  (890 mg  $kg^{-1}$ ) (Watenabe and Olsen, 1965),  $K^+$  (5.34 g  $kg^{-1}$ ); pH (H<sub>2</sub>O, 8.45; KCl, 8.01); electrical conductivity (EC = 4.63 dS m<sup>-1</sup>). The relative humidity was 60-80% and the temperature range 24  $\pm$  4 °C with extremes of 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 drip irrigated. Each treatment was replicated four times in plots of  $4 \text{ m} \times 2 \text{ m}$  wide (24 plots). Each plot contained eight plots of 4 m  $\times$  2 m while (24 plots). Each plot contained eight plants. The irrigation water had the following properties: pH, 8.05; EC, 2.03 dS m<sup>-1</sup>; Cl<sup>-</sup>, 483.90 mg L<sup>-1</sup>; Na<sup>+</sup>, 305.76 mg L<sup>-1</sup>; K<sup>+</sup>, 10.16 mg L<sup>-1</sup>; HCO, 278.15 mg L<sup>-1</sup>.

The treatments consisted of applying increasing rates of both N and P in the following manner: N in the form of KNO<sub>3</sub> (N<sub>1</sub> = 15 g m<sup>-2</sup>; N<sub>2</sub> = 22.5 g m<sup>-2</sup>; and N<sub>3</sub> = 30 g m<sup>-2</sup>) and P in the form of H<sub>3</sub>PO<sub>4</sub> (P<sub>1</sub> = 24 g m<sup>-2</sup>; and P<sub>2</sub> = 36 g m<sup>-2</sup>). Calcium (11 g m<sup>-2</sup>) and magnesium (3 g m<sup>-2</sup>) were supplied as sulfates. The rate of each nutrient was applied gradually with the irrigation water over the entire growth period of the plants.

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Fertilization–irrigation was complemented with the following micronutrients: Fe = 0.5 mg L<sup>-1</sup>; B = 0.1 mg L<sup>-1</sup>; Mn = 0.1 mg L<sup>-1</sup>; Zn = 0.075 mg L<sup>-1</sup>; Cu = 0.075 mg L<sup>-1</sup>; and Mo = 0.05 mg L<sup>-1</sup>. The pH values of the solution oscillated between 5 and 6. Fe was applied as FeEDDHA and B as H<sub>3</sub>BO<sub>3</sub>. The remaining micronutrients were applied as sulfates.

**Plant Sampling.** Leaf samples were taken only from plants with fully expanded leaves of the same size (>25 cm). Leaves were removed from a zone at about two-thirds of the total height of the plant, during flowering (95 days after transplanting until 170 days) every 2 weeks (López-Cantarero et al., 1995). Leaves were rinsed three times in distilled water after disinfection with nonionic detergent at 1% (Decon 90, Merck) (Wolf, 1982) and then blotted on filter paper. At each sampling, fresh leaf matter was used for the APA assay and carbohydrates (glucose, fructose, sucrose, and starch); a subsample was dried in a forced-air oven at 70 °C for 24 h and ground in a Wiley mill before further analyses (total P, inorganic P, and organic P).

**Plant Analysis.** Acid phosphatase activity (APA) was determined according to the method of Besford (1979). Fresh leaf matter was homogenized with 0.1 M sodium acetate buffer and then centrifuged at 30000*g*. All procedures were carried out at 4 °C. Enzyme extracts were incubated for 30 min at 30 °C. Hydrolysis of the *p*-nitrophenyl phosphate (p-NPP) was determined as APA by spectrophotometry at 405 nm. The results were expressed as micromoles of p-NPP hydrolyzed per hour at 30 °C.

Carbohydrates were measured following the method of Irigoyen et al. (1992), with appropriate adaptations being made for our plant material. A sample of 0.1 g of fresh leaf matter was homogenized twice with 95% ethanol (v/v) and washed with 70% ethanol (v/v), followed by centrifugation at 1500g for 15 min. Glucose, fructose, and sucrose were determined in the resulting supernatant by spectrophotometry at 650 nm, using the colorimetric assay with anthrone reagent. Starch was determined from the dried residue of the previous extraction, which was incubated in 4.5 M buffer acetate, 0.5 w/v  $\alpha$ -glucoamylase, and water for 48 h at 37 °C. Starch levels were calculated from the multiplication by 0.9 of the glucose obtained (Ettel, 1981). The concentrations of all carbohydrates analyzed were expressed as milligrams per gram of fresh weight (fw).

Leaf dry matter was digested with 96%  $H_2SO_4$  in the presence of hydrogen peroxide ( $H_2O_2$ ). *Total P* was analyzed according to the vanadomolybdophosphoric colorimetric method at 430 nm (Hogue et al., 1970). *Inorganic P* was determined in an aqueous extraction (Cataldo et al., 1975) and measured following the same procedure as for total P. *Organic P* levels were calculated from the difference between total and inorganic P. Results were expressed as milligrams per gram of dry weight (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 fruits lacking these qualities represent noncommercial or residual yield. Total yield (kilogram per plant) is the sum of both types of yield.

**Statistical Analysis.** The data were submitted to three statistical treatments 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 techniques were used to assess the treatment means. As there were no climatic effects on yield, 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 to 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.

 Table 1. Acumulation of Various P Forms due to

 Application of Different N Rates<sup>a</sup>

			$mg \ g^{-1} \ of \ dw$	
trea	tments	total P	inorganic P	organic P
P <sub>1</sub>	$N_1$	5.62 b	3.25 b	2.37 b
	$N_2$	5.89 b	3.49 a	2.40 b
	$N_3$	6.34 a	3.56 a	2.78 a
$P_2$	$N_1$	6.45 b	3.97 a	2.48 c
	$N_2$	7.31 a	4.01 a	3.30 b
	$N_3$	7.84 a	3.63 b	4.21 a

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

#### **RESULTS AND DISCUSSION**

**Response to the N Rate.** When the P rate was  $P_1$ , the different P fractions as well as the principal bioindicators of P were directly influenced by the N rate applied. In relation to total and organic P (Table 1), greater N rates resulted in increased parameters, maximum concentrations being recorded at the  $N_3$  rate. As indicated in the Introduction, one of the processes that facilitate the uptake of low-mobility nutrients is acidification of the rhizosphere, by the net release of H<sup>+</sup>, which takes place principally when plants are supplemented with  $NO_3^-$  (Hoffland et al., 1989b; le Bot et al., 1990). In our experiment, the N was applied in the form of  $NO_3^-$ ; thus, the increased N rates apparently boosted the P uptake by acidifying the rhizosphere.

As with total and organic P, the inorganic P rose as the N dosage increased (Table 1). There are two basic contradictory opinions concerning the behavior of inorganic P in response to more or less P uptake. Some researchers suggest that the difference in P nutritional status in a plant alters the level of inorganic P in the leaf (Crafts-Brander, 1992), while others hold that inorganic P is completely independent of P status (Foyer and Spencer, 1986). In our experiment, when the P rate was P<sub>1</sub>, the relationship between the total and inorganic P was positive and significant ( $r^2 = 0.74$  \*), implying dependence of the inorganic P on the nutritional status of P.

The rise in foliar levels of inorganic P with increased N application (Table 1) is reflected in the APA (Figure 1), given that the activity of this enzyme shows a close relationship with the nutritional status of P, and more specifically with inorganic P, showing a rise in the APA under conditions of P deficiency (Barret-Lennard et al., 1993; Tadano et al., 1993; Ruiz et al., 1996). In fact, the greater enzymatic activities correspond to the lower levels of inorganic P (N<sub>1</sub>P<sub>1</sub>), presenting a negative relationship between the two parameters ( $r^2 = -0.82$  \*\*).

The principal indicators studied for P uptake and translocation toward the leaves were carbohydrates. Contradictions persist concerning the function of carbohydrates with regard to P uptake. According to some authors, good P uptake and translocation toward the leaves involve the transport of carbohydrates toward the roots, thereby diminishing the foliar content of these carbohydrates (Qui and Israel, 1992; Ruiz et al., 1996). This is primarily because both P absorption by the plasmalemma of the roots cortex cells and the xylemloading process require carbohydrates as an energy source (Loughman, 1987). However, other researchers have indicated that the low or high P absorption is not necessarily related to an increase or decrease in foliar



**Figure 1.** Effect of the different N rates on the acid phosphatase activity. For each P rate, different letters indicate a significant effect (p < 0.05) according to Duncan's test.

 Table 2. Acumulation of Carbohydrates due to

 Application of Different N Rates<sup>a</sup>

			$\mathrm{mg}~\mathrm{g}^{-1}$ of fw		
trea	tments	glucose	fructose	sucrose	starch
P <sub>1</sub>	N <sub>1</sub> N <sub>2</sub>	28.29 a 26 53 b	26.27 a 23 46 b	40.46 a 38 23 b	37.06 a 35 46 b
	$N_3$	22.18 c	19.96 c	36.92 c	32.27 c
P <sub>2</sub>	$\begin{array}{c} N_1 \\ N_2 \\ N_3 \end{array}$	19.68 a 15.89 b 13.21 c	17.41 a 14.92 b 11.76 c	35.67 a 30.22 b 28.41 c	33.92 a 30.08 b 29.59 b

<sup>*a*</sup> Values followed by the same letter within a column and phosphorus treatment were not measurably different (p < 0.05).

carbohydrate level (Crafts-Brandner, 1992). In our experiment, under the low P dosage  $(P_1)$ , heavier N application lowered the foliar concentrations of all the sugars studied (Table 2). That is, higher N rates ( $NO_3^{-1}$ -N) increased P absorption, which in turn boosted sugar translocation and utilization by the roots. This accounts for the fact that the plants treated with N<sub>3</sub>P<sub>1</sub> registered maximum foliar concentrations of total P (Table 1) and minimum concentrations in sugars (Table 2), the relationships between these parameters being negative and significant (total P-glucose,  $r^2 = -0.83$  \*\*; total P-fructose,  $r^2 = -0.85$  \*\*; total P-sucrose,  $r^2 = -0.88$  \*\*; total P-starch,  $r^2 = -0.84$  \*\*). However, the processes of assimilation of inorganic P to organic P and of NO<sub>3</sub><sup>-</sup> to organic molecules, which are high in these plants (N<sub>3</sub>P<sub>1</sub>) (López-Cantarero et al., 1997), also depend on an energy expense, perhaps contributing to the decrease of foliar carbohydrates.

In relation to production, nitrogen fertilization plays an important role in the growth and development of most plants (Mattson et al., 1991; López-Cantarero et al., 1997). Yield (kilogram per plant) 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<sub>3</sub> treatment (Table 3). It is also noteworthy that with this treatment of maximum yield (N<sub>3</sub>P<sub>1</sub>), the highest levels of total and organic P were registered (Table 1), and these parameters can directly influence yield increases (Adalsteinsson et al., 1994; Jeschke et al., 1996). Table 3. Effect of N Rate on Yield of Eggplant<sup>a</sup>

		yield, kg plant $^{-1}$			
treatments		total	commercial	noncommercial	
P <sub>1</sub>	N <sub>1</sub>	17.7 b	15.9 с	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	

<sup>&</sup>lt;sup>*a*</sup> Values followed by the same letter within a column and phosphorus treatment were not measurably different (p < 0.05).



**Figure 2.** Effect of the different P rates on the acid phosphatase activity. For each N rate, different letters indicate a significant effect (p < 0.05) according to Duncan's test.

The P<sub>2</sub> rate, with respect to the parameters discussed above, showed a trend similar to that of P<sub>1</sub>. The increased N (N<sub>3</sub>P<sub>2</sub>) rate again raised foliar concentrations of total and organic P (Table 1). Nevertheless, in contrast to P<sub>1</sub>, a higher concentration of total P did not determine the rise in the levels of inorganic P (Table 1), since the maximum concentration of inorganic P occurred in N<sub>2</sub>P<sub>2</sub> and the minimum in N<sub>3</sub>P<sub>2</sub>, the relationship in this case being nonsignificant ( $r^2 = -0.42$ ns). This indicates the independence of the inorganic P with respect to the nutritional status of P when the rate of the P (P<sub>2</sub>) applied is increased.

The APA again shows a highly significant and negative relationship with the inorganic P ( $r^2 = -0.81$  \*\*), representing the maximum activity in N<sub>3</sub>P<sub>2</sub> with an increase of 32% compared to the minimum of N<sub>2</sub>P<sub>2</sub> (Figure 2). The relationship obtained between the APA and the inorganic P when the fertilization was P<sub>1</sub>, as well as that found for P<sub>2</sub>, defined the APA as the principal bioindicator of the nutritional status of P.

The behaviors of the sugars were similar in both  $P_1$ and  $P_2$  fertilization, foliar concentrations falling with rising N rates (Table 2). It was striking that the combination  $N_3P_2$  gave the minimum sugar concentrations, possibly because of (i) greater P uptake and translocation, reflected in the maximum foliar concentration of this nutrient, and (ii) greater assimilation or integration of inorganic P to organic P in these plants, raising the foliar levels of the latter (Table 1). The relationships between the concentrations of total P and the different foliar levels of sugars at  $P_2$  were again significant and negative (total P-glucose,  $r^2 = -0.83^{**}$ ;

 Table 4. Acumulation of Various P Forms due to

 Application of Different P Rates<sup>a</sup>

			${\rm mg}~{\rm g}^{-1}$ of dw	
treatments		total P	inorganic P	organic P
N <sub>1</sub>	P <sub>1</sub>	5.62 b	3.25 b	2.37 a
	$P_2$	6.45 a	3.97 a	2.48 a
$N_2$	$P_1$	5.89 b	3.49 b	2.40 b
	$P_2$	7.31 a	4.01 a	3.30 a
$N_3$	$P_1$	6.34 b	3.56 a	2.78 b
	$P_2$	7.84 a	3.63 a	4.21 a

<sup>*a*</sup> Values followed by the same letter within a column and nitrogen treatment were not measurably different (p < 0.05).

total P-fructose,  $r^2 = -0.81$  \*\*; total P-sucrose,  $r^2 = -0.84$  \*\*; total P-starch,  $r^2 = -0.80$  \*\*).

Finally, yields were directly dependent on the N rate applied (Table 3), given that  $N_3$  gave the maximum values in total and commercial yield. This again means that, in this treatment,  $N_3P_2$  gave the maximum foliar concentrations of total and organic P (Table 1), a situation which perhaps influenced the increase in yield. It is striking that noncommercial yield differed from the rest in that the minimal value was recorded for the  $N_3$ rate (Table 3), showing a trend completely opposite of that shown when the P rate was  $P_1$ ; 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 directly influenced the foliar concentration of P and its utilization and influenced yield, given that the treatments  $N_3P_1$  and  $N_3P_2$  presented the maximum foliar concentrations of total and organic P (Table 1) as well as maximum total and commercial yield (Table 3). The essential role of N in growth and yield (Mattson et al., 1991) is possibly the reason for the response of these parameters. On the other hand, the trends of the bioindicators of P (APA and carbohydrates) were defined principally by total and organic P. Finally, P<sub>2</sub> played a part in the foliar inorganic P content (Table 1) and noncommercial yield (Table 3).

**Response to P Rate.** Independent of the N rate applied, the  $P_2$  rate considerably boosted foliar concentrations of total and organic P, in relation to those found when  $P_1$  was applied (Table 4). Inorganic P (Table 4) increased with the application of  $P_2$  with the  $N_1$  and  $N_2$  rates, while no significant differences were found with the  $N_3$  rate. These results show a direct effect of P application on the foliar concentration of total, organic, and inorganic P, the latter only with  $N_1$  and  $N_2$  rates, while the trend of inorganic P at high NP rates was possibly due to the interaction of these elements.

The trend of APA (Figure 2) was similar to that of inorganci P, given that its activity increased when  $P_2$  was applied at the  $N_1$  and  $N_2$  rates, whereas no statistical differences were found between the  $P_1$  and  $P_2$  activities at the  $N_3$  rate.

With respect to sugars (Table 5), regardless of the N rate used, the increase in uptake, translocation, and subsequent accumulation of P in the leaves, caused by the application of the  $P_2$ , resulted in a lower foliar concentration of all sugars, due largely to their utilization in the above processes (uptake and translocation).

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 6), while for the  $N_2$  and  $N_3$  rates, an increase in P raised total

 Table 5. Acumulation of Carbohydrates due to

 Application of Different P Rates<sup>a</sup>

		mg $g^{-1}$ of fw				
treat	tments	glucose	fructose	sucrose	starch	
$N_1$	$P_1$	28.29 a	26.27 a	40.46 a	37.06 a	
	$\mathbf{P}_2$	19.68 b	17.41 b	35.67 b	33.92 b	
$N_2$	$P_1$	26.53 a	23.46 a	38.23 a	35.46 a	
	$\mathbf{P}_2$	15.89 b	14.92 b	30.22 b	30.08 b	
$N_3$	$P_1$	22.18 a	19.96 a	36.92 a	32.27 a	
	$\mathbf{P}_2$	13.21 b	11.76 b	28.41 b	29.59 b	

<sup>*a*</sup> Values followed by the same letter within a column and nitrogen treatment were not measurably different (p < 0.05).

Table 6. Effect of P Rate on Yield of Eggplant<sup>a</sup>

		yield, kg plant $^{-1}$				
trea	tments	total	commercial	noncommercial		
N <sub>1</sub>	P1	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 7. Accumulation of Various P Forms due to

 Application of the Different NP Rates<sup>a</sup>

	$\mathrm{mg}~\mathrm{g}^{-1}$ of dw		
treatments	total P	inorganic P	organic P
$N_1P_1$	5.62 c	3.25 с	2.37 d
$N_2P_1$	5.89 c	3.49 b	2.40 d
$N_3P_1$	6.34 bc	3.56 b	2.78 с
$N_1P_2$	6.45 b	3.97 a	2.48 d
$N_2P_2$	7.31 a	4.01 a	3.30 b
$N_3P_2$	7.84 a	3.63 ab	4.21 a

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

and commercial yield (Table 6). The  $P_2$  rate caused noncommercial yield to fall sharply and significantly regardless of the N level (Table 6). These results indicate an individual effect of  $P_2$ , both negative in reducing the commercial yield at  $N_1$  and positive in diminishing noncommercial yield at all the N rates.

**Response to NP Fertilization.** This aspect of our study was undertaken to compare statistically all of the fertilization treatments, with the ultimate aim of identifying the most adequate combinations. Table 7 shows the rise in total P values as the P and N rates increase, due possibly to the individual effects of the greater application of both nutrients: (i) possible increase in the acidification of the rhizosphere by N and (ii) increase in P availability because of increased fertilization. This fact indicates that the concentrations in total P obtained were not toxic in any combination, since excessive P rates resulted in constant concentrations of this element, while the integration of inorganic to organic diminished, and the inorganic form showed a tendency to accumulate (Marschner, 1995). In addition, it is worth noting that the application of the combination  $N_3P_2$  led to a greater P content in the plant, perhaps signifying a fall in P use to reach the P level obtained in this treatment.

The trend found for P was similar to that found for total P (Table 7). Both N and P are essential nutrients



**Figure 3.** Effect of NP rates on the acid phosphatase activity. Different letters indicate a significant effect (p < 0.05) according to Duncan's test.

Table 8. Accumulation of Carbohydrates due toApplication of the Different NP Rates<sup>a</sup>

		mg $g^{-1}$ of fw			
treatments	glucose	fructose	sucrose	starch	
$N_1P_1$	28.29 a	26.27 a	40.46 a	37.06 a	
$N_2P_1$	26.53 ab	23.46 b	38.23 ab	35.46 ab	
$N_3P_1$	22.18 b	19.96 c	36.92 b	32.27 bc	
$N_1P_2$	19.68 с	17.41 cd	35.67 b	33.92 b	
$N_2P_2$	15.89 d	14.92 d	30.22 c	30.08 с	
$N_3P_2$	13.21 d	11.76 d	28.41 c	29.59 с	

<sup>*a*</sup> Values followed by the same letter within a column were not measurably different (p < 0.05).

for plant growth and development (Mattson et al., 1991; Adalsteinsson et al., 1994; Jeschke et al., 1996; López-Cantarero et al., 1997), and therefore increased application of both nutrients would augment organic P. The foliar concentration of organic P was maximum at  $N_3P_2$ (Table 7), indicating a greater integration of inorganic P in this treatment. This accounts for the lowered level of inorganic P (Table 7). In short, the overall effect of NP interaction in this treatment ( $N_3P_2$ ) appears to be responsible for the independence of inorganic P with respect to total P.

With regard to the bioindicators studied, APA (Figure 3) shows a highly significant and close relationship with the foliar concentration of inorganic P ( $r^2 = -0.86$  \*\*) already reported in other works (Barret-Lennard, 1993; Tadano et al., 1993; Ruiz et al., 1996). The behavior of the sugars (Table 8) reflects, as reported by other authors (Qui and Israel, 1992; Ruiz et al., 1996), that good P uptake and translocation toward the leaves involve the transport of carbohydrates toward the roots, thereby diminishing the foliar content of these carbohydrates and increasing the foliar content of P. The relationships obtained using all of the NP combinations support this hypothesis (total P-glucose,  $r^2 = -0.86$  \*\*; total P-fructose,  $r^2 = -0.89$  \*\*; total P-sucrose,  $r^2 =$ -0.90 \*\*; total P-starch,  $r^2 = -0.82$  \*\*). Finally, this means that the predominant sugar was sucrose (Table 8). Under saline conditions, as in our experiment, the sucrose content increases, facilitating the osmoregulatory process of plants (Hawker et al., 1991).

Finally, the total and commercial yields (Table 9) were maximal in  $N_3P_2$ , with increases of 13 and 14%, respec-

Table 9. Effect of NP Rates on Yield of Eggplant<sup>a</sup>

		yield, kg plant $^{-1}$		
treatments	total	commercial	noncommercial	
$N_1P_1$	17.7 b	15.9 bc	1.83 b	
$N_2P_1$	17.9 b	16.1 b	1.86 b	
$N_3P_1$	18.3 a	16.4 b	1.97 a	
$N_1P_2$	16.3 c	15.2 c	1.16 c	
$N_2P_2$	17.9 b	16.7 b	1.16 c	
$N_3P_2$	18.7 a	17.6 a	1.10 c	

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

tively, in comparison with the minimal yield obtained in the  $N_1P_2$  treatment. Meanwhile, noncommercial yield (Table 9) registered its minimal value in  $N_3P_2$ , with a decline of 44% from the maximal value of treatment  $N_3P_1$ .

In conclusion, the results obtained show a positive effect of NP fertilization on the nutritional status of P in the plants, clearly reflected in the response of bioindicators of the P (acid phosphatase activity and carbohydrates). Taking into account the results of these experiments, we conclude that the most adequate fertilizer combination for eggplant was  $N_3P_2$ . The plants treated with  $N_3P_2$  registered greater integration or assimilation of inorganic P to organic P, a fact that may be related to the maximum total and commercial yield in these plants and minimum noncommercial yield. However, further research should be focused on increased NP fertilization to test whether higher rates could improve performance in the eggplant.

### LITERATURE CITED

- Adalsteinsson, S.; Schjorring, J. K.; Jensén, P. Regulation of phosphate influx in winter wheat: root-shoot phosphorus interactions. J. Plant Physiol. 1994, 143, 681–686.
- Barrett-Lennard, E. G.; Dracup, M.; Greenway, H. Role of extracellular phosphatases in the phosphorus-nutrition of clover. J. Exp. Bot. **1993**, 44, 1595–1600.
- Besford, R. T. Phosphorus nutrition and acid phosphatase activity in leaves of seven plant species. *J. Sci. Food Agric.* **1979**, *30*, 282–285.
- Cataldo, D. A.; Haroon, M.; Schrader, L. E.; Young, V. L. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Commun. Soil Sci. Plant Anal.* 1975, 6, 71–80.
- Crafts-Brandner, S. J. Phosphorus nutrition influence on leaf senescence in soybean. *Plant Physiol.* **1992**, *98*, 1128–1132.
- Ettel, W. Eine neue enzymatische stäskebestimmung für lebensmittel. *Alimenta* **1981**, *20*, 7–11.
- Foyer, C.; Spencer, C. The relationship between phosphate status and photosynthesis in leaves. Effects on intracellular orthophosphate distribution, photosynthesis, and assimilate partitioning. *Planta* **1986**, *167*, 369–375.
- Fredeen, A. L.; Rao, I. M.; Terry, N. Influence of phosphorus nutrition on growth and carbon partitioning in *Glycine max. Plant Physiol.* **1989**, *89*, 225–230.
- Hawker, J. S.; Jennes, C. F.; Niemietz, C. M. Sugar metabolism and compartmentation. *Aust. J. Plant Physiol.* 1991, 18, 227–237.
- Heuwinkel, H.; Kirkby, E. A.; le Bot, J.; Marschner, H. Phosphorus deficiency enhanes molybdenum uptake by tomato plants. *J. Plant Nutr.* **1992**, *15*, 549–568.
- Hoffland, E.; Findenegg, G. R.; Nelemans, J. A. Solubilization of rock phosphate by rape. II. Local root exudation of organic acids as a response to P-starvation. *Plant Soil* **1989a**, *113*, 161–165.
- Hoffland, E.; Findenegg, G. R.; Nelemans, J. A. Solubilization of rock phosphate by rape. I. Evaluation of the role of the nutrient uptake pattern. *Plant Soil* **1989b**, *113*, 155–160.

- Hogue, E.; Wilcow, G. E.; Cantliffe, D. J. Effect of soil P on phosphate fraction in tomato leaves. J. Am. Soc. Hortic. Sci. 1970, 95, 174–176.
- Irigoyen, J. J.; Emerich, D. W.; Sánchez-Diaz, M. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. *Physiol. Plant.* **1992**, *84*, 55–60.
- Jeschke, W. D.; Puke, A.; Kirkby, E. A.; Pate, J. S.; Hartung, W. Effects of P deficiency on the uptake, flows and utilization of C, N and H<sub>2</sub>O within intact plants of *Ricinus communis* L. J. Exp. Bot. **1996**, 47, 1737–1754.
- Lauer, M. J.; Blevins, D. G.; Sierzputowska-Gracz, H. <sup>31</sup>Pnuclear magnetic resonance determination of phosphate compartmentation in leaves of reproductive soybeans (*Glycine max* L.) affected by phosphate nutrition. *Plant Physiol.* **1989**, *89*, 1331–1336.
- le Bot, J.; Alloush, G. A.; Kirkby, E. A.; Sanders, F. E. Mineral nutrition of chikpea plants supplied with NO<sub>3</sub><sup>--</sup> or NH<sub>4</sub><sup>+-</sup> N. II. Ionic balance in relation to phosphorus stress. *J. Plant Nutr.* **1990**, *13*, 1591–1605.
- López-Cantarero, I.; Belakbir, A.; Romero, L. Influence of the phenological stages on ionic alterations in aubergine plants. *J. Plant Nutr.* **1995**, *18*, 1353–1370.
- López-Cantarero, I.; Ruiz, J. M.; Hernandez, J.; Romero, L. Nitrogen metabolism and yield response to increases in nitrogen-phosphorus fertilization: Improvement in greenhouse cultivation of eggplant (*Solanum melongena* cv. Bonica). J. Agric. Food Chem. **1997**, 45, 4227–4231.
- Loughman, B. C. The application of *in vivo* techniques in the study of metabolic aspects of ion absortion in crop plants. *Plant Soil* **1987**, *99*, 63–74.
- Lynch, J.; Läuchli, A.; Epstein, E. Vegetative growth of the common bean in response to phosphorus nutrition. *Crop Sci.* **1991**, *31*, 380–387.
- Marschner, H. *Mineral Nutrition of Higher Plants*, 2nd ed.; HB Publishers: London, 1995; ISBN 0-12-473542-8.

- Mattson, M.; Lundborg, T.; Larsson, M.; Larsson, C. M. Nitrogen utilization in N-limited barley during vegetative and generative growth. I. Growth and nitrate uptake kinetics in vegetative cultures grown at different relative addition rates of nitrate-N. J. Exp. Bot. **1991**, 43, 15–23.
- Qiu, J.; Israel, D. W. Diurnal starch accumulation and utilization in phosphorus-deficient soybean plants. *Plant Physiol.* **1992**, *98*, 316–323.
- Ruiz, J. M.; Belakbir, A.; Romero, L. Foliar level of phosphorus and its bioindicators in *Cucumis melo* grafted plants. A possible effect of rootstocks. *J. Plant Physiol.* **1996**, *149*, 400–404.
- Sisson, V. A.; Rufty, T. W.; Williamson, R. E. Nitrogen-use efficiency among flue-cured tobacco genotypes. *Crop Sci.* **1991**, *31*, 1615–1620.
- Tadano, T.; Ozawa, K.; Sakai, H.; Osaki, M.; Mutsui, H. Secretion of acid phosphatase by the roots of crop plants under phosphorus-deficient conditions and some properties of the enzyme secreted by lupin roots. *Plant Soil* **1993**, *155/ 156*, 95–98.
- Watenabe, F. S.; Olsen, S. R. Test of an ascorbic acid method for dertimining phosphorus in water and NaHCO<sub>3</sub> extracts from soil. *Soil Sci. Soc. Proc.* **1965**, 677–678.
- Wolf, B. A comprehensive system of leaf analysis and its use for diagnosing crop nutrients status. *Commun. Soil Sci. Plant Anal.* **1982**, *13*, 1035–1059.
- Xiong, L. M.; Zhou, Z. G. Magnesium influence on plant uptake of phosphorus in a calcareous soil. J. Plant Nutr. 1995, 18, 1251–1261.

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