Nitrogen metabolism in roots and leaves of green bean plants exposed to different phosphorus doses

Metabolismo del nitrógeno en raíces y hojas de plantas de frijol ejotero expuestas a diferentes dosis de fósforo

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Abstract. The objective of this work was to determine the effect of different P doses on nitrogen assimilation in roots and leaves of green beans plants (Phaseolus vulgaris L. cv. Strike). Phosphorus was applied in the nutrient solution as H₃PO₄, at the nutrient doses of: P₁ = 0.40 mM; P₂ = 0.80 mM; P₃ = 1.60 mM; P₄ = 3.20 mM; P₅ = 4.80 mM, and P₆ = 6.40 mM P. Our results indicate that both P toxicity and deficiency gave similar responses to N assimilation. Phosphorus and NO₃⁻ interacted on the absorption and translocation processes affecting N assimilation. The deficiency (P₁), and toxicity (P₆) treatments, diminished root absorption of NO₃⁻ in 15% and 36%, respectively, respect to the optimum dose (P₃), thus reducing nutrient availability for assimilation. This result may explain the minimum enzymatic activities observed in NO₃⁻ assimilation in P₁ and P₆. The minimum N assimilation observed in these treatments will eventually translate into a minimum synthesis of major N organic compound involved in plant growth and development. This will lead to a reduced plant biomass production and productivity of green beans plants.

Key words: Phaseolus vulgaris L., nitrogen metabolism, green bean, phosphorus, deficiency, toxicity.

Resumen. El objetivo de este trabajo fue determinar el efecto de diferentes dosis de fósforo sobre la asimilación de nitrógeno en raíces y hojas de frijol ejotero (Phaseolus vulgaris L. cv. Strike). El fósforo fue aplicado a la solución nutritiva en la forma de H₃PO₄; se usaron las siguientes dosis: P₁ = 0.40 mM; P₂ = 0.80 mM; P₃ = 1.60 mM; P₄ = 3.20 mM; P₅ = 4.80 mM, y P₆ = 6.40 mM P. Nuestros resultados indican que tanto los tratamientos de deficiencia y toxicidad de P presentaron respuestas similares en la asimilación de N. Estos resultados definen la interacción P-NO₃⁻ que se produce a nivel de absorción y movilización como el principal efecto que ejerce este macronutriente sobre la asimilación de N. Tanto la deficiencia (P₁) como la toxicidad (P₆) redujeron la absorción radical de NO₃⁻ en un 15% y 36%, respectivamente en relación a la dosis óptima (P₃), reduciendo así la disponibilidad de NO₃⁻ para ser asimilado. Nuestros resultados explicarían las actividades enzimáticas mínimas observadas para la asimilación de NO₃⁻ en P₁ y P₆. La mínima asimilación de N que se produce en estos tratamientos se traducirá eventualmente en una mínima síntesis de los principales compuestos nitrogenados orgánicos implicados en el crecimiento y desarrollo vegetal. Finalmente, esto conducirá a una menor producción de biomasa y productividad en plantas de frijol ejotero.

Palabras clave: Phaseolus vulgaris L., metabolismo nitrogenado, frijol ejotero, fósforo, deficiencia, toxicidad.
INTRODUCTION

Beans are grown and consumed in nearly all the world. In many developing countries, 20% of the available protein is provided by beans. Beans represent also an integral part of dietary protein for 50% of the world’s population (Deshpande et al., 1984). Beans are produced in large quantities in the American Continent and East Africa (Singh, 1999).

Phosphorus (P) is the second nutritional element after nitrogen that limits plant growth, having a concentration of about 0.2% of the total plant dry weight (Jeschke et al., 1996; Raghothama, 1999). This macronutrient is a key component in many molecules (i.e., nucleic acids, phospholipids and ATP) that participate in basic plant processes (Schachtman et al., 1998). In its inorganic form (Pi) it is involved in the control of many enzymatic reactions, which regulate different metabolic processes (Theodorou & Plaxton, 1993). Main effects of P deficiency include (1) the reduction in leaf number (Lynch et al., 1991) and (2) loss of photosynthetic efficiency (Lauer et al., 1989).

Nitrogen (N) and P are intimately involved in plant metabolism and growth: they interact in different biochemical processes. N participates directly in amino acid, protein, nucleic acid and other cellular component syntheses, which are needed for plant growth and development. N assimilation by plants requires NO$_3^-$ absorption, its reduction and subsequent conversion to NO$_2^-$ and NH$_4^+$, respectively, and the final NH$_4^+$ incorporation into organic compounds (Sivasankar & Oaks, 1996; Stitt, 1999). Factors influencing the enzymatic regulation responsible for N assimilation include: plant phenological stage (Ireland & Lea, 1999); light–darkness periods (Migge et al., 1996); sucrose concentration (Lam et al., 1996); N source: NO$_3^-$ and NH$_4^+$ (Ruiz & Romero, 1999); CO$_2$ level (Edwards & Coruzzi, 1989); temperature (Woodall et al., 1996); nutrient (Lopez-Lefebre et al., 2000); growth regulators (Ruiz et al., 2000), products of nitrogen assimilation (Padget & Leonard, 1996) and genetic variability (Ruiz & Romero, 1998).

Phosphorus role in N metabolism has been studied in detail, mostly related to its deficiency. Some of the effects of P deficiency on NO$_3^-$ assimilation are: (1) reduction of NO$_3^-$ absorption by roots (Rufty et al., 1991; Pilbeam et al., 1993; Jeschke et al., 1997). This might be likely caused by limited root ATP availability and limitations in the transport membrane system to NO$_3^-$ absorption (Rufty et al., 1993); (2) Decline in NO$_3^-$ translocation from roots to shoots (Rufty et al., 1991; Pilbeam et al., 1993; Jeschke et al., 1997). This is associated with the decrease of water pressure through roots and xylem (Rufty et al., 1991; Rufty et al., 1993; Jeschke et al., 1997). All these factors may diminish nitrate reductase activity, and therefore cause a decrease of NO$_3^-$ assimilation.

In general, N assimilation is affected when P-deficient plants are supplied with NO$_3^-$ However, the regulatory mechanism associated with N assimilation under nutrient (P) stress has not been completely clarified. The objective of this study was to determine the effect of different P doses on nitrogen assimilation of roots and leaves in green beans plants (Phaseolus vulgaris L. cv. Strike).

MATERIALS AND METHODS

Crop management and experimental design. Seeds of Phaseolus vulgaris cv. Strike were sown and grown in a growth chamber under controlled environmental conditions: 60-80% relative humidity, 30/20°C (day/night) temperature and 16/8 h photoperiod under a photosynthetic photon flux density of 350 μmol/m$^2$/s (measured at the top of the plants with a 190 SB quantum sensor, LI-COR Inc., Lincoln, NE). Four plants were grown in 8 l pots (25 cm upper diameter, 17 cm lower diameter, 25 cm height), filled with vermiculite. During 20 days, before the experimental treatments, all plants received a nutrient solution consisting of 6 mM NH$_4$NO$_3$; 1.6 mM H$_2$PO$_4$; 4.0 mM K$_2$SO$_4$; 4 mM CaCl$_2$·2H$_2$O; 1.4 mM MgSO$_4$·H$_2$O; 5 μM Fe-EDDHA; 2 μM MnSO$_4$·H$_2$O; 1 μM ZnSO$_4$·H$_2$O; 7H$_2$O; 0.25 μM CuSO$_4$·5H$_2$O; 0.3 μM Na$_2$MoO$_4$·2H$_2$O; and 25 μM H$_3$BO$_3$. The nutrient solution (pH= 6.0 ± 0.1) was renewed every 3 days.

Twenty days after sowing, different P treatments were applied as H$_2$PO$_4$ during 40 days (until harvest): P1: 40 mM; P2: 0.80 mM; P3: 1.60 mM; P4: 3.20 mM, P5: 4.80 mM; P6: 6.40 mM. The optimal N dose for Phaseolus vulgaris under our experimental conditions was the P3 treatment; this result is similar to previous studies (Sánchez et al., 2004). A completely randomized block experimental design was used with 6 replicates (individual pots: 24 plants in each of them) per treatment.

Sampling and plant analysis. Plants were sampled 60 days after sowing, at full pod development and maturity. Roots and leaves were sorted out for analysis. Plant material was first rinsed three times in distilled water after disinfesting with a non-ionic detergent at 1% (Wolf, 1982) and then blotted on filter paper. A fresh subsample of roots and leaves was used for analysis of nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS), glutamate synthase (GOGAT), phosphoenolpyruvate carboxylase (PEPC), amino acids and proteins. These subsamples were dried in a forced air oven at 70°C for 24 h, ground in a Wiley mill and placed in plastic bags until analysis for nitrate (NO$_3^-$) and ammonium (NH$_4^+$). Dry weights (DW) were recorded and expressed as mg DW per root or leaf. All determinations were performed in triplicate.

Statistical analysis. Data were analyzed using ANOVA. When F tests were significant, differences between treatment means were compared using LSD at the 0.05 probability level. Also, correlation analyses were made between the different variables. Levels of significance were represented by * at p<0.05, ** at p<0.01, *** at p<0.001, and NS: not significant. Data shown are mean values ± SE.
RESULTS AND DISCUSSION

Phosphorus is an important nutritional element that may limit plant growth (Raghothama, 1999). In our experiment, P treatments affected root biomass production (Fig. 1). Leaf biomass production was also influenced by increasing phosphorus concentrations. P3 showed the highest leaf biomass, with a value 15% greater than that in P6 (Fig. 1); while root biomass production attained its highest value in P1, with an increment of 61% in relation to P6. P3 was considered optimum for greater root and foliar biomass production than P6. This result coincides with those of Carbonell-Barrachina et al. (1997). Treatments below P3 concentrations (i.e., P1 and P2) can be considered P deficient, having a negative effect on leaf biomass production. Doses higher than P3 may have caused P toxic effects, which diminished production of both leaf and root bean biomasses.

In general, N assimilation is disturbed when plants growing with an adequate NO₃ concentration are limited by inadequate P levels (Rufty et al., 1993). Three different adverse effects have been identified in this regard: (i) diminished NO₃ root absorption (Rufty et al., 1991); (ii) reduction of NO₃ translocation from roots to shoots, with a subsequent NO₃ concentration increment in roots (Lee, 1982; Rufty et al., 1990); and (iii) increased amino acid accumulation in leaves (Israel & Rufty, 1988). Accumulation of amino acids has also been shown in roots as a rare event (Rabe & Lovatt, 1984; Rufty et al., 1990).

The effect of the different P doses on NO₃ concentrations in roots and leaves are shown in Fig. 2. Maximum NO₃ concentration was observed in P3. This concentration was 29% and 31% greater than concentrations on P1 and P6, respectively. These findings are similar to those reported by Lee (1982) and Rufty et al. (1990). P limitation produces a reduced both NO₃ absorption and translocation to leaves, causing a NO₃ accumulation in roots (Fig. 2). Rufty et al. (1993) showed that the diminished root NO₃ absorption and its subsequent translocation to leaves are probably caused by a drastic decrease in the Pi and ATP concentrations, which are essential in the NO₃ absorption and transportation processes.

Higher than optimum P doses showed a reduction in root and foliar NO₃ concentrations. This may be due, at least partially, to the antagonism between PO₄ and NO₃ absorption (Marschner, 1995).
Table 1. Effect of H$_3$PO$_4$ treatments (P1: 0.4 mM; P2: 0.80 mM; P3: 1.60 mM; P4: 3.20 mM; P5: 4.80 mM and P6: 6.40 mM P) on the key enzymes of nitrogen metabolism in roots and leaves of green bean plants. Data are means ± 1 s.e. (n = 6).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NR</th>
<th>NiR</th>
<th>GS</th>
<th>GOGAT</th>
<th>PEPC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Roots</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>0.020 ± 0.001</td>
<td>0.58 ± 0.04</td>
<td>0.19 ± 0.01</td>
<td>0.18 ± 0.01</td>
<td>2.68 ± 0.02</td>
</tr>
<tr>
<td>P2</td>
<td>0.024 ± 0.001</td>
<td>0.72 ± 0.06</td>
<td>0.21 ± 0.02</td>
<td>0.20 ± 0.02</td>
<td>3.56 ± 0.03</td>
</tr>
<tr>
<td>P3</td>
<td>0.035 ± 0.002</td>
<td>0.82 ± 0.07</td>
<td>0.22 ± 0.02</td>
<td>0.26 ± 0.02</td>
<td>3.94 ± 0.04</td>
</tr>
<tr>
<td>P4</td>
<td>0.026 ± 0.001</td>
<td>0.76 ± 0.06</td>
<td>0.20 ± 0.02</td>
<td>0.22 ± 0.02</td>
<td>3.64 ± 0.03</td>
</tr>
<tr>
<td>P5</td>
<td>0.020 ± 0.001</td>
<td>0.62 ± 0.05</td>
<td>0.18 ± 0.01</td>
<td>0.20 ± 0.02</td>
<td>2.96 ± 0.02</td>
</tr>
<tr>
<td>P6</td>
<td>0.018 ± 0.001</td>
<td>0.54 ± 0.04</td>
<td>0.16 ± 0.01</td>
<td>0.17 ± 0.01</td>
<td>2.41 ± 0.01</td>
</tr>
<tr>
<td><strong>Significance</strong></td>
<td>*</td>
<td>*</td>
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</table>

| **Leaves** |    |     |    |       |      |
| P1        | 0.062 ± 0.004 | 1.14 ± 0.10 | 0.48 ± 0.03 | 0.35 ± 0.02 | 5.10 ± 0.04 |
| P2        | 0.066 ± 0.004 | 1.34 ± 0.12 | 0.62 ± 0.04 | 0.42 ± 0.03 | 6.64 ± 0.05 |
| P3        | 0.071 ± 0.005 | 1.54 ± 0.14 | 0.76 ± 0.05 | 0.56 ± 0.04 | 7.87 ± 0.06 |
| P4        | 0.068 ± 0.004 | 1.50 ± 0.14 | 0.70 ± 0.05 | 0.52 ± 0.04 | 7.26 ± 0.05 |
| P5        | 0.060 ± 0.004 | 1.36 ± 0.12 | 0.64 ± 0.04 | 0.48 ± 0.03 | 5.67 ± 0.04 |
| P6        | 0.054 ± 0.004 | 1.12 ± 0.10 | 0.48 ± 0.03 | 0.33 ± 0.02 | 4.83 ± 0.03 |
| **Significance** | * | * | * | * | * |

*p<0.05.

Although statistically not significant (p>0.05), both deficient and toxic P doses resulted in negative effects on root and leaf NH$_4$ concentrations (Fig. 3).

The role of P in N assimilation has been widely studied, although mostly in experiments related to P deficiency. The results obtained so far in our study, indicate that NO$_3$ assimilation diminishes with limited P availability. Nitrate absorption and translocation have been strongly affected curtailing root and shoot biomass accumulation (Rufty et al., 1991).

Regarding the enzyme responses influencing NO$_3$ reduction (i.e., NR and NiR), we found that the activity of both enzymes was similar to the trend of NO$_3$ concentrations observed in roots and leaves (Table 1): P3 showed the highest values and the enzymatic activity increased more than 25% in both organs compared to P1 and P6. The direct relationship between both parameters (P concentration versus enzyme activity) is reflected in the high correlation coefficients obtained in different study plant parts (roots: NO$_3$ -NR, r = 0.94***; NO$_3$-NiR, r = 0.90***; leaves: NO$_3$-NR, r = 0.84***; NO$_3$-NiR, r = 0.80***; *p<0.01, ***p<0.001).

The GS/GOGAT activities showed a similar behavior, both in roots and leaves, as the activity shown by the NR and NiR enzymes. The maximum activities for GS and GOGAT were also observed in the optimum treatment (P3): increases were over 30% in roots and 35% in leaves compared to the minimum concentrations obtained in the extreme treatments (P1 and P6).

Several research works have shown that the PEPC activity is stimulated under P deficiency conditions (Kondracka & Rychter, 1997). However, Biddinger et al. (1998) found that lack of P diminishes CO$_2$ assimilation and, therefore, PEPC is reduced as well. Results of the current experiment (Table 1) are in agreement with this statement. The maximum activities of PEPC were obtained in P3 in both roots and leaves, while the minimum values were found in P1 and P6.

In relation to high molecular weight N compounds, such as amino acids and proteins (Table 2), the concentration in roots and leaves followed a similar behavior to all study enzymes activities. While P3 showed the highest concentrations of these compounds, the minimum was observed in P1 and P6.
Table 2. Amino acid and protein accumulation in roots and leaves of green bean plants in response to HPO₄²⁻ treatments (P1: 0.4 mM; P2: 0.80 mM; P3: 1.60 mM; P4: 3.20 mM; P5: 4.80 mM and P6: 6.40 mM P). Data are means ± 1 s.e. (n = 6).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Amino acids (mg/g)</th>
<th>Proteins (mg/g)</th>
<th>Amino acids (mg/g)</th>
<th>Proteins (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Roots</td>
<td>Leaves</td>
<td>Roots</td>
<td>Leaves</td>
</tr>
<tr>
<td>P1</td>
<td>0.32 ± 0.02</td>
<td>1.36 ± 0.12</td>
<td>1.42 ± 0.12</td>
<td>5.91 ± 0.35</td>
</tr>
<tr>
<td>P2</td>
<td>0.46 ± 0.03</td>
<td>1.58 ± 0.13</td>
<td>1.58 ± 0.13</td>
<td>7.11 ± 0.42</td>
</tr>
<tr>
<td>P3</td>
<td>0.62 ± 0.04</td>
<td>1.74 ± 0.14</td>
<td>1.92 ± 0.15</td>
<td>7.20 ± 0.43</td>
</tr>
<tr>
<td>P4</td>
<td>0.58 ± 0.04</td>
<td>1.68 ± 0.14</td>
<td>1.84 ± 0.14</td>
<td>7.02 ± 0.42</td>
</tr>
<tr>
<td>P5</td>
<td>0.52 ± 0.04</td>
<td>1.62 ± 0.13</td>
<td>1.76 ± 0.14</td>
<td>6.84 ± 0.41</td>
</tr>
<tr>
<td>P6</td>
<td>0.30 ± 0.02</td>
<td>1.30 ± 0.11</td>
<td>1.38 ± 0.12</td>
<td>5.82 ± 0.34</td>
</tr>
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</table>

Significance: *p<0.05.

CONCLUSION

Results proved that both P toxicity and deficiency gave similar responses in N assimilation. Phosphorus and NO₃ interacted on the absorption and translocation processes affecting N assimilation. The deficiency (P1) and toxicity (P6) treatments diminished root NO₃ absorption by 15% and 36%, respectively, in comparison to P3, the optimum dose. These treatments also reduced nutrient assimilation within the plant, which may help explain the minimum enzymatic activity for NO₃ assimilation observed in P1 and P6. The minimum N assimilation shown in these treatments, and the subsequent minimum synthesis of N organic compounds, which are keys to plant growth and development, will influence the reduction of green bean plant biomass and production.

REFERENCES


