Yield and biosynthesis of nitrogenous compounds in fruits of green bean (*Phaseolus vulgaris* L cv Strike) in response to increasing N fertilisation

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Abstract: The objective of the present work was to determine the effect of different dosages of N on the biosynthesis of organic N compounds in fruits and their influence on yield in green bean (*Phaseolus vulgaris* L cv Strike) plants. The nitrogen was applied to the nutrient solution as NH4NO3 at 1.5 (N1), 3 (N2), 6 (N3), 12 (N4), 18 (N5) and 24 mM (N6). Treatment N3 was considered optimal for efficient yield in green bean plants and also for the null presence of NO3\(^{-}\). Highest N dosages (18 and 24 mM) resulted in the accumulation of NO3\(^{-}\) in pods and seeds. This accumulation encouraged nitrate reductase (NR) and nitrite reductase (NiR) activity in both tissues and treatments, and therefore NR activity might be considered as a good bioindicator of the presence of NO3\(^{-}\) in edible fruits. The greater NH4\(^{+}\) assimilation by glutamine synthetase (GS) and glutamate synthase (GOGAT) occurred primarily in the pods of the N6 treatment, while the seeds acted as physiological sinks, these latter tissues presenting the highest concentrations of amino acids, proteins and organic N. The high accumulation of NO3\(^{-}\) and NH4\(^{+}\) in both seeds and pods could be the direct cause of the reduction in fruit production, indicating that green bean plants are very sensitive to high N levels.

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Keywords: fruits; green bean; nitrogenous compounds; *Phaseolus vulgaris*; yield

INTRODUCTION

The growth and yield of a plant often depend on N supplementation1–3 in order to form amino acids, proteins, nucleic acids and other cell constituents needed for development.4 However, the excessive use of nitrogenous fertiliser in the form of NO3\(^{-}\) can increase the concentration of this anion in foods of vegetal origin.5 An excess of N in general harms the quality of fruits and their commercial yield,3,6,7 in addition to polluting aquifers as well as surface water by leaching.8 Few works have studied N metabolism in fruits, but one of the enzymes that participate in N assimilation is known to be glutamine synthetase (GS), which catalyses glutamine synthesis from glutamate, ATP and NH4\(^{+}\). This enzyme has been detected in substantial quantities in various fruits10,11 and is therefore considered key to NH4\(^{+}\) assimilation in fruits. In contrast, the enzyme that catalyses glutamate synthesis (NADH-glutamate synthase, GOGAT), although having been detected in fruits, has proved generally less active than GS in the fruits analysed.12

The few studies on N metabolism in fruits have focused primarily on development and maturity. Here we study the effect of different N doses on the biosynthesis of organic N compounds in fruits and their influence on yield in green bean plants.

MATERIALS AND METHODS

Crop design and plant sampling

Seeds of *Phaseolus vulgaris* L cv Strike were sown and grown in a chamber under controlled environmental conditions, with relative humidity 60–80%, temperature 28/22 °C (day/night) and a photoperiod of 16/8 h (day/night) in a photosynthetic photon flux density (PPFD) of 350 µmol m\(^{-2}\)s\(^{-1}\) (measured at the top of the plants with a 190 SB quantum sensor, LI-COR Inc, Lincoln, NE, USA). Four plants were grown in 8 l pots (25 cm upper diameter, 17 cm lower diameter, 25 cm height) filled with vermiculite. For 30 days (including 8 days for germination) before the experimental treatments the plants received a nutrient solution of 6 mM NH4NO3, 1.6 mM K2HPO4, 2.4 mM K2SO4, 4 mM CaCl2·2H2O, 1.4 mM MgSO4·H2O, 5 µM Fe-EDDHA, 2 µM MnSO4·H2O, 1 µM ZnSO4·7H2O, 0.25 µM CuSO4·5H2O, 0.3 µM Na2MoO4·2H2O and 0.5 µM H3BO3. The nutrient solution (pH 6.0–6.1) was renewed every 3 days.
At 30 days after sowing, different N treatments in the form of NH₄NO₃ (N1: 1.5, N2: 3, N3: 6, N4: 12, N5: 18 and N6: 24 mM N) were applied for 30 days (until harvest), the concentration 6 mM N being considered optimal according to Carbonell-Barrachina et al. This experimental design was a complete randomised block with six replicates (individual pots) with 24 plants per treatment.

The plants were sampled at 60 days after sowing, at full pod development and maturity, and the seeds and pods were sorted for analysis. The material was rinsed three times in distilled water after it had been decontaminated with non-ionic detergent at 1% (v/v) (Decon 90, Merck, Madrid, Spain) and then blotted on filter paper. A subsample of pods and seeds was used fresh for the analysis of nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS), glutamate synthase (GOGAT), amino acids and proteins, with triplicate assays for each extraction. A second 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 pending further analyses (NO₃⁻, NH₄⁺ and organic N).

**Plant analysis**

**Extraction of NR, NiR and GOGAT**

At each sampling, portions were ground at a ratio of 1:5 (w/v) in a mortar at 0 °C with 50 mM KH₂PO₄ buffer, pH 7.5, containing 2 mM EDTA, 1.5% (w/v) soluble casein, 2 mM DTT and 1% (w/v) insoluble PVPP. The homogenate was filtered and centrifuged at 3000 × g for 5 min, after which the supernatant was centrifuged at 30000 × g for 20 min. The resulting extract was used to measure enzyme activities (nitrate reductase, nitrite reductase and glutamate synthase) and soluble protein. The extraction medium was optimised for the enzymatic activities so that these could be extracted jointly by the same method.

**Assay of NR (EC 1.6.6.1)**

The nitrate reductase assay followed the methodology of Kaiser and Lewis. The nitrite formed was determined colorimetrically at 540 nm after azocoupling with sulphamidine and naphthylenediamine dihydrochloride according to the method of Hageman and Hucklesby. The NR activity was expressed as µmol NO₂⁻ formed mg⁻¹ protein min⁻¹.

**Assay of NiR (EC 1.7.7.1)**

Nitrite reductase activity was determined by the disappearance of NO₂⁻ from the reaction medium. After incubation at 30 °C for 30 min the NO₂⁻ content was determined colorimetrically at 540 nm and expressed as µmol NO₂⁻ reduced mg⁻¹ protein min⁻¹.

**Assay of GOGAT (EC 1.4.1.14)**

NAD-glutamate synthase activity was assayed spectrophotometrically at 30 °C by monitoring the oxidation of NADH at 340 nm, essentially as indicated by Groat and Vance and Singh and Srivastava, consistently within 2 h of extraction. Two controls, without ketoglutarate and glutamine respectively, were used to correct for endogenous NADH oxidation. The decrease in absorbance (linear for at least 10 min) was recorded for 5 min. The activity was expressed as µmol NADH oxidised mg⁻¹ protein min⁻¹.

**Extraction and assay of GS (EC 6.3.1.2.)**

The extraction of glutamine synthetase followed the method of Rees et al. The assay was based on that described by Slawyk and Rodier. The reaction mixture contained 0.2 M Heps-KOH, pH 7.9, 50 mM MgCl₂, 5 mM Na₂EDTA, 100 mM potassium glutamate, 50 mM ammonium acetate and 8 mM ATP. Blanks consisted of the complete mixture minus glutamate. The reaction was started by adding ATP, and the mixture was routinely incubated at 30 °C for 15 min before the reaction was stopped by adding 0.25 ml of 0.5 M H₂SO₄. The mixture was centrifuged at 14000 × g for 1 min, and 50 µl of supernatant was added to 0.95 ml of Millipore water for P₁ determination. P₁ concentrations were determined using a sensitive malachite green assay. Absorbance values were read against a blank containing Millipore water and converted to P₁ concentrations from standard curves (0–100 µM K₂HPO₄). Overestimation of P₁ due to ATP hydrolysis by the acidic colour reagent was prevented by the addition of 50 µl of 1.3 M trisodium citrate 2 min after the colour reagent. Enzyme activities were expressed as µmol P₁ mg⁻¹ protein min⁻¹.

**Quantification of amino acids and proteins**

Amino acids and proteins were determined after homogenisation of 0.5 g fresh samples in 50 mM cold KH₂PO₄ buffer at pH 7 and centrifugation at 12000 × g for 15 min. The resulting supernatant was used for the determination of total amino acids by the ninhydrin method, total free amino acids were expressed as mg glycine g⁻¹ fresh weight (FW). Soluble protein was measured with Bradford G-250 reagent and expressed as mg g⁻¹ FW, using bovine serum albumin (BSA) as standard.

**Quantification of nitrate and ammonium**

NO₃⁻ was analysed from an aqueous extract of 0.2 g of dried and ground plant material in 10 ml of Millipore-filtered water. A 100 µl aliquot was taken for NO₃⁻ determination and added to 10% (w/v) salicylic acid in sulphuric acid at 96%, and the NO₃⁻ determination and added to 0.95 ml of Millipore water for P₁ determination. NO₃⁻ concentrations were expressed as µmol g⁻¹ dry weight (DW).

**Organic N determination**

A 0.1 g DW subsample was digested with sulphuric acid and H₂O₂. After dilution with deionised water a 1 ml aliquot of the digest was added to the reaction medium (5% potassium sodium tartrate, 100 µM...
sodium phosphate and 5.4% (w/v) sodium hydroxide) containing 15/0.03% (w/v) sodium salicylate/sodium nitroprusside and 5.35% (v/v) sodium hypochlorite. Samples were incubated at 37 °C for 45 min and organic N was measured by spectrophotometry at A630 as performed by Baethgen and Alley. 28 The results were expressed as mg g$^{-1}$ DW.

Yield

Plant yield was expressed as the mean fruit fresh weight per plant. French beans collected from each plant were weighed at sampling. Commercial yield was expressed as the mean fruit fresh weight per plant. Non-commercial yield was calculated as the mean fruit fresh weight minus the mean commercial yield per plant. The data are shown as mean ± standard error of treatment means. The results were significant at $P < 0.05$, ** significant at $P < 0.01$, *** significant at $P < 0.001$ and not significant (NS) at $P > 0.05$.

Statistical analysis

Analysis of variance was used to assess the significance of treatment means. The data are shown as mean value ± standard error. A correlation analysis was also made between the different variables. Levels of significance were represented by * at $P < 0.05$, ** at $P < 0.01$, *** at $P < 0.001$ and not significant (NS) at $P > 0.05$.

RESULTS

Significant differences were found in the total, commercial and non-commercial fresh yields of fruits (Table 1). The N3 treatment presented the highest total and commercial yields, these being 54% greater than the lowest, in N6, which registered the lowest values. With respect to non-commercial fruit yield, the N3 treatment presented the lowest value, some 260% lower than the highest, in N6.

The application of high N dosages prompted NO$_3^-$ concentration only in treatments N5 and N6 of the pods and seeds (Table 2), while this anion was not detected in the other treatments. However, ammonium N was detected at all N levels. The ammonium concentrations in pods and seeds (Table 2) were directly affected by the high N dosages, N6 presenting the highest concentrations, some 57 and 60% higher respectively than in N1, which registered the lowest values.

The first step in NO$_3^-$ assimilation is the reduction of nitrate to nitrite, which is catalysed by NR. 29 Afterwards, NiR converts nitrite to ammonium. 4, 30 In our experiment the activities of both enzymes, NR and NiR, presented a behaviour similar to that of the concentration of NO$_3^-$ in the pods and seeds (Table 3), these enzymes being detected only in N5 and N6. 

The main assimilation pathway of NH$_4^+$ is via glutamine synthetase/glutamate synthase (GS/GOGAT). 4, 30 In our experiment the activities of GS and GOGAT followed a similar pattern in the pods and seeds (Table 3), the highest activities appearing in the N6 treatment, some 70% higher than the lowest activities, in treatment N1.

Finally, with respect to nitrogenous compounds of high and low molecular weights (proteins and amino acids) and to organic N (Table 4), the concentrations in pods and seeds rose significantly with the N dosage, reaching the highest values in the N6 treatment and the lowest in N1.

DISCUSSION

The effectiveness of the N treatments in our experiment is reflected in the total, commercial and non-commercial yields of fruits (Table 1). The first two declined significantly at higher N dosages. That is, total and commercial yields rose to the N3 treatment and fell sharply in N4, N5 and N6 (a 51% drop compared with N3), indicating that these treatments were harmful to green bean cultivation. The non-commercial yield proved the reverse, as in previous works, 3, 6 in which high N application rates seriously reduced total and commercial yields but increased non-commercial yield.

Meanwhile, the treatments below N3 (N1 and N2) are considered suboptimal for their negative effect on total and commercial yields and their positive effect on non-commercial yield.

The negative effect of N toxicity could be due to the accumulation of NO$_3^-$ and NH$_4^+$ produced in the pods and seeds with treatments N5 and N6. In the case of NO$_3^-$, Andreeva et al. 31 found that heavy additions of this anion to the nutritive solution (>15 mM) produced stressful for the plants. These authors reported that greater foliar contents of NO$_3^-$ substantially depressed key photosynthetic processes, starch accumulation and synthesis, and N accumulation, thereby strongly depressing yield. In our experiment the possible alteration of these processes in the leaves of the plants treated with N5 and N6 could explain the reduction in total and commercial fruit production.

Also, NO$_3^-$ concentrations were detected only in treatments N5 and N6 in the fruits (pods and seeds) of our experiment, apparently because absorption and accumulation exceeded the assimilation capacity, 32 a situation that might explain the transport of this anion towards the fruits in these treatments. It is noteworthy.

Table 1. Total, commercial and non-commercial fresh yields of fruits from green bean plants in response to N treatments (N1: 1.5, N2: 3, N3: 6, N4: 12, N5: 18 and N6: 24 mM NH$_4$NO$_3$). Values are mean ± standard error (n = 6).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total (g per plant)</th>
<th>Commercial (g per plant)</th>
<th>Non-commercial (g per plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>0.750 ± 0.029</td>
<td>0.472 ± 0.018</td>
<td>0.278 ± 0.011</td>
</tr>
<tr>
<td>N2</td>
<td>0.859 ± 0.041</td>
<td>0.598 ± 0.023</td>
<td>0.261 ± 0.010</td>
</tr>
<tr>
<td>N3</td>
<td>1.283 ± 0.051</td>
<td>1.162 ± 0.046</td>
<td>0.121 ± 0.004</td>
</tr>
<tr>
<td>N4</td>
<td>1.201 ± 0.048</td>
<td>0.943 ± 0.037</td>
<td>0.258 ± 0.010</td>
</tr>
<tr>
<td>N5</td>
<td>0.758 ± 0.030</td>
<td>0.425 ± 0.017</td>
<td>0.333 ± 0.013</td>
</tr>
<tr>
<td>N6</td>
<td>0.587 ± 0.023</td>
<td>0.151 ± 0.006</td>
<td>0.436 ± 0.017</td>
</tr>
</tbody>
</table>

Significance: * at $P < 0.05$, ** at $P < 0.01$, *** at $P < 0.001$ and not significant (NS) at $P > 0.05$. 

Effect of N treatments (N1: 1.5, N2: 3, N3: 6, N4: 12, N5: 18 and N6: 24 mM NH₄NO₃) on enzymatic activities of NR, NiR, GS and GOGAT in pods and seeds of green bean plants. Values are mean ± standard error (n = 6).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pods</th>
<th>Seeds</th>
<th>Pods</th>
<th>Seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>ND</td>
<td>ND</td>
<td>1.48±0.04</td>
<td>2.18±0.06</td>
</tr>
<tr>
<td>N2</td>
<td>ND</td>
<td>ND</td>
<td>1.88±0.05</td>
<td>2.44±0.07</td>
</tr>
<tr>
<td>N3</td>
<td>ND</td>
<td>ND</td>
<td>2.18±0.06</td>
<td>2.76±0.08</td>
</tr>
<tr>
<td>N4</td>
<td>ND</td>
<td>ND</td>
<td>2.74±0.08</td>
<td>3.18±0.09</td>
</tr>
<tr>
<td>N5</td>
<td>0.32±0.01</td>
<td>0.51±0.02</td>
<td>3.00±0.09</td>
<td>3.70±0.11</td>
</tr>
<tr>
<td>N6</td>
<td>0.39±0.01</td>
<td>0.69±0.03</td>
<td>3.48±0.13</td>
<td>5.40±0.16</td>
</tr>
<tr>
<td>Significance</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

ND, not detected; DW, dry weight.

Table 3. Effect of N treatments (N1: 1.5, N2: 3, N3: 6, N4: 12, N5: 18 and N6: 24 mM NH₄NO₃) on enzymatic activities of NR, NiR, GS and GOGAT in pods and seeds of green bean plants. Values are mean ± standard error (n = 6).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pods</th>
<th>Seeds</th>
<th>Pods</th>
<th>Seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>ND</td>
<td>ND</td>
<td>0.09±0.002</td>
<td>0.043±0.001</td>
</tr>
<tr>
<td>N2</td>
<td>ND</td>
<td>ND</td>
<td>0.13±0.003</td>
<td>0.052±0.001</td>
</tr>
<tr>
<td>N3</td>
<td>ND</td>
<td>ND</td>
<td>0.17±0.005</td>
<td>0.261±0.007</td>
</tr>
<tr>
<td>N4</td>
<td>ND</td>
<td>ND</td>
<td>0.18±0.005</td>
<td>0.337±0.008</td>
</tr>
<tr>
<td>N5</td>
<td>0.018±0.001</td>
<td>0.039±0.005</td>
<td>0.21±0.006</td>
<td>0.465±0.010</td>
</tr>
<tr>
<td>N6</td>
<td>0.021±0.002</td>
<td>0.043±0.006</td>
<td>0.33±0.006</td>
<td>0.607±0.015</td>
</tr>
<tr>
<td>Significance</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

NR: μmol NO₃⁻ formed mg⁻¹ protein min⁻¹; NiR: μmol NO₂⁻ reduced mg⁻¹ protein min⁻¹; GS: μmol P; mg⁻¹ protein min⁻¹; GOGAT: μmol NADH oxidised mg⁻¹ protein min⁻¹.

Table 4. Accumulation of organic nitrogenous compounds in pods and seeds of green bean plants in response to N treatments N1: 1.5, N2: 3, N3: 6, N4: 12, N5: 18 and N6: 24 mM NH₄NO₃. Values are mean ± standard error (n = 6).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pods</th>
<th>Seeds</th>
<th>Pods</th>
<th>Seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>0.25±0.007</td>
<td>2.25±0.06</td>
<td>29.01±0.87</td>
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</tr>
<tr>
<td>N2</td>
<td>0.37±0.011</td>
<td>3.76±0.11</td>
<td>34.05±1.02</td>
<td></td>
</tr>
<tr>
<td>N3</td>
<td>0.55±0.016</td>
<td>4.16±0.12</td>
<td>37.95±1.13</td>
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<tr>
<td>N4</td>
<td>0.92±0.027</td>
<td>4.96±0.13</td>
<td>42.83±1.28</td>
<td></td>
</tr>
<tr>
<td>N5</td>
<td>0.99±0.029</td>
<td>5.38±0.16</td>
<td>47.44±1.72</td>
<td></td>
</tr>
<tr>
<td>N6</td>
<td>1.07±0.032</td>
<td>5.72±0.20</td>
<td>51.30±1.74</td>
<td></td>
</tr>
<tr>
<td>Significance</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

FW, fresh weight; DW, dry weight.

that NO₃⁻ accumulation (Table 2) proved higher in the seeds than in the pods, as the seeds acted as a physiological sink, while the pods functioned generally as a source or in assimilation.33,34

With respect to NH₄⁺, the accumulation of this ion was probably due to a direct effect of the application of heavy dosages of N, this perhaps being one of the factors responsible for the sharp fall in fruit yield (Table 1). It has been demonstrated in several studies that a strong supplement of NH₄⁺ harms growth and yield in plants compared with plants nourished with NO₃⁻.35–37 Toxicity in NH₄⁺ can result from the following causes: (i) induced nutrient deficiency, blocking ion uptake; (ii) inhibited secondary growth, acidifying the root zone; (iii) altered intracellular pH and osmotic balance; (iv) decoupled electron transport and photophosphorylation, followed by NH₄⁺ accumulation in leaves; and (v) altered polyamine and phytohormone metabolism.38

On the other hand, Sánchez et al.39 noted that the application of high dosages of NH₄NO₃ in green bean resulted in foliar accumulation of H₂O₂, reducing
biomass production. In the present experiment the high N dosage affected NH$_4^+$ accumulation in the leaves, as occurred with NO$_3^-$, causing these compounds to be transported towards the fruits for being the principal physiological sink, resulting in greater contents in the seeds.

The first stage in NO$_3^-$ assimilation is the reduction to NO$_2^-$ by NR, this stage being the most prone to regulation and, in turn, being limiting for NO$_3^-$ assimilation.$^{4,29,41–43}$ The next step in NO$_3^-$ assimilation is the conversion of NO$_2^-$ to NH$_4^+$ by NiR.$^{4,30}$ One of the main factors regulating NR and NiR is NO$_3^-$ availability.$^{41}$ These enzymes are key in reducing NO$_3^-$ principally in the leaves.$^{4,43}$ While, in the fruits no NO$_3^-$ reduction generally occurs under normal conditions, because the N is incorporated into the fruits in the form of amino acids.$^{44}$ Nevertheless, when excesses of NO$_3^-$ appear because of high N application, this anion can be transported via the phloem to the fruits.$^{44}$ In the present experiment this occurred only in the treatments that were subject to high N dosages (N5 and N6), where NR and NiR activity was detected in the pods and seeds, activity being greater in the pods than in the seeds (Table 3).

Both NH$_4^+$ produced in the plant by the reduction of NO$_3^-$ and the supply via fertiliser application are incorporated into organic forms primarily by the enzyme GS.$^{4,30}$ This enzyme catalyses the conversion of glutamate to glutamid. Afterwards, GOGAT catalyses the reduction of the amide group from glutamine formed by GS to 2-oxoglutarate in order to form two glutamate molecules. One of the glutamate molecules can be incorporated as a substrate for the reaction of GS.$^{8}$ In our experiment the activities of GS and GOGAT were promoted by the high N dosages applied, with the highest activity appearing in the N6 treatment and the lowest in N1. As in the reduction of NO$_3^-$, the greater activity of the enzymes in charge of NH$_4^+$ assimilation (GS and GOGAT) occurred prepared mainly in the pods rather than the seeds. Also, it is well known that one of the determining factors in the activation of GS and GOGAT is the presence of NH$_4^+$, and in our experiment a significant and positive relationship was found between NH$_4^+$ and the enzymatic activities of GS and GOGAT in both the pods (NH$_4^+$−GS, $r = 0.76^{**}$; NH$_4^+$−GOGAT, $r = 0.93^{**}$) and the seeds (NH$_4^+$−GS, $r = 0.96^{**}$; NH$_4^+$−GOGAT, $r = 0.99^{***}$).

The main end products from NH$_4^+$ assimilation which are used by the plants include amino acids, proteins and organic N.$^{46}$ In the present experiment the results show that these nitrogenous compounds increased with the N dosage (Table 2), the highest concentrations appearing in the N6 treatment in both the pods and the seeds. In addition, it is notable that the contents of these nitrogenous compounds were higher in the seeds than in the pods.

In short, the pods had an assimilation function, which was reflected by the greater enzymatic activity presented by NR, NiR, GS and GOGAT, whereas the seeds, where there was little assimilation, functioned as physiological sinks, thereby explaining the lower activities of these enzymes. These data agree with the fact that green beans contain high levels of protein.$^{47}$

**CONCLUSIONS**

According to these results, we conclude that treatment N3 (6 mM N) was considered optimal for efficient yield in green bean plants and also for the null presence of NO$_3^-$, assuring low health risk for the consumer. In contrast, the high accumulation of NO$_3^-$ and NH$_4^+$ in both seeds and pods could be the direct cause of the reduction in fruit production, indicating that green bean plants are very sensitive to high N levels. In addition, the high N dosages resulted in the accumulation of NO$_3^-$ only in the treatments N5 and N6 in pods and seeds. This accumulation encouraged the NR and NiR activity in both tissues and treatments, and therefore NR activity can be defined as a good bioindicador of the presence of NO$_3^-$ in edible fruits. Finally, in our experiment the greater NH$_4^+$ assimilation by GS and GOGAT occurred primarily in the pods of the N6 treatment, while the seeds acted as physiological sinks, these latter tissues presenting the highest concentrations of amino acids, proteins and organic N.

**REFERENCES**


30 Migge A and Becker TW, In tobacco leaves, the genes encoding the nitrate-reducing or the ammonium-assimilating enzymes are regulated differently by external nitrogen-sources. Plant Physiol Biochem 34:665–671 (1996).


