BIOSYNTHESIS OF NON-STRUCTURAL CARBOHYDRATES AND THEIR DISTRIBUTION IN GREENBEAN PLANTS (*Phaseolus vulgaris* L. Cv. Strike): DEFICIENCY *VS* TOXICITY OF NITROGEN

BIOSÍNTESIS DE HIDRATOS DE CARBONO NO ESTRUCTURALES Y SU DISTRIBUCIÓN EN PLANTAS DE FRIJOL EJOTERO (*Phaseolus vulgaris* L. Cv. Strike): DEFICIENCIA *VS* TOXICIDAD DE NITRÓGENO

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RESUMEN

Sacarosa y almidón son los dos principales productos de la fotosíntesis en las plantas superiores, y la sacarosa es el principal azúcar de transporte desde las hojas hacia el resto de la planta. Además, de aportar carbono y energía a los órganos en desarrollo, la sacarosa interviene de forma importante en respuestas de protección o tolerancia ante diferentes estreses ambientales. Uno de los principales factores que regulan tanto el metabolismo de los azúcares como la relación fuente-demanda (distribución) es el estado nutricional de N, por lo que en este trabajo se estudiaron las respuestas de estos procesos fisiológicos ante condiciones desde deficiencia hasta toxicidad de N. El N fue aplicado a la solución nutritiva en la forma de NH_4NO_3 y en dosis crecientes: 1.5, 3.0, 6.0, 12.0, 18.0, y 24.0 mM. La deficiencia de N se caracterizó por estimular la síntesis de sacarosa, y las máximas actividades se encontraron en fructosa-1,6-bifosfatasa y sacarosa fosfato sintasa. Bajo estas condiciones de estrés se produjo un aumento de la translocación de la sacarosa a las raíces, lo que explica que el crecimiento radicular no haya sido afectado. Por el contrario, la toxicidad de N provocó, además de una disminución general del crecimiento, una estimulación significativa de la degradación de sacarosa asociada con las máximas actividades enzimáticas de sacarosa sintasa y las invertasas ácida y alcalina. Finalmente, el cociente obtenido de la relación sacarosa/almidón pudiera ser considerado un buen indicador de la suficiencia de N en las plantas de frijol ejotero.

Palabras clave: *Phaseolus vulgaris*, deficiencia, frijol ejotero, nitrógeno, carbohidratos no estructurales, toxicidad.

SUMMARY

Sucrose and starch are the main products of photosynthesis in higher plants, sucrose being the main sugar transported from the leaves to the rest of the plant. Sucrose, in addition to providing carbon and energy to developing organs, is important in responses of defense and tolerance against environmental stress. One of the main factors regulating both sugar metabolism and the source-sink relationship (distribution) is the nutritional state of N. In the present study, we examined the responses of these physiological processes in relation to N conditions ranging from deficiency to toxicity. That is, N was applied to the nutrient solution as NH_4NO_3 at the following rates: 1.5, 3.0, 6.0, 12.0, 18.0 and 24.0 mM. N deficiency stimulated the sucrose synthesis, yielding the highest values for fructose-1,6-biphosphatase and sucrose phosphate synthase activities. Under these stressful conditions sucrose translocation to the roots increased, thus explaining why root growth was un affected. On the contrary, N toxicity not only stunted general growth, but also stimulated significantly sucrose degradation associated with the highest activities of sucrose synthase, as well as of the acidic and alkaline invertases. Finally, the sucrose/starch quotient could be considered a good indicator of N sufficiency in green beans.

Index words: *Phaseolus vulgaris*, deficiency, greenbean, nitrogen, non-structural carbohydrates, toxicity.

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 South, Central, and North America; México and East Africa (Singh, 1999).

Sucrose, one of the main photosynthetic products in higher plants, is the main form in which carbohydrates are translocated from the leaves to the rest of the plant, supplying these organs with carbon and energy essential for growth (ap Rees, 1987). In addition, this sugar is the main carbohydrate stored in some plants, appearing in leaves, branches, tubers and fruits (Kruger, 1999). On the other hand, sucrose is highly important in plants for acclimation

Recibido: 16 de Enero del 2004.
Aceptado: 3 de Noviembre del 2004.

to environmental stress caused by cold, salt and drought (Lunn and Furbank, 1999).

Although the mechanisms regulating sucrose biosynthesis are not yet fully understood (Cheikh and Brenner, 1992), the enzymes fructose-1,6-biphosphatase (F1,6BPase, EC 3.1.3.11) and sucrose phosphate synthase (SPS, EC 2.4.1.14) have been defined as the key limiting enzymes in the regulation of the photosynthetic pathway for the production of this sugar (Stitt *et al*., 1988).

In C_3 plants, the sucrose biosynthesis pathway begins with the export of triose-phosphate from the chloroplast towards the cytosol, where it is converted to fructose-1,6-biphosphate (Fru1,6P_2) by the aldolase enzyme. Then, Fru1,6P_2 is irreversibly hydrolyzed to fructose-6-phosphate (Fru6P) by fructose-1,6-biphosphatase (F1,6BPase), a stage being considered a crucial control point along this pathway (Stitt *et al*., 1987). Afterwards, sucrose is synthesized by the sequential action of sucrose phosphate synthase (SPS), which catalyses the synthesis of sucrose-6-phosphate from UDP-glucose and fructose-6-phosphate, releasing UDP. Finally, sucrose phosphate phosphatase (SPP) irreversibly hidrolyses sucrose-6-phosphate to produce sucrose and P_i (Lunn and ap Rees, 1990).

Sucrose accumulation in higher plants depends on its biosynthesis and degradation rates. This latter process depends on sucrose sythetase (SS), as well as on acidic and alkaline sucrose invertases (Huber and Akazawa, 1986). The enzyme SS catalyses the reversible reaction of sucrose + UDP, giving rise to UDP-glucose and fructose, while the invertases catalyse the hydrolytic reaction of sucrose, producing glucose and fructose (Schaffer, 1986).

The main factors that regulate sucrose biosynthesis are: light and darkness (Lunn and Furbank, 1999; Robinson, 2000); CO_2 level (Murchie *et al*., 1999; Demmers-Derks *et al*., 1998; Roumet *et al*., 2000; Zerihun and BassiriRad, 2000; Cabrerizo *et al*., 2001); water availability (Lunn and Furbank, 1999); temperature (Demmers-Derks *et al*., 1998; Jones *et al*., 1998); and growth regulators such as gibberellin and abscisic acid (Cheikh and Brenner, 1992; Goupil *et al*., 1998; Coruzzi and Zhou, 2001). In addition, the principal factor regulating not only sugar metabolism but also the source-sink relationship (distribution), is the nutritional state of N (Chaillou *et al*., 1994; Ciompi *et al*., 1996; Demmers-Derks *et al*., 1998; Banks *et al*., 1999; Robinson, 2000; Zerihum and BassiriRap, 2000; Coruzzi and Zhou, 2001).

Studies conducted to date have centred on the analysis of N deficiency in the distribution and biosynthesis of non-structural carbohydrates, highlighting that this nutrient can affect the synthesis of photoassimilates and thus their distribution between the source (leaves) and the different physiological sinks. The most detailed study shows that N deficiency alters the distribution of photoassimilates during the vegetative growth (Marschner *et al*., 1996). Under these conditions, translocation of photoassimilates and transport of nitrogenous compounds to the roots via phloem increase (Peuke *et al*., 1994), reflected ultimately in the increased dry-weight quotient between the root and the aerial parts (Engels and Marschner, 1995).

In view of the above, it is necessary to perform a physiological study on the influence that different levels of N (from deficiency to toxicity) exert on the metabolism and distribution of non-structural carbohydrates in shoots and roots of greenbean plants (*Phaseolus vulgaris* L. cv. Strike), with the aim of determining whether these physiological processes can be considered good indicators of N sufficiency.

MATERIALS AND METHODS

Crop management and experimental design

Seeds of *Phaseolus vulgaris* cv. Strike were sown and grown in a chamber under controlled environmental conditions and under a hydroponic system, in Southeast Spain (Granada) during the Spring of 2002. Relative humidity was 60-80 %, temperature 28/22 °C (day/night), and a photoperiod of 16/8 h (day/night) at 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 20 d (including 8 d for germination), before the experimental treatments, the plants received a nutrient solution of: NH_4NO_3 (6 mM), K_2HPO_4 (1.6 mM), K_2SO_4 (2.4 mM), $CaCl_2 \cdot 2H_2O$ (4 mM), $MgSO_4 \cdot H_2O$ (1.4 mM), Fe-EDDHA (5 μM), $MnSO_4 \cdot H_2O$ (2 μM), $ZnSO_4 \cdot 7H_2O$ (1 μM), $CuSO_4 \cdot 5H_2O$ (0.25 μM), $Na_2MoO_4 \cdot 2H_2O$ (0.3 μM), and H_3BO_3 (0.5 μM). The nutrient solution (pH 6.0-6.1) was renewed every 3 d.

At 20 d after sowing, the different N treatments in the form of NH_4NO_3 (1.5, 3.0, 6.0, 12.0, 18.0, and 24.0 mM of N) were applied for 40 d (until harvest). The concentration 6.0 mM of N was considered optimal, according to Carbonell-Barrachina *et al*. (1997). This experimental design was a complete randomized block with six replicates (individual pots), with 24 plants per treatment.

Plant sampling

Whole plants were sampled at 60 d after sowing, at full pod development. Roots and shoots were rinsed three times in distilled water with non-ionic detergent at 1 % (Wolf, 1982), then blotted on filter paper. Subsamples of root and shoot each of 5 g fresh weight, were used for the analysis of fructose-1,6-bisphosphatase (F1,6BPase), sucrose phosphate synthase (SPS), sucrose synthetase (SS), invertases (acid and alkaline), and non-structural carbohydrates (sucrose, glucose, fructose and starch).

Plant analysis

Extraction of SPS, F1,6BPase and SS. The subsamples were ground, in a mortar at 0 ºC in Hepes-NaOH buffer (50 mM, pH 7.5) at a ratio of 1:5 (w/v), containing $MgCl_2$ (5 mM), Na-EDTA (1 mM), DTT (2.5 mM), BSA (0.5 mg mL^{-1}) and Triton X-100 (0.05 %, v/v), according to Hubbard *et al*. (1989) with slight modifications. The homogenate was filtered and centrifuged at 13500 g_n for 10 min; the resulting extract was used to measure enzymes activities (SPS, SS, and F1,6BPase) and soluble protein was measured following the method of Bradford (1976), with BSA as a standard.

Assays of SPS (EC 2.4.1.14) and SS (EC 2.4.1.13). The sucrose phosphate synthase was assayed by measuring Fru6P-dependent sucrose (+sucrose-P) formation from UDP-Glc (Kerr *et al*., 1984). The SPS was determined in reaction mixtures (70 µL) containing Hepes-NaOH (50 mM, pH 7.5), $MgCl_2$ (15 mM), Fru6P (25 mM), Glc6P (25 mM), UDP-Glc (25 mM), and 40 µL of extract. Mixtures were incubated for 30 min at 37 °C, and incubation was terminated with the addition of 70 µL of 30 % KOH (w/v). Enzyme blanks were terminated with KOH at 0 min. Sucrose was assayed according to the modified anthrone method of Van Handel (1968).

Sucrose synthetase was assayed as above but using Fru (25 mM) instead of Fru6P, and in the absence of Glc6P (Cheikh and Brenner, 1992).

Assay of F1,6BPase (EC 3.1.3.11). Cytoplasmatic F1,6BPase was assayed spectrophotometrically at 630 nm, by measuring the F1,6BP-dependent P_i released under conditions that inhibited chloroplast F1,6BPase activity. The standard reaction medium contained Hepes-NaOH (50 mM, pH 7.6), $MgCl_2$ (5 mM), 2-mercaptoethanol (10 mM), NaF (10 mM), fructose-1,6-bisphosphate (0.25 mM) and an aliquot of enzyme extract, all in a final volume of 1 mL. The mixture was incubated for 20 min at 25 °C. The activity of nonspecific phosphatase was estimated by omission of $MgCl_2$ from the reaction mixture, and phosphate production was estimated by incubating the enzyme extract with the same assay mixture. The reaction was terminated by addition of 1 mL of TCA (30 %, v/v). P_i was determined by the method described by Geladopoulus *et al*. (1991).

Extraction and assays of the invertases (acid, EC 3.2.1.26 and alkaline, EC 3.2.1.27). Samples of 0.25 to 0.5 g were ground in a mortar containing sand and an extraction medium composed of KH_2PO_4-K_2HPO_4 (0.2 M, pH 7.0) and 2-mercaptoethanol (20 mM) (Hubbard *et al*., 1989). The homogenate was filtered and centrifuged at 35000 g_n for 15 min; the resulting extract was used to measure enzymes activities and soluble protein.

The extracts were assayed for acid invertase by addition of 0.2 mL enzyme preparation to test tubes containing 0.6 mL sodium acetate buffer (0.1 M, pH 4.5) and 0.2 mL sucrose (0.75 M) equilibrated in a 30 °C water bath. The reaction was allowed to proceed for 30 min and was stopped by addition of 1.0 mL dinitrosalicylic acid reagent prepared according to the Miller's method (1959). Reducing sugars released from sucrose were determined according to Miller (1959). Alkaline invertase activity was determined as described above, except that K_2HPO_4-sodium citrate buffer (0.1 M, pH 7.0) was substituted for the acetate buffer (Hubbard *et al*., 1989).

Quantification of non-structural carbohydrates. Carbohydrates were measured following the method of Irigoyen *et al*. (1992), with appropriate adaptations being made for our plant material. A sample of 0.5 g fresh matter was homogenized twice with 95 % ethanol (v/v) and washed with 70 % ethanol (v/v), followed by centrifugation at 1500 g_n at 2 °C for 15 min. Glucose, fructose, and sucrose were determined in the resulting supernatant by spectrophotometry at 650 nm, using the colorimetric assay with anthrone (Van Handel, 1968). Starch was determined from the dried residue of the previous extraction, which was incubated in buffer acetate (4.5 M), α-glucoamylase (0.5 %, w/v) and water, during 48 h at 37 °C. Starch levels were then calculated by multipliying by 0.9 the glucose obtained (Ettel, 1981).

Statistical analysis

Standard analysis of variance techniques were used to asses the significant differences among treatment means. The data shown are mean values ± standard errors. Difference between treatment means were compared using the LSD test at 0.05 probability level (SAS, 1987). A correlation analysis was also made between the different variables. Levels of significance are represented by * at $P < 0.05$, ** at $P < 0.01$, *** at $P < 0.001$, and ns: not significant.

RESULTS AND DISCUSSION

Currently, N is the most widely used nutrient in intensive agriculture in many zones of the world. This is because N is an essential component of proteins and nucleic acids in plants, and, when its concentration in the cultivation medium falls below the optimal range, plant growth diminishes (Weinhold *et al*., 1995). In our experiment, the N treatments affected the biomass production of roots ($P < 0.01$; Figure 1) and shoots ($P < 0.01$; Figure 1). Root biomass production declined as the N level rose since the lowest value appeared in the 24.0 mM treatment and the highest value in the was achieved by 1.5 mM dose. For shoot biomass, the highest values were registered in the 6.0 mM treatment, the level considered optimal by Carbonell-Barrachina *et al*. (1997), while the lowest production resulted at 1.5 mM (deficient) as well as at 18.0 mM and 24.0 mM (toxic) levels.

Thornley (1972) was the first researcher to propose a model explaining the changes in biomass distribution between roots and shoots. In this model, he proposed that growth depends on the carbon source from the shoot and of N from the root. In our experiment, the application of 6.0 mM of N proved to be the optimal treatment for an efficient production and distribution of root and shoot biomass in greenbeans. Treatments lower than 6.0 mM were considered N deficient, resulting in greater root but a lesser shoot growth, whereas the higher N levels (12.0, 18.0 and 24.0 mM) were considered toxic given the stunted growth of both root and shoot, the latter being more severely affected.

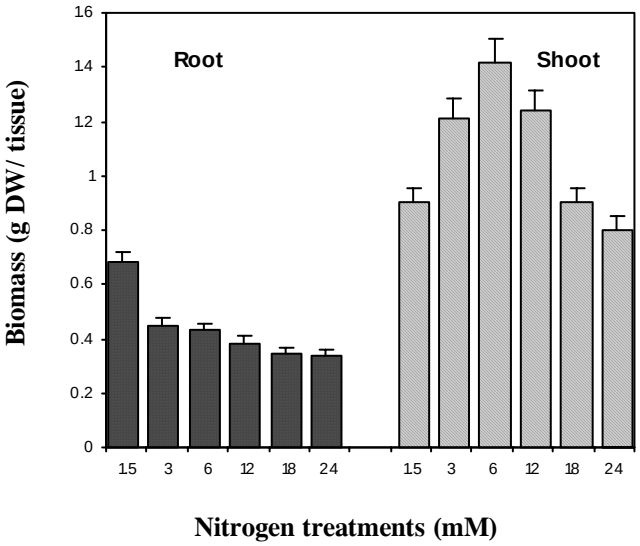

Figure 1. Effect of N treatments (1.5, 3.0, 6.0, 12.0, 18.0 and 24.0 mM) on the production of root and shoot biomass, expressed in g of dry weight in greenbean plants. The data are the means ± standard error (n = 6).

Table 1. Influence of the N treatments (1.5, 3.0, 6.0, 12.0, 18.0 and 24.0 mM) on the key enzymes in the biosynthesis and degradation of sucrose in the roots and shoot of greenbean.

Treatment (mM of N)	SPS µmol sucrose (mg protein x h)	F1,6BPase µmol Pi (mg protein x min)	SS µmol sucrose (mg protein x h)	Acid Invertase µmol glucose (mg protein x h)	Alkaline Invertase µmol glucose (mg protein x h)
Root					
1.5	10.35 ± 0.41	67.50 ± 2.70	2.58 ± 0.103	132.60± 5.30	216.70± 8.6
3.0	8.17 ± 0.32	65.83 ± 2.63	3.19 ± 0.127	194.30± 7.76	278.50± 11.1
6.0	6.43 ± 0.25	63.56 ± 2.54	4.16 ± 0.166	237.70± 9.50	310.90± 12.4
12.0	5.52 ± 0.22	59.13 ± 2.36	5.96 ± 0.238	272.50± 10.9	352.70± 14.1
18.0	4.74 ± 0.18	55.09 ± 2.20	7.16 ± 0.286	389.80± 15.5	470.70± 18.8
24.0	3.84 ± 0.15	42.84 ± 1.71	9.64 ± 0.385	407.60± 16.3	637.10± 25.4
Significance	**	**	**	***	***
Shoot					
1.5	12.79 ± 0.51	89.79 ± 3.59	1.05 ± 0.042	14.11± 0.56	26.98± 1.07
3.0	11.21 ± 0.44	82.39 ± 3.29	2.12 ± 0.084	26.00± 1.04	31.00± 1.24
6.0	9.48 ± 0.37	78.18 ± 3.12	3.32 ± 0.132	31.95± 1.27	42.05± 1.68
12.0	7.13 ± 0.28	73.69 ± 2.94	4.06 ± 0.162	35.99± 1.43	59.17± 2.36
18.0	6.61 ± 0.26	65.56 ± 2.62	5.18 ± 0.207	38.08± 1.52	67.86± 2.71
24.0	5.11 ± 0.20	58.71 ± 2.34	7.37 ± 0.294	43.72± 1.74	86.71± 3.46
Significance	**	**	**	***	***

Data are means ± standard error (n=6). The least significant difference (LSD) is given for each treatment. Levels of significance were represented by ** at $P < 0.01$ and by *** at $P < 0.001$.

SIS = Sucrose phosphate synthose; F1,6BPase= Fructose; 1,6 biophosphatse; SS = Sucrose synthose.

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Table 2. Effect of the N treatments (1.5, 3.0, 6.0, 12.0, 18.0 and 24.0 mM) on the accumulation of non-structural carbohydrates in the roots and shoot of greenbean plants.

Treatment (mM of N)	Glucose (mg g^{-1} f.w.)	Fructose (mg g^{-1} f.w.)	Sucrose (mg g^{-1} f.w.)	Starch (mg g^{-1} f.w.)	Sucrose/Starch Quotient
Root					
1.5	2.22 ± 0.088	2.71 ± 0.108	3.57 ± 0.142	14.64 ± 0.58	0.24
3.0	2.83 ± 0.113	3.28 ± 0.131	2.77 ± 0.110	12.95 ± 0.51	0.21
6.0	3.82 ± 0.151	4.18 ± 0.167	1.38 ± 0.055	10.62 ± 0.42	0.13
12.0	5.23 ± 0.209	5.71 ± 0.228	1.02 ± 0.040	8.87 ± 0.35	0.11
18.0	6.16 ± 0.246	6.55 ± 0.262	0.66 ± 0.026	6.09 ± 0.24	0.11
24.0	7.71 ± 0.308	8.43 ± 0.337	0.24 ± 0.009	4.03 ± 0.16	0.06
Significance	**	**	**	***	**
Shoot					
1.5	0.22 ± 0.008	0.26 ± 0.010	8.39 ± 0.335	22.54 ± 0.90	0.37
3.0	0.76 ± 0.030	0.78 ± 0.031	6.52 ± 0.260	20.00 ± 0.80	0.32
6.0	1.20 ± 0.048	1.04 ± 0.041	5.68 ± 0.227	18.48 ± 0.73	0.30
12.0	1.54 ± 0.061	1.40 ± 0.056	4.15 ± 0.166	16.56 ± 0.66	0.25
18.0	1.93 ± 0.077	2.80 ± 0.112	3.28 ± 0.131	15.45 ± 0.61	0.21
24.0	2.44 ± 0.097	3.10 ± 0.124	2.68 ± 0.107	13.49 ± 0.53	0.19
Significance	**	**	**	***	**

Data are means ± standard error (n=6). The least significant difference (LSD) is given for each treatment. Levels of significance were represented by ** at $P < 0.01$ and *** at $P < 0.001$

The nutritional status of N also determines the accumulation and distribution of sucrose within the plant. A high foliar accumulation of non-structural carbohydrates in N-deficient plants is due to a poor shoot growth in comparison to plants with adequate N (de Veau *et al.*, 1990; Robinson, 1996, 1997), as well as to an increase in enzymes responsible for the biosynthesis and degradation of these sugars (Champigny and Foyer, 1992; Huber *et al.*, 1994).

The key enzymes in sucrose biosynthesis are SPS and F1,6BPase (Stitt *et al.*, 1988). In our experiment, the N dosage significantly altered the activity of both enzymes in roots and shoots (Table 1). The highest activities were registered in the 1.5 mM treatment, with increases of 35 % and 63 %, respectively, in relation to the 24.0 mM dose, which produced the lowest activities.

Sucrose presented a behaviour similar to that of key enzymes involved in its biosynthesis, the 1.5 mM treatment resulting in the highest concentration in the roots and shoot, with increases of 93 % and 68 %, respectively, compared to 24.0 mM treatment, which gave the lowest value (Table 2). These results reveal a positive correlation between the activities of the enzymes responsible for sucrose biosynthesis (SPS and F1,6BPase) and the sucrose concentration in the roots (SPS-sucrose, r = 0.76**; F1,6BPase-sucrose, r = 0.82**) and in the shoots (SPS-sucrose, r = 0.92***; F1,6BPase-sucrose, r = 0.95***).

The accumulation of sucrose in higher plants depends also on its degradation rate, this brought about by a group of sucrose synthetase (SS) enzymes and acidic and alkaline invertases (Huber and Akazawa, 1986), yielding in both cases glucose and fructose (Schaffer, 1986). In our experiment, the SS and invertase activities in roots and shoots presented an effect directly proportional to the amount of N applied (Table 1), because the highest activities were found in the 24.0 mM level, which exceeded by 68 %, the activities found in both parts of the plant in the 1.5 mM treatment. Between the two invertases, the alkaline invertase presented highest activity. According to Schaffer (1986), in fully developed organs, alkaline invertase is more important for sucrose degradation, while acidic invertase is more active in less-developed organs.

The glucose and fructose concentrations resulting from the activities of SS and from the two invertases (Schaffer, 1986; Huber and Akazawa, 1986) increased significantly bottle in roots and shoot with the rising N levels (Table 2). The highest values registered in the 24.0 mM treatment were 68 % and 92 % higher than the lowest values of the 1.5 mM dose. It is noteworthy that glucose and fructose were more concentrated in the roots than in the shoots, presumably because in roots sucrose degradation was faster than its biosynthesis, while the opposite should occur in the shoot.

Finally, the triose-phosphates not used for sucrose synthesis are used to produce starch, which acts as a carbon-reserve substance (Medrano and Flexas, 2001). In our experiment, the starch concentration in roots and shoots presented the same behaviour as sucrose did (Table 2) in response to the increasing levels of N applied; that is, the 1.5 mM treatment induced the highest starch content thus overrating by 72 % (in the roots) and by 40 % (in the shoots) the 24.0 mM treatment, which registered the lowest value (Table 2). The highest sucrose/starch quotient was also found in the 1.5 mM treatment, and the lowest at 24.0 mM dose. Therefore, this quotient can be considered as a good indicator of deficiency and/or toxicity of N, since under deficient conditions (1.5 mM) this quotient was highest in the root and in the shoot, and declined as

the N level rose, with the lowest quotient corresponding to the toxic N level (24.0 mM).

CONCLUSION

Nutritional stress caused by N deficiency and toxicity largely determines the plant biomass distribution, as well as sucrose synthesis and degradation rates. The N deficiency was characterized by the stimulation of sucrose synthesis, because of the highest activities of fructose-1,6-biphosphatase and sucrose phosphate synthase. Under these stress conditions, sucrose translocation to the roots should be increased, thereby explaining why root growth was not affected. On the contrary, N toxicity diminished plant growth, prompting a glucose and fructose accumulation due to the significant stimulation of sucrose degradation associated with the highest activities of sucrose synthase, as well as those of acidic and alkaline invertases. Finally, the sucrose/starch quotient could be considered a good indicator of N deficiency and/or toxicity in green beans.

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