BIOLOGIA PLANTARUM 48 (1): 67-72, 2004

Effects of nitrate nutrition on nitrogen metabolism in cassava

J.L. CRUZ*, P.R. MOSQUIM, C.R. PELACANI**, W.L. ARAÚJO and F.M. DaMATTA[1]

Departamento de Biologia Vegetal, Universidade Federal de Viçosa, 36571-000 Viçosa, MG, Brasil

Abstract

Two experiments were conducted independently with plants of cassava (*Manihot esculenta* Crantz) growing in sand with nutrient solutions with four nitrate concentrations (0.5, 3, 6 or 12 mM). In leaves, nitrate-N was undetectable at the low nitrate applications; total-N, ammonium-N, amino acid-N, reduced-N and insoluble-N all increased linearly, while soluble proteins did it curvilinearly, with increasing nitrate supply. In contrast, soluble-N did not respond to N treatments. Total-N and soluble proteins, but not nitrate-N or ammonium-N, were much higher in leaves than in roots. Plants grown under severe N deficiency accumulated ammonium-N and amino acid-N in their roots. Further, plants were exposed to either 3 or 12 mM nitrate-N, and leaf activities of key N-assimilating enzymes were evaluated. Activities of nitrate reductase, glutamine synthetase, glutamate synthase and glutamate dehydrogenase were considerably lower in low nitrate supply than in high one. Despite the low nitrate reductase activity, cassava leaves showed an ability to maintain a large proportion of N in soluble proteins.

Additional key words: growth, *Manihot esculenta*, nitrogen-assimilating enzymes, nitrogen deficiency

Introduction

Under field conditions, nitrate-N (NO_3-N) is the main N source for most higher plants. Within the plant, NO_3-N is reduced to NH_4-N by the sequential action of nitrate reductase (NR; EC 1.6.6.1) and nitrite reductase (EC 1.7.7.1). The resulting NH_4-N is then assimilated into an organic form as glutamate and glutamine. These amino acids are the N donors in the biosynthesis of all other amino acids, nucleic acids, chlorophylls and hormones (Oliveira *et al.* 2001). The enzymes responsible for the biosynthesis of glutamine and glutamate are glutamine synthetase (GS; EC 6.3.1.2), glutamate synthase (GOGAT; EC 1.4.7.1) and glutamate dehydrogenase (GDH; EC 1.4.1.2). While GS/GOGAT cycle is recognised as the principal route of NH_4-N assimilation in higher plants, the physiological role of GDH, if it were more active in deamination rather than amination direction, is still disputed (Oliveira *et al.* 2001, Inokuchi *et al.* 2002, Miflin and Habash 2002).

To some Latin American, Asian and particularly African countries cassava is a major staple crop. Moraes *et al.* (1981) showed that yield of cassava increased substantially with increasing N supply, while Gomes and Howeller (1984) noted in some cases that N applications were not translated into economical yields; in other cases, cassava was even unresponsive to N fertilisation. Differences in experimental conditions and in cultivars investigated, from many other reasons, may be implied in this controversy. In any case, measurements of N in plant tissues would be important in order to forecast cassava's requirements for N, and how it makes use of the available N. This information is virtually unknown for cassava, as also for most other tropical crops. The main objective of this report was to study the changes in N metabolism in response to N nutrition. Concentrations of major organic and inorganic N component were measured in leaves and roots. In addition, activity of key enzymes involved in N-metabolism was also investigated.

Received 28 April 2003, *accepted* 3 September 2003.

Abbreviations: Fd - ferredoxin; GDH - glutamate dehydrogenase; GOGAT - glutamate synthase; GS - glutamine synthetase; NR - nitrate reductase.

Acknowledgements: Scholarships (J.L. Cruz, W.L. Araújo) and research fellowship (F.M. DaMatta) granted by the Brazilian Science and Technology Council (CNPq) are gratefully acknowledged.

*Present address: EMBRAPA/CNPMF, Rua Embrapa s/n, 44380-000 Cruz das Almas, BA, Brasil.

**Present address: Universidade Estadual de Feira de Santana, Rod. BR 116, 44031-460 Feira de Santana, BA, Brasil.

[1] Corresponding author; fax: +55-31-3899-2580; e-mail: fdamatta@ufv.br

Materials and methods

Two experiments were conducted independently, each one lasting 90 d, under greenhouse conditions, using plants of cassava (*Manihot esculenta* Crantz cv. Cigana Preta), in Viçosa (20°45' S, 42°15' W), Brazil. The first experiment was installed in 15 December 1998, and the second one in 20 September 2000. Growth conditions were similar in both experiments.

Two stem cuttings of cassava were planted on each 11 dm^3 pots filled with washed river sand. After 10 d, plants were thinned to one per pot. Plants were then watered daily and fertilised twice weekly with nutrient solutions modified from Hoagland and Arnon (1950) in order to contain four concentrations of NO_3-N (0.5, 3, 6 and 12 mM) in the first experiment, or two concentrations of NO_3-N (3 and 12 mM) in the second experiment. Solutions were adjusted to pH 5.7 - 6.0. Weekly, pots were thoroughly washed with tap water and subsequently with distilled water in order to avoid salinization of the substrate. All leaf samplings were made using central leaf lobes from the youngest, fully expanded leaves. Sections from the middle parts of absorbing roots were used for N analyses.

Fresh plant tissues (approximately 500 mg) were immersed in boiling 80 % ethanol and then stored at -20 °C. An aqueous extract from this material, obtained as described in DaMatta *et al.* (1999), was used for NO_3-N (Cataldo *et al.* 1975), NH_4-N (Weatherburn 1967) and amino acid-N (Moore and Stein 1954) determinations. Total-N was quantified using 100 mg dry mass according to Lang (1958).

For soluble proteins and insoluble-N, fresh plant tissues (approximately 500 mg) were immersed in liquid nitrogen and then stored at -80 °C until analysis. Frozen tissues were finely ground in methanol:chloroform:water (12:5:3, v/v/v), and the slurry was centrifuged at 1 000 *g* for 10 min. The resulting pellet was submitted to three successive extractions with 0.1 mM NaOH. The supernatants were combined, and then centrifuged at 1 000 *g* for 10 min. An aliquot of the supernatant was used for protein determination (Bradford 1976). The residue was digested with H_2SO_4 after which insoluble-N was quantified (Umbreit *et al.* 1972). Soluble-N was estimated as the difference between total-N and insoluble-N.

Activity of NR, assayed 24 and 48 h after the last addition of NO_3-N (90 and 91 d after planting), was performed using fresh tissues (about 300 mg) according to Jaworski (1971), with modifications, as follows: 5 mm^2 leaf discs were placed in vials containing 10 cm^3 incubation medium composed by 100 mM K-phosphate buffer (pH 7.5), 100 mM KNO_3 and 2 % (v/v) *n*-propanol. Vials were vacuum infiltrated three times at 87 kPa for 1 min each time, then incubated under darkness at 30 °C for 20 or 40 min. Aliquots were withdrawn to quantify the NO_2-N released into the medium. Each 0.5 cm^3 aliquot was added to a second medium containing 1 cm^3 1 % sulphanilamide in 1.5 mM HCl, 1 cm^3 0.02 % *N*-(1-naphthyl)-ethylenediamide-dihydrochloride and 1.5 cm^3 distilled water. After 30 min, the resulting absorbance was read at 540 nm. Enzyme activity was expressed as NO_2-N produced.

For GS, NADH-dependent GDH (NADH-GDH) and ferredoxin-dependent GOGAT (Fd-GOGAT), leaf tissues (approximately 1 g) previously stored at -80 °C were homogenised (0 - 4 °C) in 5 cm^3 medium containing 50 mM HEPES buffer (pH 7.5), 10 mM 2-mercapto-ethanol, 2 % polyvinylpolypyrrolidone, 1 % polyvinyl-pyrrolidone, 1 mM EDTA and 5 mM $MgCl_2$. The resulting slurry was centrifuged at 18 000 *g* for 20 min at 2 - 4 °C. For Fd-GOGAT, HEPES buffer was replaced by 100 mM K-phosphate buffer (pH 7.5).

Activity of GS was determined by the hydroximate biosynthetic method according to Ratajczak *et al.* (1981). Each 1 cm^3 reaction medium contained 100 mM Tris-HCl buffer (pH 7.5), 10 mM 2-mercaptoethanol, 40 mM $MgSO_4$, 50 mM glutamate, 10 mM hydroxylamine, 10 mM ATP and 0.32 cm^3 enzyme extract. The mixture was incubated at 30 °C for 20 min. Activity of GS was assessed at 540 nm and expressed as Fe-γ-glutamil hydroxymate produced.

Activity of NADH-GDH was assayed as described in Delú-Filho *et al.* (1998), with minor modifications. An appropriate aliquot from the enzyme extract was added to a reaction medium composed by 100 mM Tris-HCl buffer (pH 7.5), 100 mM $(NH_2)_2SO_4$, 10 mM 2-oxoglutarate and 4 mM $CaCl_2$, in a 2.7 cm^3 volume. After incubation at 30 °C for 3 min, 0.3 cm^3 0.1 mM NADH was added. Enzyme activity was measured at 340 nm and expressed as NAD^+ produced.

Activity of Fd-GOGAT was assayed following Matoh *et al.* (1979), with modifications. The reaction medium, containing 133 mM K-phosphate buffer (pH 7.5), 100 mM glutamine, 10 mM 2-oxoglutarate, 8 mM methyl viologen and 0.3 cm^3 enzyme extract, was incubated at 30 °C for 2 min. The reaction was started by adding 0.3 cm^3 reducing reagent (16 mg cm^{-3} Na-dithionite + 16 mg cm^{-3} $NaHCO_3$), in 1.5 cm^3 total volume. After 20 min, the reaction was stopped under boiling water. After cooling, centrifugation at 20 000 *g* for 5 min was performed. An aliquot of the supernatant was applied on a *Dowex* 1 × 8 (100 - 200 mesh) column in order to separate glutamate from glutamine. Glutamate was eluted with 0.3 mM acetic acid. A 2 cm^3 aliquot was added to 2 cm^3 medium (freshly prepared solutions composed by 400 mg ninhydrin, 1 g $CdCl_2$, 80 cm^3 ethanol, 10 cm^3 acetic acid and 20 cm^3 distilled water), and the reaction was allowed to occur at 80 °C for 10 min, after which it was stopped under an ice bath. Activity of Fd-GOGAT

was measured at 506 nm and expressed as glutamate produced.

The plants were distributed over a randomised block design with four and two treatments (NO_3-N applications) in the first and second experiments, respectively, both with five replicates. Each experimental plot was constituted by one plant per pot.

Results

Dry mass of the whole-plant and shoots increased sharply until 6 mM NO_3-N applications, and slightly thereafter (Table 1). Changes in dry mass of shoots were much more pronounced than that of roots and, as a consequence, shoot to root ratio enhanced with increasing N availability.

In leaves, total-N, NH_4-N, amino acid-N, reduced-N and insoluble-N increased consistently with increasing N applications (Table 2). Concentration of NO_3-N was virtually undetectable at 0.5 or 3 mM NO_3-N treatments, and then enhanced considerably with increasing N supply (Table 2). In contrast, soluble-N did not respond to N treatments, so that the ratio of soluble-N to total-N diminished as the N availability increased (Table 2). This ratio varied from 0.53 to 0.66 indicating that a large fraction of N was maintained in a soluble form. Concentration of soluble proteins, in turn, tended to respond curvilinearly to N applications. Altogether, these results suggest that a greater proportion of soluble-N should be diverted to the formation of soluble proteins in plants grown at 6 or 12 mM NO_3-N than in those of the other N treatments. In roots, total-N and soluble proteins increased consistently with increasing N availability

Table 1. Dry mass of cassava plants grown under four NO_3-N applications during 90 d (means ± SE, n = 5).

Dry matter [g]	NO_3-N [mM] 0.5	3.0	6.0	12.0
Whole-plant	20.6 ± 1.4	73.9 ± 3.8	129.5 ± 5.7	155.8 ± 3.4
Shoot	12.4 ± 0.8	40.6 ± 1.4	82.9 ± 1.2	101.6 ± 2.0
Absorbing root	8.2 ± 0.6	15.6 ± 1.2	16.8 ± 0.7	25.3 ± 1.5
Tuberous root	–	17.6 ± 2.9	29.8 ± 3.6	28.9 ± 3.3

Table 2. Nitrogenous fractions and ratio of soluble-N to total-N of cassava plants grown under four NO_3-N applications during 90 d. Results are expressed as [g kg^{-1}(f.m.)], except for amino acid-N [mmol kg^{-1}(f.m.)]. Means ± SE, n = 5; nd - not determined.

Parameter	0.5 mM NO_3-N		3.0 mM NO_3-N		6.0 mM NO_3-N		12.0 mM NO_3-N	
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
Total-N	27.2 ± 0.3	8.60 ± 0.2	33.40 ± 0.6	10.80 ± 0.9	36.90 ± 1.2	12.10 ± 0.5	43.60 ± 2.1	16.10 ± 0.2
NO_3-N	-	0.46 ± 0.04	-	0.06 ± 0.01	82.60 ± 12.5	0.52 ± 0.04	213.50 ± 20.4	4.80 ± 0.3
NH_4-N	-	0.22 ± 0.02	0.01 ± 0.001	0.10 ± 0.01	0.04 ± 0.004	0.09 ± 0.02	0.07 ± 0.003	0.12 ± 0.03
Amino acid-N	33.8 ± 2.6	50.90 ± 4.3	38.40 ± 3.1	22.30 ± 1.3	46.20 ± 1.9	35.20 ± 3.5	58.20 ± 3.3	46.90 ± 5.2
Soluble protein	38.9 ± 3.0	3.90 ± 0.7	70.90 ± 3.6	10.90 ± 2.0	110.20 ± 5.9	16.00 ± 1.7	116.40 ± 6.3	23.50 ± 1.9
Reduced-N	27.2 ± 0.3	nd	33.40 ± 0.6	nd	36.80 ± 1.3	nd	43.40 ± 2.1	nd
Soluble-N	17.6 ± 0.5	nd	21.90 ± 0.7	nd	21.30 ± 1.8	nd	23.40 ± 2.2	nd
Insoluble-N	9.6 ± 0.6	nd	11.50 ± 0.6	nd	15.50 ± 1.6	nd	20.20 ± 1.3	nd
Soluble-N to total-N ratio	0.65 ± 0.02	nd	0.66 ± 0.02	nd	0.58 ± 0.05	nd	0.53 ± 0.05	nd

Table 3. Activity of nitrate reductase (RN; assayed at 24 and 48 h after the last addition of NO_3-N), glutamine synthetase (GS), glutamate synthase (GOGAT) and glutamate dehydrogenase (GDH) in leaves of cassava grown under two NO_3-N applications during 90 days. Enzyme activities are expressed as [nmol g^{-1}(f.m.) min^{-1}] (see Material and methods for details). Means ± SE, n = 5.

Enzymes	3.0 mM NO_3-N	12.0 mM NO_3-N
NR - 24 h	3.33 ± 0.18	13.83 ± 0.42
NR - 48 h	13.83 ± 0.52	25.33 ± 1.03
GS	19.00 ± 2.67	40.50 ± 2.00
GOGAT	12.33 ± 1.00	32.00 ± 2.72
GDH	2.17 ± 0.35	5.33 ± 0.22

(Table 2). Nitrate-N concentration decreased with N nutrition at first, was undetectable at 3 mM NO_3-N and then increased at higher NO_3-N supplies (Table 2). Concentration of NH_4-N was the highest in plants grown at 0.5 mM NO_3-N. Amino acid-N concentration was large for plants nourished with 0.5 or 12 mM NO_3-N (Table 2).

Compared with roots, leaf concentrations of NO_3-N and NH_4-N were much lower; an opposite response was found for total-N and soluble proteins, regardless of the N treatments (Table 2). Leaves also exhibited a higher amino acid-N concentration than roots, except for plants grown at 0.5 mM NO_3-N in which such a concentration in roots was larger by 50 % than in leaves (Table 2). Changes in total-N concentration in both roots and leaves occurred in parallel; because shoot to root ratio decreased with declining N applications, an increase in partitioning of N to roots in N-deficient plants should have occurred.

Activity of NR was assayed 24 or 48 h after the last addition of NO_3-N. Compared with the first day, enzyme activity in the second day enhanced significantly, but much more in low-N plants (270 %) than in high-N plants (83 %) (Table 3), although absolute activity of NR was markedly larger in the last ones. Also, activity of the other N-assimilating enzymes was considerably greater in the high-N than in the low-N plants, 146 % for GDH, 113 % for GS, and 159 % for GOGAT (Table 3).

Discussion

The severity of N stress can be assessed as its effect on biomass accumulation of the whole-plant (Greenwood 1976). Thus, because total dry mass tended to increase curvilinearly, while total-N concentration did it linearly, with increasing N supply, it can be proposed that plants grown at 12 mM NO_3-N application did not suffer from any N limitation.

Root NO_3-N concentration was larger in plants grown at 0.5 mM NO_3-N than at 3 mM NO_3-N application. This might have been a consequence of increased capacity of roots to take up available NO_3-N as well as increase of NO_3-N retention in root tissues, processes occurring generally when the availability of external NO_3-N is severely limited (Talouizte *et al.* 1984, Rufty *et al.* 1990). In parallel, root concentrations of NH_4-N and amino acid-N were the largest at 0.5 mM NO_3-N. Hence, not only the capacity for NO_3-N uptake but also the root potential for assimilating NO_3-N and/or for retaining assimilation end-products apparently enhanced in the plants grown at 0.5 mM NO_3-N. Similar results were obtained in wheat by Talouizte *et al.* (1984) and in tobacco by Rufty *et al.* (1990). As stability of leaf proteins may be decreased under severe N stress (Mishra and Srivastava 1985), an increased mobilisation of reduced-N from older leaves to roots might have occurred. In any case, the accumulation of amino acid-N paralleling a very low soluble protein concentration suggests that protein synthesis was limiting, though we could not rule out that increased protein degradation has taken place in roots of severely N-deficient plants.

Since NR activity in cassava is believed to be greater in leaves than in roots (Pereira and Splittstoesser 1986), consumption of NO_3-N is expected to be larger in the former, so decreasing NO_3-N pools relatively more in leaves than in roots. In fact, concentrations of NH_4-N and amino acid-N in leaves, but not in roots, increased consistently with increasing N supply, which might be associated with an enhanced activity of N-assimilating enzymes.

The proportion of soluble-N diverted to the formation of soluble proteins decreased with the severity of N stress. In addition to the low availability of N for protein synthesis, two scenarios might be also suggested for explaining the lower protein concentration in N-starved plants: *1*) NO_3-N at relatively high concentration would lead to a marked rise of transcripts encoding various proteins (Stitt 1999); and *2*) greater activity of proteases in N-starved plants which would lead to a rise in protein degradation (Galangau *et al.* 1988). Whatever the case, the ability to maintain a large proportion of N in a soluble form and divert it to the formation of soluble proteins might help to understand, at least in part, why cassava is highly efficient in terms of carbon assimilation when contrasted with other C_3 species (El-Sharkawy *et al.* 1992).

Leaf NR activity was greater 48 h than 24 h after addition of NO_3-N. This result suggests that a time period longer than 24 h would be required for inducing maximal NR activity in cassava. Similar results were noted in tomato by Doddema *et al.* (1986), but not in maize in which maximal enzyme activity was found after 24 h following NO_3-N application to the growth medium (Purcino *et al.* 1998). Possibly, processes such as uptake, xylem load, and/or upward transport of NO_3-N are slower in cassava than in maize. Anyhow, it is well-known that NR is highly responsive to NO_3-N applications (Shankar and Srivastava 1997, Shankar *et al.* 2001), and our results confirm this.

Maximal NR activity as herein observed was similar to those of other reports for cassava (Pereira and Splittstoesser 1986) and coffee (DaMatta *et al.* 1999), but it was markedly lower than in herbaceous species such as tomato (Doddema *et al.* 1986) and spinach (Zornoza and González 1998). Since NR is known to be substrate-inducible (Campbell 1999, Stitt 1999), it might be supposed that the low enzyme activity would be associated with low leaf NO_3-N concentration. Nevertheless, even N-starved plants, in which NO_3-N was undetectable in leaves, showed NR activity. According to Campbell (1999), newly absorbed NO_3-N transported to

shoots seems to be more important to induce the activity of NR than leaf NO_3-N pools which are mostly stored in the vacuole rather than in the cytoplasm, where the first step of NO_3-N reduction takes place.

Parallel increases in activity of GS and GOGAT in the high-N versus in the low-N cassava plants show that they would act in a co-ordinate manner in order to assimilate NH_4-N, and help to explain the largest concentrations of amino acid-N and soluble proteins obtained in leaves of high-N plants. Furthermore, comparison of GDH against GS activity suggests a low contribution of the former for (re)assimilating NH_4-N.

In conclusion, increases in NO_3-N supply led to an enhanced activity of key N-assimilating enzymes. The lower activity of NR than that of GS might suggest that NO_3-N reduction would be more limiting to growth in cassava than NH_4-N assimilation, confirming the general belief that NR activity is the rate-limiting step in N-assimilation pathway (Campbell 1999). However, it should be pointed out that only measurements of metabolic fluxes across biochemical pathways could provide evidence for an imbalance between production and assimilation of NH_4-N. In any case, although analyses *in vitro* of the enzyme activities do not mean the actual activities *in vivo*, they reflect the potential and, thus, the capacity for using different substrates. For example, NR activity in cassava is apparently low in comparison with herbaceous species. Despite this, it was observed that cassava leaves exhibited high concentrations of reduced-N and soluble proteins. As already proposed, this ability might help in part to explain the high genetic potential for crop production of cassava when contrasted with other C_3 species.

References

Bradford, M.M.: Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Campbell, W.H.: Nitrate reductase structure, function, and regulation: bridging the gap between biochemistry and physiology. - Annu. Rev. Plant Physiol. Plant mol. Biol. **50**: 245-276, 1999.

Cataldo, D.A., Haroon, M., Schrader, L.E., Youngs, V.L.: Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. - Soil Sci. Plant Anal. Commun. **6**: 71-80, 1975.

DaMatta, F.M., Amaral, J.A.T., Rena, A.B.: Growth periodicity in trees of *Coffea arabica* L. in relation to nitrogen supply and nitrate reductase activity. - Field Crops Res. **60**: 223-229, 1999.

Delú Filho, N., Oliveira, L.E.M., Alves, J.S., Purcino, A.A.C.: [Nitrate reduction and ammonium assimilation in *Hevea brasiliensis* seedlings grown under increasing nitrate levels.]. - Rev. bras. Fisiol. veg. **10**: 185-190, 1998. [In Port.].

Doddema, H., Stulen, I., Hofstra, J.J.: The distribution of nitrate reductase in tomato (*Lycorpersicon esculentum*) leaves as affected by age. - Physiol. Plant. **68**: 615-619, 1986.

El-Sharkawy, M.A., Tafur, S.M., Cadavid, L.F.: Potential photosynthesis of cassava as affected by growth conditions. - Crop Sci. **32**: 1336-1342, 1992.

Galangau, F., Danielvedele, F., Moureaux, T., Thérèse, M., Dorbe, M.F., Leydecker, M.T., Caboche, M.: Expression of leaf nitrate reductase from tomato and tobacco in relation to light-dark regimes and nitrate supply. - Plant Physiol. **88**: 383-388, 1988.

Gomes, J.C., Howeller, R.: Produção de Mandioca em Solós de Baixa Fertilidade. [Production of cassava in low fertility soils.]. - EMBRAPA/DDT, Brasília 1984. [In Port.]

Greenwood, E.A.N.: Nitrogen stress in plants. - Adv. Agron. **28**: 1-35, 1976.

Hoagland, D.R, Arnon, D.I.:. The water culture method for growing plants without soil. - California agr. exp. Sta. Circular **347**: 1-32, 1950.

Inokuchi, R., Kuma, K., Miyata, T., Okada, M.: Nitrogen-assimilating enzymes in land plants and algae: phylogenic and physiological perspectives. - Physiol. Plant. **116**: 1-11, 2002.

Jaworski, E.G.: Nitrate reductase assay in intact plant tissues. - Biochem. biophys. Res. Comm. **43**: 1274-1290, 1971.

Lang, C.A.: Simple microdetermination of Kjeldahl nitrogen in biological materials. - Anal. Chem. **30**: 1692-1692. 1958.

Matoh, T., Suzuki, F., Ida, S.: Corn leaf glutamate synthase: purification and properties of enzyme. - Plant Cell Physiol. **20**: 1329-1340, 1979.

Miflin, B.J., Habash, D.Z.: The role of glutamine synthetase and glutamate dehydrogenase in nitrogen assimilation and possibilities for improvement in the nitrogen utilization of crops. - J. exp. Bot. **53**: 979-987, 2002.

Mishra, S.N., Srivastava, H.S.: Role of inorganic nitrogen in synthesis and degradation of protein in maize leaves. - Indian J. Plant Physiol. **28**: 43-52, 1985.

Moore, S., Stein, W.H.: Photometric ninhydrin method for use in chromatography of amino acids. - J. biol. Chem. **176**: 367-388. 1948.

Moraes, O., Mondardo, E., Vizzotto, V.J., Machado, M.O.: Adubação Química e Calagem da Mandioca. [Mineral Fertilisation and Liming for Cassava]. - EMPASC, Florianópolis 1981. [In Port.]

Oliveira, I.C., Brenner, E., Chiu, J., Hsieh, M.H., Kouranov, A., Lam, H.M., Coruzzi, G.: Metabolite and light regulation of metabolism in plants: lessons from the study of a single biochemical pathway. - Braz. J. med. biol. Res. **34**: 567-575, 2001.

Pereira, J.F., Splittstoesser, W.E.: Nitrate reduction by cassava. - Plant Cell Physiol. **27**: 925-927, 1986.

Purcino, A.A.C., Arellano, C., Athwat, G.S., Huber, S.C.: Nitrate effects on carbon and nitrogen assimilating enzymes of maize hybrids representing seven eras of breeding. - Maydica **43**: 83-94, 1998.

Ratajczak, L., Ratajczak, W., Mazurowa, H.: The effect of different carbon and nitrogen sources on the activity of glutamine synthetase and glutamate dehydrogenase in lupine embryonic axes. - Physiol. Plant. **51**: 277-280, 1981.

Rufty, T.W., Mackown, C.T., Volk, R.J.: Alterations in nitrogen assimilation and partitioning in nitrogen stressed plants. - Physiol. Plant. **79**: 85-95, 1990.

Shankar, N., Khan, S.R., Srivastava, H.S.: The response of nitrate reductase activity and nitrate assimilation in maize roots to growth regulators at acidic pH. - Biol. Plant. **44**: 500-601, 2001.

Shankar, N., Srivastava, H.S.: Nitrate reductase activity and uptake of nitrate and oxygen as affected by nitrate supply to detached maize organs. - Biol. Plant. **39**: 583-589, 1997.

Stitt, M.: Nitrate regulation of metabolism and growth. - Curr. Opinions Plant Biol. **2**: 178-186, 1999.

Talouizte, A., Guiraud, G., Moyse, A., Marol, C., Champigny, M.L.: Effect of previous nitrate deprivation on $^{15}NO_3^-$ absorption and assimilation by wheat. - J. Plant Physiol. **116**: 113-122, 1984.

Umbreit, W.W., Burris, R.H., Stauffer, J.F.: Manometric and Biochemical Techniques. - Burgess, Minneapolis 1972.

Weatheburn, M.W.: Phenol hypochloride reaction for determination of ammonia. - Anal. Chem. **39**: 971-974, 1967.

Zornoza, P., González, M.: Intraspecific differences in nitrogen assimilating enzymes in spinach under contrasting forms of nitrogen supply. - J. Plant Nutr. **21**: 1129-1138, 1998.