BIOLOGIA PLANTARUM (PRAHA)

33 (6): 468–474, 1991

Nitrogen Metabolism in *Erica* and Soybean, Two Species Differing by Their Sensitivity to Inorganic N Source

G. GUERRIER

FRD Biotechnologies. Ensemble Scientifique, 2 Bd Lavoisier, 490 45 Angers Cedex, France

Abstract. Growth of soybean was not altered, whatever the inorganic N-source (NO_3, NH_4 or a NO_3/NH_4 mixture); conversely, growth of *Erica* × *darleyensis* plants *in vitro* decreased more in NH_4 medium than in a NO_3 medium, compared to a NO_3/NH_4 medium. The GS/GOGAT pathway (in NH_4 medium), the nitrate and nitrite reductase activities (in NO_3 medium), as the contents in free nitrogenous forms and total nitrogen (in NO_3 and NH_4 media) were not more altered in *Erica* than in soybean, compared to a NO_3/NH_4 medium. PEPCase activity was the highest in soybean irrespective of the N-treatments; the involvement of PEPCase in N-metabolism could be explained by its function in ionic and osmotic balances rather than its function in supplying carboxylates as acceptors for NH_4-assimilation.

Additional index words: Erica × *darleyensis; Glycine max;* ionic and osmotic balances; N-assimilation; N-reduction; stress.

Nutrient solution content is an essential factor for plant growth and for exploitation of plant tissue cultures, especially for the plants *in vitro* with high economical value, such as abiotic or biotic stress-adapted clones, or selected ornamental shrubs as *Erica* × *darleyensis*, the most cultivated heather in France. Particularly the inorganic N source (NO_3 or NH_4) affects plant growth and nutrient content; several hypotheses have been made for explaining the decrease in growth caused by NH_4 nutrition.

Recent studies have shown that phosphoenolpyruvate carboxylase (PEPcase) activity in the roots of NH_4-fed plants is higher than that of NO_3-fed plants, while PEPCase activity in the leaves of NO_3-grown plants is higher than that of NH_4-grown plants (Schweizer and Erismann 1985; Salsac *et al.* 1987; Arnozis *et al.* 1988). Since NH_4 is assimilated mainly in the roots and NO_3, for a major part, in the shoots (Blackwood and Miflin 1976; Raven and Smith 1976), two functions have been decribed for the PEPCase in the N-metabolism: the PEP carboxyation would provide substrates for amination and organic acids for transfer of cations to the leaves, ionic balance and osmotic adjustments (Kirkby and Knight 1977; Salsac *et al.* 1987; Arnozis *et al.* 1988). As a matter of fact,

Received September 7, 1990; accepted January 1, 1991

Arnozis and Findenegg (1986) observed that all organic acids found in the shoots of NH_4-fed plants were practically produced in the roots, whereas those found in the shoots of NO_3-fed plants were produced in the shoots.

The purpose of these experiments was to specify, in two species differing by their sensitivity to NO_3 and NH_4, the effects of N-source on the enzymes involved in N-assimilation, including PEPCase activity.

MATERIAL AND METHODS

Plant culture: Soybean seedlings (*Glycine max* Merr, cv. Maple Arrow) and *Erica* × *darleyensis* plants *in vitro* (Guerrier *et al.*, 1985) were cultivated on perlite media under controlled irradiance and temperature (60 W m^{-2}, 18–22 °C). Nitrogen was given as either NO_3 or NH_4 or a combination of NO_3/NH_4. i) NO_3 solution [mM]: KH_2PO_4, 0.4; $MgSO_4$ $7H_2O$, 0.25; Ca $(NO_3)_2$ $4H_2O$, 1; KNO_3, 0.6; Fe-EDTA and micronutrients as in Guerrier *et al.* (1985). ii) NH_4 solution: KH_2PO_4, 0.4; $MgSO_4$ $7H_2O$, 0.25; KCl, 0.6; $CaCl_2$, 1; NH_4Cl, 2.6; micronutrients and Fe-EDTA as stated above. iii) NO_3/NH_4 solution: KH_2PO_4, 0.4; $MgSO_4$ $7H_2O$, 0.25; $Ca(NO_3)_2$ $4H_2O$, 0.5; KCl, 0.6; $CaCl_2$, 0.50; NH_4Cl, 1.6; micronutrients and Fe-EDTA as stated above. 10 ml of nutrient solution (pH 5.9) was supplied daily to aech plant.

After 28 d of culture, plants were harvested; shoots and roots were separated, rinsed with distilled water and then analysed.

Mineral analysis: Total N content was determined by colorimetry after mineralisation with H_2SO_4; free NO_3, NH_4 and NO_2 forms were determined by colorimetry after extraction with boiling water under agitation for one hour (Cataldo *et al.* 1975; Robin 1979; Guerrier *et al.* 1985). Ten replicates of 3 shoots or roots each were performed.

Enzyme assays: 5 g fresh matter was homogeneized in a mortar with 30 ml Tris-HCl buffer 0.05 M pH 7.4 containing polyvinylpyrrolidone (50% of fresh matter mass), 5 mM GSH and 1 mM $MgCl_2$. The homogenates were centrifuged at 20 000 *g* at 0 °C for 15 min, and the supernatants were directly used for the enzyme assays.

Activities of nitrate reductase (NRase) and nitrite reductase (NiRase) were assayed *in vitro* and NO_2 concentrations were measured colorimetrically (Robin 1979; Guerrier *et al.* 1985, Guerrier 1988a). Glutamine synthetase (GS) was expressed as the quantity of Pi releassed in the assay medium (Boucaud and Billard 1981; Guerrier 1988a). Glutamate synthase (GOGAT), glutamate dehydrogenase (GDH) and phoshoenolpyruvate carboxylase (PEPCase) were measured at 340 nm by coupling the reactions to the NADH-oxidation (Bergmeyer, 1974; Guerrier, 1988a,b).

All activities were given per minute per mg protein. Soluble protein content of crude extracts was determined using Bradford reagent (Bradford 1976) and bovine serum albumin as standard.

TABLE 1

Fresh matter [mg per plant] of soybean and *Erica* after 28 d of culture on NO_3, NH_4 or NO_3/NH_4 media.

		Soybean	*Erica*
Shoots	NH_4	870a	69c
	NO_3/NH_4	990a	197a
	NO_3	923a	138b
Roots	NH_4	194a	46c
	NO_3/NH_4	195a	191a
	NO_3	191a	107b

For a given organ and a given species, means not denoted by the same letter differ significantly at the $P < 0.05$.

RESULTS AND DISCUSSION

1. Plant growth.

After 28 d of culture, the fresh mass of soybean was not affected by the nitrogen source; on the contrary, growth of *Erica* was the highest on NO_3/NH_4 medium, the NH_4 medium being twice toxic than NO_3 medium (Table 1). The sole presence of NH_4 (2.6 mM) in the nutrient medium was responsible for this decrease in growth, because *Erica* biomass was not altered by nutrient solutions containing either a combination of 2.74 mM NH_4 and 2.74 mM NO_3 or 5.47 mM Cl (Guerrier *et al.*, 1985).

2. Free N-forms and enzymes involved in N-metabolism.

Changes in fresh and dry matter production in *Erica* were not linked with energy expediture or with activity of enzymes involved in N-assimilation: (i) growth of NO_3-fed *Erica* was higher than that of NH_4-plants, while the cost of N assimilation was three times higher in NO_3-fed plants (Cox and Reisenhauer 1973; Salsac *et al.* 1987); (ii) the GS/GOGAT pathway in NH_4 medium (main route of NH_4 assimilation), and the NRase and NiRase activities in NO_3 medium were not more altered in *Erica* than in soybean (Table 2).

Although the NRase activity was 5 to 10 times higher in soybean, free NO_3 supply in soybean was 10 to 50 times higher than that in *Erica* (Table 3.) But the contribution of free nitrogenous forms in osmotic balance did not seem responsible for the decrease of *Erica* biomass in NO_3 medium; as a matter of fact, free NO_3 content was higher in NO_3-fed *Erica* than in NH_4/NO_3-fed *Erica*, while in soybean the NO_3 supply was practically equal both in NO_3 or NO_3/NH_4 media.

The ratio of NRase/NiRase enzyme activity decreased in the same proportions in *Erica* and soybean from NO_3/NH_4 medium to NO_3 medium, but the NO_2 contents (Table 3) were 5 times higher in *Erica* than in soybean. In the

TABLE 2

Nitrate (NRase) and Nitrite (NiRase) reductase, Glutamine synthetase (GS), Glutamate synthase (GOGAT), Glutamate dehydrogenase (GDH) and Phosphoenol pyruvate carboxylase (PEPCase) in soybean and *Erica* after 28 d of culture on NO_3, NH_4 or NO_3/NH_4 media. All results are given per minute per milligram protein.

	NRase [ng NO_2 released]		NiRase [ng NO_2 oxidized]		GS [μmol P_i released]		GOGAT [nm NADH oxidized]		GDH [nm NADH oxidized]		PEPCase [nm NADH oxidized]	
	Soybean	*Erica*	Soybean	*Erica*	Soybean	*Erica*	Soybean	*Erica*	Soybean	*Erica*	Soybean	*Erica*
Shoots												
NH_4	–	–	–	–	3.50	3.67	5.20	3.50	27.9	115.0	1.03	0.73
NO_3/NH_4	51.9	33.5	657	54.3	1.83	1.43	3.50	3.20	15.4	77.2	6.19	1.28
NO_3	33.5	15.8	619	48.0	1.02	0.64	2.30	3.16	7.63	34.3	20.3	0.81
Roots												
NH_4	–	–	–	–	0.68	0.78	2.83	12.0	38.7	44.2	38.6	18.9
NO_3/NH_4	122.0	25.6	4660	130.0	0.76	0.90	2.16	8.50	35.1	31.5	27.1	17.7
NO_3	71.7	13.3	3847	80.2	0.62	0.71	0.36	0.47	4.71	2.20	26.2	10.2

TABLE 3

N-total, free nitrogenous form- and soluble protein contents [per gram of fresh matter] in soybean and *Erica* after 28 d of culture on NO_3, NH_4 or NO_3/NH_4 media.

	N total [mg g^{-1}]		NO_3 [µg g^{-1}]		NH_4 [µg g^{-1}]		NO_2 [ng g^{-1}]		Soluble protein [mg g^{-1}]	
	Soybean	*Erica*	Soybean	*Erica*	Soybean	*Erica*	Soybean	*Erica*	Soybean	*Erica*
Shoots										
NH_4	4.36a	3.10a	–	–	5.78a	79.6a	4.30a	26.7a	7.92a	4.25a
NO_3/NH_4	4.88a	2.63a	1880a	42.6a	5.58a	30.4b	13.40b	29.2a	6.81a	4.41a
NO_3	4.84a	2.70a	2120a	160.0b	3.96b	2.8c	17.50c	96.7b	7.60a	4.20a
Roots										
NH_4	2.06a	2.60a	–	–	110.00c	31.3c	5.12a	37.2a	2.30a	3.25a
NO_3/NH_4	1.93a	2.70a	565a	9.7a	40.10a	22.1b	15.40b	137.0b	1.85a	3.02a
NO_3	2.35a	3.00a	899b	22.1b	59.60b	12.0a	38.70c	212.0c	2.12a	3.21a

For a given organ and a given species, means not denoted by the same letter differ significantly at the $P < 0.05$.

same way, NH_4 content (Table 3) increased in shoots of NH_4-fed *Erica*, compared to NO_3/NH_4 medium. However, NO_3 and NH_4 contents did not reach critical or toxic levels (Guerrier *et al.*, 1985).

3. PEPCase activity and total nitrogen content.

Our results corroborate that PEPCase activities (Table 2) of shoots of NO_3-fed plants were higher than those of NH_4-fed plants, and that PEPCase activities of roots of NH_4-fed plants were higher than those of NO_3-fed plants (Schweizer and Erismann 1985; Salsac *et al.* 1987; Atnozis *et al.* 1988). As PEPCase activity was related with NaCl-tolerance (Guerrier 1988b), high PEPCase activity involved in N-metabolism could be also linked with plant adaptation towards NO_3 or NH_4: PEPCase activity was the highest in soybean irrespective of the inorganic nitrogen source. Such a change in PEPCase activity did not result from pH change at the root surface or within the root free space: a NH_4 nutrition decreases the pH at the root surface, but PEPCase activity decreases with the pH of the rooting medium (Arnozis *et al.* 1988).

The function of PEPCase in providing substrates for amination did not seem to be a limiting factor of growth: the total N and soluble protein contents (Table 3) were similar, irrespective of the inorganic nitrogen source, contrarily to observations of Cox and Reisenhauer (1973) or Magalhaes and Wilcox (1984). Hence, another explanation for the increased activity of PEPCase concerns the capacity for maintaining the ionic and osmotic balances, *via* the organic acid system: a low supply of organic anion impaired the absorption and translocation of cations (Salsac *et al.*, 1987), the accumulation of solutes and the possibility of osmotic adjustment – and osmolarity due to free nitrogenous forms (Table 3) and cations (Guerrier et al., 1985) was low in *Erica* compared to soybean, thus leading to internal physiological disorders: the formation of oxaloacetace *via* PEPCase or the import of malate (Oaks and Hirel 1985) could maintain the efficient functioning of the tricarboxylic acid cycle, and provide the replacement of ketoglutarate for glutamine and chlorophyll syntheses, all the more as a high GDH activity, linked with NH_4 detoxification (Fentem *et al.*, 1983) provoked a high requirement for ketoglutarate as substrate for amination. Other physiological disorders have already been reported such as inhibitions of NADP reduction, CO_2 fixation within the chloroplasts, ATP formation or modification of energy charge coupled with carboxylase activities (Davies, 1979; Magalhaes and Wilcox, 1984; Salsac *et al.*1987).

The involvement of PEPCase in ionic balance and osmotic adjustment, regardless of plant-tolerance towards abiotic streses (NH_4, NO_3 or NaCl), is currently under investigation.

Acknowledgements

Special thanks to F. Beaujard and JD Viemont for providing *Erica* plants *in vitro* and to JC Moreau for his technical assistance.

REFERENCES

Arnozis, P. A., Findenegg, G. R.: Electrical charge balance in the xylem sap of beet and sorghum plants were grown with either NO_3 or NH_4 nitrogen. – J. Plant Physiol. **125**: 441–449, 1986.

Arnozis, P. A., Nelemans, J. A., Findenegg, G. R.: Phosphoenolpyruvate carboxylase activity in plants grown with either NO_3 or NH_4 as inorganic N source. – J. Plant Physiol. **132**: 23–27, 1988.

Bergmeyer, H. U.: Methods of enzymatic analysis. Vol. 2. Pp. 613–617. Acad. Press, New York 1974.

Blackwood, C., Miflin, B. J.: The effect of nitrate and ammonium feeding on carbon assimilation in maize. – J. exp. Bot. **27**: 735–747, 1976.

Boucaud, J., Billard, J. P.: La glutamine synthetase du *Suaeda maritima*: Action *in vivo* et *in vitro* du NaCl. – Physiol. Plant. **53**: 558–564, 1981.

Bradford, M. M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. – Anal. Biochem. **72**: 248–253, 1976.

Cataldo, D. A., Hardon, M., Schrader, L. E., Youngs, V. L.: Rapid colorimetric determination of NO_3 in plant tissue by nitration of salicylic acid. – Commun. Soil Sci. Plant Anal. **6**: 71–80, 1975.

Cox, W. J., Reisenauer, H. M.: Growth and ion uptake by wheat supplied nitrogen as nitrate, ammonium or both. – Plant Soil **38**: 363–380, 1973.

Davies, D. D.: The essential role of phosphoenolpyruvate carboxylase in plant metabolism. – Annu. Rev. Plant Physiol. **30**: 131–158, 1979.

Fentem, P. A., Lea, P. J., Stewart, G. R.: Ammonia assimilation in roots of nitrate and ammonia growth *Hordeum vulgare*. – Plant Physiol. **71**: 496–501, 1983.

Guerrier, G.: Activité de quelques enzymes du cycle de l'azote lors de la germination en milieu salé. – Fyton **48**: 85–91, 1988a.

Guerrier, G.: Capacités Phosphoénolpyruvate carboxylase et Malate déshydrogénase extraites des plantules germées en milieu salé: des paramètres de l'écophysiologie de la plante? – Seed Sci.-Technol. **16**: 571–578, 1988b.

Guerrier, G., Beaujard, F., Viémont, J. D.: Influence de la concentration de la solution nutritive dans la phase de réinsertion *in vivo* des vitroplants d'*Erica* × *darleyensis*. 2) Relations entre les fractions libres azotées et les capacités nitrate réductate et glutamate déshydrogénase. – Plant Soil **84**: 337–345, 1985.

Kirkby, E. A., Knight, A.: Influence of the level of nitrate nutrition on ion uptake and assimilation, organic acid accumulation and cation-anion balance in whole tomato plants. – Plant Physiol. **60**: 349–353, 1977.

Magalhaes, J.- R., Wilcox, G. E.: Ammonium toxicity development in tomato plants, relative to nitrogen form and light intensity. – J. Plant Nutr. **7**: 1447–1496, 1974.

Oaks, A., Hirel, B.: Nitrogen metabolism in roots. – Annu. Rev. Plant Physiol. **36**: 345–365, 1985.

Raven, J. A., Smith, F. A.: Nitrogen assimilation and transport in vascular land plants in relation to intracellular pH regulation. – New Phytol. **75**: 415–431, 1976.

Robin, P.: Etude de quelques conditions d'extraction de la nitrate réductase des racines et des feuilles de plantules de mais. – Physiol. vég. **17**: 45–54, 1979.

Salsac, L., Chailou, S., Morot-Gaudry, J. F., Lesaint, C., Jolivet, E.: Nitrate and ammonium nutrition in plants. – Plant Physiol. Biochem. **25**: 805–812, 1987.

Schweizer, P., Erismann, K. H.: Effect of nitrate and ammonium nutrition of non nodulated *Phaseolus vulgaris* on PEPCase and pyruvate kinase activity. – Plant Physiol. **78**: 455–458, 1985.