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Effect of ammonium salt on enzymes of ammonium assimilation in maize seedlings

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Abstract

Glutamate dehydrogenase (GDH E.C. 1.4.1.2.4), glutamine synthetase (GS E.C. 6.3.1.2) and glutamate synthase (glutamine oxoglutarate amino transferase, GOGAT E.C. 2.6.1.53) activities, protein and organic nitrogen contents and growth of roots and shoots of maize seedlings raised in dark at 25 ± 2 °C in half strength Hoagland's solution containing different ammonium salts as source of nitrogen, were determined to assess the contribution of alternate pathways in ammonium assimilation. Ammonium nitrate or in some cases ammonium chloride appeared to be the best source for both root and shoot growth and for increase in protein, total nitrogen and the enzymes of ammonium assimilation. In roots, NH_4-nitrogen appeared to be assimilated by both GDH as well as GS-GOGAT pathways specially in the dark grown seedlings, while in shoots it was primarily by GS-GOGAT pathway.

Key words: glutamate dehydrogenase, glutamate synthase, glutamine synthetase, *Zea mays*.

Introduction

The most important reaction in ammonium assimilation is the synthesis of glutamic acid. Majority of the opinion is in favour of ammonium assimilation through the sequential action of glutamine synthetase (GS) and glutamine oxoglutarate amino transferase (GOGAT) (Lea *et al.* 1992), although some experimental findings support the operation of glutamate dehydrogenase (GDH) pathway, especially in the roots (Rhodes *et al.* 1981). Among other factors, the source of inorganic nitrogen, the nature of the plant organ, or the light-dark conditions may also influence the operation of either GDH or GS-GOGAT pathway (Srivastava and Singh 1987). A positive correlation between the total GDH activity and total Kjeldahl nitrogen is more apparent in roots than in other tissues (Singh and

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Srivastava 1982). In maize seedlings, the supply of any form of inorganic nitrogen increases NADH-GOGAT activity, although the increase in the presence of nitrate is higher than in presence of ammonium (Singh and Srivastava 1986). In the cells culture of *Ipomoea* sp. increase in either nitrate or ammonium concentration increases the levels of GS along with GDH and GOGAT activities (Zink 1989). In some cases the stimulatory effect of ammonium on the activity of GS has been observed *e.g.* in pea (Kretovich 1978) and *Cucurbita pepo* (Kretovitch *et al.* 1981). In maize roots, the activity of both GDH as well as GS is higher with NH_4^+ as compared to NO_3^- as nitrogen source (Magalhaes and Huber 1989). In contrast to this, ammonium supply decreases GS activity in *Lemna* (Rhodes *et al.* 1975) and lupine embryos (Ratajczak *et al.* 1981).

Although the effect of a few cations on GDH activity has been examined, the influence of anions accompanying ammonium on its assimilation and GDH activity has not been examined. Further, it is also not known how this response varies according to the tissues. Effects of various ammonium salts on increase in NADH-GDH, NADH-GOGAT and GS activity, protein, total nitrogen and growth of roots and shoots of maize seedlings were studied in the present investigation, with this perspective.

Materials and methods

Seeds of *Zea mays* L. cv. Ganga safed-2 were surface sterilised with 0.1 % $CaOCl_2$ for about 5 min and then washed throughly with distilled water. The seedlings were raised on moist filter paper in Petri plates, for 5 d in darkness or in light (irradiance of 70 W m^{-2}) at temperature 25 ± 2 °C. These were watered with 1/2 strength modified Hoagland's solutions which contained different ammonium salts as nitrogen source. The filter paper was always kept moist. The pH of the nutrient solution was 6.2.

Glutamate dehydrogenase was extracted in a medium containing 0.05 M sodium phosphate buffer (pH 7.4), 0.4 M saccharose and 2 mM EDTA (as sodium salt). The ratio of plant material to medium was 1:4 (m/v). The enzyme activity (NADH specific GDH) in the clear supernatant obtained after centrifugation at 12 000 *g* for 10 min was assayed by the method of Singh and Srivastava (1982).

Activity of glutamine synthetase was assayed in the crude extracts by the method of Lillo (1984) with slight modifications. Extract was prepared at 0 to 4 °C. Root and shoot tissues (0.1 g each) were homogenized with sand in a mortar with 4 cm^3 of 50 mM Tris HCl (pH 7.8) containing 15 % (v/v) glycerol, 14 mM 2-mercaptoethanol, 1 mM EDTA and 0.1 % (v/v) Triton X-100. The homogenate was squeezed through two layers of cheese cloth and then centrifuged at 10 000 *g* for 10 min. The supernatant was used as the enzyme extract. Glutamine synthetase was determined using the synthetase assay (Webster 1964).

Tissue was extracted in a mortar in a medium containing 0.2 M sodium phosphate buffer (pH 7.5), 2 mM EDTA and 50 mM KCl, 0.1 % mercaptoethanol and 0.5 % Triton X-100 (v/v) at 0 - 4 °C and filtered through 2 layers of cheese cloth. The

homogenate was centrifuged at 12 000 *g* for 20 min. The clear supernatant was used as enzyme preparation. The glutamate synthase was assayed by the method of Singh and Srivastava (1986).

Protein was measured either in the dry tissue or in the enzyme extract by the method of Lowry *et al.* (1951). The protein from the dry tissue was extracted from roots and shoots in phosphate buffer (0.1 M, pH 7.4). In each case, it was precipitated with equal volumes of 20 % trichloroacetic acid. Bovine serum albumin was used as a standard.

For nitrogen determination, an aliquot of 100 mg tissue from the oven dried (at 60 °C for 48 h) sample was digested in 2 - 3 cm^3 of concentrated sulfuric acid. Total nitrogen in the digested aliquot was measured by micro-Kjeldahl method (Lang 1958).

Dry mass of the roots and shoots from uniformly grown seedlings were measured by drying the tissues at 55 °C for 48 h.

The values presented are means ± S.D. of at least three independent experiments. The growth data are means of 3 - 4 experiments, 10 plants in each experiment.

Results

The specific activity of glutamate dehydrogenase (GDH), glutamine synthetase (GS) and glutamate synthase (GOGAT) in roots and shoots of 5-d-old seedlings in response to various ammonium salts in the nutrient solution were measured to evaluate the significance of GDH versus GS-GOGAT pathway in the assimilation of ammonium. GDH-activity in the roots increased substantially with the supply of NH_4Cl, NH_4NO_3 and ammonium acetate in the roots and in addition with $(NH_4)_2SO_4$ and $(NH_4)_2CO_3$ as well in the shoots (Fig. 1). Other salts had either no effect or inhibited the enzyme activity slightly.

The specific activities of GS in the roots increased with the supply of ammonium salts, except for ammonium acetate (Fig. 1). The maximum increase (over no nitrogen) was observed with ammonium sulphate. Similar effect of ammonium salt supply on GS activity was seen in shoots.

NADH-GOGAT in the roots increased with almost each ammonium salt except for ammonium chloride (Fig. 1). The maximum increase was observed with ammonium nitrate. In the shoots, however, an increase in enzyme activity was recorded only with NH_4Cl and NH_4NO_3. Other salts inhibited GOGAT activity.

In the root tissue, the supply of ammonium nitrate, ammonium chloride, or ammonium sulphate increased protein content on dry mass basis by 6, 14 and 11 % respectively, while the ammonium carbonate, ammonium oxalate and ammonium acetate inhibited it slightly (Fig. 1). Total root nitrogen increased with each of the ammonium salts. The protein and total nitrogen contents of shoots increased with each of the ammonium salts, except for ammonium acetate which slightly inhibited the protein content or which had no effect on total nitrogen content (Fig. 1).

Fresh mass of roots increased considerably with each of the ammonium salts, but ammonium oxalate and ammonium acetate had almost no effect (Fig. 1). Ammonium

acetate had no effect on root dry mass, while all other salts increased it. Shoot fresh mass increased only with ammonium chloride and ammonium nitrate, other salts had either no effect or decreased it slightly. Dry mass of the shoots increased slightly with ammonium nitrate, ammonium carbonate and ammonium chloride but decreased with other salts tested.

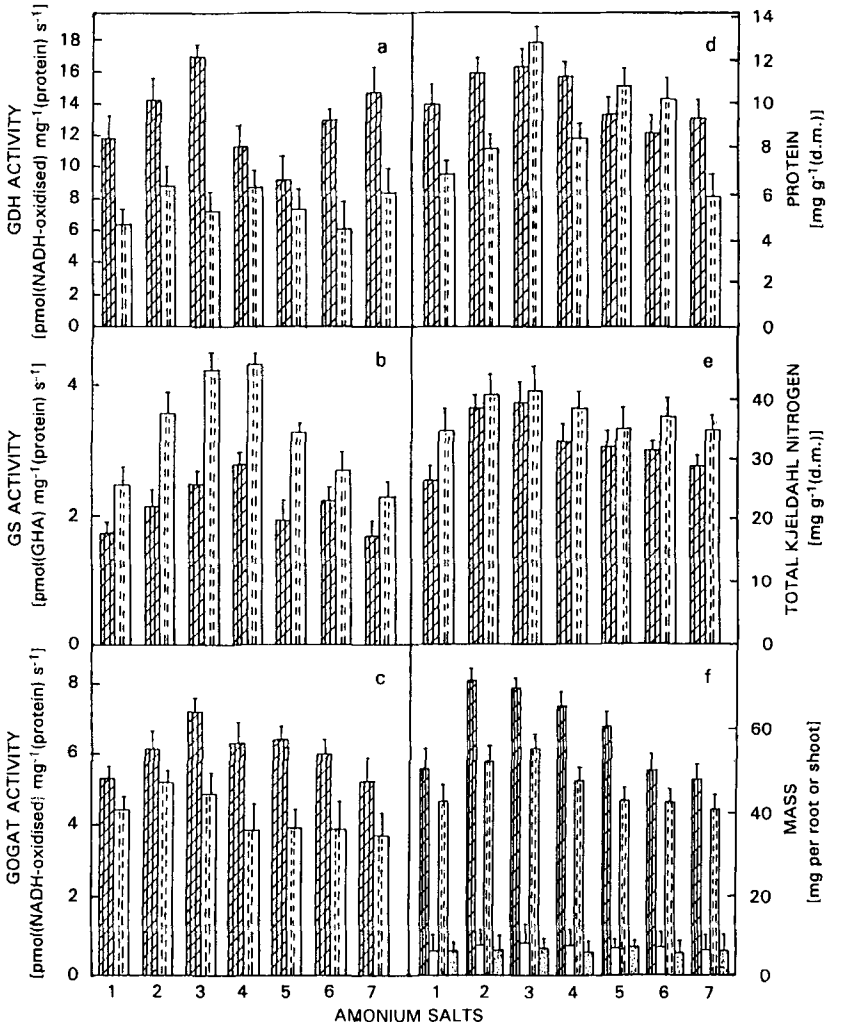

Fig. 1. Effect of different ammonium salts on (*a*) glutamate dehydrogenase activity, (*b*) glutamine synthetase activity, (*c*) glutamate synthase activity, (*d*) total protein, (*e*) total nitrogen, (*f*) growth of roots and shoots of maize seedlings. The salts are indicated by numbers *1* to *7*. *1* - control, *2* - ammonium nitrate, *3* - ammonium chloride, *4* - ammonium sulphate, *5* - ammonium carbonate, *6* - ammonium oxalate, *7* - ammonium acetate.

Ammonium assimilation in roots and shoots of dark versus light grown seedlings: In the roots, there was no difference on protein content although total nitrogen content

in the roots of dark grown seedlings was slightly smaller than that of light grown (Table 1). The GDH activity was, however, substantially higher in dark than in light, while other enzyme activities were less affected. In shoots, both protein and total nitrogen contents were lower in dark grown seedlings than in light grown, although the enzyme activities were little affected.

Table 1. Effect of light-dark conditions on enzymes of ammonium assimilation, protein and total nitrogen contents and growth of roots and shoots of 5-d-old seedlings grown with 5 mM NH_4NO_3 as nitrogen source.

Parameter	Light-grown seedlings		Dark-grown seedlings	
	root	shoot	root	shoot
GDH activity [nmol(NADH) mg^{-1}(protein) s^{-1}]	12 480.0 ± 6.8	6 240.0 ± 6.2	16 740.0 ± 2.80	7 260.0 ± 13.0
GS activity [nmol(GHA) mg^{-1}(protein) s^{-1}]	3 480.0 ± 2.5	4 560.0 ± 3.6	2 640.0 ± 2.20	3 480.0 ± 2.1
GOGAT activity [nmol(NADH) mg^{-1}(protein) s^{-1}]	6 120.0 ± 2.6	3 980.0 ± 4.1	7 140.0 ± 4.40	492.0 ± 3.8
Total protein [mg g^{-1}(d.m.)]	13.4 ± 2.8	18.3 ± 2.1	13.8 ± 0.53	14.7 ± 2.2
Total nitrogen [mg g^{-1}(d.m.)]	40.0 ± 3.5	50.3 ± 2.9	34.0 ± 6.00	41.1 ± 3.9
Dry mass [mg]	6.5 ± 1.2	7.5 ± 1.4	7.2 ± 1.30	5.5 ± 1.0

Discussion

Assimilation of ammonium by either GDH or GS-GOGAT pathway would increase amino acid, protein and other organic nitrogen components. This may ultimately lead to increased seedling growth, as nitrogen is often limiting in plant growth and productivity. Among various ammonium salts, NH_4NO_3 appears to be the best form for maize seedling growth. The induction of all the three enzymes in the roots and increase in protein and total nitrogen contents were also maximum in this salt. Thus in the roots, both pathways GDH and GS-GOGAT seem to be operating in the assimilation of ammonium when ammonium nitrate is the nitrogen source, specially, in dark grown seedlings. This is apparently because this nitrogenous compound (NH_4NO_3) contains cationic (NH_4^+) as well as anionic (NO_3^-) nitrogen. It is generally believed that GDH is the primary aminating enzyme when the source of inorganic nitrogen is the ammonium (Singh and Srivastava 1982, Cammaerts and Jacobs 1985) while GS-GOGAT pathway operates primarily when nitrogen supply is in the form of nitrate (Rhodes *et al.* 1975, Loyola-Vargas and Sanchez de Jimenez 1986, Magalhaes and Huber 1989). In some tissues, however, the pathway is not affected by the form of inorganic nitrogen. Skokut *et al.* (1978) by using ^{13}N

demonstrated that GS-GOGAT pathway was the major pathway of nitrogen assimilation in cultured tobacco cells irrespective of the nitrogen source *i.e.* nitrate, ammonium or urea.

Increase in GDH activity (Srivastava and Singh 1987) and in GOGAT (Singh and Srivastava 1986) by ammonium has been demonstrated in many tissues. A stimulatory effect of nitrate and ammonium on crude and sephadex-treated NADH-dependent GOGAT from maize endosperm has been observed by Oaks *et al.* (1979). An increase in the NADH-dependent GOGAT level in the presence of low ammonium concentrations in soybean cell suspension culture (Chiu and Shargool 1979) and maize endosperm (Sodek and da Silva 1977) have also been observed. However, in *Neurospora crassa* (Hummelt and Mora 1980) excess ammonium depressed NADH-GOGAT activity. In earlier studies with maize roots and leaves, supply of nitrate or ammonium increased NADH-GOGAT activity, but the increase was more pronounced with nitrate than with ammonium (Singh and Srivastava 1986).

In root tissues, however, the situation seems to be different. Maximum increase in GDH in this case is with ammonium chloride and ammonium sulfate. While in GS-GOGAT activity the maximum is with ammonium sulfate although some increase with ammonium chloride is also observed (Fig. 1). In most cases, the increase in enzyme activity were not correlated with the increase in protein, total nitrogen and dry mass of the shoot and hence it may be proposed that assimilation of inorganic nitrogen in the shoot tissues of young maize seedlings contributed to the seedling growth only to a limited extent. Apparently the flow of organic nitrogen from storage tissue (endosperm) was sufficient for maintaining the shoot growth.

Another important observation in the study was the inhibition of enzyme activities and growth with some of the ammonium salts. This may be related to decrease in environmental pH (Magalhaes and Wilcox 1984, Magalhaes and Huber 1989).

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