BIOLOGIA PLANTARUM 34 (1-2): 153-157, 1992

BRIEF COMMUNICATION

Seedling age and cytokinin effects on glutamate dehydrogenase activity and nitrogen assimilation in maize leaves

S. GARG and H.S. SRIVASTAVA*

Department of Plant Science, Rohilkhand University, Bareilly 243005, India

Abstract

Glutamate dehydrogenase (GDH) activity, protein and total nitrogen contents in the secondary leaves of maize (*Zea mays* L. cv. Ganga Safed-2) seedlings increased during early seedling growth and then declined after reaching a peak level at either 10 d (GDH) or 12 d (metabolites). While the effect of kinetin on enzyme activity was statistically insignificant, benzyladenine supplied with nutrient solution increased GDH activity in secondary leaves of both 10-d as well as 14-d seedlings. However, both growth regulators increased the contents of total soluble proteins, total nitrogen, chlorophyll (*a+b*) and carotenoids in both 10 and 14-d old leaves.

The effect of seedling age on glutamate dehydrogenase (GDH) activity has been observed in many cases. The enzyme activity mostly increases during initial growth phase of the seedling (Srivastava and Singh 1987), although an increase in enzyme activity during senescent phase of the tissue has also been observed (Lauriere and Daussant 1983). Cytokinins like other plant growth regulators influence the activity of various enzymes through their specific or general effect on protein synthesis (Maab and Klambt 1977). Thus, they may influence the pattern of enzyme changes during the growth of the seedlings. Although the effect of the growth regulators has been examined on nitrate reductase (NR) activity, an important enzyme of inorganic nitrogen assimilation (Kende *et al.* 1972, Schmerder and Borriss 1986), no such studies have been undertaken with GDH activity.

Received 26 October 1990, *accepted* 14 December 1991.
*To whom all correspondences should be sent.
Acknowledgement: This research was supported by a financial assistance from C.S.I.R., New Delhi, (No. 38 (719)/ 88. EMR.II) to HSS.

The enzyme catalysing the reductive amination of 2-oxoglutarate to produce glutamate is an important enzyme in nitrogen assimilation especially during stress and during ammonium supply (Srivastava and Singh 1987).

The present study of the effect of seedlings age and cytokinins on NADH-GDH activity was undertaken with this perspective. Simultaneous measurements of protein and total nitrogen contents were also made to evaluate the role of GDH in ammonium assimilation.

Seeds of *Zea mays* L. cv. Ganga Safed-2 were purchased from the National Seed Corporation, New Delhi. They were surface sterilized with 0.1 percent bleaching powder ($CaOCl_2$) for about 10 min and then washed with distilled water. Seedlings were raised in small plastic pots containing acid washed sand in a controlled environment growth chamber at 25 ± 2 °C and 12 h photoperiod (approximately 60 W m^{-2}). The plantation was watered with modified 1/2 strength Hoagland solution (pH 6.0) containing 5 mM NH_4NO_3 as the sole nitrogen source. In a preliminary investigation, NH_4NO_3 supported better plant growth than either $(NH_4)_2SO_4$ or NH_4Cl. The desired cytokinin was dissolved and mixed in nutrient solution wherever applicable. The plantations were watered with such nutrient solution on alternate days. On other days, the watering was with nutrient solution containing no cytokinins. Only secondary leaves from uniformly grown seedlings were used for analysis. NADH specific GDH was extracted and its activity assayed as described by Singh and Srivastava (1982). In few experiments, the enzyme was partially purified by employing $(NH_4)_2SO_4$ precipitation and dialysis, but the response of seedling age and cytokinin was the same as observed by measuring the activities in crude enzyme preparations. The protein in the enzyme extract was precipitated by using equal volumes of 20 % trichloroacetic acid and then estimated by the method of Lowry *et al.* (1951). Total nitrogen in oven dried (at 60 °C for 48 h) leaf samples was measured by micro-Kjeldahl method (Lang 1958). Extract of chloroplast pigments was achieved with 80 % acetone, at room temperature. The absorbance of the clear extract was measured at 663, 645 and 440 nm.

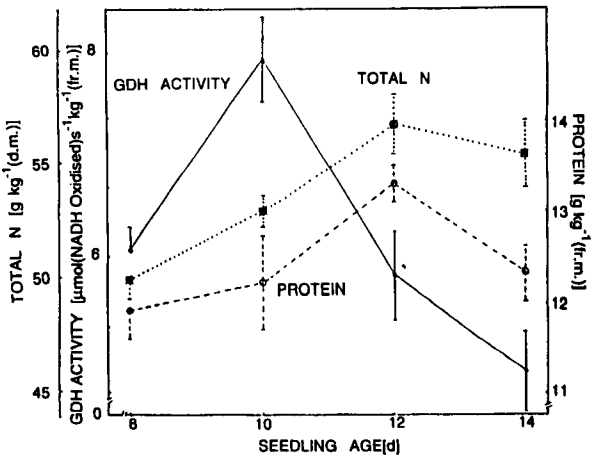

Fig. 1. Total N and protein content and NADH-GDH activity in dependence of seedling age.

Chlorophyll (*a+b*) and total carotenoid contents were calculated by the method of Strain and Svec (1966) and Ikan (1969), respectively. The data presented are average of at least two independent experiments in duplicate (n= 4) with ±S.D.

Total NADH-GDH activity in secondary leaves increased to a maximum at the age of 10 d and then declined, while the protein and total nitrogen contents peaked at the age of 12 d (Fig. 1). The effect of cytokinins on GDH activity varied to some extent according to their nature and concentrations and to the seedlings age. Kinetin at 0.5 or 1.0 μM concentration did not induce statistically significant differences in NADH-GDH activity (Table 1), while benzyladenine increased enzyme activity (17 to 35 %) at either concentration and leaf age. Protein and total nitrogen contents of the leaf tissues increased slightly with either of the cytokinins (Table 1). The increments were higher when the control values were lower. Kinetin and benzyladenine at either concentration increased also chlorophyll (*a+b*) and total carotenoid contents at both leaf age.

Table 1. Effect of cytokinins on glutamate dehydrogenase activity and contents of protein, total nitrogen and chloroplast pigments in leaves of maize. (Values relative to control are given in brackets).

	Plant age [d]	Control	Kinetin [μM]		Benzyl-adenine [μM]	
			0.5	1.0	0.5	1.0
NADH-GDH [μmol(NADH oxidised) s^{-1} kg^{-1}(f.m.)]	10	3.50±0.33 (100)	3.33±0.25 (95)	3.15±0.25 (90)	4.60±0.25 (131)	4.66±0.21 (133)
	14	2.80±0.33 (100)	3.16±0.25 (113)	2.80±0.43 (100)	3.28±0.25 (117)	3.78±0.25 (135)
Protein [g kg^{-1}(f.m.)]	10	7.66±0.20 (100)	8.50±0.21 (111)	8.42±0.30 (110)	8.27±0.23 (108)	8.35±0.40 (109)
	14	6.51±0.28 (100)	7.35±0.35 (113)	7.74±0.27 (119)	7.55±0.26 (116)	8.07±0.33 (124)
Total nitrogen [g kg^{-1}(f.m.)]	10	50.2±1.98 (100)	62.8±1.35 (125)	65.8±2.45 (131)	54.2±1.78 (108)	58.7±1.78 (117)
	14	51.4±1.78 (100)	54.5±3.89 (106)	62.2±3.53 (121)	57.1±2.25 (111)	63.8±1.78 (124)
Chlorophyll (*a+b*) [g kg^{-1}(f.m.)]	10	0.93±0.03 (100)	1.08±0.04 (106)	1.16±0.04 (126)	1.23±0.02 (133)	1.26±0.01 (136)
	14	1.14±0.02 (100)	1.31±0.08 (115)	1.49±0.01 (131)	1.36±0.05 (120)	1.65±0.03 (145)
Carotenoids [g kg^{-1}(f.m.)]	10	0.48±0.01 (100)	0.55±0.01 (115)	0.60±0.01 (125)	0.62±0.01 (131)	0.64±0.01 (135)
	14	0.54±0.02 (100)	0.59±0.02 (115)	0.67±0.03 (124)	0.65±0.01 (121)	0.78±0.01 (145)

An increase in NADH-GDH activity during early growth period of the leaf observed in the present study (Fig. 1) is similar to the increase reported in the roots of *Canavalis ensiformis* (Loyala-Vargas *et al.* 1988) and also in the root and shoot tissues of relatively younger maize seedlings (Sengar and Srivastava 1990). The similarity of changes in protein and total nitrogen contents and GDH activity indicated that NADH-GDH activity was involved in the assimilation of inorganic nitrogen. Cytokinins as anti-senescent growth regulator protect the degradation of macro-molecules including protein and chlorophyll in mature leaves (Thimann 1980, Thomas and Stoddart 1980). Thus, they may alter the pattern of changes in these metabolites and related enzyme activities during leaf maturation and senescence, as shown by their effects on these metabolites in younger leaves. Cytokinins stimulated their biosynthetic processes as well. Increase in protein, total organic nitrogen and chlorophyll during cytokinin supply may be due to increased level of nitrogen assimilation. Assimilation of NH_4NO_3, supplied as a source of nitrogen involves the activity of nitrate reductase (NR), nitrite reductase (NiR), GDH, glutamine synthetase (GS) and glutamate synthase (GOGAT). The incorporation of ammonium produced from the reduction of nitrate to glutamate, by either GDH or GS/GOGAT pathway, is essential for the eventual assimilation of inorganic nitrogen. In the present study, while the effect of kinetin was not clear cut, benzyladenine increased GDH activity both in the younger and older leaves (Table 1).

The increased GDH activity, together with increased total organic nitrogen is suggestive of the stimulatory role of benzyladenine in overall nitrogen assimilation. An increase is non-nitrogenous chloroplastic pigments, the carotenoids, indicates that cytokinins have a more general effect in the development and organisation of chloroplasts (Harvey *et al.* 1974, Woolhouse and Batt 1966).

References

Harvey, B.M.R., Lu, B.C., Fletcher, R.A.: Benzyladenine accelerates chloroplast differentiation and stimulates photosynthetic enzyme activity in cucumber cotyledons. - Can. J. Bot. **52**: 2581-2586, 1974.

Ikan, R.: Natural Products: A Laboratory Guide. - Academic Press, New York 1969.

Kende, H., Hahn, H., Kays, S.E.: Enhancement of nitrate reductase activity by benzyladenine in *Agrostemma githago*. Comparison of the inductive effects of nitrate and cytokinin. - Biochim. biophys. Acta **286**: 118-125, 1972.

Lang, C.A.: Simple microdetermination of Kjeldahl nitrogen in biological materials. - Anal. Chem. **30**: 1692-1694, 1958.

Lauriere, C., Daussant, J.: Identification of the ammonium-dependent isoenzyme of glutamate dehydrogenase as the form induced by senescence or darkness stress in the first leaf of wheat. - Physiol. Plant. **58**: 89-92, 1983.

Loyola-Vargas, V.M., Yanez, A., Caldera, J., Oropeza, C., Robert, M.L., Qviroz, J., Scvorier, K.N.: Nitrogen metabolism in *Canavalis ensiformis* (L) D.C. II. Changing activities of nitrogen assimilating enzymes during growth. - J. Plant Physiol. **132**: 289-293, 1988.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with Folin phenol reagent. - J. biol. Chem. **193**: 265-275, 1951.

Maab, H., Klambt, D.: Cytokinin effect on protein synthesis *in vivo* in higher plants. - Planta **133**: 117-120, 1977.

Schmerder, B., Borriss, H.: Induction of nitrate reductase by cytokinin and ethylene in *Agrostemma githago* L. embryos. - Planta **169**: 589-593, 1986.

Sengar, R.S., Srivastava, H.S.: Effect of seedlings age on glutamate dehydrogenase activity in maize. - Indian J. Plant Physiol. **33**: 40-45, 1990.

Singh, R.P., Srivastava, H.S.: Glutamate dehydrogenase activity and assimilation of inorganic nitrogen in maize seedlings. - Biochem. Physiol. Pflanz. **177**: 633-642, 1982.

Srivastava, H.S., Singh, R.P.: Role and regulation of L-glutamate dehydrogenase activity in higher plants. - Phytochemistry **26**: 597-610, 1987.

Strain, H.H., Svec, W.A.: Extraction, separation, estimation and isolation of chlorophyll. - In: Vernon, L.P., Seely, G.R. (ed.): Chlorophylls. Pp. 21-66. Academic Press, New York 1966.

Thimann, K.V.: The senescence of leaves. - In: Thimann, K.V. (ed.): Senescence in Plants. Pp. 85-115. CRC Press, Boca Raton 1980.

Thomas, H., Stoddart, J.L.: Leaf senescence. - Annu. Rev. Plant Physiol. **31**: 83-111, 1980.

Woolhouse, H.W., Batt, T.: The nature and regulation of senescence in plastids. - In: Sünderland, N. (ed.): Perspectives in Experimental Biology. Pp. 169-175. Pergamon Press, Oxford 1976.