



INFLUENCE OF PUTRESCINE ON ENZYMES OF AMMONIUM ASSIMILATION IN MAIZE SEEDLING

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The effect of different concentrations of putrescine on biochemical changes in root and shoot of six days old maize seedlings in terms of enzymes of ammonium assimilation was examined. The results revealed that glutamate dehydrogenase (GDH) activity enhanced at lower concentration of putrescine but at higher concentration, the activity of this enzyme declined. Glutamine synthetase (GS) activity decreased with increase in concentration of putrescine and it was highest at 1000 μM concentration. However, glutamate synthase (GOGAT) activity increased with increase in concentration of putrescine upto 100 μM in root and upto 50 μM in shoot and further increase in concentration resulted in decline of enzymatic activity. Protein and total nitrogen content increased upto 10 μM concentration of putrescine and it decreased further with increase in concentration both in root and shoot of maize seedling, indicating maintenance of NH_4^+ concentration in root and shoot by low concentration of putrescine inducing NADH dependent GDH/GOGAT system. The non-NADH dependent GS continuously goes on detoxifying excess NH_4^+ in the presence of increasing concentrations of putrescine.

Key words: GDH, GS, GOGAT, maize seedlings, putrescine, *Zea mays*.

The naturally occurring polyamines-putrescine (PUT), spermidine (SPD) and spermine (SPM) are important polycationic molecules that are ubiquitously present across the living world. These nitrogenous aliphatic compounds are known to influence a variety of biological processes. The most important characteristic of polyamines being the polycationic nature, they are implicated in electrostatic interactions with the negatively charged molecules in the cell. Since last decade, understanding of the involvement of polyamines in cellular and developmental processes across the living systems has been increased (Pandey *et al.* 2000, Talaat *et al.* 2005, Kumar *et al.* 2006). However, very little work has been done on effect of this growth regulator on enzymes of ammonium assimilation. Keeping above in view, the present investigation was carried out to study the effect of putrescine on the enzymes of ammonium assimilation viz. glutamate dehydrogenase (GDH), glutamine synthetase (GS) and glutamate synthase (GOGAT). The activities of the enzymes of N metabolism

decrease during senescence. In general, the activity of Nitrate reductase (NR) is the first to be lost. The activities of Glutamine synthetase (GS), Glutamate synthase (GOGAT) and Glutamate dehydrogenase (GDH) stay on for longer period (Storey and Beevers 1978).

Glutamate synthetase (GS) is the enzymes of glutamate synthesis in the senescent leaves (Mifflin and Lea 1977, Oaks and Hirel 1985). However, the GS activity goes down in the same way as its happens with RUBISCO (Simpson and Dalling 1985, Kamachi *et al.* 1992). Glutamate synthetase exists in at least two isoforms: GS1 is a cytosolic enzymes while GS2 is in the chloroplast (Oaks and Hirel 1985). The decrease in GS activity during senescence can be due to the decrease of the GS2 isoform. In the same way as the other chloroplastic proteins GS2 is hydrolysed during this period. In isolated chloroplast, it was observed that GS2 hydrolyse and degradation goes faster than Rubisco's and the other carbon assimilation enzymes (Crawford *et al.* 1995). On the other hand cytosolic GS1 is kept

constant or may even increase its activity during senescence (Makino *et al.* 1983). As GS1 changes glutamate into glutamine, it increases the N transport efficiency, since glutamate has a 5 C:2 N ratio.

Another indication that the cytosolic GS1 is related to the remobilization of N is the increase of the expression of the GS1 genes during senescence. Also, post translational phosphorylation of GS1 protects the enzymes against degradation. Interaction with 14-3-3 proteins can also increase GS1 activity (Finnemann and Schoerring 2000)

Although during the reproductive period total GS activity (GS1+GS2) decreases, GS1 remains active in the production of glutamine from glutamate and ammonium. In this way, cytosolic GS is closely related to the synthesis of the transport of substances, after the degradation of proteins. Besides GS1 there also have been observed increases in the activities of NADH-GOGAT and GDH, which suggest the participation of these enzymes in the remobilization of nitrogen (Hirel *et al.* 2001.). GDH is one of the few enzymes that can remove nitrogen directly from amino acids, what results in keto acids and ammonium, both of which can be remobilized to used in respiration and synthesis if the amides glutamine and asparagine (Miflin and Habash 2002). Machado and Fernades (2001) working with a maize land race (sol da manha) breed through a participatory process involving small farmers, have shown that this variety was much more efficient than the commercial hybrids when growing in soils depleted of nutrients specially nitrogen. Through studies of the enzymes of N-assimilation this ability was related by the authors to a higher capacity of "sol da manha" to take up NH_4^+ -N from the soil. Data from Machado and Fernades (2001) indicates that under NH_4^+ -nutrient GS activity is closely related to the dry matter accumulation and the reduction of N – level in the tissues. Studying the N-use efficiency in endogamic families of maize (sol da manha and cateto) in nutrient solutions using two N-levels (10 and 100 mg N/L), Machado and Fernades

(2001) have found higher activity of GS for plants under NH_4^+ , and higher NR activity for plants under NO_3^- . These authors related the higher N-use efficiency of these plants to its superior capacity to take up nitrogen under a range of environmental conditions.

MATERIALS AND METHODS

Seeds of *Zea mays* L. cv. Ganga Safed-2, procured from National Seed Corporation, New Delhi were surface sterilized with 0.1% HgCl_2 for 5 min. and then washed thoroughly with distilled water. The sterilized seeds were placed in 15 cm petriplate lined with Whatman No. 1 filter paper and allowed to germinate at $25 \pm 2^\circ\text{C}$ under 14 hr. photoperiod of approximately 70 Wm^{-2} radiant flux density. There were three replications with 30 seeds for each treatment. The first set was supplied with Hoagland's nutrient solutions (Arditti and Dunn 1969) to serve as control while set 2, 3, 4 & 5 were supplied with 10, 50, 100 and 1000 μM aqueous solutions of putrescine, respectively. All the petri-plates were kept wet by supplying respective solutions daily. Emergence of radicle was taken as a criterion for the out set of seed germination in each treatment. On 6th day of sowing, roots and shoots of maize seedlings were used separately for nitrogen, protein and enzyme analysis.

Determination of Enzyme Activity

Glutamate Dehydrogenase Activity

Glutamate dehydrogenase (GDH) from the fresh sample was extracted in a mortar in a medium containing 0.5 M sodium phosphate buffer (pH 7.4), 0.4 M sucrose and 2mM EDTA. The ratio of plant tissue and medium was 1:4 (w/v). The samples were thoroughly extracted in cold (ice bucket) and the extract was centrifuged at 6000 rpm for 15 min. The clear supernatant was used as enzyme preparation to assess the enzyme activity (Singh and Srivastava 1983).

Glutamine Synthetase Activity

Enzyme extract were prepared in cold in a mortar containing 50 mM Tris-HCl (pH 7.8), 15% (v/v) glycerol, 14 mM 2-mercaptoethanol, 1.0 mM EDTA and 0.1% (w/v) Triton X-100.

The extract was centrifuged at 6000 rpm for 10 min. The supernatant was used for determination of enzyme activity (Lillo 1984).

Glutamate Synthase Activity

Enzyme was extracted in a medium containing 0.2 M sodium phosphate buffer (pH 7.5), 2mM EDTA, 50 mM KCl, 0.1% mercaptoethanol and 0.5% Triton X-100 in a ratio of 1:4 (w/v). The homogenate was centrifuged at 4°C at 6000 rpm for 15 min. The clear supernatant was used as enzyme preparation. Glutamate synthase activity (NADH-specific) was determined using oxidation of NADH (Singh and Srivastava 1986).

Determination of Total Nitrogen and Protein

The total nitrogen was determined after digestion with concentrated sulphuric acid by a modified micro-Kjeldahl method (Lang 1958). Protein content in shoot and root of maize seedlings was also estimated (Lowry *et al.* 1951).

Table 1: Effect of Putrescine on total enzyme activity of ammonium assimilation in maize seedling.

Conc. (μ M)	GDH activity (NADH oxidized min^{-1}) g^{-1} fresh wt.		GS activity (GHA min^{-1}) g^{-1} fresh wt.		GOGAT activity (NADH oxidized min^{-1}) g^{-1} fresh wt.	
	Root	Shoot	Root	Shoot	Root	Shoot
0.0	794.84 \pm 1.93 (100)	460.09 \pm 1.92 (100)	23.96 \pm 0.08 (100)	32.99 \pm 0.08 (100)	1898.99 \pm 2.88 (100)	1456.73 \pm 4.76 (100)
10	1053.41 \pm 3.18 (140)	468.83 \pm 0.73 (109)	18.90 \pm 0.21 (79)	21.75 \pm 0.08 (66)	2154.52 \pm 4.76 (113)	1906.63 \pm 3.78 (131)
50	565.65 \pm 1.26 (75)	511.78 \pm 2.62 (111)	17.92 \pm 0.09 (75)	21.21 \pm 0.05 (64)	2501.77 \pm 2.89 (132)	2281.19 \pm 2.89 (157)
100	516.87 \pm 1.92 (68)	297.75 \pm 1.93 (64)	16.92 \pm 0.09 (71)	19.16 \pm 0.08 (58)	2928.74 \pm 3.78 (154)	1767.95 \pm 2.88 (121)
1000	337.79 \pm 1.93 (45)	250.43 \pm 1.93 (54)	19.84 \pm 0.08 (83)	20.51 \pm 0.09 (62)	1384.65 \pm 4.75 (73)	1379.19 \pm 5.00 (95)

Data \pm SE The values relative to control are given in parenthesis

Table 2: Effect of Putrescine on specific enzyme activity of ammonium assimilation in maize seedling.

Conc. (µM)	GDH activity (NADH oxidized min ⁻¹ mg ⁻¹ protein)		GS activity (GHA min ⁻¹ mg ⁻¹ protein)		GOGAT activity (NADH oxidized min ⁻¹ mg ⁻¹ protein)	
	Root	Shoot	Root	Shoot	Root	Shoot
0.0	14.21±0.01 (100)	9.72±0.02 (100)	0.472±0.01 (100)	0.601±0.02 (100)	51.5±0.26 (100)	31.65±0.24 (100)
10	19.38±0.14 (136)	9.81±0.03 (101)	0.468±0.02 (99)	0.443±0.03 (74)	56.98±0.22 (111)	40.51±0.25 (128)
50	10.11±0.03 (71)	10.54±0.07 (108)	0.447±0.02 (95)	0.442±0.01 (73)	58.94±0.46 (114)	41.23±0.12 (130)
100	8.90±0.06 (62)	6.00±0.04 (61)	0.425±0.01 (90)	0.398±0.02 (66)	64.79±0.25 (126)	34.31±0.32 (108)
1000	6.89±0.06 (48)	5.47±0.05 (56)	0.545±0.03 (115)	0.454±0.03 (75)	41.30±0.15 (80)	23.12±0.33 (73)

Data ± SE The values relative to control are given in parenthesis

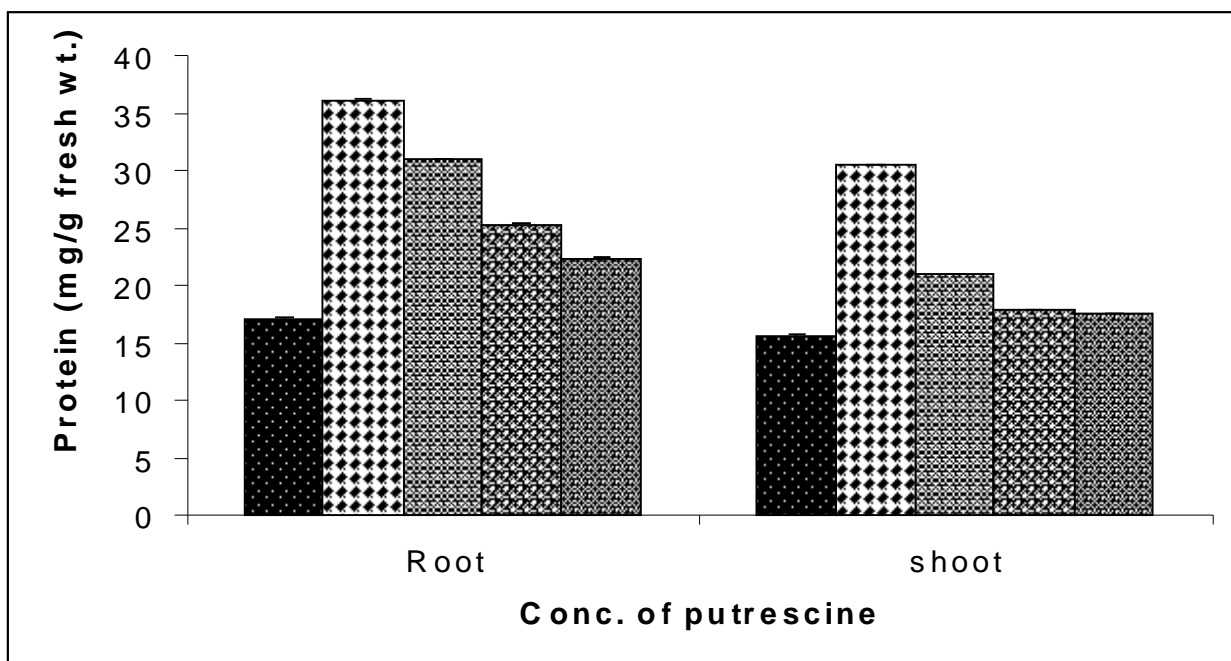


Figure 1: Effect of putrescine (µM) on protein content in root and shoot of maize seedling

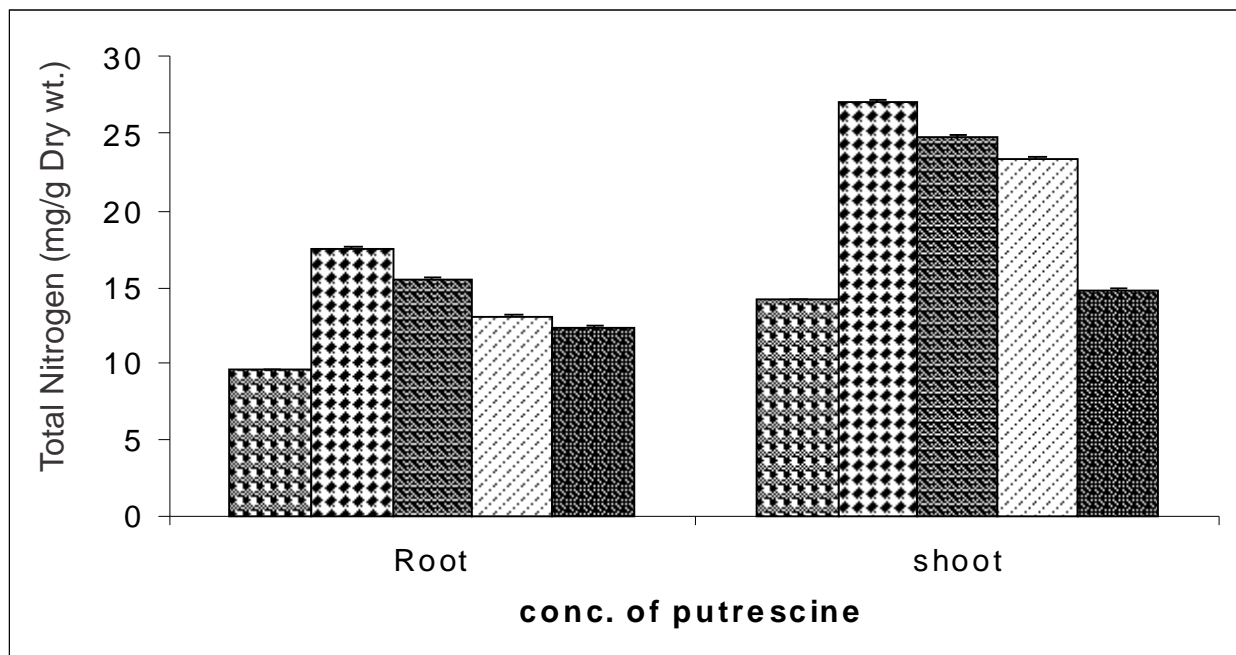


Figure 2: Effect of putrescine (μM) on Nitrogen content in root and shoot of maize seedling

RESULTS AND DISCUSSION

Concentration of putrescine and the tissue used influenced the effect of putrescine on enzymes of ammonium assimilation in root and shoot of maize seedling. Total and specific glutamate dehydrogenase (GDH) activity increased upto $10 \mu\text{M}$ concentration of putrescine i.e. 40 and 36%, respectively in root and thereafter it decreased gradually upto $1000 \mu\text{M}$, whereas in shoot it was maximum at $50 \mu\text{M}$ concentration i.e. 11 and 8% and further increase resulted in decreased activity (Table 1 and 2).

Total and specific glutamine synthetase (GS) activity decreased i.e. 29 and 10% at $100 \mu\text{M}$ concentration in root, whereas, in shoot it decreased to 42 and 34%, respectively. At higher concentration ($1000 \mu\text{M}$) it was found to be increased both in root and shoot of maize seedlings (Table 1 and 2).

Total and specific glutamate synthase (GOGAT) activity was found to increase 54 and 26% in root at $100 \mu\text{M}$ concentration and thereafter it decreased whereas in shoot these two activities increased by 57 and 30% at $50 \mu\text{M}$ concentration and further increase in concentration resulted in decline in total as well as specific GOGAT activities (Table 1 and 2).

Protein content was found to be increased 112 and 94% in root and shoot, respectively, at $10 \mu\text{M}$ concentration and further increase in concentration resulted in decline in protein content (Fig 1). An increase in total nitrogen content i.e. 82 and 91% at $10 \mu\text{M}$ concentration was observed both in root and shoot of maize seedling, respectively and further increase in concentration resulted in decrease in nitrogen content (Fig. 2).

Polyamines play a major role in cellular and developmental processes (Walden *et al.* 1997; Rajam *et al.* 1998). It is well established that growth regulator, polyamines, affect various aspects of nucleic acid, protein synthesis, membrane organization and function (Talaat *et al.* 2005; Slocum *et al.*, 1984; Kashiwagi *et al.* 1990). Exogenous supply of polyamines affects a variety of plant processes (Smith 1985). Increased GDH activity and declined GS activity were also reported in leaf protoplast from rape cv. Bronowski (Watanabe *et al.* 1998). The increase of total nitrogen in leaves of *Leucaena* seedlings by the treatment of $100 \mu\text{M}$ spermidine (family member of putrescine) was also reported (Pandey and Srivastava 1994). The maximum increase in total nitrogen using putrescine was also

obtained in *Populus deltoides* (Prakash and Srivastava 2000). In this investigation, low concentrations of putrescine have been found to induce NADH dependent NH_4^+ detoxifying enzymes (GDH and GOGAT), whereas non-NADH dependent GS continues to detoxify excess NH_4^+ with increasing concentrations of putrescine. Spermidine has been shown to induce all three enzymes alike (Zhang *et al.* 2013)

REFERENCES

- Anant Prakash & Srivastava HS 2000 Growth and nitrogen assimilation in *Populus* in response to growth regulators and varying nitrate levels. *PNAS*. **70**(1) 53-60.
- Arditti J & Dunn A 1969 *Experimental Plant Physiology*. 1st Ed. Holt Rinehart and Winston, New York. p. 265
- Crawford NM 1995 Nitrate: nutrient and signal for plant growth. *Plant Cell* **7** 858-868.
- Finnemann J & Schioerring JK 2000 Post translational regulation of cytosolic glutamine synthetase by reversible phosphorylation and 14-3-3 protein interaction. *Plant Journal* **24** 171-181.
- Hirel B, Bertin P, Quillere I, Bourdoncle W, Attagnant C, Dellay C, Gouy A, Cadiou S, Retailliau C, Falqu, M & Gallais A 2001 Towards a better understanding of the genetic and physiological basis for nitrogen use efficiency in maize. *Plant Physiol* **125** 1258-1270.
- Kamachi K, Yamaya T, Hayakawa T, Mae T & Ojima K 1992 Vascular bundle specific localization of cytosolic glutamine synthetase in rice leaves. *Plant Physiol* **99** 1481-1486.
- Kashiwagi K, Yamaguchi Y, Sakai Y, Kobayashi H & Igarashi K 1990. Identification of the polyamine-induced protein as a periplasmic oligopeptide binding protein. *J Biol Chem* **265** 8387-8391.
- Kumar SV, Sharma ML & Rajam MV 2006 Polyamine biosynthetic pathways as a novel target for potential applications in plant biotechnology. *Physiol Mol Biol Plants* **12** (1) 13-28.
- Lang CA 1958 Simple microdetermination of Kjeldahl nitrogen in biological materials. *Annal Chem* **30** 1692-1694
- Lillo C 1984 Diurnal variations of nitrite reductase, glutamine synthetase, glutamate synthase, alanine amino transferase and aspartate amino transferase in barley leaves. *Physiol Plant* **61** 214-218.
- Lowry OH, Rosebrough NJ, Farr AL & Randall RJ 1951 Protein measurement with folin-phenol reagent. *J Biol Chem* **193** 265-275.
- Machado AT & Fernandes MS 2001 Participatory maize breeding for low nitrogen tolerance. *Euphytica* **122** 567-573.
- Makino A, Mae T & Ohira K 1983 Photosynthesis and ribulose 1,5-bisphosphate carboxylase in rice leaves-change in photosynthesis and enzymes involved in carbon assimilation from leaf development through senescence. *Plant Physiol* **73** 1002-1007.
- Mifflin BJ & Habish DZ 2002 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.
- Mifflin BJ & Lea PJ 1977 Amino acid metabolism. *Annu Rev Plant Physiol* **28** 299-329
- Oaks A & Hirel B 1985 Nitrogen metabolism in roots. *Annu Rev Plant Physiol* **33** 345-365.
- Pandey S & Srivastava HS 1994 Stimulation of growth and nitrate assimilation in *Leucaena leucocephala* seedlings in response to spermidine supply. *Biol Plant* **37** 153-157.
- Pandey S, Ranada TA, Nagar PK & Kumar N 2000 Role of polyamines and ethylene as modulators of plant senescence. *Journal of Bioscience* **25** 291-299.
- Rajam MV, Dagar S, Waie B, Yadav JS, Kumar PA, Shoeb F & Kumria R 1998. Genetic engineering of polyamine and carbohydrate metabolism for osmotic stress tolerance in higher plants. *JBiosci* **23** 473-482.
- Rajam MV, Shoeb F & Yadav JS 1998 Polyamines as modulators of plant regeneration in tissue cultures. In: *Plant tissue culture and molecular biology: applications and prospects*. (ed. Srivastava P.S.). Narosa Publishing House, New Delhi, India, pp. 620-641.
- Singh RP & Srivastava HS 1983 Regulation of glutamate dehydrogenase activity by amino acids in maize seedlings. *Physiol Plant* **57** 549-554.
- Singh RP & Srivastava HS 1986 Increase in glutamate synthase (NADH) activity in maize seedlings in response to nitrite and ammonium nitrogen. *Physiol Plant*. **66** 413-416.
- Slocum RD, Kaur-Shawney R & Galston AW 1984 The physiology and biochemistry of polyamines in plants. *Arch Biochem Biophys* **235** 283-303.
- Smith TA 1985 Polyamines. *Annu Rev Plant Physiol*. **36** 117-143.
- Storey R & Beevers L 1978 Enzymology of glutamine metabolism related to senescence and seed development in pea (*Pisum sativum* L). *Plant Physiol* **61** 494-500.
- Talaat IM, Bekheta MA & Mahgoubi MH 2005 Physiological response of periwinkle plants (*Catharanthus roseus* L.) to tryptophan and putrescine. *Int J Agri Bot* **7**(2) 210-213.
- Walden E, Cordeir A & Tiburcio AR 1997 Polyamines: small molecules triggering pathways in plant growth and development. *Plant Physiol* **113** 1009-1013
- Watanabe M, Kawasaki H, Itho Y & Watanabe Y 1998 Senescence development of Brassica napus leaf protoplast during isolation and subsequent culture. *J Plant Physiol* **152** 487-493.
- Zhang Yi, Hu Xiao-Hui, Shi Yu, Zou Zhi-Rong, Yan Fei, Zhao Yan-Yan, Zhang Hao & Zhao Jiu-Zhou 2013 Beneficial Role of Exogenous Spermidine on Nitrogen Metabolism in Tomato Seedlings Exposed to Saline-alkaline Stress, *J Am Soc Hort Sc* **138**(1) 38-49.