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SHORT COMMUNICATION

EFFECT OF SALICYLIC ACID ON ENZYMES OF AMMONIUM ASSIMILATION IN MAIZE SEEDLING

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The response of salicylic acid application was studied in roots and shoot of six day old maize seedling. Glutamate dehydrogenase (GDH) activity enhanced in root and shoot at lower concentrations of salicylic acid. However, glutamine synthetase (GS) and glutamate synthase (GOGAT) activities decreased with the increase in concentration of salicylic acid. Protein and total nitrogen content showed upward trend with application of salicyclic acid upto 50 µM but further increase in its concentration resulted in lower protein and total nitrogen content.

***Key words*: GDH, GOGAT, GS, maize, salicylic acid**

Growth regulating effects of salicylic acid (SA) on plants are well documented (Lee and Skoog 1965a, b, Cleland and Ajami 1974, Deckock *et al*. 1975, Nanda *et al*. 1976, Pieterse 1976, Asthana and Srivastava 1978, Cleland and Tanaka 1979), but information on its metabolic effects are least traced. The role of salicylic acid, a naturally occurring phenolic compound is well established as a plant morphogenetic regulator and a flower inducing factor. Exogenous supply of salicylic acid affects seedling growth, stimulates or inhibits nitrate assimilation depending upon concentration supplied (Jain and Srivastava 1981a, b & c). There is, however, limited information available regarding its effect on activities of enzymes of ammonia assimilation like glutamine synthetase (GS), glutamate dehydrogenase (GDH) and glutamate synthase (GOGAT) (Jain and Srivastava 1981, Singh and Srivastava 1987, Negi and Prasad 2001). Therefore, the present study, has been planned to study the effect of salicylic acid on enzymes of ammonium assimilation viz. GS, GDH and GOGAT in roots and shoot of maize seedlings.

Seeds of *Zea mays* L. cv. Ganga Safed-2, were obtained from National Seed Corporation, New Delhi and surface sterilized with 0.1% $HgCl_2$ for 5 min. and then washed thoroughly with distilled water. The sterilized seeds were placed in petriplates (diameter 15 cm) lined with Whatman No. 1 filter paper and allowed to germinate at 25 ± 2^0C under 14 h 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 salicylic acid, respectively. All the petri-plates were kept wet by supplying respective solutions daily. Emergence of radicle was considered as a criterion for the seed germination. On 6^{th} day of germination, roots and shoots of maize seedlings were harvested and used for analysis of nitrogen, protein and enzyme analysis.

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Glutamate dehydrogenase (GDH) from the fresh samples was extracted in sodium phosphate buffer (0.5 M, pH 7.4) containing sucrose (0.4 M) and EDTA (2mM) in cold condition followed by centrifugation at 6000 rpm for 15 min. The clear supernatant was used to assay enzyme by the method of Singh and Srivastava (1983). Glutamine synthetase (GS) was extracted in Tris-HCl (50 mM, pH 7.8) containing glycerol (15%), 2-mercaptoethanol (14 mM), EDTA (1.0 mM) and Triton X-100 (0.1%) followed by centrifugation at 6000 rpm for 10 min. The supernatant was used as enzyme extract to assay by the method of Lillo (1984). Glutamate synthase (GOGAT) was extracted in sodium phosphate buffer (0.2 M, pH 7.5) containing EDTA (2mM), KCl (50 mM), mercaptoethanol (0.1%) and Triton X-100 (0.5%) followed by centrifugation at 4^0C at 6000 rpm for 15 min. The supernatant was used to determine the enzyme activity by the method of Singh and Srivastava (1986). The total nitrogen was determined after digestion with concentrated sulphuric acid by a modified micro-Kjeldahl method (Lang 1958). Protein in tissues was estimated by the method of Lowry *et al.* (1951).

The effect of salicylic acid was studied on the activities of enzymes of ammonium assimilation. The effect of salicylic acid was dependent upon the concentration of salicylic acid and the tissue used (Table 1). Glutamate dehydrogenase (GDH) activity increased from 0 to 10 µM concentration of salicylic acid and there after it decreased gradually. However, GS activity

Table 1. Effect of salicylic acid on enzymes of ammonium assimilation in maize seedlings

Salicylic acid concentration (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	9.9+0.02 (100)	5.3+0.06 (100)	0.53+0.03 (100)	0.62+0.02 (100)	18.0+0.01 (100)	20.1+0.05 (100)
10	22.2+0.11 (223)	16.5+0.1 (310)	0.50+0.04 (94)	0.59+0.03 (95)	19.8+0.01 (109)	22.2+0.24 (110)
50	19.4+0.15 (195)	13.2+0.2 (248)	0.58+0.03 (109)	0.61+0.05 (98)	17.5+0.02 (96)	21.3+0.13 (106)
100	16.5+0.20 (165)	10.8+0.1 (203)	0.64+0.02 (121)	0.65+0.04 (105)	17.1+0.02 (95)	19.7+0.06 (98)
1000	11.1+0.12 (119)	7.2+0.03 (136)	0.51+0.01 (96)	0.54+0.02 (87)	28.1+0.03 (155)	23.8+0.06 (118)

Figures in parenthesis are pre cent values taking control as 100

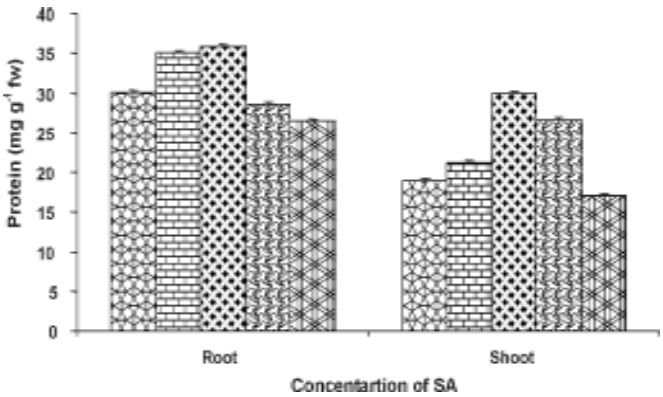

Fig. 1. Effect of different concentrations of salicylic acid (SA) on protein content in root and shoot of maize seedling

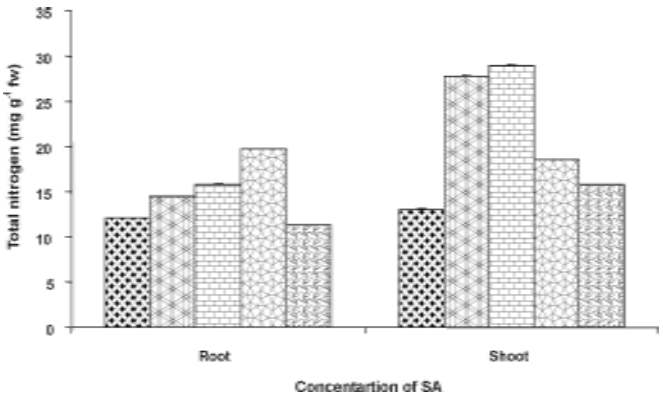

Fig. 2. Effect of different concentrations of salicylic acid (SA) on total nitrogen content in root and shoot of maize seedling

increased from 10 to 100 µM concentration of salicylic acid and after this concentration it declined. GOGAT activity decreased with increase in concentration of salicylic acid from 10 to 100 µM and further increase in concentration resulted in decline in enzyme activity (Table 1). Protein content in the root as well as shoot increased up to 50 µM concentration and thereafter decreased (Fig. 1). Whereas, nitrogen content increased up to 100 µM concentration in root while in shoot it increased up to 50 µM concentration and thereafter it decreased (Fig. 2).

Glutamine synthetase has a prominent role in plant metabolism and it is the key enzyme involved in the assimilation of ammonia either provided by nitrate reduction, direct absorption by plant roots or released from glycine via the photorespiratory nitrogen cycle (Lacuesta *et al*. 1990). Negi and Prasad (2001) also observed less GOGAT activity at lower concentration and at higher concentration it enhanced. Singh and Srivastava (1987) have suggested that salicylic acid increases GOGAT activity by inducing synthesis of the enzyme as well as by some kind of direct modulation of the enzyme molecules.

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