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TRIAZOLE INDUCED PROTEIN METABOLISM IN THE SALT STRESSED *RAPHANUS SATIVUS* SEEDLINGS

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Salt stress decreased the growth and dry weight, whereas triadimefon increased the length of the root and dry weight of the root and cotyledon but decreased it in the hypocotyl. NaCl salinity induced the accumulation of free amino acid and proline with concomitant decrease in protein content, however triadimefon treatment to the NaCl stressed seedling increased the amino acid and proline contents in the root system and cotyledons but decreased it in the hypocotyl. Sodium chloride stress decreased the protein content in the root and hypocotyl while triadimefon treatment to the NaCl stressed seedlings increased it even above the level of control. The level of protease activity coincides with the higher amino acid accumulation. The higher protein content in the root and hypocotyl and decreased protein content in the cotyledons of the triadimefon treated NaCl stressed seedlings showes that the triadimefon enhanced the protein synthesis in the root and hypocotyl with increased mobilization of amino acids from the cotyledons by the increased hydrolysis of proteins indicated by the lower protein content of the cotyledons.

Key Words : *Raphanus sativus,* NaCl stress, triadimefon, protein, amino acids, proline, protease.

The effect of NaCl stress is more harmful during early stages of germination and seedling growth. The effect is generally, concentration dependent and differs amongst different species of plants (Levitt, 1980). Application of growth retardants is reported to alleviate the deleterious effect of salinity in bean (Halevy and Kessler, 1963). It has been demonstrated that the triazole which is used as a fungicide also have growth regulatory effect and protect plants against various stresses including drought, temperature and salinity. Hence the triazole have been referred to as "plant multi-protectants" and suggested that their protective effects are mediated by shifting the balance of important plant hormones in the isoprenoid pathway (Fletcher *et al.,* 1988). Triazoles are used to protect the plants like sunflower and mungbean seedlings against salinity stress (Saha and Gupta, 1993). Present study was designed to findout the effect of triadimefon [1-(4-Chlorophenoxy)-3,3-dimethyl-1-(1 H-1,2,4 triazol - 1 -yl)-2 butanone] a triazole group of fungicide on the growth and protein metabolism of salt stressed *Raphanus sativus* seedlings.

MATERIALS AND METHODS

Seeds of *Raphanus sativus* Var. No. 8 was obtained from "Indo American Seeds Exports" Bangalore and the triadimefon (Bayleton) 25 per cent W P was obtained from bayer (India) Ltd. Bangalore. Seeds were surface sterilized with 0.1 per cent $HgCl_2$ for five minutes and thoroughly washed with distilled water. Then the seeds were soaked for 12 hours in distilled water (control), 80mM NaCl, 80mM NaCl with triadimefon at a concentration of 3 mg, 6 mg litre^{-1}, and sown in 30 x 40 x 7 cm plastic trays containing distilled water washed fluted Whatman No -1 filter paper. Fifteen seeds were sown in each row at 1.5 cm distance. The filter paper was moistened daily with 50 ml of respective solution. This experiment was conducted in a seed germinator maintained at 25±1°C and 80±3 per cent relative humidity (RH) and maintained in 3.8 x 10^4 lux of light for 16 hours per day till the end of the investigation. The seedlings were randomly harvested at 7 days after sowing (DAS) and separated into root, hypocotyl, cotyledon and analysed for the estimation of growth and biochemical parameters.

Protein was extracted and estimated following the method of Lowry *et al.* (1951) bovine serum albumin (BSA) Vth fraction was used as standard. Total free amino acid was estimated following the method of Moore and Stein (1948), using leucine as standard. Free proline content was estimated according to the method of Bates *et al.* (1973), using proline as standard. The enzyme protease (EC. 3.4.2.2) was extracted and its activity was assayed by the method of Prisco *et al.* (1975), using glycine as standard. Protease activity was expressed in terms of (units) μ

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Table 1. Effect of triadimefon on the growth behaviour of the salt stressed radish seedlings

Treatments	Root length (cm)	Hypo-cotyl length (cm)	Root/ Hypo-cotyl ratio	Dry weight (g/10 seedlings) Root	Hypo-cotyl	Cotyle-don
Control	6.73	8.86	0.75	0.021	0.047	0.078
80 mM NaCl	6.16	5.10	1.20	0.016	0.036	0.071
	(-8.46)	(-42.43)		(-23.80)	(-23.40)	(-8.97)
80 mM NaCl +	10.51	4.61	2.27	0.025	0.030	0.079
3 mg litre^{-1} Tri.	(+56.16)	(-47.96)		(+19.04)	(-36.17)	(+1.28)
80 mM NaCl +	8.93	2.36	3.78	0.027	0.016	0.083
6 mg litre^{-1} Tri.	(+32.68)	(-73.36)		(+28.57)	(-65.95)	(+6.41)

Values in parentheses indicates per cent increase (+) or decrease (-) over control.

Moles of $-NH_2$ released per hour per mg protein. Seven replicates were maintained for each treatment and SD was calculated.

RESULTS AND DISCUSSION

Salinity caused reduction in the length of root and hypocotyl. The germination and the growth of the seedling are adversely affected by salinity in *Pisum sativum* (Siddiqui and Kumar, 1985). However triadimefon increased the root length and reduced the hypocotyl elongation in the NaCl stressed seedlings over control (Table 1). Pretreatment of seeds with LAB 150978 a triazole group of fungicide [1-(4-trifluoromethyl)-2-1 (1, 2, 4-triazolyl- (1)-3-(5-methyl-1, 3- dioxan-5-yl) propen-3-01] counter-acted the inhibitory effect of salinity on root growth, but it also inhibited the hypocotyl growth of sunflower and mungbean seedlings (Saha and Gupta, 1993). Triazoles increased the root growth in cucumber and this was associated with increased level of endogenous cytokinin (Fletcher and Arnold, 1986). On the other hand, triazoles distinctly inhibited the conversion of ent-kaurine to ent-kaurenoic acid (Rademacher *et al.*, 1987), thereby, lowering the availability of gibberellins for elongation growth. Therefore, it may be stated that triadimefon is acting differently with respect to root and hypocotyl growth.

The dry weight decreased in all parts of the salt stressed seedlings over the control. The dry weight of both root and shoot of the barley were reported to be decreased substantially with increasing concentration of NaCl (Hurkman and Tanaka, 1987). Triadimefon, increased the dry weight in root and decreased it in the hypocotyl when compared with control and NaCl stressed. The decrease in dry weight was higher in hypocotyl of the seedlings treated with NaCl combined with 6 mg litre^{-1} triadimefon (Table 1). In radish the fresh and dry weight of the hypocotyl decreased with triadimefon treatment, whereas the dry weight of root increased leading to an increased root shoot ratio (Fletcher and Nath, 1984). Increase in cotyledon and root dry weight with the increase in cytokinin in the triadimefon treated cucumber seedlings was reported by Fletcher and Arnold (1986). Our results coincides with the above author's observations.

Table 2.Effect of triadimefon on protein, amino acid, proline contents and protease activity of radish seedlings under NaCl stress (Values are mean ± SD of 7 samples expressed in mg/gram fresh weight)

Treatments	Parameters	Root	Hypocotyl	Cotyledon
Control	Protein	1.55 ± 0.07	1.18 ± 0.04	10.14 ± 0.41
	Amino Acid	2.14 ± 0.10	11.51 ± 0.57	28.95 ± 1.15
	Proline	0.156 ± 0.006	0.075 ± 0.003	0.867 ± 0.043
	Protease*	41.61 ± 2.08	22.62 ± 1.51	51.12 ± 3.03
80m M NaCl	Protein	1.26 ± 0.05	1.06 ± 0.03	7.90 ± 0.31
	Amino Acid	4.17 ± 0.20	19.83 ± 0.99	32.67 ± 1.63
	Proline	0.738 ± 0.013	2.112 ± 0.105	3.468 ± 0.173
	Protease*	43.50 ± 2.95	42.71 ± 2.32	62.92 ± 3.25
80 mM NaCl + Tri. 3 mg litre	Protein	1.95 ± 0.08	1.56 ± 0.06	5.25 ± 0.29
	Amino Acid	4.19 ± 0.21	7.59 ± 0.42	35.51 ± 1.77
	Proline	0.751 ± 0.035	1.321 ± 0.091	3.910 ± 0.187
	Protease*	46.10 ± 2.92	43.62 ± 2.41	68.34 ± 3.48
80 mN NaCl Tri± 6 mg litre	Protein	1.70 ± 0.06	1.73 ± 0.08	6.68 ± 0.37
	Amino Acid	5.08 ± 0.25	8.39 ± 0.51	39.76 ± 1.95
	Proline	0.865 ± 0.043	1.614 ± 0.095	4.050 ± 0.310
	Protease*	51.81 ± 3.62	46.41 ± 2.01	65.51 ± 3.91

* units/hour mg protein.

The protein content decreased in all part of the NaCl treated seedlings, whereas the increasing concentration of triadimefon increased the protein content in the root and hypocotyl of the NaCl stressed seedling, however it decreased in the cotyledons (Table 2). Salinity adversely affected the protein metabolism and it is due to decrease in protein synthesis, accelerated proteolysis, decreases in availability of amino acids and denaturation of enzymes involved in protein synthesis (Levitt, 1972). Paclobutrazol (a triazole compound) treatment increased the protein content in the seedling of *Brassica carinata* (Seitia *et al.*, 1995). The protein content declined in the cotyledons of soybean with uniconazole (a triazole) treatment (Trevor *et al.*, 1993). The increased protein content in the root and hypocotyl of the triadimefon treated NaCl stressed seedlings may be due to the increased protein synthesis caused by triadimefon.

All three sampled organs possessed higher quantities of free amino acids and proline contents under NaCl stress. The amino acid contents increased with increasing salinity in bean (Huber *et al.*, 1977). Proline accumulation under salt stress has been observed in soybean seedlings (Durgaprasad *et al.*, 1996). Proline may serve as an intercellular osmotic solute for the maintenance of osmotic balance between cytoplasm and vacuole (Brown and Hellebust, 1978). Triadimefon treatments to the NaCl stressed seedlings showed a varied effect on different plant parts. The free amino acids and proline contents increased in the root and cotyledons whereas it decreased in hypocotyl when compared with control and NaCl treatment (Table 2). In sunflower and mungbean, LAB 150978 a triazole compound increased the free amino acid and proline contents, which might act as an osmoticum to counter-act salinity stress (Saha and Gupta, 1993). Mulberry plants treated with triadimefon showed a tremendous increase in free proline content and the increase was directly proprotional with the concentration of triadimefon (Sreedhar, 1991).

NaCl salinity increased the protease activity in all parts of the seedling which was higher than that of control and this enzyme activity showed a slight increase in the triadimefon treated NaCl stressed seedlings and it was not statistically significant. The highest protease activity was recorded in the cotyledons when compared with hypocotyl and root of the control and treatments (Table 2). In bajra salinity caused higher activity of protease than that of controlled plants (Reddy and Vora, 1985). It was reported that salinity either reduced or had no effect on protease activity in all organs except in the case of leaves in mungbean during germination and seedling growth (Sheoran and Garg, 1978).

Salinity with triadimefon treatments resulted in accumulation of protein in the root and hypocotyl and increased the hydrolysis of protein in the cotyledons of germinating radish seedlings. The level of protease activity was less in the root and hypocotyl when compared to the cotyledons for the reason that proteins stored in the cotyledons are hydrolysed by the enzymes protease and translocated to the root and hypocotyl where they were resynthesized as new proteins. The amino acids and proline contents showed a significant increase associated with the increase of protease activity in the respective parts. This shows that, the protease degrade the protein into amino acids and proline and their accumulation may serve as a osmotic protectant in the cells under NaCl stress. The results shows that triadimefon increased root growth and reduced the hypocotyl elongation. Triadimefon also executes a protective effect in the NaCl stressed *Raphanus sativus* seedlings by increasing the amino acids, proline and protein content.

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