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Influence of triadimefon on the metabolism of NaCl stressed radish

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Abstract

The effect of triadimefon (TDM) on various biochemical parameters was studied in NaCl stressed radish (*Raphanus sativus* L.). Stress imposed by 80 mM NaCl decreased the protein content and proline oxidase activity, and increased the proline and glycine betaine contents, and protease, γ-glutamyl kinase and ATPase activities. The TDM treatment alleviated the stress by increasing protein, and glycine betaine contents, and by decreasing proline accumulation, and proline oxidase and ATPase activities.

Additional key words: ATPase, glycine betaine, proline, *Raphanus sativus* L., salinity.

Introduction

Salt stress induced changes in biochemical parameters could be correlated with the degree of salt tolerance with a particular emphasis on amino acids (Stewart and Larher 1980), quaternary ammonium compounds (Gratten and Grieve 1985), and enzymes of various metabolic pathways like proteases (Ramanjulu *et al.* 1994), γ-glutamyl kinase (Delauney and Verma 1990, Hu *et al.* 1992, Rout and Shaw 1998), proline oxidase (Flowers and Hanson 1969), and phosphatases (Hasson-Porath and Poljakoff-Mayber 1971). However, the results are still insufficient to generalise salient conclusions regarding biochemical parameters which are chiefly influenced under salinity and could be taken as criteria for salt tolerance.

In recent years, information are gradually accumulating on the amelioration of NaCl stress by triazole compounds (Fletcher and Hofstra 1988, Saha and Gupta 1993). Triazoles induce a variety of morphological and biochemical responses in plants, which includes stimulated root growth, inhibited shoot elongation, reduced gibberellic acid biosynthesis, increased cytokinin and abscisic acid contents, as well as confer protection from various environmental stresses (Fletcher and Hofstra 1988, Rademacher 1992). The stimulatory effect of triadimefon (TDM) on growth and net photosynthetic rate has already been studied in NaCl stressed *Raphanus sativus* (Panneerselvam *et al.* 1997). The present work describes the influence of TDM on the metabolism of proline and glycine betaine, and ATPase activity in *Raphanus sativus* under NaCl stress.

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Abbreviations: TDM - triadimefon, GK - γ-glutamyl kinase; DAS - days after sowing; DCPIP - 2,6-dichlorophenol indophenol.

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Materials and methods

The seeds of radish (*Raphanus sativus* L. cv. 8) were obtained from "Indo American Seeds Exports", Bangalore, India. They were surface sterilized with 0.2 % $HgCl_2$ solution for 5 min and thoroughly washed with deionized water.

In preliminary tests, the plants were grown in pots with various concentrations of NaCl (40, 60, 80, 100 and 120 mM), to determine the effect of NaCl stress on the growth of the plant. 80 mM NaCl was found to decrease the dry mass to 50 % over control and this concentration was used for the induction of stress. Among the various concentrations of TDM tested (5, 10, 15, 20 and 25 mg dm^{-3}), TDM concentration 15 mg dm^{-3} exhibited the maximum amelioration of the effect of with 80 mM NaCl on growth and dry matter production (Panneerselvam *et al.* 1997). Hence this concentration was used to study the interactive effect of TDM and NaCl on various biochemical parameters.

The surface sterilized seeds were pre-soaked in distilled water (control), 80 mM NaCl, 80 mM NaCl + 15 mg dm^{-3} TDM (25 % wetable powder, *Bayer's India Ltd*, Bangalore) for 12 h and sown in to plastic pots filled with soil. Pots were irrigated with respective treatment solution at 7-d intervals. Electrical conductivity (EC) of the soil mixture was measured and adjusted with the respective solution; it was 0.10 (control), 12 (80 mM NaCl) and 10 dS m^{-1} (NaCl + TDM). The pots was irrigated to the field capacity with deionized water daily and care was taken to avoid leaching of the salts.

The experiment was designed in a completely randomised design with 40 replicates per treatment. The positions of pots were changed on 4 d, to minimise spatial effects in the greenhouse. The photoperiod was 16 ± 2 h with a photosynthetic photon flux density (PPFD) of 870 ± 60 μmol m^{-2} s^{-1}, maximum/minimum temperature was 28/22 °C, and relative humidity varied between 75 and 85 %. The plants were harvested randomly at 15, 30, 45 and 60 d after sowing (DAS). Vegetative phase of the plants persists upto 60 d, therefore, biochemical analysis were restricted upto that phase.

The soluble protein content was estimated according to the method of Bradford (1976). Free proline content was estimated following the method of Bates *et al.* (1973). Glycine betaine content was determined in dry samples using the method of Cromwell and Rennie (1953).

Protease activity (EC 3.4.2.2) was assayed by the method of Prisco *et al.* (1975), activity was expressed in U [1 U = 1 mg(α-NH_2 released) mg^{-1}(protein) h^{-1}]. Activity of γ-glutamyl kinase (EC.2.7.2.11) was assayed, using the modified method of Hayzer and Leisinger (1980) [1 U = 1 mg(γ-glutamyl hydroxamate formed released) mg^{-1}(protein) min^{-1}]. Proline oxidase (EC.1.4.3.1) activity was determined according to the method outlined by Huang and Cavalieri (1979) [1 U = 1 mg(DCPIP reduced) mg^{-1}(protein) min^{-1}]. ATPase (EC.3.6.1.3) activity was assayed by the method of Evans (1969) and the Pi liberated was determined colorimetrically by the method of Jaffe and Galston (1966) [1 U = 1 μmol (Pi liberated) mg^{-1}(protein) min^{-1}].

Data of the main and interactive effect of salt and TDM treatments were tested using analysis of variance (*ANOVA*) by the method outlined by Ridgman (1975). Least significant difference (LSD) was calculated at the $P = 0.05$ confidence level using Tuckey's (1953) test.

Results and discussion

NaCl stress significantly decreased the protein content in the root and leaf. Addition of TDM to NaCl stressed plants increased the protein content to a level higher than that of the control (Table 1). Salinity adversely affected the protein metabolism. Protein degradation under saline environment have been reported due to decrease in protein synthesis, accelerated proteolysis, decrease in availability of amino acids and denaturation of enzymes involved in protein synthesis (Poljakoff Mayber 1982). Increase in protein contents in the TDM treated salt stressed plants may be due to enhanced protein synthesis induced by TDM. Similar results were observed in salinity stressed peanut and soybean seedlings treated with TDM (Muthukumarasamy and Panneerselvam 1997, Panneerselvam *et al.* 1998).

In the roots and leaves of the NaCl stressed radish, the proline content was higher than that of the control. These increases were less during TDM treatment (Table 1). Similar findings has also been reported in sunflower and mung bean seedlings treated with a triazole compound, LAB-150978 (Saha and Gupta 1993). There are conflicting reports concerning the role of proline in salt tolerance. Higher content of proline in NaCl stressed plants of barley (Singh *et al.* 1973) or tomato (Perez-Alfocea *et al.* 1996) have been suggested as a factor conferring salt tolerance. In contrast, proline accumulation has been excluded as the mechanism of tolerance in other cases (Tall *et al.* 1979). The significant changes in proline content observed in our study supports the former.

The reduction in protein content in the NaCl stressed plants could be correlated with increased proline accumulation. It may be due to the hydrolysis of protein or due to inhibition of protein synthesis by salinity leading to the accumulation of proline. It seems that decreased protein synthesis due to NaCl stress was partially alleviated by TDM. Presumably, TDM speed up the protein turnover leading to an increased protein synthesis and decreased proline content under NaCl stress.

Table 1. Changes of protein, proline, and glycine betaine contents of radish induced by 80 mM NaCl and 80 mM NaCl + 15 mg dm^{-3} TDM (means marked by * and ** were significantly different at $P = 0.05$ and 0.01, respectively, $n = 5$).

Parameter	Organ	DAS	Control	NaCl	NaCl + TDM	LSD ($P = 0.05$)
Protein	Root	15	3.54	2.52 *	4.39	0.172
[mg g^{-1}(d.m.)]		30	8.56	4.82 **	11.58 *	0.210
		45	10.00	8.63 **	14.92 **	0.995
		60	15.35	9.10 **	18.86 **	0.776
	Leaf	15	13.70	5.89 **	14.66 **	0.090
		30	22.46	12.48 **	30.53 **	0.128
		45	29.78	19.43 **	33.51 **	0.156
		60	33.08	23.73 **	39.43 **	0.128
Proline	Root	15	73.76	206.00 **	134.40 **	0.798
[μg g^{-1}(d.m.)]		30	213.43	591.22 **	539.49 **	12.762
		45	358.72	696.75 **	615.23 **	25.532
		60	415.28	899.89 **	797.32 **	45.250
	Leaf	15	262.82	402.29 **	307.96 **	25.520
		30	715.63	977.28 **	802.27 **	25.541
		45	797.94	1011.07 **	955.42 **	25.516
		60	965.81	1215.00 **	1010.02 **	62.050
Glycine	Root	15	0.067	0.075*	0.089	0.028
betaine		30	0.141	0.163*	0.199*	0.025
[μg g^{-1}(d.m.)]		45	0.151	0.177*	0.298**	0.058
		60	0.316	0.394*	0.416**	0.032
	Leaf	15	0.157	0.170	0.196*	0.005
		30	0.328	0.453*	0.457*	0.046
		45	0.361	0.483*	0.540**	0.019
		60	0.878	1.070*	1.236**	0.112

NaCl stress significantly increased the glycine betaine content in the roots and leaves when compared to control (Table 1). The glycine betaine may serve as an intercellular osmoticum and its content can be correlated with the decrease in osmotic potential as was observed in *Spartina townsendii* (Storey and Wyn-Jones 1978, Wyn-Jones and Storey 1978). TDM treatment to the NaCl stressed plants signifcantly increased the glycine betaine content (Table 1), thereby decreased the osmotic potential of the cytoplasm. The increased content of glycine betaine in TDM treated NaCl stressed peanut seedling was reported earlier (Muthukumarasamy and Panneerselvam 1997).

NaCl stress significantly increased protease activity in roots and leaves of radish and the leaves showed higher protease activity than the roots (Table 2). The increase in protease activity in NaCl stressed radish plants coincides with the increased proline and decreased protein content. A two-fold increase in protease activity in the leaves and a lower activity in the roots of NaCl stressed mung bean seedling was reported by Sheoran and Garg (1978). They also suggested that the effect of salinity varies with the stages of plant growth, the organ of plant and type of salinity. In the present study, TDM treatment decreased the protease activity.

Compared to control and TDM treated plants the activity of γ-glutamyl kinase (GK) increased significantly under NaCl stress (Table 2). The increase in GK activity coincides with the increased proline content. The induction of proline accumulation may be due to an activation of proline synthesis through glutamate pathway involving GK (Hare and Cress 1997). The expression of Δ^1-pyrroline-5-carboxylate synthetase (GK is a constituent of it) has also been reported to be increased in response to salinity (Hu *et al.* 1992, Zhang *et al.* 1995). Addition of TDM to the NaCl stressed plants decreased the GK activity. But the activity was still higher than that of control.

Table 2. Changes in protease, γ-glutamyl kinase, and proline oxidase activities in radish, induced by 80 mM NaCl and 80 mM NaCl + 15 mg dm^{-3} TDM (means marked by * and ** were significantly different at $P = 0.05$ and 0.01, respectively, $n = 5$).

Parameter	Organ	DAS	Control	NaCl	NaCl + TDM	LSD ($P = 0.05$)
Protease	Root	15	2.08	4.53 *	3.75	0.251
[U mg^{-1}(protein) h^{-1}]		30	7.51	15.66 **	13.90 *	0.664
		45	17.25	22.14 **	21.25 *	1.043
		60	18.54	25.60 **	23.66 *	1.008
	Leaf	15	4.11	7.53 **	6.71	0.507
		30	11.58	20.81 **	19.47 **	1.271
		45	18.32	25.30 **	22.59 *	2.038
		60	23.82	29.27 **	26.27 *	1.075
γ-glutamyl kinase	Root	15	0.520	0.988**	0.895*	0.040
[U mg^{-1}(protein) min^{-1}]		30	0.605	1.050**	1.015**	0.085
		45	0.795	1.280**	1.175*	0.114
		60	1.393	2.023**	1.925**	0.096
	Leaf	15	1.075	1.987*	1.795*	0.136
		30	1.805	3.005**	2.595**	0.191
		45	2.005	3.335**	3.003**	0.242
		60	3.213	5.893**	5.001*	0.448
Proline oxidase	Root	15	0.134	0.051**	0.058*	0.005
[U mg^{-1}(protein) min^{-1}]		30	0.228	0.091**	0.096**	0.023
		45	0.242	0.114**	0.116**	0.010
		60	0.276	0.156**	0.181*	0.013
	Leaf	15	0.041	0.017*	0.025*	0.021
		30	0.116	0.043**	0.066**	0.005
		45	0.232	0.085**	0.112**	0.017
		60	0.254	0.120*	0.135**	0.013
ATPase	Root	15	0.188	0.327*	0.089*	0.023
[U mg^{-1}(protein) min^{-1}]		30	0.814	0.965*	0.292*	0.105
		45	0.935	1.390**	0.865**	0.125
		60	0.986	1.412**	0.881*	0.155
	Leaf	15	0.452	0.531	0.110*	0.064
		30	1.013	1.453**	0.661*	0.057
		45	1.212	1.754**	0.899**	0.082
		60	1.416	1.996**	0.912**	0.116

NaCl stress also decreased proline oxidase activity. The activity was slightly higher in the roots when compared with leaves (Table 2). The proline oxidase activity can be inversely correlated with the proline content of the roots and leaves of salt stressed radish plants. Addition of TDM to the NaCl stressed plants increased the proline oxidase activity. Salt stress caused a reduction in activity of proline oxidase in *Brassica juncea* (Madan *et al.* 1995). Inhibition of proline oxidation was necessary for maintaining the high content of proline under water stress in barley leaves (Stewart and Boggess 1978), or at low temperature stress in wheat (Charest and Phan 1990). In the TDM treated NaCl stressed radish the proline accumulated during salt stress was converted into other amino acids by proline oxidase and these amino acids might have been utilized for the synthesis of new proteins. Similar observation was made in salt stressed, TDM treated peanut seedlings (Muthukumarasamy and Panneerselvam 1997).

The ATPase activity increased significantly in the NaCl stressed plants (Table 2). Increased ATPase activity was also observed in the NaCl stressed *Vigna unguiculata* (Fernandes Demelo *et al.* 1994). Salt stimulated ATPase activity has been attributed to the energy dependent ion transport in the roots against the concentration gradient of ions under NaCl stress. TDM treatment reduced the ATPase activity, and was more pronounced in roots. Inhibition of plasma membrane ATPase activity by TDM has been noted in the osmotically stressed *Picea glauca* needles (Sailerová and Zwiazek 1993). Other triazoles like penconazole and flusilazol inhibited the ATPase activity in the cultured cells of *Acer pseudoplatanus* (Colombo *et al.* 1991).

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