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BRIEF COMMUNICATION

Enhancement of peroxidase, polyphenol oxidase and superoxide dismutase activities by triadimefon in NaCl stressed *Raphanus sativus* L.

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Abstract

The activities of peroxidase, polyphenol oxidase and superoxide dismutase was significantly lower in roots and leaves of NaCl stressed radish (*Raphanus sativus* L.) plants. Addition of triadimefon to the NaCl stressed plants increased peroxidase, polyphenol oxidase and superoxide dismutase activities, and thereby ameliorated the negative effect of NaCl stress.

Additional key words: radish, salt stress, triazole.

The primary effects of salt damage are the changes in membrane permeability (Colmer *et al.* 1994). Ascorbate peroxidase (AP), superoxide dismutase (SOD), and glutathione reductases (GR) are implicated in the protection of membranes from salt damage by scavenging toxic oxygen radicals (Nakano and Asada 1981, Shaaltiel and Gressel 1986). Activity of antioxidant enzymes has been correlated with resistance to salinity (Kayupova and Klyshev 1984). It has been reported that in *Vigna* and *Oryza* seedlings, $O_2^{\bullet}$-radical and H_2O_2 could play an important role in salt injury (Singha and Choudhuri 1990). Enhanced production of oxygen free radicals is responsible for peroxidation of membrane lipids and the degree of peroxidative damage of cells was controlled by the potency of peroxidase enzyme system (Sreenivasulu *et al.* 1999). However, research concerning the effect of salt stress on the activity of oxygen-related enzymes and metabolites is still scarce.

The triazole compounds are known to protect plants from the adverse effects of various stresses (Fletcher and Hofstra 1988) and may increase the antioxidant enzyme activities (Kraus and Fletcher 1994). The ameliorative effect of triadimefon [TDM; 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1,2,4-triazol-1-yl)-2-butanone], on NaCl stress has recently been demonstrated (Muthukumarasamy and Panneerselvam 1997, Muthukumarasamy *et al.* 2000). However the mechanism of amelioration is not yet carried out. The present study is aimed at to elucidate whether ameliorative effect of TDM is through its effect on the activity of peroxidase, polyphenol oxidase (PPO) and SOD.

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.

Earlier experiments indicated that 80 mM NaCl treatment decreased the dry mass by 50 % over control. Therefore this concentration was used for the induction of stress. Similarly TDM at 15 mg dm^{-3} concentration with

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Abbreviations: AP - ascorbate peroxidase, DAS - days after sowing, NBT - nitroblue tetrazolium, EC - electrical conductivities, GR - glutathione reductases, PPFD - photosynthetic photon flux density, PPO - polyphenol oxidase, RH - relative humidity, SOD - superoxide dismutase, TDM - 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1,2,4-triazol-yl)-2-butanone.
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80 mM NaCl exhibited the maximum ameliorative effect 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 enzyme activities.

The surface sterilized seeds were pre-soaked in distilled water (control), 80 mM NaCl, 80 mM NaCl + 15 mg dm^{-3} TDM (*Bayer's*, Bangalore, India) and 15 mg dm^{-3} TDM alone for 12 h and sown in plastic pots filled with soil. Pots were irrigated with the respective treatment solution at 7-d intervals. Electrical conductivities (EC) of the moistured with the respective solution were 0.10 dS m^{-1} (control), 12 dS m^{-1} (80 mM NaCl), 10 dS m^{-1} (80 mM NaCl + 15 mg dm^{-3} TDM), and 1.13 dS m^{-1} (15 mg dm^{-3} TDM. The pots were 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 change every 4 d, to minimise spatial effects in the greenhouse. The day length was 16 ± 2 h with a PPFD of 870 ± 60 µmol m^{-2} s^{-1}, maximum /minimum temperature was 28/22 °C and relative humidity (RH) varied between 75 - 85 %. The plants were harvested randomly at 15, 30, 45 and 60 d after sowing (DAS). Vegetative phase of the plants persists up to 60 d therefore, biochemical analysis were restricted up to that phase.

The roots and leaves of radish were excised and 1 g of fresh mass was ground with a pestle in an ice-cold mortar with 8 cm^3 of 0.05 M sodium phosphate buffer (pH = 7.0). The homogenates were filtered through 4 layers of cheese cloth and then centrifuged at 4 °C for 20 min at 15 000 *g*. The supernatants were used for the assays of enzyme activities. The protein content in the supernatant was estimated according to Bradford (1976), using bovine serum albumin as a standard.

Peroxidase (EC 1.11.1.7) and polyphenol oxidase (EC 1.10.3.1) activities were assayed adopting the method of Kar and Mishra (1976). The assay mixture for peroxidase contained 2.5 cm^3 of 0.1 M phosphate buffer (pH = 7), 1 cm^3 of 0.01 M pyrogallol, 1 cm^3 of 0.005 M hydrogen peroxide and 0.5 cm^3 of enzyme extract. Adding 1 cm^3 of 2.5 N sulphuric acid stopped the reaction. Similar procedure was followed for polyphenol oxidase (PPO) except that H_2O_2 was not added in the assay mixture. The amount of purpurogallin formed in peroxidase and PPO activities were estimated by measuring the absorbance at 420 nm.

Superoxide dismutase (SOD) (EC 1.15.1.1) activity, the basis of which is its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) (Beauchamp and Fridovich 1971) was determined according to the method of Giannopolitis and Ries (1977). For SOD assay, the reaction mixture contained 50 mM Hepes (pH = 7.6), 0.1 mM EDTA, 50 mM Na_2CO_3 (pH = 10.4), 13 mM methionine, 0.025 % (m/v) Triton X-100, 75 µM NBT, 2 µM riboflavin and appropriate volume of enzyme extract. The reaction mixture was illuminated for 15 min an irradiance of 350 µmol m^{-2} s^{-1}. Identical solutions that were kept under dark served as blank. Absorbance by the reaction mixture was read at 560 nm.

The data were analyzed using *ANOVA*. The differences between treatments were evaluated by LSD at the $P = 0.05$ confidence level using Tuckey's (1953) test.

Peroxidase activity was inhibited by NaCl stress and the inhibition was higher in the leaves than in the roots. Addition of TDM to the NaCl stressed and unstressed plants increased the peroxidase activity to a level higher than that of NaCl stressed and controls plants (Table 1). Similarly, uniconazole and paclobutrazol (triazole compounds) protected cucumber (Upadhyaya *et al.*1989) and maize seedlings (Pinhero and Fletcher 1994) from chilling damage and the stress protection was mediated by an increase in antioxidants such as α-tocopherols and ascorbate and enhanced activities of glutathione reductase, peroxidase and catalase (Pinhero and Fletcher 1994). Paclobutrazol also increased the ascorbate peroxidase activity when compared with control in wheat cultivars (Kraus *et al.* 1995).

NaCl treatment significantly decreased PPO activity. In TDM treated plants, PPO activity was increased in the roots and leaves of radish (Table 1). The peroxidase and PPO are the two major enzymes responsible for oxidation of phenolic compounds (Sheen and Calvert 1969). The decrease in activity of PPO might induce an accumulation of total phenols in the salt stressed plants as reported in pearl millet by Das *et al.* (1992). The increased PPO activity after TDM treatment might reduce the phenol accumulation in the plant tissues under stress.

NaCl treatment caused a decline in SOD activity in the roots and leaves of radish. TDM treatment to the NaCl stressed and unstressed plants increased this activity, even above the level of control (Table 1). SOD catalyses the dismutation of superoxide anion radical ($O_2^{\bullet}$), resulting in the production of H_2O_2 and O_2 (Winston 1990, Smirnoff 1993). Inhibition of the SOD activity in the salt stressed pea leaves was reported earlier (Hernandez *et al.* 1995). The decrease in the SOD activity may cause the accumulation of reactive oxygen species that in turn causes oxidative damage in the salt stressed radish. A decreased SOD activity was also observed in the low temperature stressed banana and treatment with paclobutrazol increased the SOD activity and improved tolerance to low temperature (Biyan *et al.* 1995). Increased SOD activity was also observed in paclobutrazol treated wheat seedlings (Kraus *et al.* 1995). It was suggested that damage caused by stress, is in part due to increased generation of active oxygen species and paclobutrazol protects plants by maintaining increased

Table 1. Changes induced by 80 mM NaCl, 80 mM NaCl + TDM (15 mg dm^{-3}) and TDM (15 mg dm^{-3}) on peroxidase, polyphenol oxidase (PPO) and superoxide dismutase (SOD) activities in radish. SOD activity was expressed also in units per mg protein. One enzyme unit was defined as the amount of enzyme required to cause 50 % inhibition of NBT reduction as monitored at 560 nm. Means marked by * and ** were significantly different at $P = 0.05$ and 0.01, respectively. $n = 3$.

Activity	Type of tissue	DAS	Control	NaCl	NaCl + TDM	TDM	LSD ($P = 0.05$)
Peroxidase [U mg^{-1}(protein)]	Root	15	0.170	0.114**	0.197**	0.205**	0.012
		30	0.476	0.310**	0.505*	0.612**	0.049
		45	0.525	0.416**	0.755*	0.923**	0.101
		60	0.635	0.493**	1.584*	2.224**	0.132
	Leaf	15	0.327	0.183*	0.346	0.406*	0.048
		30	0.870	0.512**	0.921**	0.998**	0.016
		45	0.899	0.592**	1.123**	1.961**	0.064
		60	0.910	0.613**	3.222**	4.635**	0.135
PPO [U mg^{-1}(protein)]	Root	15	1.330	0.540*	1.870**	1.990**	0.042
		30	3.430	2.800**	4.280**	4.780**	0.049
		45	4.650	3.950**	7.920**	9.690**	0.090
		60	8.520	5.720**	12.440**	15.920**	0.574
	Leaf	15	1.560	0.815**	2.090**	2.480**	0.091
		30	4.160	3.420*	4.770	5.570**	0.397
		45	5.170	4.400**	10.960*	11.590**	0.564
		60	10.110	7.340**	15.000**	17.300**	0.475
SOD [U mg^{-1}(protein)]	Root	15	1.710	1.010**	2.390*	2.870**	0.088
		30	3.750	2.310**	4.990**	6.870**	0.247
		45	4.010	2.810**	5.210**	7.100**	0.167
		60	9.220	6.240**	11.700*	15.840**	1.115
	Leaf	15	2.010	1.500**	3.460**	4.450**	0.157
		30	4.350	3.120**	7.560	9.080**	0.484
		45	4.920	3.850**	7.890*	9.910**	0.352
		60	11.280	8.200**	16.260*	20.640**	0.982

antioxidant enzyme activity in wheat seedlings (Kraus and Fletcher 1994). The increased enzyme activity in the TDM treated radish plants coincides with observations of above authors.

The activities of peroxidase, PPO and SOD were decreased by the NaCl stress, which may lead to the oxidative damage in the NaCl stressed *Raphanus sativus* plants. TDM increases the activities of these enzymes and ameliorate the effect of NaCl stress.

References

Biyan, Z., Lifeng, L., Huibai, H., Zehuai, W.: Effects of low temperature and paclobutrazol on superoxide dismutase and abscisic acid of bananas (*Musa* species). - Acta hort. sin. **22**: 331-335, 1995.

Beauchamp, C., Fridovich, I: Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. - Anal. Biochem. **44**: 276-286, 1971.

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Colmer, T.D., Fan, T.W.M., Higashi, R.M., Lauchli, A.: Interactions of Ca^{2+} and NaCl stress on the ion relations and intracellular pH of *Sorghum bicolor* root tips: An *in vivo* ^{31}P-NMR study. - J. exp. Bot. **45**:1037-1044, 1994.

Das, N., Misra, M., Narayanmisra, A.: Sodium chloride salt stress induced metabolic changes in callus cultures of pearl millet (*Pennisetum glaucum* (L.) R. Br.): Oxidases. - Proc. nat. Acad. Sci. India **62**: 263-268, 1992.

Giannopolitis, C.N., Ries, S.K.: Superoxide dismutase. I. Occurrence in higher plants. - Plant Physiol. **59**: 309-314, 1977.

Fletcher, R.A., Hofstra, G.: Triazoles as potential plant protectants. - In: Berg, D., Plempel, M. (ed.): Sterol Biosynthesis Inhibitors. Pp. 321-331. Ellis Horwood, Cambridge 1988.

Hernandez, J.A., Olmos, E., Corpas, F.J., Sevilla, F., Del-Rio, L.A.: Salt-induced oxidative stress in chloroplasts of pea plants. - Plant Sci. **105**: 151-167, 1995.

Kar, M., Mishra, D.: Catalase, peroxidase and polyphenol oxidase activities during rice leaf senescence. - Plant Physiol. **57**: 315-319, 1976.

Kayupova, G.A., Klyshev, L.K.: [Superoxide dismutase of pea roots under the influence of high NaCl concentrations.] - Fiziol. Rast. **31**: 441-445, 1984. [In Russ.]

Kraus, T.E., Mckersie, B.D., Fletcher, R.A.: Paclobutrazol induced tolerance of wheat leaves to paraquat may involve increased antioxidant enzyme activity. - J. Plant Physiol. **145**: 570- 576, 1995.

Kraus, T.E., Fletcher, R.A.: Paclobutrazol protects wheat seedlings from heat and paraquat injury. Is detoxification of active oxygen involved? - Plant Cell Physiol. **35**: 45-52, 1994.

Muthukumarasamy, M., Panneerselvam, R.: Amelioration of NaCl stress by triadimefon in peanut seedlings. - Plant Growth Regul. **22**: 157-162,1997.

Muthukumarasamy, M., Dutta Gupta, S., Panneerselvam, R.: Influence of triadimefon on the metabolism of NaCl stressed radish. - Biol. Plant. **43**: 67-72, 2000.

Nakano, Y., Asada, K.: Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. - Plant Cell Physiol. **22**: 867-880, 1981.

Panneerselvam, R., Muthukumarasamy, M., Karikalan, L.: Triadimefon enhanced growth and net photosynthetic rate in NaCl stressed plants of *Raphanus sativus* L. - Photosynthetica **34**: 605-609, 1997.

Pinhero, R.G., Fletcher, R.A.: Paclobutrazol and ancymidol protect corn seedlings from high and low temperature stresses. - Plant Growth Regul. **15**: 47-53, 1994.

Shaaltiel, Y., Gressel, J.: Multienzyme oxygen radical detoxifying system correlated with paraquat resistance in *Conyza bonariensis*. - Pestic. Biochem. Physiol. **26**: 22-28, 1986.

Sheen, S.J., Calvert, J.: Studies on polyphenol content, activities and isoenzymes of polyphenol oxidase, and peroxidase during air- curing in three tobacco types. - Plant Physiol. **44**: 199-204,1969.

Singha, S., Choudhuri, M. A.: Effect of salinity (NaCl) stress on H_2O_2 metabolism in *Vigna* and *Oryza* seedlings. - Biochem. Physiol. Pflanzen **186**: 69-74, 1990.

Smirnoff, N.: The role of active oxygen in the response of plants to water deficit and desiccation. - New Phytol. **125**: 27-58,1993.

Sreenivasulu, N., Ramanjulu, S., Ramachandra-Kini, K., Prakash, H.S., Shekar-Shetty, H., Savithri, H. S., Sudhakar, C.: Total peroxidase activity and peroxidase isoforms as modified by salt stress in two cultivars of fax-tail millet with differential salt tolerance. - Plant Sci. **141**: 1-9,1999.

Tuckey, J.W.: The Problem of Multiple Comparisons. - Princeton University Press, New York 1953.

Upadhyaya, A., Davis, T. D., Walser, R. H., Galbraith, A. B., Sankhla, N.: Uniconazole - induced alleviation of low temperature damage in relation to antioxidant activity. - Hort. Sci. **24**: 955-957, 1989.

Winston, G. W.: Physiochemical basis for free radical formation in cells. - In: Alscher, R.G., Cumming, J.R. (ed.).: Production and defenses. In Stress Responses in Plants: Adaptation and Acclimation mechanisms. Pp. 57- 86, Wiley - Liss Inc., New York 1990.