Effect of Triadimefon on antioxidant enzyme activities in Pigeon pea (*Cajanus cajan* L. Millsp.) under NaCl stress

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Abstract:

The activity of the antioxidant enzymes viz., peroxidase and superoxide dismutase were very much inhibited by the NaCl stress. While triadimefon treatment increased it to a level even higher than that of control thereby increased the antioxidant potential in the triadimefon treated plants. The enzyme polyphenol oxidase, oxidise the phenols and prevents its accumulation in plant cells. Accumulation of phenols in plant cells increases the IAA catabolism through the increased activity of IAA oxidase. Sodium chloride stress inhibited the polyphenol oxidase activity in pigeonpea, which may lead to the accumulation of phenols thereby decreased the auxin content. Triadimefon treatment increased the polyphenol oxidase activity to a level higher than that of control, thereby protected the IAA from catabolism and this increased auxin content can be correlated with the increased root and stem growth in the triadimefon treated plants.

Keywords: Antioxidant, Cajanus cajan, Peroxidase, Triadimefon.

Introduction:

The environmental factors like temperature, soil moisture, wind velocity, soil pH and concentration of salts causes stress to the plants. Among these, salt stress is one of the major global issues in respect to agricultural sector. Irrigation is the most commonly needed requirement in agriculture. Irrespective of its source, generally all irrigation water contain dissolved salts, as a result of the general tendency to use water charged with dissolved salts for irrigation, irrigated lands tend to develop salinity and sodicity thus affecting adversely the soil fertility and consequently the productivity of the irrigated agriculture. Soil salinity influences negatively the germination, seedling emergence, growth and yield of crop plants. The inability to regulate the concentration of ions such as Na^+ in metabolically active tissue of the shoot of crop plants may lead to severe biochemical and physiological disturbances (Poljakoff-Mayber, 1982).

Pigeon pea is grown mainly in the states of Uttar Pradesh, Madhya Pradesh, Maharashtra, Karnataka, Bihar, Gujarat, Tamil Nadu and Andhra Pradesh. Pigeon pea is cultivated in semi-arid region, where salinity problems can be acute. Over 90 per cent of the world's pigeon pea is produced in India, where salinity problems are becoming increasingly severe.

Growth retardants have been shown to impart tolerance to salinity in some plants. The triazole derivatives have both fungi toxic and plant growth regulating properties and they include the largest and most important group of compounds developed for the control of fungal disease in plants and animals (Fletcher *et al.,* 1988). It has been demonstrated that the triazole can also protect plants against various stresses including drought, low and high temperatures, salinity and air pollutants. Hence the triazoles have been referred to as "a plant multi-protectants" and suggested that their protective effects are mediated by shifting the balance of important plant hormones in the isoprenoid pathway (Fletcher and Hofstra, 1985). Hence an attempt has been made in this study to anlysis the effect of triadimefon in the NaCl stressed redgram seedlings on its antioxidant enzymes.

Materials and Methods

Pigeonpea [*Cajanus cajan* (L.) Millsp.] cv. ICPL 7 seeds were surface sterilized with 0.2 per cent $HgCl_2$ solution for 5 minutes with frequent shaking, and thoroughly washed with deionized water. The seeds were pre-soaked in 500 ml of distilled water (control), 100 mM NaCl, 100 mM NaCl + 20 mg litre^{-1} triadimefon 25% WP (Bayers India Ltd.) and 20 mg litre^{-1} triadimefon alone for 12 hours and sown in plastic pots (300 mm diameter) filled with 3 kg of soil mixture containing red soil, sand and farm yard manure (FYM) in the ratio of 1:1:1. Pots were also previously irrigated with the respective treatment solution, as followed for the soaking treatments and then the electrical conductivity (EC) of the soil mixture was measured and adjusted with the respective solutions and the EC levels were 0.10 dS m^{-1} (control), 12.50 dS m^{-1} (100 mM NaCl), 10.00 dS m^{-1} (100 mM NaCl + 20 mg L^{-1} triadimefon) and 2.00 dS m^{-1} (20 mg L^{-1} triadimefon) respectively. Three seeds were sown per pot and the pots were watered to the field capacity with deionized water upto 60 days after sowing (DAS) and every care was taken to avoid leaching. The initial EC level of the soil was maintained by flushing each pot with required volume of corresponding treatment solutions on 7th, 22nd, 37th and 52nd DAS and the initial EC level was maintained by the addition of required volume of respective treatment solutions. The experiment was laid out in a completely randomized block design with 50 replicates for each treatment. The position of each pot was randomized every 4 days, to minimize spatial effects in the green house, where the temperature was 28/22°C (maximum/minimum) and the relative humidity (RH) varied between 60-70 per cent. The seedlings were thinned to one per pot on the 10th day after sowing. Plants were harvested randomly on the 15th, 30th, 45th and 60th DAS and separated as root, stem and leaves and used for analysing the antioxidant enzymes like peroxidase, super oxide dismutase and polyphenol oxidase. Peroxidase activity was assayed using the method of Kumar and Khan (1982). Superoxide dismutase activity was assayed as

described by Beauchamp and Fridovich (1971). Polyphenol oxidase activity was assayed by the method of Kumar and Khan (1982).

Results and Discussion

Peroxidase (Table -1)

Inhibition of peroxidase activity was observed in NaCl stessed pigeonpea plants. All environmental stresses decreases the protection against the oxidative stress caused by free oxygen radical such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH), which caused retardation in cell division and cell elongation (Leprince *et al.,* 1990; Price and Hendry, 1991). Reduction in peroxidase activity under NaCl stress was reported in *Arachis hypogaea* (Satakopan *et al.,* 1990) and soybean (Zaidi and Singh, 1995).

Addition of triadimefon to the NaCl stressed and unstressed plants increased the peroxidase activity to a level even higher than that of control. Uniconazole treatment protected cucumber (Upadhyaya *et al.,* 1989) and corn seedlings form chilling damage and the stress protection is mediated by an increase in antioxidants such as α-tocopherols and ascorbate levels and enhanced activities of glutathione reductase, peroxidase and catalase (Pinhero and Fletcher, 1994). Paclobutrazol increased the level of ascorbate peroxidase activity in wheat cultivars (Kraus *et al.,* 1995). Similar observation was made in triazole treated *Echinochloa frumentacea* (Sankhla *et al*., 1992).

Superoxide dismutase (SOD) (Table – 2)

The activity of superoxide dismutase was lowered to a larger extent in the NaCl stressed pigeonpea plants. Superoxide dismutase catalyses the dismutation of superoxide anion radical (O_2^-) with great efficiency, resulting in the production of H_2O_2 and O_2 (Winston, 1990; Smirnoff, 1993). Inhibition of superoxide dismutase in the salt stressed pea leaves was reported by Hernandez *et al.,* (1993; 1995) and this inhibition could increase the accumulation of oxygen species which in turn causes oxidative damage in salt stressed plants. A reduction in SOD activity was observed in many plants with abiotic stresses (Zhang and Kirkham, 1994).

NaCl in the presence of triadimefon and triadimefon alone caused an increase in the level of superoxide dismutase activity which was even higher than the control plants. Paclobutrazol treatment increased the SOD activity in the low temperature stressed banana and improved its tolerance (Biyan *et al.,* 1995). Increased level of SOD activity was reported in the paclobutrazol treated wheat cultivars (Kraus *et al.,* 1995). It was suggested that damage caused by stress, is in part due to increased generation of active oxygen and paclobutrazol protects plants by maintaining increased antioxidant enzyme activity in wheat seedlings (Kraus and Fletcher, 1994).

Polyphenol oxidase (Table -3)

Sodium chloride stress inhibited the polyphenol oxidase activity in pigeonpea. Similar observation was made in salt stressed calli of pearl millet (*Pennisetum glaucum*) (Namita Das *et al.,* 1992). Accumulation of phenolics in plant tissue has been reported to induce IAA catabolism (Sirju and Wilson, 1974) by the induction of IAA oxidase (Mackakova *et al.,* 1975). Auxin is essential for the cell wall extension and cell elongation. The inhibited polyphenol oxidase activity might have induced IAA oxidase activity thereby reducing the auxin content of the cells which might have lead to the decreased growth in the NaCl stressed plants.

Triadimefon treatments to the NaCl stressed and unstressed plants increased the polyphenol oxidase activity to a level higher than that of control. This increased activity may help in the reduction of phenol accumulation thereby protecting the IAA from oxidation and this increased IAA content protected by the triadimefon might be a reason for the increased the growth in the triadimefon treated stressed and unstressed pigeonpea plants.

Table 1. **Effect of NaCl, triadimefon and their combination on the level of peroxidase activity in pigeonpea** (Values are the mean ± SE of 3 replicates expressed in units = 0.1 absorbance per minute per mg protein)

Days after sowing (DAS)	Control	Treatments			F ratio	LSD (P = 0.05)
		100 mM NaCl	100 mM NaCl + 20 mg L^{-1} Tri	20 mg L^{-1} Tri		
		ROOT				
15	1.276 ± 0.037	0.911 ± 0.027	1.302 ± 0.038	1.641 ± 0.047	**	0.124
30	1.843 ± 0.053	1.580 ± 0.046	2.106 ± 0.061	2.633 ± 0.076	**	0.196
45	2.148 ± 0.062	1.671 ± 0.048	2.255 ± 0.065	2.706 ± 0.078	**	0.210
60	2.817 ± 0.081	2.113 ± 0.061	2.902 ± 0.084	3.521 ± 0.102	**	0.272
		STEM				
15	1.665 ± 0.048	1.332 ± 0.039	1.832 ± 0.053	2.165 ± 0.062	**	0.167
30	2.098 ± 0.061	1.865 ± 0.054	2.564 ± 0.074	3.030 ± 0.088	**	0.229
45	2.338 ± 0.068	2.004 ± 0.058	2.585 ± 0.075	3.086 ± 0.089	**	0.240
60	3.098 ± 0.089	2.685 ± 0.077	3.305 ± 0.095	3.986 ± 0.115	**	0.311
		LEAF				
15	1.912 ± 0.055	1.564 ± 0.045	2.260 ± 0.065	2.607 ± 0.075	**	0.200
30	2.214 ± 0.064	2.030 ± 0.059	2.768 ± 0.080	3.505 ± 0.101	**	0.254
45	2.615 ± 0.076	2.354 ± 0.068	3.007 ± 0.087	3.661 ± 0.106	**	0.278
60	3.310 ± 0.096	2.961 ± 0.085	3.725 ± 0.107	4.388 ± 0.126	**	0.343

Means marked by ** was significantly different at P = 0.01 level.
LSD = Least Significant Differences. ± SE = Standard Error.
C - Control, N - NaCl, NT - NaCl + Triadimefon, T and Tri - Triadimefon.

Table 2. **Effect of NaCl, triadimefon and their combination on the level of superoxide dismutase activity in pigeonpea** (Values are the mean ± SE of 3 replicates expressed in units = 0.1 absorbance per hour per mg protein)

Days after sowing (DAS)	Control	Treatments			F ratio	LSD (P = 0.05)
		100 mM NaCl	100 mM NaCl + 20 mg L^{-1} Tri	20 mg L^{-1} Tri		
		ROOT				
15	2.668 ± 0.077	1.685 ± 0.048	3.370 ± 0.098	3.475 ± 0.100	**	0.273
30	5.912 ± 0.171	4.751 ± 0.137	7.514 ± 0.217	9.117 ± 0.263	**	0.663
45	8.498 ± 0.245	7.140 ± 0.206	11.364 ± 0.328	11.616 ± 0.335	**	0.928
60	10.867 ± 0.314	8.036 ± 0.232	13.243 ± 0.382	14.203 ± 0.410	**	1.115
		STEM				
15	5.847 ± 0.169	3.661 ± 0.106	6.762 ± 0.195	7.499 ± 0.217	**	0.576
30	8.847 ± 0.255	6.294 ± 0.182	10.332 ± 0.298	11.222 ± 0.324	**	0.883
45	10.968 ± 0.316	9.159 ± 0.264	13.626 ± 0.393	14.699 ± 0.424	**	1.160
60	12.965 ± 0.374	9.553 ± 0.276	14.414 ± 0.416	15.780 ± 0.456	**	1.262
		LEAF				
15	4.139 ± 0.120	2.555 ± 0.074	4.262 ± 0.123	4.877 ± 0.141	**	0.382
30	6.592 ± 0.191	4.395 ± 0.127	6.836 ± 0.197	7.446 ± 0.215	**	0.606
45	9.691 ± 0.280	7.667 ± 0.221	9.814 ± 0.283	10.531 ± 0.304	**	0.895
60	11.594 ± 0.335	7.967 ± 0.230	12.130 ± 0.350	12.457 ± 0.360	**	1.055

Means marked by ** was significantly different at P = 0.01 level.
LSD = Least Significant Differences. ± SE = Standard Error.
C - Control, N - NaCl, NT - NaCl + Triadimefon, T and Tri - Triadimefon.

Table 3. **Effect of NaCl, triadimefon and their combination on the level of polyphenol oxidase activity in pigeonpea** (Values are the mean ± SE of 3 replicates expressed in units = 0.1 absorbance per minute per mg protein)

Days after sowing (DAS)	Control	Treatments			F ratio	LSD (P = 0.05)
		100 mM NaCl	100 mM NaCl + 20 mg L^{-1} Tri	20 mg L^{-1} Tri		
		ROOT				
15	1.864 ± 0.054	1.509 ± 0.043	1.912 ± 0.055	2.130 ± 0.062	**	0.176
30	2.467 ± 0.071	2.114 ± 0.061	2.643 ± 0.076	2.907 ± 0.084	**	0.240
45	3.086 ± 0.089	2.572 ± 0.074	3.189 ± 0.092	3.600 ± 0.104	**	0.295
60	5.437 ± 0.157	4.757 ± 0.137	5.559 ± 0.161	6.117 ± 0.177	**	0.518
		STEM				
15	2.478 ± 0.072	1.918 ± 0.055	2.638 ± 0.076	3.038 ± 0.088	**	0.241
30	2.895 ± 0.084	2.461 ± 0.071	3.402 ± 0.098	3.720 ± 0.107	**	0.298
45	4.297 ± 0.124	3.915 ± 0.113	4.488 ± 0.129	5.061 ± 0.146	**	0.420
60	5.984 ± 0.173	4.934 ± 0.143	6.614 ± 0.191	7.034 ± 0.203	**	0.584
		LEAF				
15	2.983 ± 0.086	2.424 ± 0.070	3.263 ± 0.094	3.822 ± 0.110	**	0.298
30	3.572 ± 0.103	2.977 ± 0.086	4.252 ± 0.123	4.848 ± 0.140	**	0.375
45	5.148 ± 0.148	4.455 ± 0.129	5.544 ± 0.160	6.498 ± 0.188	**	0.515
60	6.697 ± 0.193	4.940 ± 0.143	7.533 ± 0.218	8.124 ± 0.234	**	0.653

Means marked by ** was significantly different at P = 0.01 level.
LSD = Least Significant Differences. ± SE = Standard Error.
C - Control, N - NaCl, NT - NaCl + Triadimefon, T and Tri - Triadimefon.

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