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EFFECTS OF NaCl-SALINITY ON GROWTH AND PEROXIDASE ACTIVITY IN *TRITICUM AESTIVUM* L. VAR. CHANAB 70

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Abstract

The effect of sodium chloride on the growth, phenolic content and peroxidase activity of *Triticum aestivum* L. var. Chanab 70 was studied. High salt concentration retarded the root and shoot growth after 4 days of treatment, suppression of root growth was more than that of shoot presumably due to accumulation of ions. Decrease in peroxidase activity at −2 and −4 bar NaCl was coupled with the increase in total phenol level in the root tissues. Electrophoretic separation revealed that the peroxidases were markedly inhibited by increasing salt concentration after 2 days of treatment. Appearance of two new iso-enzymes after 4 days of treatment with −4 bar NaCl suggest that toxicity might influence the early metabolic changes such as synthesis of enzymes.

Introduction

Plant raised in saline media exhibit considerable differences in physiological and biochemical activities than those grown in non-saline environment (Waisel, 1972; Roeb *et al* 1982). Some studies have demonstrated that the level of several key enzymes is lower in salt affected plants than the untreated ones (Weinberg, 1970; Greenway & Osmond, 1972). However, evidences so far provided are inconclusive and further studies on the enzymatic level and their isoenzyme patterns are required. NaCl-salinity increases both numbers and quantity of phenolic inhibitors in certain glycophytes like wheat, maize and cotton (Khan *et al* 1976). Consequently, the activity of the enzyme peroxidase that governs the level of phenols is expected to be influenced by salinity. This investigation reports the effect of NaCl-salinity on growth and peroxidase activity and its isoenzymes pattern in young wheat seedlings.

Material and Methods

Growth of seedlings: Seeds of *Triticum aestivum* L. var. Chanab 70 obtained from Atomic Energy Agricultural Research Centre, Tandojam were surface sterilized with 0.2% mercuric chloride solution, rinsed thoroughly with distilled water for 2h and allowed to germinate in dark in a growth chamber maintained at 25 ± 1°C and 60% R.H. Seeds placed over a nylon gauze fitted in a plastic plate were placed on plastic pots containing distilled water such that seeds remained in contact with water. After 48h of germination, 10 uniform seedlings were transplanted to saline media, using the same setup as above. Osmotic concentrations of −1, −2 and −4 bar NaCl

were prepared in half-strength Hoagland solution. Half-strength Hoagland solution served as control. Pots were kept in a growth chamber under light intensity of 6000 Lux, 10h day length at 25 ± 1°C and 60% R.H. All solutions were changed on alternate days. Two and four days after treatment shoot and root length, fresh and dry weight of seedlings and percentage water content of seedlings was determined.

Assay of peroxidase activity and peroxidase iso-enzyme pattern: Plants raised as above were used to determine the activity in the roots at 2 and 4 days after treatment. Root tissues were crushed in a mortar containing ice-chilled 3 ml of 0.02 M Tris--HCl buffer, pH 7.5; a drop of 3mM Sodium EDTA were added. The extract was centrifuged at 4000 rpm for 20 min., at 0°C in a refrigeration centrifuge. The supernatant was used for the estimation of soluble proteins and peroxidase activity by the method of Jensen (1955) as modified by Alvarez (1968). Crude enzyme extract, 0.5 ml was mixed with 2.5 ml of 0.15 M phosphate buffer, pH 5.6 and 0.25 ml of 0.1 M H_2O_2. At 0 time, 0.25 ml of 0.01 M pyrogallol was added as a substrate. The reaction mixture was taken after adjusting the total volume to 10 ml with distilled water. After 8 min. incubation at 28 ± 1°C, optical densities were recorded in SP 600 spectrophotometer at 470 nm. One unit of enzyme was considered to be the change in optical density/8 min., whereas, specific activity was expressed as unit activity per mg protein. Protein content of the extract was measured in accordance with the method of Lowry *et al* (1951).

Iso-enzymes assay: Polyacrylamide gels were prepared as described by Davis (1964). Samples of 400 8μg of soluble protein containing one drop of 0.05 M sucrose were loaded on the gels and electrophoresis was performed at a constant current of 4 mA/gel tube for 1.5h using a tris glycine buffer, pH 8.4, as an electrode buffer solution. The gels were removed from the glass tubes by gently loosening them from the gel tube at the lower end and using a long needle syringe filled with 7% acetic acid. Acetic acid was used to avoid the dissolution of water soluble proteins (Smith, *et al* 1970). Subsequently, the gels were stained following the method of Pearse (1961). A 0.5% of 3.3′ diemethoxybenzidine in 15% acetic acid and 3% H_2O_2 were mixed in the ratio 1:2 (v/v) and gels were immersed. After one min. blue bands appeared on the gel.

Results

Seedling growth:

At 2 days after treatment, growth was significantly ($P<0.01$) retarded only in –4 bar NaCl salinity (Table 1). Shoot and root growth were not influenced by –1 and –2 bar NaCl concentration (Table 1 and 2).

Table 1. Effect of NaCl on root and shoot length of wheat seedlings.

Treatments Na Cl (bar)	2 days		4 days	
	Root length cm	Shoot length cm	Root length cm	Shoot length cm
0.0	1.54 ± 0.123	2.19 ± 0.164	3.27 ± 0.210	5.84 ± 0.238
−1	1.59 ± 0.250	2.59 ± 0.187	3.29 ± 0.326	6.39 ± 0.121
−2	1.61 ± 0.281	1.96 ± 0.236	2.82 ± 0.241	5.02 ± 0.209
−4	1.42 ± 0.176	1.66 ± 0.123	1.68 ± 0.231	3.14 ± 0.186

After 4 days of treatment root growth significantly reduced over the controls at −2 and −4 bars ($P<0.01$). Whereas shoot growth showed stimulation at −1 bar but was significintly supressed at −2 and −4 bar NaCl (Table 1 and 2).

Peroxidase activity:

Two days after treatment, NaCl salinity significantly retarded peroxidase activity over the controls at −2 and −4 bars ($P<0.001$) but not at −1 bar (Fig. 1). At 4 days after treatment peroxidase activity remained suppressed in −2 and −4 bar NaCl as compared to 2 days treatment (Fig. 1).

Total phenols:

Total phenol content in all the salinity concentrations significantly increased over the controls after 2 days of treatment ($P<$at least 0.05) and at 4 days total phenol level showed an increase at −2 and −4 bars (Table 3).

Table 2. Effect of NaCl on fresh weight, dry weight and water content of wheat seedlings after 2 and 4 days of treatment.

		Root			Shoot		
Days after treatment	NaCl (in bars)	Fresh wt./pot (mg)	Dry wt./pot (mg)	% water content	Fresh wt./pot (mg)	Dry wt./pot (mg)	% water content
2	0.0	70.51 ± 3.61	7.46 ± 0.01	89.41	114.71 ± 4.66	13.10 ± 0.05	88.55
	−1	93.56 ± 4.72	6.66 ± 1.02	92.89	134.82 ± 7.32	61.08 ± 1.01	87.99
	−2	62.44 ± 2.49	7.16 ± 0.95	86.85	111.92 ± 3.91	15.50 ± 0.062	86.19
	−4	50.31 ± 3.62	8.46 ± 0.054	88.63	99.34 ± 2.85	19.02 ± 0.87	80.57
4	0.0	105.0 ± 9.42	11.99 ± 1.34	88.58	220.00 ± 17.42	24.88 ± 2.12	88.69
	−1	131.0 ± 8.36	14.50 ± 1.62	88.93	248.00 ± 21.36	26.73 ± 2.71	89.22
	−2	107.0 ± 9.10	14.22 ± 1.21	86.71	194.00 ± 16.43	26.65 ± 1.80	86.26
	−4	68.00 ± 5.26	9.64 ± 0.89	85.81	136.00 ± 13.21	22.63 ± 1.64	83.60

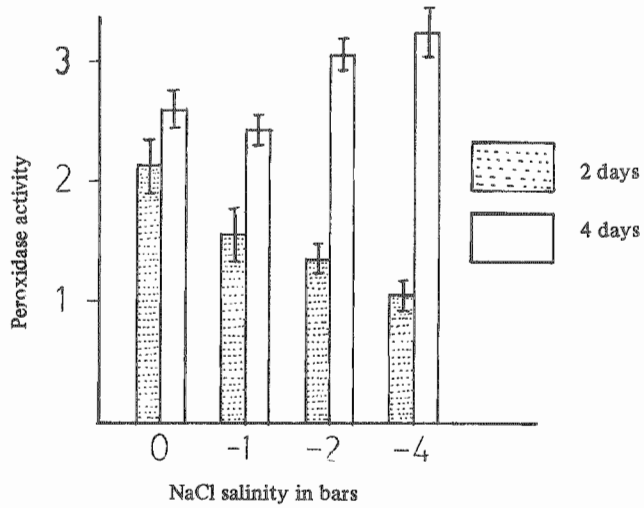

Fig. 1. Peroxidase activity in roots of wheat seedling at various levels of NaCl salinity.

Isoenzymes pattern of peroxidase

In controls 5 isoenzymes of peroxidase were found. Two days after NaCl treatment the isoenzyme pattern remained almost unchanged in –1 bar salinity but one isoenzyme disappeared in seedlings treated with –2 bar NaCl and the activity of two isoenzymes declined as indicated by the intensity of isozyme bands. At –4 bar salinity one isoenzyme disappeared but the activity of two cathodic iso-enzymes slightly increased (Fig. 2A). After 4 days of treatment the iso-enzymes pattern at – 1 bar NaCl remained unchanged but at –2 bar NaCl two iso-enzymes disappeared and the activity of two cathodic iso-enzymes declined substantially. In contrast, at –4 bar NaCl two new isoenzymes of peroxidase appeared and the activity of the two cathodic iso-enzymes that was decreased in –2 bar salinity increased (Fig. 2B).

Table 3. Effect of NaCl on total phenols of wheat roots after 2 and 4 days of treatment.

Treatments NaCl (bars)	μg of phenols/gm dry wt. 2 days	4 days
0.0	274 ± 5.87	262 ± 5.01
– 1	312 ± 6.12	275 ± 4.67
– 2	334 ± 6.71	325 ± 5.43
– 4	380 ± 7.32	317 ± 6.11

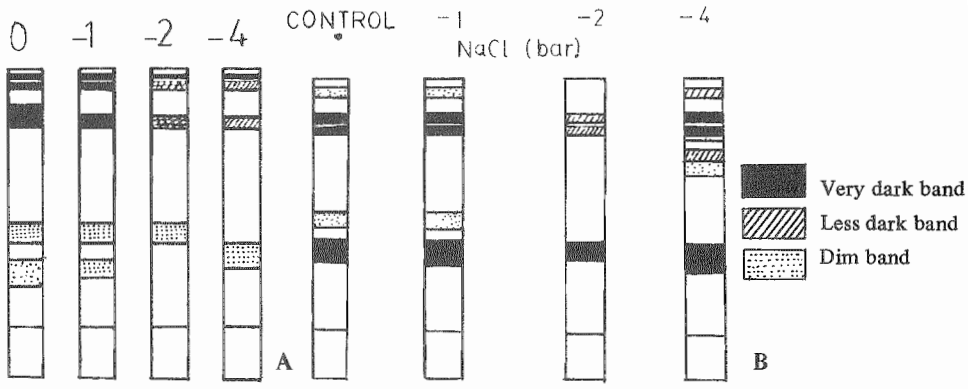

Fig. 2. Peroxidase isoenzyme patterns of roots, A): 2 days after NaCl treatment of wheat seedlings, B): 4 days after NaCl treatment of wheat seedlings.

Discussion

High salt concentration retarded the root and shoot growth of wheat seedlings after 4 days of treatment. Greater suppression of root growth is presumably due to accumulation of sodium and chloride ions. NaCl inhibition of growth suggests that salt toxicity might influence the early metabolic changes, such as synthesis of nucleic acids and proteins. Salt may affect synthesis of certain enzymes which regulate the process of growth. Growth suppression at high NaCl concentration is also attributable to the osmotic pressure (Khan & Khan, 1978; Verma, 1981). Plants raised under saline condition possess the ability to adjust their osmotic pressure (Bernstein & Hayward, 1958; Bernstein, 1963) so as to neutralize external pressure. However, even if osmotic balance is achieved, growth remains suppressed (Slatyer, 1961) due to internal ion accumulation in the cell sap (Khan & Khan, 1978) or because of the inability of certain subcellular organelles to adjust themselves to high osmotic pressure prevailing in the cell sap (Bernstein, 1961) and not due to reduced water uptake (Slatyer, 1961). Peroxidase activity of root tissues decreased at −2 and −4 bar NaCl. Our results are similar to the inhibition of peroxidase enzyme isolated from *Pisum sativum* L. and *Suaeda maritima* (L.) Dum. by 200 and 400 mM NaCl (Flower, 1972) and 18-50 percent reduction of peroxidase activity in 44 cultivars of *Cucumis, Brassica* and other plants grown in various dilution of sea water (Siegel *et al* 1982). The inhibitory effect of NaCl on activity of certain enzymes in plants has been reported (Evans & Sorger, 1966; Hansen-Porath & Poljakoff-Mayber, 1968, 1969). Although the exact nature of the effect of salts on the enzymes is not known, it may be related to a more general lyothophic effect of ions on enzyme conformation.

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