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BRIEF COMMUNICATION

Induction of oxidative stress in roots of *Oryza sativa* L. in response to salt stress

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Abstract

With the imposition of salt stress (0.5 to 3 % NaCl or $CaCl_2$) a decrease in germination rate and accumulation of proline was observed in the root tissue. Both NaCl and $CaCl_2$ solutions induced an increase in the total peroxide content and lipid peroxidation and decrease in catalase, guaiacol peroxidase and superoxide dismutase activities in root tissues suggesting an oxidative stress in the salt sensitive rice cultivar.

Additional key words: catalase, $CaCl_2$, guaiacol peroxidase, NaCl, *Oryza sativa* L., root, superoxide dismutase.

Salt stress is known to cause several physiological changes including oxidative stress (Hernandez *et al.* 1994, Alscher *et al.* 1997, Shalata and Tal 1998, Cherian *et al.* 1999). As rice is a salt sensitive crop and roots are the most affected parts for any stress, the present experiment has been undertaken to investigate the salt stress effects in root cells of rice and the possible oxidative damage caused by the salt stress.

Dry graded rice (*Oryza sativa* L. cv. Beeroin) seeds were dehusked and surface sterilized with 0.1 % mercuric chloride ($HgCl_2$) for 5 - 10 min and thoroughly washed with tap water and rinsed finally with distilled water. The seeds were germinated in Petri plates containing *Whatman No. 1* filter paper moistened with distilled water and incubated in *BOD* incubator for 3 d and then transferred to plastic flasks containing Yoshida solution (Yoshida *et al.* 1972) and kept in a growth chamber under continuous white light provided with cool fluorescent tubes (36 W, *Philips TLD*) with a photon flux density of 52 μmol m^{-2} s^{-1} (PAR), temperature of 25 ± 2 °C and relative humidity 75 %. On the 12th day, rice seedlings growing in Yoshida solution were treated in NaCl and $CaCl_2$ (0, 0.5, 1.0, 2.0 and 3.0 %) solution for 4 h. After 4 h of salt treatment, seedlings were rehydrated with fresh Yoshida solution. On the 15th day roots were sampled for various analyses. Rate of germination of rice seeds were recorded by similar treatment with different concentrations of NaCl and $CaCl_2$ to rice seeds, and on the 3rd day the germination percentage was calculated.

Extraction and estimation of proline was done by the method of Bates *et al.* (1973). Root tissue (0.5 g) was homogenised in 5 % trichloroacetic acid (TCA) and the homogenate was used for the estimation of total peroxide according to Sagisaka (1976) and malondialdehyde according to Heath and Packer (1968). Extraction and assay of catalase (CAT), guaiacol peroxidase (GPx) and superoxide dismutase (SOD) were done as per the methods described in Chance and Maehly (1955) and Giannopolitis and Ries (1977). Extraction and estimation of glutathione and ascorbate was done according to Griffith (1980) and Oser (1979), respectively. All the experiments were done in triplicates and the data represent means ± SD.

A gradual decrease in germination rate (Table 1) under different concentrations of NaCl and $CaCl_2$ was observed and concentrations 2 % for NaCl and 3 % CaCl, were found to be lethal for germination. Similar results were done for other plant species (Ozturk *et al.* 1997,

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Abbreviations: CAT - catalase; GPx - guaiacol peroxidase; MDA - malondialdehyde, SOD - superoxide dismutase; TCA - trichloroacetic acid.

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Hajar *et al.* 1996, Dash and Panda 2001). An uniform accumulation of proline content was recorded in the roots of rice seedlings treated with different concentrations of NaCl and $CaCl_2$ which suggested an osmoprotection to rice seedlings (Delauney and Verma 1993, Singh and Singh 1999). Both NaCl and $CaCl_2$ treatments induced an increase in the total peroxide and malondialdehyde (MDA) content in the root tissue proportionally with the increase in salt concentration. Increase in toxic hydrogen peroxide content and lipid peroxidation suggested an induction of a membrane damage and thus root cells were under oxidative stress (Bhattacharjee and Mukherjee 1997, Qin *et al.* 1998, Sairam *et al.* 2001).

Under different salt concentrations sharp decrease in CAT activity was seen. A similar decrease in concentration dependent manner was noticed in case of GPx and SOD activities in root tissue. This decrease in enzyme activity suggested a loss of antioxidant protection in response to salt stress (Bhattacharjee and Mukherjee 1997, Foyer 1997, Shalata and Tal 1998, Dash and Panda 2001). However, the non-enzymic antioxidants showed an accumulation in root tissue (Table 2) in plants subjected to salt stress with a maximum in NaCl salinity as reported elsewhere for other plant species (Alscher 1989, Hernandez *et al.* 1994, Foyer 1997).

Our results showed that in the roots of rice seedlings with the imposition of salt stress there is an increase in peroxide content and membrane lipid peroxidation with a concomitant decrease in the activities of antioxidant enzymes. From this it could be concluded that the rice cultivar Beeroin is salt sensitive because it gradually loose antioxidant protection in the root cells under salt stress.

Table 1. Changes in germination rate, and contents of proline, peroxide and malondialdehyde in rice roots subjected to different concentrations of NaCl and $CaCl_2$. Means ± SD.

Conc. [%]	Salt	Germination [%]	Proline [μmol g^{-1}(f.m.)]	H_2O_2 [μmol g^{-1}(f.m.)]	Malondialdehyde [μmol g^{-1}(f.m.)]
0		100 ± 4.31	1.0 ± 0.11	0.8 ± 0.11	0.10 ± 0.03
0.5	NaCl	93 ± 3.46	1.1 ± 0.09	1.2 ± 0.14	0.10 ± 0.02
0.5	$CaCl_2$	96 ± 4.12	1.3 ± 0.11	1.3 ± 0.12	0.20 ± 0.04
1.0	NaCl	89 ± 5.12	1.5 ± 0.14	1.4 ± 0.15	0.25 ± 0.04
1.0	$CaCl_2$	89 ± 5.41	1.7 ± 0.21	1.5 ± 0.19	0.29 ± 0.07
2.0	NaCl	42 ± 3.91	1.8 ± 0.15	1.6 ± 0.24	0.41 ± 0.09
2.0	$CaCl_2$	68 ± 5.56	2.0 ± 0.29	1.7 ± 0.26	0.52 ± 0.11
3.0	NaCl	27 ± 5.96	2.1 ± 0.25	1.8 ± 0.34	0.68 ± 0.09
3.0	$CaCl_2$	38 ± 5.88	2.4 ± 0.24	1.9 ± 0.32	0.73 ± 0.10

Table 2. Changes in activities of catalase, guaiacol peroxidase, and superoxide dismutase, and contents of glutathione and ascorbate in rice roots subjected to different concentrations of NaCl and $CaCl_2$. Means ± SD.

Conc. [%]	Salt	CAT [U g^{-1}(f.m.)]	GPx [U g^{-1}(f.m.)]	SOD [U g^{-1}(f.m.)]	Glutathione [μmol g^{-1}(f.m.)]	Ascorbate [μmol g^{-1}(f.m.)]
0		1200 ± 46.2	72 ± 8.12	3.6 ± 0.95	2.5 ± 0.96	2.8 ± 0.99
0.5	NaCl	560 ± 59.56	52 ± 7.59	3.0 ± 1.14	3.5 ± 0.98	3.2 ± 0.96
0.5	$CaCl_2$	420 ± 58.91	49 ± 7.26	3.4 ± 1.12	3.1 ± 0.91	2.8 ± 0.94
1.0	NaCl	410 ± 50.21	48 ± 6.56	1.8 ± 0.61	4.2 ± 1.21	4.3 ± 0.99
1.0	$CaCl_2$	290 ± 38.56	45 ± 5.15	2.8 ± 0.88	3.7 ± 1.01	3.9 ± 1.04
2.0	NaCl	180 ± 30.11	44 ± 5.26	1.6 ± 0.89	5.6 ± 1.11	4.9 ± 1.14
2.0	$CaCl_2$	270 ± 21.41	36 ± 4.91	2.2 ± 0.94	4.8 ± 0.98	4.2 ± 1.23
3.0	NaCl	110 ± 20.91	38 ± 4.89	1.4 ± 0.91	6.6 ± 1.61	6.8 ± 1.56
3.0	$CaCl_2$	90 ± 11.88	28 ± 4.01	1.7 ± 0.90	5.2 ± 1.21	6.2 ± 1.41

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