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BRIEF COMMUNICATION

Plant water status, H_2O_2 scavenging enzymes, ethylene evolution and membrane integrity of *Cicer arietinum* roots as affected by salinity

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Abstract

The chickpea genotype, CSG-8962 was raised in screenhouse to study salinity induced changes in ethylene evolution, antioxidative defence system and membrane integrity in relation to changes in plant water and mineral content. At vegetative stage (60 d after sowing), the plants were exposed to single saline irrigation (0, 2.5, 5.0 and 10.0 dS m^{-1}). Sampling was done 3 d after saline treatments. The other sets of treated plants were re-irrigated with water and sampled after further 3 d. The Ψ_w of leaf and Ψ_s of leaf and roots decreased from -0.47 to -0.61 MPa, -0.67 to -1.23 MPa and from -0.57 to -0.95 MPa, respectively, with increasing salinity. Similarly, RWC of leaf and roots reduced from 87.5 to 72.3 % and 96.7 to 84.35 %, respectively. The decline in Ψs of roots was mainly due to accumulation of proline and total soluble sugar. With salinity, increase in ethylene evolution, 1-aminocyclopropane-1-carboxylic acid (ACC) content and ACC oxidase activity was reported. Similarly, marked increase in H_2O_2 content (20 - 182 %) and lipid peroxidation (43 - 170 %) was observed. The defense mechanism activated in roots was confirmed by the increased activities of superoxide dismutase (SOD), peroxidase (POX), ascorbate peroxidase (APX), glutathione transferase (GTase), glutathione reductase (GR) and catalase (CAT) but ascorbic acid (AA) content was decreased. About 3-fold increase in Na^+/K^+ ratio and 2.5 fold increase in Cl^- content was observed. Upon desalinization, a partial recovery was observed in most of the parameters studied.

Additional key words: ascorbic acid, chickpea, lipid peroxidation, mineral content, recovery, water relations.

The harmful effects of salinity on the crop performance may be attributable to the ionic effect, osmotic effect and alteration in ionic composition leading to deficiency of nutrient ions and excess of salt ions. As a consequence of these primary effects, a secondary stress such as oxidative damage often occurs (*e.g.* Sairam *et al.* 2002). Plant cells contain an array of protective and repair systems which under any circumstances minimize the occurrence of overt oxidative damage (Demir and Oztürk 2003/04, Sairam *et al.* 2002), *i.e.*, low molecular mass antioxidants, and enzymes of ascorbate-glutathione (ASC-GSH) cycle in addition to superoxide dismutase, peroxidase, catalase, *etc*. Ethylene under different environmental constraints is one of the contributory factors for premature senescence of different plant parts (Abeles *et al.* 1992). In the soil, roots are thought of as a most sensitive organ to reactive oxygen species caused by different stresses.

The chickpea is considered sensitive to salinity (Manchanda *et al.* 1991) and is a particularly important crop in semi-arid and arid regions of the world. The mechanism by which salinity affects plant metabolism, thereby reducing growth and development are still not completely understood. Osmoprotectants such as proline and total soluble sugars are usually accumulated during exposure to salinity stress and help the plant to overcome

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Abbreviations: ACC - 1-aminocyclopropane-1-carboxylic acid, AA - ascorbic acid; CD - critical difference; C_2H_4 - ethylene; MDA - malondialdehyde; RWC - relative water content; Ψ_s - osmotic potential; Ψ_w - water potential.
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stress conditions (Nandwal *et al.* 2000a,b, Sairam *et al.* 2002, Qasim *et al.* 2003). Because of lack of sufficient information on root system as compared to the shoot portion, the present investigations were undertaken with objective to assess the effect of single saline irrigation (Cl^- dominated) and subsequent desalinization on ethylene evolution, membrane integrity and antioxidative defense system in chickpea roots along with the changes in plant water status.

Chickpea (*Cicer arietinum* L.) cv. CSG-8962 was raised in earthen pots (diameter 30 cm) filled with 5.5 kg of dune sane in screenhouse conditions. The seeds before sowing were surface sterilized and inoculated with effective *Rhizobium* culture (Ca 181). The crop was supplied with an equal quantity of nitrogen free nutrient solution at a regular interval of 15 d. The chloride dominated salinity was prepared by using a mixture of different salts such as NaCl, $MgCl_2$, $MgSO_4$ and $CaCl_2$ where Na:Ca+Mg was in the ratio of 1:1 and Ca:Mg in the ratio of 1:3, the $Cl:SO_4$ ratio was 7:3. At vegetative stage (60 d after sowing), the desired salinity was applied to saturate each pot so as to maintain four levels [0 (control), 2.5 (S_1), 5.0 (S_2) and 10.0 (S_3) dS m^{-1}]. The sampling was done 3 d after treatments. Half of the treated plants were re-irrigated with water and sampled after further 3 d and were designated as S_1R, S_2R and S_3R, respectively.

Three replicates (3 pots and each pot contained 2 plants) were used for each observation under each treatment. The data were analyzed statistically using complete randomized design (CRD) and the critical difference (CD) was tested at 5 % level.

The third fully expanded leaf from the top was used to measure water potential (Ψ_w) with a pressure chamber (*Model-3005*, *Soil Moisture Equipment Corporation*, Santa Barbara, USA). The osmotic potential (Ψ_s) of leaf and root was determined with *Vapour Pressure Osmometer* (*Model-5100*, *Wescor*, Logan, USA). The relative water content (RWC) of leaves and roots was calculated as described by Weatherley (1950). These measurements were made between 09:00 and 11:00 (local time) during a sunny day. The proline content and total soluble sugars (TSS) of roots was estimated by the methods of Trotel *et al.* (1996) and Dubois *et al.* (1956), respectively. Ascorbic acid content was measured by the method of Schopfer (1966). Free 1-aminocyclopropane-1-carboxylic acid (ACC) content was assayed following the method of Miller and Pengelly (1984). The ethylene production and activity of ACC oxidase was measured by the method described by Fearn and La Rue (1991). For extraction of protein for enzyme assays, one g of roots were washed in chilled distilled water and homogenized with a chilled pestle and mortar in 5 cm^3 of extraction buffer (0.1 M phosphate buffer, pH 7.0), containing 10 mM KCl, 1 mM $MgCl_2$ and 10 mM EDTA and centrifuged at 10 000 *g* at 4 °C for 20 min. The supernatant was used for the following enzymes assay. The protein content was determined by the method of Lowry *et al.* (1951).The specific activities of catalase (CAT - EC 1.11.1.6), peroxidase (POX - EC 1.11.1.7), glutathione reductase (GR - EC 1.11.1.9), glutathione transferase (GTase - EC 2.5.1.18), ascorbate peroxidase (APX - EC 1.11.1.11), and superoxide dismutase (SOD - EC 1.15.1.1) were measured by the methods of Aebi (1983), Shannon *et al.* (1966), Goldberg and Spooner (1983), Habig and Jakoby (1981), Nakano and Asada (1981) and Giannopolitis and Ries (1977), respectively. H_2O_2 content was determined by Patterson *et al.* (1984) method. The lipid peroxidation was measured in terms of malondialdehyde (MDA) content by thiobarbituric acid (TBA) reaction (Heath and Packer 1968). Sodium and potassium were estimated using flame photometer (*Model MK-1-121*, *Systronics*, New Delhi, India). The Cl content in the digested material was determined by *EILmV* meter (*Model CM2400A*, *Caltex Instruments*, Bradford, UK) using calomel electrode.

Salinity resulted in significant alterations in the parameters of plant water relations. Leaf Ψ_w significantly decreased from -0.47 to -0.61 MPa (Table 1). After desalinization, the values increased but none of the values reached to the level of control. Leaf and roots Ψ_s also decreased significantly from -0.65 to -1.23 MPa and from -0.57 to -0.95 MPa. Upon recovery, the values increased and were from -0.65 to -0.94 MPa and from -0.58 to -0.77 MPa in leaf and roots, respectively. The reduction in RWC of leaf and roots ranged from 87.5 to 74.3 % and from 96.70 to 84.35 %, respectively (Table 1). Improvement in RWC of leaf and roots was observed after desalinization, but complete recovery was never observed. The reduction in RWC of different plant parts under salt stress may be attributed to decreased water uptake due to low substrate water potential or to injury of root system. The reduction in osmotic potential under stress conditions is important for osmotic adjustment and can be a result of solute accumulation, *e.g.* proline, betaine, total soluble sugars (Nandwal *et al.* 2000a,b, Sairam *et al.* 2002, Kaur *et al.* 2003, Qasim *et al.* 2003, Fernandes *et al.* 2004).

In the present investigation, accumulation of proline and TSS in chickpea roots was also found. Proline content in roots increased maximally by 2-fold at 10.0 dS m^{-1}, whereas TSS content increased significantly at higher (5.0 and 10.0 dS m^{-1}) levels of stress by 70 and 129 %. Upon recovery, decrease in the content of accumulated proline and TSS was observed.

A considerable increase in C_2H_4 evolution (16 to 75 %) was noticed with salinity. Upon recovery, decrease in ethylene evolution was observed. Enhance in C_2H_4 evolution with increased salinization was accompanied by increase in ACC content (18 to 116 %) and ACC-oxidase activity above 2-fold (Table 1).

Lipid peroxidation is an attack upon unsaturated fatty acid components of the membrane (Heath and Packer 1968). It is the system most easily ascribed to oxidative

Table 1. Changes in plant water status, ethylene production, specific activity of antioxidative enzymes and lipid peroxidation in chickpea roots under salinity and upon recovery. C - control; S_1 - salinity 2.5 dS m^{-1}; S_1R - recovery of S_1; S_2 - salinity 5.0 dS m^{-1}; S_2R - recovery of S_2; S_3 - salinity 10.0 dS m^{-1}; S_3R - recovery of S_3; CD - critical difference at level 5 %.

	C	S_1	S_1R	S_2	S_2R	S_3	S_3R	CD
Leaf Ψ_w [-MPa]	0.47	0.50	0.48	0.59	0.53	0.61	0.58	0.03
Leaf Ψ_s [-MPa]	0.65	0.76	0.65	0.94	0.82	1.23	0.94	0.05
Leaf RWC [%]	87.50	82.90	85.75	76.15	79.95	74.30	78.03	3.01
Root Ψ_s [-MPa]	0.57	0.71	0.58	0.85	0.66	0.95	0.77	0.04
Root RWC [%]	96.70	92.30	94.79	87.75	90.95	84.35	87.36	3.28
Root proline content [mg g^{-1}(d.m.)]	0.29	0.32	0.30	0.38	0.36	0.59	0.34	0.02
Root TSS [mg g^{-1}(d.m.)]	15.10	16.49	15.54	25.72	20.96	34.51	23.03	2.75
Root ACC [pmol(C_2H_4)g^{-1}(d.m.) s^{-1}]	0.084	0.112	0.081	0.161	0.119	0.190	0.165	0.025
Root ACC oxidase [pmol(C_2H_4) g^{-1}(d.m.) s^{-1}]	0.251	0.339	0.301	0.563	0.388	0.624	0.449	0.058
Root ethylene [pmol g^{-1}(d.m.) s^{-1}]	0.774	0.917	0.764	1.218	0.875	1.653	1.151	0.114
Root SOD [U mg^{-1}(protein) s^{-1}]	0.114	0.139	0.123	0.170	0.147	0.233	0.175	0.016
Root CAT [µmol (H_2O_2) mg^{-1}(protein) s^{-1}]	0.470	1.027	0.810	1.655	1.270	2.068	1.709	0.111
Root ASC-POX [nmol(ascorbate) s^{-1}]	0.882	0.637	0.676	1.290	0.959	2.130	1.548	0.085
Root POX [U mg^{-1}(protein) s^{-1}]	120.50	161.50	141.50	229.0	184.83	333.83	268.66	15.83
Root GTase [µmol(*p*-nitrophenyl) mg^{-1}(protein) s^{-1}]	0.043	0.056	0.047	0.065	0.051	0.086	0.060	0.003
Root GR [nmol (NADH) mg^{-1}(protein) s^{-1}]	0.968	1.379	1.188	1.740	1.440	1.574	1.223	0.110
Root H_2O_2 content [mmol g^{-1} (d.m.)]	3.15	3.79	3.58	6.08	5.29	8.91	7.04	0.53
Root lipid peroxidation [µmol(MDA) g^{-1}(d.m.)]	0.46	0.65	0.50	1.11	0.95	1.24	0.96	0.06
Root AA [mg g^{-1}(d.m.)]	1.64	1.27	1.43	1.13	1.35	1.14	1.18	0.09
Root Na^+/K^+ ratio	0.40	0.52	0.45	0.81	0.61	1.13	0.93	0.15
Root Cl [mmol g^{-1}(d.m.)]	1.32	1.71	1.44	2.35	2.09	3.19	2.33	0.17

damage and also the most frequently measured. As expected, upon salinization, lipid peroxidation measured in terms of MDA content increased significantly from 43 to 172 %. Revival up to 23 % was observed upon desalinization but a complete recovery was never observed. Under this condition, the increase in the H_2O_2 content of roots with increased salinization might be the cause for increased lipid peroxidation.

A significant increase in the specific activity of SOD and GTase by 2 fold and 95 %, respectively, was reported at 10.0 dS m^{-1}, whereas an increase of 5-fold was observed for CAT activity (Table 1). The activity of POX and GR increased from 35 to 178 % and from 42 to 63 %, respectively. With increasing salinity up to 141 % increase in activity of APX was reported. Complete recovery in the activitieas of these enzymes was not reported upon desalinization.

With increasing salinity, 20 to 182 % increase in H_2O_2 content was observed (Table 1), however, upon recovery the values decreased to 6 and 12 % with respect to those in stressed plants. Besides being deleterious to many cells, H_2O_2 is known to induce several genes, proteins and enzymes involved in stress defenses like catalase (Prasad *et al.* 1994a) and peroxidase (Prasad *et al.* 1994b).

Ascorbic acid (AA) content generally declines under stress conditions. In chickpea roots, under salinity also, a decline from 23 to 31 % was reported (Table 1). After desalinization the roots showed slight increase in AA content.

About three times increase in Na^+/K^+ ratio was observed with increasing salinity in chickpea roots (Table 1). Upon recovery, a decrease in Na^+/K^+ ratio was noticed but the values were still higher than in control. Cl^- content increased significantly (Table 1), as was reported earlier in various crops (Manchanda *et al.* 1991, Sharma 1996, Nandwal *et al.* 2000b, Sairam *et al.* 2002). Upon recovery Cl^- content was also decreased.

It is clear that the application of saline irrigation resulted in increase in membrane injury (MDA content), Na^+/K^+ ratio and Cl^- content of roots with a simultaneous decrease in ascorbic acid content, Ψ_w of leaf, RWC and Ψ_s of leaf and roots. Activation of antioxidants enzymes could not overcome the accumulation of H_2O_2. A complete recovery after 3 d of salinization of stressed plants was never seen in these parameters.

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