DOI: 10.1007/s11099-013-0003-8 PHOTOSYNTHETICA 51 (1): 95-101, 2013

Effect of sodium chloride on gas exchange, antioxidative defense mechanism and ion accumulation in different cultivars of Indian jujube (*Ziziphus mauritiana* L.)

R. AGRAWAL[*], S. GUPTA[**], N.K. GUPTA[**,+], S.K. KHANDELWAL[*], and R. BHARGAVA[***]

SKN College of Agriculture, Jobner, Jaipur 303 329, India[*]
Swami Keshwanand Rajasthan Agricultural University, Bikaner 334 006, India[**]
Central Institute of Arid Horticulture, ICAR, Bikaner 334 006 India[***]

Abstract

An experiment was conducted to study the effect of NaCl (electric conductivity of 0, 4, 8, 12, and 16 dS m^{-1}) on growth, gas exchange parameters, water status, membrane injury, chlorophyll stability index and oxidative defense mechanisms in two cultivars (Gola and Umran) of Indian jujube (*Ziziphus mauritiana*). Results showed that the dry mass and leaf area reduced linearly with increasing levels of salinity. Net photosynthetic rate (P_N), transpiration (E), and stomatal conductance (g_s) were comparatively lower in Umran which further declined with salinity. Leaf relative water content, chlorophyll (Chl) stability and membrane stability also decreased significantly under salt stress, with higher magnitude in Umran. Superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT) activities were higher in Gola whereas hydrogen peroxide (H_2O_2) accumulation and lipid peroxidation (MDA content) were higher in control as well as salt-treated plants of Umran. The Na^+ content was higher in the roots of Gola and in the leaves of Umran, resulting in high K^+/Na^+ ratio in Gola leaves. Thus it is suggested that salt tolerance mechanism is more efficiently operative in cultivar Gola owing to better management of growth, physiological attributes, antioxidative defense mechanism, and restricted translocation of Na^+ from root to leaves along with larger accumulation of K^+ in its leaves.

Additional key words: antioxidants; chlorophyll; K/Na ratio; membrane injury; photosynthesis; salinity; *Ziziphus.*

Introduction

Salinity affects a large area of the world's arable land making the landscape either barren or unproductive. It is estimated that about 20% of the earth's land mass and nearly half of the total irrigated land is affected by salinity. Increased salinization of arable land is expected to have devastating global effects, with prediction of 30% land loss in next 25 years, and up to 50% by the year 2050 (Ebert 2000). Salt stress is a complex physical-chemical process, in which many biological molecules like nucleic acids, proteins, carbohydrates, lipids, hormones, ions, free radicals, and mineral elements are actively involved (Munns 2002, Liu and Baird 2004). In addition, salt stress reduces the photosynthesis (Pradeep and Jumbhale 2000), causes stomata closure (Hernandez and Almansa 2002) and increases the concentration of activated oxygen species (AOS), such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) *via* enhanced leakage of electrons to oxygen (Arzani 2008). To mitigate and repair the damage initiated by AOS, the tolerant plant species have been found to develop a complex antioxidant system. SOD is the primary scavenger which converts O_2^- to H_2O_2. This toxic product of SOD reaction is eliminated by ascorbate peroxidase (APX) in association with dehydro-ascorbate reductase and glutathione reductase (GR). H_2O_2 is also scavenged by CAT, though the enzyme is less efficient than APX–GR system (Sairam and Srivastava 2000). Peroxidases are often the first enzymes which alter their activity under stress conditions (Srivalli *et al.* 2003).

Indian jujube or ber (*Z. mauritiana* L.) is a common fruit indigenous to India. Its fruits are palatable and delicious with high concentrations of vitamin A, C and B

Received 9 April 2012, *accepted* 30 September 2012.
[+]Corresponding author; fax: +91-151-2250576; e-mail: nkgupta69@yahoo.co.in
Abbreviations: CAT – catalase; CD – critical difference; CSI – chlorophyll stability index; DM – dry mass; E – transpiration rate; Ece – electric conductivity; g_s – stomatal conductance; LA – leaf area; MSI – membrane stability index; POX – peroxidase; P_N – net photosynthetic rate; SOD – superoxide dismutase; RWC – relarive water content; TM – turgid mass.

complexes. Ber leaves contain 10–19% crude protein with about 40% digestibility. The leaves are commonly used as a fodder for animals (Pareek 1983). Compared to other agricultural and horticultural crops, Indian jujube (ber) is known to grow successfully under a low and erratic rainfall, temperature extremes and saline soils with low fertility (Pandey *et al*. 1991, Meena *et al*. 2003). However, systematic information with respect to relative tolerance of one cultivar over other is completely lacking. Therefore, the present investigation was carried out to find out the relative salt tolerance of two commonly used ber cultivars. The results were validated by estimating various morpho-physiological parameters, antioxidative defense mechanism and ionic regulation in roots and leaves.

Materials and methods

Plant material and experimental details: The study was conducted in the cage house under natural conditions. Six-month-old plants of *Z. mauritiana* cvs. Gola and Umran grafted on *Ziziphus rotundifolia* grown in ceramic pots of 20 × 20 cm diameter were taken for study. The pots were filled with 10 kg of loamy sandy soil having a bulk density of 1.5 g cm^{-3}, electric conductivity (Ece) 1.5 dS m^{-1}, pH 8.2, sodium absorption ratio 12.5 and $CaCO_3$ 0.14%. The field capacity and permanent wilting point of the soil were 11.8 and 2.8%, respectively. About 100 pots of each cultivar were used. The recommended doses of manures, fertilizers and other inputs were provided at the appropriate time. Saline water with ECe of 4.0, 8.0, 12.0, and 16.0 dS m^{-1} were prepared with NaCl and 500 ml of the solution was provided to each pot on alternative day. The control plants were irrigated with same amount of distilled water. The top most fully expanded leaves were sampled at 60, 120, and 180 d after grafting on both the cultivars. The pattern was almost the same at different stages of these cultivars and therefore, we presented only the data obtained at 180 d after grafting.

Leaf area and dry mass: Three plants per replication were selected for quantifying the leaf area (LA). The fully expanded green leaves of these plants were detached from the plants and LA was determined directly with the help of LA meter (*LICOR–3100,* Lincoln, USA). The average LA was then calculated by dividing the LA with the number of leaves used. For dry mass (DM), three healthy plants per replication were selected, their leaves and stems were thoroughly washed and oven-dried at 65°C till constant mass. DM was then recorded on a sensitive balance (*AB204*, *Mettler*, OH, USA).

Gas-exchange parameters: P_N, E, and g_s were measured by infrared gas analyzer (*CID 301*, *Bio-Science, Inc.*, Vancouver, USA). Five topmost fully expanded leaves from each treatment were selected randomly for the measurements. The leaf was enclosed in the assimilation chamber and P_N was monitored while CO_2 concentration changed over a definite time interval. The system automatically calculated P_N on the basis of preloaded flow and LA. E and g_s were also measured simultaneously by infrared gas analyzer (*CID 301*, Vancouver, USA) on the same leaf. All these measurements were taken at 10:00–11:00 h (Indian time), when relative humidity, temperature, photosynthetic photon flux density and CO_2 concentration ranged from 50–60%, 30–35°C, 1,200 μmol (photon) m^{-1} s^{-1} and 350–360 μmol mol^{-1}, respectively. The data presented in figures are means of three replications per treatment.

Membrane stability index (MSI): Leaves of control and salt-treated plants were collected and thoroughly washed with distilled water. 200 mg of a leaf sample was placed in 25 ml of double distilled water at 40°C for 30 min and thereafter an electric conductivity (C_1) was measured with conductivity meter (*304, Systronics*, Ahmedabad, India). Subsequently, the same samples were placed on boiling water bath (100°C) for 10 min and their electric conductivity was recorded (C_2). MSI was calculated (Gupta *et al.* 2000): $MSI = [1 – \{C_1/C_2\}] \times 100$.

Chl stability index (CSI): Two sets of 100 mg of fresh leaf samples were kept in 100 ml distilled water separately. One set was kept at room temperature and another was incubated at 45°C for 30 min. Water from all the samples was drained and Chls were extracted with mortar and pestle in 80% chilled acetone (10 ml). After centrifugation at 2,000 × *g* for 10 min, the resultant solution of Chl *a* and *b* was determined spectrophotometrically at 663 nm and 645 nm. The CSI was calculated according to Gupta *et al.* (2000).

Relative water content (RWC): Fresh mass (FM) of the leaf samples was taken and then kept in distilled water for 4 h to obtain turgid mass (TM). TM was recorded after blotting the excess water on the surfaces of leaf samples. Dry mass (DM) was obtained after drying the samples in the oven at 60°C till a constant mass. RWC was then calculated as RWC = 100 × (FM – DM)/(TM – DM).

Superoxide dismutase: Enzyme extract for SOD was prepared by grinding 0.5 g of the leaf material with 10 cm^3 of chilled 0.1 M potassium phosphate buffer (pH 7.5) containing 0.5 mM EDTA. The buffer was filtered through cheesecloth and the filtrate was centrifuged in a refrigerated centrifuge (*IEC 59-2, Thero Scientific*, USA) for 15 min at 20,000 × *g* (Dhindhsa *et al.* 1981). The 3.0 cm^3 of the reaction mixture contained 13 mM methionine; 25 mM nitroblue tetrazolium chloride (NBT), 0.1 mM EDTA, 50 mM phosphate buffer

pH (7.8), 50 mM sodium bicarbonate and 0.1 cm^3 of the enzyme extract. The reaction was started by adding 2 µm of riboflavin and placing the tubes below 2 × 15 W fluorescent lamp for 15 min. It was stopped by switching off the light and covering the tubes with black cloth. Tubes without enzyme developed the maximum colour. The nonirradiated complete reaction mixture did not develop a colour and served as a blank. The absorbance was recorded at 560 nm and one unit of enzyme activity was taken as that quantity of enzyme, which reduced the absorbance reading to 50% in comparison with the tubes lacking enzymes. Total soluble proteins were determined according to the method of Bradford (1976) with bovine serum albumin as a calibration standard.

Peroxidase: Enzyme extract for POX was prepared by grinding 0.1 g of the leaf material with 10 cm^3 of prechilled 0.1 M potassium phosphate buffer (pH 7.5) containing 0.5 mM EDTA in a prechilled mortar and pestle. The brie was filtered through cheese cloth and the filtrate was centrifuged in a refrigerated centrifuge for 16 min at 20,000 × *g*. All operations were carried out at 4°C. Peroxidase activity was estimated at 25°C in a 3 ml cuvette containing 100 mM potassium phosphate buffer (pH 6.0), 10 mM O-dianisidine 20 mM H_2O_2 and 0.1 ml of the diluted enzyme extract (10 ×). The absorbance was recorded at 470 nm and calculated according to Castillo *et al.* (1984).

Catalase was assayed by measuring the disappearance of H_2O_2 according to Teranishi *et al.* (1974). The 3.0 ml of the reaction mixture contained 50 mM phosphate buffer (pH 7.0), 20 mM H_2O_2, and 0.1 ml of the diluted enzyme. The reaction was stopped after 5 min by the addition of 2 ml of the titanium reagent, which also form coloured complex with the residual H_2O_2. Aliquot was centrifuged at 10,000 × *g* for 10 min and the absorbance of the supernatant was recorded at 410 nm in UV-visible spectrophotometer (*UV-VIS 108, Systronic Ltd.*, Ahmedabad, India). The activity was measured in [U mg^{-1}(protein) min^{-1}]. Total soluble proteins were determined with bovine serum albumin as a calibration standard (Bradford 1976).

Lipid peroxidation was measured in terms of malondialdehyde (MDA) content, a product of lipid peroxidation, following the method of Heath and Packer (1968). Leaf sample (0.5 g) was homogenized in 10 ml of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000 × *g* for 5 min. To 1.0 ml aliquot of the supernatant, 4.0 ml of 0.5% thiobarbituric acid (TBA) in 20% TCA was added. The mixture was heated at 95°C for 30 min and then quickly cooled in an ice bath. After centrifugation at 10,000 × *g* for 10 min, the absorbance of the supernatant was recorded at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The MDA content was calculated by its extinction coefficient of 155 mM^{-1} cm^{-1}.

H_2O_2 content was estimated by measuring the Ti-H_2O_2 complex at 415 nm (Mukherjee and Chaudhary 1983). For Ti reagent preparation, 1g TiO_2 and 10 g K_2SO_4 were digested in 150 cm^3 of concentrated H_2SO_4 over a hot plate for 4 h. The digested mixture was diluted to 500–600 cm^3 and stirred with a magnetic stirrer cum heater at 70–80°C till a clear transparent solution was obtained. This solution was diluted to 1.5 dm^3 and stored in a dark brown bottle. A fresh leaf sample (0.5 g) was homogenized in 10 ml of cold acetone. The homogenate was filtered through *Whatman* No. 1 filter paper. To this extract 4.0 ml of Ti reagent was added followed by 5.0 ml of concentrated ammonium solution to precipitate the Ti-H_2O_2 complex. After centrifugation for 5 min at 10,000 × *g*, the supernatant was discarded and residue was dissolved in 10 ml of 2N H_2SO_4. It was recentrifuged to remove the undissolved material and the absorbance was recorded at 415 nm against water blank. The concentration of H_2O_2 was determined with the help of the standard curve plotted with a known concentration of H_2O_2.

Na^+ and K^+ content: The concentrations of Na^+ and K^+ in roots and leaves were determined as described by Wignarajah *et al.* (1975). Dried plant material (1 g) was extracted three times with a boiling deionized water and the supernatant was collected after centrifugation. The residue was then extracted with 30% (v/v) HNO_3 for 1.0 h at 90°C. The suspension was cooled and the supernatant was collected after centrifugation. The residue was extracted twice with 30% HNO_3. All supernatants were pooled and the concentration of sodium and potassium ions estimated [g kg^{-1}(DM)] using flame photometry (*Chemito 1020*, Bangalore, India). Finally, K^+/Na^+ ratio in roots and leaves was calculated.

Statistical analysis: There were five replications for each treatment. Statistical analysis of data was processed using completely randomized block design (Gomez and Gomez 1984).

Results and discussion

Salinity reduced DM significantly in Gola and Umran cultivars of ber, particularly after salinity of 8 dS m^{-1}. The reduction was 48.17% in Gola and 60.71% in Umran at highest level of salinity treatment (16 dS m^{-1}). Cultivar Gola exhibited higher DM in control as well as salt-treated plants. LA also reduced in both the cultivars with higher magnitudes in Umran (Table 1). The reduction was more pronounced under the salinity of 12 dS m^{-1}. Reduction in DM might be due to the increased osmotic pressure in the root zone after the enhanced salt

Table 1. Effect of NaCl on dry mass (DM), leaf area (LA), relative water content (RWC), chlorophyll stability index (CSI) and membrane stability index (MSI) in *Ziziphus* cultivars at 180 d after budding. CD – critical difference.

Salinity [dS m^{-1}]	DM [g plant^{-1}]		LA [cm^2]		RWC [%]		CSI		MSI	
	Gola	Umran	Gola	Umran	Gola	Umran	Gola	Umran	Gola	Umran
0	1.638	1.596	16.38	15.46	77.33	71.34	2.18	2.05	52.92	51.34
4	1.557	1.542	15.41	15.09	71.64	67.72	2.05	1.95	48.54	48.35
8	1.374	1.179	14.65	13.99	54.29	47.52	1.02	1.06	45.68	45.36
12	1.209	0.795	12.79	11.71	45.15	39.17	0.92	0.82	43.45	42.85
16	0.849	0.627	10.42	10.16	34.19	27.48	0.81	0.69	41.55	36.67
$CD_{0.05}$	0.12		1.23		4.59		0.13		3.81	

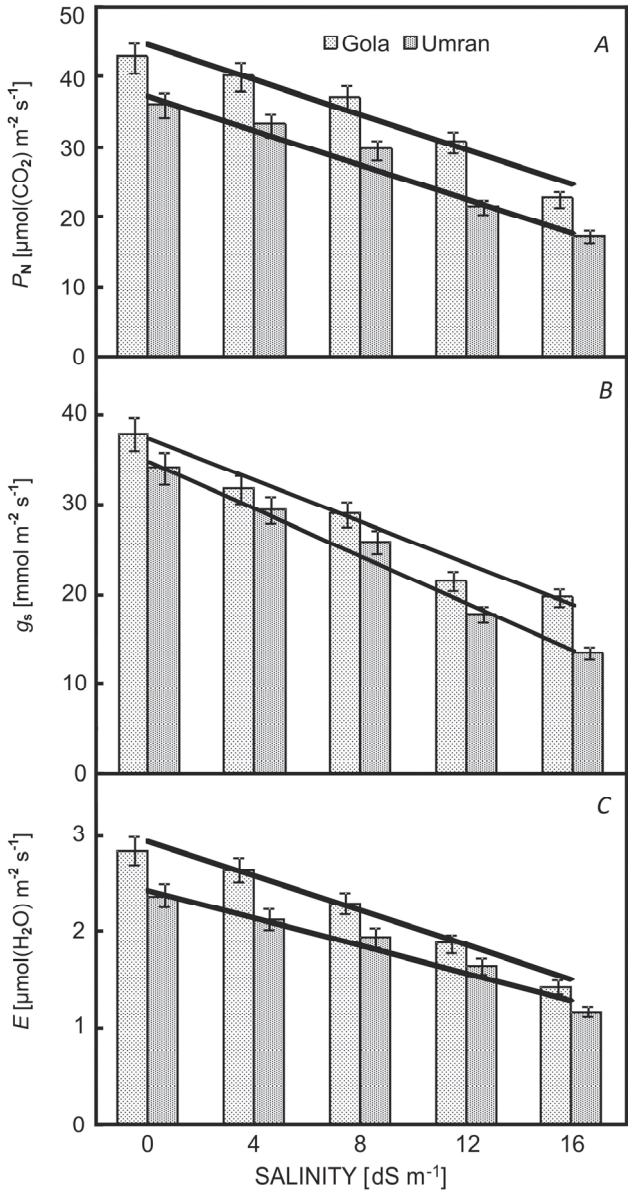

Fig. 1. P_N, g_s, and E in salt-treated *Ziziphus* cultivars at 180 d after budding. Vertical bars indicate SE of mean. Critical differences (CD) for cultivar and treatment were significant at P=0.05. The line in each graph represents linear regression with salinity as the independent variable.

concentration in the soil solution, the accumulation of ions (especially Na^+ and Cl^-) in the plant tissues to the toxic levels, and the excessive concentration of soluble ions that might have resulted in nutrient imbalance in the soil solution and plant tissues (Bhatt *et al.* 2008). The high rate of DM accumulation and better LA in control and salinity treated plants of Gola might contributed towards its salt tolerance over Umran. Variations among the species and/or genotypes of the same species for the salt tolerance owing to various morpho-physiological adjustments have already been reported in Indian jujube (Pradeep and Jumbhale 2000).

Perturbations in different gas-exchange parameters due to salt stress is considered as an important indicator of salinity-induced damage to plants. In present investigation, salinity significantly reduced P_N of both the cultivars, particularly after NaCl 8 dS m^{-1} treatment. Compared with control, the values of P_N were reduced to 47.30% in Gola and 52.30% in Umran at the salinity of 16 dS m^{-1}. The g_s and E were also reduced significantly with increasing salinity in both the cultivars (Fig. 1). Comparatively, Gola maintained higher rates of P_N, g_s, and E even at higher salinity levels (16 dS m^{-1}). The inhibition of photosynthesis under salinity is due, in part, to the closure of stomata which reduced the availability of internal CO_2 (Halliwell 1987). Decreased g_s is often related to an increased ABA concentration in the leaves or the xylem flux (Meena *et al.* 2003). High salt concentration decreases the available water resources in soil (Liu *et al.* 2005) and thus a plant senses by sending chemical signals to PSII, which plays key role in maintaining photosynthesis under saline conditions (Zheng *et al.* 2009). Our results concerning leaf RWC also support this hypothesis. It was observed that the salinity treatment significantly reduced the RWC in both the cultivars. The reduction was 55.81% in Gola and 61.48% in Umran at 16 dS m^{-1} salinity. Gola always exhibited higher water status in the control as well as in the salt-treated plants (Table 1). Salinity-induced decline in MSI was observed in both the cultivars, but the reduction was lesser in Gola (6.96–49.85%) than in Umran (8.86–56.21%). CSI was also reduced from 2.18 to 0.81 in Gola and 2.05 to 0.69 in Umran at highest salinity level (Table 1). The poor membrane integrity with salinity treatment indicates high

Table 2. Effect of NaCl on superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), H_2O_2, and lipid peroxidation in *Ziziphus* cultivars at 180 d after budding. MDA – malondialdehyde; FM – fresh mass; CD – critical difference.

Salinity [dS m^{-1}]	SOD [U mg^{-1}(protein) min^{-1}]		POX [μmol mg^{-1}(protein) min^{-1}]		CAT [U mg^{-1}(protein) min^{-1}]		H_2O_2 [μmol g^{-1}(FM)]		MDA content [nmol g^{-1}(FM)]	
	Gola	Umran	Gola	Umran	Gola	Umran	Gola	Umran	Gola	Umran
0	0.557	0.360	3.40	2.96	0.065	0.055	0.058	0.076	130.30	144.10
4	0.650	0.403	4.03	3.06	0.093	0.070	0.087	0.097	167.70	176.50
8	0.723	0.597	4.33	3.33	0.096	0.079	0.096	0.147	185.00	197.30
12	0.807	0.693	4.93	4.30	0.120	0.099	0.115	0.177	206.90	219.50
16	0.980	0.765	5.43	4.80	0.145	0.127	0.140	0.203	227.90	244.00
$CD_{0.05}$	0.059		0.75		0.009		0.510		20.56	

Table 3. Effect of NaCl on Na^+ and K^+ content in roots and leaves of *Ziziphus* cultivars at 180 d after budding. DM – dry mass; CD – critical difference.

Salinity [dS m^{-1}]	Na^+ content [g kg^{-1}(DM)]		K^+ content [g kg^{-1}(DM)]	
	Root	Leaves	Root	Leaves
Gola				
0	9.85	7.68	5.98	8.23
4	10.89	8.37	5.15	7.96
8	14.36	11.15	3.60	6.70
12	17.32	12.89	2.81	6.13
16	20.14	14.53	2.05	4.56
Umran				
0	8.93	8.19	5.46	7.93
4	10.35	9.16	5.10	7.32
8	16.17	13.17	4.17	5.97
12	15.92	15.62	3.32	4.81
16	19.81	17.42	2.89	3.76
$CD_{0.05}$	2.61	2.16	1.85	2.36

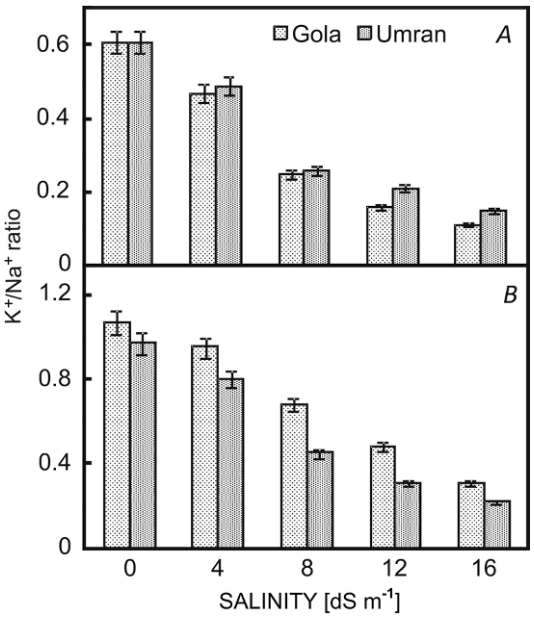

Fig. 2. K/Na ratio in salt-treated *Ziziphus* cultivars at 180 d after budding. *A*: roots, *B*: leaves. Vertical bars indicate SE of mean. Critical differences (CD) for cultivar and treatment were significant at P=0.05.

Na^+ accumulation in cells, thus disturbing ionic balance and normal plant growth (Chakraborty *et al.* 2012). Variations in CSI of two cultivars in the present investigation indicated the existence of an efficient mechanism of Chl retention in Gola.

The antioxidants also play an important role in imparting stress tolerance (Sairam and Srivastava 2000). Our results show that SOD activity enhanced linearly with increasing levels of salinity in both the cultivars (Table 2). However, the magnitude was higher in the control as well as salt-treated plants of Gola. SODs are the first line of the defense against a stress scavenging the superoxide radical (O^-). The enhancement in its activity with salinity treatments indicated its significance in imparting salt tolerance, particularly in Gola. The next step in enzymatic antioxidant defense involves H_2O_2 degrading enzymes such as POX and CAT. The POX activity was higher in Gola than in Umran under control as well as in salt-treated plants (Table 2). The enhancement in POX activity under salinity stress can be linked to protection from oxidative damage, lignification and cross linking of cell wall (Dalal and Khanna-Chopra 2001). The pattern of CAT activity was similar to that of SOD and POX activity, with Gola always having higher values than Umran. The role of CAT in imparting salt tolerance by scavenging H_2O_2 has been discussed by Takahashi *et al.* (1997). Studies on transgenic CAT-1-deficient tobacco plant reveled that it was essential for the protection of ascorbate and glutathione pool from an oxidation for maintaining the redox balance in cells (Stepien *et al.* 2005).

Salt stress resulted in an extensive lipid peroxidation. Present investigation indicates that the lipid peroxidation, estimated as MDA content, was enhanced under saline conditions (Table 2). The rise in MDA content under salinity suggests that both the cultivars induced the membrane lipid peroxidation by means of activated oxygen species (Zhang and Kirkham 1994). Accumulation of H_2O_2 reflects the damaging effects of stress since it can

inactivate various enzyme and cause protein degradation (Kholov *et al.* 2009). However, the increment in SOD, CAT, and POX with the high magnitude in Gola suggested the existence of salt tolerance mechanism which was more efficient than in Umran. Observations on gas-exchange parameters, water status, CSI and MSI also support this conclusion.

An excess of Na ions is toxic to plants because of its adverse effects on K^+ nutrition, cytosolic enzyme activities, photosynthesis and metabolism (Chakraborty *et al.* 2012). Control of intercellular ion homeostasis is essential for a normal growth and metabolism. Our results showed that the accumulation of sodium ions was higher in the roots of Gola and in the leaves of Umran. K^+ content also decreased significantly under salinity in both the cultivars. The K^+ content did not differ much in the roots of these cultivars but it was always higher in the leaves of Gola (Table 3). Salt tolerance is associated with low rates of transport of Na^+ to shoots with high selectivity for K^+ over Na^+. K^+ is essential for cell expansion; osmoregulation and cellular homeostasis (Bhatt *et al.* 2008). Our results indicate that Gola accumulated Na^+ in the roots *via* restricted translocation to maintain ionic balances in leaves. Comparatively better growth and LA of Gola might be attributed to improved K^+ status along with other physiological adaptations. K^+/Na^+ ratio, a very good indicator of plant's response to salt stress, also decreased with the increasing level of salinity (Fig 2). However, the ratio was significantly higher in the salt-treated leaves of Gola, indicating its capacity to maintain favourable cellular environment for the growth and other metabolic activities, which can be the basis of their tolerance towards soil salinity.

Thus, from the present study it was concluded that the salt tolerance mechanism was operative in both the cultivars but Gola was better equipped for salt tolerance owing to its better management of photosynthetic system, membrane retention capacity, antioxidative defense mechanism, restricted translocation of toxic Na^+ to leaves and a high K^+/Na^+ ratio.

References

Arzani, A.: Improving salinity tolerance in crop plants: a biotechnological view. – *In Vitro* Cell. Develop. Biol. - Plant **44**: 373-383, 2008.

Bhatt, M.J, Patel, A.D, Bhatti, P.M., Pandey A.N.: Effect of soil salinity on growth, water status and nutrient accumulation in seedlings of *Ziziphus mauritiana* (Rhamnacea). – J. Fruit Ornam. Plant Res. **16**: 83-401, 2008.

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

Castillo, F.J., Penel, J.C., Greppin, H.: Peroxidase release induced by ozone in *Sedum album* leaves. – Plant Physiol. **74**: 846-851, 1984.

Chakraborty, K, Sairam, R.K., Bhattacharya, R.C.: Differential expression of salt overly sensitive pathway genes determines salinity stress tolerance in *Brassica* genotypes. – Plant Physiol. Biochem. **51**: 90-101, 2012.

Dalal, M., Khanna-Chopra, R.: Differential response of antioxidant enzymes in leaves of necrotic wheat hybrids and their parents. – Physiol. Plant. **111**: 297-304, 2001.

Dhindhsa, R.S., Plumb-Dhidsa, P., Thorne, T.A.: Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. – J. Exp. Bot. **32**: 93-101, 1981.

Ebert, G.: Salinity problem in subtropical fruit production. – Acta Hort. **531**: 99-10, 2000.

Gomez, K.A., Gomez A.A.: Statistical Procedures for Agricultural Research. – John Wiley & Sons, New York 1984.

Gupta, N.K., Gupta, S. and Kumar, A.: Cytokinin application increases cell membrane and chlorophyll stability in wheat *(Triticum aestuvum* L.). – Cereal Res. Commun. **28**: 287-291, 2000.

Halliwell, B.: Oxidative damage, lipid peroxidation and antioxidant protection in chloroplasts. – Chem. Physiol. Lipids **44**: 327-340, 1987.

Heath, R.L., Packer, L.: Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. – Arch. Biochem. Biophys. **125**: 189-198, 1968.

Hernandez, J.A., Almansa, M.S.: Short term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. – Physiol. Plant. **115**: 251-257, 2002.

Kholova, J., Sairam, R.K., Meena, R.C., Srivastava, G.C.: Response of maize genotypes to salinity stress in relation to osmolytes and metal-ions contents, oxidative stress and antioxidant enzyme activity. – Biol. Plant. **53**: 249-256, 2009.

Liu, F.L., Anderson, M.N., Jacobsen, S.E., Jensen, C.R.: Stomatal control and water use efficiency of soybean (*Glycine max* L.) during progressive soil drying. – Environ. Exp. Bot. **54**: 33-40, 2005.

Liu, X.N., Baird, W.V.: Identification of a novel gene, HAABRC5, from *Helianthus annuus* (Asteraceae) that is upregulated in response to drought, salinity, and abscisic acid. – Amer. J. Bot. **91**: 184-191, 2004.

Meena, S.K., Gupta, N.K., Gupta, S., Khandelwal S.K., Sastry, E.V.D.: Effect of sodium chloride salinity on the growth and gas exchange of young *Ziziphus* seedling rootstocks. – J. Hort. Sci. Biotech. **78**: 454-457, 2003.

Mukherjee, S.P., Chaudhary, M.A.: Implications of water stress induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. – Physiol. Plant. **58**: 166-170, 1983.

Munns, R.: Comparative physiology of salt and water stress. – Plant Cell Environ. **25**: 239-250, 2002.

Pandey S.D., Pathak, R.K., Dwivedi, R.: Effect of sodicity and salinity levels on chlorophyll free proline and amino acid content in ber leaves. – Hort. J. **4**: 33-36, 1991.

Pareek, O.P.: Ber. – ICAR Publication, Indian Council of Agricultural Research, New Delhi 1983.

Pradeep, T., Jumbhale, N.D.: Ploidy level variations for stomata, Chloroplast number, pollen size and sterility in ber (*Ziziphus mauritiana* Lamk). – Indian J. Gen. **60**: 519-525, 2000.

Sairam, R.K., Srivastava, G.C.: Changes in antioxidant activity

in sub-cellular fraction of tolerant and susceptible wheat genotypes in response to long term salt stress. – Plant Sci. **162**: 897-904, 2000.

Stepien, P., Klobus, G.: Temperature response of photosynthesis and internal conductance to CO_2: results from two independent approaches. – J. Exp. Bot. **57**: 3057-3067, 2005.

Srivalli, B., Sharma, G., Khanna-Chopra, R.: Antioxidative defense system in an upland rice cultivar subjected to increasing intensity of water stress followed by recovery. – Physiol. Plant. **119**: 503-512, 2003.

Takahashi, H., Chen, Z., Du, H., Liu, Y., Klessing, D.F.: Development of necrosis and activation of disease resistance in transgenic tobacco plants with severely reduced catalase levels. – Plant J. **11**: 993-1005, 1997.

Teranishi, Y., Tanaka, A., Osumi, M., Fukui, S.: Studies on physiology and metabolism of hydrocarbon-utilizing microorganisms .6. Catalase activity of hydrocarbon-utilizing Candida yeasts. – Agr. Biol. Chem. **38**: 1213-1216, 1974.

Wignarajah, K., Jennings, D.H., Hanley, J.F.: The effect of salinity on growth of *Phaseolus vulgaris* L. II. Effect on internal solute concentration – Ann. Bot. **39**: 1039-1055, 1975.

Zhang, I., Kirkham, M.B.: Drought stress induced changes in activities of superoxide dismutase, catalase and peroxidase in wheat species. – Plant Cell Physiol. **35**: 785-791, 1994.

Zheng, Y.H., Xu, X.B., Wang, M.Y., *et al.*: Responses of salt tolerant and intolerant wheat genotypes to sodium chloride salinity: photosynthesis, antioxidant activities and yield. – Photosynthetica **47**: 87-94, 2009.