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Full Length Research Paper

Salt-induced changes in photosynthetic activity and oxidative defense system of three cultivars of mustard (*Brassica juncea* L.)

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A greenhouse experiment was conducted to assess salt-induced modulation in gas exchange attributes and some key enzymes of oxidative defense system in three cultivars of mustard (*Brassica juncea* L.), Varuna, RH-30 and Rohini, under saline conditions. After 21 days of seed germination, three salt treatments (0, 100 and 200 mM NaCl) were applied to the root growing medium. Salt treatments for three weeks significantly decreased the biomass, shoot and root lengths, CO_2-assimilation rate (*A*), transpiration rate (*E*), stomatal conductance (g_s), chlorophyll *a* and *b* pigments and relative water contents (RWC), while electrolyte leakage, proline, H_2O_2, malondialdehyde (MDA) and activities of superoxide dismutase (SOD), peroxidise (POD), catalase (CAT) and glutathione reductase (GR) enzymes increased in all three mustard cultivars. In comparison with the two other cultivars, cv. Varuna had higher shoot fresh and dry weight, proline content and activities of SOD, ascorbate peroxidase (APX), CAT and GR, while RH-30 and Rohini were higher in electrolyte leakage, H_2O_2 and MDA levels. Overall, the differential growth of all three mustard cultivars under saline conditions was found to be attributable to differential photosynthetic and transpiration rates, stomatal conductance, chlorophyll pigments and relative water contents, hence these could be used as potential selection criteria for screening mustard germplasm for salt tolerance.

Key words: Salt stress, *Brassica juncea*, antioxidant enzymes, proline.

INTRODUCTION

The world population is gradually increasing and is expected to reach 8.5 billion by 2025 (Chrispeels and Sadava, 2003). Thus, there is a dire need for a progressive increase in food crops production, but on the other hand, environmental stresses (biotic and abiotic stresses) suppress the overall yield of the agricultural crops. Plants are sessile and experience different environmental stresses like high temperature, cold, drought, salinity, UV and other biotic stresses. However, amongst these stresses, salinity is considered the most limiting factor for productivity of agricultural crops. Salinity is responsible for the induction of primary effects like ionic and osmotic stress, which in turn induce oxidative stress in plants. Reactive oxygen species (ROS) generated as a result of oxidative stress is highly deleterious for plants. ROS can destroy the structure and functions of biomolecules such as membrane lipids, proteins and nucleic acids (Hernandez et al., 2001; Ahmad et al., 2010c), and in higher concentrations,

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causes death of the plant cells (Ahmad and Sharma, 2008; Ahmad et al., 2008, 2010a, b, c).

Nonetheless, plants have mechanisms to counteract the deleterious effects of primary and secondary stresses (Devi and Prasad, 1998) through the generation of osmolytes or antioxidants (Foyer et al., 1997; Ashraf and Foolad, 2007; Ahmad et al., 2008, 2010a,b,c). Superoxide dismutase, peroxidases, catalases and glutathione reductases are major enzymatic antioxidants that help the plant to withstand deleterious effects of the environmental stresses. In plants, superoxide dismutase scavenges superoxide anions and converts them to hydrogen peroxides (Alscher et al., 2003). Catalase, the second line of defense, converts these lethal hydrogen peroxides to water and molecular oxygen. The effectiveness of oxidative defense system in plants can be measured by the activities of antioxidant enzymes and levels of non-enzymatic antioxidants (Geebelen et al., 2002).

Brassica juncea (mustard) is an important oil-seed crop, which often experiences saline stress as it is grown extensively in the arid and semi-arid regions of the world (Singh et al., 2001). Hence, it becomes necessary to examine the underlying processes involved in salt tolerance in mustard plants. While screening some cultivars of *B. juncea*, Hayat et al. (2011) have categorized cv. Varuna as salt tolerant and cv. Rohini as salt sensitive and attributed this differential salt tolerance to salt-induced changes in fresh and dry biomass. However, cv. RH-30 has not been examined earlier for salt tolerance. Taking this into consideration, the present study was undertaken to examine the salt-induced modulation in the growth, photosynthetic characteristics, chlorophyll pigments, leaf fluorescence, antioxidant enzymes and levels of non-antioxidants in salt-tolerant and sensitive mustard cultivars.

MATERIALS AND METHODS

Five seeds each of the three cultivars (Varuna, RH-30 and Rohini) of *B. juncea* were sown in pots filled with peat, perlite and sand (1:1:1, v/v/v). Thinning of the plants was done after germination of the seeds (one plant/pot) and the plants were allowed to grow for further three weeks under natural photoperiod of 12 to 13 h and temperature of 28 ± 4°C after which different NaCl levels (100 and 200 mM) were applied to the plants. The experiment was arranged in a completely randomized design with five replicates. After 45 days of salt treatment, the samples were harvested for analysis. After recording fresh weights of leaves and roots, dry weights were recorded by drying the material in an oven at 70°C to constant dry weight.

Gas exchange, chlorophyll fluorescence and pigment concentration

At plant age of 73 days, the fully expanded leaf from each plant was used for the analysis of net CO_2 assimilation rate (A), stomatal conductance (g_s) and transpiration rate (E) by using an open system LCA-4 ADC portable infrared gas analyser (Analytical Development Company, Hoddesdon, England). The above measurements were made from 10:30 to 12:00 h. At the same age as mentioned earlier, the chlorophyll fluorescence was recorded on attached leaves in a growth chamber (PAM 2000 apparatus, H. Walz, Effeltrich, Germany). The method of Li et al. (2007) was used to calculate efficiency of PSII photochemistry (F_v/F_m), quantum yield of PSII (Φ_{psII}), non-photochemical quenching (NPQ) and photochemical quenching coefficient (qP).

Hiscox and Israelstam (1979) method was employed for the extraction of chlorophyll content in leaves. The leaves (100 mg each) were chopped and immersed in dimethyl sulfoxide (DMSO) in a test tube. Tubes were kept in an oven at 65°C for 40 min. Aliquot from the tube and DMSO was then mixed in the ratio 1:2 respectively and then vortexed. Absorbance was recorded at 480, 510, 645 and 663 nm using a spectrophotometer (Beckman 640 D, USA), while DMSO was used as blank.

Relative water contents (RWC), electrolyte leakage and proline content

Leaf discs of 10 mm diameter were excised from fully expanded leaves for the determination of leaf RWC. The fresh weight of the leaf discs was recorded. The leaf discs were allowed to float on deionised water for about 7 h and then the turgid weight (TW) was recorded. The leaf discs were dried at 80°C for 24 h and the dry weight (DW) recorded. The RWC was calculated using following formula (Smart and Bingham, 1974):

RWC (%) = (FW - DW/ TW - DW) × 100

Analysis of inorganic ions in leaves of plants grown under salt stress was done by the method of Dionisio-Sese and Tobita (1998). Leaf discs (20) were immersed in deionised water in the tube and the electrical conductivity was measured (EC_a). Then the tubes were heated in a water bath for about 25 min at 50 to 60°C and the electrical conductivity (EC_b) measured. Furthermore, the tubes were then heated at 100°C for 10 min and the electrical conductivity (EC_c) was measured. The electrolyte leakage was calculated by the following formula:

$$\text{Electrolyte leakage (\%)} = \frac{EC_b - EC_a}{EC_c} \times 100$$

Proline concentration in the leaves was determined by the method of Bates et al. (1973). Leaf sample of 300 mg was ground and homogenized in 10 ml of 3% aqueous sulfosalicylic acid. After centrifugation of the homogenate at 12, 000 × g for 15 min, 2 ml of the aliquot from the supernatant was mixed with equal volumes of acetic acid and acid ninhydrin and incubated at 100°C for 1 h. Termination of the reaction was done in an ice bath and extracted with 4 ml of toluene. The extract was vortexed for 20 s, then the chromophore containing toluene was then aspirated from the aqueous phase, and its absorbance recorded at 520 nm using a spectrophotometer (Beckman 640 D, USA) with toluene as a blank.

Determination of H_2O_2 content and lipid peroxidation (MDA)

The method of Velikova et al. (2000) was used for the determination of hydrogen peroxide. Leaf sample of 500 mg was ground and homogenized with 5 ml of trichloroacetic acid (0.1%, w/v). After centrifugating the homogenate at 12,000 × g for 15 min, 0.5 ml of the aliquot was mixed with 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 M potassium iodide (KI). The optical density (OD) was recorded at 390 nm.

Formation of malondialdehyde (MDA) content (a measure of lipid peroxidation) was determined by the method of Rao and Sresty (2000). Leaf sample (500 mg) was homogenized with 2.5 ml of trichloroacetic acid (0.1%) and the homogenate was centrifuged at 10,000 × *g* for 10 min. One millilitre of the aliquot from the supernatant was taken and mixed with 4 ml of 20% trichloroacetic acid and 0.5% of thiobarbituric acid (TBA). The mixture was heated at 95°C for 30 min and then cooled in an ice bath and then centrifuged at 10, 000 × *g* for 15 min. The absorbance was measured at 532 nm. Measurements were corrected for unspecific turbidity by subtracting the absorbance at 600 nm.

Antioxidant enzymes

Extraction of the enzymes

Fresh leaves (10 g each) were homogenized with 50 volumes of 100 mM Tris-HCl (pH 7.5) containing 5 mM DTT, 10 mM $MgCl_2$, 1 mM EDTA, 5 mM magnesium acetate and 1.5% PVP-40. The material was filtered through cheesecloth and the homogenate was centrifuged at 10,000 × *g* for 15 min. The supernatant was collected and used as a source of enzyme assay. The extraction buffer was also supplemented with serine and cysteine proteinase inhibitors (1 mM PMSF + 1 µg/ml aproptinin). In addition to the above-mentioned chemicals, 2.0 mM ascorbate was also used for the extraction of ascorbate peroxidase (APX) in the homogenizing medium. Estimation of protein was done by Bradford's method (1976), with standard curves prepared using bovine serum albumin.

Enzyme assay

Superoxide dismutase

The method of Van Rossum et al. (1997) was used for the estimation of SOD (EC1.15.1.1) activity. The reaction mixture (50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionine, 75 µM nitroblue tetrazolium (NBT), 2 µM riboflavin and 100 µL of supernatant) in tubes were placed under 15 W fluorescent lamps for the initiation of reaction. After 10 min, the reaction was terminated by switching off the fluorescent lamps. For the blank, the non-illuminated reaction mixture was used. The absorbance was taken at 560 mm and the SOD activity was expressed as unit mg^{-1} protein. One unit of SOD activity was defined as the amount of enzyme that inhibited 50% of NBT photoreduction.

Catalase

CAT (EC 1.11.1.6) activity was determined by the method of Luck (1974). Fifty microliter of supernatant were mixed with 3 ml of 20 mM H_2O_2 and 50 mM phosphate buffer (pH 7.0), and then the absorbance was measured at 240 mm. CAT activity was expressed as unit mg^{-1} protein. For the calculation of CAT enzyme activity, the extinction coefficient of 30×10^3 mM^{-1} cm^{-1} was used.

Ascorbate peroxidase

The method of Nakano and Asada (1981) was used for the assay of APX (EC 1.11.1.11) activity. The assay mixture contained: 1.0 ml of reaction buffer (potassium phosphate (pH 7.0) with 0.1 mM EDTA, 0.5 mM ascorbate, 0.1 mM H_2O_2 and 0.1 ml of enzyme extract. APX was assayed as a decrease in absorbance at 290 nm of ascorbate. APX activity was expressed as unit mg^{-1} protein. For the calculation of APX enzyme activity, the extinction coefficient of 2.8 mM^{-1} cm^{-1} was used. One unit enzyme was considered as the amount necessary to decompose 1 µmol of substrate per min at 25°C.

Glutathione reductase

The method of Carlberg and Mannervik (1985) was used for the assay of GR (EC 1.6.4.2) activity. The reaction was initiated by adding 0.1 ml enzyme extract to assay mixture (0.75 µL potassium phosphate buffer (pH 7.0), 2 mM EDTA, 75 µM NADPH (2 mM), 75 µL GSSG (20 mM) and the decrease in absorbance was measured at 340 nm for 2 min. An extinction coefficient for NADPH of 6.2 mM^{-1} cm^{-1} was used for the calculation of GR activity. The GR activity was expressed as µmol NADPH oxidized min^{-1} (nits mg^{-1} protein).

Statistical analysis

Two-way analysis of variance (ANOVA) was used for the statistical analysis followed by Duncan's multiple range test (DMRT). Each value is a mean of five replications. *P* values ≤ 0.05 were considered as significant.

RESULTS

Growth attributes

Salt stress decreased the fresh and dry biomass of all three mustard cultivars (Table 1). Shoot and root lengths decreased but the decrease was more pronounced in cv. Rohini as compared to that cvs. Varuna and RH-30. Shoot and root fresh weights decreased significantly at all salt regimes, and of all three mustard cultivars, cv. Rohini was lower in shoot fresh and dry weights than the other mustard cultivars. Treatments with 200 mM NaCl evoked significant decreases in shoot dry weight in cv. Varuna (31.4%), cv. RH-30 (37.3%) and cv. Rohini (45.7%) in comparison to lower salt stress that is 100 mM as well as non-stressed plants. Root dry weight also showed the same decreasing trend (Table 1).

Gas exchange characteristics

CO_2-*A* decreased to 14.5 and 21.8% at 100 and 200 mM NaCl treatments, respectively in cv. Varuna. Similar decreasing trends were observed in cv. RH-30 (26.8%) and cv. Rohini (42.2%) at 200 mM NaCl stress (Table 1). In addition, g_s and *E* also decreased in all cultivars at all stress regimes, but a stronger decrease was observed in cvs. Rohini and RH-30 compared to cv. Varuna (Table 1). Correlation coefficient among different attributes showed that growth (shoot and root fresh weight) was significantly related to photosynthetic rate, transpiration rate, stomatal conductance, chlorophyll pigments and relative water contents (Table 2).

Leaf fluorescence

Stressed with 100 mM NaCl, all three mustard cultivars

Table 1. Mean data showing effect of NaCl stress on different growth and physio-biochemical attributes in different cultivars of mustard.

Cultivar	Shoot length (cm)			Root length (cm)			Shoot fresh wt (g/plant)			Root fresh wt (g/plant)		
	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM
Varuna	53.4	42.7	39.4	22.1	19.6	15.9	52.07	37.05	33.93	22.4	14.8	9.82
RH-30	51.2	40.01	37.07	21.2	17.5	13.2	49.2	33.82	29.26	21.6	13.01	7.88
Rohini	49.3	38.4	35.7	20.2	15.6	11.7	44.84	27.75	23.36	20.1	11.52	6.03

	Shoot dry wt (g/plant)			Root dry wt (g/plant)			*A* (µmol CO_2 m^{-2} s^{-1})			*E* (mmol H_2O m^{-2} s^{-1})		
	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM
Varuna	52.07	37.05	33.93	22.4	14.82	9.82	11.0	9.4	8.6	5.9	4.8	3.7
RH-30	49.2	33.82	29.26	21.6	13.01	7.88	10.8	8.9	7.9	5.8	4.5	3.4
Rohini	44.84	27.75	23.36	20.1	11.52	6.03	10.78	7.6	6.2	5.8	4.3	4.1

	g_s (mol m^{-2} s^{-1})			Chl. *a* (mg g^{-1} FW)			Chl. *b* (mg g^{-1} FW)			Chlorophyll *a*/*b* ratio		
	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM
Varuna	0.37	0.29	0.18	1.47	1.25	1.1	0.72	0.65	0.59	2.04	1.92	1.8
RH-30	0.37	0.27	0.17	1.43	1.2	1.07	0.68	0.58	0.51	2.1	2.0	2.0
Rohini	0.36	0.26	0.15	1.38	1.13	0.98	0.64	0.54	0.47	2.1	2.0	2.0

	RWC (%)			Electrolyte leakage (%)			Proline (µmol g^{-1} FW)			H_2O_2 (µmol g^{-1} FW)		
	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM
Varuna	87.8	69.9	64.7	8.3	15.4	22.4	58.3	108.5	142.7	52.07	37.05	33.93
RH-30	85.2	61.2	51.1	8.2	17.6	24.9	56.1	101	121.5	49.2	33.82	29.26
Rohini	85.1	52.6	45.2	8.3	17.9	25.6	56.2	95.2	105.7	44.84	27.75	23.36

	MDA (µg g^{-1} FW)			SOD (U/mg protein)			CAT (U/mg protein)			APX (U/mg protein)		
	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM	0 mM	100 mM	200 mM
Varuna	3.2	4.1	4.9	70.4	105	131.6	155	189	235	4.9	8.2	10.4
RH-30	3.2	4.6	5.9	67.5	99.2	112	141	176	209	4.5	7.3	9.0
Rohini	3.3	4.9	6.1	66.6	95.4	104	132	169	191	4.3	7.0	8.3

	GR (U/mg protein)		
	0 mM	100 mM	200 mM
Varuna	5.2	7.7	9.5
RH-30	5.0	7.4	8.5
Rohini	4.8	6.7	7.3

Table 2. Correlation of different attributes of mustard (*Brassica juncea* L.) grown under saline and non-saline conditions.

Variable	Shoot dwt	Root dwt	*A*	*E*	g_s	Chl. *a*	Chl. *b*	RWC	EL	Proline	MDA	H_2O_2	SOD	CAT	APX	GR
Shoot dwt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Root dwt	0.96***	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A	0.98***	0.94***	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E	0.94***	0.96***	0.93***	-		-	-	-	-	-	-	-	-	-	-	-
g_s	0.91***	0.94***	0.89**	0.98***	-	-	-	-	-	-	-	-	-	-	-	-
Chl. *a*	0.97***	0.96***	0.96***	-0.97***	0.94***	-	-	-	-	-	-	-	-	-	-	-
Chl. *b*	0.91***	0.9***	0.91***	0.9***	0.86**	0.91***	-	-	-	-	-	-	-	-	-	-
RWC	0.97***	0.93***	0.96***	0.89**	0.86**	0.94***	0.87**	-	-	-	-	-	-	-	-	-
EL	-0.89**	-0.86**	-0.87**	-0.9***	-0.91***	-0.91***	-0.74**	-0.897**	-	-	-	-	-	-	-	-
Proline	-0.762**	-0.8**	-0.67*	-0.81**	-0.83**	-0.77**	-0.55ns	-0.72**	0.87**	-	-	-	-	-	-	-
MDA	-0.91***	-0.86**	-0.89**	-0.88**	-0.88**	-0.91***	-0.79**	-0.93***	0.97***	0.77**	-	-	-	-	-	-
H_2O_2	-0.93***	-0.87**	-0.92***	-0.88**	-0.86**	-0.92***	-0.81**	-0.96***	0.96***	0.76**	0.98***	-	-	-	-	-
SOD	-0.751*	-0.79**	-0.66*	-0.8**	-0.82**	-0.76**	-0.53ns	-0.71**	0.86**	0.99***	0.76**	0.74**	-	-	-	-
CAT	-0.65*	-0.71**	-0.58ns	-0.76**	-0.79**	-0.69**	-0.44ns	-0.62*	0.84**	0.96***	0.74**	0.69*	0.97***	-	-	-
APX	-0.74**	-0.78**	-0.67*	-0.8**	0.82**	-0.76**	-0.52ns	-0.72**	0.89**	0.98***	0.8**	0.77**	0.99***	0.98***	-	-
GR	-0.689*	-0.733	-0.594ns	-0.77**	-0.75**	-0.7**	-0.46ns	-0.67*	0.85**	0.98***	0.75**	0.7**	0.98***	0.97***	0.98***	-

Non-significant ≥ 0.06; Significant at * (0.06), ** (0.06 - 0.08) and *** ≤ 0.08, respectively. *A*, CO2-assimilation rate; *E*, transpiration rate; g_s, stomatal conductance; a and b, chlorophyll pigments; RWC, relative water contents; MDA, malondialdehyde; SOD, superoxide dismutase; POD, peroxidise; CAT, catalase; GR, glutathione reductase; APX, ascorbate peroxidise; dwt, dry weight.

showed a non-significant decrease in F_v/F_m., Treatments with 200 mM NaCl evoked a further decrease in F_v/F_m, Φ_{psII} and qP accompanied with increase in NPQ was observed (Table 3).

Chlorophyll pigments

Salt stress led to decreased chlorophyll content in all mustard cultivars. A significant decrease in chlorophyll '*a*' was noticed in all cultivars, mostly pronounced (28.9%) by Rohini at 200 mM NaCl stress (Table 1). A significant decrease in chlorophyll '*b*' was also observed in all mustard cultivars. Maximum reduction in chlorophyll *b* was observed in cv. Rohini compared to cvs. Varuna and RH-30 (Table 1). Similarly, a decreasing trend was observed in chl. *a/b* ratio (Table 1).

Relative water content

Treating *Brassica* plants with NaCl led to decrease in RWC. Decrease in RWC was more pronounced in cv. Rohini (46.8%) than in cvs. RH-30 (40%) or Varuna (26.3%) at 200 mM NaCl stress (Table 1).

Electrolyte leakage

A very high electrolyte leakage was recorded in all three cultivars of mustard especially at 200 mM NaCl stress (cv. Varuna 62.9%, cv. RH-3067%, cv. Rohini 67.5%) (Table 1).

Free proline content

Leaf free proline was observed to increase at all stress levels in all mustard cultivars. At 200 mM NaCl stress, proline increased to about 59.1% in cv. Varuna, 53.8% in cv. RH-30 and 46.8% in cv. Rohini, respectively (Table 1).

Hydrogen peroxide (H_2O_2)

Stressing plants with 100 and 200 mM NaCl respectively, led to increase in H_2O_2 contents of 41.1 to 51.2% in cv. Varuna, 55.4 to 64.9% in cv.

Table 3. Effect of NaCl stress on chlorophyll fluorescence in different cultivars of mustard (means ± S.E.).

Parameter	Varuna			RH-30			Rohini		
	Control	100 mM	200 mM	Control	100 mM	200 mM	Control	100 mM	200 mM
Efficiency of PSII (F_v/F_m)	0.91 ± 0.2	0.90 ± 0.17	0.87 ± 0.16	0.90 ± 0.2	0.90 ± 0.2	0.84 ± 0.2	0.89 ± 0.19	0.89 ± 0.19	0.73 ± 0.17
Quantum yield of PSII (ΦpsII)	0.69 ± 0.13	0.60 ± 0.11	0.57 ± 0.10	0.68 ± 0.17	0.59 ± 0.14	0.56 ± 0.13	0.68 ± 0.12	0.57 ± 0.16	0.54 ± 0.11
Capture efficiency of PSII (Φexc)	0.68 ± 0.07	0.65 ± 0.09	0.62 ± 0.07	0.68 ± 0.08	0.65 ± 0.05	0.58 ± 0.06	0.67 ± 0.07	0.64 ± 0.8	0.53 ± 0.04
Photochemical quenching (qp)	0.99 ± 0.12	0.96 ± 0.15	0.95 ± 0.16	0.98 ± 0.13	0.93 ± 0.14	0.91 ± 0.05	0.98 ± 0.09	0.93 ± 0.13	0.90 ± 0.15
Non photochemical quenching (NPQ)	0.25 ± 0.03	0.31 ± 0.03	0.38 ± 0.06	0.24 ± 0.04	0.29 ± 0.02	0.36 ± 0.05	0.24 ± 0.05	0.28 ± 0.02	0.33 ± 0.03

RH-30 and 55.3 to 64.4% in cv. Rohini ((Table 1).

Malondialdehyde (MDA)

The lipid peroxidation (MDA) concentration was observed more in cv. Rohini (45.9%) and cv. RH-30 (45.7%) than that in cv. Varuna (34.6%) due to different salt concentrations (Table 1).

Activities of anti-oxidant enzymes

Salt stress increased the SOD activity in all three cultivars of mustard with the highest SOD activity in cv. Varuna followed by cv. RH-30 and cv. Rohini (Table 1). The cultivars showed a marked differential response to this attribute. All three cultivars of mustard also showed a significant increase in shoot catalase activity under salt stress. The highest catalase activity was observed in cv. Varuna (Table 1).

Furthermore, under both control and saline conditions, all cultivars differed significantly in peroxidase activity. Cultivars Varuna and RH-30 showed highest activity of this enzyme under saline conditions (Table 1). All three mustard cultivars showed increased activity of GR under saline conditions. Higher GR activity was observed in cvs. Varuna and RH-30 compared to cv. Rohini under saline conditions (Table 1).

DISCUSSION

Salt stress (100 and 200 mM NaCl) considerably decreased the shoot and root fresh and dry weights, and shoot and root lengths of all three (Varuna, RH-30 and Rohini) cultivars of mustard. Similar salt-induced reduction in different growth attributes have been reported in a number of crop plants e.g., tomato (Mohammad et al., 1998; Zribi et al., 2009), sunflower (Akram et al., 2009; Akram and Ashraf, 2011), pepper (Chartzoulakis and Klapaki, 2000), *Populous alba* (Imada and Tamai, 2009), strawberry (Keutgen and Pawelzik, 2009), mulberry (Ahmad and Sharma, 2010), okra (Saleem et al., 2011) and proso millet (Sabir et al., 2011), etc. In the present study, cv. Varuna was found to be relatively salt tolerant and cv. Rohini salt sensitive, whereas, CV-RH-30 moderately tolerant. The higher growth performance of the former cultivar is expected as it has already been categorized as salt tolerant (Hayat et al., 2011). This differential growth of the three mustard cultivars may have been due to differential regulation of different biochemical and physiological attributes involved in the growth processes of three cultivars. Generally, salt stress causes reduction in cell division as well as cell elongation (Yasseen et al., 1987; Pitann et al., 2009) mainly due to salt-induced perturbance in uptake of nutrients, high accumulation of reactive oxygen species (Ashraf, 2009), cytoplasmic enzyme inhibition, turgor loss (Pitann et al., 2009) and hormonal imbalance (Ashraf et al., 2010; Iqbal and Ashraf, 2011) which in turn impairs plant growth in terms of yield or biomass production.

In the present study, saline stress considerably decreased the stomatal conductance, CO_2-assimilation rate and transpiration of all the three mustard cultivars. A significantly stronger reduction was observed in cv. Rohini compared to cvs. Varuna and RH-30. It is well known that stomatal closure due to salt-induced abscisic acid (ABA) accumulation is one of the vital factors which cause retardation in vital photosynthetic processes (Noreen et al., 2010; Akram and Ashraf, 2011; Saleem et al., 2011). It was observed that, of all mustard cultivars, cv. Varuna was relatively higher and cv. Rohini lower in growth as well as photosynthetic attributes under saline conditions. Such a positive relationship between photosynthetic capacity and growth has already been reported for various plants grown under saline conditions e.g., okra (Saleem et al., 2011), wheat (James et al., 2002), maize (Crosbie and Pearce, 1982), asparagus (Faville et al., 1999), cotton (Pettigrew and Meredith, 1994), common bean (Seemann and Critchley, 1985)

and *Cynodon dactylon* (Akram et al., 2007). Under low NaCl stress, no significant change was observed in F_v/F_m, but under high salt conditions a significant reduction was observed in all three mustard cultivars. Similar pattern of reduction has already been described for different crops (Everard et al., 1994; Lu et al., 2003; Cha-um and Kirdmanee, 2009; Yang et al., 2009; Zribi et al., 2009). A decrease in photochemical processes along with a significant increase in non-photochemical quenching (NPQ) was observed in tomato (Zribi et al., 2009), okra (Saleem et al., 2011) and wheat (Zheng et al., 2009). A consistent decrease in *Fv/Fm* may indicate the occurrence of photo-inhibitory damage (Maxwell and Johnson, 2000; Colom and Vazzana, 2003). Moreover, a significant correlation of Na^+ accumulation and qP, NPQ and ΦPSII have been found by many workers and these attributes could be used as an indicator of photosynthetic disturbance in plants under salt stress (Zribi et al., 2009).

Regulation of water balance measured in terms of leaf RWC is considered as one of the most important adaptations to salt stress (Ashraf, 2004; Noreen et al., 2010). In the present study, RWC decreased in all three mustard cultivars. Such a salt-induced decrease in RWC has already been observed in *Populus cathayana* (Yang et al., 2009), pea (Ahmad and Jhon, 2005; Noreen et al. 2010), olive (Boussadia et al., 2008), mulberry (Ahmad and Sharma, 2010), and turnip (Noreen et al., 2010), etc. Salinity is known to cause water deficits, which in turn leads to decreased RWC. In addition, soluble salts in the soil hamper the uptake of water and nutrients which induce osmotic effects and ion toxicity (Duan et al., 2005). The mustard cultivars differed significantly in RWC with high RWC levels at 200 mM treatments for the salt tolerant mustard cv. Varuna compared to the other cultivars. In contrast, Noreen et al. (2010) reported high leaf RWC in salt sensitive pea and turnip cultivars compared to that in salt tolerant ones.

Proline (Pro) accumulation is thought to stabilize membranes and hence maintains the conformation of proteins under stress conditions. Pro is also reported to play a significant role in reducing the photo-damage of thylakoid membranes by scavenging the superoxide radicals (Reddy et al., 2004; Ashraf and Foolad, 2007; Banu et al., 2009; Hayat et al., 2011). Generally, in salt tolerant cultivars, a relatively high accumulation of proline has been reported e.g., in *C. dactylon* (Hameed and Ashraf, 2008), pea (Noreen and Ashraf, 2009), *B. juncea* (Hayat et al., 2011), sugarcane (Cha-um and Kirdmanee, 2009), and proso millet (Sabir et al., 2011). Similar to all these reports, our results also show that salt tolerant mustard cv. Varuna accumulated relatively higher leaf proline than did the sensitive cultivars under different saline regimes. On the other hand, hydrogen peroxide (H_2O_2) content was much higher under salt stress than the control e.g., in wheat (Zheng et al., (2009), *Catharanthus roseus* (Jaleel et al., 2007) and *P. cathayana* (Yang et al., 2009). The increase in H_2O_2 leads to enhanced lipid peroxidation that in turn leads to the leakage of the membranes (Ashraf, 2009). In the present study, salt tolerant mustard cv. Varuna was relatively lower in H_2O_2 contents under different saline regimes similar to what has earlier been reported in salt tolerant cultivars of mulberry, which accumulated less H_2O_2 as compared to salt sensitive cultivar (Ahmad et al., 2010a).

Lipid peroxidation appraised in terms of MDA contents has been used as a promising criterion for determining the sensitivity of plants to saline stress (Ashraf et al., 2010; Noreen et al., 2010). The MDA content significantly increased in all three mustard cultivars under different salty regimes. The MDA concentration was observed more in cvs. Rohini and RH-30 than that in cv. Varuna. This means that salt sensitive cultivars showed more lipid peroxidation than did the tolerant cultivars. Such a pattern of MDA accumulation has already been observed in tomato (Li, 2009), mulberry (Ahmad et al., 2010a), okra (Saleem et al., 2011), wheat (Ashraf et al., 2010) and sesame (Koca et al., 2007). Meanwhile, an increase in the expression of antioxidant enzymes like SOD, CAT, APX and GR was observed in the three mustard cultivars under saline regimes, which was analogous to what has been earlier reported in different crops such as canola (*B. napus*) (Ashraf and Ali, 2008), *P. cathayana* (Yang et al., 2009), sunflower (Noreen et al., 2009), proso millet (Sabir et al., 2011), wheat (Ashraf et al., 2010), and safflower (Siddiqi, 2010). Ali and Alqurainy (2006) reported that the levels of superoxides ($O_2^{\bullet-}$) and H_2O_2 are reduced due to the enhanced activities of different antioxidant enzymes. The first line of defense in oxidative stress is the action of SOD that converts $O_2^{-\bullet}$ to H_2O_2 (Mittler, 2002; Ashraf, 2009). The increase in H_2O_2 is very harmful for the cells; hence it gets dismutated to water and oxygen with the help of another antioxidant enzyme catalase (van Breusegem et al., 2001; Ashraf, 2009). One of the adaptive traits under saline stress is increase in catalase activity which reduces the toxic levels of H_2O_2 and protects the cell from oxidative damage (Sekmen et al., 2007; Vital et al., 2008; Ashraf, 2009; Noreen et al., 2010).

Another versatile antioxidant enzyme is ascorbate peroxidase which utilizes ascorbate (AsA) as electron donor and scavenges H_2O_2 in water-water and ascorbate glutathione cycles. Hydrogen peroxide is reduced to water by APX and plays a role in cell defense mechanism (Kangasjarvi et al., 2008; Ashraf, 2009). GR catalyses the NADPH-dependent reduction of oxidized glutathione (GSSG) to its reduced form (GSH) (Meister, 1988; Mannervik, 1987). GR activity is thought to increase the ratio of $NADP^+$/NADPH. The $NADP^+$ accepts electrons from the photosynthetic electron transport chain (Bishop, 1971). Thus, the flow of electrons to O_2 and therefore, the formation of $O_2^{\bullet-}$ can be minimized. In the present study, relatively salt tolerant mustard cv. Varuna was higher in SOD, peroxidise (POD), CAT and GR activities. Such

type of differential response of cultivars differing in salt tolerance has already been observed in a number of crops, in which salt tolerant cultivars exhibited higher antioxidant enzyme activities than did the salt sensitive cultivars (Ashraf, 2009; Noreen et al., 2009; Sabir et al., 2011). In contrast however, a study on six different pea cultivars under different saline regimes (Noreen and Ashraf, 2009) has shown that of the various antioxidant enzymes, only increase in CAT enzyme activity in salt tolerant pea cultivars was found to be a reliable marker of salt tolerance. In other studies, in different cultivars of turnip (Noreen et al., 2010) and radish (Noreen and Ashraf, 2009), increase in the activities of antioxidant enzymes did not relate to the growth performance of the cultivars under saline regimes.

Overall, salt treatments significantly decreased the biomass, shoot and root lengths, *A*, *E*, g_s, chlorophyll *a* and *b* pigments and RWC, but increased electrolyte leakage, proline, H_2O_2, MDA and activities of SOD, POD, CAT and GR enzymes in all three mustard cultivars. Of all the cultivars, cv. Varuna was found to be the highest in shoot fresh and dry weight, proline content, and activities of SOD, APX, CAT and GR, while RH-30 and Rohini showed higher electrolyte leakage, H_2O_2 and MDA levels. In general, the differential growth of all three mustard cultivars under saline conditions was significantly attributed to their differential photosynthetic and transpiration rates, stomatal conductance, chlorophyll pigments and relative water contents, so these could be used as potential selection criteria for screening mustard germplasm for salt tolerance.

Abbreviations:

***A*,** CO_2-assimilation rate; ***E*,** transpiration rate; g_s, stomatal conductance; *a* and *b,* chlorophyll pigments; **RWC,** relative water contents; **MDA,** malondialdehyde; **SOD,** superoxide dismutase; **POD,** peroxidise; **CAT,** catalase; **GR,** glutathione reductase; **APX,** ascorbate peroxidise.

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