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NaCl pretreatment alleviates salt stress by enhancement of antioxidant defense system and osmolyte accumulation in mungbean (*Vigna radiata* L. Wilczek)

Papiya Saha, Paramita Chatterjee & Asok K Biswas*

Plant Physiology and Biochemistry Laboratory, Department of Botany, University of Calcutta
35, Ballygunge Circular Road, Kolkata 700 019, India

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Enhancement of salt (NaCl) tolerance by pretreatment with sublethal dose (50 m*M*) of NaCl was investigated in *V. radiata* seedlings. NaCl stress caused drastic effects on roots compared to shoots. Accompanying reductions in length, number of root hairs and branches, roots became stout, brittle and brown in color. Salt stress caused gradual reduction in chlorophyll, carotenoid pigment contents and chlorophyll fluorescence intensity also. Superoxide dismutase and catechol peroxidase activities increased under stress in both roots and leaves. But catalase activity showed an increase in roots and decrease in leaves. In these seedlings, the oxidative stress has been observed under salinity stress and the level of proline, H_2O_2 and malondialdehyde content were increased. But pretreatment with sublethal dose of NaCl was able to overcome the adverse effects of stress imposed by NaCl to variable extents by increasing growth and photosynthetic pigments of the seedlings, modifying the activities of antioxidant enzymes, reducing malondialdehyde and H_2O_2 content and increasing accumulation of osmolytes like proline. Thus, mungbean plants can acclimate to lethal level of salinity by pretreatment with sublethal level of NaCl, improving their health and production under saline condition.

Keywords: Acclimation, Growth and metabolism, Mungbean, NaCl pretreatment

Abiotic stresses affect plant metabolism, disrupt cellular homeostasis and uncouple major physiological and biochemical processes[1,2]. Among abiotic stresses, salinity is one of the most severe problems in worldwide agricultural production. Salt affected land comprises 19% of the 2.8 billion hectares of arable land on earth and an increase in this menace is posing a serious threat to agriculture globally[3].

Mungbean (*Vigna radiata* L. Wilczek) is an important traditional crop of India characterized by a relative high content of protein and is a short summer season crop. Moreover, mungbean can be used as a crop with export potential, but soil salinity is a major limitation to legume production[4].

Salt stress causes decline in seed germination, shoot and root lengths, fresh mass and seedling vigor in mungbean[5-7]. One of the pronounced salt induced injury symptoms on mungbean is enhanced chlorosis, necrosis and decreased content of chlorophyll a, b and carotenoids[8]. The levels of stress induced markers, viz., proline, glycine, pinnitol etc. increase with increasing concentrations of salinity and their accumulation has been reported in different organs of mungbean plants to adjust osmotic potential of the cells[9-11]. The effect of salinity stress in plants is mediated at least in part by an enhanced generation of active oxygen species[12], particularly in chloroplasts and mitochondria[13] which cause lipid peroxidation and membrane injury, protein degradation and enzyme inactivation[14]. Plants have developed a complex antioxidant system which mitigate and repair the damage initiated by reactive oxygen species (ROS)[15,16], toward enzyme synthesis[17] to protect the cellular and subcellular systems from the cytotoxic effects of these active oxy-free radicals. The modulation of the activities of these enzymes may be important in the resistance of the plants to environmental stress.

Plants can improve their physiological ability to adapt to various environmental stress. This phenomenon is known as acclimation, and there have been reports on acclimation to cold, drought, salinity and other environmental changes[18-20]. Analysis of the physiological changes associated with such acclimation may help in advancing the study of plant tolerance to environmental stresses, by adopting the strategies by which plants acquire stress tolerance.

*Correspondent author
Telephone: 033-2461-5445/4959/5277 (Ext. 293)
Fax: 91-033-2461- 4849
E-mail: biswasak52@yahoo.in; piyakmail@yahoo.com

In the present study, sodium chloride induced effects in mungbean (*Vigna radiata* L. Wilczek) has been investigated by studying the morphological changes, the degree of oxidative damage, changes in the levels of chloroplastic pigments, level of organic osmolytes and other biochemical attributes. Further, whether mungbean acclimates to salt stress and overcomes the injurious effects of salt stress on prior exposure to sublethal stress has also been studied.

Materials and Methods

Plant materials—Mungbean seeds (*Vigna radiata* L. Wilczek) variety B 105 were obtained from Pulse and Oilseed Research Institute, Behrampore, West Bengal, India and used as experimental material.

Growth condition and NaCl treatment—The mungbean seeds were surface sterilized with $HgCl_2$ (0.1%, w/v) and washed thoroughly in distilled water. About 25 seeds were placed on each glassplate lined by blotting papers dipped in different concentrations of NaCl, (Merck, India). In the second phase, mungbean seeds were immersed in 50 m*M* NaCl for 2 h prior to treatment with different concentrations of NaCl. In each set, a minimum of 5 glassplates were maintained, with one set as control. The seeds were kept in well aerated place at 28° ± 2°C, exposed to 16 h photoperiod (260 µmol m^{-2} s^{-1} PFD). The seedlings were harvested after seven days for the following studies.

Morphological studies—The root and shoot lengths of control, NaCl treated and pretreated seedlings were measured.

Chlorophyll and carotenoid contents—Total chlorophyll, chlorophyll-a, chlorophyll-b contents were measured from the mungbean leaves according to Arnon[21]. Fresh leaves (1 g) were extracted with 80% alkaline acetone (v/v) and chlorophyll contents were estimated spectrophotometrically at 645 and 663 nm using Hitachi U-2000 spectrophotometer. The chlorophyll contents were expressed in terms of mg chlorophyll present g^{-1} fresh mass. The fluorescence of the chlorophyll was monitored at an excitation wavelength 640 nm and emission wavelength 680 nm with the help of Hitachi-650-40 spectrofluorometer. Carotene and xanthophyll contents were estimated according to the method of Mukherji and Biswas[22] and data were expressed in terms of optical density g^{-1} fresh weight.

Malondialdehyde contents (lipid peroxidation)—For the measurement of lipid peroxidation, the thiobarbituric acid (TBA) test was used to measure MDA level as an end product of lipid peroxidation[23]. The amount of MDA-TBA complex present was calculated using an extinction coefficient (ε) of 155 m$M^{-1}$$cm^{-1}$.

Proline content—The root and shoot tissues of mungbean seedlings were extracted with 0.1 *M* sulphosalicylic acid and centrifuged at 5000 *g* for 30 min. The proline content of the supernatant was estimated according to Bates *et al*.[24] and expressed as µg g^{-1} fresh weight.

H_2O_2 content—The H_2O_2 content from roots and leaves of mungbean seedlings was measured as described by Vellikova *et al*[25]. The H_2O_2 content was determined using an extinction coefficient (ε) of 0.28 µM^{-1} cm^{-1}.

Enzyme extraction and assays—Enzyme extraction procedures were carried out at 4°C. Plant samples were homogenized in 5 ml of pre-chilled 0.1 *M* phosphate buffer (*p*H 7.0). The homogenate was centrifuged at 12,000 *g* for 20 min and supernatant was used to assay the activities of superoxide dismutase (SOD; EC 1.15.1.1)[26], catalase (CAT; EC 1.11.1.6)[27] and catechol peroxidase (CPX; EC 1.11.1.7)[28].

Statistical analysis—The experiments were carried out in a completely randomized design (CRD) with five replicates; each replication comprised a single glassplate containing an average of 25 seeds. The data and significant differences among mean values were compared by descriptive statistics (± SE) followed by Student's '*t*'-test. The values of $P \leq 0.05$ and $P \leq 0.01$ were considered as statistically significant.

Results

Effect of NaCl on seedlings growth—Various concentrations of NaCl treatment caused a significant ($P \leq 0.01$) reduction in root and shoot lengths of mungbean seedlings (Fig. 1). This inhibitory effect was more prominent on root than on shoot length. The root length was decreased significantly ($P \leq 0.01$) in 100 and 150 m*M* NaCl treatments which was about 48% reduction, on an average. In contrast, the reduction in shoot length was slightly lower amounting to 40% reduction, on an average. Pretreatment of mungbean seeds with 50 m*M* NaCl prior to germination in different concentrations of NaCl, significantly ($P \leq 0.01$) increased the root as well as shoot lengths as compared to direct NaCl treatments (Fig. 1) whereby variable amounts of relief

of inhibition measuring about 35% and 23% increments on an average in root and shoot growth was observed respectively.

Effect of NaCl on pigment contents—There was a linear decrease in the levels of total chlorophyll, chlorophyll-a, chlorophyll-b, carotene and xanthophyll as well as the intensity of chlorophyll fluorescence under increasing concentrations of NaCl treatments. Compared to water control, the pigment contents decreased on an average, by 31% for total chlorophyll, 22% for chlorophyll-a, 45% for chlorophyll-b, 14% for carotene and 19% for xanthophyll (Table 1). Associated with the decline in pigment levels, there was an average 16% loss of the intensity of chlorophyll fluorescence also (Table 1). Maximum decrease in pigment content was recorded from the seedlings treated with 150 m*M* NaCl. But when mungbean seeds were pretreated with 50 m*M* NaCl and transferred to different concentrations of NaCl, the pigment contents and fluorescence intensity were increased as compared to non-pretreated samples (Table 1). The increase was on an average, 27% for total chlorophyll, 20% for chlorophyll-a, 52% for chlorophyll-b, 6% for carotene, 8% for xanthophylls and 5% for fluorescence intensity. But maximum increase in pigments content were recorded from the seedlings treated with 150 m*M* NaCl.

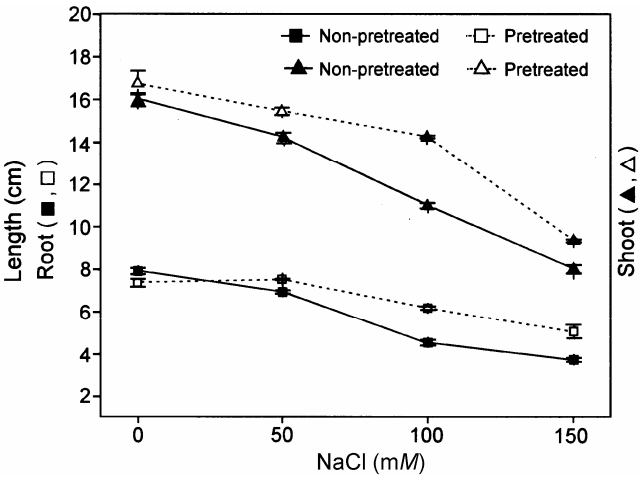

Fig. 1—Effect of sodium chloride (NaCl) on root and shoot length of seven days old non-pretreated and pretreated (with 50 m*M* NaCl) mungbean (cv. B 105) seedlings. Each data point is the mean of 5 replicates ± SE.

Effect of NaCl on antioxidant enzyme activities—In different parts of the plant, the activities of the enzyme varied greatly. Salinity significantly ($P \leq 0.01$) increased the superoxide dismutase (SOD) activity in both roots and leaves over control (Fig. 2), the increase being more significant in roots to

Table 1—Effect of sodium chloride (NaCl) on photosynthetic pigments and chlorophyll fluorescence in seven days old non-pretreated and pretreated mungbean (cv. B 105) seedlings.

[Values are mean ± SE of 5 replicates. Figures in parenthesis are % increase (+) and decrease (-) over control]

Treatment	Total Chl [mg g^{-1}(fw)]	Chl-a [mg g^{-1}(fw)]	Chl-b [mg g^{-1}(fw)]	Fluorescence intensity	Carotene [A_{425} g^{-1}(fw)]	Xanthophyll [A_{450} g^{-1}(fw)]
Non-pretreated						
Control	0.80 ± 0.07	0.50 ± 0.02	0.30 ± 0.05	701 ± 2.46	1.151 ± 0.52	1.216 ± 0.43
50 m*M* NaCl	0.72 ± 0.07 (-9.87)	0.48 ± 0.03 (-4.65)	0.24 ± 0.04 (-18.49)	634 ± 2.68[b] (-9.60)	1.081 ± 0.50 (-6.10)	1.152 ± 0.42 (-5.30)
100 m*M* NaCl	0.62 ± 0.08 (-23.03)	0.44 ± 0.04 (-12.11)	0.18 ± 0.05 (-41.17)	591 ± 2.24[b] (-15.70)	1.004 ± 0.49 (-12.80)	1.059 ± 0.40 (-12.90)
150 m*M* NaCl	0.32 ± 0.12[b] (-60.39)	0.25 ± 0.10[a] (-49.90)	0.07 ± 0.03[b] (-77.85)	552 ± 1.79[b] (-21.30)	0.879 ± 0.49 (-23.60)	0.744 ± 0.31 (-38.80)
Pretreated (50 m*M* NaCl)						
Control	0.77 ± 0.07 (-4.44)	0.48 ± 0.02 (-3.01)	0.28 ± 0.04 (-6.48)	696 ± 2.93 (-0.70)	1.134 ± 0.51 (-1.40)	1.198 ± 0.43 (-1.40)
50 m*M* NaCl	0.75 ± 0.06 (+3.66)	0.48 ± 0.03 (+0.83)	0.27 ± 0.04 (+9.68)	652 ± 1.34[d] (+2.80)	1.121 ± 0.50 (+3.80)	1.188 ± 0.42 (+3.10)
100 m*M* NaCl	0.70 ± 0.07 (+13.29)	0.47 ± 0.03 (+6.75)	0.23 ± 0.04 (+29.56)	623 ± 1.79[d] (+5.40)	1.049 ± 0.49 (+4.50)	1.094 ± 0.41 (+3.40)
150 m*M* NaCl	0.52 ± 0.12 (+64.40)	0.38 ± 0.07 (+50.93)	0.14 ± 0.05 (+115.20)	596 ± 0.89[d] (+8.00)	0.951 ± 0.51 (+8.10)	0.881 ± 0.32 (+18.40)

[a]$P \leq 0.05$ as compared to non-pretreated control, [b]$P \leq 0.01$ as compared to non-pretreated control, [d]$P \leq 0.01$ as compared to non-pretreated plants in respective NaCl concentration.

an average of 77% in contrast to 10% in leaves. In NaCl pretreated mungbean seeds prior to NaCl treatment, the SOD activity was significantly ($P \leq 0.01$) reduced in both roots and leaves on an average by 18% and 5% respectively as compared to non pretreated samples (Fig. 2). Similarly, catechol peroxidase (CPX) activity also showed an increase in both roots and leaves of NaCl treated mungbean seedlings by about 17% and 54% respectively, on an average (Fig. 3), above water control. Pretreated mungbean seedlings showed further increase of 9% in roots and 18% in leaves on an average over non pretreated mungbean seedlings (Fig. 3). Under saline conditions, catalase (CAT) activity increased in roots by 64%, but decreased very little in leaves when compared with control. On the contrary, pretreated mungbeen seedlings showed a significant ($P \leq 0.01$) increase in CAT activity over non-pretreated seedlings in both roots by 38% and very little in leaves (Fig. 4).

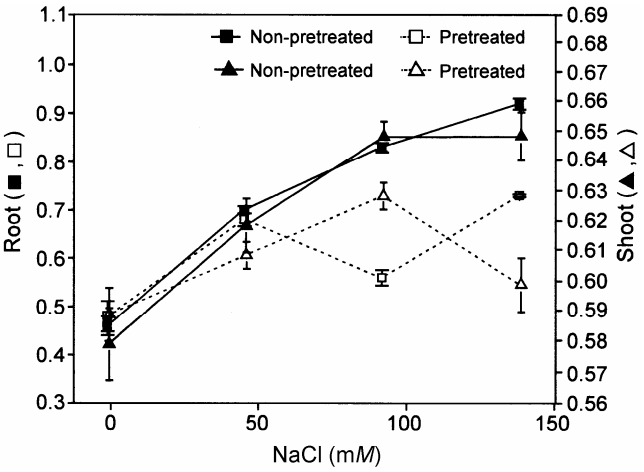

Fig. 2—Effect of sodium chloride (NaCl) on the activities of superoxide dismutase (SOD) (EU SOD $min^{-1}g^{-1}$ fw) in root and shoot of seven days old non-pretreated and pretreated (with 50 m*M* NaCl) mungbean (cv. B 105) seedlings. Each data point is the mean of 5 replicates ± SE.

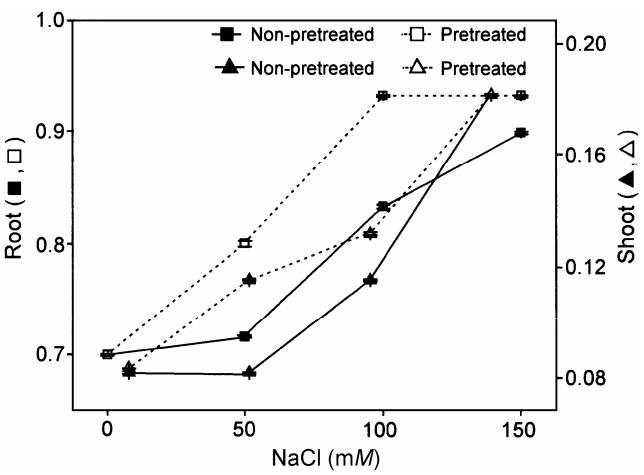

Fig. 3—Effect of sodium chloride (NaCl) on the activities of catechol peroxidase (CPX) (A_{420} $min^{-1}g^{-1}$ fw) in root and shoot of seven days old non-pretreated and pretreated (with 50 m*M* NaCl) mungbean (cv. B 105) seedlings. Each data point is the mean of 5 replicates ± SE.

Effect of NaCl on oxidative stress markers—NaCl treatments significantly ($P \leq 0.05$) increased the proline contents in roots and leaves of mungbean seedlings (Table 2). Proline contents were increased by about 0, 40 and 160% respectively in roots and by about 8, 60 and 312% respectively in leaves under 50, 100 and 150 m*M* NaCl treatments. Pretreatment of mungbean seeds with sublethal dose of NaCl prior to treatment in different concentrations of NaCl, proline contents further increased on an average by 32% in roots and 9% in leaves in comparison with non-pretreated seedlings (Table 2). But maximum increase in proline content was recorded in the seedlings treated with 100 m*M* NaCl (by 43% in roots and 13% in leaves). Enhanced rate of lipid peroxidation was recorded as indicated by gradually increasing malondialdehyde (MDA) contents in mungbean seedlings exposed to salinity (Table 2). The MDA contents increased significantly ($P \leq 0.01$) in all NaCl treated seedlings amounting to an average of about 42% in roots and 79% in leaves above water control. The elevated levels of MDA suffered reduction from

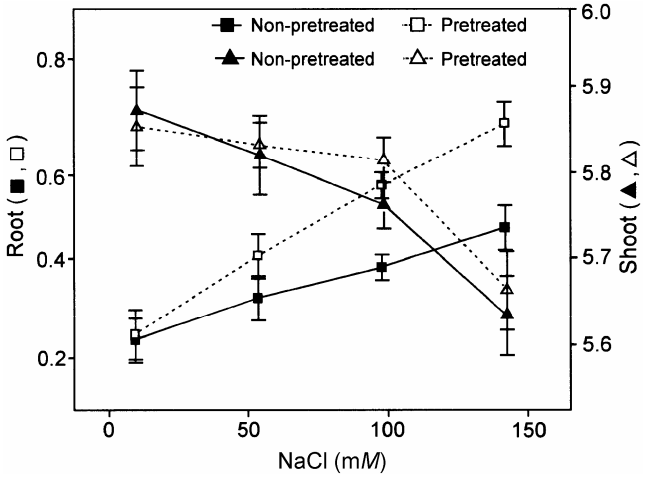

Fig. 4—Effect of sodium chloride (NaCl) on the activities of catalase (CAT) (mg H_2O_2 min^{-1}g-1 fw) in root and shoot of seven days old non-pretreated and pretreated (with 50 m*M* NaCl) mungbean (cv. B 105) seedlings. Each data point is the mean of 5 replicates ± SE.

Table 2—Effect of sodium chloride (NaCl) on proline, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents in seven days old non-pretreated and pretreated mungbean (cv. B 105) seedlings.

[Values are mean± SE of 5 replicates. Figures in parenthesis are % increase (+) and decrease (-) over control]

Treatment	Proline [µg g^{-1}(fw)]		MDA [µM g^{-1}(fw)]		H_2O_2 [µM g^{-1}(fw)]	
	Roots	Leaves	Roots	Leaves	Roots	Leaves
Non-pretreated						
Control	0.25 ± 0.003	1.25 ± 0.004	1.75 ± 0.002	2.41 ± 0.001	3.00 ± 0.002	3.86 ± 0.005
50 m*M* NaCl	0.25 ± 0.003 (0.00)	1.35 ± 0.003 (+8.00)	2.39 ± 0.002[b] (+36.41)	2.71 ± 0.001[b] (+12.43)	3.10 ± 0.001 (+3.17)	4.38 ± 0.008 (+13.50)
100 m*M* NaCl	0.35 ± 0.003[a] (+40.00)	2.00 ± 0.003[b] (+60.00)	2.50 ± 0.006[b] (+42.86)	3.03 ± 0.001[b] (+25.82)	3.60 ± 0.002[b] (+19.84)	5.00 ± 0.002[b] (+29.53)
150 m*M* NaCl	0.65 ± 0.002[b] (+160.00)	5.15 ± 0.026[b] (+312.00)	2.58 ± 0.002[b] (+47.47)	3.40 ± 0.004[b] (+40.99)	3.79 ± 0.001[b] (+26.19)	5.05 ± 0.004[b] (+30.77)
Pretreated (50 m*M* NaCl)						
Control	0.25 ± 0.003 (0.00)	1.35 ± 0.003 (+8.00)	1.81 ± 0.003 (+3.38)	2.45 ± 0.002 (+1.79)	3.04 ± 0.009 (+1.19)	3.93 ± 0.001 (+1.85)
50 m*M* NaCl	0.35 ± 0.003[d] (+40.00)	1.50 ± 0.003[c] (+11.11)	2.02 ± 0.002[d] (-15.54)	2.54 ± 0.001[d] (-6.35)	2.92 ± 0.002 (-5.77)	3.92 ± 0.001 (-10.60)
100 m*M* NaCl	0.50 ± 0.003[d] (+42.86)	2.25 ± 0.003[d] (+12.50)	2.18 ± 0.002[c] (-12.90)	2.86 ± 0.001[d] (-5.67)	3.42 ± 0.001 (-4.97)	3.99 ± 0.002[d] (-20.24)
150 m*M* NaCl	0.75 ± 0.004 (+15.38)	5.25 ± 0.013 (+1.94)	2.25 ± 0.002[d] (-12.92)	3.05 ± 0.003 (-10.13)	3.51 ± 0.004 (-7.23)	4.57 ± 0.002[d] (-9.43)

[a]$P \leq 0.05$ as compared to non-pretreated control, [b]$P \leq 0.01$ as compared to non-pretreated control, [c]$P \leq 0.05$ as compared to non-pretreated plants in respective NaCl concentration, [d]$P \leq 0.01$ as compared to non-pretreated plants in respective NaCl concentration.

42% to 15% and 79% to 7% in roots and leaves respectively when seedlings were grown after pretreatment to sublethal dose of NaCl (Table 2). Similarly, H_2O_2 contents upon NaCl treatments were increased, by about 16% in roots and 25% in leaves, as compared to water control, whereas pretreatment with sublethal dose of NaCl, reduced the production of H_2O_2 by 6% and 13% in roots and leaves respectively, as compared to non-pretreated NaCl treatments (Table 2).

Discussion

Growth and development—Salinity caused a significant effect on the normal growth and development of mungbean seedlings (Fig. 1). There was reduction of both root and shoot lengths with increasing concentrations of NaCl. The rate of root growth inhibition is more prominent compared to shoot growth inhibition. With increasing NaCl concentrations, the damage of roots enhanced with decrease in number of lateral roots, increase in girth and brittleness accompanied with browning of tissues. Similar reduction in shoot and root lengths in different plant species with progressive increase in salinity stress has been reported[5,7,29-32]. In the present study, it was observed that the significant reduction of shoot and root lengths in mungbean seedlings caused by salt stress was ameliorated by the application of sublethal concentration of NaCl (50 m*M*) to mungbean seeds prior to treatment in different concentrations of NaCl. The enhanced growth in pretreated plants over non-pretreated plants at lethal dose indicates that the low concentration of NaCl pretreatment has a stimulative effect in acclimation process as reported earlier in soybean[19] and rice[20].

Pigment contents—Photosynthetic activity of mungbean seedlings are also affected by salinity. With increasing concentrations of sodium chloride, a gradual decline in total chlorophyll, chlorophyll-a, chlorophyll-b and also intensity of chlorophyll fluorescence in mungbean seedlings was recorded and the present results lend support to several previous findings[15,20]. Also there is a decrease in other accessory photosynthetic pigments such as carotene and xanthophyll contents in mungbean leaves under salinity. This reduction of chlorophyll contents under salinity stress could be due to the increased activity of chlorophyllase enzyme or due to the disruption of fine structure of chloroplast and instability of pigment protein complexes by ions. Also the degradation of chlorophyll-b at a higher rate than chlorophyll-a can be explained by the fact that chlorophyll-b

degradation begins with its conversion to chlorophyll-a. However, the pretreated plants showed a reduced rate of chlorophyll degradation compared to non-pretreated plants. As chlorophyll-a contents are directly associated with carbohydrate production in higher plants, it may be concluded that the salinity induced reduction in growth and production of mungbean seedlings is partially due to the reduction in carbohydrate production in mungbean leaves.

Antioxidant enzyme activities—Salinity results in an enhanced generation of reactive oxygen species (ROS) such as superoxide radical $O_2^{\cdot-}$, H_2O_2, hydroxyl radical OH and singlet oxygen O_2[33]. Plants under stress adopt some defense mechanisms to protect themselves from the harmful effect of salinity induced oxidative stress. ROS scavenging is one of the common responses against abiotic stresses, which depends on the detoxification mechanisms provided by several enzymatic antioxidants[2,15]. In the present study, the salinity stress altered the activities of antioxidant enzymes compared to control. However, pretreatment with sublethal dose of NaCl prior to treatment in different concentrations of NaCl enhanced the antioxidant metabolism, and thus partially ameliorated the negative effects of salinity mediated injury. A significant increase in SOD activity in both roots and leaves of mungbean seedlings was observed in response to NaCl stress. SOD is the major superoxide ($O_2^{\cdot-}$) scavenger and provides a first line of defense against the cellular injury due to environmental stress. However, the pretreated mungbean seedlings showed decrease in SOD activity in both roots and leaves which has also been reported in phytohormone treated NaCl stressed mungbean[27]. The activity of CPX increased due to NaCl stress in both roots and leaves of mungbean seedlings when compared with control. Salinity caused increase in the content of cellular antioxidative enzymes like peroxidase was reported earlier[27,34]. An increase in CPX activity was observed in pretreated mungbean seedlings which indicate the formation of H_2O_2 much earlier, inducing the production of ROS scavenging enzymes in large amounts and production of certain secondary metabolites which enable the plant to withstand salt stress[31]. Thus, pretreated plants are more capable of effectively scavenging the ROS produced due to stress condition. There was a significant increase in CAT activity in roots and decrease in the leaves under salinity stress compared to control plants, which suggests the existence of an effective scavenging mechanism to remove ROS because roots are the primary organs which come in contact with salt and are thus thought to play a critical role in salt tolerance. In contrast to the present results, decreased CAT activity in roots and increased activity in leaves was recorded in *Withania somnifera* under salinity[35]. The changes in CAT may vary according to the intensity of stress, time of assay after stress and induction of new isozyme(s)[36]. The level of antioxidative response also depends on the species, the development and metabolic rate of the plant as well as the duration and intensity of stress[37]. An increase in CAT activity was recorded in the present study in both roots and leaves of pretreated mungbean seedlings over non-pretreated seedlings. This increase in CAT activity may be due to the fact that H_2O_2 is one of the crucial ROS produced in response to different environmental stresses including salt and ionic stress[38] and this H_2O_2 might act as an inducer of its scavenging enzymes like CAT and other enzymes which in turn result in lowering the endogenous level of H_2O_2[39].

Oxidative stress elements—Proline, an amino acid, besides acting as a cytoplasmic osmoticum, may function as a carbon and nitrogen source for post stress recovery and growth, as a stabilizer for membranes and protein synthesis machinery[40], as a scavenger of free radicals[41], as a sink for energy to regulate redox potential[42] and also serves to protect the protein against denaturation[43]. The pretreated mungbean seedlings accumulated proline at higher proportion than non-pretreated seedlings. Elevated level of proline has been reported to confer increased tolerance to hyperosmotic stress that might be the possible role of proline in pretreated plants[44].

Similarly, the peroxidation of membrane lipids is both a reflection and measure of stress-induced damage at the cellular level. In the present study, an increase in the MDA content of both roots and leaves of mungbean seedlings treated with increasing concentrations of NaCl was observed, indicating membrane damage due to peroxidation of lipids which in turn results in enhanced ROS production leading to oxidative stress. These results corroborate previous reports, where salinity causes severe lipid peroxidation in sugarcane[34] and wheat[32]. The present study also recorded that the increased levels of MDA were reduced significantly by pretreatment of mungbean seeds with sublethal dose of NaCl before

application of deleterious concentrations of NaCl. Salinity stress leads to an increased H_2O_2 content in both roots and leaves of mungbean seedlings which is in agreement with previous findings in wheat[32] and mungbean[27]. In plants, ROS like H_2O_2 is continuously produced as by-products of aerobic metabolic processes like photosynthesis and respiration localized in chloroplast, mitochondria and peroxisomes. Production and removal of ROS must be controlled to ensure normal growth of plants. Enzymatic ROS scavenging mechanisms include antioxidant enzymes like SOD, CAT and CPX. When the equilibrium between production and scavenging of ROS is perturbed by any abiotic stress factor like salinity in the present work, the plant becomes hypersensitive to stress and thus H_2O_2 level increases. Stone and Yang[45] have shown that during abiotic stress, H_2O_2 serves as a signal molecule and plays a dual role in plant defense. Also H_2O_2 effectively increased the activities of H_2O_2 scavenging enzymes. Further, pretreatment to sublethal dose of NaCl caused a marked decrease in the H_2O_2 content in both roots and leaves of mungbean seedlings. Wahid *et al*.[46] have suggested that H_2O_2 signals the activation of antioxidants in seeds, which persists in the seedlings to offset the ion-induced oxidative damage. These changes lead to the expression of stress proteins and improved physiological attributes, which support the better seedling growth under salinity[32].

From the results of the present investigation, it can be concluded that salinity affected the early growth of mungbean seedlings, particularly the root growth. Salinity also damaged the photosynthetic machinery by causing reduced chlorophyll contents and also induced oxidative stress by altering antioxidant scavenging machinery leading to membrane deterioration by lipid peroxidation. Pretreatment with sublethal dose of NaCl ameliorated the injurious effects of NaCl stress to some extent by increasing growth, photosynthetic pigments, activities of antioxidant enzymes and accumulation of osmolytes for osmotic adjustments. Therefore, plants can acclimate to lethal level of salinity by pretreatment in sublethal level of NaCl and can improve the production of plants under saline conditions.

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