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Original article

Identification of physiological and biochemical markers for salt (NaCl) stress in the seedlings of mungbean [*Vigna radiata* (L.) Wilczek] genotypes

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ABSTRACT

Salt stress, which is dominant among environmental stresses, poses challenges to global agriculture. We studied the role of exogenous application of sodium chloride (NaCl) in three arid and three semi-arid genotypes of mungbean [*Vigna radiata* (L.) Wilczek] by examining some physiological and biochemical stress indicators. Ten-day old seedlings were subjected to salt stress (00–250 mM) by split application along with the half strength Hoagland's medium. The salt stress caused a decline in the fresh weight, dry weight, relative water content, photosynthetic pigments (chlorophyll and carotenoids) and glutathione content of the seedlings. On the other hand, it increased the electrolyte leakage, lipoxygenase activity, and the proline, protein and total soluble sugar contents. Osmolyte accumulation was relatively higher in the arid genotypes revealing that they are more tolerant to NaCl stress. The physiological and biochemical screening provides a basic platform for selecting the stress-tolerant genotypes in the absence of suitable salt-tolerance markers in mungbean.

1. Introduction

The abiotic stresses limit the photosynthetic efficiency rate in plants, thus hampering the biomass production (Grime, 1977) and affecting the plant survival and yield (Qadir et al., 2014). Of these stresses, salinity poses the biggest threat leading to huge economic losses to the tune of 10–14 billion US dollars (Qadir et al., 2014; Shabala, 2013). Salt-stress effects comprise of the adverse effects caused by Na^+ and Cl^- ions on plants (Munns, 2005). The present-day irrigational practices have markedly aggravated the situation of salt stress (Zhu, 2001). As the majority of agricultural crops are salt-sensitive glycophytes (Munns and Tester, 2008), salinity causes tremendous yield losses in agriculture, posing drastic challenges to the world food security (Flowers, 2004; Ozturk et al., 2006; Godfray et al., 2010; Tester and Langridge, 2010; Agarwal et al., 2013).

Plants are able to survive in adverse environments by adapting to the prevailing conditions and/or fine-tuning their metabolic activities with the physiological changes. The regulation of plant adaptations to salinity is acquired by osmoprotectant biosynthesis, which helps plants in controlling the water flux and adjusting the cellular osmosis (Hasegawa et al., 2000; Flowers, 2004; Ashraf and Akram, 2009; Agarwal et al., 2013). The imbalance of ion homeostasis caused by salt stress is compensated by the regulation of ion influx and efflux at plasma membrane and the sequestration of ions by vacuoles (Hasegawa et al., 2000). Additionally, salt stress also causes disturbance in energy supply and redox homeostasis, which are balanced by the rearrangement of primary metabolism and alterations in cell architecture (Chen et al., 2005; Baena-González et al., 2007; Jaspers and Kangasjärvi, 2010; Miller et al., 2010; Zhu et al., 2010). Thus, the salt tolerance of plants is determined by their ability to transport Na^+ and Cl^- ions across the plasma membranes of root cells, vacuolar membranes, and salt accumulation/excretion by the specialized cells. As the world agriculture is faced with the challenge of feeding the ever-increasing human population, it is imperative that salt tolerant genotypes are identified for cultivation on the moderate to above moderate salt-infested soils (Ozturk et al., 1992, 1993, 1997).

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Mungbean [*Vigna radiata* (L.) Wilczek] is a salt-sensitive pulse and an intensive crop due to its short growing period and cultivation worldwide for its protein rich edible seeds (Ashraf et al., 2015). The present study was undertaken to screen and compare the performance of six arid and semi-arid genotypes of mungbean grown under salt stress.

2. Material and methods

2.1. Procurement of genotypes

Seeds of six Mungbean [*Vigna radiata* (L.) Wilczek] genotypes, AEM-96 (Azri Bhakkar), NCM-1 (NARC-Islamabad) and CM-6 (BARI-Chakwal) from arid region, and NFM-12 (NIFA-Peshawar), NM-92 (NIAB-Faisalabad) and NFM-6 (NIFA- Peshawar) from semi-arid region were procured from Pakistan Agriculture Research council (PARC), Islamabad Pakistan.

2.2. Determination of stress tolerance index (STI)

The seeds of each genotype were sterilized with 0.2% $HgCl_2$ solution for 5 min and washed thoroughly with tap water and then with deionized water. These were then put on Petriplates covered with Whatman filter paper for germination and growth. Ten seeds were put on each petriplate (n = 10) including the control and scored for germination. The Petriplates were kept in the dark at 25 °C until germination and later divided into five sets to be treated with different concentrations (0, 100, 150, 200, 250 mM) of sodium chloride. Initially 10 ml of salt concentrations were added to the petriplates. The germination did occur within 48 h, however, the growth of the seedlings was measured after 7 days. further 5 ml salt solution were added every two days. The experiments were repeated thrice, each with three replicates, and the values presented are the mean of three observations. The root was measured in mm scale in independent experiments. About 2 mm root was considered as the germination. Following Mustafiz et al. (2014), stress tolerance index was calculated as: STI (%) = (Average fresh weight of 10 stressed seedlings/Average fresh weight of 10 control seedlings) × 100%.

2.3. Plant growth and treatment

In the second experiment, the sterilized seeds of each genotype were sown in plastic pots (300 mm diameter) filled with moist 3 kg of acid-washed, autoclaved sand and the pots were moistened (watered) regularly till seed germination. The plants were irrigated with the half-strength Hogland's nutrient medium with pH 6.5 (Hoagland and Arnon, 1950). All pots were kept in an environmentally controlled growth chamber at 28 ± 1.5 °C at daytime and at 22 ± 1.5 °C at night. The plants were maintained at 300 μmol m^{-2} s^{-1} photosynthetic photon flux density with 60–70% relative humidity. Randomized block design was adapted for the treatments with three replicates and the sampling was completed 20 days after the start of treatments with sodium chloride (0, 200, 250 mM). The sodium chloride treatments prepared in Hoagland's solution were given in the split application of 50 mM from 1 to 5 days. The course of treatment was started 1 week after germination and the split application of 50 mM was given every day (1–5 days i.e 1–4 days for 200 mM and 1–5 days for 250 mM).

2.4. Measurement of growth parameters

After 20 days of stress imposition, the seedlings were randomly picked from the sets of the control and treated plants. The carefully uprooted seedlings were cleaned systematically with double distilled water for removing the sand particles. Root length, shoot length and fresh weight were then recorded. Dry weight was obtained after drying the material in hot air oven at 65 °C until the weight became constant. The relative water content (RWC) was calculated as: RWC (%) = [(FW − DW)/FW] × 100, as described by Chen et al. (2009).

2.5. Estimation of pigment content

In order to estimate the pigments, 0.2 g of leaf samples, collected 20 days after germination from each of the control and treated plant sets, were homogenized in 80% chilled acetone (10 ml) under dark conditions and the absorbance was measured at 663, 645 and 480 nm. The content of chlorophylls (chl_a and chl_b) was quantified by the method of Lichtenthaler (1987). The carotenoid content was determined by using the formula given by Duxbury and Yentsch (1956) and expressed in mg/g FW.

2.6. Electrolyte leakage and LPO measurements

The fresh expanded leaves from the control and treated samples (n = 3) were taken and electrolyte leakage was determined using the formula of Rodriguez-Hernandez et al. (2013), which is: MSI = (EC1/EC2) × 100, where, EC1 and EC2 are the initial and final values of electrical conductivity respectively. Lipid peroxidation of leaves was estimated by measuring the formation of thiobarbituric acid reactive substances (TBARS) as described by Heath and Packer (1968) and modified by Tanveer et al. (2018). The lipid peroxides was expressed as nmol TBARS g^{-1} fresh weight, using an extinction coefficient for MDA (ϵ = 155 mM^{-1} cm^{-1}) calculated by the formula: TBARS content (nmol g^{-1} FW) = $(A_{532} - A_{600}) \times V \times 1000/\epsilon \times W$, where V = extraction volume; W = weight of the fresh tissue.

2.7. Determination of osmolytes

The proline content in the control and treated seedlings was estimated according to Bates et al. (1973). Fresh leaves (0.5 g) were homogenized in 3% sulfosalicylic acid (10 ml) followed by centrifugation (10 min) at 10,000 rpm. Incubation (100 °C for 30 min) of 2 ml of supernatant was done with 2 ml each of acid ninhydrin and glacial acetic acid. After cooling the samples were extracted with toluene (4 ml) and their pink colour intensity was recorded at 520 nm against a standard curve of proline. The estimation of soluble sugar was performed according to Dey (1990) by extracting fresh leaves (0.5 g) in hot ethanol (90% v/v). To the exthnolic extract (2 ml), 5% phenol (1.0 ml) and concentrated H_2SO_4 (5.0 ml) were added. The final volume was adjusted to 10 ml by DDW and the absorbance was recorded at 485 nm. The reduced glutathione (GSH) content was estimated according to Anderson (1985) by homogenizing fresh leaves (0.5 g) in 5% sulphosalicylic acid, followed by centrifugation (4 °C) at 10,000 rpm for 10 min. 1.5 ml reaction buffer and 3 mM 5,5-dithio-bis(2-nitro benzoic acid) were added to 0.5 ml aliquot, and after 1 min, the absorbance was recorded at 412 nm. The total soluble protein content was determined following Bradford (1976) with Bovine Serum Albumin (BSA) taken as standard.

2.8. Statistical analysis

Results are presented as mean ± SE subjected to one-way ANOVA, using the GraphPad Prism 6.0 software. Tukey's post hoc test was performed to calculate the statistical differences in data at $p < 0.05$. All experiments were carried out in triplicate (n = 3) excluding for growth parameters (the root & shoot lengths, the fresh & dry weights, and the RWC, where n = 10).

3. Results

3.1. Effect of NaCl on germination and plant growth

Seed germination declined linearly with increase in NaCl concentrations from 100 mM to 250 mM. The effects of NaCl are presented as stress tolerance index (STI) and growth performance with respect to control (Fig. 1a and b). The STI declined in all the genotypes studied, *viz.* AEM-96 (35.24–5.87%), NCM-1 (37.16–0.00%), CM-6 (29.44–5.63%), NFM-12 (35.22–6.13%), NM-92 (36.89–7.86%), and NFM-6 (22.49–4.78%). At the highest NaCl concentration (250 mM), it declined drastically (4.78–7.86%) in different genotypes, whereas the genotype NCM-1 failed to germinate at this concentration. The shoot and root length were significantly affected under salinity stress, showing a dose-dependent decline (Table 1, Fig. 2). The maximum reduction of shoot length (24.85%

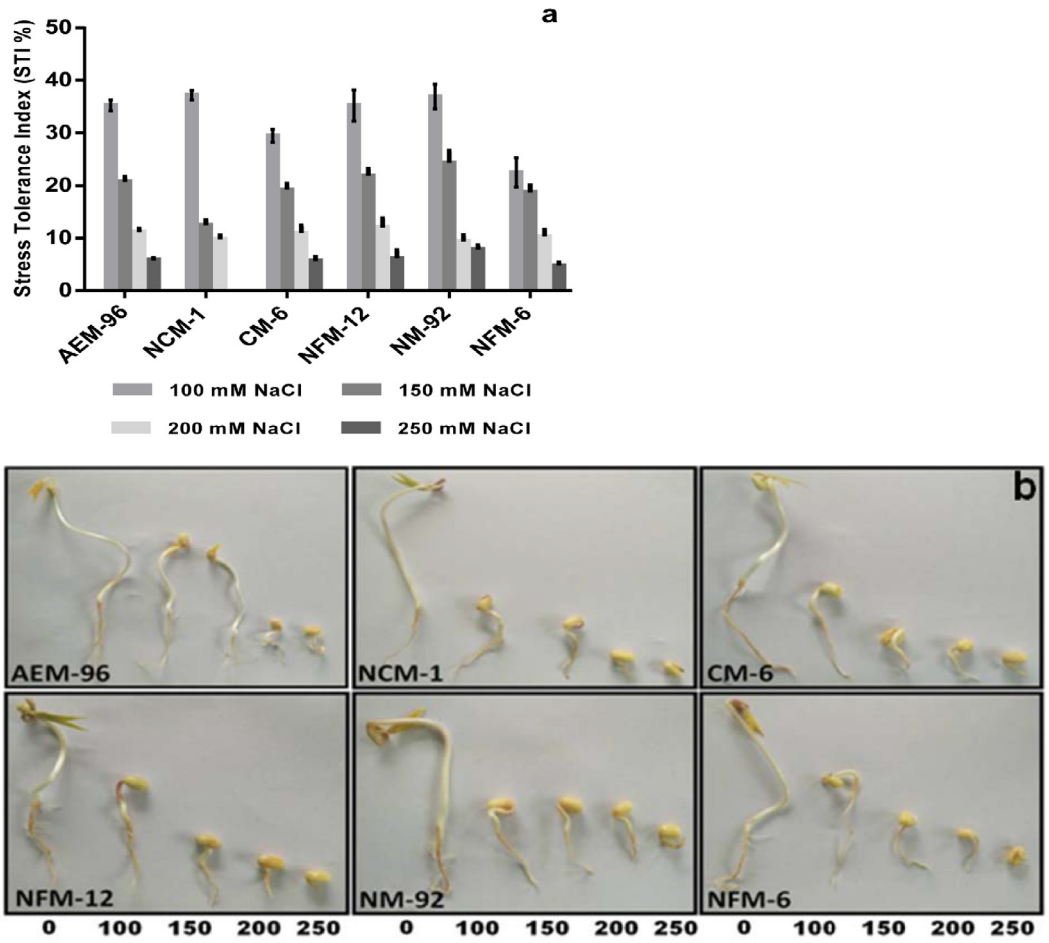

Fig. 1. Effect of NaCl on seed germination presented as stress tolerance index (a) and growth performance (b) in mungbean genotypes AEM-96, NCM-1, CM-6, NFM-12, NM-92, NFM-6 respectively. Each of the genotypes were subjected to different NaCl treatments (0, 100, 150, 200, 250 mM). Stress tolerance index (STI %) for each genotype is presented over the control. The experiment was repeated thrice (n = 10) with mean ± SE (n = 3).

Table 1
Results of one-way ANOVA (p values) from the effect of NaCl on each genotype on plant weight (fresh weight, dry weight, relative water content), Plant growth (root length, shoot length), photosynthetic pigment concentration (chlorophyll, carotenoid, chlorophyll/carotenoid), electrolyte leakage, lipid peroxidation (LPO) and osmolyte content (proline, total sugar, reduced glutathione (GSH) and total protein). For plant weight n = 10 and Plant growth n = 10: photosynthetic pigment concentration, electrolyte leakage, lipid peroxidation (LPO) and osmolyte content n = 3.

Genotype		FW	DW	RWC	RL	SL	Chl.	Car.	Chl./Car.	EL	LPO	Pro.	Sugar	GSH	Protein
AEM-96	P values	<0.0001	=0.0002	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Mean Square	0.00772	0.000056	74.42	6.154	53.18	0.0108	0.0050	0.001972	45.05	0.00311	1.015	0.0989	5.623	15.61
NCM-1	P values	<0.0001	<0.0001	<0.0001	=0.6615	<0.0001	<0.0001	<0.0001	=0.0105	<0.0001	<0.0001	<0.0001	<0.0001	=0.0003	<0.0001
	Mean Square	0.00264	0.000066	33.12	0.2528	100.7	0.0457	0.0210	0.008388	130.9	0.007082	1.920	1.064	8.067	12.48
CM-6	P values	<0.0001	=0.0043	<0.0001	=0.0343	<0.0001	=0.0043	=0.0043	=0.9002	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Mean Square	0.00539	0.000159	58.25	0.5827	62.94	0.0009	0.0011	0.000353	35.56	0.001673	4.422	4.431	8.124	9.29
NFM-12	P values	<0.0001	<0.0001	<0.0001	=0.0283	=0.0329	<0.0001	<0.0001	<0.0001	<0.0001	=0.0329	<0.0001	<0.0001	=0.0003	<0.0001
	Mean Square	0.00642	0.000101	58.52	0.6995	87.75	0.01391	0.0123	0.1076	660.1	0.001317	5.522	4.431	6.38	40.09
NM-92	P values	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Mean Square	0.00891	0.000156	105.1	2.079	69.89	0.1177	0.0779	0.1590	470.9	0.01589	4.058	3.375	8.379	40.07
NFM-6	P values	<0.0001	<0.0001	<0.0001	=0.0014	<0.0001	<0.0001	<0.0001	=0.0054	<0.0001	<0.0001	<0.0001	<0.0001	=0.0043	<0.0001
	Mean square	0.01614	0.000353	164.4	1.196	59.74	0.2267	0.1197	0.01066	166	0.00073	1.804	4.288	5.421	15.59

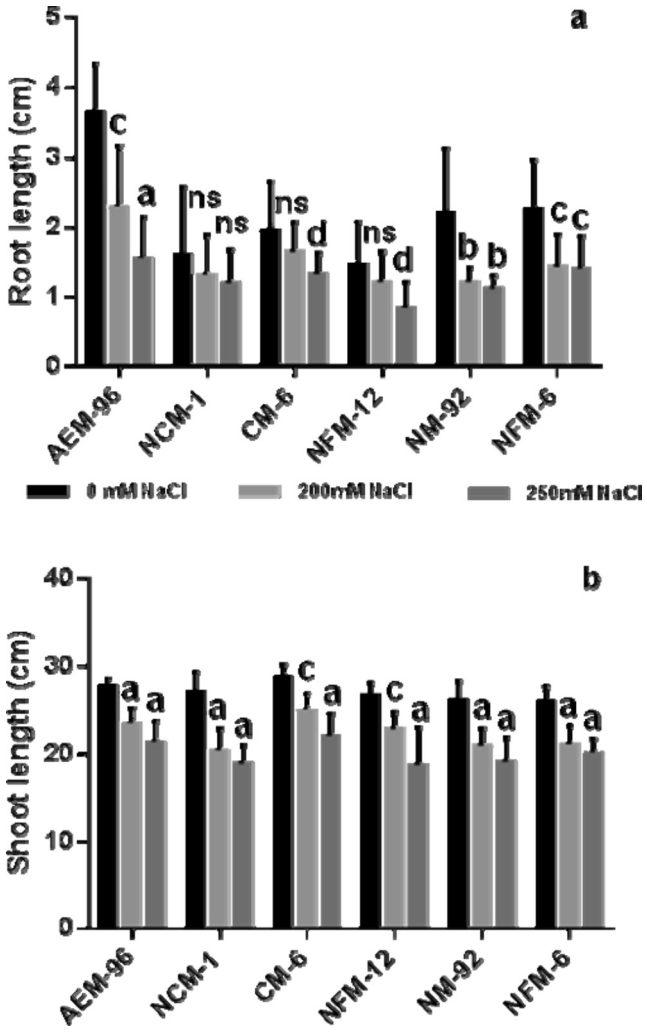

Fig. 2. Effect on shoot length and root length in salt stressed mungbean genotypes. The leaves from control and salt treated seedlings from each genotype were collected and shoot length (a) and root length (b) were recorded and data is presented as mean ± SE (n = 10). Different letters within columns represent significant differences (P < 0.05) between treatments within each genotype respectively. a = **** (highly significant), b = *** (moderately significant), c = ** (less significant) and ns (not significant) with respect to control of each genotype.

and 30.17%) was seen in NCM-1 at 200 mM and 250 mM respectively. All the genotypes taken together, the range of reduction was noted to be 13.15–24.85% at 200 mM and 22.76–30.17% at 250 mM. In semi-arid genotypes (NFM-12, NM-92, NFM-6), the effect was more prominent at 250 mM NaCl (Fig. 2a). The maximum reduction in root length was seen at 200 mM in NM-92 (45.14%) and at 250 mM in AEM-96 (57.44%) as compared to the control. All the genotypes taken together, the range of reduction in the root length varied from 15.81% to 45.14% at 200 mM and from 25.60% to 57.44% at 250 mM (Fig. 2a).

3.2. Effect of NaCl on biomass and relative water content

Fresh weight (FW), dry weight (DW) and relative water content (RWC) of mungbean genotypes were significantly affected by treatments with 200 mM and 250 mM NaCl concentrations (Tables 1 and 2). The fresh weight declined 13.56–29.40% at 200 mM and 23.51–37.69% at 250 mM, in all the genotypes studied. The maximum loss was associated with the genotype NFM-6. The range of the dry weight decline was between 6.62% and 47.52% at 200 mM and 28.37–52.30% at 250 mM. The maximum reduction occurred with NM-92 at 200 mM and NFM-6 at 250 mM. The decline in RWC ranged from 14.51% to 29.37% at 200 mM and 21.57% to 36.90% at 250 mM in various genotypes, with the maximum reduction seen in NFM-6 as compared to the control.

3.3. Effect of NaCl on photosynthetic pigment

The chlorophyll and carotenoid contents were significantly affected by salinity, showing a dose-dependent decline in the salt-treated plants as compared to the control (Tables 1 and 3). The mean chlorophyll and carotenoid content was lower in semi-arid genotypes than in the arid ones. Reduction in the chlorophyll content in genotypes was 5.93–49.44% at 200 mM and 7.28–49.11% at 250 mM. The maximum reduction in chlorophyll content was observed in NFM-6, whereas the minimum in CM-6, at both 200 mM and 250 mM treatments. The carotenoid content in the genotypes studied showed a decline ranging between of 7.13% and 46.81% at 200 mM and between 9.55% and 50.33% at 250 mM, with the maximum and minimum reductions seen in NFM-6 and CM-6, respectively, at both concentrations. Moreover, the chlorophyll:carotenoid ratio decreased in all genotypes at 200 mM but the changes were not-significant in NFM-6 and CM-6. On the contrary, the ratio increased non-significantly at 250 mM NaCl in NM-92, NFM-6 and CM6, and significantly in AEM-96 and NCM-1.

Table 2
Effect on fresh weight, dry weight and relative water content in salt stressed mungbean genotypes. The leaves from control and salt treated seedlings from each genotype were collected and fresh weight, dry weight were recorded and data is presented as mean ± SE (n = 10). The relative water content is presented as % calculated as RWC = {(FW − DW)/FW) × 100}. Different letters within columns represent significant differences (P < 0.05) between genotypes within each genotype respectively.

Parameters	Treatments (NaCl mM)	Genotypes					
		AEM-96	NCM-1	CM-6	NFM-12	NM-92	NFM-6
Fresh weight (g^{-1} Plant)	0	0.38 ± 0.0016	0.42 ± 0.0035	0.43 ± 0.0040	0.51 ± 0.0031	0.47 ± 0.0035	0.47 ± 0.0026
	200	0.31 ± 0.0036^{a}	0.36 ± 0.0015^{a}	0.36 ± 0.0108^{b}	0.41 ± 0.0022^{a}	0.36 ± 0.0079^{a}	0.33 ± 0.0033^{a}
	250	0.24 ± 0.0031^{a}	0.34 ± 0.0031^{a}	0.34 ± 0.0031^{a}	0.39 ± 0.0033^{a}	0.32 ± 0.0077^{a}	0.29 ± 0.0027^{a}
Dry weight (g^{-1} Plant)	0	0.033 ± 0.00048	0.043 ± 0.0001	0.037 ± 0.0002	0.044 ± 0.00043	0.034 ± 0.00023	0.046 ± 0.00020
	200	0.031 ± 0.0016^{ns}	0.037 ± 0.001^{c}	0.023 ± 0.0005^{a}	0.033 ± 0.00023^{a}	0.018 ± 8.819^{a}	0.025 ± 0.0006^{a}
	250	0.022 ± 0.0001^{b}	0.031 ± 0.00049^{a}	0.022 ± 0.0002^{a}	0.028 ± 0.00017^{a}	0.017 ± 0.00018^{a}	0.022 ± 0.0005^{a}
Relative Water content (%)	0	37.76 ± 1.142	41.12 ± 1.402	41.78 ± 2.007	50.40 ± 0.573	46.87 ± 0.1168	46.70 ± 0.306
	200	30.52 ± 2.072^{a}	35.15 ± 0.968^{a}	33.44 ± 1.011^{a}	40.46 ± 0.207^{a}	34.98 ± 0.277^{a}	32.98 ± 0.302^{a}
	250	23.91 ± 2.468^{a}	32.25 ± 0.747^{a}	31.22 ± 1.021^{a}	39.25 ± 0.308^{a}	30.77 ± 0.246^{a}	29.46 ± 1.284^{a}

Significance of values at P < 0.05, a = **** (highly significant), b = *** (moderately significant), c = ** (less significant) and ns (not significant) with respect to control of each genotype.

Table 3
Effect on photosynthetic pigments (total chlorophyll and carotenoid) in salt stressed mungbean genotypes. The leaves from control and salt treated seedlings from each genotype were collected and total chlorophyll (a) and carotenoids (b) were recorded and data is presented as mean ± SE (n = 3). Different letters within columns represent significant differences (P < 0.05) between genotypes within each genotype respectively.

Parameters	Treatments (NaCl mM)	Genotypes					
		AEM-96	NCM-1	CM-6	NFM-12	NM-92	NFM-6
Total chlorophyll (mg gram^{-1} FM)	0	0.754 ± 0.002	0.863 ± 0.002	0.635 ± 0.001	1.040 ± 0.013	1.340 ± 0.002	1.252 ± 0.006
	200	0.633 ± 0.0005^{a}	0.597 ± 0.002^{a}	0.597 ± 0.003^{b}	0.952 ± 0.004^{c}	0.937 ± 0.002^{a}	0.632 ± 0.012^{a}
	250	0.621 ± 0.002^{a}	0.542 ± 0.015^{a}	0.588 ± 0.002^{a}	0.895 ± 0.010^{a}	0.873 ± 0.010^{a}	0.636 ± 0.002^{a}
Carotenoid (mg gram^{-1} FM)	0	0.589 ± 0.001	0.663 ± 0.0015	0.519 ± 0.009	0.787 ± 0.011	1.061 ± 0.078	0.950 ± 0.0009
	200	0.502 ± 0.002^{a}	0.466 ± 0.0009^{a}	0.482 ± 0.0021^{d}	0.701 ± 0.002^{b}	0.690 ± 0.002^{b}	0.505 ± 0.001^{a}
	250	0.490 ± 0.001^{a}	0.467 ± 0.0077^{a}	0.470 ± 0.0013^{c}	0.613 ± 0.010^{a}	0.672 ± 0.001^{b}	0.472 ± 0.016^{a}
Chlorophyll/Carotenoid	0	1.215 ± 0.003	1.275 ± 0.033	1.254 ± 0.0467	1.694 ± 0.027	1.315 ± 0.006	1.317 ± 0.006
	200	1.264 ± 0.006^{a}	1.280 ± 0.016^{ns}	1.240 ± 0.006^{ns}	1.267 ± 0.012^{a}	1.267 ± 0.013^{c}	1.252 ± 0.023^{ns}
	250	1.270 ± 0.002^{a}	1.300 ± 0.0015^{ns}	1.253 ± 0.0049^{ns}	1.273 ± 0.014^{a}	1.305 ± 0.004^{ns}	1.352 ± 0.040^{ns}

Significance of values at at P < 0.05, a = **** (highly significant), b = *** (moderately significant), c = ** (less significant), d = * (least significant) and ns (not significant) with respect to control of each genotype.

3.4. Cell-membrane damage in response to NaCl

The electrolyte leakage and lipid peroxidation (LPO) rates were significantly higher in salt-treated plants than in the control (Table 1). The electrolyte leakage increased 0.18–1.94 fold at 200 mM and 0.43–2.1 fold at 250 mM NaCl concentration. The maximum leakage was observed in NFM-6 whereas the minimum in NM-92 (Fig. 3a). The increase in LPO rate among the genotypes ranged from 6.1% to 23.04% at 200 mM and from 13.10% to 51.76% at 250 mM NaCl respectively, with the maximum levels observed in genotype NM-92 (Fig. 3b).

3.5. Accumulation of osmolytes in response to NaCl

The salt-treated plants had significantly higher levels of osmolytes (proline, total soluble sugar and reduced glutathione) than control plants (Table 1). The proline content of the genotypes increased 0.53–2.86 fold at 200 mM and 0.87–3.38 fold at 250 mM NaCl, with the maximum elevation seen in NM-92 and the minimum in AEM-96 with both 200 mM and 250 mM NaCl treatments (Fig. 4a). The increase in total soluble protein in different genotypes was noted to be 0.05–0.83 fold at 200 mM and 0.16–0.82 fold at 250 mM. The maximum effect was observed in NM-92 and the minimum in CM-6 at both 200 mM and 250 mM NaCl concentrations (Fig. 4b). Likewise, the increase in the total soluble sugar ranged from 0.02 to 1.32 fold at 200 mM and 0.22 to 1.45 fold at 250 mM. The maximum increase was observed in CM-6 at both NaCl concentrations, while the minimum increase at 200 mM was observed in NFM-6 and at 250 mM was observed in AEM-96 (Fig. 4c). The increase in GSH ranged from 0.74 to 1.06 fold at 200 mM and 0.94 to 1.56 fold at 250 mM. The maximum GSH was observed in CM-6 and the minimum in NFM-6 at at both the NaCl concentrations used (Fig. 4d).

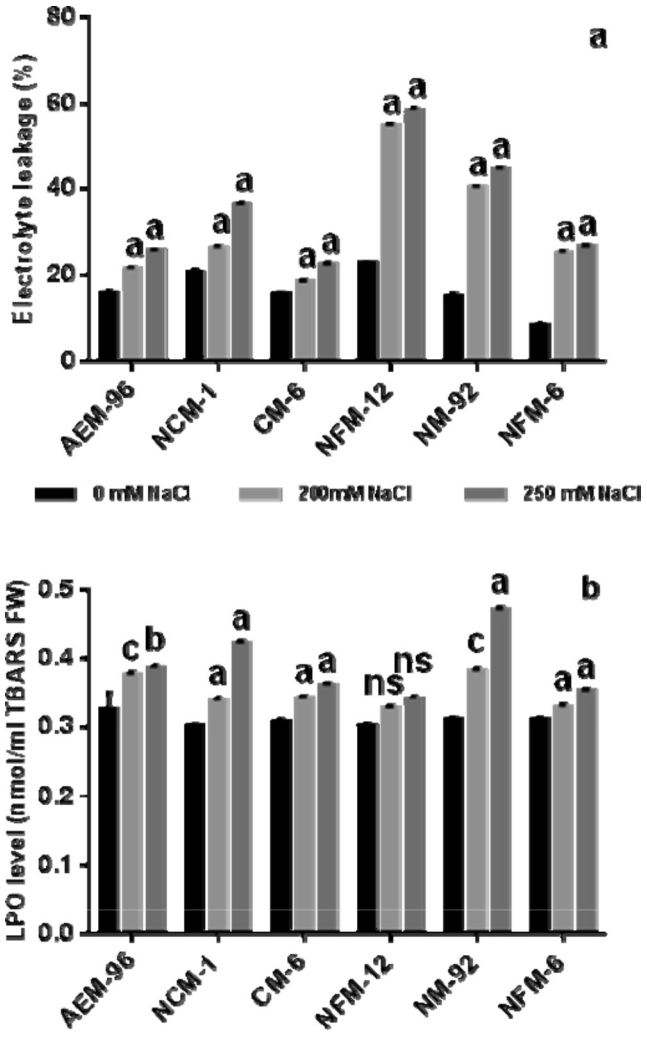

Fig. 3. Effect on electrolyte leakage (a) and LPO level (b) in salt stressed mungbean genotypes. The leaves from control and salt treated seedlings were collected. The electrical conductivity and lipid peroxides were recorded and data presented as mean ± SE (n = 3). Different letters within columns represent significant differences (P < 0.05) between genotypes within each genotype respectively. a = **** (highly significant), b = *** (moderately significant), c = ** (less significant) and ns (not significant) with respect to control of each genotype.

4. Discussion

4.1. Plant growth response

Seed germination rate declined in all the mungbean genotypes when subjected to different NaCl treatments. Seedling length also decreased in all genotypes, whereas the salinity tolerance index varied among the genotypes (Fig. 1). Plants respond to stress by adapting their morpho-physiological systems to changes in the environment so as to ensure their survival in the changed condition (Shelke et al, 2017). Under salt stress, the plant root system adapts its morpho-physiological characteristics for absorbing nutrients (Hasegawa et al., 2000). The decrease in root length is an adaptation response of plants to avoid and reduce salt absorption (Hasegawa et al., 2000). Salt stress lowers the extracellular water potential and bioavailability of water in the root zone,

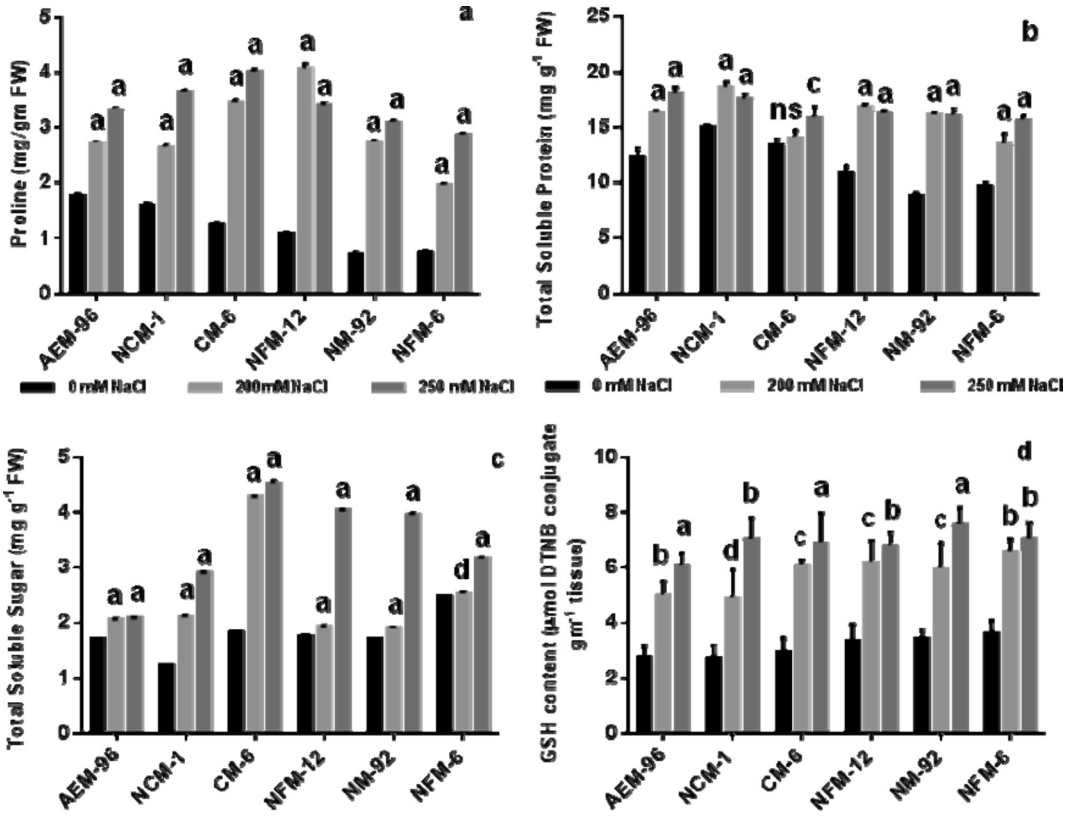

Fig. 4. Effect on osmolytes in leaves in salt stressed mungbean genotypes. The leaves from control and salt treated seedlings were collected and analyzed. The proline (a), protein (b), total sugar (c) and reduced glutathione (GSH) (d) were recorded and data presented as mean ± SE (n = 3). Different letters within columns represent significant differences ($P < 0.05$) between treatments within each genotype respectively. a = **** (highly significant), b = *** (moderately significant), c = ** (less significant) and ns (not significant) with respect to control of each genotype.

causing a low absorption of water and nutrients by plants and this hampers their adequate growth and biomass production (Zhang, 1991; Zhang et al., 2002). Some earlier studies have shown that the effect of salt stress on mungbean is dose dependent (Saha et al., 2010). Salinity stress affects the plant vigor due to reduction in imbibitions resulting in a limited hydrolysis of food reserves from the storage tissues (Ghosh et al., 2015). Our results show that the NaCl stress has a greater effect on roots than shoots with a sudden fall in the root growth (Fig. 2a), which is in accordance with some earlier reports (Saha et al., 2010). The deleterious effect of salinity on mungbean and the genotypic variation of plant response to salt stress has been seen in some earlier works also (Shakeel and Mansoor, 2012).

4.2. Photosynthetic pigment

Chlorophyll is indispensible for photosynthesis and is thus directly correlated with plant growth and health; it acts as an indicator of metabolic state at the cell level. Salt stress caused reduction in chlorophyll and carotenoid contents (Table 3), which might be caused by membrane swelling in chloroplasts and/or excess Na^+ and Cl^- ions in the leaves. The accumulation of ions results in excess ROS production, reducing the photosynthesis and plant growth, as observed in a variety of crop plants such as rice (Saha et al., 2010), soybean (Hakeem et al., 2012), Cucumber (Khan et al., 2013), sweet annie (Qureshi et al., 2013) and mungbean (Ghosh et al., 2015). Usually, there is dominance of chlorophyll *a* over chlorophyll *b*, but their values come closer when salinity goes high (Mane et al., 2010).

4.3. Cell membrane damage

The effect of salt stress causing lipid peroxidation (LPO), as observed in the present study, is supposed to lead to increased permeability of membranes causing ion leakage (Zhang et al., 2006), which is likely to exhibit genotypic variation. As the LPO rate is an important index of cell membrane permeability, the lower LPO might be due to elevated levels of antioxidants.

4.4. Accumulation of osmolytes

One of the universal responses to changes in the external osmotic potential is the accumulation of metabolites that act as compatible solutes, which do not inhibit normal metabolic reactions. Accumulation of osmolytes, which facilitates osmotic adjustment by decreasing the internal osmotic potential and hence contributes to tolerance (McCue and Hanson, 1990), is proportional to the external osmolarity (Hasegawa et al., 2000). Plants normally cope with salt stress by accumulating compatible solutes including proline and sugars (Pattanagul and Thitisaksakul, 2008), which help in the osmotic adjustments. Proline is a potential osmolyte for countering the stress and providing resistance (Arshi et al., 2002, 2004); it acts as a source of nitrogen under normal conditions (Tie et al., 2014). The accumulation of proline increased linearly under salt stress concentrations in all genotypes (Fig. 4a) which is in agreement with the previous reports by Hoque et al. (2008) and Misra and Gupta (2006) among others.

Sugar accumulation also contributes to osmotic balance permitting the plants to sustain under stressed conditions by maintaining their storage reserves (Smeekens, 2000). The total soluble sugar

increased with the salt stress in this study, as in many earlier ones (Muscolo et al., 2003). These solutes buffer the redox potential of the cell and protect the cellular structure under stress. Accumulation of these solutes might also involve alteration in the allocation of photo-assimilates. Protein is measured as one of the main indicators of stress in plants (Plata et al., 2009), and the increased protein content during salt stress may be due to enhanced activity of detoxification pathways. The high stress causes a decline of protein content due to protein oxidation. The reduced glutathione has been found to confer tolerance to drought and salt stress in Arabidopsis both endogenously (Cheng et al., 2015, Nahar et al., 2015) and exogenously (Chen et al., 2012). The increased glutathione levels may cause salt-stress tolerance and translational changes. There was an increased GSH content in all the genotypes in this study (Fig. 4d), which reflects an increased demand of GSH-metabolizing enzymes (Thounaojam et al., 2012).

4.5. Genotypic variation

There was a difference in responses of arid and semi-arid genotypes towards salt stress. Semi-arid genotypes exhibited a greater decline in the average root length, relative water content, and photosynthetic pigments, whereas a greater increase in the electrolyte leakage and the corresponding LPO levels in comparison to arid genotypes. Increase in the proline and total soluble protein accumulation under salt stress was more pronounced in arid genotypes than in the semi-arid ones. The sugar and GSH accumulation was also relatively more in arid genotypes. The differential behaviour of arid and semi-arid genotypes in response to salinity stress under uniform growth conditions merits special focus in future investigations.

4.6. Conclusion

In conclusion, salt treatments overall had a negative impact on the growth and survival of the mungbean genotypes studied. The tolerance was ensured by the linear increase in osmolytes concentrations. The arid and semi-arid genotypes displayed a differential response to the salinity stress applied.

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Conflict of interest

The authors declare no conflict of interest among them.

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