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Morpho-physiological, biochemical and developmental responses of diploid cotton (*Gossypium arboreum* L.) cultivars under varying NaCl stress

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Key words: NaCl stress, *Gossypium arboreum*, water relations, osmoregulation, biochemical analysis.

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Abstract

Salinity is one of the severe environmental factors limiting cotton productivity. The present study aimed to investigate the salt stress response of diploid cotton (*Gossypium arboreum* L.) cultivars commonly cultivated in the region. The plant developmental, biochemical and physiological responses of desi cotton varieties FDH 171 and FDH 786 under NaCl stress (100, 150 and 200 mM) were evaluated. Root and shoot length and seedling vigor were reduced, but root: shoot was gradually increased as the plants were subjected to increased stress of NaCl. Leaf relative water content and turgor potential were reduced when the NaCl stress was increased gradually. Decrease in osmotic and water potential imposed and maintained the physiological mechanism of the plants. Biochemical processes, including chlorophyll content, proline and soluble sugars under salt stress justify the reduction of cellular osmotic potential. Consequently, the genotypes FDH 171 & FDH 786 were suitably adapted under NaCl stress. These genotypes can be an excellent source for breeding and cotton improvement program for salinity tolerance.

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Introduction

Soil salinity is one of the major harms for agricultural crops in arid and semi-arid areas, however, this threat could also be experienced in areas other than the barren. The adverse effects of salinity are one of the major causes of the economic losses and hereafter reduces the yield production (Ashraf and Harris, 2004). There are many factors responsible for this challenge and major among those are, high temperature, the occurrence of low rainfall and higher surface evaporation, low quality irrigation water and improper agricultural practices (Seraj *et al.,* 2015).

Salt stress leads to a number of activities such as biochemical and physiological responses alter these pathways (Saeedipour, 2014), cellular membranes are disrupted and several enzymes are degraded (Khan, 2003) and nutrient uptake and distribution is unjustified within the host plant. Photosynthesis is one of the major physiological processes which is adversely affected and reduce plant growth due to restricted functioning of stomata (Saeed *et al.,* 2009). Other adverse effects of salinity are on the different developmental stages of the plants such as root and shoot growth, leaf size, and seed setting and maturity which may lead to a substantial decline in production and yield (Ahmad *et al*., 2002). Uneven distribution of ions and essential nutrients under salinity is another factor, which reduces the plants' growth by impaired access, transport and judicial partitioning of nutrients within the plants. Perhaps, low molecular weight organic solutes accumulate in plants under salt stress and cause most of the changes in metabolism of plants.

Plants have developed the defense mechanisms to adapt themselves under the saline environments. Some of the plants have developed the structures or modified their internal mechanisms which regulate the availability and usage of ions efficiently. This is maintained as the exclusion of the excess amount of salts through specialized secreting glands (Ashraf and Harris, 2004), or to utilize the vacuoles inside the cell to accumulate the excess ions within the cells (Siddiqi *et al*., 2009). Osmolytes are accumulated in plants to preserve cell turgidity and may play a role in defense against salinity stress (Cherki *et al*., 2002).

Cotton (*Gossypium hirsutum* L.) is considered as the *white gold*, because it provides fiber to our textile industry. Cotton industry plays an important role in the economy of the country as it earns the foreign exchange. *Gossypium arboreum*, the diploid species is the gene pool to identify the important genes and to study their salient features with the structure and functions (Shahid *et al*., 2012). Plants adapt themselves under salt stress by modifying their physiochemical processes and these studies may help to combat the crop under such circumstances such as, previously, we reported the growth and molecular responses of cotton under NaCl stress (Hassan *et al*., 2014). The aims of the present study are to screen out the morphophysiological, biochemical and plant developmental responses of the genotypes of *G. arboreum* (FDH 171 and FDH 786) under different levels of NaCl stress (100, 150 and 200 mM). Perhaps, this would lead to understand and the mechanism of cotton crop for salt tolerance.

Materials and methods

Experimental design, plant growth and salt stress

This study (based on Complete Randomized Design) was done in the green house at Centre of Excellence in Molecular Biology, University of the Punjab Lahore, Pakistan. Seeds of two local varieties of cotton (*G. arboreum*) FDH 171 and FDH 786 were germinated and grown as described earlier (Rashid *et al*., 2004). Pots were kept in green house at temperature 30 ± 2° C and at light intensity (250-300 $\mu molm^{-2} s^{1}$). Salt stress (NaCl) applied (100, 150 and 200 mM) was imposed as previously described (Hassan *et al*., 2014). Plants irrigated without NaCl treatment were considered as control. Data were analyzed for the parameters such as Morpho-physiological and biochemical variables.

Root and shoot length, root: shoot, seedling vigor index (SVI)

Growth parameters were observed and compared between the plants under different concentrations of

NaCl stress and control conditions (Shahid *et al.,* 2011). Shoot length was measured from base of stem to the apex. Root length was measured from tip of primary root to the base of hypocotyl and mean root and shoot length was expressed in inches (Fig. 1A). Seedling Vigor Index (SVI) was calculated as (Abdul-Baki and Anderson, 1973).

Root length+shoot length×germination%

Leaf relative water content

LRWC of leaves from each treatment were determined as (Shaheen and Shahbaz, 2012). Five fully developed young leaves (1 g) were selected from each replication and covered with polythene bags and FW was noted. Leaves were then immersed in double distilled water (ddH_2O) for 24 h to determine Turgor weight (TW). Samples were then oven-dried at 80˚C for 24 h to determine (DW). LRWC was determined using the following formula

Leaf Relative water content= (FW-DW)/(TW-DW)×100.

Relative membrane permeability (% ion leakage)

Leakage of ions from leaf cellular membranes was determined as (Saeed *et al*., 2009). About 1 g of fresh leaf from each treatment was detached, immersed in deionized water and electrical conductivity (EC_0) of the water was determined. Leaves were kept soaked for 12 hours in the same water and electrical conductivity of water (EC_1) was observed. Then leaves along with water were autoclaved, cooled to room temperature and final electrical conductivity was taken (EC_2). Relative membrane permeability was calculated as:

Relative Membrane permeability= EC1-ECO/EC2-ECO×100.

Water linked attributes

Leaf water potential (WP) was measured (-Mpa) as mentioned by (Kusvuran, 2012) with a Pressure chamber (Plant Water Status Console-Model 3005-1412; Soil moisture Equipment Corp., Goleta, California, USA). After determination of WP, leaf was kept at -20 ˚C for half an hour. Sap was extracted from frozen leaf by pressing it with a glass rod and leaf osmotic potential (OP) was measured (-MPa) by computerized osmometer (Multi-Osmette 2430, Precision Systems, Natick, MA, USA). The leaf turgor potential (TP) was estimated as the difference between osmotic potential (OP) and water potential as:

TP= (OP-WP).

Proline content

Proline content from leaf was extracted according to (Bates *et al*., 1973). About 1 g leaf was extracted using 10 ml of 3% Sulfosalicylic acid and filtered. Acid ninhydrin (2 ml) was added to 2 ml of filtrate (v/v) and incubated for 1 hour at 100 ˚C. The reaction was terminated by placing in an ice bath. Then mixed vigorously with 4ml toluene for 20-30 sec. Layers were separated and red color intensity was measured at 520 nm. Standard curve was obtained using a known concentration of proline and calculated as $\mu g g^{-1}$ of leaf tissue.

Total soluble sugar

Soluble sugars were estimated as (Yemm and Willis, 1954). Ground dry leaf (100 mg) was homogenized with 80% ethanol and centrifuged at 3000 rpm. This was repeated to remove traces of soluble sugars. One ml of filtrate was treated with 10 ml anthrone reagent and heated in boiling water for 12 minutes. Then cooled to room temperature and absorbance was read at 625 nm. Total soluble sugar was calculated ($\mu g g^{-1}$ DW) by the following formula:

Conc. of glucose solution/Absorbance of glucose×absorbance of sample×dilution factor.

Chlorophyll content

Chlorophyll extract was prepared from 100 mg fresh leaves by grinding with 10 ml of 80% (v/v) acetone and centrifuged at 10000 g for 10 min at 4 °C. Absorbance of the extract was read at 663 nm and 645 nm. Chlorophyll contents a, b and total (mg/gm fresh weight) was calculated using Arnon (1949) equations.

Statistical analysis

The experiment was analyzed in completely

randomized design with two factor factorial arrangement. Experimental data are the means of 5 replicates, and results were determined using analysis of variance (ANOVA) via Statistix software. Variation among treatment means were compared using least significant difference (LSD) test ($P \leq 0.05$).

Results

Shoot & root length, root:shoot and seedling vigor index

The variable length of plants under control and salt stressed condition. Shoot growth was decreased as NaCl was increased in both the varieties. Variety FDH 171 has 4.2 inch long shoot at control condition but when NaCl (100, 150 and 200 mM) was applied, length of shoots was reduced as 3.8, 3.0 and 2.7 inches respectively (Fig. 1A). Similarly the variety FDH 786 produced 4.0 inches long shoots under control conditions, but decreased to 3.3 2.9 and 2.2 inches when NaCl was applied at 100, 150 and 200 mM respectively (Table 1). Root length was 8.5 inches in FDH 171 as compared to FDH 786 i.e. 6.2 inches under the control condition. The lowest root length i.e. 4.9 inches was noticed in FDH 786. As the concentration of NaCl was increased from 100, 150 to 200 mM, the length of root was decreased as 7.8, 7.0 to 6.2 inches respectively in FDH 171 (Fig 1B). Similarly in FDH 786, NaCl at the rate of 100, 150 and 200 mM increased the reduction in the root length from 5.8, 5.2 to 4.9 inches (Table 1). Salinity level of 200mM reduced the max root length in both the varieties. It was observed that the root to shoot ratio was found to be 2.0 under control conditions in variety FDH 171 and remained constant at 100 mM NaCl but increased to 2.3 and then 2.9 at salinity level 150 and 200 mM respectively. Same root shoot ratio pattern was observed in FDH 786. The ratio was 1.5 at the control condition but rose to 1.7 and remained same at 100 and 150 mM NaCl and increased to 2.2 at 200 mM NaCl (Table 2-3). The maximum seedling index was observed in FDH 171 in control plants, i.e. 1270 and it was decreased as 1160, 1000 and 890 when the application of NaCl was increased as 100, 150 and 200 mM respectively (Table 1). Same pattern of SVI was observed in FDH 786 such as control plants showed 1020 SVI which was declined as 910, 810 and 710 while the treatment of NaCl was increased like 100, 150 and 200 mM respectively. The reduction in SVI was recorded as the increase in NaCl.

Table 1. Growth indicators of cotton varieties FDH-171 and FDH-786 under NaCl stress.

Cotton variety	NaCl Stress mM	RL	SL	Root/Shoot	SVI
FDH-786	Control	6.2CD	4.0A	1.5C	1020A
	100	5.8DE	3.3BC	1.7BC	910BC
	150	5.2E	2.9CD	1.7BC	810CD
	200	4.9E	2.2E	2.2A	710E
FDH-171	Control	8.5A	4.2A	2.0AB	1270A
	100	7.8AB	3.8AB	2.0AB	1160AB
	150	7.0BC	3.0CD	2.3A	1000CD
	200	6.2CD	2.7DE	2.9A	890D

RL: root length, SL: shoot length, SVI: seedling vigor index.

Leaf relative water content (LRWC)

LRWC under control conditions were 40 and 54% in FDH 171 and FDH 786 respectively. As NaCl was applied (100, 150 and 200 mM) to FDH 171, LRWC was found to be 35, 32 and 31% respectively. Similarly LRWC in FDH 786 was decreased relatively such as 51, 40 and 35% as NaCl was applied at the same rate (Fig. 2A). ANOVA and comparative mean study indicates variations in LRWC and highly significant impact of salt stress in both cultivars, However it was slight for genotype × treatment interaction (Table 2-3) ($P \leq 0.05$). Therefore a positive correlation

between LRWC and the salt stress level has been observed in this study.

Relative membrane permeability (RMP)

Solute leakage was increased significantly in both the varieties at all salinity levels ($P \leq 0.05$) (Table 2-3). Hence, the increase in solute leakage at higher NaCl stress represents the higher EC value which ultimately shows that RMP was decreased. Highest solute leakage (EC value) 44% was observed in FDH 171 at highest level of NaCl i.e. 200 mM and it was decreased to 39, 36 and 8.0% at the salinity level 150, 100 mM and control conditions (Fig. 2A). Similarly, FDH 786 made the RMP to 11% at non saline level and decreased the permeability to 26, 31 and 42% at the saline level 100, 150 and 200 mM respectively. ANOVA and the comparative mean study concludes that both the varieties are significantly different for RMP under different concentrations of NaCl stress (Table 2-3).

Table 2. Mean value of physiological and biochemical studies of cotton varieties FDH-171 and FDH786 under NaCl stress.

Trait	Cultivars	
	FDH-786	FDH-171
Root Shoot Ratio	1.7479B	2.2946A
Water Potential(-MPa)	0.7167B	1.0328A
Osmotic Potential	3.1656A	3.1334A
Turgor Potential	2.3014A	2.0733B
Relative Membrane Permeability (%)	27.917B	31.375A
Relative Water Content (%)	45.375A	34.563B
Chlorophyll Content	10.957B	14.623A
Total Soluble Sugar	8.7973A	7.8050B
Proline Content	36.608A	34.376A

Means followed by different alphabet are different at 5% level of significance based on least significant difference test (LSD), while those followed by same letters are statistically non-significant.

Water related attributes

While comparing two varieties under control conditions, FDH 171 was found to be having more WP i.e. 1.42 (-MPa) then FDH 786 which has 1.07 (-MPa) (Fig. 3A). As the application of NaCl was increased from 100, 150 to 200 mM, WP in FDH 171 was decreased gradually i.e. 1.03, 0.99 and 0.91 (-MPa) respectively. That was reduced similarly as 0.84, 0.78 and 0.59 (-MPa) respectively in FDH 786 at the same rate of NaCl stress. Different concentrations of NaCl are also significantly responsive to plant water potential (Table 2-3). Data showed that there is a reduction in the leaf OP with increased application of NaCl. For control plants, OP was 3.71 (-MPa) in FDH 171 that was decreased as 3.12, 2.97 and 2.85 (-MPa) with application of NaCl 100, 150 and 200mM respectively (Fig. 3B). Reduction in OP was also observed in FDH 786 such as plants grown under control conditions showed OP 3.89 (-MPa) that was reduced as 3.2, 2.9 and 2.58 (-MPa) when NaCl was increased as 100, 150 and 200 mM. Thus increasing salt stress significantly reduced the OP in both varieties (Table 2-3). This study shows that TP has been reduced under different levels of NaCl stress as 2.29 MPA and 2.82 MPA in FDH 171 & FDH 786 respectively under control conditions. But as the NaCl was applied (100, 150 and 200 mM), the same was declined to 2.09, 1.98 and 1.94 MPA respectively in FDH 171. Likewise TP was found to be decreased gradually in FDH 786 as 2.36, 2.12 and 1.99 MPA while the NaCl was increased (Fig. 3C). Difference in TP shows the physiological changes in the metabolism of plants under different treatments and we observed the two varieties are significantly different at different levels of NaCl stress (Table 2-3).

Proline content

The endogenous level of proline improved significantly under increased salinity levels as compared to control condition. FDH 786 was accumulating more proline then FDH 171 (Fig. 4A) but the difference was not significant (P ≤ 0.05) (Table 2-3). Under control condition, proline accumulation in FDH 786 was 9.1 µgg^{-1} FW and 9.47 µgg^{-1} FW in FDH 171. There is positive correlation between proline content and increasing salt stress treatment. A sharp increase in proline content was observed at 200 mM NaCl such as 101.91 µgg^{-1} FW in FDH 786 and 96.11 µgg^{-1} FW in FDH 171.

Table 3. Analysis of variance of physiological and biochemical analyses of cotton.

Trait	SOV	DF	SS	MS	F
Root Soot Ration	*Cultivars*	*1*	1.73873	1.73873	38.93**
	Treatments	*3*	1.53186	0.51062	11.43**
	Cultivars×Treatments	*3*	0.13893	0.04631	1.04
	Cultivars×Treatments	*3*	0.01711	0.00570	1.07
Water Potential(-MPa)	*Cultivars*	*1*	0.34835	0.34835	37.19**
	Treatments	*3*	0.90071	0.30024	32.06**
	Cultivars×Treatments	*3*	0.02168	0.00723	0.77
Osmotic Potential	*Cultivars*	*1*	0.00603	0.00603	0.07
	Treatments	*3*	3.81649	1.27216	14.48**
	Cultivars×Treatments	*3*	0.01793	0.00598	0.07
Turgor Potential	*Cultivars*	*1*	0.30264	0.30264	7.54*
	Treatments	*3*	1.20546	0.40182	10.02**
	Cultivars×Treatments	*3*	0.30754	0.10251	2.56
Relative Membrane Permeability(%)	*Cultivars*	*1*	69.59	69.59	6.56*
	Treatments	*3*	3642.12	1214.04	11.49**
	Cultivars×Treatments	*3*	184.07	61.36	5.79*
Relative Water Content(%)	*Cultivars*	*1*	680.205	680.205	79.30**
	Treatments	*3*	643.607	214.536	25.01**
	Cultivars×Treatments	*3*	108.579	36.193	5.22*
Chlorophyll Content	*Cultivars*	*1*	78.1867	78.1867	17.52**
	Treatments	*3*	79.161	26.3869	7.86*
	Cultivars×Treatments	*3*	12.0430	4.0143	0.90
Total Soluble Sugar	*Cultivars*	*1*	5.72	5.72	35.93**
	Treatments	*3*	21.7208	7.24027	45.41**
	Cultivars×Treatments	3	1.4304	0.47681	2.99
Proline Content	*Cultivars*	*1*	6.5	6.5	0.04
	Treatments	*3*	30593.7	10197.9	1255.45**
	Cultivars×Treatments	*3*	12.6	4.2	0.01

varieties FDH-171 and FDH786 under NaCl stress.

*, denotes significant differences at **5%** probability level (P≤0.05)

, denotes significant differences at **1% probability level (P≤0.01).

Total soluble sugars

Accumulation of total soluble sugars is found to be significantly increased as the plants were subjected to an increased concentration of NaCl stress (P ≤ 0.01). In FDH 171 under control conditions the soluble sugars were found to be lowest i.e. 6.75 µgg^{-1} of the dry matter and it was 7.16 µgg^{-1} DW in FDH 786 under the same conditions. It was noticed that the accumulation of soluble sugars was increasing like 7.6, 8.0 and 8.55 µgg^{-1} DW with the increasing stress of NaCl as 100, 150 and 200 mM respectively in FDH 171. Similarly, in FDH 786 the deposition of soluble sugars was estimated increasing gradually as 8.25, 9.6 and 10.2 µgg^{-1} DW under 100, 150 and 200 mM salt stress (Fig. 4B). Hence, FDH 786 was observed with higher accumulation of soluble sugars under stress and both the varieties were significantly different to each other at different concentrations of NaCl (Table 2-3).

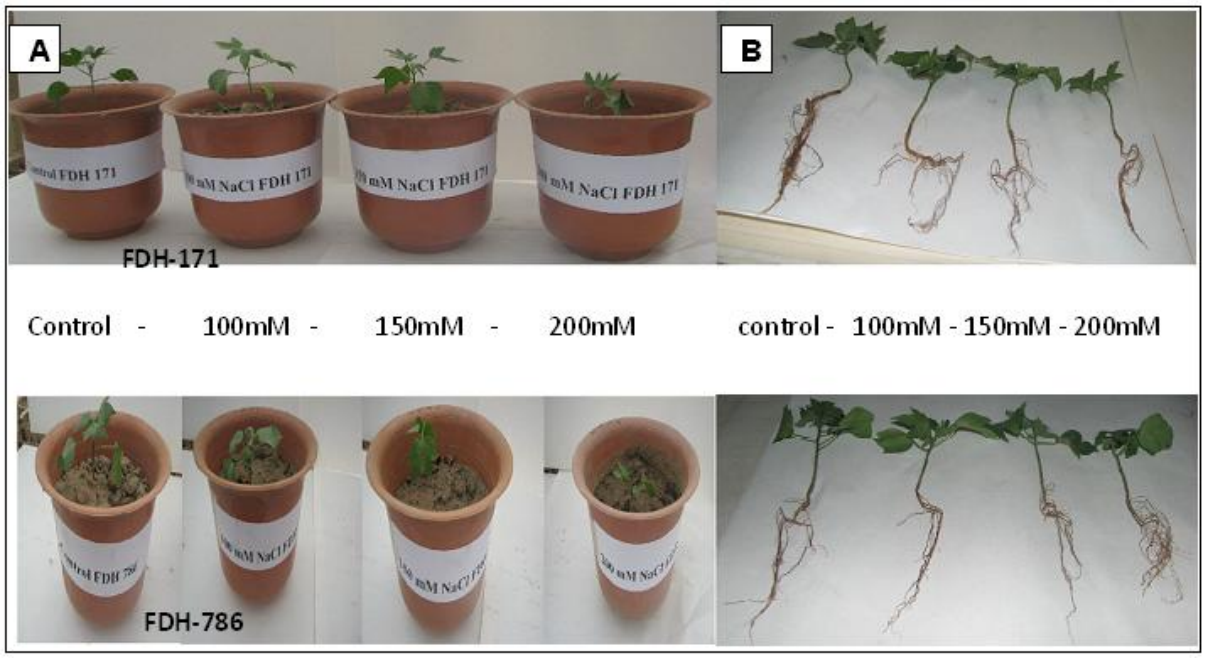

Fig. 1. (A) Cotton plants FDH 171 and FDH 786 growing under NaCl stress. (B) Root and Shoot morphology of the plants under NaCl stress.

Chlorophyll content

In this study we observed the significant fluctuation in chlorophyll content at different levels of NaCl stress (Fig. 4C). FDH 171 exhibited significantly higher chlorophyll content than FDH 786 at all levels of NaCl stress ($p \leq 0.01$). Maximum chlorophyll content 15.15 mgg^{-1} FW and 10.42 mgg^{-1} FW were achieved at 100 mM NaCl in FDH 171 and FDH 786, respectively. FDH 171 exhibited significantly higher chlorophyll content than FDH 786 at 150 and 200 mM NaCl. However when compared the control and NaCl stressed plants, chlorophyll values were higher in control plants in both the varieties.

Analysis of variance determined the interactions between cultivars and the stress treatments and it was observed that most of the interactions between genotypes and stress treatments were significant. Thus, stress treatment highlighted the optimum difference between FDH 171 and FDH 786, though interactions between varieties and NaCl stress treatment were greatest for the stress.

Discussion

Delayed response was observed for the development of seedling in cotton under salt stress (Qadir and Shams, 1997). This report states that different concentrations of NaCl (100, 150 and 200mM) reduced seedling growth, such as root and shoot length and SVI decreased except root: shoot, which was increased with the increase in salinity level. This observation has also been confirmed previously (Munns, 2002) and the differences in the growth response were also reported in cotton (Khan, 2003). It can be concluded from the available data that the seedling development is not the final stage of any cultivar to be declared as tolerant or susceptible, as the growth is restricted under salt stress (Cherki *et al.*, 2002). Roots are represented as key sensors to identify water scarcity in soil and triggers the physiological and biochemical alarms to the whole plant. They uptake the water and essential nutrients independently (Maathuis and Amtmann, 1999). Our data show that, there is maximum reduction in root and shoot length of both the varieties at 200mM NaCl and the root: shoot was increased gradually with an increase in NaCl stress. Seedling vigor has been identified as complex character which is directed by many physiological factors and is considered as an important attribute in determining the seed physiology. Initial seedling vigor identifies the high planting value of a specific seed lot and better

development of crop. We observed that SVI was decreased as the NaCl stress was increased. Higher SVI means that the plants have a higher tendency to tolerate salinity. Suriya-Arunroj *et al.*, (2004) reported that the salts create toxicity to the cells when their concentration is more in the plant growing media. That may lead to the reduced moisture content which ultimately disturb the physiological mechanism and reduced plant growth and development.

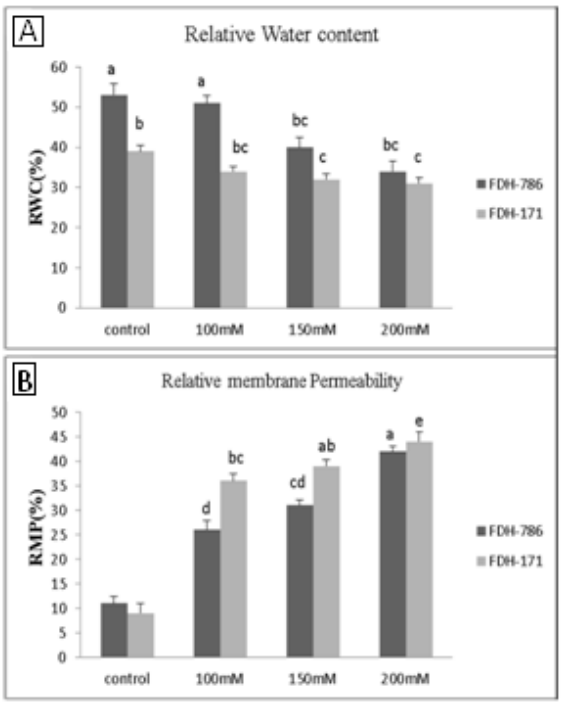

Fig. 2. A-Leaf relative water content. B- Relative membrane permeability under control and NaCl stress. Each value is the mean of five replicates and the vertical bars give the standard error of mean. Values with the same letter were not significantly different based on Fisher's Least Significant Difference (LSD) test (P ≤ 0.05).

LRWC is observed as the available water content of a leaf relative to the maximum amount of water that the leaf can take under full turgidity. Water uptake is reduced under salt stress, which affects the LRWC and many other physiological processes such as stomatal conductance, ion accumulation and photosynthesis (Saeedipour, 2014). There is correlation between LRWC and salt stress and lower water content has been observed in this study as stress was increased. Decrease in LRWC also decreases the leaf tissue elasticity due to H^+ imbalance (Munns, 2002). This concludes that, LRWC can effectively be used for screening of salt tolerant cultivars. Under salt stress, cell membrane is subjected to changes such as decrease in sustainability (Shilpi and Narendra, 2005).

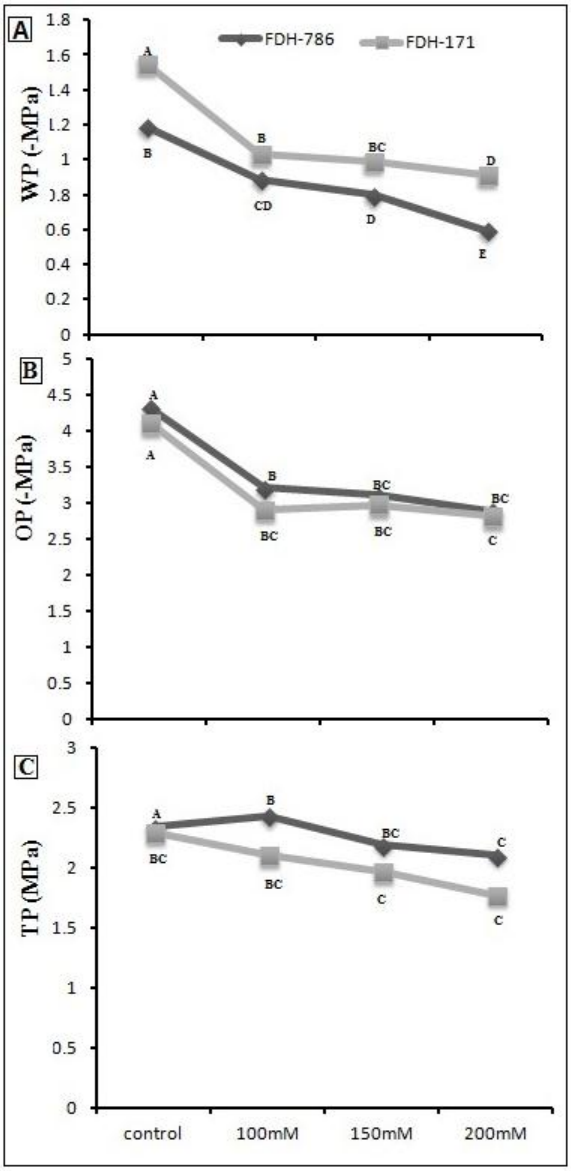

Fig. 3. Water related attributes under NaCl stress. (A) Water potential (B) Osmotic potential (C) Turgor potential. Each value is the mean of five replicates .Values with the same letter were not significantly different based on Fisher's Least Significant Difference (LSD) test (P ≤ 0.05).

Accumulation of Na^+ has toxic effects which damages the structural and functional integrity of membranes (Maathuis and Amtmann, 1999). In this study relative leakage ratio was increased with increase in salinity

which shows that the salt tolerant genotypes adjust the osmoticum by maintaining the relative ion leakage ratio in the leaves. Absisic acid the plant hormone which plays an important role when the plants are under abiotic stress and thus it transmits the caution for reduced water potential and decreaed transpiration under higher salt stress. (Zhang and Davies, 1991). Positive turgor pressure maintains the cell expansion and stomatal opening and helps the plant's survival under salt stress. Generally, the leaf water content is maintained within the plants under salt stress because of the higher ability of the osmotic adjustment of the tolerant genotypes and that means the stronger adaptation and more tolerance (Mao *et al.*, 2012). The tolerant or resistant genotypes may shield the plants with improved physiological and biochemical processes such as higher cell membrane stability and photosynthetic rate and reduced water loss and osmotic potential against environmental stresses (Bartels and Sunker, 2005). Besides, different factors such as the level and duration of stress and number of exposures and the type of species may also contribute to determine the crops' tolerance level.

Salt stress limits the plants' water use efficiency and overall environmental water potential is reduced, which induces the osmotic stress to plants. In this study, while comparing the two varieties under normal conditions, FDH 171 maintained significantly higher WP than FDH 786. Decline in WP under salt stress has been reported in crops such as safflower (Siddiqi *et al.*, 2009), *Beta vulgaris* (Dadkhah, 2011) and sunflower (Saeed *et al.*, 2009). This study has the observation OP was decreased by increased stress of NaCl, which badly influences the potential of plants to take up water. The adverse effects of osmotic stress also depend upon the degree of salt imposition (Munns, 2002). Reduction in OP in salt treated plants, mainly occurs due to high accumulation of Na^+ and K^+ (Hasegawa *et al.*, 2000). Difference between varieties is not significant due to the fact that generally cotton is tolerant to salt. Turgor potential is also reduced as the NaCl stress was increased. This is believed that water uptake and its utilization within plant tissues are maintained by roots and leaves so osmotic adjustment retains cell turgor which efficiently regulates the functioning of physiological processes (Serraj and Sinclair, 2002).

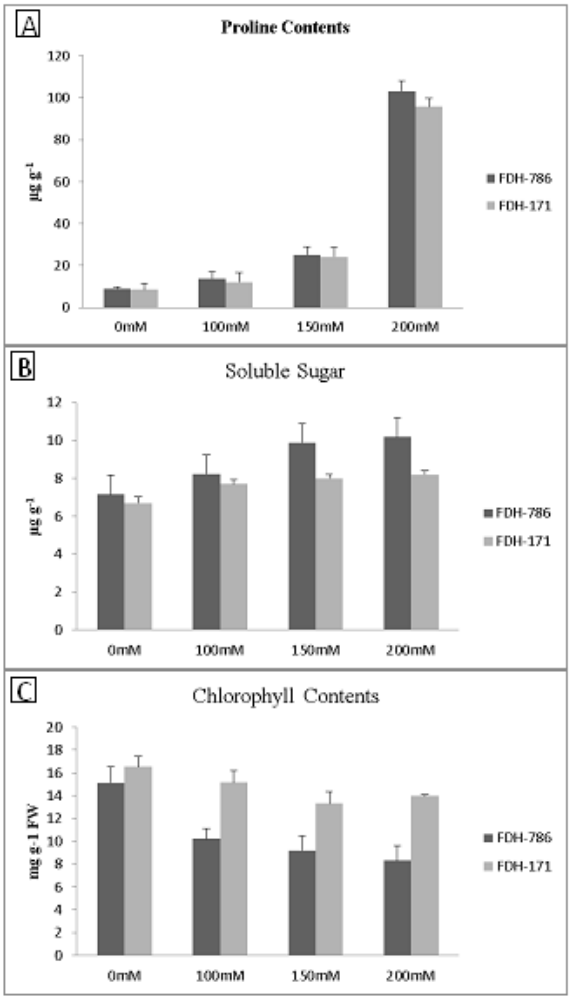

Fig. 4. Biochemical indicators in FDH-786 & FDH-171 under NaCl stress. (A) Proline content (B) Total soluble sugars (C) Chlorophyll content. Each value is mean of five replicates and vertical bars give standard error of mean. Values with same letter were not significantly different based on Fisher's Least Significant Difference (LSD) test (P≤0.05).

Osmotic adjustment measures the net increase in solute concentration within a cell that is independent of the volume changes that result from loss of water (Suriya-Arunroj *et al.*, 2004). Total soluble sugar and proline estimation are measurable biochemical parameters, suggested to be correlated with adaptation to plants' ability to tolerate the saline environment. Therefore, they are considered important to use in crop breeding programs as they

are considered main macro osmoprotectants that play a vital role during stress. These results correlate with the other reports that salt stress significantly increased the accumulation of proline and soluble sugars in different salt sensitive and tolerant species/cultivars, but the tolerant species showed more solute accumulation (Ashraf and Harris, 2004). Besides osmotic adjustments, proline and soluble sugars also protect the plants' enzymes under salt stress and maintains cell membrane stability (Mansour *et al.*, 2005). Chlorophyll is the green pigment in plants which is common to all photosynthetic cells, absorbs all wavelengths of visible light except green. Salt stress negatively affects the activity of photosynthetic enzymes, chlorophyll and carotenoids (Stępień and Kłbus, 2006). Cotton especially *arboreum* species classified as stress tolerant, variation in salt tolerance has been observed among both cultivars (Table 2-3). Reduction in chlorophyll content is perhaps due to decrease or inhibition of its biosynthesis which lead to an increase in ethylene production (Khan, 2003). Further, chlorophylase activity increases to combat with stressful condition (Myrene and Varadahally, 2009).

Plants adapt various mechanisms to cope up salinity stress. Plant genomics and biotechnology applications have made it possible to identify and correlate the genes regulating the physiological and biochemical pathways leading to the tolerance mechanism in the plants (Shahid *et al.*, 2012). Thus, in our results, the genotypes FDH 171 & FDH 786 were tolerant to the salt stress and hence could be used as a line source to develop putative genotypes for improving salinity tolerance.

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References

Abdul-Baki AA, Anderson JD. 1973 Vigor determination in soybean seed by multiple criteria. Crop Science **13,** 630-633. http://dx.doi:10.2135/cropsci1973.0011183X001300060013x

Ahmad S, Khan N, Iqbal M, Hussain A, Hassan M. 2002. Salt tolerance of cotton (*Gossypium hirsutum* L.). Asian Journal of Plant Science **1,** 715-719.

Arnon D. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiology **24,** 1-15. http://dx.doi.org/10.1104/pp.24.1.1

Ashraf M, Harris PJC. 2004. Potential biochemical indicators of salinity tolerance in plants. Plant Science **166,** 3-16. http://dx.doi.org/10.1016/j.plantsci.2003.10.024

Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of free proline for water-stress studies. Plant and Soil **39,** 203-205.

Bartels D, Sunkar R. 2005. Drought and salt tolerance in plants. Critical Reviews in Plant Science **24,** 23–58. http://dx.doi.org/10.1080/07352680590910410

Cherki G, Ahmed F, Khalid F. 2002. Effects of salt stress on growth, inorganic ions and proline accumulation to osmotic adjustment in sugar beet cultivars. Environmental and Experimental Botany **47,** 39-50. http://dx.doi.org/10.1016/S0098-8472(01)00109-5

Dadkhah AR. 2011. Effect of salinity on growth and leaf photosynthesis of two sugar beet (*Beta vulgaris* L.) cultivars. Journal of Agricultural Science and Technology **13,** 1001-1012.

Hasegawa P, Bressan R, Zhu JK, Bohnert H. 2000. Plant cellular and molecular responses to high

salinity. Annual Review in Plant Physiology and Plant Molecular Biology **51,** 463-499. http://dx.doi.org/10.1146/annurev.arplant.51.1.463

Hassan S, Sarwar MB, Sadique S, Rashid B, Aftab B, Mohamed BB, Husnain T. 2014. Growth, physiological and molecular responses of cotton (*Gossypium arboreum* L.) under NaCl stress. American Journal of Plant Sciences **5(5),** 605-614. http://dx.doi.org/10.4236/ajps.2014.55075

Khan N. 2003. NaCl-inhibited chlorophyll synthesis and associated changes in ethylene evolution and antioxidative enzyme activities in wheat. Biologia Plantarum **47,** 437-440. http://dx.doi:10.1023/B:BIOP.0000023890.01126.43

Kusvuran S. 2012. Effects of drought and salt stress on growth, stomatal conductance, leaf water and osmotic potentials of melon genotypes (*Cucumis melo* L.). African Journal of Agricultural Research **7,** 775-781. http://dx.doi.org/10.5897/AJAR11.1783

Maathuis FJM, Amtmann A. 1999. K+ nutrition and Na+ toxicity: The basis of cellular K+/Na+ ratios. Annals of Botany **84,** 123-133. http://dx.doi.org/10.1006/anbo.1999.0912

Mansour M, Salama K, Ali F. 2005. Cell and plant responses to NaCl in *Zea mays* L. cultivars differing in salt tolerance. Gennetics and Applied Plant Physiology **35,** 29-41.

Mao X, Zhang H, Qian X, Li A, Zhao G, Jing R. 2014. TaNAC2, a NAC-type wheat transcription factor conferring enhanced multiple abiotic stress tolerances in Arabidopsis. Journal of Experimetal Botany **2014,** 1-14. http://dx.doi.org/10.1093/jxb/err462.

Munns R. 2002. Comparative physiology of salt and water stress. Plant Cell and Environment **25,** 239-250. http://dx.doi.org/10.1046/j.00168025.2001.00808.x

Myrene RDS, Varadahally RD. 2009. Biochemical responses of Hyacinth bean (*Lablab purpureus*) to salinity stress. Acta physiologia plantarum **32,** 341-353. http://dx.doi.org/10.1007/s11738-009-0412-2

Qadir M, Shams M. 1997. Some agronomic and physiological aspects of salt tolerance in cotton (*Gossypium hirsutum* L.). Journal of Agronomy and Crop Science **179,** 101-106. http://dx.doi.org/10.1111/j.1439037X.1997.tb00504.x

Rashid B, Husnain T, Riazuddin S. 2004. In vitro shoot tip culture of cotton (*Gossypium hirsutum*). Pakistan Journal of Botany **4,** 817-823.

Saeed M, Ashraf M, Akram MS, Akram NA. 2009. Growth and photosynthesis of saltstressed sunflower (*Helianthus annuus*) plants as affected by foliar-applied different potassium salts. Journal of Plant Nutrition and Soil Science **172,** 884-893. http://dx.doi.org/10.1002/jpln.200900102

Saeedipour S. 2014. Effects of salinity stress on growth, chlorophyll content and ion accumulation in two indica rice (*Oryza sativa* L.) cultivars differing in salinity tolerance. International Journal of Biosciences **4(4),** 33-40. http://dx.doi.org/10.12692/ijb/4.4.33-40

Seraj RGM, Moghaddam EG, Asgharzade A. 2015. In vitro evaluation of salinity tolerance of two selected dwarf Mahaleb (Prunus mahaleb L.) genotypes. International Journal of Biosciences **6(2),** 100-105. http://dx.doi.org/10.12692/ijb/6.2.100-105

Serraj R, Sinclair T. 2002. Osmolyte accumulation: can it really help increase crop yield under drought conditions? Plant Cell and Environment **25,** 333-341. http://dx.doi.org/10.1046/j.1365-3040.2002.00754.x

Shaheen HL, Shahbaz M. 2012. Salt induced effects on some key morpho-physiological attributes of cotton (*Gossypium hirsutum* L.) at various growth stages. Soil and Environment **31,** 125-133.

Shahid MA, Pervez MA, Balal R, Ahmad R, Ayyub C, Abbas T, Akhtar N. 2011. Salt stress effects on some morphological and physiological characteristics of okra (*Abelmoschus esculentus* L.). Soil and Environment **30,** 66-73.

Shahid MN, Jamal A, Rashid B, Aftab B, Husnain T. 2012. Identification and isolation of salt stress responsive transcripts from *Gossypium arboreum* L. Turkish Jounal of Biology **36,** 746-756. http://dx.doi.org/10.3906/biy-1207-54

Shilpi M, Narendra T. 2005. Cold, salinity and drought stresses: An overview. Archives of Biochemistry and Biophysics **444,** 139-158. http://dx.doi.org/10.1016/j.abb.2005.10.018

Siddiqi EH, Ashraf M, Hussain M, Jamil A. 2009). Assessment of intercultivar variation for salt tolerance in safflower (*Carthamus tinctorius* L.) using gas exchange characteristics as selection criteria. Pakistan Journal of Botany **41,** 2251-2259.

Stępień P, Kłbus G. 2006. Water relations and photosynthesis in *Cucumis sativus* L. leaves under salt stress. Biologia Plantarum **50,** 610-616. http://dx.doi.org/10.1007/s10535-006-0096-z

Suriya-Arunroj D, Supapoj N, Toojinda T. 2004. Relative leaf water content as an efficient method for evaluating rice cultivars for tolerance to salt stress. ScienceAsia **30,** 411-415. http://dx.doi.org/10.2306/scienceasia15131874.2004.30.411

Yemm E, Willis A. 1954. The estimation of carbohydrates in plant extracts by anthrone. The Biochemistry Journal **57,** 508-514.

Zhang J, Davies W. 1991. Antitranspirant activity in maize plants. Journal of Experimental Botany **42,** 317-321. http://dx.doi.org/10.1093/jxb/42.3.317