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PHYSIOLOGICAL PARAMETERS OF SALT TOLERANCE IN THREE CULTIVARS OF *SORGHUM BICOLOR* (L.) MOENCH. AT SEEDLING STAGE UNDER SINGLE SALT (NaCl) SALINITY

Zulfiqar Ali, D. Khan and Naeem Ahmed

Department of Botany, University of Karachi, Karachi-75270, Pakistan.

ABSTRACT

Three sorghum (*Sorghum bicolor* (L.) Moench.) cultivars namely Mr. Buster, Honey Graze and Extra Sweet of Australian origin were tested for their salinity tolerance. Their seedling growth was tested in a sand culture experiment n pots under saline irrigation with 0, 50, 100, and 150 mM NaCl prepared in half strength Hoagland solution. The parameters like number of leaves per plant and total leaf area per plant declined with salinity. Salt tolerance sequence, on the basis of 50 % loss of leaf area per plant over control, of the cultivars in hand was as - Cv. Buster > Cv Extra Sweet > Cv Honey Graze. Fifty per cent reduction in growth, in terms of dry weight of seedling phytomass, corresponded with 82.895, 82.089, and 72.65 mM NaCl in cultivars Extra Sweet, Honey Graze and Mr. Buster, respectively. Salt tolerance sequence, on the basis of 50 % losses of seedling phytomass over control, of the cultivars in hand was as - Cv. Honey Graze ≈ Cv Extra Sweet > Cv. Mr. Buster

The relative turgidity of the plants remained unchanged under salinity treatments. Overall photosynthetic pigments were reduced significantly although chlorophyll – a remained statistically unchanged in concentration. Carotenoids level was reduced under salinity. The total soluble sugar contents in treated plants appeared not to vary with salinity. Protein contents declined. Although proline contents increased by 23.5% in Cv Mr. Buster under high salinity; in other varieties proline declined with salinity. There was an increase of phenolic contents up to 34.66 % in Honey Graze and 11.92% in Mr. Buster at NaCl concentration of 150 mM. The phenols, however, declined in Extra Sweet (6.14% in 50 mM NaCl and 19.67% in 100 mM NaCl. The electrolyte leakage from leaves of three varieties didn't vary significantly among the varieties and under the salinity. Sodium, Potassium and chloride ions increased greatly with NaCl concentration. The results are discussed in eco-physiological context.

Key-words: Sorghum cultivars, Mr. Buster, Honey Graze, Extra Sweet, salt tolerance, Physiological parameters of growth.

INTRODUCTION

Salt stress in soil is one of the major stresses especially in arid and semi arid regions and can severely limit plant growth and productivity (Parvaiz and Satyawati, 2008).One of the reason of salinity is the high concentration of cations such as sodium, calcium and magnesium whereas chloride, phosphate and nitrate as anions. The effect of salinity on plant growth is a complex phenomenon that involves osmotic stress, ion toxicity, mineral deficiencies, physiological and biochemical perturbations (Hasegawa *et al*., 2000).

Salinity affects plants in different ways such as osmotic effects, specific-ion toxicity and/or nutritional disorders (Laüchli and Epstein, 1990). The extent by which one mechanism affects the plant over the others depends upon many factors including the species, genotype, plant age, ionic strength and composition of the salinizing solution, and the organ in question. Plants undergo characteristic changes from the time salinity stress is imposed until they reach maturity (Munns, 2002a). Sorghum is a moderately salt-tolerant species (Greenway and Munns, 1980). Krishnamurthy *et al*., (2003) at ICRISAT have identified some elite sorghum varieties and improved lines promising for agronomic traits and also having better salinity tolerance in a series of pot culture experiment. Kulhari *et al.* (2008) tested 100 germplasms of *Sorghum bicolor* under a series of saline stress. Three genotypes- Raj 27, Raj 30 and Raj 4 were identified as salinity tolerant and stable ones. Reddy *et al.* (2010) have screened 27 hybrids and 26 varieties for their salt tolerance. Nawaz *et al.* (2010) tested two cultivars, Live Brand (V2) and Myco India (V2) at 50 mM and 100 mM levels of proline against NaCl (100 mM). Recently, Tabatabaei *et al.* (2012) tested seven elite lines (KFS1, KFS2, KFS3, KFS4, MFS1, MFS2 and LFS56) for their forage production under irrigation with water of 2 and 11 $dS.m^{-1}$. Kausar *et al.* (2012) reported Sorghum lines JS-2002 and Sandalbar to be tolerant to salinity, lines Hegari-sorghum and JSA-263 to be medium tolerant, lines Noor as medium sensitive and FJ-115 and PSV-4 as sensitive one to salinity. El-Naim *et al.* (2012) reported sorghum cultivar Wad Ahmed to be salt tolerant and CV Arfagadamak and Butana to be salt sensitive. Chauhan *et al*. (2012) reported CSV-15, HD-19 and HC-171 to be salt tolerant. The inter-cultivar salinity tolerance in sorghum, therefore, appears quite variable.

The aim of the present studies was to gain a fundamental biological understanding and knowledge of salt tolerance in three sorghum genotypes of Australian origin - Mr. Buster, Honey Graze and Extra Sweet, by collecting

data on physiological parameters of growth and biochemical and mineral parameters to assess their salinity tolerance.

MATERIALS AND METHODS

The three cultivars of *Sorghum bicolor* (L.) Moench. Viz., Cv. Mr. Buster, Extra Sweet, and Honey Graze, were evaluated for their salinity tolerance. Fungicide treated grains (seeds) of the cultivars were provided by the Dr. M. Qasim Khan, Seed Certification Department, Karachi. The seed weight 100 seeds for each cultivar were found to distribute in differentially-negatively skewed manner in cultivars – Extra sweet and Honey Graze and distributed symmetrically and normally in cultivar Mr. Buster. The mean seed weight varied substantially among the cultivars. The mean seed mass was the lowest (25.38 ± 4.11 mg) in cv. Extra Sweet, medium (29.71 ± 0.629 mg) in Honey graze and largest (35.62 ± 0.5713 mg) in cv. Mr. Buster. The seeds for testing growth were, therefore selected from the mid-region of the area of the distributions (not shown here) i.e. between 24 – 28 mg of weight in cv. Extra Sweet, 27 – 35 mg in cv. Honey Graze and 33 – 39 mg in cv. Mr. Buster.

Sand Culture Experiment

The present work was conducted during July- September 2012 in the green house of the Biosaline Research Laboratory, Department of Botany, University of Karachi.

Preparation of Pots

The sand was collected from sand dunes of Sandspit, Karachi. The sand was passed through a 2 mm sieve to remove gravels and other materials. The sand was washed with acid solution and then 5-6 times with running tap water in order to make it free from all nutrients and minerals. Approximately, one Kg of this washed sand was filled in plastic pots measuring 10 cm in diameter and 15 cm in height. At the bottom of pots holes were made for the purpose of absorption of nutrients and water. A filter paper was placed at the bottom of pots. Four replicate pots for a treatment were placed in plastic tray containing irrigation medium.

Preparation of irrigation medium

A modified Hoagland solution was prepared according to Epstein (1972). The composition of modified Hoagland solution is given in Table 1. Different NaCl solutions (50, 100 and 150 mM) were prepared in Hoagland solution. The control solution had 0 mM NaCl. The average pH and EC of irrigation medium is given in Table 2.

The seeds of three cultivars were imbibed for 30 min in distilled water. The seeds were germinated for four days into sterilized Petri plates moistened with distilled water. Four seedlings of similar sizes were selected and transplanted into pots at nearly equal distance. The seedlings were initially irrigated with ¼ strength Hoagland solution for ten days and the solution was replaced after three days interval. Then seedlings were irrigated with ½ strength Hoagland solution two times at the interval of three days. The irrigation medium was changed to full strength Hoagland solution and the seedlings were subjected to pre-conditioning for salinity treatment after their establishment.

In order to avoid the shock effects of saline irrigation, the plants were pre-conditioned by increments of 25 mM NaCl per irrigation up to the desired salinity levels. The pre-conditioned seedlings were continued to their respective NaCl treatment. They were irrigated ten times at the interval of three days. The control and treatments consisted of four replicates. Three seedlings of each pot were used for growth analysis while fourth seedling was used for relative water content and relative turgidity of leaves, electrolyte leakage and biochemical analysis.

Growth analysis

Plants were harvested after one month of desired NaCl treatment and different growth parameters were analyzed including number of leaves per plant, leaf area, fresh and dry weight of root and shoot. For dry weights, the root and shoots were dried at 60 ˚C for 48 h in oven.

Leaf Area

For leaf area determination, ten leaves of each cultivar were taken randomly from separately grown plants. Sorghum leaves are elongated and narrow at both ends. The length (L) and maximum breadth (B) of these leaves was measured with a scale of 1mm least count and the area of leaf was determined graphically (Khan, 2009). Then the value of *k* was determined for each cultivar by the following formula, $k = A / (L \times B)$.

The average *k* value for ten leaves was calculated. The calculated value of k was 0.62 for cultivars Extra Sweet and Honey Graze and 0.55 for Cv. Mr. Buster. The k values were used for the determination of total leaf area of a seedling. The leaf area for n leaves of seedling was measured as:

$$\text{Leaf area / seedling} = \sum_{i=1}^{n} (L \times B \times k)$$

Table 1. Composition of modified Hoagland nutrient solution.

Compounds		Concentration of stock solution (mM)	Volume of stock solution per liter of final solution (ml)
Macronutrients	KNO_3	1000	6
	$Ca(NO_3)_2 . 4H_2O$	1000	4
	KH_2PO_4	1000	2
	$MgSO_4 . 7H_2O$	1000	1
Micronutrients	KCL	25	2
	$H3BO_3$	12.5	
	$MnSO_4 . H_2O$	1.0	
	$ZnSO_4 . 7H_2O$	1.0	
	$CuSO_4 . 5H_2O$	0.25	
	MoO_3	0.25	
Fe Na EDTA		64	1

Table 2. The Average EC and pH of the irrigation medium.

NaCl solution (mM)	EC ($dS.m^{-1}$)	pH
0	2.1	7.60
50	8.2	7.12
100	14.2	7.16
150	21.3	7.07

$EC = 1.91 + 0.1271$ (mM NaCl) – $R^2 = 0.9993$

Relative water content OR Relative Turgidity

For the determination of relative water content (RWC), fully expanded leaf was excised from one plant of each pot. The dust particles were removed. The leaf samples were immediately weighed to take the fresh weight (FW) and then immersed in distilled water at 4 °C for 10 hours. The saturated leaf samples were removed from water and excess water was removed by tissue paper. These leaf samples were weighed to obtain turgid weight (TW) and then dried in an oven at 60 °C for 48 h to record dry weight (DW). The RWC of leaf was determined by the following formula (Singh, 1982).

$$RWC\ (\%) = [FW - DW] / [TW - DW] \times 100$$

Electrolyte leakage

The electrolyte leakage of the leaves was determined according to the method of Sullivan and Ross (1979). One particular leaf from one plant of each pot was collected and 20 discs of 6 mm diameter were made by paper punch machine. The discs were placed in test tubes and 10 ml deionized water was added. The test tubes were shaken and first electrical conductivity value (EC_a) was determined. The test tubes were warmed at 45 – 55 ˚C on water bath for 30 min and second electrical conductivity (EC_b) was determined. Then the tubes were kept at 100 ˚C on boiling water bath for 10 min and third electrical conductivity value (EC_c) was noted. The Electrolyte leakage (%) was determined by following formula.

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

Photosynthetic pigments

The leaf samples were excised from the plants and immediately frozen in liquid nitrogen and stored at -20 ˚C until used for photosynthetic pigments. The leaf samples (0.1 g) were grounded in liquid nitrogen and then homogenized in 5 ml 80% cold acetone, centrifuged at 3000 g for 5 minutes. The supernatant was separated and the residue was again dissolved in 3 ml of 80% cold acetone and centrifuged. The process was repeated until all the photosynthetic pigments were extracted. All supernatant fractions were pooled and final volume was adjusted. The absorbance of the extracts was recorded at 649 and 665 nm for chlorophylls determination while 480 and 510 nm for carotenoids determinations, respectively. The absorbance was recorded on spectrophotometer. The chlorophyll and carotenoids contents were determined according to the equations described by Strain *et al*., (1971) and Duxbury and Yentsch (1956), respectively.

$$\text{Chlorophyll a } (\mu g/ml) = 11.63\ (A_{665}) - 2.39\ (A_{649})$$
$$\text{Chlorophyll b } (\mu g/ml) = 20.11\ (A_{649}) - 5.18\ (A_{665})$$
$$\text{Total Chlorophylls } (\mu g/ml) = 6.45\ (A_{665}) + 17.72\ (A_{649})$$
$$\text{Carotenoids } (\mu g/ml) = 7.6\ (A_{480}) - 2.63\ (A_{510})$$

The chlorophyll and carotenoids contents were expressed as $mg.g^{-1}$ fresh weight of leaves.

Biochemical analysis

Proteins

The fully expanded leaf was immediately frozen in liquid nitrogen and stored at -20 °C until use. The leaf sample (0.5 g) was grounded in liquid nitrogen and homogenized in 5 ml of ice chilled potassium phosphate buffer (pH = 7, 0.1 M) containing 1mM EDTA and 1% PVP (w/v). The homogenate was filtered through a muslin cloth and then centrifuged at 21,000 x g at 4 °C for 20 min in refrigerated centrifuge. The supernatant was separated and stored at -20 °C. The protein contents were determined by using Bradford Assay reagent method (Bradford, 1976). The proteins were determination against Bovine Serum Albumin as standard and the value of proteins was calculated from a following best-fitted standard curve equation.

Proteins ($\mu g.ml^{-1}$) = -3.29196 + 114.2755 OD ± 5.3436

($t = 16.76$, $F = 280.93$, $P < 0.0001$, $R^2 = 0.9723$)

The concentration of protein contents were mentioned in $mg.g^{-1}$ fresh weight of leaves.

Total sugars

Fresh leaf samples were boiled in 80% ethanol at boiling water bath to kill the tissues. Then leaf samples were homogenized in 80% ethanol and centrifuged at 4000 g for 10 minutes. The supernatant was separated and the residue was again extracted with 80% ethanol. Both supernatants were combined and then the volume was made up to desired level by distilled water. The extract was used for the determination of total sugars by the method of Fales (1951). The total sugars were determined were determined against glucose as standard and the total sugars were calculated from a following best-fit standard curve equation.

Total sugars ($\mu g.ml^{-1}$) = $228.462.\ OD^{0.97275} \pm 0.04455$

($t = 49.28$, $F = 2428.32$, $P < 0.0001$, $R^2 = 0.9967$)

The concentration of total sugars was expressed as $mg.g^{-1}$ fresh weight of leaves.

Phenols

Soluble phenols were determined by the method of Singleton and Rossi (1965). The fresh leaf material was homogenized in 80% methanol and centrifuged. To 1 ml of diluted extract 5 ml of Folin-Ciocalteu reagent (1:9 ratio in distilled water) and 4 ml of 7.5% Na_2CO_3 were added. The absorbance was recorded at 765 nm after incubation of 30 minutes at 25 °C. The soluble phenols concentration in leaf tissues was determined against Gallic acid and calculated from a following best-fit standard curve equation.

Phenols ($\mu g.ml^{-1}$) = $1.62724 + 94.5284\ OD - 17.19352\ (OD)^2 \pm 0.3425$

($t = 35.57$) ($t = -4.17$),

($p < 0.0001$) ($p < 0.0051$) $F = 8786.10$, $P < 0.0001$ & 0.0051, $R^2 = 0.9996$)

The concentration of total phenols was mentioned in $mg.g^{-1}$ fresh weight of leaves.

Proline

The proline contents were determined by the method of Bates *et al*. (1973). The dried leaf powder sample (0.1 g) was homogenized with 5 ml of 3% (w/v) sulphosalicylic acid and centrifuged at 5000 g for 20 minutes. Two ml of extract was transferred in caped test tube, and then 2 ml glacial acetic acid and 2 ml ninhydrin reagent (prepared by dissolving 2.5 g ninhydrin in 60 ml of glacial acetic acid and 40 ml 6 M phosphoric acid) were added. The mixture was boiled for 1 hour at 100 °C, cooled and then 4 ml of toluene was added to each tube and vortex. Two layers were appeared, the chromophore layer of toluene was removed and their absorbance was recorded against reference blank of pure toluene. The proline concentration was determined from a predictive equation of the standard curve prepared from extra pure proline from Sigma.

Proline (microgram / 2 ml) = -0.740092 + 16.60767 (OD520) ±0.54031

$t = 35.07$

$p < 0.00001$

$F = 1230.16$ ($p < 0.00001$)

Mineral analysis:

The mineral ions in leaf samples were determined according to the method of Chapman and Pratt (1961). The leaves of the plants were dried at 60 ˚C for 48 h. The dried leaves (100 mg) were powdered and transferred into

porcelain crucibles. The crucibles were placed in a muffle furnace at 550 ˚C for 6 h. The ash was dissolved in 5 ml of 2 N HCl. After 20 min the solution was diluted with deionized water. This solution was filtered through a Whatman No. 1 filter paper and the concentrations of Na^+ and K^+ ions were determined with flame photometer. The best-fit standard curve equations are as follows:

Na (ppm) = $0.016135.X^{1.879824} \pm 0.04433$
($t = 49.528$, $F = 2453.01$, $P < 0.0001$, $R^2 = 0.9968$)

K (ppm) = $0.244346.X^{1.314603} \pm 0.04433$
($t = 29.47$, $F = 868.54$, $P < 0.0001$, $R^2 = 0.9909$)
Where X = Reading on the flame photometer.
The concentration of Na and K ions were expressed as $meq.g^{-1}$ dry weight of leaves.

Chlorides

One hundred mg dried leaf powder was dissolved in 20 mL deionized water. The solution was boiled for one hour. For Cl determination , 100 µL of hot water extract, 4 mL of acid reagent (900 mL deionized water, 6.4 mL conc. Nitric acid and 100 mL of glacial acetic acid) were taken in vial. Four drops of gelatin reagent (0.62 % boiling water) were also added in it. The concentration of Cl ion in the solution was determined by silver nitrate precipitation with chloridometer (HBI, model No. 4425150).

RESULTS AND OBSERVATION

Seedling growth in terms of phytomass in Sand Culture

In pot culture experiment the plants were treated with three salinity levels (50, 100 and 150 mM NaCl) after pre-conditioning them to the required salinity. The experiment was continued up around 60 days when plants were harvested. The plants of all cultivars irrigated with 150mM NaCl died after 48 days of life. The experiment was thus delimited to two salinity levels only (50 and 100 mM NaCl). The shoot and root weight of the seedlings declined progressively with salinity (Fig. 1). Two-way ANOVA indicated that main effects on shoot dry weight were significant for salinity only ($F=13.03$, $p < 0.0001$). The varietal effects were insignificant ($F=2.56$, $p < 0.09$). The variety x salinity interaction was also insignificant ($F=2.63$, $p < 0.057$). The varietal as well as salinity effects were significant in case of root weights of the seedlings ($F=20.38$, $p < 0.0001$ and $F= 3.80$, $p < 0.035$, respectively). The interaction of the two factors was also significant ($F=2.56$, p, 0.062).

The seedling growth of the three selected cultivars in terms of dry mass accumulation per seedling declined progressively substantially in 50 and 100 mM NaCl salinity (Fig. 2) as compared to the control and was well represented by the following models.

Cv. Mr. BUSTER
Seedling growth (g) = 1.83083 – 0.012600 (mM NaCl) ± 0.8457
$t = -2.21$
$p < 0.0501$
$F = 4.439$, $p < 0.0501$; $R^2 =$ 0.0.3070, $N = 12$

Cv. HONEY GRAZE
Seedling growth (g) = 4.0275 – 0.037150 (mM NaCl) ± 1.354
$t = -3.880$
$p < 0.0031$
$F = 15.055$, $p < 0.0031$; $R^2 =$ 0.6008, $N = 12$

Cv. EXTRA SWEET
Seedling growth (g) = 2.3957 – 0.014592 (mM NaCl) ± 060999
$t = -3.080$
$p < 0.0116$
$F = 9.49$, $p < 0.0116$; $R^2 =$ 0.4869, $N =12$

There was comparatively higher reduction of seedling growth over control in variety Honey Graze (-83.76 %) than other varieties which showed more or less equal reduction in 100 mM NaCl (-61.64) in Mr, Buster and -63.78% in Extra sweet) (Table 3). Two-way ANOVA indicated the variety effects to be insignificant ($F = 3.087$, $p < 0.062$) and the salinity effects to be significant ($F = 16.31$; $p < 0.00001$). The interaction of variety and salinity was also significant ($F = 2.90$; $p < 0.0405$).

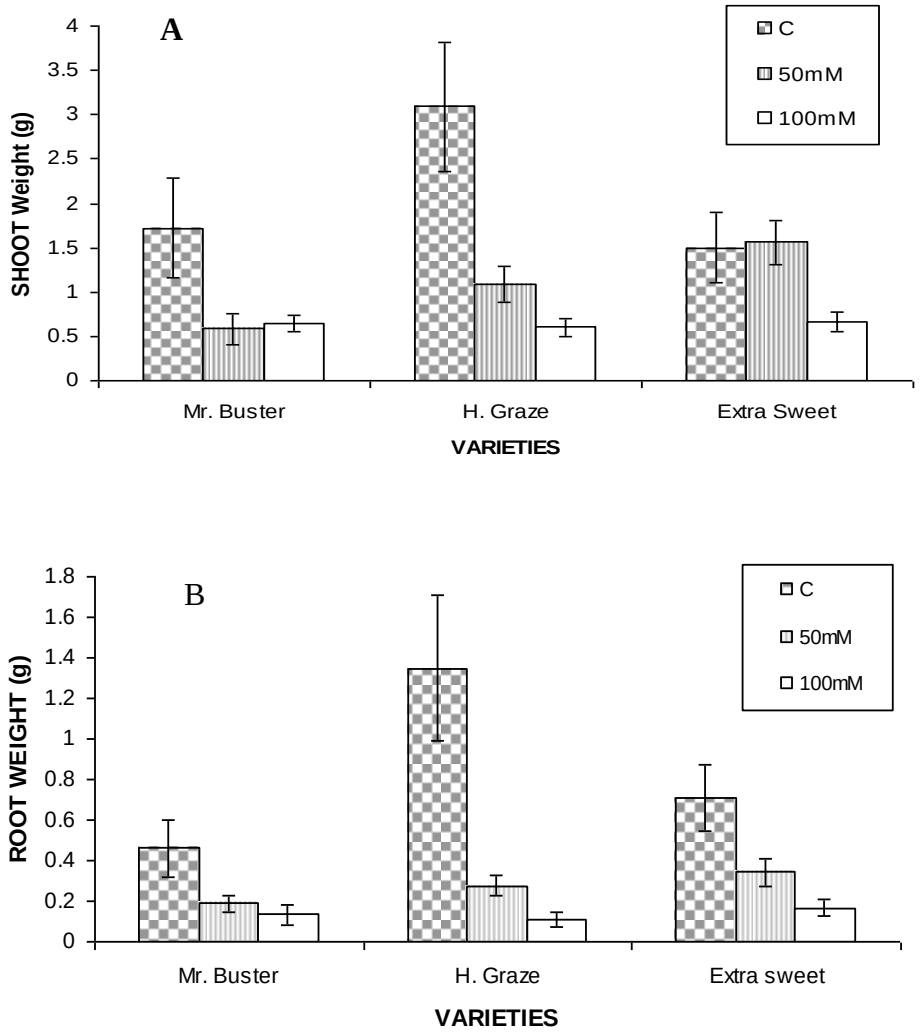

Fig. 1. Effect of saline irrigation on shoot (A) and root (B) dry wt. (g) of three sorghum varieties in a sand Culture experiment; C, Control.

Table 3. Salinity induced reduction over control in seedling growth of three sorghum cultivars.

Treatments	Cv. Mr. Buster	Cv. Honey Graze	Cv. Extra Sweet
50mM NaCl	-48.79	-69.45	-18.78
100 mM NaCl	-61.614	-83.76	-63.78

Salinity effects on number of leaves per seedling

The number of leaves per seedling (Fig. 3A) was the function of salinity in all the three cultivars. Salinity reduced the number of leaves significantly ($F = 33.39$; $p < 0.00001$). The varietal effects on number of leaves per seedling were insignificant ($F = 3.09$; $p < 0.5993$).

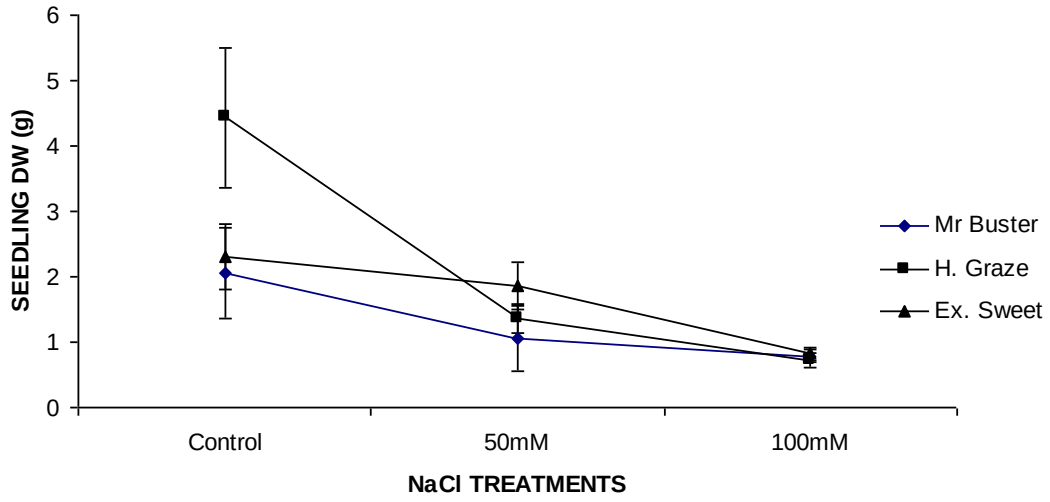

Fig. 2. Effect of saline water irrigation (NaCl) on seedling growth of three Australian cultivars of Sorghum in pot experiment.

Total leaf area per plant

The leaf area of the three varieties reduced under salinity treatments. Such a decline in leaf area was more pronounced in variety Honey Graze followed by Extra Sweet (-78.9 and 68.78%, respectively) under 100 mM NaCl concentration (Fig, 3B; Table 4). Two way ANOVA rated salinity effects to significant ($F = 19.51$; $p < 0.00001$) but the varietal effects insignificant ($F = 1.77$; $p < 0.1887$).

Relative Turgidity

Figure 4 represents the effects of saline treatments on relative turgidity of the three varieties. Maximum reduction of 29.28% was observed in Cv. Mr. Buster followed by a reduction of 21.22% in CV Extra Sweet (see also Table 5). The relative turgidity declined by only 10.54% over control in CV Honey Graze while growing in 100 mM NaCl concentration. At low salinity relative turgidity showed an increase of 18.10 % over control in CV. Honey Graze. Salinity effects on relative turgidity were insignificant ($F = 1.24$; $p < 0.3045$) but the varietal effects were significant ($F = 8.2774$; $p < 0.0016$). Mr. Honey Graze was thus more competitive cultivars in maintaining hydrature under saline conditions **(**Table 5).

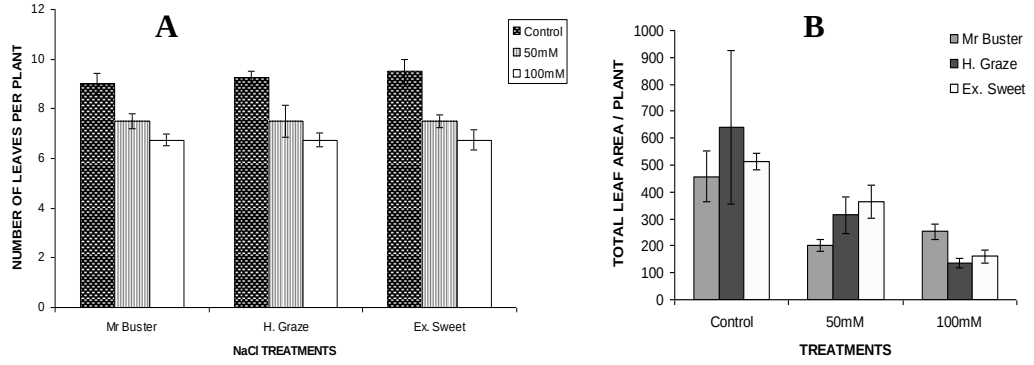

Fig. 3. Effect of NaCl salinity on number of leaves (A) and total leaf area per seedling (cm^2) (B) in three sorghum cultivars when grown in pots

Table 4. Salinity induced reduction / promotion over control in leaf area of seedlings irrigated with saline (NaCl) water

Treatment	Cv. Mr. Buster	Cv. Honey Graze	Cv. Extra Sweet
50 mM NaCl	-55.72	-50.86	-28.90
100 mM NaCl	- 44.73	-78.90	-68.78

Chlorophyll and carotenoids

Table 6 represents the contents of photosynthetic pigments in three cultivars growing under salinity. The decline in chlorophyll-a was due to varietal reasons and not salinity ($F=11.67$, $p < 0.001$ and $F=1.76$, $p < 0.019$, respectively). Chlorophyll-b didn't vary in concentration as a result of salinity ($F = 6.42$, $p < 0.004$). Total chlorophyll concentration remained unaffected with varietal effects but declined with salinity reasons ($F= 8.23$, $p < 0.0001$ and $F=1.73$, $p < 0.199$). The carotenoids contents were found to reduce with salinity significantly (Table 6). The reduction due to varietal effects was not significant at all ($F = 0.2161$; $p < 0.8070$).

Total Sugars

The total soluble sugar contents in treated plants appeared not to vary with salinity (Fig. 5). Although sugar level declined by 17.54% in Honey Graze and 22.27% in Extra Sweet (Table 7), the ANOVA, however, indicated no significant effects due to varietal or salinity reasons ($F=1.07$, $p < 0.358$ and $F=1.48$, $p < 0.241$).

Proteins

Protein contents declined with salinity in Mr. Buster up to 44.75% in 100 mM NaCl (Fig. 6). In other two varieties protein contents increased with salinity up to 27.10 % in Honey Graze and up to 71.34% in Extra Sweet at 100 mM concentration (Table 8). The main effect to such a variation was salinity, of course, marginally ($F = 1.042$; $p < 0.055$). The variety effects were not significant.

Phenol contents

Figure 7 represents the total phenol contents of the varieties under salinity. There was an increase of phenolic contents up to 34.66 % in Honey Graze and 11.92% in Mr. Buster at NaCl concentration of 50 mM. The phenols, however, declined in Extra Sweet (-6.14% in 50 mM NaCl and -19.67% in 100 mM NaCl (Table 9**).** Two-way ANOVA indicated significant varietal differences ($F = 7.50$; $p < 0.0026$) but no significant salinity effects ($F = 1.24$; $p < 0.3047$).

Proline

Although proline contents (mg per g DW leaf increased by 46% in Mr, Buster under high salinity; in other varieties proline declined with salinity (Fig. 8, Table 10). ANOVA, however, showed no significant varietal and salinity effects ($F = 1.206$; p, 0.3151 and $F = 0.36$; $p < 0.6978$, respectively).

Electrolyte Leakage

The electrolyte leakage from leaves of three varieties didn't vary significantly among the varieties and under salinity treatment (Fig. 9) and fluctuated little below or above 20%. ANOVA also did not show ant varietal or salinity effects on electrolyte leakage ($F = 0.159$; $p < 0.8537$ and $F = 1.557$; $p < 0.2289$, respectively).

Ionic contents

Na concentration increased greatly in the leaves of the three sorghum varieties especially under high salinity). Na increased by a quantum of 2768% in Honey Graze, 17.27% in Extra Sweet and 1473% in Mr. Buster (Table 11). This increase was found to be due to salinity effects ($F = 49.26$; $p < 0.0001$) and not due to varietal reasons ($F =0.6576$; $p < 0.5262$).

Similarly, K contents increased in more or less similar pattern in these varieties. Only salinity effects on K increment were significant ($F = 55.089$; $p < 0.0001$) and varietal effects were insignificant ($F= 0.3639$; $p < 0.6983$).

In all varieties tested, the chloride ion concentration increased with salinity progressively (Fig. 10**)**. There was 339.29% increase in Chloride in Mr. Buster and 253.82% in Honey Graze and 270.38% over control in Extra

Sweet (Table 12). The increase in chloride ion was attributable to salinity around the roots significantly ($F = 41.495$; $p < 0.0001$) and not to any varietal differences ($F = 2.3468$; $p < 0.1150$).

Na / K ratio in leaf didn't vary significantly with NaCl concentration in the irrigation medium (Fig. 11). The plants appeared to sodiophillic in nature as Na / K ratio remained larger than unity in all varieties and in all treatments of salinity (Table 13).

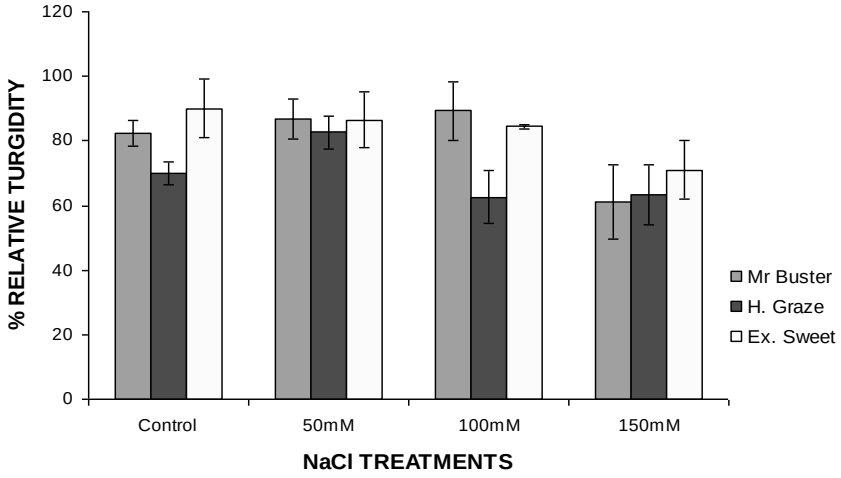

Fig. 4. Relative turgidity in leaves of three sorghum cultivars grown under NaCl salinity in pots.

Table 5. Salinity induced reduction / promotion over control in foliar relative turgidity of seedlings irrigated with saline (NaCl) water before the death of seedlings in 150 mM NaCl.

Treatment	Cv. Mr. Buster	Cv. Honey Graze	Cv. Extra Sweet
50 mM NaCl	5.30	18.10	- 4.03
100 mM NaCl	8.47	- 10.54	-6.29
150 mM	-29.28	- 9.43	-21.22

Table 6. Chlorophylls and carotenoids estimation in three cultivars of sorghum in response to the irrigation with saline water.

Treatments	Chl. A (mg/g .FW)	Chl. B (mg/g .FW)	Total chl. (A+B) Mg/g. FW)	Carotenoids (mg/g. FW)
Mr. Buster				
Control	0.2297 ± 0.044	0.1150 ± 0.036	0.3467 ± 0.065	0.2964 ± 0.036
50mM NaCl	0.2216 ± 0.038 (-3.53)*	0.1360 ± 0.068 (18.30)	0.3570 ± 0.047 (2.998)	0.3110 ± 0.033 (4.93)
100 mM	0.1978 ± 0.028 (13.89)	0.1180 ± 0.043 (2.61)	0.3160 ± 0.069 (-8.55)	0.2110 ± 0.067 (-28.81)
Honey Graze				
Control	0.2683 ± 0.013	0.1045 ± 0.0056	0.3730 ±0.013	0.3230 ± 0.013
50mM NaCl	0.3121 ± 0.037 (16.35)	0.1600 ± 0.024 (53.11)	0.4720 ± 0.056 (26.54)	0.3090 ± 0.022 (-4.33)
100 mM	0.1894± 0.014 (-29.41)	0.1270 ± 0.013 (21.53)	0.3160 ± 0.0244 -15.28)	0.2390 ± 0.022 (-26.01)
Extra Sweet				
Control	0.5220 ± 0.114	0.2020 ± 0.049	0.7096 ± 0.172	0.3570 ± 0.008
50mM NaCl	0.6640 ± 0.159 (23.37)	0.2050 ± 0.064 (1.50)	0. 8110 ± 0.229 (14.29)	0.2795 ± 0.072 (-21.71
100 mM	0.3920± 0.102 (-24.99)	0.1130 ± 0.029 (-44.06)	0.6590 ± 0.191 (-7.13)	0.1730 ± 0.045 (-51.54)

*, Figures in parenthesis denote % reduction or promotion over control.

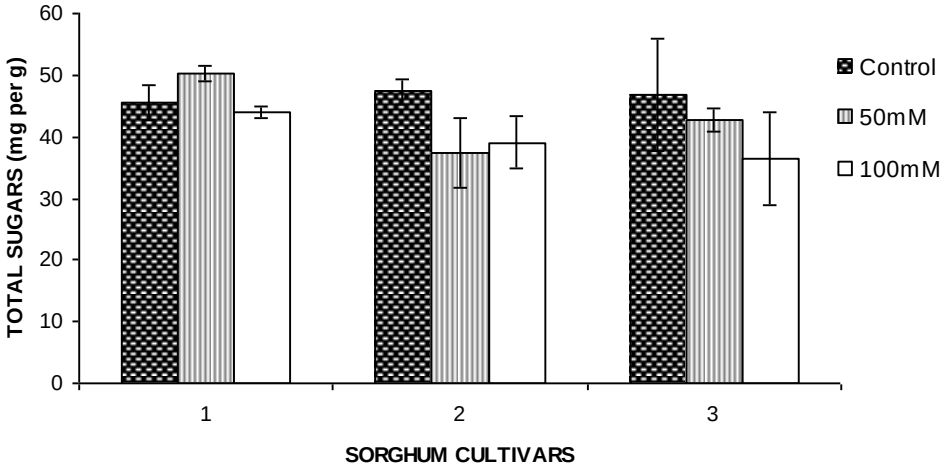

Fig. 5. Sugar contents in leaves of three Sorghum cultivars seedlings treated with NaCl salinity. Key to the acronyms: 1, Mr. Buster; 2, Honey Graze; 3, Extra Sweet.

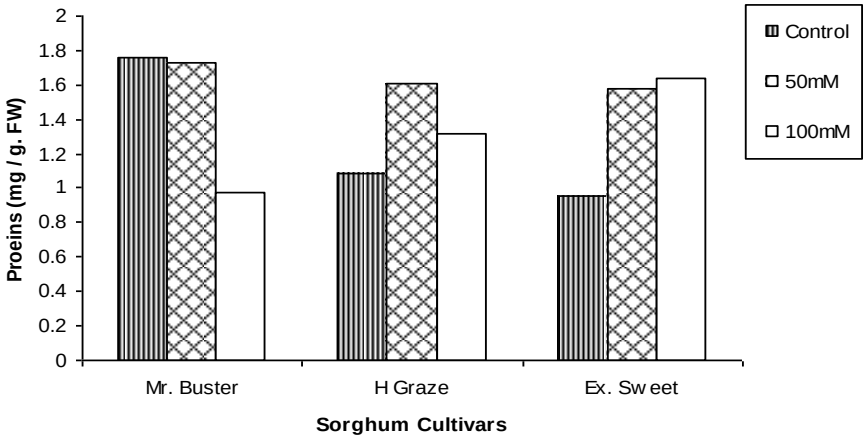

Fig. 6. Protein contents in leaves of Sorghum cultivars under saline water irrigation.

Table 7. Salinity induced reduction / promotion over control in sugar contents of seedlings irrigated with saline (NaCl) water

Treatment	Cv. Mr. Buster	Cv. Honey Graze	Cv. Extra Sweet
50 mM NaCl	10.17	-20.95	- 9.07
100 mM NaCl	3.34	- 17.54	-22.27

Table 8. Salinity induced reduction / promotion over control in protein contents of seedlings irrigated with saline (NaCl) water

Treatment	Cv. Mr. Buster	Cv. Honey Graze	Cv. Extra Sweet
50 mM NaCl	-1.704	-10.84	6.53
100 mM NaCl	-44.75	-27.10	71.34

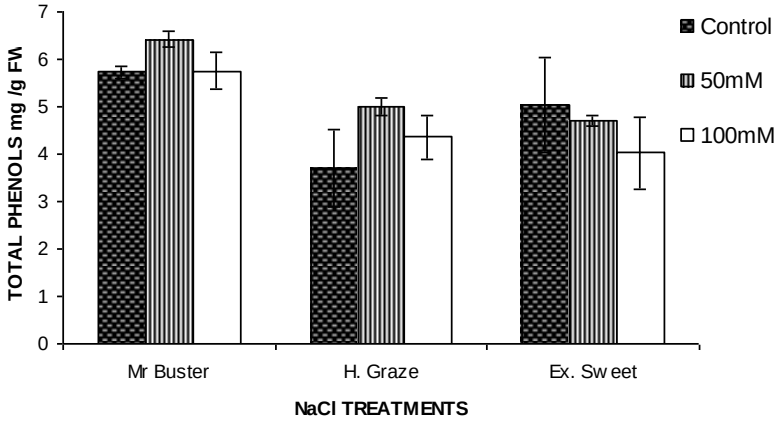

Fig. 7. Total phenol contents in leaves of three sorghum cultivars under saline water irrigation.

Table 9. Salinity induced reduction / promotion over control in phenol contents of seedlings irrigated with saline (NaCl) water.

Treatment	Cv. Mr. Buster	Cv. Honey Graze	Cv. Extra Sweet
50 mM NaCl	11.92	34.66	- 6.14
100 mM NaCl	0.192	17.62	-19.67

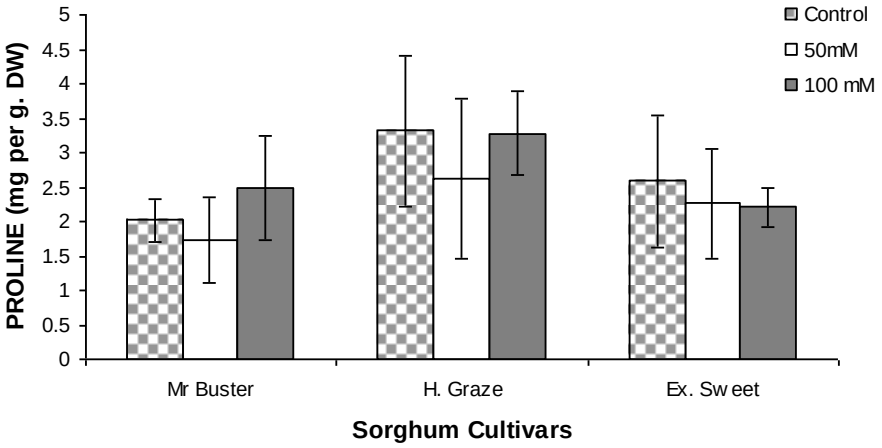

Fig. 8. Effect of saline irrigation on the proline content of three sorghum cultivars.

Table 10. Salinity induced reduction / promotion over control in proline contents of seedlings irrigated with saline (NaCl) water .

Treatment	Cv. Mr. Buster	Cv. Honey Graze	Cv. Extra Sweet
50 mM NaCl	-13.10	-20.91	-12.55
100 mM NaCl	+ 23.50	- 1.12	-14.48

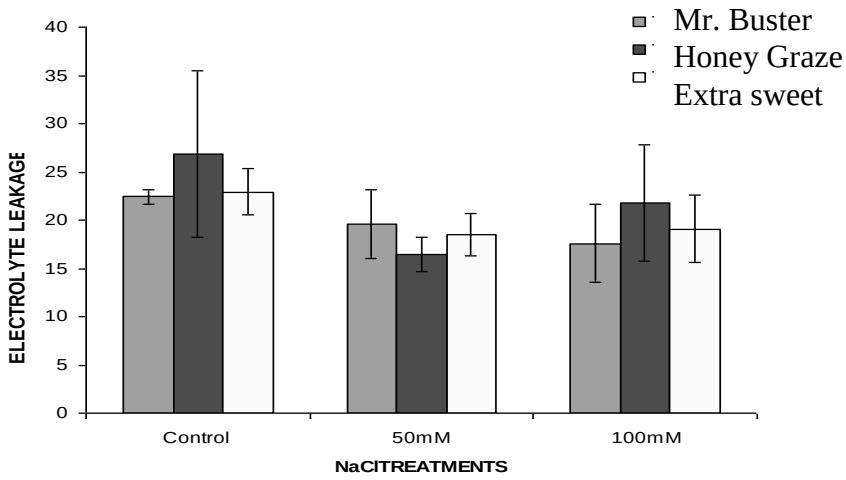

Fig. 9. Effects of NaCl salinity on electrolyte leakage from leaves. of three cultivars of *S. bicolor*.

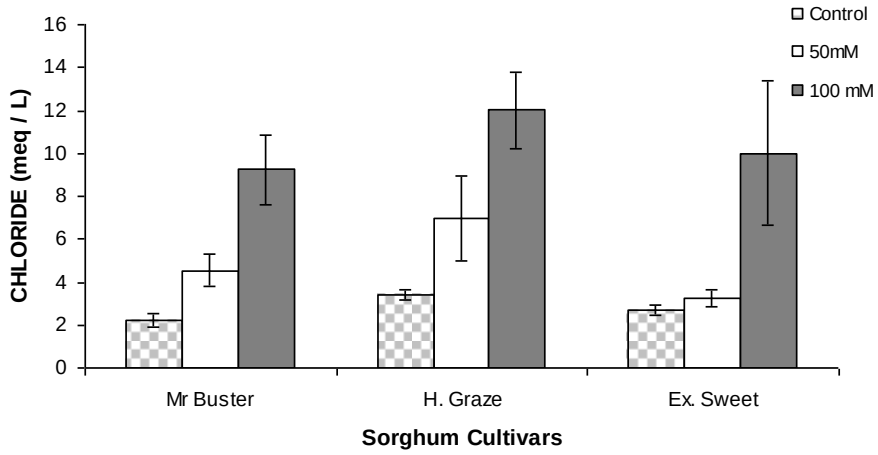

Fig. 10. Effect of saline irrigation on foliar chloride contents of three sorghum cultivars.

DISCUSSION

The experiment was conducted to observe the influence of salinity on the seedling growth and the physiological, biochemical and mineral parameters of growth in three sorghum genotypes. NaCl salinity inhibited the number of leaves, total leaf area and seedling phytomass in all the three cultivars significantly as a direct function of the NaCl concentration. A decrease in plant growth under salinity is common (Ahmad *et al.*, 1985). The reduction in leaf area under the influence of salinity has been reported in several plants – *Gossypium hirsutum* and *Phaseolus vulgaris* (Brugnoli and Lauteri, 1991), maize (Çiçek and Cakirlar, 2002), *Prosopis alpataco* (Villagra and Cavangnaro, 2005), *Catharanthus roseus* (Jaleel *et al., 2008) and wheat (*Sheldon *et al*., 2004). Growth reduction under salinity has been reported even in halophytes such as *Nitraria retusa* and *Atriplex halimus* in NaCl concentration of 400-800 mM (Boughalleb and Denden, 2011). The salt tolerance, on the basis of 50 % loss of leaf area of the plants over control, of the cultivars in hand was found to be as - Cv. Buster > Cv. Extra Sweet > Cv. Honey Graze and on the basis of 50 % loss of seedling phytomass over control, it was found to be as - Cv. Honey Graze ≈ Cv Extra Sweet > Cv. Mr. Buster. The cultivars in hand were, therefore, differentially tolerant to salinity.

The mechanisms of salt tolerance are of two main types: those minimizing the entry of salt into the plant and other minimizing the cytoplasmic concentration of salt (Munns, 2002). The later mechanism may include exclusion, succulence, transport, compartmentalization and excretion (Popp, 1995). These processes bring osmotic adjustments which is crucial for plant survival in the saline environment (Flowers and Colmer, 2008). The ability of plants to

tolerate salts involves multiple biochemical pathways that facilitates retention or acquisition of water, protect chloroplast functions and maintain ion homeostasis and scavenging of oxygen radicals (Parvaiz and Satyawati, 2008; Zielinska, 2012). The influence of abiotic stress signals on secondary metabolism in plants have been reviewed by a number of researchers recently (Ramakrishna and Ravishankar, 2011; Mane *et al.,* 2011; Aslam *et al.,* 2011; Rahdari and Hoseini, 2011).

Table 11. Foliar sodium and potassium contents in three cultivars of sorghum irrigated with saline water.
*, Figures in parenthesis denote % promotion over control.

Treatments	Na(meq / L)	K(meq / L)
Mr. Buster		
Control	0.1375 ± 0.111	0.0825 ± 0.0335
50mM NaCl	0.475 ± 0.108 (245.45)*	0.2800 ± 0.0626 (239.4)
100 mM	2.163 ± 0.48 (1473.09)	1.2675 ± 0.2792 (1436.4)
Honey Graze		
Control	0.0875 ± 0.013	0.0525 ± 0.0085
50mM NaCl	0.4725 ± 0.1765 (440)	0.2800 ± 0.104 (433.33)
100 mM	2.51 ± 0.552 (2768.6)	1.480 ± 0.324 (2719.04)
Extra Sweet		
Control	0.1100 ± 0.034	0.0675 ± 0.0213
50mM NaCl	0. 400 ± 0.100 (263.64)	0.2175 ± 0.0755 (222.22)
100 mM	2.01 ± 0.22 (1727.3)	1.185 ± 0.1256 (1655.5)

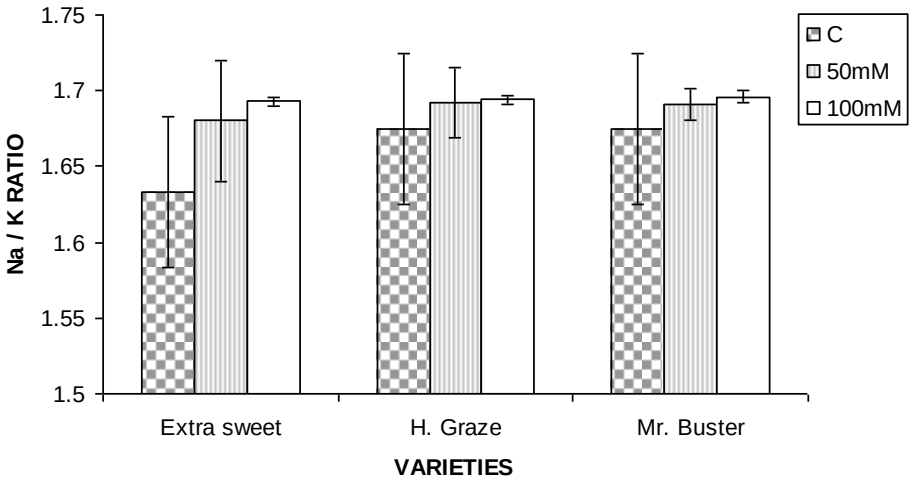

Fig. 11. Effect of saline irrigation on Na / K ratio in leaves of three sorghum varieties.

Table 12. Salinity induced reduction / promotion over control in chloride contents of seedlings irrigated with saline (NaCl) water.

Treatment	Cv. Mr. Buster	Cv. Honey Graze	Cv. Extra Sweet
50 mM NaCl	+ 116.67	+ 104.41	+ 21.30
100 mM NaCl	+ 339.29	+ 253.82	+ 270.38

Table 13. *Per cent* promotion of Na / K ratio in leaves of three sorghum varieties under saline irrigation.

Treatments	Extra Sweet	Honey Graze	Mr. Buster
50mM NaCl	+ 2.86	+ 1.10	+ 0.96
100 mM NaCl	+ 3.69	+ 1.13	+ 1.25

In our experiment the salinity effects were relatively insignificant to influence relative turgidity of the treated plants up to 100 mM NaCl. The variation in turgidity was due to the varietal reasons. Salt stress is reported to bring cellular dehydration which causes osmotic stress and removal of water from cytoplasm resulting in reduction of the cytosolic and vacuolar volumes (Ramakrishna and Ravishankar, 2011). Statistically, no significant difference was observed in relative turgidity in the tested sorghum cultivars. However, at low salinity, moisture content relatively increased in Honey Graze and Mr. Buster and at higher salinity there was some dehydration in all cultivars.

There was decline of photosynthetic pigments, particularly chlorophyll-a and carotenoids in Sorghum cultivars under salinity. A decrease in plant growth and chlorophyll contents have been reported in even halophytes such as *Nitraria retusa* and *Atriplex halimus* in NaCl concentration of 400-800 mM NaCl. In present studies chlorophyll- b didn't vary against salinity but decrease in chlorophyll-b is often reported (Ahmad *et al.,* 1985) which is suggested to be due to inhibition of iron-containing enzymes which activates the biosynthesis of chlorophyll (Rubin and Artiskhovaskaya, 1964). Anthocyanins are reported to increase under salinity (Parida and Das, 2005). In salt sensitive species anthocyanins, in contrast, are reduced (Daneshmand *et al*., 2010). Relatively better salt tolerance of *N. retusa* has been suggested to be related to higher carotenoids accumulation in *N. retusa* (Boughalleb and Denden, 2011).

The electrolyte leakage is greatly increased on exposure of plants to high salinity (DKhil and Denden, 2012; Kaya *et al.,* 2001a and b). No significant increase in electrolyte leakage was observed in the selected *Sorghum* cultivars indicating that up to 100mM NaCl, the cell membrane permeability in these cultivars was not significantly affected.

There was increase of total sugar content in Cv. Mr. Buster but it declined in Cv. Honey Graze and Cv. Extra Sweet. There are several reports where increase in sugar concentration is observed, particularly at lower salinities (Rozema, 1978; Ahmad *et al.,* 1987; Khan and Ahmad, 1998, 2002). Total sugars content has been reported to increase in *Medicago arborea* (Boughalleb *et al.,* 2011). The decrease in sugar content has, however, also been reported in *Melia azedarach* under saline conditions by Ahmad *et al*. (1985). It seems that the translocation of sugar to roots is more efficient in cultivar Honey Graze and Cv Extra Sweet. Also the breakdown of complex molecules occurs rapidly for the supply of metabolic energy for growth processes. The translocation of sugars should provide more respiratory substrate to roots and energy yielding products thus could control the ion-fluxes during mineral uptake (Chimiklis and Karlander, 1973). Rozema (1978) reported larger increase in sugar concentration under salinity stress in relatively salt sensitive species, *Juncus alpinoarticularis* ssp. *articappilus*. Shannon and Qualset (1984) reported that accumulation of sugar in leaf is generally larger in salt excluding plants. Khan (1998) reported significant promotion in sugar accumulation in salt excretive *Sporobolus arabicus*. Relatively salt tolerant legume, *Indigofera oblongifolia* (reducing growth by 50 % at ECiw: 12.05 ± 0.92 $dS.m^{-1}$) also showed increase of sugar level in leaves, which became fleshy with age under saline environment (Khan and Ahmad, 1998). A moderately salt tolerant grass *Panicum turgidum* with tendency of excluding Na from shoot, on the other hand, showed substantial decrease in foliar sugar level under salinity (Khan and Ahmad, 2007). However, it is certain that sugars not only serve as resource food materials but also serve as cellular osmoticum (Shannon, 1984; Jeffereies *et. al*., 1979), besides proline, glycinebetaine and other organic solutes.

A regards protein-amino acid metabolism, though protein content remained quite unaffected, the proline level increased (23.5% over control) under low salinity in Cv. Mr. Buster and it declined in Cv. Extra Sweet and Cv. Honey Graze. The benefits of increased proline may be thought to be available to Cv. Mr. Buster. The accumulation of proline has been reported under different stressful conditions and its accumulation in saline environment (Strogonov, 1964; Rozema, 1978 ; Rains *et al.,* 1982; Joshi *et al.,* 2005) is considered beneficial for plant growth (Rozema, 1978 ; Rains *et al.,* 1982; Nawaz *et al.,* 2012). Total proline contents were reported to increase in *Medicago arborea* by Boughalleb *et al.,* 2011). Azooz (2004) has, however, reported decrease in proline content in *Sorghum* cultivars Hagen Shandawil and Giza 113. It increased in cv. Dorado as a result of salinity stress. Aziz *et al.* (1998) reported correlation between proline accumulation and salt tolerance in *Lycopersicon esculentum* and *Aegiceras corniculatum*. Petrusa and Winicov (1997) had demonstrated that salt tolerant alfalfa plants rapidly doubled their proline contents in roots whereas such increase in salt-sensitive plants was slow. Proline accumulation

may take place either due to protein degradation or inhibition of proline conversion under salinity (Singh *et al.,* 1973). It is assumed that proline increases the protein solubility (Schobert and Tschesche, 1978), it is compatible in permeability to cytoplasm and prevents the dehydration of enzymes and other essential structures (Gorham *et al.,*,1981), it controls the ion-fluxes (Stewart and Lee, 1974) and regulates the intracellular Na distribution and storage of nitrogen (Jeffereies, 1980; Ahmad *et al.,* in Jeschke, 1984).

Na, K, and Chloride contents increased greatly under salinity in all the in hand cultivars. Na / K ratio being more than one indicated their sodiophillic nature. The results on ionic contents in *Sorghum* are varying but quite correlative with their salt tolerance. Yang *et al.* (1990) have reported Na / K ratio to be lower in halophytic *Sorghum halepense*. Greater growth reduction observed in *S. bicolor* was associated with higher level of chloride ion and higher Na / K ratio. Na exclusion mechanism was more apparent in *S. halepense* but slow in *S. bicolor* (Yang *et al.,* 1990). Increase in NaCl concentration resulted in increase of Na and decrease in K in rice (Amirjani, 2010). Leaves are more vulnerable than roots to Na because Na and Cl more accumulate in shoots than in roots (Tester and Davenport, 2003)- even in halophytes (Boughalleb and Denden, 2011). Higher levels of Na or Na / K ratio can disrupt various enzymatic processes in cytoplasm. Several studies suggest that the plasma membrane may be the primary site of salt injury (Mansour, 1997). Non-electrolytes and water permeability g*et al*tered markedly altered upon salt exposure.

Salt effects are the combined result of the complex interaction among different morphological, physiological and biochemical processes (Munns *et al.,* 2006). Sorghum is considered to be moderately salt tolerant to salinity to be almost 6.8 $dS.m^{-1}$ (Greenway and Munns, 1980). As regards to the salinity of the irrigation medium in the present investigations, the in hand cultivars appeared to tolerate NaCl salinity 72 – 82 mM NaCl concentration corresponding ECe ranging from 11.14 to12.45 dS.m-1 as per criterion of 50% reduction in phytomass. While growing under saline irrigation all these sorghum cultivar suffered from higher Na concentrations in leaf. To effectively counteract the toxic and osmotic effects of increased Na level, the role of vacuole as hypothesized by Jennings (1968) could be of utmost physiological significance in these cultivars. Under saline conditions sequestration of Na in vacuole i.e., intracellular compartmentalization of cations and Na -K exchange at cellular membrane are known processes in many halophytes and glycophytes as well (Jeschke, 1984). Furthermore, under such conditions the increased concentration of proline could not only prevent dehydration and degradation of enzymes and proteins within cytoplasm counteracting the osmotic effects of the increased vacuolar sap, but also could be important in regulating the intracellular Na distribution (cf. Ahmad *et al*., in Jeschke, 1984). At higher salinity of 150 mM NaCl, the effects of Na accumulation in the selected sorghum cultivars, as are observed here, are highly toxic and results in mortality of plants. Ionic effects bring accumulation or reduction of specific secondary metabolites (Mahajan and Tuteja, 2005) such as phenols which are known to increase under stressful conditions and help plants to bring osmotic balance. In present studies, there was an increase of phenolic contents up to 34.66 % in Honey Graze and 11.92% in Mr. Buster at NaCl concentration of 50 mM. The phenols, however, declined in Extra Sweet (-6.14% in 50 mM NaCl and -19.67% in 100 mM NaCl**.** The variation was significant due to varietal differences. The benefits of increased phenol contents may be thought to be available to Cv. Honey and Cv. Mr. Buster. Boughalleb and Denden (2011) have reported the role of higher polyphenol content in better salt tolerance of *Nitraria retusa*.

The physiological parameters such as seedling phytomass, number of leaves per seedling, leaf area per seedling, total chlorophyll, carotenoids, protein, Na, K, ratio and Chloride contents varied in the plants due to salinity reasons and not due to varietal differences. Relative turgidity, chlorophyll and total phenol contents varied due to varietal differences. Parameters such as chlorophyll b, sugar level in leaves and electrolyte leakage didn't vary at all (neither due to salinity nor varietal reasons). Proline generally declined under salinity but increased substantially in cultivar Mr. Buster. Taken together the results, the three cultivars of *Sorghum bicolor* in hand appear to be somewhat equally salt tolerant of lower level - of course not of the rank of *Sorghum halepense* (cf. Yang *et al.,* 2011). However, Cv. Honey Graze and Mr. Buster have a slight edge over cv. Extra sweet which may be attributed to its better capabilities to maintain osmoticum balance with such processes as production and accumulation of proline, and other secondary metabolite like phenols.

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