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SALT TOLERANCE OF THREE SORGHUM CULTIVARS DURING GERMINATION AND EARLY SEEDLING GROWTH

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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 germination was assayed against a series of NaCl concentrations (0, 100, 200, 300, 400, and 500 mM NaCl) in Petri plates. Seedling growth was tested in a sand culture experiment in pots under saline irrigation with 50, 100, and 150 mM NaCl prepared in half strength Hoagland solution. Germination of all cultivars was inhibited as a direct function of salinity. Salinity reduced the germination velocity relatively in higher magnitude in case of CV. Honey Graze. Fifty per cent reduction in final germination corresponded with 421.57, 335.57 and 327.46 mM NaCl in CV Mr. Buster, CV Extra Sweet and CV Honey Graze., respectively. The parameters like seedling phytomass, number of leaves per plant and total leaf area per plant declined with salinity. Fifty per cent reduction in growth, in terms of dry weight of seedling phytomass, corresponded with 82.9, 82.1, and 72.7 mM NaCl in CV Extra Sweet, CV Honey Graze and CV Mr. Buster, respectively. Obviously, sorghum appeared to be more tolerant at germination phase. Growth phase is more susceptible to the salinity in the root zone. Salt tolerance sequence, on the basis of 50 % germination loss over control, of the cultivars in hand was –

Cv. Mr. Buster > CV Extra Sweet > CV. Honey Graze.

Salt tolerance sequence, on the basis of 50 % loss of seedling phytomass over control, of the cultivars in hand was- Cv. Honey Graze ≈ CV Extra Sweet > CV. Mr. Buster

Key Words: Sorghum Cultivars – CV Mr. Buster, CV Honey Graze, CV Extra Sweet; NaCl salinity, Germination, Early Seedling Growth.

INTRODUCTION

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 experiments. 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, line 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) experimented with 13 cultivars of Sorghum and found CSV-15, HD-19 and HC-171 to be salt tolerant. The salinity tolerance in sorghum, therefore, appears quite variable.

Higher degree of salt tolerance in crops is needed to sustain food production in many regions in the world in irrigated agriculture. The focus of this experiment was to understand salinity tolerance in three selected sorghum cultivars (viz. Mr. Buster, Honey Graze and Extra Sweet) at the whole plant level so that to underpin future applications of in hand genotypes. Germination being a crucial stage in life history of a plant, this study aimed to evaluate the effects of salinity on the germination and early seedling growth of these sorghum varieties of Australian origin.

MATERIALS AND METHODS

Caryopses Source

Three Australian cultivars of *Sorghum bicolor* (L.) Moench. Viz. CV. Mr. Buster, Extra Sweet, and Honey Graze, were evaluated for their salinity tolerance. Fungicide treated caryopses (seeds) of three cultivars were kindly provided by Dr. M. Qasim Khan, In Charge, Seed Certification Department, Karachi.

Caryopses Selection Criterion

The seed weight of 100 seeds of each cultivar were found to distribute in differentially-negatively skewed manner in cultivars – Extra sweet and Honey Graze but distributed symmetrically and normally in cultivar Mr. Buster (seed mass distributions not presented here). The mean seed weight varied substantially in the three cultivars. The mean seed 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 caryopses for testing germination and growth were, therefore selected from the mid-region of the area of the distribution i.e. located 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.

Germination Experiment

In order to analyze the salinity tolerance of sorghum genotypes at germination phase, a Petri plate experiment was conducted in laboratory with 4 replications including one control and 5 levels of salinity during June, 2012. For this purpose, 7.5 cm diameter Petri plates were thoroughly washed and sterilized in autoclave. These Petri plates were lined with sterilized filter paper and moistened with 5ml of saline water (100, 200, 300, 400, 500 mM NaCl). Saline water for germination medium was prepared by dissolving the salts of NaCl into distilled water. Control sets were moistened with equal amount of distilled water. Before germination, the caryopses were soaked for 30 minutes in distilled water. Ten seeds of each genotype were placed equidistantly on the filter paper in the Petri plates and moistened with 5 ml of saline water. NaCl solution was replaced at alternate day. The Petri plates were kept at 28^0C in an incubator. The germination counts were made in each petri plates on daily basis up to seven days. A seed was considered germinated if its radicle protruded out of the seed and attained a length of not less than 1.5 mm (Taylor, 1942). To assess the effect of salinity on seedling growth, the seedlings were collected after seven days. The observations were recorded on the length of shoot and root. The seedlings were dried at 50^0C for 48 h and their dry weights were recorded cumulatively for all seedlings in a replicate. The germination velocity was calculated with the following formula (Woodstock, 1976).

Germination Velocity = N1 / 1 + N2 /2 + N3 /3 ……Nn /n,

Where N1, N2, N3 …….Nn are the new germinants (here expressed as percent) on the day 1, 2, 3 …… or n. The germination velocity is high if more seeds germinate on the fewest number of days.

Table 1. The composition of the 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
	H_3BO_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 media.

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); $R^2 = 0.9993$

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 the sand dunes of Sandspit, Karachi. The sand was passed through a 2 mm sieve to remove gravels and other material. 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. The filter paper was also placed at the bottom of pots. Four pots were placed in each plastic tray which contained irrigation medium.

Preparation of irrigation medium

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

Growth

The growth experiment was conducted in pots (sand culture experiment) filled with sand. Seeds of three cultivars were imbibed for 30 minutes 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, 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 they had established.

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 subjected to their respective NaCl treatment. They were irrigated ten times at the interval of three days. The control and treatments consisted of four replicates.

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. The leaves are elongated and narrow at both the ends. The length (L) and maximum breadth (B) of these leaves was measured with scale of 1mm least count. The area of leaf was also determined graphically (Khan, 2009). Then the value of *k* was determined for each variety as

$$k = A / (L \times B)$$

The average *k* value for ten leaves of each cultivar was calculated. The k values were used for the determination of leaf area (A). The calculated value of k was 0.62 for cultivars Extra Sweet and Honey Graze and 0.55 for CV Mr. Buster. The leaf area was measured as $A = (L \times B) \times k$.

RESULTS AND OBSERVATION

Germination

The germination in all cultivars declined with salinity though increased with time of incubation in all salinity regimes (Fig. 1). The final germination percentage varied in three cultivars – 75 ± 6.46% in Mr. Buster, 95.0 ± 5.0% in Honey Graze and 87.5 ± 5.0% in Extra sweet in control treatment. The germination declined to 25.0 ± 10.4, 30.0 ± 0 and 15.0 ± 5.0 % in these cultivars, respectively, in 500 mM NaCl treatment. Since the velocity of germination was greatly impeded with salinity (Fig. 2), there was significantly high reduction in final germination percentage even after seven days of incubation. On the basis of retardation of germination velocity Honey Graze was the most affected cultivar (regression coefficient b = -0.1819) followed by Extra Sweet (b = - 0.1458) and Mr. Buster (b = - 0.1159). Three-way ANOVA of germination data indicated that variety effects, salinity effects and time of incubation were significant to influence germination ($p< 0.0001$) and variety and treatment (salinity) interacted significantly ($p < 0.0001$) but other interactions were not significant. On the basis of overall influence of salinity Cv. Honey Graze was relatively more resistant to salinity than other two cultivars. Mr. Buster was the least resistant variety. The final germination percentage of the three cultivars related with salinity according to the following equations.

CV. Mr. BUSTER
Final Germination (%) = 71.667 – 0.08500 (mM NaCl) ± 16.839
t = -4.323
p < 0.0003
F = 17.84, p < 0.0003; R^2 = 0.4473, N = 12.

CV. HONEY GRAZE
Final Germination (%) = 98.2886 – 0.15714 (mM NaCl) ± 13.077
t = -9.64
p < 0.00001
F = 92.98, p < 0.00001; R^2 = 0.8087, N = 12

CV. EXTRA SWEET
Final Germination (%) = 89.643 – 0.13357 (mM NaCl) ± 15.480
t = -7.218
p < 0.0001
F = 52.100, p < 0.0001; R^2 = 0.7030, N = 12

Seedling growth in petri plates

The seedlings of sorghum are thin and tender. Seedling growth was recorded in terms of shoot, root length and shoot; root fresh and the cummulative dry weights of shoot and shoot at different levels of salinity. The increase in NaCl concentrations decreased all the growth parameters in all cultivars significantly so much so that growth was negligible in 400 mM and 500 mM NaCl concentration (Fig. 3, 4 and 5).

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 irrigation. 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. 6). Two-way ANOVA indicated that only salinity effects on shoot dry weight were significant ($F = 13.03, p < 0.0001$) and the varietal effects were insignificant ($F = 2.56, p < 0.096$). The variety x treatment interaction was also insignificant ($F = 2.63, p < 0.566$). The varietal ($F = 3.80, p < 0.035$) as well as salinity ($F = 20.38, p < 0.0001$) effects were significant in case of root weights of the seedlings. The interaction of the two factors was also significant ($F = 3.46, p < 0.021$).

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. 7) as compared to the control and was well represented by the following models.

CV. Mr. BUSTER
Seedling mass (g) = 1.83083 – 0.012600 (mM NaCl) ± 0.8457
t = -2.21
p < 0.0501; F = 4.439; R^2 = 0.3070, N = 12

CV. HONEY GRAZE
Seedling mass (g) = 4.0275 – 0.037150 (mM NaCl) ± 1.354
t = -3.880
p < 0.0031; F = 15.06; R^2 = 0.6008, N = 12

CV. EXTRA SWEET
Seedling mass (g) = 2.3957 – 0.014592 (mM NaCl) ± 060999
t = -3.080
p < 0.0116; F = 9.490; R^2 = 0.4869, N =12

There was comparatively higher reduction of 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; $p < 0.0001$). The interaction of variety and salinity was also significant (F = 2.90; $p < 0.0405$).

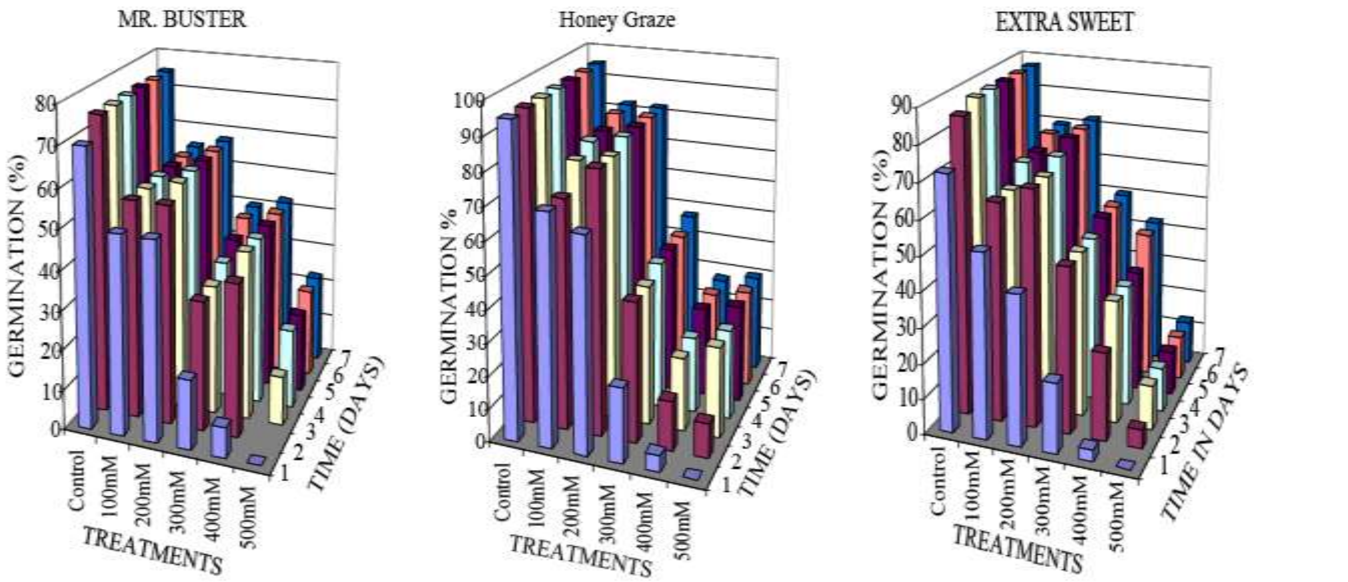

Fig. 1.Germination of three sorghum varieties against NaCl salinity.

Salinity effects on number of leaves per seedling

The number of leaves per seedling (Fig. 8A) was the function of salinity in all the three cultivars. Salinity reduced the number of leaves significantly (F = 33.39; $p < 0.0001$). The varietal effects on number of leaves per seedling were insignificant (F = 3.09; $p < 0.5993$).

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. 8B; Table 4). Two way ANOVA rated salinity effects to be significant (F = 19.51; $p < 0.0001$) but the varietal effects insignificant (F = 1.77; $p < 0.1887$).

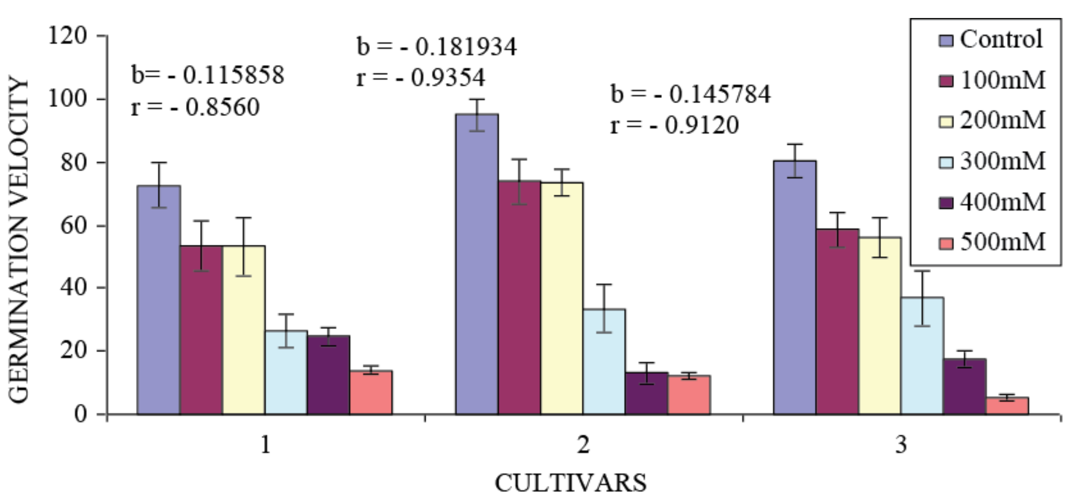

Fig. 2. Germination velocity of three sorghum cultivars as influenced by NaCl salinity. Cultivar 1 = Mr. Buster; Cultivar 2 = Honey Graze; Cultivar 3 = Extra Sweet. ‘b’ is the linear regression coefficient of slope.

Comparison salinity tolerance of the cultivars at germination and growth phases

A comparative account of 50% reduction in germination and seedling growth (biomass) calculated as per statistical models given above, of the three cultivars under NaCl salinity is presented in Table 5. All the three cultivars exhibited to be much resistant to NaCl salinity at germination phase than at the growth phase of the seedlings. Cultivar Mr. Buster appeared to be more resistant than other varieties at germination but other two cultivars were relatively more tolerant to salinity at growth phase.

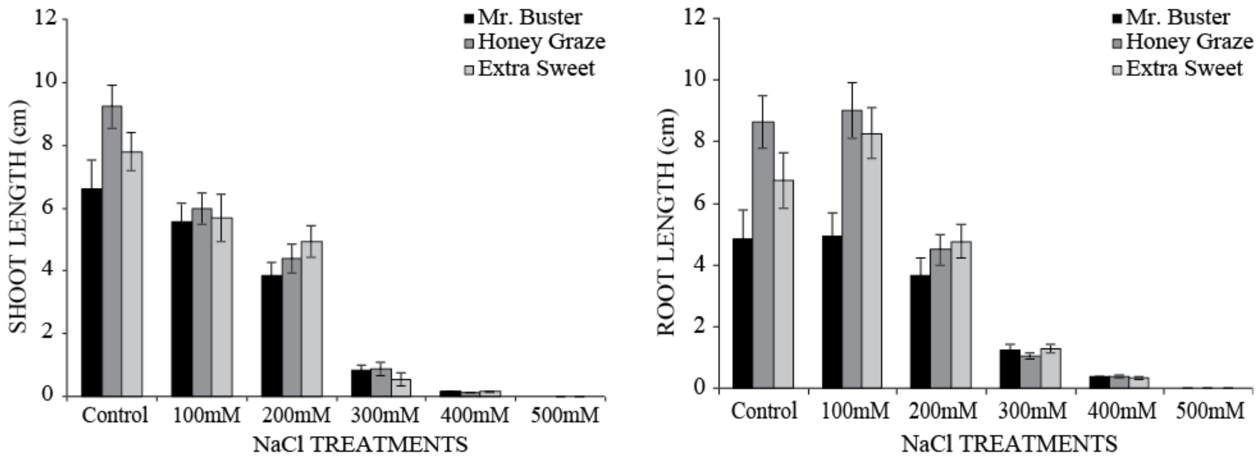

Fig. 3. Effects of NaCl salinity on mean shoot and root lengths of three sorghum cultivars.

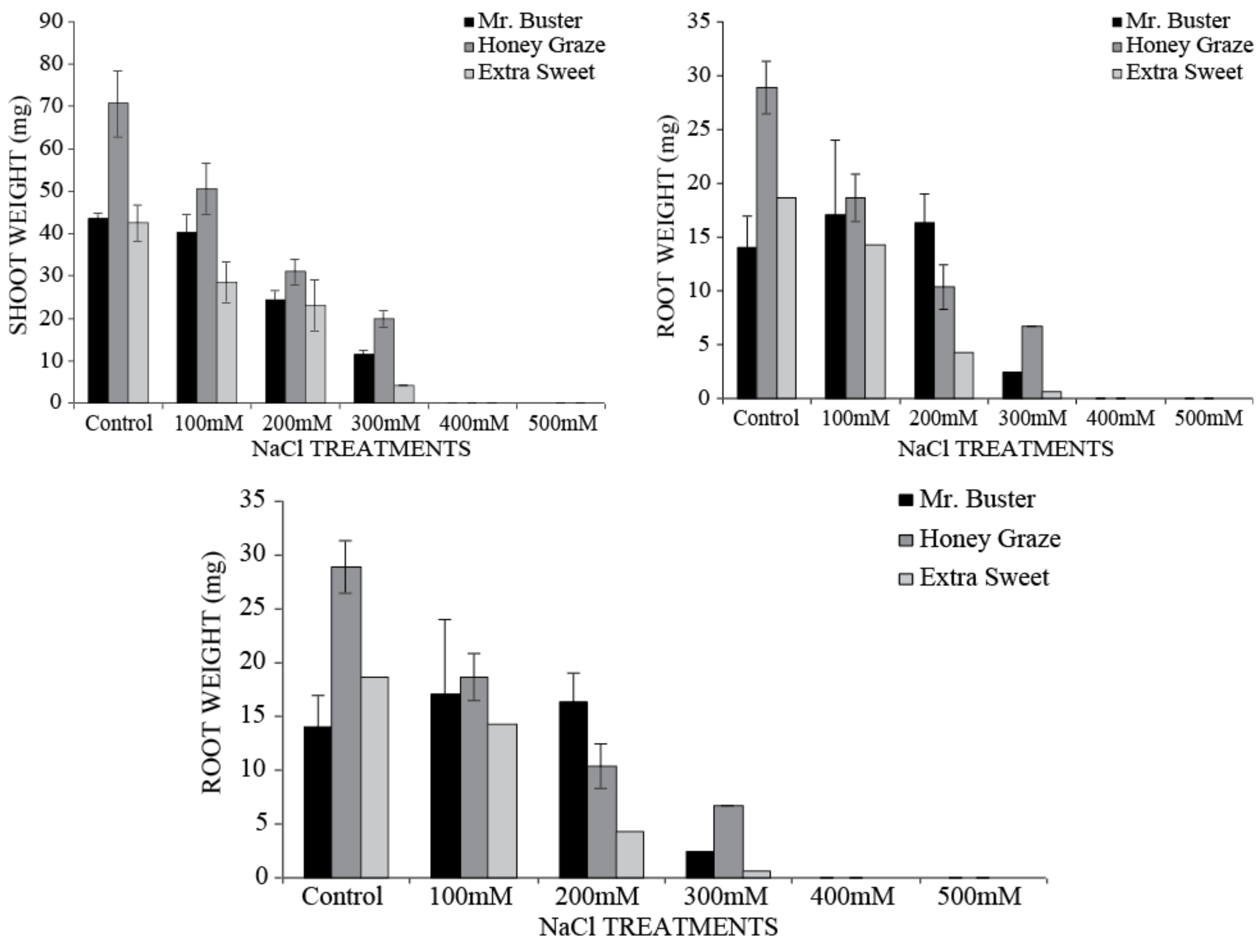

Fig.4. Effects of NaCl salinity on cummulative Shoot and root dry mass accumulation in seedling shoots and roots of three sorghum cultivars in petri plates.

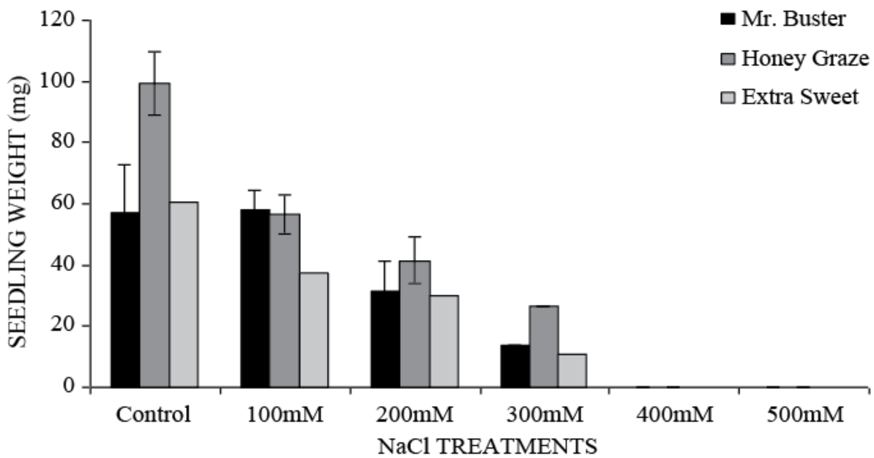

Fig. 5. Effects of NaCl salinity on cummulative seedlings dry mass accumulation (mg) in three cultivars of *Sorghum* in petri plates.

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

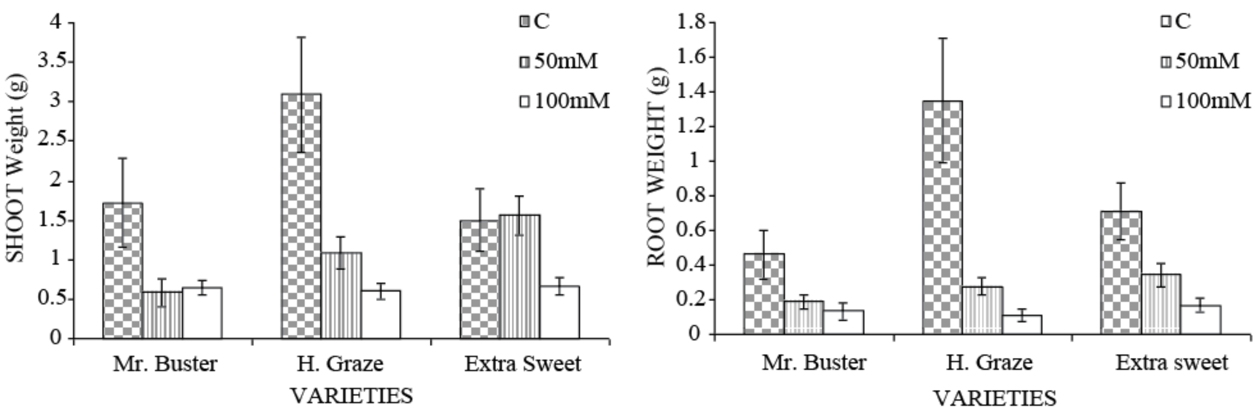

Fig. 6. Effect f saline irrigation on shoot and root dry wt. (g) of three sorghum varieties in a pot experiment. C, Control.

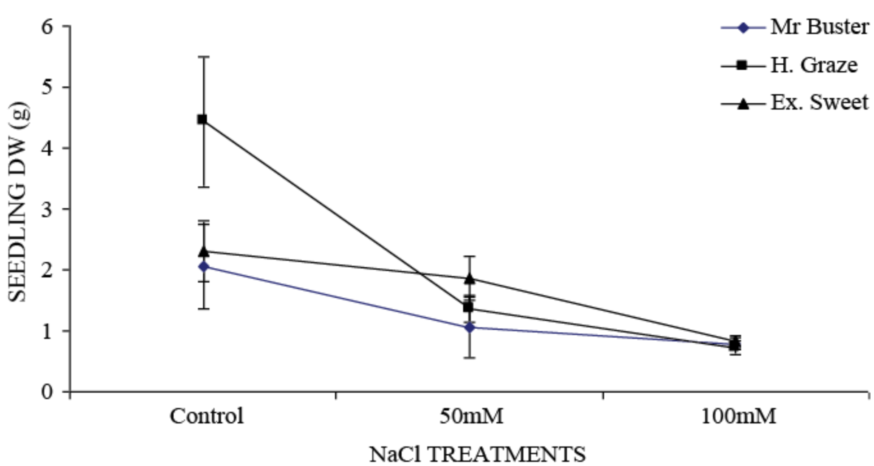

Fig. 7. Effect of saline water irrigation (NaCl) on seedling growth of three cultivars of Sorghum in pot culture experiment.

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

Table 5. The NaCl concentration (mM) corresponding to the 50% reduction in % final germination in Petri plates and seedling growth of three cultivars in pot.

Physiological Processes	**CV. Mr. Buster**	**Honey Graze**	**Extra Sweet**
Final Germination	421.57	329.46	335.57
Seedling growth	72.652	82.089	82.895

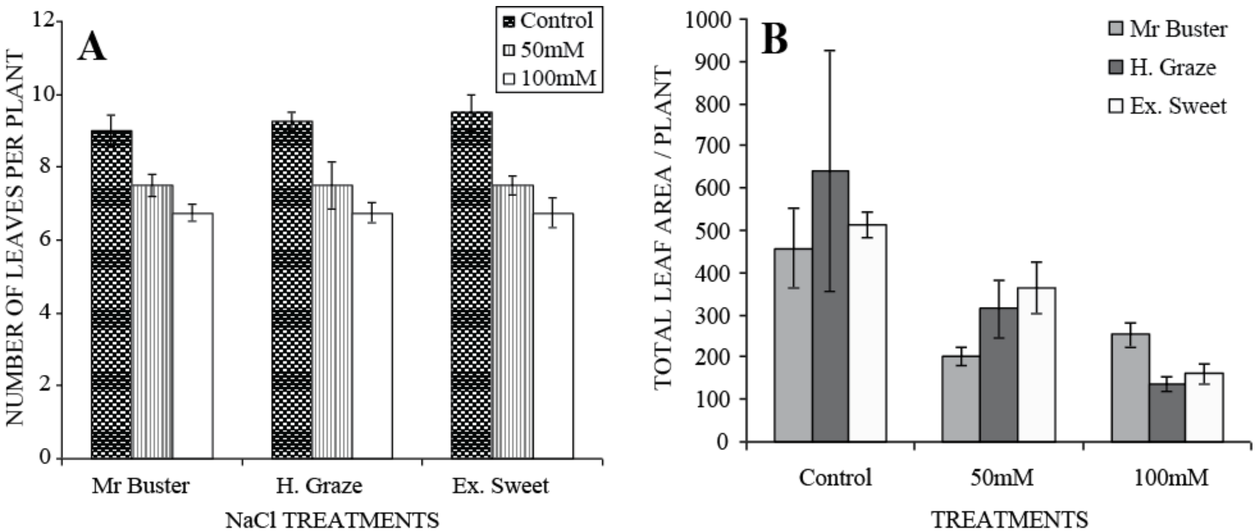

Fig. 8. Effect of NaCl salinity on number of leaves at 30 days of age (A) and total leaf area per plant (cm^2) (B) in three sorghum cultivars when grown in pots.

DISCUSSION

The experiment was conducted to determine the influence of salinity on germination and the seedling growth of three sorghum genotypes (Mr. Buster, Honey Graze and Extra Sweet). The mean final germinability varied among the cultivars even in control substantially from 75 to 95 % which is comparable to the standard laboratory germination of 89.5% (63-98%) for various lots of *Sorghum* grains reported by Baskin *et al.* (1993). The germination in all cultivars declined with salinity though increased with time of incubation in all salinity regimes but never reached to the level of the control. Since the velocity of germination was greatly impeded with salinity, there was significantly high reduction in final germination percentage under high NaCl concentration even after seven days of incubation. These results are in agreement with the results of the previous researches that salinity may affect seed germination by decreasing the ease with which water may be taken up by the seeds or toxicity of Na and Cl ions. The activities and events associated normally with germination are either delayed and / or proceed at reduced rates (Khan *et al.,* 1984; Khan and Ahmad, 1998, 2007 a and b; Kazemi and Eskandari, 2011) and even in case of salt tolerant plants (Khan and Ahmad, 2002). At the early seedling stage, percentage reduction in root and shoot lengths, root and shoot dry weights, and total dry weight have demonstrated differences in vegetative growth response to salinity among sorghum genotypes. Several researchers have found differences in salt tolerance (Krishnamurthy *et al.,* 2003; Kulhari *et al.,* 2008; Nawaz *et al.,* 2010; Kausar *et al.,* 2012) in sorghum cultivars. Increase in salt concentration decreased the seedling performance of sorghum cultivars at EC $\geq$ 10 $dS.m^{-1}$ and the performance varied with the genotypes tested (Chauhan *et al.,* 2012).

Our studies indicated that the selected *sorghum* cultivars were more tolerant to salinity at germination than at the subsequent growth stage. It is in agreement with the contention of Ayers and Hayward (1948) that salt tolerance at germination and other phases of life cycle are not necessarily correlated. In some plants germination is more resistant process whereas in others growth phase is more tolerant to salinity. Azizov (1974) has reported that seeds of sea lavender (*Limonium meyeri*) can not germinate in salts solution above 1.5%, yet the mature plant can grow even in the presence of 10% salt solution in soil. Fifty per cent reduction in germination and biomass production in *Indigofera oblongifolia* is reported to correspond with ECiw: 10.0 and 12.05 $dS.m^{-1}$, respectively (Khan and Ahmad, 1998) and in case of *Sporobolus arabicus*, such a reduction corresponded with ECiw: 32.4 and 28.6 $dS.m^{-1}$, respectively (Khan and Ahmad, 2002). Seeds of halophytic Andean Qu*inoa* (*Chenopodium quinoa*), exhibited only around 14% germination in 0.4 M NaCl compared to that in controls (87%) (Prado *et al.*, 2000). Halophytes, from marshy habitats, such as *Aeluropus lagopoide*s and *Sporobolus madraspatanum* are reported not to germinate in 8 $dS.m^{-1}$ seawater or 5 ppt NaCl (Joshi *et al.*, 2005). According to Gulzar *et al.* (2001) halophytic *Urochondra setulosa* seeds best germinate in non-saline environment and only less than 10% seeds could germinate in 500 mM NaCl salinity. Ungar (1974) has, on the other hand, reported that seed germination in *Hordeum jubatum* is more resistant process to salinity than the later growth of the seedling. Mahmood and Malik (1986) had observed greater salt tolerance at growth than germination stage of *Atriplex undulata*. Our studies indicated that germination and seedling growth of *sorghum* cultivars declined with salinity in accordance with simple linear model. Decline in germination and dry matter production of seedlings under saline environment has widely been reported in literature (Alam and Naqvi, 1991; Yurtseven, *et al.*, 2005; Mujeeb-ur-Rahman *et al.*, 2008; Prado *et al.*, 2000). The NaCl concentration corresponding to 50% decrease in germination and growth varied greatly among the cultivars. Salt tolerance sequence, on the basis of 50 % germination loss over control, of the cultivars in hand was as Cv. Mr. Buster > CV Extra Sweet > CV. Honey Graze.

Salt tolerance sequence, on the basis of 50 % losses of leaf area per plant over control, was Cv. Buster > CV Extra Sweet > CV. Honey Graze. The salt tolerance sequence, on the basis of 50 % decline of seedling phytomass (a better parameter of growth) over control was as Cv. Honey Graze ≈ CV Extra Sweet > CV. Mr. Buster.

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