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Variability in Salt Tolerance of *Sorghum bicolor* L.

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Abstract

Salt tolerance of ten sorghum (*Sorghum bicolor* L. Moench) varieties ('1790E', 'BTx642', 'Desert Maize', 'Macia', 'RTx430', 'Schrock', 'Shallu', 'Tx2783', 'Tx7078', and 'Wheatland') was evaluated in two greenhouse experiments. In the first experiment, sorghum were sown in substrates moistened with either nutrient solution (no addition of salts, control) at electrical conductivity (EC) of 1.2 $dS{\cdot}m^{-1}$ or salt solution at EC 5, 10 or 17 $dS{\cdot}m^{-1}$. Seedling emergence percentage decreased in all varieties only at EC of 17 $dS{\cdot}m^{-1}$ compared to the control. Seedling emergence percentage of sorghum 'Macia' and '1790E' irrigated with salt solution at EC of 17 $dS{\cdot}m^{-1}$ decreased by 50% and 51%, while that of 'RTx430' reduced by 97%, other varieties ranged from 64% to 90%. Both salt solution at EC of 5 and 10 $dS{\cdot}m^{-1}$ reduced the dry weight of sorghum seedlings by 29% and 72% on average, respectively, compared to control. In the 2nd experiment, plants were irrigated with nutrient solution or salt solution at EC of 5.0 or 10.0 $dS{\cdot}m^{-1}$ for 30 days. Salt solution at EC of 5.0 and 10.0 $dS{\cdot}m^{-1}$ had similar influences on dry weight (DW) of all sorghum varieties except 'Tx2783'. The relative dry weight of 'Shallu', 'Desert Maize', and '1790E' irrigated with salt solution at EC of 10 $dS{\cdot}m^{-1}$ were over 67%, those of 'Macia', 'Schrock', and 'RTx430' ranged from 30% to 33%, and other varieties were 45% to 59%. Foliar salt damage was observed on all salt-treated sorghum varieties except for 'Shallu', which had the lowest shoot DW reduction and greatest visual score. Leaf photosynthesis of all sorghum plants irrigated with salt solution at EC of 5 and 10 $dS{\cdot}m^{-1}$ was decreased by 6.0% and 10.6%, respectively. Leaf Na^+ concentration at EC of 5.0 and 10.0 $dS{\cdot}m^{-1}$ increased by 25.6% and 60.7%, respectively, compared to the control; while Cl^- concentration increased by 16.4% and 41.2%, and Ca^{2+} concentration increased by 17.8% and 34.3%. In conclusion, salt tolerance of sorghum varied with plant growing stage and varieties. 'Shallu', 'Desert Maize', and '1790E' were the most salt tolerant varieties, while 'Schrock' and 'RTx430' showed the least salt tolerance in both experiments. All varieties had high Na^+ exclusion ability.

Keywords: biofuel feedstock, gas exchange, mineral nutrient, salinity, sorghum

1. Introduction

Biofuel is expected to make a significant contribution to meet global energy needs due to diminishing availability of discoverable fossil fuel reserves and environmental consequences of exhaust gases from fossil fuel. A total of 110 billion liters of biofuel was produced worldwide in 2011, among which bioethanol accounted for 78.7% (U.S. Energy Information Administration, 2011). Bioethanol is produced from agricultural feedstocks such as corn, miscanthus, sweet potato, sugarcane, sorghum, and switchgrass (Drapcho, Nghim, & Walker, 2008; Pyter, Voigt, Heaton, Dohleman, & Long, 2007; Schmer, Vogel, Mitchell, & Perrin, 2008), several of which are food crops. Therefore, the production and use of bioethanol may compete with the food supply and with food crops for arable land in the long-term. Cellulosic ethanol made from cellulose fibers could play a critical role in promoting energy diversity and reducing carbon-dioxide emissions (International Energy Agency, 2006). The market could eventually be worth $20 billion a year, and there is enough feedstock to produce 286 billion liters of cellulosic ethanol in the U.S. (Sanderson, 2006). High-yield lignocellulose feedstock is essential for production of the large quantities of biofuel needed to sustain an efficient bioenergy processing and production chain.

Sorghum bicolor (L.) Moench (sorghum) is an economically important crop grown for grain and for forage. Sorghum is the only crop from which ethanol can be produced from grain (starch), juice (sweet sorghums) and biomass (lignocellulose). Sorghum is also promising as a bioethanol crop because it is water-use efficient and well adapted to semi-arid regions where soil salinity is too high for most common economically important crops and groundwater with high salinity is the major water source. Most of the lands in semi-arid regions are referred to as marginal lands because they have low inherent productivity and have been abandoned or degraded (Tang, Xie, & Geng, 2010). Growing sorghum on marginal lands would be an alternative way to conserve fresh water, reduce fossil fuel pollution, and secure food safety. Therefore, identifying salt tolerant sorghum genotypes and improving their salt tolerance for salt affected lands is critically important.

Salinity causes reduction in seed germination (Tabatabaei & Anagholi, 2012), seedling growth (Kausar, Ashraf, Ali, Niaz, & Abbass, 2012), yield of sorghum (Almodares & Sharif, 2005), and modifies the plant physiological and biochemical processes (Netondo, Onyango, & Beck, 2004). According to Almodares and Sharif (2005, 2007) sorghum is moderately tolerant to salt conditions. Sorghum is more sensitive to salinity at the seedling emergence stage than at any other stage (Macharia, Kamau, Gituanja, & Matu, 1994). Additionally, the salt tolerance varies with the varieties (Azhar & McNeilly, 1987; Niu, Xu, Rodriguez, & Sun, 2012b). To provide more information about such variation, the relative salt tolerance of ten sorghum varieties was determined in two greenhouse studies by evaluating their growth, gas exchange rates, and leaf ion accumulation under different salt conditions.

2. Materials and Methods

2.1 Seedling Emergence (Expt. 1)

Seedling emergence of ten sorghum varieties (‘1790E’, ‘BTx642’, ‘Desert Maize’, ‘Macia’, ‘Schrock’, ‘Shallu’, ‘Tx2783’, ‘RTx430’, ‘Tx7078’, and ‘Wheatland’) were tested on 22 August, 2013 and repeated on 26 September. Seeds were sown at a depth of 2.5 cm in insert (13.5 × 13.5 × 4.5 cm) filled with Metro-Mix 360 (SunGro Hort., Bellevue, WA), 16 seeds per insert and four inserts (replications) per treatment. The potting mix was saturated with the same amount of nutrient solution at electrical conductivity (EC) of 1.2 ± 0.1 $dS{\cdot}m^{-1}$ (control), or salt solution at EC of 5.1 ± 0.2 $dS{\cdot}m^{-1}$ (EC5), 9.9 ± 0.4 $dS{\cdot}m^{-1}$ (EC10), or 16.9 ± 0.2 $dS{\cdot}m^{-1}$ (EC17). The nutrient solution was prepared by adding 1 $g{\cdot}L^{-1}$ of 15N-2.2P-12.5K (Peters 15-5-15; Scotts, Marysville, OH) to reverse osmosis water. The salt solution was prepared by dissolving the calculated amounts of NaCl and

$CaCl_2$ salts at 2:1 molar ratio into the nutrient solution. Nutrient or salt solutions were applied through subirrigation whenever substrate surface started to dry and these were repeated five times during the experiment.

The experiment followed a completely randomized design with four replications and 16 seeds per replication per treatment per variety. Seeds began to emerge three days after sown, and seedling emergence was counted thereafter every day and continued for two weeks. A seedling was considered emerged when the hypocotyl hook was visible above the surface. Seedlings were harvested and dry weight (DW) was recorded after oven-dried at 65°C to constant weight. The greenhouse environment was maintained at average day temperature at 29.1 ± 4.4 ºC, night temperature at 23.9 ± 2.4 ºC. The substrate final ECs, as determined using saturated paste extract (Gavlak, Horneck, & Miller, 1994; U.S. Salinity Laboratory, 1954) were 4.4 ± 1.4 $dS \cdot m^{-1}$, 12.1 ± 3.7 $dS \cdot m^{-1}$, 25.0 ± 5.0 $dS \cdot m^{-1}$, and 50.0 ± 8.6 $dS \cdot m^{-1}$, respectively, for control, EC5, EC10, and EC17, respectively.

Emergence percentage (EP) was calculated using the formula:

$$\text{EP}\ (\%) = \frac{\text{Number of emerged seedlings}}{\text{Total number of seeds}} \times 100\% \quad (1)$$

Emergence index (EI) was calculated as: $\text{EI} = \sum_{i=1}^{n}(\text{EP}_\text{i}/\text{T}_\text{i})$;

where EP_i is the emergence percentages on day i, and T_i is the number of days after sowing seeds. Relative emergence percentage for each replicate in the salt treatment was calculated as:

$$\text{Relative EP}\ (\%) = \frac{\text{EP in salt treatment}}{\text{Averaged EP in control}} \times 100\% \quad (2)$$

Similarly, relative percentage for emergence index and dry weight was calculated. The relative values from both tests were pooled for data analysis using a two-way analysis of variance (ANOVA). When a significant difference among varieties or treatments existed, means were separated by Tukey's Honestly Significant Difference (HSD) multiple comparison at $P < 0.05$.

2.2 Seedling Growth (Expt. 2)

2.2.1. *Plant Materials and Treatments*

On 23 April 2012, sorghum seeds were sown at the depth of 2.5 cm in 2.3-L Poly-Tainer containers (No.1S, 16.5 x 16.5 cm) with Sunshine Mix #4 (SunGro Hort., Bellevue, WA), four seeds per container. Ten days later seedlings were thinned and two seedlings per container were grown for the experiment. Seedlings were watered with nutrient solution until treatments were initiated. The nutrient solution was prepared by adding 0.72 $g \cdot L^{-1}$ of 15N-2.2P-12.5K (Peters 15-5-15; Scotts) to tap water. The major ions in the tap water were Na^+, Ca^{2+}, Mg^{2+}, Cl^-, and SO_4^{2-} at 184.0, 52.0, 7.5, 223.6, and 105.6 $mg \cdot L^{-1}$, respectively. Treatments were initiated on 15 May and terminated on 12 June. Plants irrigated with nutrient solution (control) or salt solution with 10% to 20% leaching fraction and the nutrient or salt solutions were re-applied whenever the substrate surface started to dry. Salt solutions at EC of 5.0 and 10.0 $dS \cdot m^{-1}$ were prepared by adding calculated amounts of NaCl and $CaCl_2$ at 2:1 (molar ratio) to the nutrient solution. Salt solutions were prepared in 100-L tanks with confirmed EC for each treatment.

Leachate was collected periodically, and the EC of the leachate was measured using an EC meter (Model B-173, Horiba, Ltd., Japan). To reduce salt accumulation, plants were flushed with deionized water to lower the salinity in the root zone when the leachate EC was 1.5 times the EC of irrigation water. The temperatures in the greenhouse were maintained at 31.9 ± 5.7 °C (mean ±

standard deviation) during the day and 24.2 ± 3.5 °C at night. The daily light integral (photosynthetically active radiation) was 19.6 ± 3.6 $mol \cdot m^{-2} \cdot d^{-1}$.

2.2.2 *Growth Parameters*

Plant height from the pot rim to the sheath of the top leaf and number of leaves and tillers were recorded at the beginning and end of the experiment. Upon termination of the experiment, visual foliar salt damage (leaf edge burn, necrosis, and discoloration) was rated by giving a score to every plant from 0 to 5, where 0 = dead; 1 = over 90% foliar damage; 2 = moderate (50-90%) foliar damage; 3 = slight (<50%) foliar damage; 4 = good quality with minimal foliar damage; and 5 = excellent without any foliar damage. Shoots were severed at the substrate surface, and shoot dry weight (DW) was determined after oven-dried at 65 °C to constant weight. Leaf area of one plant per pot was determined using LI-3100C area meter (LI-COR® Biosciences, Lincoln, NE).

Relative dry weight for each plant in the salt treatment was calculated as:

$$\text{Relative dry weight (\%)} = \frac{\text{Dry weight in salt treatment}}{\text{Averaged dry weight in control}} \times 100\% \quad (3)$$

Similarly, relative percent for height, leaf area, and number of leaves were calculated. These relative values were used for data analysis.

2.2.3 *Gas Exchange*

Leaf net photosynthesis (P_n), transpiration (E), and stomatal conductance (g_s) of two plants per variety per treatment were measured at the end of the experiment using a CIRAS-2 portable photosynthesis system (PP Systems, Amesbury, MA) with an automatic universal PLC6 broad leaf cuvette. The 3rd fully expanded leaf counting from the top of the plant downward was labeled for the measurements. The environmental conditions within the cuvette were maintained at leaf temperature 25 °C, photosynthetic photon flux (*PPF*) 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and CO_2 concentration 375 $\mu mol \cdot mol^{-1}$. Data were recorded when the environmental conditions and gas exchange parameters in the cuvette became stable. These measurements were taken on sunny days between 1000 hr and 1400 hr, and plants were well watered to avoid water stress.

2.2.4 *SPAD Reading*

Leaf greenness (or relative chlorophyll content) of all plants was measured using a hand-held SPAD chlorophyll meter (Minolta Camera Co., Osaka, Japan) at the end of experiment. For each plant, the 3rd and 4th healthy, fully expanded leaf counting from the top of the plant downward were chosen for the measurements.

2.2.5 *Mineral Analysis*

Four leaf samples per treatment per variety were randomly selected for mineral analysis. Dried tissue samples were ground to pass a 40-mesh screen with a stainless Wiley mill (Thomas Scientific, Swedesboro, NJ). Plant tissues were digested by using Environmental Protection Agency method 3051 with 1 ml nitric acid and 4 ml H_2O_2 using a microwave acceleration reaction system (CEM Corporation; Mathews, NC) for determining alkaline earth metals (Na^+, K^+, Ca^{2+}) contents. The plant tissues were extracted with 2% acetic acid for determining Cl^- concentration using methods described in Gavlak *et al.* (1994). Na^+, K^+, and Ca^{2+} in the digested samples were analyzed by Inductively Coupled Plasma-Optical Emission Spectrometry (Perkin-Elmer Optima 4300 DV, Shelton, CT). The Cl^- concentration was determined by DX-120 Ion-Chromatography (Dionex Corporation, Sunnyvale, CA, EPA 300.0).

2.2.6 *Experimental Design and Statistical Analysis*

The experiment followed a split-plot design with variety as the main plot and salinity as subplot with 5 replications. All data including growth, photosynthetic parameters, and mineral nutrients were analyzed by a two-way ANOVA. When the main effect was significant, a *t*-test was performed. Means separation among varieties was conducted using Tukey's HSD multiple comparison. All statistical analyses were performed using SAS software (Version 9.1.3, SAS Institute Inc., Cary, NC).

3. Results and Discussion

3.1 Seedling Emergence

The relative seedling emergence percentage and emergence index were significantly different among varieties and salt treatments, but no interactive effects occurred (Table 1). All sorghum varieties irrigated with salt solution at EC of 5 and 10 $dS \cdot m^{-1}$ had similar relative seedling emergence percentage and emergence index. On average, the relative seedling emergence percentage of all sorghum varieties irrigated with salt solution at EC of 5 and 10 $dS \cdot m^{-1}$ was 102% and 102%, respectively, and the relative seedling emergence index was 102% and 97%. Compared with control, there was about 2% increase in the seedling emergence percentage and index at EC of 5 and 10 $dS \cdot m^{-1}$. These results might be caused by the nutrient elements in salt solution. Seedling emergence percentage and index at EC of 5 and 10 $dS \cdot m^{-1}$ did not differ from those in the control (data not shown). Dürr and Mary (1998) found that mineral availability helped the seedling emergence of sugar beet.

Salt solution at EC of 17 $dS \cdot m^{-1}$ considerably reduced the relative seedling emergence percentage and index of sorghum varieties. The relative seedling emergence percentage of sorghum 'Macia' and '1790E' irrigated with salt solution at EC of 17 $dS \cdot m^{-1}$ were 50% and 49%, respectively, indicating a reduction of 50% and 51% compared to control. 'RTx430' had the smallest relative seedling emergence percentage of 3%, corresponding to a reduction of 97% compared to control. Other sorghum varieties had the relative seedling emergence percentage of 10% (90% reduction) to 36% (64% reduction). In term of the relative seedling emergence index, '1790E' was the greatest numerically (24%), while 'RTx430' and 'Tx2783' were the smallest (2% and 4%, respectively). All other sorghum varieties had a relative seedling emergence index of 5% to 19%. Poor seedling emergence of sorghum varieties might be caused by hypocotyl mortality associated with the salts accumulation at the soil surface (Miyamoto, Piela, & Petticrew, 1985). In the study, the substrate final EC as determined using saturated paste extract were about three times that of the corresponding irrigation water.

As salinity increased, the dry weight of sorghum seedlings was reduced dramatically in all cultivars (data not shown). Sorghum seedlings irrigated with salt solution at EC of 17 $dS \cdot m^{-1}$ were too small to collect their dry weight. The relative dry weight of sorghum seedlings was significantly affected by salt solutions, but similar among varieties (Table 1). On average, the relative dry weight of sorghum seedlings irrigated with salt solution at EC of 5 and 10 $dS \cdot m^{-1}$ was 71% and 28%, respectively, indicating a reduction of 29% and 72% compared to control. Azhar and McNeilly (1987) also observed that increasing NaCl concentration caused significant reductions in the dry weight of sorghum accessions.

Based on the above results, '1790E', 'Macia', 'Desert Maize', and 'Shallu', were the most salt tolerant varieties at the stage of seedling emergence, 'Wheatland', 'Tx7078', 'BTx642', and 'Tx2783' had intermediate salt tolerance, 'Schrock' and 'RTx430' were the least salt tolerant varieties. EC of irrigation water at 5 and 10 $dS \cdot m^{-1}$ did not affect the seedling emergence. However, young seedlings right after emergence were sensitive to salinity, as evidenced by high reduction in dry weight.

Table 1. Relative seedling emergence percentage and index, and dry weight of ten sorghum varieties irrigated with salt solution [Electrical conductivity (EC) =5.0, 10.0, or 17.0 $dS \cdot m^{-1}$]. Control was nutrient solution at EC of 1.2 $dS \cdot m^{-1}$

Variety	Relative (%)								
	Emergence percentage			Emergence index			Dry weight§		
	EC 5	EC 10	EC 17	EC 5	EC 10	EC 17	EC 5	EC 10	*t*-test‡
1790E	112 aA†	110 aA	49 aB	116 aA	102 aA	24 aB	84 aA	33 aB	***
BTx642	102 aA	97 aA	14 abB	103 aA	89 aA	5 abB	73 aA	25 aB	***
Desert Maize	102 aA	113 aA	36 abB	104 aA	111 aA	16 abB	84 aA	31 aB	***
Macia	117 aA	114 aA	50 aB	115 aA	110 aA	19 abB	79 aA	23 aB	***
RTx430	98 aA	91 aA	3 bB	96 aA	85 aA	2 bB	54 aA	26 aA	NS
Schrock	105 aA	105 aA	10 abB	100 aA	94 aA	5 abB	57 aA	31 aA	NS
Shallu	88 aA	98 aA	28 abB	90 aA	95 aA	16 abB	73 aA	19 aB	***
Tx2783	92 aA	95 aA	14 abB	90 aA	92 aA	4 bB	59 aA	27 aB	*
Tx7078	102 aA	107 aA	20 abB	100 aA	100 aA	8 abB	69 aA	39 aB	*
Wheatland	103 aA	94 aA	25 abB	104 aA	89 aA	10 abB	82 aA	25 aB	***
Variety (V)		***‡			*			NS	
Treatment (T)		***			***			***	
V * T		NS			NS			NS	

† Means with same lowercase letters within column (among varieties) or with same uppercase letters within row (among treatments) are not significantly different by Tukey's HSD multiple comparison at $P < 0.05$.

‡ NS, *, **, ***: non-significant, significant at $P < 0.05$, 0.01, and 0.001, respectively.

§ Dry weight was not recorded for the treatment of EC 17 since seedlings were too small.

3.2 Plant Growth

Salt treatment significantly impacted sorghum growth in term of relative dry weight, height, leaf area, and number of leaves, and the response varied with sorghum varieties (Table 2). However, the interactions between salt treatment and variety were insignificant. Salt solution at EC of 5 and 10 $dS \cdot m^{-1}$ had similar influences on the relative dry weight of all sorghum varieties except 'Tx2783' (Table 2). When salt solution at EC of 5 $dS \cdot m^{-1}$ applied, the relative dry weight of 'Shallu', 'Desert Maize', 'Tx2783', and '1790E' were more than 66% (34% reduction), while that of all other tested varieties were less than 48% (52% reduction). However, the relative dry weight of 'Shallu', 'Desert Maize', and '1790E' irrigated with salt solution at EC of 10 $dS \cdot m^{-1}$ were more than 67%, 'Macia', 'Schrock', and 'RTx430' ranged from 30% to 33%, and other varieties were 45% to 59%. Sorghum 'BTx642', 'RTx430', 'Schrock', 'Shallu', 'Tx2783', 'Tx7078', and 'Wheatland' irrigated with salt solution at EC of 10 $dS \cdot m^{-1}$ were shorter than those at EC of 5 $dS \cdot m^{-1}$ (Table 2). The relative height of all sorghum plants irrigated with salt solution at EC of 5 and 10 $dS \cdot m^{-1}$ was 71% and 53%, respectively, corresponding to a reduction of 29% and 47% compared to the control. Boursier and Läuchli (1990) reported that sorghum dry matter production decreased substantially in response to a moderate increase in soil electrical conductivity from 2.1 to 5.9 $dS \cdot m^{-1}$.

Table 2. Relative dry weight, height, leaf area, and number of leaves of ten sorghum varieties irrigated with salt solution [Electrical conductivity (EC) = 5.0 or 10.0 dS·m^{-1}] in the greenhouse. Control was nutrient solution at EC of 1.5 dS·m^{-1}

Variety	Relative (%)											
	Dry weight			Height			Leaf area			Number of leaves		
	EC 5	EC 10	*t*-test	EC 5	EC 10	*t*-test	EC 5	EC 10	*t*-test	EC 5	EC 10	*t*-test
1790E	65.9 ab†	67.1 ab	NS	50.1 b	50.2 abc	NS	98.6 a	84.7 ab	NS	85.0 ab	89.5 a	NS
BTx642	47.1 b	45.3 cd	NS	85.2 ab	49.4 abc	*	78.7 ab	49.3 bcd	NS	87.9 ab	81.0 ab	NS
Desert Maize	84.9 a	80.0 a	NS	77.1 ab	70.2 ab	NS	100.3 a	95.4 a	NS	106.4 a	92.9 a	NS
Macia	38.8 b	29.7 d	NS	83.3 ab	72.8 a	NS	40.1 c	40.2 d	NS	78.3 b	78.3 abc	NS
RTx430	41.8 b	31.5 d	NS	78.3 ab	48.4 bc	**	52.2 bc	44.9 cd	NS	91.2 ab	71.6 abc	NS
Schrock	47.6 b	33.3 d	NS	56.1 ab	41.1 c	*	45.4 bc	31.2 d	NS	69.4 b	56.5 c	NS
Shallu	91.5 a	82.2 a	NS	86.3 a	65.7 ab	**	93.6 a	80.0 abc	NS	93.3 ab	87.1 a	NS
Tx2783	81.1 a	58.8 bc	*	70.1 ab	40.4 c	**	70.8 abc	49.7 bcd	NS	83.4 ab	57.6 bc	*
Tx7078	47.3 b	40.4 cd	NS	50.1 b	33.3 c	*	50.3 bc	39.8 d	NS	72.6 b	59.4 bc	NS
Wheatland	47.8 b	49.6 bcd	NS	76.1 ab	53.9 abc	**	51.9 bc	43.5 d	NS	90.7 ab	72.3 abc	*
Variety (V)	***‡			***			***			***		
Treatment (T)	**			***			***			***		
V * T	NS			NS			NS			NS		

† Means with same lowercase letters within column are not significantly different among varieties by Tukey's HSD multiple comparison at $P < 0.05$.

‡ NS, *, **, ***: non-significant, significant at $P < 0.05$, 0.01, and 0.001, respectively.

Salt solution at EC of 5 and 10 dS·m^{-1} had similar influences on the relative leaf area of all sorghum varieties (Table 2). The average relative leaf area of all sorghum plants irrigated with salt solution at EC of 5 and 10 dS·m^{-1} was 68% and 56%, respectively, corresponding to a reduction of 32% and 44% compared to the control. The relative leaf area of 'Shallu', 'Desert Maize', and '1790E' irrigated with salt solution at EC of 5 dS·m^{-1} was over 94% (6% reduction), while that of 'BTx642' and 'Tx2783' was 79% and to 71%, respectively, others ranged from 52% to 40%. However, when salt solution at EC of 10 dS·m^{-1} applied, the relative leaf area of 'Shallu', 'Desert Maize', and '1790E' was more than 80%, while that of 'BTx642', 'RTx430', and 'Tx2783' were 45% to 50%, others were 31% to 44%. In addition, the relative number of leaves of all sorghum plants irrigated with salt solution at EC of 5 and 10 dS·m^{-1} was 86% and 75%, respectively (Table 2). Salt solution at EC of 10 dS·m^{-1} significantly reduced the number of leaves of 'Tx2783' and 'Wheatland' compared to that at EC of 5 dS·m^{-1}. Leaf area and number of leaves of forage sorghum 'Pegah' and 'Speedfeed' also decreased with increasing salinity levels from 0 to 12 dS·m^{-1} (Sadeghi & Shourijeh, 2012).

Table 3. Number of tillers, visual score, leaf net photosynthesis (P_n), and SPAD reading of ten sorghum varieties irrigated with nutrient solution [Electrical conductivity (EC)=1.5 dS·m^{-1}; Control] and salt solution (EC = 5.0 or 10.0 dS·m^{-1}) in the greenhouse

Variety	Treatment	Number of tillers	Visual score	P_n (μmol·m^{-2}·s^{-1})	SPAD
1790E	Control	1.3 a	4.0 a	33.3 a	46.2 a
1790E	EC 5	2.0 a	2.8 b	26.8 a	51.0 a
1790E	EC 10	2.7 a	2.8 b	27.2 a	46.4 a
BTx642	Control	0.0 b	4.0 a	30.2 a	43.3 a
BTx642	EC 5	0.2 ab	3.6 a	29.4 a	49.9 a
BTx642	EC 10	1.0 a	2.9 b	30.8 a	50.9 a
Desert Maize	Control	1.6 a	4.0 a	31.9 a	45.0 b
Desert Maize	EC 5	1.9 a	2.9 b	28.6 a	51.1 a
Desert Maize	EC 10	1.7 a	2.6 b	27.5 a	50.2 ab
Macia	Control	0.0 b	4.0 a	32.9 a	48.1 a
Macia	EC 5	0.7 ab	3.0 b	29.6 a	51.9 a
Macia	EC 10	1.3 a	2.6 b	26.5 a	47.1 a
RTx430	Control	1.0 a	4.0 a	32.2 a	53.0 a
RTx430	EC 5	2.0 a	3.0 b	30.5 a	52.5 a
RTx430	EC 10	2.2 a	2.2 c	28.6 a	46.8 a
Schrock	Control	0.2 b	4.0 a	34.2 a	46.2 a
Schrock	EC 5	2.2 a	2.5 b	33.6 a	52.0 a
Schrock	EC 10	3.2 a	2.0 b	27.1 a	50.2 a
Shallu	Control	3.2 a	4.0 a	31.6 a	39.5 a
Shallu	EC 5	3.9 a	4.0 a	30.3 a	37.9 a
Shallu	EC 10	4.8 a	4.0 a	31.4 a	37.8 a
Tx2783	Control	1.0 b	4.0 a	32.1 a	44.5 a
Tx2783	EC 5	3.8 a	3.0 b	30.9 a	48.1 a
Tx2783	EC 10	5.0 a	2.8 b	27.4 a	45.9 a
Tx7078	Control	1.3 a	4.0 a	27.4 a	42.3 b
Tx7078	EC 5	2.7 a	3.0 b	25.8 a	50.9 a
Tx7078	EC 10	2.6 a	2.6 c	28.8 a	45.9 ab
Wheatland	Control	0.1 a	4.0 a	26.9 a	46.4 a
Wheatland	EC 5	1.0 a	3.0 b	26.6 a	47.3 a
Wheatland	EC 10	1.1 a	2.3 c	24.1 a	47.0 a
Variety (V)		***‡	***	NS	***
Treatment (T)		***	***	*	***
V * T		NS	***	NS	NS

† For each variety, means with same lowercase letters within column are not significantly different among treatments by Tukey's HSD multiple comparison at $P < 0.05$.

‡ NS, *, **, ***: non-significant, significant at $P < 0.05$, 0.01, and 0.001, respectively.

Salt treatment significantly affected the number of tillers and visual quality of sorghum plants, and the response varied with sorghum varieties (Table 3). However, the interaction was significant only on visual quality. Salt solution at EC of 5 and 10 dS·m^{-1} substantially increased the number of tillers of sorghum 'BTx642', 'Macia', 'Schrock', and 'Tx2783', and also numerically increased the number of tillers of other tested sorghum varieties but not significant statistically. On average, all sorghum plants irrigated with salt solution at EC of 5 and 10 dS·m^{-1} increased by 119% and 161%, respectively, compared to the control. However, Sadeghi and Shourijeh (2012) observed that total number of tillers of forage sorghum ‘Pegah’ and ‘Speedfeed’ decreased as the salinity levels increased. All sorghum plants except for ‘Shallu’ had foliar salt damage when they were irrigated with salt solution (Table 3).

These results showed that ‘Shallu’, ‘Desert Maize’, and ‘1790E’were the most salt tolerant varieties at the stage of seedling growth, ‘Tx2783’, ‘Wheatland’, ‘BTx642’, and ‘Tx7078’ had intermediate salt tolerance, ‘Macia’, ‘Schrock’, and ‘RTx430’ were the least salt tolerant varieties.

3.3 Gas Exchange and SPAD Reading

All sorghum varieties had similar leaf net photosynthesis (P_n), and leaf P_n was impacted by salt treatment (Table 3), although no statistical difference was identified among treatments for each individual variety. Compared to the control, the leaf P_n of all sorghum plants irrigated with salt solution at EC of 5 and 10 $dS \cdot m^{-1}$ was decreased by 6.0% and 10.6%, respectively, when pooled for all varieties. Sadeghi and Shourijeh (2012) also found that salinity decreased the photosynthesis rate of forage sorghum ‘Pegah’ and ‘Speedfeed’. In this study, the reduction in leaf P_n was smaller than that of dry weight, which suggested that other factors such as leaf area and number of leaves contributed to the reduction of biomass accumulation. Another reason was because the instantaneous P_n was measured in a small area of a healthy leaf, which may not represent the P_n of the whole plant. In other words, the instantaneous P_n of a healthy leave was higher than that of the whole plant. Stomatal conductance (g_s) and transpiration (E) were statistically not significant among varieties and treatments (data not shown).

All sorghum varieties except for ‘Desert Maize’ and ‘Tx7078’ had similar SPAD readings among treatments (Table 3). In our previous study, no difference in SPAD readings was found between control and salt treatment for sorghum hybrids (SS304, NK7829, Sordan79, and KS585) (Niu *et al.*, 2012b). Ibrahim, Wright, Mirbahar, and Panhwar (2007) reported that the SPAD readings remained same in the leaves of wheat varieties (Pirsabak, Inqlab-91, SARC-6 and HD-2329) in salinity treatments after 70 days of sowing. However, SPAD readings were reduced by salt stress in maize genotypes (CUBA1, B73, and BR1) (Niu *et al.,* 2012b). Khatkar and Kuhad (2000) also observed that total chlorophyll content in wheat declined with increase in salinity. Salt solution at EC of 5 10 $dS \cdot m^{-1}$ increased the SPAD readings of ‘Desert Maize’ and ‘Tx7078’ (Table 3). This result agree with previous report that SPAD reading was significantly higher in salt treated sunflower (*Helianthus annuus*) plants relative to control (Rivelli, Lovelli, & Perniola, 2002).

3.4 Mineral Nutrient

The leaf concentrations of Na^+, Ca^{2+}, and Cl^- were significantly different among varieties and salt treatments, but no interactive effects occurred (Table 4). Although the leaf Na^+, Ca^{2+}, and Cl^- concentrations in most sorghum varieties weren’t statistically significant among treatments, they still increased numerically with increasing EC in all varieties, and the magnitude of increase varied with varieties. Compared to the control, the leaf Na^+ concentration in all varieties at EC of 5 and 10 $dS \cdot m^{-1}$ increased by 25.6% and 60.7%, respectively; while Cl^- concentration by 16.4% and 41.2%. The highest Na^+ (1.13 $mg \cdot g^{-1}$ DW) and Cl^- (20.93 $mg \cdot g^{-1}$ DW) concentrations were found in ‘Shallu’ at EC of 10 $dS \cdot m^{-1}$, at which ‘Shallu’ had lowest shoot DW reduction and greatest visual score. Furthermore, the Na^+ and Cl^- concentrations in ‘Shallu’ at EC of 10 $dS \cdot m^{-1}$ were still not significantly different from that in the control and EC of 5 $dS \cdot m^{-1}$. The Na^+ and Cl^- concentrations in this study are similar to previous reports on sorghum genotypes at EC of 8.0 $dS \cdot m^{-1}$ (Niu *et al.,* 2012b). Leaf Ca^{2+} concentration at EC of 5 and 10 $dS \cdot m^{-1}$ increased by 17.8% and 34.3%, respectively, compared with the control. The highest Ca^{2+} concentration of 12.04 $mg \cdot g^{-1}$ DW was observed in ‘Macia’ at EC of 10 $dS \cdot m^{-1}$. Na^+ or Cl^- exclusion and tolerance of tissue to accumulated Na^+ or Cl^- are two mechanisms in plant adaptation to salinity (Munns & Tester, 2008). In this study, the Na^+ concentration is much lower than that in maize genotypes at EC of 8.0 $dS \cdot m^{-1}$ (Niu *et al.,* 2012b), chili peppers at both EC of 4.1 and 8.1 dS·m^{-1} (Niu, Osuna, Sun, & Rodriguez, 2012a), *Sophora secundiflora* at EC of 3.0 and 6.0 dS·m^{-1} (Niu, Rodriguez, & Gu, 2010). The Cl^- concentration is also lower than that in chile peppers at EC of 8.1 dS·m^{-1} (Niu *et al.*, 2012a), but similar to that in maize genotypes at EC of 8.0 dS·m^{-1} (Niu *et al.*, 2012b) and *Sophora secundiflora* at EC of 3.0 and 6.0 dS·m^{-1} (Niu *et al.*, 2010). Therefore, at the species level, both Na^+ exclusion (Krishnamurthy, Serraj, Hash, Dakheel, & Reddy, 2007) and tolerance to high Cl^- attributed to salt tolerance of sorghum. Although 'Shallu' had relatively high Na^+ concentrations among the tested varieties the concentration (up to 1.13 mg·g^{-1} DW) was very low as compared to those of other species as discussed above.

Table 4. Leaf ion concentrations of ten sorghum varieties irrigated with nutrient solution [Electrical conductivity (EC) =1.5 dS·m^{-1}; Control] and salt solution (EC = 5.0 or 10.0 dS·m^{-1}) in the greenhouse

Variety	Treatment	Ion concentration (mg·g^{-1})			
		Na^+	Ca^{2+}	Cl^-	K^+
1790E	Control	0.24 b†	7.37 a	10.99 a	25.04 b†
1790E	EC 5	0.29 b	8.02 a	15.49 a	31.38 a
1790E	EC 10	0.42 a	8.95 a	17.25 a	32.88 a
BTx642	Control	0.25 a	6.19 a	8.66 b	24.21 a
BTx642	EC 5	0.28 a	7.42 a	8.70 b	25.53 a
BTx642	EC 10	0.28 a	8.23 a	15.35 a	29.14 a
Desert Maize	Control	0.48 a	7.66 a	14.64 a	18.75 a
Desert Maize	EC 5	0.53 a	8.21 a	17.61 a	21.64 a
Desert Maize	EC 10	0.52 a	8.03 a	19.10 a	20.84 a
Macia	Control	0.34 a	6.83 a	7.01 a	26.50 a
Macia	EC 5	0.35 a	10.00 a	9.21 a	28.70 a
Macia	EC 10	0.31 a	12.04 a	12.83 a	29.62 a
RTx430	Control	0.47 a	6.28 b	7.25 b	33.23 b
RTx430	EC 5	0.43 a	7.11 ab	6.92 b	33.99 b
RTx430	EC 10	0.59 a	7.80 a	13.94 a	38.61 a
Schrock	Control	0.29 b	4.89 a	9.59 a	25.19 c
Schrock	EC 5	0.65 a	4.85 a	14.78 a	34.19 b
Schrock	EC 10	0.58 a	5.03 a	18.24 a	49.60 a
Shallu	Control	0.58 a	3.93 b	18.85 a	28.21 a
Shallu	EC 5	0.74 a	5.41 a	18.98 a	31.21 a
Shallu	EC 10	1.13 a	6.01 a	20.93 a	31.65 a
Tx2783	Control	0.28 a	5.67 b	17.80 a	29.21 b
Tx2783	EC 5	0.30 a	7.29 ab	19.01 a	36.61 a
Tx2783	EC 10	0.77 a	9.93 a	20.68 a	42.07 a
Tx7078	Control	0.38 b	7.47 a	14.42 a	29.41 b
Tx7078	EC 5	0.55 ab	8.50 a	16.67 a	39.49 ab
Tx7078	EC 10	0.68 a	10.31 a	16.54 a	44.34 a
Wheatland	Control	0.36 a	8.69 a	12.17 a	26.50 b
Wheatland	EC 5	0.49 a	9.76 a	13.96 a	34.67 ab
Wheatland	EC 10	0.63 a	10.95 a	16.53 a	35.99 a
Variety (V)		***‡	***	***	***‡
Treatment (T)		***	***	**	***
V * T		NS	NS	NS	***

† For each variety, means with same lowercase letters within column are not significantly different among treatments by Tukey's HSD multiple comparison at $P < 0.05$.

‡ NS, *, **, ***: non-significant, significant at $P < 0.05$, 0.01, and 0.001, respectively.

Potassium makes a significant contribution to the turgor-pressure-driven solute transport in the xylem and the water balance of plants (Marschner, 1995). Maintenance of adequate K^+ levels is essential for plant survival in salt conditions. In this study, leaf K^+ concentrations increased significantly with increasing EC in six varieties but not in 'BTx642', 'Desert Maize', 'Macia', and 'Shallu' (Table 4). Compared to the control, the K^+ concentration in '1790E', 'Schrock', 'Tx2783', 'Tx7078', and 'Wheatland' at EC of 5 $dS \cdot m^{-1}$ increased by 25.3%, 35.7%, 25.3%, 34.3%, 30.8%, respectively; while that at EC of 10 $dS \cdot m^{-1}$ by 31.3%, 96.9%, 44.0%, 50.7%, and 35.8%. Cachorro, Ortiz, and Cerdá (1993) also reported that K^+ levels in the cell sap of bean leaves increased with increasing NaCl concentration. All these results demonstrated that K^+ might be preferentially acquired and transported against a strong Na^+ concentration gradient (Grattan & Grieve, 1998).

The results indicated that significant variability of salt tolerance existed among the sorghum varieties. 'Shallu', 'Desert Maize', and '1790E' were more tolerant to salt than other tested sorghum varieties, while 'Schrock' and 'RTx430' showed the least salt tolerance in both experiments. Salt tolerance also varied at plant growth stage (Macharia *et al.,* 1994). Our results demonstrated that salinity caused more damage in the seedling emergence stage than seedling growth stage in term of dry weight. Therefore, selection of suitable sorghum varieties for salt soil should consider the difference of growth stage and variation of varieties.

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