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NOTE

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Growth and Physiological Responses of Five Cotton Genotypes to Sodium Chloride and Sodium Sulfate Saline Water Irrigation

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ABSTRACT

This study was conducted to investigate the salt tolerance of five cotton genotypes [three *Gossypium hirsutum* L. (DN 1, DP 491, and FM 989) and two *G. barbadense* L. (Cobalt and Pima S-7)] under NaCl or Na_2SO_4 salinity conditions at similar osmotic potentials (100 mM NaCl vs. 70 mM Na_2SO_4 and 150 mM NaCl vs. 111 Na_2SO_4). To investigate whether the addition of calcium sulfate could alleviate the deleterious salinity effect, two more treatments were prepared by adding 10 mM $CaSO_4$ to 150 mM NaCl and 111 mM Na_2SO_4 solutions. All genotypes had significant growth reduction in all salt treatments as compared to their respective controls. Whereas Upland and Pima cotton did not differ in response to salt, DP 491 had lower growth reduction as compared to other genotypes and was therefore more salt tolerant. Salt type did not affect the growth of FM 989 and Pima S-7; however, dry weight (DW) of all organs were reduced to a greater extent by NaCl than by Na_2SO_4 in most organs in Pima Cobalt, shoot and total DW in DP 491, and root DW in DN 1. The addition of $CaSO_4$ alleviated some detrimental effects in DN 1 caused by NaCl and in Pima Cobalt caused by Na_2SO_4. The five genotypes also responded to salt treatments differently in that DN 1 and DP 491 had higher Na^+ and Cl^- concentrations and higher leaf osmotic potentials than the other three genotypes except for higher Cl^- concentrations in Cobalt. These results indicated that diversity of salt-tolerant mechanisms existed among the five cotton genotypes.

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High soil salinity reduces agricultural productivity in many regions of the world (Rozema and Flowers, 2008). It is estimated that 397 million hectares of land throughout the world are affected by salinity (FAO, 2005). The soil salinity problem in arid and semiarid areas is exacerbated due to low rainfall and poor quality of irrigation water (Pasternak and Malach, 1994; Villa-Castorena et al., 2003). For irrigated croplands, soil salinity can be increased to a damaging level, which varies with species or even cultivar within a species, when crops are irrigated without sufficient leaching or when poor drainage occurs. The degree of salt accumulation in the root zone depends on a number of interacting factors including the amount of dissolved salts in the irrigation water (water quality), fertilization rate, and the local climate.

The demand for cotton fiber is steadily increasing worldwide and the cottonseed is used for feed or oil (Ashraf, 2002). Accompanied with cotton fiber and seed production, large amount of residues from fields and gins are generated. Due to increased focus on renewable fuels in recent years, the potential for converting cotton waste into pellets, methane, pyrolytic products, and ethanol for energy are being explored (Sharm-Shivappa and Chen, 2008). Recent trends and demographic projections suggest that the need to produce more food and fiber will necessitate effective utilization of salt-affected land and saline water resources because the nonsaline lands are inadequate (Qadir et al., 2008). Currently, at least 20% of the world's irrigated land is salt affected and/or irrigated with waters containing elevated levels of salts (Ghassemi et al., 1995).

Upland cotton (*Gossypium hirsutum* L.) is considered a moderately salt-tolerant crop with a threshold salinity of 7.7 $dS \cdot m^{-1}$ (Maas, 1986). Therefore, cotton is a good candidate crop to be grown in salt-affected lands. However, reductions in cotton growth, yield, and fiber quality due to high salinity in soil or irrigation water have been reported (Dong, 2012; Higbie et al., 2010; Khan et al., 2001; Maas and Hoffman, 1977; Qadir and Shams, 1997). Many studies have revealed the existence of genetic

variations in salt tolerance among cotton genotypes (Ashraf, 2002; Hanif et al., 2008; Khan et al., 2001). Seed emergence and young seedlings are more sensitive to salinity compared to mature cotton plants (Ashraf, 2002; Khan et al., 1995; Leidi and Saiz, 1997; Qadir and Shams, 1997).

Most saline water and saline soils are dominated by chloride or sulfate salts (Bilski et al., 1988; Manchanda and Sharma, 1989; Rogers et al., 1998). However, much of the research quantifying salt tolerance of plant species has been based on experiments in which NaCl is the predominant salt. The degree of salt tolerance depends on dominant salt type and species (Khan et al., 1995; Niu and Rodriguez, 2008; Rogers et al., 1998). For example, sulfate salts were less deleterious than chloride salts to sweet pepper (*Capsium annuum* L.) (Navarro et al., 2002), chickpea (*Cicer arietinum* L.) (Manchanda and Sharma, 1989), and Siberian larch (*Larix siberica* L.) (Carter, 1980). Chloride-dominated salinity had more growth reduction in four species of rose rootstocks, although the magnitude of growth reduction varied with species (Niu and Rodriguez, 2008). However, for fababean (*Vicia faba* L.) and potato (*Solanum tuberosum* L.), Na_2SO_4 treatments were more detrimental to growth than NaCl treatments (Al-Hamzawi, 2007; Bilski et al., 1988).

Supplemental calcium has been found to alleviate some of the detrimental effects of NaCl or Na_2SO_4 salinity in some crops including tomato and potato (Bilski et al., 1988; Cabanero et al., 2004; Carvajal et al., 2000; Lopez and Satti, 1996; Navarro et al., 2000, 2002, 2005). However, Montesano and van Iersel (2007) reported that adding $CaSO_4$ to the hydroponic solution prevented a reduction of leaf photosynthetic rate but did not restore growth of the tomato plants. Khan et al. (1998) found that NaCl alone caused more growth reduction of four Upland cotton cultivars than a mix of salts of Na_2SO_4, NaCl, and $MgCl_2$. They also reported that salt effect on growth reduction of the cotton cultivars was partially alleviated by the addition of Ca^{2+} to the rooting medium.

Pima cotton (*G. barbadense* L.) might be more salt tolerant than Upland cotton because the former was originated from the sea coast in Peru (Smith and Cothren, 1999) and is bred and grown in the arid and semiarid southwest U.S. Although the detrimental effects of different salts and efficacy of supplemental Ca^{2+} on alleviation of salinity effect depend on species and genotypes, there currently is limited information on different responses between Upland and Pima cotton to different salts and addition of Ca^{2+}. The objectives of this study were to quantify the detrimental effect of NaCl and Na_2SO_4 salinity at similar osmotic potentials on growth of five cotton genotypes including three Upland DN 1, DP 491 (PVP200100159), FM 989 (PVP009800259) and two Pima Cobalt (PVP200500112) and Pima S-7 (PI560140), and to investigate the effect of supplemental Ca^{2+} on alleviation of salinity. DN 1 is an unreleased experimental line derived from TX-0307 (PI165390) plants selected from NaCl-treated hydroponic medium at Texas A&M AgriLife Research in Lubbock. FM 989 and DP 491 are used in the study as examples of more current cotton cultivars. Pima Cobalt and Pima S-7 are two Pima representatives.

MATERIALS AND METHODS

Plant Materials and Culture. Two cottonseed from each of the five genotypes (DN 1, DP 491, FM 989, Pima Cobalt, and Pima S-7) were sown on 13 Jan. 2011 at 2 cm depth into a 1.8-L round plastic pot filled with commercial potting soil mix Sunshine Mix No. 4 (SunGro, Hort., Bellevue, WA) in the greenhouse. The potting mix was pre-wet with reverse osmosis water with electrical conductivity of nearly zero. After emergence, seedlings were thinned to one plant per pot. Before salt treatments, plants were irrigated with a nutrient solution containing 0.72 $g \cdot L^{-1}$ of 15N-2.2P-12.5K (i.e., Peters 15-5-15, Scotts, Marysville, OH). One month after sowing, treatments were initiated by irrigating plants with treatment solutions as described below.

Treatments and Experimental Design. The seven treatments including control (i.e., nutrient solution with no addition of salts), together with the respective electrical conductivity (EC) levels are listed in Table 1. The two concentrations of NaCl at 100 mM and 150 mM had similar osmotic potentials to the two concentrations of Na_2SO_4 at 70 mM and 111.2 mM at temperature of 25°C. The osmotic potentials of the solutions were determined using an osmometer (Vapro Model 5520, Wescor, Logan, UT) to determine the relationships between osmotic potential and salt concentration for both salt solutions. To determine if the addition of calcium sulfate ($CaSO_4$) would mitigate the negative salinity effect, two more treatments were included by adding 10 mM $CaSO_4$ to 150 mM NaCl and 111 mM Na_2SO_4, respectively.

Table 1. Electrical conductivity (EC), pH, and osmotic potential (Ψ) of treatment solutions.

Treatment	EC ($dS \cdot m^{-1}$)	pH	Ψ (MPa)
Control	1.8	6.9	-- [z]
100 mM NaCl	11.3	6.8	-0.43
150 mM NaCl	15.4	6.8	-0.64
70 mM Na_2SO_4	12.3	6.9	-0.42
111 mM Na_2SO_4	17.2	7.0	-0.64
150 mM NaCl+10 mM $CaSO_4$	17.0	6.0	--
111.2 mM Na_2SO_4+10 mM $CaSO_4$	18.2	6.2	--

[z] Not measured.

The greenhouse experiment was a split-plot design with the treatments as the main plots and genotypes as the subplots (eight plants per subplot), and eight replications were used. Treatment solutions were prepared in 100-L tanks with confirmed EC and pH each time. The treatments were initiated on 14 Feb. 2011 and terminated on 31 March 2011, 42 d after treatments (DAT). Plants were manually irrigated with 500 mL each time when substrate surface started to dry, which yielded a leaching fraction of approximately 20%. Irrigation intervals were adjusted according to climate, treatment, and growth stage of the plants to prevent water stress and overwatering. The air temperatures in the greenhouse ranged from 25 to 30°C during the day and 19 to 22°C at night, and the relative humidity averaged 23% during the day and 35% at night. The daily integrated photosynthetic photon flux (PPF, photosynthetically active radiation) averaged 13.6 $mol \cdot m^{-2} \cdot d^{-1}$.

Measurements. At the end of the experiment, shoots were severed at the surface of substrate. Leaves, stems, and squares were separated and weighed. Roots were washed free of substrate soil and weighed. Dry weight of all plant parts were determined after oven drying at 70 °C to a constant weight.

Dry leaf samples were ground with a stainless steel Wiley mill and sent to an analytical lab for Na^+ and Cl^- concentration measurement (SWAT laboratory, New Mexico State University, Las Cruces, NM). To reduce the analytical cost, the two low concentrations (100 mM NaCl and 70 mM Na_2SO_4) were not analyzed. The Na^+ concentrations were determined based on the Environmental Protection Agency (EPA) method 200.7 (EPA, 1983) using an inductively coupled argon plasma (ICAP) Trace Analyzer (Thermo Jarrell Ash, Franklin, MA). Chloride was determined by EPA method 300.0 (EPA, 1983) using an ion chromatograph (Dionex, Sunnyvale, CA).

Due to the nature of the potting mix, salt accumulation was inevitable (Niu and Rodriguez, 2006). To monitor the root-zone salinity over time of the salt treatments, leachates were collected three times during the experiment according to the pour-through method (Wright, 1986). Whenever salinity of leachate exceeded 20 $dS \cdot m^{-1}$, leachate solution was diluted before measurement. The EC of leachate was determined using a salinity meter (Model B-173, Horiba, Ltd., Kyoto, Japan).

Leaf osmotic potential was determined as described in Niu and Rodriguez (2006). Briefly, a part of leaf was sampled from the middle section of the shoots in the early morning at the end of the experiment, washed in de-ionized water and dried by paper towel, sealed in a plastic bag, and immediately stored in a freezer at -20 °C until analysis. Frozen leaves were thawed in a plastic bag at the room temperature before sap was pressed out with a Markhart leaf press (LP-27, Wescor, Logan, UT) and analyzed using a vapor pressure osmometer (Vapro Model 5520, Wescor, Logan, UT).

Data Analysis. To compare the effect of salt stress on the reduction of growth in DW, a relative value to the control was calculated for each plant in the salt treatments for each genotype. As an example, relative shoot DW was calculated as:

$$\text{Relative shoot DW reduction (\%)} = 100\% - \frac{\text{Shoot DW in a salt treatment}}{\text{Averaged shoot DW in control}} \times 100\%$$

Similarly, relative values for other organs, square, leaf, stem, and total DW were calculated. These relative values were used to compare the differences among genotypes in statistical analysis.

All data were subjected to a two-way analysis of variance (ANOVA) using PROC GLM to test the significance of the main effects. When the main effects were significant, Student-Newman-Keuls multiple comparisons were performed for means separations among treatments or genotypes. PROC

GLM was also used to test the significance of a contrast between the two salts (NaCl vs. Na_2SO_4). All statistical analyses were performed using SAS (Version 9.1.3, SAS Institute Inc., Cary, NC).

RESULTS

Leachate Salinity. There were salt accumulations in all treatments, including the nonsaline control irrigated with nutrient solution. The EC of the irrigation water in control was approximately 1.8 $dS \cdot m^{-1}$, but its leachate EC was increased to 3.1 to 8.0 $dS \cdot m^{-1}$ (Table 2). The EC of leachates for salt treatment solutions at 8 DAT ranged from 1.3 to 1.6 times that of their respective irrigation treatment solutions. At 20 and 32 DAT, the leachate EC of treatments reached 1.5 to 2.6 times that of their respective irrigation treatment solutions.

Dry Weight. Both salinity and genotype and their interaction affected DW of all organs except for squares based on analyses of variance. Leaf DW was reduced by salt treatments (compared to the control) in all genotypes; however, no differences in leaf DW among salt treatments were detected, regardless of genotype (Table 3). For relative leaf DW reduction, no differences were found among genotypes in 150 NaCl and 70 Na_2SO_4 treatments. However, in almost all the salt treatments especially at 100 NaCl, 111 Na_2SO_4, and Ca_111 Na_2SO_4 levels, DP 491 had the lowest leaf DW reductions (46%, average). DN 1 had the lowest relative leaf DW reduction in Ca_150 NaCl.

DP 491 had the lowest and the two Pima genotypes had the highest stem DW under the control conditions. Stem DW of Pima Cobalt was the lowest in 150 NaCl and Ca_150 NaCl and the highest in Ca_111 Na_2SO_4. In this genotype, the stem DW in 100 NaCl was not statistically different from other salt treatments. The relative reduction percentage in stem DW by salt treatments ranged from 57 to 63%, regardless of genotype or salt treatment.

Under the control conditions, the two Pima cotton cultivars and FM 989 had higher square DW due to their earlier formation of squares, whereas DN 1 had the lowest square DW due to its late squaring. Therefore, it is understandable that DN 1 had the lowest square DW reduction (11%). Salt treatment reduced square DW (by 19-34%) but the effect was not significant except for Pima S-7. The square DW in Pima S-7 was reduced by 33 to 61% with an average of 48%, but no differences were detected among salt treatments. For relative square DW reduction, no differences were found among genotypes at different salt treatments except for Ca_150 NaCl treatment in which DN 1 did not show any reduction. DN 1 and DP 491 had the lowest relative square DW reduction across the treatments.

Salt treatments also reduced shoot, root, and total DW in all genotypes with an average of 53, 32, and 51% reductions, respectively (Table 4). However, there were no treatment differences in all genotypes except for Pima Cobalt and root DW in DN 1. In Cobalt, shoot and total DW was higher in 70 Na_2SO_4 and Ca_111 Na_2SO_4 as compared with other treatments. Root DW of Pima Cobalt was higher in 70 Na_2SO_4 than in 150 NaCl. However, no differences were found in root DW among the other treatments. On average across all the treatments, shoot and total DWs were reduced by more than 50% except for DP 491 (47-48%). Root DW reduction was greatest in Cobalt (41%) and smallest in DN 1 (24%) due perhaps to their highest and lowest root DW under normal nonsaline conditions, respectively.

Table 2. Leachate electrical conductivity (EC) of treatment solutions at 8, 20, and 32 d after treatment (DAT) for cotton genotypes irrigated with nutrient solution or saline solutions at two concentrations of NaCl (100 and 150 mM) or Na_2SO_4 (70 and 111 mM) with or without addition of 10 mM $CaSO_4$ to the higher NaCl or Na_2SO_4 concentration (Ca_150 NaCl and Ca_111 Na_2SO_4).

Treatment	Treatment EC ($dS \cdot m^{-1}$)	Leachate EC ($dS \cdot m^{-1}$)		
		8 DAT	20 DAT	32 DAT
Control (nutrient solution)	1.8	3.1	8.0	6.4
100 mM NaCl	11.3	18.0	26.6	28.0
150 mM NaCl	15.4	19.0	27.8	33.0
70 mM Na_2SO_4	12.3	19.5	27.1	32.1
111 mM Na_2SO_4	17.2	25.4	26.1	37.4
150 mM NaCl+10 mM $CaSO_4$	17.0	25.3	35.7	31.8
111.2 mM Na_2SO_4+10 mM $CaSO_4$	18.2	23.0	37.9	36.2

Table 3. Dry weight (DW, in grams, relative reduction percent in parentheses in percentage) of leaves, stems, and squares of five cotton genotypes when irrigated with saline solutions at two concentrations of NaCl (100 and 150 mM) or Na_2SO_4 (70 and 111 mM) with or without addition of 10 mM $CaSO_4$ to the higher NaCl or Na_2SO_4 concentration (Ca_150 NaCl and Ca_111 Na_2SO_4).

Control	Leaf DW				
	DN 1	DP 491	FM 989	Pima Cobalt	Pima S-7
	10.7 a [z]	8.1 a	10.1 a	9.7 a	9.5 a
100 NaCl	5.2b (52AB)	4.9b (39B)	4.9b (52AB)	4.2b (57A)	4.8b (49AB)
150 NaCl	4.6b (58A)	3.6b (55A)	3.7b (63A)	3.8b (61A)	4.0b (58A)
70 Na_2SO_4	4.9b (55A)	4.3b (47A)	5.0b (50A)	5.0b (48A)	4.2b (56A)
111 Na_2SO_4	4.8b (55AB)	4.5b (45B)	3.7b (64A)	3.7b (62A)	4.4b (54AB)
Ca_150 NaCl	5.6b (48B)	3.8b (53AB)	4.0b (61A)	3.9b (60A)	3.8b (60A)
Ca_111 Na_2SO_4	4.6b (55A)	5.1b (37B)	4.6b (55A)	5.1b (47A)	4.6b (52A)
Average	5.0 (54)	4.4 (46)	4.3 (58)	4.3 (56)	4.3 (55)
	Stem DW				
Control	6.1 a	4.2 a	6.3 a	6.9 a	7.3 a
100 NaCl	2.5b (56A)	1.8b (57A)	2.5b (60A)	3.0bcd (57A)	3.1b (58A)
150 NaCl	2.2b (64A)	1.4b (66A)	2.3b (63A)	2.6d (63A)	2.9b (60A)
70 Na_2SO_4	2.5b (58A)	1.3b (68A)	2.8b (55A)	3.2bc (54A)	3.3b (55A)
111 Na_2SO_4	2.4b (61A)	1.8b (58A)	2.1b (66A)	2.7cd (61A)	3.0b (60A)
Ca_150 NaCl	3.2b (47B)	1.5b (64A)	1.9b (69A)	2.4d (65A)	2.6b (64A)
Ca_111 Na_2SO_4	2.8b (55A)	2.4b (43A)	2.4b (62A)	3.5b (50A)	3.0b (59A)
Average	2.6 (57)	1.7 (59)	2.3 (63)	2.9 (58)	3.0 (59)
	Square DW				
Control	0.8 a	1.7 a	2.6 a	2.8 a	2.9 a
100 NaCl	0.6a (24A)	1.1a (38A)	2.2a (17A)	2.1a (25A)	1.6b (46A)
150 NaCl	0.5a (44A)	1.0a (40A)	1.7a (33A)	1.8a (33A)	1.5b (48A)
70 Na_2SO_4	0.7a (14A)	1.4a (21A)	1.5a (40A)	2.3a (26A)	1.9b (33A)
111 Na_2SO_4	0.6a (31A)	1.3a (27A)	1.9a (26A)	1.7a (40A)	1.1b (61A)
Ca_150 NaCl	0.9a (-6B)	1.3a (24AB)	1.4a (46A)	1.8a (33A)	1.6b (48A)
Ca_111 Na_2SO_4	0.8a (8A)	1.7a (9A)	1.5a (43A)	2.2a (21A)	1.5b (49A)
Average	0.7 (19)	1.3 (19)	1.7 (34)	2.0 (30)	1.5 (48)

[z] Means with the same small letters in the same column are not significantly different among treatments, and means with the same capital letters in the same row are not significantly different among genotypes tested by Student-Newman-Keuls multiple comparison at $P \leq 0.05$.

Overall, higher salt concentrations reduced plant growth to a greater extent than at the lower concentrations (100 NaCl or 70 Na_2SO_4). However, in the lower concentrations of NaCl and Na_2SO_4, no differences in relative reductions of shoot, root, and total DW were detected among genotypes. For relative shoot DW reduction, genotype differences were detected in higher salt concentrations (Ca_150 NaCl and Ca_111 Na_2SO_4). In Ca_150 NaCl, DN 1 had the lowest relative shoot DW reduction, followed by DP 491, whereas in Ca_111 Na_2SO_4, DP 491 had lowest shoot DW reduction as compared with other genotypes.

For root DW, DN 1 and Pima S-7 had lower root DW reduction than Cobalt. In 111 Na_2SO_4, DN 1 had a smaller root DW reduction as compared with Cobalt, whereas no differences were found among DN 1, DP 491, and Pima S-7. In Ca_150 NaCl, Cobalt had the greatest root DW reduction compared with other genotypes. No differences were found in relative root DW reduction in Ca_111 Na_2SO_4.

Table 4. Dry weight (DW, in grams, relative reduction percent in parentheses in percentage) of shoots, roots, and total of five cotton genotypes when irrigated with saline solutions at two concentrations of NaCl (100 and 150 mM) or Na_2SO_4 (70 and 111 mM) with or without addition of 10 mM $CaSO_4$ to the higher NaCl or Na_2SO_4 concentration (Ca_150 NaCl and Ca_111 Na_2SO_4).

Control	Shoot				
	DN 1	DP 491	FM 989	Pima Cobalt	Pima S-7
	17.7 a	14.0 a	19.0 a	19.4 a	19.7 a
100 NaCl	8.5b (52A)	7.8b (44A)	9.6b (50A)	9.3c (52A)	9.4b (52A)
150 NaCl	7.2b (59A)	6.1b (57A)	7.8b (59A)	8.2c (58A)	8.4b (58A)
70 Na_2SO_4	8.1b (54A)	7.0b (50A)	9.4b (51A)	10.5b (46A)	9.4b (52A)
111 Na_2SO_4	7.8b (56A)	7.5b (46A)	7.7b (60A)	8.1c (58A)	8.5b (57A)
Ca_150 NaCl	9.8b (45C)	6.6b (55B)	7.3b (62A)	8.1c (58AB)	8.1b (59AB)
Ca_111 Na_2SO_4	8.1b (54A)	7.5b (46B)	8.5b (55A)	10.8b (44AB)	9.1b (54A)
Average	8.3 (53)	7.1 (48)	8.4 (56)	9.2 (53)	8.8 (55)
	Root DW				
Control	1.5 a	1.9 a	2.5 a	2.7 a	2.4 a
100 NaCl	1.0b (31A)	1.1b (45A)	1.8b (25A)	1.6bc (42A)	1.6b (35A)
150 NaCl	1.0b (31B)	1.1b (44AB)	1.5b (38AB)	1.3c (51A)	1.7b (32B)
70 Na_2SO_4	1.3ab (17A)	1.3b (32A)	1.9b (21A)	1.9b (30A)	1.8b (27A)
111 Na_2SO_4	1.3ab (21B)	1.3b (33AB)	1.5b (39A)	1.5bc (43A)	1.8b (25AB)
Ca_150 NaCl	1.2b (21B)	1.4b (28B)	1.8b (28B)	1.4bc (46A)	1.8b (27B)
Ca_111 Na_2SO_4	1.2b (22A)	1.1b (41A)	1.8b (29A)	1.7bc (36A)	1.7b (31A)
Average	1.2 (24)	1.2 (37)	1.7 (30)	1.6 (41)	1.7 (30)
	Total DW				
Control	19.2 a	15.9 a	21.5 a	22.0 a	22.1
100 NaCl	9.5b (50A)	8.9b (45A)	11.4b (47A)	10.9c (51A)	11.0b (50A)
150 NaCl	8.2b (57A)	7.1b (55A)	9.3b (57A)	9.5c (57A)	10.0b (58A)
70 Na_2SO_4	9.4b (51A)	8.3b (48A)	11.3b (47A)	12.4b (44A)	11.2b (56A)
111 Na_2SO_4	9.1b (53AB)	8.8b (45B)	9.2b (47A)	9.6c (56A)	10.3b (53AB)
Ca_150 NaCl	10.9b (43C)	8.0b (50B)	9.1b (58A)	9.5c (57A)	9.9b (55A)
Ca_111 Na_2SO_4	9.3b (52A)	10.2b (36B)	10.2b (52A)	12.5b (43AB)	10.8b (51A)
Average	9.4 (51)	8.6 (47)	10.1 (51)	10.7 (51)	10.8 (54)

[z] Means with the same small letters in the same column are not significantly different among treatments, and means with the same capital letters in the same row are not significantly different among genotypes tested by Student-Newman-Keuls multiple comparison at $P \leq 0.05$.

In 111 Na_2SO_4, relative total DW reduction was significantly lower in DP 491 than FM 989 and Pima Cobalt, whereas no differences were found among other comparisons. In Ca_150 NaCl, DN 1 had the smallest total DW reduction, followed by DP 491, whereas the other three genotypes had similar reduction ranging from 55 to 58%. In Ca_111 Na_2SO_4, DP 491 had smaller total DW reduction, followed by Cobalt; however, no differences were found between Cobalt and other genotypes.

Overall, DP 491 had the lowest relative reductions in leaf, stem, square, shoot, and total DW and therefore was more salt tolerant than other genotypes tested in the current study. DN 1 had the lowest relative reduction in root DW, but that did not translate to less reduction in the above-ground growth as reflected in leaf, stem, and square DW.

The type of salt did not affect the DW of any organ in FM 989 and Pima S-7 (Table 5). Na_2SO_4 had smaller reduction in root DW of DN 1, shoot and total DW of DP 491, and all organs of Pima Cobalt except for square. The addition of $CaSO_4$ to 150 NaCl alleviated the growth reduction in DN 1, but not in other genotypes, whereas addition of $CaSO_4$ alleviated the growth reduction in Pima Cobalt in most organs but not in other genotypes.

Leaf Ion Concentration. To determine if different cotton genotypes accumulated Na^+ and Cl^- differently under different salt treatments, leaf Na^+ and Cl^- concentrations were analyzed for the control, 150 NaCl, 111 Na_2SO_4, Ca_150 NaCl, and Ca_111 Na_2SO_4 treatments (Table 6). Both salinity and genotype and their interaction affected leaf Na^+ and Cl^- concentrations. As expected, the control had the lowest Na^+ and Cl^- concentrations in all genotypes. For DN 1, the highest Na^+ concentration was found in 150 NaCl (32.6 $mg \cdot g^{-1}$), followed by Ca_150 NaCl at 25.5 $mg \cdot g^{-1}$ and Ca_111 Na_2SO_4 at 20.7 $mg \cdot g^{-1}$. For DP 491, the highest Na^+ concentration was found in Ca_150 NaCl at 31.3 $mg \cdot g^{-1}$. For FM 989, no differences were found in salt treatments with Na^+ concentrations ranging from 14.3 to 18.6 $mg \cdot g^{-1}$. For Pima Cobalt and Pima S-7, Ca_150 NaCl, and 150 NaCl had higher Na^+ concentrations as compared with that in the control. There were no differences in Na^+ concentrations among genotypes under the control conditions and Ca_111 Na_2SO_4 treatments. DN 1 in 150 NaCl and DP 491 in Ca_150 NaCl had the highest Na^+ concentrations among the five genotypes. FM 989, Pima Cobalt, and Pima S-7 had relatively lower Na^+ concentrations.

Table 5. Summary of contrast (100 NaCl, 150 NaCl, Ca_150 NaCl vs. 70 Na_2SO_4, 111 Na_2SO_4, Ca_111 Na_2SO_4) and t-test (150 NaCl vs. Ca_150 NaCl and 111 Na_2SO_4 vs Ca_111 Na_2SO_4) on dry weight (DW) of cotton genotypes irrigated with nutrient solution or saline solutions at two concentrations of NaCl (100 and 150 mM) or Na_2SO_4 (70 and 111 mM) with or without addition of 10 mM $CaSO_4$ to the higher NaCl or Na_2SO_4 concentration (Ca_150 NaCl and Ca_111 Na_2SO_4).

	Genotype				
Contrast	**DN 1**	**DP 491**	**FM 989**	**Pima Cobalt**	**Pima S-7**
Leaf DW	**NS**	**NS**	**NS**	**0.0221**	**NS**
Stem DW	**NS**	**NS**	**NS**	**0.0023**	**NS**
Square DW	**NS**	**NS**	**NS**	**NS**	**NS**
Root DW	**0.0071**	**NS**	**NS**	**0.0081**	**NS**
Shoot DW	**NS**	**0.0545**	**NS**	**0.0002**	**NS**
Total DW	**NS**	**0.0453**	**NS**	**0.0002**	**NS**
t-test: 150 NaCl vs. Ca_150 NaCl					
Leaf DW	**0.0154**	**NS**	**NS**	**NS**	**NS**
Stem DW	**0.0083**	**NS**	**NS**	**NS**	**NS**
Square DW	**NS**	**NS**	**NS**	**NS**	**NS**
Root DW	**0.0177**	**NS**	**NS**	**NS**	**NS**
Shoot DW	**0.0123**	**NS**	**NS**	**NS**	**NS**
Total DW	**0.0117**	**NS**	**NS**	**NS**	**NS**
t-test: 111 Na_2SO_4 vs. Ca_111 Na_2SO_4					
Leaf DW	**NS**	**NS**	**NS**	**0.02**	**NS**
Stem DW	**NS**	**NS**	**NS**	**0.05**	**NS**
Square DW	**NS**	**NS**	**NS**	**NS**	**NS**
Root DW	**NS**	**NS**	**NS**	**NS**	**NS**
Shoot DW	**NS**	**NS**	**NS**	**0.0052**	**NS**
Total DW	**NS**	**NS**	**NS**	**0.0075**	**NS**

NS: not significant.

Table 6. Leaf Na^+ and Cl^- uptake of five cotton genotypes when irrigated with nutrient solution, saline solutions at 150mM NaCl, 111 mM Na_2SO_4, 150 mM NaCl +10 mM $CaSO_4$, or 111 mM Na_2SO_4 + 10 mM $CaSO_4$.

Genotype	Control	150 mM NaCl	111 mM Na_2SO_4	Ca + 150 mM NaCl	Ca + 111 mM Na_2SO_4
			Na^+ ($mg \cdot g^{-1}$)		
DN1	3.3 d A [z]	32.6 a A	15.8 c B	25.5 ab AB	20.7 bc A
DP 491	3.0 c A	16.8 b B	20.8 b A	31.3 a A	19.8 b A
FM 989	7.6 b A	17.2 ab B	14.3 ab B	18.6 a BC	14.8 ab A
Pima Cobalt	2.0 c A	18.2 ab B	13.6 b B	20.9 a BC	14.1 b A
Pima S-7	2.1 c A	16.5 a B	13.3 b B	15.0 ab C	12.6 b A
			Cl^- ($mg \cdot g^{-1}$)		
DN1	16.0 c A	76.2 a A	9.3 c A	55.6 b AB	13.6 c A
DP 491	16.4 c A	38.9 b C	9.7 c A	68.3 a A	11.1 c A
FM 989	10.7 b A	50.7 a BC	8.4 b A	44.9 a B	9.9 b A
Pima Cobalt	13.9 b A	57.8 a B	10.9 b A	58.6 a AB	11.9 b A
Pima S-7	12.6 b A	49.2 a BC	9.3 b A	45.1 a B	8.9 b A

Analysis of Variance

	Na^+	Cl^-
Genotype	<0.0001	<0.0001
Treatment	<0.0001	<0.0001
Genotype x Treatment	0.0005	<0.0001

[z] **Means with the same small letters in the same row are not significantly different among treatments, and means with the same capital letters in the same column are not significantly different among genotypes tested by Student-Newman-Keuls multiple comparison at $P \leq 0.05$.**

Averaged over the five genotypes, leaf Cl^- concentration was the highest in 150 NaCl, and/or Ca_150 NaCl, and no differences were found among the rest of the salt treatments. No genotypic differences in Cl^- concentrations were detected under the control conditions, and at the 111 Na_2SO_4 and Ca_111 Na_2SO_4 levels. At the 150 NaCl level, DN 1 had the highest Cl^- concentration (76.2 $mg \cdot g^{-1}$) and DP 491 had the lowest (39.9 $mg \cdot g^{-1}$). At the Ca_150 NaCl level, DP 491 had higher Cl^- concentration than FM 989 and Pima S-7.

Supplemental Ca^{2+} reduced leaf Na^+ and Cl^- concentrations in DN 1 in the NaCl salinity but not in Na_2SO_4 salinity. In contrast, supplemental Ca^{2+} to NaCl salinity increased leaf Na^+ and Cl^- concentrations in DP 491. For other genotypes, supplemental Ca^{2+} to either NaCl or Na_2SO_4 did not affect leaf Na^+ and Cl^- concentrations.

Leaf Osmotic Potential. The accumulation of the ions in leaves might also affect osmotic potential. Both salinity and genotype, and their interaction affected leaf osmotic potential ($P = < 0.0001$). As compared to the control, which was usually the highest, salt treatments reduced leaf osmotic potentials. The exception was DN 1, in which salt treatments did not affect osmotic potential (Table 7). For DP 491, the osmotic potential was reduced at 100 NaCl and the reduction was significant when NaCl concentration was increased to 150 mM. The same trend was found for Na_2SO_4 salinity. The addition of $CaSO_4$ further decreased leaf osmotic potential and the effect was significant in Na_2SO_4 salinity. For FM 989, osmotic potential in the two NaCl concentrations and lower (70 mM) Na_2SO_4 concentration reduced leaf osmotic potentials from the control, but differences were insignificant. The leaf osmotic potentials in 111 Na_2SO_4, Ca_150 NaCl, and Ca_111 Na_2SO_4 were significantly lower. For Pima Cobalt and Pima S-7, all the salt treatments significantly reduced leaf osmotic potential and 111 Na_2SO_4 was significantly lower than other salt treatments in Cobalt. Among the five genotypes tested, DN 1 and DP 491 had the highest osmotic potentials under the control conditions and various salt treatments (except for DP 491 at Ca_111 Na_2SO_4), indicating that these two genotypes had the least osmotic adjustment, whereas the other three genotypes had similar lower osmotic potentials.

Table 7. Osmotic potential in MPa of cotton genotypes irrigated with nutrient solution or saline solutions at two concentrations of NaCl (100 and 150 mM) or Na_2SO_4 (70 and 111 mM) with or without addition of 10 mM $CaSO_4$ to the higher NaCl or Na_2SO_4 concentration (Ca_150 NaCl and Ca_111 Na_2SO_4).

	DN 1	DP 491	FM 989	Pima Cobalt	Pima S-7
Control	-2.5 a A [z]	-2.2 a A	-3.2 a B	-3.1 a B	-3.1 a B
100 NaCl	-2.4 a A	-2.6 a A	-3.6 ab B	-3.8 bc B	-4.0 b B
150 NaCl	-3.2 a A	-3.5 b A	-3.8 ab A	-3.6 b A	-4.0 b A
70 Na_2SO_4	-2.7 a A	-3.4 b B	-3.9 ab BC	-4.1 bc BC	-4.2 b C
111 Na_2SO_4	-2.3 a A	-3.7 b A	-4.2 b C	-4.9 d D	-4.2 b C
Ca_150 NaCl	-2.7 a A	-3.8 b B	-4.0 b B	-4.2 c B	-3.8 b B
Ca_111 Na_2SO_4	-2.4 a A	-4.3 c B	-4.0 b B	-3.8 bc B	-3.7 b B

[z] **Means with the same small letters in the same column are not significantly different among treatments, and means with the same capital letters in the same row are not significantly different among genotypes tested by Student-Newman-Keuls multiple comparison at $P \leq 0.05$.**

In control and at the 100 NaCl, 70 Na_2SO_4, and 111 Na_2SO_4 levels, osmotic potentials in DN 1 and DP 491 were significantly higher than those in other genotypes, whereas Pima Cobalt and Pima S-7 had the lowest leaf water potential. However, no genotypic differences in osmotic potential were observed in 150 NaCl.

DISCUSSION

Although cotton is classified as one of the most salt-tolerant crops and considered as a pioneer crop in reclamation of saline soils (Maas, 1986), its tolerance to salinity is far from that of halophytes (Dong, 2012). Therefore, growth and yield reduction is inevitable under high salinity conditions, which often reduces vegetative growth of cotton plants to a great degree with more reduction in shoots than roots (Ashraf, 2002; Dong, 2012). This is consistent with the current study, where shoot DW was reduced by more than 50% as compared to control for all genotypes in most saline water treatments, whereas the reduction of root DW ranged from 21 to 46%. However, the magnitude of reduction varied with genotype, salinity level, and with or without the addition of calcium sulfate. The greatest reductions (> 55%) were seen in stem DW and leaf DW at the higher salinity levels, as expected. The significant growth reduction under high salinity also agreed with previous studies in other cotton genotypes (Khan et al., 1995; Leidi and Saiz, 1997; Qadir and Shams, 1997). It should be noted that these high percentages of DW reductions were caused by excessive salts in the root zone as evidenced by high leachate EC (Table 2), which lessened the differences among treatments. The small differences in growth reduction among the five genotypes might be due to the small differences in tolerance to salinity among these genotypes. DP 491 might be slightly more tolerant as compared with the other four genotypes, because its average relative DW reductions in leaf, shoot, and total were the smallest among the five. Salt-tolerant cotton lines had higher shoot biomass than salt-sensitive lines (Ashraf, 2002; Ashra and Ahmad, 2000). In horticultural crops, salt-tolerant species also had smaller growth reduction under salinity stress conditions (Niu, 2012; Niu and Cabrera, 2010). Nevertheless, under these high salinity levels with leachate EC ranging from 18 to 38 $dS \cdot m^{-1}$ (significantly higher than the threshold of 7.47 $dS \cdot m^{-1}$), the fact that plants did not exhibit any visual foliar damage indicated the high salt tolerance of these cotton genotypes.

Responses to salt type and the efficacy of supplemental calcium sulfate on alleviation of growth reduction caused by high salinity differed among genotypes. Salt type and the addition of calcium sulfate to either NaCl or Na_2SO_4 did not affect the growth of FM 989 and Pima S-7. Growth of Pima Cobalt was reduced more by NaCl salinity as compared with Na_2SO_4 salinity, and the addition of calcium sulfate to Na_2SO_4 increased DW of leaf, stem, shoot, and total, although adding calcium sulfate to NaCl did not affect DW of any organ. For DN 1, NaCl had slightly more reduction in root DW. Supplemental calcium sulfate to NaCl alleviated some growth reduction of DN 1 but no effect was seen when calcium sulfate was added to Na_2SO_4. Although NaCl salinity caused lower shoot and total DWs of DP 491 as compared with Na_2SO_4, the addition of calcium sulfate to either NaCl or Na_2SO_4 solution did not alleviate any growth reduction.

Plant adaptations to salinity are of three distinct types: osmotic stress tolerance, Na^+ or Cl^- exclusion, and the tolerance of tissue to accumulation of Na^+ or Cl^- (Munns and Tester, 2008). Preferential accumulation of either Na^+, Cl^-, and/or both is known to associate with salt tolerance in crop species, and specific injury due to accumulation of these ions rather than osmotic stress are the major factor for sensitivity (Grattan and Grieve, 1999). In the current study, among the five genotypes, DN 1 and DP 491 had higher Na^+ concentrations than the other three genotypes, whereas these two cultivars and Pima Cobalt had higher Cl^- concentrations than the other two genotypes. The similar growth reductions by high salinity among all genotypes indicated similar tolerance to salinity. The differences in leaf Na^+ and Cl^- concentrations among the genotypes in this study are not surprising because of the diverse mechanisms and the resulting complexity involving in adaptation. Salt-tolerant cotton genotypes have evolved many mechanisms to adapt salinity (Ashraf, 2002; Dong, 2012). Previous studies indicated that the complexity in leaf Na^+ and Cl^- concentrations was associated with salt tolerance in different cotton species. For example, in Upland cotton (*G. hirsumtum*), Läuchli and Stelter (1982) found that salt tolerance appeared to be related to accumulation of Na^+ and Cl^- in the shoot. However, Pima cotton (*G. barbadense*) (Rathert, 1982) and in some wild *Gossypium* species (Gorham and Young, 1996), Na^+ accumulation was related to salt sensitivity. The leaf Na^+ and Cl^- concentrations of cotton genotypes found in this study and previous studies by other researchers (Akhtar et al., 2010; Leidi and Saiz, 1997) were much higher than other crops such as sorghum (Netondo et al., 2004; Niu et al., 2012) and wheat (Goudarzi and Pakniyat, 2008).

Osmotic adjustment is another mechanism in tolerance to salt and drought stresses in many crops. Plants are able to tolerate salinity by reducing the cellular osmotic potential as a consequence of a net increase in inorganic and solute accumulation (Hasegawa et al., 2000). Differences in osmotic potentials were detected in the five cotton genotypes. Interestingly, the osmotic potentials in genotypes DN 1 and DP 491, which had relatively high Na^+ and Cl^- concentrations, were higher (less negative) than those in other three genotypes. These results further indicate the diversity of the salt-tolerant mechanisms of these genotypes.

In summary, the five cotton genotypes had good salt tolerance as evidenced by no foliar salt damage under high salinity in the substrate ranging from 18 to 36 $dS \cdot m^{-1}$, although significant growth reductions were observed in all treatments. DP 491 had slightly smaller growth reduction among the five. No differences in salt tolerance were observed between the two species in this study. Salt type did not affect the growth of FM 989 and Pima S-7, whereas that of Pima Cobalt and DP 491 was reduced more with NaCl salinity as compared with Na_2SO_4 salinity. The addition of calcium sulfate alleviated the growth reduction in DN 1 caused by NaCl and in Pima Cobalt caused by Na_2SO_4 salinity. The five genotypes appeared to have different salt tolerant mechanisms as evidenced by their differences in leaf Na^+ and Cl^- concentrations and osmotic potentials and the efficacy of calcium in alleviation of growth reduction by salinity.

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