

BREEDING AND GENETICS

Salinity Responses of Selected Introgressed Cotton Lines Grown in Two Soils from Organic and Conventional Cotton Production

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ABSTRACT

Cotton yield is adversely affected by various stresses including salinity. Genetic variation in salt tolerance is often evaluated in potting soil media irrigated with saline solutions but not a field soil. This study evaluated the efficacy of screening four introgressed Upland genotypes from a backcross *Gossypium hirsutum* L. × *G. barbadense* L. inbred line population grown in two soils, i.e., an organic farm loam soil or a conventional farm clay soil. The genotypes were treated with NaCl for three wk at concentrations of 0 or 200 mM starting at the second true leaf stage. Chlorophyll content and fluorescence, plant height, leaf length, main stem node number and internode length, shoot biomass, and number of fruiting sites at three, six and nine week intervals (WT) were measured. Significant genotypic variation at three and six WT was observed, suggesting that salt tolerance screening in early cotton establishment is optimal. The salt treatment had negative effects on all growth traits except chlorophyll content and fluorescence. The organic farm loam soil amended with dairy manure had higher pre-treatment salinity content than the conventional farm clay, resulting in greater initial growth suppression at zero WT. However, the manure-amended soil showed less reduction in vegetative and reproductive growth after more prolonged salt treatment, suggesting that more scrutiny may be needed in soils treated with carbon-based fertilizers. The lack of genotype by soil or treatment interactions suggests that soil type had little bearing in screening cotton genotypes for salt tolerance and that

control (non-saline) treatments may not be needed for cotton salt tolerance screening. Finally, this pot study demonstrates that three to six weeks of salt treatments after the second true leaf stage is an adequate duration of time to screen for cotton salt tolerance.

Estimates of the world's irrigated land affected by salinity range from 20% upwards to 50% (Pitman and Läuchli, 2002). Cotton is a moderately salt tolerant crop (Hemphill et al., 2006) with an initial yield decline (i.e., threshold) at 7.7 dS m⁻¹ in the soil saturation extract (Maas and Hoffman, 1977). Nevertheless, cotton yield can adversely be affected through variable interactions of plant responses to salinity, with Na⁺ and Cl⁻ toxicity being the most detrimental (Ashraf, 2002; Higbie et al., 2010; Maas and Hoffman, 1977). Deleterious effects of salinity are particularly pronounced in arid and semiarid climates, where secondary salinization is a consequence of evapotranspiration, improper crop management, and poor irrigation water quality (Carter, 1975; Flower and Flowers, 2005; Munns, 2002; Pitman and Läuchli, 2002; Sharma and Goyal, 2003). Considering the prevalence of these problems, exploitation of salt tolerant cultivars is one of the most viable solutions to improving cotton yield in the Southwestern United States.

Screening for salt tolerance based on yield in field conditions is problematic due to the spatial and temporal variability of soil salinity and local environmental conditions (Akhtar et al., 2010; Ashraf, 2004; Flowers, 2004; Mittler, 2006; Munns, 2002; Neumann, 1997; Sharma and Goyal, 2003). Additionally, field trials may involve lengthy and expensive evaluations. Thus, screening attempts have relied on assessments of genetic markers and *in vivo* physiological responses. Despite advances in methods for detecting genetic variation, there have been few salt tolerant cultivars released through the use of quantitative trait locus (QTL) mapping using genetic markers, transgenic plants, wide-crossing, or exploitation of molecular mechanisms (Flowers, 2004; Flowers and Flowers, 2005; Lubbers et al., 2007;

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Noble and Rogers, 1992; Sharma and Goyal, 2003). In cotton, studies in salt tolerance have been based on inter- and intraspecific variation in physiological characteristics conferring tolerance in controlled environments (Ashraf, 2002 and 2004; Epstein, 1976; Flowers, 2004; Hanif et al., 2008; Highbie et al., 2005 and 2010; Niu et al., 2013; Rodriguez-Uribe et al., 2011; Tiwari et al., 2013a and b).

Cotton genotypes vary in their responses to NaCl, and this variation may be ontogeny-dependent (Ashraf, 2002; Bajaj et al., 2008; Barrick et al., 2012; Flowers, 2004; Flowers and Flowers, 2006; Hemphill, 2006; Highbie et al., 2005 and 2010; Niu et al., 2013; Qadir and Shams, 1997; Tiwari et al., 2013a and b). Tiwari et al. (2013b) recently reported genetic variation in salt tolerance in a backcross inbred line (BIL) population of an interspecific hybrid between *Gossypium hirsutum* L. and *G. barbadense* L. using a commercial potting medium and a two-wk duration of salt treatment. However, it is unknown if that variation is affected by soil type or experimental duration. The objectives of this study were to evaluate salt tolerance of four of these cotton BILs in two different farm soils using larger pots, and over a longer period of plant growth. We evaluated the effects of genotype, soil, salt treatment, and their interactions.

MATERIALS AND METHODS

Plant Materials. Four introgressed Upland cotton genotypes (NMHT-50, NMHT-59, NMHT-70, and NMHT-80) were selected from a backcross inbred line (BIL) population derived from an interspecific hybrid between *G. hirsutum* L. cv. Sure-Grow 747 and *G. barbadense* L. cv. Pima S-7 (Tiwari et al., 2013a and b). Based on that study, NMHT-50 and NMHT-80 were more salt tolerant during early (two-wk) seedling growth, while NMHT-59 and NMHT-70 were relatively salt sensitive.

Soil Collection, Preparation, and Characterization. The two soils that were used in this study were collected from the upper 30 cm of planting beds from each respective field. The loam (Anthony-Vinton Loam, and Harkey Loam soil series) was collected from a 15-yr certified organic cotton farm in La Union, NM (<http://www.newfarm.org/features/0104/organicotton.shtml>), and the clay soil (Armijo Clay Loam, and Harkey Loam soil series) from a conventional (non-organic) cotton field at New Mexico State University's Leyendecker Plant Science Research Center, south of Las Cruces, NM.

The two sites where soils were collected are situated in the Mesilla valley and are 40 km apart. Generally, these thermal Typic Torrifluent (Anthony-Vinton Loam and Harkey Loam soil series) and thermal Typic Torrtent (Armijo Clay Loam) soils are calcareous, and range from moderate to low permeability, and moderate to high shrink-swelling as the soil composition moves from coarse, i.e. loam, to fine texture, i.e. clay (Bullock and Neher, 1980; Soil Survey Staff, 2009).

Soil was air dried, crushed and sieved with a two mm sieve and texture was determined using the hydrometer method (Gee and Bauder, 1979; USDA-SCS, 1972). The soil that was potted for this experiment was crushed, sieved in a two mm sieve, and homogenized in a cement mixer. The soil was sifted into 3.8 L blow molded, tapered nursery pots up to approximately 2.54 cm from the upper rim. Saturated paste extracts were prepared and analyzed to determine the initial soil salinity (USDA, 1954; Rhoades, 1996), sodium adsorption ratio (Gavlak et al., 1994; Rhoades, 1996) and pH (USDA-SCS, 1972).

Pot Preparation, Planting, and Growing Conditions. At the bottom of the 3.8 L nursery pots, all but the center drain hole was plugged with cotton fibers to allow leaching but prevent soil loss. Pots were moistened daily with tap water with electrical conductivity (EC) of ~ 0.5 dS m^{-1} for a 10-d period to allow soil to settle and cotton seed to germinate in favorable conditions. To ensure a uniform emergence, seeds were pre-soaked in tap water at room temperature for 24 h (Hegarty, 1978; Stiles, 1948), and then sowed in three hills per pot and two seeds per hill. After emergence, seedlings were thinned to one plant per hill. Plants were grown in a shadehouse from July 16, 2013 to October 7, 2013. The shadehouse received only natural sunlight, and the temperature ranged from a daytime mean of 29.4 to a nighttime mean of 21.8°C. The roof and walls of the shadehouse were covered with 70% shade-cloth allowing a maximum photosynthetic photon flux of 1149 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Model LI-189, LI-COR Inc., Lincoln, NE, USA).

Salt Treatment. At the second true leaf stage, i.e., three weeks after planting (WAP), the salt treatment was initiated at 200 mM NaCl (Highbie et al., 2010; Tiwari et al., 2013b), equivalent to an electrical conductivity (EC) of 19.48 dS m^{-1} . The control treatment was tap water (0.5 dS m^{-1}). Five-hundred mL of each treatment were applied to the top of each pot every other day up to the first sampling date, i.e.,

three weeks after treatment (WT) or 6 WAP. On the off-treatment days, a predetermined amount of 200 mL of tap water was applied to the top of pots, which minimized the shrink/swell of the soil while maintaining pot water storage capacity without leaching. The soil leachate from the NaCl treatment reached 37 dS m⁻¹ at three WT. Therefore, following three WT, NaCl was no longer applied and soils were maintained at pot water storage capacity with tap water, without leaching, to ensure continued plant growth and exposure to the salt introduced at three WT. At three WAP and three WT, Miracle-Gro fertilizer (Scotts Miracle-Gro Co., Marysville, OH, USA) was dissolved into, and applied with the saline and non-saline treatments.

Plant Measurements and Analyses. Individual plant height, leaf length, the number of main stem nodes, main stem internode length, number of fruiting sites (including squares, flowers or young bolls), total shoot biomass, chlorophyll content, and chlorophyll fluorescence (measured as described below) were recorded at three, six and nine WT or six, nine and 12 WAP. The growing degree days (base = 60°C) for the three sampling dates were 539, 712, and 829°C, respectively, based on method two of McMaster and Wilhelm (1997). Pre-treatment plant height was measured at two WAP. At three WT, the center plant in each pot was measured and destructively sampled, and for the six and nine WT sampling dates, the outer two plants were sequentially sampled.

Plant height was measured from the soil surface to the apical meristem. The number of main stem nodes between the first true leaf and the topmost leaf (newest fully expanded leaf) was determined, and used to calculate the average internode length. The third leaf from the topmost leaf was used to measure leaf length, chlorophyll content, and chlorophyll fluorescence (measured as described below). Leaf length was measured from the base of the petiole along the midrib to the tip of the main lobe. Chlorophyll content was obtained with a Konica Minolta SPAD-502 (Konica Minolta Sensing Americas, Inc., Ramsey, NJ, USA), averaged from three readings on the interveinal tissue for each individual, and hereafter referred to as SPAD chlorophyll reading. The quantum efficiency of photosystem II (F_v/F_m) was determined using dark adaptation with a chlorophyll fluorometer OS-30P (Opti-Sciences, Inc., Tynsboro, MA, USA), and hereafter referred to as chlorophyll fluorescence. By six and nine WT, flower buds had emerged, and the number of fruit-

ing sites per plant was counted. Plant shoots were destructively sampled by cutting the plant at the soil surface. Shoot fresh weight was recorded, and then shoot dry weight was determined after oven drying at 65°C for three to four d.

Experimental Design and Statistical Analysis.

The study was laid out as a split-split plot design with four replications. The main plot was the cotton genotype, the subplot was the soil type, and the sub-subplot was the salt treatment. Analysis of variance (ANOVA), was conducted using SAS version 9.3 (SAS Institute Inc., 2012), at three, six, and nine WT. The ANOVA was also performed on plant height at two WAP prior to initiation of saline treatment. Means were separated by least significant difference (LSD) at $P=0.05$, and were the average of the two plants (sampled at three, six, and nine WT) from each replication, within each sub-subplot for both salt treatment and control.

RESULTS AND DISCUSSION

Genotypic Effects. Genotype interacted with neither salinity nor soil type except at six WT when there was a genotype \times salt treatment interaction on shoot dry weight. This interaction (data not shown) resulted from NMHT-59, which had a positive cross-over interaction, having the lowest mean of the non-saline treatment (9.31 g), but the highest mean (4.16 g) of the salt treatment with NMHT-70. In addition, NMHT-50 had a negative cross-over interaction, having the largest mean of the non-saline treatment (11.14 g), and the lowest mean of the saline treatment (3.72 g). These interactive differences between saline and non-saline treatment in shoot dry weight means may suggest a combination of increased osmotic adjustment and preferential anti-oxidant response by NMHT-59, and the inverse response for NMHT-50 at six WT (Desingh and Kanagaraj, 2007; Gossett et al., 1994; Qadir and Shams, 1997).

Genotype effects on chlorophyll fluorescence, leaf length, the number of main stem nodes, and internode length were significant only at three and six WT but not nine WT (Tables 1-3). At three WT, NMHT-50 had the highest chlorophyll fluorescence (Fig. 1A), NMHT-70 the largest leaf length (Fig. 1B), and NMHT-59 and NMHT-70 the longest internodes (Fig. 1C). By six WT, the leaf length continued to be the longest on NMHT-70 and NMHT-50, and NMHT-80 had the highest number of main stem nodes (Fig. 1D), although NMHT-80 had shortest internodes.

Table 1. Analysis of variance for four introgressed cotton genotypes grown in two soil types under salt treatment (200 mM NaCl) and control conditions after six wk (three WT).

Source	SPAD chlorophyll (reading)	Chlorophyll florescence (<i>Fv/Fm</i>)	Plant height (cm)	Leaf length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Main stem nodes (no)	Internode length (cm)
Genotype (Geno)		**		**				*
Soil	***		**			**		**
Geno x Soil								
Treatment (Trt)			***	***	***	***	***	**
Geno x Trt								
Soil x Trt	*			*				
Geno x Soil x Trt								

^z *, **, ***, Significance of F-test at $p \leq 0.05, 0.01, \text{ or } 0.0001$, respectively; blank cells denote non-significance.

Table 2. Analysis of variance for four introgressed cotton genotypes grown in two soil types under salt treatment (200 mM NaCl) and control conditions after nine wk (six WT).

Source	SPAD chlorophyll (reading)	Chlorophyll florescence (<i>Fv/Fm</i>)	Plant height (cm)	Leaf length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Main stem nodes (no)	Fruiting sites (no)	Internode length (cm)
Genotype (Geno)				**			**		*
Soil		*		**		**			
Geno x Soil									
Treatment (Trt)	*			***	***	***	***	***	***
Geno x Trt						*			
Soil x Trt				**		**			
Geno x Soil x Trt									

^z *, **, ***, Significance of F-test at $p \leq 0.05, 0.01, \text{ or } 0.0001$, respectively; blank cells denote non-significance.

Table 3. Analysis of variance for four introgressed cotton genotypes grown in two soil types under salt treatment (200 mM NaCl) and control conditions after 12 wk (nine WT).

Source	SPAD chlorophyll (reading)	Chlorophyll florescence (<i>Fv/Fm</i>)	Plant height (cm)	Leaf length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Main stem nodes (no)	Fruiting sites (no)	Internode length (cm)
Genotype (Geno)									
Soil	*			**	*		**	**	
Geno x Soil									
Treatment (Trt)	**		***	***	***	***	***	***	***
Geno x Trt									
Soil x Trt									
Geno x Soil x Trt									

^z *, **, ***, Significance of F-test at $p \leq 0.05, 0.01, \text{ or } 0.0001$, respectively; blank cells denote non-significance.

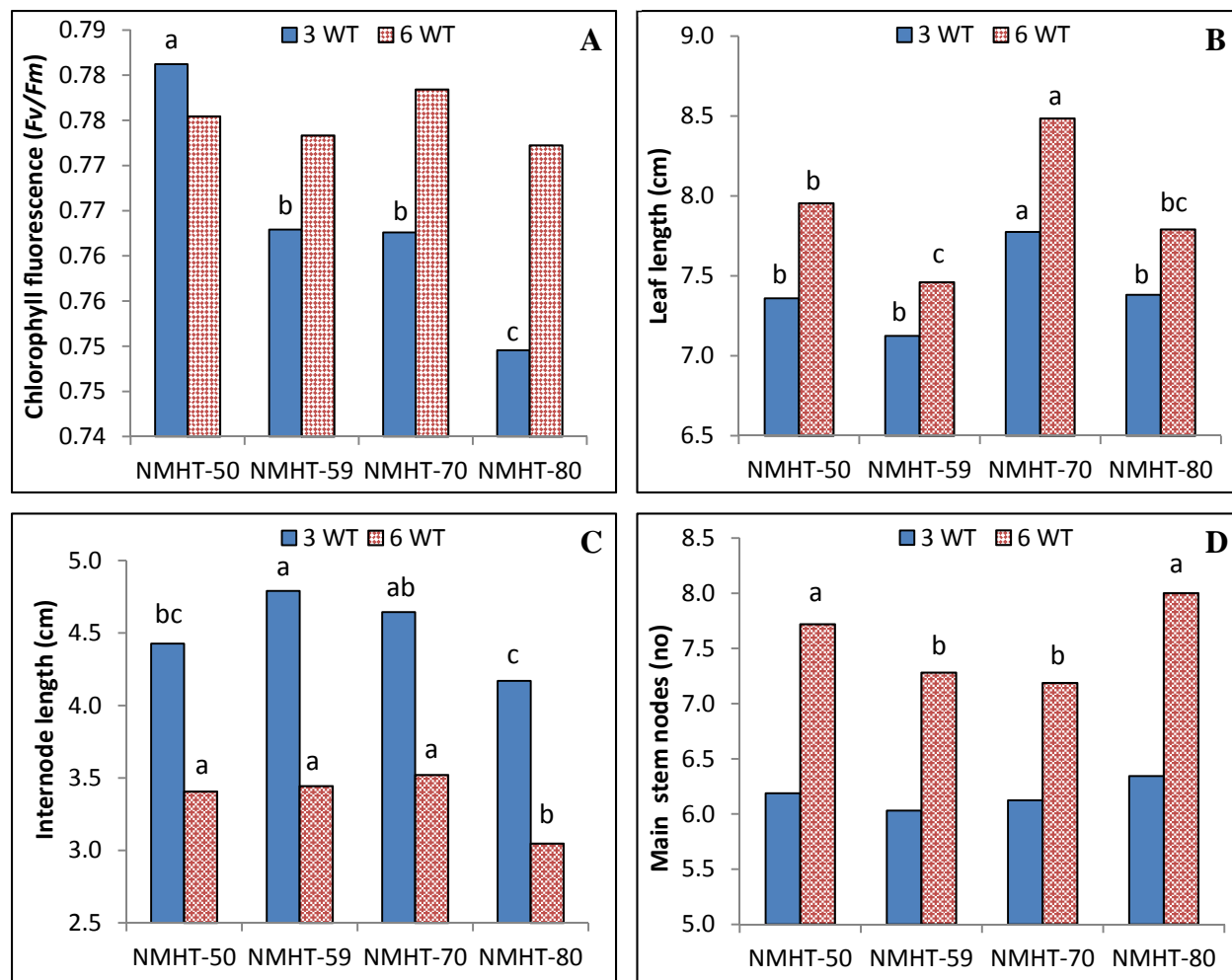


Figure 1. Average chlorophyll fluorescence (A), leaf length (B), main stem internode length (C), and number of nodes on the main stem (D), of four introgressed cotton genotypes at three and six wk of salt treatment (WT) with 200 mM NaCl and 0 mM NaCl. Mean separation by LSD at $\alpha = 0.05$; means within WT and across genotypes with different letters are significantly different and lack of letters within WT and across genotypes indicates that there were no significant genotype effects.

These genotypic effects were apparent in the early stages of cotton growth (nine WAP), and were consistent with other reports (Ashraf, 2002; Hemphill et al., 2006; Higbie et al., 2010; Qadir and Shams, 1997). Thus, the results suggest that three-six wk of salt treatment may be adequate for screening cotton genotypes for salt tolerance, and that additional salt treatment may not reveal genetic variation in salt tolerance.

Cross-examination of the above salt tolerance or sensitivity of the BILs evaluated in this study, with the results reported by Tiwari et al. (2013b), revealed some incongruities. Tiwari et al. (2013b) classified NMHT-50 and NMHT-80 as more salt tolerant than NMHT-59 and NMHT-70, based on shoot dry weight at two-wk of salt treatment. The divergent plant response of these salt-affected genotypes to similarly segregate by dry weight in the present study, in

otherwise similar experimental conditions (three to six WT and 200 mM NaCl), may be due to the use of different soil types.

Soil Type Effects. Effect due to soil on plant height was statistically significant even before salt treatment was initiated, at two WAP. On average, seedlings grown in the organic farm loam soil were 12.6% shorter than those grown in the conventional farm clay soil (10.95 vs. 12.53 cm). This difference may have been due to pretreatment salinity levels, since the EC_e of the organic farm loam soil was 8.4 $dS\ m^{-1}$ (higher than the salinity threshold, 7.7 $dS\ m^{-1}$ for cotton) compared to 4.8 $dS\ m^{-1}$ in the conventional farm clay soil. The higher salinity in the organic farm loam soil may have resulted from long-term application of dairy manure, which introduces monovalent cations, particularly Na^+ and K^+ (Hao and Chang, 2003; Haynes and Naidu, 1998; Mendoza et al., 2011).

Soil type interacted with salt treatment on SPAD chlorophyll and leaf length at three WT, and on leaf length and shoot dry weight at six WT (Tables 1 and 2). These interactions are presented and discussed later. Soil type main effect was significant for numerous plant response variables during salt treatment (Tables 1-4). At three WT, seedlings grown on the conventional farm clay soil were taller (Fig. 2A), and had longer internodes (Fig 2B), higher mean shoot dry weight (Fig. 2C) and SPAD chlorophyll (Fig. 2D) than seedlings grown in the organic farm soil. At six WT, mean shoot dry weight of the plants grown on the conventional farm clay soil was still higher than that on the organic farm loam soil, but there was shorter plant height and leaf length (Fig. 2E), and lower chlorophyll fluorescence (Fig. 2F) on the conventional farm clay soil as compared with the organic farm loam soil. At the third sampling date (nine WT), plants grown on the conventional farm clay had lower SPAD chlorophyll readings, shorter leaf length, lower fresh weight (Fig. 2G), and smaller number of main stem nodes (Fig. 2H) and fruiting sites (Fig. 2I) compared with plants grown on the organic farm loam soil. The only consistent difference between the two soils was for leaf length at the last two sampling periods, i.e., plants grown on the clay soil had smaller leaves, which may have resulted in reduced number of fruiting sites and fresh shoot weight.

Nonetheless, higher plant vigor was observed on the conventional farm clay soil at the first sampling period, possibly due to its initial lower level of soil salinity, whereas the overall growth response was higher on the organic farm loam soil after a prolonged salt treatment (six and nine WT). This improved growth response may be attributable to increased availability of soil organic carbon and improved soil physical

properties imparted by the dairy manure fraction of this soil, ameliorating ionic deficiencies outside of the plant root. The increased soil organic matter acts as a labile pool of soil organic carbon, giving rise to microbial activity, which in turn facilitates greater synthesis and exchange of plant available nutrients. Concurrently, dairy manure soil amendments can also reduce soil bulk density, improve porosity (especially pore spaces <30 μm) and aggregate stability. The promulgation of these soil physical characteristics is increased hydraulic conductivity and water holding capacity, which helps to offset the increasingly negative solute potential (ψ_s) of the saline soil solution by lowering soil water tension thereby alleviating salt induced osmotic stress outside the root (Haynes and Naidu, 1998). In this study, calcium concentration of the pre-treatment soil was 34.7 meq/L for the organic farm loam soil and 25.15 meq/L for the conventional clay soil. Whether the higher calcium concentration in the organic farm soil had any effect in mitigating the negative effect of Na is currently unknown.

Salt Treatment Effects. The salt treatment at 200 mM NaCl resulted in significant reductions in plant height, leaf size, the number of main stem nodes, the number of fruiting sites, internode length, chlorophyll content and shoot biomass, as reported in other cotton studies (Ashraf, 2002; Highbie et al., 2010; Hemphill et al., 2006; Qadir and Shams, 1997). Out of the eight traits evaluated at every sampling period, plus fruiting sites at six and nine WT, the NaCl treatment reduced all plant-growth-related responses except SPAD chlorophyll reading at three WT and chlorophyll fluorescence (Tables 1-3 and Fig. 3). The overall percent reductions due to the 200 mM NaCl treatment (all statistically significant between control and treatment plant responses,

Table 4. Physiological and agronomic characters by soil type (conventional farm clay soil vs. organic farm loam soil) inequality relationships at three, six, and nine weeks of salt treatment (WT).

Physiological and agronomic character	3 WT		6 WT		9 WT	
Plant height, cm (Fig. 2A)		<		>		ns
Internode length, cm (Fig. 2B)		<		ns		ns
Shoot dry weight, g (Fig. 2C)		<		<		ns
SPAD chlorophyll, reading (Fig. 2D)		<		ns		>
Leaf length, cm (Fig. 2E)		ns		>		>
Chlorophyll fluorescence, F_v/F_m (Fig. 2F)		ns		>		ns
Shoot fresh weight, g (Fig. 2G)		ns		ns		>
Main stem nodes, no (Fig. 2H)		ns		ns		>
Fruiting sites, no (Fig. 2I)		n/a		ns		>

^z <, > denote inequality trends of the mean of four double pot, double plant replications between two soil types; ns denotes non-significance; n/a denotes not applicable.

and depending on WT) ranged from 28.0-45.3% for plant height (Fig. 3A), 7.9-29.3% for internode length (Fig. 3B), 19.1-26.8% for the number of main stem nodes (Fig. 3C), 22.1-26.7% for leaf length (Fig. 3D), 7.7-8.1% for SPAD chlorophyll reading (Fig. 3E), 44.8% for number of fruiting sites (Fig. 3F, 6 and 9 WT only), 53.2-59.8% for shoot fresh weight (Fig. 3G), and 52.8-70.5% for shoot dry weight (Fig. 3H). However, differences in percent reduction of treatment from one sampling date to another ranged from -3 to 10%

with a trend of decreasing reduction, suggesting plant acclimation to salt over time, or differences in plant response at differing growth periods. For most response variables, deleterious effects of salt were broadly similar regardless of salt treatment duration except for shoot fresh and dry weight, for which the negative salt effects increased over time. SPAD chlorophyll reading may be reduced due to chloroplast degradation and inhibition of chloroplast synthesis by salt (Desingh and Kanagaraj, 2007; Munns and Tester, 2008).

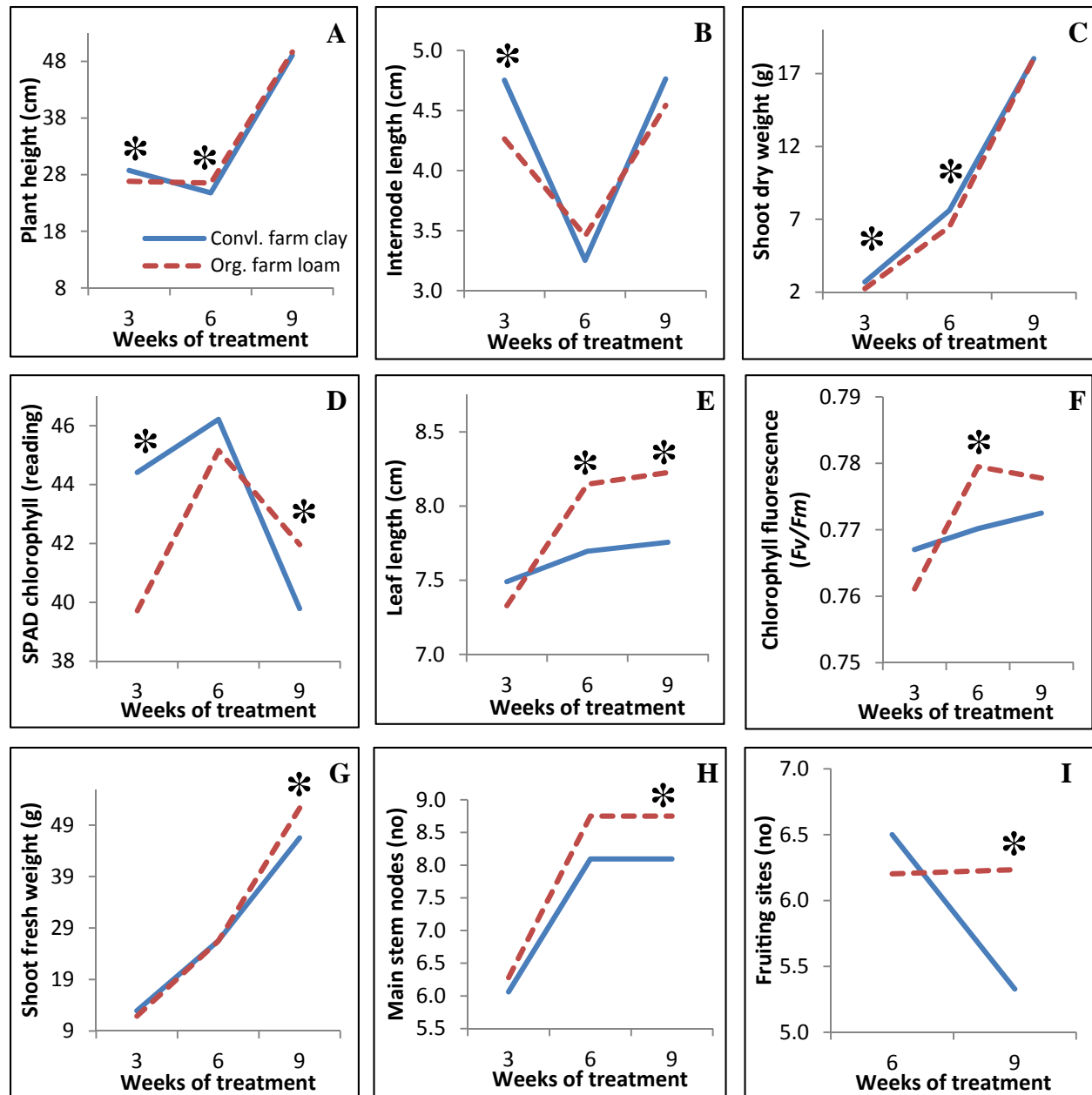


Figure 2. Influence of organic farm loam or conventional farm clay soil type (pooled across salt treatment and genotype) on plant height (A), main stem internode length (B), shoot dry weight (C), SPAD chlorophyll (D), leaf length (E), chlorophyll fluorescence (F), shoot fresh weight (G), number of main stem nodes (H), and number of fruiting sites (I) of four cotton genotypes (BILs) at three, six, and nine wk of salt treatments (WT) with 200 mM NaCl (fruiting sites (I) were not present at three WT). Significant differences at a given WT (*) at $p \leq 0.05$ by LSD.

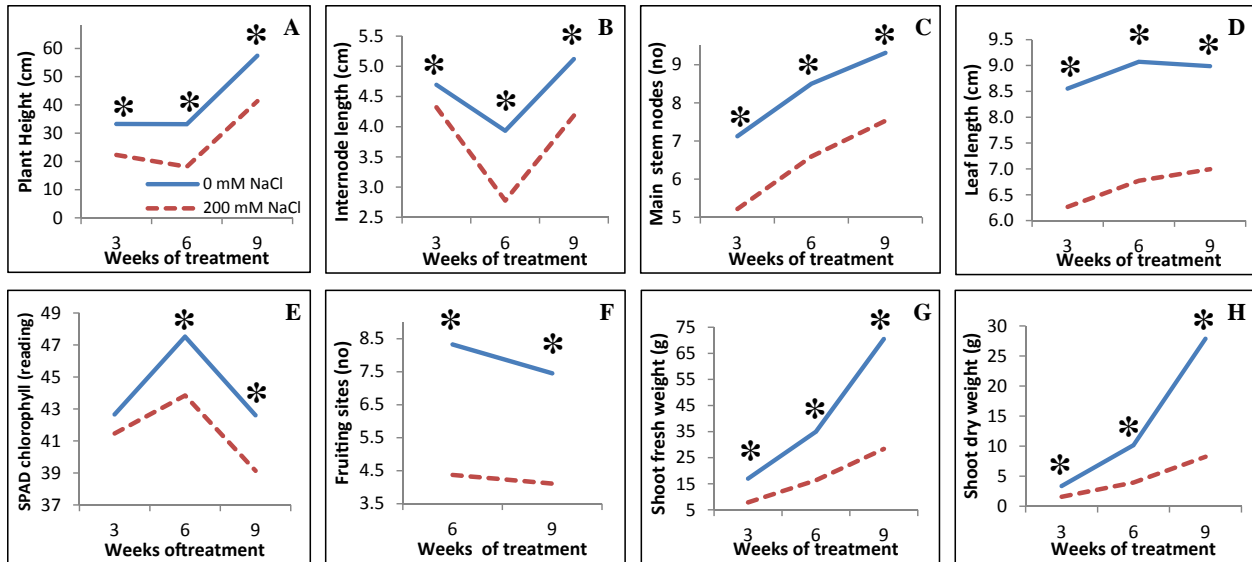


Figure 3. Influence of salt treatment (tap water with no added NaCl or 200 mM NaCl; pooled across soil type and genotype) on plant height (A), main stem internode length (B), number of main stem nodes (C), leaf length (D), SPAD chlorophyll (E), number of fruiting sites (F), shoot fresh (G) and dry weight (H) of four introgressed cotton genotypes at three, six, and nine wk (fruiting sites were not present at three WT). Significant differences at a given WT (*) at $p \leq 0.05$ by LSD (chlorophyll fluorescence was not significant for three, six, and nine WT).

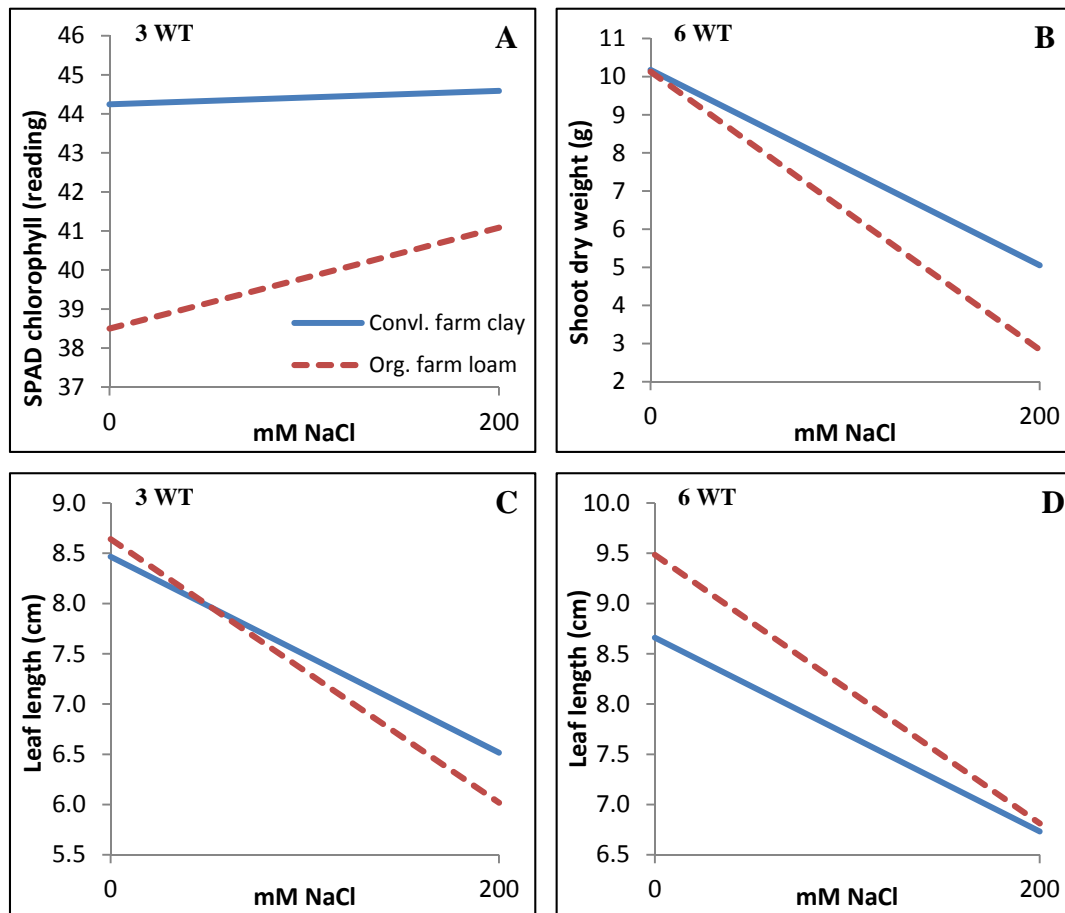


Figure 4. Interactive effects of soil type (organic farm loam or conventional farm clay) and salinity (0 to 200 mM NaCl) on SPAD chlorophyll (A; six WT), shoot dry weight (B; six WT), and leaf length (C and D; three and six WT, respectively) of four introgressed cotton genotypes. Data were pooled across genotype.

Interactions. Soil \times salt treatment interactions were observed for SPAD chlorophyll reading and leaf length at three WT (Fig. 4A and C), and for shoot dry weight and leaf length at six WT (Fig. 4B and D). In each case, the differences between the salt-treated and control plants grown in the clay soil were not as large as those in the loam soil. The fact that such soil \times salt treatment interactions were not detected at nine WT may be, in part, due to adaptive physiological changes of plants in the loam soil.

The lack of genotype \times soil interactions for any of the nine traits measured at any of the three sampling dates after the salt treatment began (Tables 1-3) indicates that the genotypes tested performed similarly in both soils. Also, genotype \times treatment interaction was detected for only shoot dry weight at six WT (Tables 1-3), indicating that the genotypes tested had similar growth trends between salt treatment and the control. This study also did not detect any genotype \times soil \times salt treatment interactions.

Tiwari et al. (2013a and b) showed that genotype \times salt treatment interactions were very low or non-existent for seed germination, plant height, and shoot and root weight when a BIL population of 142 lines (including the four lines used in the current study) was evaluated in a potting soil medium. However, there was no evaluation of the interactions from genotype \times soil, soil \times salt treatment or genotype \times soil \times salt treatment in those studies.

CONCLUSIONS

In this study, four introgressed Upland cotton lines were selected from previous research and tested in two soils for responses to salinity. Evaluation of these genotypes in two soils adds important information for these contrasting BILs, since as we show here, the trends in plant growth between two soils and salinity levels were broadly similar. The findings have practical implications for cotton salt tolerance studies and screening, particularly in view of the lack of genotype \times soil type and genotype \times salt treatment interactions. If the objective is to provide preliminary salt tolerance evaluations of numerous, closely related cotton genotypes in the salinity range tested here, the use of different soil types and control (non-saline) treatments may not be necessary. Further, there is a growing trend in organic cultivation practices. As of 2007, the percentage of global agricultural land practicing, or in conversion to organic farming is 7.2% (Willer et al., 2009). This

study demonstrates that further scrutiny of plant-salinity relations in soils managed with carbon-based fertilizers may be warranted.

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REFERENCES

- Ashraf, M. 2002. Salt tolerance of cotton: some new advances. *Crit. Rev. Plant Sci.* 21:1-30.
- Ashraf, M. 2004. Some important physiological selection criteria for salt tolerance in plants. *Flora* 199:361-376.
- Bajaj, S., W. Wang, E. Hughs, R. Percy, M. Ulloa, and J.F. Zhang. 2008. Evaluation of cotton germplasm and breeding populations for salt tolerance. *Proc. Beltwide Cotton Conf.* p. 876-880.
- Barrick, B., R. Tiwari, A. Ulery, and J.F. Zhang. 2012. Salt tolerance evaluation in commercial cotton cultivars. *Proc. Beltwide Cotton Conf.* p. 706-712.
- Bullock, H.E., and R.E. Neher. 1980. Soil survey of Doña Ana county area New Mexico. Washington, D.C.. U.S. Government Printing Office.
- Carter, D.L. 1975. Problems of salinity in agriculture. p. 25-30. *In: Poljakoff A. and J. Cales (eds.) Plants in Saline Environments.* Springer Verlag, Heidelberg.
- Desingh, R., and G. Kanagaraj. 2007. Influence of salinity stress on photosynthesis and antioxidative systems in two cotton varieties. *Gen. Appl. Plant Physiol.* 33: 221-234.
- Epstein, E. 1976. Genetic potentials for solving problems of soil mineral stress: Adaption of crops to salinity. p. 73-82. *In: Wright M.J. (ed.) Plant Adaptation to Mineral Stress in Problem Soils.* Special Publication of Cornell University Agricultural Experiment Station, Ithaca, NY.
- Flowers, T.J. 2004. Improving crop salt tolerance. *J. Exp. Bot.* 55:307-319.
- Flowers, T.J., and S.A. Flowers. 2005. Why does salinity pose such a difficult problem for plant breeders? *Agr. Water Manage.* 78:15-24.
- Gavlak, R.G., D. A. Horneck, and R.O. Miller. 1994. Plant, soil and water reference methods for the western region. *W. Reg. Ext. Publ.* 125.

- Gee, G.W., and J.W. Bauder. 1979. Particle size analysis by hydrometer: a simplified method for routine textural analysis and a sensitivity test of measured parameters. *Soil Sci. Soc. Am. J.* 43:1004-1007.
- Gossett, D.R., E.P. Millhollon, and M.C. Lucas. 1994. Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. *Crop Sci.* 34:706-714.
- Hanif, M., A. Qayyum, W. Malik, E. Noor, and N. Murtaza. 2008. Assessment of variability for salt tolerance at seedling stage in *Gossypium hirsutum* L. *J. Food Agric. Environ.* 6:134-138.
- Hao, X., and C. Chang. 2003. Does long-term heavy cattle manure application increase salinity of clay loam soil in semi-arid southern Alberta? *Agric. Ecosyst. Environ.* 94: 89-103.
- Haynes, R.J., and R. Naidu. 1998. Influence of lime, fertilizer, and manure application on soil organic matter content and soil physical conditions: A review. *Nutr. Cycl. Agroecosys.* 51:123-137.
- Hegarty, T. 1978. The physiology of seed hydration and dehydration, and the relation between water stress and the control of germination: a review. *Plant Cell Environ.* 1:101-119.
- Hemphill, J.K., H. Basal, and W.C. Smith. 2006. Screening method for salt tolerance in cotton. *Am. J. Plant Pathol.* 1:107-112.
- Higbie, S.M., T.M. Sterling, J.M. Stewart, and J.F. Zhang. 2005. Physiological response and genetic diversity of tetraploid cotton to salt stress. *Proc. Beltwide Cott. Conf.* p. 944-945.
- Higbie, S.M., G. Wang, J.McD. Stewart, T.M. Sterling, W.C. Lindemann, E. Hughs, and J. Zhang. 2010. Physiological response to salt (NaCl) stress in selected cultivated tetraploid cottons. *Intl. J. Agron.* 2010:1-12.
- Lubbers, E.L., P.W. Chee, Y. Saranga, and A.H. Paterson. 2007. Recent advances and future prospective in molecular breeding of cotton for drought and salinity stress tolerance. p. 775-796. *In: Jenks M.A., P.M. Hasegawa, and S.M. Jain (eds.) Advances in Molecular Breeding toward Drought and Salt Tolerant Crops.* Springer, Netherlands.
- Maas, E.V., and G.J. Hoffman. 1977. Crop salt tolerance – current assessment. *J. Irrig. Drain. Div. Am. Soc. Civ. Eng.* 103:115-134.
- McMaster, G.S., and W.W. Wilhelm. 1997. Growing degree-days: one equation, two interpretations. *Agric. Forest Meteorol.* 87:291-300.
- Mendoza, G.O., M.K. Shukla, J.G. Mexal, D.M. VanLeeuwen, and Y. Ikemura. 2011. Assessment of properties of a Harkey soil under organic and conventional farming systems. *Commun. Soil. Sci. Plan.* 42:1791-1808.
- Mittler, R. 2006. Abiotic stress, the field environment and stress combination. *Trends Plant Sci.* 11:15-19.
- Munns, R. 2002. Comparative physiology of salt and water stress. *Plant Cell Environ.* 25:239-250.
- Munns, R., and M. Tester. 2008. Mechanisms of Salinity Tolerance. *Annu. Rev. Plant Bio.* 59:651-681.
- Neumann, P. 1997. Salinity resistance and plant growth revisited. *Plant Cell Environ.* 20:1193-1198.
- Niu, G.H., D. Rodriguez, J. Dever, and J.F. Zhang. 2013. Responses of five cotton genotypes to sodium chloride and sodium sulfate saline water irrigation. *J. Cotton Sci.* 17:233-244.
- Noble, C.L., and M.E. Rogers. 1992. Arguments for the use of physiological criteria for improving the salt tolerance in crops. *Plant Soil* 146:99-107.
- Pitman, M.G., and A. Läuchli. 2002. Global impacts of salinity and agricultural ecosystems. p. 3-20. *In: Läuchli A. and U. Lüttge (eds.) Salinity: Environment - Plants – Molecules.* Springer Sciences, New York, NY.
- Qadir M., and M. Shams. 1997. Some agronomic and physiological aspects of salt tolerance in cotton (*Gossypium hirsutum* L.). *J. Agron. Crop Sci.* 179:101-106.
- Rhoades, J.D. 1996. Salinity: electrical conductivity and total dissolved solids. p. 417-435. *In: Sparks D.L. (ed.) Methods of Soil Analysis Part 3 Chemical Methods.* No. 5 SSSA Book Series. SSSA, ASA, Inc. Madison, WI.
- Rodriguez-Urbe, L., S.M. Higbie, J.M. Stewart, T. Wilkins, W. Lindemann, C. Sengupta-Gopalan, and J.F. Zhang. 2011. Identification of salt responsive genes using comparative microarray analysis in Upland cotton (*Gossypium hirsutum* L.). *Plant Sci.* 180:461-469.
- SAS Institute Inc. 2012. SAS/STAT® 9.3 User's Guide. Cary, NC: SAS Institute Inc.
- Sharma, S.K., and S.S. Goyal. 2003. Progress in plant salinity resistance research: need for an integrative paradigm. p. 387-407. *In: Goyal S.S. S.K. Sharma, D.W. Rains (eds.) Crop Production in Saline Environments: Global and Integrative Perspectives.* Haworth Press, NY.
- Soil Survey Staff. 2009. Custom soil resource report for Doña Ana county area, New Mexico. Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online at <http://websoilsurvey.nrcs.usda.gov/>. Accessed [05/18/2012].
- Stiles, I.E. 1948. Relation of water to the germination of corn and cotton seeds. *Plant Physiol.* 23:201-222.

- Tiwari, R.S., G.A. Picchioni, R.L. Steiner, D. Jones, S.E. Hughs, and J.F. Zhang. 2013a. Genetic variation in salt tolerance during seed germination in a backcross inbred line population and advanced breeding lines derived from *Gossypium hirsutum* x *G. barbadense*. *Crop Sci.* 53:1974-1982
- Tiwari, R.S., G.A. Picchioni, R.L. Steiner, D. Jones, S.E. Hughs, and J.F. Zhang. 2013b. Genetic variation in salt tolerance at the seedling stage in an interspecific backcross inbred line population of cotton. *Euphytica* 194:1-11.
- USDA. 1954. Diagnosis and improvement of saline and alkaline soils. *In*: Richards (eds.) USDA Agriculture Handbook 60. L.A. U.S. Gov. Print. Office, Washington, D.C.
- USDA-SCS. 1972. Soil survey laboratory methods and procedures for collecting soil samples. 1972. U.S. Gov. Print. Office, Washington, D.C.
- Willer, H., M. Rohwedder, and E. Wynen. 2009. Organic agriculture worldwide: current statistics. pp. 25-58. *In*: H. Willer, and L. Kilcher (Eds.) *The World of Organic Agriculture-Statistics and Emerging Trends*. IFOAM, Bonn, and FiBL, Frick.