<u>Abstract</u>

Cotton is one of the major crops grown in New Mexico. Identification and development of cotton cultivars with abiotic stress tolerance is vital to sustainable cotton production. Genetic variation of salt tolerance in cotton genotypes grown in saline conditions can be isolated for selective breeding. This study evaluated 64 commercial cotton cultivars and 66 elite breeding lines for salt tolerance in five tests in the greenhouse. Significant genotypic variation was detected in two tests including most commercial cultivars in one test and advanced breeding lines from the New Mexico Cotton Breeding Program in another. Notably, two breeding lines developed from Upland x Pima exhibited a significantly higher level of salt tolerance than Acala 1517-08.

Introduction

Estimates of World's irrigated land affected by salinity are from 20% upwards to 50% (Pitman and Lauchli, 2002). Cotton is a moderately salt tolerant crop with a threshold of 7.7 dS m^{-1} (Higbie et al., 2010). Nevertheless, cotton yield can adversely be affected through variable interactions of plant responses to salinity, with Na⁺ and Cl⁺ toxicity being the most detrimental (Hoffman et al., 1971; Ashrif, 2002). Effects are particularly pronounced in the arid and semi-arid climate of the Southwestern United States where secondary salinization is a consequence of improper crop management schemes, poor irrigation water and saline soils (Pitman and Lauchli, 2002). Considering the prevalence of these problems, use of salt-tolerant cultivars is one of the most viable solutions to maximizing cotton yield in Southwestern United States agriculture.

Materials and Methods

Plant Growth and Salt Treatments

A total of 130 genotypes were divided into four trials: Trial 1- High Fiber Quality Test with 32 genotypes, including mostly commercial cotton cultivars; Trial 2- National Variety Test with 32 commercial cotton cultivars; Trial 3-Regional Breeders' Testing Network with 34 genotypes, including advanced breeding lines from most public cotton breeding programs in the U.S.; and Trial 4- Advanced Yield Test with 32 genotypes including advanced breeding lines developed from New Mexico State University. Each trial was arranged using a randomized complete block (RCB) design. Plants were grown in the greenhouse during July through December 2011. Three seeds were sown per 4" plastic pot filled with Metro-Mix 360®. After emergence seedlings were thinned to one plant per pot, for a total of 3 control plants and 3 plants receiving the treatment per replication. Three replications of control (without salt) and three replications of saline treatment were arranged separately using the same RCB design (576-588 plants in total). Soil was watered to saturation using tap water (~0.5 dS m⁻¹) and allowed to drain to field capacity. Upon emergence of the second true leaf, tap water (control) or salt solution (100 mL/pot) was hand applied to the surface of the pots every other day for three weeks. The temperature range in the greenhouse was maintained between 21 and 32°C, and the relative humidity ranged from 35%-50%. Since 150 mM - 250 mM of NaCl are the most frequently used concentrations in cotton salt tolerance studies, a concentration of 200 mM NaCl (approximately 18 dS m⁻¹) was used in this study (Higbie et al., 2010). Each replication for control and treatment had 2 pots (for a total of 12), that were sampled for salinity measurement using saturated paste at the end of the experiment without concern for preserving roots.

For comparison, a system using Ray-Leach Cone-tainers, SC10RI® (1.5" x 8.25" cells of recycled plastic) filled with Metro-Mix 360® (with 98 cells in a tray) was tested (Trial 5) using the same genotypes and same experimental design as in Test 2. In this system however, treatments were done by submerging trays under water or salt solution for approximately 10 min instead of only surface hand irrigation. It was difficult to keep the individual Con-tainers

submerged due to buoyancy, so to ensure saturation of each cell, solution was also hand applied to the surface during treatment.

Plant Measurements

Plant height was measured from the soil surface to the meristem at the end of the three week treatment period. Plant shoots were harvested by cutting the plant at the soil surface and then oven dried at 60-65°C for 3 d. Roots were separated from the growth media by gentle washing in tap water and weighed for total fresh and dry weights.

Saline Measurements

Saturated pastes (Rhoades, 1996) were made from the four blanks (2 for control and 2 for treatment) in each replication at the end of each trial. It was assumed that the electrical conductivity of the saturated paste (ECe) reflected the salinity experienced by the cotton plants.

<u>Data Analysis</u>

The heights and weights of the three plants of each cultivar, in each replication, were averaged. Shoot to root ratio was also calculated. Analysis of variance (ANOVA) was performed on plant height, shoot, root and total plant weight, and shoot-to-root ratio. Variances of these traits for each test due to replications, treatment, cultivars, and cultivars x treatment were calculated using the SAS version 9.2 (SAS Institute Inc., 2008). Least significant difference (LSD) values were computed at the P=0.05 level.

Results and Discussion

Plant Growth and Salt Treatments

Application of 200 mM NaCl for three weeks at the early seedling growth stages significantly reduced plant height, and fresh shoot, root and total plant weights, compared with the non-saline controls. Although it was presumed that salt accumulation in the soil would be unlikely due to leaching, the 4-inch surface-irrigated control pots had an average ECe of 0.8 dS m⁻¹ while the salt treated pots were >20 dS m⁻¹. In contrast, the Cone-tainers irrigated by submergence averaged similar ECe for control but only 10-12 dS m⁻¹ in the salt treatments. In addition to leaching of excess salt, irrigation every other day minimized water deficit stress. At the concentration of NaCl used in this experiment it was assumed that cotton plants would exhibit reduction in growth while limiting symptoms of salt toxicity.

Commercial Upland Cotton Cultivars

Numeric genotypic differences were noted in salt tolerance when reduction in plant growth parameters including total plant weight was used as the criteria. However, ANOVA did not detect significant genotypic variation in plant growth traits in the two trials (Trial 1 and 2) with commercial cotton cultivars (Table 1 for Trial 1 as an example) when seedlings were grown in 4" pots and treated with NaCl by hand irrigation for three weeks.

However, significant genotypic differences in salt tolerance were detected in Trial 5 (Table 2), when the same genotypes in Trial 2 were tested under an improved salt treatment system. In this system, Cone-tainers were submerged in tap water or salt solution for approximately 10 min. This improved system has several advantages. First, seedlings were treated uniformly with minimal experimental errors (resulting in a lower coefficient of variation) as compared with hand irrigation of individual pots. Second, this system was much faster and more convenient than the hand irrigation method. However, due to the repeated use of the salt solution, the concentration in solution was later found to be reduced (ca. 10-12 dS m⁻¹), which resulted in no significant reduction due to salt treatment in many genotypes. In fact, although insignificant, a positive effect of salt on total seedling weight was noted for some genotypes. This problem could be avoided by using freshly made salt solutions when plants are treated.

Advanced Breeding Lines

Advanced breeding lines developed from most of the public cotton breeding programs were tested in Trial 3 and no significant genotypic variation was detected (Table 3). Again, numeric differences between genotypes were seen as plant weight reductions in this test. Similar to Trials 1 and 2, higher experimental errors prevented detection of significant differences in salt tolerance between the advanced breeding lines.

Genotype	Control ²	Treatment ²	Difference ³
		(g)-	
Ark9803-23-04	15.97	4.84	11.12
ST4554B2RF	17.09	6.26	10.83
FM9170B2RF	15.10	4.82	10.28
TAM06WE-62-4	14.41	4.17	10.24
TAM03-WZ-37	14.70	4.72	9.98
DP1048B2RF	14.79	4.88	9.91
ST4288B2F	14.52	5.48	9.04
PHY72* ⁵	14.38	5.47	8.91
PHY755WRF	14.84	5.97	8.88
DP1050B2RF	13.96	5.58	8.38
DP555BG/RR*	12.46	4.29	8.17
PHY375WRF	13.76	5.64	8.11
Acala 1517-08	12.83	5.10	7.73
PHY72	12.10	4.48	7.62
MD25y	13.30	5.70	7.60
Acala 1517-09R	13.26	5.67	7.59
PHY499WRF	12.49	4.91	7.58
PHY565WRF	12.72	5.21	7.51
MD25ne	11.86	4.41	7.44
TAM04WB-33s	13.14	5.98	7.17
DP161B2RF	12.28	5.19	7.09
PHX4912WRF	11.67	4.77	6.90
FM9160B2RF	11.97	5.11	6.86
DP555BG/RR	11.32	4.63	6.69
FM1773LLB2	11.36	4.67	6.69
DP1032B2RF	11.02	5.40	5.62
DP1034B2RF	11.67	617	5.50
FM1845LLB2	9.61	4.18	5.43
Ark0102-48	8.98	3.57	5.41
PHY367WRF	10.59	5.23	5.36
MD10	8.84	4.38	4.47
FM9058F	8.30	5.20	3.10
F			1.04 ns^4
Pr > F			0.433
LSD (0.05)			5.17

Table 1. Total¹ fresh plant weights in commercial cultivars (Trial 1) under control and salt treatment.

¹Shoot plus root weight measured immediately after three weeks of treatment. ²Each value represents the weight of three plants measured together and divided by three to calculate an individual average plant weight. ³LSD means of the difference between control and treatment plants calculated from SAS. ⁴ Not significant. ⁵Tested using two different entries.

Genotype	Control ²	Treatment ²	Difference ³
		(g)	
NM08N1564	3.45	2.70	0.74
DP1028B2RF	2.87	2.29	0.59
PHY569WRF	3.09	2.58	0.50
DP1032B2RF	3.15	2.65	0.49
DP1048B2RF	2.86	2.37	0.49
PHY565WRF	2.71	2.23	0.48
BCSX1040F	3.12	2.67	0.45
NM08N1084	2.81	2.41	0.40
PHY499WRF	2.85	2.47	0.37
FM9160B2F	2.71	2.35	0.36
DP0912B2RF	2.97	2.61	0.36
FM1740B2F	2.44	2.14	0.30
Acala GLS	3.05	2.84	0.20
BCSX1030F	2.98	2.78	0.20
NM07N1185	2.51	2.37	0.14
PHY519WRF	2.76	2.66	0.10
DP1050B2RF	2.64	2.55	0.09
ST4288B2F	2.38	2.30	0.09
Acala 1517-08	2.48	2.41	0.07
PHY367WRF	2.33	2.35	-0.01
DP1044B2RF	2.46	2.48	-0.02
NM08N1562	2.52	2.59	-0.07
BCSX1010F	2.70	2.78	-0.08
ST5288B2F	2.37	2.47	-0.10
FM1845LLB2	2.50	2.60	-0.10
ST5458B2RF	2.05	2.17	-0.12
FM1773LLB2	2.57	2.70	-0.13
DP0949B2RF	2.79	2.94	-0.15
FM9170B2F	2.84	3.05	-0.21
NM07N1295	2.51	2.73	-0.21
PHY375WRF	2.39	2.70	-0.31
NM07N1189	2.24	2.57	-0.33
F			5.79** ⁴
$\Pr > F$			0.007
LSD (0.05)		0.63	

Table 2. Total¹ fresh plant weights in commercial cultivars (Trial 5) under control and salt treatment.

⁴ Significant at the P=0.01 level.

¹Shoot plus root weight measured immediately after three weeks of treatment.
²Each value represents the weight of three plants measured together and divided by three to calculate an individual average plant weight.
³LSD means of the difference between control and treatment plants calculated from SAS.

Genotype	Control ²	Treatment ²	Difference ³
NC05AZ06	16.47	6.09	10.38
NMW1218	16.22	6.12	10.10
LBB-07-21-311	14.19	5.96	8.23
0033-6	13.49	5.39	8.10
TAM03WZ-37	14.34	6.31	8.03
NM03012	12.90	5.01	7.89
AU6001	13.30	5.64	7.65
AU3202	12.84	5.56	7.29
SG105	13.68	6.47	7.21
LA07307111	13.51	6.61	6.90
LA07307122	11.97	5.21	6.76
PD05041	12.31	5.67	6.64
PX03202-65-1	12.97	6.34	6.62
MD25Y	12.20	5.60	6.60
Ark0219-15	13.98	7.83	6.14
MD25ne	13.59	7.60	5.99
DP393	11.50	5.69	5.81
NM06N1104	11.73	6.29	5.45
FM958	10.99	5.83	5.16
PD05035	11.29	6.20	5.09
NM06N1166	11.26	6.27	4.99
GA2006106	10.07	5.09	4.98
Ark0203-11	11.90	7.01	4.89
AU3111	11.42	6.60	4.82
GA2006053	11.28	6.50	4.77
LA07307106	11.91	7.40	4.51
Ark0232-24	11.27	6.91	4.36
PX03201-66-1	11.92	7.71	4.21

Table 3. Total¹ fresh pl alt treatment.

¹Shoot plus root weight measured immediately after three weeks of treatment.

10.09

10.87

10.62

9.12

10.42

9.71

²Each value represents the weight of three plants measured together and divided by three to calculate an individual average plant weight.

5.92

7.09

6.88

5.90

7.53

7.03

4.17

3.78

3.74

3.22

2.89 2.68

1.52 ns⁴

0.072

5.19

³LSD means of the difference between control and treatment plants calculated from SAS.

⁴ Not significant.

F

Pr > F

GA2007095

Ark0222-12

LA06307025

NC09AZ09

GA2004143

LSD (0.05)

AU6202

However, in Trial 4, 29 breeding lines developed at New Mexico State University (NMSU), their two parents (Acala 1517-99 and Pima Phytogen 76) and the check Acala 1517-08 were tested (Table 4). ANOVA indicated that genotypic variance in plant weight reduction was significant within the 32 genotypes tested, indicating the existence of genetic variation in salt tolerance. Further analysis indicated that the 29 lines tested did not significantly differ from their parents in salt tolerance as measured by difference in total seedling weight between the control and treatment. Interestingly, two breeding lines (08N1782 and 08N1547) showed significantly lower plant biomass reduction and therefore more salt tolerance in comparison with Acala 1517-08 (Table 4). The results indicated that the NMSU Cotton Breeding Program has made some improvement in salt tolerance in cotton.

Summary

- 1. In this study, 64 commercial cotton cultivars and 66 advanced breeding lines were divided into four trials, grown in 4" plastic pots and hand irrigated with 200 mM NaCl solution for three weeks after the second true leaf stage. The results showed that salt treatment by hand irrigation using 4" pots introduced high experimental errors, thereby rendering no significant genotypic difference in salt tolerance in three of the four trials.
- 2. However, when 32 genotypes in one of the four trials were also grown in Ray-Leach Cone-tainers, SC10RI® (1.5" x 8.25" cells of recycled plastic) and irrigated by submerging trays into water or salt solution for approximately 10 min, significant genotypic variation was detected, indicating a better system for salt treatment because of lower experimental error and reduced variability.
- 3. Two advanced breeding lines (08N1782 and 08N1547) developed at NMSU showed better salt tolerance than Acala 1517-08, a newly released cotton cultivar in New Mexico.
- 4. Further studies are needed to reduce experimental errors while imposing salt treatments in the greenhouse.

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Genotype	Control ²	Treatment ²	Difference ³
		(g)	
08N1803	7.27	2.04	5.23
08N1823	7.14	2.53	4.63
08N1762	6.93	2.37	4.57
08N1717	6.67	2.22	4.43
08N1789	6.50	2.08	4.43
08N1736	6.18	1.94	4.20
08N1747	6.64	2.63	4.03
08N1786	6.12	2.21	3.93
Acala 1517-08	6.04	2.16	3.90
08N1773	6.32	2.46	3.87
08N1745	6.58	2.94	3.63
08N1770	6.41	2.86	3.57
08N1718	6.21	2.67	3.53
08N1739	5.77	2.31	3.47
08N1735	5.79	2.38	3.43
08N1810	5.98	2.54	3.43
Pima Phy 76	5.83	2.44	3.40
08N1805	6.02	2.64	3.37
Acala 1517-99	5.81	2.51	3.30
08N1740	5.97	2.66	3.30
08N1724	5.51	2.29	3.23
08N1755	5.56	2.42	3.13
08N1742	5.92	2.97	2.97
08N1825	5.72	2.74	2.97
08N1817	5.94	3.04	2.90
08N1835	5.57	2.66	2.90
08N1787	5.13	2.32	2.80
08N1749	5.28	2.51	2.77
08N1792	4.88	2.48	2.40
08N1722	5.32	3.17	2.13
08N1782	4.97	3.03	1.93
08N1547	4.53	2.80	1.73
F			2.41** ⁴
$\Pr > F$			0.001
LSD (0.05)			1 93

Table 4. Total¹ fresh plant weights in advanced breeding lines (Trial 4) under control and salt treatment.

⁴ Significant at the P=0.01 level.

¹Shoot plus root weight measured immediately after three weeks of treatment.
²Each value represents the weight of three plants measured together and divided by three to calculate an individual average plant weight.
³LSD means of the difference between control and treatment plants calculated from SAS.