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Shoot Biomass Production of Converted Race Stocks of Upland Cotton (*Gossypium hirsutum* L.) Exposed to Salt Stress

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Abstract: Increasing soil salinity is becoming a serious problem in the agriculture areas of all over the world. Although cotton is considered a moderately salt tolerant crop, it would be useful to use exotic cotton accessions as a new genetic resource for increasing salt tolerant level of cotton genotypes. The objective of this research was to identify salt-tolerant CRS accession(s) that would serve as parental material for further salt-tolerant research in cotton. Seedling of four putative salt-tolerant (M-9044-0031, M-9044-0061, M-9044-0140 and M-9044-0150) and three putative salt-sensitive CRS accessions (M-9044-0060, M-8744-0091 and M-8744-0175) plus Acala 1517-77, Deltapine 50, TAM94L-25, Stonville-453 and Nazilli-84 were exposed to two different salt concentrations (125 and 250 mM NaCl) and control in completely randomized design with ten replications. Significant differences were observed among cotton genotypes for reduction ratios in Shoot Dry Weight (SDW), Total Dry Weight (TDW) Leaf Area (LA) and Plant Water Content (PWC). SDW, TDW and PWC were correlated positively regardless of treatment, indicating that either trait could be used as a selection criterion. With TAM94L-25, M-9044-0060, M-8744-0091 and M-9044-0140 exhibited less reduction in SDW and TDW. It was concluded that CRS lines, M-9044-0060, M-8744-0091 and M-9044-0140 may provide parental material for salt tolerance in upland cotton breeding.

Key words: Cotton (*Gossypium hirsutum* L.), salt stress, biomass production

INTRODUCTION

Soil salinity, as an abiotic stress, is one of the major problems limiting the yield of a wide variety of crops all over the world. About 5% (77 million ha) of the 1.5 billion cultivated area is affected by salt (Munns *et al*., 1999). The salinity is still becoming a serious problem in the agriculture areas due to low rainfall, heavy irrigation or poor water management and high evaporation, especially, in hot and dry areas where an extensive water loss occurs through combination of evaporation and transpiration. Since salinity limits the agriculture production (up to 40%) all over the world (Serrano and Gaxiola, 1994), salt tolerant cultivars need to be improved to utilize of saline soils and to meet the demands of the world's increasing population (Holmberg and Bulow, 1998). Extensive studies focused on different strategies to over come salinity during the last decade include morpho-physiological characteristics, cellular and molecular mechanisms, the regulation of ion uptake and translocation, the novel genes function as a tonoplast Na^+/H^+ antiporter and antioxidant enzyme activity induced under stress conditions (Gossett *et al*., 1994; Ashraf, 2002; Pessarakli, 2002; Garratt *et al*., 2002; Meloni *et al*., 2003; Wu *et al*., 2004).

Cotton is considered to be moderately tolerant to salinity ranked second behind barley (Soltanpour and Follett, 1995). However, variation in salt-tolerance has been observed among different cotton cultivars (Gossett *et al*., 1994; Khan *et al*., 1995; Leidi, 1994). Ashraf and Ahmad (1999) reported that seed germination and early seedling development are the stages in plant development that are the most sensitive to salt accumulation. The accumulation of specific ions in shoots or leaves, or the the production of a specific metabolite have been proposed for selection criteria (Gossett *et al*., 1994; Ashraf and Ahmad, 2000; Garratt *et al*., 2002; Munns *et al*., 2002). However, under saline condition absolute or relative growth or yield is usually the ultimate goal (Shannon, 1984). Ashraf and Ahmad (2000) reported that salt-tolerant cotton varieties (*G. hirsutum* L.) had higher shoot biomass production than salt-sensitive varieties at the vegetative stage. Munns *et al*. (2002) reported that developmental pattern of wild population is different from that of the cultivars; and assessment of the actual salt tolerance may be determined by comparing of their biomass production.

In some crops, wild species exhibit a wide variation in their salinity tolerance compared to their cultivated

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species such as barley (Mano and Takeda, 1998) and cowpea (Gulia and Jaiwal, 1996). The Converted Race Stocks (CRS) of upland cotton (*G. hirsutum* L.) have been identified as potential sources of traits including drought tolerance (Quisenberry *et al*., 1981; Basal *et al*., 2003) since they were collected in the arid regions of Mexico and Central America (Percival and Kohel, 1990). Both drought and salt stress induce similar physiological dysfunctions and their recovery mechanisms, in part, have been shown to overlap (Munns, 2002). Since the CRS lines evolved under semi-arid conditions, it is anticipated that both drought- and salt-tolerant related genes could, theoretically, would exist within these lines. In the study reported herein, robust and non-robust rooting CRS were evaluated for salt-tolerance during early seedling growth. The main objectives of this research were (i) to determine the response of selected CRS accessions to salt stress, (ii) to identify salt-tolerant CRS accession(s) or individual plants within these accessions that would serve as parental material for further salt-tolerant research in cotton.

MATERIALS AND METHODS

Seeds of Acala 1517-77, Deltapine 50 (DPL), TAM94L-25 (Smith, 2003), Stonville-453, Nazilli-84 and four putative salt-tolerant accessions (M-9044-0031, M-9044-0061, M-9044-0140 and M-9044-0150) and three putative salt-sensitive CRS accessions (M-9044-0060, M-8744-0091 and M-8744-0175) based on germination test (unpublished data) were planted in a walking-greenhouse on May 16, 2005. Four seeds were planted in pots (26 cm ht x 11 cm diam; volume: 1.5 L) filled with a 3:1 mixture of sand:perlit. After emergence, plants were thinned to one plant per pot. Ten pots of each genotype were established in completely randomized experimental design. The experiment was conducted in a walking greenhouse at the Adnan Menderes University with 30/17°C and 57/59% relative humidity (day/night) conditions. The experimental design consists of control (0 mM NaCl) and two salinity treatments (125 and 250 mM NaCl). The plants in pots were watered every day with 300 mL half strength Hoagland solution (Hougland and Arnon, 1950). Fourteen days after planting, when the plants reached the beginning first true-leaf stage seedlings were subjected to the salt stress. Seedlings were exposed to salt stress by adding 50 mM NaCl at 24 h intervals until the final concentrations of 125 and 250 mM NaCl were reached. After 7 weak (50 days after planting) fully expanded 3rd main stem leaves were excised from each plant and each leaf area was measured by using scanner with Flaeche packing programme. Half of the plants from each genotype were harvested 50 days after planting (DAP) and half were harvested 51 DAP on 04-05 July 2005, respectively. Plants were removed from pots and washed free of sand: perlit mixture, then plants were cut into root and shoot and shoot fresh weight measured. Shoot and roots were dried for 48 h at 90°C and dry weight recorded. Plant water content (in g H_2O g dry weight^{-1}) was obtained from shoot fresh and dry weight measurement. Genotypes were evaluated for Shoot Dry Weight (SDW), Total Dry Weight (TDW) Leaf Area (LA) and Plant Water Content (PWC).

The experiment was run based on completely randomized design. Data of reduction ratios were normally distributed and thus analyzed statistically without transformation. The analyses of all data from this study were carried out utilizing the MIXED procedure of SAS (SAS, 1999). Main effects in the model included salt, genotype and the interaction of salt x genotype and were assessed using the Type III F-test. Differences between Tukey-Kramer adjusted LSMeans from this analysis were assessed by t-test and declared significant when $p \leq 0.05$.

RESULTS

The interaction between salt treatments and genotypes was found to be significant for biomass production components including Shoot Dry Weight (SDW), Total Dry Weight (TDW), Leaf Area (LA) and Plant Water Content (PWC).

The reduction in the values of the measured characters of cotton genotypes grown with and without salt and genotypic differences at 125 and 250 mM NaCl were compared (Table 1). As the concentration of NaCl increased from control to 125 and to 250 mM NaCl levels, mean shoot biomass of cotton genotypes decreased 9.70 and 46.84% relative to control, respectively. Genotypes varied for investigated characters regardless of salt concentrations. When the cotton genotypes were grown in 125 mM NaCl salt level, the highest reduction in SDW was in Nazilli-84 (35.46%). In contrast, M-8744-0091 (-6.57%) and M-9044-0140 (-2.71%) showed an increase in SDW and TAM94L-25 and M-9044-0060 showed comparatively moderate reduction of shoot dry weight, 5.49 and 5.95%, respectively. No differences were observed in SDW reduction ratio among cotton genotypes when salinity level increased from control to 250 mM NaCl level. However, the result for CRS line M-9044-0150 was interesting since it had less SDW reduction than Stonville-453 and M-8744-0091 when salt level increased from 125 to 250 mM NaCl.

Increasing salinity decreased mean TDW by an average 14.03 and 46.46% under 125 and 250 mM NaCl treatment relative to that under control. Significant genotypic variation was found among cotton genotypes

Table 1: Least square means of reduction ratios of investigated characteristics in 12 genotypes of upland cotton when exposed to 2 salt concentration levels

	SDW‡			TDW		
Genotype	0-125 mM NaCl	0-250 mM NaCl	125-250 mM NaCl	0-125 mM NaCl	0-250 mM NaCl	125-250 mM NaCl
Nazilli-84	35.46a†	57.91a	33.03ab	31.79a	55.47a	33.38ab
Stonville-453	11.17abc	54.95a	48.07a	15.10abc	53.13a	44.09a
Acala 1517-77	6.23bc	54.25a	42.29ab	14.42abc	55.44a	39.09ab
Deltapine 50	16.57abc	55.67a	40.91ab	18.89abc	46.79a	38.68ab
TAM94L-25	5.49c	39.60a	39.59ab	-3.60c	37.79a	37.07ab
M-9044-0031	25.05ab	36.56a	25.72ab	27.87ab	46.25a	22.68ab
M-9044-0060	5.95c	45.53a	46.20ab	4.97bc	48.53a	44.13a
M-9044-0061	12.38abc	48.96a	39.35ab	17.20abc	48.77a	36.06ab
M-8744-0091	-6.57c	46.73a	47.67a	-1.86bc	45.08a	41.81a
M-9044-0140	-2.71c	34.99a	34.32ab	-0.43c	35.50a	34.46ab
M-9044-0150	31.19ab	44.97a	17.72b	33.00a	43.26a	13.20b
M-8744-0175	9.01abc	41.87a	27.40ab	14.30abc	41.41a	24.15ab
Overall mean	9.70	46.84	36.86	14.03	46.46	34.07

Table 1: Continued

	LA			PWC		
Genotype	0-125 mM NaCl	0-250 mM NaCl	125-250 mM NaCl	0-125 mM NaCl	0-250 mM NaCl	125-250 mM NaCl
Nazilli-84	19.49a†	36.16abc	18.76abc	45.03a	59.79a	25.87a
Stonville-453	8.81abc	49.94a	44.35a	17.22abcd	58.08a	47.38a
Acala 1517-77	14.98ab	41.63ab	21.16abc	11.36bcd	56.42a	42.25a
Deltapine 50	14.63ab	42.96ab	38.59ab	30.01ab	55.23a	48.26a
TAM94L-25	4.47abc	41.10ab	39.44ab	6.25bcd	49.44a	42.67a
M-9044-0031	-5.51bc	8.08d	11.93c	26.15abc	46.26a	24.14a
M-9044-0060	1.89bc	33.20abc	29.91abc	-12.07d	42.11a	44.31a
M-9044-0061	3.22abc	23.93bcd	25.67abc	8.50bcd	50.19a	44.00a
M-8744-0091	-10.91c	23.65bcd	33.27abc	-0.83cd	48.02a	42.41a
M-9044-0140	-6.05bc	22.92bcd	29.88abc	3.89cd	46.53a	34.20a
M-9044-0150	2.91bc	25.55bcd	25.50abc	31.55ab	47.22a	21.00a
M-8744-0175	0.93bc	14.76bcd	14.29c	14.73abcd	42.87a	32.87a
Overall mean	0.85	30.75	27.73	13.49	50.18	37.45

†Values within columns followed by the different letter are different at $p \leq 0.05$, ‡Shoot Dry Weight: SDW, Total Dry Weight: TDW, Leaf Area: LA, Plant Water Content: PWC

for reduction ratio in TDW. Total (shoot+root) dry weight decreased progressively as the salinity level increased from control to 125 mM NaCl salinity level, except for TAM94L-25, M-8744-0091 and M-9044-0140. The highest reduction in TDW was found in Nazilli-84 and M-9044-0150 and the lowest reduction in TDW was observed for TAM94L-25 and M-9044-0140. When salinity level increased from control to 250 mM NaCl level, differences in TDW reduction ratios of cotton genotypes was not significant. However, when salt level increased from 125 to 250 mM NaCl, Stonville-453, M-9044-0060 and M-8744-0091 had more reduction in TDW than M-9044-0150.

As the salt level increased leaf area of cotton cultivars reduced and the overall LA reduction ratios relative to the control were 0.85% and 30.75% at 125 and 250 mM NaCl salinity stresses, respectively. Within salt treatment there were significant differences among cotton genotypes for LA reduction ratio. The largest reduction in LA under 125 mM NaCl treatment was in Nazilli-84 (19.49%). On the other hand, LA of 3 converted race stocks, M-8744-0091, M-9044-0140 and M-9044-0031, increased under 125 mM NaCl salt stress. However, when salt level increased from control to 250 mM NaCl, M-9044-0031 had less LA reduction than all cotton cultivars and one CRS line, M-9044-0060. Under increasing NaCl application, 125 to 250 mM NaCl, the smallest LA reduction was occurred for M-9044-0031 and M-9044-0175 CRS lines.

Irrigation with 125 and 250 mM NaCl solutions decreased PWC by over 13.49 and 50.18% relative to that with non-saline irrigation. The reduction of PWC in cotton genotypes were significantly affected by increased salt level from 0 to 125 mM NaCl. However, significant variations were not observed among cotton genotypes for PWC neither increased salt level from 0 to 250 mM NaCl nor from 125 to 250 mM NaCl. M-9044-0060 and M-8744-0091 and Acala 1517-77 exhibited less PWC decrease, while Nazilli-84 had highest PWC decrease in 125 mM NaCl salt level.

Shoot dry weight, total dry weight, leaf area and plant water content all were positively and significantly

Table 2: Pearson correlation coefficients among shoot dry weight, total dry weight, leaf area and plant water content under non-stressed and salt-stressed growing conditions

		SDW‡		TDW			LA			PWC		
		125 mM NaCl	250 mM NaCl	Control	125 mM NaCl	250 mM NaCl	Control	125 mM NaCl	250 mM NaCl	Control	125 mM NaCl	250 mM NaCl
SDW	Control	0.1052	0.1742	0.9536**	0.1195	0.1493	0.3441**	0.0288	0.2266*	0.8604**	0.0471	0.1530
	125 mM NaCl		0.0385	0.1185	0.9664**	0.0699	0.1185	0.4171**	0.0419	0.0688	0.8405**	0.0120
	250 mM NaCl			0.1318	0.0365	0.9284**	0.0871	0.0863	0.5201**	0.1905*	0.0281	0.8048**
TDW	Control				0.1464	0.1312	0.3530**	0.0071	0.2422*	0.7753**	0.0849	0.1112
	125 mM NaCl					0.0513	0.0537	0.3737**	0.0218	0.0731	0.7820**	0.0216
	250 mM NaCl						0.1341	0.1396	0.5374**	0.1690	0.0787	0.7918**
LA	Control							0.3301**	0.0165	0.2717**	0.1591	0.1122
	125 mM NaCl								0.0023	0.0086	0.4817**	0.1303
	250 mM NaCl									0.1532	0.0088	0.4970**
PWC	Control										0.1276	0.2174*
	125 mM NaCl											0.0468

*and **indicates significance at $p = 0.05$ and 0.01, ‡Shoot Dry Weight: SDW, Total Dry Weight: TDW, Leaf Area: LA, Plant Water Content: PWC

correlated regardless of salt stress (Table 2). The highest positive and significant correlation was observed between SDW and TDW, (r = 0.9536, for control; r = 0.9664 and 0.9284 for 125 and 250 mM NaCl salt stressed) and SDW was associated with PWC (r = 0.8604, for control; r = 0.8405 and 0.8048 for 125 and 250 mM NaCl salt stressed) (Table 2). Leaf area at 250 mM NaCl was positively correlated with SDW and TDW at control level. A positive association was found between SDW (in 250 mM NaCl application) and PWC (in control).

DISCUSSION

Data, based on reduction ratio of SDW, TDW, LA and PWC at the early growth stage, demonstrated that genetic variation exist among the CRS accessions for response to different salinity levels. In previous study (unpublished data) the germination test suggested that CRS lines M-9044-0031, M-9044-0061, M-9044-0140 and M-9044-0150 were salt-tolerant while the other CRS lines in this study were more salt-sensitive. However, among four CRS lines mentioned above only M-9044-0140 showed consistent result for investigated characters. These results indicated that response of some accessions to salinity could be different at seed germination and seedling stages in contrast to previous research results (Ashraf and Ahmad, 2000). Among CRS accessions M-9044-0060, M-8744-0091 and M-9044-0140 exhibited low SDW and TDW reduction ratio, which would result from keeping plant water potential under salt stress (125 mM NaCl) allowing plant to maintain turgor. These CRS accessions would be more salt tolerant since Ashraf and Ahmad (2000) reported that salt-tolerant cotton varieties (*G. hirsutum* L.) had higher shoot biomass production than salt-sensitive varieties at the vegetative stage. However, cotton genotypes did not show any differences to an additional 125 mM NaCl. Leaf area per plant of all cotton genotypes progressively decreased with the increasing salinity level from control to 125 mM NaCl except for M-8744-0091, M-9044-0140 and M-9044-0031. Salt tolerance can be expressed in terms of absolute or relative growth under saline conditions (Shannon, 1984). Since salinity also may decrease biomass production due to the reduction in plant water potential, it is important to maintain turgor by less water reduction (Greenway and Munns, 1980). Among CRS accessions M-9044-0060 and M-8744-0091 increased PWC at 125 mM NaCl level. Compare across both salt concentrations, Nazilli-84 and Stonville-453 are displayed sensitivity to salt stress while TAM94L-25 appear to be more salt tolerant relative to all biomass production reduction ratios. The positive correlation of SDW and LA (under 250 mM NaCl) with PWC and, SDW and TDW (under control), indicate that accessions with good vegetative growth under salt stress also had good vegetative growth without salt stress, which supported similar findings by Foolad (1996). SDW, TDW and PWC were correlated positively and significantly regardless of salt treatment, indicating that either trait could be used as a selection criterion. With TAM94L-25, three CRS accessions, M-9044-0060, M-8744-0091 and M-9044-0140, exhibited less reduction in SDW and TDW. These results indicated that CRS accessions have genotypic variation in terms of response to salt stress and suggest that CRS accessions M-9044-0060, M-8744-0091 and M-9044-0140 would be used parental material for salt tolerance breeding in upland cotton. In addition, selected single plants from salt-tolerant CRS lines were transferred to pots to obtain seeds for further investigation of salt tolerance in cotton.

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