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Research Article

Physiological Response to Salt (NaCl) Stress in Selected Cultivated Tetraploid Cottons

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In the southwestern and western Cotton Belt of the U.S. soil salinity can reduce cotton productivity and quality. This study was conducted to determine the physiological responses of six genotypes including five Upland cotton (*Gossypium hirsutum* L.) cultivars and one Pima cotton line (*G. barbadense* L.) to NaCl under greenhouse conditions. Seeds were germinated and grown for 14 days prior to salt treatment (daily 100 ml of 200 mM NaCl) for 21 days. Compared with the control (daily 100 ml tap water), the NaCl treatment significantly reduced plant height, leaf area, fresh weight, and dry weight. The NaCl stress also significantly increased leaf chlorophyll content, but did not affect leaf fluorescence. Of the six genotypes, Pima 57-4 and SG 747 had the most growth reduction, and were most sensitive to NaCl; DP 33B, JinR 422 and Acala Phy 72 had the least growth reduction and were most NaCl tolerant. Although all the six genotypes under the salt treatment had significantly higher Na and Cl accumulation in leaves, SG 747 and Pima 57-4 accumulated more Na and Cl than DP 33B. Increases in leaf N, Zn, and Mn concentrations were also observed in the NaCl-treated plants. While leaf P, Ca, and S concentrations remained unchanged overall in the genotypes tested, leaf K, Mg, Fe, and Cu concentrations significantly decreased during salt stress. Reduction in plant height is a simple, easy, sensitive, non-destructive measurement to evaluate salt tolerance in cotton.

1. Introduction

In the southwestern and western Cotton Belt of the U.S., soil salinity can ultimately lead to reduced crop productivity. In many areas secondary salinization, as a result of irrigation practices, drainage, or water quality, are primary factors contributing to the loss of productive agricultural land [1]. Three viable options are plausible to solve the problem of saline growing environments: (1) cease the agronomic usage of salinized soils, (2) desalinize soil, or (3) use salt-tolerant cultivars. Options (1) and (2) may not be agronomically or financially viable. Salt tolerance is measured by the relative decrease in yield of cultivars grown under saline conditions relative to nonsaline conditions [2]. Even though many studies have demonstrated salt tolerance in crops including cotton, high yielding and high fiber-quality cultivars with known salt tolerance are not commercially available [3]. Identification of salt-tolerant genotypes from the cotton germplasm pool is needed.

High salinity reduces plant growth by affecting the plant's osmotic or ionic homeostasis [4]. Many studies have investigated phenotypic and physiological responses of cotton to salinity under controlled conditions using soil, potting soil, or hydroponics [5–14]. Even though the negative effects of salt (NaCl) on cotton can vary depending on growth stage, salt concentration, and duration of salt treatment, cotton seed germination is delayed and reduced. During seedling and vegetative stages, cotton plants can exhibit reduced

stomatal conductance, transpiration rate, photosynthesis, water use efficiency, and increased respiration rate [15–21]. Salinity also reduces primary and lateral root growth, leaf expansion and size, stem thickness, plant height, and shoot and root weight [6–9, 11, 22–24]. The negative effects on cotton are more profound with a longer exposure to salt. As a result, mature cotton plants may have delayed fruit initiation, reduced fruit node number, increased fruit shed, and late maturity; boll and seed weight, fiber length, fiber strength, lint percentage, and yield are reduced, especially when cotton is under extended long period of salt conditions or during the full life cycle [11, 25–28].

High salt is deleterious to cotton by competing or severely limiting the uptake of ions leading to cellular ion toxicity and imbalance of osmoregulation [29]. Many studies have reported the effects of salt on ion concentrations in cotton, but the results are contradictory [6–11, 22, 30–32]. Salinity increased Na and Cl concentrations in leaves and roots [9, 33, 34] but not in fruit parts [35]. Through comparative analysis, some cultivars were considered salt-tolerant, such as Acala 1517-88 and Acala 1517-SR2 [36], NIAB-78 [30], and MNH-93 [32]. As compared with NaCl susceptible (S) lines, tolerant (T) lines had lower Na and Cl concentrations [32], especially at lower K/Na ratio soil conditions [37]. However, Ashraf and Ahmad [11] reported that T and S lines did not differ in Na concentration, while Leidi and Saize [23] and Stelter et al. [38] indicated that tolerance was associated with Na accumulation in leaves. Salt inhibited the uptake of other ions, leading to decreased N, P, K, Ca, and Mg concentrations in leaves and roots [9, 19, 34, 39–41]. However, several reports indicated that K, Ca, or S are stable in leaves, leading to lower K/Na or K/Ca ratios [33, 34, 42]. Thomas [33] reported that salt increased Ca and Mg concentration in leaves. Because salt tolerance is a relative measure, the inconsistency might be related to the use of different cotton species, genotypes, growth stages, or evaluation methods. Further studies using more cotton genotypes are needed in developing a comprehensive understanding of the elements in cotton during salt stress. A simple, reliable criterion for evaluating salt tolerance of cotton is also needed.

The objectives of the present study were to compare the physiological responses of six cotton genotypes from the two cultivated tetraploid species, Upland cotton (*Gossypium hirsutum* L.) and Pima cotton (*G. barbadense* L.) and to determine a reliable measure of salt tolerance/susceptibility among genotypes in future screens for salt tolerance.

2. Materials and Methods

2.1. Plant Growth and Salt Treatment. Five cultivars of Upland cotton (*G. hirsutum* L.) including DP 33B, SG 747, Acala Phy 72, Acala 1517-88, and JinR 422 and one Pima cotton (*G. barbadense* L.) genotype 57-4, were grown in the greenhouse from June 24, 2004 to July 28, 2004. Seeds were sown in individual 10-cm diameter pots filled with Metro-Mix 360 containing vermiculite, peat moss, processed bark ash, and composted pine bark (Scott-Sierra Horticultural Products Co., Marysville, OH) and allowed to germinate. After germination, five pellets of Osmocote fertilizer (N : P : K = 14 : 14 : 14, Scott-Sierra Horticultural Products Co., Marysville, OH) were placed in each pot for long-term fertilization. Seedlings were thinned to 1 plant per pot after emergence, for a total of 36 seedlings per genotype (216 plants in total). All plants were watered daily with 20 to 50 ml of tap water (0.57 dS m^{-1}) for two weeks before treatment. The plants were then arranged in a randomized complete block design with 3 replications (6 pots per replication per genotype) resulting in 18 plants for each genotype and treatment. Salt-treated plants (n = 108) were treated daily for 21 days with 100 ml of 200 mM NaCl (20 dS m^{-1}), while control plants (n = 108) were watered daily with 100 ml of tap water. Since 150 to 250 mM of NaCl were the most frequently used concentration for salt tolerance studies in cotton (see "Section 4" for more details), a step wise salt treatment or more salt concentrations were not considered. A large-scale salt tolerance study screening a number of germplasm would not be possible if a step wise salt treatment or multiple salt treatments were implemented. In this experiment, accumulation of NaCl in the pots was unlikely because excess NaCl or water (~30 ml) was well leached out from the bottoms of the pots and, in fact, the pots were still wet when irrigated the following day. However, the exact NaCl content was unknown since soil salinity was not measured. The greenhouse received only natural sunlight with no supplemental or artificial lighting; the average light intensity at 0700 MDT was 25 W/m^2 with a maximum average light intensity of 1008 W/m^2 occurring at 1400 MDT, daily. The temperature in the greenhouse ranged from 25 to 35°C and relative humidity ranged from 35%–50%. To verify the results of NaCl on plant growth including plant height, fresh and dry weight, the same experiment was repeated in the greenhouse using the same genotypes, same experimental design under similar greenhouse conditions; similar results were obtained (data not included).

2.2. Plant Measurements. Individual plant height was recorded at 0, 7, 14, and 21 days after (onset of) treatment (DAT). At 21 DAT, plants were measured for height (cm), chlorophyll content index (CCI) and fluorescence before a destructive harvest for fresh weight (g), leaf area (cm^2), dry weight (g), and mineral concentration.

Height was measured from the soil surface to the base of petiole of youngest fully expanded leaf. Total leaf area was determined on excised leaves with a Li-Cor model LI-3000 portable area meter (Li-Cor Biosciences, Lincoln, NE). Chlorophyll content index was obtained using an Opti-Sciences CCM-200 chlorophyll content meter (Opti-Sciences, Inc., Tyngsboro, MA). The fourth leaf from the topmost leaf was used for measurement and two readings per leaf (one on each side of the main vein) were recorded. Fluorescence was also measured on the fourth leaf without dark adaptation using an Opti-Sciences OS5-F1 modulated fluorometer (Opti-Sciences, Inc., Tyngsboro, MA) and photosynthetic quantum yield (Fv/Fm) was calculated. Readings were taken between 1.5 and 2.5 hours after sunrise on the day of harvest. The above-ground green plant parts were cut at the soil surface and fresh weight was then measured before removing leaves for further study. Leaves were then removed with stems and leaves weighed separately. Only leaves were

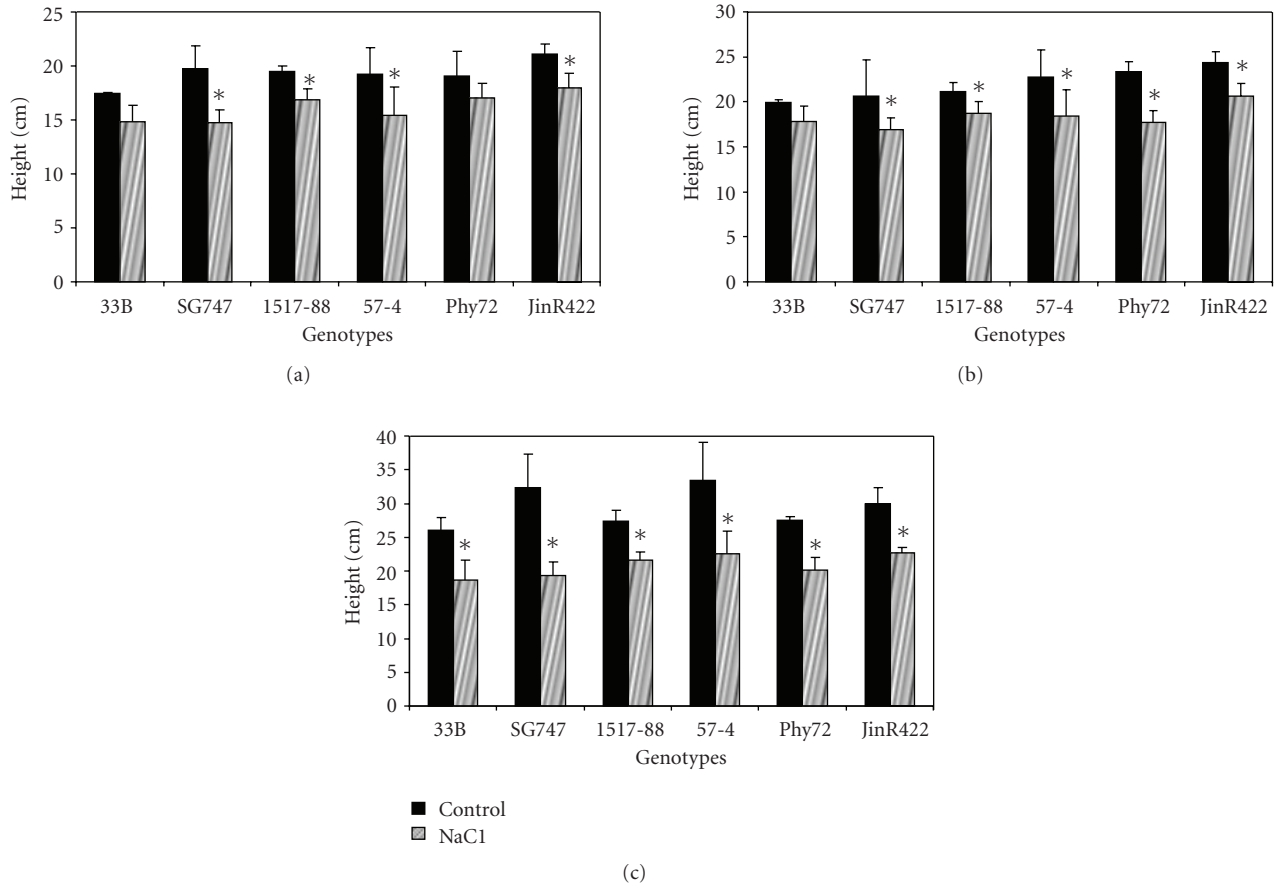

Figure 1: Plant height of six genotypes of cotton at 7 (a), 14 (b) and 21 (c) days after treatment (DAT) with 200 mM NaCl (NaCl) or tap water (Control). Bars represent standard error. *Significance between control and NaCl-treated within a genotype at $P \leq .05$.

bulked on plant or replicate basis and dried in an oven at 65°C for 48 hours and weighed.

2.3. Elemental Analysis. Dried leaf samples were sent to Ward Laboratories, Inc. (Kearney, NE: http://www.wardlab.com/) for analysis of elemental concentrations including: sodium (Na), potassium (K), zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), calcium (Ca), magnesium (Mg), phosphorus (P), sulfur (S), chloride (Cl), and nitrogen (N).

2.4. Data Analysis. Analysis of variance (ANOVA) was conducted on collected data using SAS version 9.1 (2002, SAS Institute, Inc.) to separate variation into replicate, treatment, cultivar, and cultivar x treatment interaction. Means between the NaCl-treated and nonsaline control plants within genotype were compared using *t*-test at the 5% level. Coefficients of correlation between growth reduction and plant height or leaf area were calculated using Excel.

3. Results and Analysis

3.1. Analysis of Variance. The analysis of variance (ANOVA) indicated that except for leaf chlorophyll fluorescence readings and leaf concentrations for P, S, and Ca, all the measurements showed significant differences between NaCl treatment and control. Also, except for leaf chlorophyll fluorescence readings and leaf concentrations for N, P, Ca, Mn, Cu, and Na, significant genotypic variations existed for other traits. However, genotype x treatment interaction was only detected for plant height measured at 14 and 21 DAT, and leaf Cl concentration. This result indicates that genotypic differences in response to NaCl treatment in cotton have similar trends to the genotypic differences under nonNaCl conditions for most physiological traits measured in this investigation.

3.2. Plant Height. Over the 21 days of 200 mM NaCl treatment, the six genotypes responded to the NaCl stress similarly. NaCl significantly reduced plant height as early as 7 DAT for four genotypes, that is, SG 747 (by 5.0 cm and 25.3%), Pima 57-4 (by 3.8 cm and 19.6%), JinR 422 (by 3.1 cm and 14.6%), and Acala 1517-88 (by 2.6 cm and 13.3%) (Figure 1(a)). Plant height in DP 33B and Acala Phy 72 was not significantly affected by salt stress at 7 DAT.

NaCl significantly reduced plant height in five genotypes at 14 DAT (Figure 1(b)). NaCl treatment did not significantly affect DP 33B plants, although the trend for NaCl to shorten plant height was apparent at both 7 and 14 DAT.

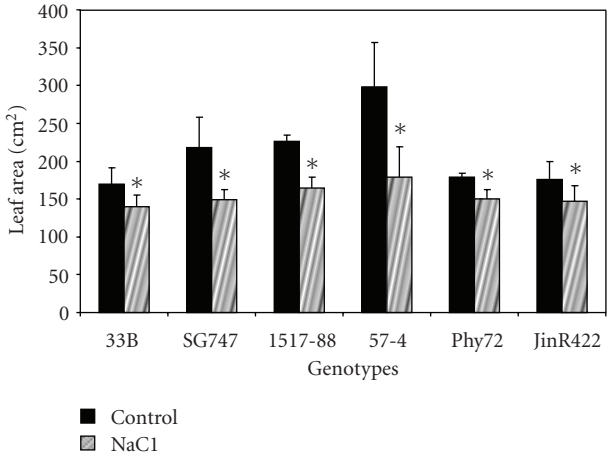

Figure 2: Total leaf area of six genotypes of cotton at 21 days after treatment (DAT) with 200 mM NaCl (NaCl) or tap water (Control). Bars represent standard error. *Significance between control and NaCl-treated within a genotype at $P \leq .05$.

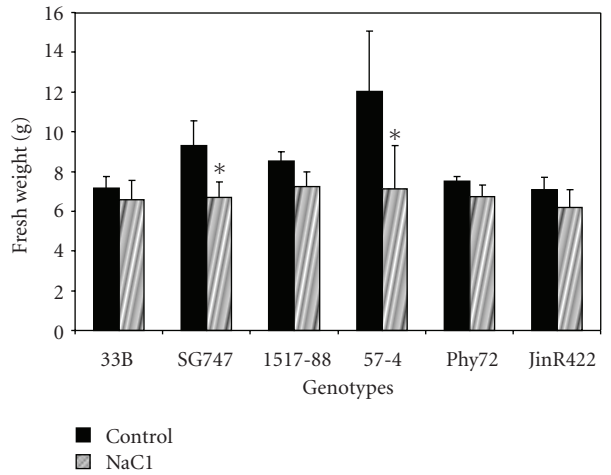

Figure 3: Fresh weight of six genotypes of cotton at 21 days after treatment (DAT) with 200 mM NaCl (NaCl) or tap water (Control). Bars represent standard error. *Significance between control and NaCl-treated within a genotype at $P \leq .05$.

At 21 DAT, NaCl treatment significantly reduced plant height in all genotypes (Figure 1(c)). SG 747 and Pima 57-4 had the tallest plants under control conditions and also had greatest reduction in plant height under NaCl stress (by 10.9 and 13.0 cm, and 40.3 % and 32.6%, resp.). Reduction in plant height in other four genotypes was similar (5.8–7.4 cm and 21.0%–26.8%).

The difference in plant height over the course of the experiment suggests that reduction in plant height is a sensitive method for determining cotton responses to salt stress. Even though noticeable genotypic differences were seen as early as 7 DAT, plant response was more profound at 21 DAT. Our data also demonstrated that evaluating salt tolerance of germplasm without comparison with their respective nonNaCl control would be misleading. For example, DP 33B, SG 747, and Pima 57-4 at 7 DAT, and DP 33B, SG 747, and Acala Phy 72 at 14 and 21 DAT had the shortest plant height under salt stress. However, when sensitivity to salt stress was measured by height reduction and reduction percentage, DP 33B, Acala Phy 72 and JinR 422 were found as the most tolerant among the six lines tested; SG 747 and Pima 57-4 were the most sensitive to NaCl stress. It is interesting to point out that, at 21 DAT under no-NaCl conditions, DP 33B was the shortest, while SG 747 and Pima 57-4 were the tallest. The results indicate that NaCl tolerance measured by height reduction percentage is negatively correlated with plant height (-0.757, $P > .05$) under nonNaCl conditions, that is, cotton genotypes with known vigorous growth, such as Pima 57-4 and SG 747, might be more salt sensitive due to the need for faster water, nutrient and mineral uptake through the roots, resulting in a faster accumulation of salt in various tissues.

3.3. Leaf Area per Plant. Under nonNaCl control conditions, Pima 57-4 had the highest leaf area per plant, followed by SG 747 and Acala 1517-88. NaCl significantly reduced leaf area in all genotypes (Figure 2). The reduction in the leaf area due to NaCl was greatest and in Pima 57-4 (40.1%), followed by SG 747 (31.8%), Acala 1517-88 (27.2%), DP33B (18.0%), Acala Phy 72 (16.2%), and JinR 422 (16.4%). As with plant height, leaf area reduction percentage is also negatively correlated with leaf area per plant under nonNaCl conditions (-0.968; $P < .01$). This result suggests that genotypes with larger leaf area have a greater response to NaCl treatment than those with smaller leaf areas.

3.4. Fresh Weight and Leaf Dry Weight per Plant. NaCl treatment significantly reduced plant biomass in two genotypes, SG 747 (28.0% for fresh weight and 25.4% for leaf dry weight) and Pima 57-4 (40.7% for fresh weight and 39.1% for dry leaf weight). Similar trends were also noted for the other genotypes (Figures 3 and 4). However, DP 33B, Acala 1517-88, Acala Phy 72, and JinR 422 had the least difference in fresh weight between control and NaCl-treated plants. DP 33B did not exhibit significant reduction in leaf dry weight (-2%) (Figure 4).

In summary, our results suggest that the 200 mM NaCl treatment was sufficient to elicit a physiological response as determined by reduced plant height, leaf area, and biomass accumulation. Taken together, these measurements indicate that DP 33B, Acala 1517-88, Acala Phy 72 (all three were bred in the southwest arid region of the U.S.) and JinR 422 are more salt-tolerant, while Pima 57-4 and SG 747 are more salt sensitive.

3.5. Chlorophyll Content Index (CCI) and Fluorescence. Leaf color in salt-treated plants appeared visually greener than in the nonNaCl control plants as early as 3 DAT. At 21 DAT, NaCl treatment significantly increased CCI in 3 of the 6 cultivars (SG 747, 1517-88, and JinR 422) (Figure 5). Acala Phy 72 did not exhibit any detectable change in CCI after NaCl stress.

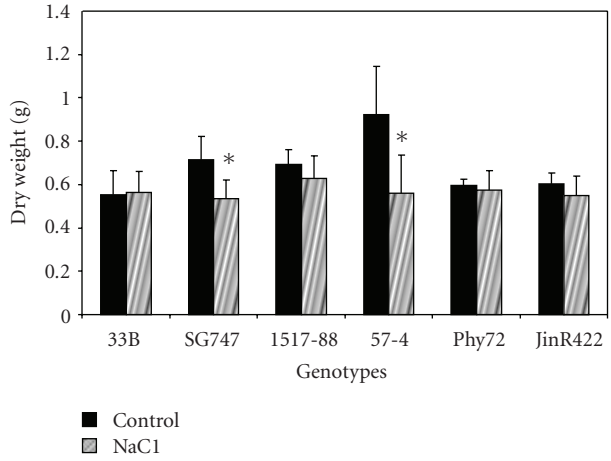

Figure 4: Dry weight of total leaves of six genotypes of cotton at 21 days after treatment (DAT) with 200 mM NaCl (NaCl) or tap water (Control). Bars represent standard error. *Significance between control and NaCl-treated within a genotype at $P \leq .05$.

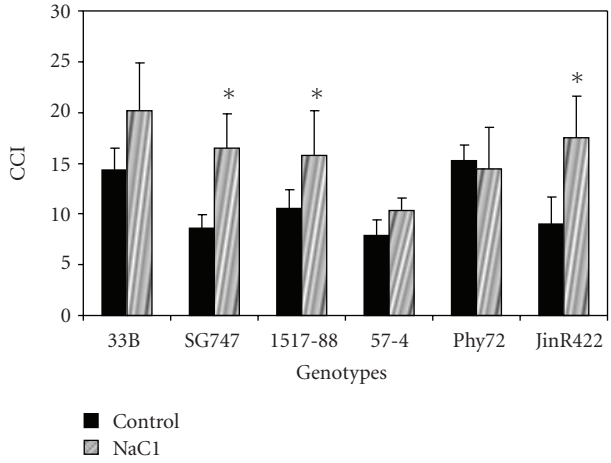

Figure 5: Chlorophyll Content Index (CCI) of six genotypes of cotton at 21 days after treatment (DAT) with 200 mM NaCl (NaCl) or tap water (Control). Bars represent standard error. *Significance between control and NaCl-treated within a genotype at $P \leq .05$).

The chlorophyll fluorescence readings under nondark adapted conditions ranged from 0.55 to 0.65 and did not significantly differ between salt-treated and control plants (data not shown). This result implies that NaCl treatment did not affect the efficiency of light use at PSII during photosynthesis.

3.6. Elemental Analysis. All six genotypes exhibited similar trends in the concentrations of 12 elements in leaves after salt treatment (Figures 6–8). Under nonsaline control conditions, based on values published by Marschner [43], the mineral concentrations were within the normal ranges for Na, K, P, Mg, and Zn. The concentrations of N and Co were below the normal ranges, while Cl, Mn, Fe, Ca, and S were above the normal adequate ranges.

Based on the trends, the changes in the concentrations of elements during salt treatment can be classified into 3 categories: (1) increased in response to salt treatment–Na, Cl, Mn, N, and Zn; (2) unchanged during salt treatment–Ca, P, and S; (3) decreased in response to salt treatment–Cu, Fe, Mg, and K.

3.7. Elements Increased in Response to NaCl. AT 21 DAT, Na and Cl concentrations were significantly increased in leaf tissues of NaCl-treated plants (Figures 6(a) and 6(b)). Leaf Na concentration in NaCl-treated plants increased by 450%–840% compared to the control plants, while Cl increased by 230%–340%. The two susceptible lines, SG 747 and Pima 57-4 had the greatest accumulation of Na (750%–840%) and Cl (330%–340%) among the six genotypes compared to controls. The other four genotypes had similar Na (450%–620%) and Cl (220%–270%) concentrations in leaves.

Nitrogen concentration in leaves increased significantly (on average by 15.1%) in NaCl-treated plants compared to controls. The increases were significant in three genotypes: SG 747 (24%), Pima 57-4 (28%), and Acala 1517-88 (17%), while the increase in leaf N concentration was not significant (5%–10%) for the other three genotypes, among which, DP 33B had the least increase (Figure 6(c)). Overall across the six genotypes, leaf Zn and Mn concentrations increased significantly due to NaCl treatment (by 28.3% and 23.8%, resp.), but their increases were significantly above the nonNaCl control within only SG 747 and DP 33B (Figures 6(d) and 6(e)).

3.8. Elements Decreased in Response to NaCl. At 21 DAT, overall concentrations of four (K, Mg, Fe, and Cu) elements in leaves decreased significantly below that of the nonNaCl controls after NaCl treatment (Figures 7(a)–7(d)). Leaf K and Mg concentrations in all the six cultivars decreased significantly below the control levels (by 21%–35% for K and 16%–36% for Mg) during salt treatment (Figures 7(a) and 7(b)). The trends in reduction of leaf Fe and Cu concentrations were apparent after NaCl stress since the overall difference averaged from the six genotypes was significant. Leaf Fe concentration was reduced (by 22%–47%) after salt stress, but the reduction was only significant within DP 33B and Pima 57-4 (Figure 7(c)). Salt stress also reduced leaf Cu concentrations (by 5%–39%); however, the difference only reached to a significant level in Acala Phy 72 (Figure 7(d)).

3.9. Elements Unchanged in Response to NaCl. In response to NaCl, Acala 1517-88 showed a significant decrease in Ca concentration, while SG 747 had significantly higher S concentration. However, on average across the six genotypes, NaCl treatment did not affect leaf concentrations of Ca, P, and S (Figure 8(a)–8(c)).

4. Discussion

4.1. Physiological Responses and Criteria in Salt Tolerance of Cotton. Even though cotton is classified as a more salt-tolerant crop with a soil salinity threshold of 7.7 dS m^{-1}, its

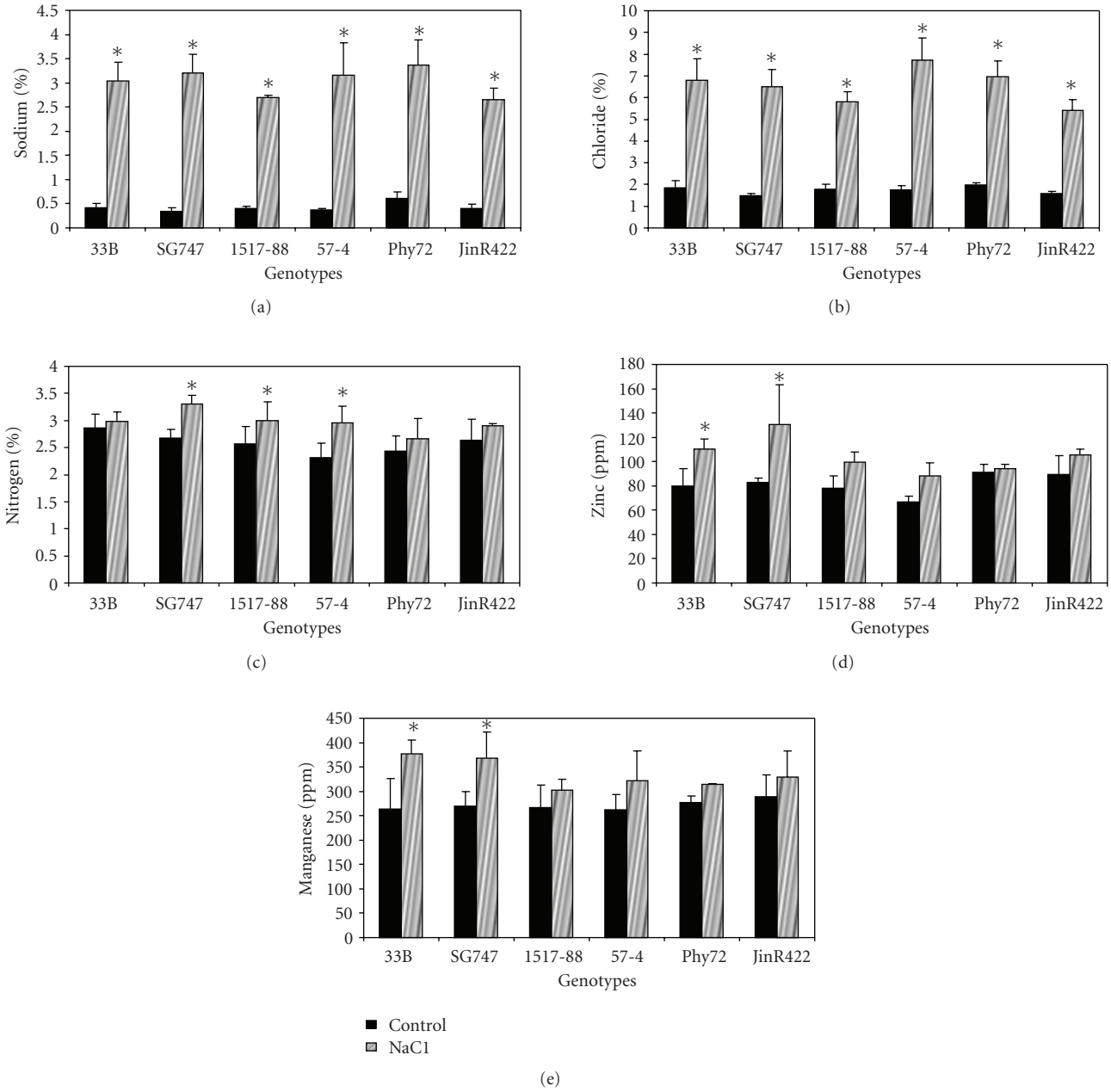

Figure 6: Elemental concentrations of the leaves of six cotton genotypes which increased after 21 days of treatment (DAT) with 200 mM NaCl (NaCl) or tap water (Control). (a) sodium; (b) chloride; (c) nitrogen; (d) zinc; (e) manganese. Bars represent standard error. *Significance between control and NaCl-treated within a genotype at $P \leq .05$.

yield decreases by 5.2% per unit dS m^{-1} increase beyond the threshold [44]. Therefore, the 200 mM NaCl (20 dS m^{-1}) used for salt stress treatment in our test, could simulate an approximate 60% yield reduction under field conditions. The severe saline stress was designed as a method to more rapidly, and visibly, help identify salt-tolerant versus sensitive plant on an individual basis. Cotton responses were noticeable with NaCl treatment as early as 3 DAT with a significant plant height reduction at 7 DAT in most of the genotypes tested. At 21 DAT, all plants treated with NaCl had reduced plant height, leaf area, and biomass accumulation. Reduction in plant height was also found in cotton plants grown at varying salt concentrations [22], suggesting that reduction in plant height may be a viable indicator of salt tolerance, or sensitivity. A preliminary test, conducted by our lab, indicated that 400 mM (40 dS m^{-1}) treatment caused cotton seedlings to wilt immediately and die (data not shown). Therefore, 200 mM NaCl was not only found to elicit visible, and measurable phenotypic differences among salt-tolerant and salt sensitive cotton plants over a short period of time, but regardless of salt stress status all plants were also able to recover, after treatment, and survive to maturity.

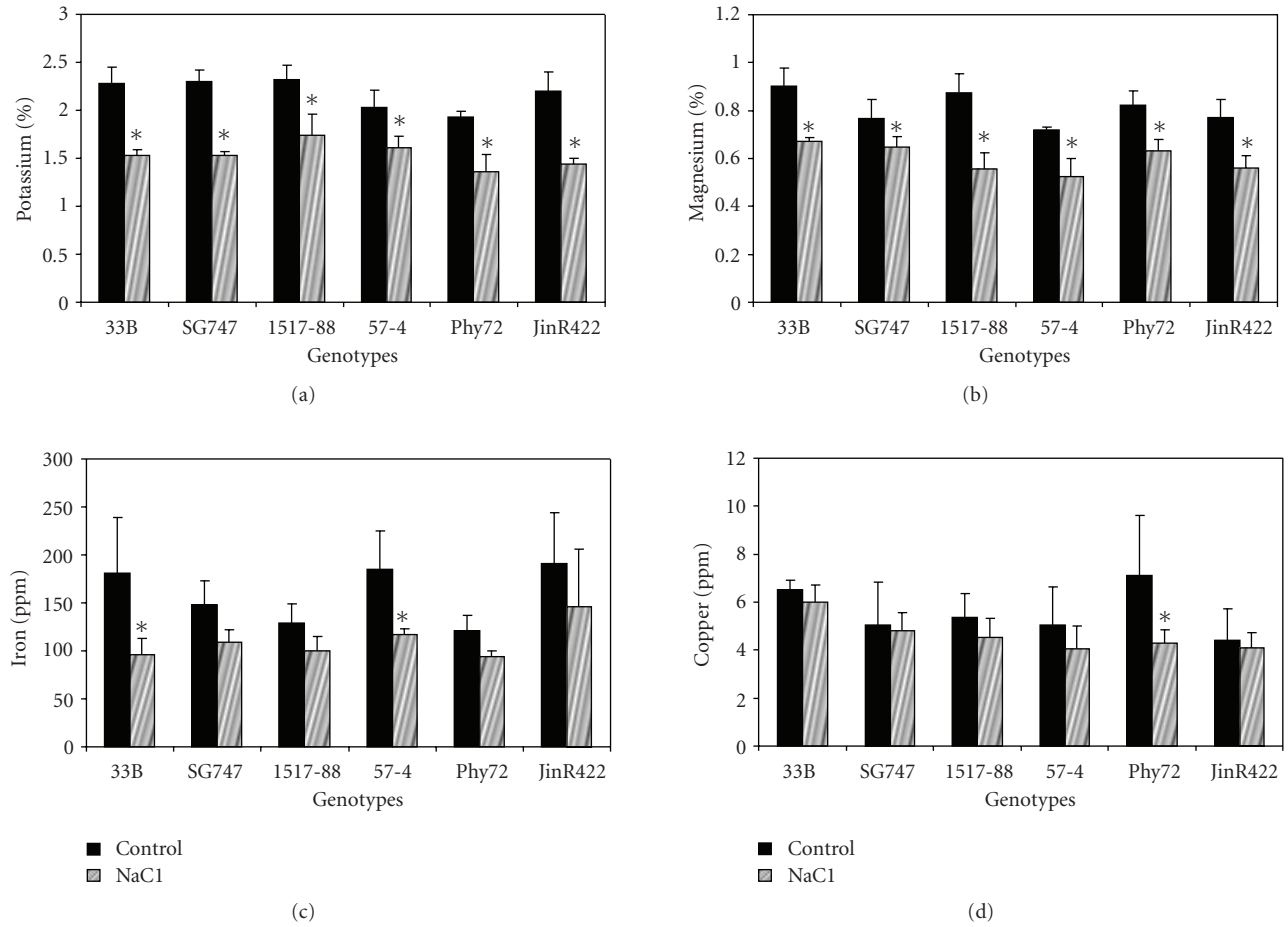

Figure 7: Elemental concentrations of the leaves of six cotton genotypes which decreased after 21 days treatment (DAT) with 200 mM NaCl (NaCl) or tap water (Control). (a) potassium; (b) magnesium; (c) iron; (d) copper. Bars represent standard error. *Significance between control and NaCl-treated within a genotype at $P \leq .05$.

Salt tolerance selection must be based on plant growth over a period of time since individual cultivars within the same self-pollinated species are nearly genotypically homozygous, but not homogeneous and differences between individuals may only be seen over a longer period of time [45]. A short-term study may show decreases in growth rate; however, these decreases might be the same for tolerant and sensitive individuals within a population. Only after a prolonged period of time can tolerance or sensitivity be accurately measured, in an individual plant, or can identification be made of specific mechanisms that aid certain plants to withstand NaCl conditions at different stages of growth [45]. Since the first true leaf in cotton emerges 10–15 days after seedling emergence, NaCl treatment right after seedling emergence might suppress the development of the first true leaf. Therefore, genotypic differences in plant height, leaf area, and biomass might not be detected after a short period of salt stress. In our study, we initiated the salt treatment after the emergence of the first true leaf, that is, 14 days after seedling emergence. Of the six genotypes used in our study, Pima 57-4 and SG 747 had the greatest reductions in plant height, leaf area, fresh plant weight, and dry weight at 21 DAT and were considered the most NaCl-sensitive. Genotypes DP 33B, JinR 422 and Acala Phy 72 had the least growth reduction and were classified as most salt-tolerant among the genotypes tested. The early genotypic differences were detected by 7 DAT. SG 747 and Pima 57-4 had the highest leaf area under both NaCl and nonNaCl conditions at 21 DAT, while DP 33B was shorter with smaller leaf area. Our data indicates that NaCl stress tolerance, as measured by reduction in plant height and leaf area, is negatively correlated with plant height and leaf area under nonNaCl conditions. This disagrees with Leidi and Saiz [23] who reported that leaf area was correlated with salt tolerance and a salt-tolerant cultivar, Z407, had a higher total leaf area than the susceptible one. Slama [5] reported that Acala cotton had the least height reduction after 20 days of salt treatment, while Pima cotton had the greatest height reduction.

In salt stress studies in other crop plants, accumulation of both Na and Cl slowed olive growth rate, resulting in altered leaf morphology and nutrient concentrations, ultimately affecting the nutritional composition of the tree [31]. Netondo et al. [46] suggested that a decrease in total leaf area might affect photosynthesis leading to a decrease

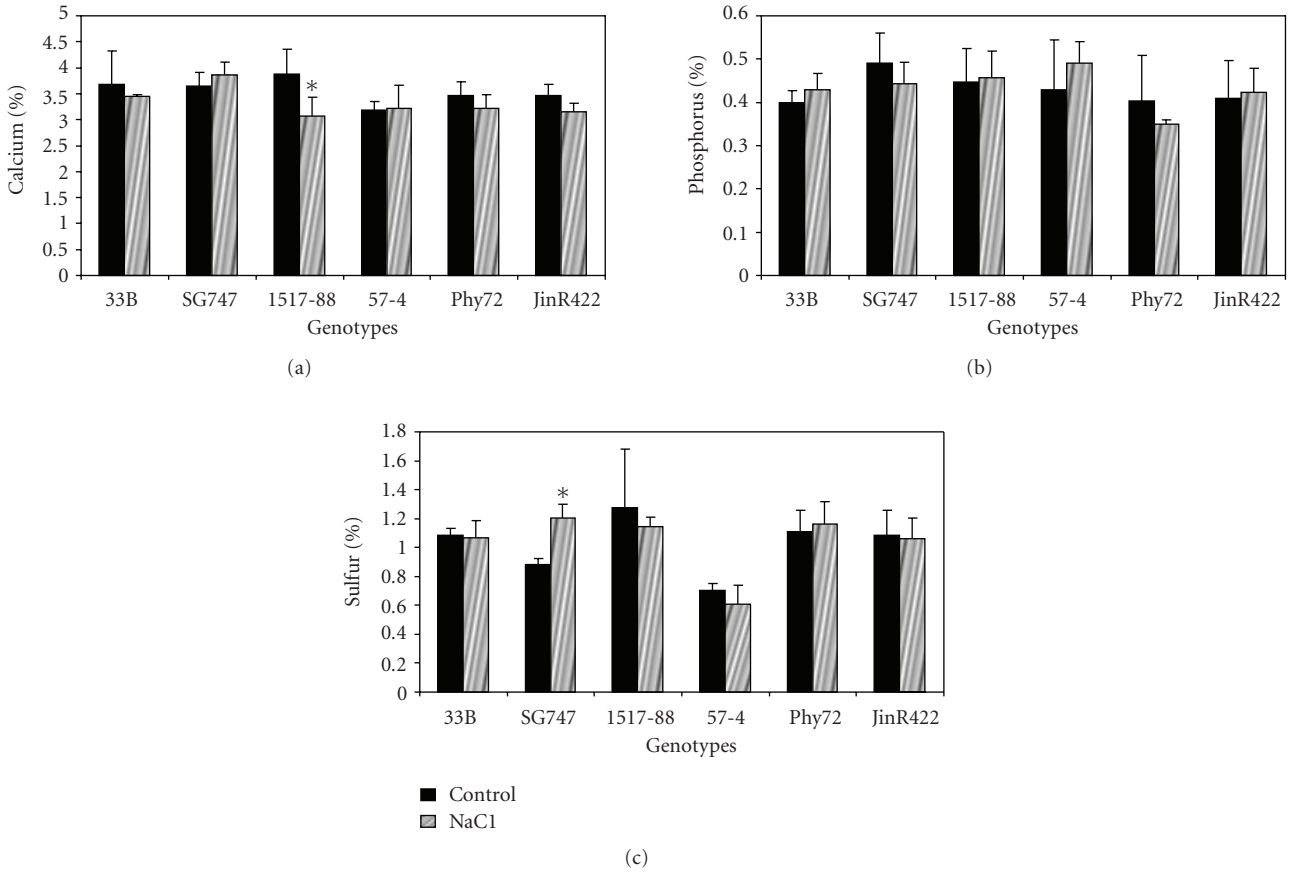

Figure 8: Elemental concentrations of the leaves of six cotton genotypes which remained unchanged after 21 days of treatment (DAT) with 200 mM NaCl (NaCl) or tap water (Control). (a) calcium; (b) phosphorus; (c) sulfur). Bars represent standard error. *Significance between control and NaCl-treated within a genotype at $P \leq .05$.

in biomass accumulation. Increased salinity reduced dry weight, indicating the loss of carbon gain, presumably from a shift in growth to combating the salt conditions [46]. In this study both salt-treated Pima 57-4 and SG 747, identified as more salt sensitive, had the largest decrease in biomass at 21 DAT, versus control and compared to all other genotypes, suggesting the growth potential of these two cultivars was more compromised by salt treatment. Since this particular study was limited by total sample number the absolute relationship between salt tolerance/susceptibility by the reduction in plant height and biomass cannot be completely recognized. In a subsequent salt tolerance trial involving 95 separate accessions of tetraploid cotton, repeating the experimental parameters of this study, the correlation between height reduction and biomass accumulation, across 3 independent trials, was found to be highly significant ($r = 0470$, $r_{0.001} = 0.271$), suggesting that height reduction and decrease in biomass might be a viable indicator of salt tolerance/susceptibility [47]. Of the parameters measured, reduction in leaf area was the most severe. This result indicates that leaf initiation and expansion is the most sensitive to NaCl stress and that leaf area might also be used as an indicator for NaCl tolerance in cotton. Dry matter accumulation would also appear to be a reliable method of determining salt tolerance in cotton, since reduction in salt sensitive plants is greater than salt-tolerant plants. However, destructive harvests need to be conducted in order to measure leaf area and dry weight which would be counterproductive in a situation where testing was being conducted to determine salt tolerance status prior to field planting of seedlings. Additionally, terminal measurements are time consuming, labor intensive, and not practical if testing for tolerance in the field or where a large number of plants are involved. Our experiment demonstrated that reduction in plant height represents an easy, nondestructive and reliable criterion to gauge NaCl tolerance in cotton.

Chlorophyll fluorescence of excised leaves, leaf discs, cell culture, or isolated chloroplasts have been measured in barley [48, 49], rice [50], and sorghum [51, 52]. Few experiments used intact leaves leading to the question of whether an in-vivo difference of fluorescence occurs in intact leaves. Even though some experiments indicated fluorescence difference between salt-treated versus control plants [53–55], others and our study did not confirm the result [17, 48, 49, 56, 57], indicating that NaCl treatment does not affect the efficiency of light used during photosynthesis. Additionally, other stresses such as temperature can contribute to systemic response to NaCl stress leading to a change in fluorescence

[49, 51]. Therefore, chlorophyll fluorescence does not appear to be a reliable indicator for NaCl tolerance.

4.2. Elemental Analysis. Analysis of 12 elements in leaves in this study suggests that along with the above response to NaCl, element concentrations in leaves are affected. The elements that increased during NaCl treatment (Na, Cl, N, Mn, and Zn) suggest that besides the expected accumulation of Na and Cl, this increase in N-containing compounds was consistent with the increased concentration in Mg-containing chlorophyll. Na in the six genotypes showed a significant increase in Na concentration after 21 days of NaCl treatment, but no significant difference was detected in leaf Na concentration between the salt sensitive (SG 747 and Pima 57-4) and NaCl-tolerant (DP 33B) genotypes, even though the former group accumulated Na and Cl to higher levels. This result is consistent with Ashraf and Ahmad [11] but contradictory to Leidi and Saiz [23] and Slama [5] where Na accumulated to higher levels in the leaves of the NaCl-tolerant cultivars than in the NaCl-sensitive cultivars. The effect of NaCl was more pronounced on newer leaves than on older leaves [46]. The accumulation of NaCl was higher in the older leaves, a response seen in glycophytic plants [4]. Older leaves might have a higher level of salt because their longer growth period allows for accumulation of salt [46].

The Cl concentrations in the NaCl-sensitive and tolerant genotypes were variable at 21 DAT. In previous studies involving salt-tolerant and salt sensitive genotypes, NaCl-sensitive lines accumulated Cl to a higher level than the NaCl-tolerant cultivars [11]. But Leidi and Saiz [23] reported that Cl accumulated to similar levels in both the NaCl-tolerant and NaCl-sensitive cultivars at 100 mM and 200 mM of NaCl in the soil. In our study, the most sensitive genotypes, Pima 57-4 and SG 747 had the highest increase in Cl when treated with NaCl. However, the absolute concentrations were similar to these for the other genotypes. In *Poncirus*, Cl accumulated to a higher level than did Na in the leaves. This result led to the conclusion that *Poncirus* had no control in Cl sequestration or exclusion [13]. However, in our experiment, Na accumulated to a much relative higher level than Cl, because cotton possibly possesses a mechanism for sequestration. Our data indicated that the salt-tolerant cotton genotype (DP 33B) did not exclude or eliminate Na and Cl ions better than in the salt susceptible genotypes (SG 747 and Pima 57-4).

In addition to Na and Cl, leaf N levels in half of the six cultivars increased significantly during salt treatment which is contradictory to other results [11, 40]. In our study, a significant increase in Mn concentration in response to NaCl treatment was also seen. This result is consistent with Thomas [33] but inconsistent with those of Meloni et al. [34] that showed that increasing salinity decreased Mn concentration in cotton leaves. In NaCl-tolerant and NaCl-sensitive sunflowers, no significant difference in Mn was detected [58]. Our results also indicated that significant increase in N, Mn and Zn due to salt stress was genotype specific.

The significant increase in Na concentration in all the six cultivars was related to a significant decrease in K. This result is in agreement with the findings from most of the published results [9, 11, 19, 40]. Since K has been implicated in turgor regulation [59], a decrease in K in the cotton genotypes may have also contributed to the decrease in plant height growth and leaf expansion in the NaCl-treated plants. However, several investigations did not detect changes in leaf K concentration [33, 34, 42]. In a study involving *P. trifoliata*, the level of K increased in leaves of the NaCl-treated plants [13]. According to Leidi and Saiz [23] and Ashraf and Ahmad [11], leaf K decreased significantly in both NaCl S and T cultivars, but the T cultivars had a higher concentration of K in the leaves compared to S lines. However, in our test, the two most NaCl-sensitive genotypes, SG 747 and Pima 57-4, did not show significantly lower levels of K in the leaves.

Abd-Ella and Shalaby [42] reported that high NaCl did not change Ca concentration in cotton leaves. This research is consistent with the results from our study that none of the six genotypes showed any significant decrease in leaf Ca concentration after salt treatment. In *P. trifoliata*, Ca and P levels were not significantly different in the NaCl-treated and control plants [13]. Others reported a decrease in Ca concentrations due to NaCl stress in cotton, sorghum, barley, wheat, and maize [33, 34, 39, 60–63]. No significant difference in Ca concentration was also seen between NaCl-tolerant and NaCl-sensitive sunflowers [58]. However, Ca was found to be higher in NaCl-tolerant cotton lines [11].

Leaf S concentration also did not change in cotton at 21 DAT. We demonstrated that Zn and Mn concentrations tended to be increased while Fe and Cu decreased after NaCl stress. No significant change in P concentration was noted in the present study. However, Martinez and Läuchli [41, 64] reported that NaCl increased P concentration in old leaves and inhibited P uptake within the roots and its translocation from roots and cotyledons to young leaves in cotton.

As indicated by ANOVA and in Figures 1 to 8, significant genotypic differences were detected under the nonNaCl conditions. Our study reported here demonstrates that plant height, leaf area, chlorophyll content, biomass, and nutrient concentrations in cotton are not only affected by NaCl stress tolerance, but also more importantly by other genetic factors unrelated to salt tolerance. To evaluate cotton germplasm for salt tolerance, control (normal nonsalt treatment) for each genotype should be concurrently used for a comparison.

The different results between our study and previous reports may be from the use of different species, genotypes, and tests at different growth stages and leaf ages (i.e., leaves at different mainstem positions). Physiological responses of different genotypes at different growth stages and leaf ages under different NaCl stress conditions should be further investigated. Currently, no comprehensive data is available in cotton germplasm pool with regard to salt tolerance. This lack of information has hindered researchers from choosing appropriate genotypes with various levels of salt tolerance for comparison purposes in order to better understand the mechanisms of salt tolerance in cotton. An immediate task is to evaluate sufficient cotton germplasm to identify salt tolerance genotypes for physiological, genetic, and molecular mechanisms of salt tolerance and breeding for salt tolerance in cotton.

In conclusion, this study did offer a direction for further study into a more rapid method of identifying the salt tolerance/susceptibility of individual plants. From the data presented, it is clear that larger trials need to be conducted to confirm the use of reduction in plant height as a viable indicator of salt susceptibility in individual cotton plants. In addition, while this study did show statistically significant differences in salt-treated versus nontreated control plants, the use of only one concentration did not address the minimum concentration needed to elicit a statistically significant response and additional studies using multiple salinity treatments should be conducted.

Abbreviations

ANOVA: Analysis of variance
DAT: Days after treatment
LSD: Least significant difference
ppm: Parts per million
S: Susceptible
T: Tolerant

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