

SALT TOLERANCE IN UPLAND COTTON GENOTYPES TO NaCl SALINITY AT EARLY GROWTH STAGES

Z. Bibi^{1,2}, N. U. Khan^{3,*}, A. Latif⁴, M. J. Khan⁵, G. Manyuan², Y. Niu², Q. U. Khan¹, M. J. Khan¹, I. U. Khan¹, S. Shaheen¹ and G. U. Sadozai⁶

¹Department of Soil and Environmental Sciences, Gomal University, Dera Ismail Khan, Pakistan

²Institute of Soil Science, Chinese Academy of Sciences (CAS), Nanjing, China

³Department of Plant Breeding and Genetics, The University of Agriculture, Peshawar, Pakistan

⁴Agricultural Research System, The University of Agriculture, Peshawar, Pakistan

⁵Department of Soil and Environmental Sciences, The University of Agriculture, Peshawar, Pakistan

⁶Department of Agronomy, Gomal University, Dera Ismail Khan, Pakistan

* Corresponding author e-mail: nukmarwat@yahoo.com, nukmarwat@aup.edu.pk

ABSTRACT

Eight upland cotton genotypes (CIM-446, CIM-473, CIM-496, CIM-499, CIM-506, CIM-554, CIM-707 and SLH-284) were screened for salt tolerance with five NaCl salinity concentrations [0 mM (control), 50, 100, 150 and 200 mM] at early growth stages. Highly significant differences were observed among genotypes, salinity concentrations, and genotype vs. salinity interactions for growth variables. In the control (0 mM NaCl), genotypes showed enhanced vigor, however, response of genotypes at 50 mM NaCl was similar to the control, and for some traits, the response was increased as a result of stimulation by low salinity. Growth variable response gradually decreased as salinity concentrations increased (50 > 100 > 150 > 200 mM), while significant decrease were observed at 200 mM NaCl due high Na⁺ ion toxicity. The uptake of K⁺ and Na⁺ were inversely proportional, and Na⁺ accumulation was least in control and gradually increased as salinity application increased. Absorption of K⁺ was highest in the control and gradually decreased through increased salinity in shoot and root tissues. Genotype CIM-707 was most tolerant to salinity and exhibited the best performance for majority variables, followed by CIM-446. Identification of salt tolerant cotton genotypes for salt affected areas can benefit the farming community through increased crop yield.

Key words: Cotton shoot and root tissues; growth variables; NaCl salinity concentrations; Na⁺ / K⁺ accumulation; salt tolerance; *Gossypium hirsutum* L.

INTRODUCTION

Soil salinity is a common abiotic stress which affects the yield of agricultural crops in various ways including increased levels of certain toxic ions, reduced activity levels for water and essential nutrients, nutritional problems, and reduction in crop yields and qualities (Ibrahim *et al.*, 2007). Salinity is usually confined to arid and semi-arid regions of the world, and Pakistan is no exception to this problem because of its semi arid climate (Ashraf, 2002). Approximately 1000 million hectares, or seven percent of the world's land-area, is salt-affected either by salinity or sodicity (Anonymous, 2005). In Pakistan, about 5.8 million hectares of land are salt-affected, and every year 0.2-0.4% of total arable land is left out of cultivation due to salinity and water logging.

Salinity is serious problem and one of the major hazards affecting irrigated agriculture. Improper irrigation practices and lack of drainage have generally led to accumulation of salts in the soil which are harmful to crops. Each year about 120 million tons of salt is added to the land from canal water and brackish underground water (Munns, 2002). Only about 1/5th of this salt finds its

way to the sea. The remainder accumulates in the soil and continues to reduce the growth and survival of crops. There is a major imbalance in the amount of salt entering and leaving the soil. It is evident that some plant species can tolerate high levels of salinity by either partial salt exclusion or salt inclusion (Pessaraki, 2001; Hou *et al.*, 2009). The mechanism of ion uptake and pattern of accumulation of ions in various parts of a plant is very important in making a distinction between salt-tolerant and salt-sensitive genotypes.

Limited land and water resources increase the demand and necessitate the use of poor quality soils and waters to increase production. Salinity is inimical to plant growth through specific ion effects, osmotic effects and induced nutrient deficiency. One easy way to cope with the problem of salinity is to exploit the genetic potential of plants for their adaptability to adverse soil conditions. This approach prompted crop cultivation on salt affected fields; however, considerable variability for salt tolerance was observed among and even within plant species. Salt tolerance improvement might be achieved through screening and selection of existing germplasm (Akhtar *et al.*, 2003).

Salinity affects the plant growth at all stages of the plant's life cycle at different salinity concentrations. Salts presence near root zone does not allow the plants to flourish and may be one of the reasons for lower crop yields. Seed germination and seedling stages are very sensitive to salinity (Qadir and Shams, 1997). Salinity adversely affects the growth of primary roots, leaf area, shoot length, shoot and root fresh weight, and root growth; however, there are reports that show an increase in root growth at moderate salinity levels (Akhtar and Azhar, 2001).

Cotton is one of the more salt tolerant crops (Maas, 1990), and observed with enhanced growth at low salinity concentration (Pessarakli, 2001). Cotton growth, nutrition absorption and yield were improved by adding appropriate amounts of K and Na (Zhang *et al.*, 2006). Moderate salinity with adequate nutrition did not have adverse effects on growth but at higher salt concentration, shedding and premature leaf senescence were observed (Chen *et al.*, 2010). Although considerable variation exists between species, cotton is generally considered to be relatively tolerant to both salinity and sodicity (Maas, 1990). A number of authors have used hydroponic experiments to investigate the effect of sodium chloride (NaCl) on cotton growth and nutrition (Kent and Lauchli, 2006).

Salinity stress causes a series of negative effects on cotton growth, yield, and fiber quality. It is also important to point out that cotton seedlings are sensitive to soil salinity with EC_e levels of 4 dS/m threshold salinity level, and its seed germination, yield and quality are affected by different salinity levels. Researchers suggested looking for naturally occurring salt tolerance in crop genotypes for improving growth under saline conditions. Identification of salt-tolerance in cotton germplasm is an important goal for further improvement in cotton production. Previous studies conducted on this issue revealed genotypic variability for salinity tolerance in cotton (Azhar and Ahmad, 2000). Therefore, the present research was planned with the objectives i.e. a) to determine the effect of various NaCl salinity concentrations on growth variables, and b) to identify genotypes most tolerant to salinity at early growth stages in upland cotton.

MATERIALS AND METHODS

Plant material and procedure: Eight newly bred upland cotton genotypes (CIM-446, CIM-473, CIM-496, CIM-499, CIM-506, CIM-554, CIM-707 and SLH-284) were evaluated for salt tolerance to five NaCl salinity concentrations [0 mM (control), 50 mM, 100 mM, 150 mM and 200 mM] for 40 days at early growth stage. The pot experiment using sand culture was carried out in a complete randomized design (CRD) with two factors and three replications in net house during May-July, 2011 at

The University of Agriculture, Peshawar, Pakistan. The coarse sand was collected from River Kabul banks near Peshawar and was thoroughly washed (3-4 times) with tap water to make it free from all the nutrients and clay particles. After sun drying, the pots (having size of 18 cm height \times 21 cm dia.) were filled with five kg of sand at required intensity. Overall, 15 pots were used for each genotype and 120 pots were arranged for the whole experiment. Four seeds of each cotton genotype were sown in pots during last week of May, 2011.

Seed germination was completed in a week time, and thinning was performed after one week of germination to ensure a single plant per pot. Pots were fully saturated every day with required volume of Half (50%) Strength Hoagland Solution (HSHS) (Hoagland and Arnon, 1950) containing no NaCl until the plants reached the beginning first true-leaf stage, and that was subsequently achieved after 14 days of planting and then the cotton seedlings were subjected to the salt stress. Salinity treatments beyond 50 mM were applied stepwise starting from 50 mM salt first and then increased to the desired concentration to avoid osmotic shock. An initial of 50 mM was raised one day later to 100 mM and then onward to final 200 mM in HSHS. After 40 days of imposition of NaCl stress, plants were uprooted and various growth variables were measured.

Traits measurement and statistical analysis: Stem diameter was determined with help of "Vernier Caliper" in cm. Total number of true leaves were counted on shoots and recorded as leaves per plant. After uprooting the plants and washing with water, plants were cut at the juncture of shoots and roots, and then actual root length was recorded in cm. Fresh shoots and roots were weighed and recorded in grams. Shoots and roots were placed in separate paper envelopes, dried in an oven at 70 °C for 72 hours, weighed and recorded in grams as dry shoot and root weight. All the data were analyzed using completely randomized design (CRD) with two factors (Steel *et al.*, 1997). For each variable, the three mean groups (genotypes, salinity concentrations and their $G \times S$ interactions) were further compared by using the least significant difference (LSD) test at 5% level of probability.

RESULTS AND DISCUSSION

According to analysis of variance, highly significant ($p < 0.01$) differences were observed among genotypes, salinity concentrations, and genotype vs. salinity interactions ($G \times S$) for stem diameter, leaves per plant, root length, fresh and dry shoot weight, and fresh and dry root weights (Table 1). The physical growth of plant such as shoot and root length, stem thickness, fresh and dry shoot and root weight, leaves per plant, and ions concentrations (K^+ , Na^+) in shoot and root tissues

contributed more towards salt tolerance at the early growth stage and could be used as selection criteria for salt tolerance (Ashraf, 2002).

Stem diameter: Stem diameter was influenced by various salinity concentrations resulting in significantly decreased stem diameter. However, maximum and alike stem diameter (0.30 and 0.29 cm) was recorded in genotypes CIM-446 and CIM-496, respectively and indicating their superiority over all other genotypes (Table 2). These two genotypes were followed by genotype CIM-707 (0.28 cm). Genotypes SLH-284, CIM-506 and CIM-499 showed similar and medium stem diameter (0.26 cm). Genotypes CIM-554 and CIM-473 produced the thinner stem of 0.24 cm and were designated sensitive to salinity. In case of salinity concentration means, surely thicker stems were observed in control (0.32 cm) followed by genotypes with 50 mM (0.29 cm) (Table 2). Increased salinity concentrations had a greater impact on plant stems and hence the stem diameter was gradually decreased to 0.26 (100 mM) > 0.23 (150 mM) > 0.22 cm (200 mM). In response of cotton genotypes to salinity, higher salt stresses (125 and 250 mM NaCl) revealed significantly reduced stem diameter (Basal, 2010).

In genotype by salinity interactions, genotype CIM-707 with highest salinity (200 mM) provided medium stem diameter and bold stem (0.28 cm) and was tolerant to salinity (Table 2). Higher salinity concentrations severely affected and reduced the stem diameter of genotypes i.e. CIM-473, CIM-506 and CIM-554 with 200 mM, and CIM-473, CIM-554 and CIM-499 with 150 mM ranging from 0.16 to 0.21 cm. However, genotype CIM-446 with 0 mM (control) manifested maximum stem diameter (0.37 cm) followed by CIM-496 (0.34 cm) and CIM-446 (0.33 cm) with 50 mM, and CIM-707 (0.34 cm), CIM-496 (0.33 cm), CIM-473 (0.32 cm) and CIM-499 (0.32 cm) in control pots. With lowest salinity concentration of 50 mM, the stem growth of some genotypes stimulated, and two genotypes (CIM-496 and CIM-446) showed some tolerance to salinity. Remaining genotypes with salinity concentrations of 100 and 150 mM revealed medium stem diameter.

Qadir and Shams (1997) reported increased salinity stress exhibited harmful effects on vegetative growth with significant differences among cotton genotypes, and stem thickness decreased with increases in salinity concentration. Jafri and Ahmad (1994) found that higher salinity concentrations reduced stem diameter in upland cotton genotypes and suppress other growth variables. Stem diameter and plant height were significantly reduced by higher salinity concentrations in upland cotton (Azhar and Ahmad, 2000).

Leaves per plant: Genotype means revealed that increased and similar number of leaves per plant were recorded in genotypes CIM-446 (7.60) and CIM-554

(7.53) and designated as tolerant to salinity because these genotypes were more healthy than other genotypes at all salinity concentrations (Table 3). These genotypes were followed by five other genotypes (CIM-496, CIM-707, SLH-284, CIM-506 and CIM-499) having alike number of leaves per plant ranged from 6.87 to 7.07 and were found moderate sensitive to salinity. A severe effect of the salinity concentration was observed in genotype CIM-473 with the least number of leaves per plant (6.13) and was indicated as the genotype most sensitive to salinity. In salinity concentration means, salinity concentrations reduced the plant growth and number of leaves (Table 3). However, the highest number of leaves per plant (8.63) was recorded in control with zero NaCl followed by NaCl concentration of 50 mM (8.17). Salinity concentrations of 100 and 150 mM showed medium number of leaves per plant i.e. 6.63 and 6.33, respectively. NaCl concentration of 200 mM severely affected the growth of all the genotypes and on average the number of leaves reduced to 5.42.

Growth traits (leaves and their area) were severely affected and significantly reduced at highest salinity than control in cotton (Ashraf, 2002). In upland cotton, the leaves per plant, general growth and reproductive variables were significantly reduced at higher concentration of salinity (Jabeen and Ahmad, 2009). Significant differences were reported among the upland cotton genotypes with increased salinity level and reduced vegetative growth including leaves per plant (Basal, 2010).

In genotype by salinity concentration interactions (Table 3), the salinity concentration of 200 mM severely and 150 mM less affected the plant growth of all genotypes. CIM-446 with NaCl concentrations of 200 mM (6.33) and 150 mM (7.67) was the leading genotype for leaves per plant as compared to other genotypes with same salinity concentrations, and these results authenticated also the genotypes means over NaCl concentrations. Genotypes CIM-506, CIM-707, CIM-499, SLH-284, CIM-473 and CIM-554 with 200 mM NaCl exhibited least number of leaves per plant ranged from 4.67 to 5.67 and none of the genotypes exhibited tolerance in term of said trait to salinity. At control, highest number of leaves per plant was recorded in genotypes CIM-506 (9.33) and CIM-496 (9.00) followed by CIM-554 (9.33) at 50 mM. These associations were pursued by genotypes CIM-446, CIM-707 and SLH-284 at salinity concentration of 50 mM. Other interactions have medium effect of salinity on genotypes leaves per plant.

Similar results were reported by Akhtar *et al.* (2010) that tolerant genotypes provided more fresh weight with increased leaves than susceptible cotton genotypes. However, Chen *et al.* (2010) mentioned that cotton was found more sensitive to salinity during the emergence and early growth stages than later

developmental stages. In upland cotton, the lower relative growth rate (RGR) at early stage under salt stress also prolonged the vegetative growth period and as a result reproductive growth carried the plants to relatively colder season and maturity become late (Jafri and Ahmad, 1994).

Root length: In case of root length, the genotype means manifested that salinity concentrations suppressed and decreased the genotypes root length (Table 4). However, the vital and alike root length was obtained in four genotypes CIM-707, SLH-284, CIM-506 and CIM-499 ranged from 18.40 to 19.07 cm at all salinity concentrations, and CIM-707 was leading genotype numerically and has more tolerance to salinity in term of root development. Genotypes CIM-554 (17.33 cm) and CIM-496 (16.13) were having moderate root length. However, least root lengths of 14.33 and 14.53 cm were observed in genotypes CIM-446 and CIM-473, respectively at all salinity concentrations. Means of salinity concentrations revealed that higher salinity levels severely affected and decreased the root length (Table 4). However, on average, maximum root length of 19.54 cm was observed in control followed by 50 mM NaCl (18.71 cm). Salinity concentrations of 150 and 200 mM severely affect the root length and decreased to 15.13 and 15.00 cm, respectively.

NaCl effects were studied on root growth in upland cotton, and their findings revealed that ionic activities of low NaCl stimulate the root growth; however, at higher NaCl concentrations the root growth was inhibited (Basal, 2010). The responses of *G. hirsutum* were determined under different NaCl concentrations, and concluded that salinity caused significant reduction in root length (Akhtar and Azhar, 2001). NaCl concentrations of 150 and 200 mM significantly reduced the root length and growth in upland cotton genotypes (Basal *et al.*, 2006). At seedling stage, the *G. hirsutum* genotypes manifested significant variation in root length and at higher salinity the root growth was severely affected (Azhar *et al.*, 2007).

In genotype \times salinity interactions, salinity concentrations of 100 to 200 mM significantly decreased the root length (Table 4). However, the promising genotype CIM-707 (20.00 cm) at salinity concentration of 200 mM NaCl showed above moderate root length and was found the most tolerant genotype than other genotypes with highest NaCl concentrations in terms of root length. Least root length was observed in genotypes CIM-473, CIM-496 and CIM-446 at 200 mM NaCl ranged from 11.00 to 13.33 cm, followed by CIM-473 and CIM-446 at 150 mM NaCl. The supreme root length was also monitored in CIM-707 (22.00 cm) followed by CIM-506 with control, CIM-499 and CIM-496 with 50 mM and CIM-506 at 100 mg/l from 21.00 to 21.67 cm.

Results revealed that low concentrations of NaCl stimulate the root growth of these genotypes.

Response of cotton to various salinity concentrations showed significant variations in radical elongation and seedling maturation (Hemphill *et al.*, 2006). Rajashekar (2006) investigated that germination % and root length were decreased relatively in cotton genotypes due to NaCl application. Basal (2010) studied the response of upland cotton at different salt concentrations and reported significant reduction in root length with increased salinity concentrations. However, three different cotton species (*G. hirsutum* L., *G. tomentosum* L. and *G. darwinii* L.) to NaCl concentrations (50, 75, 100, 125, 150, 175, 200 and 250 mM) exhibited that at low salinity concentration the root growth was stimulated and root length was increased in *G. hirsutum* L. genotypes (Tiwari and Stewart, 2008).

Fresh shoot weight: Genotype means revealed that maximum fresh biomass was noticed in genotypes CIM-707 (4.32 g) and CIM-496 (4.09 g) followed by SLH-284 (3.76 g) (Table 5). These genotypes produced more fresh biomass and tolerance to salinity. Genotypes CIM-554, CIM-506 and CIM-446 enunciated medium and similar values (3.37 to 3.48 g) for fresh shoot weight. However, genotypes CIM-473 and CIM-499 got least average values of fresh biomass i.e. 2.97 g and 2.95 g at all salinity concentrations and control. In salinity concentration and control means, salinity concentrations revealed significant differences in genotypes means with varying values (Table 5). However, on average, the utmost fresh shoot weight was obtained with 50 mM (4.91 g) for all the genotypes pursued by control (4.62 g), means that low concentration of NaCl stimulated the vegetative growth of genotypes and showed an increase in fresh shoot weight than control. Fresh shoot weight of genotypes was gradually decreased as the salt concentration increases i.e. 3.62 g in 100 mM > 2.71 g in 150 mM, and the least fresh shoot weight (1.88 g) was obtained in salinity concentration of 200 mM for all the genotypes.

Physiological responses to low salt concentrations (2.7 and 27 mM NaCl) was reported in cotton genotypes and observed that there were no differences in growth with no salt, whereas under salt stress the tolerant genotype had higher fresh/dry matter accumulation and leaf area than susceptible genotypes (Leidi and Saiz, 1997). Similarly, Azhar and Ahmad (2000) studied the response of upland cotton genotypes with five NaCl concentrations (0, 50, 75, 100 and 150 mM) which revealed that increased salinity cause significant reduction in fresh shoot and root weight and genotypes were classified as most tolerant, moderately tolerant and susceptible to salinity.

Genotype by salinity concentration interactions displayed that cv. CIM-496 (2.29 g) at 200 mM NaCl

revealed maximum fresh shoot weight followed by SLH-284 and CIM-707 with mean values of 2.24 and 2.19 g, respectively (Table 5). Genotype CIM-707 was founding the leading genotype at 150 mM as compared to other genotypes. However, the genotypes CIM-496, CIM-707 and CIM-554 with 50 mM NaCl revealed maximum mean values for fresh shoot weight ranged from 5.49 to 7.77 g. The said range was found alike and even maximum than other genotypes at control and 50 mM. Results revealed that low concentration NaCl also stimulated growth and 50 mM NaCl showed even improvement in fresh shoot biomass than control. Other genotypes were drastically affected and showed least fresh shoot weight at 200 mM ranged from 1.28 to 2.29 g.

Crop maintains its degree of salt tolerance consistently throughout its entire developmental phases; however, effective selection for salt tolerance can be made at any growth stage of cotton crop (Ashraf, 2002). In upland cotton, the fresh shoot weight and germination were more affected and reduced at salinity concentration of 200 mol m⁻³ (Kent and Lauchli, 2006; Basal, 2010). Jafri and Ahmad (1994) reported that increased salinity concentrations reduced the cotton growth with higher inhibition in shoot than root. Tiwari and Stewart (2008) mentioned that fresh shoot weight was significantly reduced with increased salinity in three cotton species.

Dry shoot weight: Dry shoot weight of different genotypes was significantly influenced by various salinity concentrations (Table 6). However, highest dry shoot weight was recorded in cv. CIM-707 (1.09 g) followed by genotypes CIM-496, SLH-284 and CIM-506 with mean values of 0.97, 0.83 and 0.82 g, respectively. Genotype CIM-707 was found again most tolerant in term of dry biomass overall all salinity concentrations. Genotypes CIM-446 and CIM-554 revealed medium dry shoot weight of 0.79 and 0.78 g, respectively. CIM-499 and CIM-473 showed least shoot dry biomass of 0.67 and 0.72 g, respectively and were found largely sensitive to salinity. In salinity concentrations and control means, on average, the control certainly exhibited maximum dry shoot weight (1.18 g) followed by salinity concentrations of 50 mM NaCl (1.11 g) (Table 6). Salinity concentrations of 100 and 150 mM reduced dry shoot biomass to 0.82 and 0.60 g, respectively. However, 200 mM NaCl severely affected the genotypes dry shoot weight (0.45 g).

Cotton genotypes were screened with salinity (EC 15 dS m⁻¹) and control, and observed that salinity significantly decreased the shoot fresh and dry weight (Akhtar *et al.*, 2005). In upland cotton, drastic reduction was noted in dry shoot weight at 200 mol m⁻³ NaCl salinity stress, and observed significant variations among cotton genotypes in terms of growth and salinity tolerance (Ibrahim *et al.*, 2007). Basal (2010) studied the response of upland cotton genotypes to salt stresses (125 and 250

mM NaCl) and recorded significant reduction for dry shoot weight under increasing salt stress.

Genotypes and salinity interactions revealed that promising cv. CIM-707 showed medium dry shoot weight (0.69 g) at 200 mM NaCl and was found leading genotype as compared to all other genotypes at highest NaCl concentration (Table 6). Genotype CIM-707 again confirmed its salt tolerance at 50 mM and showed 3rd ranking value (1.76 g) for dry shoot weight and surpassed other genotypes even at control and 50 mM NaCl. Genotype CIM-707 (2.00 g) with control also revealed maximum dry shoot weight followed by cvs. CIM-496, CIM-707, CIM-554, CIM-499 and CIM-446 at 50 mM with dry shoot weight mean values of 1.76, 1.36, 1.28, 1.06 and 1.00 g, respectively. However, other genotypes with salinity concentration of 200 mM were drastically affected with least magnitude of dry shoot weight (0.34 to 0.52 g). Other associations of genotypes with NaCl concentrations of 100 and 150 mM showed medium dry shoot weight.

In upland cotton, the dry matter, seed cotton yield, N uptake, and plant 15N recovery were significantly increased as soil salinity concentration increased from 2.5 to 6.3A dSA m⁻¹, but decreased markedly at higher concentration of salinity (Hou *et al.*, 2009). In response of upland cotton at different salinity concentrations, revealed significant differences among cotton genotypes with reduced vegetative growth and dry biomass with increased salinity (Basal, 2010). The decrease in shoot length and seedling dry weight was relatively low in salt tolerant genotypes than susceptible in upland cotton (Rajashekar, 2006), however, dry matter and stem diameter were significantly reduced by increased salinity concentrations in *G. hirsutum* L (Ibrahim *et al.*, 2007).

Fresh root weight: For fresh root weight, the genotype means expounded that cv. CIM-707 (1.06 g) revealed best performance in root development, followed by cvs. CIM-496, CIM-554 and SLH-284 with alike fresh root weight of 0.89, 0.86 and 0.81 g, respectively (Table 7). CIM-707 by having more vigorous root growth was found to be more tolerant to salinity in terms of fresh root weight. Genotypes CIM-506 and CIM-499 were having medium values (0.70 g; 0.58 g), respectively for fresh root weight over all salinity concentrations. Genotypes CIM-446 (0.49 g) and CIM-473 (0.52 g) revealed least and alike fresh root weight and were indicated as most sensitive to salinity in root growth. Means of salinity concentrations revealed that salinity concentration of 50 mM and control has highest and similar fresh root weight of 1.02 and 0.98 g, respectively (Table 7). It elucidated that low salinity concentration inspired the root growth and has no adverse effect on root development. Salinity concentrations of 100 and 150 mM followed above genotypes with medium fresh root weight of 0.82 and 0.52 g, respectively.

However, 200 mM of NaCl drastically reduced the genotypes fresh root weight (0.36 g).

In previous studies, increasing concentrations of salinity caused significant reduction in fresh root weight of cotton genotypes (Azhar and Ahmad, 2000). Higher salinity concentrations reduced the growth in upland cotton; however lower salinity concentrations promoted root development (Jafri and Ahmad, 1994). Both length and weight of the primary root were enhanced by moderate salinities; however, roots became thinner than control plants in upland cotton (Basal *et al.*, 2006).

Genotypes by salinity interactions illustrated that cvs. SLH-284 (0.68 g, 0.79 g) and CIM-707 (0.78 g, 0.52 g) with salinity concentration of 150 and 200 mM showed more than medium range of fresh root weight values, respectively (Table 7). In above genotypes, cv. CIM-707 showed more encouraging performance and even with 50 and 100 mM of salinity, the root growth was not affected and excelled other genotypes. CIM-707 better confirm its position for tolerance to salinity in fresh root weight. Genotypes CIM-473, CIM-446, CIM-499, CIM-506, CIM-554 and CIM-496 at salinity concentration of 200 mM were harshly suppressed in root development and their fresh root weight reduced to 0.21 to 0.33 g. Genotype CIM-496 and CIM-554 at 50 mM NaCl showed vital fresh root weight of 1.91 and 1.52 g followed by CIM-707 at control (1.60 g). Other combinations of various genotypes and salinity concentrations revealed medium values for fresh root weight.

Relative growth rate (RGR) of root and shoot was reduced to 24, 47 and 70% at salt concentrations of 75, 150 and 250 mol m⁻³, respectively as compared to control in upland cotton (Khan *et al.*, 2004). Response of upland cotton to NaCl salinity revealed that root growth was greatly disturbed with significant variations at 200 mol m⁻³ (Kent and Lauchli, 2006). Various cotton genotypes were examined with two NaCl concentrations (100 and 200 mol m⁻³) and reported that root and shoot fresh/dry weights were decreased with increased salinity concentrations and greatest reduction was observed at 200 mol m⁻³ NaCl (Ibrahim *et al.*, 2007; Basal, 2010). Saqib *et al.* (2002) screened five cotton genotypes with NaCl salinity @ 75, 150 and 225 mol m⁻³ and reported that rising concentrations of salinity (225 mol m⁻³) significantly reduced the root and shoot growth of cotton genotypes.

Dry root weight: Salinity concentrations and control brought significant variations in genotypes mean values for dry root weight (Table 8). However, highest dry root weight (0.37 g) was provided by cv. CIM-707 followed by genotypes SLH-284 and CIM-496 with values of 0.29 and 0.28 g, respectively. Genotype CIM-707 was found more tolerant to salinity by having best root growth.

Genotypes CIM-554, CIM-506 and CIM-473 exhibited medium values for dry root weight ranged from 0.24 to 0.27 g. However, genotypes CIM-446 and CIM-499 have shown least magnitude of dry root weight (0.18 and 0.22 g) and both genotypes were severely affected by salinity. Salinity concentrations and control means showed that on average, control having zero NaCl has maximum dry root weight (0.37 g) for genotypes followed by NaCl concentrations of 50 and 100 mM with dry root weight of 0.33 and 0.29 g, respectively (Table 8). However, 150 and 200 mM NaCl exhibited least values of 0.19 and 0.14 g for dry root weight.

Soil salinity is a dominated factor affecting cotton's root development and above-ground dry mass (Chen *et al.*, 2010). In upland cotton, dry root weight and yield components were highly affected by NaCl, however, the salt-tolerant genotypes yielded more than salt-sensitive genotypes (Ashraf and Ahmad, 2000). In study of root and shoot traits with salt stresses of 150 and 200 mM NaCl, observed significant differences among cotton genotypes about reduction in root and shoot dry weight with increasing salt concentrations (Basal *et al.*, 2006; Basal, 2010).

Genotypes and salinity levels interactions revealed that cv. CIM-707 (0.23 g) with 200 mM manifested medium dry root weight followed by SLH-284 (0.21 g) with same concentration of NaCl (Table 8). CIM-707 was found most tolerant genotype to salinity and excelled all other genotypes in dry root weight at highest salinity. Genotypes CIM-473, CIM-446, CIM-554, CIM-499, CIM-506 and CIM-496 were severely affected by NaCl concentration of 200 mM and their dry root weights dropped to range of 0.09 to 0.13 g and were found extra sensitive to salinity. In control, the genotype CIM-707 (0.64 g) obtained highest dry root weight followed by SLH-284 (0.43 g). These two combinations were followed by genotypes CIM-496, CIM-707 and CIM-554 at salinity concentration of 50 mM NaCl, and CIM-506 and CIM-554 at 100 mM NaCl ranged from 0.34 to 0.54 g. Other combinations of genotypes with salinity concentrations of 100 and 150 mM showed medium dry root weight.

Cotton growth was studied under various salinity stresses and observed significant reduction in root growth and plant dry weight (Akhtar *et al.*, 2005). In response of cotton genotypes of three species (*G. hirsutum* L., *G. tomentosum* L., *G. darwinii* L.) to NaCl concentrations (50, 75, 100, 125, 150, 175, 200 and 250 mM) revealed that root length and weight was increased in *G. hirsutum* L genotypes at low salinity concentration (Tiwari and Stewart, 2008). Salinity stress exhibited adverse effect on vegetative growth and significantly reduced root and shoot fresh/dry weights in cotton (Qadir and Shams, 1997).

Table 1. Mean squares of genotypes, NaCl salinity concentrations and their interaction for various growth variables in upland cotton.

Variables	Mean Squares				CV %
	Genotypes	Salinity levels	Interactions (C × S)	Error	
Stem diameter	0.007**	0.039**	0.002**	0.000238	5.79
Leaves plant	3.029**	42.529**	1.020**	0.400	8.99
Root length	56.495**	101.792**	11.311**	1.492	7.11
Fresh shoot weight	3.598**	39.021**	3.057**	0.122	9.85
Fresh root weight	0.602**	2.066**	0.242**	0.007375	11.59
Dry shoot weight	0.275**	2.367**	0.166**	0.001388	4.47
Dry root weight	0.045**	0.227**	0.021**	0.00045	8.04

** Significant at $p \leq 0.01$.**Table 2. Effect of NaCl salinity concentrations on stem diameter in upland cotton.**

Genotypes	NaCl Salinity Concentrations					Means (cm)
	Control	50 mM	100 mM	150 mM	200 mM	
CIM-446	0.37	0.33	0.30	0.25	0.23	0.30
CIM-473	0.32	0.30	0.23	0.19	0.16	0.24
CIM-496	0.33	0.34	0.28	0.25	0.23	0.29
CIM-499	0.32	0.29	0.24	0.21	0.23	0.26
CIM-506	0.29	0.27	0.27	0.25	0.19	0.26
CIM-554	0.25	0.26	0.28	0.20	0.20	0.24
CIM-707	0.34	0.30	0.25	0.25	0.28	0.28
SLH-284	0.31	0.25	0.24	0.25	0.25	0.26
Means (cm)	0.32	0.29	0.26	0.23	0.22	

LSD ($p \leq 0.05$) Genotypes = 0.011, Salinity Levels = 0.009, Genotypes × Salinity Levels = 0.025**Table 3. Effect of NaCl salinity concentrations on leaves per plant in upland cotton.**

Genotypes	NaCl Salinity Concentrations					Means
	Control	50 mM	100 mM	150 mM	200 mM	
CIM-446	8.33	8.67	7.00	7.67	6.33	7.60
CIM-473	7.67	7.00	6.00	4.67	5.33	6.13
CIM-496	9.00	8.00	5.67	6.67	6.00	7.07
CIM-499	8.67	8.00	6.67	6.00	5.00	6.87
CIM-506	9.33	7.33	7.00	6.67	4.67	7.00
CIM-554	8.67	9.33	8.00	6.00	5.67	7.53
CIM-707	8.67	8.67	6.33	6.67	5.00	7.07
SLH-284	8.67	8.33	6.33	6.33	5.33	7.00
Means	8.63	8.17	6.63	6.33	5.42	

LSD ($p \leq 0.05$) Genotypes = 0.460, Salinity Levels = 0.363, Genotypes × Salinity Levels = 1.028**Table 4. Effect of NaCl salinity concentrations on root length of upland cotton.**

Genotypes	NaCl Salinity Concentrations					Means (cm)
	Control	50 mM	100 mM	150 mM	200 mM	
CIM-446	18.00	13.67	14.00	12.67	13.33	14.33
CIM-473	20.33	15.67	14.00	11.67	11.00	14.53
CIM-496	18.00	23.00	16.00	14.00	11.67	16.13
CIM-499	18.00	21.67	19.00	18.33	15.00	18.40
CIM-506	21.33	20.00	21.33	15.00	15.33	18.60
CIM-554	18.00	18.33	18.67	14.33	17.33	17.33
CIM-707	22.00	20.67	16.00	16.67	20.00	19.07
SLH-284	20.67	18.67	20.67	18.33	16.33	18.93
Means (cm)	19.54	18.71	17.46	15.13	15.00	

LSD ($p \leq 0.05$) Genotypes = 0.888, Salinity Levels = 0.702, Genotypes × Salinity Levels = 1.985

Table 5. Effect of NaCl salinity concentrations on fresh shoot weight of upland cotton.

Genotypes	NaCl Salinity Concentrations					Means (g)
	Control	50 mM	100 mM	150 mM	200 mM	
CIM-446	4.62	4.02	3.63	2.74	1.82	3.37
CIM-473	4.96	4.10	1.84	2.67	1.28	2.97
CIM-496	4.02	7.77	3.84	2.53	2.29	4.09
CIM-499	2.59	4.65	3.46	2.42	1.63	2.95
CIM-506	4.70	3.55	4.21	2.93	1.88	3.46
CIM-554	3.02	6.26	3.96	2.47	1.67	3.48
CIM-707	7.29	5.49	3.63	3.01	2.19	4.32
SLH-284	5.75	3.47	4.43	2.92	2.24	3.76
Means (g)	4.62	4.91	3.62	2.71	1.88	

LSD ($p\%0.05$) Genotypes = 0.254, Salinity Levels = 0.201, Genotypes \times Salinity Levels = 0.568**Table 6. Effect of NaCl salinity concentrations on dry shoot weight of upland cotton.**

Genotypes	NaCl Salinity Concentrations					Means (g)
	Control	50 mM	100 mM	150 mM	200 mM	
CIM-446	1.16	1.00	0.82	0.60	0.38	0.79
CIM-473	1.29	0.86	0.54	0.56	0.34	0.72
CIM-496	1.19	1.76	0.84	0.56	0.49	0.97
CIM-499	0.66	1.06	0.75	0.52	0.34	0.67
CIM-506	1.19	0.80	0.95	0.68	0.48	0.82
CIM-554	0.74	1.28	0.92	0.60	0.37	0.78
CIM-707	2.00	1.36	0.81	0.59	0.69	1.09
SLH-284	1.20	0.78	0.96	0.71	0.52	0.83
Means (g)	1.18	1.11	0.82	0.60	0.45	

LSD ($p\%0.05$) Genotypes = 0.027, Salinity Levels = 0.021, Genotypes \times Salinity Levels = 0.060**Table 7. Effect of NaCl salinity concentrations on fresh root weight of upland cotton.**

Genotypes	NaCl Salinity Concentrations					Means (g)
	Control	50 mM	100 mM	150 mM	200 mM	
CIM-446	0.73	0.46	0.58	0.48	0.21	0.49
CIM-473	0.97	0.63	0.37	0.46	0.21	0.52
CIM-496	1.05	1.91	0.73	0.45	0.33	0.89
CIM-499	0.58	0.81	0.87	0.39	0.26	0.58
CIM-506	1.17	0.57	1.03	0.45	0.26	0.70
CIM-554	0.91	1.53	1.16	0.45	0.28	0.86
CIM-707	1.60	1.40	0.99	0.78	0.52	1.06
SLH-284	1.18	0.57	0.85	0.68	0.79	0.81
Means (g)	1.02	0.98	0.82	0.52	0.36	

LSD ($p\%0.05$) Genotypes = 0.062, Salinity Levels = 0.049, Genotypes \times Salinity Levels = 0.140**Table 8. Effect of NaCl salinity concentrations on dry root weight of upland cotton.**

Genotypes	NaCl Salinity Concentrations					Means (g)
	Control	50 mM	100 mM	150 mM	200 mM	
CIM-446	0.34	0.13	0.19	0.16	0.09	0.18
CIM-473	0.38	0.27	0.31	0.16	0.09	0.24
CIM-496	0.34	0.54	0.25	0.15	0.13	0.28
CIM-499	0.21	0.30	0.30	0.18	0.12	0.22
CIM-506	0.41	0.21	0.35	0.18	0.12	0.25
CIM-554	0.25	0.42	0.34	0.21	0.11	0.27
CIM-707	0.64	0.47	0.29	0.21	0.23	0.37
SLH-284	0.43	0.27	0.27	0.29	0.21	0.29
Means (g)	0.37	0.33	0.29	0.19	0.14	

LSD ($p\%0.05$) Genotypes = 0.015, Salinity Levels = 0.012, Genotypes \times Salinity Levels = 0.034

Conclusion: Genotypes, salinity concentrations, and genotype vs. salinity interactions were observed with highly significant differences for growth variables. Genotypes performance at 50 mM NaCl followed the control and revealed alike/increased growth over control because some of the growth traits were stimulated by low salinity. Growth variables were gradually decreased as the salinity concentration increased (50 > 100 > 150 > 200 mM), and significant decrease was observed for all the cotton genotypes at 200 mM NaCl. Absorption of K⁺ and Na⁺ ions were inversely proportional, and Na⁺ buildup was least in control and gradually increased with highest salinity concentrations. However, K⁺ accretion was highest in control and gradually decreased through increased salinity concentrations in cotton shoot and root tissues. Genotype CIM-707 was found the most tolerant to salinity and showed best performance for majority variables at highest salinity concentrations followed by CIM-446. Identification of salt tolerant cotton genotypes for salt affected areas can benefit the farming community through increased seed cotton yield.

REFERENCES

- Akhtar, J., and F.M. Azhar (2001). Response of *G. hirsutum* hybrids to NaCl salinity at seedling stage. *Int. J. Agric. Biol.* 6(5): 233-235.
- Akhtar, J., M.A. Haq, K. Ahmad, M. Saqib and M.A. Saeed (2005). Performance of cotton genotypes under saline conditions. *Caderno de Pesquisa. série Biologi.* 17(1): 29-36.
- Akhtar, J., T. Haq, A. Shahzad, M.A. Haq, M. Ibrahim and N. Ashraf (2003). Classification of different wheat genotypes in salt tolerance categories on the basis of biomass production. *Int. J. Agric. Biol.* 5(3): 322-325.
- Akhtar, J., Z.A. Saqib, M. Sarfraz, I. Saleem and M.A. Haq (2010). Evaluating salt tolerant cotton genotypes at different levels of NaCl stress in solution and soil culture. *Pakistan J. Bot.* 42(4): 2857-2866.
- Anonymous (2005). Global network on integrated soil management for sustainable use of salt affected soils. Rome, Italy: FAO Land and Plant Nutrition Management Service. Available online at <http://www.fao.org/ag/agl/agll/spush>.
- Ashraf, M. (2002). Salt tolerance of cotton: some new advances. *CRC Crit. Rev. Plant Sci.* 21(1): 1-30.
- Ashraf, M. and S. Ahmad (2000). Influence of sodium chloride on ion accumulation, yield components and fiber characteristics in salt-tolerant and salt-sensitive lines of cotton (*G. hirsutum* L.). *Field Crops Res.* 66 (2): 115-127.
- Azhar, F.M. and R. Ahmad (2000). Variation and heritability of salinity tolerance in upland cotton at early stage of plant development. *Pakistan J. Biol. Sci.* 3(12):1991-1993.
- Azhar, F.M., A.A. Khan and N. Saleem (2007). Genetic mechanisms controlling salt tolerance in *G. hirsutum* seedlings. *Pakistan J. Bot.* 39(1): 115-121.
- Basal, H. (2010). Response of cotton (*G. hirsutum* L.) genotypes to salt stress. *Pakistan J. Bot.* 42(1): 505-511.
- Basal, H., J.K. Hemphill and C.W. Smith (2006). Shoot and root characteristics of converted race stocks accessions of upland cotton (*G. hirsutum* L.) grown under salt stress conditions. *Am. J. Plant Path.* 1(1): 99-106.
- Chen, W., Z. Hou, L. Wu, Y. Liang and C. Wei (2010). Effects of salinity and nitrogen on cotton growth in arid environment. *Plant and Soil* 326(1-2): 61-73.
- Hemphill, J.K., H. Basal and C.W. Smith (2006). Screening method for salt tolerance in cotton. *Am. J. Plant Path.* 1(1): 107-112.
- Hoagland, D.R. and D.I. Arnon (1950). The water culture method for growing plants without soil. Circular 347, College of Agric., Univ. of California, USA.
- Hou, Z., W. Chen, X. Li, L. Xiu and L. Wu (2009). Effects of salinity and fertigation practice on cotton yield and 15N recovery. *Agric. Water Manag.* 96(10): 1483-1489.
- Ibrahim, M., J. Akhtar, M. Younis, M.A. Riaz, M.A.U. Haq and M. Tahir (2007). Selection of cotton (*G. hirsutum* L.) genotypes against NaCl stress. *Soil Environ.* 26(1): 59-63.
- Jabeen, R. and R. Ahmad (2009). Alleviation of the adverse effects of salt stress by foliar application of sodium antagonistic essential minerals on cotton (*G. hirsutum*). *Pakistan J. Bot.* 41(5): 2199-2208.
- Jafri, A.Z. and R. Ahmad (1994). Plant growth and ionic distribution in cotton (*G. hirsutum* L.) under saline environment. *Pakistan J. Bot.* 26(1): 105-114.
- Kent, L.M. and A. Lauchli (2006). Germination and seedling growth of cotton: salinity-calcium interactions. *Plant, Cell Environ.* 8(2): 155-159.
- Khan, A.N., R.H. Qureshi and N. Ahmad (2004). Salt tolerance of cotton cultivars in relation to relative growth rate in saline environments. *Int. J. Agric. Biol.* 6(5): 784-785.
- Leidi, F.O. and J.F. Saiz (1997). Is salinity tolerance related to Na⁺ accumulation in upland cotton (*G. hirsutum* L.) seedlings. *Plant and Soil* 190(1): 67-75.
- Maas, E.V. (1990). Crop salt tolerance. In: *Agricultural salinity assessment and management*. Tanji

- (Editor). Am. Soc. Civil Eng., New York. pp. 262-304.
- Munns, R. (2002). Comparative physiology of salt and water stress. *Plant Cell, Environ.* 25(2): 239-250.
- Pessaraki, M. (2001). Physiological responses of cotton (*G. hirsutum* L.) to Salt Stress. In: *Handbook of Plant and Crop Physiology*. 2nd Ed., Revised and Expanded (M. Pessaraki, Ed.). Marcel Dekker, Inc., New York, pp. 681-696.
- Qadir, M. and M. Shams (1997). Some agronomic and physiological aspects of salt tolerance in cotton (*G. hirsutum* L.). *J. Agron. Crop Sci.* 179: 101-106.
- Rajashekar R.B.H. (2006). Physiological and molecular studies of salinity tolerance in cotton (*G. herbaceum* L.). M.Sc Thesis, Univ. Agric. Sci. Dharwad, India.
- Saqib, M., J. Akhtar, S. Pervaiz, R.H. Qureshi and M. Aslam (2002). Comparative growth performance of five cotton (*G. hirsutum* L.) genotypes against different levels of salinity. *Pakistan J. Agric. Sci.* 39(2): 69-75.
- Steel R.G.D., J.H. Torrie and D.A. Dickey (1997). *Principles and Procedures of Statistics - A Biometrical Approach*, 3rd ed. McGraw-Hill Pub. Co. New York, USA.
- Tiwari, R.S. and J.M.D. Stewart (2008). Effect of salt on several genotypes of *Gossypium*. *Summaries of Arkansas Cotton Res.* 2008.
- Zhang, Y., Q. Li, X. Zhou, C. Zhai and R. Li (2006). Effects of partial replacement of potassium by sodium on cotton seedling development and yield. *J. Plant Nutr.* 29(10): 1845-1854.