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RESPONSE OF UPLAND COTTON GENOTYPES TO SALINITY AT EARLY GROWTH STAGES

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Abstract

Salt tolerance was studied in eight upland cotton cultivars (CIM-446, CIM-473, CIM-496, CIM-499, CIM-506, CIM-554, CIM-707 and SLH-284) under four NaCl salinity concentrations (50, 100, 150 and 200 mM) and control. Significant ($p \leq 0.01$) variations were observed among cultivars, salinity concentrations, and cultivar by salinity interactions for various growth traits and ions (K^+ and Na^+) accumulation in dry shoot and root tissues. As compared to control, the growth variables were decreased by increasing concentrations of NaCl; however, greatest reduction was observed at salinity stress of 200 mM. The uptake of K^+ and Na^+ were inversely proportional, and Na^+ accumulation was least in control and gradually amplified as the salinity increased. The K^+ absorption was highest in control and gradually decreased through increased salinity in shoot and root tissues. The foliage has more capacity for Na^+ accumulation than roots which ensured increased K^+ absorption in roots. The K^+/Na^+ ratio of various genotypes differed significantly at various NaCl concentrations. Cultivar CIM-707 by having medium K^+/Na^+ ratio, showed better K^+ utilization, and identified as most tolerant genotype to salinity. Such studies are useful in identification of salt tolerant cotton genotypes for salt affected areas, and farming community can benefit by getting optimum yield from cultivar CIM-707.

Keywords: *Gossypium hirsutum* L., growth variables, K^+ and Na^+ accumulation, K^+/Na^+ ratio, NaCl salinity, shoot and root tissues

Received: June, 2014; Revised final: January, 2015; Accepted: February, 2015; Published in final edited form: August, 2018

1. Introduction

Salinity is one of the major soil problems of arid and semi-arid regions of the world. In soils, the vast buildup of salts generate a stern reduction in the yield of various crops. Worldwide, over 800 million hectares of land are salt affected either by salinity (397 million ha) or sodicity (434 million ha). (Marklund and Batello, 2005). In Pakistan, about 6.30 million hectares of land are salt-affected, while 0.56 million hectares in province of Khyber Pakhtunkhwa. Since salinity limits the agriculture production (up to 40%) all over the world, and salt tolerant cultivars need to be improved to utilize saline soils to meet the food

demand of the world's increasing population (Holmberg and Bulow, 1998).

Past studies revealed that some species can abide high regimes of salinity with limited salt elimination and insertion (Hou et al., 2009; Pessarakli, 2001). The ion uptake and deposition in different parts of a plant is making a distinction between salt-tolerant and salt-sensitive genotypes. In general, salt tolerance has been positively correlated with ion exclusion in some crop species, e.g., wheat (Ashraf and O'Leary, 1996), sunflower (Ashraf and Tufail, 1995), legumes and *Brassica carinata* (Ashraf and Sharif, 1997). In contrast, a negative correlation between ion exclusion and salt tolerance has been reported in other crops,

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e.g., alfalfa, *Lupinus* spp., *Lolium perenne*, *Vigna* spp. and lentil. Past findings reported the effects and response mechanisms of salinity on plants (Cha-Um and Kirdmanee, 2009; Munns and James, 2003).

Soil salinity problems are of frequent occurrence in irrigated areas of the world and in Pakistan due to the presence of different salts species in the soil profile (Al-Zou'by et al., 2017; Malik and Shah, 1996). Salts presence near root zone does not allow the plants to flourish and this may be one of the reasons of low yield of different crops in Pakistan. Installation of tube wells and use of gypsum had proved to be effective in ameliorating the problems of salinity, but escalating costs of laborers and energy does not allow the continuous running of the projects to reclaim the soils. Therefore, the researches looking for natural salt tolerance in crop cultivars for successful growing under saline soil conditions. Previous studies showed that cultivars of rice, barley, maize and sorghum differed from each other for their response to salinity stress (Azhar and Khan, 1997).

Cotton (*Gossypium hirsutum* L.) is considered to be moderately tolerant to salinity (Bibi et al., 2016; Maas, 1990; Taghizadeh et al., 2018), ranked second behind barley (Tiwari and Stewart, 2008; Zhang et al., 2006). However, variation in salt-tolerance has been observed among different cotton cultivars (Leidi and Saiz, 1997). Under saline conditions the absolute/relative growth and yield is usually the ultimate goal. Biomass production at high salinity (up to 250 mM NaCl) has been proposed as a selection criterion for salt tolerant genotypes and high accumulation of Na⁺ in the leaves of salt-tolerant cultivars of cotton has also been found (Ali et al., 2009; Akhtar et al., 2005). In contrast, no clear correlation between salt tolerance and Na⁺ accumulation was found in cotton (Jafri and Ahmad, 1994). Cotton being major cash and industrial crop, and an important crop of the areas affected by salinity in Pakistan. Past studies conducted on this aspect of cotton plant revealed genotypic variability among upland cotton genotypes for salinity tolerance (Bibi et al., 2016; Nabi et al., 2011).

Plants are sensitive to salinity during germination and early seedling development. It is due to extreme spatial and temporal variability in soil salinity under field conditions that selection of large number of genotypes under saline field conditions is not feasible (Ibrahim et al., 2007). Therefore, the crop gene stocks are often screened/selected in nutrient solution by adding different amounts of salts to develop the desired salinity concentrations. This method is relatively quick and reliable for selecting the crop genotypes against salinity (Qureshi et al., 1990). Therefore, the present research was planned with the objectives i.e., a) to formulate the effect of different NaCl salinity concentrations on growth variables of upland cotton genotypes, and to screen out the best tolerant cultivars to salinity, b) to quantify the K⁺ and Na⁺ ions accumulations and their ratio K⁺/Na⁺ in dry shoot and root tissues of upland cotton genotypes during early seedling growth.

2. Material and methods

2.1. Plant material and procedure

The pot experiment was laid out in a complete randomized design (CRD) with three replications in net house during May-July, 2011 at The University of Agriculture, Peshawar, Pakistan. Eight newly bred upland cotton cultivars (CIM-446, CIM-473, CIM-496, CIM-499, CIM-506, CIM-554, CIM-707, SLH-284) were studied for salt tolerance through control and four salinity concentrations of NaCl (50 mM, 100 mM, 150 mM, 200 mM) for 40 days at early growth stage.

The coarse sand was completely washed (3-4 times) with tap water to make it free from all the nutrients and clay particles, collected from banks of River Kabul, Peshawar, Pakistan. The pots with required size were filled with dried sand. During last week of May, 2011, four seeds of each cotton cultivar were sown in pots. The germination was completed in one week and thinning was made to make sure a single plant per pot. The Half Strength Hoagland Solution (HSHS) was applied every day to fully saturate the pots with required volume containing no NaCl until the plants attained the first true-leaf stage (Table 1) (Hoagland and Arnon, 1950). After 14 days of planting, subsequently the true-leaf stage was achieved and then the cotton seedlings were subjected to salt stress (Table 2).

The salinity treatments beyond 50 mM were applied stepwise (starting from 50 mM and then increased to the desired concentration) to avoid osmotic distress (Bibi et al., 2016). An initial of 50 mM was raised to 100 mM on the second day and then onward to final 200 mM in HSHS. After 40 days of imposition of NaCl stress, the plants were uprooted and the following growth traits and ions intensity were determined.

2.2. Growth variables

The plant height was recorded in cm from the base to the tip of the plant in pot with the help of meter rod. Leaf area was calculated with the help of "Leaf Area Meter CI-202, CID Inc". The leaf blade of fully expanded three leaves was placed on the plate of Leaf Area Meter, scanned and the mean was recorded as leaf area in cm².

2.3. Ions concentration

The K⁺ and Na⁺ ions were determined according to wet digestion procedure following USDA HB 60 (Richard, 1954). The oven dried shoots and roots was crushed and dissolved separately in 20 mL of concentrated Nitric Acid in flasks.

The flasks were kept in hot sand tray (with continuous shaking) and cooked with help of gas burner. After drying the nitric acid and getting the white precipitation in flasks, 20 mL of 10% nitric acid was added and the solution was prepared in test tubes.

Table 1. Half Strength Hoagland solution

Stock	Compound	Stock g 500 ml ⁻¹	Volume in ml of stock for 10 liters of Half Strength Hoagland Solution
A	KH ₂ PO ₄	17 g	20 mL
	KNO ₃	53 g	
	MgSO ₄ .7H ₂ O	64 g	
B	Ca(NO ₃) ₂ .4H ₂ O	118 g	20 mL
C	H ₃ BO ₃	125 mg	10 mL
	ZnSO ₄ .3H ₂ O	12.5 mg	
	CuSO ₄ .3H ₂ O	5.0 mg	
	Na ₂ MoO ₄ .H ₂ O	14.5 mg	
	MnCl ₂ .4H ₂ O	125 mg	
D	Na ₂ EDTA	3.73 g	50 mL
	FeSO ₄ .7H ₂ O	2.78 g	

Table 2. Application methodology of various NaCl concentrations through Half Strength Hoagland Solution (HSHS)

Time of App.	T1	T2	T3	T4	T5	Remarks
	HSHS (Control)	HSHS + 50 mMNaCl	HSHS + 100 mMNaCl	HSHS + 150 mMNaCl	HSHS + 200 mMNaCl	
First day	Plain HSHS	50 mM	50 mM	50 mM	50 mM	The plants were acclimatized slowly to different saline environments.
2 nd day	-do-	-do-	100 mM	100 mM	100 mM	
3 rd day	-do-	-do-	-do-	150 mM	150 mM	
4 th day	-do-	-do-	-do-	-do-	200 mM	
5 th day	-do-	-do-	-do-	-do-	-do-	

Note: From 4th day, the same NaCl doses were continuously applied for crop life period.

For ions determination, 1% solution (one ml of 10% Nitric Acid solution + 99 mL of distilled water) was prepared and the ions K⁺ and Na⁺ were determined with the help of flame photometer. The ratio of K⁺/Na⁺ from the above values of K⁺ and Na⁺ in dry shoot/root tissues was also calculated.

2.4. Statistical analysis

Data were analyzed using completely randomized design with two factors (Steel et al., 1997). The means of the three groups (cultivars, salinity concentrations and their interactions) for each variable were further separated and compared by using the least significant difference (LSD) test at 5% level of probability.

3. Results

According to analysis of variance (Table 3), significant ($p \leq 0.01$) differences were observed among cultivars, salinity concentrations and cultivar vs. salinity interactions (C × S) for all the growth variables and accumulation of K⁺ and Na⁺ in dry shoot and root tissues.

3.1. Growth variables

3.1.1. Plant height

Plant height of various cultivars was significantly influenced by different concentrations of salinity. On average, tallest plants were observed in cultivar CIM-707 (24.93 cm) with different salinity concentrations and control, and the said cultivar was

found tolerant in term of plant height to salinity followed by SLH-284 (24.27 cm) and CIM-496 (23.53 cm) (Fig. 1). Least shoot length was noted in cultivar CIM-499 (20.73 cm) with all salinity concentrations and was recorded as most sensitive cultivar to salinity. Other four cultivars (CIM-446, CIM-554, CIM-606 and CIM-473) manifested medium plant height ranged from 22.98 to 23.20 cm. In case of salinity concentrations and control means, salinity concentrations decreased the shoot length gradually as the salt concentrations increased from 50 to 200 mM. Average maximum plant height (31.24 cm) was observed in control pots with zero NaCl followed by 50 mM NaCl (26.25 cm). Salinity concentrations of 100 and 150 mM showed nearly at par plant height of 21.63 cm and 19.58 cm, respectively. However, the salinity concentration of 200 mM (17.50 cm) had more adverse impact on plant height that resulted in stunted growth.

The interactions of cultivars and salinity concentrations were significant ($p \leq 0.01$) and salinity concentrations clearly affect and decreased shoot length of all cultivars with varying degrees (Fig. 1). However, the cultivar CIM-707 even at 200 mM confirmed superiority over the rest of cultivars and showed medium plant height (20.00 cm) and was found tolerant to salinity. Salinity concentration of 200 mM severely affected growth and reduced shoot length of other cultivars to least values (CIM-473, 15.33 cm; CIM-496, 16.00 cm; CIM-499, 16.67 cm). Cultivars CIM-496 (30.00 cm), CIM-554 (29.00 cm), CIM-707 (29.00 cm) and CIM-446 (27.67 cm) with salinity concentration of 50 mM followed the control for plant height.

Table 3. Mean squares for cultivars, salinity and their interaction for various traits in upland cotton

Variables	Mean Squares				CV %
	Cultivars	Salinity concentrations	Interactions (C × S)	Error	
Plant height	21.276**	654.717**	19.812**	0.733	3.69
Leaf area	101.728**	1492.726**	29.757**	0.780	4.33
K ⁺ in dry shoots	0.762**	14.198**	0.239**	0.046	7.77
K ⁺ in dry roots	4.726**	9.684**	0.472**	0.112	12.17
Na ⁺ in dry shoots	0.140**	2.884**	0.063**	0.004	11.36
Na ⁺ in dry roots	0.069**	0.877**	0.010**	0.001	8.02

** Significant at $p \leq 0.01$

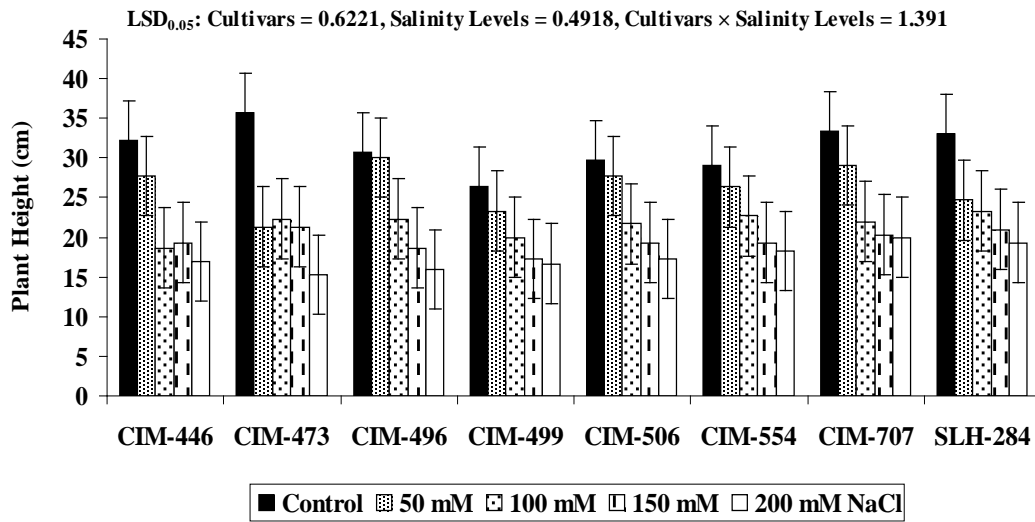


Fig. 1. NaCl salinity effect on plant height of upland cotton

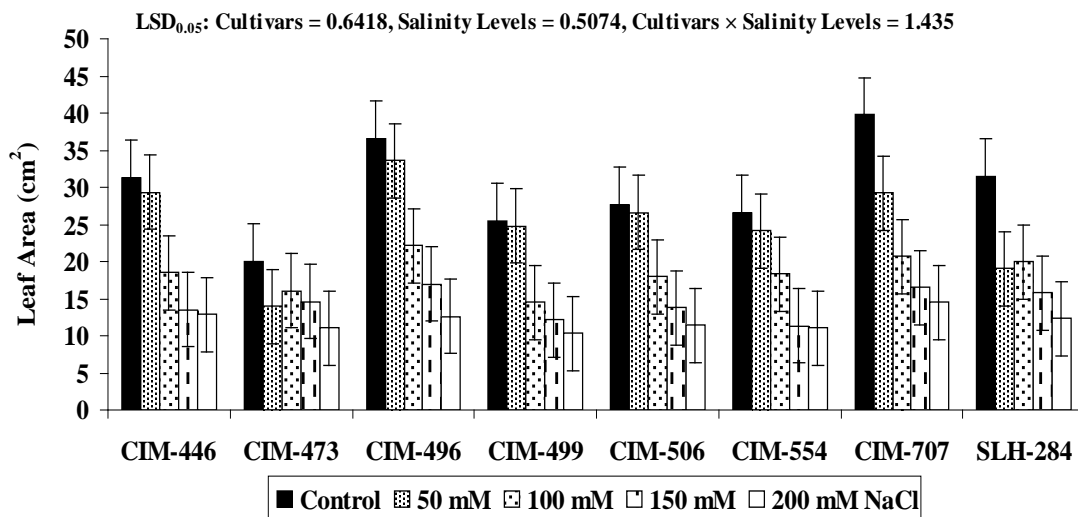


Fig. 2. NaCl salinity effect on leaf area of upland cotton

Salinity concentrations of 100 and 150 mM also affect the plant height and exhibited moderate values for shoot length. However, control pots revealed maximum plant height with cultivars CIM-473 (36.67 cm), CIM-707 (33.33 cm), CIM-446 (32.33 cm) and SLH-284 (31.00 cm).

3.1.2. Leaf area

Result pertaining to leaf area for cultivar means showed that salinity concentrations significantly

influenced the leaf area (Fig. 2). However, larger leaf area displayed by cultivars CIM-496 (24.38 cm²) and CIM-707 (24.12 cm²) was being significantly different than all other cultivars when averaged across salinity concentrations. These two cultivars were comparatively tolerant as larger leaf area means healthier plants with the capacity of producing more photosynthate resulting higher yield. Cultivars CIM-499, SLH-284, CIM-506 and CIM-554 were having medium leaf area ranged from 17.44 to 19.73 cm² with

all salinity concentrations. Smallest leaves with lowest values for leaf area were noticed in cultivar CIM-473 (15.74 cm²) at all salinity concentrations and found more sensitive to salinity at this stage. In salinity concentrations and control means, the salinity concentrations harshly affected the leaf area and condensed the leaves. The leaf area was decreased linearly with the increase in salinity concentrations as expected. Certainly bigger leaves were observed in control (29.87 cm²) followed by genotypes with NaCl concentration of 50 mM (25.08 cm²). Salinity concentrations of 100 and 150 mM reduced leaf area with values of 18.53 and 14.32 cm², respectively. However, 200 mM showed extreme decrease in average leaf area (12.01 cm²) for all the genotypes.

The interactions of cultivars and salinity concentrations (Fig. 2) manifested that cultivar CIM-707 with salinity concentration of 200 mM showed maximum leaf area (14.50 cm²) than other cultivars. However, CIM-496 with NaCl concentrations of 50 (33.63 cm²), 100 (22.18 cm²) and 150 (16.94 cm²) mM excelled all other genotypes. Cultivar CIM-707 and CIM-496 were found healthy and more tolerant to salinity as compared to other cultivars. However, cultivars CIM-499, CIM-554, CIM-473, CIM-506 and SLH-284 at 200 mM NaCl revealed least leaf area ranging from 10.31 to 12.33 cm² and were assumed as most sensitive to salinity. Cultivars CIM-707 (39.79 cm²) and CIM-496 (36.59 cm²) with control also exhibited maximum leaf area. Other cultivars with salinity concentrations of 100 and 150 mM claimed the medium leaf area.

3.2. Ions concentration

3.2.1. Potassium (K⁺) absorption in dry shoot

The cultivar means about ionic concentrations of K⁺ in dry shoot revealed that paramount concentration of K⁺ was noticed in cv. CIM-707 (3.00

g 100 g⁻¹) followed by cultivars CIM-496, CIM-499 and CIM-506 with K⁺ accumulation of 2.99, 2.89 and 2.84 g 100 g⁻¹, respectively (Fig. 3). Cultivar CIM-707 was healthier by accumulation of more K⁺ at various salinity concentrations and was found more tolerant to salinity. Cultivars SLH-284 and CIM-446 showed medium K⁺ absorption with values of 2.77 and 2.65 g 100 g⁻¹, respectively. Least and at par K⁺ absorption values of 2.41 and 2.46 g 100 g⁻¹ were recorded in genotypes CIM-554 and CIM-473, respectively. In salinity levels and control means, control showed maximum K⁺ concentration of 3.82 g 100 g⁻¹ in dry shoot, followed by salinity concentrations of 50 and 100 mM with K⁺ values of 3.13 and 2.69 g 100 g⁻¹, respectively. However, the K⁺ ion concentrations were gradually decreased in dry shoot tissues as the salinity concentration increased i.e., 2.32 g 100 g⁻¹ with 150 mM > 1.81 g 100 g⁻¹ with 200 mM. Results revealed that by increasing salinity concentrations, the toxic ions Na⁺ accumulation was increased which inhibits and reduced K⁺ accumulation in shoot tissues.

Means over cultivars and salinity concentrations (Fig. 3) revealed that cultivar CIM-707 with NaCl concentrations of 100, 150 and 200 mM revealed maximum values (3.02, 2.81, 2.04 g 100 g⁻¹) for K⁺ accumulation and was found more tolerant cultivar as compared to other genotypes at those salinity concentrations. In control, the K⁺ concentration was utmost in CIM-496 (4.28 g 100 g⁻¹) followed by cultivars CIM-446, CIM-506, CIM-499, CIM-707 and CIM-473 ranged from 3.77 to 4.10 K⁺ g 100 g⁻¹. The K⁺ was significantly reduced in cultivars through increased salinity concentration of 200 mM and least K⁺ concentration was found in CIM-554 (1.35 g 100 g⁻¹) followed by five other genotypes ranging from 1.74 to 1.94 g 100 g⁻¹. Other interactions of cultivars and salinity concentrations (100 and 150 mM) showed medium concentration of K⁺ accumulation in dry shoot tissues.

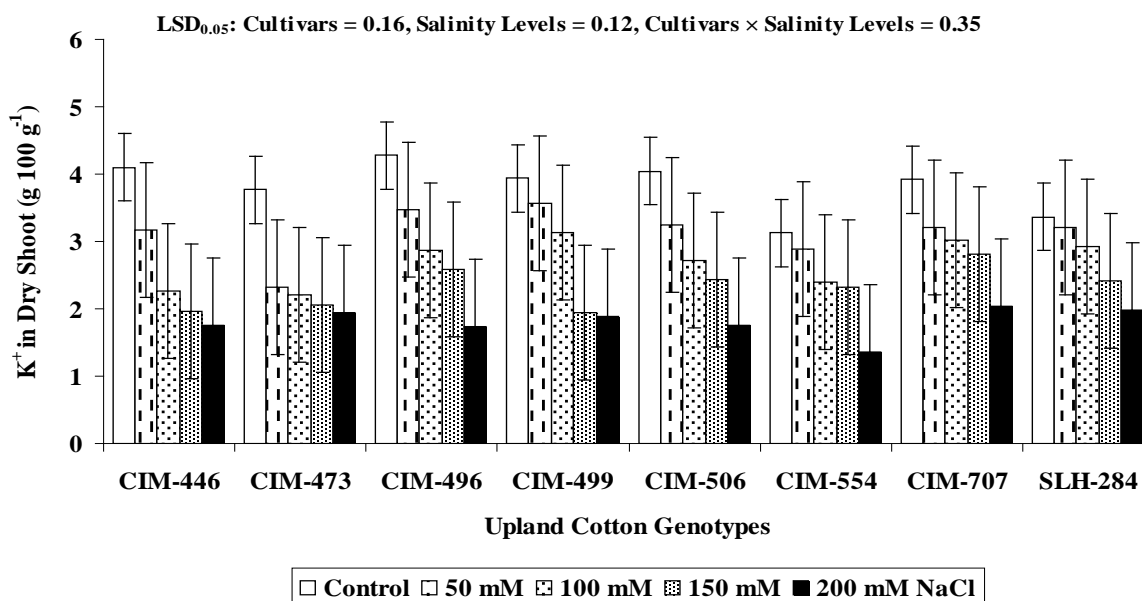


Fig. 3. NaCl salinity effect on K⁺ accumulation in dry shoot tissues of upland cotton

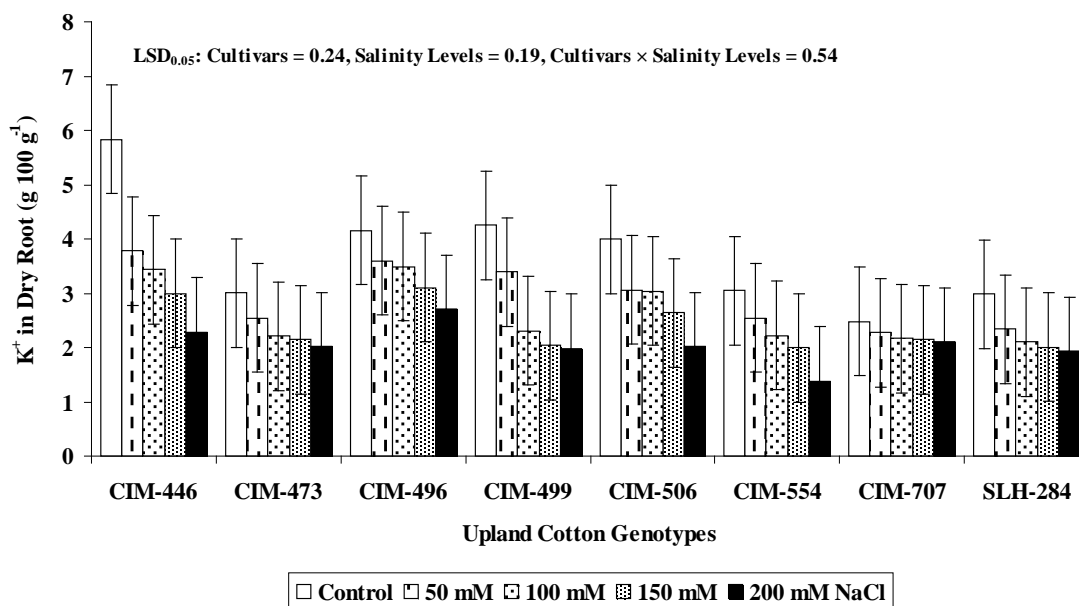


Fig. 4. NaCl salinity effect on K⁺ accumulation in dry root tissues of upland cotton

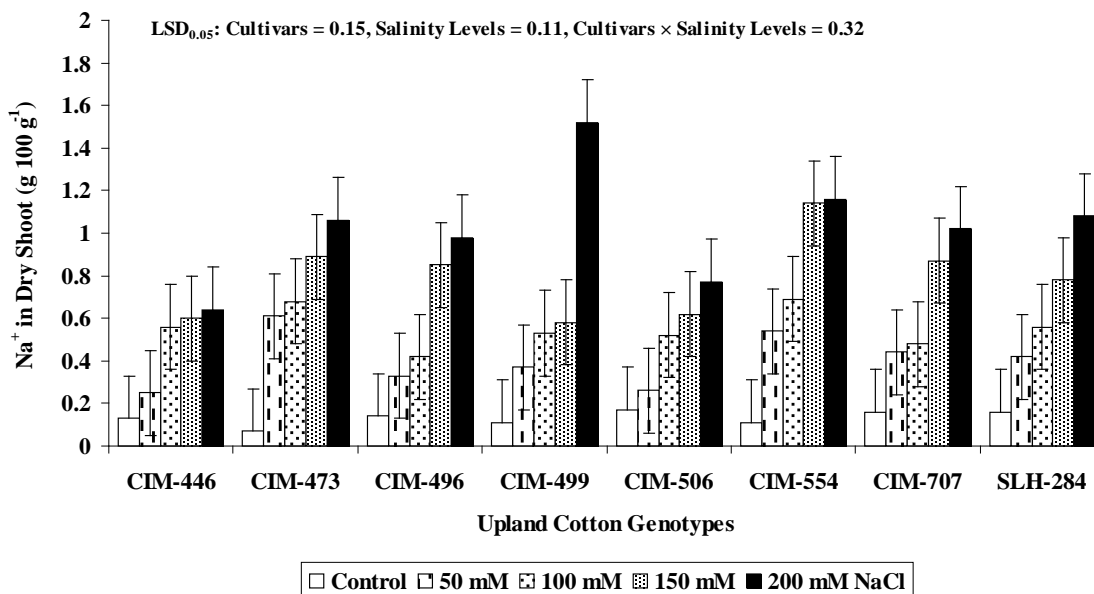


Fig. 5. NaCl salinity effect on Na⁺ accumulation in dry shoot tissues of upland cotton

3.2.2. Potassium (K⁺) absorption in dry root

On average in cultivar means (Fig. 4), the K⁺ accumulation was highest in genotype CIM-446 (3.67 g 100 g⁻¹) in dry root tissues. However, it was followed by three genotypes viz; CIM-496, CIM-506 and CIM-499 with K⁺ concentration of 3.41, 2.95 and 2.79 g 100 g⁻¹, respectively. Genotypes CIM-707, CIM-554, SLH-284 and CIM-473 showed least and at par K⁺ concentration (2.23 to 2.38 g 100 g⁻¹) in dry roots. However, cv. CIM-707 was promising cultivar for having maximum concentration of K⁺ ion in shoot tissues but came to bottom for K⁺ accumulation in roots in all genotypes. In salinity concentrations and control means for dry root, the K⁺ concentration was certainly highest at control (3.72 g 100 g⁻¹) and was gradually decreased as the salinity increased.

The K⁺ values of 2.94, 2.62 and 2.38 g 100 g⁻¹ were obtained at NaCl concentrations of 50, 100 and

150 mM, respectively for all cultivars. The lowest K⁺ (2.05 g 100 g⁻¹) accumulation was recorded at 200 mM NaCl for all cultivars in dry root tissues which might be due to inhibitory effect of Na⁺.

Average over interactions of cultivars and salinity concentrations (Fig. 4), cultivar CIM-496, CIM-446 and CIM-707 at 200 mM revealed maximum K⁺ concentration (2.70, 2.28, 2.10 g 100 g⁻¹) in dry root tissues. Other genotypes revealed least values for K⁺ accumulation at 200 mM ranging from 1.38 to 2.02 g 100 g⁻¹ in root tissues. CIM-496 obtained highest values for K⁺ concentration at NaCl concentrations of 100 and 150 mM with values of 3.49 and 3.10 g 100 g⁻¹, respectively than other cultivars. CIM-446 showed maximum K⁺ concentration at control (5.83 g 100 g⁻¹) followed by CIM-499, CIM-496 and CIM-506 at control (3.99 to 4.25 g 100 g⁻¹). Lowest K⁺ concentration (1.38 g 100 g⁻¹) was noted in cultivar

CIM-554 at 200 mM, followed by SLH-284 and CIM-499 at 200 mM with K^+ values of 1.93 to 1.98 g 100 g⁻¹, respectively. Other formulations of cultivars and various salinity concentrations showed medium absorption of K^+ in dry root tissues.

3.2.3. Sodium (Na^+) absorption in dry shoot

In cultivar means (Fig. 5), lowest absorption of toxic ion Na^+ was exhibited by cv. CIM-446 (0.44 g 100 g⁻¹) followed by CIM-506 (0.47 g 100 g⁻¹) in dry shoot tissues. These two cultivars were found more tolerant to salinity and desirable by storing minimum Na^+ even with high salinity environment. Genotypes CIM-707, SLH-284 and CIM-496 showed medium values of Na^+ concentration (0.54 to 0.60 g 100 g⁻¹) at all salinity concentrations. However, maximum concentration of said toxic ion was observed in cv. CIM-554 (0.73 g 100 g⁻¹) followed by CIM-473 and CIM-499 (0.66 and 0.62 g 100 g⁻¹) at all salinity concentrations in dry shoot tissues. Salinity concentrations and control means revealed that control owned least value of Na^+ (0.13 g 100 g⁻¹) followed by increasing order of Na^+ ion at NaCl levels of 50 (0.40 g 100 g⁻¹) and 100 mM (0.56 g 100 g⁻¹). Due to osmotic pressure, the NaCl concentrations of 150 and 200 mM showed maximum accumulation of Na^+ i.e., 0.79 and 1.03 g 100 g⁻¹, respectively in dry shoot for all the cultivars. Results revealed that as the salinity concentration increased the Na^+ accumulation was also increased.

Means over interactions of cultivars and salinity concentrations (Fig. 5) exhibited that minimum Na^+ accumulation was noticed in cultivar CIM-446 (0.64 g 100 g⁻¹) followed by CIM-506 (0.77 g 100 g⁻¹) at salinity concentration of 200 mM in dry shoot tissues. The same two genotypes also showed lowest restoration of said toxic ion at NaCl concentration of 150 mM with values of 0.60 and 0.62 g 100 g⁻¹. However, other six cultivars revealed maximum Na^+ accumulation i.e., CIM-499 (1.52 g 100 g⁻¹), CIM-554 (1.16 g 100 g⁻¹), SLH-284 (1.08 g 100 g⁻¹), CIM-473 (1.06 g 100 g⁻¹), CIM-707 (1.03 g 100 g⁻¹) and CIM-496 (0.98 g 100 g⁻¹) at salinity concentration of 200 mM in dry shoot tissues. All the cultivars at control showed least and at par concentration of toxic ion Na^+ ranged from 0.07 to 0.17 g 100 g⁻¹ in dry shoot tissues. Other cultivars in association with different salinity concentrations showed medium accumulation of Na^+ in dry shoot tissues.

3.2.4. Sodium (Na^+) absorption in dry root

Cultivar means revealed that least absorption of toxic ion Na^+ was observed in cultivar CIM-496 (0.35 g 100 g⁻¹) followed by cultivars CIM-499, CIM-554, CIM-473 and CIM-506 ranging from 0.38 to 0.45 g 100 g⁻¹ (Fig. 6). Cultivar CIM-496 was found desirable by storing less Na^+ ion in their root tissues even with high salinity concentrations. Maximum absorption of the said toxic ion was observed in cv. CIM-446 (0.54 g 100 g⁻¹) followed by cultivars SLH-284 and CIM-707 (0.50 g Na^+ 100 g⁻¹) at all salinity

concentrations. Salinity levels and control means exhibited that in control, the Na^+ value was least (0.20 g 100 g⁻¹) followed by 0.34 g 100 g⁻¹ (50 mM) < 0.41 g 100 g⁻¹ (100 mM) for all cultivars. Maximum accumulation of Na^+ was observed with salinity concentration of 200 mM (0.70 g 100 g⁻¹) followed by 150 mM (0.54 g 100 g⁻¹) in root tissues of cultivars.

The interactions of cultivars and salinity concentrations (Fig. 6) also confirmed that CIM-496 at NaCl concentration of 200 mM showed lowest Na^+ accumulation (0.50 g 100 g⁻¹) in roots, as compared to other genotypes with same highest NaCl level. The CIM-496 along with CIM-473 at NaCl levels of 50 and 100 mM revealed lowest values for Na^+ concentration (0.29, 0.33 g 100 g⁻¹) followed the control treatments. Maximum Na^+ concentration was observed in root tissues of CIM-707 (0.87 g 100 g⁻¹) at salinity concentration of 200 mM followed by six other genotypes with range of 0.61 to 0.77 g 100 g⁻¹ with same highest salinity concentration. Certainly the least accumulation of toxic ion Na^+ was also recorded in cv. CIM-496 (0.12 g 100 g⁻¹) followed by seven other cultivars in control ranged from 0.13 to 0.27 g 100 g⁻¹. Other combinations of cultivars and salinity concentrations showed medium Na^+ concentration in dry root tissues.

3.2.5. K^+/Na^+ ratio

The K^+/Na^+ ratios ranged from 2.53 to 4.68 and 3.68 to 7.96 in shoot and root tissues among six genotypes, respectively (Table 4). In shoot tissues, the maximum K^+/Na^+ ratio was observed in genotypes CIM-506 (4.68) and CIM-446 (4.47), while least K^+/Na^+ was observed in CIM-554 (2.53) and CIM-473 (2.63). Other four genotypes (CIM-496, CIM-499, CIM-707 and SLH-284) have sodium range of K^+/Na^+ ratios (3.51 to 4.14), respectively in shoot tissues.

In case of root tissues, the maximum K^+/Na^+ ratio was observed in genotypes CIM-496 (7.96) and CIM-499 (5.72), while least K^+/Na^+ was observed in SLH-284 (3.68) and CIM-707 (3.82). Other four genotypes (CIM-446, CIM-473, CIM-506 and CIM-554) have medium range of K^+/Na^+ ratios (4.68 to 5.33), respectively in root tissues.

The K^+/Na^+ was also calculated through control and various concentrations of NaCl in dry shoot and root tissues of upland cotton genotypes (Table 5). In dry shoot tissues of different cultivars, the K^+/Na^+ ratios for various treatments were ranging for control (21.00 to 53.86), 50 mM (3.80 to 12.68), 100 mM (3.24 to 6.81), 150 mM (2.04 to 3.94) and 200 mM NaCl (1.16 to 2.75).

However, in root tissues in control and salinity treatments, the K^+/Na^+ ratio ranges were 11.27 to 34.67, 6.14 to 12.41, 4.12 to 10.58, 2.83 to 6.20 and 2.12 to 5.40, respectively. Overall, the highest K^+/Na^+ ratios were revealed CIM-496 and CIM-499 among control and various salinity treatments (Tables 4 and 5). Cultivar CIM-707 revealed medium and least ratio K^+/Na^+ ratios in shoot and root tissues.

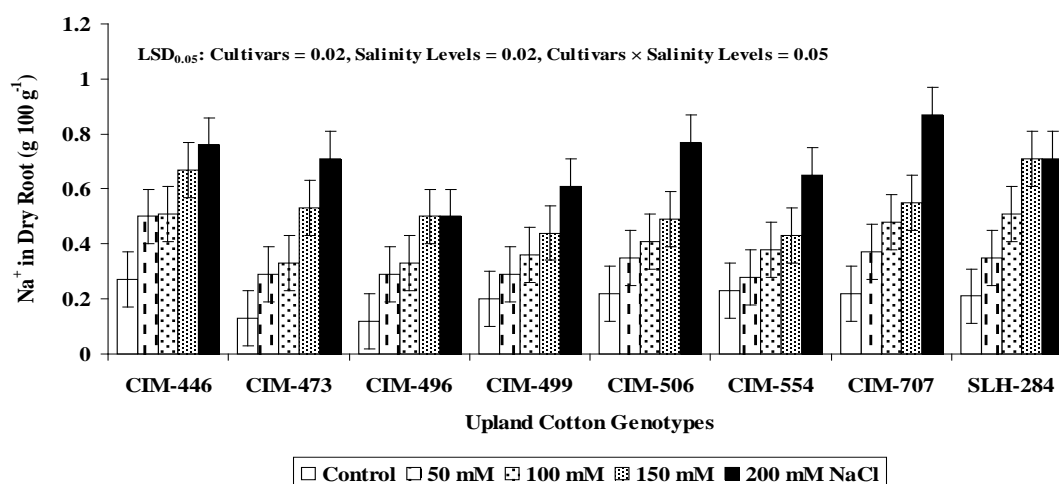


Fig. 6. NaCl salinity effect on Na⁺ accumulation in dry root tissues of upland cotton

Table 4. NaCl salinity effect on K⁺/Na⁺ ratio in shoot and root tissues of upland cotton

Cultivars	Shoot tissues			Root tissues		
	K ⁺	Na ⁺	K ⁺ /Na ⁺	K ⁺	Na ⁺	K ⁺ /Na ⁺
CIM-446	2.29	0.51	4.47	3.13	0.61	5.12
CIM-473	2.13	0.81	2.63	2.23	0.47	4.79
CIM-496	2.67	0.65	4.14	3.22	0.41	7.96
CIM-499	2.63	0.75	3.51	2.43	0.43	5.72
CIM-506	2.54	0.54	4.68	2.69	0.51	5.33
CIM-554	2.24	0.88	2.53	2.04	0.44	4.68
CIM-707	2.77	0.70	3.94	2.17	0.57	3.82
SLH-284	2.63	0.71	3.71	2.10	0.57	3.68

However, cultivar CIM-707 at 200 and 150 mM NaCl also showed best performance and was found as most tolerant genotype as compared to other cultivars having least K⁺/Na⁺ ratio at highest salinity. Some genotypes used K more efficiently than others and CIM-707 better utilized the K⁺ ion and revealed best performance under saline conditions. Cultivars had higher K⁺/Na⁺ ratios might be due to osmotic pressure, but those genotypes not showed best performance which may be due unbalanced values of K⁺ and Na⁺ and due to which K⁺ was not better utilized by cotton plants because the higher Na⁺ ratio also upset the plants nutrition.

The uptake of K⁺ and Na⁺ were inversely proportional. The toxic ion Na⁺ accumulation was least in control and was gradually increased as salinity increased, and highest uptake was observed at NaCl concentration of 200 mM in both shoot and root tissues (Tables 4 and 5). However, K⁺ absorption was in reverse to Na⁺ and highest K⁺ uptake was noted in control (having zero NaCl) with no inhibition power and was gradually decreased through increased salinity concentrations (50, 100, 150 and 200 mM NaCl), and least K⁺ uptake was recorded in NaCl concentration of 200 mM for both shoot and root tissues. The Na⁺ uptake itself in shoot and root was also varied with increased quantity in shoots as compared to roots (Tables 4 and 5). It means that foliage (leaves and shoots) store more Na⁺ and has

more capacity for said toxic ion as compared to roots. Therefore, the K⁺ absorption was also more in roots as compared to shoots and this is due to less accumulation of Na⁺ in roots than shoots. Results further enunciated that by comparing grand mean values (overall genotypes and salinity concentrations including control), the K⁺ absorption was five times greater than Na⁺ accumulation in both shoot and root tissues which generate the K⁺:Na⁺ ratio of 5:1.

4. Discussions

The physical growth of plant such as shoot and root length, leaf area, leaves per plant, and ions concentrations (K⁺, Na⁺) in shoot and root tissues contribute more towards salt tolerance of crop at early growth stages and can be used as selection criteria for salt tolerance (Bibi et al., 2016; Qureshi et al., 1990). Different concentrations of salinity significantly influenced plant height of various cultivars and decreased the shoot length gradually as the salt concentrations increased from 50 to 200 mM.

The increased NaCl concentrations revealed significant reduction in shoot length in cotton cultivars (Jafri and Ahmad, 1994; Akhtar and Azhar, 2001; Wang et al., 2017). The control pots (with zero NaCl) were followed by 50 mM NaCl, however, the 200 mM NaCl had more adverse impact on plant height that resulted in stunted growth.

Table 5. NaCl salinity various concentrations and control effect on K⁺/Na⁺ ratio in dry shoot and root tissues of upland cotton

Cultivars	Control			50 mM NaCl			100 mM NaCl			150 mM NaCl			200 mM NaCl		
	K ⁺	Na ⁺	K ⁺ /Na ⁺	K ⁺	Na ⁺	K ⁺ /Na ⁺	K ⁺	Na ⁺	K ⁺ /Na ⁺	K ⁺	Na ⁺	K ⁺ /Na ⁺	K ⁺	Na ⁺	K ⁺ /Na ⁺
K⁺/Na⁺ ratio in dry shoot															
CIM-446	4.10	0.13	31.54	3.17	0.25	12.68	2.27	0.56	4.05	1.96	0.60	3.27	1.76	0.64	2.75
CIM-473	3.77	0.07	53.86	2.32	0.61	3.80	2.20	0.68	3.24	2.05	0.89	2.30	1.94	1.06	1.83
CIM-496	4.28	0.14	30.57	3.48	0.33	10.55	2.86	0.42	6.81	2.59	0.85	3.05	1.74	0.98	1.78
CIM-499	3.94	0.11	35.82	3.57	0.37	9.65	3.13	0.53	5.91	1.95	0.58	3.36	1.88	1.52	1.24
CIM-506	4.04	0.17	23.76	3.25	0.26	12.50	2.71	0.52	5.21	2.44	0.62	3.94	1.75	0.77	2.27
CIM-554	3.13	0.11	28.45	2.88	0.54	5.33	2.39	0.69	3.46	2.32	1.14	2.04	1.35	1.16	1.16
CIM-707	3.92	0.16	24.50	3.20	0.44	7.27	3.02	0.48	6.29	2.81	0.87	3.23	2.04	1.02	2.00
SLH-284	3.36	0.16	21.00	3.20	0.42	7.62	2.92	0.56	5.21	2.42	0.78	3.10	1.99	1.08	1.84
K⁺/Na⁺ ratio in dry root															
CIM-446	5.83	0.27	21.59	3.78	0.5	7.56	3.44	0.51	6.75	3	0.67	4.48	2.28	0.76	3.00
CIM-473	3.01	0.13	23.15	2.54	0.29	8.76	2.21	0.33	6.70	2.14	0.53	4.04	2.02	0.71	2.85
CIM-496	4.16	0.12	34.67	3.6	0.29	12.41	3.49	0.33	10.58	3.1	0.5	6.20	2.7	0.5	5.40
CIM-499	4.25	0.2	21.25	3.39	0.29	11.69	2.31	0.36	6.42	2.04	0.44	4.64	1.98	0.61	3.25
CIM-506	3.99	0.22	18.14	3.06	0.35	8.74	3.04	0.41	7.41	2.64	0.49	5.39	2.02	0.77	2.62
CIM-554	3.05	0.23	13.26	2.54	0.28	9.07	2.22	0.38	5.84	2	0.43	4.65	1.38	0.65	2.12
CIM-707	2.48	0.22	11.27	2.27	0.37	6.14	2.17	0.48	4.52	2.14	0.55	3.89	2.1	0.87	2.41
SLH-284	2.98	0.21	14.19	2.34	0.35	6.69	2.1	0.51	4.12	2.01	0.71	2.83	1.93	0.71	2.72

The growth and seed cotton yield were severely reduced at high salinity and affected the cotton growth to a variable extent (Ashraf, 2002). Salinity level of EC 15 dS m⁻¹ significantly reduced the shoot length in upland cotton (Akhtar et al., 2005). On average, the tallest plants were observed in cultivar CIM-707 with all salinity concentrations, and was found tolerant genotype in term of plant height to salinity followed by SLH-284 and CIM-496. At highest salinity (200 mM), the CIM-707 confirmed superiority over the rest of cultivars which were severely affected. Different salt concentrations have significantly influenced cotton cultivars, and the shoot length was specifically reduced with increased concentration of salts (Basal et al., 2006; Hou et al., 2009; Basal, 2010). Similarly, the cotton species showed reduced growth through increased salinity levels (Chen et al., 2010; Tiwari and Stewart, 2008). In another study, the application of NaCl over a range of 100-1000 mM resulted in 0% survival (Sattar et al., 2010).

Salinity concentrations harshly influenced the leaf area and condensed the leaves. The leaf area was decreased linearly with the increase in salinity levels as expected. The interactions of cultivars and salinity concentrations showed that cultivar CIM-707 with salinity concentration of 200 mM showed maximum leaf area as compared to other cultivars. However, CIM-496 with NaCl levels of 50, 100 and 150 mM, excelled all other genotypes. Cultivar CIM-707 followed by CIM-496 were found healthy and more tolerant to salinity than other cultivars. Significant effects of salinity on growth and stomatal conductance of *G. hirsutum* were observed and the plant growth and leaf area development were drastically reduced by salinity (Brugnoli and Lauteri, 1991). The cotton growth under continuous salinity stress revealed reduced leaf area due to various salinity levels (Brugnoli and Bjorkman, 1992; Hou et al., 2009). However, in present studies, the tolerant cultivars (CIM-496, CIM-707) revealed larger leaf area means healthier plants with the capacity of producing more photosynthate resulting higher yield. Certainly, larger leaves were observed in control plants followed by genotypes with least concentration of NaCl (50 mM). Tolerant genotypes had higher leaf area and dry matter accumulation as compared to susceptible cultivars in cotton (Leidi and Saiz, 1997). Salinity stress exhibited deleterious effect on vegetative growth with decreased leaf area and significant differences among cotton genotypes (Qadir and Shams, 1997; Tiwari and Stewart, 2008).

Present studies revealed that paramount concentration of K⁺ and medium values for Na⁺ were noticed in cv. CIM-707 in dry shoot tissues. In interactions at 200 mM of NaCl, CIM-707 also accumulates more K⁺ than other cultivars. However, other genotypes at same highest concentration of NaCl did not show salt tolerance by accumulating less K⁺ and maximum Na⁺. All the genotypes in control pots showed maximum concentration of K⁺ due to having no inhibition effect of Na⁺ in both shoot and root

tissues. Cultivar CIM-707 was in good health by accumulation of more K⁺ at various salinity concentrations and found more tolerant to salinity. The cotton genotypes exhibited that salinity (EC 15 dS m⁻¹) significantly decreased K⁺ concentration and K⁺/Na⁺ ratio (Akhtar et al., 2005), however, Na⁺ concentration was amplified with increased salinity. Ion accumulation of K⁺ and Na⁺ and physiological responses in cotton genotypes to salt stress were illuminated (Leidi and Saiz, 1997), and the tolerant genotype with higher accumulation of Na⁺ in leaves had an apparent capacity for K⁺ redistribution to younger leaves.

Cultivars CIM-446 and CIM-496 showed maximum accumulation of K⁺, however, at 200 mM of NaCl, the CIM-707 was alike with CIM-446 for K⁺ accumulation in root tissues. The cultivars CIM-446, CIM-707 and CIM-496 also showed maximum Na⁺ accumulation in root tissues. Other cultivars with same concentration of salt, not stored the desired level of K⁺ which may due to their susceptibility to salt. In highest NaCl concentrations, the salt-tolerant genotypes had higher concentrations of K⁺, and K⁺/Na⁺ ratios in leaves than those of the salt-sensitive cultivars (Ashraf and Ahmad, 2000). The K⁺:Na⁺ ratio of cotton genotypes differed significantly at 100 and 200 mol m⁻³ NaCl and revealed significant differences among cotton genotypes regarding growth and salinity tolerance (Ibrahim et al., 2007). The K⁺ and Ca²⁺ contents were reduced in both roots and shoots by the NaCl treatments, however, the Na⁺ contents were not affected by the supplemental Ca²⁺ (Kent and Lauchli, 2006). Higher concentrations of Na⁺ and Cl⁻ ions inhibit the cotton plant nutrition, and use of K significantly increased the cotton yield in saline (13%) and non-saline (6%) soils (Keshavarz et al., 2004).

Present findings revealed that uptake of K⁺ and Na⁺ were inversely proportional. The accumulation of toxic ion Na⁺ was least in control and its ratio was gradually augmented as the salinity increased. However, K⁺ assimilation was highest in control and gradually decreased through amplified salinity in shoot and root tissues. The increased salinity also enhanced the net ionic uptake with more affinity to K⁺ and K⁺/Na⁺ ratio > one which was greater than one in all cotton cultivars (Jafri and Ahmad, 1994). Leaf sap of upland cotton showed increased Na⁺ and Cl⁻ concentrations and decreased K⁺ concentration with increased salinity (Qadir and Shams, 1997; Chen et al., 2010). Lower K/Na ratio (1:9) was of beneficial effects on most agronomic traits with increased K⁺ to certain extent; and could be useful in irrigating cotton plant with high salinity water (Abd-Elia and Shalaby, 1993). The foliage has more capacity for Na⁺ accumulation than roots which revealed more K⁺ absorption in roots. The concentration of Na⁺, Cl⁻ and Ca²⁺ in plant tissues increased with salinity and highest absorption were observed in the cotton leaves than other plant parts including roots (Chen et al., 2010). Salinity effect on K concentration revealed that high concentrations of Na⁺ and Cl⁻ ions upset the

cotton plant nutrition (Keshavarz et al., 2004; Wang et al., 2017).

Results revealed that salt-tolerant and salt-sensitive genotypes were significantly differed in ions (K^+/Na^+) uptake which effect their performance in terms of various growth variables. However, salt tolerant genotypes better utilized the K^+ even in presence of toxic ion Na^+ . The leaf/root Na^+ ratios did not differ and showed no relationship with crop salt tolerance (Ashraf and Ahmad, 2000), however, accumulation of Cl^- , K^+ and Ca^{2+} in leaves has positive association with salt tolerance of cotton. Maintenance of high K/Na and Ca/Na ratios were suggested to be an important selection criteria for salt tolerance in cotton (Ashraf, 2002).

Results further enunciated that cultivar CIM-707 by having medium K^+/Na^+ ratio revealed better K^+ utilization even in presence of toxic ion (Na^+). By comparing grand mean values (overall genotypes and salinity levels) for K^+ and Na^+ , the K^+ absorption was five times greater than Na^+ accumulation in both shoot and root tissues which generate the $K^+:Na^+$ ratio of 5:1. The uptake of toxic ions (Na^+/Cl^-) in plant tissues subjected to saline conditions appeared to be due to partial ion exclusion (Na^+/Cl^-) in cotton (Ashraf, 2002). Maintenance of high K/Na ratio was suggested to be an important selection criterion for salt tolerance in cotton. The $K^+:Na^+$ ratio of cotton genotypes differed significantly at different $NaCl$ concentrations, while the uptake of K^+ decreased with increased salinity, and observed significant differences among genotypes regarding growth and salinity tolerance (Akhtar et al., 2005; Chen et al., 2010; Ibrahim et al., 2007).

Growth variables at 50 mM of $NaCl$ had similar values with control and somewhere even excelled the control, revealed that growth variables have been stimulated by less quantity of salts. Cotton being the salt tolerant crop showed increase in growth with low concentration of salts (Maas, 1990; Pessarakli, 2001). Low salinity with optimal supply of nutrients increased the growth and seed cotton yield (Bibi et al., 2016). The adding of appropriate amounts of K increased its use efficiency and K^+ substitution for Na^+ which can substantially increase the cotton crop productivity (Ali et al., 2009; Zhang et al., 2006). Passive accumulation of Na^+ caused the low $K:Na$ ratio in root and shoot tissues, however, the K^+ counteracts Na^+ stress while Na^+ in turn to a certain extent alleviates K^+ deficiency (Maser et al., 2002). Sodium can replace K^+ due to osmotic function, and thus under K^+ starvation, the addition of Na^+ can promote plant growth.

5. Conclusions

Cultivar CIM-707 by having medium K^+/Na^+ ratio, showed better K^+ utilization, and identified as most tolerant genotype to salinity. Farming community can get more net return through optimum yield of such genotype in salt affected areas. In future,

such studies can guide the researchers in screening the crop plants for salt tolerance.

Acknowledgments

Authors are grateful to Prof. Dr. Safdar Hussain Shah and Dr. Muhammad Sayyar Khan, Institute of Biotechnology and Genetic Engineering (IBGE), The University of Agriculture, Peshawar, Pakistan for their help and providing the laboratory facilities.

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