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ORIGINAL ARTICLE

Effect of salt stress (NaCl) on biomass and K^+/Na^+ ratio in cotton

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A pot experiment was conducted to evaluate performance of five Upland cotton (*Gossypium hirsutum* L.) varieties, Aleppo118 (A118), Aleppo33/1 (A33/1), Aleppo90 (A90), Raqqa5 (Raq5) and Deir-Ezzor22 (DE22), grown under different salinity concentration 0, 50, 100 and 200 mM NaCl for 56 days. Results indicated that increasing salinity decreased fresh and dry leaf and root weights. Also, leaf K^+/Na^+ ratio was decreased as increasing salinity levels in all tested varieties. This reduction for the all indicators tested was relatively more pronounced in A118 than DE22. Thereby, DE22 variety relatively performed better under salinity compared to the other tested varieties. Based on this investigation, it can be concluded that DE22 and Raq5 are relatively characterized as salt tolerant, while A90 as moderate salt tolerant. On the other hand, A118 and A33/1 could be considered as salt sensitive.

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Salinity is one of the most serious factors that limiting crop production, especially for the sensitive species (Manivannan et al 2007). Plants show variable capacity to salinity tolerance that could be range from negligible effect to plant death. Considerable differences are found between plant species. For example, after exposure to 200 mM NaCl, a salt- tolerant species such as sugarbeet might have a reduction of only 20% in dry weight, whereas, a moderately tolerant species such as cotton might have a 60% reduction, and a sensitive species such as soybean might die (Munns 2002). Different strategies commonly used by plants to improve salt stress tolerance: maintain desirable K^+/Na^+ ratio in the cytosole, regulation of K^+ uptake and prevention of Na^+ entry, efflux of Na^+ from cell or compartmentalize Na^+ into vacuole (Munis et al 2010, Munns 2002). The ionic toxicity of the salt could be attributed to the replacement of K^+ by Na^+ through biochemical interactions as well as structural changes, lack of protein functions as a result of uptake of ions Cl^- and Na^+ and interference of interactions between amino acids (Zhu 2003). Many researches reported that K^+/Na^+ ratio might be considered as useful indicator for plant salinity tolerance (Ahmad et al 2002, Khan et al 2009, Munis et al 2010).

In Syria, about 50% of irrigated soil suffers from salinity that has been considered as the main limitation of agricultural production. Cotton is a very economically important crop in Syria, with a cultivated area amount to 175.000 ha, and a production of 700.000 t of seed cotton and lint production is estimated at 235,000 t (USDA 2009).

This study aimed to investigate the biomass, leaf and root Na^+ and K^+ content and leaf K^+/Na^+ ratio in some cultivated cotton varieties grown in Syria under different salinity concentration 0, 50, 100 and 200 mM NaCl for 56 days.

MATERIALS AND METHODS

Plant materials

Seeds of Upland cotton (*G. hirsutum*. L) Aleppo 118, Aleppo 33/1, Aleppo 90, Raqqa 5 and Deir-Ezzor 22 were provided by the GCSAR – Syria (Table 1). The seeds were soaked in distilled H_2O for 24 h and then planted in pots filled with a 1/3:2/3 (v/v) mixture of perlite:peat mosse. Germination was carried out in the greenhouse at temperature of 18^0C, 12 h photoperiod and relative humidity of 80%. Seedlings were allowed to grow in the greenhouse under controlled conditions (temperature of 25^0C, 12 h photoperiod and relative humidity of 80%).

Table 1. Descriptive of 5 certificated cotton varieties used in this study

Variety	Agro-ecological zone	Yield (kg /ha) Upon certification	Certification year	Origine
Aleppo118	Aleppo - Idleb	6252	2004	Hybride (Syrian var. Aleppo40 x American var. BW 76-31)
Aleppo1/33	Hama, Homs, Ghab	5166	1987	Created from selected line Acala SG-4
Aleppo90	Hassakeh	5130	1977	Hybride (Russian var. Tashkand-3 x American var. Deltapine 70)
Raqqa5	Raqqa	4840	1988	Created from selected Russian var. Tashkand-3
Deir Ezzor22	Deir Ezzor	5420	1988	Created from selected American var. Deltapine 41

Source: General Commission for Agricultural Research Damascus – Duma, Syria (GCSAR).

Seedlings were watered for 1 week with 0.1 Hoagland nutrient solution before the initiation of NaCl treatment. The seedlings were subjected to salt stress by adding NaCl (0, 50, 100 and 200 mM) to the nutrient solution. The same environmental conditions were maintained during the experimental period. All solutions were changed twice a week. Plants were harvested 56 days after salt application (three replicates by treatment). Plants were up-rooted carefully and washed properly with tap water. Then, they were separated into roots and leaves for mineral analysis.

Statistical analysis

All statistical analyses were performed using Statview 4.5 statistical package (ABACUS 1996) significance level (P = 0.05). Data were subjected to analysis of variance (ANOVA) for the determination of differences in means between tested plants of each concentration of NaCl applied. Differences between means were tested for significance by Fisher's PLSD test. Data are expressed as mean of three replicates.

Plant sampling and mineral analysis

Leaf and root fractions were oven-dried at 70°C for 48 h, weighed, crushed in a hammer-mill and stored at room temperature. Nutrient analyses were carried out on dried leaves and roots. Samples were ground and 0.5 g of a fine powder was burnt at

400^{0}C for 4 h. The resulting ashes were dissolved in 100 ml of 0.5N concentrated nitric acid. Determination of Na^+ and K^+ was carried out using flame photometer procedure.

RESULTS AND DISCUSSION

Analysis of variances of data for the different investigated parameters for the five cotton varieties tested in this study was summarize in Table 2.

Table 2. Analysis of variances (mean squares) of data for the five cotton varieties after 56 days growth at 0, 50, 100 and 200 mM NaCl

S.O.V	df	FWR	FWL	DWR	DWL	Biomass	K^+/Na^+ ratio
V	4	1.047*	5.758*	0.138^{ns}	0.611*	3.391*	42.666*
T	3	5.099*	7.793*	0.496*	0.667*	11.428*	89.958*
V x T	12	0.102^{ns}	0.653^{ns}	0.095^{ns}	0.077^{ns}	1.189*	32.271*
Error	40	0.242	0.487	0.067	0.039	0.3	6.496

* : significant at 0.05 level, ns: Non-significant, V: variety, T: treatment.

Table 3. Fresh weight (g) of five cotton varieties 56 days after NaCl (0, 50, 100 and 200 mM) treatment

Variety	Control	50 mM	100 mM	200 mM	Control	50 mM	100 mM	200 mM
	Leaf				Root			
A118	5.25 aX	3.25 bY	2.54 cZ	2.56 bZ	3.45 aX	2.64 bY	2.43 aY	2.02 aZ
A33/1	4.14 bX	3.28 bY	2.95 bY	2.63 bZ	3.57 aX	3.26 aX	2.69 aY	2.23 aZ
A90	5.49 aX	4.79 aY	4.71 aY	3.31 aZ	3.33 aX	2.78 bY	2.56 aY	1.77 bZ
Raq5	3.29 cX	2.66 cY	2.67 cY	2.06 bZ	3.42 aX	3.07 aX	2.55 aY	1.73 bZ
DE22	3.71 bX	3.79 bX	3.46 bX	2.55 bY	2.69 bX	2.07 cY	1.98 bY	1.76 bY
	$LSD_{0.05}$ (T) = 0.52 $LSD_{0.05}$ (V) = 0.58				$LSD_{0.05}$ (T) = 0.36 $LSD_{0.05}$ (V) = 0.41			

Mean with the same letters in each column (a-c) and each row (X-Z) don't differ significantly at *P* = 0.05. (T): treatment; (V): variety.

Table 4. Dry weight (g) of five cotton varieties 56 days after NaCl (0, 50, 100 and 200 mM) treatmen

Variety	Control	50 mM	100 mM	200 mM	Control	50 mM	100 mM	200 mM
	Leaf				Root			
A118	1.14 bX	0.54 cY	0.58 bY	0.49 bY	1.09 aX	0.89 bY	0.75 aY	0.75 aY
A33/1	0.96 cX	0.61 bY	0.35 cZ	0.37 cZ	1.05 aX	1.18 aX	0.46 bY	0.53 bY
A90	1.34 aW	1.18 aX	0.95 aY	0.54 bZ	1.13 aX	0.91 bY	0.79 aY	0.42 bZ
Raq5	0.45 eX	0.42 cX	0.42 cX	0.23 cY	0.68 bY	1.05 aX	0.79 aY	0.57 aZ
DE22	0.77 dX	0.76 bX	0.63 bX	0.75 aX	0.61 bX	0.64 cX	0.53 bX	0.57 aX
	$LSD_{0.05}$ (T) = 0.15 $LSD_{0.05}$ (V) = 0.16				$LSD_{0.05}$ (T) = 0.19 $LSD_{0.05}$ (V) = 0.21			

Mean with the same letters in each column (a-e) and each row (W-Z) don't differ significantly at *P* = 0.05. (T): treatment; (V): variety.

Fresh and dry weights biomass

Salinity significantly ($p<0.001$) reduced the fresh weight of leaf (FWL) and root (FWR) (Table 3). This reduction was more pronounced for A118, A33/1, A90 and Raq5 than for DE22.

On the other hand, decline in dry weight of leaf (DWL) and root (DWR) (Table 4) was recorded for all tested varieties regardless the concentration of NaCl applied. A significant reduction ($P<0.001$) in DWR and DWL was observed in all varieties due to salt stress as salinity level increased. However, no significant decrease in both leaf and root dry weight was recorded for DE22 (Table 4) which seems to be the most salt tolerant variety relative to the other varieties in this study. While, DWR increased significantly above the control plants in Raq5 by 55% and 16% at 50 mM and 100 mM respectively.

The observed reduction in DW resulting from the increasing in salinity might be attributed to the combination effect of osmotic and specific ion of Cl^- and Na^+ (Basal 2010, Hajer et al 2006). It was noticed that, the reduction in DWR index exceeded 62% with increasing salt level from 0 to 200 mM, and differences among cotton varieties were significant. These results are in agreement with Munns (2002). The highest reduction in DWR was found for A90 (63%), and the lowest reduction was observed for DE22 (7%) at high NaCl salinity level (200 mM). At the same range of NaCl (200 mM), a reduction in DWL was also observed among the different tested cotton varieties es. The lowest decrease in this parameter was recorded for DE22 (3%), whereas, the highest reduction was found in A33/1 (62%). Several researchers reported that fresh and dry weights of cotton plants might be affected under saline conditions (Ahmad et al 2002, Akhtar et al 2010, Basal 2010, Khan et al 1995). Deir-Ezzor 22 variety is differed by showing high salt tolerance relative to Aleppo 118 and Aleppo 33/1 varieties (unpublished). Similar observation has also been recorded in tomato (Hajer et al 2006) and Maize (*Zea mays* L.) (khatoon et al 2010).

Total biomass production

Salt stress significantly (p<0.001) impaired total plant dry weight (leaf, root and stem) for all tested varieties below the control (Fig.1). This inhibition was less pronounced in Raq5 and DE22 compared to the other tested varieties. At low NaCl concentration (50 mM), this reduction ranged between 5% (DE22) to 44% (A118). While, at moderate NaCl treatment (100 mM), the reduction rate varied from 34% (DE22) to 62% (A33/1).

It is worth noting that the previous parameter increased in Raq5 at 50 mM and 100 mM NaCl by 29% and 10% respectively over the control. Whereas, the highest reduction was recorded at high NaCl concentration (200 mM). It could be concluded that the decline in this parameter is can be arranged as the following: Raq5 (25%) < DE22 (34%) < A118 (53%) < A33/1 (56%) < A90 (64%). These values fall within the same range of Munns (2002) who reported that the reduction in dry weight of cotton tissues reach 60%.

Similarly, Ahmad et al (2002), Akhtar et al 2010, Basal (2010) and Munis et al (2010) found that biomass production was negatively affected by NaCl application in cotton. However, based on our study, Raq5 and DE22 could relatively be classified as salt tolerant varieties compared to other tested varieties.

Na^+ and K^+ content

Differences in Na^+, K^+ content in leaves and roots showed different patterns of ion accumulation in all tested varieties (Fig. 2). Sodium content was higher in leaves than in roots for all tested varieties in both the control and the salt stressed plants. Data revealed that an increase of leaf Na^+ accumulation with increasing salinity was evident in all varieties. In leaves, DE22 and Raq5 varieties showed the lowest Na^+content while, A33/1 has the highest one (Fig. 2A). This result was in agreement with Akhtar et al (2010) and Munis et al (2010) who mentioned that the cotton tolerant cultivars had lower concentration of Na^+ in leaves than the sensitive ones.

In root, A90 accumulates the highest Na^+ value compared to the tested varieties (Fig. 2B). Based upon the estimated K^+ content, genotypic variation in leaf K^+ content was observed for all tested varieties (Fig. 2C). This previous parameter was constant in Raq5 regardless to salinity level. While at root level, slight decrease was recorded for A118 (Fig. 2D). A slight increase in this parameter was recorded for Raq5. However, this parameter was constant for A33/1 and remarkably for DE22 (Fig. 2D). This observation was in accordance with Ahmad et al (2003), Akhtar et al (2010) and Munis et al (2010) in cotton. In this respect, DE22 and Raq5 varieties could be relatively considered as salt tolerant variety compared to other tested varieties.

Leaf K^+/Na^+ ratio

Estimated leaf K^+/Na^+ ratio was negatively affected by NaCl application (Table 5). At 100 mM NaCl, DE22 and Raq5 were classified in the first rank according to this previous index while the other tested varieties were in the least rank (Table 5). To avoid the toxicity resulting from an excess salt, plants tend to accumulate more K^+ over Na^+ at leaf level and thus maintained high K^+/Na^+ ratio. These plants could be characterized as salt-tolerants relative to the other treated plants. According to Abdullah and Ahmad (1986), Ahmad et al (2002), Khan et al (2009), Khorshidi et al (2009), Munis et al (2010), Wyn Jones et al (1979) and Zhu (2003), K^+/Na^+ ratio has been considered as a critical indicator for salt tolerance selection in some crops.

Genotypic variation exists among the five cotton varieties for investigated characters under control and salt treatments. In general, NaCl application reduced leaf K^+/Na^+ ratio (Table 5) in all tested varieties. Leaf K^+/Na^+ ratio was decreased to less than 1.0 in A118 (about 0.39), A33/1 (about 0.41) and slightly increased to more than 1.0 in DE22 and Raq5 (about 1.23 and 1.38 respectively).

These findings available at all external NaCl regimes except at the highest NaCl treatment (200 mM) where this ratio became below 1.0 in all tested varieties. It is worth to note that, K^+/Na^+ ratio decreased with increasing salinity in all tested varieties. However, it was found that K^+/Na^+ ratio is higher in DE22, i.e., 0.62 than in A118 (0.27).

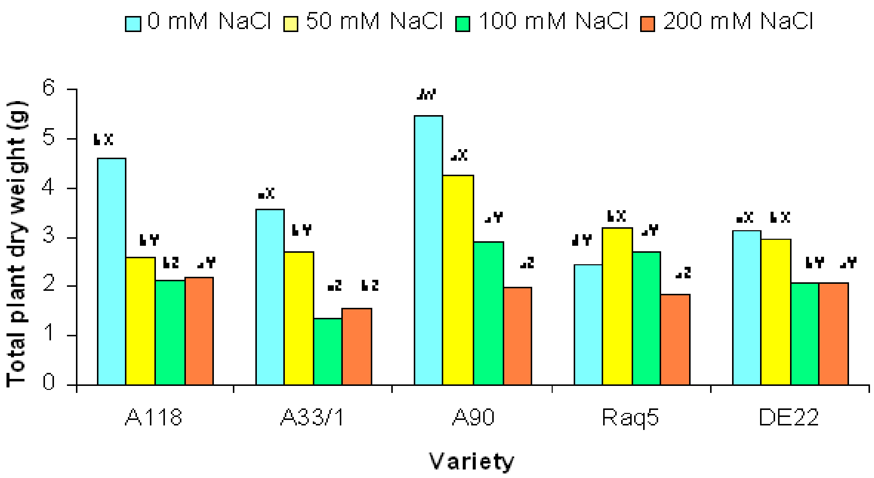

Figure 1. Total plant dry weight (g) 56 days growth at 0, 50, 100 and 200 mM NaCl. Mean with the same letters among varieties (a-d) and (W-Z) within the same variety don't differ significantly at $P = 0.05$

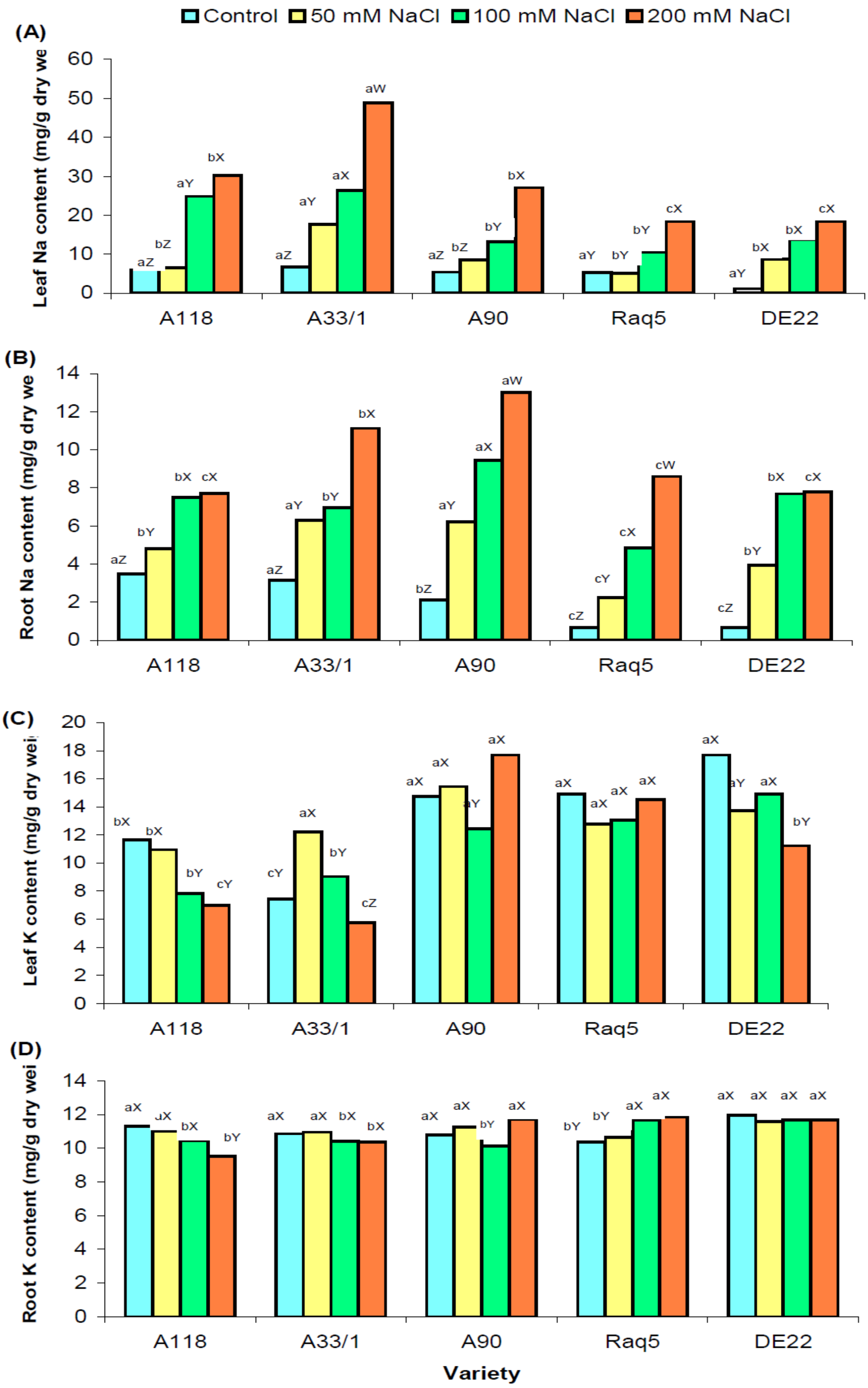

Figure 2. Leaf Na^+ (A), root Na^+ (B), leaf K^+ (C) and root K^+ (D) content of five cotton varieties after 56 days growth at 0, 50, 100 and 200 mM NaCl. Mean with the same letters among varieties (a-c) and (W-Z) within the same variety don't differ significantly at $P = 0.05$

Table 5. Leaf K^+/Na^+ ratio of five cotton varieties 56 days after NaCl (0, 50, 100 and 200 mM) treatment

Variety	Control	50 mM	100 mM	200 mM
A118	2.27 bX	2.07 aX	0.39 aX	0.27 aY
A33/1	1.26 cX	1.03 aX	0.41 aX	0.12 aX
A90	4.04 bX	1.91 aY	1.04 aY	0.71 aY
Raq5	3.95 bX	2.52 aX	1.38 aY	0.82 aY
DE22	17.79 aX	2.48 aY	1.23 aY	0.62 aY
	$LSD_{0.05}$ (T) = 1.88 $LSD_{0.05}$ (V) = 2.10			

Mean with the same letters in each column (a-c) and each row (X-Y) don't differ significantly at $P = 0.05$. (T): treatment; (V): variety.

This study revealed that Raq5 and DE22 varieties retained low Na^+ concentration and maintained high K^+/Na^+ ratio and thus it's reflecting a better salt tolerance. While A118 and A33/1 varieties could be declared as a salt-sensitive variety due to there high accumulation of Na^+ and maintaining lower K^+/Na^+ ratio. Similar finding was reported in cotton (Ahmad et al 2003, Akhtar et al 2010, Munis et al 2010) and in wheat (Khan et al 2009).

These results are also in accordance with the previous studies of Abdullah and Ahmad (1986) and Ahmad et al (2002) who found that a positive relationship between high K^+/Na^+ ratio and salt tolerance in cotton. Maintenance of higher K^+/Na^+ ratio in salt tolerant cultivars may have been one of the factors for their relative superiority in growth under saline conditions, since Wyn Jones et al (1979) suggested a minimum value for K^+/Na^+ of 1.0 for normal growth of plants subjected to saline substrate.

It is obvious to notice that at highest salinity levels (200 mM) this index was higher in A90, Rak5 and DE22 comparing to the other tested varieties. Where, Raq5 and DE22 restricted the Na^+ and accumulated K^+ and thus maintained high K^+/Na^+. While A118 and A33/1 behaved conversely. The increased of K^+ observed in some previous varieties towards salt application could be attributed to efficient K^+ absorbance by selective inclusion of Na^+ by cortical cells. Our results were in accordance of Ahamad et al (2003) and Munis et al (2010) in cotton. A high leaf K^+/Na^+ ratio in the cytosol is essential for normal cellular functions of plants. Na^+ competes with K^+ uptake through Na^+/K^+ cotransporters, and may block the K^+ specific transporters of root cells under salinity (Zhu 2003).

In conclusion, biomass production and leaf K^+/Na^+ ratio could be considered as useful parameters for screening salt tolerance among different cotton varieties cultivated in Syria. Thereby, cotton varieties that could be considered as salt tolerants will help in boosting crop production in salt affected regions. Ionic analysis performance (another ions and cations analyses), further determinants such as e.g. transpiration rate and osmolytes accumulation, are needed for a better understanding of the response towards salt treatment among these tested varieties.

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