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**ORIGINAL ARTICLE**

# Ion partitioning and $Mg^{2+}/Na^{+}$ ratio under salt stress application in cotton

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The response of five cotton (*Gossypium hirsutum* L.) varieties Aleppo118 (A118), Aleppo33/1 (A33/1), Aleppo90 (A90), Raqqa5 (Raq5) and Deir-Ezzor22 (DE22) to salt stress (0, 50, 100 and 200 NaCl) was studied in a pot experiment, in terms of their ionic distribution for 56 days. Leaves and roots of DE22 exhibited the lowest $Na^{+}$ and $Cl^{-}$ and the highest leaf $Mg^{2+}$ contents conversely to A118. based on this investigation, there was no relationship between $Ca^{2+}$ content and salt tolerance of different cotton varieties. Leaf and root $Mg^{2+}/Na^{+}$ ratios were decreased as salinity level increased for all tested varieties. In this respect, the highest value for the previous indicators was recorded in DE22 while the lowest one was recorded in A118. Thereby, among all tested varieties, DE22 variety relatively performed better under salinity compared to the other varieties.

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Lands surfaces submitted to salt stress are in large extension day by day in arid, semiarid regions and Mediterranean area. This increase is caused by unsuitable irrigation management and the high salt content of the irrigation water. Currently, one third of all irrigated lands in the world are affected to a greater or lesser degree by salinity and the salinity problem continues to increase (Munns 2005). The effect of high salt concentration on growth and yield has been mentioned in many reports (Akhtar et al 2010, Ahmad et al 2002, Munis et al 2010). Salinity is one of the most serious factors that limiting crop production, especially for the sensitive ones (Manivannan et al 2007). Cotton is one of the major fiber crops of the world which is classified as a salt tolerant crop (Ahmad et al 2002). The mechanism of ion uptake and pattern of ions accumulation in different parts of plant is very important for discriminate between salt tolerant and salt sensitive genotypes. For instance, less accumulation of $Na^+$ in leaves of salt tolerant varieties of cotton has also been reported by different researchers (Ahmad et al 2003, Akhtar et al 2010, Khan et al 2004, Munis et al 2010). Excess of salt content in the soil could prevent the uptake of essential nutrients such as $Ca^{2+}$, $K^+$, $Mg^{2+}$ and $NO_3^-$ (Khatoon et al 2010). However, investigating the role of magnesium as a good criteria in salt tolerance plants is poorly documented. 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). Thus, this investigation focused on leaf and root $Mg^{2+}/Na^+$ ratios as critical indicators for screening of salt tolerance capacity in some cultivated cotton varieties in Syria.

## 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 GCSAR – Syria (Table 1). 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 a greenhouse at temperature of 18єC, 12 h photoperiod and relative humidity of 80%. Seedlings were allowed to grown in a greenhouse under controlled conditions (temperature of 25єC, 12 h photoperiod and relative humidity of 80%). Seedlings were watered for 1 week with 0.1 *Hoagland* nutrient solution befor the intiation 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 this period. All solutions were changed twice a week. The pot experiment (three replicates by treatment) was carried out in the greenhouse for 56 days. Plants were up-rooted 56 days after salt application carefully and washed properly with tap water. Then, plants were fractionated into roots and leaves for mineral analysis.

### Statistical analysis

Statistical analyses were performed using Statview 4.5 statistical package (ABACUS 1996) at 5% 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.

### Plants sampling and mineral analysis

Leaves and roots 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 ◦C for 4 h. The resulting ashes were dissolved in 100 ml of 0.5N concentrated nitric acid. Determination of $Na^+$ was carried out using flame photometer procedure, while that for $Ca^{2+}$ and $Mg^{2+}$ cations using calibration by EDTA methods. While, chloride concentration was determined using an Ion Selective Crystal Membrane Electrode $Cl^-$ (ISE 6.0502.120 Metrohm- Switzerland). For all cations and ions, their content was expressed in mg/g of dry material.

**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).

**Table 2.** Leaf and Root $Na^+$ content 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** | 6.01 aZ | 6.64 bZ | 24.84 aY | 30.26 bX | 3.49 aZ | 4.79 bY | 7.5 bX | 7.73 cX |
| **A33/1** | 6.78 aZ | 17.58 aY | 26.37 aX | 48.92 aW | 3.15 aZ | 6.28 aY | 6.95 bY | 11.13 bX |
| **A90** | 5.4 aZ | 8.51 bZ | 13.22 bY | 27.04 bX | 2.12 bZ | 6.22 aY | 9.43 aX | 13.00 aW |
| **Raq5** | 5.35 aY | 5.07 bY | 10.49 bY | 18.36 cX | 0.65 cZ | 2.23 cZ | 4.85 cX | 8.59 cW |
| **DE22** | 1.17 aY | 8.72 bX | 13.69 bX | 18.39 cX | 0.67 cZ | 3.95 bY | 7.69 bX | 7.78 cX |
| | **$LSD_{0.05}$ (T) = 6.569**<br>**$LSD_{0.05}$ (V) = 7.344** | | | | **$LSD_{0.05}$ (T) = 1.125**<br>**$LSD_{0.05}$ (V) = 1.258** | | | |

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

**Table 3.** Leaf and Root $Cl^-$ content 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** | 6.31 bZ | 16.06 cZ | 65.64 aYX | 79.09 aX | 0.69 aY | 1.92 aY | 2.93 bY | 6.76 aX |
| **A33/1** | 7.96 bY | 31.32 aY | 49.12 aY | 78.5 aX | 0.69 aX | 1.19 bX | 6.36 aX | 8.51 aX |
| **A90** | 16.24 aY | 29.19 abY | 32.49 aY | 74.15 aX | 0.59 aY | 1.86 aY | 3.26 bY | 9.51 aX |
| **Raq5** | 7.61 bZ | 16.04 cZ | 30.39 aY | 60.09 aX | 1.1 aY | 0.91 bcY | 2.25 bY | 4.94 aX |
| **DE22** | 5.64 bZ | 12.74 cZ | 30.57 aY | 43.64 aX | 0.5 aY | 0.99 bcY | 2.49 bX | 2.48 aX |
| | $LSD_{0.05}$ (T) = 13.723<br>$LSD_{0.05}$ (V) = 15.343 | | | | $LSD_{0.05}$ (T) = 2.205<br>$LSD_{0.05}$ (V) = 2.465 | | | |

Mean with 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.** Leaf and Root $Ca^{2+}$ content 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** | 42.31 aX | 41.28 aX | 46.46 aX | 49.08 aX | 5.97 aY | 5.12 bY | 6.08 aX | 5.96 aY |
| **A33/1** | 43.79 aX | 46.81 aX | 53.15 aX | 54.37 aX | 6.81 aX | 7.03 aX | 6.82 aX | 5.82 aX |
| **A90** | 48.94 aX | 37.82 aX | 48.71 aX | 28.16 aY | 5.97 aX | 5.47 bX | 5.53 aX | 5.55 aX |
| **Raq5** | 8.16 bX | 7.44 bY | 5.72 bY | 4.28 bY | 1.99 bX | 1.13 cY | 1.14 bY | 1.12 bY |
| **DE22** | 6.51 bX | 4.99 bY | 6.59 bX | 5.05 bX | 1.92 bX | 1.88 cX | 1.67 bX | 1.79 bX |
| | $LSD_{0.05}$ (T) = 7.564<br>$LSD_{0.05}$ (V) = 8.457 | | | | $LSD_{0.05}$ (T) = 0.861<br>$LSD_{0.05}$ (V) = 0.963 | | | |

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

**Table 5.** Leaf and Root $Mg^{2+}$ content 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** | 1.55 bX | 1.39 bX | 1.04 cX | 0.91 bX | 4.19 bY | 6.88 bX | 6.08 bY | 5.96 bY |
| **A33/1** | 1.91 bX | 1.75 bX | 1.37 cY | 1.25 bX | 6.12 aX | 6.65 bX | 4.23 bX | 3.48 bX |
| **A90** | 1.98 bY | 3.66 bX | 5.25 cX | 1.36 bY | 2.87 bX | 5.61 bX | 5.77 bX | 5.41 bX |
| **Raq5** | 7.35 bY | 11.52 aY | 17.03 bY | 28.97 aX | 4.98 bX | 4.83 bX | 4.25 bX | 7.06 bX |
| **DE22** | 36.85 aX | 37.61 aX | 22.95 aX | 22.24 aX | 4.51 bY | 16.89 aX | 15.17 aX | 19.59 aX |
| | $LSD_{0.05}$ (T) = 7.802<br>$LSD_{0.05}$ (V) = 8.802 | | | | $LSD_{0.05}$ (T) = 3.487<br>$LSD_{0.05}$ (V) = 3.899 | | | |

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

**Table 6.** Leaf and root $Mg^{2+}/Na^{+}$ ratio of five cotton varieties 56 days after NaCl (0, 50, 100 and 200 mM) treatment

| Variety | Control | 50 mM | 100 mM | 200 mM | Control | 50 mM | 100 mM | 200 mM |
|---|---|---|---|---|---|---|---|---|
| | Leaf | | | | Root | | | |
| **A118** | 0.26 bX | 0.21 aX | 0.04 aX | 0.03 aX | 1.20 bX | 1.44 aX | 0.81 aX | 0.77 aX |
| **A33/1** | 0.28 bX | 0.10 aX | 0.05 aX | 0.03 aX | 1.94 bX | 1.06 bX | 0.61 aX | 0.31 aX |
| **A90** | 0.37 bX | 0.43 aX | 0.40 aX | 0.05 aX | 1.35 bX | 0.90 bX | 0.61 aX | 0.42 aX |
| **Raq5** | 1.37 bX | 2.27 aX | 1.62 aX | 1.58 aX | 7.66 aX | 2.17 aY | 0.88 aY | 0.82 aY |
| **DE22** | 31.5 aX | 4.31 aY | 1.68 aY | 1.21 aY | 6.73 aX | 4.28 aX | 1.97 aY | 2.52 aY |
| | $LSD_{0.05}$ (T) = 5.158<br>$LSD_{0.05}$ (V) = 5.766 | | | | $LSD_{0.05}$ (T) = 2.796<br>$LSD_{0.05}$ (V) = 3.126 | | | |

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

## RESULTS AND DISCUSSION

### $Na^{+}$ content

Sodium content was higher in leaves than in roots for all tested varieties in both the control and the salt stressed plants. Our results showed that $Na^{+}$ accumulation progressively increased for both leaf and root as increasing salinity levels for all tested varieties (Table 2). Analysis of variance indicated that the effect of variety and NaCl levels on $Na^{+}$ accumulation was highly significant ($P<0.001$)

regardless plant parts.

At leaf level, A33/1 variety exhibited the highest $Na^+$ content, conversely to DE22 and Raq5 varieties that exhibited the lowest one (Table 2). This result was in agreement with Akhtar et al (2010), Khan et al (2004) and Munis et al (2010) who mentioned that the cotton tolerant cultivars had lower concentration of $Na^+$ in leaves than the sensitive ones. Earlier, Ramanjulu et al (1999) found less $Na^+$ content in leaves in salt-tolerant barley plants under 100 mM NaCl. This finding confirmed the results obtained previously by Saleh (2011) who found that DE22 and Raq5 were also the most salt tolerant varieties compared to the other tested based on plant growth and chlorophyll content. However, at root level, A90 accumulates the highest $Na^+$ value compared to the tested varieties (Table 2). It was noticed that DE22 and A118 varieties have the same $Na^+$ content (7.78 and 7.73 mg/g dry weight) at 200 mM NaCl and this accumulation was 1.7 folders higher in A118 compared to DE22 at leaf level under the same range of NaCl (200 mM). Based on this observation it can be conduced that salt tolerance among the previous cotton varieties tested don't correlated only to the $Na^+$ rate accumulation, but few other factors (will be mentioned latter in this investigation) could be explain this sensitivity towards salt application.

**$Cl^-$ content**

Salinity increased leaf $Cl^-$ content as salinity level increased for all tested varieties (Table 3). At high concentration of NaCl (200 mM NaCl), A118 variety accumulated the highest leaf $Cl^-$ content value (79 mg/g dry weight), while, DE22 exhibited the lowest index value (44 mg/g dry weight). In the present study, salinity significantly ($P<0.001$) increased leaf and root $Cl^-$ content as salinity level increased in all tested varieties. At 100 and 200 mM NaCl levels, A118, A33/1 and A90 had the highest leaf $Cl^-$ content value (66, 49 and 33 at 100 mM and 79, 79 and 74 mg/g dry weight at 200 mM NaCl respectively), while Raq5 and DE22 exhibited the lowest index value (31, 31 at 100 mM and 60, 44 mg/g dry weight at 200 mM ). This finding is in agreement with an earlier findings of Qadir and Shams (1997) who found that, the salt sensitive cultivars had more concentration of $Cl^-$ in leaves than those of the salt tolerant lines at the highest salt level. Moreover, same trend of $Cl^-$ concentration was observed at root level where A118, A33/1 and A90 varieties were the highest in root $Cl^-$ concentration (7, 9 and 10 mg/g dry weight respectively) of all the varieties at the highest external salt concentration (200 mM). While, DE22 and Raq5 were in the last rank with values of 3 and 5 mg/g dry weight respectively at the same range of NaCl (Table 3). Similar finding was obtained by Ahmad et al (2003) in cotton. Data reaveled that chloride content increased in similar pattern to sodium at roots for all tested varieties.

**$Ca^{2+}$ content**

Leaf $Ca^{2+}$ content was increased as salinity level increased in A118 and A33/1 varieties which exhibited the heighest value. However, A90, Raq5 and DE22 showd opposite trend in showing lowest values (Table 4). Based upon the measurement of root $Ca^{2+}$ content, the same trend was recorded for the five cotton tested varieties.

Decrease in $Ca^{2+}$ and $Mg^{2+}$ content of leaves has been reported by salt accumulation in mangrove *Bruguiera parviflora* species (Parida et al 2003). In contrast, Ahmad et al (2003) reported high $Ca^{2+}$ accumulation in cotton salt tolerant cultivars than in sensitive one at 210 mM NaCl. While, Mudgal et al (2009) reported that salinity enhances the content of $Na^+$, $Ca^{2+}$ and $Cl^-$ in Chickpea. However, there was no relationship between $Ca^{2+}$ content and salt tolerance of different cotton varieties.

**$Mg^{2+}$ content and $Mg^{2+}/Na^{+}$ ratio**

It was noticed that $Mg^{2+}$ accumulation in leaves and roots was also conversely related to $Ca^{2+}$ (Table 5). Data regarding leaf and root $Mg^{2+}/Na^{+}$ ratios were also followed the same trend (Table 6). Regarding to leaf $Mg^{2+}$ content, our data revealed that treated plants in DE22 variety was in the first rank while A118 and A90 were in the last rank. It was found that this indicator was 22 and 25 fold higher on DE22 compared to A118 at 100 mM and 200 mM NaCl respectively.

In the root, tested cotton varieties showed different pattern in $Mg^{2+}$ content. This index was constant in A90, with non significant differences in A118, A33/1 and Raq5 varieties, while, a significant increased ($P$<0.001) was recorded in DE22. Therefore, it seems that the decrease of leaf $Mg^{2+}$ content, which observed in A118, A33/1 and A90 varieties could lead to a decreased in the export of photosynthesis in cotton varieties. This observation was in agreement of Hadi et al (2008) in wheat (*Triticum turgidum* L.). In this respect, leaf and root $Mg^{2+}/Na^{+}$ ratios were investigated to evaluate the salt tolerance capacity of different cotton varieties.

Many investigations mentioned that $K^{+}/Na^{+}$ ratio might be considered as useful indicator for plant salinity tolerance. However, not much attention has been paid to the role of magnesium in plants salt tolerance. In spite of its important role in chlorophyll structure and as an enzyme cofactor, another important role of $Mg^{2+}$ in plants is in the export of photosynthates, which is impaired and leads to enhanced degradation of chlorophyll in magnesium deficient source leaves, resulting in increased oxygenase activity of ribolose bisphspate carboxylase (Marschner and Cakmak 1989, Ramoliya et al 2004).

Our data revealed that the both ratios (leaf and root $Mg^{2+}/Na^{+}$ ratios) were negatively affected by NaCl stress (Table 6). Increasing NaCl concentration leads to the decrease of these previous indices. The decrease may be attributed to the fact that increasing $Na^{+}$ ions inhibited the absorption of both $K^{+}$ and $Mg^{2+}$ due to antagonistic interaction (Correia et al 2010). A decline in leaf and root $Mg^{2+}/Na^{+}$ ratios was observed as increased salinity (Table 6). The heighest value was recorded for DE22 wille the lowest one was recorded for A118. This observation could explain the differences cotton varieties response towards salt stress. This previous index was significantly ($P$<0.001) reduced by the interaction between salt treatment and variety effect. This ratio at leaf level amounted to be 42 and 40 fold higher on DE22 compared to A118 at 100 mM and 200 mM NaCl respectively and at root level to be 3 fold higher on DE22 compared to A118 for the both salinity leveles. So, $Mg^{2+}/Na^{+}$ ratio could be used for salt tolerance screening ability among these tested varieties. Our results were in accordance of Karadge and Chavan (1983) whose metioned the important role of $Mg^{2+}$ in salinity tolerance in *Sesbania aculeate* Poir.

In Conclusion, various physiological indices were evaluated in this study, among these parmeters leaf and root $Mg^{2+}/Na^{+}$ ratios could be considered as a usefull parameters for screnning salt tolerance among different cotton varieties cultivated in Syria. Based on this investigation it was suggested that A118 and A33/1 could be considered as salt sensitive. While, DE22 and Raq5 relatively characterized as salt tolerant, whereas, A90 as moderate salt tolerant. Further determinants such as e.g. antioxidant systems and osmolytes accumulation and *GhNhx1* gene expression investigation are needed for a better understanding of the response towards salt treatment among these tested varieties.

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