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Mineral Nutrient Acquisition by Cotton Cultivars Grown under Salt Stress

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ABSTRACT

Physiological responses were investigated in two cotton cultivars grown at various concentrations of sodium chloride (NaCl) in order to determine the degree of the tolerance of the cultivars to salt stress and understand the physiological responses with respect to utilization of mineral nutrients. After germination of the seeds of cotton cultivars, they were transferred into standard pots with 210 g sterilized compost and watered with 30 ml Hoagland's solution containing different concentrations (0, 50, 100, 200, and 400 mM) of NaCl at two-day intervals for 3 months. Growth parameters were measured and the mineral nutrient analyses were done using inductively coupled plasma optical emission spectrometry (ICP-OES, Thermo Fisher Scientific, Waltman, MA). It was observed that plant growth and mineral nutritional status of both cultivars were altered extensively in those grown with NaCl. Excess NaCl reduces the concentrations of certain mineral nutrients and increases that of others, the patterns depending on the mineral nutrient and the plant part and varieties being compared to the control.

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Introduction

Since plants do not have mobility, exposure to stresses cannot be avoided in their environment but rather plants must adapt to ambient conditions. Low or elevated temperature, high salinity, drought, and presence of heavy metals, as well as pathogen and insect attacks are common stresses that plants encounter. Even with the best use of available land and sustainable farming practices, the impact of these abiotic and biotic stresses can severely reduce both food and fiber crop yields.

High soil salinity is a major limitation to crop production. Plant physiologists and breeders have long sought to develop efficient approaches to identify tolerant genotypes and understand their genetic and physiological mechanisms coping with salt. To this end, a great deal of research into impacts of salinity on plant physiology and development has generated a wealth of information in recent decades. The response of plants to salinity stress occurs as morphological, physiological, and metabolic responses and modifications in plant organs such as decreased seed germination, shoot and root length, and alteration of hydrolytic enzyme activity during germination and other metabolic processes (Akbarimoghaddam et al., 2011; Seckin, Sekmen, and Turkan 2009). Disrupted plant-water relations and ion balance due to the reduced water potential and high sodium levels in soil cause water loss and nutritional limitation in plants (Ali et al. 2001), leading to impairment of

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photosynthetic capacity (Bor, Ozdemir, and Turkan 2003; Netondo, Onyango, and Beck 2004; Tiwari et al. 2010) and cellular metabolic processes (Javid et al. 2011; Seckin, Sekmen, and Turkan 2009; Tabur and Demir 2010). Limited or excess of nutrient availability can result in retardation of plant growth (Berry 2010; Donohue 2001; Kudoyarova et al. 2015). Salinity influences the metabolism of mineral nutrients leading to reduction in plant growth indirectly as a result of nutrient imbalance and physiological disorders (Bano and Fatima 2009; Munns 2002). The most common salt found in the environments is sodium chloride (NaCl), which competes with the uptake of other nutrients causing nutrient deficiency and specific toxicity in plants (Bano and Fatima 2009; Tester and Davenport 2003).

Cotton is an important commercial fiber crop widely cultivated throughout the world (Chachar, Solangi, and Verhoef 2008). Besides being utilized in the textile industry, cotton is also used for the production of various goods such as hulls, oil, linters, and food for animals (Aragao et al. 2005; Mishra et al. 2003). The cotton, which belongs to the genus Gossypium from Malvaceae family, is a deciduous, indeterminate perennial plant. Gossypium genus includes 51 species with tropical and subtropical distributions. The cotton is distributed worldwide and its wild members are inhabited in all continents, with exceptions of Europe and Antarctica (Ozyigit and Gozukirmizi 2008; Aguilera and Aguilera-Gomez, 2016).

There are large differences in plant species' response to salinity (Flowers, Munns, and Colmer 2014; Shrivastava and Kumar 2015). Plants exhibiting tolerance to salt can still manage to grow when grown on soils containing high levels of salt. Although cotton is considered a salt tolerant crop (Dong 2012; Mahajan and Tuteja 2005), its growth and yield are retarded markedly under high salinity stress, especially during germination and emergence stages (Ashraf 2002). Comparisons among cotton species have shown varietal differences in the levels of salt tolerance (Ashraf 2002; Hussain et al. 2012).

The two cotton cultivars (Nazilli 84S and Cukurova 1518) used in this study are widely planted in Turkey (Ozyigit and Gozukirmizi 2008). Physiological responses of these two cotton cultivars grown at various concentrations of NaCl were investigated with respect to utilization of mineral nutrients to determine the degree of the tolerance of these cultivars to salt stress and to gain a better understanding of the physiological responses in terms of how mineral nutrition uptake could be used to develop efficient strategies for minimizing the detrimental effects of salt stress.

Materials and methods

The seeds of cotton cultivars Nazilli 84S and Cukurova 1518 were surface-sterilized by immersion of ethyl alcohol (70%) for 1 min and then washed with distilled water several times. They were germinated on wet filter paper in a growth chamber for 7 days and watered with full strength Hoagland nutrient solution (Hoagland and Arnon 1950). After 7 days, they were transferred into standard plastic pots containing 210 g of sterilized compost (Gardol® pH: 6–7, electrical conductivity (EC): 1–2 m S/cm, safety: min. 95%). Plants were grown under 5000 μmol m^{-2} s^{-1} fluorescent light, 23 ± 2 °C temperature and 45–50% relative humidity at 16h-day / 8h-night regime. Upon appearance of second mature leaves, each experimental group (10 replicated seedlings) was watered with 30 ml Hoagland's solution containing different concentrations (0, 50, 100, 200, and 400 mM) of NaCl at two-day intervals for 3 months. The pH value was in range of 6 to 7 in control and experimental groups (NaCl treatment).

At the end of three-month experimental period, the seedling were harvested for analysis. The growth parameters such as stem length, leaf area, and fresh and dry weight of leaves/stems were measured. Followingly, leaf and stem samples were separated and then they were oven-dried at 80 °C for 24 h, ground in a micro-hammer cutter and passed through a 1.5-mm sieve. Plant samples (0.5 g) were placed in Teflon vessels and then 8 ml 65% (v/v) nitric acid (HNO_3), 3 ml 37% (v/v) hydrochloric acid (HCl) and 2 ml 48% (v/v) hydrogen fluoride (HF) were added. Samples were mineralized in a microwave oven as follows: at 145 °C for 5 min., at 165 °C for 5 min. and at 175 °C

for 20 min. After cooling, the samples were filtered with Whatman filters (Macherey-Nagel, 640de/125 mm), and diluted to 50 ml with ultra pure water. Mineral elements boron, calcium, iron, potassium, magnesium, manganese, sodium, and zinc (B, Ca, Fe, K, Mg, Mn, Na, and Zn) were measured by Inductively Coupled Plasma Optical Emission Spectroscopy.

All calculations were based on the parameters including the concentrations of the elements of leaves and stems, fresh and dry weights of leaves, lengths of stems and leaf areas of both cotton varieties. Using IBM SPSS 20 statistical software, the multivariate analysis of variance (MANOVA) with Tukey's post-hoc honest significant difference (HSD) and Pearson correlation analyses were performed. Statistically significant levels are given as $^{**}p < 0.01$ and $^{*}p < 0.05$ (2-tailed).

Results and discussion

Figures 1–6 show the effects of NaCl concentration on the plant stem length, leaf area and fresh and dry weights of the two cotton cultivars. As shown in Figures 1–6 increasing NaCl concentration gradually inhibited the growth rate of both cultivars. The inhibition of growth pattern was similar in both cultivars but Cukurova 1518 showed a better tolerance to NaCl (Reduction rates in growth parameters were lower for Cukurova 1518 indicating better survival ability). Decreases were seen in the stem length of both cultivars (Figure 1). For leaf area, notable decreases were also observed for both cultivars (Figure 2). Overall there were significant decreases by ~65% for Cukurova 1518 and ~73.98% for Nazilli 84S in stem length and by ~84.53% for Cukurova 1518 and ~89.87% for Nazilli 84S in leaf area under severe salt stress (400 mM NaCl). Data obtained from Figure 1 and 2 revealed that stem lengths and leaf areas of both cultivars substantially decreased with the increase of NaCl concentrations compared with the control seedlings. After 90 days of NaCl exposure, growth rates decreased from 0.808 g to 0.144 g (~82.18%) and 0.963 g to 0.067 g (~93.04%) for leaf fresh weights and from 2.684 g to 0.312 g (~88.37%) and 3.064 g to 0.300 g (~90.2%) for stem fresh weights and from 0.174 g to 0.0355 g (~79.6%) and 0.161 g to 0.024 g (~85.09%) for leaf dry weights and from 0.600 g to 0.132 g (~78.0%) and 0.682 g to 0.190 g (~72.14%) for stem dry weights of Cukurova 1518 and Nazilli 84S, respectively (Figures 3–6). Also, for some growth parameters, there were fluctuations. For example, for stem dry weight, increases at low level (50 mM) of NaCl treatment (from 0.600 g to 0.732 g, ~22.0% for Cukurova 1518 and from 0.682 to 0.853 g, ~25.1% for Nazilli 84S) and reductions at high level (400 mM) of NaCl treatment (from 0.600 to 0.132 g, ~78.0% for Cukurova 1518 and from 0.682 to 0.190 g, ~72.1% for Nazilli 84S) were observed (Figure 6).

Previous studies showed that deleterious effects of salt ions on plant growth may result largely from altered metabolic activities, carbon-use efficiency, protein synthesis or enzymatic activities and

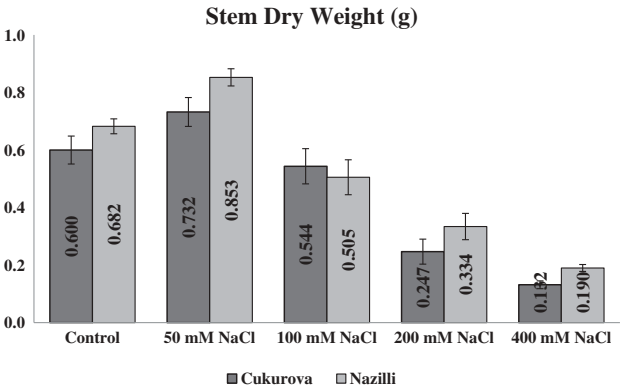

Figure 1. Stem lengths of cotton cultivars in different NaCl levels (0, 50, 100, 200 and 400 mM) in three months of growing period. According to the results of variance analysis and Tukey's test, the mean difference is significant at $p < 0.01$ (*) and $p < 0.05$ (**) levels.

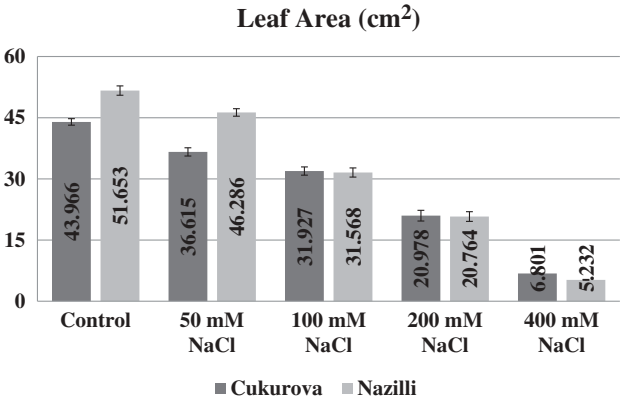

Figure 2. Leaf areas of cotton cultivars in different NaCl levels (0, 50, 100, 200 and 400 mM) in three months of growing period. According to the results of variance analysis and Tukey's test, the mean difference is significant at $p < 0.01$ (*) and $p < 0.05$ (**) levels.

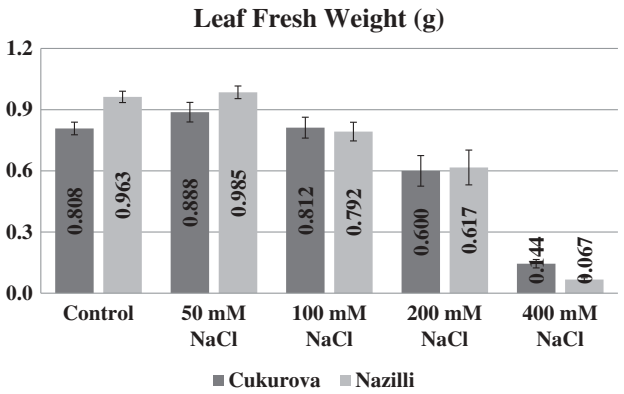

Figure 3. Leaf fresh weights of cotton cultivars in different NaCl levels (0, 50, 100, 200 and 400 mM) in three months of growing period. According to the results of variance analysis and Tukey's test, the mean difference is significant at $p < 0.01$ (*) and $p < 0.05$ (**) levels.

osmotic potential leading to disruption of cell wall extension and cellular expansion (Glenn, Brown, and Blumwald 1999; Munns 1993; Neumann 1997; Staple and Toenniessen 1984; Zhu 2001). Under osmotic stress, absorbing water and micronutrient uptake by the roots of plants are reduced, and root-to-shoot transportation of micronutrients becomes problematic due to the impaired active transport and membrane permeability, and restricted transpiration rates (Alam 1999; Pasternak 1987). Overall, NaCl is known to have negative effects on plant growth and our results are consistent with the information given above.

Table 1 shows Na^+ concentrations after 3 months in leaves and stems of the two cotton cultivars, Cukurova 1518 and Nazilli 84S, grown in different NaCl levels. Na^+ concentrations in Cukurova 1518 and Nazilli 84S increased dramatically with increasing NaCl levels. There was a difference in Na^+ concentrations among the leaves and stems of Cukurova 1518 (Table 1, see Na). The concentrations of Na^+ increased significantly in leaves (from 62.03 to 8698.5 mg/kg dw, ~140.23 fold) and stems (from 416.38 to 9608.2 mg/kg dw, ~23.08 fold) of Cukurova 1518 (from control to 400 mM treatment) (Table 1). Nazilli 84S exhibited a similar trend (from 164.9 to 24470.0 mg/kg dw, ~148.4 fold in leaves and from 352.7 to 18417.8 mg/kg dw, ~52.22 fold) (from control to 400 mM treatment) (Table 1). The increase in Na^+ concentrations between plants receiving no NaCl and those with

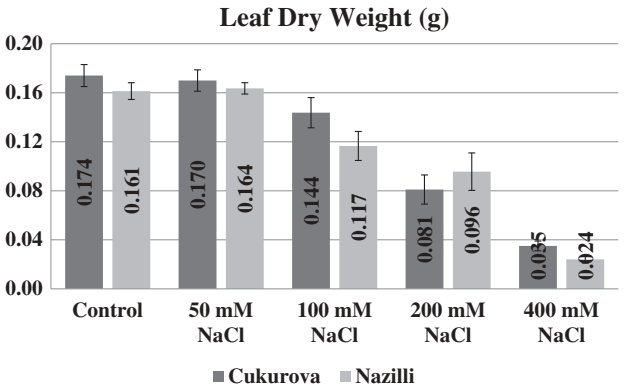

Figure 4. Leaf dry weights of cotton cultivars in different NaCl levels (0, 50, 100, 200 and 400 mM) in three months of growing period. According to the results of variance analysis and Tukey's test, the mean difference is significant at $p < 0.01$ (*) and $p < 0.05$ (**) levels.

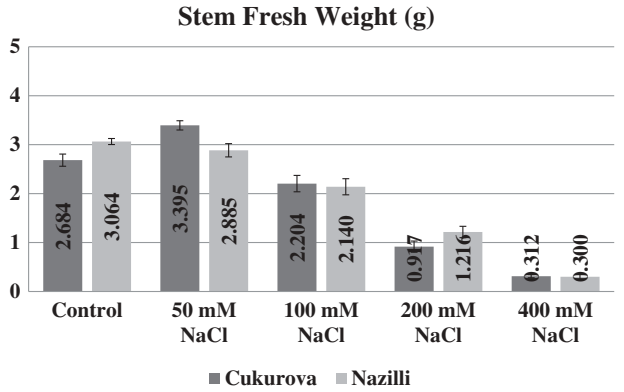

Figure 5. Stem fresh weights of cotton cultivars in different NaCl levels (0, 50, 100, 200 and 400 mM) in three months of growing period. According to the results of variance analysis and Tukey's test, the mean difference is significant at $p < 0.01$ (*) and $p < 0.05$ (**) levels.

400 mM NaCl were about 148 fold in leaves and 52 fold in stems of Nazilli 84S. Sodium accumulated excessively in leaf and stem parts of both cultivars, but the accumulation rates were different in two cultivars (Cukurova 1518 and Nazilli 84S) indicating the difference in the adaptive response to salinity of the cultivars. In Nazilli 84S, Na accumulated more rapidly with increasing amount of exogenous salt than the Cukurova 1518 (Table 1, see Na).

Mineral nutrient status of both cultivars was altered by salinity resulting in significant differences in the concentrations of mineral nutrients (Table 1). The concentrations of several mineral nutrients increased at a low level of NaCl but gradual reductions were observed with increasing NaCl concentrations in leaves and stems of the both cultivars. For example, for K, Mg, Zn, and B, following the increases at low levels of NaCl, reductions were observed in leaves of Nazilli 84S at higher levels of NaCl (from 14873.7 to 13428.6 mg/kg dw, ~9.72% for K, from 2242.1 to 1600.9 mg/kg dry weight (dw), ~28.6% for Mg, from 7.9 to 2.96 mg/kg dw, ~62.54% for Zn and from 10.8 to 4.3 mg/kg dw, ~60.19% for B) while a similar pattern was observed for Mg and Mn in leaves of Cukurova 1518 (from 2368.3 to 1502.7 mg/kg dw, ~36.55% for Mg and from 0.446 to 0.057 mg/kg dw, ~87.22% for Mn) (Table 1). Potassium, Ca, and Zn concentrations in leaves of Cukurova 1518 fluctuated. For example, reductions at the lowest level of NaCl were observed whereas increases were

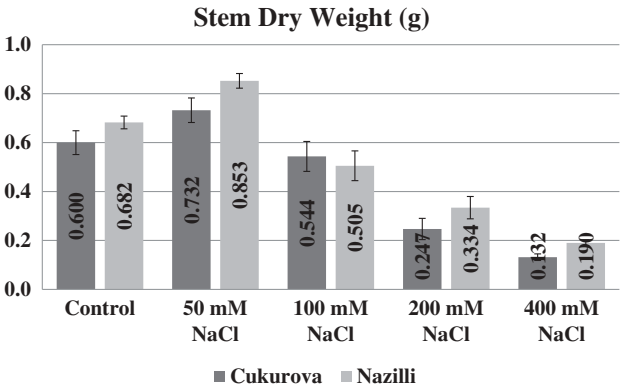

Figure 6. Stem dry weights of cotton cultivars in different NaCl levels (0, 50, 100, 200 and 400 mM) in three months of growing period. According to the results of variance analysis and Tukey's test, the mean difference is significant at $p < 0.01$ (*) and $p < 0.05$ (**) levels.

observed at the highest level of NaCl for Ca (Table 1). A similar pattern was noticed for Mn in leaves of Nazilli 84S and vice versa for Ca in leaves of Nazilli 84S. For K in leaves of Cukurova 1518, increases were seen at 50 and 200 mM NaCl levels whereas reductions were observed at 100 and 400 mM NaCl levels, comparing with the control. For B in leaves of Cukurova 1518, reductions were noticed at all levels of NaCl except 400 mM level, comparing with the control. Content of Fe was reduced by NaCl treatment, with the greatest reduction observed at higher levels of NaCl in leaves of both cultivars. Contents of Mg, Fe, Mn, Zn, and B in stems of both cultivars decreased in the presence of NaCI, with the greatest decrease observed at higher levels of NaCI. Increases in stem concentrations of K and Ca, were seen at all levels of NaCl in Nazilli 84S, relative to the control. Following the reductions at low level of NaCI, the increments were observed but at the highest level of NaCI, once again the reductions followed the increments in stems of Cukurova 1518.

It is thought that Na^+ is transported into plant cells passively via K^+ transport systems (Schroeder, Ward, and Gassmann 1994). There is an increase in Na^+ influx and a decrease in K^+ uptake during salt stress in plants. K^+ deficiency is considered to be a result of excessive uptake of Na^+ resulting in the inhibition of K^+ uptake into plant cells. Its reason could be that Na^+ ions are more available than K^+ ions in the influx transport systems. Besides, in high Na^+ and low K^+ soils, plants can accumulate Na^+ more than K^+. Thus, herein findings are corroborated by above-earlier reports. K^+ accumulation increased in leaves and stems until a point but after that point (400 mM NaCl level) reductions were seen in K concentration in both cultivars because of detrimental effects of Na. This demonstrates that Na^+ more favorably binds to the influx K^+ transport systems than K^+. Therefore, an increased level of Na^+ was observed in leaves and stems. The cells could act in preserving the K^+/Na^+ ratio to keep ionic strength and osmotic pressure in balance. So, K^+ transportation could have been increased from roots to leaves to counteract the limited K^+ uptake. Perhaps this is why K^+ concentration level was above the baseline (control) in cells.

There was fluctuation for Ca in leaves of Cukurova 1518. Reductions were observed at 50 mM (from 15.743 to 11.938 g/kg dw, ~24.17%) and 400 mM (from 16.787 to 12.054 g/kg dw, ~28.2%) NaCl whereas increases was observed at 100 mM (from 11.938 to 13.831 g/kg dw, ~15.86%) and 200 mM (from 11.938 to 16.787 g/kg dw, ~40.63%) NaCl and a similar trend was observed for Nazilli 84S (increases from 337.3 to 587.7 mg/kg dw, ~74.24% at 100 mM NaCl and from 376.4 to 659.2 mg/kg dw, ~75.13% at 400 mM NaCl and a reduction from 587.7 to 376.4 mg/kg dw, ~64.05%). There was a decrease in stems of Cukurova 1518 whereas there was an increase in stems of Nazilli 84S at all levels of NaCl. It is widely recognized that Ca^{2+} plays a regulatory role involving the passive entry of Na^+ and in K^+/Na^+ selectivity (Davies 2014; Ma et al. 2014). So, it is considered that the integrity of

Table 1. Concentrations of B, Ca, Fe, K, Mg, Mn, Na, and Zn (mg/kg DW) in leaf and stem samples of cotton cultivars grown in different NaCl (0, 50, 100, 200 and 400 mM) levels for three months.

			Control	50 mM NaCl	100 mM NaCl	200 mM NaCl	400 mM NaCl
B	Leaf	Cukurova 1518	14.620 ± 1.279^{a}	9.994 ± 0.134^{**b}	8.159 ± 0.759^{**c}	6.462 ± 0.333^{**d}	8.490 ± 0.522^{**cd}
		Nazilli 84S	10.291 ± 1.032^{a}	10.848 ± 1.343^{**b}	7.104 ± 0.188^{**c}	4.177 ± 0.197^{**d}	4.307 ± 0.233^{**cd}
	Stem	Cukurova 1518	5.346 ± 0.147^{a}	3.292 ± 0.546^{**b}	2.402 ± 0.484^{**c}	0.952 ± 0.033^{**d}	1.082 ± 0.078^{**cd}
		Nazilli 84S	3.597 ± 0.176^{a}	1.831 ± 0.085^{**b}	1.065 ± 0.092^{**c}	1.948 ± 0.287^{**d}	1.367 ± 0.082^{**cd}
Ca	Leaf	Cukurova 1518	15743.296 ± 858.641^{a}	11937.994 ± 239.582^{**b}	13831.245 ± 732.932^{ab}	16787.294 ± 823.630^{b}	12053.731 ± 856.441^{**c}
		Nazilli 84S	337.305 ± 22.265^{a}	378.445 ± 59.326^{**b}	587.733 ± 72.137^{ab}	376.394 ± 63.284^{b}	659.171 ± 43.511^{**c}
	Stem	Cukurova 1518	12810.099 ± 493.214^{a}	11270.712 ± 633.915^{**b}	11605.533 ± 784.809^{ab}	7968.391 ± 272.697^{b}	3210.204 ± 201.588^{**c}
		Nazilli 84S	255.824 ± 17.022^{a}	328.898 ± 24.531^{**b}	395.403 ± 30.972^{ab}	431.488 ± 23.909^{b}	518.800 ± 34.552^{**c}
Fe	Leaf	Cukurova 1518	19.996 ± 1.622^{a}	15.144 ± 1.412^{**b}	11.244 ± 1.468^{**c}	8.461 ± 1.047^{**d}	5.350 ± 0.203^{**d}
		Nazilli 84S	21.185 ± 0.418^{a}	16.005 ± 0.595^{**b}	15.620 ± 0.410^{**c}	9.138 ± 0.432^{**d}	8.260 ± 0.490^{**d}
	Stem	Cukurova 1518	5.589 ± 0.214^{a}	3.706 ± 0.259^{**b}	2.430 ± 0.211^{**c}	1.982 ± 0.272^{**d}	1.740 ± 0.033^{**d}
		Nazilli 84S	6.647 ± 0.125^{a}	5.146 ± 0.634^{**b}	4.683 ± 0.293^{**c}	4.021 ± 0.361^{**d}	3.551 ± 0.102^{**d}
K	Leaf	Cukurova 1518	9879.726 ± 542.506^{b}	10324.101 ± 410.855^{ab}	8742.123 ± 762.743^{**a}	10029.462 ± 590.234^{**a}	8698.507 ± 523.124^{*a}
		Nazilli 84S	10710.984 ± 109.250^{b}	11117.437 ± 599.775^{ab}	14873.723 ± 114.688^{**a}	14125.157 ± 503.654^{**a}	13428.571 ± 742.473^{*a}
	Stem	Cukurova 1518	4413.448 ± 431.096^{b}	4217.393 ± 398.794^{ab}	4837.536 ± 332.317^{**a}	3806.512 ± 310.689^{**a}	3586.122 ± 202.674^{*a}
		Nazilli 84S	8607.306 ± 673.434^{b}	12355.540 ± 796.154^{ab}	13350.852 ± 635.416^{**a}	14316.511 ± 451.230^{**a}	13902.222 ± 966.431^{*a}
Mg	Leaf	Cukurova 1518	2101.966 ± 135.661^{a}	1912.390 ± 72.764^{ab}	2368.296 ± 209.172^{ab}	2434.929 ± 296.383^{*b}	1502.687 ± 70.422^{**c}
		Nazilli 84S	2110.891 ± 99.702^{a}	2242.113 ± 105.295^{ab}	2072.709 ± 75.446^{ab}	1902.954 ± 104.378^{*b}	1600.922 ± 94.445^{**c}
	Stem	Cukurova 1518	761.886 ± 45.414^{a}	412.996 ± 33.354^{ab}	308.996 ± 53.870^{ab}	194.672 ± 18.664^{*b}	148.204 ± 8.441^{**c}
		Nazilli 84S	653.961 ± 46.890^{a}	367.925 ± 17.281^{ab}	251.868 ± 23.436^{ab}	184.958 ± 16.732^{*b}	114.667 ± 6.558^{**c}
Mn	Leaf	Cukurova 1518	2.495 ± 0.206^{a}	3.319 ± 0.147^{*b}	0.446 ± 0.097^{**c}	0.073 ± 0.003^{**c}	0.057 ± 0.002^{**c}
		Nazilli 84S	2.895 ± 0.265^{a}	0.968 ± 0.269^{*b}	1.402 ± 0.100^{**c}	2.322 ± 0.841^{**c}	1.674 ± 0.071^{**c}
	Stem	Cukurova 1518	0.683 ± 0.095^{a}	0.376 ± 0.033^{*b}	0.298 ± 0.076^{**c}	0.237 ± 0.044^{**c}	0.218 ± 0.011^{**c}
		Nazilli 84S	0.919 ± 0.041^{a}	0.416 ± 0.032^{*b}	0.395 ± 0.028^{**c}	0.338 ± 0.007^{**c}	0.284 ± 0.013^{**c}
Na	Leaf	Cukurova 1518	62.030 ± 4.660^{e}	605.933 ± 17.926^{**d}	2538.522 ± 316.413^{**c}	4829.123 ± 176.061^{**b}	8698.507 ± 512.644^{**a}
		Nazilli 84S	164.928 ± 8.805^{e}	1572.391 ± 90.190^{**d}	6342.324 ± 839.086^{**c}	11100.860 ± 598.226^{**b}	24470.046 ± 1132.558^{**a}
	Stem	Cukurova 1518	416.376 ± 8.819^{e}	2305.611 ± 323.214^{**d}	3303.157 ± 532.216^{**c}	6354.735 ± 605.887^{**b}	9608.163 ± 503.221^{**a}
		Nazilli 84S	352.707 ± 18.363^{e}	4679.750 ± 316.074^{**d}	8345.978 ± 346.237^{**c}	14339.602 ± 965.702^{**b}	18417.778 ± 996.311^{**a}
Zn	Leaf	Cukurova 1518	6.972 ± 0.457^{a}	6.473 ± 0.284^{a}	6.498 ± 0.259^{**b}	6.337 ± 0.265^{**c}	3.275 ± 0.162^{**d}
		Nazilli 84S	6.268 ± 0.670^{a}	7.914 ± 0.511^{a}	4.932 ± 0.764^{**b}	3.271 ± 0.176^{**c}	2.963 ± 0.122^{**d}
	Stem	Cukurova 1518	4.475 ± 0.500^{a}	2.807 ± 0.223^{a}	2.453 ± 0.114^{**b}	1.491 ± 0.214^{**c}	1.325 ± 0.074^{**d}
		Nazilli 84S	5.556 ± 0.325^{a}	4.472 ± 0.550^{a}	3.716 ± 0.225^{**b}	3.006 ± 0.283^{**c}	2.496 ± 0.106^{**d}

The mean difference is significant at 0.01 (**) and 0.05 (*) levels by the Tukey's test and multivariate analysis of variance (MANOVA). Means for group in homogeneous subsets are displayed. Based on observed means (Tukey HSD^{a,b,c,d}).

cell membranes is preserved through the maintenance of the cell levels of Ca^{2+}, allowing the change in K^+/Na^+ and the selective absorption of K^+ (Meloni et al. 2001). It seems that the ability of plants to retain Ca^{2+} is associated with their salt resistance. In our work, both of our cultivars kept the Ca^{2+} levels stable at all levels of NaCl, although there were reductions and increases. But, it was obvious that the accumulation amount in leaves and stems was much higher than Nazilli 84S for Cukurova 1518. For example, Ca concentration in leaves for Cukurova 1518 (15.743 g/kg dw) at control level was higher than Nazilli 84S (337.3 mg/kg dw) and similar data were seen for all other levels.

Nonselective cation channels in the plasma membranes, which allow passive fluxes of cations into the cells (Demidchik, Davenport, and Tester 2002; Demidchik & Maathuis, 2007; Very and Sentenac 2003) are typically permeable to a wide range of monovalent cations like Na^+ (Very and Sentenac 2003). These channels were also proposed by many studies as being a major pathway for Na^+ into the plants (Furini and Domene 2013) and many studies have confirmed the proposal (Demidchik and Tester 2002; Essah, Davenport, and Tester 2003; Maathuis and Sanders 2001). In our study, reductions were observed in concentrations of Mg, Fe, Mn, Zn, and B in leaves of both cultivars at high levels of NaCl but for Mg and Mn, following the increments at low level of NaCl, the reductions were seen at higher levels of NaCl in leaves of Cukurova 1518. A similar pattern was noticed for Mg, Zn, and B in leaves of Nazilli 84S. There were reductions in concentrations of Mg, Fe, Mn, Zn, and B in stems of both cultivars in the presence of NaCl. Once again, Na ions are considered able to bind to nonselective cation channels more favorably than other cations. Therefore, Mg, Fe, Mn, Zn, and B uptakes were highly disrupted in leaves and stems because of high Na^+.

The data for correlation coefficients between elements' concentrations in leaves and stems of both cotton varieties were given in Table 2. Based on the obtained data from the elements' concentrations in both leaves of Nazilli and Cukurova and the elements' concentrations in both stems of Nazilli and Cukurova, relative positive correlations (>0.52, >0.89) were found between B and Fe, and Mg, Mn and Zn, and Fe and B, and Mg, Mn and Zn, and Mn and B, and Zn and Fe. Relative negative correlations (->0.61, ->0.95) existed between B, Fe, Zn and Na were noticed. High correlations (>0.50, >0.79) were detected in Cukurova groups whereas negative correlations (->0.58, ->0.86) were detected in Nazilli groups for Fe and Ca, and Zn and Ca. The data showing negative relationship existed between Na and other elements (in Table 3) supports the data showing negative relationship existed between Na

Table 2. Correlation matrix of mineral nutrients in leaf and stem samples of cotton cultivars grown in different NaCl (0, 50, 100, 200 and 400 mM) levels for three months.

Correlation Matrix (R)								
Pearson Correlation	B NL	Ca NL	Fe NL	K NL	Mg NL	Mn NL	Na NL	Zn NL
B NS	.461*	−.553**	.610**	−.530**	.180	.630**	−.507**	.305
Ca NS	−.710**	.577**	−.864**	.441*	−.593**	−.294	.833**	−.746**
Fe NS	.594**	−.447*	.774**	−.311	.528**	.303	−.706**	.664**
K NS	−.607**	.324	−.750**	.559**	−.342	−.396	.556**	−.582**
Mg NS	.771**	−.501*	.865**	−.546**	.461*	.366	−.758**	.634**
Mn NS	.549**	−.456*	.800**	−.454*	.381	.450*	−.634**	.423*
Na NS	−.816**	.496*	−.950**	.513**	−.707**	−.140	.930**	−.760**
Zn NS	.594**	−.553**	.811**	−.434*	.719**	.078	−.762**	.610**
Pearson Correlation	B CL	Ca CL	Fe CL	K CL	Mg CL	Mn CL	Na CL	Zn CL
B CS	.716**	.112	.697**	.116	−.140	.672**	−.762**	.540**
Ca CS	.472*	.264	.772**	.115	.408*	.606**	−.927**	.791**
Fe CS	.790**	.120	.805**	.215	.098	.748**	−.748**	.521**
K CS	.225	−.074	.213	−.122	.201	.208	−.408*	.504*
Mg CS	.870**	.240	.892**	.213	.199	.674**	−.754**	.495*
Mn CS	.707**	.218	.644**	−.055	.020	.542**	−.616**	.427*
Na CS	−.617**	−.191	−.886**	−.385	−.330	−.751**	.950**	−.780**
Zn CS	.865**	.218	.848**	.244	.169	.662**	−.760**	.583**

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed) (NL: the leaves of Nazilli 84S, NS: the stems of Nazilli 84S, CL: the leaves Cukurova 1518, and CS: the stems of Cukurova 1518).

Table 3. Some growth parameters of cotton cultivars in different NaCl levels (0, 50, 100, 200 and 400 mM) in three months of growing period.

		Control	50 mM NaCl	100 mM NaCl	200 mM NaCl	400 mM NaCl
Stem Lenght (cm)	Cukurova 1518	16.638 ± 0.490^{a}	16.188 ± 0.565^{**ab}	12.800 ± 0.524^{**b}	8.575 ± 0.248^{**c}	5.675 ± 0.260^{**d}
	Nazilli 84S	16.375 ± 0.347^{a}	13.450 ± 0.117^{**ab}	10.213 ± 0.501^{**b}	8.175 ± 0.433^{**c}	4.260 ± 0.535^{**d}
Leaf Fresh Weight (gr)	Cukurova 1518	0.808 ± 0.031^{b}	0.888 ± 0.048^{a}	0.812 ± 0.051^{b}	0.600 ± 0.075^{**c}	0.144 ± 0.021^{**d}
	Nazilli 84S	0.963 ± 0.028^{b}	0.985 ± 0.031^{a}	0.792 ± 0.046^{b}	0.617 ± 0.085^{**c}	0.067 ± 0.008^{**d}
Leaf Dry Weight (gr)	Cukurova 1518	0.174 ± 0.007^{a}	0.170 ± 0.008^{a}	0.144 ± 0.012^{**b}	0.081 ± 0.010^{**c}	0.035 ± 0.004^{**d}
	Nazilli 84S	0.161 ± 0.007^{a}	0.164 ± 0.005^{a}	0.117 ± 0.012^{**b}	0.096 ± 0.015^{**c}	0.024 ± 0.003^{**d}
Stem Fresh Weight (gr)	Cukurova1518	2.684 ± 0.125^{ab}	3.395 ± 0.074^{a}	2.204 ± 0.168^{**b}	0.917 ± 0.114^{**c}	0.312 ± 0.032^{**d}
	Nazilli 84S	3.064 ± 0.021^{ab}	2.885 ± 0.135^{a}	2.140 ± 0.164^{**b}	1.216 ± 0.116^{**c}	0.300 ± 0.063^{**d}
Stem Dry Weight (gr)	Cukurova 1518	0.600 ± 0.049^{ab}	0.732 ± 0.050^{*a}	0.544 ± 0.061^{**b}	0.247 ± 0.043^{**c}	0.132 ± 0.011^{**d}
	Nazilli 84S	0.682 ± 0.026^{ab}	0.853 ± 0.030^{*a}	0.505 ± 0.061^{**b}	0.334 ± 0.046^{**c}	0.190 ± 0.012^{**d}
Leaf Area (cm^2)	Cukurova 1518	43.966 ± 0.801^{a}	36.615 ± 1.012^{**ab}	31.927 ± 1.006^{**b}	20.978 ± 1.306^{**c}	6.801 ± 0.112^{**d}
	Nazilli 84S	51.653 ± 1.146^{a}	46.28 ± 0.926^{**ab}	31.568 ± 1.124^{**b}	20.764 ± 1.197^{**c}	5.232 ± 0.098^{**d}

The mean difference is significant at 0.01 (**) and 0.05 (*) levels by the Tukey's test and multivariate analysis of variance (MANOVA). Means for group in homogeneous subsets are displayed. Based on observed means (Tukey HSD^{a,b,c,d}).

concentration and height, leaf—stem weights and leaf area of the cotton varieties (Note: explanation of the statistical results of Tables 1 and 2 (sentence by sentence) will be too long. Therefore, using Tukey's post-hoc tests for concentration groups will be enough for those who know the expression of Manova. The article is designed for carrying out support the relationship exists between elements).

Finally, overcoming salt stress, and achieving recovery and growth seem to necessitate more complex responses by plants. From our data, it could be said that the cotton var. Cukurova 1518 used in this work exhibited better salt tolerance. It seems that Ca is the most important mineral nutrient in establishing high degree salt tolerance and the amount of Ca in cells is critical in response to salt stress. The Ca accumulation was much higher in Cukurova 1518 than Nazilli 84S. Our data suggest that Ca may prevent excessive Na^+ accumulation in the cells. The accumulation rate of Na^+ was significantly higher in Nazilli 84S than Cukurova 1518.

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