

MINERAL ELEMENT DISTRIBUTION OF COTTON (*GOSSYPIUM HIRSUTUM* L.) SEEDLINGS UNDER DIFFERENT SALINITY LEVELS

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Abstract

Cotton (*Gossypium hirsutum* L.) is the world's leading natural fiber and second largest oilseed crop. In addition to textile manufacturing, cotton and cotton-by products are the sources of wealth of consumer based products, livestock feed, fertilizer, foodstuff and paper. High concentrations of NaCl in soils account for large decreases in the yield of a wide variety of crops all over the world. The present study was conducted to evaluate NaCl stress on mineral nutrient composition of cotton due to its economic importance. Cotton seeds were germinated in Magenta vessels containing Murshige and Skoog (MS) media for 15 days and then transferred in sterile jars containing MS exposed to different levels of NaCl (50, 100, 200 and 400 mM) treatments for 1 month. Uptake of some mineral nutrients (B, Ca, Fe, K, Mg, Mn, Na and Zn) by the plants was examined in roots and leaves by using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). The data proved that plant growth and uptake and accumulation of microelements are altered extensively in cotton grown with NaCl. Excess NaCl reduces the uptake pattern of certain elements and increases that of others, the patterns depending on the element and the plant part being compared to the control.

Introduction

Salinity is a very common stress condition to many plants. Survival, growth and development of plants are affected negatively due to salinity, which causes ion imbalance and hyperosmotic stress (Zhu, 2001). As a consequence of disturbed ion balance and plant-water relations resulting from lowered water potential and high amounts of sodium in the soil, water and mineral nutrition uptakes are disrupted in plants, leading to impairment of photosynthetic capacity (owing to stomatal closure and consequently limited CO₂ uptake) (Delfine *et al.*, 1999; Dolek *et al.*, 2001; Bor *et al.*, 2003) and cellular metabolic processes (related to the accumulation of Na⁺ and Cl⁻ or K⁺ and Ca²⁺ depletion) (Kingsbury & Epstein, 1986; Rengel, 1992; Perez-Alfocea *et al.*, 1996; Al-Karaki, 2000). Following these primary effects, secondary stresses such as oxidative damage (Fridovich, 1986; Wise & Naylor, 1987; Davis, 1987; McKersie & Leshem, 1994; McCord, 2000) linked to the production of toxic reactive oxygen intermediates like superoxide (O₂⁻), hydrogen peroxide (H₂O₂), singlet oxygen and hydroxyl radicals often occur, resulting in mutation, protein destruction, and peroxidation of membrane lipids (Dat *et al.*, 2000; McCord, 2000; Imlay, 2003). More importantly, salinity stress may inhibit cell division and expansion directly (Sharp *et al.*, 1988; Neumann, 1993; Frensch & Hsiao, 1994). Because of these changes at both the cellular and whole plant level, growth arrest (and even death) can be observed (Zhu, 2001). The volume of the reduction in growth depends on the species of plant, salinity level, and the ionic composition of the soil (Hurkman *et al.*, 1988).

Salt stress has been under intense study. Due to intense practices and irrigation, salinization of agricultural areas occurs and high concentrations of salts in soils account for large decreases in the yield of a wide variety of crops (Maiale *et al.*, 2004; Koca *et al.*, 2007; Sekmen *et al.*, 2007; Basal, 2010; Mahmood *et al.*, 2010). The most abundant salt found in the environments is NaCl

which competes with various nutrients resulting in nutrient deficiency and specific toxicity (Tester & Davenport, 2003) and because of this, NaCl is one of the biggest environmental threats to cultivated plants. Cotton is one of the most important fiber crops, which is widely cultivated throughout the world (Chachar *et al.*, 2008). In addition to textile manufacturing, it produces seeds with a potential multiproduct base such as hulls, oil, linters and food for animals (Song & Yamaguchi, 2003). The distribution of cotton species is world-wide and wild species are found in all the continents except Europe. The genus *Gossypium* consists of 51 species (Sun *et al.*, 2006), distributed in tropical and subtropical regions.

It has been known for many years that there are large differences in salt tolerance between species of crop species (McKersie & Leslem, 1994). Although cotton is classified as a salt tolerant crop (Reinhardt & Rost, 1995), its growth and yield are severely inhibited in higher salinity soil, especially at the germination and emergence stages (Ashraf, 2002). Comparisons among the cotton species have shown varietal differences in the levels of salt tolerance (Lauchi *et al.*, 1981; Gossett *et al.*, 1992, 1994).

The growth, uptake and accumulation of mineral nutrients are altered extensively in plants grown under salinity stress. Plant growth is reduced when essential mineral nutrients become limited or are in excess (Clark, 1970). The reduction of plant growth due to salinity could be the indirect consequence of its influence on the metabolism of mineral nutrients, resulting in nutrient imbalance and physiological disorders. Hence, the aim of this study was to investigate the effects of salinity on mineral nutrition uptake of cotton. A better understanding of mineral nutrition uptake responses under salinity stress may help us to take account possible measurements of water relations and mineral composition on salt stress tolerance.

Materials and Methods

Seeds of cotton var. Nazilli 84S were obtained from Nazilli Cotton Research Institute, Aydin-Turkey. Before surface sterilization, cotton seeds were kept under flowing tap water for 1 h. They were surface sterilized by immersion in 70% ethanol (Sigma Chemical Co.) for 3 min, followed by stirring in 20% commercial bleach (ACE Lever Co.) for 20 min. The surface sterilized seeds were rinsed 3 times with sterile distilled water for 5 min and they were dried on filter papers. Seed coats were removed with a sterile scalpel and tweezers prior to germination. The seeds were germinated on hormone free-MS (Murashige & Skoog, 1962) medium. The medium contained 1 mL MS vitamin solution (Sigma Chemical Co.), 30 g sucrose (Sigma Chemical Co.) and 2.2 g phytigel (Sigma Chemical Co.). The pH of the media was adjusted to 5.7 with 1 M NaOH (Merck) before autoclaving. After autoclaving, 4.3 g basal salt mixture (Sigma Chemical Co.) sterilized by micro filter was added into MS media. 20 mL MS media was poured into Magenta vessels (Sigma Chemical Co.) and 1 seed was germinated in each Magenta vessel. Seeds were kept in a growth chamber with a photoperiod of 16 h light (7500 lux) and 8 h dark, at 25°C and 70% humidity. 15 days into the germination period, the young plants were transferred into sterile jars with MS (250 mL) containing different levels of NaCl (0, 50, 100, 200 and 400 mM) for an incubation period of 1 month.

Plant parts (roots, stems and leaves) were isolated and oven-dried at 80°C for 24 h, milled in micro-hammer cutter and fed through a 1.5-mm sieve. Samples were weighed as 0.5 g and transferred into Teflon vessels and then 8 ml 65% HNO₃, 3 ml 37% HCl and 2 ml 48% HF (Merck) was added. Samples were mineralized in a microwave oven (Erghof-MWS2) as follows: in 145°C for 5 min, in 165°C for 5 min and in 175°C for 20 min. After cooling, the samples were filtered by Whatman filters, and made up to 50 ml with ultra pure water in volumetric flasks and then stored in falcon tubes. Standard solutions were prepared by using multi element stock solutions-1000 ppm (Merck) and mineral element (B, Ca, Fe, K, Mg, Mn, Na and Zn) measurements were done by Inductively Coupled Plasma Optical Emission Spectroscopy (PerkinElmer-Optima 7000 DV).

The standard error values of the means were calculated to compare the site categories. Statistical analysis was performed using a one-way ANOVA (for $p < 0.05$). Based on the ANOVA results, a Tukey test for mean comparison was performed, for a 95% confidence level, to test for significant differences among treatments.

Results

Table 1 shows B, Ca, Fe, K, Mg, Mn, Na and Zn (mg/kg dw) concentrations in roots, stems and leaves of cotton var. Nazilli 84S grown in different NaCl levels (0, 50, 100, 200, and 400 mM NaCl). Na concentration in cotton increased dramatically with increasing NaCl levels. There was a slight difference in Na concentrations among the roots and stems whereas in leaves it was lower than roots and stems at all levels of NaCl treatments. The concentration of Na was increased significantly in roots and stems, and to a less extent in leaves at all levels of NaCl treatments. The degree of increments increased with

the increasing level of NaCl treatment in roots, stems and leaves. The data shows that Na itself mainly accumulated in all parts of the plant suggesting that the roots, stems and leaves are the sites of Na accumulation and large amounts of Na were transported into the leaves.

The effects of salinity stress on mineral nutrient concentrations in cotton var. Nazilli 84S seedlings:

In cotton seedlings grown under different NaCl levels, the concentrations of some micronutrients were examined in roots, stems, and leaves at one month of NaCl exposure. It is clear from the results that micronutrient composition in roots, stems and leaves was altered by NaCl. The micronutrient concentrations in cotton leaves, stems and roots are shown in Table 1. There existed significant differences in the accumulation of some micronutrients in roots, stems and leaves of cotton seedlings under NaCl stress. The concentration of several micronutrients was reduced by the presence of NaCl in roots, stems and leaves of cotton seedlings. Root, stem and leaf concentrations of Fe, Mn, Zn, and B were reduced by the NaCl treatment, with the greatest reduction observed at higher levels of NaCl (Table 1). Contents of Ca and Na in roots, stems and leaves were increased in the presence of NaCl, with the greatest increase observed at higher levels of NaCl (Table 1). The concentration of micronutrient K was reduced in roots and stems while it was actually increased in leaves in the presence of NaCl (Table 1). For micronutrient Mg, an increase was observed in roots whereas in stems and leaves a reduction was observed at all levels of NaCl treatment (Table 1). For K and Mg, the reductions and increments in roots, stems and leaves showed an increase with increasing NaCl level, comparing with the controls.

Discussion

High salt concentrations in soils are the result of high evaporative demand during the dry periods and insufficient leaching of ions due to the low precipitation (Kerepesi & Galiba, 2000; Meloni *et al.*, 2003). Excessive salt concentrations cause inhibition of plant development and finally lead to plant death due to salt ion accumulation, K⁺/Na⁺ ionic imbalance and osmotic stress. Salt ions can have deleterious effects on plasma membranes or after uptake into cytoplasm, may alter metabolic activities such as photosynthesis and respiration, decrease carbon-use efficiency, and inhibit protein synthesis or modify enzymatic activities (Flowers & Yeo, 1995; Neumann, 1997; Glenn *et al.*, 1999; Zhu, 2001). Besides ion toxicity, secondary effects are related to lowering osmotic potential of the soil solution that reduces plant-available water. Lowered osmotic potential may lead to inhibition of cell wall extension and cellular expansion (Staple & Toenniessen, 1984). As a consequent of osmotic stress, plants have difficulty in absorbing water, difficulty with the uptake of micronutrients by the roots and difficulty transporting micronutrients from the roots to the shoots due to restricted transpiration rates and impaired active transport and membrane permeability (Viets, 1972; Pasternak, 1987; Alam, 1999). Also, the diffusion rate of nutrients is dependent on soil moisture. Absorbing root surface shows decline in the diffusion rate of nutrients when soil moisture drops gradually (Pinkerton & Simpson, 1986; Alam, 1999).

Table 1. Concentrations of B, Ca, Fe, K, Mg, Mn, Na and Zn (mg/kg dw) in leaf, stem and root samples of cotton var. Nazilli 84S grown in different NaCl (0, 50, 100, 200 and 400 mM) levels for 1 month.

According to the results of variance analysis and Tukey test, the mean difference is significant at $p < 0.01$ (*) and $p < 0.05$ (**) levels.

		Control	50 mM NaCl	100 mM NaCl	200 mM NaCl	400 mM NaCl
B (mg/kg)	Leaf	7.202 ± 0.277**	6.565 ± 0.252**	5.934 ± 0.228**	4.672 ± 0.180**	4.087 ± 0.157**
	Stem	2.171 ± 0.084**	1.845 ± 0.071**	1.528 ± 0.059**	1.287 ± 0.050**	1.049 ± 0.040**
	Root	4.680 ± 0.180**	1.938 ± 0.075**	1.546 ± 0.059**	1.213 ± 0.047**	1.069 ± 0.041**
Ca (mg/kg)	Leaf	2083.721 ± 130.233**	2846.809 ± 177.926**	2997.531 ± 187.346**	3103.817 ± 193.989**	3975.000 ± 248.438**
	Stem	1969.730 ± 123.108**	2833.645 ± 177.103**	3157.303 ± 197.331**	3396.239 ± 212.265**	3737.736 ± 233.609**
	Root	1112.500 ± 69.531	1372.211 ± 85.763**	1412.784 ± 88.299**	1615.733 ± 100.983**	1751.667 ± 109.479**
Fe (mg/kg)	Leaf	48.419 ± 3.725**	38.809 ± 2.985**	34.650 ± 2.665**	27.305 ± 2.100**	21.981 ± 1.691**
	Stem	56.216 ± 4.324**	38.822 ± 2.986**	28.283 ± 2.176**	22.000 ± 1.692**	19.962 ± 1.536**
	Root	995.750 ± 35.563*	670.947 ± 23.962*	312.577 ± 11.163*	212.000 ± 7.571*	165.250 ± 5.902**
K (mg/kg)	Leaf	575.581 ± 35.974**	1076.596 ± 67.287**	1409.053 ± 88.066**	2213.359 ± 138.335**	2848.077 ± 178.005**
	Stem	7859.459 ± 224.556**	6342.056 ± 181.202**	5896.629 ± 168.475**	5317.949 ± 151.941**	5018.868 ± 143.396**
	Root	3997.500 ± 114.214**	3427.368 ± 97.925**	2856.907 ± 81.626**	2490.667 ± 71.162**	2085.833 ± 59.595**
Mg (mg/kg)	Leaf	801.628 ± 33.401**	610.638 ± 25.443**	543.210 ± 22.634**	401.527 ± 16.730**	365.962 ± 15.248**
	Stem	765.405 ± 31.892*	691.215 ± 28.801*	597.978 ± 24.916**	560.342 ± 23.348**	471.321 ± 19.638**
	Root	649.500 ± 27.063**	798.526 ± 33.272**	1075.258 ± 44.802**	1136.533 ± 47.356**	1225.583 ± 51.066**
Mn (mg/kg)	Leaf	136.977 ± 5.707**	108.823 ± 4.534**	105.267 ± 4.386**	70.527 ± 2.939**	63.135 ± 2.631**
	Stem	42.141 ± 3.242**	28.467 ± 2.190**	24.090 ± 1.853**	20.855 ± 1.604**	17.151 ± 1.319**
	Root	53.775 ± 4.137**	25.432 ± 1.956**	24.412 ± 1.878**	18.952 ± 1.458**	17.483 ± 1.345**
Na (mg/kg)	Leaf	112.233 ± 3.301*	5531.915 ± 131.712*	6106.996 ± 119.745*	7503.817 ± 153.139*	9471.154 ± 197.316*
	Stem	389.189 ± 11.447**	7233.645 ± 172.230*	11550.562 ± 226.482*	13880.342 ± 283.272*	16396.226 ± 341.588**
	Root	464.000 ± 13.647**	8042.105 ± 191.479*	9711.340 ± 190.418*	13893.333 ± 283.537*	18116.667 ± 377.431*
Zn (mg/kg)	Leaf	48.674 ± 3.744**	31.574 ± 2.429**	27.877 ± 2.144**	24.328 ± 1.871**	22.769 ± 1.751**
	Stem	56.270 ± 4.328**	53.738 ± 4.134**	37.978 ± 2.921**	31.060 ± 2.389**	28.000 ± 2.154**
	Root	130.825 ± 5.451**	90.189 ± 3.758**	84.887 ± 3.537**	72.267 ± 3.011**	67.750 ± 2.823**

Table 1 shows concentrations of B, Ca, Fe, K, Mg, Mn, Na, and Zn (mg/kg dw) in cotton grown at 0, 50, 100, 200 and 400 mM NaCl. The present study showed that micronutrient status of roots, stems and leaves was altered by NaCl stress.

In the present study, concentration of K^+ was increased in leaves but decreased in stems and roots at all levels of NaCl treatments in cotton seedlings. K^+ is the major solute contributing to osmotic pressure and ionic strength. Most cells maintain relatively high K^+ and low Na^+ concentrations in the cytosol. This is achieved through coordinated regulation of transporters for H^+ , K^+ and Na^+ . A family of P-type H^+ -ATPases performs as the primary pump that establishes a proton motive force on the plasma membrane so that active transport of solutes including Na^+ and K^+ can be carried out (Sze *et al.*, 1999). A number of K^+ channels and transporters have been recognized (Sentenac *et al.*, 1993; Nakamura *et al.*, 1995; Rubio *et al.*, 1995; Hirsch *et al.*, 1998; Gaymard *et al.*, 1998; Kim *et al.*, 1998; Fu & Luan, 1998). Na^+ is taken up into plant cells passively, presumably through K^+ transport systems (Schroeder *et al.*, 1994). There is an increase in Na^+ influx whereas in K^+ uptake there is a decrease during salt stress in plants. K^+ deficiency occurs as a result of external Na^+ by inhibiting K^+ uptake into plant cells. The reason could be that Na ions occupy the influx transport systems more than K ions. So, plants accumulate much more Na^+ instead of K^+ in such soils containing high Na^+ and low K^+ . On the other hand, Na^+ efflux is accomplished by the activities of Na^+/K^+ antiporters found on the plasma membrane. Much of Na^+ enters the cell is resulted in compartmentalization of Na^+ achieved through the action of vacuolar Na^+/H^+

antiporters (Gaxiola *et al.*, 1999; Apse *et al.*, 1999). Hence, the toxic effect of Na^+ accumulation in the cytosol is reduced by its sequestration into the vacuole. Also, because of an increased level of cytosolic Ca^{2+} , the activity of K^+ influx channels in the plasma membranes may be reduced at pH 5 (Clarkson *et al.*, 1988; Evans *et al.*, 1991). Our results are consistent with the information given above. K^+ entrance was decreased in roots and the amount of the K^+ was inversely proportional to increasing NaCl treatments. This shows that Na^+ binds to the influx K^+ transport systems more avidly than K^+ . Therefore, an increased level of Na^+ was observed in roots, stems and leaves, and excess Na^+ accumulated in vacuoles. The cells could take action to preserve the K^+/Na^+ ratio, which is important for keeping osmotic pressure and ionic strength in balance. So, K^+ transportation could have been increased from roots to leaves to counteract the limited K^+ uptake.

Passive fluxes of cations into the cells are achieved through nonselective cation channels found on plasma membranes (reviewed by Hedrich & Schroeder, 1989; Tester, 1990; White, 1998; Very & Sentenac, 2003). Nonselective cation channels typically are permeable to a wide range of monovalent cations like Na^+ (Very & Sentenac, 2003). They have been proposed to form a major pathway for Na^+ into plants (Tyerman *et al.*, 1997) and the many studies have confirmed the proposal (Maathuis & Sanders, 2001; Demidchik & Tester, 2002; Essah *et al.*, 2003). In our study, significant reductions were observed in concentrations of Fe, Mn, Zn, and B in the presence of NaCl. Once again, the reason could be that Na ions bind nonselective cation channels more avidly than other cations. Therefore, Fe, Mn, Zn and B

uptake was highly disrupted in roots, stems and leaves because of high Na^+ . Concentration of Mg was increased in roots but decreased in stems and leaves at all levels of NaCl in cotton seedlings. The pH and Ca content of the surrounding soil influence the uptake of Mg by plants grown under field conditions (Christenson *et al.*, 1973; Marschner, 1995). This may explain the increased level of Mg^{2+} in roots and concurrent reductions in stems and leaves observed in our study. Mg^{2+} has many functions including contribution to structural stability of the plasma membrane, governing permeability and transport and osmotic and ion balance (Clarkson & Hanson, 1980). In order to protect and maintain their function, plants might increase the amount of Mg^{2+} in root cells by activation of certain mechanisms.

Because of its role in membrane and stomatal functions, cell division and cell-wall synthesis, cellular stress recovery, rates of respiratory metabolism (McLaughlin & Wimmer, 1999), Ca is especially an important nutrient in plants. Increased cytoplasmic Ca regulates hyperosmotic stress and salt stress responses in plants (Knight *et al.*, 1997). One mechanism of Ca increase is based on activation of Ca channels in the plasma membrane (Blatt, 2000). Ca binds to the plasma membrane and by that it controls the permeability of the plasma membrane and prevents Ca efflux from the cells (Rengel, 1992). Concentration of Ca was significantly increased in roots, stems and leaves at all levels of NaCl treatments in cotton seedlings in the present study. Plants subjected to salt stress display complex responses including the increment of Ca^{2+} . Salinity stress induces transient Ca influx into the cell cytoplasm by either influx from the apoplastic space or release from internal stores (Sanders *et al.*, 1999; Knight, 2000). Unno *et al.*, (2002) reported that in the salt sensitive plants under salt stress the concentration of Ca was decreased in the shoots while a reduction in Ca concentration was not observed in the salt tolerant plants, suggesting that the ability of plants to retain Ca^{2+} is associated with their salt resistance. Also, this is consistent with our results. Contradictory to our results, numerous studies reported that NaCl application decreased the concentration of Ca in roots and shoots of several species (Lynch & Lauchli, 1985; Maas & Grieve, 1987; Grieve & Maas, 1988; Cramer, 1992; Awada *et al.*, 1995; Hu & Schmidhalter, 1997; Meloni *et al.*, 2001; Unno *et al.*, 2002; Netondo *et al.*, 2004).

Re-establishing homeostasis in plants is achieved by either preventing Na^+ accumulation in cytosol or in organelles other than vacuoles or managing Na^+ efflux to a lower concentration in high salinity environment (Jeschke, 1984; Pitman, 1984). Accomplishing salt stress survival, recovery and growth by plants depends on displaying complex responses. From our data, it could be said that the cotton var. Nazilli 84S used in this work exhibited salt tolerance in some degree and Ca^{2+} , K^+ and Mg^{2+} played significant roles in the response to salt stress. The results of this study suggest that a new fertilizer can be designed to support proper growth of our species in such a saline environment. Addition of Ca^{2+} , K^+ and Mg^{2+} into fertilizer may facilitate the growth of cotton plants such as those used in our study under saline conditions along with a suitable irrigation regime.

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