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IONOMIC AND ANTIOXIDANT SYSTEM RESPONSES TO Na_2SO_4 STRESS OF TWO COTTON CULTIVARS DIFFERING IN SALT TOLERANCE

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Abstract

Understanding the impact of salt stress on cotton growth, the antioxidant system, and ionomic responses is of great importance for maintaining cotton productivity and sustainability in arid regions. In this study, we investigated the physiological and ionomic responses of different cotton cultivars (salt-sensitive X45 and salt-tolerant L24) to Na_2SO_4 stress under greenhouse conditions. We found a low concentration of Na_2SO_4 mainly inhibited cotton shoot growth, whereas high concentrations of Na_2SO_4 significantly inhibited cotton shoot and root growth. The growth inhibition rate of Na_2SO_4 stress was higher for X45 than for L24. Na_2SO_4 stress increased relative electrical conductivity (REC) and malondialdehyde (MDA) and proline (Pro) contents. The REC and MDA content in L24 leaves were significantly lower than those in X45 leaves. As the Na_2SO_4 stress increased, the SOD, POD, and CAT activities increased first and then decreased; Pro in L24 continued to increase, but in X45, it first increased and then decreased. The activities of SOD, POD, and CAT, and the PRO content in L24 leaves were significantly higher than those in X45. The contents of Ca, Cu, B, and Mo in roots and Ca in leaves in both cultivars decreased significantly; L24 stored Na in cotton leaves under salt stress and transported more mineral elements to leaves (P, Cu, Ca, Mg, Zn, and B). However, X45 tended to accumulate more mineral elements in roots (Ca, Fe, and Mo). Correlation analysis showed that cotton mainly absorbed mineral elements in roots to resist Na_2SO_4 stress. It was concluded that cotton cultivars showed different reaction to Na_2SO_4 stress, and salt-tolerant cotton has a stronger antioxidant enzyme system, which allows ion homeostasis through root absorption of more mineral elements.

Key words: Salt stress, Cotton growth, Antioxidant system, Osmotic adjustment, Ionomics.

Introduction

Soil salinization is a global ecological problem and is one of the most serious abiotic stress factors limiting crop production. It is estimated that salinized soil covers 1–10 billion ha in more than 100 countries (Jesus *et al*., 2015; Qadir & Oster, 2002), exceeding 7% of the global land area and with a potential increase of 10–16% per year (Aydemir & Sünger, 2011; Panta *et al*., 2014), Therefore, utilization og salt-affected soils has become a vital aspect in sustainable agricultural development. Salinized soils are not only chlorinated but also sulfated. Although NaCl stress is one of the most common types of salt stress, the salt composition is mainly sulfate in the saline soil area of northwestern China. In addition, secondary pollutants formed due to the weathering of the earth's crust, increased industrial SO_2 emissions, factory effluent discharge, and application of soil amendments (such as gypsum) will lead to an increase in soil sulphate. The damage caused by salt stress to crop growth includes osmotic stress, nutrient and hormone imbalance, ion toxicity, oxidative damage, cell membrane structure damage, production of toxic metabolites, reduced nutrient uptake, and ultimately crop death (Ahmad *et al*., 2013). Most reports have focused on the effect of NaCl stress on crop growth (Moud & Maghsoudi, 2008; Hussain *et al*., 2012; Hassan *et al*., 2014); however, few studies were conducted on the effects of Na_2SO_4 on crop growth.

Crop salt tolerance involves a range of defense mechanisms, for example, ionic homeostasis, osmotic equilibrium, and reactive oxygen species scavenging (Endler *et al*., 2015; Gupta & Huang, 2014). In high salinity environments, crops respond to nutritional disequilibrium by regulating ion transport and maintaining ionic homeostasis. This in turn produces osmotic regulators, such as proline and betaine to resist ions and osmotic stress, protect cell redox homeostasis against salt-induced ROS stress, and participate in some non-enzymatic antioxidant and enzymatic antioxidant reactions (Mostofa *et al*., 2015). Although different crops have different methods and mechanisms for salt tolerance, maintaining a stable intracellular mineral ionic content (ion homeostasis) is the key mechanism for crops to adapt to salt stress (Xu *et al*., 2014). Some reports showed that the physiology of salt-tolerant plants was associated with mineral nutrition, and the mechanisms of absorption, distribution, and regulation of different ions by plants should be studied from the perspective of mineral nutrition (Cheeseman, 1988). Salt stress destroys the equilibrium state between the water potential relationship and ion distribution between crops and soils, thus leading to nutrient imbalances and cell metabolism disorders (Munns & Tester, 2008; Tavakkoli *et al*., 2010; Sall *et al*., 2015). This in turn seriously hinders the normal development of plants (Ali *et al*., 2001). Salt stress not only inhibits macroelement absorption by crops but also limits the absorption of trace elements (Wu *et al*., 2013). Although each element has its unique physiological function in crop growth, its main function is to maintain intracellular ionic homeostasis. Maintaining intracellular ionic homeostasis is an important mechanism for crops to adapt to salt stress, and all of the physiological activities associated with salt tolerance in crops are aimed at maintaining ionic homeostasis. Therefore, in the process of plant mineral nutrient absorption under salt stress, the ionic homeostasis mechanism of crops remains an important aspect for revealing the mechanism of salt tolerance in crops.

Cotton is considered a pioneer crop for the development and utilization of salt-alkali soil due to its relatively high salt tolerance ability. Under salt stress conditions, Na enters the plant cells through K channels and K transport proteins, thus destroying the original ionic homeostasis in plants (Zhang *et al*., 2013a). Cotton responds to salt stress by maintaining the balance of Na and K ions in tissues; Na ions inhibit K uptake by plants through competitive action (Rodriguez-Navarro, 2000). Na can replace K at the binding sites, thus inhibiting the normal metabolism of plants. As more Na accumulates in the shoots than in the roots, it becomes more sensitive to salt stress conditions (Shabala *et al*., 2010; Sanchez *et al*., 2011). In addition to K, Ca and Mg are also important for improving cotton salt tolerance (Dogan *et al*., 2012; Rabhi *et al*., 2018). However, Severino *et al*., (2014) showed that Ca and Mg could not reduce Na toxicity at the seedling stage of cotton. Salt tolerance in plants is the result of the interaction of all ions. Although many studies have been carried out on the response of cotton to salt stress and the improvement of salt tolerance by mineral elements (Dai *et al*., 2014; Habib *et al*., 2014), but most of these have focused on the effects of salt stress on one or several mineral elements, and understanding the responses to other elements and their relationship with salt stress is still fragmented and incomplete.

Ionomics refers to mineral nutrients and trace elements in plants and is used to characterize the inorganic components of cells and biological systems. To adapt to salt stress, cotton must change the ionome of cells to achieve a new equilibrium. Therefore, clarifying the ionomic response characteristics of crops under Na_2SO_4 stress remains the basis to understand the mechanism of ionic homeostasis in cotton. In our study, the effect of Na_2SO_4 stress on: (1) physiological indexes of salt tolerance (REC, MDA, and Pro) and biochemical indexes (SOD, POD, and CAT activities) of cotton, the mechanism of organic osmotic regulation and enzyme protection in cotton under different Na_2SO_4 stress level were analyzed, and the physiological mechanism of salt tolerance in different salt-tolerant cotton genotypes was discussed, and (2) the distribution of the main mineral elements in cotton plants (in roots, stems, and leaves) revealed the response characteristics of the cotton ionome under Na_2SO_4 stress, as well as the correlations between Na and other elements, providing a theoretical basis for the ionic regulation and rational fertilization of cotton in saline soil.

Materials and Methods

Materials: The experiment was carried out in the greenhouse of the experimental station of the College of Agriculture of Shihezi University, China in 2017. The experimental soil was collected from the experimental station, and the depth of the soil was 0-30 cm. The soil type used was grey desert soil with a loam texture, and the basic soil properties were as follows: salt content: 0.53 $g \cdot kg^{-1}$; pH: 8.16; total nitrogen: 0.57 $g \cdot kg^{-1}$; organic matter: 6.77 $g \cdot kg^{-1}$; available phosphorus: 7.21 $mg \cdot kg^{-1}$; available potassium: 182 $mg \cdot kg^{-1}$. The two cotton cultivars used were Xin-lu-zao No. 45 (salt-sensitive cotton, X45) and Lu-mian-yan No. 24 (salt-tolerant cotton, L24).

Experimental design: The experiment consisted of three soil salinity levels [0.17, 1.07, and 2.01 dS m^{-1} (EC1:5), representing CK, SL, and SH, respectively]. The detailed steps are as follows: different concentrations of Na_2SO_4 solutions were added to soil that was naturally dried, crushed, and passed through 2-mm sieves (the salt/dry soil weight ratio was 0, 0.30, and 0.60%, respectively) to produce a supersaturated state (the same volume of deionized water was added to the control plants) for 1 month to achieve the balance of the soil; three of these salt soils were formed. Then, the soil with the three salinity levels was naturally dried, crushed and passed through 2-mm sieves. A 20-cm diameter and 60-cm height soil column was used. The experimental soil was layered at 50-cm depth according to the soil bulk density of 1.25 g cm^{-3}, with 10 cm for each layer, and 20 kg for each soil column. Each treatment had 6 replications. The drip irrigation method was adopted; the dripper-flow rate was 2.1 $L \cdot h^{-1}$, and the dripper spacing was 40 cm. The drip irrigation pipe was laid flat on the soil column, with each soil column supplied by an emitter, and the emitter was fixed at the center of the top of the soil column.

Cotton sowing started on May 6, 2017, and 20 seeds were sown in each soil column. In order to ensure cotton emergence, each soil column was irrigated with 3 L of water after sowing. When the cotton seedlings grew to the "2 leaves and 1 heart" stage, 2 cotton seedlings with uniform growth were retained in each soil column. To ensure water supply, water replenishment was conducted at regular intervals during the experiment so that the soil moisture content was maintained at 60%–80% of field capacity. After sowing, the 60-day experiment was considered to be complete.

Sample collection and treatment: Sixty days after cotton seedling emergence, all of the samples of functional leaves on the main stem (the 3rd leaves on the main stem were completely unfolded) were used for each treatment and were placed in an ice box for transport to the laboratory. The dust and dirt on the surface of the leaves were removed, the moisture on the surface was wiped with an absorbent paper, and the main vein was removed. The REC of leaves, MDA, Pro, SOD, POD, and CAT were measured. The REC of leaves was measured by the conductance method. The MDA and Pro content were measured according to the methods as described by Wu *et al*., (2012) and Bates *et al*., (1973). The activities of SOD, POD, and CAT were measured according to the Becana *et al*., (1998), Tan *et al*., (2008), and Cakmak & Marschner (1992), respectively.

When the dry matter of cotton was sampled and measured, 3 representative cotton plants were selected for each treatment. The samples (roots, stems, and leaves) were separated and were green killed at 105°C. After 30 minutes, they were dried at 70°C in an oven to bring them to a constant weight, and then the dry matter was weighed.

The specific steps of plant ionomics are as follows: the roots, stems and leaves were crushed and passed through sieves. Then, 0.25 g of each sample was taken, and 10 ml concentrated nitric acid was added, followed by digestion in a microwave digestion instrument (Milestone, ETHOS A). After microwave digestion, the samples were placed on a 230°C electric heating plate for acid driving for about 20 minutes. After the digestion tank was removed, the solution was then transferred to a 25-ml colorimetric tube with ultrapure water. The microwave digestion tank and the lid were rinsed 3–5 times, the buffer solution was transferred to a colorimetric tube, diluted to volume, and then shaken evenly. The ion contents (Na, P, K, Ca, Mg, S, Fe, B, Mn, Zn, Cu, Mo, and Si) in roots, stems, and leaves were measured using inductively coupled plasma mass spectrometry (ICP-MS, ICAP-Q series, Thermo Fisher Scientific, USA).

Statistical analyses

The data were analyzed using SPSS 17.0 software. Means (n=3) and standard errors (SD) were calculated. One-way ANOVA was used to determine significant differences between each treatment. Duncan multiple range tests were carried out to determine if there were significant differences between individual treatments at $p<0.05$. CANOCO 4.5 software was used for principal component analysis (PCA) of the ionome.

$$\text{Relative biomass} = \frac{\text{Measured value under stress treatment}}{\text{Measured value under the control treatment (Tian } \textit{et al.}\text{, 2009)}} \text{---------- 1}$$

$$\text{Growth inhibition rate (\%)} = \frac{\text{(Dry weight of control plants-Dry weight of treated plants)}}{\text{Dry weight of control plants} \times 100\% \text{ (Li } \textit{et al.}\text{, 2010)}} \text{---------- 2}$$

Results

Relative biomass and growth inhibition rate: The relative biomass of the root, stem, and leaves of L24 and X45 cultivars decreased significantly with increasing Na_2SO_4 stress (Fig. 1a, b and c), and the relative biomass of L24 tissues was significantly higher than those of X45. Averaged across the two soil salinities, the relative biomass of the root, stem and leaves of L24 was significantly higher than that of X45 by 19.6%, 5.7%, and 5.4%, respectively.

Na_2SO_4 stress significantly inhibited cotton growth, and the growth inhibition rates of the root, stem, and leaves of L24 and X45 tissues increased significantly with increasing Na_2SO_4 stress. In general, the growth inhibition rate of salt stress of X45 was significantly higher than that of L24 (Fig. 1 d, e and f). Averaged across the two soil salinities, the growth inhibition rates of the root, stem, and leaves of L24 were significantly lower than those of X45 by 28.6%, 5.5%, and 6.3%, respectively. Regardless of the cotton genotype, under SL treatment, the growth inhibition rate of the root, stem, and leaves was 9.3%, 46.9%, and 41.3%, respectively. Under SH treatment, the growth inhibition rate of the root, stem, and leaves was 50.2%, 52.4%, and 48.3%, respectively. The above results indicate that Na_2SO_4 stress mainly inhibited the growth of shoots (stem and leaves) at a low concentration. Under high concentrations, Na_2SO_4 mainly inhibited shoot and root growth.

REC and MDA: The REC and MDA content of leaves tended to increase with increasing Na_2SO_4 stress (Fig. 2a, b). In general, the REC and MDA content of L24 leaves were significantly lower than those of X45. The average REC of L24 and X45 leaves was 92.4% and 131.4% higher, respectively, than that of the CK treatment. The average MDA content of L24 and X45 leaves was 84.8% and 117.0% higher, respectively, than that of the CK treatment. The above results indicated that the salt-tolerant cotton genotype was less damage from ROS compared with the salt-sensitive genotype under Na_2SO_4 stress.

Antioxidant enzyme activity: Na_2SO_4 stress significantly increased the SOD activity in the leaves, and SOD activity in L24 leaves was significantly higher than that in X45 (Fig. 3a). The SOD activity of L24 leaves under SL and SH treatments was 179.3%, and 159.9% higher than that of the CK treatment, and the SOD activity of X45 leaves under SL and SH treatments was 112.03% and 75.6% higher, respectively, than that of the CK treatment. The SOD activities in leaves of the two cotton genotypes initially increased and then decreased.

Na_2SO_4 stress significantly increased the POD activity of leaves (Fig. 3b). The POD activity in L24 leaves showed a continuous upward trend, however, the POD activity in X45 leaves tended to increase first and then decrease. Under the SL treatment, POD activities were not significantly different between L24 and X45. However, under the SH treatment, the POD activity in L24 leaves was significantly higher than that in X45.

Na_2SO_4 stress significantly increased the CAT activity of leaves, and the CAT activity in L24 leaves was significantly higher than that in X45 (Fig. 3c). In general, the CAT activities in the leaves of L24 and X45 tended to increase initially and then decrease. Under the SL and SH treatments, the CAT activity in L24 leaves was 217.4% and 116.7% higher than that of CK, and the CAT activity in X45 leaves was 140.0% and 38.5% higher, respectively, than that of CK.

Proline: Na_2SO_4 stress significantly increased the Pro content in L24 leaves; however, the Pro content in X45 leaves increased initially and then decreased with increasing Na_2SO_4 stress (Fig. 4). In general, the Pro content in L24 leaves was significantly higher than that in X45 leaves. Averaged across the two soil salinities, the Pro contents in the leaves of L24 and X45 were 60.9% and 12.0% higher, respectively, than those under CK treatment.

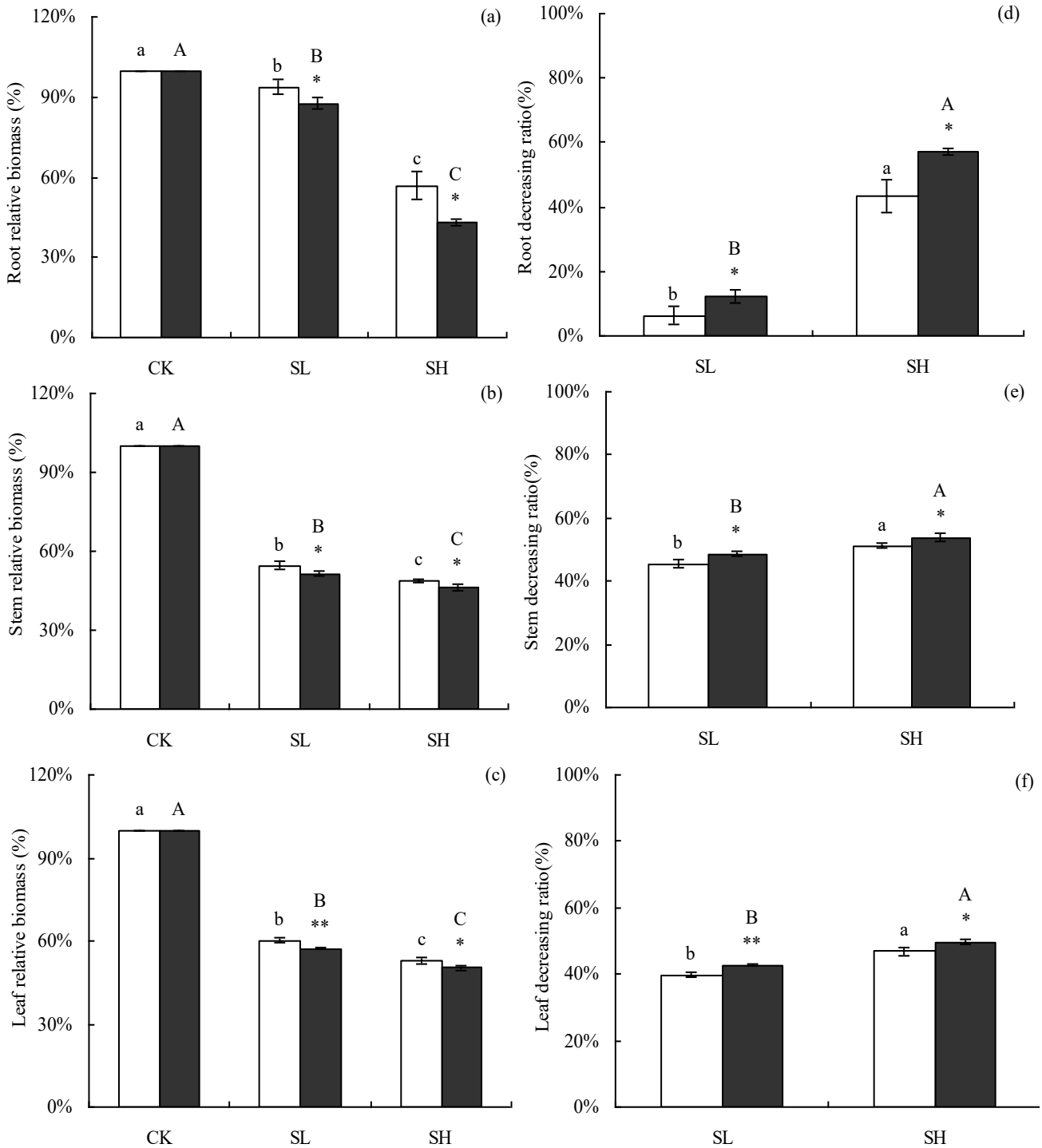

Fig. 1. Effect of Na_2SO_4 stress on relative biomass and decreasing ratio of cotton. Symbols indicate L24 cultivar (white bar) and X45 cultivar (black bar). Vertical bars represent ± standard error (n = 3). Asterisks indicate a significant difference between L24 and X45 (*p<0.05; **p<0.01). Bars labeled with the different lowercase letters on open square bars or uppercase letters on closed square bars are significant difference (p<0.05).

Ionome: To demonstrate the effect of Na_2SO_4 stress on element distribution in the seedling stage of cotton, we analyzed the contents of ions (Na, P, K, Ca, Mg, S, Zn, Mn, Fe, Cu, B, Mo, and Si) in the root, stem and leaves under different Na_2SO_4 stress levels (Fig. 5a, b, c). The results of PCA showed that it could significantly distinguish between different Na_2SO_4 stress treatments and cotton cultivars. Different Na_2SO_4 stress levels were well separated on the first principal component, accounting for 53.1%, 50.3%, and 56.4% of the total variation in root, stem, and leaves, respectively. The elements that mostly contributed to the first principal component were Na, S, Mg, and Zn in roots, Na, S, P, Zn, and Mn in stems, and Na, S, P, and Ca in leaves. The second principal components of the different cotton cultivars (L24 and X45) were well distinguished and explained 20.6%, 24.1%, and 18.3% of the total variation in the ionome of roots, stems and leaves, respectively. The elements that contributed mostly to the second principal component were Fe, Mo, Ca, Mn, and K in roots, K, Ca, Cu, Mo, and Fe in stems, and Mg, Cu, and Mn in leaves.

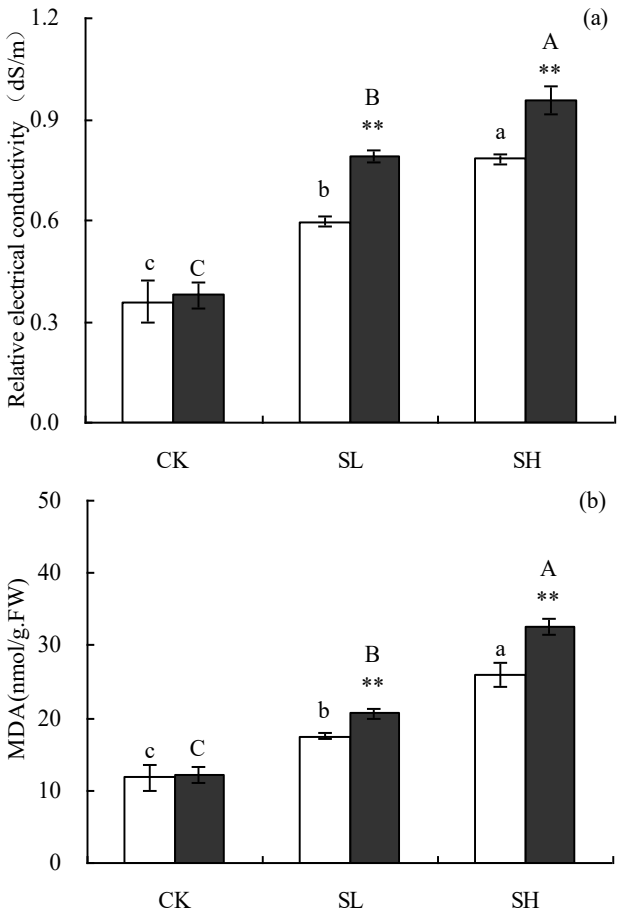

Fig. 2. Effect of Na_2SO_4 stress on relative electrical conductivity and malondialdehyde content of cotton leaves. Symbols indicate L24 cultivar (white bar) and X45 cultivar (black bar). Vertical bars represent ± standard error (n = 3). Asterisks indicate a significant difference between L24 and X45 (*p<0.05; **p<0.01). Bars labeled with the different lowercase letters on open square bars or uppercase letters on closed square bars are significant difference (p<0.05).

In response to salt stress, compared with CK, the Na content in the respective roots, stems and leaves significantly increased 2.02, 5.94, and 2.54 times under SL treatment, and 3.87, 13.68, and 8.641 times under SH treatment, averaged over the two cultivars (Table 1). In addition, the contents of S, Mg, P, and Zn in roots were significantly increased by 65.7%, 11.5%, 25.1%, and 16.6% in the SL treatment and by 137.5%, 28.5%, 50.8%, and 84.0% in the SH treatment, respectively. The contents of S, Mg, P, Zn, Mn, and Fe in stems were significantly increased by71.3%, 21.8%, 30.3%, 61.7%, 59.3%, and 85.8% in the SL treatment and by 140.5%, 10.2%, 70.8%, 78.9%, 57.4%, and 33.9% in the SH treatment, respectively. The contents of S, P, Zn, Mn, Fe, Cu, Mo, and Si in leaves were significantly increased by 49.2%, 19.2%, 31.3%, 25.0%, 4.3%, 7.3%, 0.8%, and 2.1% in the SL treatment and by 142.8%, 45.6%, 44.4%, 36.8%, 14.4%, 18.2%, 35.7%, and 19.6% in the SH treatment, respectively. However, the contents of Ca, Cu, B, and Mo in roots were significantly reduced by 8.4%, 20.7%, 37.7%, and 5.8% in the SL treatment and by 16.7%, 35.2%, 40.1%, and 31.8% in the SH treatment, respectively, and the contents of Ca in leaves were significantly reduced by 7.1% in the SL treatment and by 12.1% in the SH treatment. Generally, L24 had much higher contents of K and P in roots, K, Ca, and Cu in stems, and P and Cu in leaves than X45. On the contrary, L24 had much lower contents of Ca, Fe, and Mo in roots, Mo in stems, and Mg, Mn, and Si in leaves than X45.

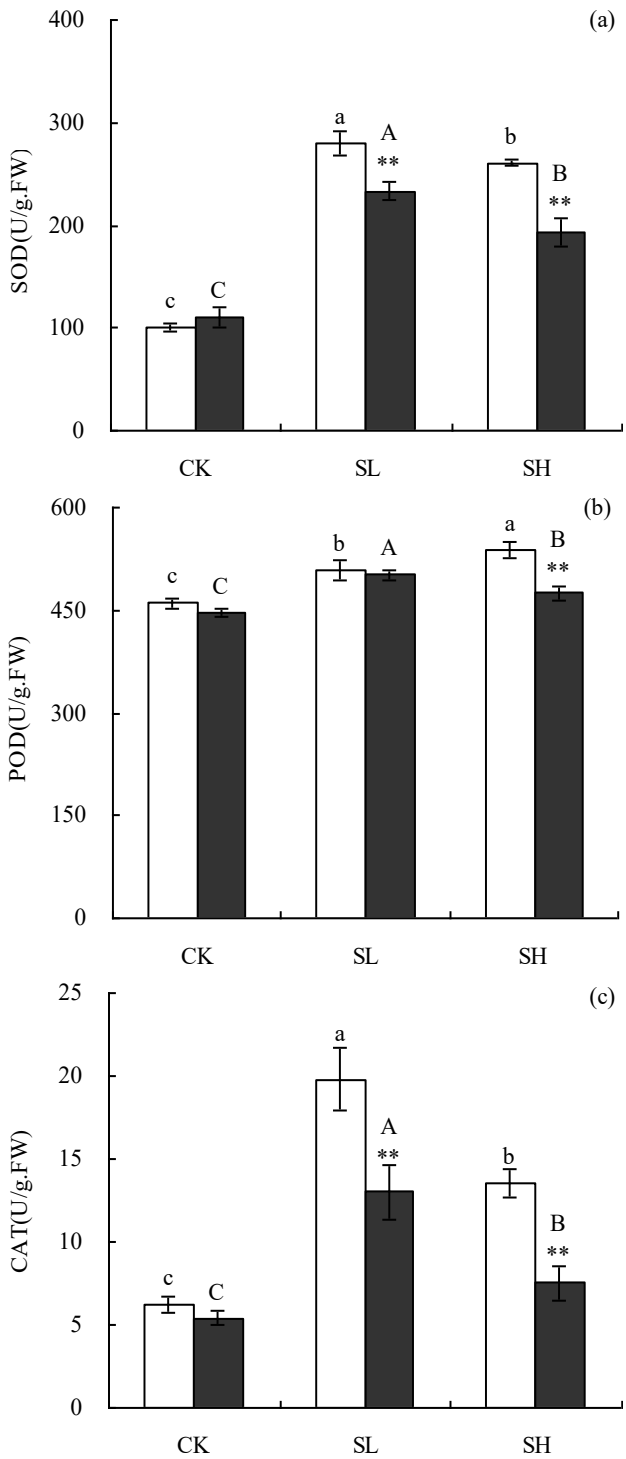

Fig. 3. Effect of Na_2SO_4 stress on antioxidant enzyme activities of cotton leaves. Symbols indicate L24 cultivar (white bar) and X45 cultivar (black bar). Vertical bars represent ± standard error (n = 3). Asterisks indicate a significant difference between L24 and X45 (*p<0.05; **p<0.01). Bars labeled with the different lowercase letters on open square bars or uppercase letters on closed square bars are significant difference (p<0.05).

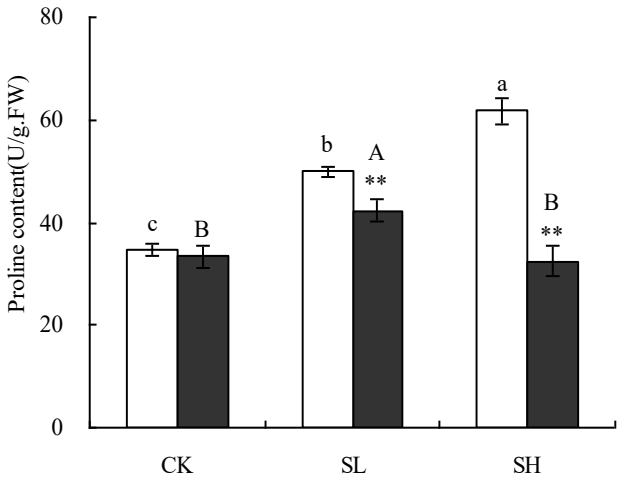

Fig. 4. Effect of Na_2SO_4 stress on Proline of cotton leaves. Symbols indicate L24 cultivar (white bar) and X45 cultivar (black bar). Vertical bars represent ± standard error (n = 3). Asterisks indicate a significant difference between L24 and X45 (*p<0.05; **p<0.01). Bars labeled with the different lowercase letters on open square bars or uppercase letters on closed square bars are significant difference (p<0.05).

Correlation analysis: The correlations among Na and other elements in the root, stem, and leaves were analyzed (Fig. 6). In L24 roots, Na was significantly negatively correlated with B, Ca, Mo, and Cu and was significantly positively correlated with S, P, Fe, Zn, Mg, and Mn (Fig. 6a). In X45 roots, Na was significantly negatively correlated with Ca, K, Cu, Mn, B, and Mo but was significantly positively correlated with S, Mg, Zn, and P (Fig. 6b). In L24 stems, Na was significantly negatively correlated with Mo and was significantly positively correlated with S, P, Fe, Zn, Si, Mn, and Cu (Fig. 6c). In X45 stems, Na was significantly negatively correlated with Ca and was significantly positively correlated with Fe, S, P, Zn, and Mn (Fig. 6d). In L24 leaves, Na was significantly negatively correlated with Mg and Ca and was significantly positively correlated with S, P, Si, Mo, B, Zn, Fe, and Cu (Fig. 6e). In X45 leaves, Na was significantly negatively correlated with Ca and was significantly positively correlated with Mn, S, P, Fe, Mo, Cu, and Si (Fig. 6f).

Discussion

Soil salinization is one of the most serious abiotic stress factors limiting crop production. Excessive salt mainly causes damage to the plants through osmotic stress and ion toxicity, and growth inhibition is the most common physiological response to saline habitats (Freitas *et al.*, 2011; Abbas *et al.*, 2015). Many growth and physiological parameters are used to verify salt tolerance of crops at the seedling stage, including the relative biomass and Na and K uptake (Chen *et al.*, 2007; Qiu *et al.*, 2011). The relative biomass of plants is considered to be a more reliable factor for indicating the growth performance under salt stress (Wang *et al.*, 2017). This study showed that the relative biomass of salt-tolerant cultivar L24 and salt-sensitive cultivar X45 decreased significantly with increasing Na_2SO_4 stress. Na_2SO_4 stress significantly inhibited the growth of cotton, and the growth inhibition rate of X45 under Na_2SO_4 stress was significantly higher than that of L24. The inhibition effect of salt stress on crop growth might be due to the toxicity of sodium ions (Lokhande *et al.*, 2011; Munns, 2010).

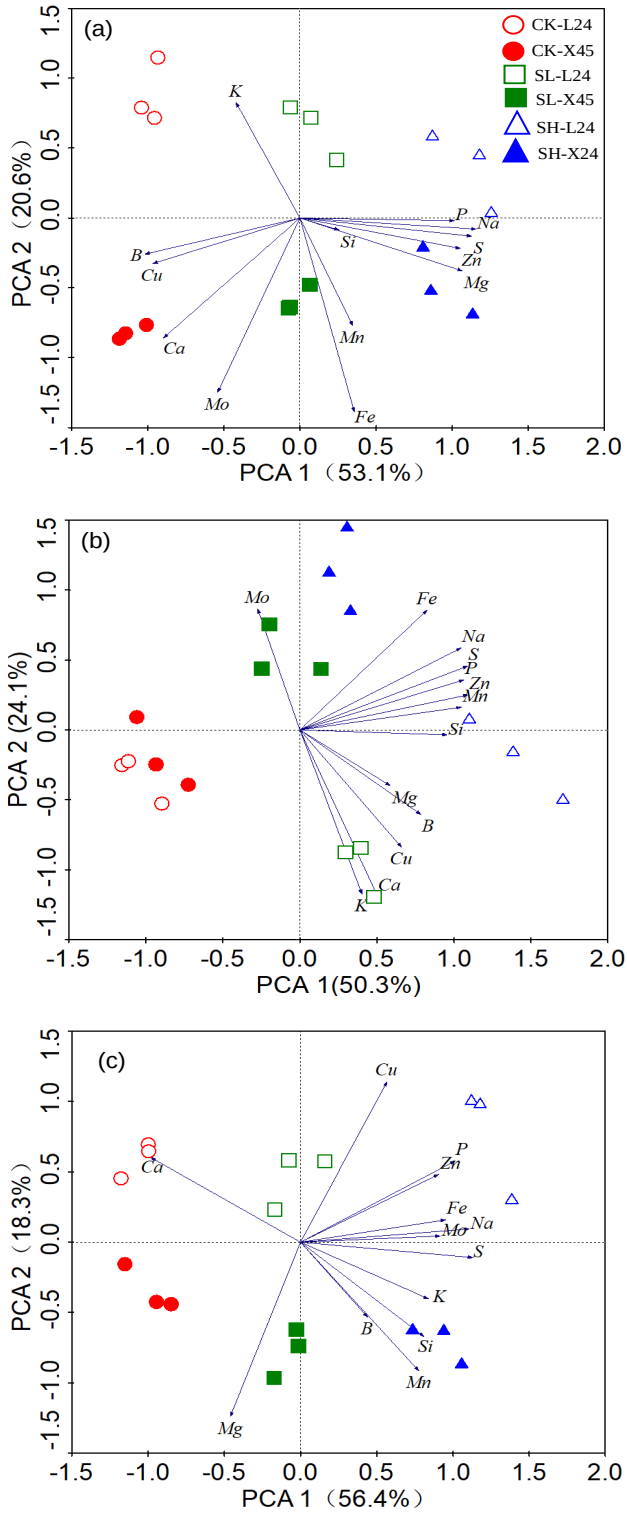

Fig. 5. Tissue ionome variation analysis using PCA at the seeding stage and the loadings of elements to the PC1 and PC2 under Na_2SO_4 treatment. (A) Root ionome variation among samples and the loadings of elements to the PC1 and PC2 in roots; (B) stem ionome variation among samples and the loadings of elements to the PC1 and PC2 in shoots. (C) leaves ionome variation among samples and the loadings of elements to the PC1 and PC2 in shoots.

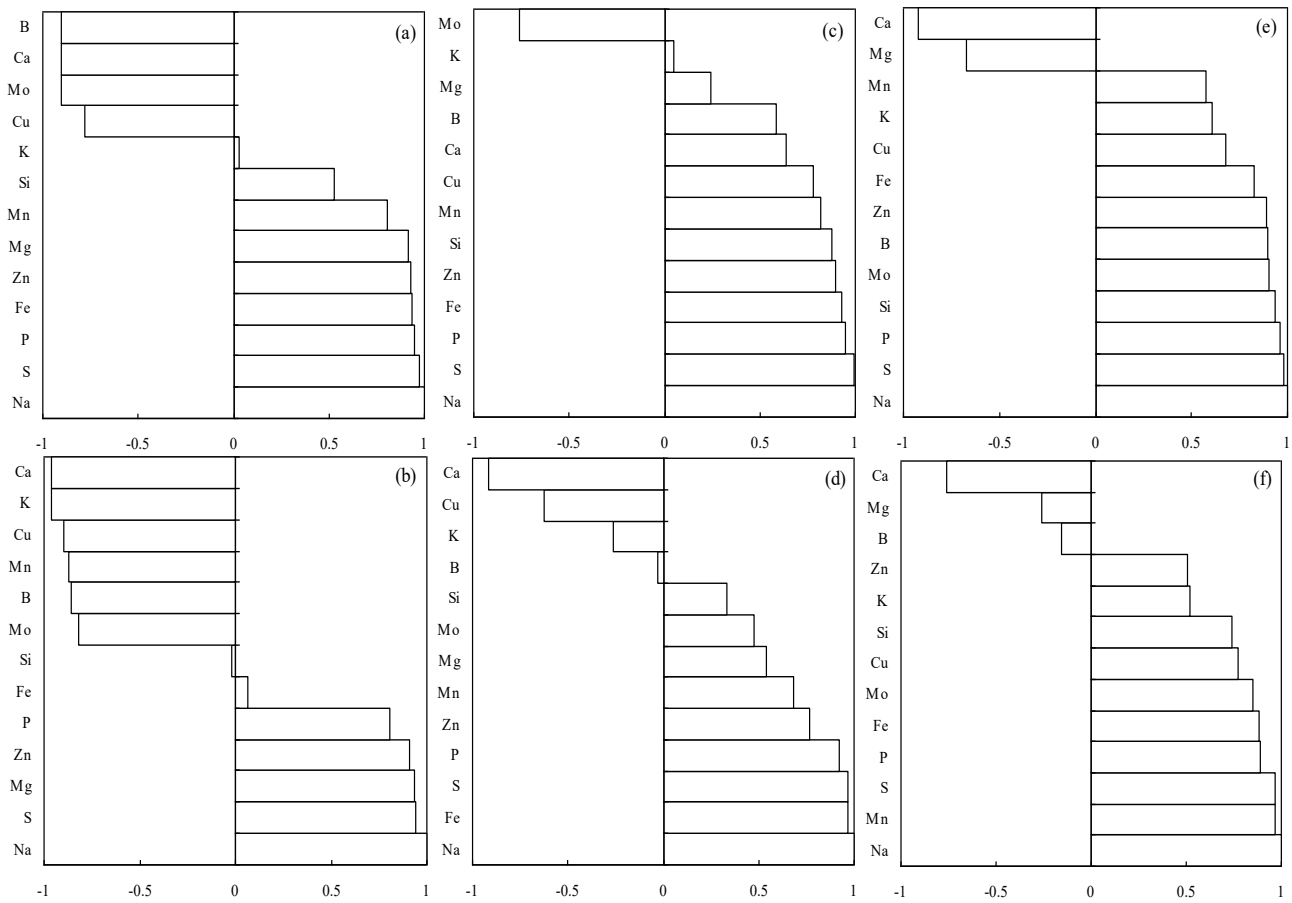

Fig. 6. The correlation among Na elements and other elements in root, stem, and leaves of L24 and X45 at the seedling stage under Na_2SO_4 stress conditions. (a), (c), and (e) indicate a correlation in root, stem, and leaves of L24, (b), (d), and (f) indicate a correlation in root, stem, and leaves of X45l, respectively.

The structure and function of the plant cell membrane play an important role in plant adaptability to adversity. MDA is one of the most important markers of membrane injury. Its content is usually used to assess the severity of oxidative injury of membrane lipids and the sensitivity of plants (Lokhande *et al*., 2011). This study showed that Na_2SO_4 stress significantly increased the MDA content and REC of leaves, indicating that Na_2SO_4 stress was harmful to the cell membrane permeability of leaves. From the perspective of cultivars, the MDA content in X45 was much higher than that in L24. The reason for this might be that salt-sensitive genotype does not effectively scavenge excessive ROS from the leaves, and this is closely related to the activities of non-enzymatic antioxidants and antioxidant enzymes (Huang *et al*., 2015). Salt stress destroys the dynamic equilibrium of ROS, resulting in the peroxidation and deacylation of membrane lipids. This subsequently damages the membrane system and metabolic process of plants and further damages the biological macromolecules such as proteins and nucleic acids, resulting in cell death (Wang *et al*., 2004). Plants respond to salt stress by up-regulating protective enzymes, such as SOD, POD, and CAT, enhancing the ability to scavenge ROS (Eraslan *et al*., 2007; Koca *et al*., 2007; Li, 2009). It is an important way to improve the salt tolerance of plants by increasing the activity of antioxidant enzymes and the level of antioxidant metabolism. This study revealed that Na_2SO_4 stress increased the activities of SOD, POD, and CAT. This agreed with Ibrahim *et al*., (2018), who found that salt stress increased the activities of SOD, POD, and CAT. From the perspective of cultivars, the activities of SOD, POD and CAT in L24 were significantly higher than those in X45, indicating that the salt-tolerant cotton genotype had better ROS scavenging ability. Zhang *et al*., (2013b) showed that the SOD activity of salt-tolerant cotton cultivars was significantly higher than that of salt-sensitive cultivars. Meloni *et al*., (2003) also found that salt-tolerant cotton cultivars had a strong antioxidant system.

Pro is one of the main organic osmotic regulators in plants that helps to resist salt stress and plays an important role in osmotic regulation, biological macromolecule protection, nitrogen metabolism and energy metabolism. The results of this study showed that the Pro content in L24 leaves under Na_2SO_4 stress was significantly higher than that in X45. Several studies showed that the Pro content increased as soil salt increased (Amirjani, 2010; Azarmi *et al*., 2016; Iqbal *et al*., 2018). In addition, the Pro content in X45 leaves tended to increase initially and then decreased with increasing Na_2SO_4 stress. The reason for this is that high concentrations of salt (Na_2SO_4) stress exceeded the tolerance range of the plants (Liu & Zhu, 1997).

Table 1. Effects of Na_2SO_4 stress on the concentration of mineral elements (mg g^{-1} DW) in roots, stems and leaves of two cotton varieties.

Tissue	Genotype	Treatment	Na	P	K	Ca	Mg	S	Zn	Mn	Fe	Cu	B	Mo	Si
Root	L24	CK	0.666c	0.957d	12.20a	3.158bc	2.126c	2.674c	0.028e	0.016d	0.639d	0.013b	0.021b	0.006c	0.194c
		SL	2.174b	1.271b	11.73a	2.889d	2.436b	4.117b	0.034c	0.017cd	0.759c	0.011bc	0.014c	0.006c	0.221b
		SH	3.529a	1.688a	12.28a	2.68e	2.750a	6.571a	0.050b	0.021a	0.874b	0.010cd	0.013c	0.004d	0.213b
	X45	CK	0.755c	1.057cd	11.77a	3.633a	2.261bc	2.711c	0.029de	0.020ab	0.987a	0.017a	0.024a	0.012a	0.195c
		SL	2.097b	1.240bc	10.64b	3.333b	2.451b	4.590b	0.033cd	0.019abc	0.970a	0.012bc	0.014c	0.011a	0.240a
		SH	3.357a	1.323b	9.48c	2.959cd	2.887a	6.216a	0.055a	0.018bcd	0.980a	0.009d	0.014c	0.009b	0.193c
Stem	L24	CK	0.417d	1.114d	13.02bc	4.857b	2.965c	2.979d	0.015c	0.004b	0.040c	0.005b	0.010b	0.003bc	0.035c
		SL	2.687c	1.392c	15.38a	5.874a	3.907a	5.327c	0.024b	0.007a	0.075bc	0.006a	0.012ab	0.003bc	0.043bc
		SH	5.791a	2.106a	13.41b	5.711a	3.309bc	8.040a	0.030a	0.007a	0.188a	0.007a	0.012a	0.002c	0.065a
	X45	CK	0.355d	1.085d	11.75d	4.497b	3.087bc	3.297d	0.015c	0.004b	0.055c	0.005b	0.010b	0.003c	0.044bc
		SL	2.637c	1.473bc	12.14cd	4.073c	3.451b	5.401c	0.024b	0.007a	0.100b	0.004b	0.011ab	0.005a	0.041bc
		SH	5.490b	1.656b	11.34d	3.766c	3.360b	6.960b	0.024b	0.006a	0.224a	0.004b	0.010b	0.004ab	0.046b
Leaves	L24	CK	0.917d	1.399c	14.67b	26.67a	6.705ab	18.14c	0.028c	0.036d	0.348bc	0.005bc	0.039d	0.013b	0.165e
		SL	2.818c	1.765b	21.65a	25.15b	6.305bc	28.14b	0.037b	0.047bc	0.357b	0.005ab	0.044c	0.014b	0.179de
		SH	7.889a	2.305a	21.31a	23.50c	6.071c	48.28a	0.046a	0.047bc	0.384a	0.006a	0.049b	0.017a	0.207b
	X45	CK	0.651d	1.402c	14.98b	25.91ab	6.887a	20.96c	0.027c	0.042c	0.330c	0.003e	0.043cd	0.013b	0.197bc
		SL	2.612c	1.573c	22.80a	23.70c	7.072a	30.01b	0.035b	0.050b	0.349bc	0.004de	0.057a	0.012b	0.189cd
		SH	6.954b	1.773b	21.25a	22.71c	6.820a	45.98a	0.033b	0.060a	0.390a	0.004cd	0.043cd	0.018a	0.226a

Note: Different letters in the same column of root, stem, or leaves indicate significant differences ($p<0.05$) between individual treatments

Absorption of nutrients by plants is inhibited under salt stress (Chen *et al*., 2010; Tavakkoli *et al*., 2011; Kirmizi & Bell, 2012; Kopittke, 2012). Salt stress produces osmotic and ionic stresses, inhibits the uptake of ions, and changes soil nutrient availability, thus leading to disequilibrium of crop ions and mineral nutrients. Our study showed that cotton accumulated large amounts of Na under Na_2SO_4 stress. Previous studies also reported that plants frequently accumulated large amounts Na to lower the cell water potential under salt stress (Yang *et al*., 2007). In addition, the Na content in leaves was higher than that in roots under high salt stress, indicating that neither salt-tolerant nor salt-sensitive cotton could prevent Na transport from the roots to the leaves. In this study, the K contents in L24 roots under Na_2SO_4 stress were significantly higher than those in X45. Although there was no significant difference in the Na content between the roots of L24 and X45, the K/Na ratio of L24 roots was higher than that of X45 roots. Therefore, a high K/Na ratio is considered to be a salt tolerance mechanism used by crops (Abbas *et al*., 2010).

Jafri & Ahmad, (1994) also found that moderate accumulation of Na in leaves would cause a slight increase in the K content. Salt stress is essentially caused due to excess salt ions in the soil, and rebuilding ion homeostasis under salt stress remains an important salt tolerance strategy for plants (Blumwald, 2000). Wu *et al*., (2013) used ICP-AES to investigate the composition of 10 elements in three barley cultivars and found that the ionome in the roots and shoots of barley was rearranged under moderate and high salt stresses. Our study found that the contents of S, Mg, P, and Zn in roots, S, Mg, P, Zn, Mn, and Fe in stems, and S, P, Zn, Mn, Fe, Cu, Mo, and Si in leaves were significantly increased under Na_2SO_4 stress. However, the contents of Ca, Cu, B, and Mo in roots and the contents of Ca in leaves were significantly reduced. The results of PCA showed that the elements that most strongly contributed to the first principal component were Na, S, Mg, and Zn in roots and Na, S, P, and Ca in leaves. In addition, Na_2SO_4 stress increased the contents of Na, S, Mg, and Zn in roots and increased Na, S, and P in leaves; however, Na_2SO_4 stress decreased the content of Ca in leaves. In our study, cotton plants absorbed more S because of the presence of SO_4^{2-}. However, Azevedo *et al*., (2000) found that P uptake efficiency in maize decreased with increasing salt concentration, and the uptake efficiency in salt-tolerant maize was higher than that of salt-sensitive maize. We also found that the P contents in L24 leaves were higher than those in X45 under Na_2SO_4 stress. Ca and Na have a certain antagonistic effect, and excessive Na intake leads to a relative deficiency of Ca in cotton. The results in this study were similar to findings of Zhang *et al*., (2014), who reported that salt stress significantly decreased the Ca content in plants. Correlation analysis showed that Na_2SO_4 stress reduced Ca absorption of the salt-sensitive genotype X45. Zn is involved in the synthesis of auxins, and a lack of Zn causes stagnation in crop growth and development. To maintain their growth in a stressed environment, plants might promote the absorption of Zn. This study found that Na_2SO_4 stress significantly increased the Zn content in roots. Moreover, a cultivar

difference could be found in the changes in the contents of these elements under Na_2SO_4 stress. The results of PCA showed that the elements that most strongly contributed to the second principal component were Fe, Mo, Ca, Mn, and K in roots and Mg, Cu, and Mn in leaves. Generally, Na_2SO_4 stress increased the contents of Fe and Mn in the roots of L24 and increased the contents of Cu and Mn in the leaves of L24 and X45; however, Na_2SO_4 stress decreased the contents of Mo and Ca in the roots of L24 and X45 and decreased the content of Mg in the roots of L24. The significant decrease in the Mg content in leaves might be due to the lower chlorophyll content in the leaves after salt stress (Zhang *et al*., 2014). In this study, Na_2SO_4 stress increased the Fe content in leaves, and the reasons for this might be that in order to maintain growth, cotton synthesizes chlorophyll to improve photosynthesis to respond to high Na_2SO_4 stress. Mn plays a catalytic role in chlorophyll synthesis, which is closely related to photosynthesis and respiration in plants. This study found that the Mn content in cotton leaves increased significantly under Na_2SO_4 stress, however, Karimi *et al*., (2005) reported that excessive accumulation of Na reduced Mn absorption. Salt stress also significantly affected the absorption of Si in cotton. Our study showed that Na_2SO_4 stress significantly increased the Si content in the leaves. Li *et al*., (2015) studied the effects of Si on tomato seedling growth under salt stress and found that Si significantly alleviated the adverse effects of salt stress on tomato seedling growth, photosynthetic performance, and soluble protein content.

Conclusion

In general, the growth inhibition rate caused by Na_2SO_4 stress in X45 was significantly higher than that in L24. Low concentrations of Na_2SO_4 mainly inhibited the growth of cotton shoots, and a high concentration of Na_2SO_4 inhibited the growth of shoots and roots. According to the antioxidant enzyme system and ionome analysis, we suggest that the mechanism of ionome response to salt (Na_2SO_4) stress during the seedling stage of cotton might involve the following: (1) salt-tolerant cotton has a strong antioxidant enzyme system, and cotton resists Na_2SO_4 stress by increasing the antioxidant enzyme activity; (2) there was no significant difference in the K content in the leaves. However, the content of K in the roots of L24 was significantly higher than that of X45, therefore, the root system of salt-tolerant cotton had a higher K/Na ratio to response to salt stress conditions. Moreover, salt-tolerance was achieved by storing Na in cotton leaves under salt stress; (3) salt-tolerant cotton roots absorb more mineral elements than salt-sensitive cotton, and the transport of Ca and Cu to the shoots is of great significance for resistance to Na_2SO_4 stress.

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