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Photosynthesis, ion accumulation, antioxidants activities and yield responses of different cotton genotypes to mixed salt stress

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This study analyzes the effects of soil salinity on photosynthetic character, osmoregulation, content of pigment, K^+/Na^+ ratio, lipoxygenase and antioxidants activities in functional leaves during the flowering and boll-forming stages of two cotton cultivars, namely, CCRI-44 (salt-tolerant) and Sumian12 (salt-sensitive), grown under different soil salinity conditions. In the control plants, non-significant differences were found in gas exchange, saturation irradiance (SI) and carotenoid (Car) content between the two cultivars. However, it showed higher K^+/Na^+ ratio, antioxidant enzyme activities, soluble sugar and protein contents, and lower chlorophyll (Chl) content and yield in CCRI-44. Salinity stresses remarkably increased soluble sugar and protein contents, lipoxygenase and the antioxidant activities, but decreased K^+/Na^+ ratio, Chl and Car contents, SI, photosynthetic capacities and yield, the extent being considerably larger in Sumian12 than CCRI-44. Although the soluble sugar, protein contents and the antioxidant activities of Sumian12 elevated more evidently under salt stresses, those variables never reached the levels of CCRI-44. Thus, CCRI-44 could maintain higher seed cotton yield than Sumian12 by sustaining higher osmoregulation and antioxidative abilities, which led to higher photosynthetic capacity. Hence, the salt-tolerant cotton cultivars could harmonize the relationship between CO_2 assimilation (source) and the seed cotton yield (sink) under the experimental conditions.

Key words: Cotton (*Gossypium hirsutum* L.), mixed salt stress, salt tolerance, photosynthesis, ion accumulation, antioxidant enzyme activity, seed cotton yield.

INTRODUCTION

Salinity is considered one of the major limiting factors for plant growth and agricultural productivity (Munns, 2005). Currently this stress is becoming even more prevalent as the intensity of land use increases in the world (Meloni et al., 2003). It reduces the growth of crops, at least partially, by leading to specific ion toxicity and enhances the generation of reactive oxygen species (ROS), which resulting in a decrease of photosynthetic capacity. The K^+/Na^+ ratio is important for the adjustment of cell osmoregulation, stomatal function, activation of enzymes, protein synthesis, oxidants metabolism, and photosynthesis

(Glenn et al., 1999). Normally, the K^+/Na^+ ratio tends to decrease under salinity stress as a result of either excessive Na^+ accumulation in plant tissue or enhanced K^+ leakage from the cell by activating K^+ efflux channels (Cuin and Shabala, 2007).

Although the adverse symptoms caused by salinity could be partially alleviated by implementing schemes, such as plastic film mulching (Dong et al., 2009), KNO_3 supply (Zheng et al., 2008), or foliar application of coronatine (Xie et al., 2008), the most promising strategy to overcome the problems of salty soil, is the use of salt-

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Table 1. Selected physical and chemical properties of the soil used. Means \pm SE ($n = 3$).

Characteristic	Value
Bulk density (g cm^{-3})	1.25 \pm 0.09
Field water capacity (%)	28.6 \pm 0.19
pH (H_2O)	7.3 \pm 0.04
Organic matter (OM, g kg^{-1})	15.3 \pm 0.14
Total nitrogen (N, g kg^{-1})	0.96 \pm 0.08
Available phosphorus (Olsen-P, mg kg^{-1})	32.43 \pm 0.51
Available potassium (K, mg kg^{-1})	202 \pm 4.83
ECe (dS m^{-1})	1.25 \pm 0.02

tolerant species. Thus, investigating the metabolism characteristics of salinity tolerant genotypes will be extremely important both for understanding the mechanism of salinity tolerance and selecting salt-tolerant cultivars.

Cotton is one of the most important economic crops in China. In the Yangtze River Valley, one of the largest cotton-growing areas in China, high temperature and evapotranspiration in summer and autumn, inadequate water management as well as saltwater intrusion close to the coastal area has contributed to an increase in soil salinity. Although cotton is classified as a salt-tolerant crop, its tolerance of salinity is not only limited, but also varied according to genotypes (Ashraf and Ahmad, 2000). Several past studies have been done analyzing physiological variations at seedling stage under either hydroculture or sand culture by NaCl treatment (Yang et al., 2010; Zhang et al., 2011). However, less information is available about the responses of different cotton genotypes grown in saline field soil (the major solutes comprising dissolved mineral salt are the cations Na^+ , Ca^{2+} , Mg^{2+} , and K^+ and the anions Cl^- , SO_4^{2-} , HCO_3^- , CO_3^{2-}) at flowering and boll-forming stage which is the key yield determinant period of cotton. The physiological responses caused by mixed salt stress differ from only NaCl stress since there is a significant interaction among ions (Ashraf and Ahmad, 2000; Chen et al., 2010). Thus, a careful study on the responses of cotton to mixed salt stress at this stage is urgently needed.

In order to understand why salt-tolerant cotton could relieve the saline adverse effects and obtain higher grain yield than the salt-sensitive one in saline field, two cotton genotypes (CCRI-44 (salt-tolerant) and Sumian12 (salt-sensitive)) were treated with various mixed salts (NaCl, MgCl_2 , Na_2SO_4 , MgSO_4 , CaCl_2 , Na_2CO_3 , and NaHCO_3 at even molar ratio) levels (1.25, 5.80, 9.61, 13.23, and 14.65 dS m^{-1}). Photosynthetic capacities, K^+/Na^+ ratios, soluble sugars, and proteins, and antioxidant activities of both cultivars were measured at 70 days after transplanting (when all the cotton plants were at flowering and boll-forming stage). The objectives were to compare the differential responses of two genotypes to mixed salt stress at flowering and boll-forming stage, and try to

demonstrate a determinant of growth and yield determination under mixed salt conditions.

MATERIALS AND METHODS

Plant materials and growth conditions

Pot experiments were conducted in the summer of 2009 in a greenhouse at the Pailou experimental station of the Nanjing Agricultural University, located at Nanjing (32°02'N and 118°50'E), Jiangsu Province of China. The minimum and maximum air temperature was 19 and 34°C, respectively. The relative humidity ranged from 40 to 68%. Cotton (*Gossypium hirsutum* L.) cultivars planted were CCRI-44 (salt-tolerant) and Sumian12 (salt-sensitive) (Zhang et al., 2011), which are grown widely in the Yangtze River Valley in China. Cotton seeds were planted on 25 April 2009.

When the seedlings had three true leaves, individual healthy, uniform plants were transplanted into plastic pots, 50 cm high and 33 cm diameter, filled with 30 kg air dry soil. The yellow brown soil collected from the 0 to 30 cm topsoil layer from the experiment station was passed through a 2 mm sieve and packed in the pots. Selected physical and chemical properties of the soil are presented in Table 1. For each cotton cultivar, there were five salinity treatments. Seven kinds of salts (sodium carbonate, sodium bicarbonate, sodium chloride, calcium chloride, magnesium chloride, magnesium sulfate and sodium sulfate) were mixed into natural dried, sieved, selected soils at an even molar ratio before the experiment, forming soils with five levels of salinity (ECe, electrical conductivity of 1:5 soil/water extract), (CK) with 1.25 dS m^{-1} soil salinity, (S1) with 5.80 dS m^{-1} soil salinity, (S2) with 9.61 dS m^{-1} soil salinity, (S3) with 13.23 dS m^{-1} soil salinity, and (S4) with 14.65 dS m^{-1} soil salinity, respectively. The resulting ion compositions in the treated soil were similar to those observed in the local Coastal saline soil. Before filling the pots, 4.5 g N, 0.36 g P_2O_5 , and 0.9 g K_2O per pot was applied into the soil. Another 4.5 g N per pot was top-dressed at the early flowering stage (35 days after transplanting) in every treatment.

The experiment was arranged in a completely random design, each treatment had 20 replications and one pot with single plant represented one replication.

Gas exchange and irradiance response

Simultaneous measurements of gas exchange and irradiance response were taken on functional leaves (4th leaf from top) at flowering and boll-forming stage (70 days after transplanting), with

a portable open-flow gas exchange system LI-6400 (LI-COR Biosciences, Lincoln, USA). Photosynthetic rate (P_N), stomatal conductance (G_s) were measured at PAR of $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ of internal light source, $65 \pm 5\%$ RH, $32 \pm 2^\circ\text{C}$ leaf temperature and $380 \mu\text{mol mol}^{-1} \text{CO}_2$ concentration.

The irradiance response curve was recorded automatically in the same leaf by means of operation program. During irradiance response measurements, CO_2 concentration was maintained at $380 \mu\text{mol mol}^{-1}$. Photosynthesis versus PAR of 1 800, 1 600, 1 400, 1 200, 1 000, 800, 600, 400, 200, and $0 \mu\text{mol m}^{-2} \text{s}^{-1}$ was measured. Each PAR step lasted 3 min. The data obtained for each leaf were analyzed with the program photosynthetic assistant (Version 1.1, Dundee Scientific, Dundee, UK) to obtain saturation irradiance (SI).

Pigment analyses

After determined the gas exchange and irradiance response, six leaves (4th leaf from top) were harvested. Half of the samples were immediately frozen in liquid N_2 and stored at -70°C for enzyme activity analysis; others were dried in an oven at 80°C to a constant weight. Frozen leaf samples (0.1 g) were extracted with pure acetone in the dark for 48 h at 4°C in order to guarantee the complete extraction of pigment from leaf. The concentration of Chlorophyll (Chl) and carotenoid (Car) was determined spectrophotometrically according to Shabala et al. (1998).

Determination of soluble sugar content

Soluble sugars were determined based on the method of phenolsulfuric acid (Dubois et al., 1956). Frozen leaf samples (0.3 g) were homogenized with deionized water, extract was filtered and the extract treated with 5% phenol and 98% sulfuric acid, mixture remained for 1h and then absorbance at 485 nm was determined by spectrophotometer (UV-2401, Shimadzu Corporation, Japan). Content of soluble sugar was determined by using glucose as standard.

Determination of Lipoxygenase activity

Lipoxygenase (LOX) was assayed spectrophotometrically at 234 nm according to You et al. (2009). Frozen leaf samples (0.2 g) were crushed into fine powder in a mortar in an ice-bath. 5.0 ml of 0.1 mol L^{-1} phosphate buffer (including 0.5 mmol L^{-1} PMSF, 0.6 mmol L^{-1} EDTA, pH 7.0) was used as an extraction buffer. The homogenate was centrifuged at $15,000 \times g$ for 15 min at 4°C , then the supernatants were used to measure. LOX activity was analyzed in 2.8 ml of 0.1 mol L^{-1} phosphate buffer containing 0.1 ml of 100 mmol L^{-1} sodium linoleate. The increase in absorbance at 234 nm was recorded after adding 0.2 ml enzyme extract.

Determination of antioxidant enzymes activity and soluble proteins and malondialdehyde (MDA) Content

Frozen leaf samples (0.3 g) were crushed into fine powder in a mortar in an ice-bath. To each sample, 5 ml of 0.05 mol L^{-1} phosphate buffer (pH 7.8) with 1% Poly vinyl pyrrolidone (PVP) was used as an extraction buffer. The homogenate was centrifuged at $15000 g$ for 15 min at 4°C . The supernatant was used to measure protein and malondialdehyde (MDA) and antioxidant enzyme activities.

Protein content was determined according to Bradford (1976) with bovine serum albumin as the standard. Superoxide dismutase (SOD) activity was determined according to the method of Foster

and Hess (1980): One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of nitro blue tetrazolium (NBT) reduction, measured with a spectrophotometer (UV-2401, Shimadzu Corporation, Japan) at 560 nm. Catalase (CAT) activity was determined by potassium permanganate titration (Giannopolitis and Ries, 1977). The action mixture contained 2.9 ml of 50 mM phosphate buffer (pH 7.0), 1.0 ml of 10 mM H_2O_2 , and 100 ml of enzyme extract in tubes. Peroxidase (POD) activity was analysed in 2.9 ml of 0.05 mol L^{-1} phosphate buffer containing 1.0 ml of 0.05 mol L^{-1} guaiacol and 1.0 ml of 2% H_2O_2 (Tan et al., 2008). The increase in absorbance at 470 nm was recorded after adding 2.0 ml of 20% chloroacetic acid. Content of MDA content was made according to the method of Du and Bramlage (1992). Determination of K^+ , Na^+ and Cl^- contents.

The content of Na^+ and K^+ were determined in the extract obtained after digestion with $\text{HNO}_3:\text{HClO}_4$ (10:1, v/v) of dry leaf powder (0.2 g) by atomic absorption spectrophotometer (Liu et al., 2010). Cl^- content was determined after treated with 10 ml of deionized water at 100°C for 60 min by ion chromatography (DX-300, Sunnyvale, CA, USA) (Liu et al., 2010). Growth parameters and yield components.

The measurements of plant height, leaf area and leaf water content were performed soon after the measurements of gas exchange and irradiance response. Leaf area per plant was measured with a portable area meter (Li 3000-A, Li-COR Inc., NE, USA). From fresh weight (FW) and dry weight (DW) measurements, leaf water contents $((\text{FW}-\text{DW})/\text{FW} \times 100\%)$ were obtained. Number of boll was counted at boll-opening stage. Boll weight and seed cotton yield were obtained after harvest. Statistical analysis.

Each determination was carried out with three replicates. Statistical treatment of the data was performed by One-way ANOVA method. Differences between means were established using a Duncan test ($p < 0.05$). For these analyses SPSS 11.0 software (SPSS, Chicago, IL, USA) was used.

RESULTS

Gas exchange and SI Non-significant differences ($p < 0.05$) were observed in net photosynthetic rate (P_N) between CCRI-44 and Sumian12 in CK plants (Figure 1). Mixed salt stress remarkably reduced the P_N of both cultivars, with the largest reductions occurring under S4 treatment. Such adverse effects were more serious in Sumian12 than in CCRI-44. In CCRI-44, P_N was reduced by 3.4, 13.6, 34.2 and 43.9% relative to CK when plants were under S1, S2, S3 and S4 treatments, respectively, whereas in Sumian12, P_N decreased by 5.4, 23.1, 48.6 and 50.1%, respectively. The responding trends of stomatal conductance (g_s) to mixed salt stress were consistent with P_N . The reductions of g_s were always smaller in Sumian12 than in CCRI-44 relative to CK. P_N and g_s in CCRI-44 were consistently higher than in Sumian12 under all treatments. Distinct PAR- P_N curves were established after analyzing the data set measured in different treatments (Figure 2). For CCRI-44, two PAR- P_N curves monitored under S1 and S2 treatments were closed to CK, with SI of about $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$. However, the slope of PAR- P_N curve was much lower under S3 and S4 treatments, with SI of about 1300 and $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$. The extent of variation was larger in Sumian12 than CCRI-44. Two PAR- P_N curves of Sumian12 measured under CK and S1 treatment were

Table 2. Contents of soluble sugars and proteins (g kg^{-1}), K^+ , and Na^+ (g kg^{-1}) and K^+/Na^+ ratio in functional leaves of salt-tolerant CCR-44 and salt-sensitive Sumian12 under salt treatments at flowering and boll-forming stage. Means \pm SE ($n = 3$).

Cultivar	Treatments	Soluble sugars	Soluble proteins	Na^+	K^+	Cl^-	K^+/Na^+
CCR-44	CK	87.83 \pm 2.51 ^c	8.90 \pm 0.46 ^c	5.36 \pm 1.03 ^d	16.46 \pm 0.98 ^a	18.81 \pm 0.93 ^e	3.14 \pm 0.51 ^a
	S1	89.76 \pm 3.26 ^c	9.10 \pm 0.65 ^c	7.47 \pm 0.71 ^d	16.11 \pm 0.79 ^{ab}	22.15 \pm 1.12 ^d	2.17 \pm 0.31 ^b
	S2	94.10 \pm 4.03 ^c	11.12 \pm 0.48 ^b	13.16 \pm 1.75 ^c	14.78 \pm 0.95 ^b	29.22 \pm 1.08 ^c	1.13 \pm 0.21 ^c
	S3	113.90 \pm 3.33 ^b	13.28 \pm 0.52 ^a	15.48 \pm 1.21 ^b	12.75 \pm 0.91 ^c	37.46 \pm 1.50 ^b	0.82 \pm 0.09 ^{cd}
	S4	120.78 \pm 4.23 ^a	12.57 \pm 0.30 ^a	18.74 \pm 1.02 ^a	9.51 \pm 0.56 ^d	42.23 \pm 2.16 ^a	0.51 \pm 0.11 ^d
Sumian12	CK	74.37 \pm 1.85 ^e	7.99 \pm 0.21 ^e	5.89 \pm 0.56 ^d	15.55 \pm 0.70 ^a	18.12 \pm 1.03 ^e	2.75 \pm 0.20 ^a
	S1	79.49 \pm 2.37 ^d	8.69 \pm 0.14 ^d	10.07 \pm 1.46 ^c	14.97 \pm 0.51 ^a	24.04 \pm 0.74 ^d	1.57 \pm 0.15 ^b
	S2	85.78 \pm 1.89 ^c	9.96 \pm 0.33 ^c	14.88 \pm 1.27 ^b	12.40 \pm 0.46 ^b	33.84 \pm 2.04 ^c	0.84 \pm 0.07 ^c
	S3	102.75 \pm 3.52 ^b	11.39 \pm 0.38 ^b	16.39 \pm 0.79 ^b	10.47 \pm 0.86 ^c	42.05 \pm 1.62 ^b	0.65 \pm 0.11 ^c
	S4	115.56 \pm 3.41 ^a	12.06 \pm 0.22 ^a	21.15 \pm 1.55 ^a	8.36 \pm 0.69 ^d	46.74 \pm 2.34 ^a	0.39 \pm 0.09 ^d

Different letters in the same column indicate significant difference ($p < 0.05$).

close, with SI of about $1300 \mu\text{mol m}^{-2} \text{s}^{-1}$. The SI of Sumian12 was about 1300, 1100 and $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ in S2, S3 and S4 treatment, respectively.

Contents of pigment

Chlorophyll (Chl) content of CCR-44 was lower (by 5 %) than that of Sumian12 under CK (Figure 3). Mixed salt stress caused decreases in Chl contents of both cultivars. However, the reductions in CCR-44 were lower than those in Sumian12. In fact, the Chl contents of CCR-44 were 7.2, 7.6, 13.2 and 8.6% higher than Sumian12 under S1, S2, S3 and S4 treatments, respectively. There was no significant difference in Car content between the two varieties under CK condition. Car content in Sumian12 kept on decreasing with increasing salinity levels while CCR-44 maintained a steady content under S1 and S2 treatments compared with CK, however, a considerable decrement was observed in S3 and S4 treatments.

Contents of soluble sugars, and proteins

As important osmoregulatory compounds, soluble sugars and proteins accumulated significantly in both cultivars under salinity stresses (Table 2). Soluble sugar content of CCR-44 was considerably higher (by 18.2%) than that of Sumian12 in CK plants. Non-significant changes were noted in CCR-44 under S1 and S2 treatments compared with CK, however, considerable increment were observed in S3 and S4 treatments. Although the increments in Sumian12 were more significant considerable than those in CCR-44 under mixed salt stress, they never reached the high levels of CCR-44. The variation tendency of the soluble protein contents was similar to the trends of soluble sugar contents.

Contents of K^+ , Na^+ and Cl^-

Even in CK plants, the Na^+ content was remarkably higher and K^+ was considerably lower, resulting in a higher K^+/Na^+ ratio in CCR-44 than Sumian12 (Table 2). Mixed salt stress decreased K^+ content, but increased Na^+ content, with reduced K^+/Na^+ ratio in both cultivars. However, the variable extent of Na^+ and K^+ content was smaller in CCR-44 than that in Sumian12, especially in CK and S1 treatments. With further increase of salinity level, the K^+ content and the K^+/Na^+ ratio decreased significantly in both cultivars, but they were still higher in CCR-44 than Sumian12. There was no significant difference in Cl^- content between the two varieties under CK condition. Mixed salt stress resulted in a higher accumulation of Cl^- in CCR-44 than Sumian12.

Antioxidant enzyme and lipoxygenase (LOX) activities, MDA content

Figure 4 shows that, the activity of SOD was significantly higher (by 23.1%) in CCR-44 than that in Sumian12 even in CK plants. Mixed salt stress remarkably elevated the activities of those antioxidant enzymes in both cultivars. Although the SOD activities of Sumian12 increased considerably before the salinity level approached S4 (14.65 dS m^{-1}), they were always lower than those of CCR-44.

POD activity in CCR-44 was lower (by 10.8%) than Sumian12 in CK. POD activity increased with increasing salinity levels only in CCR-44, whereas in Sumian12, it remained constant under all salinity levels. In fact, POD activity was higher in CCR-44 than that in Sumian12 under all salinity levels (Figure 4).

Non-significant differences ($p < 0.05$) were observed in CAT activity between CCR-44 and Sumian12 in CK plants. A continuous increase in CAT activity was associated with increased salinity in CCR-44. However,

Table 3. Plant height (cm), leaf area ($\text{m}^2 \text{plant}^{-1}$), leaf water content(%) and yield components, *i.e.* number of boll per plant, boll weight (g) and seed cotton yield (g plant^{-1}) in salt-tolerant CCRI-44 and salt-sensitive Sumian12 under control conditions (CK) or increased salinity levels (S1, S2, S3 and S4). Means \pm SE ($n = 3$).

Cultivar	Treatments	Plant height	Leaf area	water content	Number of bolls	Boll weight	Seed cotton yield
CCRI-44	CK	111.79.83 \pm 4.01 ^a	0.45 \pm 0.02 ^a	83.81 \pm 0.43 ^a	19.40 \pm 1.81 ^a	4.41 \pm 0.27 ^a	86.74 \pm 5.05 ^a
	S1	106.27 \pm 3.03 ^a	0.43 \pm 0.02 ^a	83.54 \pm 0.28 ^a	18.20 \pm 1.10 ^a	4.30 \pm 0.24 ^{ab}	80.21 \pm 4.73 ^a
	S2	83.74 \pm 2.03 ^b	0.36 \pm 0.02 ^b	82.04 \pm 0.44 ^b	15.40 \pm 1.51 ^b	3.92 \pm 0.18 ^{bc}	63.20 \pm 4.03 ^b
	S3	63.88 \pm 3.50 ^c	0.28 \pm 0.01 ^c	81.06 \pm 0.26 ^c	10.80 \pm 1.30 ^c	3.72 \pm 0.24 ^c	41.30 \pm 3.53 ^c
	S4	51.71 \pm 2.52 ^d	0.22 \pm 0.01 ^d	78.74 \pm 0.24 ^d	8.60 \pm 1.14 ^d	3.20 \pm 0.17 ^d	26.73 \pm 3.80 ^d
Sumian12	CK	117.18.37 \pm 2.02 ^a	0.47 \pm 0.03 ^a	83.14 \pm 0.38 ^a	19.80 \pm 2.04 ^a	4.56 \pm 0.20 ^a	90.11 \pm 7.17 ^a
	S1	98.24 \pm 3.52 ^b	0.40 \pm 0.02 ^b	82.83 \pm 0.50 ^a	17.60 \pm 1.14 ^b	4.17 \pm 0.30 ^b	73.80 \pm 5.68 ^b
	S2	75.07 \pm 2.60 ^c	0.29 \pm 0.02 ^c	81.52 \pm 0.39 ^b	12.20 \pm 1.48 ^c	3.63 \pm 0.21 ^c	41.16 \pm 4.68 ^c
	S3	54.75 \pm 2.54 ^d	0.24 \pm 0.02 ^d	80.25 \pm 0.39 ^c	8.80 \pm 1.10 ^d	3.34 \pm 0.31 ^{cd}	28.58 \pm 2.62 ^d
	S4	48.46 \pm 2.50 ^e	0.20 \pm 0.01 ^e	78.57 \pm 0.33 ^d	7.40 \pm 1.14 ^d	3.02 \pm 0.09 ^d	21.96 \pm 2.21 ^d

Different letters in the same column indicate significant difference ($p < 0.05$).

for Sumian12, CAT activity increased under S1, S2 and S3 treatments followed by a decline under S4 treatment. Anyway, CAT activity was higher in CCRI-44 than that in Sumian12 under all salinity levels (Figure 4).

LOX activity and MDA content increased gradually with increasing salinity levels in both cultivars (Figure 5). When salinity level was lower than S1, they did not show significant difference between CCRI-44 and Sumian12, while higher than S1, LOX activity and MDA content in CCRI-44 were markedly higher than that in Sumian12.

Growth and yield component

Plant height and leaf area were lower, but leaf water content was higher in CCRI-44 than Sumian12 under CK treatment (Table 3). Mixed salt stress caused decreases in the above mentioned parameters of both cultivars. However, the extents of reduction were larger in Sumian12 than those in CCRI-44. Number of bolls, boll weight and seed cotton yield of CCRI-44 were lower than those of Sumian12 under CK treatment. However, salt-induced decreases in yield components of CCRI-44 were lower than those of Sumian12, especially at S2 and S3 treatment. There was no significant change in seed cotton yield of CCRI-44 under S1 treatment compared with CK, whereas it decreased significantly (being 19.2% lower than that in CK) in Sumian12. Nevertheless, the seed cotton yield of both cultivars decreased significantly under higher salinity. Although the yield of CCRI-44 was lower by 4.4% than that of Sumian12 in CK, it was higher than that of Sumian12 under each salt treatment.

DISCUSSION

Previous studies have shown that, CCRI-44 could

maintain higher net photosynthetic rate and dry mass than Sumian12 after stressed by 150 mmol L^{-1} NaCl at seeding stage (Zhang et al., 2011). According to Kao et al. (2006) and Moradi and Ismail (2007), the relatively higher salt-tolerant species would have less reduced in P_N , our results proved that salinity induced less reduction in P_N , leaf area and seed cotton yield (Figure 1 and Table 3) conferred CCRI-44 higher salt-tolerance at flowering and boll-forming stage. Significant positive correlations existed between P_N and g_s (Ma et al., 2006). Mixed salt stress drastically reduced g_s , this might be attributed to the lower leaf water potential and a reduction in leaf water content, which resulted in loss of turgor, which leads to reduced photosynthetic rate. However, the salt-tolerant cotton cultivar CCRI-44 performed higher ability in maintaining leaf water content (Figure 3). Thus, it could maintain higher g_s and P_N under salinity.

The value of SI reflects the efficiency of photon energy utilization (Evans et al., 1993). SI of the salt-sensitive cotton cultivar Sumian12 decreased more significantly than that of salt-tolerant cotton cultivar CCRI-44 under salinity stress (Figure 2). Several studies suggested that photon energy efficiency mostly determines the ability of photosystem II, which plays a key role in response of photosynthesis to salinity stress (Xu et al., 1995; Didenko and Suslick., 2002). The salt-tolerant cotton cultivar CCRI-44 had higher ability in catching photon energy which might be attributed to lower salt-induced degradation of CP43 or by higher salt-enhanced synthesis of D1 protein than those in Sumian12 (Sairam et al., 2002).

The decrease in Chl and Car content under mixed salt stress in both cotton cultivars were consistent with those results obtained from results by NaCl treatment at seeding stage (Meloni et al., 2003; Yang et al., 2010; Zhang et al., 2011), which could be attributed to increased activity of the chlorophyll-degrading enzyme

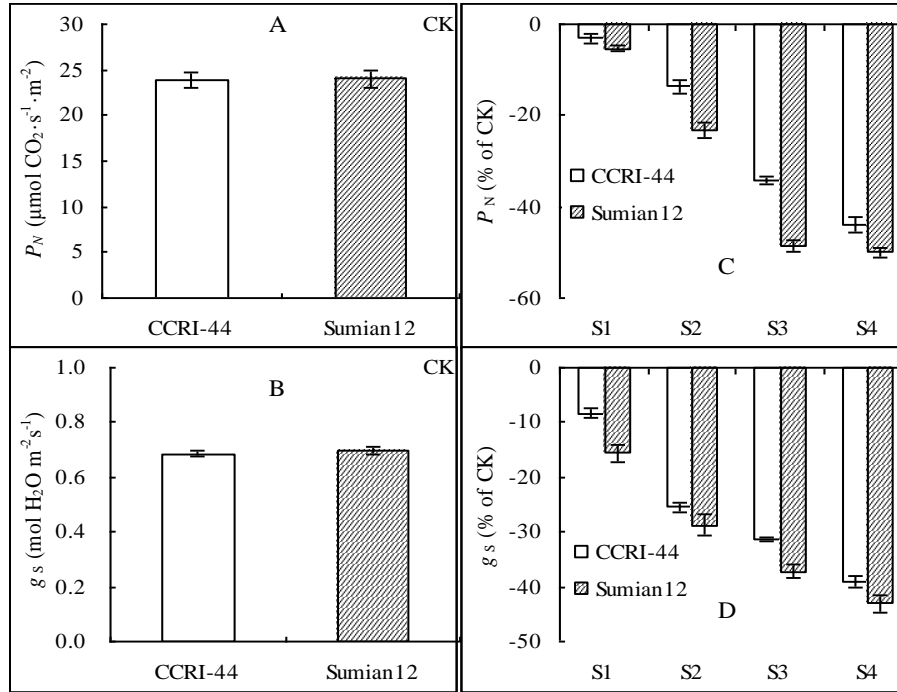


Figure 1. Net photosynthetic rate (P_N) and stomatal conductance (g_s) in functional leaves of salt-tolerant CCRI-44 and salt-sensitive Sumian12 at flowering and boll-forming stage. (A, B) Plants grown under control conditions (CK), (C, D) changes of contents (%) under S1, S2, S3 and S4 in relation to CK, Vertical bars indicate SE ($n = 3$).

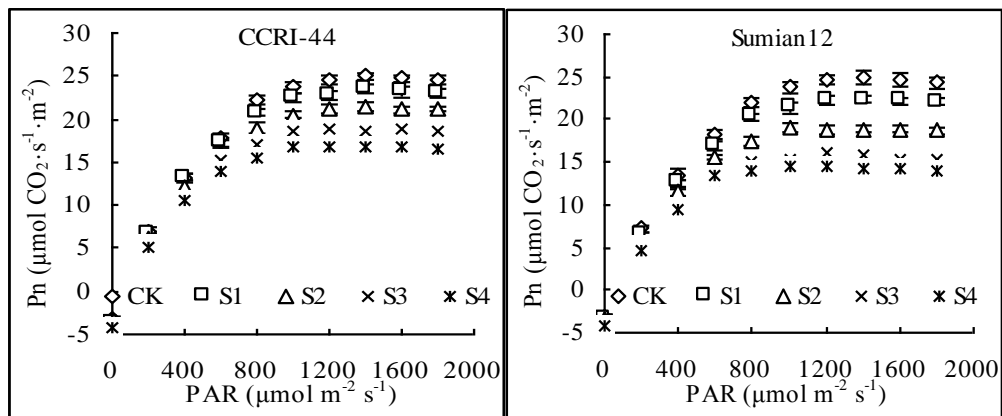


Figure 2. Functional relationship between net photosynthetic rate (P_N) and photosynthetically active radiation (PAR) in functional leaves of salt-tolerant CCRI-44 and salt-sensitive Sumian12 at flowering and boll-forming stage. Error bars show S.E., $n=3$.

such as chlorophyllase and Mg-chelatase, and toxic ion accumulation in leaves (Garcia-Sanchez et al., 2002). Parida and Das (2005) reported Chl content as one of the parameters of salt tolerance in crop plants. Car is responsible for quenching of singlet oxygen (Koyro, 2006), the decrease in Car under salinity stress leads to degradation of β -carotene and formation of zeaxanthins,

which are apparently involved in protection against photoinhibition (Sultana et al., 1999). The salt-tolerant cotton cultivar CCRI-44 retained higher Chl and Car content than salt-sensitive cotton cultivar Sumian12 under salinity stress. Hence, their comparative levels in Chl and Car may determine its relative tolerance.

Salinity stress can impair plant growth by specific ion

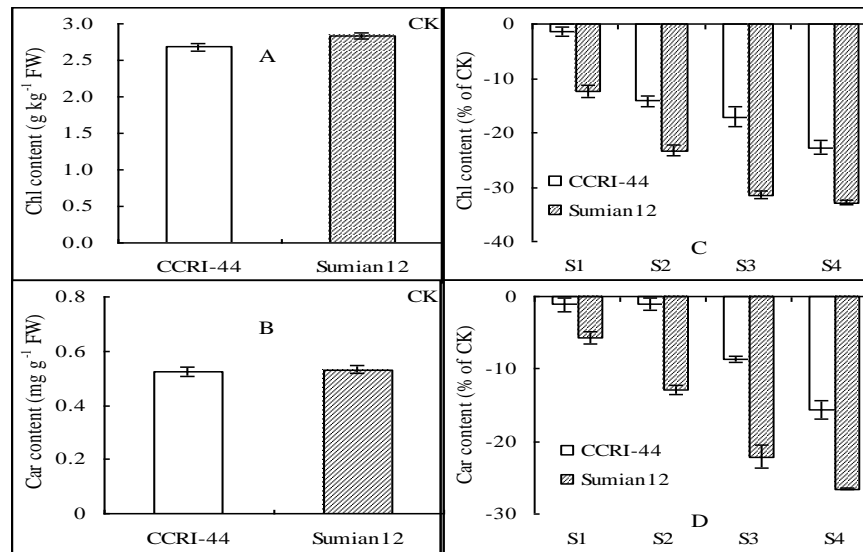


Figure 3. Content of Chlorophyll (Chl) and carotenoid (Car) in functional leaves of salt-tolerant CCRI-44 and salt-sensitive Sumian12 at flowering and boll-forming stage. (A, B) Plants grown under control conditions (CK), (C, D) changes of contents (%) under S1, S2, S3 and S4 in relation to CK, Vertical bars indicate SE ($n = 3$).

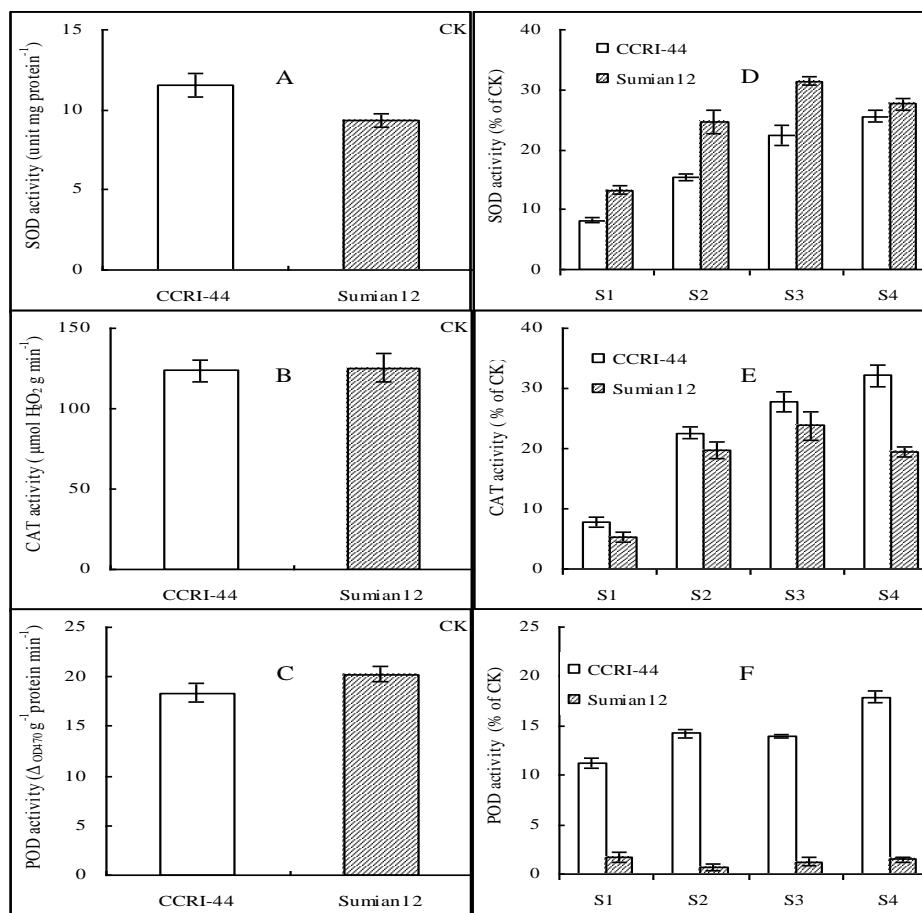


Figure 4. Superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities in functional leaves of salt-tolerant CCRI-44 and salt-sensitive Sumian12 at flowering and boll-forming stage. (A-C) Plants grown under control conditions (CK), (D-F) changes of contents (%) under S1, S2, S3 and S4 in relation to CK, Vertical bars indicate SE ($n = 3$).

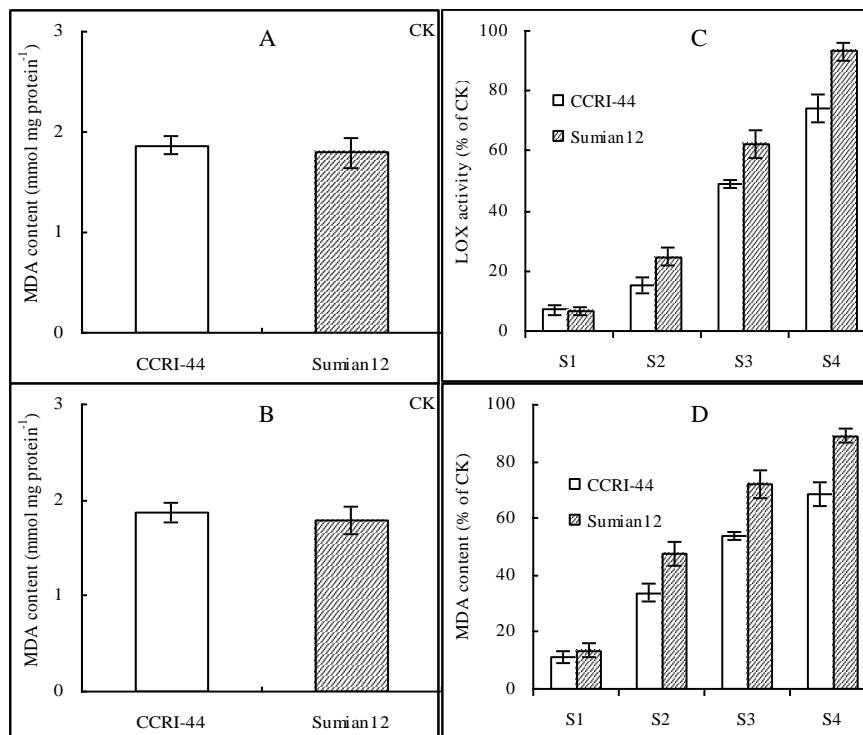


Figure 5. Malondialdehyde (MDA) content and lipoxygenase (LOX) activities in functional leaves of salt-tolerant CCRI-44 and salt-sensitive Sumian12 at flowering and boll-forming stage. (A, B) Plants grown under control conditions (CK), (C, D) changes of contents (%) under S1, S2, S3 and S4 in relation to CK, Vertical bars indicate SE ($n = 3$).

toxicity (Munns, 2005). In this study, Na^+ and Cl^- content was increased in both cultivars with increasing salinity levels, while K^+ content and K^+/Na^+ ratio were decreased (Table 2). These results were similar with that treated with NaCl (Ashraf and Ahmad, 2000) or mixed salts of NaCl and CaCl_2 (1:1 weight ratio) (Chen et al., 2010) in cotton. High Na^+ and Cl^- content may change composition and function of thylakoid membrane (Wang et al., 2008). Deficiency of K^+ may decrease activities of photosynthetic enzyme (Mahajan and Tuteja, 2005).

All these mentioned could decrease the stability of photosystem II reaction center. So maintaining a lower Na^+ and Cl^- content and higher K^+ content might be one of the reasons accounting for a high level of P_N in cotton under salinity stress. In addition, the K^+/Na^+ ratio has been used as a nutritional indicator to select salt-tolerant plants (Glenn et al., 1999). The reason for more significant decrease of K^+/Na^+ ratio in salt-sensitive cotton cultivar Sumian12 than that in salt-tolerant cotton cultivar CCRI-44 might be that, the K^+ selectivity of cell membrane in CCRI-44 was better than that in Sumian12, which was testified by always higher K^+ content in CCRI-44 than that in Sumian12, even in CK plants (Table 2). Soluble sugar and protein plays an important osmotic role in plants (Parida and Das, 2005). Higher soluble sugar and protein accumulation in CCRI-44 than those in

Sumian12 indicated that CCRI-44 had higher ability of osmotic adjustment under salinity. A direct consequence of higher organ osmolyte concentration in CCRI-44 is the maintenance of comparatively higher water and pigment content in leaf. However, Paul and Pellny (2003) observed that, higher soluble sugar, especially glucose, might repress the photosynthesis in most plants by disturbing the balance of carbon to nitrogen balance.

Salinity stress causes oxidative stress by inhibiting the CO_2 assimilation, exposing chloroplasts to excessive excitation energy, which in turn increases the generation of ROS from triplet chlorophyll (Tan et al., 2008). These ROS are all very reactive and cause severe damage to membranes, DNA and proteins (Demidchik et al., 2003). To alleviation of oxidative stress, plants detoxify ROS by up-regulating antioxidative enzymes (Mandhania et al., 2006). Higher activities of antioxidant enzymes in salt-tolerant cotton cultivar CCRI-44 than in salt-sensitive cotton cultivar Sumian12 indicated that CCRI-44 had higher ability to eliminate free active radicals than Sumian12 (Figure 5). A sharp decline observed in antioxidative enzymes activities of Sumian12 in S4 treatment could be due to formation of ROS beyond the critical limit which might pose serious threat to plant cell, causing higher membrane lipid peroxidation (Kumar et al., 2010). In addition, lipoxygenase can catalyzed

polyunsaturated fatty acid oxygenation, producing MDA. Mixed salt stress activated LOX activity in cotton leaves, as observed by Elkahoui et al. (2005) in *Catharanthus roseus* suspension cells. With increasing LOX activity, MDA content also increased, indicating that LOX catalyzed PUFA peroxidation under mixed salt stress. The MDA content and LOX activity in Sumian12 were higher than those in CCRI-44, which provided evidence of a higher lipid peroxidation in Sumian12 in comparison to CCRI-44.

Conclusion

In conclusions, mixed salt stress significantly inhibited the growth of both cotton cultivars by reducing their gas exchange and leaf area. The salt-tolerant cotton cultivar CCRI-44 was better equipped than salt-sensitive cotton cultivar Sumian12 in maintaining gas exchange, SI, pigment content, K^+/Na^+ ratio, and in mechanisms resistant to secondary oxidative stress under salinity stress. CCRI-44 could effectively relieve the inhibition of salt stress and obtain high grain yield. The result of this study was similar with previous studies obtained from treatment with NaCl, indicating that, Na^+ and Cl^- were still the main factors causing salinity stress under the mixed salt treatment.

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