DOI: 10.1007/s11099-011-0050-y PHOTOSYNTHETICA 49 (3): 417-425, 2011

Comparative effects of osmotic-, salt- and alkali stress on growth, photosynthesis, and osmotic adjustment of cotton plants

W. CHEN, C. FENG, W. GUO, D. SHI, and C. YANG+

Key Laboratory of Molecular Epigenetics of MOE, Northeast Normal University, Changchun 130024, Jilin Province, China

Abstract

In this study, cotton seedlings were subjected to osmotic-, salt- and alkali stresses. The growth, photosynthesis, inorganic ions, and organic acids in the stressed seedlings were measured, to compare the mechanisms by which plants adapt to these stresses and attempt to probe the mechanisms by which plants adapt to high pH stress. Our results indicated that, at high stress intensity, both osmotic and alkali stresses showed a stronger injurious effect on growth and photosynthesis than salt stress. Cotton accumulated large amount of Na^+ under salt and alkali stresses, but not under osmotic stress. In addition, the reductions of K^+, NO_3^-, and $H_2PO_4^-$ under osmotic stress were much greater than those under salt stress with increasing stress intensity. The lack of inorganic ions limited water uptake and was the main reason for the higher injury from osmotic- compared to salt stress on cotton. Compared with salt- and alkali stresses, the most dramatic response to osmotic stress was the accumulation of soluble sugars as the main organic osmolytes. In addition, we found that organic acid metabolism adjustment may play different roles under different types of stress. Under alkali stress, organic acids might play an important role in maintaining ion balance of cotton; however, under osmotic stress, malate might play an important osmotic role.

Additional key words: alkali stress; cotton; osmotic stress; salt stress.

Introduction

Abiotic stresses such as salinity, drought, and alkalinity limit crop productivity worldwide (Hu and Schmidhalter 2005). In many agricultural areas of Asia, especially north China, high pH is also an important factor that limits plant growth. Soil alkalization (high-pH stress) causes severe problems in some areas; in northeast China, more than 70% of grassland is alkalinized (Kawanabe and Zhu 1991), sometimes with a soil pH > 10 (Zheng and Li 1999). In saline and sodic soils, Na^+, Ca^{2+}, Mg^{2+}, and K^+ are the main cations of dissoluble mineral salts and Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-} and NO_3^- are the corresponding main anions (Läuchli and Lüttge 2002); all these ions come from neutral or alkaline salts. Previous studies have suggested that salt stress is defined as the stress of neutral salts, and alkali stress as the stress of alkaline salts (Shi and Sheng 2005, Shi and Wang 2005). However, to date, most reports have emphasized salt stress (Munns and Tester 2008) with little attention to alkali stress (Shi *et al*. 2002, Shi and Sheng 2005, Shi and Wang 2005, Gao *et al*. 2008, Wang *et al*. 2008, Yang *et al*. 2007, 2008a, b). The deleterious effect of salt stress mainly results from osmotic stress and ions toxicity (Munns 2002). Comparison of alkali stress with salt stress reveals an added high-pH effect of alkali stress. Namely, both salt- and alkali stresses are at the base of osmotic stress and ion injury; however, alkali stress is due to high pH. High pH due to alkali stress can cause the loss of the normal physiological functions of the roots, destruction of the root cell structure (Yang *et al*. 2008a, b), inhibit the absorption of ions such as Cl^-, NO_3^-, and $H_2PO_4^-$, thus greatly affecting the metabolism of K^+ and Na^+ and disrupt metabolism homeostasis (Yang *et al*. 2007).

Cotton (*Gossypium hirsutum* L.) is an important industrial crop, with some cultivars tolerant to salt and water stresses. In north China, salinity, drought, and high pH are three important factors in limiting cotton productivity. Osmotic stress (water stress) is the dominant stress factor of soil drought. In this study, we used increasing

Received 17 October 2010, *accepted* 26 June 2011.
+Corresponding author; tel.: +86 431 85269590, fax: +86 431 85098103, e-mail: chunwuyang@gmail.com
Abbreviations: DM − dry mass; *E* − transpiration rate; FM − fresh mass; g_s − stomatal conductance; OA − organic acid; P_N − net photosynthetic rate.
Acknowledgments: This work was supported by the National Natural Science Foundation of China (Nos. 31072078 and J0830627-2) and the Basic Research Project by Jilin Provincial Government (No. 20090576).

mannitol concentration to simulate osmotic stress (Chen and Xiong 2005, Saavedra *et al.* 2006). Alkali stress is combination of osmotic stress, ion injury, and high pH stress. The differential response of plants to salt- and osmotic stresses is mainly due to ion injury. Similarly, the differential response of plants to salt- and alkali stresses is mainly due to high pH stress. Therefore, comparing osmotic-, salt- and alkali stresses is important for understanding of high pH tolerance.

In this study, cotton seedlings were subjected to osmotic-, salt- and alkali stresses. The growth, photosynthesis, inorganic ions, and organic acids in the stressed seedlings were then measured to compare the mechanisms by which plants adapt to these stresses and attempt to probe the mechanisms by which plants adapt to high-pH stress.

Materials and methods

Plant growth conditions: Seeds of Yiluzao-7, a major cotton cultivar in north China, were immersed in deionized water for 2 d in a growth chamber (30°C during the day and 25°C at night). Then the seeds were sown in 24-cm diameter plastic pots containing washed sand. The pots were well drained with holes at the bottom. Each pot contained five seedlings that were sufficiently watered with Hoagland nutrient solution daily. All pots were placed outdoors and were kept out of rain. Temperatures during the experiment were 23–27°C during the day and 19–22°C at night.

Stress treatment: Two neutral salts (NaCl and Na_2SO_4) and two alkaline salts ($NaHCO_3$ and Na_2CO_3) were selected based on the salt components and pH in the majority of salt-alkaline soils in northeast China. The salts were mixed in a 9:1 molar ratio for the two treatments. Within the salt-stress group, four concentration treatments were applied: 60, 120, 180, and 240 mM (labeled S1–S4, respectively; pH 6.40–6.57). Within the alkali-stress group, four concentration treatments were applied: 30, 60, 90, and 120 mM (labeled A1–A4, respectively; pH 9.01–9.09). Mannitol was used for osmotic stress (pH 6.5). Within the osmotic stress group, four concentration treatments were applied: 63, 126, 252, and 378 mM (labeled O1–O4, respectively; pH 6.45–6.55). The stress factors of each treatment are shown in Table 1. The osmotic pressures of each treatment solution were detected by a vapour pressure osmometer (*Wescor 5520, VAPRO*, USA). When the seedlings were five weeks old, they were subjected to stress treatments. A pot containing 5 seedlings represented one replicate, and there were three replicates (three pots) per treatment. Out of 80 pots, 39 pots with seedlings growing uniformly were selected and randomly divided into 13 sets, with three pots per set. One set was used as a control, and the remaining 12 sets used for the different stress treatments. Each pot including 5 seedlings was considered a single replicate; therefore three pots per treatment (15 seedlings) were three replicates. Stress treatments were applied once daily around 17:00–18:00 h with nutrient solutions containing the appropriate stress salts. All pots were watered thoroughly with 2 L of treatment solution divided in three portions. Control plants were maintained by watering with nutrient solution. The pots were well drained with holes at the bottom. Thus, the treatment solution was free to run through the pots. The treatment duration was 10 d.

Measurement of physiological indices: Net photosynthetic rate (P_N), stomatal conductance (g_s) and transpiration rate (E) of leaves were determined during 08:30–10:30 h in fully expanded third blades, using a portable open flow gas-exchange system *LI-6400* (*LI-COR*, Lincoln, USA). The photosynthetically active radiation (PAR) was 1,200 μmol m^{-2} s^{-1}. The ambient CO_2 concentration was set at 360 μmol mol^{-1}, the leaf temperature in the cuvette was about 25°C. The water content (WC) of shoots was calculated using the formula WC = (FM – DM)/DM, and expressed as g g^{-1}(DM) (Khan *et al*. 2000, Yang *et al*. 2007), where DM is dry mass and FM is fresh mass. Plants were harvested in the morning after the final treatment, and were first washed with tap water followed by distilled water. Roots and shoots were separated and freeze-dried. Then all dry samples of five seedlings in each pot were levigated and mixed for physiological index measurements. Dry samples of plant material (100 mg) were treated with 20 mL of deionized water at 100°C for 1 h, and the resulting extract used to determine the contents of free inorganic ions and organic acids (OAs). In this study, we considered the roles of phosphorus (P) in ion balance and osmoregulation. The free phosphates in plant tissues were principally in the form of $H_2PO_4^-$. Therefore, we determined $H_2PO_4^-$ instead of total P. The contents of NO_3^-, Cl^-, $H_2PO_4^-$, and oxalic acid were determined by ion chromatography using a *DX-300* ion chromatographic system with an *AS4A-SC* ion-exchange column and a *CDM-II* electrical conductivity detector (mobile phase: $Na_2CO_3/NaHCO_3$ = 1.7/1.8 mM; *DIONEX*, Sunnyvale, USA). The levels of other OAs (malate, citrate, succinate, acetate, formate, tartrate, glycolate, and lactate) were also determined by ion chromatography using the *DX-300* ion chromatographic system with an *ICE-AS6* ion-exclusion column, *CDM-II* electrical conductivity detector, and an *AMMS-ICE II* MicroMembrane suppressor (mobile phase: 0.4 mM heptafluorobutyric acid; *DIONEX*, Sunnyvale, USA). An atomic absorption spectrophotometer (*TAS-990*, *Purkinje General*, Beijing, China) was used to determine the levels of Na^+ and K^+. The contents

Table 1. The stress factors of various stress treatments.

Treatment		Osmotic pressure [MPa]	Solution concentration [mM]	Na^+ concentration [mM]	Total ion concentration [mM]	pH
	Control	–0.098	0	0	0	6.40
Salt stress	S1	–0.337	60	66	126	6.41
	S2	–0.657	120	132	252	6.45
	S3	–0.929	180	198	378	6.54
	S4	–1.213	240	264	504	6.57
Alkali stress	A1	–0.211	30	33	63	9.01
	A2	–0.330	60	66	126	9.04
	A3	–0.505	90	99	189	9.09
	A4	–0.623	120	132	252	9.08
Osmotic stress (mannitol)	O1	–0.208	63	0	0	6.45
	O2	–0.412	126	0	0	6.46
	O3	–0.570	252	0	0	6.55
	O4	–0.710	378	0	0	6.54

of proline and total soluble sugars were measured using ninhydrin and anthrone, respectively (Zhu 1993).

Statistical analysis was performed using *SPSS 13.0* (*SPSS*, Chicago, USA). All data were represented by an average of three replicates and standard errors (SE). Statistical difference between different stress intensities of the same stress type was tested by one-way analysis of variance. The significance level was $P<0.05$.

Results

Photosynthesis and growth: Under 378 mM mannitol stress, all cotton seedlings died. The trends of shoot and root masses, P_N, g_s, and E in cotton exposed to the different stresses were similar. With increasing stress intensity, their values decreased under the three stresses, with reductions under osmotic- and alkali stresses greater than under salt stress ($P<0.05$; Fig. 1). Salt stress only had a small effect on water content of cotton shoots, while osmotic and alkali stresses significantly reduced shoot water content, with greater reductions under osmotic than under alkali stress ($P<0.05$; Fig. 1*C*).

Inorganic cations: Na^+ did not accumulate under osmotic stress, while its content increased sharply with increasing stress intensity under salt- and alkali stresses ($P<0.05$; Fig. 2*A*,*B*). With increasing stress intensity, K^+ contents in both shoots and roots decreased under the three stresses, with the extents of reduction greater under osmotic and alkali stresses than under salt stress ($P<0.05$; Fig. 2*C*,*D*).

Inorganic anion: Under salt stress, the Cl^- content increased with increasing stress intensity ($P<0.05$; Fig. 2*E*,*F*). However, alkali- and osmotic stresses did not influence the Cl^- accumulation in cotton shoots (Fig. 2*E*), but reduced Cl^- contents in the roots (Fig. 2*F*). The NO_3^- content in roots was much higher than in shoots (Fig. 2*G*,*H*). With increasing stress intensity, the NO_3^- contents decreased with the three stresses ($P<0.05$; Fig. 2*G*,*H*), with a greater reductions under osmotic- and alkali stresses greater than with salt stress. The effects of the three stresses on $H_2PO_4^-$ contents were small (Fig. 2*I*,*J*).

Compatible solutes: The three stresses all stimulated proline accumulation in both shoots and roots. With increasing stress intensity, in the shoots, the increases of proline under three stresses were similar. However, in roots, at high stress intensity (> –0.6 MPa), the increases under osmotic- and alkali stresses were much greater than for salt stress of the same osmotic potential (Fig. 1*G*,*H*). Salt- and alkali stresses produced only small effects on the accumulation of soluble sugars in shoots and roots, but osmotic stress strongly stimulated accumulation in shoots and roots ($P<0.05$; Fig. 1*I*,*J*).

Organic acids in shoots: Malate, citrate, tartrate, oxalate, formate, and lactate were detected in cotton shoots. Of these OAs, malate and citrate were clearly the dominant components in both shoots and roots of cotton (Fig. 3). Salt stress decreased the contents of citrate and total OA in cotton shoots, but only little affected other OAs. Although all stresses decreased the citrate content in shoots, the reduction under osmotic stress was greater than under salt- and alkali stresses (Fig. 3). In addition, both osmotic- and alkali stresses strongly stimulated malate accumulation in cotton shoots ($P<0.05$; Fig. 3*E*).

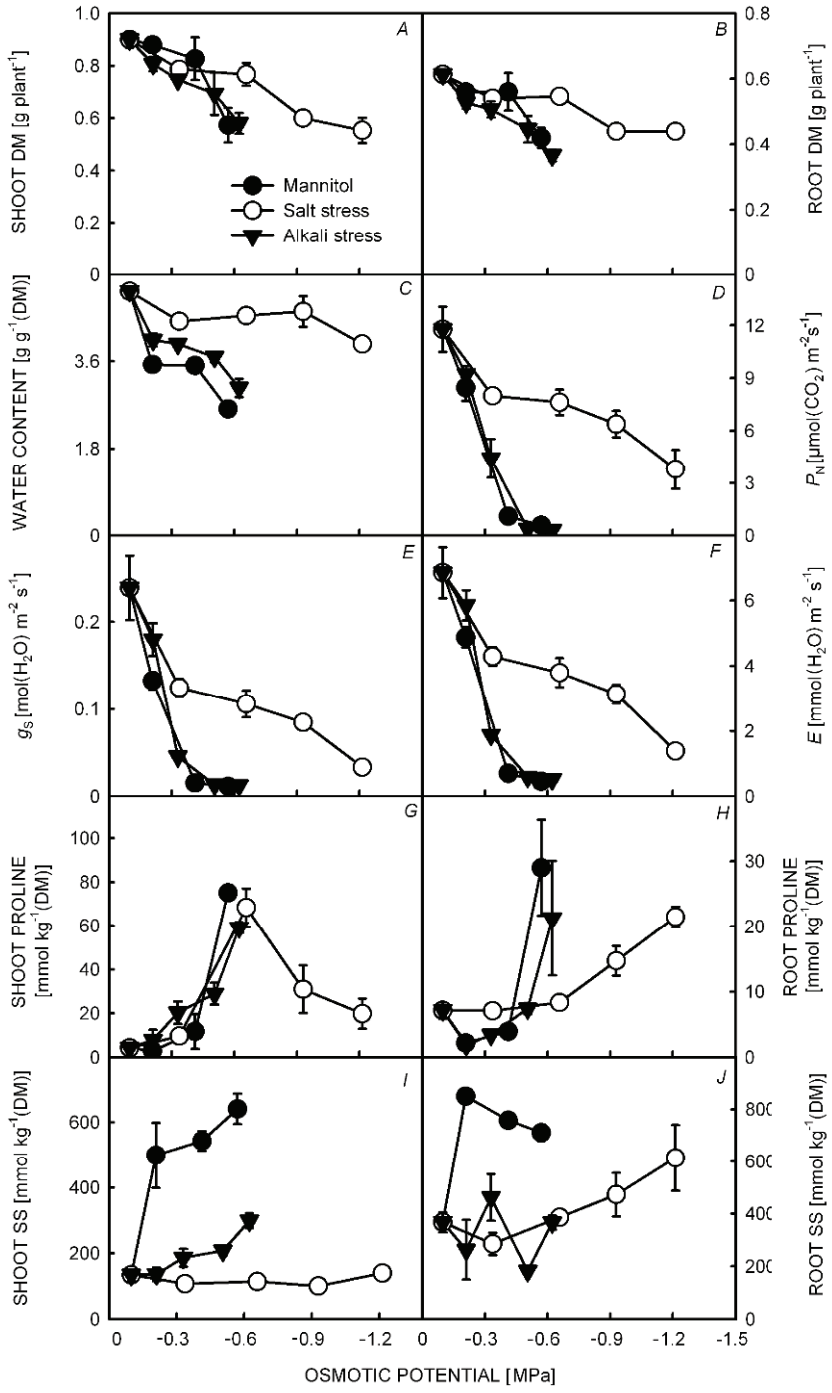

Fig. 1. Effect of osmotic- (mannitol), salt- and alkali stresses on (*A* and *B*) dry mass (DM), (*C*) shoot water content, (*D–F*) photosynthesis, (*G* and *H*) proline and (*I* and *J*) soluble sugars (SS) of cotton plants. The values are means (± SE) of three replicates. Five-week-old seedlings were subjected to osmotic stress (mannitol; pH 6.45–6.55), salt stress ($NaCl:Na_2SO_4$ = 9:1; pH 6.40-6.57), and alkali stress ($NaHCO_3:Na_2CO_3$ = 9:1; pH 9.01–9.09) for 10 d. *E* − transpiration rate; g_s − stomatal conductance; P_N − net photosynthetic rate.

Organic acids in roots: Malate, citrate, tartrate, oxalate, formate, and lactate were also detected in cotton roots (Fig. 6). Salt stress only produced a small effect on accumulation of OAs in cotton roots (Fig. 3). Similarly, both osmotic- and alkali stresses also stimulated malate accumulation in cotton roots (P<0.05; Fig. 3*F*). Under osmotic- and alkali stresses, with increasing stress intensity, the citrate content initially increased gradually, and then declined (Fig. 3*C*,*D*).

Discussion

Growth and photosynthesis: It has been documented that salt stress (Ashraf and Ahmad 2000, Meloni *et al.* 2003) and osmotic stress (Turner *et al.* 1986, Nepomuceno *et al.* 1998, Pettigrew 2004, Ullah *et al.* 2008) limit growth and photosynthesis of cotton plants. Our results indicated that, at low stress intensity, the

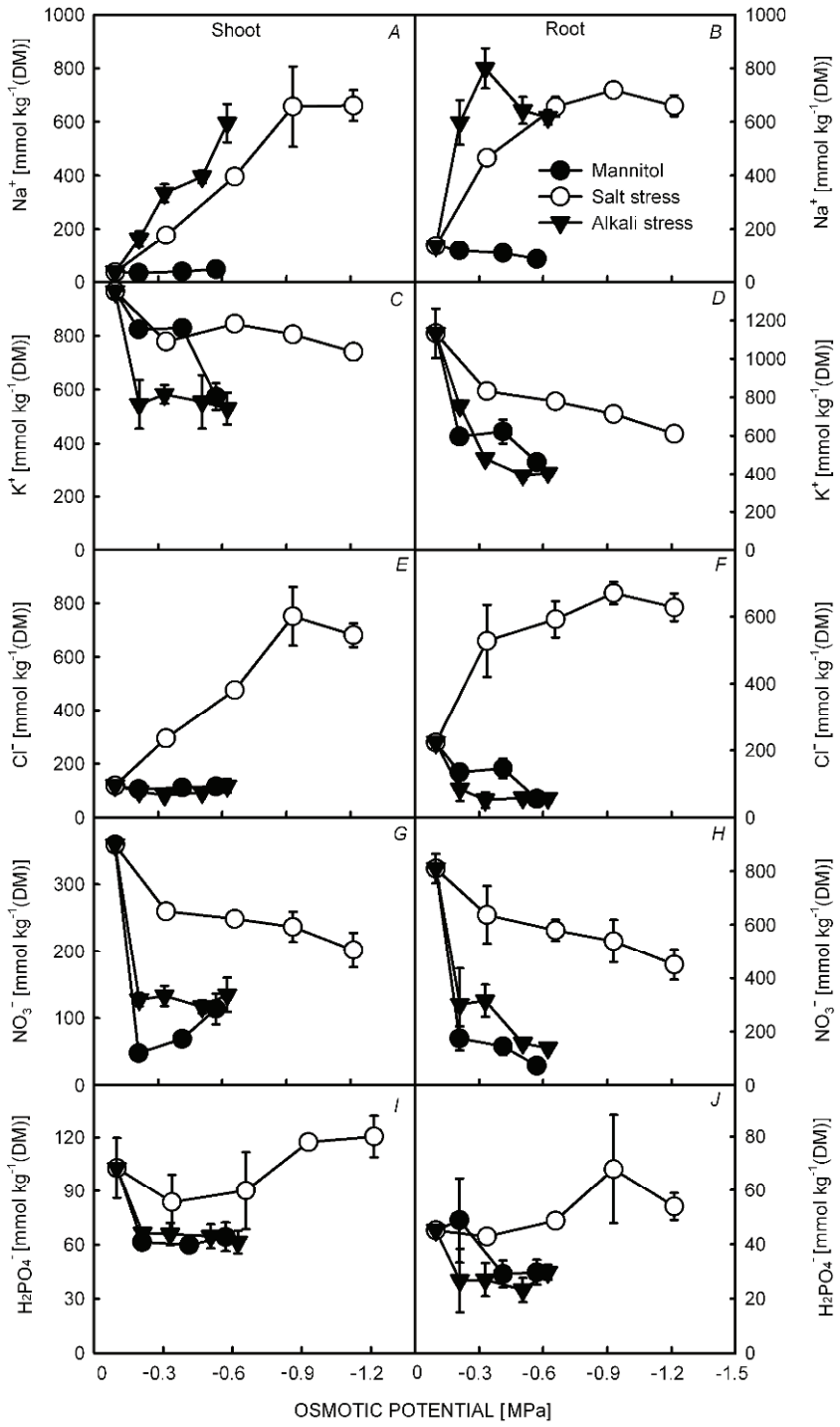

Fig. 2. Effect of osmotic- (mannitol), salt- and alkali stresses on the contents of (*A* and *B*) Na^+, (*C* and *D*) K^+, (*E* and *F)* Cl^-, (*G* and *H*) NO_3^- and (*I* and *J*) $H_2PO_4^-$ in cotton plants. The values are means (± SE) of three replicates. Five-week-old seedlings were subjected to osmotic stress (mannitol; pH 6.45–6.55), salt stress ($NaCl:Na_2SO_4$ = 9:1; pH 6.40-6.57), and alkali stress ($NaHCO_3:Na_2CO_3$ = 9:1; pH 9.01–9.09) for 10 d.

effects of the three stresses on cotton were similar. However, at high stress intensity both osmotic- and alkali stresses showed a stronger injurious effect on growth and photosynthesis than salt stress in cotton plants (Fig. 1). Under moderate stress, the harmful effects of osmotic- or alkali stresses were alleviated by adjustment outside the roots and consequently the intracellular environment was not affected.

Osmotic regulation and ion balance: Under salt- and alkali stresses, plants frequently accumulate large amounts of Na^+ to lower the cell water potential (Yang *et al*. 2007, Munns and Tester 2008). Under osmotic stress (mannitol), lack of Na^+ in the external environment will reduce the frequency of Na^+ absorption by roots, and influence the osmotic regulation of plants. In agreement with previous studies, we found that cotton also accumulated large amounts of Na^+ under salt- and alkali stresses, but not under osmotic stress (Fig. 2*A*,*B*). In addition, the reductions of K^+, NO_3^-, and $H_2PO_4^-$ under

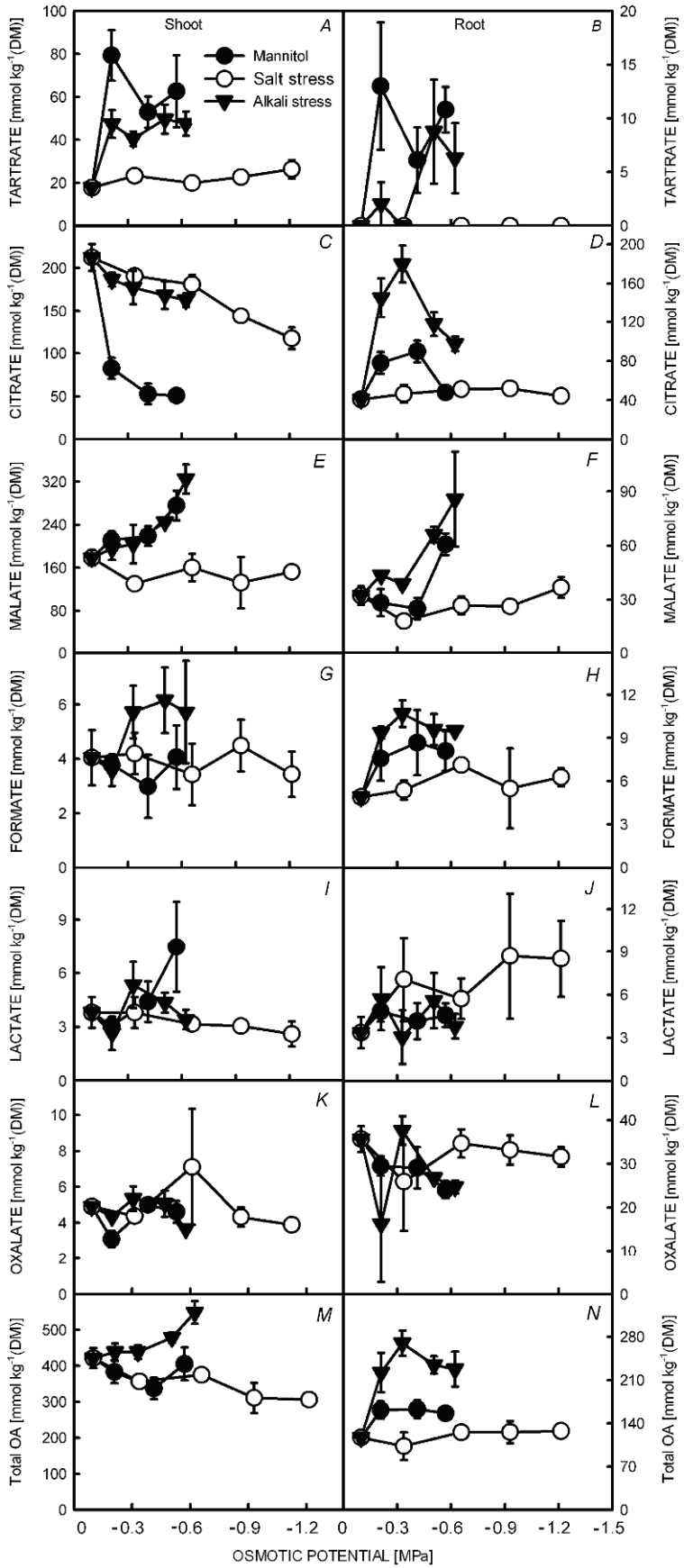

Fig. 3. Effect of osmotic- (mannitol), salt- and alkali stresses on the contents of organic acids (OAs) in shoots and roots of cotton plants. The values are means (± SE) of three replicates. Five-week-old seedlings were subjected to osmotic stress (mannitol; pH 6.45–6.55), salt stress ($NaCl:Na_2SO_4$ = 9:1; pH 6.40–6.57), and alkali stress ($NaHCO_3:Na_2CO_3$ = 9:1; pH 9.01–9.09) for 10 d.

Table 2. Percent contribution of each solute molarity to total determined molarity in cotton shoots under osmotic-, salt-, and alkali stresses. Percentage is calculated according to means of each solute.

Treatment		Na^+ [%]	K^+ [%]	Cl^- [%]	NO_3^- [%]	$H_2PO_4^-$ [%]	Proline [%]	Soluble sugars [%]	Organic acids [%]
	Control	1.7	45.0	5.5	16.8	4.8	0.2	6.3	19.7
Salt stress	S1	8.5	37.7	14.3	12.6	4.0	0.5	5.2	17.3
	S2	15.2	32.3	18.2	9.5	3.4	2.6	4.4	14.4
	S3	21.8	26.7	25.0	7.9	3.9	1.0	3.3	10.3
	S4	23.0	25.8	23.7	7.0	4.2	0.7	4.9	10.7
Alkali stress	A1	10.3	34.5	6.1	8.1	4.2	0.5	8.6	27.8
	A2	18.1	31.6	4.5	7.2	3.6	1.1	10.1	23.8
	A3	20.4	28.6	4.8	6.0	3.3	1.5	10.7	24.7
	A4	25.4	22.6	5.0	5.7	2.6	2.5	12.7	23.4
Osmotic stress	O1	1.7	42.1	5.4	2.4	3.1	0.1	25.5	19.6
(mannitol)	O2	2.0	41.4	5.6	3.5	3.0	0.6	27.1	16.9
	O3	2.4	28.2	5.7	5.6	3.2	3.7	31.5	19.9

Table 3. Percent contribution of each solute molarity to total determined molarity in cotton roots under osmotic-, salt-, and alkali stresses. Percentage is calculated according to means of each solute.

Treatment		Na^+ [%]	K^+ [%]	Cl^- [%]	NO_3^- [%]	$H_2PO_4^-$ [%]	Proline [%]	Soluble sugars [%]	Organic acids [%]
	Control	4.8	39.9	7.9	28.5	1.6	0.3	12.9	4.1
Salt stress	S1	16.1	28.7	18.2	22.0	1.5	0.2	9.8	3.5
	S2	20.7	24.5	18.6	18.3	1.5	0.3	12.1	4.0
	S3	21.6	21.5	20.2	16.2	2.0	0.4	14.2	3.8
	S4	20.8	19.3	19.8	14.2	1.7	0.7	19.4	4.0
Alkali stress	A1	26.6	33.5	3.8	13.4	1.2	0.1	11.6	9.9
	A2	33.2	20.0	2.2	13.1	1.1	0.1	19.1	11.2
	A3	37.8	23.2	3.4	9.2	1.4	0.4	10.7	13.8
	A4	33.1	21.7	3.1	7.5	1.6	1.1	19.7	12.2
Osmotic stress	O1	5.7	28.6	6.4	8.4	2.3	0.1	40.8	7.7
(mannitol)	O2	5.5	31.5	7.4	7.3	1.5	0.2	38.4	8.2
	O3	5.4	28.9	3.5	4.6	1.8	1.8	44.3	9.7

osmotic stress were much greater than those under salt stress with increasing stress intensity (Fig. 2). As the energy consumption of synthesizing organic osmolytes is far larger than for absorbing inorganic ions (Munns 2002), the lack of inorganic ions in cotton under osmotic stress might limit water uptake, and reduce growth and photosynthesis. In addition, the increased Na^+ in cotton roots and shoots under alkali stress might be related to decreased Na^+ exclusion. It is well known that many plant species have a Na^+ exclusion mechanism that is dependent on a Na^+/H^+ antiport, such as salt overly sensitive 1 types (SOS1), which exchanges cytoplasmic Na^+ with external H^+ (Zhu 2003, Munns and Tester 2008). The exchange activity relies on the transmembrane proton gradient achieved by H^+-ATPase (Zhu 2003). Under alkali stress, the lack of external protons might weaken the exchange activity of the Na^+/H^+ antiport on the root plasma membrane, possibly reducing the exclusion of Na^+ into the rhizosphere and enhancing *in vivo* accumulation of Na^+, even to toxic levels. This may be the basis of alkali injury.

Plants generally compartmentalize Na^+ into vacuoles to avoid Na^+ toxicity in the cytosol. At the same time, plants will also synthesize compatible solutes, such as betaine, proline, free sugar, and polyalcohols in cytoplasm to prevent cytoplasm dehydration (Munns and Tester 2008). In this study, we tested proline and soluble sugars in shoots and roots of cotton. Salt- and alkali stresses produced only small effects on the accumulation of soluble sugars in shoots and roots of cotton, but osmotic stress strongly stimulated its accumulation in shoots and roots (Fig. 1*I,J*). Under osmotic stress, the contents of soluble sugars in cotton was very high (17–21% of DM), and was significantly higher than that of proline, revealing that soluble sugars might be the main organic osmolytes in the response of cotton to osmotic stress. Under osmotic stress the contribution of soluble sugars to total molarity of determined solutes in both shoots and roots was much higher than that of proline and OAs (Tables 2, 3), indicating that soluble sugars might

play an important role in osmotic regulation of cotton, and that the osmotic role of proline was small. This conclusion might differ from that of Gadallah (1995), who reported that proline played important role in osmotic regulation of cotton. Although the mannitol, salt- and alkali stresses can all result in water stress, cotton plants had different responses to these stresses in terms of osmotic regulation mechanisms (Tables 2, 3). This might be due to different stress factors (Table 1): under salt stress, the main osmolytes were Na^+, K^+, and Cl^-; while under alkali stress, the main osmolytes were Na^+, K^+, and OA. However, under osmotic stress, except for soluble sugars, K^+ and OAs also played important osmotic roles (Tables 2, 3).

A striking feature of plant tissues is that the total content of OAs is higher than that in animals and microorganisms (López-Bucio *et al*. 2000). OAs have a potential role as metabolically active solutes for osmotic adjustment, balance of cation excess and pH homeostasis (López-Bucio *et al*. 2000). The OAs are key components in mechanisms that some plants use to cope with drought (Timpa *et al*. 1986), Al^{3+} toxicity (Li *et al*. 2000), P deficiency (Koyama *et al*. 2000), Fe deficiency (López-Millán *et al*. 2000), heavy-metal stress (López-Bucio *et al*. 2000), and plant–microbe interactions at the root–soil interface (López-Bucio *et al*. 2000). In recent years, reports have shown that some halophytes accumulate high concentration of OAs under alkali stress to remedy the deficit of inorganic anions and maintain intracellular ion balance (Yang *et al*. 2007, 2008c, 2010). The OA change in cotton (Fig. 3) is unique compared with other plants under osmotic stress (Timpa *et al*. 1986) and alkali stress (Shi and Sheng 2005, Shi and Wang 2005, Yang *et al*. 2007, 2008c, 2010, Chen *et al*. 2009). Our results indicated that malate and citrate were clearly the dominant components in both shoots and roots of cotton (Fig. 3). Malate did not accumulate under salt stress, while its content increased sharply under alkali- and osmotic stresses with increasing stress intensity (Fig. 3). For citrate, the three stresses reduced its content in shoots, and the reductions under osmotic stress were much greater than those under salt- and alkali stresses with increasing stress intensity. Under alkali stress, the change of citrate in cotton was different from other plants, *e.g*. *Aneurolepidium chinense* (Shi and Wang 2005), *Hippophae rhamnoides* (Chen *et al*. 2009), sunflower (Shi and Sheng 2005), *Puccinellia tenuiflora* (Shi *et al*. 2002) and *Chloris virgata* (Yang *et al*. 2010). In these other species, citrate contents in shoots increased sharply with increasing alkali stress intensity. Under osmotic stress, the change of citrate in cotton also contradicted the previous report of Timpa *et al*. (1986), who reported that the most dramatic response of cotton to osmotic stress was the accumulation of citrate. This difference might be attributed to stress treatment method or genotypic differences. There was water stress in the experiment of Timpa *et al*. (1986), and plants were grown in a rainout shelter to prevent rainfall; they also found that the effects of field water stress on the OAs and carbohydrate compositions in various cotton genotypes were different. These data indicate that OA metabolism adjustment may play different roles under different stress types or in different plant species, and even in different genotypes. Our results showed that, under alkali stress, OAs might play an important role in maintaining ion balance of cotton; however, under osmotic stress, malate might play an important osmotic role. Moreover, during response to stresses, the roles of various OAs in different organs might also be different. This study confirmed that OA metabolism adjustment is important in the adaptive response of plants to abiotic stress, and should be an important future research direction for plant stress physiology.

References

Ashraf, M., Ahmad, S.: In uence of sodium chloride on ion accumulation, yield components and ®bre characteristics in salt-tolerant and salt-sensitive lines of cotton (*Gossypium hirsutum* L.). Field Crops Res. **66**: 115-127, 2000.

Chen, W., Cui, P., Sun, H., Guo, W., Yang, C., Jin, H., Fan, B., Shi, D.: Comparative effects of salt and alkali stresses on organic acid accumulation and ionic balance of seabuckthorn (*Hippophae rhamnoides* L.). – Ind. Crop. Prod. **30**: 351-358, 2009.

Chen, H., Xiong, L.: Pyridoxine is required for post-embryonic root development and tolerance to osmotic and oxidative stresses. – Plant J. **44**: 396-408, 2005.

Gao, C., Wang,Y., Liu, G., Yang, C., Jiang, J., Li, H.: Expression profiling of salinity–alkali stress responses by large-scale expressed sequence tag analysis in *Tamarix hispid*. – Plant Mol. Biol. **66**: 245-258, 2008.

Gadallah, M.A.A.: Effect of water stress, abscisic acid and proline on cotton plants. J. Arid Environ. **30**: 315-325, 1995.

Hu, Y., Schmidhalter, U.: Drought and salinity: A comparison of their effects on mineral nutrition of plants. – J. Plant Nutr. Soil Sci. **168**: 541-549, 2005.

Kawanabe, S., Zhu, T.C.: Degeneration and conservation of *Aneurolepidium chinense* grassland in Northern China. – J. Jap. Grassland Soc. **37**: 91-99, 1991.

Khan, M.A., Ungar, I.A., Showalter, A.M.: The effect of salinity on the growth, water status, and ion content of a leaf succulent perennial halophyte, *Suaeda fruticosa* (L.) Forssk. – J. Arid Environ. **45**: 73-84, 2000.

Koyama, H., Kawamura, A., Kihara, T., Hara, T., Takita, E., Shibata, D.: Overexpression of mitochondrial citrate synthase in *Arabidopsis thaliana* improved growth on a phosphorus-limited soil. – Plant Cell Physiol. **41**: 1030-1037, 2000.

Läuchli, A., Lüttge, U.: Salinity in the soil environment. – In: Tanji, K.K. (ed.): Salinity: Environment-Plants-Molecules. Pp. 21-23. Kluwer Academic Publ., Boston 2002.

Li, X.F., Ma, J.F., Matsumoto, H.: Pattern of aluminum-induced secretion of organic acids differs between rye and wheat. – Plant Physiol. **123**: 1537-1544, 2000.

López-Bucio, J., Nieto-Jacobo, M.F., Ramírez-Rodríguez, V., Herrera-Estrella, L.: Organic acid metabolism in plants: from adaptive physiology to transgenic varieties for cultivation in extreme soils. – Plant Sci. **160**: 1-13, 2000.

López-Millán, A.F., Morales, F., Andaluz, S., Gogorcena, Y., Abadía, A., Rivas, J.D.L., Abadía, J.: Responses of sugar beet roots to iron deficiency. Changes in carbon assimilation and oxygen use. – Plant Physiol. 1**24**: 885-898, 2000.

Meloni, D.A., Oliva M.A., Martinez, C.A., Cambraia, J.: Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. – Environ. Exp. Bot. **49**: 69-76, 2003.

Munns, R.: Comparative physiology of salt and water stress. – Plant Cell Environ. **25**: 239-250, 2002.

Munns, R., Tester, M.: Mechanisms of Salinity Tolerance. – Annu. Rev. Plant Biol. **59**: 651-681, 2008.

Nepomuceno, A.L., Oosterhuis, D.M., Stewart, J.M.: Physiological responses of cotton leaves and roots to water deficit induced by polyethylene glycol. – Environ. Exp. Bot. **40**: 29-41, 1998.

Pettigrew, W.T.: Physiological consequences of moisture deficit stress in cotton. – Crop Sci. **44**: 1265-1272, 2004.

Saavedra, L., Vensson, J., Carballo, V., Izmendi, D., Welin, B., Vidal, S.: A dehydrin gene in *Physcomitrella patens* is required for salt and osmotic stress tolerance. Plant J. **45**: 237-249, 2006.

Shi, D., Sheng, Y.: Effect of various salt-alkaline mixed stress conditions on sunflower seedlings and analysis of their stress factors. – Environ. Exp. Bot. **54**: 8-21, 2005.

Shi, D., Wang, D.: Effects of various salt-alkali mixed stresses on *Aneurolepidium chinense* (Trin.) Kitag. – Plant Soil **271**: 15-26, 2005.

Shi, D.C., Yin, S.J., Yang, G.H., Zhao, K.F.: Citric acid accumulation in an alkali-tolerant plant *Puccinellia tenuiflora* under alkaline stress. – Acta Bot. Sin. **44**: 537-540, 2002.

Timpa, J.D., Burke, J.J., Quiseberry, J.E., Wendt, C.W.: Effect of water stress on organic acid and carbohydrate composition of cotton plant. – Plant Physiol. **82**: 724-730, 1986.

Turner, N.C., Hearn, A.B., Begg, J.E., Constable, G.A., 1986. Cotton (*Gossypium hirsutum* L.); physiological and morphological responses to water deficit and their relation to yield. – Field Crops Res. **14**: 153-170, 1986.

Ullah, I, Rahman, M., Ashraf, M., Zafar, Y.: Genotypic variation for drought tolerance in cotton (*Gossypium hirsutum* L.): Leaf gas exchange and productivity. – Flora **203**: 105-115, 2008.

Wang ,Y., Ma, H., Liu, G., Xu, C., Zhang, D., Ban, Q.: Analysis of gene expression profile of *Limonium bicolor* under $NaHCO_3$ stress using cDNA microarray. – Plant Mol. Biol. Rep. **26**: 241-254, 2008.

Yang, C., Guo, W., Shi, D.: Physiological roles of organic acids in alkali-tolerance of the alkali-tolerant halophyte *Chloris virgata.* – Agron. J. **102**: 1081-1089, 2010.

Yang, C., Shi, D., Wang, D.: Comparative effects of salt stress and alkali stress on growth, osmotic adjustment and ionic balance of an alkali resistant halophyte *Suaeda glauca* (Bge.). – Plant Growth Regul. **56**: 179-190, 2008c.

Yang, C.W., Chong, J.N., Kim, C.M., Li, C.Y., Shi, D.C., Wang, D.L.: Osmotic adjustment and ion balance traits of an alkali resistant halophyte *Kochia sieversiana* during adaptation to salt and alkali conditions. – Plant Soil **294**: 263-276, 2007.

Yang, C.W., Jianaer, A., Li, C.Y., Shi, D.C., Wang, D.L.: Comparison of the effects of salt-stress and alkali-stress on photosynthesis and energy storage of an alkali-resistant halophyte *Chloris virgata*. – Photosynthetica **46**: 273-278, 2008a.

Yang, C.W., Wang, P., Li, C.Y., Shi, D.C., Wang, D.L.: Comparison of effects of salt and alkali stresses on the growth and photosynthesis of wheat. – Photosynthetica **46**: 107-114, 2008b.

Zheng, H.Y., Li, J.D.: Form and dynamic trait of halophyte community. – In: Zheng, H.Y., Li, J.D. (ed.): Saline Plants in Songnen Plain and Restoration of Alkaline-Saline Grass. Pp. 137-138. Science Press, Beijing 1999.

Zhu, G.L.: Determination of free proline and sugar. –In: Zhu, G.L. (ed.): Laboratory Manual of Plant Physiology. Pp. 10-60. Beijing University Press, Beijing 1993.

Zhu, J.K.: Regulation of ion homeostasis under salt stress. – Curr. Opin. Cell Biol. **6**: 441-445, 2003.