Effects of salt and alkali stresses on growth and ion balance in rice (*Oryza sativa* L.)

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ABSTRACT

Rice seedlings were stressed with salt stress or alkali stress. The growth, organic acids (OAs) and inorganic ions in stressed seedlings were measured to investigate the physiological adaptive mechanism by which rice tolerates alkali stress. The results showed that the injury effect of alkali stress on rice was stronger than salt stress. Under salt stress, inorganic anions were dominant in maintaining intracellular ionic equilibrium; however, under alkali stress, the contents of inorganic anions decreased, which caused a severe deficit of negative charge. The deficit of negative charge was remedied by greatly accumulated OAs, especially malate and citrate, and OAs were the dominant components and contributed to 61–81% of total negative charge, indicating that OA accumulation might be necessary for intracellular ion balance in rice. In addition, the OA metabolism adjustment of rice differed from that of other plant species, implying that rice may have a special alkali-tolerance mechanism.

Keywords*:* ion accumulation; osmotic regulation; inorganic ion; organic acid; change balance

Soil salinity and alkalinity seriously affect about 932 million hectares of land globally, reducing productivity in about 100 million hectares in Asia (Rao et al. 2008). In northeast China, > 70% of the land area is alkaline grassland (Yang et al. 2007), where the soil becomes alkaline as a result of hydrolysis of two carbonates ($NaHCO_3$ and Na_2CO_3). Some reports have clearly demonstrated alkaline salts ($NaHCO_3$ and Na_2CO_3) are more destructive to plants than neutral salts (NaCl and Na_2SO_4) (Shi and Sheng 2005, Shi and Wang 2005, Yang et al. 2008a,b,c). In previous studies, we suggested that salt stress be defined as the stress of neutral salts; and alkali stress as the stress of alkaline salts (Shi and Wang 2005). While salt stress in a soil generally involves osmotic stress and ion-induced injury (Munns 2002), there is an added high pH effect with alkali stress. A high-pH environment surrounding the roots can cause metal ions and phosphorus to precipitate, with loss of the normal physiological functions of the roots and destruction of the root cell structure (Li et al. 2009). Alkali stress can inhibit absorption of inorganic anions such as Cl^-, NO_3^- and $H_2PO_4^-$, greatly affect the selective absorption of K^+-Na^+, and break the ionic balance (Yang et al. 2007, 2008b, 2009). Thus, plants in alkaline soil must cope with physiological drought and ion toxicity, and also maintain intracellular ion balance and regulate pH outside the roots.

In many agricultural areas of Asia, especially north China, alkalinity (high pH) is an important factor limiting rice (*Oryza sativa* L.) productivity. In this study, rice seedlings were treated with a range (15–60 mmol/L) of salt stress (molar ratio of NaCl:Na_2SO_4 = 9:1, pH 5.40–5.69) or alkali stress (molar ratio of $NaHCO_3$:Na_2CO_3 = 9:1, pH 8.97–9.13). The growth, organic acids (OAs) and inorganic ions in stressed seedlings were measured to investigate the physiological adaptive mechanisms by which rice tolerates alkali stress.

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MATERIALS AND METHODS

Plant materials. The seeds of rice (*O. sativa* L.) cv. Longdun 97-1 were germinated in Petri dishes for 4 days in a growth cabinet (30°C during the day and 25°C at night, 16/8 h photoperiod at 50 μmol/m^2/s). Seedlings were then transferred to barrels containing 2000 mL of aerated sterile nutrient solution. The barrels were placed in a growth chamber (27.0 ± 1.5°C during the day and 20.0 ± 1.5°C at night, 16/8 h photoperiod at 250 μmol/m^2/s). The nutrient solution used in this work accorded to the components described by the International Rice Research Institute (Mao 2001), and contained 1.44 mmol NH_4NO_3, 0.32 mmol NaH_2PO_4, 0.6 mmol K_2SO_4, 1.0 mmol $CaCl_2$, 1.6 mmol $MgSO_4$, 0.072 mmol Fe-EDTA, 0.2 mmol Na_2SiO_3, 9.1 μmol $MnCl_2$, 0.154 μmol $ZnSO_4$, 0.156 μmol $CuSO_4$, 18.5 μmol H_3BO_3, and 0.526 μmol H_2MoO_4 at pH 5.2. 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 salt-alkaline soil of northeast China. The two neutral salts were mixed in a 9:1 molar ratio (NaCl:Na_2SO_4), and applied to the salt stress group. The two alkaline salts were also mixed in a 9:1 molar ratio ($NaHCO_3$:Na_2CO_3), and applied to the alkali stress group. Within each group, four total salt concentrations were applied: 15, 30, 45, and 60 mmol/L. In the salt and alkali stress groups, pHs were 5.40–5.69 and 8.97–9.13, respectively. After two weeks of growth in hydroponic medium, rice plants were subjected to salt or alkali stress by transferring them to another barrel containing 2000 mL of the treatment solution. A barrel containing 20 seedlings was one replicate, with four replicates per treatment. Control plants were maintained with nutrient solution. Treatment solutions were changed daily. The entire treatment duration was 6 days.

Analyses. Plants were harvested in the morning after the final treatment, and were first washed with tap water followed by distilled water. Relative growth rates (RGR) were determined as described in Kingsbury et al. (1984). Dry samples of plant material (100 mg) were treated with 20 mL of deionized water at 100°C for 30 min, and the extract used to determine the contents of free inorganic ions and OAs. The contents of $H_2PO_4^-$, NO_3^-, Cl^-, SO_4^{2-}, and oxalic acid were determined by ion chromatography (DX-300 ion chromatographic system; AS4A-SC ion-exchange column, mobile phase: Na_2CO_3/$NaHCO_3$ = 1.7/1.8 mmol; Dionex Company, Sunnyvale, USA). The contents of OAs (except oxalic acid) were also determined by ion chromatography (DX-300 ion chromatographic system, Dionex Company, Sunnyvale, USA; ICE-AS6 ion-exclusion column, mobile phase: 0.4 mmol/L heptafluorobutyric acid; Dionex Company, Sunnyvale, USA). A flame photometer was used to determine K^+ and Na^+ contents. Statistical analysis of the data, which involved data processing and variance analysis (ANOVA), was performed using the statistical program SPSS 13.0. All data were represented by an average of four replicate measurements and standard errors (SE). The significance level was $P < 0.05$.

RESULTS

Growth and inorganic ions. With increasing salinity, the RGR of rice decreased, and their reductions under alkali stress ($P < 0.01$; Figure 1)

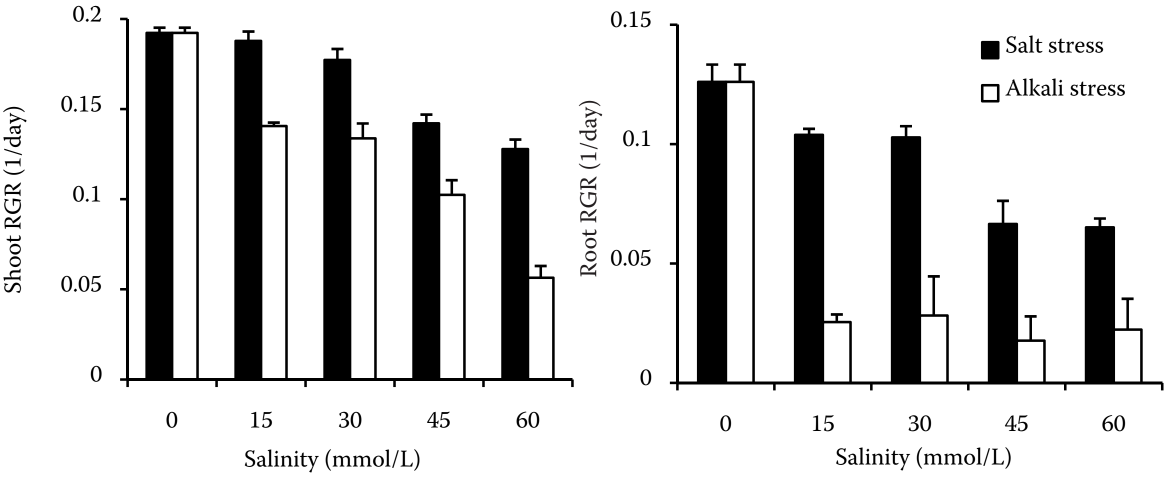

Figure 1. Effects of salt and alkali stresses on the growth of rice seedlings. The values are means (± SE) of four replicates; RGR – relative growth rate

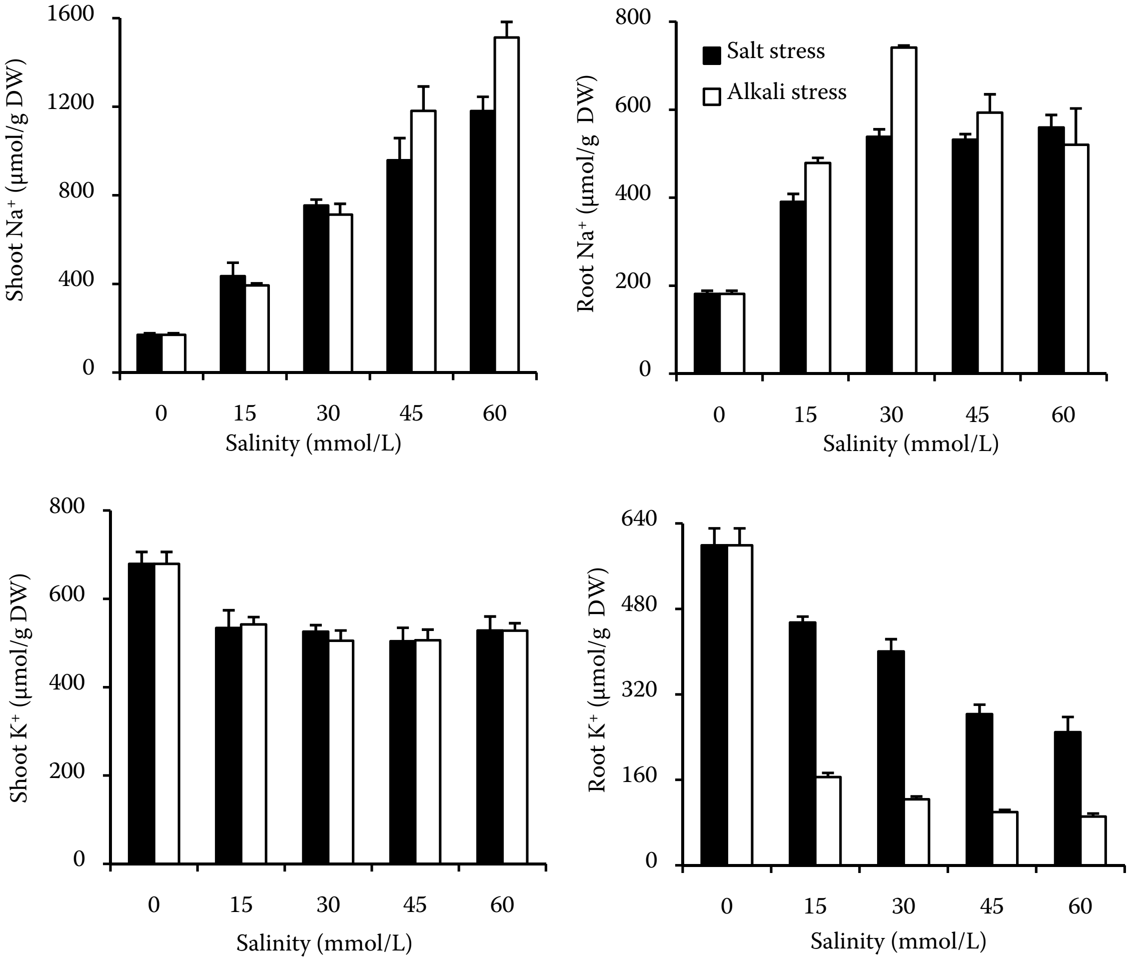

Figure 2. Effects of salt and alkali stresses on contents of Na^+ and K^+ in rice seedlings. The values are means (± SE) of four replicates

were greater than under salt stress. At lower stress intensity, the increase of Na^+ content in shoots was similar for both stresses, increasing slightly with increasing salinity ($P < 0.01$; Figure 2). However, at higher stress intensity (> 30 mmol/L) the increase in Na^+ under alkali stress was higher than under salt stress. Neither increasing salt stress nor increasing alkali stress decreased the K^+ content in rice shoots; although in roots the K^+ contents decreased sharply with increasing salinity under both stresses, with greater reductions under alkali than under salt stress ($P < 0.01$; Figure 2). Under salt stress, the Cl^- contents increased with increasing salinity ($P < 0.01$; Figure 3). However, the Cl^- contents were lower than for controls under alkali stress (Figure 3). The NO_3^- contents in roots were much higher than in shoots (Figure 3). With increasing salinity, the NO_3^- content in roots decreased under both stresses ($P < 0.01$; Figure 3), with greater reductions for alkali than for salt stress. $H_2PO_4^-$ contents under alkali stress were lower than those under salt stress. SO_4^{2-} in shoots under salt stress was also higher than under alkali stress (Figure 3).

Organic acids. Malate, citrate, succinate, acetate, oxalate, formate and lactate were detected in rice shoots (Figure 4), while malate, citrate, acetate, oxalate, formate, and lactate were detected in rice roots (Figure 5). The changing trends of malate, citrate, succinate, acetate, oxalate, and total OAs in shoots were similar to each other (Figure 4). Salt stress did not increase their contents in shoots, and even decreased them slightly; e.g. malate, citrate, and acetate contents in shoots decreased slightly with increasing salt stress (Figure 4). However, alkali stress strongly stimulated the accumulations in the five OAs and total OAs in shoots ($P < 0.01$, Figure 4). Both stresses only slightly affected formate and lactate levels (Figure 4). Salt stress did not elevate OAs accumulation in roots, whereas their accumulation was significantly stimulated by alkali stress, especially for malate and citrate (Figure 5). However, when salinity > 30 mmol/L, malate and citrate in roots decreased with increas-

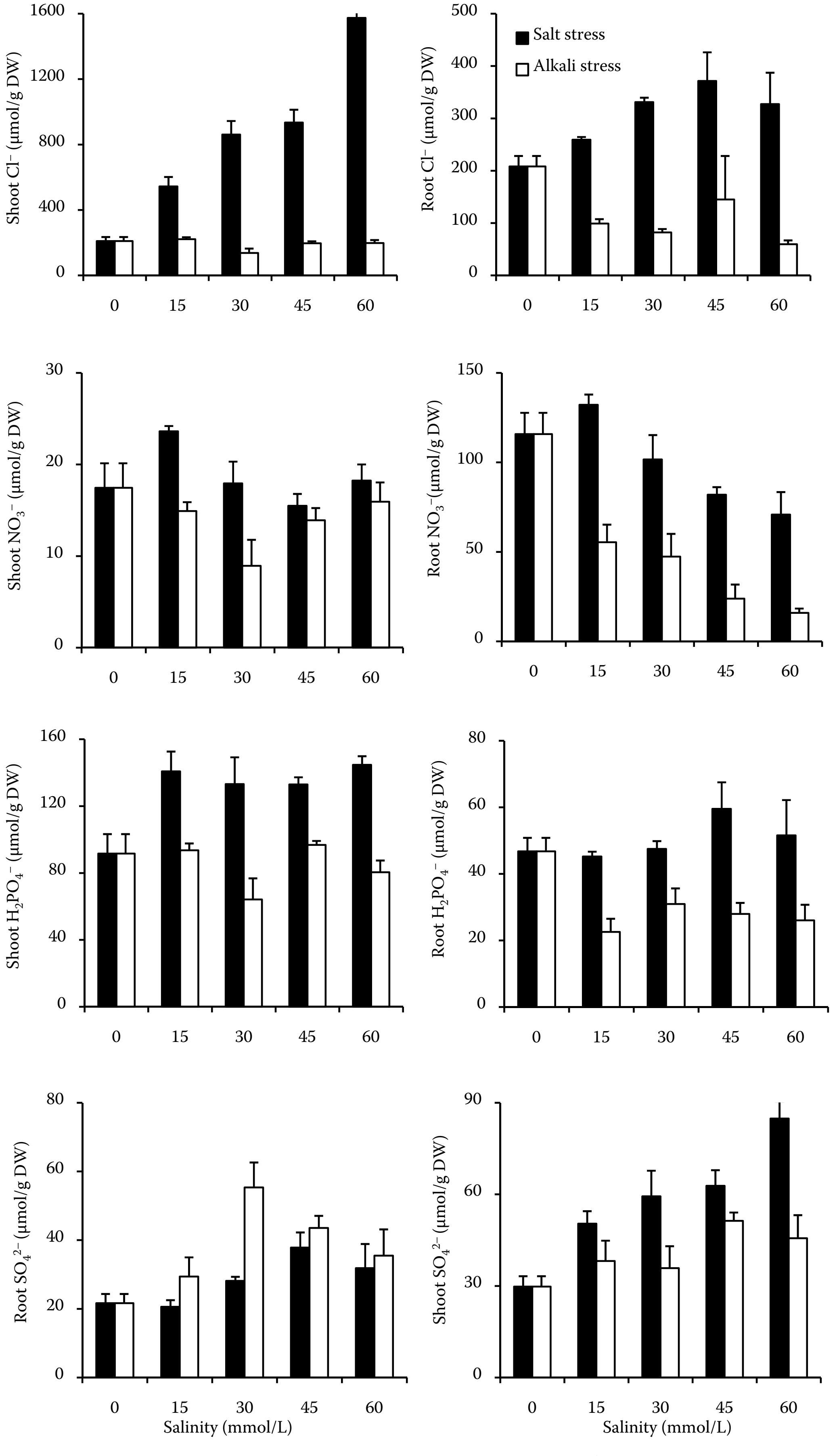

Figure 3. Effects of salt and alkali stresses on contents of Cl^-, NO_3^-, SO_4^{2-} and $H_2PO_4^-$ in rice seedlings. The values are means (± SE) of four replicates

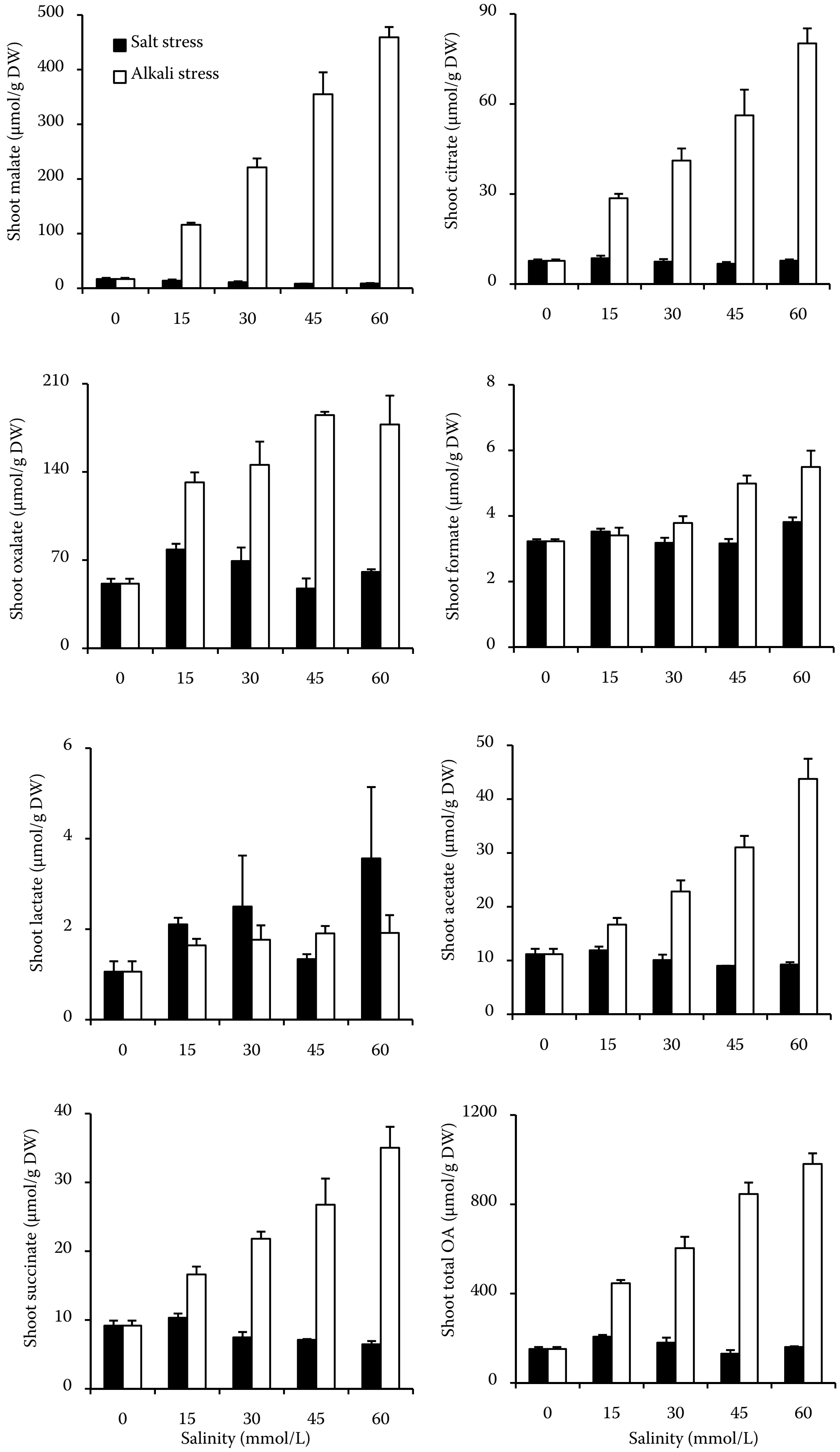

Figure 4. Effects of salt and alkali stresses on contents of organic acids (OAs) in the shoots of rice seedlings. The values are means (± SE) of four replicates

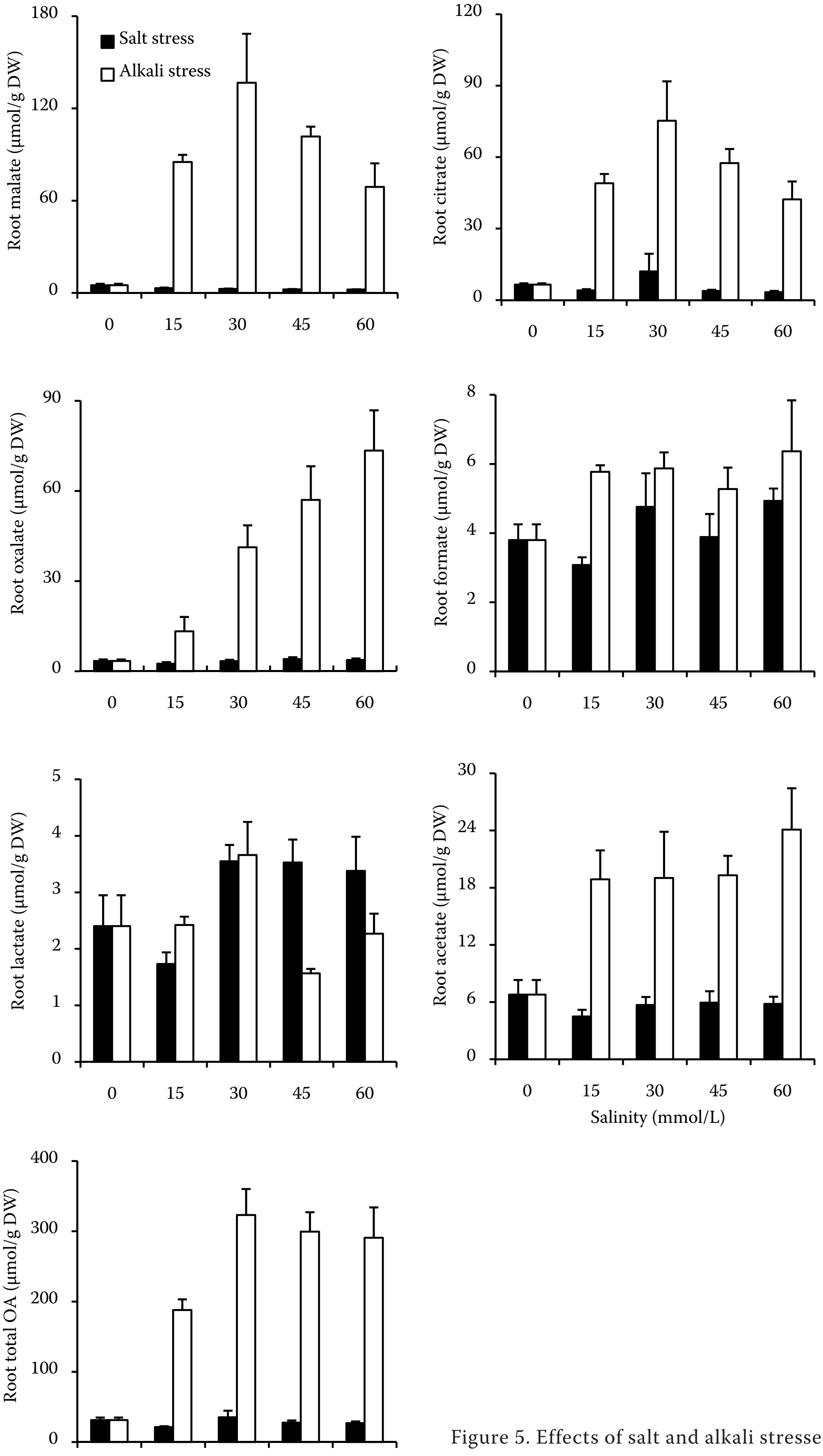

Figure 5. Effects of salt and alkali stresses on contents of organic acids (OAs) in the roots of rice seedlings. The values are means (± SE) of four replicates

ing alkali stress (Figure 5). Both stresses had little effect on the contents of formate and lactate in roots (Figure 5). Under control and salt conditions, oxalate was the dominant component of OA in shoots. Under alkali stress, after oxalate, malate was the major component of OA in shoots. For alkali stressed-rice roots, after malate and oxalate, citrate was a major OA component (Figure 5).

DISCUSSION

High salt-stress generally leads to growth arrest and even plant death (Munns and Tester 2008). However, in the present study, the injurious effect of alkali stress was greater than that of salt stress (Figure 1). Alkali stresses clearly inhibited rice growth, especially root growth (Figure 1). The different extents of injury caused by salt and alkali stresses might be due to different stress factors. Alkali stress exerts the same stress factors as salt stress but with the added influence of high-pH stress, and so with greater harmful effects (Yang et al. 2008c, 2009). The response of rice root growth to alkali stress differed from that of the alkali-tolerant halophyte *Chloris virgata* (manuscript in preparation). As little as 15 mmol/L alkali stress strongly inhibited rice root growth (Figure 1); however, for *C. virgata*, only severe alkali stress inhibited root growth, indicating that root adjustment may play a vital role in alkali tolerance.

Na^+ is the main toxic ion in salinized soil. Low Na^+ and high K^+ in the cytoplasm are essential for maintenance of a number of enzymatic processes (Blumwald 2000, Zhu 2003). Under salt stress, Na^+ competes with K^+ for uptake into roots (Munns and Tester 2008). In reports concerning other plants, such as *Aneurolepidium chinense* (Shi and Wang 2005), wheat (Yang et al. 2008c), barley (Yang et al. 2009), *Suaeda glauca* (Yang et al. 2008b) and *Helianthus annuus* (Shi and Sheng 2005), Na^+ content increased with increasing alkalinity (pH). However, in rice, there was no significant difference between the effects of salt and alkali stresses on the Na^+ content in shoots at low stress intensity (< 45 mmol/L). This implies that the adaptive mechanism of rice roots to the alkali stress (high pH) may differ to other plants, and should be further investigated. Increased salinity generally reduces K^+ content in plants (Munns and Tester 2008). However, our results showed that neither increasing salt stress nor increasing alkali stress decreased the K^+ content in rice shoots; although, in roots, the K^+ contents decreased sharply with increasing salinity under both stresses, with greater reductions under alkali than under salt stress (Figure 2). This indicated that alkali stress (high pH) might greatly affect the absorption of K^+ by rice roots. Under both stresses, the K^+ in root might be transported into shoots to maintain normal metabolism. The response of K^+ content in rice shoots to alkali stress differed from that of *C. virgata* (Yang et al. 2010) and wheat (Yang et al. 2008c); in these two other species, K^+ content in shoots under alkali stress was much lower than for salt stress of the same salinity. In rice, K^+ content in shoots of the alkali stress treatment was similar to salt stress (Figure 2). These data showed that, under alkali stress, rice might have a specific ion absorption or transport mechanism for K^+, which should be further investigated. The contents of inorganic anions under alkali stress were significantly lower than those under salt stress of the same intensity, suggesting that the high pH caused

Table 1. Contributions of inorganic anions and organic acids (%) to total negative charge (ion balance) in rice shoots under salt and alkali stresses

	Salinity (mmol/L)	Cl^-	NO_3^-	SO_4^{2-}	$H_2PO_4^-$	Organic acids
Salt stress	control	36.03	2.99	10.22	15.71	35.04
	15	50.87	2.21	9.42	13.16	24.34
	30	63.57	1.32	8.76	9.83	16.52
	45	67.87	1.12	9.12	9.66	12.23
	60	74.69	0.87	8.05	6.86	9.53
Alkali stress	15	20.90	1.41	7.21	8.84	61.65
	30	11.10	0.72	5.81	5.20	77.16
	45	11.02	0.78	5.76	5.44	77.00
	60	9.59	0.77	4.42	3.90	81.32

Table 2. Contributions of inorganic anions and organic acids (%) to total negative charge (ion balance) in rice roots under salt and alkali stresses

	Salinity (mmol/L)	Cl^-	NO_3^-	SO_4^{2-}	$H_2PO_4^-$	Organic acids
Salt stress	control	44.30	24.62	9.21	9.95	11.92
	15	50.34	25.66	8.00	8.79	7.22
	30	54.80	16.82	9.32	7.86	11.20
	45	58.77	12.96	11.97	9.42	6.88
	60	58.96	12.76	11.48	9.29	7.51
Alkali stress	15	15.82	8.85	9.40	3.61	62.32
	30	9.12	5.26	12.29	3.44	69.89
	45	17.69	2.91	10.63	3.42	65.35
	60	9.28	2.49	11.07	4.06	73.10

by alkali stress might inhibit uptake of anions such as NO_3^- and $H_2PO_4^-$ (Figure 3).

Ionic imbalance in plants is mainly caused by the influx of excess Na^+ (Munns and Tester 2008). Plants in saline conditions usually accumulate inorganic anions or synthesized organic anions, to neutralize large amounts of cations and maintain stable intracellular pH (Yang et al. 2007, 2008b). In rice, under salt stress, Cl^- played a dominant role in maintaining intracellular ion balance, whereas the contribution of OA to ion balance (total negative charge) was very low (Tables 1 and 2). However, under alkali stress, contents of inorganic anions decreased, especially of Cl^-; OA became the dominant component and contributed to 61–81% of total negative charge (Tables 1 and 2). The results showed that the negative charge deficit caused primarily by the lack of exterior Cl^-, was equalized by greatly accumulated OA in rice. Therefore, we suggest that OA accumulation is necessary to maintain the intracellular ion balance of rice. OA accumulation was a central mechanism by which rice maintained intracellular ion balance under alkali stress. Salt stress did not increase the contents of OAs in both shoots and roots of rice (Figures 4 and 5), and even reduced their contents slightly. However, alkali stress significantly stimulated the OAs accumulation in both roots and shoots, especially of malate and citrate. Under strong alkali stress, the contents of malate and citrate in shoot increased up to 27.3 and 10.4 times the control values, respectively. Contrasting with our previous studies, the adjustment mechanism of OA metabolism in rice was significantly different from plants such as *Kochia sieversiana* (Yang et al. 2007) and *S. glauca* (Yang et al. 2008b) – in both these species accumulated oxalate represented about 90% of total OAs, under both alkali and salt stresses. OA was the major basal metabolite for *K. sieversiana* and *S. glauca*, and their contents were 7–10% of DW even under non-stressed conditions. For rice, the OA content in control treatments was only 0.9% of DW, whereas under alkali stress of 60 mmol the OA content reached > 10% of DW. These data indicate that OA metabolism adjustment may play different roles in different plant species. However, OA metabolism adjustment is still an important *in vivo* adaptive response of plants to alkali stress, and should be an important future research direction for plant alkali stress physiology.

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