

# Calcium regulates $K^+/Na^+$ homeostasis in rice (*Oryza sativa* L.) under saline conditions

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## ABSTRACT

To investigate the effects of  $Ca^{2+}$  on cation accumulation and  $K^+/Na^+$  selectivity, in this study, two-week-old rice (*Oryza sativa* L.) plants were exposed to 25 or 125 mmol/L NaCl with or without 10 mmol/L  $CaCl_2$ . At low salinity (25 mmol/L NaCl),  $Ca^{2+}$  significantly decreased  $Na^+$  accumulation in roots, increased  $K^+$  accumulation in shoots, and maintained higher  $K^+/Na^+$  ratios in both roots and shoots of rice plants. At high salinity (125 mmol/L NaCl), however,  $Ca^{2+}$  did not have any effects on  $Na^+$ ,  $K^+$  accumulation and  $K^+/Na^+$  ratios in plants. Further analysis showed that, at low salinity, the addition of  $Ca^{2+}$  significantly enhanced the selective absorption and transport capacity for  $K^+$  over  $Na^+$  in rice. Although  $Na^+$  efflux and  $Na^+$  influx were remarkably reduced by  $Ca^{2+}$  under both low and high salt stresses, their ratio was lowered only under low salt stress. In summary, these results suggest that  $Ca^{2+}$  could regulate  $K^+/Na^+$  homeostasis in rice at low salinity by enhancing the selectivity for  $K^+$  over  $Na^+$ , reducing the  $Na^+$  influx and efflux, and lowering the futile cycling of  $Na^+$ .

**Keywords:** ion accumulation; selectivity;  $Na^+$  influx; futile cycling; salt stress

Salinity affects approximately 20% of the cultivated land and nearly half of all irrigated lands and is one of the major environment factors limiting quality and productivity of crop plants worldwide (Zhu 2001). High salinity in soil disturbs intracellular ion homeostasis, leads to cell membrane damage, disrupts the metabolic activity, and thus finally causes growth inhibition and even plant death (Rains and Epstein 1967). To overcome the toxicity of salinity, many curative and management practices have been adopted by soil scientists. One of these methods is to apply  $Ca^{2+}$  in soil with high exchangeable  $Na^+$  (Khan et al. 1992).

$Ca^{2+}$  is a crucial regulator of growth and development in plants. It is reported that  $Ca^{2+}$  can alleviate the negative effects of salinity on root elongation (Ashraf and Naqvi 1991) and shoot growth of plants (Al-Khateeb 2006, Nedjimi and Daoud 2009). Besides, the addition of  $Ca^{2+}$  can not only protect cell membranes from adverse effect of  $Na^+$ , but also minimize the leakage of cytosolic potassium (Maathuis and Amtmann

1999). The uptake and transport of  $Na^+$  can also be decreased by the presence of  $Ca^{2+}$  in the NaCl solution (Rubio et al. 2003). Furthermore, application of  $Ca^{2+}$  in saline medium can prevent  $Na^+$  from binding to cell walls (Kurth et al. 1986). However, the responses vary depending not only on  $Na^+$  and  $Ca^{2+}$  concentrations but also on plant species.

Rice is a staple food crop in southern and southeastern Asia, and its yield potential is very sensitive to soil salinity. Salinity damage to rice plants occurs as a result of excessive transport of  $Na^+$  and  $Cl^-$  to the shoots (Yeo et al. 1999). Approximately a third of the ions reaching the shoots of rice have been estimated to arrive via the apoplastic pathway (so-called bypass flow) (Faiyue et al. 2010). It was suggested that  $Ca^{2+}$  or silicon can reduce  $Na^+$  transport to shoots by reducing the bypass flow in rice (Anil et al. 2005, Gong et al. 2006). Although the mitigating effect of supplemental  $Ca^{2+}$  on  $Na^+$  toxicity was confirmed in some researches on rice (Alama et al. 2002, Anil et al. 2005), others found little (Song and Fujiyama 1996) or no significant

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effect of  $\text{Ca}^{2+}$  (Yeo and Flowers 1985) on rice under saline conditions. Therefore, whether  $\text{Ca}^{2+}$  has any regulatory role in  $\text{K}^+/\text{Na}^+$  homeostasis of rice needs to be further investigated.

The objective of this study was to determine the effects of  $\text{Ca}^{2+}$  on cation accumulation and  $\text{K}^+/\text{Na}^+$  selectivity in rice exposed to 25 or 125 mmol/L NaCl. The results showed that supplemental  $\text{Ca}^{2+}$  could maintain higher  $\text{K}^+/\text{Na}^+$  ratio in rice at low salinity by enhancing the selectivity for  $\text{K}^+$  over  $\text{Na}^+$ , reducing the  $\text{Na}^+$  influx and efflux, and lowering the futile cycling of  $\text{Na}^+$ .

## MATERIAL AND METHODS

**Plant materials, growth conditions and treatments.** Seeds of rice (*Oryza sativa* L. ssp. *japonica*) were germinated in the dark at  $30^\circ\text{C}$  on filter paper wetted with sterile water in Petri dishes (2 cm high  $\times$  15 cm diameter) – germination took 2–3 days. After 7 days, seedlings were transferred to black-painted containers with the modified Hoagland's solution containing 2 mmol/L  $\text{KNO}_3$ , 0.5 mmol/L  $\text{KH}_2\text{PO}_4$ , 0.25 mmol/L  $\text{Ca}(\text{NO}_3)_2$ , 0.25 mmol/L  $\text{CaCl}_2$ , 0.5 mmol/L  $\text{MgSO}_4$ , 60  $\mu\text{mol/L}$  Fe-citrate, 50  $\mu\text{mol/L}$   $\text{H}_3\text{BO}_3$ , 10  $\mu\text{mol/L}$   $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 1.6  $\mu\text{mol/L}$   $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.6  $\mu\text{mol/L}$   $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , and 0.05  $\mu\text{mol/L}$   $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ . All the seedlings were grown in the same chamber. The environmental conditions were as follows: temperature  $30^\circ\text{C}$  at day and  $28^\circ\text{C}$  at night, photon flux density 600  $\mu\text{mol/m}^2/\text{s}$ , photoperiod 16/8 h for day/night cycle, and relative humidity 75%. Two-week-old rice plants were used for following treatments. Plants were treated with the modified Hoagland's solution supplemented with 25 or 125 mmol/L NaCl together with or without 10 mmol/L  $\text{CaCl}_2$  for 4 days. Twenty-four plants were grown in each treatment. The treatment solution was changed everyday to maintain constant  $\text{CaCl}_2$  and NaCl concentrations.

**Determination of  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$  concentrations.** At the end of each treatment, plant roots were washed twice for 8 min in ice-cold 20 mmol/L  $\text{LiNO}_3$  to exchange cell wall-bound  $\text{Na}^+$  and  $\text{Ca}^{2+}$ , and the shoots were rinsed in deionized water to remove surface salts. Roots and shoots were separated and blotted; fresh weights were determined immediately and then dried at  $80^\circ\text{C}$  for 48 h to obtain dry weights.  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$  were extracted from dried plant tissue in 100 mmol/L acetic acid at  $90^\circ\text{C}$  for 2 h and ion analysis was performed using a flame spectrophotometer (2655-00, Cole-Parmer Instrument Co., Vernon hills, USA).

**Calculation of SA and ST values.** Values of the selective absorption (SA) and selective transport (ST) for  $\text{K}^+$  over  $\text{Na}^+$  were estimated according to the following equations as described by Wang et al. (2009):

$$\text{SA} = (\text{K}^+/\text{Na}^+ \text{ in whole plant}) / (\text{K}^+/\text{Na}^+ \text{ in medium})$$

The higher SA value indicates stronger roots' ability to absorb  $\text{K}^+$  over  $\text{Na}^+$ .

$$\text{ST} = (\text{K}^+/\text{Na}^+ \text{ in shoots}) / (\text{K}^+/\text{Na}^+ \text{ in roots})$$

The greater ST value indicates stronger roots' ability to transport  $\text{K}^+$  over  $\text{Na}^+$  to shoots.

**$^{22}\text{Na}^+$  influx experiment.** Two-week-old rice plants were transferred to modified Hoagland's solution supplemented with 25 or 125 mmol/L NaCl for 24 h, then transferred to 25 or 125 mmol/L NaCl together with or without 10 mmol/L  $\text{CaCl}_2$  treatment for 10 min before the estimation of influx.  $^{22}\text{Na}^+$  influx was estimated according to the method as described by Wang et al. (2007, 2009). Briefly, plants were transferred to the above solutions (20 mL; 25 or 125 mmol/L NaCl together with or without 10 mmol/L  $\text{CaCl}_2$ ) labeled with 185 to 370 kBq/L of  $^{22}\text{Na}^+$ . After 2 min, plants were removed from the uptake solution, blotted and transferred to 200 mL of ice-cold NaCl (25 or 125 mmol/L plus 10 mmol/L  $\text{CaCl}_2$ ) for two successive rinses of 2 min and then a further rinse of 3 min. Finally, the roots were blotted gently, weighed, and transferred to glass vials containing 2 mL of Biodegradable Counting Scintillant (Amersham Co., Arlington Heights, USA) and the specific activity of  $^{22}\text{Na}^+$  was determined using a scintillation counter (Beckman 6500, Fullerton, USA).  $\text{Na}^+$  influx was calculated as: counts/specific activity/time/root fresh weight (RFW) and expressed as  $\mu\text{mol/kg RFW}/\text{min}$ .

**Calculation of  $\text{Na}^+$  efflux and net  $\text{Na}^+$  uptake rate.**  $\text{Na}^+$  efflux and net  $\text{Na}^+$  uptake rate were calculated according to the methods as described by Wang et al. (2007, 2009).

**Statistical analysis.** Cation concentrations,  $\text{K}^+/\text{Na}^+$  and  $\text{Ca}^{2+}/\text{Na}^+$  ratios, SA and ST values, and  $^{22}\text{Na}^+$  influx are presented as means with standard error (SE). Statistical analyses, one-way ANOVA, and Duncan's multiple range tests were performed by using statistical software (SPSS 12.0).

## RESULTS

In preliminary experiments, we tested the effects of 5 and 10 mmol/L  $\text{Ca}^{2+}$  on  $\text{K}^+$  and  $\text{Na}^+$  accumulation in rice under 25 and 125 mmol/L NaCl, respectively. It is shown that 10 mmol/L  $\text{Ca}^{2+}$  was more

efficient than 5 mmol/L  $\text{Ca}^{2+}$  to regulate  $\text{K}^+/\text{Na}^+$  selectivity in rice under salt stresses (data not shown). In this study, therefore, we focus on the effects of 10 mmol/L  $\text{Ca}^{2+}$  on  $\text{K}^+/\text{Na}^+$  selectivity in rice at 25 and 125 mmol/L NaCl. At low salinity (25 mmol/L NaCl), the addition of  $\text{Ca}^{2+}$  significantly decreased roots  $\text{Na}^+$  concentrations by 23% and increased shoots  $\text{K}^+$  concentration by 21% in rice (Figure 1a, d);  $\text{Ca}^{2+}$  concentrations in roots and shoots of  $\text{Ca}^{2+}$ -treated plants were 2.3- and 1.4-fold of those in control plants (Figure 1e, f), respectively;  $\text{K}^+/\text{Na}^+$  ratios in roots and shoots of plants treated with  $\text{Ca}^{2+}$  significantly increased by

27% and 42% (Figure 2a, b), respectively. At high salinity (125 mmol/L NaCl), however,  $\text{Ca}^{2+}$  did not significantly change  $\text{Na}^+$  and  $\text{K}^+$  concentrations, and  $\text{K}^+/\text{Na}^+$  ratios in both roots and shoots of plants (Figures 1–2). These results suggest that the addition of  $\text{Ca}^{2+}$  maintains higher  $\text{K}^+/\text{Na}^+$  ratios in rice at low salinity by reducing  $\text{Na}^+$  accumulation in roots and increasing  $\text{K}^+$  accumulation in shoots.

In the presence of 25 mmol/L NaCl, SA and ST values in rice plants treated with  $\text{Ca}^{2+}$  were 53% and 39% higher than those in control plants, respectively (Figure 3a, b). In the presence of 125 mmol/L NaCl, although  $\text{Ca}^{2+}$  did not have any

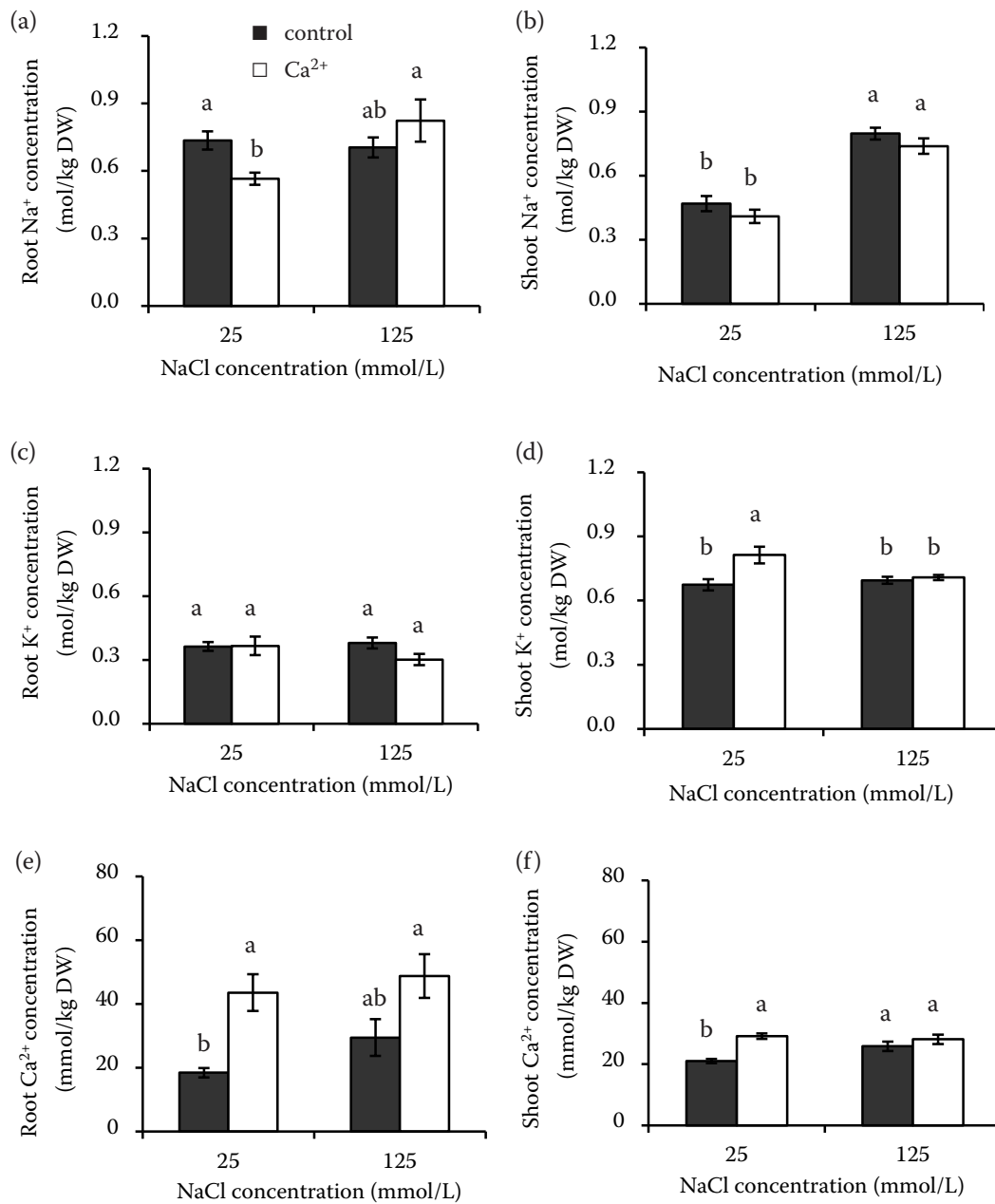


Figure 1. Effects of  $\text{Ca}^{2+}$  on  $\text{Na}^+$  (a, b),  $\text{K}^+$  (c, d), and  $\text{Ca}^{2+}$  (e, f) concentrations in rice under saline conditions. Two-week-old rice plants were exposed to 25 or 125 mmol/L NaCl together with or without 10 mmol/L  $\text{CaCl}_2$  for 4 days. Three rice plants were pooled in each replicate ( $n = 8$ ). Values are means  $\pm$  SE and bars indicate SE. Columns with different letters indicate significant differences at  $P < 0.05$  (Duncan's test)

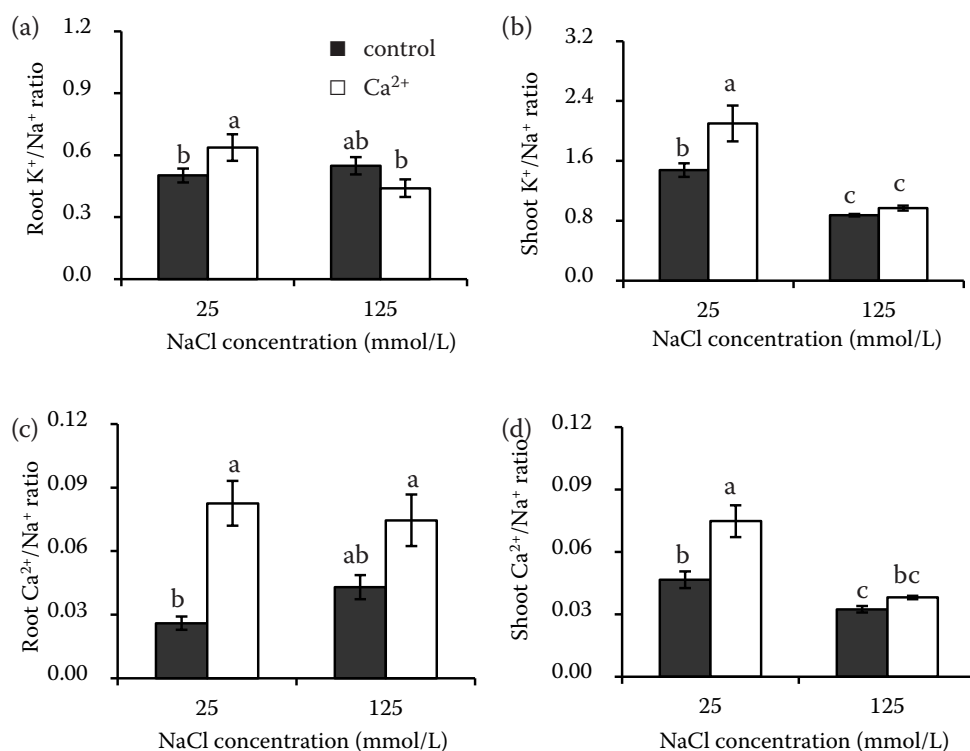


Figure 2. Effects of Ca<sup>2+</sup> on K<sup>+</sup>/Na<sup>+</sup> (a, b) and Ca<sup>2+</sup>/Na<sup>+</sup> (c, d) ratios in rice under saline conditions. Two-week-old rice plants were exposed to 25 or 125 mmol/L NaCl together with or without 10 mmol/L CaCl<sub>2</sub> for 4 days. Three rice plants were pooled in each replicate (*n* = 8). Values are means ± SE and bars indicate SE. Columns with different letters indicate significant differences at *P* < 0.05 (Duncan's test)

effects on the SA value, ST value in plants exposed to Ca<sup>2+</sup> was significantly increased by 58% (Figure 3b). These results suggest that Ca<sup>2+</sup> enhances the selective absorption and transport capacity for K<sup>+</sup> over Na<sup>+</sup> in rice at low salinity, while it increases only selective transport capacity at high salinity.

It is observed that in the presence of 25 or 125 mmol/L NaCl, unidirectional <sup>22</sup>Na<sup>+</sup> influx in plants treated with Ca<sup>2+</sup> was significantly lower (50% or 53%) than that in corresponding control plants, respectively (Table 1). Moreover,

although net Na<sup>+</sup> uptake rate was not affected, Ca<sup>2+</sup> significantly reduced Na<sup>+</sup> efflux – by 48% and 51% – under 25 and 125 mmol/L NaCl, respectively (Table 1). These results imply that supplemental Ca<sup>2+</sup> reduces not only Na<sup>+</sup> uptake by roots but also Na<sup>+</sup> efflux in rice plants under saline conditions. Further analysis showed that Ca<sup>2+</sup> also decreased Na<sup>+</sup> efflux: influx ratios in rice – by 4% – at low salinity; however, no significant difference was found for their ratios at high salinity (Table 1).

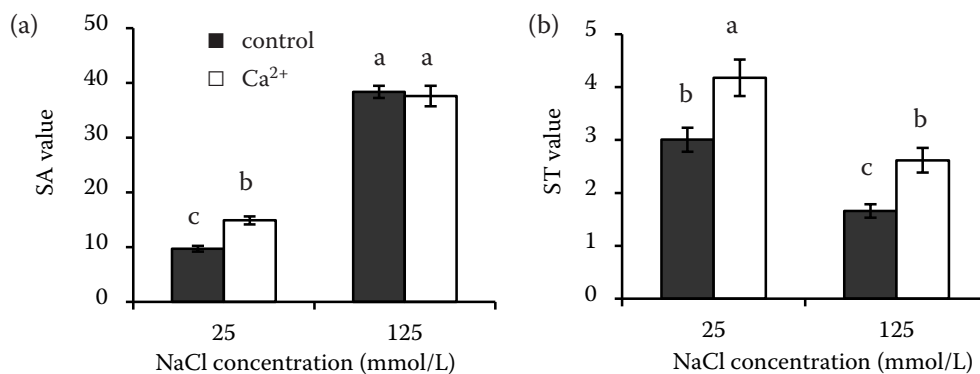


Figure 3. Effects of Ca<sup>2+</sup> on selective absorption (SA) (a) and selective transport (ST) (b) values in rice under saline conditions. Two-week-old rice plants were exposed to 25 or 125 mmol/L NaCl together with or without 10 mmol/L CaCl<sub>2</sub> for 4 days. Three rice plants were pooled in each replicate (*n* = 8). Values are means ± SE and bars indicate SE. Columns with different letters indicate significant differences at *P* < 0.05 (Duncan's test)

Table 1. Effect of Ca<sup>2+</sup> on unidirectional <sup>22</sup>Na<sup>+</sup> influx, net Na<sup>+</sup> uptake rate, and Na<sup>+</sup> efflux in rice under saline conditions

NaCl (mmol/L)	CaCl <sub>2</sub> (10 mmol/L)	Unidirectional <sup>22</sup> Na <sup>+</sup> influx	Net Na <sup>+</sup> uptake rate (μmol/kg RFW/min)	Calculated unidirectional Na <sup>+</sup> efflux	Percent
25	–	230 ± 3 <sup>c</sup>	13 ± 1 <sup>b</sup>	217 ± 4 <sup>c</sup>	94 ± 0.6 <sup>b</sup>
	+	115 ± 8 <sup>d</sup>	11 ± 2 <sup>b</sup>	103 ± 7 <sup>d</sup>	90 ± 1.2 <sup>c</sup>
125	–	1619 ± 73 <sup>a</sup>	22 ± 1 <sup>a</sup>	1593 ± 140 <sup>a</sup>	98 ± 0.2 <sup>a</sup>
	+	808 ± 37 <sup>b</sup>	25 ± 2 <sup>a</sup>	782 ± 69 <sup>b</sup>	97 ± 0.2 <sup>ab</sup>

'Percent' represents the value of unidirectional Na<sup>+</sup> efflux/unidirectional <sup>22</sup>Na<sup>+</sup> influx. Values are means ± SE ( $n = 6$ ). Columns with different letters indicate significant differences at  $P < 0.05$  (Duncan's test)

## DISCUSSION

One of the plants responses to salinity is the reduction in K<sup>+</sup> accumulation, and thus the substitution of K<sup>+</sup> by Na<sup>+</sup> may lead to ions imbalances (Nedjimi and Daoud 2009). These two ions might compete for entry into root cells of plants. This competition can result in significant adverse effects on development and growth of plants, where contents of Na<sup>+</sup> often exceed those of K<sup>+</sup> (Tester and Davenport 2003). Therefore, the maintenance of a high K<sup>+</sup>/Na<sup>+</sup> ratio in cells is essential for plants tolerance to salt stress (Maathuis and Amtmann 1999). Although mitigation of Na<sup>+</sup> toxicity by supplemental Ca<sup>2+</sup> was confirmed, the responses varied with different plants species. It was shown that, under saline conditions, Ca<sup>2+</sup> remarkably reduced Na<sup>+</sup> content and increased K<sup>+</sup>/Na<sup>+</sup> selectivity of both roots and shoots in *Cenchrus penmsetiformis* (Ashraf and Naqvi 1991), *Medicago sativa* (Al-Khateeb 2006), *Atriplex halimus* (Nedjimi and Daoud 2009), and *Cornus sericea* (Renault and Affifi 2009). However, study from *Suaeda maritima* (Wang et al. 2007) showed that the addition of Ca<sup>2+</sup> did not have any effects on Na<sup>+</sup> accumulation and growth of plants exposed to salinity. In addition, the responses of Ca<sup>2+</sup> to Na<sup>+</sup> also varied with Na<sup>+</sup>/Ca<sup>2+</sup> ratio in solution in the same plant species. For example, in rice, Ca<sup>2+</sup> did not have any effects on Na<sup>+</sup> content and growth of plants when subjected to high Na<sup>+</sup>/Ca<sup>2+</sup> ratio (5–25) solution (Yeo and Flowers 1985). This is consistent with the results obtained at high salinity in the present study: Ca<sup>2+</sup> did not significantly changed Na<sup>+</sup> and K<sup>+</sup> concentrations in rice at 125 mmol/L NaCl (Na<sup>+</sup>/Ca<sup>2+</sup> ratio: 12.5) (Figure 1a–d). In contrast, at low salinity (Na<sup>+</sup>/Ca<sup>2+</sup> ratio: 2.5), Ca<sup>2+</sup> significantly decreased roots Na<sup>+</sup> accumulation and increased shoots K<sup>+</sup> accumulation in rice (Figures 1a–d). It is also found that the addition of Ca<sup>2+</sup> to 25 mmol/L NaCl solution caused a little increase in both root and shoot dry weight of rice (data not shown). These findings are consistent with the results as described by Alama

et al. (2002), who reported that Ca<sup>2+</sup> mitigated the toxicity of salinity and reduced Na<sup>+</sup> accumulation in rice at low Na<sup>+</sup>/Ca<sup>2+</sup> ratio (> 5) solution. It is generally accepted that under NaCl treatment, high concentrations of Na<sup>+</sup> can displace both Ca<sup>2+</sup> involved in pectin-associated cross-linking and Ca<sup>2+</sup> present at the plasma membrane binding sites, thereby interfering with Ca<sup>2+</sup> function and leading to cell wall and membrane instability (Essah 2002, Zhang et al. 2010). The mitigating effect of supplemental Ca<sup>2+</sup> on the toxicity of Na<sup>+</sup> is probably due to the replacement of displaced Ca<sup>2+</sup>, thus restoring cell wall stability and plasma membrane integrity, facilitating higher K<sup>+</sup>/Na<sup>+</sup> selectivity, and so improving plant salt tolerance (Zhang et al. 2010). Furthermore, our data indicate that Ca<sup>2+</sup>-treated rice plants show stronger selective absorption and transport capacity for K<sup>+</sup> over Na<sup>+</sup> at low salinity (Figure 3a, b), and that this mechanism is related to maintain high potassium concentrations under low salt stress. However, at high external NaCl treatment, high Na<sup>+</sup>/K<sup>+</sup> and Na<sup>+</sup>/Ca<sup>2+</sup> ratios occur in the solution, plants subjected to such environments, take up high amounts of Na<sup>+</sup>, whereas the uptake of K<sup>+</sup> is considerably reduced. It is proposed that the presence of Ca<sup>2+</sup> could enhance K<sup>+</sup>/Na<sup>+</sup> selectivity and regulate ion homeostasis in rice under low saline condition.

It was shown that the Na<sup>+</sup> influx varied with plant species under saline conditions (Wang et al. 2009). The unidirectional Na<sup>+</sup> influx rates (812 and 1.443 μmol/kg RFW/min) in *Puccinellia tenuiflora* were lower than those (1.282 and 2.077 μmol/kg RFW/min) in wheat under 100 and 150 mmol/L NaCl (Wang et al. 2009). A similar low unidirectional Na<sup>+</sup> influx rate was obtained from the halophyte *Spergularia marina* (about 700 μmol/kg RFW/min under 90 mmol/L NaCl) (Lazof and Cheeseman 1986). In contrast, values of unidirectional Na<sup>+</sup> influx rates from several glycophytes were similar to those (230 and 1.619 μmol/kg RFW/min under 25 and 125 mmol/L NaCl) we recorded for rice (Table 1). *Arabidopsis* showed 1.880 μmol/kg



RFW/min under 50 mmol/L NaCl, and almost 2.000  $\mu\text{mol/kg}$  RFW/min under 100 mmol/L NaCl (Essah et al. 2003); influx in *Hordeum vulgare* L. was about 1.400  $\mu\text{mol/kg}$  RFW/min under 100 mmol/L NaCl (Kronzucker et al. 2006). These results suggested that glycophytes (such as *Arabidopsis*, barley, and rice) showed higher  $\text{Na}^+$  influx than halophytes (such as *P. tenuiflora* and *S. marina*) did under saline conditions.

In rice, one-third of the ions reaching the shoots of plant are the consequence of leakage along the transpirational bypass flow to the xylem (Faiyue et al. 2010). The movement of ions in the apoplast is usually blocked at the endodermis by Casparian bands (Gong et al. 2006). However, this blockage is not complete. It was shown that the endodermis, which contains passage cells in rice roots, is permeable to  $\text{Na}^+$  ions (Gong et al. 2006). Silicon deposition in the endodermis can enhance the endodermal integrity and block leakage of ions to the stele (Gong et al. 2006). Similarly,  $\text{Ca}^{2+}$  also can reduce the bypass flow of rice by 3-fold under 200 mmol/L NaCl (Anil et al. 2005). This reduction in the bypass flow is positively related with the concomitant reduction in the shoot  $\text{Na}^+$  uptake (Anil et al. 2005). In addition, approximately two-third of the ions reaching the shoots of rice should be transported via the symplast pathway. As for  $\text{Na}^+$  transport in higher plants, it is proposed that NSCCs (non-selective cation channel) or VICs (voltage-independent channel), LCT1 (low-affinity cation transporter), HKTs (high-affinity  $\text{K}^+$  transporter), members of the KUP ( $\text{K}^+$  uptake transporter)/HAK (high-affinity  $\text{K}^+$  transporter)/KT ( $\text{K}^+$  transporter) group of proteins, AKT1 (*Arabidopsis*  $\text{K}^+$  transporter), and CCCs (cation- $\text{Cl}^-$  cotransporter) may be the candidates (Kronzucker and Britto 2011). Of these, NSCCs or VICs, LCT, and HKTs are reported to be sensitive to  $\text{Ca}^{2+}$ , while KUP/HAK/KT and AKT1 are shown to be insensitive to  $\text{Ca}^{2+}$  (Kronzucker and Britto 2011).  $\text{Ca}^{2+}$  was demonstrated to inhibit  $\text{Na}^+$  transport through NSCCs and LCT1 (Davenport and Tester 2000, Amtmann et al. 2001). Recent research showed that  $\text{Ca}^{2+}$  inhibited OsHKT2; 1-mediated  $\text{Na}^+$  influx into plant cells (Yao et al. 2010). Therefore,  $\text{Ca}^{2+}$  plays important role in regulating apoplast and symplast pathways involved in  $\text{Na}^+$  transport. In the present study, the application of  $\text{Ca}^{2+}$  significantly reduced unidirectional  $\text{Na}^+$  influx under 25 and 125 mmol/L NaCl (Table 1). Similar results were observed in pepper (Rubio et al. 2003) and barley (Kronzucker et al. 2006). However, Rains and Epstein (1967)

reported that  $\text{Ca}^{2+}$  did not have any effects on  $\text{Na}^+$  influx in barley under 50 mmol/L NaCl. It is worthy noting that net  $\text{Na}^+$  uptake rate seemed to be not affected, although  $\text{Ca}^{2+}$  reduced significantly  $\text{Na}^+$  influx (Table 1). Furthermore,  $\text{Ca}^{2+}$  remarkably lowered  $\text{Na}^+$  efflux by 48% and 51% under 25 and 125 mmol/L NaCl (Table 1). Taken together, these results suggest that  $\text{Ca}^{2+}$  could decrease  $\text{Na}^+$  influx and efflux to maintain ion homeostasis in rice under saline conditions.

The ratio of  $\text{Na}^+$  efflux to influx represents rate of cellular ion cycling (Britto and Kronzucker 2006, Kronzucker et al. 2006). It was shown that the efflux:influx ratio showed the increased trend with the increase of external NaCl concentrations (Britto and Kronzucker 2006, Kronzucker et al. 2006). Furthermore, rate of  $\text{Na}^+$  cycling varied with plant species under saline conditions. For example, rate in *Arabidopsis* it was 77% (Wang et al. 2006); salt-tolerance wheat showed higher ratio of efflux to influx (99%) compared with salt-sensitive wheat (93%) (Davenport et al. 2005). In the present study, rice also showed high ratios of efflux to influx 94% and 98% under 25 and 125 mmol/L NaCl (Table 1). It was proposed that prolonged maintenance of futile cellular ion cycling can be energetically unfavorable and detrimental to plant growth (Britto and Kronzucker 2006). Interestingly, the addition of  $\text{Ca}^{2+}$  significantly reduced the ratio of  $\text{Na}^+$  efflux to influx by 4% at low salinity. These results suggest that  $\text{Ca}^{2+}$  could decrease the futile cycling of  $\text{Na}^+$  to adapt to saline conditions. However, how  $\text{Ca}^{2+}$  regulates the futile cycling of  $\text{Na}^+$  needs to be further investigated.

In conclusion, at low salinity,  $\text{Ca}^{2+}$  decreased roots  $\text{Na}^+$  accumulation, increased shoots  $\text{K}^+$  accumulation, and enhanced the selective absorption and transport capacity for  $\text{K}^+$  over  $\text{Na}^+$  in rice. At high salinity, however,  $\text{Ca}^{2+}$  did not have any effects on  $\text{Na}^+$  and  $\text{K}^+$  accumulation, and  $\text{K}^+/\text{Na}^+$  ratios in plants. Although  $\text{Na}^+$  efflux and  $\text{Na}^+$  influx were remarkably reduced by  $\text{Ca}^{2+}$  under both low and high salt stresses, their ratio was lowered only under low salt stress. Therefore, it is proposed that  $\text{Ca}^{2+}$  could regulate  $\text{K}^+/\text{Na}^+$  homeostasis in rice at low salinity by enhancing the selectivity for  $\text{K}^+$  over  $\text{Na}^+$ , reducing the  $\text{Na}^+$  influx and efflux, and lowering the futile cycling of  $\text{Na}^+$ .

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