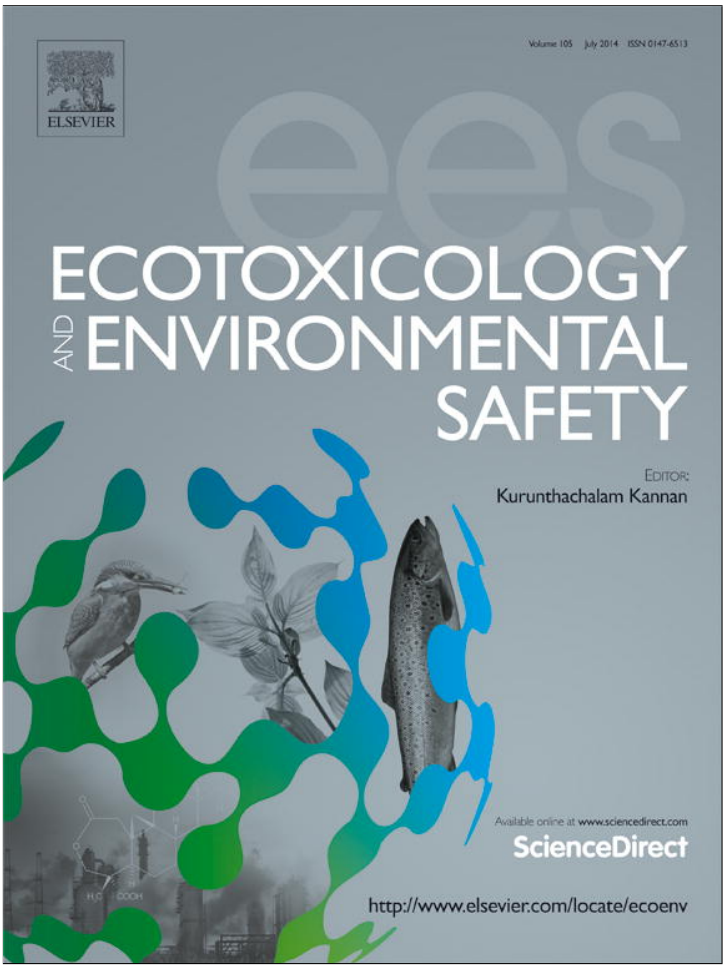

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# Sodium chloride salinity reduces Cd uptake by edible amaranth (*Amaranthus mangostanus* L.) via competition for Ca channels

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ABSTRACT

Soil salinity is known to enhance cadmium (Cd) accumulation in crops. However, the mechanism by which this occurs independent of the surrounding soil remains unclear. In this study, root adsorption and uptake of salt cations and Cd by edible amaranth under NaCl salinity stress were investigated in hydroponic cultures with 0, 40, 80, 120, and 160 mM of NaCl and 27 nM Cd. The dominant Cd species in the nutrient solution changed from free $Cd^{2+}$ to Cd chlorocomplexes as NaCl salinity increased. High salinity significantly reduced K, Ca, and Cd root adsorption and K, Ca, Mg, and Cd uptake. High salinity decreased root adsorption of Cd by 43 and 58 percent and Cd uptake by 32 and 36 percent in salt-tolerant and salt-sensitive cultivars, respectively. Transformation of Cd from free ion to chlorocomplexes is unlikely to have significantly affected Cd uptake by the plant because of the very low Cd concentrations involved. Application of Ca ion channel blocker significantly reduced Na, K, Ca, Mg, and Cd uptake by the roots, while blocking K ion channels significantly reduced Na and K uptake but not Ca, Mg, and Cd uptake. These results suggest that Na was absorbed by the roots through both Ca and K ion channels, while Cd was absorbed by the roots mainly through Ca ion channels and not K ion channels. Salinity caused a greater degree of reduction in Cd adsorption and uptake in the salt-sensitive cultivar than in the salt-tolerant cultivar. Thus, competition between Na and Cd for Ca ion channels can reduce Cd uptake at very low Cd concentrations in the nutrient solution.



## 1. Introduction

Cadmium (Cd) is one of the most common toxic pollutants found in soil. Cd in the soil poses health risks to humans because of its potential to enter the food chain (Li et al., 2012a). Cd accumulates in crops more readily than most other metals and can also be translocated into edible plant parts before any signs of phytotoxicity are observed (Tudoreanu and Phillips, 2004). Phytoavailability of Cd in the soil is controlled by its chemical speciation, soil properties, and genetic features of crops (Peris et al., 2007). Previous studies have demonstrated that chloride salinity in the soil, particularly NaCl, enhances the accumulation of Cd in crops (McLaughlin et al., 1994; Weggler-Beaton et al., 2004; Usman et al., 2005; Li et al., 2012b). McLaughlin et al. (1994) reported that the concentration of Cd in potato tubers grown in soil is increased by saline water irrigation. Soil salinity significantly enhances Cd accumulation in muskmelon leaves but does not affect Cd accumulation in muskmelon fruits (Gabrijel et al., 2009). This effect has been attributed to increase in Cd availability in the soil. Salinity increases the mobility of heavy metals in the soil (Ghallab and Usman, 2007; Acosta et al., 2011). High concentration of $Cl^-$ in the soil can result in formation of the stable compounds $CdCl^+$ and $CdCl_2^0$, which can increase desorption and mobility of Cd in the soil (Norvell et al., 2000; Usman et al., 2005). High salinity also increases the concentrations of other major cations (i.e., Na, K, Ca, and Mg) that compete with Cd for sorption sites of the solid phase. Such competition could result in desorption of Cd and promotion of its phytoavailability (Du Laing et al., 2009).

It is well known that soil salinity affects several physiological and biochemical processes, such as plasma membrane permeability and transpiration rate, in plants (Mühling and Läuchli, 2003; Amer, 2010). This effect is closely related to the uptake and translocation of heavy metals in crops. Many previous studies focused on the effect of salt on Cd availability in the soil. However, only a few studies have considered the effects of salinity on the

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uptake and accumulation of Cd by crops independent of the properties of surrounding soil and the desorption processes from clay particles (Smolders and McLaughlin, 1996a, 1996b; Mühling and Läuchli, 2003; Huang et al., 2007; Lefèvre et al., 2009). In addition, the mechanism underlying this effect remains unclear. Understanding this effect is important for both cultivated crops and phytoextraction plants. Therefore, this study sought to investigate the adsorption and uptake of salt cations and Cd by the roots of edible amaranth under NaCl salinity stress using hydroponic culture. More specifically, the role of Ca and K ion channels in Cd and Na uptake was examined using channel blockers, and the effects of crop salt tolerance on Cd adsorption and uptake under NaCl salinity stress were investigated.

## 2. Materials and methods

### 2.1. Determination of cadmium in soil

Since very high doses of Cd are commonly used in physiological studies, undermining their direct relevance in the field, we used realistic Cd doses in plant hydroponic culture. The same Cd concentration as that found in soil solution separated from field soil was used in plant hydroponic culture. Based on our previous investigation on Cd contamination of farmland soil in the Pearl River Delta (Li et al., 2012a), a sample of typical Cd contaminated farmland soil was collected from this region after soil moisture was brought to field capacity using the method of Dewis and Freiras (1976). The collected fresh soil sample was divided into two parts. One part was centrifuged at 4000g for 5 min for separation of soil solutions. Another part was air dried at room temperature, and ground to pass through a 100-mesh nylon sieve. The total Cd content of the ground soil was determined using $HCl–HNO_3–HF$ extraction. The concentration of Cd in each solution was determined by graphite furnace atomic absorption spectroscopy (GFAAS) (Shimadzu AA-7000, Japan) with Cd detection limit of 0.006 μM. The measured total Cd content of soil was $16 \pm 2.7$ μmol $kg^{-1}$ dry weight soil, which is 6-fold higher than the farmland soil standard of China (National Standard of PR China, 2006). The measured Cd concentration in separated soil solution was $19 \pm 2$ nM. Therefore, 27 nM (19 nM divided by 70 percent) Cd was arbitrarily used in plant hydroponic culture to simulate the concentration of Cd in soil solution of a typical Cd-contaminated farmland soil in the Pearl River Delta.

### 2.2. Plant material and growth conditions

The salt-tolerant cultivar Taiwanbai (cultivar A) and the salt-sensitive cultivar Jianyeqing (cultivar B) were used in this study. Seeds of these commercially available cultivars were obtained from shops in Guangzhou city.

A hydroponic culture-based experiment was conducted. Four salinity treatment conditions with 40 (T1), 80 (T2), 120 (T3), and 160 (T4) mM of NaCl and one control (CK) condition with 0 mM of NaCl were designed for each cultivar. Each treatment and control experiment had five replicates. In all treatment and control conditions, the concentration of Cd, applied as $Cd(NO_3)_2$, was 27 nM. Since nutrient concentrations vary significantly in different soils, Hoagland nutrient solution was used to standardize the concentrations of other important compounds and thus make the results comparable with those of previous studies. The composition of full-strength Hoagland nutrient solution used in this study was 4.0 mM $Ca(NO_3)_2 \cdot 4H_2O$, 2.0 mM $MgSO_4 \cdot 7H_2O$, 5.0 mM $KNO_3$, 1.0 mM $NH_4NO_3$, 1.0 mM $KH_2PO_4$, 0.132 mM $MnSO_4 \cdot 4H_2O$, 0.1 mM $H_3BO_3$, 0.03 mM $ZnSO_4 \cdot 7H_2O$, 0.1 μM $CuSO_4 \cdot 5H_2O$, 0.1 μM $CoCl_2$, 1.0 μM $Na_2MoO_4 \cdot 2H_2O$, 5.0 μM KI, and 0.1 mM EDTA–FeNa.

Seeds were germinated at 25 °C in fine sand irrigated with 0.1 strength Hoagland nutrient solution. At 2 weeks after germination, five uniform seedlings with four leaves each were transferred to 1.5 L of 0.3 strength Hoagland nutrient solution (pH = 5.5, buffered with Mes–Tris) for 4 d and then to 0.5 strength solution for 8 d. At 12 d after hydroponic culture, the seedlings were transferred to 0.8 strength solution to which 27 nM Cd and 40 mM NaCl were added. At 20 d after hydroponic culture, the seedlings were transferred to full-strength solution. In order to allow plants to adapt gradually to salt, the salt concentration was increased by 40 mM NaCl every 4 d until the targeted concentration was reached. The nutrient solution was aerated continuously and replaced every 4 d. The plants were grown in a glasshouse. The temperature range during the growth season was 20–34 °C, and the relative humidity was 60–85 percent. The plants were harvested after 50 d in hydroponic culture.

### 2.3. Desorption of elements from the roots

After washing with deionized water, fresh weights (FWs) of the roots, stems, and leaves of the vegetables in each vessel were recorded. The roots of each replicate were cut into 1.5 cm long pieces and put into a 50 mL centrifuge tube. The roots were then immersed in 10 mM EDTA–$NH_4$ and sonicated for 10 min. The supernatant was transferred to a 50 mL flask. The above process was repeated three times to completely remove adsorbed metals as previously described (Liu et al., 2011). The desorption solution was diluted to 50 mL with deionized water for determination of metal content. Samples of desorbed roots, stems, and leaves of vegetables were oven-dried at 60 °C until constant weight was achieved. After the dry weights were recorded, the samples were ground to fine powder and passed through a 60-mesh sieve in a pre-cleaned steel grinder. The fine powders were then stored in polythene zip bags.

### 2.4. Speciation and complexation modeling

Speciation and complexation of Cd, Na, Ca, and Mg with (in)organic ligands in the hydroponic culture solution under different NaCl salinity conditions were predicted using the chemical equilibrium model in Visual MINTEQ 3.0. EDTA was included by selecting the "show organic components" option when the program was run.

### 2.5. Treatment with ion channel blockers

Treatment and control experiments were conducted in Hoagland nutrient solution as follows: Ca ion channel blocker treatment, 2 μM $Cd(NO_3)_2$ + 50 mM NaCl + 1 mM $LaCl_3$; K ion channel blocker treatment, 2 μM $Cd(NO_3)_2$ + 50 mM NaCl + 5 mM tetraethyl ammonium chloride; and control treatment, 2 μM $Cd(NO_3)_2$ + 50 mM NaCl. All experiments were repeated three times.

Only cultivar A was used for this set of experiments. The hydroponic culture conditions were similar to those described in Section 2.2. Each replicate consisted of five uniform seedlings with four leaves each in Hoagland nutrient solution. Cd $(NO_3)_2$, NaCl, and ion channel blocker were added to the nutrient solution after culturing the plants for 50 d. The plants were harvested after 8 h of exposure to ion channel blockers. Plant roots were cleaned with deionized water and desorbed with EDTA–$NH_4$ for further uptake analysis.

### 2.6. Determination of metals

The ground soil sample was digested with 2 mL HCl, 6 mL $HNO_3$ and 2 mL HF, and the ground plant samples were digested with 10 mL $HNO_3$ in a microwave digestion system (MARS; CEM, USA) (Wang et al., 2011). The samples were heated for 5 min to 120 °C and maintained at that temperature for 3 min, then heated another 5 min to 150 °C and maintained for another 3 min, and were further heated for 6 min to 180 °C and maintained for 10 min for digestion. The concentrations of Na, K, Mg, and Ca in each digested solution and desorption solution were determined using flame atomic absorption spectrometry (FAAS) (Shimadzu AA-7000, Japan). The concentrations of Cd in the samples were determined using GFAAS (Shimadzu AA-7000, Japan).

Analytical reagent blanks were used in each batch of digestion and analyzed for the same elements as the samples. A plant standard reference material (GBW07602 (GSV-1)) was subjected to digestion and then analyzed to comply with the quality control protocol. Results of our analysis were accepted when the measured concentrations in the reference materials were within one standard deviation of the certified values. Average recoveries of Na, K, Mg, Ca, and Cd in certified reference materials were 103.1, 102.5, 96.5, 97.4, and 108.4 percent, respectively.

### 2.7. Data analysis and statistics

The amount of metals desorbed from the roots reflects metal adsorption by the roots. Likewise, the concentration of metals in the desorbed roots and shoots indicates metal uptake by the crop. For each metal, its uptake by the crop was calculated from weighted average of its concentrations in the desorbed roots, stems, and leaves relative to its biomass. Statistical analysis was performed using SPSS v17.0. The outcomes among different treatment conditions were compared by one-way ANOVA at the 0.05 significance level.

## 3. Results and discussion

### 3.1. Biomass

The biomasses of edible amaranth in the control and treatment groups are presented in Fig. 1. The biomasses of both cultivars decreased significantly with increasing salinity. Under control conditions, the biomasses of the two cultivars were similar. However, the biomass of cultivar A was significantly higher than that of cultivar B in the treatment groups. Zong et al. (2007) and

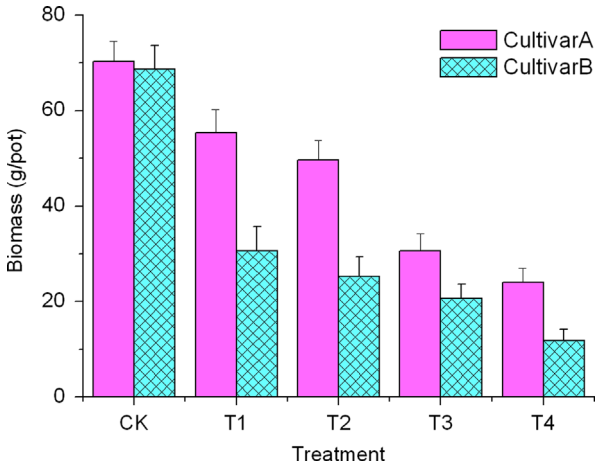


**Fig. 1.** Biomass of edible amaranth under salinity stress. CK, T1, T2, T3, and T4 represent 0, 40, 80, 120, and 160 mM NaCl treatments, respectively. Cultivar A represents a salt-tolerant cultivar, Taiwanbai; cultivar B represents a salt-sensitive cultivar, Jianyeqing. Each treatment and control was replicated five times. The error bars represent standard deviation of replicates.

Liao et al. (2010) reported that low concentrations of Cd promoted vegetable growth. Consistent with these reports, our previous study also demonstrated that treatment with low concentration of Cd in hydroponic cultures increased the biomass of edible amaranth (Zhang et al., 2012). In the current study, Cd concentration in the solution was very low (only 27 nM). Therefore, the observed difference in plant biomasses between the two cultivars is unlikely to have resulted from deleterious effects of Cd.

### 3.2. Predicted speciation distribution in the hydroponic culture solution

The chemical speciation and distribution of Cd, Ca, Mg and EDTA predicted by a computational approach using Visual MINTEQ are presented in Table 1. The most dominant sodium and chloride species in all modeled treatment conditions were $Cl^-$ and $Na^+$ ($>95$ percent; data not shown). Cd speciation was significantly affected by NaCl salinity. In the control group, Cd was mainly distributed as free $Cd^{2+}$ ($>83$ percent). However, in NaCl salinity treatment conditions, the most dominant Cd species were dissolved Cd chlorocomplex species ($CdCl^+$ and $CdCl_2$). The percentages of Cd chlorocomplex species in the control, T1, and T4 groups were 0, 60.57, and 85.18 percent, respectively. The percentage of free $Cd^{2+}$ decreased from 83.59 percent in the control group to 13.64 percent in the T4 group. One caveat of these results is that the solution in our experiment was EDTA-buffered, but EDTA is not present in real field soil solutions. However, since EDTA complexes more strongly with Fe than with other heavy metals such as Cd, more than 99.5 percent of EDTA was found in the form of EDTA–Fe in all control and treatment groups (Table 1). Only 1.52 and $<1$ percent of Cd were found to be bound to EDTA in the control and NaCl treatment groups, respectively. Therefore, EDTA is unlikely to have affected the impact of chloride on Cd speciation transformation and our results likely to reflect the natural Cd speciation pattern in field soil.

The most dominant Ca and Mg species in all modeled treatments were free $Ca^{2+}$ and $Mg^{2+}$, followed by complex sulfate species ($CaSO_4$ (aq) and $MgSO_4$ (aq)) and chlorocomplex species ($CaCl^+$ and $MgCl^+$). NaCl salinity treatment decreased the contents of free cations and complex sulfate species, and increased those of chlorocomplex species. With decrease in free Ca and Mg content in high salinity conditions, competition of these ions with Cd for binding sites on plant cell membranes decreased, resulting in enhanced uptake of Cd by crops.

### 3.3. Amounts of metals adsorbed by roots and their concentrations in desorbed roots and shoots

As shown in Table 2, the amount of Na adsorbed by roots, concentrations in desorbed roots, stems and leaves, and their uptake by edible amaranth increased with increasing salinity (data for K is not shown). Compared with the control, the average rates of Na adsorption and uptake in both cultivars increased 14.8- and 19.8-fold in T4, respectively. Na uptake by the roots was higher than Na adsorption by the roots under salinity stress (Table 2). The concentrations of Na in the controls of both cultivars were in the order leaves > stems > desorbed roots. In contrast, the concentrations of Na in the groups treated with high salinity followed the order stems > leaves > desorbed roots. These results indicate that most of Na was absorbed by the roots and then transferred to shoots.

The amount of K adsorbed by roots, concentrations in desorbed roots, stems and leaves, and uptake by edible amaranth decreased with increasing salinity (data not shown). Compared with the control, average K adsorption and uptake by roots in both cultivars decreased by 70 and 52 percent, respectively, in T4. The observed decrease in K root adsorption was greater than that of K uptake, implying that Na has greater effect on root adsorption of K than root uptake of K.

Compared with the control, average Ca root adsorption and uptake in both cultivars decreased by 25 and 58 percent, respectively, in T4. This indicates that Na has greater effect on root uptake of Ca than root adsorption of Ca. Likewise, compared with the control, average Mg root adsorption and uptake in both cultivars decreased by 17 and 38 percent, respectively, in T4. Thus, Na has greater effect on root uptake of Mg than root adsorptionof Mg.

Salinity decreased Cd root adsorption, concentrations in desorbed roots, stems and leaves, and uptake in the two cultivars. Compared with the controls, Cd root adsorption decreased by 43 percent in cultivar A and by 58 percent in cultivar B in T4. Competition by Na for binding sites in the cell wall may explain the observed reduction in Cd root adsorption with increased salinity. On the other hand, Cd crop uptake decreased by 32 percent in cultivar A and by 36 percent in cultivar B in T4. This result is in agreement with the findings of Huang et al. (2007) and Lefèvre et al. (2009). Huang et al. (2007) found that addition of NaCl to Cd-containing medium (2 μM Cd + 150 mM NaCl) significantly decreased Cd concentrations in barley grown in hydroponic culture. Lefèvre et al. (2009) also reported that chloride salinity reduced Cd accumulation in the Mediterranean halophyte species *Atriplex halimus* L. exposed to 50 μM Cd in hydroponic culture. However, these results are inconsistent with the findings of several other soil culture-based studies (McLaughlin et al., 1994; Weggler-Beaton et al., 2004; Gabrijel et al., 2009) that demonstrated that NaCl in the soil enhances Cd accumulation in crops because of increased Cd availability in the soil.

Normally, Cd chlorocomplexes can be absorbed by plants less than free Cd at the same concentration in hydroponic culture. Smolders and McLaughlin (1996a) reported that Cd concentration in Swiss chard tissue decreased significantly as Cl concentration in the nutrient solution increased even when Na concentration and ionic strength in the solution were maintained by compensating with $NaNO_3$. However, this does not occur at low free Cd concentrations. Uptake of Cd by plants is rate-limited by diffusion, and Cd chlorocomplexes can be absorbed by roots after their dissociation (Degryse et al., 2006). The findings of Oporto et al. (2009) suggest that Cd uptake by roots from free Cd and Cd

**Table 1**
Cd, Ca, Mg and EDTA species distribution (percent) in the hydroponic culture solution after commencement of salinity treatment, as estimated by a chemical equilibrium model in Visual MINTEQ 3.0.

| | Species | 0 mM NaCl | 40 mM NaCl | 80 mM NaCl | 120 mM NaCl | 160 Mm NaCl |
|---|---|---|---|---|---|---|
| Cd | $Cd–NO_3$ | 2.01 | 0.64 | 0.37 | 0.25 | 0.19 |
| | $Cd–SO_4$ | 9.29 | 2.4 | 1.19 | 0.72 | 0.49 |
| | $CdHPO_4$ (aq) | 3.54 | 1.02 | 0.54 | 0.35 | 0.25 |
| | $Cd^{2+}$ | 83.59 | 34.73 | 22.89 | 17.15 | 13.64 |
| | Cd–Cl | 0 | 60.57 | 74.59 | 81.22 | 85.18 |
| | Cd–EDTA | 1.52 | 0.62 | 0.4 | 0.3 | 0.24 |
| | $CdI^+$ | 0.04 | 0.01 | 0.01 | 0.01 | 0 |
| | Total | 100 | 100 | 100 | 100 | 100 |
| Ca | $Ca^{2+}$ | 87.34 | 87.96 | 87.22 | 86.13 | 84.91 |
| | $CaCl^+$ | 0 | 3.65 | 6.27 | 8.44 | 10.37 |
| | $CaH_2PO_4^+$ | 0.96 | 0.72 | 0.6 | 0.52 | 0.47 |
| | $CaHPO_4$ (aq) | 0.34 | 0.23 | 0.19 | 0.16 | 0.14 |
| | $CaNO_3^+$ | 2.08 | 1.62 | 1.4 | 1.26 | 1.16 |
| | $CaSO_4$ (aq) | 9.28 | 5.81 | 4.32 | 3.48 | 2.94 |
| | Total | 100 | 100 | 100 | 100 | 100 |
| Mg | $Mg^{2+}$ | 91.75 | 89.12 | 86.48 | 84.03 | 81.74 |
| | $MgCl^+$ | 0 | 5.87 | 9.85 | 13.05 | 15.82 |
| | $MgHPO_4$ (aq) | 0.48 | 0.33 | 0.26 | 0.21 | 0.18 |
| | $MgMoO_4$ (aq) | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | $MgSO_4$ (aq) | 7.75 | 4.68 | 3.4 | 2.7 | 2.25 |
| | Total | 100 | 100 | 100 | 100 | 100 |
| EDTA | Cu–EDTA | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 |
| | Fe–EDTA | 99.54 | 99.55 | 99.55 | 99.56 | 99.57 |
| | Mn–EDTA | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| | Zn–EDTA | 0.38 | 0.37 | 0.36 | 0.35 | 0.34 |
| | Total | 100 | 100 | 100 | 100 | 100 |

**Table 2**
The amounts of Na, Ca, Mg, and Cd adsorbed by roots, their concentrations in desorbed roots, stems and leaves, and uptake by edible amaranth.

| | Na (mmol $kg^{-1}$ FW) | | | | | Ca (mmol $kg^{-1}$ FW) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Root adsorption | Desorbed roots | Stems | Leaves | Uptake | Root adsorption | Desorbed roots | Stems | Leaves | Uptake |
| CKA | 3.6a | 3.8a | 6.3a | 8.2a | 7.2 a | 2.6a | 2.4a | 4.9a | 14.0a | 10.0a |
| T1A | 50.1b | 76.5b | 141.6b | 66.4b | 91.9b | 2.6a | 1.7b | 2.7b | 10.7b | 7.1b |
| T2A | 51.3b | 74.4b | 184.7c | 132.5c | 149.4c | 2.4ab | 1.6b | 2.4b | 10.0b | 6.4b |
| T3A | 58.7c | 84.6c | 211.5d | 134.4c | 158.3c | 2.2b | 1.8b | 2.2b | 8.8cb | 5.3c |
| T4A | 56.8c | 79.9c | 245.0e | 188.7d | 196.2d | 2.2b | 1.9b | 2.0b | 7.8c | 5.3c |
| CKB | 4.4a | 6.1a | 7.4a | 25.8a | 18.1a | 3.4a | 2.0a | 6.0a | 17.5a | 12.4a |
| T1B | 46.7b | 84.6b | 119.2b | 64.7b | 94.3b | 2.4b | 2.1a | 2.6b | 14.9b | 7.5b |
| T2B | 46.7b | 97.4c | 194.3c | 110.5c | 146.7c | 1.8b | 1.7b | 1.6c | 12.9c | 6.4c |
| T3B | 62.9c | 104.9d | 232.8d | 167.2d | 189.2d | 2.4b | 1.6b | 1.8c | 7.8d | 4.7d |
| T4B | 60.7c | 106.4d | 256.3e | 210.5e | 220.5e | 2.2b | 1.6b | 1.7c | 5.9e | 3.8e |
| | Mg (mmol $kg^{-1}$ FW) | | | | | Cd (μmol $kg^{-1}$ FW) | | | | |
| | Root adsorption | Desorbed roots | Stems | Leaves | Uptake | Root adsorption | Desorbed roots | Stems | Leaves | Uptake |
| CKA | 5.1a | 3.2a | 3.1a | 20.2a | 13.2a | 0.745a | 0.423a | 0.426a | 1.046a | 0.792a |
| T1A | 5.1a | 3.5a | 2.0b | 17.4b | 10.8b | 0.419b | 0.293b | 0.337b | 0.910b | 0.655b |
| T2A | 4.3b | 2.7ab | 2.3b | 16.4b | 9.8b | 0.323b | 0.227b | 0.304bc | 0.747c | 0.534c |
| T3A | 3.9b | 2.1b | 2.3b | 16.9b | 9.2b | 0.327b | 0.270b | 0.278c | 0.828c | 0.539c |
| T4A | 4.3b | 2.4b | 2.3b | 14.7c | 9.5b | 0.424b | 0.148c | 0.238c | 0.777c | 0.540c |
| CKB | 5.0a | 3.2a | 4.9a | 27.4a | 18.0a | 0.933a | 0.409a | 0.397a | 1.347a | 0.959a |
| T1B | 5.6a | 3.0a | 3.2b | 22.7b | 11.0b | 0.506b | 0.273b | 0.368ab | 1.104b | 0.654b |
| T2B | 3.9b | 3.2a | 2.1c | 21.0b | 10.3b | 0.501b | 0.268b | 0.348ab | 1.210ab | 0.704b |
| T3B | 4.5b | 3.2a | 2.5c | 18.6c | 10.4b | 0.439bc | 0.254b | 0.371ab | 0.999b | 0.665b |
| T4B | 4.1b | 3.7a | 2.7c | 15.7d | 9.3c | 0.391c | 0.282b | 0.323b | 0.913c | 0.614b |

Lower case letters a, b, c, d, and e indicate significant differences by one-way ANOVA at $p < 0.05$ within column of each cultivar for a specific element. A and B represent Taiwanbai and Jianyeqing cultivars, respectively. CK, T1, T2, T3, and T4 represent 0, 40, 80, 120, and 160 mM NaCl treatments, respectively. Each treatment and control experiment was replicated five times. For a specific element, its uptake by the crop was calculated from weighted average of its concentrations in the desorbed roots, stems, and leaves based on biomass (fresh weight).

chlorocomplexes was almost the same at 1 nM free Cd concentration in the culture solution. In our experiment, the free Cd concentration in T4 was only 3.7 nM, so Cd chlorocomplexes may have been easily absorbed by plants after dissociation. Therefore, transformation of Cd from free ion to chlorocomplexes may not have significantly reduced Cd uptake by plants in our experiments.

Competition between Na and Cd for binding sites on root plasma membrane is the most likely explanation for the observed reduction of Cd uptake by plants.

### 3.4. Effect of ion channel blockers on metal uptake by roots

The concentrations of Na, K, Ca, Mg, and Cd in the desorbed roots treated with ion channel blockers are listed in Table 3. Compared with the control, treatment with Ca ion channel blocker significantly decreased the concentrations of Na, K, Ca, Mg, and Cd in the desorbed roots, indicating reduced uptake of these ions. Treatment with K ion channel blocker significantly decreased the concentrations of Na and K but not Ca, Mg, and Cd in the desorbed roots. These results indicate that Na was absorbed by the roots through both Ca and K ion channels. Furthermore, Ca, Mg, and Cd were absorbed by the root mainly through Ca ion channels and not K ion channels, although Cd uptake can also occur through other routes, such as those used for Zn and/or Fe plasma membrane transport (Clemens, 2006). Several studies have shown that K ion channels permit diffusion of Na through the cytoplasmic membrane of root cells (Blumwald, 2000; Zhang et al., 2010; Kronzucker and Britto, 2011). Likewise, Ca ion channels allow other divalent cations, such as $Mg^{2+}$ and $Cd^{2+}$, to pass through the cytoplasmic membrane and also permit large flux of monovalent cations such as $Na^{+}$ in the absence of strong competition from divalent cations (Li and Huang, 2007; Li et al., 2010; Lu et al., 2010). However, since ion channel blockers are destructive to crop growth, duration of exposure to such blockers must be kept short to minimize impairment of cytoplasmic membrane permeability. With this in mind, high dose of Cd and short exposure to ion channel blockers was used in our experiments. The response of plants to Cd under such conditions may be different from those observed with the hydroponic approach.

### 3.5. Relationships between Na, K, Ca, Mg, and Cd adsorption and uptake

The cross-correlation coefficients of Na, K, Ca, Mg, and Cd adsorption and uptake for both the control and treatment cultivars are listed in Table 4. Adsorption of Na by the roots showed significant negative correlation with adsorption of K, Ca, and Cd. This result suggests that high affinity of Na to the sorption sites on root cell walls under salinity caused the other ions to be displaced. In addition, Na uptake was found to be significantly negatively correlated with K, Ca, Mg, and Cd uptake in the plants. This suggests that large Na influx competed with K, Ca, Mg, and Cd for diffusion through K or Ca ion channels and thus reduced their uptake by the crop under NaCl salinity stress. These results are in agreement with reports by Liu and Zhao (2005) and Huang et al. (2007) that addition of NaCl in the medium significantly reduced the concentrations of K, Ca, and Mg in plant shoots.

On the other hand, uptake of Cd was found to be significantly positively correlated with those of Ca and Mg by the plants. The concentrations of Cd, Ca and Mg in the solution were the same in all control and treatment groups. Since all three of these ions are mainly absorbed through Ca ion channels by plant roots, our results suggest that Na inhibited uptake of these ions by plant roots.

### 3.6. Effect of crop salt tolerance on Cd adsorption and uptake

In the control group, Cd root adsorption and uptake of cultivar A were significantly lower than those of cultivar B (Table 2). The total amount of Na, K, Ca, and Mg adsorbed by roots was significantly lower in cultivar A (52.2 mmol $kg^{-1}$ FW) than in cultivar B (68.9 mmol $kg^{-1}$ FW). Thus, the adsorption capacity of root cell wall was significantly lower in cultivar A than in cultivar B. As a result, cultivar A had lower Cd adsorption via roots than cultivar B. The combined uptake of Ca and Mg was only 23.2 mmol $kg^{-1}$ FW in cultivar A but 30.4 mmol $kg^{-1}$ FW in cultivar B. Since Cd is a non-essential element and is non-specifically absorbed by the root through Ca ion channels, the cultivar with less Ca and Mg uptake is also likely to absorb less Cd through Ca ion channels under identical concentrations of Cd, Ca and Mg in the solutions.

At the highest NaCl concentration (T4), no significant difference in Cd root adsorption was observed between the two cultivars (Table 2). Total uptake of Cd in cultivar A was only slightly lower than that in cultivar B. Higher amount of Na was found to be adsorbed and absorbed in the salt-sensitive cultivar compared to the salt-tolerant cultivar, which corresponded with greater inhibition of Cd adsorption and uptake in the salt-sensitive cultivar than in the salt-tolerant cultivar.

**Table 4**
Cross-correlation coefficients of Na, K, Ca, Mg, and Cd adsorption and uptake by edible amaranth.

| | Na | K | Ca | Mg | Cd |
|---|---|---|---|---|---|
| Na | | | | | |
| Pearson | | −0.968** | −0.946** | −0.814** | −0.781** |
| Sig. (two-tailed) | | 0 | 0 | 0.028 | 0.094 |
| *N* | | 10 | 10 | 10 | 10 |
| K | | | | | |
| Pearson | −0.903** | | 0.957** | 0.861** | 0.772** |
| Sig. (two-tailed) | 0.000 | | 0 | 0.01 | 0.031 |
| *N* | 10 | | 10 | 10 | 10 |
| Ca | | | | | |
| Pearson | −0.695* | 0.845** | | 0.937** | 0.870** |
| Sig. (two-tailed) | 0.026 | 0.002 | | 0 | 0.004 |
| *N* | 10 | 10 | | 10 | 10 |
| Mg | | | | | |
| Pearson | −0.509 | 0.590 | 0.605 | | 0.942** |
| Sig. (two-tailed) | 0.133 | 0.073 | 0.064 | | 0 |
| *N* | 10 | 10 | 10 | | 10 |
| Cd | | | | | |
| Pearson | −0.926** | 0.863** | 0.741* | 0.509 | |
| Sig. (two-tailed) | 0.000 | 0.001 | 0.014 | 0.133 | |
| *N* | 10 | 10 | 10 | 10 | |

Shadowed entries are coefficients of metal root adsorption, and non-shadowed entries are coefficients of metal uptake.

* Significance at the 0.05 level.

** Significance at the 0.01 level.

**Table 3**
Concentration of metals in the desorbed roots of edible amaranth (Taiwanbai, cultivar A) treated with ion channel blockers (mmol $kg^{-1}$ FW root).

| Treatment | K | Na | Ca | Mg | Cd |
|---|---|---|---|---|---|
| Control | 8.62 ± 0.421a | 1.48 ± 0.12a | 2.15 ± 0.45a | 4.72 ± 0.26a | 0.010 ± 0.002a |
| $LaCl_3$ | 7.49 ± 0.37b | 1.01 ± 0.19b | 1.22 ± 0.32b | 3.58 ± 0.59b | 0.003 ± 0.001b |
| TEA | 4.85 ± 0.35c | 0.96 ± 0.22b | 1.66 ± 0.18ab | 4.96 ± 0.30a | 0.008 ± 0.002a |

Lower case letters a, b, and c indicate significant differences by one-way ANOVA at $p < 0.05$ within column. Each treatment and control experiment was replicated three times.

## 4. Conclusion

The dominant Cd species in the nutrient solution changed from free $Cd^{2+}$ to Cd chlorocomplexes with increase in NaCl salinity. However, this transformation of Cd species is unlikely to have significantly affected Cd uptake by plants because of the very low free Cd concentration involved. NaCl salinity significantly reduced K, Ca, and Cd root adsorption and K, Ca, Mg, and Cd uptake. Blocking Ca ion channels significantly decreased the root uptake of Na, K, Ca, Mg, and Cd. Blocking K ion channels significantly decreased Na and K uptake but did not affect Ca, Mg, and Cd uptake. These results indicate that Na was absorbed by the roots through both Ca and K ion channels. Cd was absorbed by the roots mainly through Ca ion channels and not K ion channels, although Zn and/or Fe transporters have also been reported previously to mediate Cd uptake. Therefore, competition between Na and Cd for passage through Ca ion channels may explain the low Cd uptake at very low Cd concentrations in the nutrient solution. Greater degree of reduction in Cd adsorption and uptake can be achieved by increasing NaCl salinity in salt-sensitive cultivars. The results of this study provide new insights into the transport mechanisms and fate of Cd in saline environments.

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