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Impact of osmoregulation on the differences in Cd accumulation between two contrasting edible amaranth cultivars grown on Cd-polluted saline soils☆

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ABSTRACT

This study aimed to investigate the difference of osmoregulation between two edible amaranth cultivars, *Liuye* (high Cd accumulator) and *Quanhong* (low Cd accumulator), under salinity stress and determine the effects of such difference on Cd accumulation. A pot experiment was conducted to expose the plants to sewage-irrigated garden soil (mean 2.28 mg kg^{-1} Cd) pretreated at three salinity levels. Under salinity stress, the concentrations of Cd in the two cultivars were significantly elevated compared with those in the controls, and the Cd concentration in *Liuye* was statistically higher than that in *Quanhong* ($p < 0.05$). Salinity-induced osmoregulation triggered different biogeochemical processes involved in Cd mobilization in the rhizosphere soil, Cd absorption, and translocation by the two cultivars. Rhizosphere acidification induced by an imbalance of cation over anion uptake was more serious in *Liuye* than in *Quanhong*, which obviously increased soil Cd bioavailability. Salinity-induced injuries in the cell wall pectin and membrane structure were worse in *Liuye* than in *Quanhong*, increasing the risk of Cd entering the protoplasts. The chelation of more cytoplasmic Cd^{2+} with Cl^- ions in the roots of *Liuye* promoted Cd translocation into the shoots. Furthermore, the less organic solutes in the root sap of *Liuye* than in that of *Quanhong* also favored Cd translocation into the shoots. Hence, osmoregulation processes can be regarded as important factors in reducing Cd accumulation in crop cultivars grown on saline soils.

1. Introduction

About 4 million km^2 land and 20 percent of the arable land in the globe are salt-effected soils, and the areas are increasing (Ravindran et al., 2007; Rozema and Flowers, 2008). Among them, many saline soils are also contaminated with heavy metals such as Cadmium (Duarte et al., 2010; Gabrijel et al., 2009). Cd in edible crops may accumulate in food chains and trigger serious health risks to humans after long-term exposure (Li et al., 2006; Nabulo et al., 2011). Cd uptake by crops is substantially elevated in Cd-contaminated saline soils (Li et al., 2012; Manousaki et al., 2008), especially in vegetable garden soil undergoing long-term fertilization (Duan et al., 2012; Wang et al., 2014). An increase in Cd accumulation can be attributed to the formation of multivalent Cd–Cl complexes in the rhizosphere because chloro-complexes of Cd in soils exhibit strong mobility and can be efficiently absorbed by the roots (López-Chuken et al., 2010; Smolders and Mclaughlin, 1996). Many studies also reported that Cd mobility is increased by Na competing for sorption sites on soil clay (Acosta et al., 2011).

Salinity stress imposes water deficiency and ion toxicity in plants, which induce adverse growth or even death by disturbing cellular structure and function. Plant osmoregulation is an important self-protective mechanism that may alleviate osmotic stress by regulating the amounts and types of osmolytes in response to salinity stress (Mišić et al., 2012; Silveira et al., 2009). In this case, imbalance in the uptake of inorganic osmolytes by the roots can cause H^+/OH^- excretion (Fang et al., 2008; Perez et al., 2007). This condition may influence the release of inactive Cd from the solid

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phase to the rhizosphere solution. At the cellular level, variations in osmolytes can lead to a series of modifications in the active constituents (e.g., pectin, lipid, and protein) of the cell wall and cytomembrane (An et al., 2014; Eshghizadeh et al., 2012). These active constituents can bind Cd^{2+} ions and reduce the amounts of Cd entering the protoplasts (Krzesłowska, 2011; Xin and Huang, 2014). In addition, complexation of major osmolytes with intracellular Cd^{2+} ions can convert chemical forms of Cd in cells, which are closely associated with the translocation and subcellular distribution of Cd (Sharma and Dietz, 2006; Wali et al., 2015; Xin and Huang, 2014).

To reduce the health risk of consuming crops grown on Cd-polluted soils, many crops were screened for Cd-safe cultivars (He et al., 2015; Liu et al., 2009; Xin et al., 2010). The results verified that Cd-accumulating ability varies greatly among different cultivars. Edible amaranth (*Amaranthus mangostanus* L.), an annual leafy vegetable with distinct culinary–medicinal properties, is widely cultivated in China, India, and Southeast Asia. The vegetable is an abundant source of micronutrients and dietary fiber for humans, particularly for the health of bone and blood (Han and Xu, 2014). Edible amaranth is also a relatively salt-tolerant crop and is commonly grown in saline soils. Our previous investigations on various leafy vegetables in the Pearl River Delta of China showed that edible amaranth is a high Cd accumulator in Cd-contaminated saline soil and may cause health risk to humans (Li et al., 2012). To reduce its health risk, a few low-Cd cultivars of edible amaranth were screened. However, the mechanism of the difference in Cd accumulation between high- and low-Cd cultivars of edible amaranth grown on Cd-polluted saline soils is poorly understood.

The two contrasting edible amaranth cultivars may respond differently in their osmoregulation to salinity stress. The different osmoregulation may further result in the differences in rhizosphere soil pH, pectin in root cell walls, and major osmolytes in protoplasts, which are relevant to Cd mobilization in the rhizosphere soil, plant Cd uptake and internal metal redistribution processes. The aforementioned studies provided several valuable conclusions associated with Cd accumulation but did not clarify the effects of different osmoregulation processes within crop cultivars on Cd accumulation. Therefore, in the present study, for a better mechanistic understanding of the differences in Cd accumulation between two contrasting edible amaranth cultivars grown on Cd-polluted saline soils, we reconfirmed the steady difference in Cd accumulation between high- and low-Cd cultivars of edible amaranth. Then, we further investigated the difference in rhizosphere soil pH, pectin in root cell wall, cytomembrane permeability, and osmolyte concentrations from the perspective of salinity-induced osmoregulation and determined the effects of such differences on Cd mobilization in rhizosphere soil, Cd absorption, and translocation by the two cultivars.

2. Materials and methods

2.1. Plant materials and soil properties

Soil-pot experiments were carried out using two amaranth cultivars, *Liuye* (high Cd accumulator) and *Quanhong* (low Cd accumulator), in accordance with our previous research. Seeds were commercially available and bought from local shops in China.

Potting soils were collected from the 0–15 cm arable soil layer of a farm in Guangzhou suburb, China, which was sewage-irrigated 20 years ago and contaminated with Cd. Soil samples were transported into a glasshouse, air dried, and passed through a plastic sieve (10 mm) to remove bulky debris, mixed sufficiently, and then used for pot experiments. The soil had a pH of 6.64, total salt content of 0.47 g kg^{-1}, total organic carbon content of 35.4 g kg^{-1}, cation exchange capacity of 20.86 cmol kg^{-1}, total Cd content of 2.28 mg kg^{-1} DW, and exchangeable Cd content of 0.29 mg kg^{-1} DW. The Cd content exceeded the limit set by the national standards of China (Farmland environmental quality evaluation standards for edible agricultural products, HJ332-2006).

2.2. Experimental design

The cultivation trial was conducted in a greenhouse at Jinan University campus, Guangzhou (Guangdong Province, China). The potting soils were pretreated with NaCl, including a control (CK, no NaCl added) and two salt treatments (NaCl levels: 2.0 and 4.0 g kg^{-1} dry soil). Soils were thoroughly mixed with salt solution and then incubated for 4 weeks. A total of 108 rhizobags were used during the trial (2 cultivars × 3 treatments × 6 replicates × 3 rhizobags). The seeds were soaked in a disinfection solution (0.05% carbendazim) for 25 min and then washed with tap water and deionized water before they were finally sown into nylon-mesh rhizobags of polyvinyl chloride pots (2.5 kg soil·pot^{-1}). The soil moisture was maintained between 50% and 70% of the field moisture capacity. No extra fertilizers and pesticides were added.

At 7 days after seed germination, seedlings were thinned to 2 plants per rhizobag to avoid excessive competition for limited space and nutrient elements. Pot cultures were watered to saturated water content 24 h before harvesting to obtain more rhizosphere solution. Water that leaked at the bottom of the pots was collected using a plastic dish and then placed into the pot soils again to prevent salinity losses. The crops were harvested at 60 days after seed germination (harvested on 11 August 2015) on the basis of the typical growth period for commercial edible amaranth cropping.

2.3. Measurement of osmotic potential (OP,Ψ)

Plant samples were collected in the morning, separated from adhesive soil by shaking and stripping, cleaned with tap water and deionized water, and then weighed to determine the fresh weight. Some samples were dried to constant weight in an oven at 80 °C to calculate the moisture content, whereas the other samples were transferred to a liquid nitrogen canister (−196 °C) and an ultra-low temperature freezer (−80 °C) to determine the OP(Mišić et al., 2012; Silveira et al., 2009).

The determination of OP included several steps. First, frozen roots and shoots (stems and leaves) were placed in 25 mL syringes and squeezed to obtain tissue sap as much as possible. The obtained sap was centrifuged for 10 min at 9280 × g at 4 °C. Afterwards, 40 μL of the supernatant was employed to determine the OP using a freezing-point osmometer (Gonotec Osmomat 030, Germany). The total OP (Ψ_t) was calculated using the Van't Hoff formula:

$$\Psi(\text{MPa}) = -\text{iC}*R*T \quad (1)$$

where iC is the reading on the digital display instrument (mosmol·kg^{-1} tissue fluid), R is the ideal gas constant (0.008314 MPa mol^{-1} K^{-1}), and T is the indoor temperature (K) (Mišić et al., 2012).

2.4. Analyses of cell wall pectin, chlorophyll, malondialdehyde (MDA), and cytomembrane permeability

The cell walls of fresh roots were extracted in accordance with the method of Konno et al. (2010). The pectin content in the cell wall was determined following the procedure of Colzi et al. (2012). The chlorophyll content was determined after extraction with 96% ethanol, and the MDA concentration was determined using the TCA-TBA method (Buege and Aust, 1978). Relative membrane

permeability (RMP) was calculated using the following equation:

$$RMP(\%) = (A1 - A0)/(A2 - A0) \times 100 \quad (2)$$

where A_0, A_1, and A_2 are the electrical conductivities of deionized water, soaked solution, and boiled solution, respectively (Ashraf and Ali, 2008).

2.5. Determination of total Cd concentrations (Cd_{Total}) in plant

Fresh samples were dried to constant weight in an oven at 80 °C. Dried samples were mixed with 10 mL of concentrated HNO_3 in polytetrafluoroethylene tubes and then digested at 120°C–180 °C in a microwave digestion system (CEM MARs5, USA). The digested solution was then poured into a volumetric flask to determine the Cd concentration by graphite furnace atomic absorption spectrometry (GFAAS, Shimadzu AA-7000, Japan).

2.6. Determination of adsorbed Cd content in root cell wall

Fresh roots were cut into 1.5 cm long pieces and placed in a 50 mL centrifuge tube. The roots were then washed in 10 mM EDTA–NH_4 to completely remove adsorbed metals using the method of Mei et al. (2014). The desorbed solution was used for determination of Cd concentration by GFAAS (Shimadzu AA-7000, Japan).

2.7. Determination of inorganic ion concentration in plant sap

Fresh roots and shoots were thoroughly ground into homogenates in a quartz mortar, transferred to 50 mL heat-resistant tubes, boiled in a 100 °C water bath for 90 min, centrifuged and filtered to collect the filtrate, and finally diluted with deionized water to 25 mL (initial solution) to determine inorganic osmolytes in the tissue sap (Silveira et al., 2009). The concentrations of cations (K^+, Na^+, Ca^{2+}, and Mg^{2+}) were determined by ICP–AES (PE OPTIMA200DV, USA). The concentrations of anions (Cl^- and NO_3^-) were determined with an ion chromatograph (Dionex ICS-900, USA). The Cd concentration in the tissue sap (Cd_{sap}) of the roots and shoots was determined by GFAAS.

2.8. Determination of organic osmolyte concentration in plant sap

Proline (Pro) concentration was determined through the salicylic acid–ninhydrin method (Bates et al., 1973). Total free amino acid (TFAA) concentrations were determined according to their color reaction with ninhydrin (Rosen, 1957). Total soluble protein (TSP) concentration was determined using the Coomassie Brilliant Blue G-250 method (Snyder and Desborough, 1978). Total soluble sugar (TSS) was determined using the sulfuric acid–anthrone colorimetric method (Irigoyen et al., 1992).

2.9. Determination of Cd concentration in rhizosphere solution and pH in rhizosphere soils

The soil adhering to the roots in rhizobags was shaken down, loaded in a syringe, and then centrifuged at 5939×*g* for 30 min to collect the rhizosphere solution. The soil solution was filtered through a 0.45 μm membrane and then refrigerated in a freezer at 4 °C to determine the Cd concentration (He et al., 2015). Some soil samples in the rhizobags were air dried for soil pH determination. The pH was measured in a 1:5 mixture of water and soil using a pH meter (Salazar et al., 2012).

2.10. Data analysis

Experimental data were recorded in Microsoft Excel 2010 and analyzed with SPSS 19.0 (Chicago, IL, USA). Statistical analysis was performed with one-way ANOVA (Duncan test and Independent-Samples T test) and two-way ANOVA. All graphs were charted using Origin 9.2 (MA, USA). The data on Cd in the plant samples include total Cd concentrations (Cd_{Total}) in the roots and shoots, adsorbed Cd content in root cell wall, as well as Cd and osmolyte concentrations in the tissue sap extracts, which were expressed on a plant fresh weight basis (mg kg^{-1}/μg kg^{-1}/g kg^{-1} FW). The data on Cd in the rhizosphere solution samples were expressed on a soil dry weight basis (μg kg^{-1} DW soil). Cd translocation factor ($TF_{Cd\text{-}Total}$) from root to shoot was calculated based on equation (3). The total offtake of Cd by plant was calculated based on equation (4).

$$TF_{Cd-Total} = Cd_{Total} \text{ in shoot}/Cd_{Total} \text{ in root} \quad (3)$$

$$\text{Total offtake of Cd} = Cd_{Total} \text{ in plant} \times \text{biomass per pot} \quad (4)$$

3. Results and discussion

3.1. Concentrations of Cd in cultivars under salinity stress

Cd_{Total} are presented in Fig. 1(a). The average contents of Cd in the shoot of the two cultivars grown on saline soil were 0.45 and 0.63 mg kg^{-1} FW, respectively, and exceeded the maximum allowable levels (0.2 mg kg^{-1} FW) of the national standards of China (GB 2762–2012, China). The Cd_{Total} concentrations in all treatments were significantly higher in *Liuye* than in *Quanhong*. Evidently, *Liuye* had a relatively higher consumption risk under such conditions. The experiment reconfirmed that the difference in Cd accumulation between the previously identified high-Cd cultivar (*Liuye*) and low-Cd cultivar (*Quanhong*) was repeatable and stable.

The Cd_{Total} in both cultivars significantly increased ($p < 0.05$) with increasing soil salinity. The reasons are that both of Cd–Cl complexes and Na competing for sorption sites on soil clay can mobilize Cd in soil (Acosta et al., 2011; López-Chuken et al., 2010). Salinity may also acidify rhizosphere soil through the unbalanced absorption of inorganic osmolytes and H^+ release by root (Abbas et al., 2014; Zhang et al., 2011), and result in soil Cd mobilization. Besides, salinity stress can modify root cell wall and cytomembrane permeability (Keutgen and Pawelzik, 2007) and facilitate Cd uptake by root. The total offtake of Cd by the two cultivars under salinity stress is listed in **Table S2**. It showed that the total offtake of Cd by cultivars under salinity stress did not increase as much as the Cd_{Total} concentrations did. This suggested that biomass decrease under salinity stress possibly some extent concentrated Cd in plants.

3.2. Osmotic potential and cation–anion balance in cultivars, rhizosphere acidification and soil Cd mobilization

The measured osmotic potential (MOP) and calculated osmotic potential (COP) of the two cultivars under salinity stress are listed in Table 1. The COPs from each investigated osmolyte accounted for over 90% of MOPs, indicating that almost all the osmolytes actually occurred in both cultivars were investigated. In each treatment, both COPs and MOPs of *Liuye* were significantly lower than those of *Quanhong* ($p < 0.05$), implying that the two cultivars adopted different osmoregulation approaches in response to salinity stress.

The relationship between cation–anion balance in the two cultivars and rhizosphere soil pH is listed in Table 2. The Cd

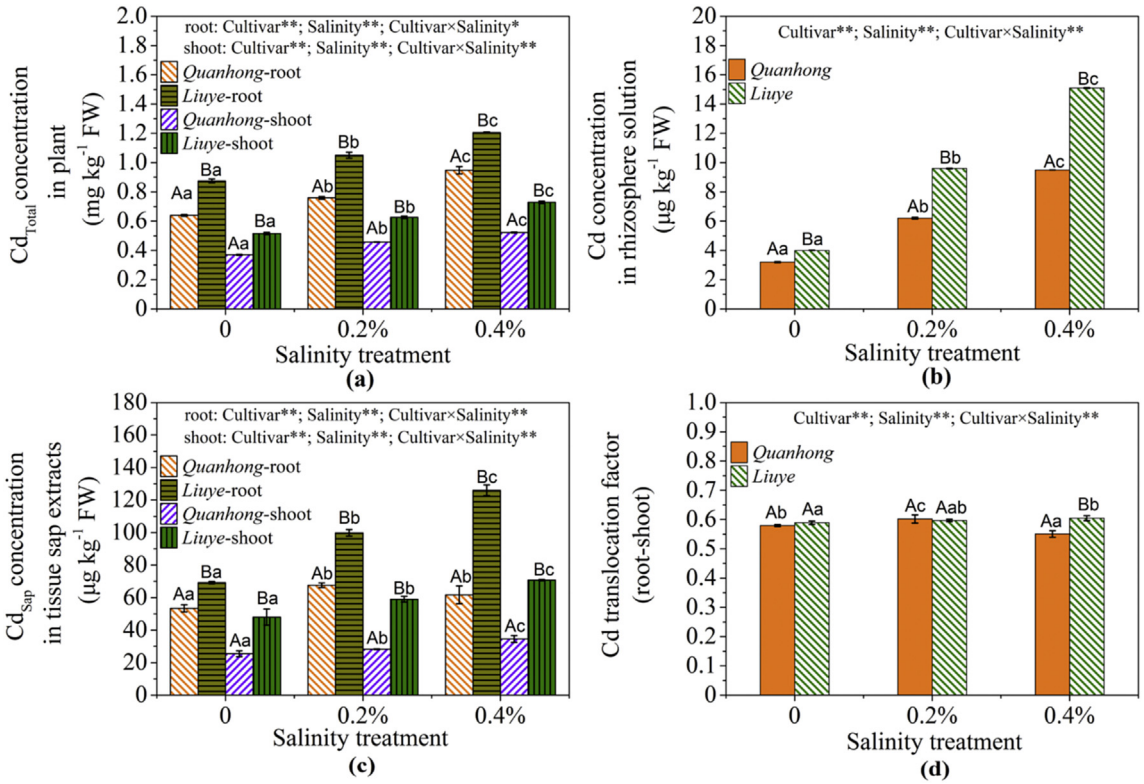

Fig. 1. Cd concentrations in plant and tissue sap extracts of the roots and shoots, rhizosphere solution, and Cd translocation factor (from root to shoot) of the two cultivars under salinity stress. Fig. 1 (a): total Cd concentration in plant; Fig. 1 (b): Cd concentration in rhizosphere solution; Fig. 1(c): Cd concentration in sap; Fig. 1 (d): Cd translocation factor. The capital letters (A and B) above the histograms indicate significant differences between cultivars under the same salinity treatment (one-way ANOVA test, $p < 0.05$), and the lowercase letters (a, b, and c) indicate significant differences between treatments of the same cultivars (one-way ANOVA test, $p < 0.05$). Asterisk **, * means significant effects of cultivar, salinity and their interaction on the indicator at p < 0.01, 0.05 by two-way ANOVA tests. Values refer to the means of three replicates with standard deviation (SD).

Table 1
Measured osmotic potential (MOP) and calculated osmotic potential (COP) in the roots and shoots sap of cultivars under salinity stress.

	Salinity treatment	Measured osmotic potential (MOP, -MPa)		Calculated osmotic potential (COP, -MPa)	
		Quanhong	*Liuye*	*Quanhong*	*Liuye*
Root	0	0.67 ± 0.01 Aa	0.83 ± 0.01 Ba	0.61 ± 0.02 Aa	0.79 ± 0.02 Ba
	0.2%	1.06 ± 0.01 Ab	1.19 ± 0.01 Bb	1.02 ± 0.02 Ab	1.14 ± 0.06 Bb
	0.4%	1.24 ± 0.01 Ac	1.45 ± 0.01 Bc	1.18 ± 0.02 Ac	1.41 ± 0.01 Bc
	ANOVA F ratio				
	Cultivar	2040**		155.31**	
	Salinity	9314**		578.38**	
	Cultivar × Salinity	44.96**		5.28**	
Shoot	0	0.83 ± 0.01 Aa	0.87 ± 0.02 Ba	0.76 ± 0.02 Aa	0.83 ± 0.00 Ba
	0.2%	1.21 ± 0.00 Ab	1.36 ± 0.01 Bb	1.09 ± 0.01 Ab	1.34 ± 0.01 Bb
	0.4%	1.37 ± 0.01 Ac	1.54 ± 0.01 Bc	1.25 ± 0.03 Ac	1.47 ± 0.02 Bc
	ANOVA F ratio				
	Cultivar	646.51**		469.07**	
	Salinity	5.54**		1637**	
	Cultivar × Salinity	5536**		44.79**	

MOP represents the measured osmotic potential measured by a freezing-point osmometer. COP represents the calculated osmotic potential calculated by the Van't Hoff formula. The different capital letters indicate significant difference of a specific organ between cultivars under the same salinity treatment (one-way ANOVA test, $p < 0.05$), and the lowercase letters indicate the significant difference of a specific organ between treatments of the same cultivars (one-way ANOVA test, $p < 0.05$). Asterisk **, * means significant effect of cultivar, salinity and their interaction on the indicator at p < 0.01, 0.05 by two-way ANOVA tests. Values refer to the means (±SD) of three replicates.

concentrations of the rhizosphere soil solution are presented in Fig. 1 (b). Salinity significantly increased the Cd concentration in the rhizosphere soil solution of each cultivar ($p < 0.05$) (Fig. 1 (b)). However, compared with the control treatments, the increments in the Cd concentrations of the rhizosphere soil solution under each salinity treatment for *Liuye* were significantly higher than those for *Quanhong* ($p < 0.05$). The cation−anion balance (charge balance) in plants can influence the H^+/OH^- release of roots. A value ($\sum$Cation − $\sum$Anion; total C−A) greater than 0 indicated more net release of H^+ from the roots; a value less than 0 indicated more net release of OH^- from the roots. Under salinity stress, the total C-A values in *Liuye* were significantly higher than those in *Quanhong* ($p < 0.05$). The total amounts of H^+ released by the roots of *Liuye* were possibly greater than those of *Quanhong*. Interestingly, the

Table 2
Relationship between cation–anion balance in cultivars and rhizosphere soil pH under salinity stress.

Salinity treatment	Total C-A (mmol kg^{-1}FW)		Rhizosphere soil pH	
	Quanhong	*Liuye*	*Quanhong*	*Liuye*
0	138.58 ± 4.73 Ab	135.31 ± 1.39Aa	6.87 ± 0.02 Ac	6.85 ± 0.02 Ac
0.2%	123.80 ± 2.54 Aa	142.80 ± 2.54Bb	6.77 ± 0.04 Bb	6.65 ± 0.01 Ab
0.4%	149.85 ± 3.95 Ac	173.77 ± 7.49Bc	6.58 ± 0.01 Ba	6.44 ± 0.01 Aa
ANOVA F ratio				
Cultivar	29.73**		55**	
Salinity	49.77**		261.51**	
Cultivar × Salinity	11.82**		8.75**	

$\sum$Cation−$\sum$Anion value represents the difference in the electric charges of the cation and anion, which were calculated according to the charge-balanced formula $c(Na^+)+c(K^+)+2*c(Mg^{2+})+2*c(Ca^{2+})-c(Cl^-)-c(NO_3^-)$. Total C−A represents the average $\sum$Cation−$\sum$Anion value weighted by the root and shoot biomass. The different capital letters indicate significant difference between cultivars under the same salinity treatment (one-way ANOVA test, $p < 0.05$), and the lowercase letters indicate the significant difference between treatments of the same cultivars (one-way ANOVA test, $p < 0.05$). Asterisk **, * means significant effect of cultivar, salinity and their interaction on the indicator at $p < 0.01$, 0.05 by two-way ANOVA tests. Values refer to the means (±SD) of three replicates.

rhizosphere soil pH of *Liuye* was also lower than that of *Quanhong* ($p < 0.05$) in each treatment. The results implied that the rhizosphere acidification resulted in the difference in soil Cd mobilization between the two cultivars.

According to the theory of ionic balance, when more cations than anions are absorbed, the root cytoplasm releases an equivalent amount (mmol of charge) of H^+ by proton pump to maintain the electric neutrality of cells (Curtin and Wen, 2004; Haynes, 1990; Hinsinger et al., 2003). The present results showed that amounts of excess cations over anions uptake by the roots of *Liuye* were significantly higher ($p < 0.05$) than those of *Quanhong*. A stronger rhizosphere acidification ($p < 0.05$) under salinity stress was observed in *Liuye* than in *Quanhong*. In this case, the release of insoluble Cd from the solid phase to the rhizosphere solution was accelerated by rhizosphere acidification (Dessureault-Rompré et al., 2010; Yanai et al., 2006). This phenomenon resulted in increased Cd concentration in the rhizosphere solution of *Liuye* and the significantly positive correlation between rhizosphere soil pH and Cd concentration in the rhizosphere solution ($p < 0.05$).

3.3. Cultivar differences in root cell wall pectin and cytomembrane permeability and their effects on Cd absorption under salinity stress

The biomass, contents of pectin, MDA, and chlorophyll (a, b), as well as the relative membrane permeability (RMP), of the cultivars are listed in Table 3. The whole-plant biomass (FW) and cell wall pectin concentrations of the two cultivars decreased under salinity stress. In all treatments, the cell wall pectin contents in the roots of *Liuye* were lower than those in the roots of *Quanhong* on average ($p < 0.05$). The MDA contents in *Liuye* were higher than in *Quanhong* ($p < 0.05$). The RMP of *Liuye* was greater than that of *Quanhong*. The chlorophyll concentration in *Liuye* was lower than that in *Quanhong* ($p < 0.05$). The chlorophyll *a*/b ratios under 0.4% salinity treatment dramatically declined by 50.3% in *Liuye* and by 24.3% in *Quanhong* compared with the controls. Therefore, although the two cultivars grew well on Cd-contaminated saline soil without visible symptoms of toxicity (i.e., withered and yellow leaves) in the pot experiment, they did exhibit different levels of salinity tolerance.

The adsorbed Cd content in root cell walls and its proportion in the total Cd content of roots are listed in Table 4. Cd_{Sap} is presented in Fig. 1 (c). The adsorbed Cd content in the root cell wall of both cultivars and its proportion in the total Cd content of root significantly decreased ($p < 0.05$) with increasing soil salinity. The adsorbed Cd content in the root cell wall was not significantly different between the two cultivars at control treatment, but significantly lower in *Liuye* than in *Quanhong* ($p < 0.05$) at salinity treatments. The proportions of adsorbed Cd in root cell wall of *Liuye* were substantially lower than those of *Quanhong* ($p < 0.05$) at all treatments. Contrary, Cd_{Sap} significantly increased ($p < 0.05$) with increasing soil salinity. The Cd_{Sap} concentrations were higher in the roots of *Liuye* than in those of *Quanhong* ($p < 0.05$). These results suggested that Cd adsorption in the root cell wall reduced Cd absorption in the root.

The correlative relationship between contents of pectin in the root and the adsorbed Cd content in the root cell wall is presented in Fig. 2. The cell wall pectin contents in the root were significantly

Table 3
Concentrations of pectin, MDA, and chlorophyll (a, b), as well as RMP of different cultivars under salinity stress.

Salinity treatment		Biomass (g pot^{-1})	Pectin (g kg^{-1} FW)	MDA (mg kg^{-1} FW)		RMP (%)		Chlorophyll (mg kg^{-1} FW)		
			Root	Root	Shoot	Root	Shoot	Chla	Chlb	Chla/b
Quanhong	0	62.22 ± 1.11Bc	16.63 ± 0.29Bc	0.88 ± 0.05Aa	3.07 ± 0.08Aa	18.58 ± 0.88Aa	20.08 ± 0.98Aa	88.91 ± 0.73Bc	37.11 ± 1.08Ac	2.40 ± 0.08Ac
	0.2%	55.31 ± 0.92Bb	14.31 ± 0.55Bb	1.06 ± 0.04Ab	3.22 ± 0.07Ab	22.64 ± 1.12Ab	18.34 ± 0.87Aa	75.22 ± 0.31Bb	34.85 ± 1.74Bb	2.16 ± 0.01Bb
	0.4%	48.20 ± 1.31Ba	11.99 ± 0.04Ba	1.50 ± 0.02Ac	3.60 ± 0.05Ac	34.77 ± 1.05Ac	37.65 ± 3.78Ab	43.35 ± 0.79Ba	23.93 ± 1.26Aa	1.82 ± 0.12Ba
Liuye	0	57.81 ± 0.90Ac	15.30 ± 0.30Ac	0.93 ± 0.09Aa	3.24 ± 0.04Ba	38.54 ± 1.09Ba	43.03 ± 1.28Ba	84.07 ± 0.81Ac	35.79 ± 2.11Ac	2.36 ± 0.16Ac
	0.2%	48.93 ± 1.01Ab	12.77 ± 0.16Ab	1.34 ± 0.03Bb	3.78 ± 0.03Bb	52.76 ± 0.69Bb	52.30 ± 1.39Bb	62.20 ± 1.53Ab	31.70 ± 1.22Ab	1.96 ± 0.04Ab
	0.4%	43.70 ± 1.05Aa	10.78 ± 0.18Aa	1.54 ± 0.05Ac	4.95 ± 0.10Bc	56.21 ± 1.35Bc	58.52 ± 1.55Bc	33.76 ± 1.07Aa	28.89 ± 1.08Ba	1.17 ± 0.07Aa
ANOVA F ratio										
Cultivar		104.36**	92.83**	25.50**	2207**	2317**	865.42**	419.05**	$0.056^{n.s}$	37.48**
Salinity		265.13**	350.19**	208.36**	370.80**	390.50**	129.03**	3923**	73.40**	115.01**
Cultivar × Salinity		$1.645^{n.s}$	$0.48^{n.s}$	10.79**	719.71**	41.02**	21.13**	28.20**	12.60**	14.05**

MDA and RMP represent malondialdehyde and relative membrane permeability, respectively. The different capital letters indicate significant difference between cultivars under the same salinity treatment (one-way ANOVA test, $p < 0.05$), and the lowercase letters indicate the significant difference between treatments of the same cultivars (one-way ANOVA test, $p < 0.05$). Asterisk **, * means significant effect of cultivar, salinity and their interaction on the indicator at $p < 0.01$, 0.05 by two-way ANOVA tests. Values refer to the means (±SD) of three replicates.

Table 4
Adsorbed Cd content in the root cell wall and its proportion relative to the total Cd content of root (mg kg^{-1} FW).

Salinity treatment	Adsorbed Cd (mg kg^{-1} FW)		Proportion	
	Quanhong	*Liuye*	*Quanhong*	*Liuye*
0	0.466 ± 0.012Ab	0.460 ± 0.003Ac	72.80%Bc	52.61%Ac
0.2%	0.422 ± 0.014Ba	0.390 ± 0.009Ab	55.28%Bb	37.15%Ab
0.4%	0.408 ± 0.011Ba	0.366 ± 0.006Aa	43.08%Ba	30.40%Aa
ANOVA F ratio				
Cultivar	32.21**		440.32**	
Salinity	95.83**		378.53**	
Cultivar × Salinity	5.42*		8.25**	

The different capital letters indicate significant difference of a specific indicator between cultivars under the same salinity treatment (one-way ANOVA test, $p < 0.05$), and the lowercase letters indicate the significant difference of a specific indicator between treatments of the same cultivars (one-way ANOVA test, $p < 0.05$). Asterisk **, * means significant effect of cultivar, salinity and their interaction on the indicator at $p < 0.01$, 0.05 by two-way ANOVA tests. Values refer to the means (±SD) of three replicates.

correlated with the adsorbed Cd content in the root cell wall of both cultivars ($r^2 = 0.94$ for *Liuye*, and $r^2 = 0.87$ for *Quanhong*), indicating that cell wall pectin substantially affected Cd adsorption in root cell wall and further influencing Cd uptake by root. Osmoregulation alters pectin components in the cell wall of the roots through changing polysaccharide metabolism (Wu et al., 2010). Among them, many chemical functional groups (e.g., −COOH, −OH) can bind to Cd ions (Brunner et al., 2008; Davis et al., 2003; Pelloux et al., 2007). Thus, a decrease in the pectin content of the root cell wall under salinity stress clearly reduced Cd adsorption in the root cell wall of both cultivars and weakened the interception capability of the root to soil Cd, which greatly increased the risk of Cd entering the protoplasts. The lower cell wall pectin contents in the roots of *Liuye* facilitated its Cd uptake, thereby partly explaining its higher Cd concentration in the root sap than that of *Quanhong*.

The MDA concentration and RMP of root cells describe the salinity stress-related oxidative damage in the root plasma membrane, which somewhat slackens the root selectivity and may permit the non-selective entry of soil Cd (Helal et al., 1999). The higher MDA contents and RMP in *Liuye* than in *Quanhong* might have also resulted in higher Cd uptake by *Liuye* than *Quanhong*. However, literature also reported that Cd uptake by crop roots mainly depends on the Ca, Fe, and Zn channels in the root plasma membrane under non-saline or slightly saline conditions (He et al., 2015; Mei et al., 2014; Vaculík et al., 2012). Thus, the non-selective entry in the root plasma membrane might only slightly contribute to Cd uptake by the crops.

3.4. Cultivar differences in major osmolyte concentrations and their effects on root-to-shoot Cd translocation under salinity stress

Cd translocation factors ($TF_{Cd\text{-}Total}$; root-to-shoot) are presented in Fig. 1 (d). The $TF_{Cd\text{-}Total}$ (root-to-shoot) in the control and low-salinity treatment (0.2%) showed no significant difference between the two cultivars, but the $TF_{Cd\text{-}Total}$ of *Liuye* under 0.4% salinity treatment was significantly higher than that of *Quanhong* ($p < 0.05$). The Cd concentrations were also higher in the shoot sap of *Liuye* than in those of *Quanhong* ($p < 0.05$) (Fig. 1 (c)). The results suggested that *Liuye* could transfer more Cd to the shoot than *Quanhong* did.

The concentrations of Na^+ and K^+ and K^+/Na^+ ratios in the roots and shoot sap of the cultivars are presented in Fig. S1. Upon salinity treatments, the Na concentrations in the roots and shoot sap of *Liuye* were significantly higher than those in *Quanhong* ($p < 0.05$). The K/Na ratio in the shoot sap of *Liuye* was higher than that in *Quanhong* under control treatment, but significantly lower than that in *Quanhong* under 0.4% salinity treatment. The high Na concentration in the sap of *Liuye* at high salinity levels might have partly contributed to the root-to-shoot Cd translocation because Na exchanged with Cd in a 5:1 M ratio at xylem binding sites (Petit and Van de Geijn, 1978; Xu et al., 2010).

The Cl^- concentrations in the roots and shoot sap, as well as the Cl^- transfer factor, of the cultivars are presented in Fig. S2. Upon salinity treatments, the shoot sap Cl^-concentrations in *Liuye* were respectively 1.26-and 1.28-fold higher than those in *Quanhong*. The

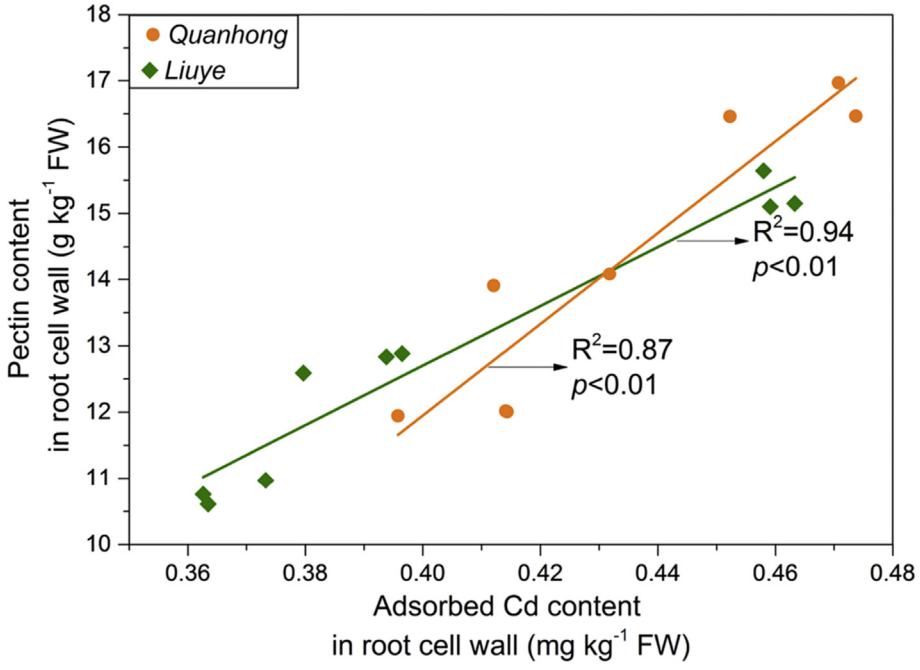

Fig. 2. Correlative relationship between contents of pectin in the root and the adsorbed Cd content in the root cell wall. $P < 0.01$ indicates significance at the 0.01 level.

Table 5
Concentrations of major organic osmolytes in the roots and shoot sap of cultivars under salinity stress.

	Salinity treatment	Pro (g kg^{-1} FW)		TFAA (g kg^{-1} FW)		TSP (g kg^{-1} FW)		TSS (g kg^{-1} FW)	
		Quanhong	*Liuye*	*Quanhong*	*Liuye*	*Quanhong*	*Liuye*	*Quanhong*	*Liuye*
Root	0	0.18 ± 0.01 Aa	0.45 ± 0.01 Ba	0.14 ± 0.02 Aa	0.63 ± 0.01B	4.46 ± 0.13 Ba	3.37 ± 0.38 Aa	0.42 ± 0.03 Aa	3.98 ± 0.40 Ba
	0.2%	0.24 ± 0.01 Ab	0.45 ± 0.02 Ba	0.26 ± 0.02 Ac	0.44 ± 0.02Bb	7.54 ± 0.08 Bc	3.67 ± 0.33 Aa	1.03 ± 0.09 Ab	6.31 ± 0.63 Bb
	0.4%	0.42 ± 0.01 Ac	0.45 ± 0.06 Ba	0.17 ± 0.00 Ab	0.32 ± 0.01 Ba	6.93 ± 0.15 Bb	6.62 ± 0.12 Ab	1.30 ± 0.05 Ac	14.04 ± 0.41 Bc
	ANOVA F ratio								
	Cultivar	191.30**		1544**		260.62**		1913**	
	Salinity	36.01**		153.02**		232.40**		395.57**	
	Cultivar × Salinity	33.93**		238.99**		98.67**		294.34**	
Shoot	0	0.17 ± 0.00 Aa	0.23 ± 0.01 Ba	0.22 ± 0.01 Aa	0.80 ± 0.02 Bc	14.42 ± 0.30Ba	10.10 ± 0.50Aa	1.24 ± 0.10 Ac	2.70 ± 0.07 Bb
	0.2%	0.19 ± 0.00 Ab	0.25 ± 0.01 Ba	0.30 ± 0.01 Ab	0.48 ± 0.01 Ba	15.50 ± 0.08Bb	15.05 ± 0.28Ac	0.98 ± 0.07 Ab	6.53 ± 0.14 Bc
	0.4%	0.47 ± 0.01 Ac	0.49 ± 0.01 Bb	0.31 ± 0.01 Ab	0.59 ± 0.03 Bb	15.97 ± 0.27Bc	14.37 ± 0.43 Ab	0.61 ± 0.03 Aa	1.96 ± 0.10 Ba
	ANOVA F ratio								
	Cultivar	110.86**		2510**		180.09**		4181**	
	Salinity	1611**		103.68**-		155.84**		1164**	
	Cultivar × Salinity	10.22**		300.02**		52.16**		1026**	

Pro, TFAA, TSP, and TSS represent proline, total free amino acid, total solute protein, and total solute sugar, respectively. The different capital letters indicate the significant difference between cultivars under the same salinity treatment (one-way ANOVA test, $p < 0.05$), and the lowercase letters indicate significant difference between treatments of the same cultivars (one-way ANOVA test, $p < 0.05$). Asterisk **, * means significant effect of cultivar, salinity and their interaction on the indicator at $p < 0.01$, 0.05 by two-way ANOVA tests. Values refer to the means (±SD) of three replicates.

Cl^- concentration in the root sap of *Liuye* was significantly higher than that in the root sap of *Quanhong* under control treatment, but significantly lowers under 0.4% salinity treatment. The Cl transfer factor $TF_{Cl\text{-}rs}$ (root-to-shoot) of *Liuye* displayed a conspicuous increase (ranging from 1.68 to 3.77) with the increase in soil salinity, whereas an opposite trend was observed in *Quanhong* (ranging from 4.53 to 1.59). Results showed that *Liuye* translocated much less Cl to the shoots than *Quanhong* under control treatment; however, *Liuye* translocated much more Cl to the shoots than *Quanhong* under salinity stress, especially under higher salinity treatment. López-Chuken et al. (2010) and Ozkutlu et al. (2007) reported that Cl^- transport considerably promoted the migration of Cd within plant organs by forming mobilized $CdCl_n^{2-n}$ complexes. Loon and Duffy (2010) and Mariem et al. (2014) expounded that uncharged or electronegative Cl–Cd complexes are more weakly adsorbed to the xylem cell wall compared with the free Cd^{2+} ions, which may be a crucial factor for accelerating xylem Cd transport.

The concentrations of major organic osmolytes in the roots and shoot sap of the cultivars are listed in Table 5. TSP concentration was the highest among the organic osmolytes in *Quanhong*. The TSP concentration in the roots was 3.67-fold of the total concentrations of Pro, TFAA, and TSS under 0.4% salinity treatment. However, TSS concentration was the highest among the organic osmolytes, followed by TSP concentration in the roots of *Liuye* under 0.4% salinity treatment. The TSP concentration was significantly higher in the roots and shoots of *Quanhong* than in those of *Liuye* ($p < 0.05$). These possibly caused the greater impediment of Cd root-to-shoot translocation by TSP in *Quanhong* than in *Liuye* under salinity stress because TSP, unlike TSS, could form a complex with Cd^{2+}. Mühling and Läuchli (2003) and Wang et al. (2015) reported that solute protein contributes to the precipitation of more ionic Cd^{2+} in the cytochylema and limits Cd translocation from the roots to shoots.

Although the concentrations of Pro and TFAA in both cultivars were much lower than those of TSP and TSS, their increments were greater in *Quanhong* than in *Liuye* under salinity stress (Table 5). Zouari et al. (2016a, 2016b) reported that Pro can slow down the upward transport of Cd in young date palms through the formation of Cd–proline complexes. Amino acids can also reduce the concentration of free Cd^{2+} ions in root cells by chelation (Sharma and Dietz, 2006) and influence their transport across cytomembranes. Zhang et al. (2009) discovered that amino acids significantly decrease Cd concentrations in plant leaves. Therefore, the greater increase in the concentrations of Pro and TFAA in *Quanhong* than in *Liuye* under salinity stress might have partly contributed to the lower Cd translocation from the roots to shoots in *Quanhong*.

4. Conclusions

Two amaranth cultivars cultivated in Cd-contaminated saline soils showed significantly different Cd-accumulating abilities. The experiments on determining cultivar differences in Cd accumulation were repeatable and stable. The high-Cd and low-Cd cultivars exhibited different osmoregulation strategies in response to salinity stress. The different behaviors triggered different biogeochemical processes involved in Cd mobilization in the rhizosphere soil, Cd absorption, and translocation by the two cultivars. Inorganic osmoregulation was more involved in salinity stress response in the high-Cd cultivar than in the low-Cd cultivar. Therefore, the high-Cd cultivar absorbed greater amounts of excess cations over anions than the low-Cd cultivar, allowing the roots of the high-Cd cultivar release more H^+ than those of the low-Cd cultivar for mobilizing soil Cd in the rhizosphere. The high-Cd cultivar has less pectin and adsorbed Cd in the root cell wall, facilitating Cd absorption. The high-Cd cultivar also transported more Cl^- from the roots to shoots and had less organic complex in the root sap. Both of them facilitated Cd translocation in the high-Cd cultivar. Our results suggest that inorganic osmoregulation in edible amaranth cultivars can cause greater Cd health risk in crops grown in Cd-contaminated saline soil. On the contrary, organic osmoregulation reduces the health risks of Cd accumulation. The results of this study are potentially useful for screening low-Cd cultivars grown on Cd-contaminated saline soils.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.12.067.

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