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RESEARCH ARTICLE

The regulatory role of root in cadmium accumulation in a high cadmium-accumulating rice line (*Oryza sativa* L.)

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Abstract

There are some key processes that regulate cadmium (Cd) accumulation in rice. Understanding the characteristics and mechanisms of Cd accumulation in high Cd-accumulating rice lines benefits for excavating relevant genes. Cd accumulation and distribution in roots of Lu527-8, a high Cd-accumulating rice line, were investigated by a hydroponic experiment, with a control of a normal rice line (Lu527-4). Lu527-8 showed significantly higher Cd concentrations in roots than Lu527-4. More than 81% of Cd in roots of two rice lines is distributed in soluble fraction and cell wall. In soluble fraction, there were more organic acids, amino acids, and phytochelatins in Lu527-8, benefiting Cd accumulation. Pectin and hemicellulose 1 (HC1), especially pectin, were main polysaccharides in cell wall. Lu527-8 showed more pectin and HC1 along with higher pectin methylesterase (PME) activity compared with Lu527-4, promoting Cd accumulation. Besides, Lu527-8 showed higher Cd translocation from root to shoot due to more amounts of ethanol-extractable Cd in roots than Lu527-4. In conclusion, specific characteristics of Cd chemical forms and subcellular distribution in roots of high Cd-accumulating rice line are important for Cd accumulation and translocation.

Keywords Rice (*Oryza sativa* L.), · Cadmium, · Subcellular fraction, · Chemical form

Introduction

Cadmium (Cd) has been considered a global toxic trace pollutant, which is readily absorbed and accumulated in plants, thus influencing a series of physiological processes, such as nutrient element assimilation, enzyme activity, respiration, and photosynthesis (Uraguchi and Fujiwara, 2013; Feng et al. 2017). To alleviate Cd toxicity, some plants, especially phytoextractors, have developed complex mechanisms for Cd detoxification and translocation (Clemens et al. 2013; He et al. 2015; He et al. 2019). Those plants pose a strong potential for Cd accumulation and transportation by mediating Cd distribution and chemical forms (Wang et al. 2008; Fu et al. 2011). Some *indica* rice varieties are considered potential phytoextractors for Cd-contaminated paddy fields because of their high biomass and Cd accumulation in shoots (Ibaraki et al. 2009). An understanding of Cd chemical form and subcellular distribution in a high Cd-accumulating rice line is beneficial to clarify the mechanism of Cd detoxification and accumulation.

The subcellular distribution of Cd is closely related to Cd accumulation and detoxification in plants (Wu and Wang 2011; Su et al. 2014; Wang et al. 2015). Plant accumulates Cd in cell wall or soluble fraction to achieve Cd detoxification (Su et al. 2017; Zhou et al. 2017). As the first barrier, cell wall poses a critical capacity of protecting Cd from being transported into the protoplast (Krzesłowska, 2011). The major components of cell wall are polysaccharides, including pectin and hemicellulose (Xiong et al. 2009). Both of them contain abundant functional groups, such as carboxyl, hydroxide radical, and sulfhydryl, which can efficiently bind metal cations (Li et al. 2015). Under Cd stress, cell wall components are actively modified to enhance Cd binding capacity (Krzesłowska, 2011). With the demethylation catalysis of pectin methylesterase (PME), more and more exposure of free carboxyl groups is observed in pectin, resulting in an increase for Cd binding capacity (Krzesłowska, 2011; Li et al. 2015). Meanwhile, there are amounts of ligands, such as

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polypeptides and organic acids, which can chelate Cd ions in soluble fraction. Cd complexes are then transported into vacuoles to compartment, resulting in Cd detoxification and reducing Cd transport (Li et al. 2016; Mwamba et al. 2016; Xin et al. 2017).

Chemical forms have attracted great attention because of their importance in ecotoxicology and translocation (Lai, 2015; Wang et al. 2016). Cd uptake by plants is usually with different chemical forms, thereby influencing Cd tolerance and translocation (Lai et al. 2015; Li et al. 2016). Cd in water-soluble and ethanol-extractable forms shows more mobility and poison to plant cell, compared with Cd in protein-extractable and pectate-extractable forms, undissolved phosphate forms, and oxalate forms (Zhan et al. 2015; Guan et al. 2018). Moreover, Cd chemical forms usually vary greatly among plant species or genotypes, suggesting different capacities of Cd mobilization and immobilization (Li et al. 2016; Yang et al. 2018).

A rice line designated Lu527-8 was selected as a high Cd-accumulating rice line due to great Cd uptake and translocation to shoots as well as greater Cd tolerance (Tang et al. 2016; Fu et al. 2019). Root is an important tissue for Cd uptake and translocation, while little information available on the characteristics and mechanisms of Cd accumulation and distribution in root of Lu527-8. Therefore, this study would comprehensively elucidate the physiological mechanisms of Cd accumulation and detoxification by clarifying Cd chemical forms and Cd subcellular distribution in the roots of Lu527-8.

Materials and methods

Plant material

A high Cd-accumulating rice line (*Oryza sativa* L.) designated Lu527-8 was used in this study, and a normal rice line designated Lu527-4 was used as a control. Both of them are *indica* varieties with similar growth period.

Hydroponic culture and plant sampling

A hydroponic experiment was conducted with the following treatments: 0 (CK), 2, and 5 mg L^{-1} Cd. Cd was supplied as a $CdCl_2 \cdot 2.5H_2O$ solution. The experiment was conducted in an entirely random permutation with five replications. The formulation recommended by the International Rice Research Institute was used as the basal nutrient medium (Ponnamperuma, 1977).

The seeds were sterilized in 30% H_2O_2 for 30 min and then with 0.1% sodium hypochlorite for 24 h and then sowed in watered perlite at 25 °C and 60% of relative humidity until germination, during which period 1/4 nutrient solution was used. At the third-leaf stage, uniform seedlings were selected and transplanted into basal nutrient solution (pH 5.5) treated with different concentrations of Cd as designed. The seedlings were cultivated in a growth room under natural light at 30 °C/25 °C (day/night temperature). The nutrient solution was renewed every 5 d.

The samples were collected at tillering stage. The roots were soaked in 20 mmol·L^{-1} Na_2-EDTA for 0.5 h and rinsed with deionized water to remove surface-adsorbed Cd. Then the samples were divided into roots and shoots. Half of the samples were immediately frozen by liquid nitrogen for the analysis of fresh sample, and the others were oven-dried at 75 °C for Cd analysis.

Determination of Cd concentrations in roots and shoots

About 250-mg dry samples were digested with $HNO_3/HClO_4$ (5:1, *v*/v) for 8 h and diluted with deionized water to a total volume of 50 mL. Flame atomic absorption spectrophotometer (AA900T, PerkinElmer, America) was used to analyze Cd concentrations in digested solutions.

Cd subcellular fraction

Subcellular fractions were processed according to previous method with minor modifications (Fu et al. 2011; Zhu et al. 2012). The samples were divided into four fractions: cell wall, organelle, membrane, and soluble fractions. About 1-g fresh root samples were homogenized with 75% ethanol for 20 min in an ice bath. The homogenates were centrifuged at 4579×*g*, 4 °C for 10 min. The sediments were gradually homogenized with 5-mL acetone, methanol:chloroform (1:1), and methanol for 20 min in an ice bath and centrifugated at 4579×*g*, 4 °C for 10 min to obtain the residue as "cell wall fraction." Meanwhile, the supernatants were centrifuged at 10000×*g*, 4 °C for 30 min and the residues were identified as "organelle fraction." The supernatants were then centrifuged at 100000×*g*, 4 °C for 30 min and the residues were identified as "membrane fraction." The final supernatants were considered "soluble fraction," including vacuoles and cytoplasm. The four fractions were dried at 75 °C and then digested with $HNO_3/HClO_4$ (5:1, *v*/v). The digested solutions were then diluted with deionized water up to a total volume of 10 mL. Cd concentrations in the digested solutions were analyzed by graphite furnace atomic absorption spectrophotometer (AA900T, PerkinElmer, America).

Determination of organic acid and amino acid concentrations

About 0.5-g fresh root samples were ground and transferred into 2 ml 0.5 mol L^{-1} HCl at 60 °C for 1 h. The solutions were centrifuged at 8000×*g* for 10 min and filtered with a 0.45-μm

millipore filter to obtain organic acids. The organic acids concentrations were determined by HPLC (Shimadzu, Japan) with the procedure described by Fu et al. (2018).

About 0.3-g fresh root samples were ground and transferred to 30 ml 0.1 mol L^{-1} HCl for 24 h. The solutions were mixed with 10% sulfonylsalicylic acid solution (1:3, *v*/v) and centrifuged at 14000×*g* for 20 min and filtered with a 0.45-μm millipore filter to obtain amino acids. The amino acid concentrations were determined by L-8800 amino acid analyzer (Hitachi, Japan) with the procedure described by Fu et al. (2018).

Cell wall preparation and polysaccharides extraction

Cell wall preparation referred to the method of Sharifi et al. (2016). The fresh root samples were ground to powder and further homogenized with ice-cold 75% ethanol in an ice bath for 20 min. Then the homogenates were centrifuged at 5000×*g* for 10 min. The residues were washed with the following solutions: ice-cold acetone (1:5, *w*/*v*), methanol-chloroform mixture (1:1, *v*/v), and methanol three times. The final residues were obtained as crude cell walls and freeze-dried and stored at 4 °C for subsequent extraction.

The extraction of cell wall polysaccharides was carried out according to Yang et al. (2008) with minor modifications. Cell wall was divided into three parts: pectin, hemicellulose 1 (HC1), and hemicellulose 2 (HC2). About 10-mg cell wall was extracted thrice with 5-mL deionized water at 100 °C for 1 h to obtain pectin. After centrifugation at 16800×*g* for 10 min, the three supernatants were pooled and diluted with deionized water to a total volume of 15 mL. Then, the residues were extracted twice with 5-mL solutions containing 4% (*w*/*v*) KOH and 0.1% (*w*/*v*) $NaBH_4$ for 12 h and centrifuged at 16800×*g* for 10 min to obtain HC1, followed by a similar extraction procedure with 24% (w/v) KOH and 0.1% $NaBH_4$ to obtain HC2. Three hundred microliter of the above extracted solutions were digested with HNO_3/$HClO_4$ (5:1, *v*/v). Cd concentrations in the solutions were determined by graphite furnace atomic absorption spectrophotometer (AA900T, PerkinElmer, America).

Determination of uronic acid and total sugar concentrations in cell wall polysaccharides

Uronic acid concentration in polysaccharides was determined according to Zhu et al. (2012) with galacturonic acid (Sigma) as a standard. One-milliliter pectin solutions were incubated with 5 mL 98% H_2SO_4 (containing 0.0125 mol L^{-1} $Na_2B_4O_7 \cdot 10H_2O$) at 100 °C for 5 min. One hundred microliter 0.15% M-hydro-dipheny was added after the solution chilled. Subsequently, the solutions were incubated at indoor temperature for 20 min. Spectrophotometer was used to determine the absorbance at 520 nm. Total sugar concentration in hemicellulose was determined according to phenol sulfuric acid method and expressed as glucose equivalents. Five-milliliter 98% H_2SO_4 and 10 μL 80% phenol were added into 1-mL HC1 and HC2 extracting solutions and incubated at indoor temperature for 15 min and then at 100 °C for 15 min, respectively. Spectrophotometer was used to determine the absorbance at 490 nm.

Determination of PME activity

PME activity was determined according to Zhu et al. (2012). Ten-milligram freeze-dried cell walls were extracted with 1 mL 1 mol L^{-1} NaCl solution in an ice bath for 20 min and mixed every 10 min. Extracts were centrifuged at 13000×*g* for 10 min to obtain the supernatants containing PME. Two hundred fifty microliter PME extracts were added into 50-μL alcohol oxidase (pH 7.5) and 500 μL 0.64 mg mL^{-1} pectin (Sigma) and incubated at 30 °C for 10 min. Subsequently, 1-mL solutions containing 5 mg mL^{-1} purpald and 0.5 mol L^{-1} NaOH were added and incubated at 30 °C for 30 min. Then, the solutions were diluted with deionized water to a total volume of 5 mL and their absorbance was measured at 550 nm by spectrophotometer.

The extraction of Cd chemical forms

Cd chemical forms were determined according to Zhao et al. (2015) with minor modifications. Cd was extracted with the following extraction solutions in sequence: 80% (*v*/v) ethanol (F_E), deionized water (F_W), 1 mol L^{-1} NaCl (F_{NaC}), 2% (v/v) acetic acid (F_{HAc}), and 0.6 mol L^{-1} HCl (F_{HCl}). About 0.3-g fresh samples were ground and transferred into extraction solutions. Corresponding supernatants were obtained after being suspended with different extraction solutions and then shaken at 25 °C for 22 h and centrifuged at 5000×*g* for 10 min. The above steps were repeated, and then all the supernatants were collected. Cd concentrations in the supernatant solutions were determined as described in the "Cd subcellular fraction" section.

Statistical analysis

Translocation factor (TF) was calculated according to Zhang et al. (2012) as follows:

$$\mathrm{TF}\ (\%) = \mathrm{Cd\ concentration\ in\ shoot}/\mathrm{Cd\ concentration\ in\ root} \times 100.$$

Data were analyzed with DPS 11.0. The least significant difference (LSD) test was used to determine differences among the treatments or rice lines at 0.05 level. Graphical work was exported by OriginPro 9.0 and Excel 2013.

Table 1 Subcellular distribution of Cd in roots of high Cd-accumulating rice line (Lu527-8) and normal rice line (Lu527-4) grown in different Cd treatments

Cd treatment (mg L^{-1})	Cell wall (mg kg^{-1} FW)		Soluble fraction (mg kg^{-1} FW)		Organelle (mg kg^{-1} FW)		Membrane (mg kg^{-1} FW)	
	Lu527-8	Lu527-4	Lu527-8	Lu527-4	Lu527-8	Lu527-4	Lu527-8	Lu527-4
0	nd	nd	nd	nd	nd	nd	nd	nd
2	29.9±0.17 b*	23.4±1.20 b	37.8±1.59 b*	27.6±0.76 b	8.33±0.59 b*	6.51±0.91 b	4.14±0.55 b	3.31±1.12 b
5	45.9±0.08 a*	34.1±0.74 a	55.8±1.65 a*	36.8±2.51 a	10.1±0.34 a	9.79±0.40 a	5.61±0.53 a	6.98±0.09 a*

The data mean the average of three replicates ± standard deviations (SDs). Different letters back of the digital indicate significant level at $p < 0.05$ among the Cd treatments, * indicates significant level at $p < 0.05$ among the rice lines. nd means not detected

Results

Cd subcellular distribution in roots

Cd concentrations of subcellular fractions in roots of the two rice lines markedly increased with increasing Cd doses (Table 1). Cd mainly distributed in soluble fraction and cell wall fraction in roots. A small amount of Cd distributed in organelle and membrane fractions. There were 47–48% and 37–39%, 42–45%, and 39% of Cd in soluble fraction and cell wall in Lu527-8 and Lu527-4, respectively. Cd concentrations in soluble fraction and cell wall of Lu527-8 were 1.37–1.52 times and 1.28–1.34 times higher than those of Lu527-4, respectively.

Organic acid and amino acid concentrations in roots

There were eight organic acids detected in roots of the two rice lines (Table 2). Oxalate, tartaric, malate, citrate, and acetic acid accounted for the most among organic acids. The concentrations of five organic acids significantly decreased with increasing Cd doses. The concentrations of tartaric, malate, citrate, and acetic acid in Lu527-8 were much more than those in Lu527-4 under Cd treatments.

There were nine amino acids detected in roots of the two rice lines (Table 3). The concentrations of proline, phenylalanine, histidine, lysine, and methionine in roots of the two rice lines markedly increased with increasing Cd doses. The concentrations of glutamic acid, proline, phenylalanine, glycine, histidine, lysine, and methionine in Lu527-8 were markedly higher than those in Lu527-4 under Cd treatments.

Cd accumulation in cell wall polysaccharides

The concentrations of cell wall polysaccharides, including pectin, HC1, and HC2, markedly increased with increasing Cd doses (Fig. 1). Pectin concentrations in Lu527-8 were markedly higher than Lu527-4 when exposed to 2 mg L^{-1} Cd. HC1 and HC2 concentrations in Lu527-8 were markedly higher than those in Lu527-4 under different Cd treatments, up to 1.11 times and 1.51 times, respectively.

As for Cd concentrations in cell wall polysaccharides, Cd concentrations in pectin were the highest, followed by HC1 and HC2 (Fig. 2). Cd concentrations in the three cell wall fractions increased by 117–229% with increasing Cd doses. Cd concentrations in the three cell wall fractions of Lu527-8 was 1.25–2.42 times higher than Lu527-4.

Table 2 The organic acids concentrations in roots of high Cd-accumulating rice line (Lu527-8) and normal rice line (Lu527-4) grown in different Cd treatments

Line	Cd treatment	Organic acids concentration (mg g^{-1} FW)							
	(mg L^{-1})	Oxalate	Tartaric acid	Malate	Citrate	Acetic acid	Succinic acid	Fumaric acid	Malonic acid
Lu527-8	0	14.3±0.28 a	11.2±0.62 a*	9.64±1.51 a*	8.47±0.53 a*	5.21±0.50 a*	0.51±0.03 a	0.53±0.06 a	0.31±0.04 a
	2	10.1±0.91 b	5.17±0.27 b*	5.37±0.44 b*	6.09±0.21 b*	2.54±0.25 b*	0.55±0.07 a	0.51±0.03 a	0.28±0.05 a
	5	3.88±0.01 c	2.76±0.10 c*	3.60±0.32 c*	2.98±0.35 c*	1.10±0.05 c	0.49±0.12 a	0.53±0.04 a	0.29±0.03 a
Lu527-4	0	14.1±0.06 a	2.40±0.06 a	3.44±0.18 a	2.89±0.53 a	3.12±0.08 a	0.47±0.06 a	0.52±0.06 a	0.33±0.04 a
	2	10.3±0.86 b	1.52±0.20 b	2.83±0.24 ab	2.43±0.16 ab	2.01±0.23 b	0.53±0.05 a	0.48±0.05 a	0.32±0.03 a
	5	3.93±0.12 c	1.15±0.13 b	2.21±0.22 b	1.84±0.09 b	1.37±0.03 c	0.51±0.03 a	0.49±0.03 a	0.28±0.04 a

The data mean the average of three replicates ± standard deviations (SDs). Different letters back of the digital indicate significant level at $p < 0.05$ among the Cd treatments, * indicates significant level at $p < 0.05$ among the rice lines

Table 3 The amino acids concentrations in roots of high Cd-accumulating rice line (Lu527-8) and normal rice line (Lu527-4) grown in different Cd treatments

Line	Cd treatment (mg L^{-1})	Amino acids concentration (mg g^{-1} FW)								
		Glutamic acid	Proline	Phenylalanine	Glycine	Histidine	Lysine	Alanine	Methionine	Tyrosine
Lu527-8	0	26.4±0.78 a	18.2±1.03 c	8.91±0.22 c	8.26±0.14 a*	8.02±0.60 c	7.86±0.25 c	5.29±0.28 a	4.97±0.02 c	2.94±0.07 a
	2	26.2±0.89 a*	23.6±0.80 b*	19.5±0.69 b*	8.65±0.56 a*	13.0±0.87 b*	12.8±0.65 b*	5.44±0.46 a*	8.05±0.26 b*	2.80±0.11 a
	5	25.7±0.75 a*	27.1±0.61 a*	30.0±2.07 a*	8.26±0.26 a*	20.0±0.31 a*	18.2±0.92 a*	5.28±0.16 a	10.8±0.44 a*	2.82±0.06 a
Lu527-4	0	27.0±1.66 a	17.2±0.90 c	8.32±0.30 c	7.26±0.56 a	9.03±0.34 c	8.06±0.67 b	4.89±0.70 a	4.80±0.08 b	2.94±0.22 a
	2	17.4±1.09 b	21.2±1.16 b	12.4±0.52 b	4.12±0.29 b	11.3±0.66b	10.0±0.28 a	4.28±0.78 a	5.64±0.12 a	2.88±0.04 a
	5	11.9±0.52 c	25.5±0.37 a	15.8±0.83 a	2.62±0.13 c	12.8±0.57a	10.7±0.45 a	4.66±0.81 a	5.67±0.15 a	2.86±0.07 a

The data mean the average of three replicates ± standard deviations (SDs). Different letters back of the digital indicate significant level at $p < 0.05$ among the Cd treatments, * indicates significant level at $p < 0.05$ among the rice lines

PME activity

PME activity significantly increased in roots of the two rice lines with increasing Cd doses (Fig. 3). Compared with CK, PME activity increased 69 and 36% in Lu527-8 and Lu527-4, respectively, when exposed to 5 mg L^{-1} Cd. The activity in Lu527-8 was 1.25–1.27 times higher than Lu527-4 under different treatments.

Cd chemical forms in roots

Cd concentrations in all chemical forms in the two rice lines enhanced with increasing Cd doses (Table 4). F_E was the predominant chemical form in the two rice lines, accounting for 53–55% in Lu527-8 and 44–48% in Lu527-4, respectively, followed by F_{NaC} (26% in Lu527-8 and 27–31% in Lu527-4, respectively) and F_{HAc} (15–18% in Lu527-8 and 20–21% in Lu527-4, respectively). Cd concentrations in the other chemical forms were much lower. In general, Cd concentrations in all chemical forms in the roots of Lu527-8 were greater than those in Lu527-4 under different Cd treatments.

Discussion

Subcellular distribution involved in Cd accumulation and detoxification in roots

As reported, 49–79% of Cd ions are retained in rice roots which are the primary tissues of Cd absorption, thus influencing xylem-mediated Cd transport from root to shoot and eventually Cd accumulation in shoot (Uraguchi et al. 2009; Nocito et al. 2011; Ricachenevsky et al. 2018). In this study, Lu527-8 accumulated more Cd in roots than Lu527-4 with more Cd translocation to shoots (Table S1), indicating that root shows a strong ability of Cd accumulation and translocation in Lu527-8. However, the mechanisms of Cd accumulation and distribution in the roots of Lu527-8 remain unclear.

Once into cells, large amounts of Cd ions are bound to cell walls, with many binding sites that show high affinity to metals, or transferred into soluble fraction (Nocito et al. 2011; Zhao et al. 2015). Cell wall and soluble fraction are the major binding sites for Cd in plants, thus being vital for Cd accumulation and detoxification (Xin et al. 2013; Zhang et al. 2015; Mwamba et al. 2016). In this study, soluble fraction and cell wall were the primary fractions for Cd accumulation in roots of the two rice lines. Cd concentrations in the soluble fraction and cell wall of Lu527-8 were much higher than Lu527-4 (Table 1). It suggests that soluble fraction and cell wall contributed greatly to high Cd accumulation in the roots of Lu527-8. In soluble fraction, there are amounts of phytochelatins, organic acids and amino acids, benefiting Cd detoxification and accumulation (Degola et al. 2014; Osmolovskaya et al. 2018; Zhu et al. 2018). Phytochelatins are rich in thiol moieties which have positive effect on Cd chelation (Degola et al. 2014; Hazama et al. 2015). Similarly, organic acids and amino acids can form strong bond with Cd through their functional groups, such as carboxyl groups (Osmolovskaya et al. 2018; Zhu et al. 2018). Our previous study proved that soluble fraction played an important role in Cd accumulation in the roots of Lu527-8 due to being with more phytochelatins, glutathione and nonprotein thiols than Lu527-4 (Tang, 2016). Organic acids and amino acids in the roots of Lu527-8 were also detected in this study. There were more amino acids, especially glutamic acid, proline, and henylalanine, in Lu527-8 under Cd stress (Table 3).

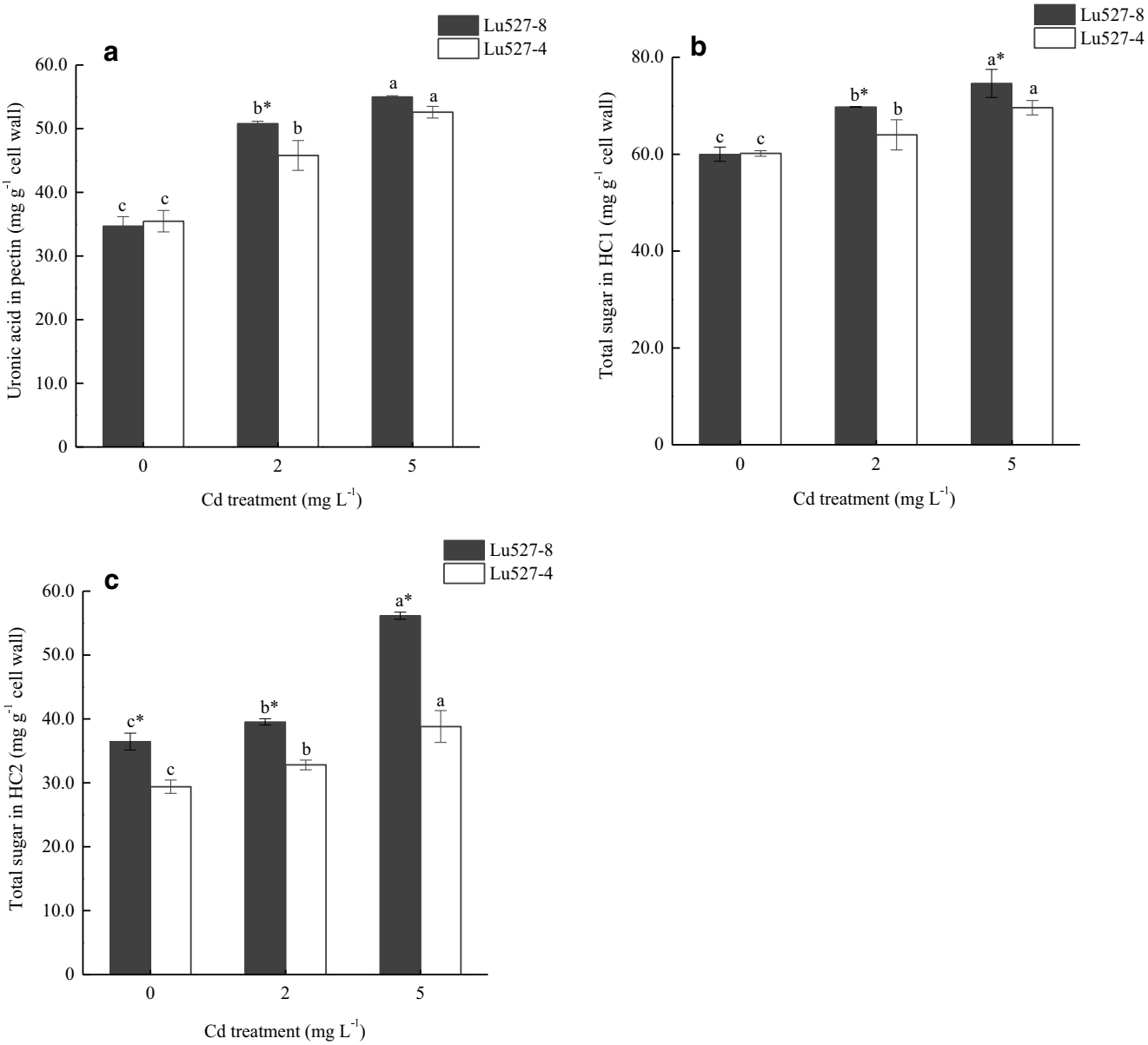

Fig. 1 Pectin, hemicellulose 1 (HC1), and hemicellulose 2 (HC2) concentrations in the root cell wall of high Cd-accumulating rice line (Lu527-8) and normal rice line (Lu527-4) grown in different Cd treatments. The data mean the average of three replicates ± standard deviations (SDs). Different letters above the bars indicate significant level at $p < 0.05$ among the Cd treatments, * indicates significant level at $p < 0.05$ among the rice lines

Glutamic acid, cysteine, and glycine are precursors of glutathione and phytochelatins (Nocito et al. 2008). Proline plays an important role in protecting plants from heavy metal stress through scavenging of free radicals, stabilizing protein synthesis, and regulating cytosolic acidity (Choudhary et al. 2007). Meanwhile, the concentrations of tartaric, malate, citrate, and acetic acid were significantly higher in Lu527-8 compared with Lu527-4 (Table 2). All of these were conducive to Cd detoxification and Cd accumulation in the soluble fraction of Lu527-8. Besides, cell wall was the subdominant domain for Cd accumulation in the roots of Lu527-8. It may attribute to its abundant polysaccharides with functional groups, and the underlying mechanism was further explored.

Cell wall polysaccharides involved in cd accumulation in roots

Cell wall is rich in polysaccharides (Gallego et al. 2012; Chen et al. 2013). Pectin and hemicellulose are the major components of cell wall and play an important role in binding Cd (Zhu et al. 2013; Li et al. 2015). There are amounts of negative charge functional groups with highly effective ability of Cd binding in pectin and hemicellulose (Li et al. 2015; Loix et al.

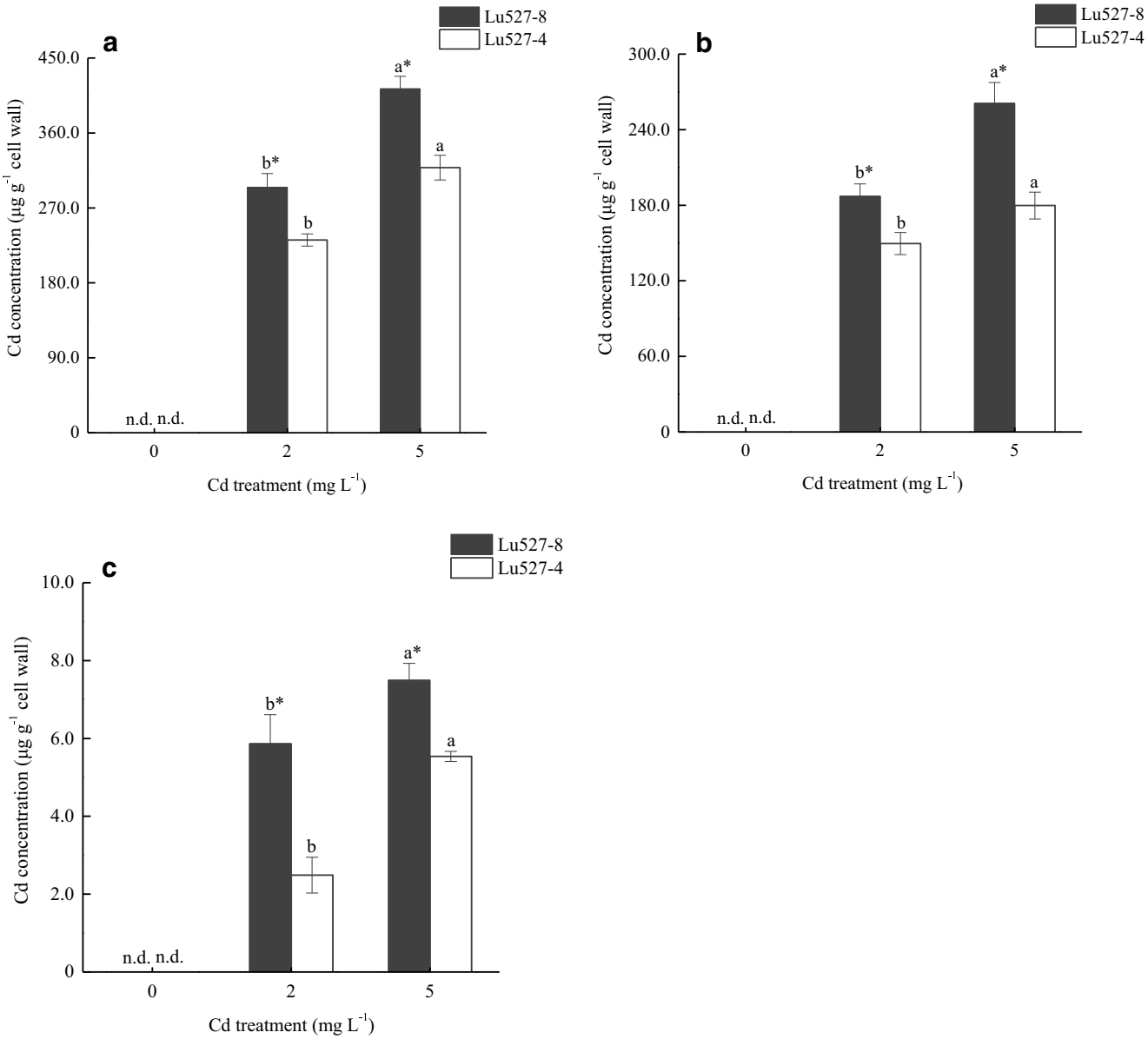

Fig. 2 Cd concentrations in different cell wall fractions extracted from the roots of high Cd-accumulating rice line (Lu527-8) and normal rice line (Lu527-4) grown in different Cd treatments. The data mean the average of three replicates ± standard deviations (SDs). Different letters above the bars indicate significant level at $p < 0.05$ among the Cd treatments, * indicates significant level at $p < 0.05$ among the rice lines

2017). Thus, more specific polysaccharides show greater potentiality to Cd binding. In this study, Cd induced more polysaccharides in cell walls of the two rice lines, and Lu527-8 showed more polysaccharides in cell walls than Lu527-4 (Fig. 1). Thus, there were more Cd bound to cell wall polysaccharides in the roots of Lu527-8 (Fig. 2). Meanwhile, Cd concentrations in pectin and HC1 showed positive linear correlations with Cd concentrations in roots of the two rice lines (Fig. S1), indicating that pectin and HC1 were important for Cd binding in root cell wall of the two rice lines. Particularly, Cd accumulation in pectin accounted for more than 60% of the total Cd in cell wall polysaccharides, suggesting that pectin plays a dominant role in binding Cd in the root cell wall of Lu527-8. Cd stress commonly induced some modifications to pectin in root cell wall (Krzesłowska et al. 2016). Pectin undergoes demethylation with the catalysis by PME, leading to an increase for free carboxyl groups available to bind Cd ions (Li et al. 2015; Chebli and Geitmann, 2017; Gutsch et al. 2019). In this study, Cd stress induced an increase for PME activity in root cell wall of Lu527-8, which shows higher PME activity than Lu527-4 (Fig. 3). Therefore, Lu527-8 presented more Cd accumulation in root cell walls than Lu527-4 when exposed to Cd. It could be demonstrated that abundant polysaccharides, especially pectin, and high PME activity in root cell walls contributed greatly to high Cd accumulation in the roots of Lu527-8.

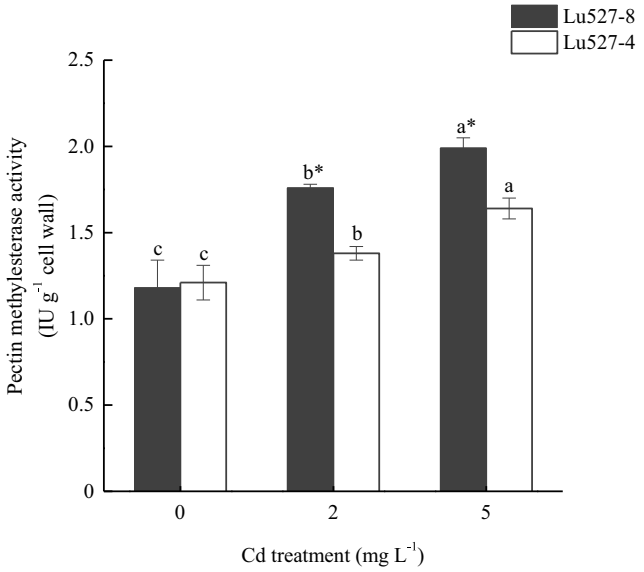

Fig. 3 Pectin methylesterase activity in the root cell wall of high Cd-accumulating rice line (Lu527-8) and normal rice line (Lu527-4) grown in different Cd treatments. The data mean the average of three replicates ± standard deviations (SDs). Different letters above the bars indicate significant level at $p < 0.05$ among the Cd treatments, * indicates significant level at $p < 0.05$ among the rice lines

Cd chemical forms in roots and their role in Cd translocation

Cd is usually present in chemical forms with different bioavailability and mobility in plants (Wang et al. 2015; Li et al. 2016; Zhao et al. 2015). Among Cd chemical forms, water-soluble and ethanol-extractable Cd showed the highest mobility and thus can be easily transported to aboveground tissues, followed by NaCl-extractable, HAC-extractable, and HCl-extractable Cd (Lu et al. 2017). In this study, Cd mainly presented as ethanol-extractable form, followed by NaCl-extractable and HAC-extractable forms in two rice lines (Table 4). About 53–55% of the total Cd was present in ethanol-extractable form in the roots of Lu527-8, which was more than that of Lu527-4. This fraction is some Cd bound to nitrate/nitrite, chloride, and aminophenol, showing high mobility to transport to shoot (Wang et al. 2009; Su et al. 2014; Lai et al. 2015; Xin et al. 2017). NaCl-extractable Cd refers to Cd bound to pectate and protein, playing an important part in Cd tolerance (Lu et al. 2019; Xue et al. 2019). The chemical forms of Cd in the roots of Lu527-8, especially those with high mobility, were markedly more than those in Lu527-4, thus making a great contribution to high Cd translocation to shoots in Lu5278. In addition, Cd in the shoots of Lu527-8, especially in leaves, was mainly in NaCl-extractable and HAc-extractable forms (Table S2). More than 70% of the total Cd were NaCl-extractable and HAc-extractable Cd in leaves of Lu527-8, which were more than those of Lu527-4, showing great benefit to Cd accumulation as well as Cd detoxification in the shoots of Lu527-8. It suggests that most of Cd in the roots of Lu527-8 shows high mobility, resulting in Cd translocation to shoot.

Table 4 Chemical forms of Cd in roots of high Cd-accumulating rice line (Lu527-8) and normal rice line (Lu527-4) grown in different Cd treatments

Cd treatment (mg L^{-1})	F_E (mg kg^{-1} FW)		F_W (mg kg^{-1} FW)		F_{NaC} (mg kg^{-1} FW)		F_{HAc} (mg kg^{-1} FW)		F_{HCl} (mg kg^{-1} FW)		F_R (mg kg^{-1} FW)	
	Lu527-8	Lu527-4	Lu527-8	Lu527-4	Lu527-8	Lu527-4	Lu527-8	Lu527-4	Lu527-8	Lu527-4	Lu527-8	Lu527-4
0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
2	52.3 ± 0.41 b*	29.0 ± 1.95 b	1.61 ± 0.14 b	1.49 ± 0.48 b	25.7 ± 1.04 b*	20.2 ± 0.32 b	17.4 ± 1.62 b*	13.5 ± 0.86 b	1.18 ± 0.09 b	0.98 ± 0.02 b	0.60 ± 0.11 b	0.52 ± 0.02 b
5	97.9 ± 1.52 a*	50.1 ± 1.10 a	3.58 ± 0.36 a*	2.81 ± 0.23 a	46.8 ± 1.59 a*	28.2 ± 0.29 a	26.6 ± 0.61 a*	20.3 ± 0.63 a	1.74 ± 0.20 a*	1.33 ± 0.13 a	0.87 ± 0.01 a*	0.64 ± 0.04 a

F_E, F_W, F_{NaC}, F_{HAc}, and F_{HCl} represent Cd extracted by 80% (v/v) ethanol, deionized water, 1 mol L^{-1} NaCl, 2% (v/v) acetic acid, and 0.6 mol L^{-1} HCl, respectively. F_R represents Cd in residue. The data mean the average of three replicates ± standard deviations (SDs). Different letters back of the digital indicate significant level at $p < 0.05$ among the Cd treatments, * indicates significant level at $p < 0.05$ among the rice lines. nd means not detected

Meanwhile, there are amounts of Cd ions with high immobility in the shoots, which is beneficial to high Cd tolerance and accumulation.

Conclusion

The roots of high Cd-accumulating rice line Lu527-8 show a strong ability of Cd accumulation and translocation due to its specific characteristic of Cd subcellular distribution and chemical forms. Cd was mainly distributed in soluble fraction and cell wall in the roots of Lu527-8, benefiting Cd accumulation. In soluble fraction, organic acids and amino acids as well as thiols played important roles in Cd detoxification and accumulation. Meanwhile, there were amounts of polysaccharides, especially pectin, in the roots of Lu527-8. Under the catalysis of PME, pectin posed a strong potential for binding Cd, resulting in alleviating Cd toxicity and promoting Cd accumulation in the root cell wall of Lu527-8. Furthermore, a large proportion of Cd in the roots of Lu527-8 were present in water-soluble and ethanol-extractable form with high mobility, contributing to high Cd translocation to the shoots. Therefore, Lu527-8 accumulates amounts of Cd not only in roots but also in shoots, which is beneficial to excavate relevant genes.

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Data availability The data and materials will be available on request.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Abbreviations Cd, cadmium; PME, pectin methylesterase; HC1, hemicellulose 1; HC2, hemicellulose 2; TF, translocation factor

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