Cellular Distribution and Chemical Speciation of Exogenous Cadmium in Different Rice Cultivar Tissues (Postprint)

Authors: Fu Shuolan, Wang Changquan, Li Bing, Xu Qiang, Zhang Jingsheng, LI Meng, Tang Jie, He Yuting, Shen Jie, Zeng Jiexi, Yan Xun

Date: 2017-11-09T00:00:00+00:00

Abstract

A hydroponic experiment was conducted using low cadmium (Cd) accumulating rice variety 'D83A/R527' and high Cd accumulating rice variety 'Fuyou 838' as test materials, with three Cd concentration treatments (5 mol·L ^{1}, 10 mol·L ^{1}, 25 mol·L ^{1}) to investigate the Cd accumulation characteristics of different genotypic rice varieties from the perspectives of subcellular and chemical form distribution of Cd, providing a scientific basis for exploring the physiological mechanisms of Cd uptake and accumulation in rice. The results showed: (1) The Cd content in roots and shoots and the root-to-shoot translocation factor of rice 'D83A/R527' were significantly lower than those of 'Fuyou 838' ($P<0.05$). (2) In both rice varieties, Cd content in various subcellular fractions of roots exhibited the order: soluble fraction (F3) > cell wall (F1) > organelles (F2), while in shoots it showed: cell wall (F1) > soluble fraction (F3) > organelles (F2); The mass fraction of Cd in cell walls of roots and shoots of 'D83A/R527' (36.76%–51.75%) was higher than that of 'Fuyou 838' (31.29%–49.07%). (3) The content of Cd chemical forms in both rice varieties exhibited the order: sodium chloride extractable form (FNaCl-Cd) > acetic acid extractable form (FHAc-Cd) > deionized water extractable form (FW-Cd) > ethanol extractable form (FE-Cd) > hydrochloric acid extractable form (FHCl-Cd); With increasing Cd treatment concentration, the mass fraction of FE-Cd and FW-Cd (active Cd) in roots of 'D83A/R527' gradually decreased (24.75%–18.34%), while that in 'Fuyou 838' gradually increased (27.18%–28.68%); The mass fraction of FHAc-Cd and FHCl-Cd (inactive Cd) in shoots (32.41%–38.98%) gradually increased and was higher than that in 'Fuyou 838' (28.44%–31.22%); The mass fraction of FNaCl-Cd in roots and shoots of 'D83A/R527'(32.71%–51.17%) was higher than that of 'Fuyou 838' (32.14%–47.63%). In summary, the Cd accumulation in rice seedlings of 'D83A/R527'was low; Compared with 'Fuyou 838', 'D83A/R527'rice seedlings had higher mass fractions of cell walls in roots and shoots, lower mass

fraction of 'active' Cd, and higher 'inactive' Cd, indicating that 'D83A/R527' rice has a stronger Cd sequestration capacity.

Full Text

Preamble

Chinese Journal of Eco-Agriculture, Jun. 2017, 25(6): 903-910
ChinaXiv Cooperative Journal
DOI: 10.13930/j.cnki.cjea.161041

Histocyte Distribution and Cadmium Forms in Different Rice Cultivar Seedlings with Exogenous Cadmium Supply

FU Shuolan, WANG Changquan**, LI Bing, XU Qiang, ZHANG Jingsheng, LI Meng, TANG Jie, HE Yuting, SHEN Jie, ZENG Jiexi, YAN Xun
(College of Resources, Sichuan Agricultural University, Chengdu 611130, China)

Abstract

A hydroponic experiment was conducted to study cadmium (Cd) accumulation characteristics in different rice genotypes—low Cd accumulation ('D83A/R527') and high Cd accumulation ('Fuyou838'). The subcellular distribution and chemical forms of Cd in the root and shoot of different rice genotypes were investigated after exposure to Cd concentrations of 5 mol·L ^{1}, 10 mol·L ^{1} and 25 mol·L ^{1}. The aim of the study was to explore subcellular distribution and chemical forms change of Cd in rice to provide reference for research on Cd absorption mechanisms of rice. The results showed that: (1) the concentrations of Cd in roots and shoots and the transfer rate in 'D83A/R527' were significantly lower than those in 'Fuyou838' ($P < 0.05$). (2) The contents of Cd in subcellular fractions of two rice cultivars were in the orders of soluble fraction (F3) > cell wall (F1) > organelle (F2) in roots, but cell wall (F1) > soluble fraction (F3) > organelle (F2) in shoots. The proportions of Cd in cell walls (36.76%-51.75%) in both roots and shoots of 'D83A/R527' were higher than those in 'Fuyou838' (31.29%-49.07%). (3) The order of contents of Cd chemical forms revealed by different processing methods was sodium chloride extraction state (FNaCl-Cd) > acetic acid extractable state (FHAc-Cd) > deionized water extraction (FW-Cd) > ethanol extracted state (FE-Cd) > HCl extractable state (FHCl-Cd) in both cultivars. When Cd concentration increased, the proportions of FE-Cd and FW-Cd (active forms) from root of 'D83A/R527' declined gradually (24.75%-18.34%), but increased gradually in 'Fuyou838' (27.18%-28.68%). The percentages of FHAC-Cd and FHCl-Cd (inertia forms) in shoot of 'D83A/R527' (32.41%-38.98%) were higher than those in 'Fuyou838' (28.44%-31.22%). The proportions of FNaCl-Cd in both root and shoot of 'D83A/R527' (32.71%-51.17%) were higher than those in 'Fuyou838' (32.14%-47.63%). In short, Cd accumulation in 'D83A/R527' was lower than that in 'Fuyou838' . Then cell wall mass fractions of both root and shoot systems were higher in 'D83A/R527'

than that in 'Fuyou838' . Also there was lower accumulation of active forms of Cd and higher accumulation of inertia forms of Cd in 'D83A/R527' than that in 'Fuyou838' . Therefore, rice cultivar 'D83A/R527' had a better Cd fixation capacity than 'Fuyou838' .

Keywords: Exogenous cadmium; Rice; Subcellular distribution; Chemical form; Active form of cadmium; Inertia form of cadmium

Introduction

Rice (*Oryza sativa*) is a major food crop for humans [1], and ensuring rice safety is an important issue for current food security in China [2]. Cadmium (Cd) is one of the "five toxic elements" with strong mobility, easily contaminating the food chain through plant enrichment [3]. Rice has a strong ability to accumulate Cd, which can easily lead to excessive Cd levels in rice grains and harm human health [4]. In recent years, Cd-contaminated farmland in China has reached 1.3×10 hm^2, with annual Cd-contaminated agricultural products exceeding 1.5×10 t, affecting 25 regions in 11 provinces [5]. Large-scale farmland Cd contamination has seriously threatened China' s food security. Therefore, reducing Cd absorption and accumulation in rice is of great practical significance for safeguarding national food security and improving people' s quality of life [6].

Cd accumulation in rice is influenced by multiple physiological processes, including root activation and absorption, vacuolar compartmentalization and retention, xylem loading and transport, distribution between stems and leaves, and further migration to grains via phloem [7]. Studies have shown that macromolecular substances such as cellulose, hemicellulose, and pectin on plant cell walls can bind heavy metals and immobilize them [8]. When the heavy metal binding sites on plant cell walls become saturated, heavy metals in the protoplast are transported to vacuoles, where they combine with numerous organic ligands to form stable chelates that are stored, reducing the concentration of free heavy metal ions in the cytoplasm [9]. The vacuolar compartmentalization process is closely related to Cd chemical forms [10], and different chemical forms of Cd exhibit significant differences in mobility and activity [11-12]. Howden et al. [13] found that thiol-bound Cd is closely associated with vacuolar compartmentalization. Currently, many studies have investigated Cd subcellular distribution and chemical forms, but few have comparatively examined the interactive effects between these two aspects. Therefore, this study used the high-Cd accumulation rice cultivar 'Fuyou 838'and low-Cd accumulation rice cultivar 'D83A/R527'previously screened by our research group, with three different Cd concentrations (5 $mol \cdot L^{1}$, 10 $mol \cdot L^{1}$, and 25 $mol \cdot L^{1}$) to investigate changes in Cd subcellular distribution and chemical forms and their interrelationships, providing a scientific basis for exploring the physiological mechanisms of Cd absorption and accumulation in rice.

Materials and Methods

1.1 Plant Materials

The experiment was conducted from May to July 2015 in a greenhouse at Sichuan Agricultural University. The test materials consisted of two conventional rice cultivars selected by our research group for their significant differences in grain Cd concentration: 'Fuyou 838' (high Cd accumulation cultivar) and 'D83A/R527' (low Cd accumulation cultivar), provided by the Rice Research Institute of Sichuan Agricultural University.

1.2 Experimental Design and Treatments

Seeds of the two rice cultivars were selected, sterilized with 30% H O , washed, and then germinated. After 10 days, uniformly growing seedlings were transplanted into black plastic buckets with a volume of 15 L. Each bucket was divided into four compartments by wooden boards, with a plastic bag placed in each compartment. A bottomless plastic cup (approximately 6 cm in diameter and 5 cm in height) wrapped with nylon mesh at the bottom was fixed in an appropriate position for rice cultivation, with five uniformly growing seedlings per cup. Rice plants were cultured in complete nutrient solution prepared according to the formula recommended by the International Rice Research Institute (IRRI). The initial pH of the nutrient solution was 5.5–6.0. Seedlings were first cultured in 1/2-strength nutrient solution for 7 days, followed by full-strength nutrient solution and different Cd concentration treatments. Three Cd treatment levels were established: 5 mol · L 1, 10 mol · L 1, and 25 mol · L 1, with three replicates per treatment. Cadmium was added to the nutrient solution in the form of CdCl · 2.5H O. The culture solution was replaced every 4 days, and pH was adjusted daily to 5.5–6.0 using 0.1 mol · L 1 NaOH or 0.1 mol · L 1 HCl. Plants were harvested after 20 days of culture. Rice roots were first rinsed with tap water, then washed several times with deionized water, soaked in 20 mmol · L 1 Na -EDTA for 15 min to remove surface-adsorbed divalent Cd ions, and finally blotted dry with gauze. Roots and leaves were separated and stored at -20 °C for later use.

1.3 Determination of Subcellular Cd Content

The determination of subcellular Cd content was performed according to reference [14] with modifications: Approximately 1.0 g of fresh root or leaf samples were weighed, and 10 mL of subcellular extraction buffer (Tris-HCl buffer containing 0.154 g · L 1 DTT and 0.25 mol · L 1 sucrose, pH 7.4, sample-to-solution ratio 1:10) was added. The samples were rapidly ground into homogenate in an ice bath and filtered through a funnel with nylon cloth into a centrifuge tube. The residue on the nylon cloth (cell wall fraction, F1) was transferred to a triangular flask. The filtrate was placed in a centrifuge tube and centrifuged at 15,000 r · min 1 for 40 min. The supernatant and precipitate were separately transferred to triangular flasks, representing the soluble fraction (F3) and the

organelle fraction (F2), respectively. Finally, 10 mL of mixed acid (nitric acid and perchloric acid, 4:1) was added to the flasks containing F2 and F3, and 20 mL to the flask containing F1. After complete digestion and clarification, the solutions were diluted to volume with distilled water, and Cd content was determined using a MKii M6 graphite furnace atomic absorption spectrometer (Thermo Elemental, USA).

1.4 Analysis of Cd Chemical Forms

The determination of chemical forms was performed according to the method of Wu et al. [14] with modifications. Accurately weighed 0.4 g of dried root or shoot samples were placed in 100 mL plastic centrifuge tubes, and extraction reagent was added at a sample-to-reagent ratio of 1:100. After soaking at 25 °C for 22 h, the samples were shaken in a constant temperature shaker at 25 °C for 1 h, then centrifuged at 5,000 $r \cdot min^{-1}$ for 10 min. The supernatant was decanted, 10 mL of extraction reagent was added again, shaken for 1 h, and centrifuged at 5,000 $r \cdot min^{-1}$ for 10 min. The two supernatants were combined in a 150 mL triangular flask, evaporated to near dryness on an electric furnace, and 10 mL of mixed nitric acid and perchloric acid (4:1) was added. After complete digestion and clarification, the solution was diluted to volume with 10% nitric acid, and Cd content was determined using a MKii M6 graphite furnace atomic absorption spectrometer (Thermo Elemental, USA).

The following five extraction reagents were used for sequential extraction: (a) 80% ethanol to extract inorganic salts (mainly nitrates and chlorides) and amino acid salts, representing ethanol-extractable Cd (FE-Cd); (b) deionized water to extract water-soluble organic acid salts and primary phosphates of heavy metals, representing water-soluble Cd (FW-Cd); (c) 1 $mol \cdot L^{-1}$ NaCl to extract pectates and heavy metals bound to proteins or in adsorbed forms, representing NaCl-extractable Cd (FNaCl-Cd); (d) 2% acetic acid to extract water-insoluble heavy metal phosphates, including secondary phosphates and orthophosphates, representing insoluble phosphate-bound Cd (FHAC-Cd); (e) 0.6 $mol \cdot L^{-1}$ HCl to extract oxalates, representing oxalate-bound Cd (FHCl-Cd).

1.5 Data Analysis

Transfer coefficient = Cd content in shoots / Cd content in roots (1)

All data were compiled and statistically analyzed using Microsoft Excel 2010. Significant differences among treatments were analyzed using SPSS 19.0.

Results

2.1 Cd Content and Transfer Coefficients in Different Rice Seedlings

[Figure 1: see original paper] shows that the Cd contents in both roots and shoots of 'D83A/R527' were significantly lower than those of 'Fuyou 838' ($P<0.05$). Under each Cd concentration treatment, the shoot Cd content of

'Fuyou 838' was 1.42, 1.93, and 1.79 times that of 'D83A/R527', respectively, while the root Cd content was 1.32, 1.60, and 1.49 times that of 'D83A/R527', respectively. The differences in Cd absorption and accumulation characteristics between the two rice cultivars were more pronounced in the shoots.

As shown in [Figure 2: see original paper], the transfer coefficients of both rice cultivars ranged from 0.30 to 0.52. Under different Cd concentration treatments, the transfer coefficient of 'D83A/R527' was significantly lower than that of 'Fuyou 838' ($P<0.05$), indicating that 'D83A/R527' had a weaker ability to transport Cd and could reduce Cd transport from roots to shoots. At the 25 mol · L ^{1} treatment, both cultivars showed the lowest transfer coefficients, suggesting that roots had stronger Cd immobilization capacity under high Cd concentration treatment.

2.2 Subcellular Distribution of Cd in Rice Seedlings Under Different Cd Concentrations

As shown in , under different Cd concentration stresses, the subcellular distribution of Cd in roots of both rice cultivars followed the order: soluble fraction (F3) > cell wall (F1) > organelle fraction (F2). In the comparison between the two cultivars, 'Fuyou 838' showed the highest Cd content in F3. At 5 mol · L ^{1} Cd treatment, the F3 Cd content in 'D83A/R527' was significantly higher than the F1 Cd content in 'Fuyou 838', while the opposite was observed at 10 mol · L ^{1} and 25 mol · L ^{1} Cd concentrations. Under different Cd concentrations, the mass fractions of Cd in F1 and F3 of 'D83A/R527' roots were higher than those of 'Fuyou 838' ([Figure 3: see original paper]), while the organelle Cd mass fraction was lower than that of 'Fuyou 838', indicating that Cd had less impact on the vital activities of root cells in 'D83A/R527'. With increasing Cd treatment concentration, the cell wall Cd mass fraction in 'Fuyou 838' roots gradually decreased (32.55%–31.29%), while that in 'D83A/R527' gradually increased (36.76%–38.55%), indicating that 'D83A/R527' roots had a stronger ability to immobilize Cd in cell walls than 'Fuyou 838'.

The subcellular distribution of Cd in rice seedling shoots differed substantially from that in roots. At 5 mol · L ^{1} and 10 mol · L ^{1} Cd concentrations, 'Fuyou 838' showed the highest Cd content in F1, followed by F3; at 25 mol · L ^{1} Cd treatment, 'Fuyou 838' had the highest Cd content in F3, followed by F1. Under different Cd concentrations, 'D83A/R527' showed the Cd content order of F1 > F3 > F2. With increasing Cd treatment concentration, the increase in subcellular Cd content in both roots and shoots of 'D83A/R527' was smaller than that of 'Fuyou 838', demonstrating low Cd accumulation characteristics.

Under all Cd concentration treatments, both rice cultivars showed the highest cell wall Cd mass fraction in shoots, and the cell wall mass fraction of 'D83A/R527' (51.75%–47.52%) was higher than that of 'Fuyou 838' (49.07%–37.05%), indicating that 'D83A/R527' could accumulate most of the Cd in cell walls.

2.3 Chemical Form Distribution of Cd in Rice Seedlings Under Different Cd Concentrations

Under different Cd concentration stresses, the distribution of different Cd chemical forms in rice seedlings is shown in . With increasing Cd concentration, the contents of all Cd chemical forms increased, with all chemical forms being higher in 'Fuyou 838' than in 'D83A/R527' . Under the same Cd concentration and cultivar, the contents of different Cd chemical forms in roots differed significantly, following the order: NaCl-extractable Cd (FNaCl-Cd) > acetic acid-extractable Cd (FHAc-Cd) > deionized water-extractable Cd (FW-Cd) > ethanol-extractable Cd (FE-Cd) > HCl-extractable Cd (FHCl-Cd).

The Cd chemical forms in shoots differed slightly from those in roots. At 5 mol · L ^{1} Cd concentration, FE-Cd and FW-Cd in 'Fuyou 838' showed no significant difference, with FNaCl-Cd being the highest, followed by FHAc-Cd, and FHCl-Cd being the lowest. At 25 mol · L ^{1} Cd concentration, FNaCl-Cd and FHAc-Cd were the highest, with significant differences among other chemical forms, following the order: FW-Cd > FE-Cd > FHCl-Cd. Overall, under different Cd concentration treatments, Cd in both roots and shoots of the two rice cultivars was dominated by FNaCl-Cd, FHAc-Cd, and FW-Cd. The average contents of these three forms accounted for 47.73%, 23.35%, and 15.74% in roots, and 34.63%, 29.49%, and 18.83% in shoots, respectively. In contrast, FE-Cd and FHCl-Cd contents were relatively low, with average values of 9.37% and 3.80% in roots, and 14.16% and 2.89% in shoots, respectively, across different concentrations and cultivars.

As shown in [Figure 4: see original paper], with increasing Cd concentration, the distribution proportions of FE-Cd and FW-Cd in 'D83A/R527' roots showed an overall decreasing trend (24.75%–18.34%), while those in 'Fuyou 838' showed an increasing trend (27.18%–28.68%). At 5 mol · L ^{1} and 10 mol · L ^{1} Cd concentrations, the total distribution proportions of FHAc-Cd and FHCl-Cd in 'D83A/R527' (24.08% and 26.10%) were lower than those in 'Fuyou 838' (25.19% and 27.19%). At 25 mol · L ^{1} Cd treatment, the sum of distribution proportions of FHAc-Cd and FHCl-Cd in 'D83A/R527' (32.47%) was higher than that in 'Fuyou 838' (27.89%), indicating that with increasing Cd concentration, the proportion of immobilized Cd in 'D83A/R527' roots gradually increased relative to 'Fuyou 838' .

Under different concentration treatments, the total distribution proportions of FHAc-Cd and FHCl-Cd in 'D83A/R527' shoots gradually increased (32.41%–38.98%) and were consistently higher than those in 'Fuyou 838' (28.44%–31.22%). The total distribution proportions of FW-Cd and FE-Cd (30.29%–28.31%) were lower than those in 'Fuyou 838' (37.01%–36.02%), indicating that 'D83A/R527' had a stronger ability to transform Cd into less mobile forms in shoots than 'Fuyou 838' , thereby reducing interference of Cd with physiological and biochemical processes in rice seedlings. In both rice cultivars, FNaCl-Cd accounted for the highest proportion, which decreased with increasing concentration, sug-

gesting that FNaCl-Cd may play an important role in Cd accumulation and detoxification in rice under different Cd treatments, and its distribution in roots affects Cd accumulation in rice seedlings.

Discussion

3.1 Differences in Cd Absorption and Transport Among Different Rice Cultivars

Cd has extremely high biological toxicity and greatly affects crop physiological performance, yield, and quality [15]. This study demonstrated that the low-Cd accumulation cultivar 'D83A/R527' had significantly lower Cd content than the high-Cd accumulation cultivar 'Fuyou 838', indicating less Cd toxicity. The transfer coefficient reflects the ability of plants to transport heavy metals from roots to shoots; a larger transfer coefficient indicates stronger heavy metal transport capacity [16-17]. The results of this study showed that 'D83A/R527' had a significantly lower ability to transport Cd from underground parts to shoots than 'Fuyou 838', indicating stronger Cd retention capacity in roots and reduced risk of Cd entering the food chain through edible parts. This may be due to differences in chemical forms and subcellular distribution among different rice genotypes, leading to variations in Cd absorption and transport capabilities.

3.2 Subcellular Distribution of Cd in Different Rice Seedlings

Roots are the first barrier for plants to resist heavy metal toxicity [18], and the cell wall, as the first protective membrane of protoplasts [19], is the primary barrier protecting protoplasts from heavy metal toxicity [20]. After Cd is absorbed by plant roots, positively charged Cd ions are adsorbed and immobilized by negatively charged Cd-affine substances in the cell wall [10], which can effectively reduce free Cd ions in cells [21]. In this study, the proportion of cell walls in 'D83A/R527' roots was significantly higher than that in 'Fuyou 838', indicating that the cell walls of 'D83A/R527' roots had stronger Cd immobilization capacity. This is an important subcellular mechanism in roots that leads to differences in shoot Cd accumulation among different rice genotypes. Similar results have been reported in watercress (*Nasturtium officinale* L. R. Br.) [22] and pakchoi (*Brassica chinensis* L.) [19]. Cell wall immobilization is the primary defense mechanism in response to increasing Cd stress. Zhang et al. [23] reported that under different treatment conditions, the percentage of cell wall components showed a pattern of high Cd concentration > low Cd concentration. Studies have shown that after plants absorb Cd, most of it is stored in the soluble fraction of roots, followed by the cell wall. For example, the soluble fraction Cd content in wheat roots accounted for 58.5%-63.4% [24], and in pepper (*Capsicum annuum* L.) roots, it accounted for 77%-87% [25]. This experiment yielded similar results, with the highest Cd content in the soluble fraction of roots (46.12%-52.13%), followed by the cell wall (31.29%-38.55%). The percentage of Cd in the soluble fraction of 'D83A/R527' roots was lower than that of

'Fuyou 838' , which explains why the transfer coefficient of 'D83A/R527' was lower than that of 'Fuyou 838', similar to the report by Yu et al. [26] that low-Cd genotypes had lower transfer coefficients than high-Cd genotypes. Due to the limited number of functional polysaccharides (such as cellulose, hemicellulose, and pectin) in cell walls, their Cd immobilization capacity is limited [27]. When Cd stress concentration increases, the Cd immobilization capacity of cell walls becomes insufficient to prevent Cd from entering the protoplast [22], and most Cd in the protoplast is subsequently transferred to vacuoles. Sylwia et al. [28] reported that Cd in vacuoles accounted for 92% of Cd in the protoplast of wild tobacco (*Nicotiana tabacum* L.). This indicates that cell walls and vacuoles are the main sites of Cd accumulation in plants. The results of this experiment showed that most Cd was bound to cell walls and the soluble fraction, consistent with previous results. With increasing Cd stress concentration, the total Cd content in all subcellular fractions also increased. The increase in all subcellular fractions of 'Fuyou 838' at the three concentrations was mostly higher than that of 'D83A/R527' , exhibiting high Cd accumulation characteristics.

3.3 Chemical Form Distribution of Cd in Different Rice Seedlings

The activity, toxicity, and mobility of heavy metals in plants are related to their chemical forms in plant tissues [15]. Different chemical extractants extract different chemical forms of Cd. Inorganic Cd (FE-Cd, extracted with 80% ethanol) and water-soluble Cd (FW-Cd, extracted with deionized water) have higher mobility and greater toxicity to plant cells, and are commonly referred to as "active forms." Insoluble phosphate-bound Cd (FHAC-Cd, extracted with 2% acetic acid) and oxalate-bound Cd (FHCl-Cd, extracted with 0.6 mol · L ^{1} HCl) are less mobile, relatively less active, and less toxic, and are referred to as "inert forms" [15,21]. The results of this experiment showed that under high Cd concentration, the content and percentage of "active form" Cd in 'D83A/R527' roots were lower than those in 'Fuyou 838' , while the percentage of "inert form" Cd was higher than that in 'Fuyou 838' , indicating that Cd absorbed and accumulated in 'D83A/R527' roots was relatively less mobile than that in 'Fuyou 838' . In both rice cultivars, the total distribution proportions of acetic acid-extractable and HCl-extractable forms increased with increasing Cd stress, suggesting that these two chemical forms are associated with Cd detoxification. "Active form" Cd is more easily transported from roots to shoots than other forms, which may explain why the transfer coefficient of 'Fuyou 838' was higher than that of 'D83A/R527' . Similar results have been reported in pakchoi [21].

Cd binding to pectin/protein (i.e., pectin/protein-bound form, also called NaCl-extractable form, extracted with 1 mol · L ^{1} NaCl solution) may be an important process for low Cd accumulation in Cd-tolerant plants [21]. Wu and Clemens et al. reported [14,29] that phytochelatins (PCs), a type of glutathione, are involved in Cd absorption, transport, and detoxification in plants. Similar to the results of Qiu et al. [21], this experiment showed that among all Cd chemical forms, the percentage of NaCl-extractable Cd was the highest, indicating its involvement

in Cd absorption, transport, and detoxification in rice seedlings.

Previous studies have reported that there is an inherent relationship between subcellular distribution of Cd and its chemical forms in plants. Jiang et al. [30] reported that insoluble phosphate-bound Cd is located in cell walls, while pectin/protein-bound Cd is mainly located in vacuoles [21]. The results of this experiment are consistent with this conclusion. Both rice cultivars showed the highest percentage of Cd in the soluble fraction and NaCl-extractable Cd in roots, followed by cell walls and insoluble phosphate-bound Cd. Similar results have been reported in watercress [22]. PCs play a key role in plant Cd tolerance [29]. After Cd enters the protoplast, low-molecular-weight PC-Cd complexes can enter the vacuole, where they combine with S^2 to form high-molecular-weight PC-Cd chelates, ultimately leading to intracellular compartmentalization of Cd. Therefore, the percentage of pectin/protein-bound Cd is higher in low-Cd accumulation cultivars than in high-Cd accumulation cultivars [21]. This may be related to the low mobility of Cd in low-Cd accumulation cultivars.

Compared with 'Fuyou 838', 'D83A/R527' rice had lower Cd accumulation and lower Cd transfer characteristics. Subcellular analysis showed that all subcellular Cd contents in 'D83A/R527' were lower than those in 'Fuyou 838', with higher cell wall mass fraction in roots, lower soluble fraction and organelle mass fractions than 'Fuyou 838', and lower soluble fraction Cd mass fraction in shoots than 'Fuyou 838', indicating that 'D83A/R527' roots had stronger Cd retention capacity and lower Cd translocation to aboveground parts. Chemical form results showed that the mass fraction of "inert form" Cd in 'D83A/R527' seedlings was higher than that in 'Fuyou 838', while the mass fraction of "active form" Cd was lower than that in 'Fuyou 838', indicating that Cd in 'D83A/R527' was less mobile than in 'Fuyou 838'.

NaCl-extractable form (pectin/protein-bound form) accounted for the highest mass fraction in both rice cultivars and is an important Cd detoxification form in rice. 'D83A/R527' rice had low Cd accumulation and strong Cd retention capacity in root cell walls. Its root "active form" Cd mass fraction was lower than that of 'Fuyou 838', and its shoot "active form" Cd mass fraction was lower than that of 'Fuyou 838' with a higher distribution proportion of "inert form" Cd, thus 'D83A/R527' rice had stronger Cd immobilization capacity than 'Fuyou 838'.

References

[1] Sha Z M, Yuan J, Zhao Z, et al. Ionome of rice seed response to rice cultivation patterns[J]. Chinese Journal of Eco-Agriculture, 2016, 24(5): 600–607
[2] Li H Y, Tang S, Wang Y Q, et al. Effects of selenium on cadmium content and subcell distribution in rice[J]. Ecology and Environmental Sciences, 2016, 25(2): 320–326
[3] Wu C B, Wang L, Guo J C, et al. Distribution and chemical forms of Cd in

Paspalum vaginatum SW.[J]. Environmental Chemistry, 2016, 35(2): 330-336
[4] Yin J, Zhao Y L, Xu Y, et al. Effects of zinc supply on absorption and translocation of cadmium in rice seedlings[J]. Journal of Agro-Environment Science, 2016, 35(5): 834-841
[5] Xu L J, Zhang M L, Yang H. Research progress of bioremediation technology of cadmium polluted soil[J]. Journal of Nanjing Normal University: Natural Science Edition, 2011, 34(1): 102-106
[6] Li B, Wang C Q, Li Z, et al. Absorption of Cd by hybrid rice under the Cd stress and its dynamic change[J]. Ecology and Environmental Sciences, 2014, 23(2): 312-316
[7] Li P, Ge Y, Wu L H, et al. Uptake and translocation of cadmium and its physiological effects in two rice cultivars differed in grain cadmium concentration[J]. Chinese Journal of Rice Science, 2011, 25(3): 291-296
[8] Francine M K, Louise W A, Pythagore F S, et al. Antioxidant properties of cell wall polysaccharides of *Stevia rebaudiana* leaves[J]. Journal of Coastal Life Medicine, 2014, 2(12): 918-923
[9] Brunetti P, Zanella L, De Paolis A, et al. Cadmium-inducible expression of the ABC-type transporter AtABCC3 increases phytochelatin-mediated cadmium tolerance in *Arabidopsis*[J]. Journal of Experimental Botany, 2015, 66(13): 3815-3829
[10] Huang B F, Xin J L. Mechanisms of heavy metal accumulation in plants: A review[J]. Acta Prataculturae Sinica, 2013, 22(1): 300-307
[11] Devriese M, Tsakaloudi V, Garbayo L, et al. Effect of heavy metals on nitrate assimilation in the eukaryotic microalga *Chlamydomonas reinhardtii*[J]. Plant Physiology and Biochemistry, 2001, 39(5): 443-448
[12] Hart J J, Welch R M, Norvell W A, et al. Characterization of cadmium binding, uptake, and translocation in intact seedlings of bread and durum wheat cultivars[J]. Plant Physiology, 1998, 116(4): 1413-1420
[13] Howden R, Goldsbrough P B, Andersen C R, et al. Cadmium-sensitive, cad1 mutants of *Arabidopsis thaliana* are phytochelatin deficient[J]. Plant Physiology, 1995, 107(4): 1059-1066
[14] Wu F B, Dong J, Qian Q Q, et al. Subcellular distribution and chemical form of Cd and Cd-Zn interaction in different barley genotypes[J]. Chemosphere, 2005, 60(10): 1437-1446
[15] Bai X, Chen Y H, Geng K, et al. Accumulation, subcellular distribution and chemical forms of cadmium in *Viola tricolor* L.[J]. Acta Scientiae Circumstantiae, 2014, 34(6): 1600-1605
[16] Li Y, Zhang S R, Zhang S Q, et al. Cadmium tolerance and accumulation characteristics of *Crassocephalum crepidioides*[J]. Journal of Agro-Environment Science, 2012, 31(7): 1296-1302
[17] Lu Z Y, Liu Z Q, Song Z G, et al. Subcellular distribution and chemical forms of Cd and the synthesis of Phytochelatins (PCs) in different barley genotypes[J]. Journal of Agro-Environment Science, 2013, 32(11): 2125-2131
[18] Qu R H, Zhang X, Li H L, et al. Effects of zinc level on low dose cadmium transport in rice plant[J]. Chinese Journal of Eco-Agriculture, 2016, 24(4): 517-523

[19] Xue M, Zhou Y H, Yang Z Y, et al. Comparisons in subcellular and biochemical behaviors of cadmium between low-Cd and high-Cd accumulation cultivars of pakchoi (*Brassica chinensis* L.)[J]. Frontiers of Environmental Science & Engineering, 2014, 8(2): 226–238
[20] Fan J L, Wei X Z, Wan L C, et al. Disarrangement of actin filaments and Ca^{2} gradient by CdCl alters cell wall construction in *Arabidopsis thaliana* root hairs by inhibiting vesicular trafficking[J]. Journal of Plant Physiology, 2011, 168(11): 1157–1167
[21] Qiu Q, Wang Y T, Yang Z Y, et al. Effects of phosphorus supplied in soil on subcellular distribution and chemical forms of cadmium in two Chinese flowering cabbage (*Brassica parachinensis* L.) cultivars differing in cadmium accumulation[J]. Food and Chemical Toxicology, 2011, 49(9): 2402–2406
[22] Wang J B, Su L Y, Yang J Z, et al. Comparisons of cadmium subcellular distribution and chemical forms between low-Cd and high-Cd accumulation genotypes of watercress (*Nasturtium officinale* L. R. Br.)[J]. Plant and Soil, 2015, 396(1/2): 345–354
[23] Zhang W, Lin K F, Zhou J. Effects of selenium foliar spray on subcellular distribution and chemical forms of cadmium in rice seedlings in different sulfur concentrations[J]. Journal of Agro-Environment Science, 2014, 33(5): 844–852
[24] Wan M, Zhou W, Lin B. Subcelluar and molecular distribution of cadmium in two wheat genotypes differing in shoot/root Cd partitioning[J]. Scientia Agricultura Sinica, 2003, 36(6): 671–675
[25] Xin J L, Huang B F. Subcellular distribution and chemical forms of cadmium in two hot pepper cultivars differing in cadmium accumulation[J]. Journal of Agricultural and Food Chemistry, 2014, 62(2): 508–515
[26] Yu H, Xiang Z X, Zhu Y, et al. Subcellular and molecular distribution of cadmium in two rice genotypes with different levels of cadmium accumulation[J]. Journal of Plant Nutrition, 2012, 35(1): 71–84
[27] Fu X P, Dou C M, Chen Y X, et al. Subcellular distribution and chemical forms of cadmium in *Phytolacca americana* L.[J]. Journal of Hazardous Materials, 2011, 186(1): 103–107
[28] Sylwia W, Anna R, Ewa B, et al. The role of subcellular distribution of cadmium and phytochelatins in the generation of distinct phenotypes of AtPCS1- and CePCS3-expressing tobacco[J]. Journal of Plant Physiology, 2010, 167(12): 970–979
[29] Clemens S. Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants[J]. Biochimie, 2006, 88(11): 1707–1719
[30] Jiang H M, Yang J C, Zhang J F. Effects of external phosphorus on the cell ultrastructure and the chlorophyll content of maize under cadmium and zinc stress[J]. Environmental Pollution, 2007, 147(3): 750–756

Note: Figure translations are in progress. See original paper for figures.