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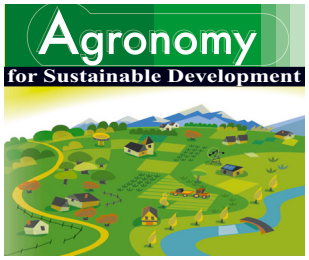

Research article

Cadmium fate and tolerance in rice cultivars

Jie ZHANG[1,2], Wanchun SUN[1], Zhaojun LI[1], Yongchao LIANG[1,3]*, Alin SONG[2]

[1] Ministry of Agriculture Key Laboratory of Crop Nutrition and Fertilization, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, P.R. China
[2] College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, P.R. China
[3] Key Laboratory of Oasis Eco-Agriculture, College of Agriculture, Shihezi University, Shihezi, Xinjiang 832003, P.R. China

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Abstract – Cadmium (Cd) is present in all soils, usually as a trace constituent, but it can reach higher levels in agricultural soils. Cd can then be absorbed by plants and become a potential risk to human health. Once taken up by a plant, there are mechanisms for heavy metal detoxification in the plant. Here, a cadmium-tolerant and a cadmium-sensitive rice cultivars were grown hydroponically to investigate the effects of cadmium (Cd) applied at low levels on uptake and transport, subcellular distribution and binding forms of Cd in rice plants. Our results showed that increasing the Cd treatment from 1.0 μM to 5.0 μM Cd increased the shoot Cd content by 55% in the cadmium-tolerant cultivar, and by 108% in the cadmium-sensitive cultivar. For the cadmium-tolerant cultivar, increasing Cd treatment from 1.0 μM to 5.0 μM increased the root Cd content by 116%, whereas for the cadmium-sensitive cultivar, increasing Cd treatment from 1.0 μM to 5.0 μM increased the root Cd content by 80%. Further, the ratio of Cd accumulation in shoots over roots decreased from 0.19 to 0.14 in the cadmium-tolerant cultivar, while it increased from 0.20 to 0.26 in the cadmium-sensitive cultivar, showing that the transportation ability for Cd was different between the two tested rice cultivars. At the higher Cd level of 5.0 μM, most of the Cd in the plants was localized in cell walls and vacuoles in both cultivars, whereas small portions of Cd were distributed in the cytoplasm, suggesting that the important metabolic and physiological processes were not impaired under Cd stress. Furthermore, the major portions of Cd in the cells were combined with organic acids, proteins and polysaccharide, and were consequently detoxified. The difference in the distribution of cadmium in rice plants resulted in the difference in Cd tolerance between the two rice cultivars used. It can be concluded that the retention of Cd in root cell walls, compartmentation of Cd into vacuoles and the suppressed transportation of Cd from roots to shoots are the most important mechanisms involved in the detoxification of Cd in rice plants.

cadmium / rice / subcellular distribution / binding form

1. INTRODUCTION

Cadmium (Cd) is a widespread pollutant of the environment and a potentially toxic heavy metal with no known benefit to plants, animals or humans. In agricultural soils, Cd can reach a higher level due to (1) application of fertilizers, manures and sewage sludge, (2) irrigation with industrial wastewater and (3) atmospheric fallout from industrial and urban activities (Liu et al., 2007; Yang et al., 2006). Contamination by heavy metals such as Cd in agricultural soil has become increasingly serious in some parts of China, such as in Pearl River areas (Yang et al., 2006), and also in some other Asian countries (Herawati et al., 2000; Sharma et al., 2007). Soils are the main source of Cd available to plants, which can become the main source of Cd in plant-derived foods (such as edible leaves, fruits and seeds) in human diets (Liu et al., 2007). Furthermore, rice from uncontaminated soil could also accumulate Cd, depending on both water management and climatic conditions (Cattani et al., 2008). Therefore, Cd, after entering a food chain, becomes a major public concern.

Cadmium is an extremely toxic element for plants, even at a low concentration, and the visible symptoms of Cd toxicity in plants have been described as, e.g., chlorosis, necrosis, wilting, leaf roll and growth reduction. In most cases, inhibition of root elongation is the most sensitive and visible symptom of Cd toxicity (Guo and Marschner, 1995; Prasad, 2004), but there are differential physiological mechanisms of cadmium tolerance among plant species and varieties (Ghani et al., 2008). Once taken up by plants, heavy metals are bound to the cell walls (polysaccharides), and/or complexed by the low-molecular-weight compounds (phytochelatins and organic acids) (Wójcik et al., 2005a; Han et al., 2006). Hence,

* Corresponding author: ycliang@caas.ac.cn

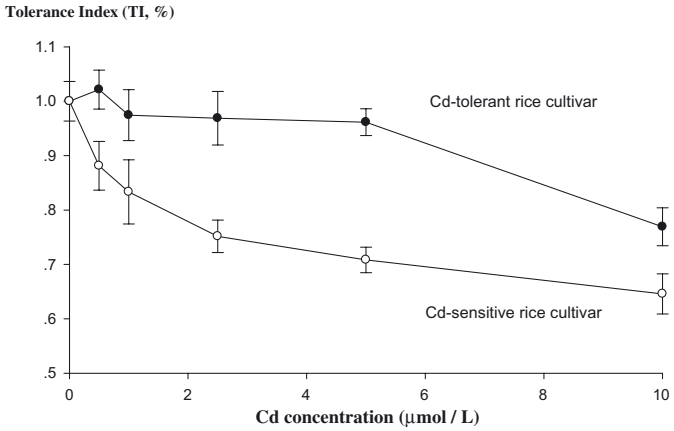

Figure 1. Tolerance index (TI) of roots in two rice cultivars treated with increasing concentrations of Cd. Tolerance index (TI) = Response at elevated test metal concentration / Response at control concentrations (Prasad, 2004). Each value is the mean of six replicates ± S.E.

the distribution of heavy metals in the plant cells is uneven, which has been reported to be a cellular mechanism for heavy metal detoxification in plants (Vögeli-Lange and Wagner, 1990; Brune et al., 1994; Wójcik et al., 2005a, b).

Rice (*Oryza sativa* L.) is one of the most important crops worldwide, especially in Asia, and also one of the crops which accumulates Cd most easily in grains (Wani et al., 2007; Liu et al., 2007). Many studies have been performed on Cd toxicity to rice, but the Cd concentrations used in these studies are much higher than the levels of Cd contamination representing the realistic environmental conditions (Sanità di Toppi and Gabbrielli, 1999; Ramos et al., 2002; Liu et al., 2007). Such studies cannot reflect the real picture and consequence of Cd contamination in agricultural soils. Little information is available in the literature about the effect of its realistic low concentrations on plant growth in the natural environment (Milone, et al., 2003). Moreover, Cd in soil can be taken up and is not generally phytotoxic at plant tissue concentrations that can significantly increase human exposure (Adriano, 1986), which has been associated with various illnesses in humans, including gastroenteritis, renal tubular dysfunction, hypertension, cardiovascular disease, pulmonary emphysema, cancer and osteoporosis (Wagner, 1993).

Based on the current knowledge in the literature (Diao et al., 2005; Wójcik et al., 2005a; Han et al., 2006) and the results of our preliminary studies, it is hypothesized that the difference in both cellular distribution and binding forms of Cd occurs in different cultivars to varying extents, which might play a crucial role in their tolerance and accumulation of Cd. To test this hypothesis, we performed a series of hydroponic experiments with two rice genotypes, cv. WYJ3 and cv. SY63, differing in their tolerance (Fig. 1) and accumulation of Cd, to examine the possible mechanisms for the differences in Cd accumulation and tolerance in rice seedlings subjected to realistic Cd concentrations in natural soil at both tissue and cellular levels.

2. MATERIALS AND METHODS

2.1. Plant material

Based on previous work (Liu et al., 2007; Sun et al., 2008), two rice (*Oryza sativa* L.) cultivars differing in Cd tolerance were used in the present experiment, Wuyujing No. 3 (a cadmium-tolerant cultivar, WYJ3) and Shanyou 63 (a cadmium-sensitive cultivar, SY63). Seeds were surface-sterilized with H_2O_2 (10%) for 30 min, rinsed thoroughly with distilled water and germinated on moist filter paper for 3 days in an incubator at 37 °C. Germinated seeds were sown in plastic containers filled with quartz sand and watered with 1/2-strength Kimura B nutrient solution (Liang et al., 2006). Uniform 10-day-old rice seedlings (with two expanded leaves and one expanding leaf) were transplanted to the hydroponic plastic pots filled with 1000 mL of Kimura B nutrient solution supplied with different Cd concentrations (added as $CdCl_2$). The plants were grown in a chamber with a 25/20 °C day/night regime, a 14h/10h (day/night) photoperiod, light intensity of 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and a relative humidity of 70%. The nutrient solution was renewed every 2 days and the pH of the nutrient solution was adjusted to 5.5 daily (Liang et al., 2006).

As estimated by Wagner (1993), Cd concentrations range from 0.04 to 0.32 μM in non-polluted soil solutions. Thus, two Cd levels, 1.0 and 5.0 μM, respectively, were used in the experiments to study the accumulation of Cd in plant tissues, while the higher Cd concentration (5.0 μM Cd) was further used to study the cellular distribution and binding forms of Cd in rice seedlings. After 10 days of Cd treatments, the plants were harvested and separated into roots and shoots for both assay of Cd and compartmental analyses. Roots were washed with 0.5 mM $CaCl_2$ several times to remove the Cd in the root free space, followed by thoroughly washing with deionized H_2O (Guo et al., 2007). Each experiment was performed in triplicate to ensure the reliability of the data.

2.2. Compartmental analysis of Cd

The subcellular distribution of cadmium in rice plants was analyzed according to the method of Weigel and Jâger (1980), with minor modifications. Weighed shoots and roots were homogenized at 4 °C using a pre-cooled mortar and pestle and extracted with 0.4 M sucrose, 50 mM Tris-HCl (pH 7.5) and 1.0 mM dithioerythritol at a ratio of 1:10 (fresh tissue weight/extraction medium volume). The homogenate was filtered through a nylon cloth (ϕ 120 μm) and the residue was washed twice with the extraction medium. The pooled washes, together with the first filtrate, were centrifuged at 300 *g* for 30 s. The resulting pellet combined with the residue of the nylon-cloth filtration, which mainly contained cell walls and wall debris, was considered as the cell wall fraction (CW). The supernatant of the third centrifugation step was then centrifuged at 20000 *g* for 30 min. The resultant pellet was considered as the cytoplasm fraction (C), while the supernatant containing mainly vacuole solution was referred to as the soluble fraction (S). The soluble fraction was used for Cd analysis

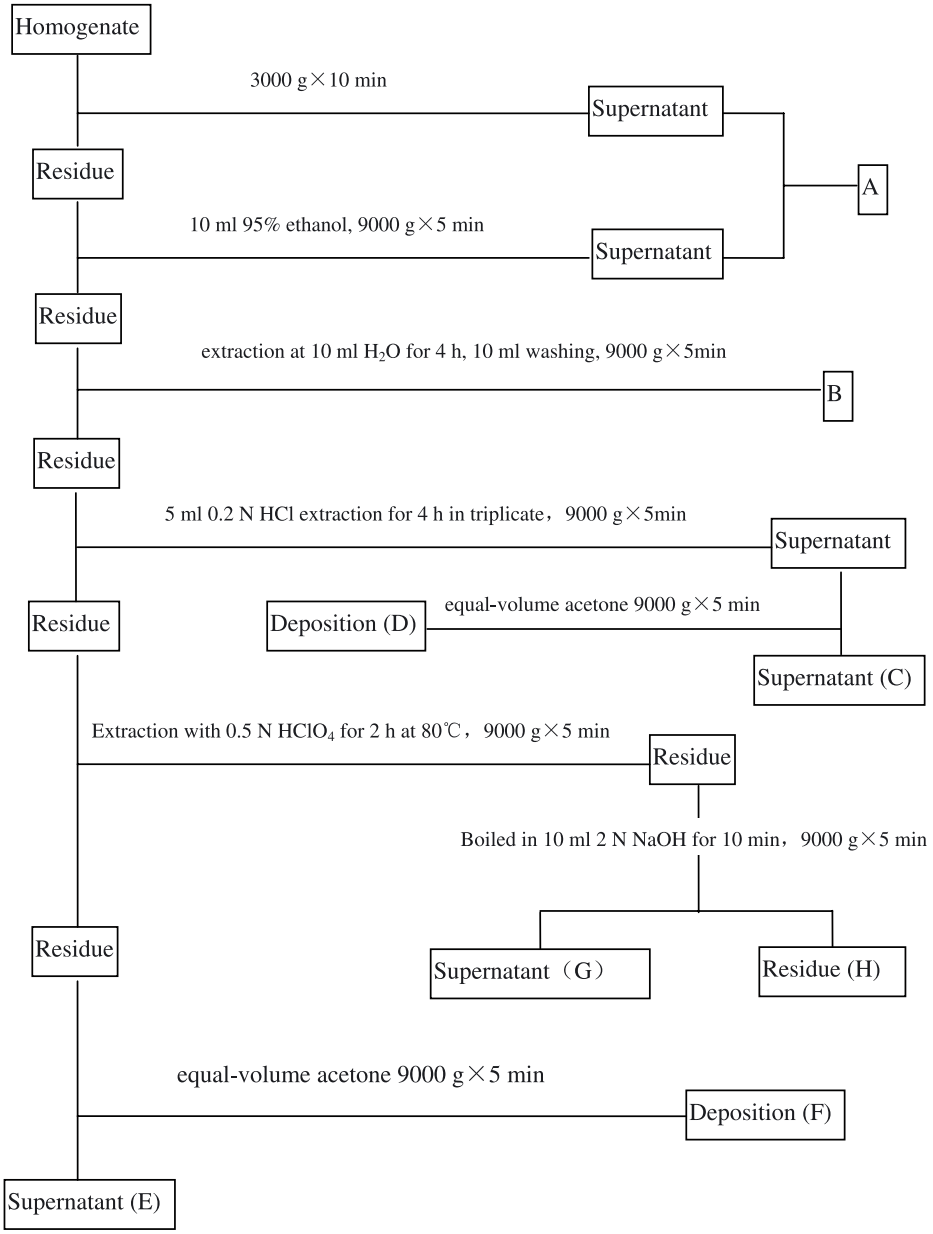

Figure 2. Schematic representation of the protocol for Cd extraction in plant tissues.

directly, while the other two fractions were dried in an oven at 60 °C prior to Cd analysis.

2.3. Binding forms of Cd

The rice tissues were extracted according to the method of Tang (2000), with slight modifications. Weighed shoots and roots were homogenized using a mortar and pestle in 10 mL 95% ethanol at a ratio of 1:5 (w/v). The homogenate was subsequently extracted following the protocol described in Figure 2. In the process, the fractions were expressed as follows: A: small neutral molecules, including amino acids and pigment; B: water-soluble low-molecular-weight polar compounds; C: exchangeable acidic polar compounds and ions in the cell wall; D: protein and pectin; E: polar compounds and structural clusters, such as cellulose and lignin; F: nucleic acid; G: remaining proteins and polysaccharides; H: cellulose, lignin and the non-active portion in the cell wall.

2.4. Determination of Cd

Cadmium concentration was determined by atomic absorption spectroscopy (Varian SpectrAA 220FS, USA) in the samples, which were predigested overnight in 5 mL

Table I. Cd content (μg/g dw), Cd accumulation (μg/Plant) and S/R values in plants of two cultivars of rice treated with cadmium.

	WYJ3		SY63	
	1.0 μM	5.0 μM	1.0 μM	5.0 μM
Shoots				
Cd content	39.13 ± 2.10 c	60.62 ± 2.11 b	34.75 ± 4.26 c	72.12 ± 1.57 a
Cd accumulation	5.39 ± 0.29 c	7.88 ± 0.27 b	5.62 ± 0.39 c	10.81 ± 0.24 a
Roots				
Cd content	310.16 ± 39.43 b	669.99 ± 25.30 a	337.43 ± 20.19 b	606.47 ± 72.12 a
Cd accumulation	27.91 ± 2.05 c	55.60 ± 1.21 a	26.99 ± 1.61 c	41.40 ± 3.68 b
S/R				
Cd content	0.13 ± 0.02 a	0.09 ± 0.01 b	0.10 ± 0.00 b	0.12 ± 0.01 a
Cd accumulation	0.19 ± 0.02 b	0.14 ± 0.01 c	0.20 ± 0.02 b	0.26 ± 0.01 a

Each value is the mean of three replicates ±/S.E. Means in rows followed by different letters are significantly different at $P \leqslant 0.05$. Differences in Cd uptake and translocation were found between WYJ3 and SY63.

concentrated HNO_3, and then digested with added $HClO_4$ at 170 °C for 3 h.

2.5. Statistical Analysis

Data were subjected to analysis of variance using SigmaStat for Windows 2.03 (SPS Inc., Chicago, USA), and the means were compared by LSD test at the 0.05 probability level.

3. RESULTS AND DISCUSSION

3.1. Cd accumulation in rice plants

Cadmium-treated plants of both cultivars, WYJ3 and SY63, demonstrated a marked increase in Cd content and accumulation in shoots and roots as the Cd concentration in the nutrition solution rose. However, the S/R values of Cd content and accumulation differed between the two rice cultivars with the treatments (Tab. I). In the cultivar WYJ3, the Cd contents in shoots and roots were 39.13 and 310.16 μg/g dw in the lower Cd treatment (1.0 μM), respectively, while they were 60.62 and 669.99 μg/g dw in the higher Cd treatment (5.0 μM). Similar results were obtained in the shoots and roots of cultivar SY63, which were 34.75 and 337.43 μg/g dw in the lower Cd treatment, and 72.12 and 606.47 μg/g dw in the higher Cd treatment, respectively. Under similar Cd stress, the Cd contents in plants were different between the two rice cultivars both in shoots and in roots. The Cd content in shoots exposed to 5.0 μM was significantly higher in WYJ3 than in SY63, while no significant differences in Cd contents were found in roots of either genotype at both Cd concentrations. Accumulation of Cd in shoots was 5.39 and 7.88 μg/plant in WYJ3 at the 1.0 and 5.0 μ M Cd levels, respectively, compared with 5.62 and 10.81 μg/plant in SY63. Accumulation of Cd in roots was 27.91 and 55.60 μg/plant in WYJ3 at the 1.0 and 5.0 μM Cd levels, respectively, compared with 26.99 and 41.40 μg/plant in SY63. At 5.0 μM Cd, significant differences were noted between the two cultivars in the amount of Cd accumulation in the same tissues, while the difference was not significant at 1.0 μM Cd.

The effects of Cd on the plant growth depended not only on stress intensity, but also the plant genotype (Milone, et al., 2003). In this study, the rice plants were subjected to low Cd concentration for 10 days. In the plants treated with the same Cd concentration, a higher tolerance index (TI) of roots (evaluated by inhibition of root elongation) was observed in WYJ3 (Fig. 1), showing the higher Cd tolerance in this cultivar (see also Milone, et al., 2003).

Genotypical differences have been characterized in uptake and accumulation of heavy metal in rice (Liu et al., 2007), wheat (Milone, et al., 2003; Page and Feller., 2005), vegetables (Alexander et al., 2006) and other crops (Gárate et al., 1993; Arao et al., 2003). Using lettuce (*Lacuca* spp.), Gárate and his group observed the difference in Cd uptake and accumulation among different cultivars under different Cd levels (Gárate et al., 1993; Ramos et al., 2002). In the present study, similar behaviors were also observed. The majority of Cd absorbed by rice plants was accumulated in the roots of both rice cultivars. Approximately 88% and 90% of total Cd absorbed by plants were located in the roots of WYJ3 and SY63 at 1.0 μM Cd, respectively, compared with 91% and 88% at 5.0 μM Cd. In the plants treated with Cd, more Cd was absorbed, but less Cd was transported into shoots by WYJ3 plants than by SY63, suggesting that the root uptake ability was stronger in cultivar WYJ3 than in cultivar SY63. It was reported that characterization of Cd uptake and accumulation in rice cultivars was related to their root oxidation abilities (Liu et al., 2006), root acidification and root organic acid secretions (Liu et al., 2007). The results in the present study showed that the roots of WYJ3 had a higher tolerance to Cd stress than the roots of SY63 (Fig. 1), which suggested that the roots of cultivar WYJ3 have higher growth activity than the roots of cultivar SY63 when cultivated in nutrient solution containing the same Cd levels.

It has been proven that the roots play an important role in heavy metal tolerance as the first barrier through selective uptake, metal excretion and metal retention (Sanità di Toppi and Gabbrielli, 1999; Ramos et al., 2002). The results in the present study also show that the uneven distribution of Cd among plant organs is an important strategy in response to Cd stress, which improves the tolerance to Cd and, to some

Table II. The subcellular distribution of Cd in roots and shoots of the rice plants treated with 5.0 μM Cd.

Genotypes	Fraction	Shoots		Roots	
		Cd concentration (μg/g dw)	Percentage (%)	Cd concentration (μg/g dw)	Percentage (%)
WYJ3	CW	49.56 ± 3.33 b	39.54	310.42 ± 9.08 a	52.96
	C	15.33 ± 4.33 e	12.23	85.42 ± 1.67 d	14.57
	S	60.44 ± 0.33 b	48.23	190.33 ± 11.17 c	32.47
	Total	125.33		586.17	
SY63	CW	51.16 ± 1.37 bc	36.38	261.38 ± 11.31 b	48.94
	C	24.00 ± 3.05 d	17.07	74.23 ± 5.46 d	13.90
	S	65.47 ± 3.47 a	46.56	198.31 ± 36.46 c	37.13
	Total	140.63		533.92	

Each value is the means of three replicates ±/S.E. Means in the same columns followed by different letters are significantly different at $P \leqslant 0.05$. Compartmentation of Cd in the cell wall (CW) and vacuoles (S) is an essential strategy to prevent injury induced by Cd.

extent, prevents whole plants from Cd toxicity. The Cd distribution and accumulation in plants depend on two major steps: uptake of Cd into root cells and translocation of Cd from root to shoot, and the heavy metal binding capacity of roots can influence the root-to-shoot transportation of Cd in rice plants (Arao et al., 2003). In the present study, the ratios of Cd in shoots to that in roots (S/R), which may represent the root-to-shoot translocation of Cd in plants, were significantly different between the two rice cultivars tested at the higher level of Cd (Tab. I). With 5.0 μM Cd supplied, the S/R values of Cd content on a dry weight basis were 0.09 in cultivar WYJ 3, and 0.12 in cultivar SY63. With increasing external Cd concentration, the S/R values of Cd content and accumulation significantly decreased in cultivar WYJ3, but the opposite was true in cultivar SY63. These results indicate that the root-to-shoot Cd transport ability was lower in cultivar WYJ3 than in cultivar SY63; namely, the binding capacity of roots for Cd was stronger in cultivar WYJ3 than in cultivar SY63.

3.2. Compartmentation of Cd

The subcellular distributions of Cd in the rice plants supplied with 5.0 μM Cd are shown in Table II. In both cultivars, Cd was unevenly distributed among subcellular fractions, with differences between shoots and roots. The major portions of Cd absorbed by the rice plants were localized in the cell walls (CW) and vacuoles (S), and a small portion of Cd was retained in their cell organs in shoots and roots (C). For example, the content of Cd in fraction C of WYJ3 is significantly lower than that of SY63.

Recent studies have demonstrated the presence of a highly selective, high-affinity Cd transporter in roots of the Cd hyperaccumulating ecotype (Zhao et al., 2002), and root-to-shoot translocation associated with phytochelatin (PCs) induction (Larsson et al., 2002). Once Cd in medium is absorbed into a plant, the distribution of Cd occurs not only among tissues, such as roots and shoots, but also among cells and subcellular organelles, which is an essential strategy to prevent injury induced by Cd. The reduction of the cytosolic concentration of free Cd ions in plants is one of the defense strategies against Cd toxicity. In this way, the plant cell avoids Cd accumulation in the cytosol organelle by compartmentalizing Cd into subcellular compartments (Wu et al., 2005).

This distribution pattern helped protect the major metabolic processes from Cd damage in the rice plants, and was a typical feature for both rice cultivars, with an exception that the distribution ratio in cell organs in shoots of cultivar SY63 was higher than in those of cultivar WYJ3 (Tab. II). The Cd contents in fraction C were 15.3 and 24.0 μg/g dw in shoots of WYJ3 and SY63, respectively, which accounted for 12.2% and 17.1% of the total Cd contents. By contrast, Cd contents in fraction C were 85.4 and 74.2 μg/g dw in roots of WYJ3 and SY63, respectively, which represented 14.6% and 13.9% of the total Cd contents. As the major storage sites of Cd, Cd in the cell wall fraction (CW) and soluble fraction (S) accounted for more than 80% of Cd accumulation in both shoots and roots of rice seedlings treated with 5.0 μM Cd. However, there were significant differences in Cd in the CW and S fractions between shoots and roots of the two rice cultivars used, suggesting that the two fractions played different roles in Cd distribution in shoots and roots in the same rice cultivar. The maximum portion of Cd was distributed in the cell wall fraction in roots, while the vacuole was the main storage site of Cd in shoots. Both in shoots and in roots of WYJ3, 39.5% and 53.0% of the total Cd were immobilized by cell walls, respectively, compared with 48.2% and 32.7% of the total Cd compartmented into vacuoles. A similar trend of subcellular Cd distribution was observed in the cultivar SY63, with 36.4% and 48.9% of the total Cd in the cell wall fraction of shoots and roots, compared with 46.6% and 37.1% of the total Cd in the soluble fraction of shoots and roots.

The cell wall of the root is the first barrier against Cd stress, which can restrict Cd uptake by the root cells (Nishizono, 1987; Sanità di Toppi and Gabbrielli, 1999). In this way, the plants can prevent the metal ions from entering the cytoplasm, which is one of the mechanisms of tolerance of rice plants to Cd. In the present study, significant difference was found in the Cd concentration in the cell wall fraction (CW) of roots between WYJ 3 and SY63 (Tab. II), suggesting that higher Cd concentration in the root cell wall may lead to higher tolerance to Cd. It is possible that the cell wall exhibits significant

Table III. Binding forms of Cd in rice plants.

Fraction	Shoots		Roots	
	Cd concentration (μg/g fw)	Percentage (%)	Cd concentration (μg/g fw)	Percentage (%)
WYJ3				
A	0.59 ± 0.12 d	5.52	8.18 ± 1.13 e	11.93
B	2.09 ± 0.49 c	19.55	18.64 ± 0.66 c	27.19
C	4.41 ± 0.39 b	41.25	19.87 ± 2.22 bc	28.93
D	0.23 ± 0.02 d	2.15	1.87 ± 0.35 ghi	2.73
E	0.25 ± 0.04 d	2.34	4.29 ± 1.41 fg	6.26
F	0.03 ± 0.03 d	0.28	0.03 ± 0.00 i	0.04
G	2.84 ± 0.63 c	26.57	4.53 ± 0.74 fg	6.61
H	0.25 ± 0.01 d	2.34	11.15 ± 1.35 d	16.26
Total	10.69		68.56	
SY63				
A	0.39 ± 0.08 d	2.89	8.58 ± 1.04 de	12.42
B	2.88 ± 0.66 c	21.36	24.37 ± 0.36 a	35.28
C	6.19 ± 0.03 a	45.92	22.10 ± 0.19 ab	31.99
D	0.52 ± 0.04 d	3.86	2.40 ± 0.45 fghi	3.47
E	0.58 ± 0.14 d	4.30	3.01 ± 0.22 fgh	4.36
F	0.51 ± 0.46 d	3.78	0.25 ± 0.10 h	0.36
G	2.08 ± 0.29 d	15.43	3.53 ± 0.15 fg	5.11
H	0.33 ± 0.02 d	2.45	4.84 ± 0.10 f	7.01
Total	13.48		69.08	

Each value is the means of three replicates ± S.E. Means in the same columns followed by different letters are significantly different at $P \leqslant 0.05$.A: small neutral molecules, including amino acids and pigment; B: water-soluble low-molecular-weight polar compounds; C: exchangeable acidic polar compounds and ions in the cell wall; D: protein and pectin; E: polar compounds and structural clusters, such as cellulose and lignin; F: nucleic acid; G: remaining proteins and polysaccharides; H: cellulose, lignin and the non-active portion in the cell wall. In rice plants, the concentrations of different Cd forms are different between tissues, and a difference is also found between genotypes.

metal accumulation, but there is a limited capacity for metal ion accumulation. Thus, the higher external Cd concentration can cause damage to the cell wall, which may be a reason why the role of cell walls in Cd detoxification is limited in certain conditions (Nishizono, 1987).

The vacuole is another important storage site of Cd in cells, which plays a main role in the intracellular compartmentalization of Cd ions. After entering into the cytoplasm through the cell walls, various forms of Cd are transported against the concentration gradient across the tonoplast by means of specific carriers (Sanità di Toppi and Gabbrielli, 1999). In this study, the major portions of Cd entering into the cell (Cd in fractions C and V) were compartmentalized in the vacuole: 79.8% and 73.2% in roots and 69% and 72.8% in shoots of WYJ3 and SY63, respectively (Tab. II). These results indicate that the higher accumulation of Cd in roots of WYJ3 depends not only on the stronger binding capacity of the root cell wall for Cd, but also on the higher storage capacity of the vacuole for Cd. It has been reported that Cd stress may be alleviated by sequestration of Cd in the cell wall or the vacuole in Cd-tolerant barley genotypes, especially in a short-term Cd-exposed experiment (Wu et al., 2005).

3.3. Binding forms of Cd

Under the Cd exposure, the Cd concentrations were different in different binding forms both in shoots and in roots in the two cultivars used, but there was some difference in the main binding forms of Cd between shoots and roots (Tab. III). In shoots, higher Cd concentrations were found in fractions B (water-soluble low-molecular-weight polar compounds), C (the exchangeable acidic polar compounds and ions in the cell wall) and G (remaining proteins and polysaccharides). The Cd concentrations in fractions B, C and G were 2.09, 4.41 and 2.84 μg/g fw in WYJ3 and 2.88, 6.19 and 2.08 μg/g fw in SY63, respectively. Significant differences in Cd concentrations in fractions B and G between WYJ3 and SY63 were found. The proportions of the Cd in different binding forms in shoots are also shown in Table III. The main Cd binding form was the Cd in fraction C, which accounted for 41.25% in WYJ3 and 45.92% in SY63, respectively. The fractions B and G were also the major binding forms of Cd absorbed by rice plants, which may be localized in the vacuoles and cell walls. The proportions of Cd in fraction B were similar in the two cultivars used, being 20% and 21% in WYJ3 and SY63, respectively, whereas the Cd in fraction G was significantly higher in WYJ3 (26.57%) than in SY63 (15.43%).

In roots, fractions B and C were still the main forms of Cd absorbed by rice plants, which were 18.64 μg/g fw and 19.87 μg/g fw in WYJ 3 and 24.37 μg/g fw and 22.10 μg/g fw in SY63 (Tab. III). In addition, the Cd concentration in fraction A (small neutral molecules, including amino acids and pigment) was highest among the remaining fractions in both cultivars, which were 8.18 μg/g fw (WYJ3) and 8.58 μg/g fw

(SY63). The Cd concentration in fraction A in WYJ3 is significantly lower than that in SY63. For fraction H (cellulose, lignin and the non-active portion in the cell wall) in roots, an interesting result was found: the Cd concentrations in fraction H were significantly different between the two rice cultivars used, which were 11.15 μg/g fw in WYJ3, compared with 4.84 μg/g fw in SY63. The proportions of the Cd in different binding forms are shown in Table III. As the major forms of binding Cd in roots, the Cd in fractions B and C accounted for 56.12% and 67.27% of their respective total Cd concentration in WYJ3 and SY63. Compared with the proportions of Cd concentration in fractions B and C, the Cd in fraction A was also an important binding form of Cd in roots, which represented 12% of the respective total Cd in both rice cultivars. However, proportions of Cd in fraction H in roots were significantly different between WYJ3 and SY63, with 16% of the total Cd in roots of WYJ3 being located in fraction H, compared with 7% in roots of SY63.

Chelation with organic compounds in the cell and conversion into a less toxic form are the strategies of inactivation of Cd absorbed in plants, which is the essence of compartmentalization and protects the functional units and metabolic processes in plants from Cd damage (Sanità di Toppi and Gabbrielli, 1999). In the present study, the results indicate that the Cd stress on rice plants was alleviated through chelating with organic compounds and storing in the vacuole and cell walls (Tabs. II, III). These may be due to the fact that the main constituent of the plant root cell wall (polysaccharide), including cellulose, semi-cellulose, lignin and pectin, etc., contains many ligands such as hydroxyl, carboxyl and amino groups as well, which can complex metal ions, restrict their transmembrane transportation (Wissenmeier et al., 1987; Ni and Wei, 2003), and thus help improve the tolerance of rice cultivars.

4. CONCLUSION

Plant roots played an important role in improving the tolerance to Cd and regulating the translocation of Cd in the plant. With increasing Cd concentration applied, the Cd concentration and accumulation in rice increased. However, root-to-shoot Cd transportation was significantly lower in the Cd-tolerant cultivar than in the Cd-sensitive cultivar at the higher Cd level (5.0 μM). Under the Cd exposure, the Cd was mainly bound with water-soluble low-molecular-weight polar compounds, and exchangeable acidic polar compounds and ions in the cell wall. However, binding differences existed between shoots and roots for both cultivars used. The proportions of Cd binding with small neutral molecules were significantly lower in shoots than in roots. The Cd concentrations in fraction G (remaining proteins and polysaccharides) in the Cd-tolerant cultivar were significantly higher than those in the Cd-sensitive cultivar. The Cd concentrations in fraction C in shoots, and in fractions A and F, were significantly lower in roots of the Cd-tolerant cultivar than in roots of the Cd-sensitive cultivar. The results suggest that the Cd stress on rice plants was alleviated through chelating with organic compounds and storage in the vacuole and cell walls. It is also suggested that the toxicity or risk of Cd to plants or human health could be decreased considerably by planting Cd-tolerant rice cultivars in the fields contaminated with Cd.

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