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Uptake and translocation of Cd in different rice cultivars and the relation with Cd accumulation in rice grain

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Abstract

The variations among six rice cultivars in cadmium (Cd) uptake and translocation were investigated with pot soil experiments. The results showed that only a very small portion (0.73%) of Cd absorbed by rice plant was transferred into grain. With regard to plant total Cd uptake, Cd concentrations and quantity accumulations in roots, stems and leaves, the differences among the cultivars (between the largest one and the smallest one) were less than one time. But for Cd concentrations and Cd quantity accumulations in the grains, the differences were more than five and eight times, respectively. With respect to Cd distribution portions in plant organs, the diversities among the cultivars were also small in roots, stems and leaves, but much larger in grains. Grain Cd concentrations correlated positively and significantly ($P < 0.01$) with Cd quantity accumulations in plant, Cd distribution ratios to aboveground parts, and especially with Cd distribution ratios from aboveground parts to the grain. The results indicated that Cd concentration in rice grain was governed somewhat by plant Cd uptake and the transport of Cd from root to shoot, and in a greater extent, by the transport of Cd from shoot to grain. Cd was not distributed evenly in different products after rice grain processing. The average Cd concentration in cortex (embryo) was five times more than that in chaff and polished rice. With regard to Cd quantity accumulation in the products, near 40% in cortex (embryo), 45% in polished rice and 15% in chaff averagely.

Keywords: Cadmium (Cd); Rice (*Oryza sativa* L.); Cultivars; Translocation

1. Introduction

Cadmium (Cd) is a toxic metal to plants and animals. Highly polluted soils containing over 100 mg/kg Cd were reported in China, France and some other countries [1–3]. Although plants do not require Cd for growth and reproduction, the bioaccumulation index of Cd in plants is high and may exceed many essential elements [4]. Moreover, Cd may pose risk to human and animal health at plant tissue concentrations that are not generally phytotoxic [5]. Therefore, Cd is one of the most important metals to consider in terms of food-chain contamination.

Genetic differences of plant in mineral uptake was observed more than 20 years ago [6], and genotypic variations in Cd contamination in food crops have also been observed [7]. The uptake and translocation of Cd in plants vary greatly not only among plant species but also among cultivars within the same species [8,9]. Based on these genetic differences, the manipulation of Cd uptake and translocation to edible tissues of sunflower and durum wheat by breeding and screening has been achieved [10,11]. This will provide a long-term effective and economical means in reducing Cd contamination in crops.

Paddy rice is one of the most important crops in the world, especially in Asia. The amount of Cd that enters human diet from a crop depends on the amount of Cd accumulated in the parts that are consumed, so the translocation of Cd within rice plant, especially into grain, is very important for human Cd intake through diet. However, the differences among rice cultivars in Cd uptake, translocation and the relation with grain Cd accumulation are poorly understood.

Based on our previous studies [12,13], six rice cultivars with different Cd accumulation abilities were used in the present experiment, and the following issues were addressed: (1) variations among rice cultivars in Cd uptake and translocation in

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different organs of rice plant; (2) the relations between plant Cd uptake, distribution and Cd concentration in grain; (3) Cd distribution in different parts of rice grain. The information will be useful in the selection and breeding of rice cultivars for reducing Cd in the diet in Cd-contaminated areas.

2. Materials and methods

2.1. Soil preparation

This study was performed as a pot trial. The trial was carried out during rice growing season (from early June to the end of September) under open-air conditions. The soil used in our previous experiments ($CdCl_2$ was added to the soil to obtain a Cd level of 100 mg Cd kg^{-1} soil, dry weight) [13] was also used in this experiment. It was a sandy loam with a high portion of sand (62.7%) and a neutral pH (7.87). It also contained moderate level of organic matter (2.85%), cation exchange capacity (11.6 cmol kg^{-1}) and nitrogen content (0.12%). After air-drying, sieving through a 2 mm sieve and mixed thoroughly, the total Cd concentration in the soil was tested with AAS following H_2O_2–HF–HNO_3–$HClO_4$ digestion [14], and the EDTA exchangeable Cd in the soil were extracted with 0.025 M $(NH_4)_2$EDTA (pH 4.6) and tested with AAS [15]. They were 91.28 and 17.35 mg kg^{-1} (dry weight), respectively. The soil receiving no Cd served as control.

About 10 kg of the soils were placed in each pot (25 cm in diameter and 30 cm in height) and submerged in water (2–3 cm above the soil surface) for a month before rice seedlings were transplanted.

2.2. Rice plant materials

Based on our previous studies [12,13], six rice cultivars of different types with varying Cd accumulation abilities were used in this experiment. These were *Liang you pei jiu* (hybrid indica, high Cd accumulating cultivar), *CV6* (new plant type, medium Cd accumulating cultivar), *Shan you 63* (hybrid indica, high Cd accumulating cultivar), *Yang dao 6* (indica, high Cd accumulating cultivar), *Wu yun jing 7* (the formal name of the cultivar 9520 in reference #12, japonica, low Cd accumulating cultivar) and *Yu 44* (japonica, low Cd accumulating cultivar). Rice seeds were soaked in water for about 48 h at room temperature (20–25 °C) and germinated under moist condition at 32 °C for another 30 h and the germinated seeds were grown in uncontaminated soils. After 30 days, the seedlings with two tillers were transplanted into the pots (three plants per pot). The pot soil was maintained under flooded conditions (with 2–3 cm of water above soil surface) during the whole growth period of 110–115 days (depending upon cultivars).

2.3. Experimental design

The pots were arranged in a randomized complete block design with five replicates. Pots received N, P and K fertilizers thrice, i.e. the third day before seedling transplant, the 20th and 70th day after the transplant. One gram of urea (N content 46%) and 1 g of $K_2HPO_4{\bullet}3H_2O$ were applied to each pot on each occasion.

2.4. Sample preparation and analytical methods

At maturity, whole rice plants were harvested and washed thoroughly with tap water and then with deionized water. The plants were divided into roots, leaves, stems and grains. The roots, stems, leaves were oven-dried at 70 °C to constant weight. The oven-dried samples were ground with a stainless steel grinder (FW-100, China) to pass through a 100 mesh sieve. The Cd concentrations of the samples were determined with AAS following HNO_3–$HClO_4$ (4:1) digestion procedures [16].

The grains were air-dried to constant weights, and processed according to the standard “the Testing Methods of Rice Qualities” issued by China Ministry of Agriculture (NY147-88). The chaff of the grains was removed with machine (OHYA-25, Japan), and the brown rice was polished with rice polishing machine (CPC 96-3, China) until the cortex (embryo) was removed from the brown rice (2 min). The samples of the cortex (embryo), brown rice and polished rice were oven-dried at 60 °C to constant weight. The oven-dried samples were ground through 100 mesh. The Cd concentrations of the samples were determined with AAS following HNO_3–$HClO_4$ (4:1) digestion procedures [16].

Data were analyzed with the statistical package SPSS 10.0 and Excel 2000 for Win. The significant levels 0.05 and 0.01 were used in presenting the results.

3. Results

3.1. Variations among rice cultivars in Cd accumulations and distributions

The total Cd accumulations in whole plant, Cd concentrations and accumulations in different organs (roots, stems, leaves and grains) of the six rice cultivars are showed in Table 1. Generally, the results of this experiment were consistent with the results of our previous researches with regards to the differences among the six rice cultivars in Cd uptake and accumulations.

On the total Cd accumulations in rice plant, the differences among the six cultivars were not large (less than one time difference between the largest one and the least one). But the differences between rice types were significant ($P < 0.05$), with the cultivars of indica consanguinity (*Liang you pei jiu*, *CV6*, *Shan you 63* and *Yang dao 6*) significantly higher than the cultivars of japonica consanguinity (*Wu yun jing 7* and *Yu 44*). The differences were mostly not significant ($P > 0.05$) between the cultivars within a type.

With regard to Cd concentrations and quantity accumulations in different organs of rice plant, they fell rapidly from root to grain. The average ratio of root:stem:leave:grain were 358.6:14.5:5.1:1 for Cd concentrations, and 125.5:12.4:1.1:1 for Cd accumulations. So absolute majority of Cd absorbed by rice plant was restrained in root and stem (more than 98%), and only a very small portion was transferred into grain (averagely 0.73%) (Table 2).

Table 1
Variations among rice cultivars on Cd concentrations and quantity accumulations in different organs of rice plant

Cultivar	Root		Stem		Leaf		Grain		Total Cd accumulation ($mg\,pot^{-1}$)
	Cd concentration ($mg\,kg^{-1}$)	Cd accumulation ($mg\,pot^{-1}$)	Cd concentration ($mg\,kg^{-1}$)	Cd accumulation ($mg\,pot^{-1}$)	Cd concentration ($mg\,kg^{-1}$)	Cd accumulation ($mg\,pot^{-1}$)	Cd concentration ($mg\,kg^{-1}$)	Cd accumulation ($mg\,pot^{-1}$)	
Liang you pei jiu	507.03 a	14.62 a	16.55 a	1.43 ab	6.51 a	0.125 a	1.44 b	0.151 b	16.32 a
CV6	454.25 a	13.63 ab	17.10 a	1.39 ab	6.39 a	0.120 a	0.97 d	0.064 d	15.18 ab
Shan you 63	364.84 b	14.80 a	15.98 a	1.60 a	4.86 b	0.097 bc	1.84 a	0.191 a	16.68 a
Yang dao 6	333.79 bc	12.36 b	17.20 a	1.20 b	6.39 a	0.110 ab	1.15 c	0.110 c	13.78 b
Wu yun jing 7	276.39 c	8.04 c	12.13 b	0.74 c	6.41 a	0.114 ab	0.42 e	0.033 e	8.93 c
Yu 44	298.12 c	8.11 c	11.71 b	0.70 c	4.40 b	0.085 c	0.28 e	0.020 e	8.91 c
$LSD_{0.05}$	60.26	2.10	2.35	0.24	1.22	0.022	0.17	0.019	2.30
Average	365.74	11.92	14.78	1.18	5.24	0.109	1.02	0.095	13.29

Different letters in a column indicate significant differences between the cultivars at the 0.05 level.

Table 2
Variations among rice cultivars on Cd quantity distribution portions (%) in different organs

Cultivar	Organs			
	Root	Stem	Leaf	Grain
Liang you pei jiu	89.56 ab	8.75 b	0.77 c	0.92 b
CV6	89.76 ab	9.16 ab	0.65 d	0.42 d
Shan you 63	88.51 b	9.79 a	0.56 d	1.14 a
Yang dao 6	89.66 ab	8.74 b	0.79 c	0.8 0 c
Wu yun jing 7	90.00 a	8.33 bc	1.24 a	0.43 d
Yu 44	90.97 a	7.84 c	0.93 b	0.26 e
$LSD_{0.05}$	1.46	0.84	0.11	0.09
Average	89.99	8.51	0.76	0.73

Different letters in a column indicate significant differences between the cultivars at the 0.05 level.

Respecting the variations among cultivars in Cd concentrations and quantity accumulations of different organs, the differences were large and significant ($P < 0.05$) between most of the cultivars for those in grains (more than five-time difference between the largest Cd concentration and the least one, and more than eight-time difference between the largest Cd accumulation and the least one), but the differences were smaller and not significant ($P > 0.05$) between most of the cultivars for those in roots, stems and leaves (generally less than one-time difference between the largest one and the least one) (Table 1). The variations among the cultivars in Cd distribution portions in different organs were also lager in grains, but smaller in vegetative organs (Table 2).

3.2. Correlations between grain Cd concentrations and plant Cd accumulations and distributions

Fig. 1 showed that grain Cd concentrations correlated positively and significantly ($P < 0.01$) with Cd quantity accumulations in rice plant. There also existed positive and significant correlations ($P < 0.01$) between grain Cd concentrations and Cd distribution ratios to aboveground parts, and between grain Cd concentrations and Cd distribution ratios from aboveground parts to grain (Figs. 2 and 3). Especially, grain Cd concentrations correlated highly with Cd distribution ratios from aboveground parts to grain ($r = 0.9713$, $n = 18$).

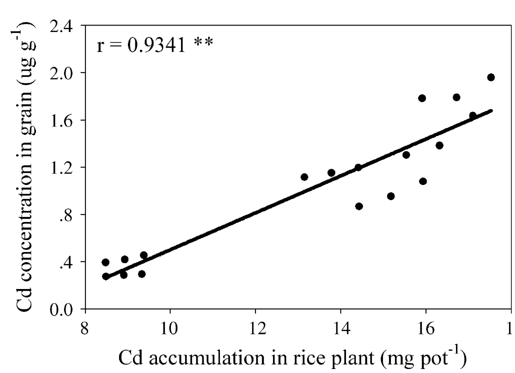

Fig. 1. Correlation between Cd concentrations in grain and total Cd accumulations in rice plant.

Table 3
Variations among rice cultivars on Cd distribution in different products of rice grain processing

Cultivar	Cd concentration ($\mu g\ g^{-1}$)			Cd quantity distribution (%)		
	Chaff	Cortex (embryo)	Polished rice	Chaff	Cortex (embryo)	Polished rice
Liang you pei jiu	0.77 b	6.53 ab	1.03 b	10.69 d	37.54 b	51.76 ab
CV6	1.25 a	3.12 c	0.58 d	24.30 a	33.14 c	42.55 c
Shan you 63	0.85 b	7.29 a	1.43 a	9.50 d	35.71 bc	54.79 a
Yang dao 6	0.73 b	5.41 b	0.80 c	13.71 c	37.13 b	49.15 b
Wu yun jing 7	0.39 c	1.80 d	0.22 e	14.95 bc	46.85 a	38.20 d
Yu 44	0.27 c	1.53 d	0.14 e	16.40 b	47.97 a	35.63 d
$LSD_{0.05}$	0.28	1.15	0.19	2.57	3.26	3.51
Average	0.71	4.28	0.70	14.93	39.72	45.35

Different letters in a column indicate significant differences between the cultivars at the 0.05 level.

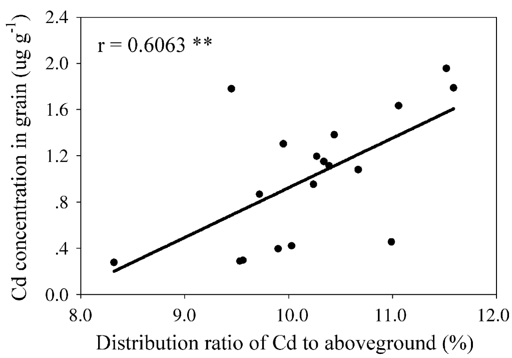

Fig. 2. Correlation between Cd concentrations in grain and distribution ratios of Cd to aboveground.

3.3. *Cd distribution in different products of rice grain processing*

Cd was not distributed evenly in different products after rice grain processing (Table 3). On Cd concentrations, they were much higher in cortex (embryo) than those in chaff and polished rice. The average Cd concentration in cortex (embryo) was five times more than those in chaff and polished rice. With regard to Cd quantity accumulations in the products, near 40% of the Cd in the grain was accumulated in cortex (embryo) although its dry weight was only 9% of the grain averagely. The polished rice occupied 71% of the grain dry weight, but it accumulated only 45% of the Cd in the grain (the data of dry weight were not shown).

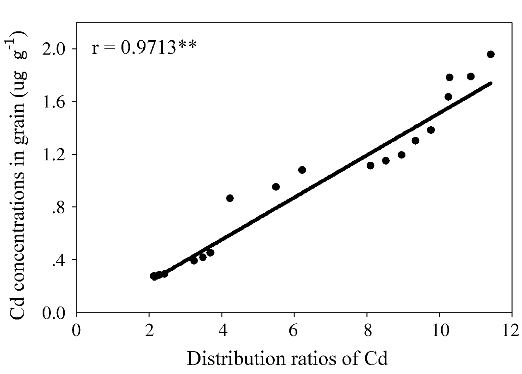

Fig. 3. Correlation between Cd concentrations in grain and distribution ratios of Cd from aboveground to grain.

There were also variations among the six cultivars in grain Cd distribution. With respect to Cd concentrations in different parts, the cultivar with the highest Cd concentration in chaff was *CV6*, but the cultivar with the highest Cd concentrations in cortex (embryo) and polished rice was *Shan you 63*. On the Cd distribution portions, it ranged from 9.5% to 24.3% in chaff, from 33.1% to 48.0% in cortex (embryo) and from 35.6% to 54.8% in polished rice, for the cultivars tested (Table 3).

4. Discussion and conclusions

It has been noted that the uptake and translocation of Cd to consuming parts of crops varied greatly with plant species and with cultivars within a species [17,18]. According to Wu et al. [19], more than two-fold difference (ranging from 0.48 to 1.17 $mg\ kg^{-1}$) was observed among 20 rice cultivars grown in South China (indica and hybrid indica) for Cd concentrations in brown rice.

Former reports also showed that the translocation of Cd and other heavy metals from roots to shoots is the main reason for high concentration in shoots [20], and the differences among plant species in seed Cd concentrations depend on the translocation of Cd from vegetative to generative plant parts [21].

In our present experiments, the variations among rice cultivars were relatively small in plant total Cd uptake and Cd accumulation in vegetative organs, but these variations were much larger in grain Cd accumulation. On Cd accumulations in whole rice plants, roots, stems and grain, *Shan you 63* (hybrid indica) was the highest cultivars, and the two japonica cultivars (*Wu yun jing 7* and *Yu 44*) were the lowest cultivars. The differences between the highest and lowest were less than one-fold for Cd accumulations in whole plants, roots and stems, but more than eight-folds for that in grains.

With regards to Cd concentrations and Cd accumulations in whole plants, roots, stems and grains, there also existed significant variations between rice types, with the cultivars of indica consanguinity significantly higher ($P < 0.05$) than the cultivars of japonica consanguinity.

Our other experiments indicated that Cd uptake and accumulation characters of rice cultivars were related to their root oxidation abilities [22], root acidifications and root organic acid secretions (data not shown). But the magnitude of the variations among the rice cultivars were much higher for grain Cd concentrations than for Cd uptake in whole rice plant and other organs. So Cd level in rice grain must related to Cd distribution in rice plant after its uptake.

In our present research, grain Cd concentrations correlated positively and significantly ($P < 0.01$) with total Cd quantity accumulations in plant, Cd distribution ratios to aboveground parts, and highly with Cd distribution ratios from aboveground parts to grain. The results indicated that Cd concentration in rice grain was governed somewhat by plant Cd uptake and the transport of Cd from root to shoot, and to a greater extent, by the transport of Cd from shoot to grain. Former reports also showed that there were relations between seed Cd concentrations and Cd distributions in the plants of solin and flax cultivars. The cultivar *Vimy* contained higher levels of Cd in seed than did the other cultivars, and the seed Cd:tissue Cd ratio was generally higher for *Vimy* than for the other two cultivars [23].

So the further research in the selection and breeding of rice cultivars for reducing Cd in the grain should not be focused solely on Cd uptake characters, especially at early stages of plant growth. It must be focused mainly on the translocation of Cd from vegetative organs to generative organ at reproductive stages.

Our present research also indicated that Cd was not distributed evenly in rice grain, with Cd concentrations in cortex (embryo) significantly higher than that in chaff and endosperm. Our former study revealed that Cd concentrations in brown rice correlated significantly with nitrogen concentration [13]. Cd may bind to Class 2 metallothioneins in developing crop seeds because the genes for the expression of these sulphydryl-rich proteins have been reported in the seeds of maize and rice [24,25]. So for the people in Cd-contaminated areas, polished rice may be safer than brown rice, although brown rice is better than polished rice in nutrition.

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