

RESEARCH ARTICLE

Genotypic Variation of Cadmium Accumulation and Distribution in Rice

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Abstract

An effective way to reduce the risk of cadmium (Cd) entering the food chain is to use low Cd-accumulation rice cultivars, particularly in Asia. The fundamental requirement for breeding low grain Cd-accumulation cultivars is to know the genotypic variation in Cd accumulation and the physiological processes and genetic basis governing the Cd accumulation in rice grain. In this experiment, genotypic variation in Cd accumulation and distribution among rice organs was studied using thirty-five rice varieties. They were grown with irrigation water containing 2 ppm Cd throughout rice growing season under field condition in 2007. At harvest, plants were sampled and analyzed for Cd concentration and accumulation in each rice organ. Significant variation of Cd concentration and accumulation in rice organs were found among thirty-five rice cultivars, revealing more than 8-fold varietal differences in grain Cd concentration and shoot Cd accumulation. Cd concentration and accumulation in grain were significantly different among cultivar groups, showing the highest in indica and the lowest in temperate japonica. Tongil-type and tropical japonica rice showed a Cd concentration intermediate to that of temperate japonica and indica rice. The higher Cd accumulation in grain of indica rice was attributable to the greater ability of Cd uptake. The greater ability of root-shoot translocation in tropical japonica and shoot-grain redistribution in tongil-type resulted in the significantly higher grain Cd concentration in these cultivar groups than in temperate japonica. For over 35 cultivars tested, grain Cd concentration revealed a significant positive correlation with root Cd concentration and shoot Cd concentration and accumulation while no significant correlation with root-shoot translocation factor and shoot-grain redistribution ratio. However, correlation analyses within each cultivar group showed that grain Cd concentration was significantly correlated with root-shoot translocation factor in indica, with root Cd concentration in tongil-type, with shoot Cd concentration and accumulation in tropical japonica, and with shoot Cd accumulation and shoot-grain redistribution ratio in temperate japonica. These results indicate that genotypic variation in grain Cd accumulation, in general, is controlled by all the three physiological processes but the major physiological process governing its genotypic variation within cultivar group is different depending on cultivar groups.

Key words: accumulation, cadmium, distribution, genotypic variation, rice

Introduction

The fast development of industry has increased the contamination of soil and water with heavy metals. Cadmium (Cd), a toxic heavy metal, is not an essential element for plant growth, but can be absorbed by crops and accumulate in edible parts (Cataldo et al. 1983; Cie li ski et al. 1996; Guo and Marschner 1996). Cd poses a serious risk to human health through the food chain (Liu et al. 2003; Shah et al. 2001).

Rice is a staple food in Asia. A higher Cd accumulation was reported in rice compared to soybean and maize (Murakami et

al. 2007). Rice containing Cd is the largest source of dietary intake of Cd, especially in Asia (Ueno et al. 2009). It is necessary to reduce the Cd concentration in rice grain for human health. Several approaches for the reduction of Cd accumulation in rice grain have been proposed. For example, in order to reduce Cd availability in the soil, soil dressing, application of alkaline amendments, and water management have been attempted (Ishikawa et al. 2005) but these techniques are costly or ineffective in some soils under some weather conditions. Many studies have shown that there is marked difference of Cd accumulation ability not only among crops but also among crop cultivars (Chen et al. 2007; Kurz et al. 1999; Liu et al. 2007a;

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Liu et al. 2009; McLaughlin et al. 2000; Murakami et al. 2007; Zeng et al. 2008; Zhang et al. 2002). Also, genotypic variation of Cd uptake and translocation in rice has been reported (He et al. 2006; Liu et al. 2007b). Based on the genotypic variation, it is possible to breed or screen the low-Cd accumulation crop cultivars. Moreover, phytoextraction, i.e. utilizing high-Cd accumulation plant species to remove Cd from soil, has been considered as a potentially efficient method to remediate the Cd-contaminated soil (Sun et al. 2008).

Root uptake is the major source of Cd in crops rather than the pathway absorbing Cd from atmosphere via shoots (Smolders 2001). Cd accumulation in rice grain is regulated by three physiological processes including Cd uptake by root, xylem translocation from root to shoot, and then phloem movement from shoot into grain (Clemens et al. 2002; Hart et al. 1998). An understanding of the correlation between these three processes and Cd accumulation in grain is necessary for breeding or engineering low-grain-Cd rice cultivars. However, which of these is the major process governing the genotypic variation in grain Cd accumulation of rice is still controversial. Liu et al. (2005, 2007b) reported that shoot-grain redistribution was the most important process revealing the highest correlation with its genotypic variation. Conversely, He et al. (2006) reported that grain Cd concentration was not correlated with shoot-grain redistribution but was significant with shoot Cd accumulation and concluded that genotypic variation in grain Cd accumulation is governed mainly by Cd uptake but not by differential Cd partitioning between shoot and grain. Uraguchi et al. (2009) reported Cd levels in xylem sap revealed a strong correlation with Cd levels in grain among 69 rice core collections and concluded that the major physiological process determining the Cd accumulation in shoot and grain of rice plant is the root-to-shoot Cd translocation via xylem. These different results suggest that there may be different mechanisms regulating the Cd translocation into grain according to rice genotypes and environment and requires more in-depth studies.

This study was designed (1) to examine the genotypic variation of Cd accumulation and distribution in organs of rice irrigated with Cd-contaminated water and (2) to evaluate the relationship of physiological processes with genotypic variation in Cd accumulation in rice grain.

Materials and Methods

Experimental design and soil

A field experiment was conducted at the experimental farm of Seoul National University (37°16' N and 126°59' E), Suwon, Korea, in 2007. Thirty-five rice cultivars belonging to different cultivar groups (temperate japonica, tropical japonica, tongil-type, and indica) were grown with irrigation water containing 2 ppm CdCl₂ throughout the rice growing season. Plots were laid out in randomized complete block design with three replications. Rice 30-day-old seedlings were transplanted with a machine transplanter at a spacing of 15 x 30 cm on 24th of May in 2007. In total, 282 kg N ha⁻¹ as urea, 40 kg P₂O₅ ha⁻¹ as fused

super-phosphate, and 130 kg K₂O ha⁻¹ as potassium chloride were applied. The experimental field had a sandy clay loam, pH of 5.4, CEC of 12.7 cmol⁺ kg⁻¹, O.M of 20.2 g kg⁻¹, total N of 11.6 g kg⁻¹, and available P of 35.8 mg kg⁻¹. The soil Cd concentration was 0.15 mg kg⁻¹.

Sample preparation and analytical method

At harvest, four plants from each replication were harvested and divided into root, straw, and grain. The samples were oven-dried at 70 °C to constant weight, weighed, and then ground to powder for heavy metal analysis. Then, 2.0 g of plant samples were digested with a 20 ml solution containing 87% of concentrated HNO₃ and 13% of concentrated HClO₄ (Ince et al. 1999). The concentrations of Cd in digested solutions were determined using an atomic absorption spectrophotometer (AA-6401, Shimadzu, Japan).

Statistical analysis

Data were analyzed with statistical program SAS version 9.1 (SAS Inc., USA). Differences among genotypes and cultivar groups were analyzed by two-way nested ANOVA and least significant difference (LSD)/Duncan's multiple range test (DMRT). Pearson correlation coefficients were calculated to determine the relationships between Cd concentration, accumulation, and distribution of different rice organs.

Results

Genotypic variation in Cd concentration, accumulation, and distribution

Cd concentration, accumulation, and distribution in rice organs were significantly different among thirty-five rice genotypes, revealing very wide genotypic variations (Table 1). Cd concentration ranged from 4.60 to 45.20 mg kg⁻¹ in root, from 0.83 to 4.41 mg kg⁻¹ in shoot, and from 0.25 to 1.70 in grain. The highest and the lowest grain Cd concentrations were observed in an indica cultivar 'IR7' and a temperate japonica cultivar 'Chucheong', respectively. The Cd accumulation varied between 0.69 and 7.94 mg m⁻² in shoot and between 0.12 and 1.38 mg m⁻² in grain. An indica cultivar 'IR64' showed the highest Cd accumulation in rice shoot and grain. On average, Cd concentration in root was seven times higher than that in shoot. As an indirect indicator for translocation ability from root to shoot, the root-shoot translocation factor was calculated as the ratio of shoot Cd concentration to root Cd concentration. The root-shoot translocation factor ranged from 0.13 to 0.51, revealing four-fold genotypic variation in root-shoot translocation ability. A part of Cd accumulated in shoot is redistributed into grain during the ripening period. The redistribution ability from shoot to grain as calculated by a ratio of shoot Cd accumulation to grain Cd accumulation was very different among cultivars. The shoot-grain redistribution ratio ranged from 9.8 to 33.7%.

Significant differences in concentration, accumulation, and

Table 1. Cadmium concentration (mg kg⁻¹ DW), accumulation (mg m⁻²), translocation factor, redistribution ratio (%) and biomass (kg m⁻²) of thirty-five rice cultivars.

Cultivar	Root		Shoot			S-G ⁴⁾	Devel. Site		
	Conc. ²⁾	R-S ³⁾	Conc.	Bio.	Accu.		Conc.	Bio.	Accu.
IR36(I) 1)	21.87	0.13	1.54	1.16	1.79	32.29	1.04	0.56	0.55
IR64(I)	22.01	0.33	4.41	1.81	7.94	17.70	1.68	0.85	1.38
IR7(I)	12.28	0.40	3.07	1.30	3.96	23.12	1.70	0.52	0.91
IR8(I)	11.44	0.28	1.86	1.39	2.63	29.90	1.28	0.58	0.78
Lunhui422(I)	9.85	0.24	1.35	0.74	1.08	25.74	0.57	0.35	0.28
SMR(I)	45.20	0.14	3.52	1.73	5.93	10.59	1.17	0.45	0.63
Namcheon(TI)	8.73	0.29	1.51	1.48	2.23	24.91	0.74	0.75	0.56
Dasan(TI)	13.84	0.20	1.50	1.44	2.13	33.31	0.95	0.71	0.71
Milyang23(TI)	9.41	0.34	1.91	1.57	3.05	19.93	0.70	0.81	0.62
Anda(TI)	6.68	0.26	0.98	1.12	1.10	24.47	0.53	0.52	0.27
Hangangchal(TI)	14.26	0.22	1.80	1.21	2.18	22.35	1.08	0.45	0.49
Hanaruem(TI)	13.54	0.18	1.32	1.71	2.32	27.88	0.71	0.83	0.63
IR31917(RJ)	9.66	0.38	2.24	1.47	3.37	15.68	0.95	0.50	0.52
IR69860(RJ)	9.54	0.46	2.85	1.07	3.11	15.65	0.86	0.52	0.48
IR71204(RJ)	10.60	0.24	1.44	1.47	2.05	21.09	0.54	0.67	0.41
IR71451(RJ)	19.40	0.22	2.39	1.18	2.93	15.22	0.64	0.51	0.43
IR71682(RJ)	10.04	0.33	1.95	1.29	2.54	14.00	0.51	0.63	0.35
IR72225(RJ)	6.61	0.51	2.29	0.87	2.03	9.76	0.47	0.33	0.20
IR73111(RJ)	10.01	0.23	1.29	1.22	1.60	25.92	0.62	0.58	0.41
IR76911(RJ)	10.56	0.45	3.05	1.11	3.49	15.45	0.92	0.47	0.54
OS4(RJ)	7.54	0.20	0.84	1.41	1.19	16.74	0.29	0.69	0.19
Namil(TJ)	4.60	0.31	0.83	0.89	0.76	21.51	0.33	0.42	0.16
Nampyeong(TJ)	17.09	0.14	1.26	1.30	1.71	26.30	0.66	0.59	0.45
Suwon468(TJ)	9.76	0.19	1.05	0.69	0.75	23.95	0.46	0.32	0.18
Suwon476(TJ)	8.32	0.26	1.27	1.79	2.26	33.74	1.02	0.75	0.74
Suwon490(TJ)	11.77	0.36	2.55	1.17	2.99	17.01	0.80	0.58	0.50
Yangjo(TJ)	10.40	0.21	1.20	0.94	1.16	21.36	0.52	0.44	0.24
Jongnam(TJ)	7.91	0.23	1.03	0.70	0.69	20.31	0.38	0.34	0.13
Juan(TJ)	4.89	0.41	1.25	1.15	1.48	26.57	0.69	0.53	0.39
Chucheong(TJ)	12.23	0.20	1.37	1.03	1.46	10.62	0.25	0.43	0.15
Hwajin(TJ)	13.28	0.20	1.49	1.08	1.61	16.47	0.56	0.47	0.26
Balilla(TJ)	9.21	0.27	1.43	1.12	1.68	13.24	0.36	0.41	0.18
Kunjing4(TJ)	11.79	0.19	1.22	0.83	1.07	24.11	0.57	0.37	0.26
SNU-SG1(TJ)	5.19	0.29	0.88	1.14	1.01	14.88	0.30	0.47	0.15
Zheng5-2(TJ)	8.75	0.20	0.98	0.99	0.95	12.17	0.28	0.43	0.12
Average	11.95	0.27	1.74	1.22	2.24	20.7	0.72	0.54	0.44
LSD0.05	4.77	0.06	0.57	0.31	0.86	6.69	0.20	0.14	0.16
Range	4.60-45.20	0.13-0.51	0.83-4.41	0.69-1.81	0.69-7.94	9.8-33.7	0.25-1.70	0.32-0.85	0.12-0.52

¹⁾ I, *Indica*; TI, Tongil-type; TJ, Temperate *japonica*; RJ, Tropical *japonica*.

²⁾ Conc., Cd concentration; Bio., biomass; Accu., Cd accumulation.

³⁾ R-S, Root-shoot translocation factor.

⁴⁾ S-G, Shoot-grain redistribution ratio.

distribution of Cd were also found among four rice cultivar groups (Table 2). *Indica* rice showed the highest Cd concentration and accumulation in root, shoot, and grain, while temperate *japonica* showed the lowest Cd concentration and accumulation in all of the rice organs. Tongil-type rice that was developed from the crosses between *japonica* and *indica* showed the in-between Cd concentration and accumulation of *japonica* (temperate and tropical *japonica*) and *indica* rice. Tongil-type cultivars showed the highest shoot-grain redistribution ratio while tropical *japonica* the lowest. Root-shoot translocation factor was significantly higher in tropical *japonica* than in the other cultivar groups.

Correlation of Cd concentration, accumulation, and

Table 2. Cadmium concentration (mg kg⁻¹ DW), accumulation (mg m⁻²), translocation factor, and redistribution ratio (%) of four rice cultivar groups.

Cultivar group	Root	R-S ²⁾	Shoot		S-G ³⁾	Grain	
	Conc. ¹⁾		Conc.	Accu.		Conc.	Accu.
<i>Indica</i>	20.44 a ⁴⁾	0.25 b	2.62 a	3.87 a	23.23 ab	1.24 a	0.76 a
Tongil-type	11.08 b	0.25 b	1.50 c	2.17 b	25.48 a	0.79 b	0.55 b
Tropical <i>japonica</i>	10.44 b	0.33 a	2.04 b	2.48 b	16.61 c	0.65 bc	0.39 c
Temperate <i>japonica</i>	9.66 b	0.25 b	1.27 c	1.40 c	20.16 bc	0.52 c	0.28 c

¹⁾ Conc., Cd concentration; Accu., Cd accumulation.

²⁾ Values with the same letters in each column are not significantly different by Duncan's Multiple Range Test at the 0.05 probability level.

³⁾ R-S, Root-shoot translocation factor.

⁴⁾ S-G, Shoot-grain redistribution ratio.

Table 3. Correlations among Cd concentration, accumulation, and translocation characteristics of thirty-five rice varieties.

Item	Grain Conc. ¹⁾	Root Conc.	R-S ²⁾	Shoot Conc.	Shoot Accu.
Root Conc.	0.48**	-			
R-S	0.20	-0.38*	-		
Shoot Conc.	0.71**	0.56**	0.45**	-	
Shoot Accu.	0.78**	0.63**	0.27	0.94**	-
S-G ³⁾	0.31	-0.12	-0.29	-0.31	-0.20

* and **: $P < 0.05$ and $P < 0.01$, respectively.

¹⁾ Conc., Cd concentration; Accu., Cd accumulation.

²⁾ R-S, Root-shoot translocation factor.

³⁾ S-G, Shoot-grain redistribution ratio.

Table 4. Correlation of grain Cd concentration with Cd concentration, accumulation, and translocation characteristics in the other organs of rice cultivars within each cultivar group.

Cultivar group	Root Conc. ¹⁾	R-S ²⁾	Shoot Conc.	Shoot Accu.	S-G ³⁾
<i>Indica</i>	0.26	0.83*	0.71	0.71	-0.37
Tongil-type	0.83*	-0.37	0.37	-0.09	0.26
Tropical <i>japonica</i>	0.20	0.45	0.75*	0.88**	-0.00
Temperate <i>japonica</i>	0.22	0.25	0.49	0.71**	0.75**

* and **: $P < 0.05$ and $P < 0.01$, respectively.

¹⁾ Conc., Cd concentration; Accu., Cd accumulation.

²⁾ R-S, Root-shoot translocation factor.

³⁾ S-G, Shoot-grain redistribution ratio.

distribution among rice organs

Significant positive correlations between Cd concentration in grain and Cd concentration in root and shoot were found among 35 rice cultivars while no significant correlation was found between Cd concentration in grain and Cd root-shoot translocation factor and shoot-grain redistribution ratio (Table 3). A significant positive correlation was also found between root and shoot Cd concentrations. In addition, correlation between Cd concentration in grain and Cd concentration and accumulation in the other organs were also analyzed among cultivars within each cultivar group (Table 4). Grain Cd concentration was found to be significantly correlated with root-shoot translocation factor in *indica*, with root Cd concentration in tongil-type, with shoot Cd concentration and accumulation in tropical *japonica*, and with shoot Cd accumulation and shoot-grain redistribution ratio in temperate *japonica*.

Discussion

An effective way to reduce the risk of Cd entering the food chain is to use low Cd-accumulation cultivar as reported by Zeng et al. (2008) in rice. The fundamental requirement for breeding low-grain Cd-accumulation cultivars is to know the genotypic variation in Cd accumulation and the physiological processes and genetic basis governing Cd accumulation in rice grain.

As in previous reports (He et al. 2006; Liu et al. 2006; Liu et al. 2007b; Morishita et al. 1987; Uruguchi et al. 2009; Wu et al. 1999; Zeng et al. 2008), significant variation of Cd concentration and accumulation in rice organs was found among 35 rice cultivars that were grown with irrigation water containing 2 ppm CdCl₂ throughout growing season in lowland rice field, revealing more than eight-fold varietal differences in grain Cd concentration and shoot Cd accumulation (Table 1). The significant differences in Cd concentration and accumulation in organs were also observed among four rice cultivar groups (Table 2). Indica rice showed the highest Cd concentration and accumulation in root, shoot, and grain while temperate japonica had the lowest Cd concentration and accumulation in all of the rice organs. Other studies (He et al. 2006; Liu et al. 2005; Liu et al. 2007b; Morishita et al. 1987) also reported consistent results that indica rice accumulated more Cd in grain than japonica rice. As indicated by the results that indica rice exhibited significantly higher Cd concentration and accumulation in root and shoot but similar root-shoot translocation factor and shoot-grain redistribution ratio compared to the other cultivar groups (Table 2), the higher Cd accumulation in grain of indica rice was attributable to the greater ability of Cd uptake rather than the greater Cd translocation ability between organs. Tongil-type rice that was developed from the crosses between temperate japonica and indica, and tropical japonica showed the in-between grain Cd concentration of temperate japonica and indica rice. The significantly higher grain Cd concentration in these cultivar groups than in temperate japonica could be attributed to the higher ability of root-shoot translocation in tropical japonica and shoot-grain redistribution in tongil-type. Tropical japonica revealed the highest root-shoot translocation factor among four cultivar groups while tongil-type the highest shoot-grain redistribution ratio (Table 2). Liu et al. (2005) also reported that tropical japonica (new plant type) exhibited the a grain Cd concentration intermediate to that of temperate japonica and indica.

Cd accumulation in plant seed is governed by three physiological processes: (1) Cd uptake from the soil solution by root, (2) xylem translocation from root to shoot, and (3) phloem translocation from shoot to seed (Clemens et al. 2002). However, there is still controversy regarding the major process governing the genotypic variation in grain Cd accumulation of rice. Liu et al. (2005, 2007b) reported that all the three processes were significantly related to genotypic variation in grain Cd accumulation but shoot-grain redistribution was the process revealing the highest correlation with its genotypic variation. On the contrary, He et al. (2006) reported that grain Cd concentration was not correlated with shoot-grain redistribution but was significant with shoot Cd accumulation among 38 rice cultivars

including japonica and indica. From these results, they concluded that genotypic variation in grain Cd accumulation is governed mainly by Cd uptake but not by differential Cd partitioning between shoot and grain. Uruguchi et al. (2009) concluded that the major and common physiological process determining the Cd accumulation in shoot and grain of rice plant is the root-to-shoot Cd translocation via xylem as Cd levels in xylem sap revealed strong correlation with Cd levels in grain among 69 rice core collections. As suggested by our results (Tables 3, 4), these different conclusions might have resulted from the different genotypes selected in the experiments, the generalized conclusions without regard to the differential responses among ecosppecies of rice, the different experimental environments, etc. In our experiment, grain Cd concentration revealed significant positive correlation with root Cd concentration and shoot Cd concentration and accumulation among 35 cultivars including four cultivar groups while no significant correlation was found with root-shoot translocation factor and shoot-grain redistribution ratio (Table 3). However, correlation analyses within each cultivar group (Table 4) produced different results from the correlation analysis among all the cultivars pooled across four cultivar groups. That is, grain Cd concentration was significantly correlated with root-shoot translocation factor in indica, with root Cd concentration in tongil-type, with shoot Cd concentration and accumulation in tropical japonica, and with shoot Cd accumulation and shoot-grain redistribution ratio in temperate japonica. These results indicate that genotypic variation in grain Cd accumulation, in general, is controlled by all the three physiological processes but the major physiological process governing its genotypic variation within cultivar group is different depending on cultivar groups.

It can be concluded that genotypic variation in grain Cd accumulation is wide enough for breeding low grain Cd-accumulator in rice and different breeding strategies are required depending on cultivar group as the main physiological process governing the genotypic variation in grain Cd accumulation is different according to cultivar group.

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