

RESEARCH ARTICLE

Absorption, Translocation, and Remobilization of Cadmium Supplied at Different Growth Stages of Rice

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Abstract

Cadmium (Cd) is absorbed by rice root and transferred into the other rice organs including grain. A solution-culture experiment was conducted to investigate the absorption and distribution of Cd supplied at different growth stages of rice. Two rice cultivars, a japonica 'Chucheong' and a tongil-type 'Milyang23' that exhibit high and low ability of Cd absorption by root and accumulation in grain were grown in culture solution and subjected to 2 ppm CdCl₂ treatment for 2 weeks at four different growth stages: before panicle initiation stage (BPI), after panicle initiation stage (API), early ripening stage (ER), and mid-ripening stage (MR). Cd concentration and accumulation in rice organs were measured at harvest. The two rice cultivars accumulated two to three times greater amounts of Cd in grain in the two Cd treatments before heading (BPI and API treatments) than in the Cd treatment after heading (ER and MR treatment). The higher grain Cd accumulation in BPI and API treatments was not attributed to the higher Cd uptake but to the higher translocation from root to shoot and the higher redistribution from shoot to grain than ER and MR treatments. These results imply that the remobilization of Cd through phloem during leaf senescence is the major process for Cd accumulation in rice grain rather than direct transport of absorbed Cd through the xylem-phloem transfer to grain. 'Milyang23' absorbed significantly smaller amount of Cd than 'Chucheong'. However, 'Milyang23' accumulated more than a three times larger amount of Cd in grain compared to 'Chucheong' as the former exhibited the higher root-shoot translocation and shoot-grain remobilization as well. It indicates that the greater Cd translocation from root to shoot and subsequent higher Cd remobilization from shoot to grain, not the higher absorption ability, have led to the higher Cd accumulation and concentration in grain of 'Milyang23'.

Key words: absorption, accumulation, cadmium, distribution, growth stage, remobilization, rice

Introduction

Cd has a toxic effect on human health. In addition, high levels of Cd in soil inhibits the growth of crops. Crops can absorb Cd from polluted soil and irrigation water by root and transfer it into shoot, including seed or the other edible parts (Guo and Marschner 1996; Patel et al. 1980; Zhang et al. 2000). Hence, Cd can accumulate in human body through the food chain. Rice is one of the most frequently consumed cereals in Asia and the major source of Cd intake for Asian people (Watanabe et al. 2004). To reduce Cd accumulation in rice grain through safe rice production, it is required to clearly understand the physiological processes regulating the uptake and translocation of Cd in rice.

There are three physiological processes most likely to mediate Cd accumulation into plant seeds: Cd uptake from the soil solution by root, xylem translocation from root to shoot, and phloem translocation into the seed (Clemens et al. 2002). Cd uptake by roots is the major source of Cd accumulated in plants and the initial processes that plants absorb Cd (Smolders 2001). The variation of Cd uptake and accumulation characters are related to their different root oxidation abilities (Liu et al. 2006), root acidifications, and root organic acid secretions (Liu et al. 2007). Symplastic influx of Cd across plasmalemma is controlled by some transport proteins in the plasmalemma in root (Kochian 1991). Several results showed that Cd might share the carriers or channels with other divalent cations, such as Zn⁺², Cu⁺², Fe⁺², Mn⁺², and Ca⁺² (Cataldo et al. 1983; Zhao et al. 2002). Recent evidence indicated that Cd adsorption on root apoplast

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represented 90% of the total root uptake at high Cd concentration conditions and significantly contributed to the amount of Cd taken up in alpine pennycress and maize root (Redjala et al. 2009). It is possible that both symplastic and apoplastic influx mediate root Cd uptake (Redjala et al. 2009).

Cd absorbed by root is translocated into shoot *via* xylem (Clemens et al. 2002). Interestingly, in our prior study, some rice cultivars exhibited a high Cd concentration in root, while it showed a low Cd concentration and accumulation in shoots. Uruguchi et al. (2009) also reported that a japonica rice cultivar, which had a low Cd shoot concentration, had a higher Cd uptake rate than an indica cultivar and suggested that the Cd translocation via xylem was the key process determining shoot Cd accumulation rather than root uptake ability. The Cd accumulation in rice grain was reported to be greatly related to Cd accumulation in shoot (He et al. 2006; Liu et al. 2007). Uruguchi et al. (2009) showed a significant correlation between the Cd level in xylem sap and that in rice shoot and grain among 69 rice core collections. Xylem root-shoot translocation was considered to be an important factor mediating the Cd accumulation in rice grain (Uruguchi et al. 2009). However, contrary evidence from wheat suggested that Cd accumulated in grains may be related to phloem-mediated Cd transport, not root-shoot translocation (Hart et al. 1998). Shoot-grain Cd translocation ratio was also related to the grain Cd concentration in rice (Liu et al. 2007). Kashiwagi et al. (2009) indicated that Cd translocation from root to culms and ear after heading did not influence the Cd accumulation in brown rice. Root-shoot translocation may affect Cd accumulation in brown rice by controlling the Cd pools in vegetative tissues that can be remobilized into brown rice (Kashiwagi et al. 2009).

It has been recognized in several crops, including rice, that Cd in the grain was predominately deposited from the phloem (Becher et al. 1997; Hart et al. 1998; Herren and Feller 1997; Popelka et al. 1996; Tanaka et al. 2003). Tanaka et al. (2007) concluded that 91 - 100% of Cd in rice grains was accumulated via phloem. Cd loaded to xylem can be transferred to the phloem and transported directly into grain via the phloem without passing through the leaf (Harris and Taylor 2001; Uruguchi et al. 2009). However, Cieslinski et al. (1996) showed that very little Cd accumulated in the newly emerged spikes at heading stage in durum wheat. They suggested that high accumulation of Cd in grain probably reflected the redistribution of Cd. It has been reported that the remobilization of several metals, such as Cu, Zn, Fe, and Mn, contributed to the metals accumulation in grain during the grain-filling stage in wheat (Garnett and Graham 2005). Harris and Taylor (2001) suggested that the elevated remobilization of Cd from leaf and stem to the maturing grain may result in the high accumulation of Cd in durum wheat grain. Kashiwagi et al. (2009) also reported the rapid decrease of Cd content in rice leaves and suggested that leaves were the most important source of Cd accumulated in grain. However, there is no direct evidence that Cd remobilization contributes to Cd accumulation in rice grain at harvest.

In the present study, two rice cultivars that exhibit different abilities of Cd accumulation in grain were evaluated for the

accumulation and distribution of Cd absorbed at different growth stages to investigate how Cd absorbed at different growth stages is distributed among rice plant organs and what processes mediate the Cd accumulation in rice grain at harvest.

Materials and Methods

Plant materials and growth conditions

A *japonica* rice cultivar 'Chucheong' selected as a low Cd accumulating cultivar in grain and a *tongil*-type cultivar 'Milyang23' as a high Cd-accumulating cultivar were used for sand culture experiment. Rice seeds were soaked and disinfected in water containing 1/12,000 prochloraz for 48 h at 25 °C, germinated under moist conditions at 30 °C for another 30 h, and then the germinated seeds were seeded on April 20, 2008 and grown in uncontaminated soil. After 40 days, the seedlings at about the 7-leaf stage were transplanted into the 1/5000a Wagner pot (three plants per pot) containing 2.5 kg of siliceous sand that was sieved with 1.5 mm mesh, washed several times to remove small sand and organic matters, and then air-dried. After transplanting, three pots were placed in a plastic container (51 x 35 x 30 cm) filled with 25 L nutrient solution. Nutrient solution was prepared according to Yoshida et al. (1971): NaH₂PO₄·2H₂O (10 ppm), K₂SO₄ (20 ppm), CaCl₂ (40 ppm), MgSO₄·7H₂O (40 ppm), MnCl₂·4H₂O (0.5 ppm), (NH₄)₂MoO₇·4H₂O (0.05 ppm), H₃BO₃ (0.2 ppm), ZnSO₄·7H₂O (0.01 ppm), CuSO₄·5H₂O (0.01 ppm), and FeCl₃·6H₂O (2 ppm). The nitrogen was applied as NH₄NO₃. Nitrogen level was varied as: 40 ppm until 3 weeks after transplanting; 80 ppm at maximum tiller number stage; 40 ppm at 2 weeks after flowering and 0 ppm at maturity (Yoshida et al. 1971). The culture solution was adjusted to pH 5 with 1.0 N NaOH. Rice was grown in a side-opened plastic house with natural temperature and light.

Cadmium treatment

After transplanting, rice plants were grown in the culture solution until harvest. Cd was treated for 2 weeks by replacing the culture solution with culture solution containing 2 ppm CdCl₂ at four different growth stages: vegetative growth stage before panicle initiation (3 to 5 weeks after transplanting, BPI), panicle initiation stage (5 to 7 weeks after transplanting, API), early ripening stage (heading to 2 weeks after heading, ER), and

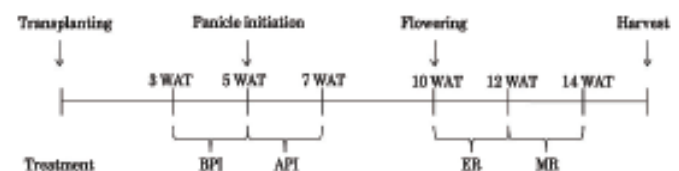


Fig. 1. Cd-treatment stage in the present experiment. Cd was treated by replacing the culture solution with that containing 2 ppm CdCl₂ for 2 weeks at four different growth stages. WAT, weeks after transplanting. BPI, vegetative growth stage before panicle initiation (3 to 5 weeks after transplanting); API, panicle initiation stage (5 to 7 weeks after transplanting); ER, early ripening stage (heading to 2 weeks after heading); MR, middle ripening stage (2 to 4 weeks after heading).

Table 1. Biomass of rice plant (g DW pot⁻¹) treated with 2 ppm Cd for 2 weeks at different growth stages.

Cultivar	Treatment stage ¹⁾	Total	Plant				
			Root	Shoot	Shoot		
					Stem ⁴⁾	Leaf	Grain
Chucheong	Control	86.2 ab ³⁾	10.9 a	75.4 a	28.2 b	35.6 a	
	BPI	64.4 d	7.3 c	57.1 c	23.0 c	25.8 c	
	API	74.6 c	8.1 c	66.5 b	29.0 ab	30.5 bc	
	ER	80.5 bc	8.5 bc	72.0 ab	31.1 ab	33.2 ab	
	MR	89.4 a	10.1 ab	79.3 a	32.7 a	35.3 ab	
	Mean ²⁾	77.3	8.5	68.8	28.9	31.2	
Milyang23	Control	82.4 a	8.5 a	73.9 b	23.4 b	38.9 a	
	BPI	51.9 d	6.1 c	45.8 e	14.3 b	22.5 d	
	API	59.9 c	6.5 bc	53.4 d	18.2c	26.5 c	
	ER	72.7 b	7.6 ab	65.1 c	22.1 b	33.4 b	
	MR	89.4 a	7.7 a	81.7 a	32.8a	36.7 a	
	Mean	68.5	7.0	61.5	21.8	29.8	
F value	Cultivar (C)	14.6**	16.3**	13.5**	51.9**	2.1	
	Treatment (T)	33.7**	6.2**	39.4**	35.8**	27.6**	
	CxT	2.0	0.8	3.0*	6.2**	1.7	

¹⁾BPI; before panicle initiation; API; at panicle initiation, ER; early ripening stage, MR, middle ripening stage.

²⁾ Mean was calculated as the average value of four treatments, not including control.

³⁾ Values followed by the same letters within each column are not different significantly at the 0.05 probability level among treatments within cultivar.

* and ** denote significance at 0.05 and 0.01 probability level, respectively.

⁴⁾Include leaf sheath

middle ripening stage (2 to 4 weeks after heading, MR) as in Fig. 1. Each treatment has four replications, i.e. four pots, and the nutrient solution was renewed every 4 to 5 days during and after Cd treatment until harvest.

Sample preparation and Cd analysis

At maturity, whole rice plants were harvested and washed with tap water and rinsed with distilled water. The plants were divided into root, stem (sheath + culm), leaf blade, and grain, oven-dried at 70 °C to constant weight, and their weights were recorded. All samples were ground to powders for heavy metal analysis. Plant samples (2.0 g) were digested with a 20 ml solution containing 87% of concentrated HNO₃ and 13% of concentrated HClO₄ (Ince et al. 1999). The concentrations of heavy metals in digested solutions were determined using an atomic absorption spectrophotometer (AA-6401, Shimadzu, Japan).

Statistical analysis

Statistical analyses for ANOVA and LSD were performed using SAS 9.1 (SAS Inc. USA).

Results

Growth

Plant growth was affected differentially according to the treatment stage of 2 ppm Cd, while no differential response of plant growth to Cd treatment stage between the two tested cultivars (Table 1). The two rice cultivars showed no significant difference in total biomass of plant between the control and the Cd

Table 2. Cd concentration (mg kg⁻¹ DW) in whole plant and each organ of rice plant treated with 2 ppm Cd for 2 weeks at different growth stages.

Cultivar	Treatment stage ¹⁾	Total	Plant				
			Root	Shoot	Shoot		
					Stem ³⁾	Leaf	Grain
Chucheong	BPI	11.26 a ²⁾	74.77 b	3.14 a	6.54 a	0.97 a	0.87 a
	API	10.67 a	77.43 b	2.65 b	5.10 b	0.94 a	0.70 b
	ER	11.10 a	85.29 a	2.46 b	4.96 b	0.66 b	0.60 c
	MR	8.31 b	61.92 c	1.48 c	3.25 c	0.44 b	0.19 d
	Mean	10.33	74.85	2.43	4.96	0.75	0.59
	Milyang23	BPI	9.63 a	37.49 b	5.97 a	12.73 a	1.26 a
	API	9.85 a	44.46 a	5.68 a	12.06 a	1.18 a	2.83 b
	ER	7.93 b	42.01 ab	3.98 b	9.73 a	0.80 b	1.05 c
	MR	5.65 c	38.75 ab	2.54 c	5.14 b	0.67 b	0.86 c
	Mean	8.27	40.68	4.54	9.92	0.98	2.09
F value	Cultivar (C)	19.7**	153.2**	71.3**	48.1**	16.3**	342.0**
	Treatment (T)	11.8**	4.5*	20.5**	11.1**	23.2**	97.5**
	CxT	1.3	2.4	3.8*	2.5	0.3	47.3**

¹⁾ BPI; before panicle initiation; API; at panicle initiation, ER; early ripening stage, MR, middle ripening stage..

²⁾ Values followed by the same letters within each column are not different at the 0.05 level among treatments within cultivar.

* and ** denote significance at 0.05 and 0.01 probability level, respectively.

³⁾Include leaf sheath

treatment at middle ripening stage (MR). However, total biomass of plant was significantly reduced by Cd treatments at the other growth stages, the reduction being greater at the treatment of earlier growth stage. The biomass of each organ of rice plant was affected by Cd treatment similarly to the total plant biomass with minor variation.

Cd concentration

Cd concentrations in whole rice plant and each organ were significantly different between cultivars and among Cd treatment stages, and showed no interaction between cultivar and Cd treatment stage in all rice organs except grain (Table 2). A japonica cultivar 'Chucheong' was significantly higher in Cd concentration in root than a tongil-type cultivar 'Milyang23' while vice versa in shoot and shoot organs. Among shoot organs, grain especially showed a marked varietal difference in Cd concentration. Grain Cd concentration on average over treatment stages was 3.5 times higher in 'Milyang23' than in 'Chucheong'.

Cd concentration in shoot and shoot organs tended to increase as Cd was treated at earlier growth stage, showing similar tendency in both cultivars. Cd concentration in grain was 4.2 ('Milyang23') and 4.7 ('Chucheong') times higher in Cd treatment before panicle initiation (BPI treatment) than in Cd treatment at middle grain-filling stage (MR treatment). Contrary to shoot Cd concentration, root Cd concentration was significantly lower in the BPI treatment.

Cd accumulation and distribution

Cd accumulations in whole rice plants and each organ were significantly different between cultivars and among Cd treatment stages, and exhibited no cultivar x treatment stage interactions except for grain Cd accumulation (Table 3).

On average over Cd treatment stages, 'Chucheong' accumu-

Table 3. Cd accumulation ($\mu\text{g pot}^{-1}$) in whole plant and each organ of rice plant treated with 2 ppm Cd treatment for 2 weeks at different growth stages.

Cultivar	Treatment stage ¹⁾	Total	Plant				
			Root	Shoot	Shoot		
					Stem ³⁾	Leaf	Grain
Chucheong	BPI	723.7 b ²⁾	545.3 b	178.4 a	148.0 a	7.9 a	22.5 a
	API	799.4 ab	624.2 ab	175.2 a	147.1 a	6.7 ab	21.4 a
	ER	890.7 a	717.9 a	172.8 a	147.8 a	5.0 b	20.0 a
	MR	740.0 b	622.8 ab	117.3 b	105.4 b	5.1 b	6.8 b
	Mean	788.4	627.5	160.9	137.1	6.2	17.7
Milyang23	BPI	494.6 c	223.5 b	271.1 a	179.2 ab	11.5 a	80.4 a
	API	583.4 a	281.8 a	301.6 a	216.3 a	10.2 ab	75.0 a
	ER	570.6 ab	313.8 a	256.8 a	214.1 a	7.6 c	35.0 b
	MR	502.0 bc	294.7 a	207.3 b	167.6 b	8.1 bc	31.6 b
	Mean	537.6	278.5	259.2	194.3	9.4	55.5
F value	Cultivar (C)	86.7**	202.2**	109.5**	40.6**	40.8**	322.0**
	Treatment (T)	4.6*	4.8**	12.6**	5.6**	10.6**	55.6**
	CxT	0.8	0.6	1.0	1.0	0.2	25.2**

¹⁾ BPI, before panicle initiation; API, after panicle initiation; ER, early ripening stage; MR, middle ripening stage.

²⁾ Values followed by the same letters within each column are not different at the 0.05 level among treatments within cultivar.

* and ** significant at 0.05 and 0.01 level, respectively.

³⁾ Include leaf sheath

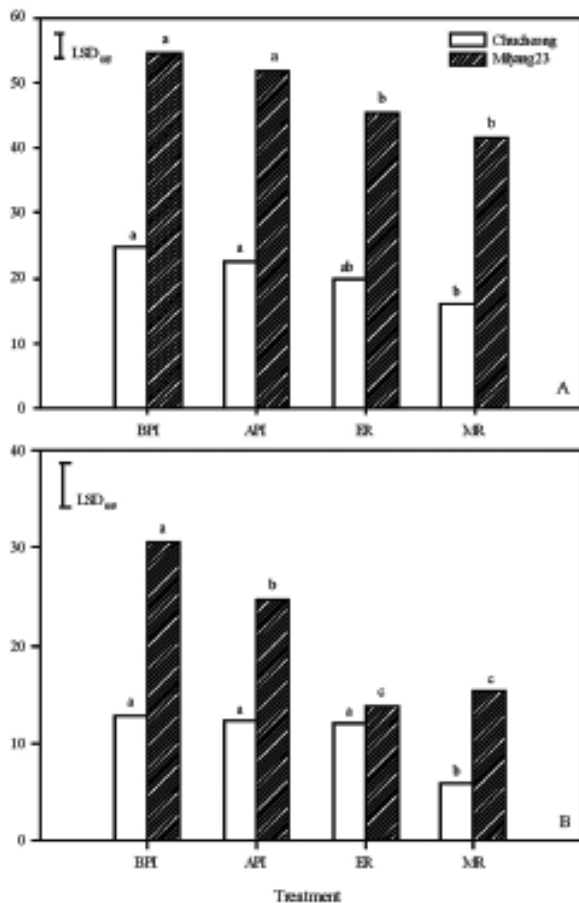


Fig. 2. Root-shoot translocation (A) and shoot-grain remobilization (B) of Cd in two rice cultivars 'Chucheong' and 'Milyang23' subjected to 2 ppm Cd treatment for 2 weeks at different growth stages. The same letters above the bars denotes no significant difference at the 0.05 probability level among treatments within cultivar. The LSD bar in each graph is for the comparison between two cultivars.

lated, (absorbed) a greater amount of Cd into whole rice plants than 'Milyang23'. However, 'Milyang23' accumulated significantly greater amounts of Cd in shoot compared to 'Chucheong' as the former translocated much higher proportion of the absorbed Cd to shoot than the latter as shown in Fig. 2. The average translocation to shoot was 20.4 and 48.2% in 'Chucheong' and 'Milyang23', respectively. Cd accumulated in shoot was redistributed into grain differentially between the two cultivars. As in Fig. 2-2, 'Milyang23' redistributed 15.2 to 29.7% (on average, 21.4%) of shoot-accumulated Cd to grain while 'Chucheong' only 5.8 to 12.6% (on average, 11.0%). Therefore, 'Milyang23' accumulated Cd into grain more than three times compared to 'Chucheong'.

Cd accumulations in whole rice plants were lower in Cd treatments before panicle initiation (BPI) and at the late ripening stage (MR) than those in Cd treatment at panicle initiation (API) and early ripening stage (ER). However, the Cd accumulation in shoot was not significantly different among Cd treatment stages except MR treatment that showed the lowest Cd accumulation in shoot as the proportion of Cd translocated to shoot tended to decrease with Cd treatment at later growth stages (Fig. 2). In the two cultivars, Cd accumulation in grain was greater in Cd treatments at early growth stages rather than in Cd treatments at late growth stages because not only the translocation to shoot but also the remobilization to grain was greater in Cd treatments at earlier growth stages (Table 3). Cd accumulation into grain was not different among Cd treatments before early ripening stage (BPI, API, and ER treatments) in 'Chucheong' and before the heading stage (BPI and API treatments) in 'Milyang23', while decreased drastically in the Cd treatments later than those stages.

Discussion

Cd uptake by root, xylem translocation from root to shoot, and phloem remobilization from shoot to seed are three physiological processes that regulate the Cd accumulation in plant seed (Clemens et al. 2002). Based on the results that a strong correlation was found between Cd levels in xylem sap and shoot and grain among 69 rice accessions, Uruguchi et al. (2009) concluded that root-shoot Cd translocation via the xylem is the major and common process determining the Cd accumulation level in grain and shoot of rice plant. However, there was very large variation of brown rice Cd concentration among rice cultivars with similar Cd concentration in xylem sap in their report, suggesting that root-shoot translocation might not be the only major process causing the genotypic variation of Cd accumulation in rice grain. In our experiment, the japonica cultivar 'Chucheong' accumulated a 1.5 times greater amount of Cd in whole rice plant, while it showed more than three times lower Cd concentration and accumulation in grain compared with tongil-type cultivar 'Milyang23' (Tables 2 and 3). Although 'Milyang23' showed a lower ability of Cd uptake, the absorbed Cd was translocated more than two times to shoot, leading to the significantly greater accumulation of Cd in shoot compared to

'Chucheong' (Table 3 and Fig. 2A). Moreover, on average across Cd treatment stages, 'Milyang23' remobilized 21.4% of shoot-accumulated Cd to grain while 'Chucheong' was only 11.0%. Therefore, 'Milyang23' accumulated Cd into grain more than three times compared to 'Chucheong'. Our previous study also indicated that the significantly higher grain Cd concentration in tongil-type rice than in temperate japonica rice could be attributed to the higher shoot-grain remobilization of tongil-type. These results indicated that both root-shoot translocation and shoot-grain remobilization involved in the regulation of Cd accumulation in grain and resulted in the higher grain Cd concentration of 'Milyang23' than that of 'Chucheong', but not the Cd uptake.

Leaf is the most important source of Cd accumulated in the panicle; the lower leaf shows the largest negative translocation but the flag leaf is always the Cd sink (Kashiwagi et al. 2009). It is evident that cell death during leaf senescence accelerates the release of elements from leaf tissue. It has been reported that leaf senescence significantly enhances the redistribution of several elements, such as N, Fe, Cu, Mn, and Zn, from vegetative tissue into rice and wheat grain (Garnett and Graham 2005; Mae 2004; Sheehy et al. 2004). This study and our previous experiment indicated that tongil-type cultivars showed significantly higher shoot-grain Cd remobilization ratio than temperate japonica (Fig. 2B). Considering the report that tongil-type rice cultivars, particularly 'Milyang23', exhibited markedly rapid leaf senescence compared to temperate japonica (Park et al. 2006), it is possible to infer that higher shoot-grain Cd remobilization ratio of tongil-type would be associated with the more rapid leaf senescence.

The evidence from wheat suggested that Cd accumulation in grain is related to phloem-mediated Cd transport (Hart et al. 1998). In rice, 91 - 100% of grain-accumulated Cd is transported via the phloem (Tanaka et al. 2007). There are two possible pathways that translocate Cd into rice grain via the phloem: one pathway is that Cd is accumulated first in vegetative tissues and then redistributed into grain during grain ripening stage (remobilization); the other one is that Cd absorbed in root is transported directly into grain after the xylem-phloem transfer (direct transport). It has been reported that Cd loaded to xylem could be transferred to the phloem at nodes and transported directly into grains via the phloem without passing through the leaf (Harris and Taylor 2001; Uraguchi et al. 2009). Although it has been reported that the remobilization of several metals, such as Cu, Zn, Fe, and Mn, contributed to the metals accumulation in grain during grain-filling stage in wheat (Garnett and Graham 2005), there was little direct evidence of Cd remobilization in rice. In this experiment, we grew rice plants in nutrient solution containing 2 ppm Cd for 2 weeks at four different growth stages, and hence rice plants did not absorb any Cd except for these four growth stages (Fig. 1). Cd absorbed by rice plants before panicle initiation stage (BPI treatment) was detected in rice grain at harvest (Tables 2 and 3). Due to the fact that no reproductive organ emerged before panicle initiation stage, Cd accumulation in grain at harvest must be derived from the remobilization of Cd absorbed at the vegetative stage. Ciesli skinet al. (1996) reported

that very little Cd accumulated in newly emerged spikes in maturing durum wheat. Kashiwagi et al. (2009) also reported that Cd translocation from root to shoot after heading did not influence the Cd accumulation in brown rice. In this experiment, Cd concentration in grain tended to increase as Cd was treated at earlier growth stage (Table 2). Although the higher grain Cd concentration in Cd treatment at earlier growth stage might have been partly attributable to the lower grain biomass (Table 1), Cd accumulation into grain tended to increase as Cd was treated at earlier growth stage (Table 3). Remobilization was the only pathway of grain Cd accumulation in Cd treatment before heading (BPI and API treatments), while both direct transport and remobilization might have contributed to Cd accumulation in grain in Cd treatment after heading (ER and MR treatments) (Fig. 1). In fact, the Cd accumulation in shoot was not significantly different among Cd in treatment stages except MR treatment in both tested cultivars (Table 3). However, the significant smaller proportion of shoot-accumulated Cd after heading in 'Milyang23' and after early ripening stage in 'Chucheong' was distributed into grain. It indicated that the rapidly decreasing translocation of shoot-accumulated Cd after heading resulted in the higher grain Cd concentration and accumulation of Cd treatment before heading (BPI and API treatments) compared to Cd treatment after heading (ER and MR treatments) (Tables 2 and 3; Fig. 2B). Kashiwagi et al. (2009) reported that root-shoot translocation did not influence Cd accumulation in brown rice after heading and suggested that restricted root-shoot translocation might limit Cd accumulation in brown rice by controlling the shoot Cd pools that can be remobilized to grain. Taken together, Cd absorbed before heading stage is the more important source of Cd accumulated in rice grain at harvest rather than that after heading stage and remobilization during leaf senescence is the major process regulating Cd accumulation in rice grain.

Cd accumulated in rice seeds mainly bind with protein (Yang et al. 1999). Increase of supplied N level increases Cd uptake in rice (Du et al. 2009). The Cd distribution pattern observed in our study was very similar to that of N reported by Sheehy et al. (2004) (Table 3 and Fig. 2B). Specific proteins and phytochelatins are related to the heavy metals transport and tolerance (Belouchi et al. 1997; DalCorso et al. 2008; Klaassen et al. 1999; Thomine et al. 2000; Vazquez et al. 2009). Additionally, Weigel et al. (1980) reported that most of the Cd was bound to peptides and/or some low molecular weight proteins. Based on this evidence, it can be supposed that Cd absorbed at the vegetative growth stage might accumulate in vegetative organs binding with protein and then transport into grain following the process of protein remobilization. However, further study is required to understand the correlation between Cd remobilization and the protein binding process.

In conclusion, our experiment demonstrated that not only xylem root-shoot translocation but also Cd remobilization via phloem play important roles in Cd accumulating in rice grain, and due to the high remobilization of Cd absorbed before heading stage it is required that Cd must be isolated from the rice plant in the entire rice growing season for safe rice production.

Acknowledgments

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