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Article *in* Plant and Soil · October 2011

DOI: 10.1007/s11104-011-0829-4

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REGULAR ARTICLE

The timing of grain Cd accumulation in rice plants: the relative importance of remobilisation within the plant and root Cd uptake post-flowering

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Received: 1 December 2010 / Accepted: 17 May 2011 / Published online: 27 May 2011

Abstract The pathways by which Cd is accumulated in rice grain are not well understood, in particular the components attributable to direct transfer from the root, and to remobilisation of Cd previously accumulated in other plant parts. In order to observe the timing of Cd accumulation in rice plants and determine the major period for accumulation of Cd which can be translocated to the grain, Cd was supplied to the roots of rice plants grown under static hydroponic conditions at a non-toxic, environmentally relevant concentration (50 nM), according to three different timing regimes: (1) Pre-flowering Cd, (2) Post-flowering Cd, or (3) Continuous Cd. The rate of accumulation of Cd in the developing grain was monitored by harvesting immature rice panicles at four time points prior to a final harvest. Nearly all grain Cd was accumulated within 16 days of anthesis and the contribution of post-flowering Cd uptake was evident from 7 days after flowering. It was estimated that 60% of the final grain Cd content was remobilised from that accumulated by the plant prior to flowering and the other 40% came from uptake during grain maturation. This study shows that Cd uptake from the root to the grain in rice is indeed possible post-flowering and it is an important source of grain Cd.

Keywords Cadmium · Rice · Heavy metal · Translocation

Responsible Editor: Jian Feng Ma.

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Introduction

Contamination of rice paddy fields is a human health concern because rice plants are able to take up the metal from soils and accumulate it in the grain at concentrations that can lead to accumulative toxicity in humans. Rice has been found to be the primary source of Cd intake for populations exposed to locally polluted soils in the documented cases in Japan and China (Jin et al. 2004; Nordberg et al. 2002). Nordberg et al. (2002), Nordberg (2003) also found that in China, even populations not living in areas of high soil Cd concentrations were still commonly exposed to levels greater than that of Europe. There is therefore a need to reduce the human intake of Cd through rice. The translocation of Cd around the

plant, which enables grain accumulation, is important to understand for the goal of reducing human exposure to Cd in food.

The mechanisms determining translocation of Cd within plants are not fully understood, however, it is known that Cd is both phloem and xylem mobile (Reid et al. 2003; Riesen and Feller 2005; Uraguchi et al. 2009). Direct quantitative measurement of the contribution of xylem and phloem is difficult, but insight into the relative amounts and likely pathways may be inferred by examining the timing of Cd loading into the grain.

Sankaran and Ebbs (2008) demonstrated with Indian mustard that the Cd supplied to the roots during seed maturation was most significant in determining seed Cd concentration. They observed that when Cd supply was removed prior to seed set, seed content was low; and for the same reason, seed Cd content of plants supplied with Cd for their whole lifecycle was not significantly different to plants that only received Cd during seed set. In contrast, the results of Chan and Hale (2004) from studies on durum wheat suggested that there was no additional uptake of Cd from roots to grain during heading stage, and rather, remobilisation from the shoots was the greatest source.

Kashiwagi et al. (2009) conducted a study on the above ground remobilisation of Cd in rice. They concluded that post-heading, when the rice panicle has already formed, translocation of Cd only occurs in two distinct pathways: (1) from leaves to brown rice and (2) from roots to aerial parts (including the ears, but in components other than brown rice). However, these results were collected from soil grown plants, which while providing realistic conditions, gave no ability to manipulate timing of Cd uptake.

The study reported here was designed to test the theory that grain Cd content is purely a product of Cd accumulation prior to heading/flowering, which would indicate remobilisation in phloem of Cd previously accumulated in other tissues. While other studies have been reported on Cd applied to rice hydroponically, these frequently use very high concentrations of Cd so that the accumulation characteristics are clouded by potential Cd toxicity to the plant. The concentration of Cd used in this study was 50 nM (McLaughlin et al. 1998; Smolders and McLaughlin 1996), which is at least three orders of magnitude lower than that of many hydroponic studies, and 40–50 times lower than the concentrations used in some similar reports on nutrition and Cd uptake in rice (Nakanishi et al. 2006; Nwugo and Huerta 2008).

Although it is known that the availability of Cd in the soil can vary with paddy-field redox changes associated with flooding and drainage (Arao et al. 2009), this study tested hydroponically grown rice plants using one concentration of Cd, supplied according to three different timing regimes, to determine in which period of the lifecycle most of the grain Cd is taken up as a result of physiological processes. Cd was supplied (1) from transplanting until beginning of flowering, (2) from beginning of flowering until harvest, or (3) continuously. As seed development in rice begins immediately after flowering (Krishnan and Dayanandan 2003), these treatments allowed the relative importance of shoot Cd remobilisation and root Cd uptake during grain development to be compared for rice. In addition to the final grain Cd content, individual seed panicles of the plants grown in this experiment were harvested during grain development in order to observe the accumulation of cadmium into the brown rice. This influx of cadmium into the grain was then contrasted with carbohydrate loading into the grain by comparison with the increase in grain dry weight.

Materials and methods

Germinating seed and plant culture

A Chinese rice breeding line from the Jiaxing Academy of Agricultural Sciences, Zhejiang, was chosen for use in this experiment. This was a rice variety designated N07-60 (*Oryza sativa* L., subsp. *Japonica*). Relative to other Chinese rice varieties, when grown in Cd-contaminated paddy soil in pot experiments, this variety was shown to be a lower accumulator of grain Cd. However, when tested in Cd radioinflux experiments and potting mix experiments in this lab, its Cd uptake characteristics to shoots and grain were seen to be similar to other varieties with reportedly higher grain Cd accumulation.

Seeds were surface sterilised with 1.5% hypochlorite bleach solution for 20 mins and then rinsed four times with reverse-osmosis (RO) water before being placed out on trays with paper towel moistened with deionised water (dH_2O). The seeds were germinated

in the dark for 7 days (20–28°C). Following germination, the seedlings were initially grown on a 20% modified Hoagland's Solution in a single tub for 3 weeks, with 12 h of light at 27°C±1°C, and 12 h darkness at 20°C.

Twenty eight days after germination (28 DAS), the plants were transferred to individual tall pots (100 mm wide) with 2.5 L of the following hydroponic solution: 1.5 mM NH_4NO_3; 1 mM KNO_3; 1 mM $Ca(NO_3)_2$; 1 mM $MgSO_4$; 0.1 mM KH_2PO_4; 50 μM FeNaEDTA; 5 μM $ZnSO_4$; 0.5 μM $CuSO_4$; 5 μM $MnCl_2$; 50 μM H_3BO_3 and 0.1 μM MoO_4. To more closely match soil conditions, Si was also added at a rate of 0.83 mM, equivalent to 50 $mg.kg^{-1}$ SiO_2 (Yoshida 1976).

Plants were then grown in a controlled-environment growth room, with 12 h of light, at 30°C±1°C, and 12 h darkness, at 25°C. Relative humidity was not controlled, but daytime measurements were in the range 60–75%. The nutrient solutions were changed approximately every 6 days in the first month after transplanting, and then every fourth or fifth day until maturity. Pots were topped up with deionised water every 1–2 days, as required, to replace loss by transpiration. Nitrogen was supplied in the growth solution as both ammonium and nitrate, which was found to moderate the pH at a fairly stable level (Yoshida 1976).

Cd, at 50 nM, was applied according to the following treatment groups, (1) Pre-flowering Cd, (2) Post-flowering Cd, or (3) Continuous Cd. The level of micronutrients in this solution was relatively high to ensure a realistic ratio between those elements and Cd; for example, the Zn:Cd ratio was 100:1, which is known to be close to the conditions found in the soil environment (Chaney et al. 1999). The hydroponic solutions used were analysed using GEOCHEM-PC (Parker et al. 1995) and no significant Cd precipitation was found. There were 12 plants in total, with 4 replicates per treatment group. The solutions were made up with deionised water (dH_2O) and adjusted to pH 5.0 using H_2SO_4.

Cd treatment began from the day of transplanting for the Pre-flowering and Continuous Cd treatments (28 DAS). The first of the rice panicles began to flower around 74 DAS. The Cd treatment was switched at the first nutrient solution change after the beginning of flowering, which was 3 days after the flowering of the first panicles for the majority of plants (77 DAS). As per Yoshida (1976), the nutrient solution was scaled-back 2 weeks after flowering (89 DAS) to reduce excessive vegetative growth; all the macronutrients were removed, leaving Fe, Si, the other micronutrients and Cd in the solutions. These treatments continued until maturity, with the plants harvested at 120 DAS (92 days after transplanting). In total, during this experiment the Pre-flowering Cd treatment received Cd in the nutrient solution for 49 days, and the Post-flowering Cd treatment received Cd for 43 days.

Mid-flowering panicle harvesting

In order to observe the accumulation of Cd in the developing rice grains, panicles were harvested from each plant at multiple time points prior to maturity. Panicles were excised from each plant on four occasions, at 10, 16, 21 and 34 days after their approximate flowering date. After oven drying at 60°C for 4 days, a representative subsample of the immature rice (with glumes removed) was taken from the panicle to analyse for Cd content. The subsamples were weighed and counted to provide an average dry weight per rice grain at each time point. The Cd content of these samples was thereafter compared on a Cd per grain basis. The third flowering panicle of each plant was preserved until the final harvest to provide a representative panicle at maturity.

Final plant harvest

One week prior to harvest, 1 mM $CaCl_2$ was added to each of the nutrient solutions to aid in the removal of extracellular Cd. On the day of harvest, the roots of all plants were rinsed briefly with dH_2O and then desorbed, with some agitation, for 30 min in a solution containing 5 mM $CaCl_2$ and 0.5 mM citric acid, adjusted to pH 3.5 with NaOH. Following this, the roots and the remainder of the plants were dried at 70°C for 5 days. After drying, the plants were separated and weighed in the following plant tissue categories for analysis: roots, lower stem, upper leaf blades, rice grain (of remaining panicles), and flag leaves (adjacent to the panicles harvested last). To avoid any complication caused by shoot material that had occasionally been submerged in the nutrient solution, the bottom 10 cm of the stem samples was discarded after weighing.

Plant tissue analysis

Plant tissue samples (except the grain) were ground in a mechanical grinder to <2 mm and then sieved using a no. 60 sieve (0.3 mm) to obtain very fine material. The brown rice samples were ground to a powder using either mortar and pestles, or an electric household grinder. The rice was not milled to obtain polished rice because it has been confirmed in previous studies that there is no significant difference between the Cd concentrations of rice bran and endosperm (Williams et al. 2009). Prior to weighing for chemical digestion, all the samples were re-dried in an oven to ensure minimum reabsorption of ambient moisture.

Cd content was analysed using graphite furnace atomic absorbance spectroscopy (GF-AAS) for all samples except the root material, for which flame-AAS was used. Plant material was digested prior to analysis following the microwave oven method of Adomako et al. (2009). The samples were digested in batches of 32 samples. For quality control, two replicate digests were performed for each set of 16, plus one blank digest, one with a Cd spike (+0.5 μg), and one containing rice powder certified-reference-material (GBW10010; Cd concentration=0.087 $mg.kg^{-1}$). The amount of tissue digested depended on the expected Cd concentration, with 0.1 g used for root and shoot samples, 0.3 g used for the rice samples, and 0.4 g for the rice CRM. After digestion with 2 ml of concentrated HNO_3, the digests were diluted by weight with 18 MΩ-cm water to 50.0 g. The shoot samples were then further diluted as required to match the calibrated range of the GF-AAS (0.1–2.5 μg/L). The grain digests were used without further dilution. The root digests were also used undiluted for FAAS (calibrated for the range of 50–800 μg/L).

Results

There was a clear trend of decreasing Cd concentration from bottom to the top of the plants (Table 1). This trend began in the roots, with Cd concentrations in the range 30–80 mg/kg, whereas shoot concentrations were 0.35–1.8 mg/kg, and rice grain Cd concentrations were 0.05–0.3 mg/kg. The actual distribution of Cd is more closely represented by the total Cd content of the various plant parts (Table 2). The majority of the plant Cd accumulated in the roots, despite the shoots comprising the most of the biomass. No significant differences between plant biomass were found between the three treatments.

There was an immediate effect of the switch in Cd treatments on the rice grain Cd content in the three different treatments. The Pre-flowering Cd treatment stopped receiving Cd approximately 3 days after the commencement of flowering of the first panicle. When contrasted with the Continuous treatment, the Cd supplied in the first 7 days after the treatment change produced a difference in grain Cd concentration that was visible from the first harvest date, as seen in Fig. 1. Due to replicate variation in grain weight at the first two harvest dates, when looking at Cd content per grain (Fig. 2), the Pre-flowering treatment was not significantly different to the Continuous treatment (by L.S.D.) until 21 days after flowering (DAF). Nevertheless, over the four sampling dates there is a trend of difference in Cd content. The Cd supplied during the post-flowering phase produced an approximate 40% difference in Cd concentration between the Pre-flowering Cd and Continuous Cd plants at 10 DAF as well as at 34 DAF (Figs. 1 and 3).

In the same way, within 7 days of Cd supply to the Post-flowering treatment (i.e. 10 DAF), Cd was measurable in the grain. In Fig. 2, it can be seen that the arithmetic addition of the Cd accumulation of the Pre-flowering Cd and the Post-flowering Cd treatments matches that of the Continuous treatment fairly closely.

Using the Continuous Cd treatment as the 'full Cd treatment,' the contribution of the pre- and post-flowering periods of Cd application was calculated. It can be seen that pre-flowering Cd uptake made a greater contribution to shoot Cd concentration than it did to total root Cd and grain Cd concentration, 74%, 46% and 60% respectively (Fig. 3). Conversely, post-flowering Cd uptake favoured grain and stems over the leaves in the above ground parts. The stem/sheath Cd concentration of the Post-flowering Cd plants was approximately double the leaf-blade Cd concentration, compared to both the other treatments where it was only 50% greater (Table 1). In the same way, there were differences in the grain-to-shoot Cd ratio between the three treatments. The Pre-flowering Cd treatment had a grain Cd to shoot Cd concentration ratio of only 0.1 and for the Continuous Cd it was

Table 1 Distribution of Cd in hydroponically grown rice plants receiving Cd for different portions of their lifecycle (Pre-flowering Cd; Post-flowering Cd; and Continuous Cd supply). Roots were rinsed with dH_20 following harvest and then desorbed with $CaCl_2$-citrate solution of pH 3.5 for 30 mins (with some agitation). Plant tissue Cd concentration in mature rice plants ($mg.kg_{DW}^{-1}$). Plant parts from final harvest except for grain Cd which is mean concentration 34 days post-anthesis. Different letters within individual columns represent means that are significantly different according to the L.S.D. method (5% level)

	Roots		Stems/sheaths		Leaf blades		Flag-leaves		Average Shoot		Rice grain	
Pre-flowering Cd	33.4	a	1.38	b	0.91	b	0.19	b	1.2	b	0.12	a
Post-flowering Cd	37.0	a	0.49	a	0.24	a	0.06	a	0.4	a	0.09	a
Continuous Cd	72.6	b	1.88	c	1.25	c	0.30	c	1.6	c	0.20	b

0.13 (both±0.01 SE), while post-flowering Cd uptake led to much less shoot accumulation; that treatment had a grain-to-shoot Cd ratio of 0.23±0.02.

The loading of Cd into the developing rice grains was compared with total dry weight per grain (approximating the flow of carbohydrates) using the Cd data from the Continuous Cd treatment. The total Cd content per grain increased rapidly for the first 16 days after anthesis and reached a maximum after 21 days (Fig. 4). This coincided approximately with the "yellow-grain" stage, when the grains began to harden and change from green to yellow. The accumulation of Cd in the grain somewhat preceded full grain carbohydrate loading, although only by a few days (Fig. 4). This difference in accumulation rate is significant at 10 DAF, where the average grain dry weight was only 37% of mature grain, but average Cd content was 55% of the final level.

In contrast to the other treatments, there was a gradual increase in grain Cd content in the Post-flowering Cd plants (Fig. 2). The Cd level of these plants did not reach its maximum content until 34 DAF. This indicates that some amount of Cd accumulation must still be possible after 21 DAF.

Table 2 The average Cd content per plant found in the roots and shoots (not including grain), and the distribution between roots and shoots. Different letters within a single column represent means which are significantly different according to L.S.D. (5% level)

	Roots (μg)		Shoots (μg)		Root/shoot ratio
Pre-flowering Cd	121	a	30.1	b	4.0
Post-flowering Cd	139	a	10.2	a	13.6
Continuous Cd	280	b	39.3	c	7.1

Discussion

Plant Cd accumulation

This study has shown that Cd uptake by rice roots post-flowering contributes to the grain Cd content of the developing seed. This uptake is relatively fast, and shows that grain Cd concentration is not only a function of shoot Cd content, contrary to the assertions of Kashiwagi et al. (2009). In the rice plant, the majority of Cd content was actually found in the roots and so this is logically an important reservoir of Cd that could contribute to grain Cd content. While Cd accumulation in the shoot tissues slows considerably after the heading phase (when the majority of above ground tissue is present), the Cd accumulation in the roots continues. This accumulation is known to, in part, occur in root vacuoles whereby plants are able detoxify Cd; moving it actively across the tonoplast by transporters such as OsHMA3, a P_{1B}-type ATPase (Miyadate et al. 2011; Ueno et al. 2011; Ueno et al. 2010). In this experiment, only approximately half the root Cd content accumulated prior to flowering (Fig. 3).The mid-flowering harvesting of panicles in this experiment meant that the total final grain Cd, as a proportion of plant Cd, could not be determined. However, the amount present in the grain was very low. An estimate based on the final Cd grain Cd concentration would put it at less than 1%, similar to the calculated percentage found for the soil-grown rice plants by Yoneyama et al. (2010), where grain Cd was only 0.8% of plant Cd, despite accounting for 9.7% of the dry weight biomass.

There is an additive effect of Cd supplied during the pre and post-flowering phases of plant growth for

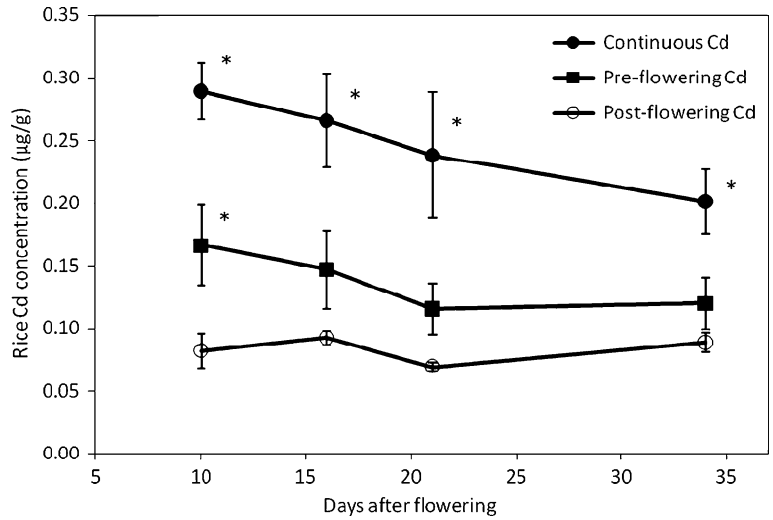

Fig. 1 Measured Cd concentration in the developing caryopses of hydroponic rice plants under three different Cd application timings (at 50 nM): Continuous Cd supply (*Closed circles*); Pre-flowering Cd (*Closed squares*); and Post-flowering Cd (*Open Circles*). Mean values are shown±SE. *Asterisks* represent means which were significantly different according to the L.S. D. method (5% level; the data from some sampling dates was log transformed prior to ANOVA)

the roots, shoots and grain of the plant (Figs. 1 and 3). The additive effect shows that Cd uptake in roots and translocation to the above-ground parts of plant continues throughout the plant's lifecycle.

It is important to note that in these experiments the transport characteristics of Cd were not compromised by Cd toxicity. No symptoms of toxicity were visible and the Cd concentrations of shoots and roots (Table 1) were both considerably lower than the concentrations reported to be toxicity threshold for rice, 5 $mg.kg^{-1}$ and 100 $mg.kg^{-1}$, respectively (Chino 1981). The tissue Cd concentrations found in these

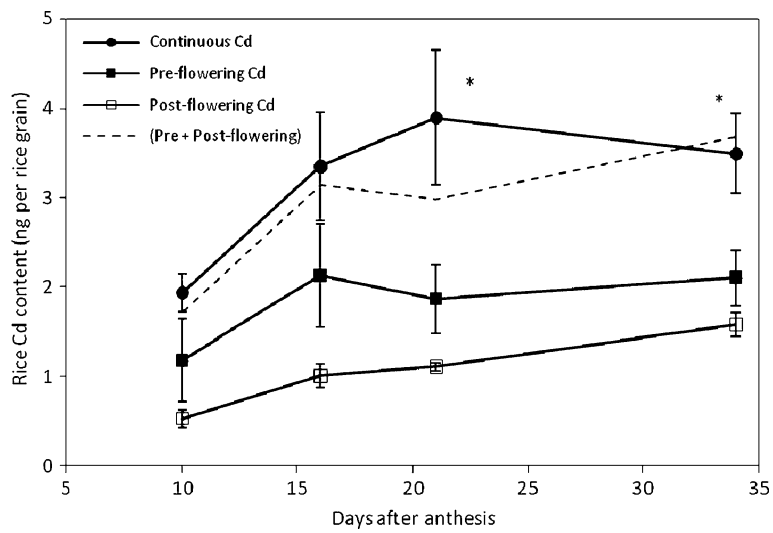

Fig. 2 Rice Cd accumulation in developing caryopses, expressed per grain, of hydroponic plants under three different Cd application timings (at 50 nM): Continuous Cd supply (*Closed circles*); Pre-flowering Cd (*Closed squares*); and Post-flowering Cd (*Open Squares*). Cd treatment was switched at the first nutrient solution change after the beginning of flowering, which was 3 days after the flowering of the first panicles for the majority of plants. Mean values are shown ± SE (n=4). Grain Cd accumulation was measured the rice caryopses of single panicles harvested per plant at four harvest dates. The *dashed line* shows the theoretical addition of the Pre-flowering and the Post-flowering Cd treatments

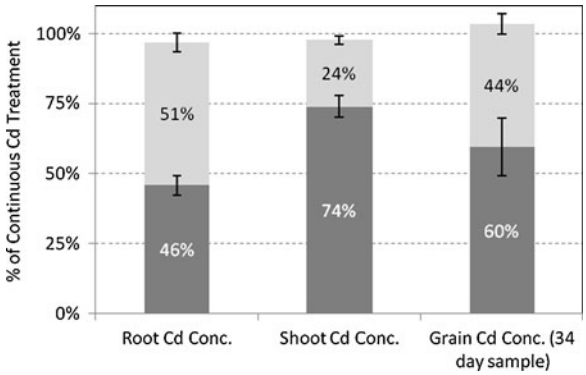

Fig. 3 Proportional contribution of pre-flowering and post-flowering Cd uptake to tissue Cd content of mature hydroponically grown plants (± SE, n=4). The Cd concentration in plants that received Cd before or after flowering is expressed as a percentage of the Cd accumulated with continuous Cd treatment. Dark bars represent Pre-flowering Cd treatment, i.e. Cd which was mobilised from shoot and root tissue into the grain. Lighter bars represent Post-flowering Cd treatment. The Continuous Cd treatment therefore equals 100% on this scale

plants (Table 1) shows a trend of decreasing Cd concentration from the roots to the grain in the plant, with relatively low transfer from root to shoot, commonly seen for Cd (Kukier and Chaney 2002). This result is indicative of both the vast capacity of the roots to accumulate Cd and the considerable lateral transfer of elements that occurs from the vascular pathways to the surrounding tissues. For example, during phloem translocation, 'leakage' of Cd is known to occur from the phloem as solutes move from source to sink (Harris and Taylor 2001). Fujimaki et al. (2010) demonstrated that, with root supplied Cd, shoot concentrations were greatest at the base of rice stems and decreased moving up the plant. They suggested that the binding of Cd to chelators within the xylem was one reason for this pattern.

Through sampling of rice at time points prior to maturation, it was seen here that the majority of rice grain Cd was accumulated during the early period of grain development, that is, within approximately 16 days after flowering (DAF) under these conditions. This result is in contrast to that of Zn, for which Jiang et al. (2007) found that significant grain Zn accumulation continued in the period from 22 DAF until maturity.

The measured timing of Cd accumulation in the rice grain showed that the majority of Cd content reached the grain prior to the yellow-grain stage of maturity. This result indicates that late-season drainage of paddy fields prior to harvest, which is associated with increased Cd availability, would not have a big impact on grain Cd content if it occurred after the yellow-grain stage (2–3 weeks after flowering). This conclusion is in agreement with the results of Arao et al. (2009), who concluded that flooding is only needed until 3 weeks after heading to effectively reduce rice Cd concentrations.

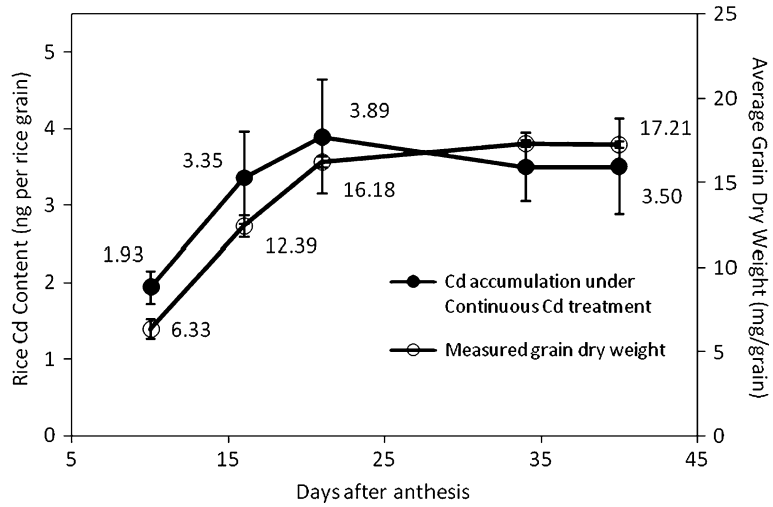

Fig. 4 Rice grain carbohydrate loading (measured by average dry weight per grain; *Open circles*) and concomitant Cd accumulation per grain of hydroponically grown plants (with continuous Cd supply; *Closed circles*). A single panicle harvested per plant (n=4) at each harvest date (until the final harvest) from which a representative sample of rice grains was taken, glumes removed. Mean values are shown ± SE. Data labels show the value of the respective adjacent data point

Phloem versus xylem

There are two main possible pathways of root to grain movement: (1) Cd is taken up directly through the xylem to the developing grain; or (2) Cd is taken up to actively transpiring parts such as culms, rachis, flag leaves and external parts of the panicles, and then relatively quickly remobilised via the phloem to the grain.

The pericarp of the developing rice grain is green tissue and, unlike wheat and barley (Oparka and Gates 1984), there is xylem continuity into the caryopsis of rice (Krishnan and Dayanandan 2003; Zee 1972). Therefore, direct xylem uptake into the grain is possible (Stomph et al. 2009), but it seems that this is limited to when the developing (green) seed is exposed, to allow transpiration (Zee 1972), or when transpiration of glumes is slowed by high humidity (>85%; Oparka and Gates 1984). Early in the seed development, when the glumes are not tight against the developing grain, transpiration of the green seed tissue could occur.

The route by which the applied Cd makes its way to the grain was not distinguished in this study. However, Tanaka et al. (2007) estimated that 91–100% of the rice grain Cd entered the developing caryopsis via the phloem. They calculated this using a mathematical model based on the measured Cd movement to parts of the panicle 7–8 days after anthesis. Their model was necessarily based on the assumption that the rate of Cd flow to different parts of the panicle was constant during grain maturation. Based on our results, approximately 40% of the grain Cd had already accumulated at 7–8 DAF. It is possible that some of the Cd in the seed comes from the maternal tissue or green tissue that is present before the main period of grain loading via the phloem. Nevertheless, given that Cd accumulation in the grain was only slightly ahead of increases in grain dry weight (Fig. 4), the phloem probably was the main route of entry into the grain.

The results of Tanaka et al. (2007) do not necessarily imply that nearly 100% of the Cd coming to the grain is remobilised from storage in the shoot. There is good evidence from other cereals that xylem-to-phloem transfer in the rachis or head is a major mechanism in the transfer of micronutrients like Zn and Mn to the grain (Wolswinkel 1999). In experiments with detached, cultured wheat ears, Pearson et al. (1995; 1996) found that the xylem was crucial in transporting Zn and Mn to the spikelet despite the phloem being the point of entry into the caryopsis.

In the results presented here, the accumulation that occurred in the grain from post-flowering Cd supply supports a fairly direct route of uptake, rather than remobilisation via the shoot. This is in agreement with the results of Fujimaki et al. (2010) that showed minimal short-term uptake of Cd into leaf blades during grain-filling stage, while considerable accumulation occurred in the stems and grain. It is logical that most of the grain Cd taken up during the post-flowering phase moves via the xylem and then this Cd is transferred between the vascular pathways before reaching the developing caryopsis. Fujimaki et al. (2010) demonstrated the importance of the nodes of the culm as sites where Cd is concentrated in the plant. As they concluded, in the pathway from the root to the grain, these nodes are likely key locations of xylem to phloem transfer.

Conclusions

In this study, conducted at environmentally relevant concentrations of Cd, the overall proportion of plant Cd content that was accumulated in the rice grains was very small, and approximately 40% of this was taken up by the plant in the period post-flowering. Therefore, both shoot accumulation until heading and uptake during the post-flowering phase are important determinants of Cd concentration in brown rice. The results presented here also fit well with the proposal originally put forward by Chino (1973), that there is a direct transport pathway of Cd from roots to grain during the early rice grain ripening stage. When interpreted against known patterns of movement for Cd and similar heavy metal micronutrients in other cereals, it is most likely that in this pathway to the grain, a high proportion of transference of Cd takes place from the xylem to the phloem in the culm nodes, rachis or pedicle.

Acknowledgements This research was supported under Australian Research Council's *Discovery Projects* funding scheme (project number DP0773638). Some experimental work in this project was also financially supported by the Chinese Academy of Sciences, International Collaboration Fund (GJHZ200828).

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