RESEARCH PAPER

Role of the node in controlling traffic of cadmium, zinc, and manganese in rice

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Abstract

Heavy metals are transported to rice grains via the phloem. In rice nodes, the diffuse vascular bundles (DVBs), which enclose the enlarged elliptical vascular bundles (EVBs), are connected to the panicle and have a morphological feature that facilitates xylem-to-phloem transfer. To find a mechanism for restricting cadmium (Cd) transport into grains, the distribution of Cd, zinc (Zn), manganese (Mn), and sulphur (S) around the vascular bundles in node I (the node beneath the panicle) of Oryza sativa ‘Koshihikari’ were compared 1 week after heading. Elemental maps of Cd, Zn, Mn, and S in the vascular bundles of node I were obtained by synchrotron micro-X-ray fluorescence spectrometry and electron probe microanalysis. In addition, Cd K-edge microfocused X-ray absorption near-edge structure analyses were used to identify the elements co-ordinated with Cd. Both Cd and S were mainly distributed in the xylem of the EVB and in the parenchyma cell bridge (PCB) surrounding the EVB. Zn accumulated in the PCB, and Mn accumulated around the protoxylem of the EVB. Cd was co-ordinated mainly with S in the xylem of the EVB, but with both S and O in the phloem of the EVB and in the PCB. The EVB in the node retarded horizontal transport of Cd toward the DVB. By contrast, Zn was first stored in the PCB and then efficiently transferred toward the DVB. Our results provide evidence that transport of Cd, Zn, and Mn is differentially controlled in rice nodes, where vascular bundles are functionally interconnected.

Key words: Cadmium, manganese, μ-XANES, μ-XRF, node, rice, zinc.

Introduction

Chronic intake of Cd causes human health problems such as renal dysfunction. The Joint Food and Agriculture Organization – World Health Organization Expert Committee on Food Additives has set the tolerable monthly intake of Cd at 25 μg kg⁻¹ body weight. According to a 2001 national survey by the Ministry of Agriculture, Forestry and Fisheries of Japan (2002), the average monthly intake of Cd through food by Japanese was 17.7 μg kg⁻¹ body weight, approximately half of which was attributed to rice consumption. Therefore, reducing Cd concentrations in rice grain, which is a staple food for half of the world’s population, is important.

Cd uptake by rice plants varies by genotype (Arao and Ae, 2003; Ishikawa et al., 2005), and the efficiency of xylem loading explains the differences in shoot Cd concentration between rice genotypes (Uraguchi et al., 2009). OsHMA3, the gene that regulates root-to-shoot Cd transport, has recently been discovered (Ueno et al., 2010; Miyadate et al., 2011). OsHMA3 encodes a heavy metal ATPase 3, which is responsible for the sequestration of Cd in the vacuoles of root cells in rice; thus, a lack of OsHMA3 causes an increase in Cd transport from the root to the shoot. After transport to the shoot, Cd is translocated to the panicle via the phloem (Tanaka et al., 2007; Fujimaki et al., 2010;...
Yoneyama et al., 2010). Tanaka et al. (2007) found that the phloem is the main route for Cd transport to rice grains. These findings indicate that xylem-to-phloem transfer is necessary for Cd to reach rice grains. In the phloem sap, Cd is bound mainly to a 13 kDa protein and to low-molecular-weight SH compounds (Kato et al., 2010). The chelators responsible for Cd transport in the phloem are different from those that transport Fe, Zn, Cu, Mn, Ni, and Co, which bind to nicotianamine, 2’-deoxymugineic acid, citrate, and histidine. It is likely that Cd is discriminated from the other metals during transport to the phloem.

Ishikawa et al. (2005) showed that shoot concentrations of Cd and Zn and of Cd and Mn were positively correlated in 11 cultivars of rice grown at relatively moderate levels of Cd contamination. Root uptake and root-to-shoot transfer of Cd may be related in some way to the corresponding processes for Zn and Mn, although cell-specific accumulation patterns can vary (Conn and Gilliham, 2010). Nonetheless, how genotype differences in grain Cd concentration are related to Zn and Mn transport is not well understood. Zn is readily transported toward the rachis; however, large amounts of Zn accumulate in the stem when Zn is present in excess (Wang et al., 2011). In this way, an overload of Zn in the panicle is prevented. When rice plants are deficient in Zn, OsZIP4, the gene for a Zn-selective plasma membrane transporter, is highly expressed in rice roots and shoots (Ishimaru et al., 2005). Lack of specificity in the uptake and transport of an essential metal such as Zn affects the absorption of Cd, which is a non-essential element. The imperfect control of metal absorption is compensated for by detoxification processes, including sequestration in vacuoles (Miyadate et al., 2011; Ueno et al., 2010) and binding to cell wall compounds (Wojcik et al., 2005).

In rice plants, elements absorbed from the roots through the transpiration stream are redirected in the nodes, where vascular bundles linked with the roots, leaves, and panicles are interconnected. Remarkable accumulations of Cd (Fujimaki et al., 2010), as well as increased transcription levels of a gene that encodes metallothioneins (Yu et al., 1998), have been observed in nodes; these findings imply the importance of nodes in metal transport. Use of a positron-emitting tracer imaging technique with organ-level resolution showed that Cd (Fujimaki et al., 2010), Mn (Tsukamoto et al., 2006), and Zn (Suzuki et al., 2008) accumulate in the non-elongated nodes at the shoot bases of graminaceous plants. In the node beneath the panicle (node I) of rice plants, there are five types of vascular bundles: enlarged elliptical vascular bundles (EVBs), diffuse vascular bundles (DVBs), large vascular bundles (LVBs), small vascular bundles, and peripheral cylinder vascular bundles. These vertically oriented vascular bundles are horizontally linked to one another via vascular anastomoses in the basal part of the node (Chonan, 1993). Vascular bundles in the flag leaf become enlarged and elliptical-shaped when they reach the node, where they become the EVBs. Xylem parenchyma cells located at the periphery of the EVBs have many plasmodesmata connected to the vascular bundle sheath cells. Therefore, the xylem parenchyma between the EVB and DVB serves as a bridge for horizontal intervacular transfer of metals and is referred to as the parenchyma cell bridge (PCB). Metals transferred to the DVB go up toward the panicle (Chonan, 1993). Yamaji and Ma (2009) found that Lsi6, a gene for a Si transporter, is expressed in the xylem transfer cells that surround the EVB. Knockout of Lsi6 results in a decrease in the Si concentration in the panicle but an increase in the Si concentration in the flag leaf. The presence of Lsi6 in the xylem transfer cells around the EVB is responsible for the efficient transport of Si toward the panicle by facilitating Si transfer from the EVB to the DVB. As in the case of Si, the intervacular transfer of metals in the node is probably an important pathway for the redirection of metals from the xylem through the transpiration stream to the panicle. However, little is known about how Cd is differentiated from the essential metals in each vascular bundle of the rice node.

Metals accumulate in a particular part of a tissue when they are left behind the flow because of the lack of a mechanism for transporting them into the adjacent cells. In this way, Cd accumulates around the endodermis and the pericycle in the roots of Solanum torvum, because the transporter required for Cd efflux into the xylem is weak or in short supply (Yamaguchi et al., 2011). In addition to poor transport, storage and sequestration processes also cause metal accumulation (Wojcik et al., 2005; Isaure et al., 2006; Van Belleghem et al., 2007). Fujimaki et al. (2010) recently reported that Cd accumulation occurs within 8 h after the elongated node at the grain-filling stage is spiked with 107Cd. The complex connections of vascular systems can retard the flow of metals in the rice node (Chonan, 1993), and this retardation is followed by intervacular and xylem-to-phloem transfer, resulting in the redirection of the metals toward the leaf, upper culms, and panicle. In the process of intervacular or xylem-to-phloem transfer in the node, metals are likely to be selectively absorbed, sequestered, or stored, depending on the physiological requirements for them.

In this study, the distributions of Cd, Zn, Mn, and S around the vascular bundles in node I of rice plants were compared to determine where these elements were differentiated. By using a synchrotron micro-X-ray fluorescence spectrometer equipped with a recently developed mirror system that allows higher-brilliance microfocusing (Terada et al., 2010), Cd, Zn, and Mn were detected simultaneously to find the tissues in which Cd and the essential elements were differentiated. Understanding the mechanisms governing Cd transport to rice grains is important for engineering rice cultivars with reduced Cd in the grains.

Materials and methods

Sample preparation

Hydroponically grown 2-week-old seedlings of Oryza sativa ‘Koshikari’ were transplanted to pots filled with soil (Gleysols) taken from a paddy field. The concentrations of 0.1 M HCl-extractable Cd, Zn, and Mn in the soil were 1.8, 16.4, and
Role of the node in controlling traffic of metals | 2731

46.1 mg kg$^{-1}$ dry soil, respectively (Ishikawa et al., 2006). The rice plants were grown in a greenhouse under natural light conditions without flooding.

One week after heading, a node I beneath a panicle of each plant was sampled and immediately frozen in hexane cooled by dry ice. Fifty-micrometer-thick cross-sections were prepared with a cryomicrotome (CM1850, Leica, Wetzlar, Germany). The sections were placed on adhesive tape precooled in a cryomicrotome chamber and then freeze-dried. The sections taken from the vertically central parts of the node and from 2 mm above those parts were analysed.

To avoid disruption of the distribution of soluble metals at the cellular level of resolution, high-pressure freezing followed by freeze-substitution with resin has been applied for sectioning (Van Belleghem et al., 2007). However, cryosectioning followed by freeze-drying was used for sample preparation because preliminary investigations had shown that resin impregnation altered the speciation of Cd. At subcellular to tissue-scale resolutions, redistribution of metals due to freeze-drying is negligibly small (Yamaguchi et al., 2010).

Elemental mapping

The two-dimensional distributions of Cd, Zn, and Mn in the node cross-sections were measured by means of synchrotron micro-X-ray fluorescence spectrometry (SXRF) at the undulator beamline 37XU of SPring-8 (Terada et al., 2004). A 30 keV incident X-ray beam was focused with a recently developed mirror system that allows higher-brilliance microfocusing (Terada et al., 2010).

The beam spot size was 1.3 μm in the vertical direction and 1.9 μm in the horizontal direction. The Cd Kα (23.11 keV), Zn Kα (8.62 keV), and Mn Kα (5.90 keV) lines were detected with a Si(Li) solid-state detector. The fluorescence yield was normalized by the incident photon intensity ($I_0$). The SXRF analyses were repeated at least twice with each of the node sections taken from different plants to confirm reproducibility. As a standard for rough conversion of fluorescence counts into metal concentrations, a 50-μm-section was prepared from Cryo Mount I (a low-viscosity embedding substance, Muto Pure Chemicals, Tokyo, Japan) containing 0, 0.1, 1, and 2 mg cm$^{-3}$ of Cd, Zn, and Mn and was then freeze-dried (Homma-Takeda et al., 1999, 2009). Elemental maps of three different parts of 110-μm squares were obtained in 10-μm steps under the measurement conditions used for the samples; then the fluorescence yields were normalized by $I_0$ and averaged. The normalized fluorescence yields and metal concentrations were linearly related ($R^2$=0.99). Data processing for the reproduction of elemental maps and the calculation of average fluorescence counts in each vascular bundle were performed with IGOR Pro 6.0.3.1 software (Wavemetrics, USA). The region of interest for the average count calculation was selected by tracing on the optical micrograph of the node section used for SXRF mapping. After SXRF measurement, the sections were coated with carbon, and the distribution of S was determined with an electron probe microanalyzer (EPMA; JXA-8500F, JEOL, Tokyo) at an acceleration voltage of 15 kV and a current of 20 nA.

Microfocused X-ray absorption near-edge structure

Selected points on the xylem and phloem of the EVB and the PCB, Cd K-edge (26 711 eV) microfocused X-ray absorption near-edge structures (μ-XANES) were obtained. While the sample remained fixed in the beam path at a point of interest identified on a 3-μm-step SXRF image of the area including an EVB and the surrounding PCB, an energy range of 26 650–26 780 eV was scanned at 1-eV-step intervals. Cd speciation was evaluated by linear combination fitting (LCF) of μ-XANES spectra to the spectra of model compounds. CdS was used as a model compound for Cd bound to S, and Cd(OH)$_2$ was used as model compound for Cd bound to O. The Athena software (version 0.8.0.56; Ravel and Newville, 2005) was used to subtract the background and to normalize the spectra and LCFs. In the LCFs, the fraction of each reference compound was set as an adjustable parameter, and optimization of fitting was achieved by minimizing the residual of the fit, defined as the normalized root-square difference between the data and the fit ($R$).

Chemical analysis of metal concentrations

Node I, the culm between node I and the rachis, the flag leaf blade, and the panicle were dried separately in an oven at 75 °C, and the dried tissues were ground to fine powders. The powders were digested with HNO$_3$ and HClO$_4$ in a Teflon beaker heated to 120 °C. Concentrations of Cd, Zn, and Mn were determined with an inductively coupled plasma optical emission spectrometer (VistaPro, Varian, CA, USA).

Results

Concentrations of metals in rice tissues

The concentrations of Cd, Zn, and Mn in node I, the flag leaf blade, the culm between node I and the rachis, and the panicle, sampled 1 week after heading, are shown in Table 1. Chino (1973) showed that Cd and Zn applied to the water culture medium within 10 d of heading are most likely transferred to the grain; and, therefore, in this study, the growth period when Cd and Zn were most actively transported to the grain was investigated. The concentrations of Cd and Zn were highest in node I; by contrast, the concentration of Mn was highest in the flag leaf blade, and that in the node was higher than the concentrations in the culm and the panicle. Compared with the other metals, Mn was preferentially transported to the flag leaf. Our results showing that Cd and Zn accumulated in the rice node were in accordance with the results of previous studies (Obata and Kitagishi, 1980; Fujimaki et al., 2010). The accumulation of Cd and Zn in node I, which branches into the flag leaf and the panicle, suggests that Cd and Zn transport via the transpiration stream was retarded in the node.

Spatial distribution of metals and sulphur in node I

A representative optical micrograph and SXRF and EPMA maps of a cross-section of the vertically central part of node I are shown in Fig. 1. Because the optical micrograph of the freeze-dried section used for SXRF and EPMA mappings lacks colour and contrast (Fig. 1A), a micrograph of a fresh

### Table 1. Cd, Zn, and Mn concentrations in rice tissues collected 1 week after heading

|           | Cd (μg g$^{-1}$ dry weight) | Zn (μg g$^{-1}$ dry weight) | Mn (μg g$^{-1}$ dry weight) |
|-----------|-----------------------------|-----------------------------|-----------------------------|
| Node I    | 440±39                      | 2204±455                    | 2531±228                    |
| Flag leaf |                            | 53±8.7                      | 79±7                        | 3014±318                    |
| blade     |                            | 62±7.7                      | 156±13                      | 70±7                        |
| Culm      | 19±0.3                      | 55±5                        | 321±17                      |
| Panicle   |                            |                             |                             |                             |
section stained with toluidine blue is also shown (Fig. 1B; note that the stained section was taken from a separate plant that was used for SXRF and EPMA mappings). Cd, Zn, Mn, and S were detected in both the vascular bundles and the parenchyma, but the distribution patterns of the metals were different (Fig. 1C–F). Cd was distributed both in the xylem of the EVB and in the surrounding PCB (Fig. 1C; see Supplementary Fig. S1 at JXB online), whereas Zn was localized in the PCB (Fig. 1D; see Supplementary Fig. S1 at JXB online). Mn accumulated near the protoxylem of the EVB (Fig. 1E). The distribution pattern of S resembled that of Cd (Fig. 1C, F). The concentrations of both Cd and S tended to be higher in the xylem of the EVB than in the PCB. The Cd/Zn ratios were high in the xylem of the EVB and in the LVB and the DVB (Fig. 1G). This result suggests that Cd tended to stay in the vascular bundles more than Zn did. The Cd/Mn ratio tended to be high in the parenchyma of the peripheral part of the node (Fig. 1H).

Figure 2A and B shows optical micrographs of a cross-section taken 2 mm above the vertically central part of node I. In this part of the node, the LVB coming from the DVB is directed to the panicle (Chonan, 1993). The highest fluorescence counts for Cd were observed in the area around the protoxylem lacuna in which xylem parenchyma cells with thin cell walls are present (Fig. 2D). Cadmium was also located in the border area between the phloem and xylem, in the bundle sheath, and in the phloem. The distribution of Cd in the peripheral cylinder vascular bundles was similar to that in the LVB. Zinc was found mostly in the bundle sheath and the fundamental parenchyma (Fig. 2E), whereas Mn was localized in the phloem (Fig. 2F). Sulphur was localized in the phloem and the fundamental parenchyma surrounding the LVB (Fig. 2G).

In the LVB of the basal part of the flag leaf (which wraps around node I; Fig. 2A, C), Cd was localized in the area surrounding the protoxylem and metaxylem where xylem parenchyma cells are present. Cd was also localized in the area enclosing the phloem (Fig. 2H) in which xylem parenchyma cells and phloem parenchyma cells are interconnected by plasmodesmata (Chonan, 1993). By contrast, Zn and Mn were localized in the bundle sheath cells, and Mn was also found in the fundamental parenchyma surrounding the LVB (Fig. 2J). Sulphur in the LVB was localized mostly in the border area between the phloem and xylem (Fig. 2K).
Figure 3 shows the average concentrations of metals in the area of each vascular bundle. The location of each vascular bundle is marked on the micrographs in Figs 1A and 2A. The concentrations were extrapolated from the standard curve obtained from the relationships between the fluorescence yields of the samples and standard compounds containing known concentrations of metals. Note that the concentrations themselves include large uncertainties but can still be used to compare the metal distributions among the vascular bundles. The Cd concentration was highest in the EVB, whereas the Zn concentration was highest in the DVB; the Mn concentration was highest in the LVB connected to the flag leaf. Metal concentrations in the phloem portion of the EVB and LVB were lower than the concentrations in the whole vascular bundle, except in the case of Mn in the LVB of the flag leaf. In the DVB, distinguishing the xylem from the phloem was difficult because of their complex configuration.

μ-XANES
Figure 4A shows the normalized Cd K-edge μ-XANES spectra of reference compounds [CdS and Cd(OH)$_2$] and of selected points on the thin section from the vertically central part of node I. Because of the low Cd concentration, the μ-XANES spectrum could not be obtained for the section taken 2 mm above the vertically central part of node I shown in Fig. 2. CdS and Cd(OH)$_2$ are not common Cd species in plant tissues; however, μ-XANES is not sensitive for the differentiation of second neighbouring atoms of Cd; for example, the μ-XANES spectra of CdS and Cd(OH)$_2$ are similar to the spectra of Cd co-ordinated with metallothionein and Cd citrate, respectively (Yamaoka et al., 2010). Point 1 is on the xylem of the EVB, point 2 is on the xylem close to the border of the phloem of the EVB, point 3 is on the phloem of the EVB, and point 4 is on the PCB (Fig. 4B). The XANES spectra obtained from all the...
positions on a particular tissue were similar to one another (see Supplementary Fig. S2 at JXB online). Distinct differences in the post-edge features of Cd co-ordinated with S and O allowed it to be determined whether Cd was co-ordinated with S or with O. At points 1 and 2, the post-edge region was featureless, suggesting that Cd was mostly co-ordinated with S. The results of the LCF fitting are shown in Table 2. In the xylem of the EVB, the contributions of the species co-ordinated with S were 95% and 84%. By contrast, approximately half of the Cd in the phloem of the EVB and the xylem parenchyma cells surrounding the EVB was co-ordinated with O. The Cd concentrations in the xylem in the EVB (Fig. 4, point 1) and in the xylem near the phloem in the EVB (Fig. 4, point 2) were higher than the concentrations in the phloem of the EVB (Fig. 4, point 3) and the PCB surrounding the EVB (Fig. 4, point 4).

Discussion

Trafficking of metals in the EVB

The different distribution patterns of Cd and Zn around the EVB (Fig. 1C, D; see Supplementary Fig. S1 at JXB online) indicate that Zn transport from the EVB to the PCB was more efficient than Cd transport. In contrast to the bulk concentrations of Cd and Zn, which were higher in node I than in the flag leaf blade, the Mn concentration was lower in node I than in the flag leaf blade (Table 1). Most of the Mn was localized in the xylem parenchyma around the protoxylem (Fig. 1E). On average, the Mn concentration in the entire EVB was lower than the Zn concentration (Fig. 3), even though the bulk concentration of Mn in node I was higher than the Zn concentration (Table 1). Mn transport toward the flag leaf blade through the EVB was likely more efficient than Cd and Zn transport.

In the xylem of the EVB, where Cd and S coexist (Fig. 1C, F; see Supplementary Fig. S1 at JXB online), many tracheids and xylem parenchyma cells are intertwined. This configuration enables metals in the xylem vessels to be

Table 2. Fractions (%) of Cd co-ordinated with S and O

| Point | Cd-S (%) | Cd-O (%) | R-factora |
|-------|----------|----------|-----------|
| Point 1: xylem in the EVB | 95 | 5 | 0.00058 |
| Point 2: xylem near the phloem in the EVB | 84 | 16 | 0.0022 |
| Point 3: phloem of the EVB | 55 | 45 | 0.0012 |
| Point 4: PCB | 67 | 33 | 0.0011 |

a R-factor = \( \frac{\sum (\text{data} - \text{fit})^2}{\sum \text{data}^2} \).
transported via the transpiration stream and then selectively absorbed by the xylem parenchyma cells. The xylem parenchyma cells in the centre of the xylem have large vacuoles (Chonan, 1993). Yu et al. (1998) reported that transcript levels of cDNA that encodes metallothionein-like proteins are enhanced in node I. In the vacuoles and cytoplasm, sequestration of Cd by chelators with S-containing ligands, such as phytochelatin, is an important mechanism for Cd detoxification in the root of Arabidopsis thaliana (Van Belleghem et al., 2007). In rice roots, expression of OsHMA3 is a key factor in determining root-to-shoot transfer of Cd, because this gene is responsible for the sequestration of Cd in vacuoles. OsHMA3 is expressed in both the root and the shoot (Ueno et al., 2010). The μ-XANES analysis indicated that in the xylem of the EVB where Cd accumulated, most of the Cd was co-ordinated with S (Fig. 4A; Table 2). In contrast, some of the Cd was co-ordinated with O in the PCB and the phloem of the EVB where less Cd was accumulated than in the xylem of the EVB (Fig. 4A; Table 2). These results suggest that more Cd was co-ordinated with S in the tissue where more Cd was accumulated. Although the sample preparation method of this study was not precise enough to reveal the Cd distribution on a cellular-scale resolution, it was sufficiently reliable to allow us to conclude that the Cd in the xylem was sequestered in the vacuoles in a form bound to chelators with S-containing ligands.

Zinc being transported in the xylem vessels via the transpiration stream was selectively absorbed by the xylem parenchyma cells in the EVB and horizontally transferred to the parenchyma that encloses the EVB. Obata and Kitagishi (1980) showed that Zn accumulates in the PCB in rice nodes. The DVB becomes the LVB in the upper part of node I. Intervascular transfer of metals from the EVB to the DVB followed by xylem-to-phloem transfer in the DVB would be an important process controlling metal concentrations in rice grains.

The DVB becomes the LVB in the upper part of node I and is then directed to the panicle (Chonan, 1993). The maximum concentrations of Cd, Zn, and S detected in the LVB of node I (Fig. 2) were lower than those in the EVB (Fig. 1), whereas the concentration of Mn was higher in the LVB. The average concentrations of Cd, Zn, and Mn showed the same trends (Fig. 3). The fact that the concentrations of Cd and Zn were higher in the node than in the other tissues (Table 1) was due to the accumulation of Cd and Zn in the vertically central part of node I, where the EVB is located. By contrast, Mn transport from the DVB to the LVB in node I was probably more efficient than Cd and Zn transport. As a result of xylem-to-phloem transfer in the DVB, Cd, Zn, and Mn were detected in the phloem of the LVB (Figs 2, 3). This finding supports the results of previous studies showing that xylem-to-phloem transfer occurs in the node and that metals are thereby transferred to the grains (Fujimaki et al., 2010). Sulphur was also detected in the phloem (Fig. 2G); therefore, certain metals in the phloem may have been complexed with chelators with S-containing ligands. The most probable ligands for Zn and Mn in the phloem sap are nicotianamine, 2'-deoxymugineic acid, citrate, and histidine, which do not contain S. By contrast, Cd binds with low-molecular-weight SH compounds in the phloem sap of rice (Kato et al., 2010). In the phloem of the EVB of the vertically central part of node I, approximately half of the Cd was co-ordinated with S (Fig. 4A). However, the additional XANES spectra shown in Supplementary Fig. S2 at JXB online indicate that the fraction of Cd co-ordinated with S was inconsistent, probably because the Cd species depended on the positions selected for XANES scans, owing to the coexistence of O- and S-co-ordinated Cd in the phloem. Nonetheless, the
presence of Cd co-ordinated with S suggested that some of the Cd was transported to the panicle by complexation with S. In the upper part of node I, the major tissues that accumulated Cd and Zn in the LVB were similar to those in EVB, despite the different morphological features of the two types of vascular bundles. Transfer of Cd was restricted in the parenchyma cells surrounding the xylem, whereas Zn transfer toward the fundamental parenchyma that encloses the LVB was more efficient than Cd transfer.

Transport of metals toward the flag leaf

The EVB is connected with the LVB of the flag leaf. Metals that are not redirected toward the panicle through the DVB are transferred toward the flag leaf. Retarded flow or storage of Cd around the protoxylem lacuna and metaxyvle (Fig. 2H) indicates that the xylem parenchyma surrounding the xylem sequestered Cd in a manner similar to that observed for the xylem parenchyma of the EVB. Cd was also detected in the area surrounding the phloem in which the xylem parenchyma cells and the phloem parenchyma cells are interconnected by plasmodesmata. Sulphur accumulation in the border area of the xylem and phloem may imply the storage of Cd in chelators with S-containing ligands. Zinc and Mn were more efficiently transferred to the fundamental parenchyma than was Cd.

 Trafficking of Cd, Zn, and Mn in node I

Intervascular transfer from the EVB to the DVB in node I is an important pathway governing metal concentrations in rice grains. The finding that accumulation of Mn in node I was minimal suggests that redirection of Mn in the node was not affected by any retardation processes such as sequestration or insufficient transfer from cells of certain tissues to adjacent ones. By contrast, the restricted flow of Cd and Zn resulted in their accumulation in node I (Fujimaki et al., 2010), indicating that the node redirected Cd and Zn. The SXRF and EPMA mappings provided evidence that Cd and S were sequestered in the xylem of the EVB and that Zn was stored in the PCB between the EVB and the DVB. The XANES analyses indicated that Cd was co-ordinated with S, probably in a form bound to an S-containing ligand. Cd and Zn were clearly discriminated in the node by regulation of the transport of Cd, which is non-essential.

In this study, the first evidence that transport of Cd, Zn, and Mn is controlled in rice nodes at a tissue-level resolution is presented. This control of metal transport is likely to have been achieved by the functional interconnection between vascular bundles, as was predicted by Chonan et al. (1985). The rice cultivar used in this study, *Oryza sativa* ‘Koshihikari’, has a genetic tendency toward a low absorption of Cd. ‘Koshihikari’ was subjected to high Cd concentrations by growing it on Cd-contaminated soil without flooding. Therefore, the metal distribution patterns and Cd speciation in node I probably resulted from the cultivar’s response to high-Cd conditions. For a more detailed understanding of the molecular mechanism that governs intervascular metal transfer, the speciation and distribution of metals and associated ligands must be investigated at a cellular scale by comparing several varieties of rice at several growth stages.

Measures taken to reduce Cd absorption in rice grains may be associated with reduced absorption of Zn and Mn, which are essential for plant growth and human health. Breeding rice cultivars with low grain-Cd concentrations, while maintaining sufficient concentrations of essential elements, will be a challenge. We believe that our results will help to overcome this challenge by improving our understanding of the physiological mechanism that differentiates Cd transport from the transport of essential metals in rice.

Supplementary data

Supplementary data can be found at JXB online.

**Supplementary Fig. S1.** Optical micrographs and overlapped SXRF maps of Cd and Zn in cross-sections of the vertically central part of node I scanned in 3-μm-steps.

**Supplementary Fig. S2.** (A) Normalized Cd K-edge μ-XANES spectra and fraction of Cd co-ordinated with S (percentages in parentheses) calculated from the linear combination fittings; (B) locations of the selected points for XANES acquisitions on the SXRF maps of Cd in 3 μm steps; (C) relationships between Cd concentration and fraction of Cd co-ordinated with S on each spot used for XANES measurements.

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