The Role of Node Restriction on Cadmium Accumulation in the Brown Rice of 12 Chinese Rice (Oryza sativa L.) Cultivars

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Supporting Information

ABSTRACT: For selection or breeding of rice (Oryza sativa L.) cultivars with low Cd affinity, the role of node Cd restriction on Cd accumulation in brown rice was studied. A pot experiment was conducted to investigate the concentration of Cd in different sections of 12 Chinese rice cultivars. The results indicated that the Cd accumulation in the brown rice was mainly dependent on the root or shoot Cd concentration. Among the cultivars with nearly equal shoot Cd concentrations, Cd accumulation in brown rice was mainly dependent on the transport of Cd in the shoot. However, the Cd transport in the shoot was significantly restricted by the nodes, especially by the first node. Furthermore, the area of the diffuse vascular bundle in the junctional region of the flag leaf and the first node was a key contributor to the variations in Cd restriction by the nodes.

KEYWORDS: cadmium, rice, node restriction, translocation, vascular bundles

INTRODUCTION

Cadmium (Cd) is a highly toxic metal and is regarded as a human carcinogen by the National Toxicology Program.1,2 According to the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the tolerable weekly intake of Cd is 7 μg/kg for a human body.3 Long-term exposure to Cd causes many chronic diseases,4 including itai-itai disease, cancer, and kidney disease.5–7 For nonsmokers, approximately 90% of the Cd in the human body reportedly comes from food.8 As one of the main cereal types, rice (Oryza sativa L.) is widely consumed by half of the world’s population. However, rice has a higher Cd-bioaccumulation ability than most other cereals,9 and brown rice is considered to be a major source of the dietary intake of Cd for humans in rice-planting regions.10 Therefore, reducing the Cd level in brown rice is vital to human health.

Cd accumulation in grain differs among different cultivars,11–15 which is affected by many factors, such as the transporter protein,16 tissue biomass,17 and translocation characteristics.18 A better understanding of the mechanisms that contribute to this variability is helpful to identify cultivars with low Cd accumulation. Cd accumulation in brown rice is related to the Cd uptake by the root from the soil. The ability of the root to take up Cd differs among different cultivars,13,19 and this variability is mainly attributed to the different Cd transporters in the root.20,21 Once Cd is taken into the root, upward translocation plays an important role in the allocation of Cd to brown rice.22,23 The upward translocation of Cd from the shoots to brown rice passes through several nodes where the elemental redistribution processes occur.24 A node is the junction between the leaves or branches and the stem in rice, and there are generally 13–18 nodes on a stem. However, only a small number of aboveground nodes are elongated in the jointing stage, depending on the genotypes.25 Inside the nodes, there are three main phases of vascular bundles (VBs) as follows: enlarged or large VBs (EVBs or LVBs), transit VBs (TVBs), and diffuse VBs (DVBs). An EVB is enlarged at the node by increasing the number of xylem vessels and sieve tubes; the TVB is a transit phase that occurs without extra enlargement in the node; the DVB surrounds the EVB and assembles just above the node.26 The VBs in nodes also play an important role in Cd translocation,27,28 e.g., most Cd in the uppermost node of rice is accumulated on EVBs and DVBs, which inhibit Cd transportation to the internodes. However, few studies have investigated the role of different nodes and internodes on Cd translocation in rice and the role of different VB systems on Cd transport inside the nodes.

Therefore, the objectives of this work were (1) to investigate the variability of the node restriction of Cd among 12 Chinese rice cultivars and analyze its impacts on Cd allocation to the brown rice and (2) to test one hypothesis, which is that differences in node restriction rely on differences in node vasculatures.

MATERIALS AND METHODS

Soil Preparation and Pot Trial. An acid paddy soil (0–20 cm) was collected from Xinghua county, Jiangou province, China. The principal physical and chemical properties of the soil were pH (H2O), 4.90; organic matter (OM), 28.3 g kg⁻¹; cation exchange capacity (CEC), 16.3 cmol kg⁻¹; total nitrogen (TN), 1.59 g kg⁻¹; available N (AN), 0.19 g kg⁻¹; total phosphorus (TP), 0.94 g kg⁻¹; available P...
(AP), 0.03 g kg⁻¹; total potassium (TK), 15.2 g kg⁻¹; available K (AK), 0.28 g kg⁻¹; total Cd, 0.14 mg kg⁻¹; available Cd (0.01 M CaCl₂ extractable Cd), 0.024 mg kg⁻¹; and soil texture as follows: sand (10.0%), silt (68.4%), clay (21.6%). After air-drying and passing through a 2 mm sieve, the soil was spiked to the soil (background Cd, 0.14 mg kg⁻¹), and the soil was maintained at 80% of the field water holding capacity for about 6 months to simulate long-term Cd-contaminated field soil. After the aging process, the measured soil Cd concentration was 0.71 mg kg⁻¹, and the soil was used for the pot experiment after air-drying and passing through a 2 mm sieve again.

The pot experiment was carried out in the greenhouse of the Institute of Soil Science Chinese Academy of Sciences, Nanjing, Jiangsu province, China. Each pot (25 cm diameter and 22 cm high) was kept moist for 7 days before planting the rice. The seeds of all cultivars were surface sterilized in 15% H₂O₂ solution for 15 min, fully rinsed with deionized water, and then soaked in deionized water in the dark for 24 h at 25 °C. The germinated seeds of each cultivar were then pregrown in the nursery tray for 20 days in the greenhouse, and uniform seedlings were prepared for the pot experiment on May 26, 2016. Each cultivar had three pots as replicates, and each pot planted six uniform seedlings. All pots were arranged randomly in the greenhouse and kept submerged (2 °C) during the entire growth stage.

One week before harvesting at the mature stage of each rice cultivar, three similar plants of each cultivar from three of the replicate pots were selected to observe the VB structure in the first node (N1). The first node beneath the panicle of each plant was sampled using a razor blade and immediately stored in an MES (2-(N-morpholino)-ethanesulfonic acid) buffer to observe its cellular structure. When the rice was ripe, the remaining five rice plants in each pot were harvested. The rice plants were sampled separately from top to bottom, including the brown rice (BR), flag leaf (FL), first internode (IN1), first node (N1), second leaf (L2), second internode (IN2), second node (N2), third leaf (L3), third internode (IN3), third node (N3), fourth leaf (L4), fourth internode (IN4), fourth node (N4), and roots (Figure 1). Each part was rinsed three times with tap water followed by deionized water and then oven-dried to a constant weight at 75 °C. After weighing of the dry weight (DW), all the samples were milled to a fine powder using a blender (A11 basic, IKA, Germany) to prepare for the chemical analysis of Cd.

### Cellular Structure Observation

The fresh first nodes in the MES buffer were cut into 0.8–0.9 mm thick sections by a razor blade, the sections were then placed into a planchette coated with hexadecane, and another planchette was used to cover the sections. The sections were precooled by a high-pressure freezer (HPM100, Leica, Wetzlar, Germany) with a pressure of 210 MPa at −196 °C for 30 s and then freeze-dried. Because the junction of the flag leaf and N1 (JFLN) is the most important region, which is directly connected to the panicle and flag leaf in the first node, 10 μm thick sections in this junctional region were sectioned by a Leica RM 2265 rotary microtome (Leica, Wetzlar, Germany). These thick sections were stained with safranine and fast green to observe the cellular structure using a digital slide scanner (Pannoramic MIDI, 3D HISTECH, Hungary). The scanning images were analyzed using the professional software Pannoramic viewer, and the images were enlarged at 1–400× magnification.

### Chemical Analysis of Plant and Soil

All plant samples were digested with HNO₃ and H₂O₂. Approximately 0.15 g samples were placed into polytetrafluoroethylene tubes, and then 4 mL of high-purity HNO₃ was added for cold digestion overnight. Thereafter, 3 mL of high-purity H₂O₂ was added to the tubes for digestion in high pressure sealed digestion vessels (HTLAB, HR-25, Shanghai) at 140 °C for 4 h according to the Determination of Cadmium in Foods, National Food Safety Standard of China (GB/T 5009.15-2003).
tubes were removed when the system cooled. Finally, the digestive solution was transferred to centrifuge tubes after an acid evaporation and was passed through a 0.45 µm filter membrane before the Cd measurement by graphite furnace atomic absorption spectrometry (SpectrAA 220Z, Varian, USA) was performed. The detection limit is 0.001 µg kg⁻¹, which equates to 0.1 µg kg⁻¹ in the brown rice, and a standard reference material, green Chinese onion material (GBW10049, National Research Center for Certified Reference Materials, China), was used for the quality control. The recovery rate ranged from 95 to 106%.

The soil pH was measured using a 1:2.5 soil-to-water ratio. The soil organic matter (K₂CrO₇–H₂SO₄ oil-bath heating), cation exchange capacity (1 M ammonium acetate leaching method at pH 7.0), soil texture (pipette method), TN (N/C soil analyzer (Flash, EA, 1112 series, Italy)), TP (H₂SO₄–H₂O₂ digestion, molybdenum–antimony colorimetric method), TK (H₂SO₄–H₂O₂ digestion, flame atomic absorption spectrophotometry), AN (Conway method), AP (Olsen method), and AK (1 M ammonium acetate extraction) were analyzed according to the routine analytical methods of agricultural chemistry in soil.32

**Data Analysis.** The translocation factors (TFs, concentration ratio between the upper parts and the lower parts) of Cd from the root to the shoot (the part between the root and the ear of rice) or brown rice (BR), from Nn to INn or Ln (n = 1, 2, 3, 4) and the translocation efficiency of Cd from IN4 to IN1 (TEIN4−IN1) were calculated based on the following equations:

\[
\text{TF}_{n-1} = \frac{C_n}{C_n} \\
\text{TF}_{IN4-IN1} = \frac{C_{IN1}}{C_{IN4}} \times \frac{C_{IN4}}{C_{IN1}}
\]

where a represents the root, N1, N2, N3, or N4; b represents shoot, BR, IN1, IN2, IN3, or IN4; \( C_a \) and \( C_b \) represent the Cd concentrations in a and b, respectively; and \( C_{IN1} \) and \( C_{IN4} \) represent the Cd concentrations in IN1 and IN4, respectively.

The total dry weight from N4 to the IN1 accounts for more than 90% of the dry weight of the shoot, so other parts in the shoot were not taken into consideration, and the weighted average concentration of Cd in the shoot was calculated based on the following equation:

\[
\text{Cd}_{\text{shoot}} = \frac{\sum_{i=1}^{n} (C_{DNi} \times DW_{Ni} + C_{DNi} \times DW_{INi} + C_{Dli} \times DW_{li})}{\sum_{i=1}^{n} (DW_{Ni} + DW_{INi} + DW_{li})}
\]

where the value of \( n \) was 4; \( i \) represents 1, 2, 3, 4; \( C_{DNi} \), \( C_{DNi} \) and \( C_{Dli} \) represent the concentration of Cd in Ni, INi, and Li, respectively; \( DW_{Ni} \), \( DW_{INi} \) and \( DW_{li} \) represent the dry weight of Ni, INi, and Li, respectively.

**Statistical Analysis.** The statistical relationships among all test data were analyzed by one-way analysis of variance (ANOVA) through SPSS 19.0, and the least significant difference (LSD) was used to check the significant differences between mean values (\( P < 0.05 \)). The data were expressed as the mean ± SD (\( n = 3 \)). All figures were generated using the SigmaPlot 10.0 software.

**RESULTS**

**Cd Accumulation in the Root, Shoot, and Brown Rice of Different Rice Cultivars.** As shown in Figure 2, the Cd concentrations in the root significantly differed among the 12 rice cultivars, ranging on average from 0.27 to 5.22 mg kg⁻¹ DW. ZXY146 and CLY4418 had the lowest and the highest root Cd concentrations among all cultivars, respectively.

Cd concentration in the shoot also differed significantly among the 12 cultivars (Figure 2), ranging on average from 0.08 to 1.27 mg kg⁻¹ DW. NXY8 and CLY4418 had the lowest and the highest shoot Cd concentrations among all cultivars, respectively.

In the BR, Cd concentration shows a significant (\( P < 0.05 \)) difference among the 12 cultivars, ranging on average from 0.020 to 0.222 mg kg⁻¹ DW (Figure 2). TYJ1 and NJ9108 had the lowest Cd concentrations in the BR, and CLY4418 had the highest Cd concentration in the BR, which exceeded the Chinese food safety standard (0.20 mg kg⁻¹ DW) and was 11 times higher than that of TYJ1 and NJ9108 (Figure 2). In addition, the tendency of the concentrations of Cd in the BR was consistent with that of the concentrations of Cd in the root in the cultivars of YYJ2, SJ9, NJ4, ZD18, WYJ27, and CLY4418, while no positive relationship between BR Cd and root Cd concentration was found in the other cultivars.

**Cd Accumulation in the Nodes and Internodes of the Cultivars with Similar Cd Levels in the Shoots.** The concentrations of Cd differed in the nodes at different locations as well as in the internodes (Figure 3). From N4 to N1 in the cultivars with similar shoot Cd (TYJ1, NJ9108, YY2640, NJ52, and ZXY146), Cd concentrations in the nodes ranged from 0.46 to 1.12 mg kg⁻¹ DW, which were even higher than those of the root from the same cultivar. The concentration of Cd in N1 was the highest among all the nodes and approximately 1.5 to 2 times higher than that of the other nodes, but no obvious
difference was found among the lower nodes (N2, N3, and N4). Cd concentrations in the internodes were approximately 3 to 10 times lower than those of nodes located at both sides of the internodes and gradually decreased as the location rose.

Cd concentrations in the nodes and internodes significantly differed among the cultivars. TYJ1 had the highest Cd concentration in N1 and the lowest Cd concentration in IN1, with values of 1.12 and 0.03 mg kg\(^{-1}\) DW, respectively, whereas the lowest Cd concentrations in N1 and the highest Cd in IN1 were both in ZXY146, with values of 0.87 and 0.07 mg kg\(^{-1}\) DW, respectively (Figure 3).

### Internal Transport Characteristics of Cd in the Cultivars with Similar Cd Levels in the Shoots.

The translocation factors from the root to the shoot (TF\(_{\text{root}\rightarrow\text{BR}}\)) were significantly different among the five rice cultivars with similar shoot Cd levels, with concentration ratios ranging from 0.036 to 0.153 (Figure 4). In the shoot, the translocation factors from the root to the shoot (TF\(_{\text{root}\rightarrow\text{BR}}\)) were significantly different among different cultivars at \(P < 0.05\).

![Figure 4](image.png)

**Figure 4.** Translocation characteristics of Cd in rice. TF\(_{\text{root}\rightarrow\text{brown rice}}\): translocation factor from the root to the brown rice. TF\(_{\text{root}\rightarrow\text{shoot}}\): translocation factor from the root to the shoot. TF\(_{\text{N1}\rightarrow\text{IN1}}\): translocation efficiency from the fourth internode to the first internode. Values are given as the means with standard deviations, as shown by the vertical bars (\(n = 3\)). Bars with the same letter(s) indicate no significant differences among the different cultivars at \(P < 0.05\).

The lowest TF\(_{\text{root}\rightarrow\text{BR}}\) was observed in ZXY146 and TYJ1, with values of 0.153 and 0.08, respectively. Among the different rice cultivars, the TF\(_{\text{root}\rightarrow\text{BR}}\) was significantly different. This variability was greatest in N4 among different rice cultivars, the TF\(_{\text{N1}\rightarrow\text{IN1}}\) of Cd was significantly different (Figure 4), respectively.

The upward translocation of Cd in the shoot passes through the nodes, internodes, and leaves. The translocation characteristics of the five rice cultivars are shown in Table 2. As shown, the TF\(_{\text{Nn}\rightarrow\text{INn}}\) ranged on average from 0.03 to 0.41, and a decreasing tendency occurred as the location rose. A similar result was observed in the translocation from the node to its leaf (TF\(_{\text{Nn}\rightarrow\text{Ln}}\)) (the concentration of Cd in the leaves are shown in Figure S2), and TF\(_{\text{Nn}\rightarrow\text{Ln}}\) ranged on average from 0.09 to 0.62. Among the different rice cultivars, the TF\(_{\text{Nn}\rightarrow\text{INn}}\) of Cd was significantly different. This variability was greatest in TF\(_{\text{N1}\rightarrow\text{IN1}}\) with a range from 0.03 (TYJ1) to 0.08 (ZXY146). Generally, Cd upward translocation was the most difficult in TYJ1, which had the lowest TF\(_{\text{N3}\rightarrow\text{IN3}}\), TF\(_{\text{N2}\rightarrow\text{IN2}}\), and TF\(_{\text{N1}\rightarrow\text{IN1}}\) among all cultivars, and the upward translocation was easiest in ZXY146, which had the highest TF\(_{\text{N4}\rightarrow\text{IN4}}\), TF\(_{\text{N3}\rightarrow\text{IN3}}\) and TF\(_{\text{N1}\rightarrow\text{IN1}}\).

#### Vascular Bundle Differences in the First Node among Three Typical Cultivars.

N1 had the strongest ability to restrict the upward translocation of Cd among all nodes, and this ability differed among the rice cultivars. Thus, three cultivars—NJ9108, NJ52 and ZXY146, which have a high, middle, and low N1 Cd-restriction ability, respectively—were selected to observe the VBs in the JFLN region. As shown in Figure 5a, the EVB was located in the leaf sheath and showed no obvious difference among the three cultivars. In the central region, there are two VB phases, i.e., TVB and DVB. The areas of the TVB and DVB differed among the three cultivars, both of which followed the order of ZXY146 > NJ52 > NJ9108, as well as the total area of VBs (Table 3), which was contrary to their ability to restrict the upward translocation of Cd. Interestingly, the variability of the DVB area was greater than that of the TVB area; the DVB area in ZXY146 was 2 times higher than that in NJ52 and 13 times higher than that in NJ9108. The variability in the TVB area was smaller, and the TVB areas in ZXY146 and NJ52 were similar and were approximately 31.3% higher than that in NJ9108. The VB (TVB, DVB, and total VB) area was significantly positively correlated with the BR Cd or TF\(_{\text{N1}\rightarrow\text{IN1}}\) (Table 3).

### DISCUSSION

Cd transportation to brown rice from the soil will pass through three main processes, including the root uptake from soil, transport from the root to the shoot, and transport from the shoot to the brown rice. Root Cd concentration differed significantly among different cultivars in this work, which

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**Table 2. TF\(_{\text{Nn}\rightarrow\text{INn}}\) and TF\(_{\text{Nn}\rightarrow\text{Ln}}\) in the Cultivars with Similar Shoot Cd Values**

<table>
<thead>
<tr>
<th></th>
<th>TYJ1</th>
<th>NJ9108</th>
<th>YY2640</th>
<th>NJ52</th>
<th>ZXY146</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF(_{\text{N1}\rightarrow\text{IN1}})</td>
<td>0.41 ± 0.02</td>
<td>0.31 ± 0.03</td>
<td>0.32 ± 0.03</td>
<td>0.32 ± 0.03</td>
<td>0.38 ± 0.03</td>
</tr>
<tr>
<td>TF(_{\text{N2}\rightarrow\text{IN2}})</td>
<td>0.21 ± 0.01</td>
<td>0.25 ± 0.02</td>
<td>0.28 ± 0.02</td>
<td>0.24 ± 0.02</td>
<td>0.28 ± 0.02</td>
</tr>
<tr>
<td>TF(_{\text{N3}\rightarrow\text{IN3}})</td>
<td>0.15 ± 0.01</td>
<td>0.15 ± 0.01</td>
<td>0.15 ± 0.01</td>
<td>0.20 ± 0.02</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>TF(_{\text{N4}\rightarrow\text{IN4}})</td>
<td>0.03 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>TF(_{\text{N1}\rightarrow\text{Ln}})</td>
<td>0.23 ± 0.02</td>
<td>0.26 ± 0.02</td>
<td>0.49 ± 0.06</td>
<td>0.16 ± 0.02</td>
<td>0.30 ± 0.02</td>
</tr>
<tr>
<td>TF(_{\text{N2}\rightarrow\text{Ln}})</td>
<td>0.10 ± 0.01</td>
<td>0.21 ± 0.02</td>
<td>0.62 ± 0.04</td>
<td>0.18 ± 0.01</td>
<td>0.35 ± 0.03</td>
</tr>
<tr>
<td>TF(_{\text{N3}\rightarrow\text{Ln}})</td>
<td>0.08 ± 0.01</td>
<td>0.18 ± 0.02</td>
<td>0.28 ± 0.02</td>
<td>0.21 ± 0.02</td>
<td>0.31 ± 0.01</td>
</tr>
<tr>
<td>TF(_{\text{N4}\rightarrow\text{Ln}})</td>
<td>0.15 ± 0.02</td>
<td>0.15 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.11 ± 0.01</td>
<td>0.09 ± 0.01</td>
</tr>
</tbody>
</table>

*TF\(_{\text{Nn}\rightarrow\text{INn}}\): translocation factor of Cd from Nn to INn (n = 1, 2, 3, or 4). TF\(_{\text{Nn}\rightarrow\text{Ln}}\): translocation factor of Cd from Nn to Ln (n = 1, 2, 3, or 4). Different letters in the same row indicate significant (\(P < 0.05\)) difference among cultivars.*
mainly resulted from the interaction of the three physiological processes. First, Cd uptake by the roots differed among the different cultivars because of the different expression of the Nramp5 (natural resistance-associated macrophage protein) transporter protein.\(^{20,34}\) Second, Cd sequestration in iron plaque depends on the ability to produce radial oxygen loss (ROL) involved in the formation of iron plaque on the root surface,\(^{35,36}\) and Cd sequestration in root cells depends on the expression of the transporter protein (like OsHMA3) involved in the sequestration of Cd in the vacuolar membrane.\(^{37}\) Third, Cd loading in the xylem depends on the expression of the transporter protein (like OsHMA2).\(^{38}\) The present work found a significant positive linear correlation between brown rice and root Cd concentration ($R^2 = 0.96$, $P < 0.01$, $n = 36$) (Figure 6a), and this correlation was still significantly positive ($R^2 = 0.67$, $P < 0.01$, $n = 33$), excluding CLY4418 (Figure S5), suggesting that the Cd concentration in brown rice was significantly affected by the root Cd concentration. However, the Cd accumulation in the brown rice of the cultivars with similar root Cd (i.e., TYJ1, NJ9108, NXY8, YY2640, NJ52, and ZXY146) mainly depended on the transport of Cd from the root to the brown rice, including root−shoot transport and shoot−brown rice transport. In the processes of upward Cd transport, xylem loading and phloem transport are the two main pathways.\(^{39−41}\) Xylem loading mediates Cd translocation from the root to the shoot and is considered to be the main and most common physiological factor accounting for the different allocations of Cd to different rice parts.\(^{40,42}\) The translocation factors from the root to the shoot were similar in the cultivars with similar root Cd levels, except for NXY8, in this work. Therefore, Cd accumulation in the brown rice of the five cultivars with similar shoot Cd amounts mainly depended on

![Diagram of vascular bundle systems](image-url)

**Figure 5.** Vascular bundle (VB) systems in the junction of the flag leaf and the first node (JFLN) of NJ9108, NJ52, and ZXY146; scale bars = 500 μm (a). Selected area by the dotted line, scale bars = 100 μm (b). Three different VB phases are shown as follows: the enlarged VB (EVB, yellow), transit VB (TVB, red), and diffuse VB (DVB, purple).

<table>
<thead>
<tr>
<th>Area of VBs in the Central Part of the Junction of the Flag Leaf and the First Node and Its Correlation Coefficients with TF(_{N1−IN1})</th>
<th>TVB(^a) (mm(^2))</th>
<th>DVB(^b) (mm(^2))</th>
<th>Total VBs(^c) (mm(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>NJ9108</td>
<td>0.65 ± 0.02 b</td>
<td>0.04 ± 0.01 c</td>
<td>0.69 ± 0.03 c</td>
</tr>
<tr>
<td>NJ52</td>
<td>0.84 ± 0.03 a</td>
<td>0.29 ± 0.03 b</td>
<td>1.13 ± 0.06 b</td>
</tr>
<tr>
<td>ZXY146</td>
<td>0.84 ± 0.02 a</td>
<td>0.53 ± 0.02 a</td>
<td>1.38 ± 0.04 a</td>
</tr>
</tbody>
</table>

\(^a\)TVB: transit vascular bundle. \(^b\)DVB: diffuse vascular bundle. \(^c\)VBs: vascular bundles. \(^d\)r between TF\(_{N1−IN1}\) and each VB: Correlation coefficients ($r$) between the translocation factor (the first node to the first internode) and each VB ($n = 9$). Different letters in the same column indicate significant ($P < 0.05$) difference among VBs; * and ** indicate significant difference at $P < 0.05$ and $P < 0.01$, respectively.
the shoot—brown rice transport (Figure 6b) mediated mainly by phloem transport.

Nodes are the central organ where xylem–phloem transfer occurs, and the totally organized vascular systems in the node includes the EVB, TVB, and DVB. An axial vascular system includes three nodes and a leaf (Nn+2, Nn+1, Nn, and Ln), transport of elements (including Cd) from the DVB of Nn+2 to the TVB of Nn+1, then to the EVB of Nn and finally to the DVB of Nn via nodal vascular anastomosis or to Ln via the xylem of EVB. In this way, Cd was finally transferred to the panicle and the brown rice through several circular translocations. The nodes had strong Cd accumulation ability in the shoot and controlled the translocation efficiency of Cd (Table 2 and Figure 4). Moreover, the TFN1−IN1 was the lowest among all nodes (Table 2), indicating that the first node played the most important role in restricting the transport of Cd to the brown rice. This phenomenon most likely occurred because Cd accumulated in the node would be readily transported to the leaves via the EVB, the nodes showed no significant obstruction of Cd transport to the leaves.

The Cd restriction in the nodes differed among the different rice cultivars, which was associated with the difference in the vascular bundles in the node. The morphological variation in the VB in the junction of the flag leaf and the first nodes (JFLN) was considered to contribute greatly to this variability in the present work. There are three phases of VB in a node. At the bottom of the node, only EVB and TVB exist in the VB system, and they are connected by nodal vascular anastomosis or to Ln via the xylem of EVB. In this way, Cd was finally transferred to the panicle and the brown rice through several circular translocations. The nodes had strong Cd accumulation ability in the shoot and controlled the translocation efficiency of Cd (Table 2 and Figure 4). Moreover, the TFN1−IN1 was the lowest among all nodes (Table 2), indicating that the first node played the most important role in restricting the transport of Cd to the brown rice. This phenomenon most likely occurred because Cd accumulation in the node mainly depends on the chelation of metallothionein, and the transcript levels of cDNA that encode metallothionein-like proteins are enhanced in the first node, and, thus, more Cd will be chelated in the first node. These results suggested that the upward translocation of Cd in the shoots was mainly restricted by the nodes, especially by the first node, and, thus, Cd accumulation in the brown rice was much lower than in the other parts of the rice plant. However, because Cd accumulated in the node would be readily transported to the leaves via the EVB, the nodes showed no significant obstruction of Cd transport to the leaves.

The Cd restriction in the nodes differed among the different rice cultivars, which was associated with the difference in the vascular bundles in the node. The morphological variation in the VB in the junction of the flag leaf and the first nodes (JFLN) was considered to contribute greatly to this variability in the present work. There are three phases of VB in a node. At the bottom of the node, only EVB and TVB exist in the VB system, and they are connected by nodal vascular anastomosis (NVA). Cd in the node mainly accumulated on the EVB surface due to the metallothionein, and it can be partly transported to the TVB by transporters via the NVA or to the EVB in the upper region. In the middle section of the node, the NVA was changed to an axial DVB, and thus, three VB phases—EVB, TVB, and DVB—constitute the basic structure of each node. Cd is also sequestered on the EVB and transported to the DBV or the upper EVB. At the upper part of the node, where the leaf sheath is located (the junction of the flag leaf and the first nodes, JFLN), the EVB is located in the leaf sheath, and only TVB and DVB exist in the central organ (Figure 5). In the JFLN region, Cd can be directly transported to the flag leaf via the EVB or transported to the first internode and panicle via the DVB, indicating that Cd transport in this

Figure 6. Linear correlation between the Cd concentration in the brown rice and in the root of the 12 rice cultivars (a). Linear correlation between the Cd concentration in brown rice and the translocation efficiency of Cd from internode 4 to internode 1 in the cultivars TYJ1, NJ9108, YY2640, NJ52, and ZXY146 (b). The dashed lines indicate 95% confidence intervals.

Figure 7. Linear relationship between the concentration of Cd in brown rice and the concentration of Cd in the first internode (a) and TFN1−IN1 and TFN1−FL (b) in the 12 cultivars. Linear relationship between the concentration of Cd in brown rice and the concentration of Cd in the flag leaf in the cultivars with similar shoot Cd values (c). The dashed lines indicate 95% confidence intervals.
region plays an important role in Cd allocation to the brown rice, which was confirmed by the significant positive correlation ($R^2 = 0.96, P < 0.01$) between the Cd concentration in the first internode and the concentration of Cd in brown rice (Figure 7a). Therefore, the morphological difference of the DVB in the JFLN region may contribute to the variability of the brown rice Cd concentration in cultivars with similar shoot DVB areas. In this work, we found that the Cd transport to the brown rice was easier in the cultivars with larger DVB areas in the JFLN region. This was because the phloem transport of Cd through the DVB is the main pathway to the panicle or brown rice, and a larger DVB area may provide more space for the transport of Cd, so more Cd will be transferred to the brown rice.

In addition, Cd in the first node was mainly sequestrated on the surface of EVB, which would be partly transported to the DVB via nodal vascular anastomosis and then to the panicle via the phloem of DVB; another portion would be transported to the flag leaf via the xylem of EVB. When the DVB area was too small to load sufficient Cd, such as in NJ9108, only a small proportion of Cd might be transferred to the first internode, and more Cd might be transferred to the flag leaf compared with the cultivars with larger DVB areas (such as in ZXY146). The significant negative correlation between TF$_{N1-N1}$ and TF$_{N1-fl}$ (Figure 7b) further confirmed this assumption. As a result, Cd transport to the flag leaf seemed to play a negative role in Cd accumulation in brown rice (Figure 7c).

Therefore, selecting or breeding cultivars with lower root Cd or a smaller DVB area in the first node is promising to reduce Cd accumulation in brown rice, therefore lowering the health risks associated with the consumption of rice. However, the factors controlling the DVB area of the node require further study.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.7b03333.

Cd concentrations in nodes and internodes and in leaves, Cd translocation characteristics, dry weight of rice root, shoot, and brown rice, and linear correlation between Cd concentration in brown rice and in root (PDF)

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Notes

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REFERENCES


(2) NTP *National toxicology program, tenth report on carcinogens*; Department of Health and Human Services: Research Triangle Park, NC, 2000; pp III-42–III-44.


