Effective reduction of cadmium accumulation in rice grain by expressing OsHMA3 under the control of the OsHMA2 promoter

Ji Feng Shao1,2,*, Jixing Xia1,3,*, Naoki Yamaji1, Ren Fang Shen2 and Jian Feng Ma1,†

1 Institute of Plant Science and Resources, Okayama University, Chuo 2-20-1, Kurashiki 710-0046, Japan
2 State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China
3 State Key Laboratory of Conservation and Utilization of Subtropical Agro-bioresources, College of Life Science and Technology, Guangxi University, Nanning 530005, China

* These authors contributed equally to this work.
† Correspondence: maj@rib.okayama-u.ac.jp

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Abstract

Reducing cadmium (Cd) accumulation in rice grain is an important issue for human health. The aim of this study was to manipulate both expression and tissue localization of OsHMA3, a tonoplast-localized Cd transporter, in the roots by expressing it under the control of the OsHMA2 promoter, which shows high expression in different organs including roots, nodes, and shoots. In two independent transgenic lines, the expression of OsHMA3 was significantly enhanced in all organs compared with non-transgenic rice. Furthermore, OsHMA3 protein was detected in the root pericycle cells and phloem region of both the diffuse vascular bundle and the enlarged vascular bundle of the nodes. At the vegetative stage, the Cd concentration in the shoots and xylem sap of the transgenic rice was significantly decreased, but that of the whole roots and root cell sap was increased. At the reproductive stage, the concentration of Cd, but not other essential metals, in the brown rice of transgenic lines was decreased to less than one-tenth that of the non-transgenic rice. These results indicate that expression of OsHMA3 under the control of the OsHMA2 promoter can effectively reduce Cd accumulation in rice grain through sequestering more Cd into the vacuoles of various tissues.

Keywords: Cadmium, node, OsHMA2, OsHMA3, transgenic rice, vacuolar sequestration.

Introduction

Cadmium (Cd) is a class 1 carcinogen that can be accumulated in the human body over time from ingestion of Cd-containing food (Clemens and Ma, 2016). Excessive intake may result in damage to kidney tubules, rhinitis, emphysema, as well as other chronic disorders (McLaughlin et al., 1999). Therefore, Cd intake through the food chain poses a serious threat to human health and food security (Clemens and Ma, 2016). Rice is an important staple food and a major source of Cd intake (Watanabe et al., 2004; Cheng et al., 2006). ‘Itai-itai disease’ is the result of intake of Cd-containing rice. The Codex Alimentarius Commission/World Health Organization with responsibility for the safety of food and human health has set 0.4 mg Cd kg⁻¹ in polished rice grain as the maximum permissible limit (Codex Alimentarius Commission of Food and Agriculture Organization, 2006), but rice produced in some areas often exceeds this. Therefore, it is a very important issue for human health to limit transfer of Cd from the soil to the grain in rice.
There are several steps for Cd transfer from soil to grain including at least uptake by the roots, root-to-shoot translocation, and distribution to the grain (Clemens and Ma, 2016). Several transporters involved in these processes have been identified in rice. The uptake of Cd is mediated by OsNramp5, a member of the natural resistance-associated macrophage protein family (Ishimaru et al., 2012; Sasaki et al., 2012). It is polarly localized at the distal side of both root exodermis and root endodermis (Sasaki et al., 2012). The expression of OsNramp5 is not induced by Cd. Knockout of OsNramp5 resulted in complete loss of Cd uptake, indicating that OsNramp5 is a major transporter for Cd uptake and is responsible for transporting Cd from soil solution to rice root cells. After the uptake, part of the Cd is subsequently sequestered into the vacuoles by OsHMA3, a P-type heavy metal ATPase (Ueno et al., 2010; Miyadate et al., 2011). OsHMA3 is localized to the tonoplast of all root cells (Ueno et al., 2010). The expression of OsHMA3 is also not induced by Cd. Loss of function of this gene resulted in high Cd accumulation in the shoots and grains (Ueno et al., 2010). On the other hand, the remaining part of the Cd taken up by OsNramp5 is translocated to the shoots, which is mediated by OsHMA2, a homolog of OsHMA3 (Satoh-Nagasawa et al., 2012; Takahashi et al., 2012; Yamaji et al., 2013). However, differing from OsHMA3, OsHMA2 is a plasma membrane-localized transporter for Cd and is localized at the pericycle of the roots (Yamaji et al., 2013). OsHMA2 is constitutively expressed in the roots at the vegetative stage, and knockout of this gene resulted in lower root-to-shoot translocation of Cd (Satoh-Nagasawa et al., 2012; Takahashi et al., 2012; Yamaji et al., 2013).

On the other hand, at the reproductive stage, two transporters (OsHMA2 and OsLCT1) responsible for the distribution of Cd to the grains have been identified. In addition to the roots, OsHMA2 is also expressed in the nodes (Yamaji et al., 2013). Especially, it is highly expressed in node I, the uppermost node at the reproductive stage. In nodes, OsHMA2 is localized at the phloem of enlarged vascular bundles and diffuse vascular bundles (Yamaji et al., 2013). Knockout of OsHMA2 resulted in decreased concentration of Cd in the upper nodes and reproductive organs compared with wild-type rice. Therefore, OsHMA2 is involved in reloading Cd from the intervening parenchyma tissues into the phloem of diffuse vascular bundles (Yamaji et al., 2013). On the other hand, OsLCT1 (Oryza sativa low-affinity cation transporter 1) seems to be also involved in the intervascular transfer of Cd (Uraguchi et al., 2011). OsLCT1 is localized to the plasma membrane and shows efflux transport activity not only for Cd but also for K, Mg, Ca and Mn (Uraguchi et al., 2011). OsLCT1 is expressed around the enlarged vascular bundles and diffuse vascular bundles in the node at the reproductive stage (Uraguchi et al., 2011). Knockdown of OsLCT1 resulted in decreased Cd concentration in phloem and grains (Uraguchi et al., 2011). However, in contrast to OsHMA2, which is highly expressed in the nodes throughout reproductive growth (Yamaji et al., 2013), expression of OsLCT1 in the nodes was only observed at the ripening stage (Uraguchi et al., 2011). OsLCT1 might be involved in efflux of Cd from the phloem within the nodes at this stage.

Several attempts have been made to reduce Cd accumulation in the rice grain by manipulating the transporter genes described above. However, although Cd accumulation was reduced in the grain of the genetically modified rice, a penalty of reduced growth and yield was observed in some cases. This is because some transporters identified for Cd are also involved in transporting essential metals (Clemens and Ma, 2016). For example, OsNramp5 is also a major transporter for Mn (Sasaki et al., 2012). Therefore, knockout of its gene also reduces Mn uptake, resulting in decreased rice yield especially under Mn-limited condition (Sasaki et al., 2012), although contradictory results were also reported due to different growth conditions (Ishikawa et al., 2012; Tang et al., 2017). Knockout of OsHMA2 also resulted in decreased rice yield because it is also required for Zn transport (Yamaji et al., 2013). An exception is overexpression of OsHMA3 in rice. The expression of OsHMA3 is very low in native rice cultivars (Ueno et al., 2010). Overexpression of OsHMA3 selectively reduced accumulation in the grain of Cd but not of Zn and Fe (Ueno et al., 2010; Sasaki et al., 2014). Although OsHMA3 also transports Zn, Zn homeostasis in the shoots seems to be maintained by up-regulating five genes related to Zn transport (Sasaki et al., 2014).

The aim of this study was to find a way to efficiently reduce Cd accumulation in rice grains by utilizing different expression pattern and properties of two Cd transporter genes, OsHMA3 and OsHMA2. OsHMA3 is mainly expressed in the roots and is involved in vacuolar sequestration of Cd (Ueno et al., 2010), while OsHMA2 is expressed in the roots and nodes (Yamaji et al., 2013). Therefore, if OsHMA3 is expressed under the control of the OsHMA2 promoter, there is a possibility that Cd will be sequestered into the vacuoles at the roots and nodes before loading to the grain, resulting in decreased Cd accumulation in rice grain. Our results support this possibility, and this provides an effective way to reduce Cd accumulation in the grains of rice grown in Cd-contaminated soils.

Materials and methods

Generation of transgenic plants

To construct the ProOsHMA2-OsHMA3 DNA fusion, the promoter of OsHMA2 (2.13 kb upstream of the translational start) was amplified by PCR from cv. Nipponbare genomic DNA using primers 5′-AAGCTTCACCTCTTTTCTCGTTGTGT-3′ (HindIII site italicized) and 5′-GGATCCCTCTCTCCTCACCTCTCTCTCTCT-3′ (BamHI site italicized). Using HindIII and BamHI, the amplified fragment was cloned into pZP2PH-lac carrying the terminator of the nopaline synthase gene, producing the OsHMA2 promoter construct. The OsHMA3 cDNA containing a BamHI restriction site was amplified from Nipponbare by RT-PCR using the primers 5′-AgaIATGGCCCAAGGATGAGG-3′ (BamHI site italicized) and 5′-TgaIAGACAATCATCACTTTTCACTTTACC-3′ (BamHI site italicized). Using BamHI, the amplified fragment was cloned into pZP2PH-lac carrying the OsHMA2 promoter and the terminator of the nopaline synthase gene, resulting in the OsHMA2 promoter-OsHMA3 cDNA construct (ProOsHMA2-OsHMA3) (see Supplementary Fig. S1 at JXB online). This construct was transformed into Agrobacterium tumefaciens (strain EHA101), followed by transforming to calluses derived from Nipponbare according to Hiei et al. (1994).
RNA isolation and gene expression analysis

To investigate the expression level of OsHMA3 in the transgenic lines, roots, shoot basal region (0.5 cm from the root-to-shoot junction), and shoots of 28-day-old seedlings grown hydroponically were separately sampled. The total RNA was extracted with an RNAsesy Plant Mini Kit (Qiagen). One microgram of total RNA was used for first strand cDNA synthesis using a SuperScript II kit (Invitrogen), following the manufacturer’s instructions with an oligo (dT)20 primer. The expression was determined with SYBR Premix Ex Taq™ (Takara) by Mastercycler ep realplex (Eppendorf). The primer sequences for quantitive RT-PCR were 5′-CATAGTGAAGCTGCCTGAGATC-3′ and 5′-GATCAAACGCATAGCAGCATCG-3′ for OsHMA2, 5′-TCCATCCAACCAACCCCGAAA-3′ and 5′-TGCCAAATGCTCCCTGTCCAACCCCA-3′ for OsHMA3, 5′-CGCTATGGCTGTCGTCGTAATCAGG-3′ and 5′-GCGACGTGAGCTAATTTGGA-3′ for ZIP1, 5′-GCAATTCGCAATGTCG-3′ and 5′-AACCGAAATCCGAAAGCCGACGTG-3′ for ZIP4, 5′-GGGCTCTGTAATGCTGAGATC-3′ and 5′-AATTTCTTCATTGCAATGTCG-3′ for ZIP9, and 5′-GCTCAGTTAAAGAA for OsHMA2. The expression pattern of OsHMA2 and OsHMA3 in different organs was compared with semi-quantitative PCR.

Immunostaining of OsHMA3 in transgenic rice

To investigate the localization of OsHMA3 in the transgenic line, we performed immunostaining with an antibody against OsHMA3 used previously (Ueno et al., 2010). The root segments, shoot basal region, and node 1 were sampled for immunostaining. The procedures for immunostaining were the same as described before (Yamaji and Ma, 2007). Fluorescence of secondary antibody (Alexa Fluor 555 goat anti-rabbit IgG; Molecular Probes) was observed with a confocal laser scanning microscope (LSM700; Carl Zeiss).

Phenotypic analysis of transgenic plants at vegetative stage

Seeds of two transgenic lines (B9 and B17) and non-transgenic rice (cv. Nipponbare) were soaked in water for 2 d at 30 °C in the dark and the germinated seeds were transferred to nylon nets floating on a solution containing 0.5 mM CaCl2 in a 1.2 liter pot and grown for another 5 d. The seedlings were then transferred to a 3.5 liter pot containing 3.5 kg soil supplemented with 0.5 mM CaCl2 and were grown for another 5 weeks. The seedlings were then transplanted to a pot containing 3.5 kg soil supplemented with 1.0 mg kg−1 Cd as CdSO4 with three replicates. The plants were grown in a controlled glasshouse at 25–30 °C under natural light from 19 July to 7 November 2012. The plants were grown under submerged conditions until flowering time and then changed to upland conditions by watering with tap water every day. At harvest, the plants were separated into brown rice, node 1, flag leaf, and the remaining part of straw. The concentration of Cd in these organs was determined with inductively coupled plasma mass spectrometry (ICP-MS) after digestion as described below.

Determination of Cd and other metals

All samples were dried at 70 °C in an oven for at least 3 d. The dried samples were digested with concentrated HNO3 (60%) at a temperature up to 140 °C. The metal concentration in the digested solution, xylem sap, and root cell sap was determined by ICP-MS (7700X; Agilent Technologies) after appropriate dilution.

Statistical analysis

The analysis of significance was performed by one-way ANOVA, followed by Tukey’s test. Significance of differences at P<0.05 is shown by different letters in the figures.

Results

Expression of OsHMA3 in transgenic line

We obtained two independent transgenic lines (B9 and B17) carrying OsHMA3 under the control of the OsHMA2 promoter (see Supplementary Fig. S1). Expression analysis showed that the expression level of OsHMA3 was significantly increased in the roots, shoot basal region including basal nodes, and shoots of two transgenic lines compared with non-transgenic line (Fig. 1A). Among the transgenic lines, B17 showed a higher OsHMA3 expression than B9. By contrast, the expression of OsHMA2 did not differ between transgenic and non-transgenic lines (Fig. 1B), indicating that introduction of OsHMA2 promoter only enhanced the expression of OsHMA3. These expression patterns were similar to their native ones; OsHMA2 was highly expressed in the roots, shoots, and shoot basal region, while OsHMA3 was mainly expressed in the roots at a low level (Supplementary Fig. S2).

Tissue localization of OsHMA3 in transgenic line

To investigate whether OsHMA3 localization was altered under the control of the OsHMA2 promoter, we performed

Collection of xylem sap and root cell sap

For collection of xylem sap, seedlings (17 d old) were exposed to a nutrient solution containing 1 mM Cd. The solution was changed every 2 d. After 8 d, the shoot (2 cm above the root) was excised with a razor, and the xylem sap was collected with a micropipette for 45 min after decapitation of the shoot.

For collection of root cell sap, the roots of the seedlings after Cd exposure as described above were washed with 5 mM cold CaCl2 three times and blotted with a paper towel, followed by placing on a filter in a tube. The samples were frozen at −80 °C till use. Before collection of root cell sap, the sample was thawed at room temperature, followed by centrifugation at 20,000 g for 10 min. The concentration of Cd and other metals in the xylem sap and root cell sap was determined as described below.

Analysis of Cd accumulation in transgenic rice

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immunostaining using an antibody specific for OsHMA3 (Ueno et al., 2010). To make all samples comparable, we observed all cross sections under the same conditions due to the detection condition used (Fig. 2A–C). By contrast, a strong signal was detected in the root pericycle and phloem region of both the diffuse vascular bundle and the enlarged vascular bundle of basal nodes of the transgenic line (Fig. 2D–E). In node I of the transgenic line, a strong signal was also detected in the phloem region of the diffuse vascular bundle and the enlarged vascular bundle (Fig. 2F). These results indicate that introduction of the OsHMA2 promoter altered the tissue localization of OsHMA3.

Phenotypic analysis of transgenic lines at vegetative growth stage

To evaluate Cd accumulation, the transgenic lines were grown in a nutrient solution containing 0, 0.1, and 1 µM Cd for 8 d. The growth was almost similar between the transgenic and non-transgenic lines at either Cd concentration although B17 showed slightly less growth (Fig. 3A). The Cd concentration of the shoots was significantly decreased by 55% and 58%, respectively, at 0.1 and 1 µM Cd in the two transgenic lines compared with the non-transgenic line (Fig. 3B). By contrast, the Cd concentration in the roots of transgenic lines was significantly increased compared with the non-transgenic line at both Cd concentrations (Fig. 3C). The root-to-shoot translocation rate (shoot Cd content /total Cd content×100) of Cd was much lower in the transgenic lines than in the non-transgenic line (8% versus 20%) (Fig. 3D).

There was no significant difference in the concentration of Zn, Cu, Fe, and Mn in the shoots between the transgenic and non-transgenic lines in both the absence and the presence of Cd (Fig. 4A, C, E, G). By contrast, the Zn concentration in the roots was higher in the transgenic lines than in the non-transgenic line (Fig. 4B) although the concentrations of Fe and Cu did not differ between the transgenic and non-transgenic lines at either Cd concentration. However, the Mn concentration in the roots of transgenic lines showed slight increase compared with the non-transgenic lines in the presence of Cd (Fig. 4D, F, H). This slight increase in Mn concentration of the roots of transgenic lines may be caused by unknown indirect effect. Overall, the concentration of Zn, Cu, and Mn was decreased by high Cd in both the roots and the shoots of the transgenic and non-transgenic lines (Fig. 4).

**Cd concentration in the xylem sap and root cell sap of transgenic lines**

The Cd concentration in xylem sap was compared between the transgenic and non-transgenic lines. The concentration of Cd in the xylem sap of the transgenic lines was only two-fifths of the non-transgenic line (Fig. 5A). However, there was no significant difference in the concentration of Fe, Zn, and Cu between the transgenic and non-transgenic lines. The concentration of Mn was slightly higher in the xylem sap of the transgenic lines than that of the non-transgenic line (Fig. 5E), but the reason for this is unknown.

Comparison of root cell sap showed that the Cd concentration in the root cell sap was 1.65-fold higher in the two transgenic lines than in the non-transgenic line although the difference was not significant due to large variation (Fig. 6A). The concentration of Zn in the root cell sap was also higher in the two transgenic lines than in the non-transgenic line (Fig. 6B), but there was no difference in the concentration of Mn, Fe, and Cu between lines (Fig. 6C–E).

**Cd accumulation in straw and brown rice of transgenic lines**

To investigate the effect of the OsHMA2 promoter on Cd accumulation in rice grain, both transgenic lines were cultivated in a Cd-contaminated soil and the Cd concentration was compared with the non-transgenic line. There was no significant difference in the grain yield between B9 and the non-transgenic line (Fig. 7A), but the grain yield of B17 was smaller compared with the non-transgenic line (Fig. 7A). There was no difference in the straw
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The Cd concentration in brown rice of the non-transgenic line was 549 µg kg⁻¹, whereas that in the transgenic lines was only 45 µg kg⁻¹ in B9 and 34 µg kg⁻¹ in B17 (Fig. 8A). The Cd concentration was also much lower in the node I, flag leaf, and remaining straw part of the transgenic lines compared with the non-transgenic line (Fig. 8B–D). However, there was no large difference in the concentration of Zn, Fe, Mn, and Cu between the transgenic lines and non-transgenic line in all organs (Fig. 8B–D).

Expression of ZIP genes in the transgenic lines

Since Zn accumulation was increased in the roots but unchanged in the shoots of the transgenic lines compared
with the non-transgenic lines, we investigated the expression level of six genes belonging to the ZIP family in the roots. Among them, the expression level of OsZIP9 in the roots was significantly higher in the transgenic lines than in the non-transgenic lines (see Supplementary Fig. S3), whereas there was no significant difference in the expression of other ZIP genes between lines.

**Discussion**

There are several ways to reduce Cd accumulation in rice grain, such as field remediation, water and nutrient management, and cultivation of low-Cd cultivars (Sebastian and Prasad, 2014). In the present study, we tested a novel transgenic approach for reducing Cd accumulation in the rice grain by expressing OsHMA3 under the control of OsHMA2 promoter. OsHMA3 encodes a tonoplast-localized transporter for Cd and Zn (Ueno et al., 2010; Sasaki et al., 2014). It is mainly expressed in the roots, but shows lower expression (Ueno et al., 2010; Supplementary Fig. S2). Therefore, its capability for vacuolar sequestration of Cd in the roots is limited. On the other hand, OsHMA2 is highly expressed not only in the roots, but also in the nodes (see Supplementary Fig. S2), and is one of the major translocation pathways of Zn and Cd in both roots and shoots (Yamaji et al., 2013). Expression of OsHMA3 under the control of the OsHMA2 promoter resulted in significant increase of OsHMA3 expression in the roots, shoots, and shoot basal region (including nodes) (Fig. 1A). This expression pattern is similar to native OsHMA2 (Supplementary Fig. S2; Yamaji et al., 2013). Furthermore, OsHMA3 localization in the transgenic line was altered; it was strongly localized at the root pericycle and phloem of enlarged and diffuse vascular bundles in the nodes (Fig. 2D–F). These results indicate that introduction of the OsHMA2 promoter not only enhanced the expression level of OsHMA3, but also altered the tissue localization of OsHMA3. As a result, the Cd concentration in the grains of
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the transgenic lines was decreased to less than one-tenth that of the non-transgenic line when grown in a Cd-contaminated soil (Fig. 8A). The Cd concentration in the straw was also decreased to one-third of the non-transgenic line (Fig. 8D). Since rice straw is often fed to livestock, reduced Cd in the straw of transgenic lines will also contribute to limiting Cd transfer to the food chain.

The reduced Cd accumulation in the grain and straw could be mainly attributed to increased OsHMA3 expression in the roots through sequestration of Cd into the vacuoles. This is supported by findings that the Cd in the root cell sap was increased (Fig. 6A) and that the Cd in the xylem sap was decreased (Fig. 5A). Localization of OsHMA3 in the nodes could also further reduce the accumulation of Cd to the shoots and grain. Recently, the node has been demonstrated to play a critical role in distribution of mineral elements including Cd in rice (Yamaji and Ma, 2014, 2017). Nodes have a complex, but well-organized vascular system, which consists of two major vascular bundles, enlarged vascular bundles and diffuse vascular bundles (Yamaji and Ma, 2014, 2017). To deliver mineral elements to the developing tissues such as young leaves and panicles, an inter-vascular transfer from enlarged vascular bundles to diffuse vascular bundles is required (Yamaji and Ma, 2014, 2017). To deliver mineral elements to the developing tissues such as young leaves and panicles, an inter-vascular transfer from enlarged vascular bundles to diffuse vascular bundles is required (Yamaji and Ma, 2014, 2017). Localization of OsHMA3 in the phloem of enlarged and diffuse vascular bundles of transgenic lines will help to sequester Cd into the vacuoles before reloading Cd from the intervening parenchyma tissues into the phloem of diffuse vascular bundles (Fig. 2F). This results in further efficient reduction of Cd in rice grain.

Expression of OsHMA3 under the control of the OsHMA2 promoter did not significantly affect growth at the vegetative stage (Fig. 3A). The grain yield of the transgenic line B9 was comparable to the non-transgenic line although B17 showed a reduced grain yield (Fig. 7A). The exact reason for this negative effect is unknown, but one possibility is that other mutations occurred during transformation.

OsHMA3 is also involved in the sequestration Zn into the vacuoles of root cells (Sasaki et al., 2014). We found that similar to Cd, the Zn concentration in the roots and root cell sap was higher in the transgenic lines than in the non-transgenic line (Figs. 4B, 6B). However, different from Cd, the Zn concentration in the shoots, xylem sap, and brown rice was similar between the transgenic and non-transgenic lines (Figs. 4A, 5B, 8A). Unlike Cd, Zn is an essential element for plant growth and its homeostasis is tightly regulated (Krämer et al., 2007). It was reported previously that overexpression of OsHMA3 resulted in up-regulation of genes related to Zn uptake/translocation including OsZIP4 (ZRT, IRT-like protein), OsZIP5, OsZIP8, OsZIP9, and OsZIP10 (Sasaki et al., 2014). In the present study, we found that OsZIP9 is highly...
enhanced in the transgenic lines (see Supplementary Fig. S3). Although the exact role of OsZIP9 is unknown, its up-regulation may contribute to maintaining Zn homeostasis in the shoots. The difference in up-regulation of ZIP genes between overexpression lines and OsHMA2 promoter–OsHMA3 transgenic lines may be attributed to different tissue specificity of OsHMA3 expression. Different from OsHMA2 promoter–OsHMA3 transgenic lines (Fig. 2), OsHMA3 was expressed in all tissues, which may cause more expression of ZIP genes to compensate Zn.

There was no difference in the concentration of Fe, Mn, and Cu in the shoots, roots, and brown rice between the transgenic and non-transgenic lines (Figs. 4, 8). However, high Cd in the external solution decreased the concentration of Mn and Cu in the shoots and roots of both transgenic and non-transgenic lines (Fig. 4C, G). Since Mn uptake is also mediated by OsNramp5 (Sasaki et al., 2012), the decreased Mn accumulation is caused by competition between Mn and Cd for the OsNramp5. The mechanism for Cd-decreased Cu uptake remains to be further investigated since the transporter for Cu uptake in rice has not been identified.

In conclusion, our results show that expression of OsHMA3 under the control of the OsHMA2 promoter enhanced the expression and altered the tissue localization of OsHMA3,

Fig. 6. Concentration of metals in the root cell sap. Seedlings (17 d old) of transgenic lines carrying OsHMA3 under the control of OsHMA2 promoter were exposed to 1 µM Cd in a 1/2 Kimura B nutrient solution. After 8 d, the root cell sap was collected by centrifugation. Concentration of Cd (A), Zn (B), Fe (C), Cu (D), and Mn (E) in the root cell sap was determined by ICP-MS. Data represent means ±SD (n=3). Statistical comparison was performed by one-way ANOVA, followed by Tukey’s multiple comparison test. Different lower-case letters indicate significant difference at P<0.05.

Fig. 7. Growth and grain yield of transgenic lines. Both transgenic lines carrying OsHMA3 under the control of OsHMA2 and the non-transgenic line were cultivated in a Cd-contaminated soil till ripening. The grain yield (A) and straw (B) were recorded. Data represent means ±SD (n=3). Statistical comparison was performed by one-way ANOVA, followed by Tukey’s multiple comparison test. Different lower-case letters indicate significant difference at P<0.05.
resulting in efficient and significant reduction of Cd in rice grain through sequestering more Cd into vacuoles in the roots and nodes. This approach provides an effective way to selectively reduce Cd accumulation in the rice grain, which will ultimately contribute to reducing health risk.

Supplementary data

Supplementary data are available at JXB online.

Fig. S1. Scheme of the construct used for the transformation.

Fig. S2. Expression pattern of OsHMA2 and OsHMA3 in different organs of rice.

Fig. S3. Expression of OsZIP genes in the roots of transgenic and non-transgenic lines.

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Conflict of interest

All the authors have declared no conflict of interest.

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