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The critical role of the shoot base in inhibiting cadmium transport from root to shoot in a cadmium-safe rice line (*Oryza sativa* L.)

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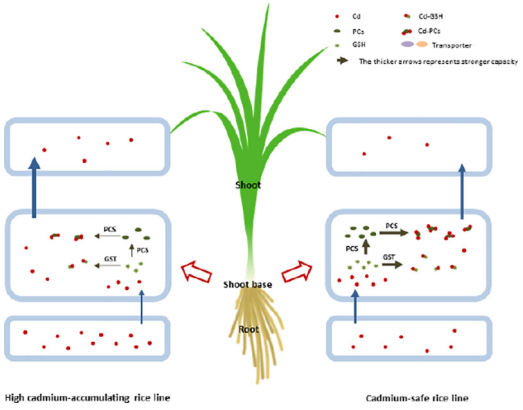
HIGHLIGHTS

- Shoot base played an important role as switch in Cadmium (Cd)-safe rice line D62B.
- Shoot base retained amounts of Cd, resulting in low Cd translocation to shoot.
- Glutathione (GSH) and phytochelatins (PCs) biosynthesis was vital for Cd chelation.
- Exogenous GSH further promoted Cd retention in shoot base.

GRAPHICAL ABSTRACT



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ABSTRACT

Cadmium (Cd) is harmful to rice and human, thus screening and understanding the mechanism of Cd-safe rice lines, which accumulate little Cd in brown rice, is necessary. D62B was screened as a Cd-safe rice line with low Cd translocation from roots to shoots, and there must be a switch restricting Cd transport from roots to shoots. Here we found that shoot base played the role as switch. Cd concentration in the shoot base of D62B was 1.57 times higher compared with a high Cd-accumulating rice line (Wujin4B) and lower Cd translocation under Cd stress. Glutathione (GSH) and phytochelatins (PCs) were important in this process. GSH and PCs concentrations in the shoot bases of D62B were 1.01– 1.83 times higher than Wujin4B as well as the glutathione S-transferase (GST) and phytochelatin synthase (PCS) concentrations, keeping in consistent with up-regulation of the genes *OsGST* and *OsPCS1*. PCs synthesis was further promoted by exogenous GSH. Our results prove the role of shoot bases as switch for restricting Cd transport in D62B due to its great potential for GSH and PCs biosynthesis, and thereby Cd chelation. This could be considered a key mechanism for low Cd accumulation in brown rice of the Cd-safe rice line.

1. Introduction

Soil contamination with metals, especially with cadmium (Cd), has become a severe global concern. Cd is a non-essential metal that can inhibit plant growth and entails a potential risk for human health (Astolfi et al., 2012; Luo et al., 2016). Rice (*Oryza sativa* L.) is the main food

Abbreviations: Cd, cadmium; NPTs, non-protein thiols; PCs, phytochelatins; GSH, glutathione; PCS, phytochelatin synthases; GST, glutathione S-transferase; TF, translocation factor.

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related to human Cd intake, accounting for more than half of the total Cd intake among the Chinese population (Song et al., 2017). Thus, it is imperative to reduce Cd accumulation in brown rice. Four major sequential processes determine the probability of Cd entry into grains: Cd uptake by the root; Cd xylem-loading-mediated translocation to the shoot; Cd redistribution through the vascular system at the nodes; remobilization from leaf blades via phloem and transport into grain (Kobayashi et al., 2013; Yoneyama et al., 2015; Ricachenevsky et al., 2018). Therefore, Cd transport from the root to the shoot influences Cd accumulation in the shoot and eventually in brown rice grains (Uraguchi et al., 2009; Nocito et al., 2011; Ricachenevsky et al., 2018). The junction of the root with the shoot, known as the shoot base, constitutes an important tissue for Cd loading into xylem sap (Fujimaki et al., 2010; Shao et al., 2018). Therefore, the shoot base may be a key tissue for inhibiting Cd transport to the shoot, but its role has not been fully explored.

Once absorbed, Cd can be bound to chelators and transported into vacuoles, thus affecting xylem loading and Cd transport from roots to shoots (Lux et al., 2011; Yoneyama et al., 2015; Ricachenevsky et al., 2018). Non-protein thiols (NPTs) such as glutathione (GSH) and phytochelatins (PCs) are key metal chelators involved in metal transport and homeostasis in cells (Hazama et al., 2015; Gao et al., 2016). GSH, an indispensable tripeptide, is a key component for Cd scavenging due to the high affinity of Cd to its thiol group (Zhang and Ying, 2008; Delalande et al., 2010). PCs are a series of polypeptides synthesised from GSH through the action of phytochelatin synthases (PCS); PC_{2-4} are reported as the predominant PCs (Hasan et al., 2016; Uraguchi et al., 2017; Maria et al., 2018). PCs are considered the most effective Cd chelators (Gupta et al., 2013; Degola et al., 2014); under Cd stress, PCs synthesis is stimulated to metal homeostasis. In the plant cell, Cd ions can bind to PCs immediately, and the Cd-PCs complexes formed are typically transported into the vacuole for compartmentalisation. In the vacuoles, the low-molecular-weight (LMW) complexes are converted to high-molecular-weight (HMW) complexes with free Cd ions (Szalai et al., 2013). The conjugation of Cd to the thiol groups of GSH and PCs synthesis are catalysed by the enzymes glutathione S-transferase (GST) and PCS, respectively (Zhang and Ying, 2008; Delalande et al., 2010; Szalai et al., 2013), and their encoding genes, *GST* and *PCS*, have been found in rice and other species (Kabir et al., 2017; Srivastava et al., 2018; Li et al., 2019a). Some reports document that the addition of exogenous GSH to roots significantly improved the concentrations of GSH and PCs in that tissue, with the concomitant mitigation of Cd toxicity and a decreased Cd transport from root to shoot (Nakamura et al., 2013; Kim et al., 2017). In maize seedlings, increased *PCS* expression and decreased GST activity after exogenous GSH application were also found (Li et al., 2017). Exogenous GSH can induce PCs synthesis and inhibit Cd transport to the shoot, but its effect on the shoot base has not been explored yet.

D62B is one of the Cd-safe rice lines which were selected from 146 rice lines in our previous study (Zhang et al., 2014). When grown in Cd-contaminated soils, D62B accumulates less than 0.2 mg kg^{-1} of Cd in the brown rice, which is within the limit established by the National Food Hygienic Standard of China (GB2762-2017) (Zhang et al., 2014). A high Cd concentration was observed in the roots of the line D62B along with a low Cd concentration in the shoots, suggesting a low Cd translocation from roots to shoots. Besides, increased amounts of GSH and PCs, which may favour Cd retention in the roots, were documented in this rice line (Li et al., 2019b). Based on these observations, we hypothesised that shoot bases take part in inhibiting Cd transport from roots to shoots as a switch in the D62B line. Moreover, we speculated that GSH and PCs biosynthesis might be critical for Cd retention in rice shoot bases. To verify the critical role of the shoot bases in Cd retention and understand the basis of the reduced Cd transport to shoots in the rice line D62B, we investigated: (1) Cd accumulation in the roots, shoot bases, and shoots; (2) GSH and PCs accumulation in the shoot bases; and (3) GSH and PCs biosynthetic potential in the shoot bases of the Cd-safe rice line D62B.

2. Materials and methods

2.1. Plant material

Our work was focused on the Cd-safe rice (*Oryza sativa* L.) line D62B, and the high Cd-accumulating rice line Wujin4B was used as a control. These two rice lines were selected from 146 rice lines evaluated in our previous studies (Zhang et al., 2014). D62B is a maintainer rice line with a growth period of 150 ± 5 d. Wujin4B is also a maintainer rice line with a growth period of 160 ± 5 d. Both are *indica* varieties and were provided by the College of Agronomy, Sichuan Agricultural University.

2.2. Hydroponic experiment and plant sampling

A hydroponic experiment was conducted with following treatments: no Cd (CK), 20 μmol L^{-1} Cd (Cd), and a mixture of 20 μmol L^{-1} Cd and 50 μmol L^{-1} GSH (Cd + GSH). Cd was supplied as a $CdCl_2 \cdot 2.5H_2O$ solution. Five replicates were arranged for each treatment. As the basal nutrient medium, the formulation recommended by the International Rice Research Institute was used (Ponnamperuma, 1977). The seeds were soaked in 30% H_2O_2 for 30 min and sterilised with 0.1% sodium hypochlorite for 24 h. Then, the seeds were germinated in a floating tray filled with watered perlite at 25 °C and 60% of relative humidity. After germination, the seedlings were cultivated in 1/4 of the nutrient solution. Uniform and healthy seedlings were selected and transplanted to the hydroponic containers at the third-leaf stage (9th July 2018). The hydroponic containers (80 cm × 45 cm × 10 cm, 36 L) were covered with foam plates in which 20 uniform round holes were previously made to allow the emergence of the seedlings, resulting in 20 seedlings per container and a total of 6 containers were used. One week later (16th July 2018), the Cd solutions and the mixed solutions of Cd and GSH were added to the basal nutrient solutions, as previously designed. No additions were made to the control units. The seedlings were cultivated in a glasshouse under natural light at 30 °C/25 °C (day/night temperature), and the nutrient solution was renewed every 5 d.

The plant samples were collected at the tillering stage (26 days after transplanting, 10th August 2018). The roots of all collected seedlings were immersed in 20 mmol L^{-1} of Na_2-EDTA for 15 min and then rinsed with deionized water. Afterwards, plants were separated into the root, shoot base (0.7 cm from the root-to-shoot junction), and shoot, as shown in Fig. 1. The plant material obtained was stored at −80 °C for further GSH and PCs analysis. For subsequent RNA extraction, some samples were stored in DNase-free, RNase-free EP tubes at −80 °C. For Cd analysis, other plant samples were dried at 105 °C for 2 h and then 85 °C for another 48 h.

Additionally, some seedlings were cut to collect the xylem sap according to the method of Ghnaya et al. (2013), with minor modifications. The seedlings were cut at 2 cm above the shoot base and the exudation from cut surface was collected as the xylem sap. The xylem sap was collected with degreasing cotton for 12 h; this material was subsequently squeezed into centrifuge tubes. Xylem sap samples collected from individual plants were pooled and taken as one replication.

2.3. Cd concentrations in plants and xylem sap

Dry plant samples were digested with HNO_3 using a microwave digestion system (TOPEX+, PreeKem, China), and the Cd concentrations were determined by inductively coupled plasma optical emission spectrometry (ICP-OES, X Series 2, Thermo Fisher, Finland). Besides, 200 μL-samples of xylem sap were diluted to 4 mL with 2% HNO_3, and Cd concentrations were determined by an inductively coupled plasma mass spectrometry (ICP-MS, 8900, Agilent Technologies, USA).

Fig. 1. The division of root, shoot base and shoot.

2.4. GSH and PCs analysis

GSH and PCs concentrations were determined according to the methods of Hasan et al. (2016), with minor modifications. Fresh shoot bases (about 100 mg) were frozen in liquid nitrogen, ground to homogenous powders, and extracted with 1 mL of an extraction buffer containing 0.1% of trifluoroacetic acid (TFA) and 6.3 mmol L^{-1} of DTPA. After standing for 15 min on ice-cold water, the homogenates were centrifuged (12,000 ×*g*, 10 min, 4 °C), and the supernatants collected. GSH, total PC, PC_2, PC_3, and PC_4 contents in the supernatants were determined with the corresponding reagent kits (Baolai, China), following the manufacturer's instructions. A microplate reader (Multiskan GO 1510, Thermo Fisher, Finland) was used to carry out the spectrophotometric measurements.

2.5. GST and PCS analysis

GST concentration was measured according to the method of Roxas et al. (2000), with minor modifications. Fresh shoot bases (about 100 mg) were frozen in liquid nitrogen, ground to homogenous powders, and extracted with 200 μL of an extraction buffer containing 0.2 mol L^{-1} of Tris-HCl buffer (pH 7.8), 1 mmol L^{-1} of EDTA, and 5 mmol L^{-1} of dithiothreitol (DTT). Following centrifugation (12,000 ×*g*, 10 min, 4 °C), the supernatants were collected.

PCS concentration was determined according to the method of Kühnlenz et al. (2014), with minor modifications. Homogenates were prepared in a similar way as described above using Fresh shoot bases (about 100 mg) were frozen in liquid nitrogen and ground to homogenous powders, and then extracted by 200 μL as extraction solution 50 mmol L^{-1} of sodium phosphate buffer (pH 8.0) containing 0.5 mmol L^{-1} of dithiothreitol, 0.1× of protease inhibitor mix, 0.1% (w/v) of sodium dodecyl sulfate, and 0.1% (v/v) of Triton-X-100. Following centrifugation (12,000 ×*g*, 10 min, 4 °C), the supernatants were collected.

Both GST and PCS concentrations in the supernatants were determined with the corresponding reagent kits (Baolai, China), following the manufacturer's instructions. Spectrophotometric readings were performed using a microplate reader (Multiskan GO 1510, Thermo Fisher, Finland).

2.6. RNA isolation and gene expression analysis

The expression levels of three genes, *OsHMA3*, *OsPCS1*, and *OsGST*, were measured according to the methods of Shao et al. (2018). Total RNA was extracted with a Trizol Reagent Kit (Sangon Biotech, China), following the manufacturer's instructions. The first strand cDNA was synthesised using a PrimeScript™ RT Reagent Kit (Takara, Japan) with an oligo $(dT)_{20}$ prirmer. The expression was determined with TB Green™ Premix Ex Taq™ II (Takara, Japan) by a real-time PCR detection system (CFX96, Bio-Rad, USA). Primers' sequences are shown in Table S1. The expression levels were calculated using the equation $2^{-\delta\delta Ct}$, with *UBQ5 RT* as the internal reference gene.

2.7. Statistical analysis

The translocation factor (TF) for Cd was calculated according to Li et al. (2019b). The following equations were used to obtain the TF from root to shoot base ($TF_{R\text{-}SB}$) and the TF from shoot base to shoot ($TF_{SB\text{-}S}$):

$$TF_{R-SB} = \text{Cd concentration in the shoot base/Cd concentration in the root;}$$

$$TF_{SB-S} = \text{Cd concentration in the shoot/Cd concentration in the shoot base.}$$

Data were analysed by a two-way ANOVA with the Data Processing System (DPS) statistical software package. The least significant difference (LSD) test was employed to determine differences among the treatments at $p = 0.05$ level.

3. Results

3.1. Cd concentration in different tissues

Cd concentrations in the roots and shoot bases of both rice lines significantly increased when the mixed solutions of Cd and GSH was added to the hydroponic solution; however, no increases were found in the shoots (Fig. 2). Cd concentrations in the roots of D62B were significantly lower than those found in the roots of Wujin4B under all treatments. Cd concentrations in the shoot bases of D62B exposed to Cd and Cd + GSH were 1.57 times and 1.43 times higher than those detected in the shoot bases of Wujin4B, respectively.

Translocation factors decreased in the treatment Cd + GSH, especially the $TF_{SB\text{-}S}$ (Table 1). The $TF_{R\text{-}SB}$ was significantly higher in D62B than in Wujin4B. However, the $TF_{SB\text{-}S}$ in D62B was significantly lower than in Wujin4B. These results suggest that shoot bases play an important role in inhibiting Cd transport to the shoot in the line D62B, and exogenous GSH further improves this capacity for Cd retention.

3.2. GSH and PCs concentration in the shoot base

GSH and total PC concentrations significantly increased in the shoot bases of both rice lines subjected to Cd treatment alone. Exogenous GSH further increased the GSH and total PC concentrations in the shoot bases by 24% to 107% (Fig. 3). GSH and total PC concentrations in the shoot bases of D62B were 1.14– 1.61 times higher than those found in the shoot bases of Wujin4B except for the GSH concentration in shoot bases without exogenous GSH.

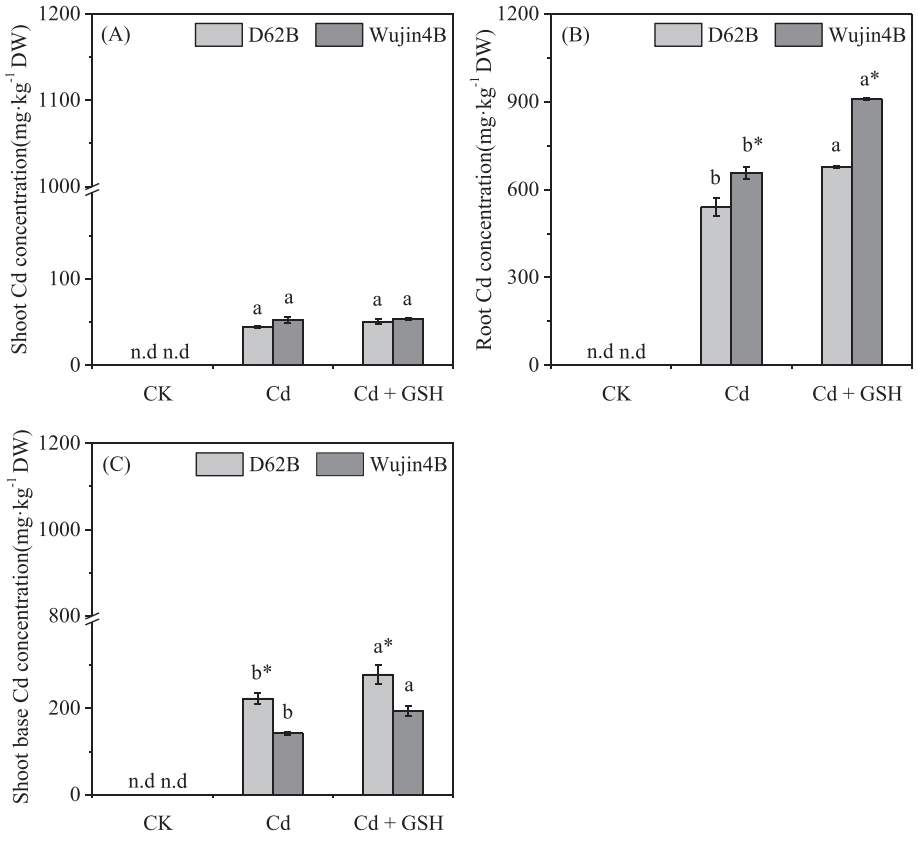

Fig. 2. The Cd concentrations of two rice lines grown in the media containing 20 μmol L^{-1} Cd or exogenous 50 μmol L^{-1} GSH. (A) The Cd concentration in shoot. (B) The Cd concentration in root. (C) The Cd concentration in shoot base. GSH represents glutathione. Data are mean ± SE, n = 5, and statistical analysis was performed by two-way ANOVA followed by LSD test. Different letters indicate statistically significant differences between different treatments at $p \leq 0.05$ and * indicates statistically significant differences between different rice at $p \leq 0.05$.

Cd treatment increased PC_2 and PC_3 concentrations and decreased PC_4 concentration in the shoot bases of both rice lines (Fig. 4). PC_2 was the predominant phytochelatin form, accounting for 46% to 70% of total PCs content. The exposure to Cd + GSH significantly enhanced PCs concentration in the shoot bases of both rice lines, with increases of 61% to 141%. In this tissue, PCs concentrations in D62B were 1.01– 1.83 times higher than those found in Wuijn4B under Cd treatment alone and 1.34– 1.90 times higher than that detected in Wuijn4B exposed to Cd + GSH. These results evidence a stronger capacity of D62B for GSH and PCs synthesis as compared to Wuijn4B.

3.3. GST and PCS activity in the shoot base

Cd treatment alone increased GST concentrations by 103% to 176% in the shoot bases of both rice lines (Fig. 5A), while the combined addition of Cd with exogenous GSH significantly decreased GST concentration in the shoot bases of D62B. GST concentrations in D62B were 1.09– 2.50 times higher than those found in Wujin4B, considering all treatments.

Table 1
The Cd translocation factor between different tissues of rice grown on the media containing 20 μmol L^{-1} or exogenous 50 μmol L^{-1} GSH.

Treatment	Root-shoot base		Shoot base-shoot	
	D62B	Wujin4B	D62B	Wujin4B
CK	–	–	–	–
Cd	0.41 ± 0.00*	0.22 ± 0.02	0.20 ± 0.02	0.37 ± 0.02*
Cd + GSH	0.41 ± 0.05*	0.21 ± 0.03	0.18 ± 0.04	0.28 ± 0.03*

Note: Data are mean ± SE, n = 5. "–" indicates not detectable.
* Indicates statistically significant differences between different rice at $p \leq 0.05$.

PCS concentrations in the shoot bases of D62B and Wujin4B significantly increased under Cd treatment alone, and exogenous GSH inclusion led to increases in PCS concentrations even higher (Fig. 5B). Thus, PCS concentrations in the shoot bases of D62B were 1.31 times and 1.36 times higher than those recorded for the shoot bases of Wujin4B, respectively. The stronger capacity of D62B for Cd binding by GSH and PCs was found to be coupled to greater concentrations of GST and PCS.

3.4. Expression of OsGST and OsPCS1 in the shoot base

OsGST expression levels in the shoot bases of both rice lines were down-regulated under Cd + GSH treatment compared with Cd treatment alone, while the expression levels of *OsPCS1* were significantly up-regulated. The expression levels of *OsPCS1* in D62B and Wujin4B exposed to Cd + GSH were 311.26 times and 201.65 times higher than those found in the shoot bases of plants subjected to Cd treatment alone, respectively (Fig. 6). Besides, the expression levels of *OsGST* and *OsPCS1* in the shoot bases of D62B were significantly higher than those detected in the same tissue of Wujin4B plants grown under different treatments. The expression levels of *OsPCS1* in the shoot bases of D62B were 1.82 times and 1.54 times higher than those in Wujin4B exposed to Cd alone and Cd + GSH, respectively.

4. Discussion

4.1. The role of the shoot base in Cd retention

It has been reported that the presence of Cd induces structural changes leading to restrict Cd diffusion, (Chen et al., 2018); therefore, Cd is expected to be mainly distributed in the roots, from which it

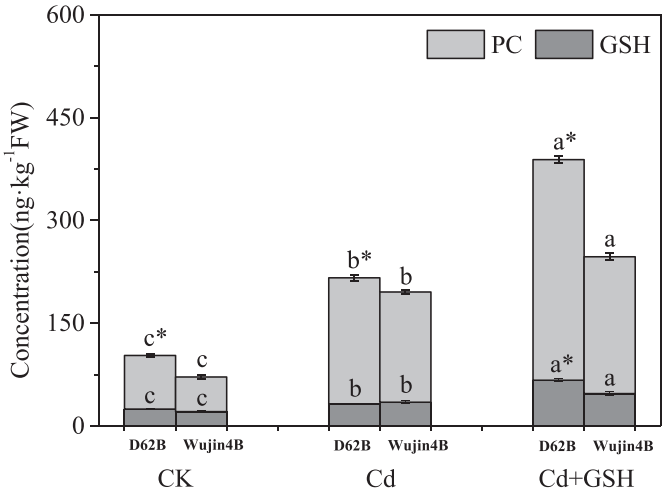

Fig. 3. The GSH and total PC concentrations in shoot base of two rice lines grown in the media containing 20 μmol L^{-1} Cd or exogenous 50 μmol L^{-1} GSH. PC represents phytochelatin. GSH represents glutathione. Data are mean ± SE, n = 5, and statistical analysis was performed by two-way ANOVA followed by LSD test. Different letters indicate statistically significant differences between different treatments at $p \le 0.05$ and * indicates statistically significant differences between different rice at $p \le 0.05$.

would be transferred to shoots. In our previous studies, we found that most of the Cd absorbed is retained in the root in the rice line D62B, and a little proportion is transported to the shoot (Zhang et al., 2014; Li et al., 2019b). In this study, we found that higher Cd concentrations in the shoot base of D62B with lower Cd concentrations in the root and lower translocation compared with Wujin4B (Fig. 2; Table 1). Thus, the shoot base plays an important role in Cd retention in the Cd-safe rice line D62B by inhibiting Cd transport to shoots. According to previous reports, some transporters are involved in the process of Cd transport from roots to shoots (Satoh-Nagasawa et al., 2013; Shao et al., 2018). OsHMA3, an ATPase-coupled heavy metal transmembrane transporter, has been found to exert a key role in limiting Cd transport from root to shoot by sequestering Cd into root vacuoles (Ueno et al., 2010; Miyadate et al., 2011; Yan et al., 2016). In our work, however, there was no difference in *OsHMA3* expression levels between D62B and Wujin4B rice lines under Cd treatment alone (Fig. S1). Different *OsHMA3* expression levels were related to different promoters' sequences, and some authors postulated that the *OsHMA3* allele might have no function in high-Cd *indica* cultivars (Uraguchi and Fujiwara, 2013; Sun et al., 2019; Liu et al., 2020). In this sense, we highlight the fact that Cd concentrations in brown rice of D62B and Wujin4B were found to be quite different, despite being both *indica* subspecies. Differences in the OsHMA3 transporter function between D62B and Wujin4B lines may exist; nevertheless, further research is necessary to confirm this hypothesis.

Exogenous GSH can inhibit Cd translocation to the shoot, but its local effects on the shoot base tissue and the underlying mechanisms, which result in the reduced translocation, are still unclear. GSH added to hydroponic media can be absorbed by the roots and then complexed with cytosolic Cd ions, thus regulating the transcription of genes related to Cd uptake and accumulation (Ding et al., 2017; Uraguchi et al., 2017). In this study, Cd accumulation in the roots and shoot bases of both rice lines assessed significantly increased with exogenous GSH application, whereas Cd translocation was inhibited, especially from shoot bases to

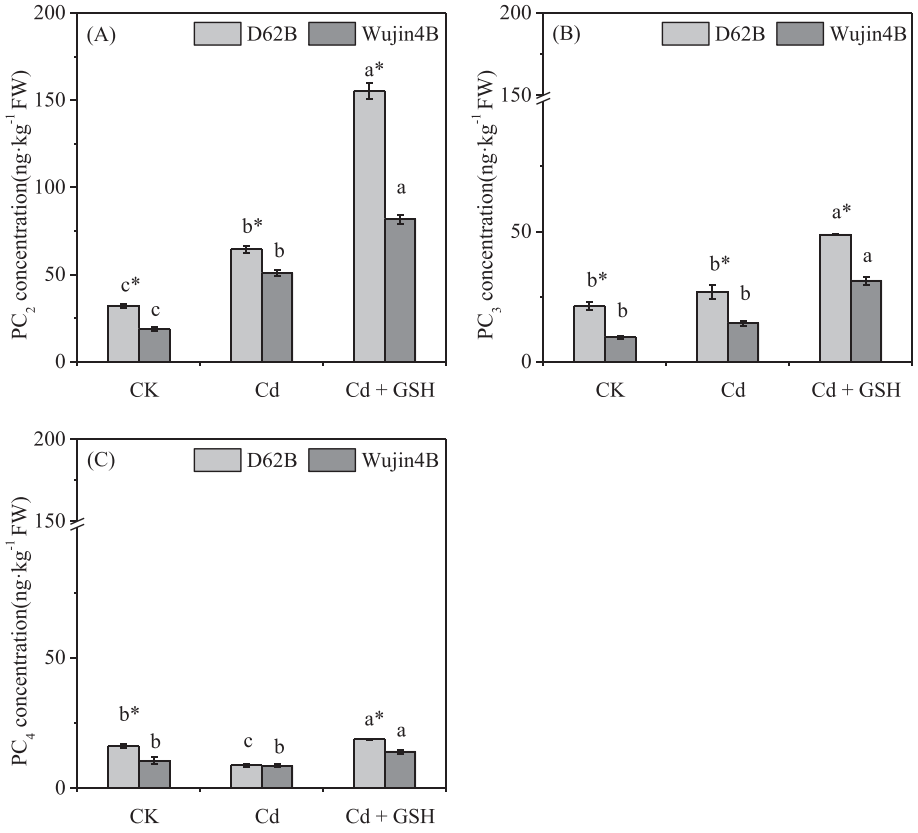

Fig. 4. The PCs concentrations in shoot base of two rice lines grown in the media containing 20 μmol L^{-1} Cd or exogenous 50 μmol L^{-1} GSH. (A) The PC_2 concentration in shoot base. (B) The PC_3 concentration in shoot base. (C) The PC_4 concentration in shoot base. PCs represent phytochelatins. GSH represents glutathione. Data are mean ± SE, n = 5, and statistical analysis was performed by two-way ANOVA followed by LSD test. Different letters indicate statistically significant differences between different treatments at $p \le 0.05$ and * indicates statistically significant differences between different rice at $p \le 0.05$.

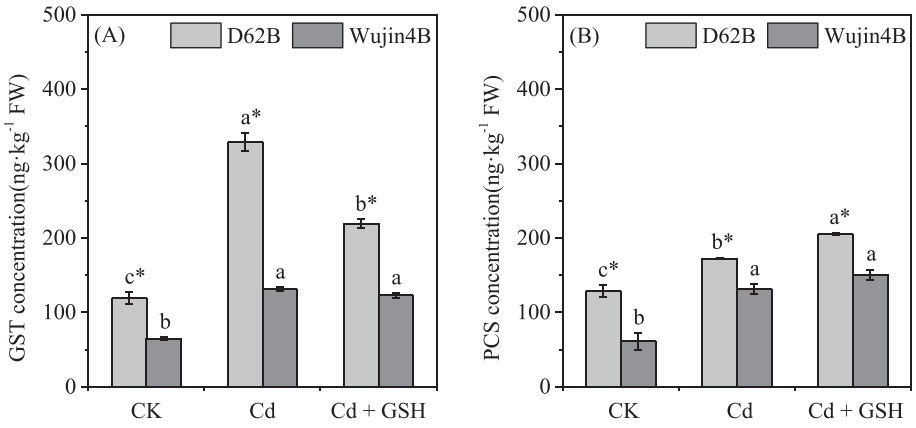

Fig. 5. The GST and PCS enzyme activity in shoot base of two rice lines grown in the media containing 20 μmol L^{-1} Cd or exogenous 50 μmol L^{-1} GSH. (A) The GST concentration in shoot base. (B) The PCS concentration in shoot base. GST represents glutathione S-transferase. PCS represents phytochelatin synthases. GSH represents glutathione. Data are mean ± SE, n = 5, and statistical analysis was performed by two-way ANOVA followed by LSD test. Different letters indicate statistically significant differences between different treatments at $p \leq 0.05$ and * indicates statistically significant differences between different rice at $p \leq 0.05$.

shoots (Fig. 2; Table 1). It may be assumed that exogenous GSH promoted Cd chelation under the catalysis of the related enzymes and stimulated the formation of stable complexes in the cytosol. Thus, the amounts of Cd ions in the symplastic sap may have decreased, resulting in lower amounts of Cd translocated from roots to shoots (Nakamura et al., 2013; Ding et al., 2017; Kim et al., 2017). These findings confirmed the central role of shoot base tissues in Cd retention displayed by the rice line D62B, as well as the positive effect of exogenous GSH addition on this capacity.

4.2. GSH and PCs involvement in Cd retention in shoot base

Chelation with GSH and PCs is the most common strategy to accomplish Cd detoxification (Fan et al., 2018; Zhang et al., 2018). It is known that PCs synthesis can be stimulated by Cd stress, to allow the binding of Cd ions to their thiol moieties (Degola et al., 2014; Hazama et al., 2015). The PC -SH moiety ($2\times PC_2 + 3\times PC_3 + 4\times PC_4$) binds to Cd at a stoichiometric ratio of 2–49 in *Thalassiosira weissflogji* (Wu et al., 2016), indicating that more thiol moieties in PCs benefit Cd chelation. In this study, Cd stress promoted GSH and PCs synthesis (except for PC_4) in the shoot bases of both rice lines evaluated (Figs. 3, 4). GSH supply provides precursors for PCs synthesis and triggers the transcription of genes involved in GSH and PCs biosynthesis (Ding et al., 2017). In line with these concepts, we found significantly higher GSH and PCs concentrations in the shoot bases of D62B as compared to the shoot bases of Wujin4B regardless of the treatment applied (Figs. 3, 4), indicating that D62B has stronger capacity for GSH and PCs synthesis in their shoot bases.

The presence of Cd—S complexes, such as PCs, allows roots to retain greater amounts of Cd, thus reducing the proportion of Cd readily available to be translocated to shoots (Wong and Cobbett, 2009; Cheng et al., 2016). It has been proved that more than 75% of the intracellular Cd can be complexed with PCs (Figueira et al., 2014), and the number of Cd ions loaded into the xylem sap of barley decreased linearly as PCs concentrations in the root tissue increased (Akhter et al., 2012; Sghayar et al., 2015). On the other hand, it has been reported that Cd-PCs complexes are transported into the vacuoles through ABC transporters (Verbruggen et al., 2009; Sharma et al., 2016; Cao et al., 2018), resulting in decreased Cd translocation. We corroborated the occurrence of higher PCs concentrations in the shoot bases and lower Cd concentrations in the xylem sap of D62B plants as compared to those measured in the Wujin4B line. Cd translocation from the shoot base to the shoot was also lower in D62B (Fig. S2; Table 1). Meanwhile, significant positive correlations were observed between Cd concentration and PCs

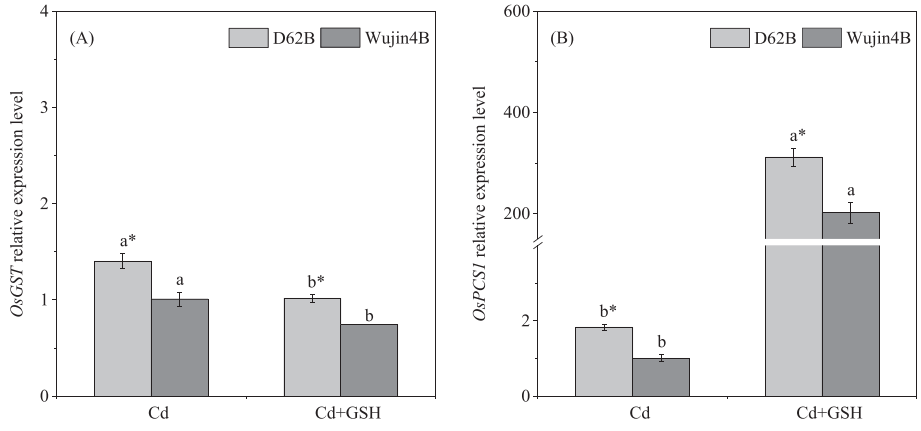

Fig. 6. Response pattern of *OsGST* and *OsPCS1* in shoot base of two rice lines grown in the media containing Cd or exogenous GSH for 4 weeks. (A) The *OsGST* relative expression level in shoot base. (B) The *OsPCS1* relative expression level in shoot base. GSH represents glutathione. Data are mean ± SE, n = 3, and statistical analysis was performed by two-way ANOVA followed by LSD test. Different letters indicate statistically significant differences between different treatments at $p \leq 0.05$ and * indicates statistically significant differences between different rice at $p \leq 0.05$.

concentrations in shoot base of both rice lines (Table S2). From these results, we conclude that PCs in shoot base play a predominant role in Cd chelation in the rice line D62B.

4.3. The capacity of PCs synthesis and Cd chelation in shoot base

Biosynthesis and metabolism of PCs and Cd chelation depend on two key enzymes: GST and PCS. GST can catalyse the conjugation of the thiol group of GSH with numerous xenobiotics, such as Cd (Zhang and Liu, 2011; Hossain and Komatsu, 2012), thereby facilitating the sequestration of Cd ions and their subsequent transfer to the vacuole (Anjum et al., 2014). GST biosynthesis was induced by Cd stress in *Brassica campestris* and *Urtica dioica* (Anjum et al., 2014; Tarhan and Kavakcıoğlu, 2016). Similarly, we found significantly higher GST concentration after Cd treatment when applied alone (Fig. 5A). Conversely, there was a significant decrease in GST concentration in the shoot bases of both rice lines under the simultaneous addition of GSH and Cd, which may be attributed to the down-regulated expression of the *OsGST* gene compared to that detected under Cd treatment alone (Fig. 6A). *GSTs* genes are observed in response to different heavy metals in various plants (Kumar and Trivedi, 2018), but few research focus on the effect of exogenous GSH on *GSTs* expression under heavy metal stress. It is assumed that the down-regulation of *OsGST* may result from the decrease of free Cd ion with the increase of PCs according to the pathway of GST involved in PCs synthesis (Kumar and Trivedi, 2018). GST concentrations in the shoot bases of D62B plants were always higher than those in Wujin4B as well as the GSH concentrations, indicating that Cd-chelating capacity in D62B is stronger than in Wujin4B; in this way, more Cd ions can be conjugated with the thiol of GSH. Consistently, the expression levels of *OsGST* in the shoot bases of D62B were higher than in those of Wujin4B under all treatments considered (Fig. 6A). Taken together, these findings demonstrate that D62B has a stronger ability than Wujin4B for GSH biosynthesis, which would contribute to Cd chelation and PCs synthesis.

PCS is a determinant enzyme for PCs biosynthesis under Cd stress (Kühnlenz et al., 2014). The main role of PCS in the process is catalysis, including two periods. Firstly, the carboxyl groups of PCS detect signal of Cd^{2+} and immediately combine with Cys to form special space structure with catalytic activity. Secondly, the γ-Glu-Cys moiety of GSH is transferred to a second GSH molecule (or an existing PCs molecule) to form a higher molecular weight PCs by the function of activated amino group of PCS (Liu et al., 2015). With the exogenous addition of GSH, tomato plants showed significantly elevated *PCS* expression and PCs accumulation, and the addition of buthionine sulfoximine (BSO), a GSH biosynthesis blocker, reversed these effects (Hasan et al., 2016). We verified that the PCS concentration in rice shoot bases significantly increased when plants were exposed to Cd alone (Fig. 5B). Cd induced the formation of PCS and Cd-PCs complexes by activating the expression of several genes (Pál et al., 2017; Prasad, 2017). It is noteworthy that *OsPCS1* was significantly up-regulated after exogenous GSH addition, in concordance with enhanced PCS concentrations (Fig. 6B). Irrespective of the treatment, PCs and PCS concentrations in the shoot bases of D62B were significantly higher than those in the shoot bases of Wujin4B, in accordance with the expression levels of *OsPCS1* and Cd concentrations detected. Positive correlations between the expression of phytochelatin synthase genes of other plant species such as *NnPCS1* or *CdPCS1* and Cd accumulation potential in plant tissues were already reported (Prasad, 2017; Talebi et al., 2019). To sum up, we found that D62B has a remarkable capacity for Cd retention in the shoot base due to its stronger potential for GSH biosynthesis and conversion into PCs, under the catalysis of GST and PCS. Besides, there may be amounts of influx transporters on tonoplast, facilitating the transfer of Cd ions and Cd-complexes into vacuoles (Fig. 7).

5. Conclusion

The shoot base plays a critical role in the Cd-safe rice line D62B, as this tissue accumulates and retains greater amounts of Cd. In addition,

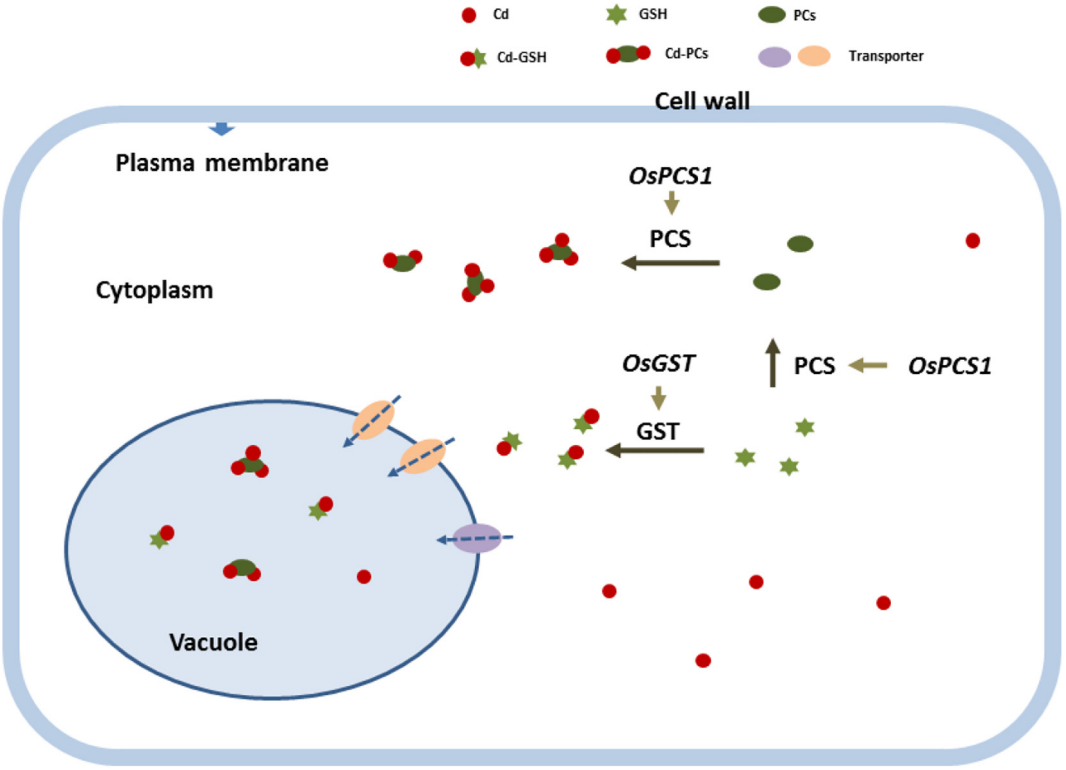

Fig. 7. The potential model of Cd retention in shoot base of rice under Cd stress.

this rice line demonstrated a stronger potential for GSH biosynthesis and conversion into PCs. Under the regulation of *OsGST* and *OsPCS1*, increased amounts of glutathione S-transferase (GST) and phytochelatin synthases (PCS) are synthesised in the shoot base of the line D62B, allowing enhanced PCs synthesis and Cd chelation. Exogenous GSH addition further promoted PCs synthesis and inhibited Cd transport from roots to shoots. As compared to Wujin4B (another high Cd-accumulating *indica* variety), the rice line D62B shows a greater capacity for GSH and PCs biosynthesis under Cd stress and, thereby, for Cd retention in the shoot base, resulting in a reduced Cd translocation to brown rice.

CRediT authorship contribution statement

Keji Wang: Investigation, Formal analysis, Writing - original draft. **Haiying Yu:** Conceptualization, Writing - review & editing. **Daihua Ye:** Visualization. **Yongdong Wang:** Investigation, Formal analysis, Visualization. **Xizhou Zhang:** Supervision, Validation. **Huagang Huang:** Methodology, Software. **Zicheng Zheng:** Formal analysis, Investigation. **Tingxuan Li:** Supervision, Resources, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.142710.

References

Akhter, M.F., Mcgarvey, B., Macfie, S.M., 2012. Reduced translocation of cadmium from roots is associated with increased production of phytochelatins and their precursors. J. Plant Physiol. 169, 1821–1829.

Anjum, N.A., Umar, S., Iqbal, M., 2014. Assessment of cadmium accumulation, toxicity, and tolerance in Brassicaceae and Fabaceae plants-implications for phytoremediation. Environ. Sci. Pollut. R. 21, 10286–10293.

Astolfi, S., Zuchi, S., Neumann, G., Cesco, S., di Toppi, L.S., Pinton, R., 2012. Response of barley plants to Fe deficiency and Cd contamination as affected by S starvation. J. Exp. Bot. 63, 1241–1250.

Cao, Z.Z., Qin, M.L., Lin, X.Y., Zhu, Z.W., Chen, M.X., 2018. Sulfur supply reduces cadmium uptake and translocation in rice grains (*Oryza sativa* L.) by enhancing iron plaque formation, cadmium chelation and vacuolar sequestration. Environ. Pollut. 238, 76–84.

Chen, X., Ouyang, Y., Fan, Y., Qiu, B., Zhang, G., Zeng, F., 2018. The pathway of transmembrane cadmium influx via calcium-permeable channels and its spatial characteristics along rice root. J. Exp. Bot. 69, 5279–5291.

Cheng, M., Wang, P., Kopittke, P.M., Wang, A., Sale, P.W.G., Tang, C., 2016. Cadmium accumulation is enhanced by ammonium compared to nitrate in two hyperaccumulators, without affecting speciation. J. Exp. Bot. 67, 5041–5050.

Degola, F., De Benedictis, M., Petraglia, A., Massimi, A., Fattorini, L., Sorbo, S., Basile, A., di Toppi, L.S., 2014. A Cd/Fe/Zn-responsive phytochelatin synthase is constitutively present in the ancient liverwort *Lunularia cruciata* (L.) Dumort. Plant. Cell. Physiol. 55, 1884–1891.

Delalande, O., Desvaux, H., Godat, E., Valleix, A., Junot, C., Labarre, J., Boulard, Y., 2010. Cadmium - glutathione solution structures provide new insights into heavy metal detoxification. FEBS J. 277, 5086–5096.

Ding, S., Ma, C., Shi, W., Liu, W., Lu, Y., Liu, Q., Luo, Z.B., 2017. Exogenous glutathione enhances cadmium accumulation and alleviates its toxicity in *Populus* × *canescens*. Tree Physiol. 37, 1697–1712.

Fan, W., Guo, Q., Liu, C.Y., Liu, X., Zhang, M., Long, D., Xiang, Z., Zhao, A., 2018. Two mulberry phytochelatin synthase genes confer zinc/cadmium tolerance and accumulation in transgenic Arabidopsis and tobacco. Gene. 645, 95–104.

Figueira, E., Freitas, R., Guasch, H., Almeida, S.F.P., 2014. Efficiency of cadmium chelation by phytochelatins in *Nitzschia palea* (Kützing) W. Smith. Ecotoxicology. 23, 285–292.

Fujimaki, S., Suzui, N., Ishioka, N.S., Kawachi, N., Ito, S., Nakamura, C.S.I., 2010. Tracing cadmium from culture to spikelet: noninvasive imaging and quantitative characterization of absorption, transport, and accumulation of cadmium in an intact rice plant. Plant Physiol. 152, 1796–1806.

Gao, L., Chang, J., Chen, R., Li, H., Lu, H., Tao, L., Xiong, J., 2016. Comparison on cellular mechanisms of iron and cadmium accumulation in rice: prospects for cultivating Fe-rich but Cd-free rice. Rice. 9, 39.

Ghnaya, T., Zaier, H., Baioui, R., Sghaier, S., Lucchini, G., Sacchi, G.A., Lutts, S., Abdelly, C., 2013. Implication of organic acids in the long-distance transport and the accumulation of lead in *Sesuvium portulacastrum* and *Brassica juncea*. Chemosphere. 90, 1449–1454.

Gupta, D.K., Vandenhove, H., Inouhe, M., 2013. Role of Phytochelatins in Heavy Metal Stress and Detoxification Mechanisms in Plants. In Heavy metal stress in plants. Springer, Berlin, pp. 73–94.

Hasan, M.K., Liu, C., Wang, F., Ahammed, G.J., Zhou, J., Xu, M.X., Yu, J.Q., Xia, X.J., 2016. Glutathione-mediated regulation of nitric oxide, S-nitrosothiol and redox homeostasis confers cadmium tolerance by inducing transcription factors and stress response genes in tomato. Chemosphere. 161, 536–545.

Hazama, K., Nagata, S., Fujimori, T., Yanagisawa, S., Yoneyama, T., 2015. Concentrations of metals and potential metal-binding compounds and speciation of Cd, Zn and Cu in phloem and xylem saps from castor bean plants (*Ricinus communis*) treated with four levels of cadmium. Physiol. Plantarum. 154, 243–255.

Hossain, Z., Komatsu, S., 2012. Contribution of proteomic studies towards understanding plant heavy metal stress response. Front. Plant Sci. 3, 43–48.

Kabir, A.H., Hossain, M.M., Khatun, M.A., Sarkar, M.R., Haider, S.A., 2017. Biochemical and molecular mechanisms associated with Zn deficiency tolerance and signaling in rice (*Oryza sativa* L.). J. Plant Interact. 12, 447–456.

Kim, Y.O., Bae, H.J., Cho, E., Kang, H., 2017. Exogenous glutathione enhances mercury tolerance by inhibiting mercury entry into plant cells. Front. Plant Sci. 8, 683.

Kobayashi, N.I., Tanoi, K., Hirose, A., Nakanishi, T.M., 2013. Characterization of rapid intervascular transport of cadmium in rice stem by radioisotope imaging. J. Exp. Bot. 64, 507–517.

Kühnlenz, T., Schmidt, H., Uraguchi, S., Clemens, S., 2014. Arabidopsis thaliana phytochelatin synthase 2 is constitutively active in vivo and can rescue the growth defect of the *PCS1*-deficient *cad1-3* mutant on Cd-contaminated soil. J. Exp. Bot. 65, 4241–4253.

Kumar, S., Trivedi, P.K., 2018. Glutathione s-transferases: role in combating abiotic stresses including arsenic detoxification in plants. Front. Plant Sci. 9, 751.

Li, M., Hao, P., Cao, F., 2017. Glutathione-induced alleviation of cadmium toxicity in *Zea mays*. Plant. Physiol. Bioch. 119, 240–249.

Li, M., Stragliati, L., Bellini, E., Ricci, A., Saba, A., Toppi, L.S.D., Varotto, C., 2019a. Evolution and functional differentiation of recently diverged phytochelatin synthase genes from *Arundo donax* L. J. Exp. Bot. 70, 5391–5405.

Li, Q., Guo, J., Zhang, X., Yu, H., Huang, F., Zhang, L., Zhang, M., Li, T., 2019b. Changes of non-protein thiols in root and organic acids in xylem sap involved in cadmium translocation of cadmium-safe rice line (*Oryza Sative* L.). Plant Soil 439, 475–486.

Liu, W., Zhang, X., Liang, L., Chen, C., Wei, S., Zhou, Q., 2015. Phytochelatin and oxidative stress under heavy metal stress tolerance in plants. In: Corpas, F. (Ed.), K. Gupta, D., M. Palma, J., J. Reactive Oxygen Species and Oxidative Damage in Plants Under Stress, Springer, Berlin, pp. 191–217.

Liu, C.L., Gao, Z.Y., Shang, L.G., Yang, C.H., Ruan, B.P., Zeng, D.L., Guo, L.B., Zhao, F.J., Huang, C.F., Qian, Q., 2020. Natural variation in the promoter of *OsHMA3* contributes to differential grain cadmium accumulation between *Indica* and *Japonica* rice. J. Integr. Plant Biol. 62, 314–329.

Luo, Z.B., He, J., Polle, A., Rennenberg, H., 2016. Heavy metal accumulation and signal transduction in herbaceous and woody plants: paving the way for enhancing phytoremediation efficiency. Biotechnol. Adv. 34, 1131–1148.

Lux, A., Martinka, M., Vaculík, M., White, P.J., 2011. Root responses to cadmium in the rhizosphere: a review. J. Exp. Bot. 62, 21–37.

Maria, D.B., Cecilia, B., Brauer, E.K., Andrea, A., Popescu, S.C., Mauro, C., Flavia, G., Adriano, S., Monica, R., Vatamaniuk, O.K., di Toppi, L.S., 2018. The Arabidopsis thaliana knockout mutant for *Phytochelatin Synthase1* (*cad1-3*) is defective in callose deposition, bacterial pathogen defense and auxin content, but shows an increased stem lignification. Front. Plant Sci. 9, 19.

Miyadate, H., Adachi, S., Hiraizumi, A., Tezuka, K., Nakazawa, N., Kawamoto, T., Katou, K., Kodama, I., Sakurai, K., Takahashi, H., Satoh-Nagasawa, N., Watanabe, A., Fujimura, T., Akagi, H., 2011. OsHMA3, a P_{1B}-type of ATPase affects root-to-shoot cadmium translocation in rice by mediating efflux into vacuoles. New Phytol. 189, 190–199.

Nakamura, S.I., Suzui, N., Nagasaka, T., Komatsu, F., Ishioka, N.S., Ito-Tanabata, S., Kawachi, N., Rai, H., Hattori, H., Chino, M., Fujimaki, S., 2013. Application of glutathione to roots selectively inhibits cadmium transport from roots to shoots in oilseed rape. J. Exp. Bot. 64, 1073–1081.

Nocito, F.F., Lancilli, C., Dendena, B., Lucchini, G., Sacchi, G.A., 2011. Cadmium retention in rice roots is influenced by cadmium availability, chelation and translocation. Plant Cell Environ. 34, 994–1008.

Pál, M., Csávás, G., Szalai, G., Tímea, O., Khalil, R., Yordanova, R., Gella, G., Birinyia, Z., Németha, E., Janda, T., 2017. Polyamines may influence phytochelatin synthesis during Cd stress in rice. J. Hazard. Mater. 340, 272–280.

Ponnamperuma, F.N., 1977. Screening rice for tolerance to mineral stresses. International Rice Research Institute (IRRI). 25, 67–72.

Prasad, R., 2017. Role of phytochelatins (PCs), metallothioneins (MTs), and heavy metal ATPase (*HMA*) genes in heavy metal tolerance. Mycoremediation and Environmental Sustainability. Springer, Berlin, pp. 39–60.

Ricachenevsky, F.K., de Araújo Junior, A.T., Fett, J.P., Sperotto, R.A., 2018. You shall not pass: root vacuoles as a symplastic checkpoint for metal translocation to shoots and possible application to grain nutritional quality. Front. Plant Sci. 9, 412.

Roxas, V.P., Lodhi, S.A., Garrett, D.K., Mahan, J.R., Allen, R.D., 2000. Stress tolerance in transgenic tobacco seedlings that overexpress glutathione S-transferase/glutathione peroxidase. Plant. Cell. Physiol. 41, 1229–1234.

Satoh-Nagasawa, N., Mori, M., Sakurai, K., Takahashi, H., Watanabe, A., Akagi, H., 2013. Functional relationship heavy metal P-type ATPases (OsHMA2 and OsHMA3) of rice (*Oryza sativa*) using RNAi. Plant. Biotechnol. 30, 511–515.

Sghayar, S., Ferri, A., Lancilli, C., Lucchini, G., Abruzzese, A., Porrini, M., Ghnaya, T., Nocito, F.F., Abdelly, C., Sacchi, G.A., 2015. Analysis of cadmium translocation, partitioning and tolerance in six barley (*Hordeum vulgare* L.) cultivars as a function of thiol metabolism. Biol. Fert. Soils. 51, 311–320.

Shao, J.F., Xia, J.X., Yamaji, N., Shen, R.F., Ma, J.F., 2018. Effective reduction of cadmium accumulation in rice grain by expressing *OsHMA3* under the control of *OsHMA2* promoter. J. Exp. Bot. 69, 2743–2752.

Sharma, S.S., Dietz, K.J., Mimura, T., 2016. Vacuolar compartmentalization as indispensable component of heavy metal detoxification in plants. Plant Cell Environ. 39, 1112–1126.

Song, Y., Wang, Y., Mao, W., Sui, H., Yong, L., Yang, D., Jiang, D., Zhang, L., Gong, Y., 2017. Dietary cadmium exposure assessment among the Chinese population. PLoS One 12, e0177978.

Srivastava, S., Srivastava, S., Bist, V., Awasthi, S., Chauhan, R., Chaudhry, V., Singh, P.C., Dwivedi, S., Niranjan, A., Agrawal, L., Chauhan, P.S., Tripathi, R.D., Nautiyal, C.S., 2018. *Chlorella vulgaris* and *Pseudomonas putida* interaction modulates phosphate trafficking for reduced arsenic uptake in rice (*Oryza sativa L.*). J. Hazard. Mater. 351, 177–187.

Sun, C., Yang, M., Li, Y., Tian, J., Zhang, Y., Liang, L., Liu, Z., Chen, K., Li, Y., Lv, K., Lian, X., 2019. Comprehensive analysis of variation of cadmium accumulation in rice and detection of a new weak allele of *OsHMA3*. J. Exp. Bot. 70, 6389–6400.

Szalai, G., Krantev, A., Yordanova, R., Popova, P., Janda, T., 2013. Influence of salicylic acid on phytochelatin synthesis in *Zea mays* during Cd stress. Turk. J. Bot. 37, 708–714.

Talebi, M., Tabatabaei, B.E.S., Akbarzadeh, H., 2019. Hyperaccumulation of Cu, Zn, Ni, and Cd in *Azolla* species inducing expression of methallothionein and phytochelatin synthase genes. Chemosphere. 230, 488–497.

Tarhan, L., Kavakcıoğlu, B., 2016. Glutathione metabolism in *Urtica dioica* in response to cadmium based oxidative stress. Biol. Plantarum. 60, 163–172.

Ueno, D., Yamaji, N., Kono, I., Huang, C.F., Ando, T., Yano, M., Ma, J.F., 2010. Gene limiting cadmium accumulation in rice. P. Natl. Acad. Sci. 107, 16500–16505.

Uraguchi, S., Fujiwara, T., 2013. Rice breaks ground for cadmium-free cereals. Curr. Opin. Plant Biol. 16, 328–334.

Uraguchi, S., Mori, S., Kuramata, M., Kawasaki, A., Arao, T., Ishikawa, S., 2009. Root-to-shoot Cd translocation via the xylem is the major process determining shoot and grain cadmium accumulation in rice. J. Exp. Bot. 60, 2677–2688.

Uraguchi, S., Tanaka, N., Hofmann, C., Abiko, K., Ohkama-Ohtsu, N., Weber, M., Kamiya, T., Sone, Y., Nakamura, R., Takanezawa, Y., Kiyono, M., Fujiwara, T., Clemens, S., 2017. Phytochelatin synthase has contrasting effects on cadmium and arsenic accumulation in rice grains. Plant. Cell. Physiol. 58, 1730–1742.

Verbruggen, N., Hermans, C., Schat, H., 2009. Mechanisms to cope with arsenic or cadmium excess in plants. Curr. Opin. Plant Biol. 12, 364–372.

Wong, C.K.E., Cobbett, C.S., 2009. HMA P-type ATPases are the major mechanism for root-to-shoot Cd translocation in *Arabidopsis thaliana*. New Phytol. 181, 71–78.

Wu, Y., Guo, Z., Zhang, W., Tan, Q., Zhang, L., Ge, X., Chen, M., 2016. Quantitative relationship between cadmium uptake and the kinetics of phytochelatin induction by cadmium in a Marine Diatom. Sci. Rep. 6, 35935.

Yan, J., Wang, P., Wang, P., Yang, M., Lian, X., Tang, Z., Huang, C., Salt, D.E., Zhao, F.J., 2016. A loss-of-function allele of *OsHMA3* associated with high cadmium accumulation in shoots and grain of *Japonica* rice cultivars. Plant Cell Environ. 39, 1941–1954.

Yoneyama, T., Ishikawa, S., Fujimaki, S., 2015. Route and regulation of zinc, cadmium, and iron transport in rice plants (*Oryza sativa* L.) during vegetative growth and grain filling: metal transporters, metal speciation, grain cd reduction and Zn and Fe biofortification. Int. J. Mol. Sci. 16, 19111–19129.

Zhang, Y., Liu, J., 2011. Transgenic alfalfa plants co-expressing glutathione s-transferase (GST) and human CYP2E1 show enhanced resistance to mixed contaminates of heavy metals and organic pollutants. J. Hazard. Mater. 189, 357–362.

Zhang, C., Ying, G.E., 2008. Response of glutathione and glutathione S-transferase in rice seedlings exposed to cadmium stress. Rice Sci. 15, 73–76.

Zhang, H.J., Zhang, X.Z., Li, T.X., Huang, F., 2014. Variation of cadmium uptake, translocation among rice lines and detecting for potential cadmium-safe cultivars. Environ. Earth Sci. 71, 277–286.

Zhang, X., Rui, H., Zhang, F., Hu, Z., Xia, Y., Shen, Z., 2018. Overexpression of a functional *Vicia sativa* PCS1 homolog increases cadmium tolerance and phytochelatins synthesis in Arabidopsis. Front. Plant Sci. 9, 107.