RESEARCH ARTICLE

Phenomic and metabolomic responses of roots to cadmium reveal contrasting resistance strategies in two rice cultivars (*Oryza sativa* L.)

Chong Liu¹, Mo-ming Lan¹, Er-kai He^{1,2}, Ai-jun Yao³, Guo-bao Wang¹, Ye-tao Tang^{1,2,*}, Rong-liang Qiu^{1,2,4,*}

¹ School of Environmental Science and Engineering, Guangdong Provincial Key Laboratory for Environmental Pollution Control and Remediation Technology, Sun Yat-sen University, Guangzhou 510006, China

² Guangdong Provincial Engineering Research Center for Heavy Metal Contaminated Soil Remediation, Sun Yat-sen University, Guangzhou 510006, China

³ School of Geography and Planning, Sun Yat-sen University, Guangzhou 510275, China

⁴ Guangdong Laboratory for Lingnan Modern Agriculture, College of Natural Resources and Environment, South China Agricultural University, Guangzhou 510642, China

HIGHLIGHTS

- We compared the phenomic and exudate metabolomic responses of roots of two rice cultivars to Cd.
- JY841 suffered serious root membrane damage and up-regulated phenylethanoid glycosides.
- TY816 up-regulated lipids and fatty acids to actively cope with oxidative stress.
- Reprogramming of root architecture and exudates contributed to contrasting Cd uptake.

ARTICLE INFO

Article history:

Received December 10, 2020

Revised March 15, 2021

Accepted March 20, 2021

Keywords:

Root morphology

Exudates

Cadmium

Rhizosphere

Metabolite

GRAPHICAL ABSTRACT

ABSTRACT

To cope with heavy metal stress, plant root systems undergo root structure modification and release of multifarious metabolites. Elucidation of the resistance strategies to heavy metals mediated by the root system is crucial to comprehend the resistance mechanisms of plants. Here two rice cultivars with contrasting grain cadmium (Cd) accumulation traits were selected and the responses of their root systems to Cd stress were evaluated by morphological and metabolomics analysis. The phenomic and metabolomic responses of the root system varied between the two cultivars under Cd stress. The low-Cd accumulation rice cultivar (TY816) had a more highly developed root system that coped with Cd stress (10 μ M) by maintaining high root activity, while the root cells of the high-Cd accumulation cultivar (JY841) lost viability due to excessive Cd accumulation. TY816 upregulated lipids and fatty acids to reduce Cd uptake, whereas JY841 upregulated phenylethanoid glycosides to cope with Cd-induced oxidative stress. The combination of metabolomics and phenomics revealed that rice roots employ multiple strategies to increase their tolerance of Cd-induced oxidative stress. Differing capacities to shape the root system architecture and reprogram root exudate metabolites may contribute to the contrasting Cd accumulation abilities between JY841 and TY816.

© Higher Education Press 2021

* Corresponding authors

E-mail address: eestyty@mail.sysu.edu.cn (Y.T. Tang);

eesqrl@mail.sysu.edu.cn (R.L. Qiu)

1 Introduction

Heavy metal contamination in soil is of growing concern in China due to the potential risks to food security. According to a national soil contamination survey, 82% of China's farmland contains inorganic pollutants, among which cadmium (Cd) is the most prevalent (Zhao et al., 2015). Cd contamination in soil has resulted in Cd accumulation in cereal crops grown in China (Hu et al., 2016). Rice, which is a staple food for more than 50% of the world's population, has a tendency to accumulate Cd. Subsequently, eating rice contaminated by Cd has become the main contribution to dietary Cd intake in rice-dependent populations, especially in tropical and sub-tropical countries that consume ~90% of the rice grown in the world (Hu et al., 2016).

Due to the toxic potential of Cd to humans, the physiological processes of Cd behavior in the soil-rice plant system have been well studied. Cd is translocated from the soil to rice grains via several processes including root uptake, xylem loading, intervascular transfer, and remobilization via phloem (Ueno et al., 2010; Li et al., 2017). This basic knowledge has led to further research on internal and external mechanisms for regulating Cd levels in rice grains, including reducing Cd bioavailability in soil, screening for low-accumulation cultivars, and manipulating transporter gene expression. As the first step for Cd entry into rice grain, root uptake is affected by variation in root morphology, which plays a key role in the Cd pool in the root system (Harris and Taylor, 2013). Huang et al. (2019) found that low-Cd rice cultivars have a lower number of root tips (NRTs) per root surface area (RSA). Positive correlations between root morphology and Cd accumulation have been reported for spring wheat and hot peppers (Huang et al., 2015; Liang et al., 2017). However, there is limited research focusing on the potential function of the root system in regulating Cd resistance ability in various rice cultivars. In addition, there is a lack of comprehensive evaluation of root morphology in response to Cd.

The chemical behavior of Cd in the rhizosphere is the main factor influencing Cd uptake by plants. Root exudates contribute to the difference between the rhizosphere and bulk soil by altering soil biochemistry (Walker et al., 2003). As the first barrier to Cd uptake, plant root exudates are altered to adapt to Cd stress (Chen et al., 2017). Root exudates play crucial roles in the detoxification and accumulation of Cd in plants. However, the interaction between root exudates and Cd uptake by plants is still a matter of debate. For example, root exudates of oxalate and tartaric acid are thought to reduce phytotoxic Cd²⁺ and promote the transport of Cd in the hyperaccumulating ecotype *Sedum alfredii* (Tao et al., 2016; Tao et al., 2019). Nevertheless, low-Cd tomato cultivars may enhance Cd resistance and prevent Cd entry into roots by secreting oxalate (Zhu et al., 2011). Using two rice cultivars with different Cd accumulating abilities, Fu et al. (2018) observed more amino acids in the root exudates of high-Cd-accumulating rice than in low-Cd-accumulating rice. Thus, an

assembly of various root exudate metabolites, which are plant and stress dependent, may contribute to Cd resistance in plants. Some studies have examined the relationships between specific root exudates and Cd uptake in rice (Lin et al., 2003; Liu et al., 2007). However, the role of various root exudate metabolites in Cd resistance and uptake in rice, as well as the underlying mechanism, remain less explored. Untargeted metabolomics allows profiling of the global metabolome, i.e., the identification and (semi-) quantification of thousands of metabolites in a plant. Metabolomics, being “downstream” from genomics and proteomics, has the closest link to phenotype (Zhang et al., 2019). Thus, it is potentially a powerful tool for revealing the molecular response of root exudate-mediated Cd resistance in rice.

This work aimed to reveal the phenomic and metabolomic responses of the rice root system under Cd stress to gain a deeper understanding of the Cd-induced response in the rhizosphere from a “downstream” level. In this study, two rice varieties with distinct Cd accumulation capacities were selected and the effects of Cd stress on their root architecture and root exudates were investigated. Root phenomics and nontargeted UPLC-ESI-Q-TOF-MS-based root exudate metabolomics were employed to evaluate and compare the physiological responses induced by Cd in the root system between the two rice cultivars. Our findings could provide new insight for understanding the resistance mechanisms and abilities of different rice cultivars to tolerate Cd.

2 Material and methods

2.1 Plant culture and growth conditions

Two rice cultivars with contrasting grain Cd accumulation traits, namely Jianyou841 (*Xian/Indica*, JY841) and Teyou816 (*Xian/Indica*, TY816), were selected for this study. In a previous study, JY841 was identified as a stable low-Cd cultivar while TY816 was identified as a high-Cd cultivar among 51 rice cultivars by a field plot test performed over two crop seasons (Chi et al., 2018). These two cultivars were used to explore the mechanisms of cultivar-specific differences in Cd accumulation and root systems.

Rice seeds were sterilized with 30% H₂O₂ for 30 min and then 0.5% sodium hypochlorite solution for 24 h. Seeds were then soaked in Milli-Q water and placed in darkness at 26°C for 24 h to germinate. One-week-old seedlings of a homogeneous size were transplanted to 2.0 L pots and raised on half-strength nutrient solution for 7 days (Yoshida et al., 1976) (Table S1), after which it was replaced with full-strength nutrient solution. Nutrient solutions were renewed every 3 days and adjusted to a pH of 5.1 using 1 M NaOH/HCl to simulate paddy soil conditions. Seedlings were allowed to grow for five weeks before treatment was initiated. For Cd treatment, seedlings were transferred to full-strength nutrient solution with 0.1, 1.0, or 10.0 µM Cd added to mimic low, moderate, and high-Cd stress conditions, respectively. Cd

treatment was applied for three days. For the control condition, seedlings continued to receive full-strength nutrient solution without Cd. The seedlings were grown in pairs in a hole with a diameter of 20 mm in a black foam plate. Each pot contained four pairs of seedlings for each cultivar, and three replicates were conducted for each treatment. The rice plants were allowed to grow eight weeks before root exudate collection. All pots were laid out in a phytotron with the following growth condition: 14 h/26°C day ($250 \mu\text{mol m}^{-2}\text{s}^{-1}$), 10 h/20°C night, and 65% relative humidity.

2.2 Root exudate collection and UPLC-ESI-Q-TOF-MS analysis

After the Cd treatment, the rice root exudates were sampled according to Widodo et al. (2010). Briefly, three pairs of plants were collected from each pot and the roots were carefully rinsed with Milli-Q water. Each plant was then placed in a separate brown glass bottle containing 150 mL Milli-Q water and wrapped with aluminum foil to avoid degradation. After a 4 h collection period, a 40 mL aliquot was immediately lyophilized (FreeZone 12, LabConco, USA). An extracting solution of methanol/water (200 μL , 50:50, v/v) was added to the dried aliquots, sonicated (20 min, 4°C), and centrifuged (15 min, 14000 g, 4°C). A quality control sample was employed by mixing aliquots of all samples, which showed good reproducibility (Fig. S1). The total organic carbon (TOC) concentration and pH value of the root exudates were determined using a TOC analyzer (Vario TOC, Elementar, Germany) and a pH meter (FE28, Mettler Toledo), respectively.

Untargeted metabolic profiling was performed using an UPLC (ACQUITY, Waters, USA) coupled to a quadrupole time-of-flight mass spectrometry (Q-TOF-MS) instrument (SYNAPT G2-Si, Waters, USA). An electrospray ionization (ESI⁺) source was used for mass detection. Samples of root exudate metabolite extracts (10 μL) were injected into the LC system and separated on a BEH C18 column ($2.1 \times 100 \text{ mm}$, $1.7 \mu\text{m}$, Waters). The detailed workflow for metabolic profiling and the operating conditions for the equipment are described in Text S1.

2.3 Morphological analysis

After collection of the exudates, the roots were imaged using a scanner (V750 Pro, Epson) set to high accuracy (800 dpi). The scanned images were analyzed using WinRHIZO software (version 2008a Pro, Regent Instruments Inc., Canada) to obtain root morphology parameters, including root tips (RT), total root length (RL), crossings (RC), average diameter (RAD), forks (RF), surface area (RSA), and volume (RV). Next, the root tips/surface area ratio (NRT/SA) was calculated based on the measured root parameters. Lastly, the dry biomass of the plant was weighed after oven-drying at 70°C for 72 h.

The viability of the RT was determined by staining the roots for 10 min with a fluorescent dye (Pan et al., 2001) comprising fluorescein diacetate (12.5 g mL^{-1}) and propidium iodide (5 g mL^{-1}). Fluorescence images of the RT were obtained using a fluorescence microscope (AF6000, Leica, Germany).

2.4 Cd and nutrient element analysis

Dried rice tissue was digested with $\text{HNO}_3\text{--H}_2\text{O}_2$ solution (3:1, v:v) by a microwave digestion system (MARS 6, CEM, USA) at 180°C for 30 min. Cd and nutrient elements were analyzed using ICP-OES (5300DV, PerkinElmer, USA) or ICP-MS (NexION, 350D PerkinElmer, USA). For ICP-MS analysis, $10 \mu\text{g L}^{-1}$ indium (In) was added to the samples as an internal standard and had a recovery rate of 95%–105%. Certified reference samples GBW07602 (GSV-1) were employed and the recovery rates for metals were 88%–105%.

2.5 Statistical analysis

Cd translocation from root to shoot was determined as the ratio of shoot Cd concentration to root Cd concentration. One-way analysis of variance with Duncan's test was performed using SPSS 19.0 to determine the statistical significance of root morphological indexes and abundance of root exudate metabolites. The Pearson correlation analysis between root Cd concentration and TOC concentration in root exudates was performed by Origin (Pro 2021b, OriginLab Corporation, USA).

Based on the membership function, the rice root morphological indexes were integrated to calculate the Cd tolerance index of the rice roots (Khan et al., 2020). The Cd tolerance index (CTI) was calculated using the following formula:

$$CTI_i = \frac{X - X_{\min}}{X_{\max} - X_{\min}} \quad (1)$$

$$CTI = \frac{1}{n} \sum_{i=1}^n CTI_i \quad (2)$$

where CTI_i is the subordinate value, X is the measured value of the root morphological indexes, and $X_{\max}$ and $X_{\min}$ are the maximum and minimum values of the root morphological indexes, respectively.

UPLC-ESI-Q-TOF-MS data processing was performed according to Petriacq et al. (2017). Prior to multivariate analyses, the raw UPLC-Q-TOF peaks were processed by normalization using the median, logarithm transformation, and Pareto scaling using MetaboAnalyst 4.0 (Xia et al., 2015). Supervised partial least square discriminant analysis (PLS-DA) was employed to determine the separation of different treatments. A variable importance in projection (VIP) > 1.0 and $P < 0.05$ were selected as the criteria for separation, identified as significantly differential metabolites (SDMs). The SDMs were identified by referencing public databases (e.g., METLIN, MassBank).

3 Results

3.1 Effects of Cd on rice growth and root Cd accumulation

The response of rice biomass to Cd was different between JY841 and TY816 plants (Fig. S2). Compared with the control condition, moderate (1.0 μM) Cd treatment significantly inhibited rice growth in TY816 but not in JY841; by contrast, biomass increased in both cultivars in the low-Cd (0.1 μM) treatment. However, when Cd stress was increased from moderate (1.0 μM) to high (10 μM), root and shoot biomass increased significantly (23.1% and 20.6%, respectively) in TY816, whereas JY841 showed no significant changes.

TY816 had significantly lower root Cd concentrations compared to JY841 under all Cd treatments (Fig. 1). To determine the effect of Cd on nutrient element uptake interference, the interactions of 10 elements in the roots of the two rice cultivars were analyzed. Intriguingly, the correlations of Cd with mineral elements in the roots differed between JY841 and TY816 (Fig. S3). For TY816, root Cd was positively correlated with Ca and Zn but negatively correlated with Mg. By contrast, for JY841, root Cd was negatively correlated with S and positively correlated with Ca.

Fig. 1 Root Cd concentration in rice roots under different Cd treatments. CK-JY/TY, Cd0.1-JY/TY, Cd1.0-JY/TY, and Cd10-JY/TY represent the control, 0.1 μM , 1.0 μM , and 10 μM Cd treatment conditions in JY841 or TY816 cultivars, respectively. *, $P < 0.05$.

3.2 Phenomic response of the rice root systems

To reveal the effects of Cd on the root systems of the two cultivars, a root morphology analysis and Cd tolerance evaluation were conducted (Table 1, Fig. 2). The Cd hormesis to the root morphological characteristics of TY816 was marginal, whereas low Cd (0.1 μM) significantly promoted root traits in JY841. Root length, surface area, root volume, root forks, and root crossings in TY816 were significantly promoted under 10 μM Cd compared with 1.0 μM Cd

Fig. 2 Cd tolerance index of the roots of the two rice cultivars after three days of exposure to various levels of Cd stress.

treatment, while these measures were not significantly changed in JY841. The CTI of TY816 was significantly promoted under 10 μM Cd compared with 1.0 μM Cd treatment whereas that of JY841 was unchanged, which is consistent with the biomass results.

To further dissect differences between the root systems of the two cultivars, the vitality of the root tips was determined. The root tip cells of both cultivars showed the highest viability in the control treatment, and some loss of viability was observed in the high-Cd treatment (Fig. 3). The root tip cells of TY816 showed no obvious damage, while most cells of JY841 lost viability.

3.3 Response of root exudates to Cd

To reveal the effects of Cd on the root exudates of the two rice cultivars, the TOC concentration and pH value of the root exudates were determined. The TOC concentration in TY816 increased with increasing Cd stress, while only marginal changes were observed in JY841. There was a sharp reduction in the pH value of the root exudates of JY841, whereas the pH increased significantly in TY816 (Fig. S4). Correlation analysis showed no significant relationship between root Cd and TOC in JY841, while root Cd was positively correlated with TOC in the root exudate of TY816 ($R^2 = 0.82$, $P < 0.0001$) (Fig. S5). To further evaluate the different effects of Cd on the root exudates of the two rice cultivars, untargeted metabolic profiling analysis was employed. The overall variation in the metabolites of the root exudates between different treatments was visualized by principal component analysis (PCA) and PLS-DA, showing high correlation ($R^2 = 0.99$) and predictability ($Q^2 = 0.94$) (Fig. 4). Analysis revealed a clear separation in the first principal component for the composition of the root exudate metabolites, explaining 58.4% of the variation in the data. There were distinct differences in the polar metabolite

Table 1 Root morphological characteristics in different treatments.

Treatment	RL (cm)	SA (cm ²)	AD (mm)	RV (cm ³)	RF	RC	RT/SA
CK-JY	9314±460d	680±27e	0.234±0.021c	3.984±0.522c	124613±8940d	22365±2327bc	139±16a
Cd0.1-JY	10010±771cd	819±48d	0.261±0.029b	5.415±0.889b	151614±8497abc	26423±4398abc	107±19bc
Cd1.0-JY	10191±425bc	696±41e	0.217±0.011c	3.795±0.372c	148578±14480abc	30261±2584a	123±18ab
Cd10-JY	9496±1275bc	702±75e	0.236±0.007c	4.15±0.314c	132039±15664cd	25429±4350abc	114±11ab
CK-TY	11674±1152ab	1105±93bc	0.301±0.004a	8.347±0.568a	171645±14192a	26860±1866ab	84±5d
Cd0.1-TY	10810±600bc	1085±87a	0.318±0.01a	8.708±0.869a	156674±11367ab	23447±1043bc	85±6d
Cd1.0-TY	9859±1045bc	934±154c	0.298±0.022a	7.138±1.604b	139260±15942bcd	21505±1539c	95±14cd
Cd10-TY	11336±740a	1058±99ab	0.296±0.01a	7.907±1.027a	162820±12017a	26035±1631ab	89±5cd

RL, RSA, RV, RT, AD, RF and RC presents total root length, root surface area, root volume average, diameter, root forks, and root crossings, respectively. Means within rows followed by different letters are significantly different according to Duncan's multiple test ($P \leq 0.05$).

Fig. 3 Visualization of the viability of root tips in rice seedlings exposed to Cd (10 μ M). The intense fluorescence indicates high activity of the cells.

composition of the root exudates between the two rice cultivars under control or 10 μ M Cd treatment conditions in our cultivation system.

To further distinguish the Cd-induced root exudates, PLS-DA analysis of the metabolic data was carried out separately for the two rice cultivars under Cd stress. As a result, cultivar-specific SDMs and SDMs shared by the two cultivars were identified (Fig. S6). Under Cd stress, the majority of affected compounds were common to both rice varieties (18 SDMs,

75%), while several were cultivar-specific (6 SDMs, 25%). For the shared Cd-induced SDMs, there were 12 shared down-regulated SDMs and 6 shared upregulated SDMs. For the cultivar-specific Cd-induced SDMs, 5 SDMs (2-hydroxy-nonadecanoic acid, PC(18:3(6Z,9Z,12Z)/0:0), PS(22:2(13Z,16Z)/21:0), PA(O-20:0/16:0), and oryzalexin B) were downregulated in JY841, but upregulated in TY816 (Fig. 5). Only one SDM (Orobanchoside) was upregulated by Cd in JY841, whereas it was downregulated by Cd in TY816.

4 Discussion

4.1 Contrasting root phenotypes regulate Cd uptake in two rice cultivars

Root phenotypes are the result of evolutionary selection for efficient acquisition of endogenous soil resources and resistance to environmental stresses (Neumann and Martinoia, 2002; Wang et al., 2015; Lynch, 2019). Cd uptake has been reported to affect root phenotypes and vice versa (Redjala et al., 2011). Exposure to Cd can modify root architecture by affecting a range of processes, including nutrient uptake, hormonal signal transport, and gene expression, consequently affecting lateral root growth (Boursiac et al., 2013; Wen et al., 2019). Correspondingly, under Cd stress, plants improve their metal tolerance ability by reconstructing their root architecture (Lu et al., 2013). We found that rice with a lower root tips/surface area ratio (RT/SA) had lower Cd uptake, suggesting that RT/RSA rather than RSA alone is pivotal for Cd absorption and migration (Huang et al., 2019). RT/RSA or apical surface area may affect the absorption of Cd, thus determining Cd concentration in roots (Redjala et al., 2011). The root tip is not only the target toxic action site for Cd, it is also the most active position for Cd²⁺ influx into roots (Tao et al., 2016). We also observed that the TY816 cultivar had a greater RSA, average diameter, and volume compared to the JY841 cultivar. Liang et al. (2017) found that the RSA and volume of spring wheat under Cd treatment were positively

Fig. 4 Variation in root exudate metabolites under different treatment conditions. (A) Principal component analysis (PCA) and (B) supervised partial least square discriminant analysis (PLS-DA). CK-JY/TY and Cd-JY/TY represent the control and 10 μM Cd treatment for JY841/TY816, respectively.

correlated with grain Cd concentration, from which they concluded that Cd retention in roots did not contribute to differences in Cd grain concentration. However, Harris and Taylor (2013) showed that root system traits were negatively correlated with Cd concentration in the grains and shoots of durum wheat. Compared to JY841, TY816 is privileged with a stronger root system characterized by a greater surface area, average root diameter, and volume, but a lower apical surface area, under high-Cd (10 μM) stress. The low-Cd cultivar TY816 had a lower apical surface area and lower maximum uptake rate of Cd relative to the high-Cd JY841, which resulted in lower Cd accumulation in its root (Huang et al., 2019). Plants with well-developed roots possess a wider attachment area, leading to greater oxygen release, metal adsorption, and/or coprecipitation (Rajkumar et al., 2010; Wang et al., 2015). Furthermore, a stronger root system can release various hormones and induce fluctuations in the rhizosphere and root architecture reconstruction, thereby influencing Cd uptake by roots (Li et al., 2009).

4.2 Contrasting regulation of root exudates of two rice cultivars under Cd stress

Root exudates have proved to play an important role in Cd detoxification in plants. The present study found contrasting regulation of root exudates between the two rice varieties differing in their Cd accumulation ability. Significant rhizosphere acidification was observed in the high-Cd accumulation cultivar JY841, while alkalization was observed in the low-Cd accumulation cultivar TY816 (Fig. S4). Sun et al. (2019) found that rhizosphere acidification promotes Cd uptake via the Cd hyperaccumulator *Sedum plumbizincicola*. Therefore,

root exudate-induced changes in rhizosphere pH may contribute to differences in Cd uptake by different rice varieties. In addition, a positive correlation of root Cd concentration with TOC of root exudates was observed in TY816, indicating that this cultivar may exhibit a detoxification or defensive reaction in response to Cd stress (Zhao et al., 2016). The root exudates of fatty acids and lipids including, 2-hydroxy-nonadecanoic acid, PC(18:3(6Z,9Z,12Z)/0:0), PS (22:2(13Z,16Z)/21:0), and PA(O-20:0/16:0), were all downregulated by Cd in JY841 but upregulated in TY816. When roots are stressed by heavy metals, the cellular responses that occur include fatty acid release from membranes and variation in lipid amounts (Upchurch, 2008). The concentrations of fatty acids and lipids in the root exudate of TY816 were significantly upregulated, indicating active defense of root membranes to Cd stress. Cd-induced disturbances in membrane lipid turnover and lipid composition might promote the metabolism of fatty acids (Nouairi et al., 2006; Debiante et al., 2012). The oxidative stress caused by Cd is known to accelerate the desaturation of membrane lipids and increase the level of unsaturated fatty acids, which may help resist membrane damage induced by Cd (Zemanová et al., 2015). Orobanchoside was upregulated by Cd in JY841, whereas it was downregulated in TY816. This difference could be due to the depletion of sugar, which acts as a reactive oxygen species scavenger in root tissue (Keunen et al., 2013). This study also found that the secondary metabolite oryzalexin B was downregulated by Cd in JY841 but upregulated in TY816. Growing evidence suggests that flavonoid compounds play an active role in metal stress response and anti-lipid peroxidation via free radical scavenging and metal chelation (Li et al., 2015).

Fig. 5 Box plots of the relative abundance of root exudate metabolites in the two rice cultivars under 10 μM Cd treatment ($n = 3$). The mean concentration of each group is indicated by a diamond.

4.3 Root architecture and exudates mediate the contrasting responses of two rice cultivars to Cd

Roots present the first barrier to Cd, which accumulates in roots and is translocated to shoots (Uraguchi and Fujiwara, 2013). Since most of the accumulated Cd is reserved in the rice root system, variation in the sink has a great influence on Cd levels in shoots and grains (Harris and Taylor, 2013). In this study, we found that some root traits of TY816 were significantly enhanced by 10 μM Cd and that lower Cd uptake was observed, indicating that root barriers may contribute to

lower levels of Cd in TY816. Plants shape their roots and secrete a wide variety of metabolites into the rhizosphere to cope with heavy metal stress. Therefore, contrasting Cd accumulation abilities among different rice cultivars may be due to differences in the reprogramming of root architecture and root exudates (Xin et al., 2017). It has been reported that improvement in root traits can accelerate the uptake of mineral elements and the transpiration rate (Ding et al., 2014). Although RT lack a Casparian strip, making them the most active region for Cd^{2+} influx into plant roots (Tao et al., 2016), most root exudates are localized at the root tip (Canarini et al.,

2019). Thus, the difference in Cd accumulation between JY841 and TY816 may be determined not only by the root architecture but also by the root exudates. Although there have been many studies on root architecture and root exudates under heavy metal stress (Redjala et al., 2011; Zhao et al., 2018; Huang et al., 2019), these issues have rarely been linked to predict rhizosphere resistance strategies. Our study found contrasting responses of root traits and root exudate metabolites to Cd stress between different rice cultivars. This finding suggests that rice roots can increase their tolerance to Cd stress by simultaneously employing multiple strategies involving metabolomics and phenotypes. Despite the fact that the rhizosphere is an active area with high dynamics, the extent and shape of root growth, element fluxes, and exudates are quasi-stationary due to the opposite formation process (Kuzyakov and Razavi, 2019). Cd exhibits a rhizosphere extent < 2 mm, whereas root exudates have a rhizosphere extent of 2–4 mm (Duan et al., 2018). Thus, it appears that there is no intersection between the extent of Cd and root exudates in the rhizosphere. However, root exudation metabolites are driven by diffusion. RT can sense variation in exudation concentration and change the architecture of the root system through signal transduction (Canarini et al., 2019). Therefore, changes in root morphology induced by Cd stress can in turn affect root Cd uptake by influencing the mobilization of Cd in the rhizosphere (Li et al., 2009).

5 Conclusions

Different rhizospheric resistance strategies to cope with Cd stress were identified between two rice varieties differing in their Cd accumulation ability. The combination of metabolomic and phenomic responses revealed that the roots of the low-Cd rice cultivar (TY816) employ multiple strategies to enhance their tolerance of Cd-induced oxidative stress, including alteration of root system architecture, adjustment of membrane lipid turnover, and release of secondary metabolites to prevent lipid peroxidation by scavenging free radicals and metal chelation. Compared with TY816, the high-Cd cultivar (JY841) suffered serious root membrane damage and showed upregulation of phenylethanoid glycosides to cope with Cd-induced oxidative stress. Taken together, these findings have further increased our understanding of root architecture and exudate-mediated Cd resistance in rice. These findings will assist not only in supplementing external Cd resistance mechanisms in the rhizosphere, but also in manipulating rhizosphere architecture and metabolites to mitigate Cd contamination in rice.

Acknowledgments

This work was supported by the National Key R&D Program of China (2018YFD0800700), the National Natural Science Foundation of China (41877121, 41920104003), and the 111 Project (B18060).

Electronic supplementary material

Supplementary material is available in the online version of this article at <https://doi.org/10.1007/s42832-021-0088-0> and is accessible for authorized users.

References

- Boursiac, Y., Lérans, S., Corratgé-Faillie, C., Gojon, A., Krouk, G., Lacombe, B., 2013. ABA transport and transporters. *Trends in Plant Science* 18, 325–333.
- Canarini, A., Kaiser, C., Merchant, A., Richter, A., Wanek, W., 2019. Root exudation of primary metabolites: mechanisms and their roles in plant responses to environmental stimuli. *Frontiers in Plant Science* 10, 1–19.
- Chen, Y.T., Wang, Y., Yeh, K.C., 2017. Role of root exudates in metal acquisition and tolerance. *Current Opinion in Plant Biology* 39, 66–72.
- Chi, Y., Li, F., Tam, N.F.y., Liu, C., Ouyang, Y., Qi, X., Li, W.C., Ye, Z., 2018. Variations in grain cadmium and arsenic concentrations and screening for stable low-accumulating rice cultivars from multi-environment trials. *Science of the Total Environment* 643, 1314–1324.
- Debiane, D., Calonne, M., Fontaine, J., Laruelle, F., Grandmougin-Ferjani, A., Lounès-Hadj Sahraoui, A., 2012. Benzo[a]pyrene induced lipid changes in the monoxenic arbuscular mycorrhizal chicory roots. *Journal of Hazardous Materials* 209–210, 18–26.
- Ding, Y., Feng, R., Wang, R., Guo, J., Zheng, X., 2014. A dual effect of Se on Cd toxicity: evidence from plant growth, root morphology and responses of the antioxidative systems of paddy rice. *Plant and Soil* 375, 289–301.
- Duan, C., Fang, L., Yang, C., Chen, W., Cui, Y., Li, S., 2018. Reveal the response of enzyme activities to heavy metals through *in situ* zymography. *Ecotoxicology and Environmental Safety* 156, 106–115.
- Fu, H., Yu, H., Li, T., Zhang, X., 2018. Influence of cadmium stress on root exudates of high cadmium accumulating rice line (*Oryza sativa* L.). *Ecotoxicology and Environmental Safety* 150, 168–175.
- Harris, N.S., Taylor, G.J., 2013. Cadmium uptake and partitioning in durum wheat during grain filling. *BMC Plant Biology* 13, 103.
- Hu, Y., Cheng, H., Tao, S., 2016. The challenges and solutions for cadmium-contaminated rice in China: a critical review. *Environment International* 92–93, 515–532.
- Huang, B., Xin, J., Dai, H., Liu, A., Zhou, W., Yi, Y., Liao, K., 2015. Root morphological responses of three hot pepper cultivars to Cd exposure and their correlations with Cd accumulation. *Environmental Science and Pollution Research International* 22, 1151–1159.
- Huang, L., Li, W.C., Tam, N.F.Y., Ye, Z., 2019. Effects of root morphology and anatomy on cadmium uptake and translocation in rice (*Oryza sativa* L.). *Journal of Environmental Sciences (China)* 75, 296–306.
- Keunen, E., Peshev, D., Vangronsveld, J., Van Den Ende, W., Cuypers, A., 2013. Plant sugars are crucial players in the oxidative

- challenge during abiotic stress: extending the traditional concept. *Plant, Cell & Environment* 36, 1242–1255.
- Khan, J., Yang, Y., Fu, Q., Shao, W., Wang, J., Shen, L., Huai, Y., Malangisha, G. K., Ali, A., Mahmoud, A., 2020. Screening of watermelon varieties for lead tolerance at the seedling stage. *HortScience* 55, 858–869.
- Kuzyakov, Y., Razavi, B.S., 2019. Rhizosphere size and shape: Temporal dynamics and spatial stationarity. *Soil Biology & Biochemistry* 135, 343–360.
- Li, H., Luo, N., Li, Y.W., Cai, Q.Y., Li, H.Y., Mo, C.H., Wong, M.H., 2017. Cadmium in rice: Transport mechanisms, influencing factors, and minimizing measures. *Environmental Pollution* 224, 622–630.
- Li, J., Lu, H., Liu, J., Hong, H., Yan, C., 2015. The influence of flavonoid amendment on the absorption of cadmium in *Avicennia marina* roots. *Ecotoxicology and Environmental Safety* 120, 1–6.
- Li, T., Yang, X., Lu, L., Islam, E., He, Z., 2009. Effects of zinc and cadmium interactions on root morphology and metal translocation in a hyperaccumulating species under hydroponic conditions. *Journal of Hazardous Materials* 169, 734–741.
- Liang, X., Strawn, D.G., Chen, J., Marshall, J., 2017. Variation in cadmium accumulation in spring wheat cultivars: uptake and redistribution to grain. *Plant and Soil* 421, 219–231.
- Lin, Q., Chen, Y.X., Chen, H.M., Yu, Y.L., Luo, Y.M., Wong, M.H., 2003. Chemical behavior of Cd in rice rhizosphere. *Chemosphere* 50, 755–761.
- Liu, J., Qian, M., Cai, G., Zhu, Q., Wong, M.H., 2007. Variations between rice cultivars in root secretion of organic acids and the relationship with plant cadmium uptake. *Environmental Geochemistry and Health* 29, 189–195.
- Lu, Z., Zhang, Z., Su, Y., Liu, C., Shi, G., 2013. Cultivar variation in morphological response of peanut roots to cadmium stress and its relation to cadmium accumulation. *Ecotoxicology and Environmental Safety* 91, 147–155.
- Lynch, J.P., 2019. Root phenotypes for improved nutrient capture: an underexploited opportunity for global agriculture. *New Phytologist* 223, 548–564.
- Neumann, G., Martinoia, E., 2002. Cluster roots—an underground adaptation for survival in extreme environments. *Trends in Plant Science* 7, 162–167.
- Nouairi, I., Ghnaya, T., Ben Youssef, N., Zarrouk, M., Habib Ghorbel, M., 2006. Changes in content and fatty acid profiles of total lipids of two halophytes: *Sesuvium portulacastrum* and *Mesembryanthemum crystallinum* under cadmium stress. *Journal of Plant Physiology* 163, 1198–1202.
- Pan, J., Zhu, M., Chen, H., 2001. Aluminum-induced cell death in root-tip cells of barley. *Environmental and Experimental Botany* 46, 71–79.
- Pétriacq, P., Williams, A., Cotton, A., McFarlane, A.E., Rolfe, S.A., Ton, J., 2017. Metabolite profiling of non-sterile rhizosphere soil. *Plant Journal* 92, 147–162.
- Rajkumar, M., Ae, N., Prasad, M.N.V., Freitas, H., 2010. Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. *Trends in Biotechnology* 28, 142–149.
- Redjala, T., Zelko, I., Sterckeman, T., Legu, V., Lux, A., 2011. Relationship between root structure and root cadmium uptake in maize. *Environmental and Experimental Botany* 71, 241–248.
- Sun, X., Li, Z., Wu, L., Christie, P., Luo, Y., Fornara, D.A., 2019. Root-induced soil acidification and cadmium mobilization in the rhizosphere of *Sedum plumbizincicola*: evidence from a high-resolution imaging study. *Plant and Soil* 436, 267–282.
- Tao, Q., Hou, D., Yang, X., Li, T., 2016. Oxalate secretion from the root apex of *Sedum alfredii* contributes to hyperaccumulation of Cd. *Plant and Soil* 398, 139–152.
- Tao, Q., Zhao, J., Li, J., Liu, Y., Luo, J., Yuan, S., Li, B., Li, Q., Xu, Q., Yu, X., Huang, H., Li, T., Wang, C., 2020. Unique root exudate tartaric acid enhanced cadmium mobilization and uptake in Cd-hyperaccumulator *Sedum alfredii*. *Journal of Hazardous Materials* 383, 121177.
- Ueno, D., Yamaji, N., Kono, I., Huang, C.F., Ando, T., Yano, M., Ma, J. F., 2010. Gene limiting cadmium accumulation in rice. *Proceedings of the National Academy of Sciences of the United States of America* 107, 16500–16505.
- Upchurch, R.G., 2008. Fatty acid unsaturation, mobilization, and regulation in the response of plants to stress. *Biotechnology Letters* 30, 967–977.
- Uraguchi, S., Fujiwara, T., 2013. Rice breaks ground for cadmium-free cereals. *Current Opinion in Plant Biology* 16, 328–334.
- Walker, T.S., Bais, H.P., Grotewold, E., Vivanco, J.M., 2003. Root exudation and rhizosphere biology. *Plant Physiology* 132, 44–51.
- Wang, X., Tam, N.F.Y., He, H., Ye, Z., 2015. The role of root anatomy, organic acids and iron plaque on mercury accumulation in rice. *Plant and Soil* 394, 301–313.
- Wen, Z., Li, H., Shen, Q., Tang, X., Xiong, C., Li, H., Pang, J., Ryan, M. H., Lambers, H., Shen, J., 2019. Tradeoffs among root morphology, exudation and mycorrhizal symbioses for phosphorus-acquisition strategies of 16 crop species. *New Phytologist* 223, 882–895.
- Widodo., Broadley, M.R., Rose, T., Frei, M., Pariasca-Tanaka, J., Yoshihashi, T., Thomson, M., Hammond, J.P., Aprile, A., Close, T. J., Ismail, A.M., Wissuwa, M., 2010. Response to zinc deficiency of two rice lines with contrasting tolerance is determined by root growth maintenance and organic acid exudation rates, and not by zinc-transporter activity. *New Phytologist* 186, 400–414.
- Xia, J., Sinelnikov, I.V., Han, B., Wishart, D.S., 2015. MetaboAnalyst 3.0—making metabolomics more meaningful. *Nucleic Acids Research* 43, W251–7.
- Xin, J., Huang, B., Dai, H., Mu, Y., 2017. Characterization of root morphology and root-derived low molecular weight organic acids in two sweet potato cultivars exposed to cadmium. *Archives of Agronomy and Soil Science* 63, 723–734.
- Yoshida, S., Forno, D., Cock, J., Gomez, K., 1976. Laboratory manual for physiological studies of rice. Manila, Philippines.
- Zemanová, V., Pavlík, M., Kyjaková, P., Pavlíková, D., 2015. Fatty acid profiles of ecotypes of hyperaccumulator *Nocca caerulea* growing under cadmium stress. *Journal of Plant Physiology* 180, 27–34.
- Zhang, H., Lu, L., Zhao, X., Zhao, S., Gu, X., Du, W., Wei, H., Ji, R., Zhao, L., 2019. Metabolomics reveals the “invisible” responses of spinach plants exposed to CeO₂ nanoparticles. *Environmental Science & Technology* 53, 6007–6017.
- Zhao, F.J., Ma, Y., Zhu, Y.G., Tang, Z., McGrath, S.P., 2015. Soil

- contamination in China: current status and mitigation strategies. *Environmental Science & Technology* 49, 750–759.
- Zhao, J.Y., Ye, Z.H., Zhong, H., 2018. Rice root exudates affect microbial methylmercury production in paddy soils. *Environmental Pollution* 242, 1921–1929.
- Zhao, L., Huang, Y., Hu, J., Zhou, H., Adeleye, A.S., Keller, A.A., 2016. ^1H NMR and GC-MS based metabolomics reveal defense and detoxification mechanism of cucumber plant under nano-Cu stress. *Environmental Science & Technology* 50, 2000–2010.
- Zhu, X.F., Zheng, C., Hu, Y.T., Jiang, T., Liu, Y., Dong, N.Y., Yang, J.L., Zheng, S.J., 2011. Cadmium-induced oxalate secretion from root apex is associated with cadmium exclusion and resistance in *Lycopersicon esulentum*. *Plant, Cell & Environment* 34, 1055–1064.