Putrescine modulates cadmium fixation ability of the cell wall to decrease cadmium accumulation in rice via a NO dependent manner

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Research Article

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Abstract

Although putrescine (PUT) has been linked to plants’ responses to cadmium (Cd) stress, the exact mechanism is yet elusive. The endogenous PUT concentration in the rice roots was enhanced by Cd stress in the current investigation, and exogenous PUT increased root cell wall hemicellulose level, which in turn increased its Cd binding capacity, concurrently decreasing the transcription level of genes such as *Natural Resistance-Associated Macrophage Protein 1* (*OsNRAMP1*) and a major facilitator superfamily gene-*OsCd1* that responsible for root Cd absorption. Finally, less Cd was accumulated in the rice as a result of the higher expression of *Heavy Metal ATPase 3* (*OsHMA3*), and Cation/Ca exchanger 2 (*OsCCX2*) that were responsible for separating Cd into vacuole and getting Cd out of cells, respectively. Additionally, PUT enhanced endogenous NO levels, and its alleviatory effect was disappeared by a NO scavenger-cPTIO. In conclusion, PUT enhanced rice’s Cd resistance through regulating the generation of the NO and the binding capacity of the cell wall to Cd.

Introduction

Via the food chain, cadmium (Cd), a hazardous heavy metal element, can endanger human health and impair plant growth[1]. The Agency for Toxic Substances and Disease Registry (ATSDR) has identified it as one of the ten most hazardous compounds[2]. When an excessive amount of Cd enters the cells cytoplasm in plants, it triggers toxicity by inducing various oxidative stress reactions, including protein denaturation, an increment in reactive oxygen species, and etc.[3–5], all of which could impact plants’ growth. Therefore, it is crucial to fully known the ways in which plants tolerate Cd, and or reduce Cd accumulation.

Intake of Cd by roots, storage of Cd in vacuoles, and transportation of Cd from roots to shoots, all are key contributors to Cd accumulation in plant shoots and grains[6]. When suffering from Cd toxicity, plants have developed a variety of mechanisms to withstand its detrimental effects, these mechanisms include vacuole compartmentalization and sequestration of Cd through cytoplasmic organic acids or peptides, as well as cell wall polysaccharides[7–9]. In fact, more evidences have shed light on the role of roots cell wall as a storage pool of the Cd[10], as cell wall not only could serve as the first defense against Cd, but it also could act as a protective barrier for root cells to minimize the cellular damage brought by Cd toxicity through trapping the Cd outside the cell[11]. Pectin, cellulose, and hemicellulose, along with a few functional proteins and trace amounts of aromatic chemicals, make up the majority of the primary cell wall[12–14]. Pectin undergo modifications by pectin methyl esterases (PMEs), and could act as a significant cell wall component to bind Cd[15], although its binding capacity is much lower than the hemicellulose[16–18]. For example, by raising the hemicellulose levels in their root cell wall, exogenous auxin could improve Cd tolerance in Arabidopsis[19]. In dicotyledonous and monocotyledonous non-grass plants, xyloglucan makes up the majority of the hemicellulose. In plant cell walls, the *XTH* genes, which encoded the enzymes called xyloglucan endotransglycosylases/hydrolases that could catalyze the transglycosylation and hydrolysis of xyloglucan polymers[20], were crucial for Al toxicity in...
Arabidopsis\textsuperscript{[21–22]}. In order to decrease the Cd accumulation in the shoot and even grains when rice cultivated in Cd-contaminated soil, it is essential to increase the fixation of the Cd in the root cell wall.

Plants typically employ two alternative pathways that required arginine decarboxylase and ornithine decarboxylase for polyamine synthesis\textsuperscript{[23]}, and polyamine affect plant growth and resilience to outer challenges including drought stress\textsuperscript{[24]}, hazardous materials stress\textsuperscript{[25]} (Al and Cd), and chilling stress\textsuperscript{[26]}. For example, after treatment with 0.1 mM Cd, the amount of putrescine (PUT) in oat (\textit{Avena sativa}) leaf was increased to 20-fold in comparison to the normal condition, demonstrating that PUT is a component that could quick react to Cd toxicity, while under situations of Al toxicity, PUT addition could considerably alleviate root elongation in wheat\textsuperscript{[27]}. Nonetheless, the aforementioned studies only concentrated on the physiological phenotype by which PUT mitigated Cd stress, and it is important for us to understand the underlying molecular mechanisms.

Rice is an important food crop in Asia, where soil acidity is increasingly serious and Cd toxicity is prevalent in most areas\textsuperscript{[28–29]}. This study used the common rice cultivar Nipponbare (Nip) to investigate the role of PUT in Cd stress. Our findings showed that Cd quickly promoted the accumulation of PUT, and the increased PUT could mitigated rice's Cd stress through two strategies. One is to increase root cell wall/hemicellulose' fixation capacity to Cd, thus inhibited the uptake and translocation of Cd, whereas the other is to decrease oxidative stress through decreasing the production of NO, and enhancing Cd detoxification through vacuole compartmentation, which could establish a theoretical foundation for further studies to decrease Cd accumulation in rice to produce safe grains.

**Materials and methods**

**Conditions for rice culture and experimental design**

Rice seeds of Nipponbare (Nip) were first steeped in deionized water for 1 day before being moved to 0.5 mM CaCl$_2$ with a pH of 5.6, and then incubated for a further 2 days at 30°C in complete murk until it reached a length of around 2 cm. 1/2 kimura B solution (-Cd; control solution), addition of putrescine (PUT) to the control solution (+ PUT), addition of Cd to the control solution (+ Cd), and addition of PUT to the + Cd solution (+ Cd + PUT) were the four treatments that were studied, although the concentration of PUT varied from 0.01 to 100 µM. The Cd concentration used here was 1 µM, and the pH of the solution was 5.6.

**Assay for total Cd content**

Samples were collected and baked in an oven until their weight remained steady. The aforesaid samples were then broken down at 120 °C in 2 mL of HNO$_3$. After the solution became clear, the digestion process was stopped and 8 mL of ultrapure water was added. Using an inductively-coupled plasma mass spectrometer (ICP-MS), Cd concentration in above solutions were determined.

**Assay for xylem sap Cd concentration**
Once the treatments were complete, seedlings were transported to a shaded area, and the overground parts were removed from about 2 cm above the junction of roots and stems. Then, the extra sap was gathered in 200 µL tubes for 1 h. Finally, ICP-MS was used to assess the level of Cd in the xylem solution. There were 4 replicates total, each containing 5 seedlings.

**Extraction of the root cell wall, especially hemicellulose**

We extracted the cell wall according to the manner in which Brummell et al.\textsuperscript{[65]} described. The materials were immediately homogenized in 80% ethanol for 20 min after being grounded in liquid nitrogen, and after centrifuged at 8000 g for 10 min, the precipitates were then washed by 6 mL 80% ethanol and acetone for 20 min each. Finally, the precipitates were incubated with 3 mL methanol and 3 mL chloroform for another 20 min, and after centrifugation, the precipitates were regared as the cell wall materials, and then dried for further use.

Then, according to Huang et al.\textsuperscript{[18]}, hemicellulose was isolated. Each cell wall sample was weighed, and 1 mL deionized water was added at 100°C for 1 h. Then the mixture was centrifuged at 10,000 g for 20 min. Repeat above steps for three times, and the combined supernatants were regarded as the pectin, while the residues were further extracted by 24 M KOH (containing 1% NaBH\textsubscript{4}) for 12 h. After centrifuged at 10,000 g for 20 min, the supernatants were collected and residues were extracted as the aforementioned steps again. The combined supernatants were regarded as hemicellulose.

**Measurement of the total sugar**

The absorbance value at OD\textsubscript{490} was calculated after a 20 min incubation with 10 µL phenol and 1 mL H\textsubscript{2}SO\textsubscript{4} with a 200 µL hemicellulose solution at 100°C. Glucose was chosen as the control to create a standard curve.

**Assay for cell wall hemicellulose Cd content**

For Cd detection in hemicellulose, ICP-MS was used to measure Cd levels in the hemicellulose solution after centrifugation at 13200 g for 20 min.

**Assay for nitric oxide (NO) production in root tips**

According to Hu et al.\textsuperscript{[66]}, 4,5-diaminofluorescein diacetate (DAF-FM DA), a fluorescent probe, was used to stain the root to detect nitric oxide (NO). Shortly after being chopped into 1-cm-long pieces, root tips were treated for 1 h in the dark with 10 µM DAF-FM DA in 20 mM HEPES-NaOH buffer with a pH of 7.4. Root tips were then rinsed for three times. An epifluorescence microscope was used to examine and record the NO fluorescence (Eclipse 80i, Nikon, Japan). The identical region of the root tips’ fluorescence signals was captured, and ImageJ software was used to analyze them. Three replications were used for each treatment, with three root tips being pooled to represent one replication.

**RNA Extraction and gene expression level determination**
Liquid nitrogen was used to preserve samples of roots and shoots after harvested, and total RNA was extracted using RNAiso Plus. The HiScript II 1st Strand cDNA Synthesis Kit was then used to create complementary DNA (cDNA) templates (TOYOBO, Japan), and quantitative real-time PCR (qRT-PCR) was used to measure expression of genes through using SYBR Premix Ex-Taq™ II (TOYOBO, Japan). Below is a description of the qRT-PCR reaction mixture: 1 µL of cDNA that has been diluted 10 times, 5 µL SYBR Premix ExTaq, 0.2 µL forward primer, 0.2 µL revise primer, and 3.6 µL RNA-free water. Table 1 listed the primers used here, and OsUBQ was utilized as a reference gene.

### Table 1
Primers used in the study.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward (5’-3’)</th>
<th>Reverse (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OsUBQ</td>
<td>GCTCCGTGGCGGTATCAT</td>
<td>CGGCAGTTGACAGCCCTAG</td>
</tr>
<tr>
<td>OsHMA2</td>
<td>CATAGTGAAGCTGCCTGAGATC</td>
<td>GATCAAAACGCATAGCAGCATCG</td>
</tr>
<tr>
<td>OsHMA3</td>
<td>TCCATCAAACCAAAACCGGAAGAAGAAGAAG</td>
<td>TGCCAATGTCTCTTCTGTTCACA</td>
</tr>
<tr>
<td>OsZIP5</td>
<td>CATGAAGACCAAGGGTGAGAGAGAGAAGAGA</td>
<td>TCACGCCCAGATGGCGATCA</td>
</tr>
<tr>
<td>OsZIP7</td>
<td>GCTTGGCGGATGCGATTG</td>
<td>CTCCACCACTAGAGCCCGTA</td>
</tr>
<tr>
<td>OsZIP9</td>
<td>ATCTTCTTCTCTGCTAAACCACAC</td>
<td>GCAGCCGCTGGCTGGAGAACACAC</td>
</tr>
<tr>
<td>OsCd1</td>
<td>TCAGCTGCACTCAACAGCAACT</td>
<td>TCTCTTGTGTGCTGCCGCA</td>
</tr>
<tr>
<td>OsLCT1</td>
<td>ATGCTTCTGATGATGCTGTG</td>
<td>ACGGCATTCTGCTCTCTGTG</td>
</tr>
<tr>
<td>OsNRAMP5</td>
<td>CAGCAGCAGTAAGAGCAAGATGAAGATG</td>
<td>GTGCTCAGGAAGTACATGTGGTTA</td>
</tr>
<tr>
<td>OsIRT2</td>
<td>GTCGTCGGCTGCTGCTCA</td>
<td>AGGAGGATGCGGAGGGA</td>
</tr>
<tr>
<td>OsIRT1</td>
<td>AGGTGCGGCGCGGTCTCCTTCTTCTTCTTCT</td>
<td>TGTCCTGTACACCCCTGGTC</td>
</tr>
<tr>
<td>OsCCX2</td>
<td>ATCTACCTCGCCTCTCGTCA</td>
<td>CGAGACAGCGATAGGGTTT</td>
</tr>
<tr>
<td>OsABCG36</td>
<td>ATTCTAGCAAGAGAGCAAGAG</td>
<td>GGTCTCATTGAGGAGGAGAGA</td>
</tr>
<tr>
<td>OsNRAMP1</td>
<td>GGATTCTCCTGGTGCTGGGTT</td>
<td>GCAACAAATCTACTCCCCATGGGCC</td>
</tr>
</tbody>
</table>

**Measurement of malondialdehyde (MDA)**

The amount of MDA was examined through following to the instructions of a commercial kit (Solarbio, Beijing), and at 600 nm wavelength, the absorption values were calculated.

**Statistical analysis**

At least two repetitions are run through each experiment. Data analysis employs a one-way ANOVA, and Duncan's multi-range testing is utilized for pairwise comparisons. Different letters in the histograms represent significant differences ($P < 0.05$). For the Student's $t$-test, asterisks in the histogram represent significant differences at $P < 0.05$. 

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RESULTS

Cd stress elevated endogenous PUT level

Nipponbare (Nip) was used in this study to determine whether PUT is associated with rice’s response to Cd stress. It’s interesting to note that root PUT accumulation was significantly boosted by Cd stress (Fig. 1A), even 30 min after Cd treatment (Fig. 1C), while Cd almost has no effect on the shoot PUT accumulation (Fig. 1B and 1D), suggested that Cd could quickly raise the endogenous PUT level in rice roots.

PUT increased rice's Cd resistance

To clarify how the elevated PUT affected Cd stressed rice, the impact of PUT on rice’s development was studied through using various PUT concentrations. Surprisingly, as shown in Fig. 2, the presence of PUT at concentrations of 0.1, 1, and 10 µM caused substantial root damage in the absence of Cd, as seen by the inhibition of root elongation and root biomass. However, when 0.001 or 0.01 µM PUT was used, this harmful effect in root did not manifest. Moreover, Cd considerably reduced the biomass of the shoot and root as well as the elongation of the root (Fig. 2). Nevertheless, these inhibitions were significantly reduced following the application of 0.01 µM PUT rather than other concentrations, hence 0.01 µM PUT was used here.

Then, one-week-old Nip seedlings were exposed to 0 and 0.01 µM PUT for 5 days to better explore the involvement of PUT in reducing Cd toxicity in rice. Intriguingly, exogenous PUT significantly reduced shoot and root Cd accumulation (Fig. 3A and 3B), along with a corresponding decrease in root to shoot Cd transportation (Fig. 3C), a process that was supported by the significantly lower Cd concentration in the xylem sap (Fig. 3D), suggesting the mitigating effect of PUT in reducing Cd absorption and translocation.

In order to prevent a direct contact between PUT and Cd, rice was first prepared with PUT before being exposed to Cd treatment. As seen in Fig. S1, there was almost no difference between the PUT pretreatment and PUT cotreatment in reducing Cd accumulation (Fig. 3), excluded the possibility of the formation of PUT-Cd complex in decreasing the Cd activity in the solution.

PUT enhanced Cd fixation in the cell wall

Hence, we examined both hemicellulose content and its Cd retention in the presence or absence of Cd and PUT. Cell wall, particularly in terms of hemicellulose, served as the initial barrier to confront Cd. Encouragingly, the application of PUT significantly increased Cd retention in the root hemicellulose fraction (Fig. 4A), along with a increment in hemicellulose level (Fig. 4B). However, there was almost no significant difference in shoot cell wall hemicellulose, and its Cd retention (Fig. 4C and 4D), suggesting that PUT could reduce Cd toxicity through increasing the root cell wall’s Cd fixation capacity, thus less Cd was entered to the roots and made the rice more Cd resistance.
PUT controlled the genes’ mRNA level of Cd uptake, transport, and compartmentation

The involvement of OsCd1[30] (a transporter belongs to the major facilitator superfamily), OsZIP5/7/9[31] (Zinc Transporter 5), OsNRAMP1/5[32–33] (Natural resistance associated macrophage protein 1/5), OsCAL1[34] (Cadmium accumulation in Leaf 1), OsHMA2/3[35] (Heavy Metal ATPase 2/3), and OsCCX2[36] (Cation/Ca exchanger 2), and others, likely plays a significant role in mitigating Cd toxicity in rice. The expression of these genes were analyzed in the to determine whether they played a role in the PUT-mediated Cd tolerance mechanism. As predicted, in Cd stressed rice, PUT significantly reduced the transcription level of OsCd1 (Fig. 5C), OsZIP5 (Fig. 5D), OsZIP7 (Fig. 5E), OsZIP9 (Fig. 5F), OsNRAMP1 (Fig. 5G), OsIRT2 (Fig. 5I), OsLCT (Fig. 5K) and OsHMA2 (Fig. 5L), while PUT significantly increased the mRNA level of OsHMA3 (Fig. 7A), and OsCCX2 (Fig. 5B), suggesting that PUT could decrease Cd stress not only by reducing Cd absorption and decreasing roots to shoots Cd translocation, but also by chelating Cd into the vacuole and elevating Cd efflux outside the cells.

Exogenous PUT reduced the lipid peroxidation brought on by Cd

Since lipid peroxidation is often caused by Cd poisoning, thus the accumulation of MDA, a typical indicator of lipid peroxidation, was examined[37–38]. Interestingly, Cd significantly accelerated MDA accumulation in rice, and exogenous PUT treatment significantly reduced this Cd induced MDA accumulation (Supplemental Fig. 2), implying PUT could alleviate Cd induced lipid peroxidation in rice roots, which further confirmed the beneficial role of PUT in Cd stressed rice (Fig. 2).

3.6. NO buildup is necessary for PUT to reduce Cd toxicity

Earlier studies have shown that gibberellic acid (GA) reduced Cd toxicity by reducing NO buildup, which in turn downregulated the transcript level of IRT1. Here, exogenous PUT dramatically increased the amount of NO that induced by Cd in rice root (Fig. 6), suggesting that NO may play a role in the PUT reduced Cd toxicity. Further investigation found that the application of a NO scavenger, cPTIO, was able to mimic this decrease in Cd toxicity in rice caused by PUT because it caused the Cd accumulation to be similar in both the + Cd + cPTIO and + Cd + PUT + cPTIO treatments (Fig. 7), indicating that PUT’s ability to mitigate Cd toxicity in rice was dependent on the buildup of NO.

Discussion

PUT, a key component of polyamines, controls how plants react when exposed to heavy metal treatment[25]. For instance, in saffron[39] (Crocus sativus L.) and Salvinia[40] (Salvinia natans Linn), exogenous putrescine greatly boosts the antioxidant enzymes activities and lowers the Al induced peroxidation damage. PUT also enhances the formation of NO and citrate in red kidney bean plants,
which reduces the amount of Al that accumulates in the roots. In wheat and rice, PUT dramatically reduces the production of hemicellulose and pectin, leading to a reduction in the Al deposition in the cell walls and promoted growth in the Al-toxic circumstances. In fact, the majority of the heavy metals associated with cell walls are coupled with polygalacturonic acids, whose metal ion abundances varied depending on the metal. Both pectin and hemicellulose that rich in polygalacturonic acid, make up the bulk of plant cell walls, and play significant roles in conferring Cd tolerance. Nevertheless, precise mechanism by which PUT modifies cell wall polysaccharide level and the underlying mechanism through which it alleviates Cd toxicity in rice is still unknown, although both Cd and PUT were demonstrated that could influence cell wall production in plants. For instance, PUT could reduce tea pollen tubes' cellulose content and rice's hemicellulose level. On the other hand, Cd also could impact the cell wall polysaccharide level in rice. In the current investigation, we detected a remarkable increment in hemicellulose level in the combination of PUT and Cd treatment when compared with Cd treatment alone, accompanied by an increment of its Cd retention, which is consistent with earlier findings, indicating that PUT positively regulated Cd accumulation in rice by increasing cell wall's ability to bind Cd, thus less Cd was entered in to the roots.

Then, a question raised how PUT could decrease Cd accumulation in the rice? Jasmonic acid (JA), for example, was demonstrated could diminish Cd accumulation in Arabidopsis by inhibiting Cd absorption and translocation through reducing the transcript level of HMA2/4 and IRON-REGULATED TRANSPORTER 1 (IRT1), while GA, a pivotal phytohormone that could respond to abiotic stress, were involved in Arabidopsis's Cd sensitivity through regulating the transcript level of IRT1 that was involved in Cd absorption. In past years, great progress has been made in the identification of genes that associated with Cd accumulation. For example, transporters such as OsZIP5/9, OsIRT1/2, OsCd1, OsNRAMP1/5, were demonstrated to be required for Cd adsorption, although OsZIP5 and OsZIP9 functionally redundantly in the absorption of Cd and Zn in rice. Deletion of OsNRAMP5 decreased grains’ Cd accumulation in rice. Additionally, OsHMA2 was a gene responsible for reloading Cd and Zn from intermediate parenchyma into the scattered phloem of the bundle, and OsCCX2 was responsible for efflux of the Cd outside the cells, while OshMA3 was responsible for sequestration of the Cd to the vacuoles. Overexpression of OsHMA3 could decrease Cd accumulation in rice grains. To investigate if the aforementioned genes contribute to the PUT reduced Cd tolerance, their transcript level were analyzed. Interestingly, PUT decreased the expression of OsCd1, OsNRAMP1, OsZIP5/9 and OsHMA2 (Fig. 5), which was supported by the reduced xylem Cd concentration (Fig. 3D), and decreased shoot to root Cd ratio and in the PUT treated Cd stressed rice (Fig. 3C), suggesting that PUT restraining Cd uptake and translocation, a conclusion further supported by the lower Cd accumulation in their roots and shoots (Fig. 3). Moreover, PUT also enhanced the sequestration of Cd to vacuole and the efflux of Cd through increasing transcription level of OshMA3 and OsCCX2 (Fig. 5), implying that PUT protected rice seedlings from Cd-toxic conditions by accelerating Cd efflux and sequestration to the vacuole.
NO acts as a signal molecular that could stimulate stomatal closure, decrease transpiration rates, and improve plants’ ability to adapt to abiotic stress such as drought stress\textsuperscript{[56]}. Evidence showed that NO functioned downstream of ABA in a physiological process of regulating stomatal transpiration\textsuperscript{[57–58]}, and it has been speculated that transpiration is responsible for the translocation of Cd from roots to shoots.\textsuperscript{[59]} Additionally, NO also played a role in reducing Cd accumulation through regulating the expression of \textit{IRT1} in Arabidopsis\textsuperscript{[50]}, or regulating the cell wall pectin and hemicellulose content in rice\textsuperscript{[60]}, or interacting with phytohormones including abscisic acid (ABA), auxin, salicylic acid (SA), JA, and ethylene\textsuperscript{[61–64]}. However, the specific role of the NO in PUT regulated Cd resistance in rice remains largely unexplored. The current study here demonstrated that PUT could further elevate the endogenous NO level that induced by Cd (Fig. 6), and this alleviatory role could be disappear after the addition of NO scavenger cPTIO (Fig. 7), suggesting that the alleviatory role of PUT in Cd stressed rice was dependent on the accumulation of NO.

\textbf{Conclusion}

In conclusion, according to Fig. 8, we demonstrated that PUT mitigated Cd toxicity in rice due to a decrease in Cd influx into cells caused by the higher cell wall hemicellulose level, and PUT also downregulated the mRNA level of the genes that in charge of Cd uptake and transportation, a pathway relied on the accumulation of NO.

\textbf{Abbreviations}

\textbf{PUT}: Putrescine

\textbf{Cd}: Cadmium

\textbf{OsNRAMP1}: Natural resistance-associated macrophage protein 1

\textbf{OsCCX2}: Cation/Ca exchanger 2

\textbf{OsHMA3}: Heavy Metal ATPase 3

\textbf{ATSDR}: The Agency for Toxic Substances and Disease Registry

\textbf{PMEs}: Pectin methyl esterases

\textbf{Nip}: Nipponbare

\textbf{GA}: Gibberellic acid

\textbf{JA}: Jasmonic acid

\textbf{IRT1}: IRON-REGULATED TRANSPORTER 1
**SA:** Salicylic acid

**ABA:** abscisic acid

**qRT-PCR:** Real time quantitative polymerase chain reaction

**MDA:** Measurement of malondialdehyde

**ICP-MS:** Inductively-coupled plasma mass spectrometer

**OsCAL1:** Cadmium accumulation in Leaf 1

**OsZIP5/7/9:** Zinc Transporter 5

**DAF-FM DA:** Diaminofluorescein diacetate

## Declarations

### Acknowledgements

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### Author Contributions

DLZ, XFZ and RFS designed the experiments; HYW, LS and HJ performed the experiments; LS, HJ and XFZ analyzed the data; HYW and XFZ wrote the manuscript; XFZ and DLZ revised the manuscript. All authors read and approved the manuscript.

### Availability of Data and Materials

All data supporting the findings of this study are available within the paper and within its supplementary materials published online.

### Ethics Approval and Consent to Participate

Not applicable.
Consent for Publication

Not applicable.

Competing interests

All authors declare no competing interest.

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18. Huang J, Jing HK, Zhang Y, Chen SY et al (2023) Melatonin reduces cadmium accumulation via mediating the nitric oxide accumulation and increasing the cell wall fixation capacity of cadmium in rice. J Hazard Mater 130529


Figures

![Graphs and Data Plots]

Figure 1
Effect of Cd on PUT accumulation in rice. Two-week-old rice were treated with or without 1 μM Cd for different times (7 d for A and B, while 0, 0.5, 1, 3, 6, 9 h for C and D), and PUT level in root (A, C) and shoot (B, D) were measured. Values are means ± S.D. (n = 4). Different letters and asterisk indicate significant differences at $P < 0.05$.

Figure 2

Effect of PUT on Cd stressed rice. After two-week-old rice were treated with or without 1 μM Cd and 0-10 μM PUT for 7 days, the growth phenotype were captured (A), root elongation (B), root biomass (C) and shoot biomass (D) were examined. Data are means ± SD (n = 10). Scale bar = 5 cm. Different letters indicate significantly different at $P<0.05$. 
Figure 3

Effect of PUT on Cd accumulation. Two-week-old rice were treated with or without 0.01 μM PUT and 1 μM Cd for 7 days, and Cd content in roots (A), shoots (B), and xylem (D) were measured. The ratio of shoot Cd to root Cd content was calculated (C). Data are means ± S.D. (n = 4). Asterisk indicate significant differences at $P < 0.05$. 
Figure 4

Effect of PUT on root cell wall Cd fixation capacity in rice. Two-week-old rice were treated with or without 1 μM Cd and 0.01 μM PUT for 7 days. Cd content in cell wall hemicellulose in root (A), and shoot (C), and total sugar in hemicellulose in root (B), and shoot (D) were detected. Data are means ±SD (n = 4). Different letters and asterisk indicate significant differences at $P < 0.05$. 
Figure 5

Effect of PUT on transcript level of genes that associated with Cd uptake, transportation, and compartmentation. Two-week-old rice were treated with or without 1 μM Cd and 0.01 μM PUT for 7 days, the transcript level of OsHMA3(A), OsCCX2 (B), OsCd1 (C), OsZIP5 (D), OsZIP7 (E), OsZIP9 (F), OsNRAMP1 (G), OsNRAMP5 (H), OsIRT2 (I), OsIRT1 (J), OsLCT (K), and OsHMA2 (L) were measured. Data are means ±SD. (n = 4). Asterisk indicate significant differences at $P < 0.05$. 
Figure 6

Effect of Put on NO production in rice. Two-week-old rice were treated with or without 1 μM Cd and 0.01 μM PUT for 7 days, the fluorescence images of rice roots were captured by epifluorescence microscopy (A), and relative fluorescence density (% of minimal production) was calculated (B). Data are mean ± SD (n=10). Scale bar = 1 mm. Different letters and asterisk indicate significant differences at \( P < 0.05 \).
Figure 7

Effect of PUT and c-PTIO on rice's Cd accumulation. Two-week-old rice were treated with or without 0.01 μM Put and 10 μM c-PTIO in the presence of 1 μM Cd for 7 days, and Cd content in roots (A) and shoots (B) were examined. Data are means ± SD (n = 4).
Figure 8

Proposed model of PUT in alleviating Cd stress in rice. Application of exogenous PUT on the one way decreased the absorption of Cd and increased the ability of the cell wall to bind Cd, while on the other way altered the transcript level of genes that responsible for Cd absorption and transportation.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- floatimage9.png
- floatimage10.png