Salicylic acid application alleviates Cadmium accumulation in brown rice by modulating its shoot to grain translocation in rice

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Abstract

Cadmium (Cd) contamination has been recognized as a major threat to the agricultural system and crop production which posing serious threat to human health. Salicylic acid (SA) serves as an important signaling molecule and plays an important role in against Cd toxicity. In the previous field experiments, we found SA spraying could reduce the Cd accumulation of rice grain grown in Cd-contaminated soil. This study investigated the effects and mechanisms of SA spraying on leaves of rice seedlings under Cd stress. Results showed that SA treatment could alleviate the Cd toxicity of rice not by changing the physical and chemical properties of the soil, but by increasing the activities of antioxidant enzymes to reduce the H_2O_2 accumulation in rice. And the key factor of SA treatment reducing Cd accumulation in rice grain was the decreasing of Cd contents in rice leaves at the flowering stage. This indicated that SA could modulate the Cd accumulation of shoots to reduce the Cd translocation to rice grain. Furthermore, SA could increase the H_2O_2 contents in a short-term to activate the SA-signaling pathway, and modulate the expression levels of Cd transporters (OsLCT1 and OsLCD) in rice leaves to raise Cd tolerance and reduce Cd accumulation in rice grain. Thus, SA spraying can be used as an effective measure to cope with Cd contamination in paddy soils.

Introduction

Growing population and fast industrialization coincide together, results in the generation and dissemination of huge amount of toxic metals in the environment (Hasanuzzaman et al., 2012). Cd is one of the most phytotoxic heavy metals which have no biological role in plants, and its contamination in agricultural soil has become a great problem for crop production in recent decades. As a common soil pollutant, Cd can accumulate in plant tissues to cause toxicity and enter human through the food chain and drinking water to cause diseases in kidney, bone and respiratory system and cancers (Zhou et al., 2017). Rice is an important staple food for nearly half of the world's population (Valipour et al. 2015) and is also a major source of Cd uptake for people. Consumption of Cd-contaminated rice caused the outbreak of "Itai-Itai disease" in the mid-20th century in Japan. Recently, Cd-contaminated rice has also occurred in China, which poses important public health risks (Liu et al., 2016b). Therefore, it is essential to produce low-Cd rice to reduce the potential risks that Cd poses to human health.

Defense mechanisms induced by Cd stress in plants have been revealed by focusing on mechanisms of its toxicity, tolerance and accumulation. The rapid production and accumulation of reactive oxygen species (ROS), especially H_2O_2 was often accompanied with Cd stress (Muhammad et al., 2019; Zoltán et al., 2018; Wei et al., 2018). Previous studies indicated that SA could alleviate the Cd toxicity (Ahmad et al. 2019; Wei et al. 2018; Guo et al., 2018; Qiao et al., 2015) and reduce Cd uptake and its distribution (Yotsova et al, 2018; Liu et al. 2016a; Shakirova et al. 2016). SA is a ubiquitous plant phenolic compound that has been used in many plant species to alleviate Cd toxicity by participating in the signal transduction pathways (Yotsova et al, 2018; Zoltán et al., 2018; Jini and Joseph, 2017; Liu et al., 2016a; Wani et al., 2017; Agami and Mohamed, 2013; Vlot et al., 2009). A number of reports indicated that SA not only plays a pro-oxidant role, but also controls cellular redox homeostasis through the regulation of antioxidant enzyme activities under stress conditions (Emad et al, 2019; Zoltán et al., 2018; Herrera-Vásquez et al., 2015; Xia et al., 2015). SA could increase the activity of superoxide dismutase (SOD), the main scavenger of superoxide radicals, which catalyses the conversion of superoxide to H_2O_2 in different cell compartments, and SA also could inactivate catalases (CAT) and ascorbate peroxidases (APX), two types of H_2O_2 scavengers, thus leading to H_2O_2 accumulation (Amit et al., 2015; Rao et al., 1997).

Many studies have reported that H_2O_2 can act together with SA in a self-amplifying feedback loop where SA induced H_2O_2 accumulation, which then enhanced the accumulation of SA (Zoltán et al., 2018; Xia et al., 2015; Wi et al., 2010). And endogenous SA could enhance and bind to CAT to increase the level of H_2O_2 , the H_2O_2 serves as secondary

messenger to induce the expression of tolerance-related genes (Amit et al., 2015; Guo et al., 2009). Furthermore, H₂O₂ also could induce the accumulation of SA (Guo P et al., 2017; Leon et al., 1995). Therefore, the production of H₂O₂ during Cd stress served as part of the signaling cascade, and increased levels of ROS might interact with SA (Orsolya et al, 2016). Pape et al. (2010) reported that SA promoted the redox modification of specific Cys residues and the monomerisation of the oligomeric form of the NPR1 (NON-EXPR- ESSOR OF PR GENES 1) transcription coactivator, a central compound of SA signaling pathway, which was transported to the nucleus and interacted with certain TGA transcription factors (TGA2 and TGA3), the regulators of SA-dependent late genes (e.g. *PATHOGENESIS RELATED1*, *PR1* gene). Class II TGA transcription factors (TGA2/5/6) were also essential regulators in SA-induced gene expression, but they mediated the NPR1-independent induction of early defense genes including antioxidant enzymes (Zoltán et al., 2018; Blanco et al., 2009). Hence, SA was not only known to induce a wide range of cellular defense reactions, but also could modulate the gene expression of plants responded to different stresses (Biswaset al. 2019).

In the previous field experiments, we found that SA spraying could reduce the Cd accumulation of rice grain grown in Cd-contaminated soil. However, the study on the reduction of Cd accumulation in rice grain by SA application is still blank. Thus, we conducted a pot experiment in the laboratory to screen the best concentration of SA treatment, and declare its molecular and physiological mechanism in regulating Cd accumulation of rice.

Materials And Methods

1.1 Growth Conditions and Experimental Design

Seeds of the pre-screened as a high grain-Cd-accumulating rice (Xiushui 110) was used in the pot experiment. After germination, rice seedlings were grown in a grid until to 4-leaf stage (Wang et al., 2009), then the plants were transplanted into the plastic pots filled with 10.0kg soil, four seedlings were grown in each pot. After treated with Cd for 1 week, the pre-screened 0.1mM SA solutions and 0.1mM AOPP (L-2-aminooxygen-3-phenyl acrylic acid, synthetic inhibitors of SA) were sprayed on the seedlings' leaves until the whole leaves were covered by the solutions at the tillering stage, heading stage and flowering stage, respectively. The experimental groups were set up 6 experimental groups: CK (control, without Cd or SA treatment), Cd (single Cd treatment), SA (single SA treatment), SA+Cd (treated with SA under Cd stress), AOPP (treated with AOPP), AOPP+Cd (treated with AOPP under Cd stress).

1.2 Estimation of the agronomic traits of rice

Samples were collected from each pot at the growth stages after sprayed with SA (or AOPP) for 5 days and at the maturity stage, respectively. The rice plants height and root length were measured by a ruler. The dry matter accumulation was determined as follows: The plants were divided into roots and shoots, then dried in the oven for 2h at 105°C and baked at 70°C to constant weight. The 1000-grain weight was also analyzed.

1.3 Estimation of the pH, available Cd and total Cd in soil

The soils at different stages of rice plants were used in the determination, and the sampled soil was naturally air-dried and removed through a 2.5 mm nylon screen to remove grit and plant debris, then passed through a 100-mesh screen and stored. The pH, available Cd and total Cd were determined according to Wang et al. (2018).

1.4 Estimation of Cd contents in different parts of rice

Different parts of rice samples were *taken at* heading and flowering stages *after sprayed with SA for 5 days and at the maturity stage, respectively*. Roots of rice samples were immersed in 20mM disodium ethylenediamine tetra-acetic acid (Na₂-EDTA) for 20min, and then rinsed three times with deionized water. All the rice samples were dried at 105°C for 2h

firstly, then baked at 70°C to achieve a constant weight, and finally ground to powders. Each 0.3g dried rice powders were treated with 6mL HNO₃ (brown rice) or 6mL HNO₃ plus 0.5ml H₂O₂ (other rice samples) to digest in a microwave digester (CEM-MARS, Boston, USA). After digestion, all the mixtures were driven acid to 1mL at elevated temperature. The solutions were then diluted into 25mL volumetric flasks with ultra-pure water, and the clarified samples thus obtained were kept in a refrigerator at 4 °C for further analysis. The Cd contents were determined by an atomic absorption spectrometer (AA7000, SHIMADZU, and Kyoto, Japan).

1.5 Estimation of endogenous SA content and PAL activity

Fresh leaves (0.1g) were ground with liquid nitrogen into power, and added 0.5mL sterile water, then carried out ultrasonic 30min. The homogenate was placed in centrifuge at 10,000rpm for 10min, and the supernatant obtained was the sample extract. Finally, the endogenous SA content was measured by the Plant Salicylic acid (SA) ELISA Kit (JM-E100150, U.S.A TSZ biological Trade Co., Ltd). The determination of PAL activity was followed with the instructions of the specific Kit (A173, Nanjing Institute of Bioengineering).

1.6 Estimation of H₂O₂ and antioxidant enzymes

Fresh leaves (0.1g) were ground with liquid nitrogen into power, then ground with 3ml precooled extraction buffer solution (50mM PBS, pH 7.0), and finally the homogenate was used for the determination of *H₂O₂ and the activity of POD, CAT and SOD*. All the indexes were measured using Amplex® Red Hydrogen Peroxide/Peroxidase Assay Kit (A22188, invitrogen), POD test kit (A084-3, Nanjing jiancheng Bioengineering Research Institute), CAT test kit (A007-1, Nanjing jiancheng Bioengineering Research Institute) and SOD test kit (A001-3, Nanjing jiancheng Bioengineering Research Institute), respectively.

1.7 Gene expression analysis using qRT-PCR

Total RNA isolated from the top 2nd leaves of rice plants after SA sprayed for 5days at the flowering stage, and the different cDNA were synthesized with the primerscript^M RT reagent kit (Takara, Japan). Primer sequences used were listed in Table 1, *β-actin* was chosen as the internal control. The qRT-PCR was performed using a Rotor-Gene Q machine (parameters: 10min at 95°C, followed by 45 cycles of 10S at 95°C, 15S at 58°C, and 15S at 72°C). All the reactions were performed in triplicate (technical replicates) for each biological replicate (three for each treatment). Quantification of gene expression was calculated using $\Delta\Delta$ CT method (Wang et al., 2012).

1.8 Statistical analysis

The experimental data was analyzed using the Prism software (Graphpad Prism 6.0, GraphPas Software Inc., SanDiego, California, USA) and presented as the mean ± standard deviation (SD) which calculated with at least three replicates. Student's *t*-test was adopted for the analysis of significant differences among the treatments. *P*-value less than 0.05 were considered as significant.

Results

2.1 SA application enhances the tolerance to Cd in rice

Preliminary experiments showed that SA played an important role at the reproductive growth period (data not shown). Therefore, our experiments were conducted mainly at the reproductive growth stages (heading stage, flowering stage and mature stage). Cd stress obviously reduced seedlings growth, which mainly reflected in the following aspects: root length, plant height, shoot Dw plant-1, and1000-grain weight. SA application could relieve the Cd toxicity obviously,

increased the plant height and shoot Dw plant⁻¹ evidently of the Cd-exposed rice plants, but there was no significant difference between Cd and AOPP+Cd groups (Table 2 and 3), the same trends were observed at all the three stages of rice plants. In addition, application of SA or AOPP to non-stressed seedlings did not result in any difference in agronomic traits.

2.2 SA application alleviates the Cd accumulation in rice grain by the reduction of Cd content in leaves at flowering stage

2.2.1 SA application changed the physical and chemical properties the soil

From the analysis of the effects of SA and AOPP application on the total Cd content, available Cd content and pH value of the soil, we found the total Cd and the available Cd contents of the soil were decreased continuously during the growth of rice. And the exogenous SA or AOPP treatment did not affect the total Cd and the available Cd contents at the four different stages obviously except there was a significant difference between SA+Cd group and AOPP+Cd group at the mature stage. Furthermore, the pH value of the Soil also was not changed after the treatment with exogenous SA or AOPP (data not shown).

2.2.1 SA application induces low Cd accumulation in rice grain

Cd accumulation of rice grain (0.29mg/kg) in Cd-exposed rice plants was significantly greater than the regulatory Cd limit (0.2mg/kg), but SA application could reduced the accumulation of Cd to 0.12mg/kg. Meanwhile, the AOPP+Cd group increased Cd accumulation (0.34mg/kg) significantly which was higher than that of the Cd group (Figure 1).

2.2.3 SA application decreases the Cd contents in other parts of rice

The rice plants accumulated significant amount of Cd in different parts during Cd stress, and the roots accumulated the most Cd by nearly 80%. Compared with the Cd-exposed rice plants, application of SA could reduce the Cd accumulation of leaves and flag leaves remarkable, especially the leaves at flowering stage (around 45% reduction), resulted in around 53% reduction of Cd accumulation in panicle. However, SA application had no significant impact on Cd accumulation in roots, stems and nodes. Furthermore, AOPP application significantly enhanced the total Cd accumulation in panicle, and the main reason was that the Cd contents in leaves at flowering stage were increased (Figure 2). Therefore, we indicated the main reason of SA treatment could effectively reduce Cd content in brown rice and panicles was SA could significantly reduce Cd contents in rice leaves at flowering stage.

Different treatments could affect the mobility of Cd in rice. From the analysis of effect of the three groups (Cd, SA+Cd and AOPP+Cd) on the Cd mobility in rice, we only found the Cd mobility at flowering stage (stem-to-leaf and leaf-to-panicle) and mature stage (leaf-to-panicle) were shown obvious significance (Table 4). SA application could markedly reduce the Cd mobility, as well as AOPP application could markedly increase the Cd mobility in stem-to-leaf at flowering stage. Furthermore, the different Cd mobility resulted in the different Cd accumulation in rice panicles, SA application both reduced the Cd mobility in leaf-to-panicle approximately both 41% at flowering stage and mature stage, and AOPP application increased the Cd mobility in leaf-to-panicle about 47% at flowering stage and 29% at mature stage separately. Therefore, leaves at flowering stage were the critical parts for SA regulate Cd accumulation in rice grain.

2.3 SA application boosts endogenous SA accumulation and activates the SA-signaling pathway

2.3.1 Application of SA boosts endogenous SA accumulat in rice

As leaves at the flowering stage were the key parts for SA regulating Cd accumulation in rice grain, we determined the content of SA in rice leaves at flowering stage. SA and AOPP applications both had a significant impact on SA contents

in leaves. Without Cd stress, SA treatment could obviously increase the SA contents in rice and AOPP treatments could obviously reduce the SA contents in rice. During Cd stress, SA application showed a time dependent increase of the SA contents in leaves, reached the maximum on the 3rd day by 94% than control, and then began to decrease on the 5th day; AOPP treatment reduced by about 80% than control (Figure 3).

Moreover, the differently biosynthesis of SA in different plant species were contribute to the activities of PAL and ICS enzymes (Xu et al. 2017). We found Cd stress could decrease the expression of PAL enzyme, and SA application could induce higher *PAL* expression (Figure 4). The expression of *OsPAL* in SA group were higher than that of CK group, it could arrived at more than 2 times at 6 hours after SA application at the flowering stage, as well as the expression level of *OsPAL* in SA+Cd group were more than 2.5 times than that of Cd group at 6 hours. In contrast to the changes of *OsPAL* expression level, there were no significance of the expression levels of *OsICS* between CK and Cd group, but the expression levels of *OsICS* in SA (or SA+Cd) group could increase by more than 4 times than that of WT (or Cd) group, respectively.

2.3.2 SA application regulates the expression of key genes in SA-signaling pathway

It was generally known that SA signaling in rice was mediated by NPR1 and the WRKY sub-pathway (Xu et al. 2017; Nakayama et al. 2013). We found the expression of *OsNPR1*, *OsWRKY45*, *OsWRKY7*, and *OsWRKY70* were all modulated by Cd stress, and the SA application could increase the expression of *OsNPR1* and *OsWRKY45* obviously (Figure 5). The trends of the *OsWRKY45* expression which represented the SA level in rice in different experimental groups were consistent with the SA levels (Amit et al., 2015; Shimono et al., 2007; Ryu et al., 2006), these results suggest that SA enhanced *OsWRKY45* expression resulted in an raise of endogenous SA content. *OsWRKY7* and *OsWRKY70* were also found related with the SA level in rice seedlings (Xu et al., 2017). Our results indicated that the expression levels of *OsWRKY7* and *OsWRKY70* were negatively correlated with the SA contents in rice.

2.4 SA application sustains a high level of H₂O₂ and boosts antioxidant enzymes' activities under Cd stress

In order to examine whether SA induces H₂O₂ production in rice leaves during Cd stress, the contents of H₂O₂ and enzyme activities of CAT, POD, and APX in the rice leaves at the flowering stage were determined. Results showed that Cd stress could result in a high H₂O₂ accumulation. The H₂O₂ content in SA group was reduced in a short period of time after SA application, the H₂O₂ content was lowest on the 1st day, then increased continuously, and returned to the same level as the control on the 5th day, similar trend was observed in SA+Cd group. Furthermore, the H₂O₂ content in SA+Cd group was higher than that of the Cd group temporary, but after SA application it decreased with time to the lowest level on the 3rd day, and then increased to the level similar with the Cd group on the 5th day (Figure 6).

Furthermore, compared with the Cd group, the activities of antioxidant enzymes (such as SOD, POD and CAT) in SA group and Cd+SA group showed a similar enhancement moderated by SA treatment (Figure 6). It was obvious that SA application on rice leaves during Cd stress could increase the H₂O₂ content in a short-term and improve the Cd tolerance of rice.

2.5 SA application modulates the expression level of genes related to Cd accumulation

Two genes related to Cd accumulation of rice grain in rice were detected in this experiment. *OsLCT1* which was reported could mediate Cd transport in the phloem of rice (Uraguchi et al., 2014). As shown in figure 7, Cd group could decrease the expression level of *OsLCT1* by around 80% than that of the control, and SA group enhanced the *OsLCT1* expression significantly in comparison to control. Compared with the Cd group, SA+Cd group could obviously enhance the *OsLCT1* expression though the level was lower than the control.

OsLCD was found related to the Cd tolerance and accumulation (Shimo et al., 2011), there was no significant change of the *OsLCD* expression in Cd group in comparison to the control, except for SA application (SA group or SA+Cd group) (Figure 7). SA group and SA+Cd group could increase the *OsLCD* expression in a short time, and then quickly dropped to its original level.

Discussion

Cd stress has been recognized as a major threat to the agricultural system and crop production. SA serves as an important signaling molecule in plant system which has been shown to play role in against Cd toxicity (Ahmad et al. 2019; Liu et al. 2016a; Abdul et al. 2016; Amit et al., 2015; Qiao et al. 2015; Noriega et al., 2012; Metwally et al., 2003). However, few studies regarding SA modulates Cd accumulation in rice have been reported. Here we found SA spraying could not only effectively improve the Cd tolerance in rice, but also reduce the Cd accumulation in rice grain.

SA spraying could effectively improve the Cd tolerance. The main manifestations were as follows: SA treatment increased the H₂O₂ contents temporary as well as boost antioxidant enzymes activities under Cd stress; Increased the expression level of PAL and ICS enzymes to boost the activities of PAL and ICS enzymes, then boost the endogenous SA accumulation as a signaling molecule, which will activate the SA-signaling pathway to induce the expressions of specific proteins or defense-related enzymes (Liu et al. 2016a; Zawoznik et al. 2007); SA was a scavenger of hydroxyl radical (OH[•]), the increased endogenous SA induced by SA application could reduce the Cd toxicity in rice (Abdul et al. 2016). That phenomenon may confirm that relationship between H₂O₂ and SA (Liu et al. 2016a; Abdul et al. 2016; Qiao et al., 2015; Amit et al. 2015). It was known that SA could act together with H₂O₂ in a self-amplifying feedback loop where SA induce H₂O₂ accumulation, which then enhanced the accumulation of SA (Zoltán et al., 2018; Xia et al., 2015; Wi et al., 2010). Our results indicated that SA treatment had no change on the physical and chemical properties of the soil, but significantly increased the activities of SOD, POD and CAT in rice under Cd stress, which could relieve the damage of a short-term increased H₂O₂. These results were consistent with the previous reports (Ahmad et al. 2019; Liu et al. 2016a; Guo et al. 2007, 2009). However, some reports showed that SA could indirectly decrease H₂O₂ accumulation by elevating antioxidant enzymes activities (Bai et al. 2014; Wang et al. 2013a), SA application could decreased the activities of antioxidant enzymes (SOD, CAT and POD) (Jini and Joseph, 2017), and SA could interact with CAT to inhibit its activity, then causing excessive H₂O₂ accumulation (Qiao et al., 2015; Sajitha and Murugan 2010). Therefore, the protective impact of SA on Cd tolerance in rice was time-dependent and dose-dependent.

In addition, SA could modulate the Cd accumulation of shoots to reduce the Cd translocation to rice grains. Our results showed that SA treatment had no significant impact on Cd accumulation in roots, stems and nodes, except the Cd contents in leaves at flowering stage. Furthermore, SA could significantly reduce the Cd mobility of stem-to-leaf and leaf-to-panicle in rice at flowering stage which resulted in low Cd accumulation in rice grain. It was not exactly consistent with the previous studies reported that SA regulated mainly the Cd uptake in rice, and then mediated the Cd translocation of root-to-shoot (Bai et al. 2014; Wang et al. 2013b; Noriega et al. 2012). Such as Bai et al. (2014) report that SA treatment reduced the Cd accumulation both in roots and shoots in perennial ryegrass, Wang et al. (2013b) report that SA treatment increased the Cd accumulation in roots but significantly reduced it in the shoots in rice seedlings, Saidi et al. (2013) report SA application could decrease the Cd uptake and transport in Bean. Thus, the difference between our experiment and the previous studies is the spraying of SA at a certain concentration on the leaves in our experiment. Therefore, this might be due to SA-mediated the Cd transporters in rice leaves which result in the low-Cd in rice grain.

In present studies, *OsLCT1* and *OsLCD* were usually recognized as two key genes which could mediate Cd transport in the phloem of rice (Uraguchi et al., 2014; Shimo et al., 2011). Our results showed that Cd

contents in leaves at flowering stage decreased were the critical factor in the low Cd accumulation in rice grain under Cd stress. Through analyzing the expression of the two Cd transporters, results indicated SA could induce higher expression level of *OsLCT1* and *OsLCD* under Cd stress while the expression level of *OsLCD* was enhanced briefly. Therefore, it might be possible that most of Cd accumulated in rice plants with SA treatment was sequestered in root vacuoles, as a result less Cd could be transported to the shoot, and then low Cd accumulated in leaves. Second, although the outward transport capacity of Cd in leaves increased, more Cd in leaves were sequestered in leaves vacuoles or SA act as an Cd-chelation (Abdul et al. 2016), led to the Cd transport was less in leaves of SA+Cd group, finally lower Cd accumulation in rice grain.

It was reported that SA was a defense hormone, the SA signaling in rice could mediate many kinds of resistance in plants, and SA signaling in rice was mediated by NPR1 and the WRKY sub-pathway (Zhao et al. 2019; Yotsova et al. 2018; Jini and Joseph, 2017; Xu et al. 2017; Xu et al. 2017; Abdul et al. 2016; Qiao et al. 2015; Nakayama et al. 2013). Our results indicated SA application up-regulated two important regulatory factors (*OsNPR1*, *OsWRKY45*) and down-regulated the expression of other two transcription factors (*OsWRKY7* and *OsWRKY70*) in SA signaling pathway. It was consistent with Chao et al. (2010) which showed that SA application enhanced *OsWRKY45* expression, which resulted in an increased endogenous SA content. Results of *OsNPR1* expression determination confirmed that *OsNPR1* was a positive regulator of SA signaling pathway (Lai et al. 2018; Xu et al. 2017; Nakayama et al., 2013). *OsWRKY7* and *OsWRKY70* were also found negatively correlated with the SA contents in rice. These results supported the hypothesis that SA application could activate the SA-signaling pathway to induce the Cd response in rice.

Conclusion

In conclusion, it is evident from the present work that SA can effectively boost the antioxidant enzymes activities and SA signaling pathway to response to Cd stress in rice. SA spraying modulates the Cd mobility of stem-to-leaf and leaf-to-panicle in rice at flowering stage to reduce the Cd accumulation in rice grain which is helpful in developing the low-Cd rice grain (Figure 8).

Declarations

Ethics approval and consent to participate: No applicable.

Consent for publication: No applicable.

Availability of data and material: All essential data are part of the article.

Conflict of interest: The authors declare that they have no competing interests.

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Authors' Contributions: This study was designed by Cheng Zhu, Zhixiang Chen and Feijuan Wang. Pot experiment was conducted by Haifeng Tan and Yiting Zhang. The data analysis was performed by Haifeng Tan, Yanfei Ding and Lihong Huang. Feijuan Wang wrote this manuscript; Cheng Zhu and Zhixiang Chen polished the manuscript.

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Abbreviations

Cadmium, Cd; Salicylic acid, SA; reactive oxygen species, ROS; superoxide dismutase, SOD; catalases, CAT; ascorbate peroxidases, APX; NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES 1, NPR1; L-2-aminooxy-3-phenyl acrylic acid, AOPP; disodium ethylenediamine tetra-acetic acid, Na₂-EDTA.

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Tables

Table 1 qRT-PCR primers for Gene expression analysis in leaves of rice plants

Primer names	Sequence (5'-3')
<i>OsLCT1-F</i>	GAGTTCTTCGTCAGAGCTAC
<i>OsLCT1-R</i>	CAGTGCTGGATGACGAATTG
<i>OsLCD-F</i>	ACCGACTGCACAGTAATCAAGC
<i>OsLCD-R</i>	TTACTTCTGCGAGTGTGAGCAA
<i>OsMTP1-F</i>	GGTCCTGATGGAGAGCACGC
<i>OsMTP1-R</i>	CTACTCGCGCTCAATCTGAA
<i>OsPAL-F</i>	GAGCTTGACTACGGCTTCAA
<i>OsPAL-R</i>	CGCTCTGGACATGGTTGGT
<i>OsICS1-F</i>	TCTGCGATGATGTGGTTGTC
<i>OsICS1-R</i>	GCTCGGATGAAGAGTATTTAGGA
<i>OsNPR1-F</i>	TGGACAGGTTATCACCATTGGT
<i>OsNPR1-R</i>	CCGCAGCTTCCATTCTATG
<i>OsWRKY45-F</i>	AGCTGAGCTGCGAGGAAGA
<i>OsWRKY45-R</i>	CGAAAGCGGAAGAACAGGA
<i>OsWRKY7-F</i>	TGATCGAGATAGCAGCCTCTT
<i>OsWRKY7-R</i>	CCAGCTACATCCAAAATGACC
<i>OsWRKY70-F</i>	CCGCTGCTGTTTTGATCATCT
<i>OsWRKY70-R</i>	GGAGCTAAGCTAACTCACTCCACA

Table 2 Effects of different treatments on agronomic traits of rice under Cd stress at the reproductive stages (heading and flowering stage)

Different treatment	Heading Stage				Flowering stage			
	Root length (cm)	Plant height (cm)	Root Dw plant ⁻¹ (g)	Shoot Dw plant ⁻¹ (g)	Root length (cm)	Plant height (cm)	Root Dw plant ⁻¹ (g)	Shoot Dw plant ⁻¹ (g)
CK	12.84±0.67a	86.20±4.32a	0.43±0.02a	11.69±0.27a	12.94±0.85a	88.60±3.05a	0.43±0.01a	12.50±0.14a
SA	12.88±0.46a	86.40±2.7a	0.42±0.02a	11.51±0.26a	12.60±0.96a	90.00±4.74a	0.43±0.05a	12.52±0.14a
AOPP	12.88±0.72a	84.60±3.91a	0.43±0.03a	11.54±0.26a	12.64±0.62a	85.60±4.04a	0.43±0.03a	12.36±0.26a
Cd	9.22±0.65b	76.40±3.21b	0.42±0.02a	9.36±0.21b	8.94±0.41b	76.60±1.82b	0.42±0.01a	11.04±0.18b
SA+Cd	8.92±0.61b	84.80±2.39a	0.42±0.05a	11.46±0.31a	8.64±0.50b	88.80±4.32a	0.43±0.03a	12.54±0.13a
AOPP+Cd	9.06±0.55b	72.80±2.59b	0.43±0.05a	9.27±0.17b	8.70±0.72b	76.60±3.51b	0.42±0.02a	11.01±0.13b

Note: Values are mean ± SD (n=5); different small letters in the same column indicate significant difference at $P<0.05$.

Table 3 Effects of different treatments on agronomic traits of rice under Cd stress at mature stage

Different treatments	Mature Stage					
	Root length (cm)	Plant height (cm)	Root Dw plant ⁻¹ (g)	Shoot Dw plant ⁻¹ (g)	Panicle number per plant	1000-grain weight (g)
CK	12.88±0.54a	88.80±3.11a	0.43±0.02a	12.27±0.26a	5.20±0.45a	27.77±0.56a
SA	12.84±0.43a	90.40±3.21a	0.43±0.04a	12.17±0.20a	5.00±0.00a	28.18±0.60a
AOPP	12.84±0.38a	87.80±4.32a	0.45±0.04a	12.31±0.22a	5.60±0.89a	28.40±0.79a
Cd	8.94±0.54b	79.20±4.49b	0.43±0.03a	10.04±0.31b	5.20±0.45a	25.86±0.58b
SA+Cd	8.84±0.48b	89.60±2.30a	0.42±0.03a	12.26±0.07a	5.20±0.45a	25.70±0.45b
AOPP+Cd	8.72±0.61b	75.00±3.08b	0.43±0.04a	10.02±0.39b	5.40±0.89a	25.33±0.82b

Note: Values are mean ± SD (n=5); different small letters in the same column indicate significant difference at $P<0.05$.

Table 4 Effects of different treatments on Cd mobility in rice

Growth stages	treatments	Mobility (%)		
		Roots-to-Stems	Stems-to-Leaves	Leaves-to-panicles
Heading stage	Cd	91.27±1.98a	6.52±0.57a	12.73±0.74a
	SA+Cd	91.62±0.39a	6.49±0.15a	12.40±0.96a
	AOPP+Cd	91.43±3.36a	6.31±0.61a	12.48±0.73a
Flowering stage	Cd	127.15±7.31a	17.16±1.24b	30.34±0.96b
	SA+Cd	127.43±0.34a	12.63±1.31c	17.98±0.65c
	AOPP+Cd	127.63±1.30a	26.02±3.08a	44.73±3.28a
Mature stage	Cd	195.71±19.25a	2.11±0.14a	212.03±25.85b
	SA+Cd	195.60±9.61a	2.05±0.12a	125.37±22.68c
	AOPP+Cd	195.17±11.04a	2.14±0.17a	274.34±8.84a

Note: Values are mean ± SD (n=5); different small letters in the same column indicate significant difference at $P<0.05$.

Figures

Figure 1
Effects of different treatments on Cd accumulation in brown rice at mature stage

Figure 2

Effects of different treatments on the accumulation of Cd in different parts of rice at the reproductive growth stage. A, roots; B, stems; C, leaves; D, flag leaves; E, nodes; F, panicles

Figure 3

Effects of SA treatment on SA content of leaves at flowering stage

Figure 4

Effects of SA treatment on the expression levels of *OsPAL* and *OsICS* in rice leaves at flowering stage

Figure 5

Effects of SA treatment on the expression levels of *OsNPR1*, *OsWRKY45*, *OsWRKY7*, and *OsWRKY70* in rice leaves at flowering stage

Figure 6

Effects of SA treatment on the H₂O₂ contents and antioxidant enzymes' activities (CAT, POD, and APX) in rice leaves at flowering stage

Figure 7

Effects of SA treatment on the expression levels of OsLCT1 and OsLCD in rice leaves at flowering stage