Journal of Hazardous Materials 398 (2020) 122860

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Selenium enhances iron plaque formation by elevating the radial oxygen loss of roots to reduce cadmium accumulation in rice (*Oryza sativa* L.)

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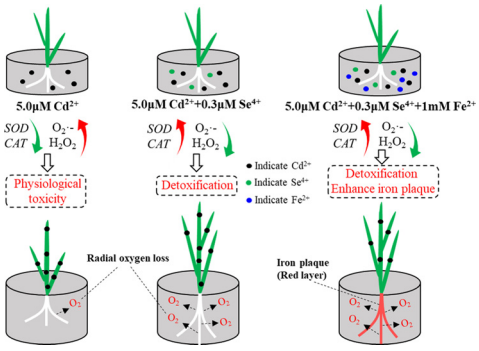
GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: R. Debora

Keywords:
Antioxidant enzyme
Reactive oxygen species
Oxygen profile
Ferrous ion
Barrier

ABSTRACT

The inhibition of cadmium (Cd) absorption by selenium (Se) in rice may be associated with iron plaque (IP) formation, but the driving mechanisms are still unclear. This study investigated the effects of Se on the growth, oxidative toxicity, radial oxygen loss (ROL), IP formation, and Cd absorption of rice exposed to Cd. The results of this study showed that Cd stress elevated the levels of $O_2^{\cdot-}$ and H_2O_2 and depressed superoxide dismutase (SOD) and catalase (CAT) activities. The maximum ROL and IP were reduced by 43.3 % and 74.5 %, respectively. However, Se alleviated Cd toxicity by stimulating SOD and CAT activities by scavenging $O_2^{\cdot-}$ and H_2O_2 and enhancing the ROL profiles. Under culture conditions without Fe^{2+}, Se had no impact on the total Cd levels in rice (T_{Cd}). However, with the addition of Fe^{2+}, T_{Cd} was significantly reduced by 23.3 % due to the enhancement of IP formation by Se. These results indicated that Se can reduce Cd accumulation in rice in the presence of Fe^{2+} treatments. However, Se just alleviated Cd toxicity in the absence of Fe^{2+} treatments. The enhancement of ROL was a potential reason for the elevated IP formation induced by Se.

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https://doi.org/10.1016/j.jhazmat.2020.122860
Received 2 February 2020; Received in revised form 7 April 2020; Accepted 2 May 2020
Available online 17 May 2020

1. Introduction

Cadmium (Cd), as a chronic carcinogen, can enter farmlands via anthropogenic activities, such as irrigation with smelting wastewater (Zhao et al., 2015). According to a 2014 report from the Ministry of Ecology and Environment (MEE) and the Ministry of Natural Resources (MNR) of the People's Republic of China, 7.0 % of soil samples (collected from more than 70 % of China's land area) exceeded the environmental quality standard for Cd (GB15618-1995) set by the MEE. Cd contamination has attracted public attention due to the frequent occurrence of "Cd rice" events in China (Du et al., 2013). Rice, as a predominant food in southern China, plays a critical role in the transport of Cd from soil to the human body (Arao et al., 2009). Excessive accumulation of Cd in human organs (e.g., kidney, liver, and skeleton) may cause many incurable diseases, such as Itai-Itai disease (Jarup and Akesson, 2009; Baba et al., 2013). Thus, reducing the absorption of Cd in rice is important to protect people who depend on rice as a staple food from excessive Cd accumulation.

Unlike other cereal crops such as wheat, rice plants have a specific aerenchyma that can transport atmospheric oxygen from the aerial parts to the roots (Bailey-Serres et al., 2012). As a result, the rhizospheric microscale of rice roots becomes an aerobic region (i.e., rhizospheric micro-oxidation zone) even though the paddy soil is generally flooded. Ferrous ions (Fe^{2+}), the dominant form of iron in waterlogged soil, can oxidize to ferric ions (Fe^{3+}) in the rhizosphere of rice roots and then be partially deposited on the root surface to form a red layer called iron plaque (IP) (Liu et al., 2006). IP is mainly composed of iron oxides and hydroxides, which can provide large surfaces to bind metals (Hansel et al., 2001). Many studies have demonstrated that IPs can sequester many heavy metals, such as Cd and copper (Cu), on root surfaces and thus act as a barrier for rice in the absorption of heavy metals (Liu et al., 2006, 2008; Cui et al., 2019). Accordingly, we presumed that enhancing the formation of IPs on the root surface is a potential method of reducing Cd accumulations in rice.

Selenium (Se) is a beneficial element in higher plants, in which its antioxidant properties are generally utilized to alleviate environmental stresses, such as heavy metal exposure (Han et al., 2015; Natasha et al., 2018). Furthermore, Se also serves to inhibit the absorption of Cd and mercury (Hg) by rice plants (Zhang et al., 2012). The antagonism and antioxidation of Se have been considered the main mechanisms accounting for Cd reduction and detoxification (Lin et al., 2012; Huang et al., 2017, 2018c). Additionally, regulation of the expression of Cd-related genes, such as *OsNramp5* (*Oryza sativa* Natural resistance-associated macrophage protein 5) and *OsHMA3* (*Oryza sativa* Heavy Metal ATPase 3), has also been reported as a potential mechanism (Cui et al., 2018). Moreover, IP formation on the root surface may also be an important cause. We have previously found that IPs form quickly during the pre- to late-tillering stage and peak at the booting stage of rice. Additionally, the application of Se at the pre-tillering stage strongly enhances the formation of IPs at the booting stage (Huang et al., 2018b), but the underlying mechanisms have not been determined. It is known that the formation of IPs is mainly controlled by two factors: the soil Fe^{2+} level and radial oxygen loss (ROL) in rice roots. ROL, which is associated with the oxidation of Fe^{2+} in the rhizosphere (Cheng et al., 2014), may be related to the Se levels in rice tissues (Gill and Tuteja, 2010; Lin et al., 2012). Cd exposure induces oxidative stress in rice tissues by generating reactive oxygen species (ROS), such as superoxide anions ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2). As a component of many antioxidant enzymes, Se can enhance the activities of superoxide dismutase (SOD) and catalase (CAT), which are involved in scavenging $O_2^{\cdot-}$ and H_2O_2 in rice tissues in response to the oxidative stress induced by Cd (Seppanen et al., 2003). Oxygen is one of the main products during the ROS catalytic degradation process and may directly affect radial oxygen loss (ROL) and the formation of IPs and Cd absorption in rice (Gill and Tuteja, 2010). Accordingly, we considered that the effects of Se on antioxidant enzymes, ROS, ROL, and IP may be associated with Cd accumulations in rice, but those complex correlations have not been elucidated. Exploration of the underlying mechanism of the positive effects of Se on IP is highly significant for reducing the risk of Cd accumulations in rice through Se fertilizer.

Therefore, several hydroponic experiments in this study were conducted to 1) investigate the role of Se on physiological and biochemical parameters (e.g., growth status, antioxidant enzymes, and ROS) of rice exposed to Cd stress; 2) explore the effects of Se on the ROL of rice roots by monitoring oxygen profiles in the rhizospheric micro-oxidation zone using a microprobe; and 3) clarify the mechanisms driving Se in IP formation and its correlation to Cd accumulations in rice. We aimed to improve the understanding of the effects of Se on Cd accumulations in rice and provide a theoretical basis for reducing Cd risks with applications of Se fertilizer.

2. Materials and methods

2.1. Rice cultivation and experimental design

2.1.1. Rice materials and cultivation

Wuyunjing21 is one of main planting cultivars in eastern China and was selected as the experimental cultivar. Wuyunjing21 is a conventional japonica rice obtained from the Wujin District Institute of Agricultural Sciences in Changzhou City and its growth period is 151 d. After surface sterilization with 30 % (v/v) H_2O_2 for 15 min and rinsing with tap and deionized water, rice seeds were presoaked in deionized water for 24 h. Thereafter, the seeds were germinated in moist gauze for 48 h in darkness at 28 °C at a relative humidity of 85 % controlled by an artificial climate chest (PRX-1000, Saifu Experimental Instrument, Ningbo, China). To cultivate rice seedlings, seeds with similar growth statuses were transferred to suspension plates in plexiglass containers containing 10 L of Kimura B nutrient solution. The Kimura B nutrient solution contained 0.36 mmol L^{-1} $(NH_4)_2SO_4$, 0.55 mmol L^{-1} $MgSO_4{\cdot}7H_2O$, 0.18 mmol L^{-1} KNO_3, 0.37 mmol L^{-1} $Ca(NO_3)_2{\cdot}4H_2O$, 0.18 mmol L^{-1} KH_2PO_4, 20 μmol L^{-1} $FeSO_4{\cdot}7H_2O$, 20 μmol L^{-1} EDTA-2Na, 0.50 μmol L^{-1} $MnCl_2{\cdot}4H_2O$, 3.0 μmol L^{-1} H_3BO_3, 1.0 μmol L^{-1} $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0.40 μmol L^{-1} $ZnSO_4{\cdot}7H_2O$, and 0.2 μmol L^{-1} $CuSO_4{\cdot}4H_2O$. The pH of the nutrient solution was adjusted to 5.6 using HCl and NaOH before the first use and at each replacement. To avoid seedling burning, 1/2 of Kimura B nutrient solution was used for the first 7 d and was then replaced with a full nutrient solution for another 21 d of cultivation. The nutrient solution was refreshed every 4 d.

2.1.2. Experimental design

According to some previous studies, the addition levels of Se ranged from 0 − 60 μmol L^{-1} and for Cd ranged from 0 − 100 μmol L^{-1} in hydroponics (Lin et al., 2012; Feng et al., 2013; Cui et al., 2018). Accordingly, we conducted a hydroponic experiment to examine the alleviating effects of Se at different levels (e.g., 0, 0.1, 0.3, 0.5, 1.0, 2.0, 3.0, 5.0, 10, and 30 μmol L^{-1}) on Cd (e.g., 0, 1.0, 5.0, and 30 μmol L^{-1}) toxicity to rice. The results showed that the optimal alleviation of rice toxicity occurred for 0.3 μmol L^{-1} Se and 5.0 μmol L^{-1} Cd (Fig. S1). Therefore, the addition levels of Se and Cd in this study were set to 0.3 μmol L^{-1} and 5.0 μmol L^{-1}, respectively. Healthy and similar seedlings were selected for four treatments with Cd ($3CdSO_4{\cdot}8H_2O$) and Se (Na_2SeO_3) as follows: 1) only the nutrient solution as the control treatment (called "CK"); 2) the nutrient solution + 0.3 μmol L^{-1} Se as the Se-only treatment (called "Se"); 3) the nutrient solution + 5.0 μmol L^{-1} Cd as the Cd-only treatment (called "Cd"); and 4) the nutrient solution + 0.3 μmol L^{-1} Se + 5.0 μmol L^{-1} Cd as the interactive treatment with both Se and Cd (called "Se + Cd"). Each treatment was replicated four times and each replicate had 16 seedlings. After treatment for 25 d, 12 seedlings in each replicate were sampled for physicochemical analysis (e.g., 3 seedlings for the determination of dry weights and Cd concentrations, 6 seedlings for the determination of SOD and CAT activities and H_2O_2 and $O_2^{\cdot-}$ levels, and 3 seedlings for determinations of

the oxygen profiles in the rhizospheric micro-oxidation zone). The remaining 4 seedlings in each replicate were used for the IP formation experiment.

2.1.3. IP formation experiment

We added 1.0 mmol L^{-1} Fe^{2+} ($FeSO_4 \cdot H_2O$) to each treatment and the solution was again refreshed every 4 d. To minimize disturbance by dissolved oxygen and ensure that Fe^{2+} was oxidized mainly by ROL, the solution in each treatment was flushed with nitrogen to remove dissolved oxygen and the solution surface was protected from the air with liquid paraffin. After 12 d of cultivation, the rice plants were sampled for IPs extractions and determinations of the Cd concentrations in tissues.

2.2. Chemical analysis of plant samples

2.2.1. Extraction of IPs

The rice plants were divided into roots and shoots and the IPs on the root surfaces were extracted using the dithionite–citrate–bicarbonate (DCB) method (Taylor and Gowder, 1983; Otte et al., 1989): fresh roots were soaked in a mixed solution of 0.03 mol L^{-1} sodium citrate and 0.125 mol L^{-1} sodium bicarbonate for 30 min followed by the addition of approximately 1.2 g of sodium dithionite to reduce Fe^{3+} for a duration of 70 min. After filtering through a 0.45-μm membrane, 2 mL of the extract was digested with 2 mL of HNO_3 (GR) and 2 mL of 6.0 mol L^{-1} HCl in a 100 °C water-bath for 2 h; the Fe and Cd concentrations in the digested solution were then measured using flame and graphite furnace atomic absorption spectrometry (SpectrAA 220FS and 220Z, Varian, USA). The roots without iron plaque were prepared for the Cd determination.

2.2.2. Dry weights and Cd concentrations in rice tissues

The roots were soaked in a 0.5 mmol L^{-1} $CaCl_2$ solution for 20 min to remove the Cd adsorbed on the root surfaces. Afterward, the roots and shoots were rinsed with tap and deionized water at least three times and were then oven-dried to constant weights at 75 °C. The dry weight of each sample was recorded. To measure the Cd concentration, dry samples were milled into powders using a blender (A11 basic, IKA, Germany) and were then digested with 5 mL of HNO_3 (GR) and 3 mL of H_2O_2 (GR) using a DigiBlock ED54-iTouch Digester system (Labtech, Beijing). The detailed processes have been described previously (Huang et al., 2018a). The Cd concentrations were measured using flame and graphite furnace atomic absorption spectrometry (SpectrAA 220FS and 220Z, Varian, USA). A standard plant material (GBW10049, National Research Center for Certified Reference Materials, China) was added to control the quality and the recovery rate ranged from 95 % to 106 %.

2.2.3. SOD and CAT activities and H_2O_2 levels

Frozen root and leaf samples were homogenized with a mortar in 0.1 mol L^{-1} sodium phosphate buffer (pH 6.8) (Chao et al., 2010). The homogenate was centrifuged at 4000 rpm min^{-1} for 10 min and the supernatant fraction was used to determine protein content using the bicinchoninic acid method (A045-3 assay kit, Jiancheng Bioengineering Institutes, China). The SOD activity was measured using the hydroxylamine method (A001-1 assay kit, Jiancheng Bioengineering Institutes, China). The CAT activity was measured using the visible light method (A007-1 assay kit, Jiancheng Bioengineering Institutes, China). The H_2O_2 level was measured using the colorimetric method (A064 assay kit, Jiancheng Bioengineering Institutes, China), and the manufacturer's instructions for each assay kit were referenced. The final measurements were performed using a microplate reader (BioTek, USA).

2.2.4. $O_2^{\cdot-}$ levels

Frozen root and leaf samples were homogenized with a mortar in 65 mmol L^{-1} potassium phosphate buffer (pH 7.8) and were then centrifuged at 5,000 rpm min^{-1} for 10 min. Then, 1.0 mL of supernatant was incubated with 0.9 mL of 65 mmol L^{-1} potassium phosphate buffer (pH 7.8) and 0.1 mL of 10 mmol L^{-1} hydroxylamine hydrochloride at 25 °C for 20 min and the same volume of ethyl ether was then added; centrifugation at 1,500 rpm min^{-1} for 5.0 min was then performed. The absorbance of the supernatant was determined at 530 nm using a microplate reader (BioTek, USA) and the $O_2^{\cdot-}$ – levels were calculated using a standard curve of different nitrite concentrations (Hu et al., 2008).

2.2.5. Oxygen profiles in the region of the rhizosphere

A Clark-type oxygen microelectrode system (Unisense, Denmark) was used to monitor the rhizospheric oxygen profiles (Colmer et al., 2019). The tested rice seedlings were placed in a container with complete Kimura B nutrient solution for 2 h. A newly formed root with a length of approximately 45 mm was inserted into a Petri dish with a nutrient solution containing 0.9 % agar (with dissolved oxygen removed). Liquid paraffin was poured onto the surface of the solidified agar to exclude air. Next, an oxygen microelectrode controlled by an automatic motor with a diameter of 25 μm was used to longitudinally penetrate the root (25 mm from the root tip). The oxygen concentrations were measured three times at each interval from the air to the root (50-μm depth intervals); the waiting and measuring times were both 3 s. To translate the electrical signal of the microelectrode into an oxygen concentration value, the microelectrode should be calibrated using a zero calibration solution (0.1 mol L^{-1} sodium ascorbate mixed with 0.1 mol L^{-1} NaOH of the same volume) and an air-saturation calibration solution (nutrient solution inflated for 15 min using an air pump) before measurement (Li and Wang, 2013). A graph showing the oxygen measurements is provided in Fig. S2.

2.3. Data analysis

The Fe and Cd concentrations in the IP ($C_{Fe/Cd}$) on the root surface were calculated using the following equation (Huang et al., 2018a):

$$C_{Fe/Cd} = T_{Fe/Cd} / DW_{Root}$$

where $T_{Fe/Cd}$ denotes the total amounts of Fe or Cd in the IPs and DW_{Root} denotes the dry weight of the root.

The total amounts of Cd (T_{Cd}) in the rice plants were calculated using the following equation:

$$T_{Cd} = C_{Root} \times DW_{Root} + C_{Shoot} \times DW_{Shoot}$$

where C_{Root} and C_{Shoot} denote the concentrations of Cd in the roots and shoots, respectively, and DW_{Root} and DW_{Shoot} denote the dry weights of the roots and shoots, respectively.

2.4. Statistical analysis

Data are expressed as the mean ± standard deviation (SD). The differences for each parameter between different treatments in the same tissue were determined using one-way ANOVA (analysis of variance, P value of 0.05) after testing the homogeneity of variances (Levene's test) followed by the post hoc test of LSD (least significant difference). SPSS 19.0 software was utilized to perform the statistical analysis and SigmaPlot 10.0 software was used to produce the graphs.

3. Results

3.1. Effects of Se and Cd on rice growth

As shown in Fig. 1, before the addition of Fe^{2+}, 0.3 μmol L^{-1} Se-only had no obvious impact on the growth of rice plants when compared with the CK treatment. While 5.0 μmol L^{-1} Cd-only clearly inhibited rice plant growth and resulted in reductions of 54.3 % and 41.1 % in the dry weights of the roots and shoots, respectively. Se clearly alleviated the growth inhibition induced by Cd stress, although the dry weights of the

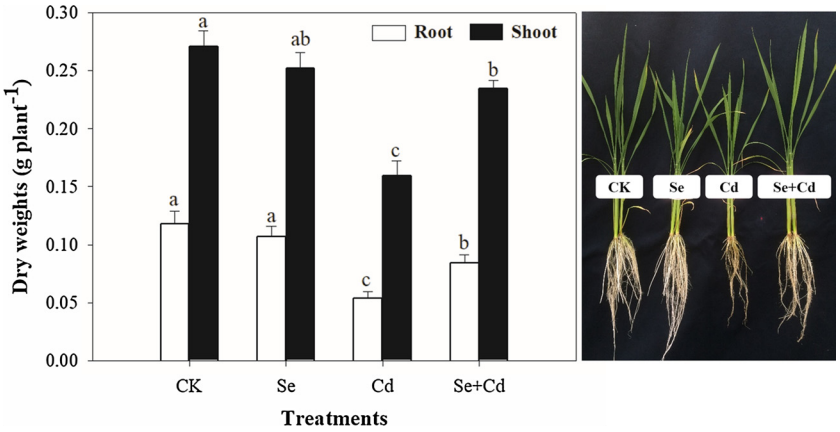

Fig. 1. Dry weights and growth graph of rice roots and shoots before treating with Fe^{2+}. CK, Se, Cd, and Se + Cd indicate the control, Se addition only, Cd addition only, and addition of both Se and Cd treatments, respectively. Significance analysis was conducted for the same tissues and the bars with the same lowercase letter(s) in the same tissue indicate no significant differences between different treatments at $P < 0.05$.

roots and shoots in the Se + Cd treatment were still less than those in the CK treatment. The dry weights of the roots and shoots were 56.5 % and 46.9 % higher than those in the Cd-only treatment, respectively (Fig. 1). Compared with rice seedlings before treating with Fe^{2+}, the dry weights of the roots and shoots increased with the growth of rice seedlings treated with Fe^{2+}, but the differences between different treatments were similar (Fig. S3).

3.2. Effects of Se and Cd on antioxidant enzyme activities and ROS levels in rice tissues

In the roots, no significant differences were observed in the SOD and CAT activities for the Se-only treatment compared with the CK treatment. However, the Cd-only treatment significantly decreased SOD and CAT activities with reductions of 46.7 % and 54.2 %, respectively. When Se was added to the culture solution containing Cd, the inhibition of SOD and CAT activities induced by Cd was alleviated, with increases of 33.2 % and 135 %, respectively, compared with the Cd-only treatment (Fig. 2). Similar results were observed in the leaves compared with the CK treatment for which the SOD and CAT activities were decreased by 17.5 % and 24.4 %, respectively, in the Cd-only treatment. These inhibitions were also mitigated by Se addition (Fig. 2).

Compared with the CK treatment, the Se-only treatment had no significant impact on the $O_2^{\cdot-}$and H_2O_2 levels in roots and leaves. However, the effects of Cd on $O_2^{\cdot-}$and H_2O_2 were completely opposite to the effects on the activities of antioxidant enzymes (Fig. 3). Compared with the CK treatment, the Cd-only treatment greatly stimulated the production of $O_2^{\cdot-}$and H_2O_2 in rice tissues, the $O_2^{\cdot-}$ levels were increased by 62.4 % and 61.1 %, and the H_2O_2 levels increased by 39.2 % and 29.5 % in the roots and leaves, respectively. Compared with the Cd-only treatment, the Se + Cd treatment significantly decreased the $O_2^{\cdot-}$ levels by 31.7 % and 35.1 % and those of H_2O_2 by 19.5 % and 12.8 % in the roots and leaves, respectively.

3.3. Effects of Se and Cd on radial oxygen loss from rice roots

As shown in Fig. 4, the O_2 concentration in the environment gradually decreased with the downward movement of the microelectrode from the air to agar. The top boundary of the rhizospheric micro-oxidation zone was at a depth of approximately 7,100 μm. Within the depth range from 7,100 μm to 9,500 μm, the O_2 concentrations quickly increased and reached a peak value. From 9,500 μm to 10,300 μm, the microelectrode penetrated the root and the O_2 concentrations were relatively stable. From 10,300 μm to 11,150 μm, the tip of the microelectrode was outside of the root and the O_2 concentrations rapidly decreased to 0 μmol L^{-1}.

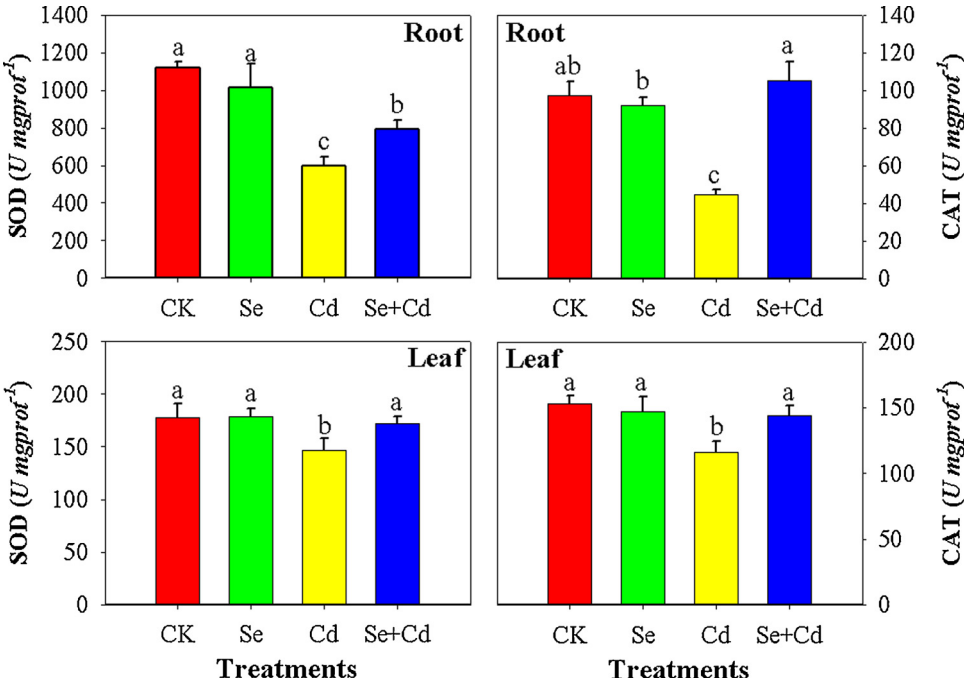

Fig. 2. Activities of antioxidant enzymes (e.g., SOD and CAT) in rice roots and leaves before treating with Fe^{2+}. CK, Se, Cd, and Se + Cd indicate the control, Se addition only, Cd addition only, and addition of both Se and Cd treatments, respectively. Bars with the same lowercase letter(s) indicate no significant difference between the different treatments at $P < 0.05$.

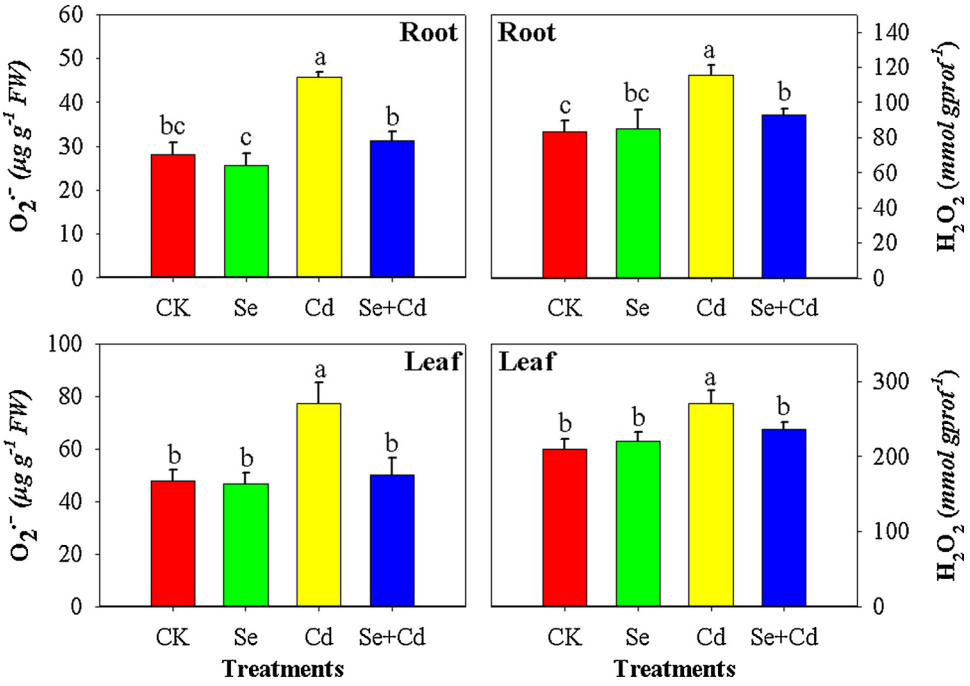

Fig. 3. Concentrations of reactive oxygen species (e.g., $O_2^{\cdot-}$and H_2O_2) in rice roots and leaves before treating with Fe^{2+}. CK, Se, Cd, and Se + Cd indicate the control, Se addition only, Cd addition only, and addition of both Se and Cd treatments, respectively. Bars with the same lowercase letter(s) indicate no significant differences between different treatments at $P < 0.05$.

Significant differences in the oxygen profiles were observed following the different treatments (Fig. 4). In the CK treatment, the maximum O_2 concentration reached 149 μmol L^{-1} and the longitudinal depth of the rhizospheric micro-oxidation zone was approximately 4,050 μm (e.g., from 7,100 μm to 11,150 μm). A similar oxygen profile was observed for the Se-only treatment. However, the profile of O_2 concentrations was significantly lower in the Cd-only treatment than in the CK treatment; the maximum O_2 concentration was only 104 μmol L^{-1} and the longitudinal depth of the rhizospheric micro-oxidation zone was only 3,100 μm (e.g., from 7,800 μm to 10,900 μm). Compared with the Cd-only treatment, the O_2 concentration profile significantly increased with the Se + Cd treatment; the maximum O_2 concentration increased by 29.8 % and the longitudinal depth of the rhizospheric micro-oxidation zone widened by 22.6 %.

3.4. Effects of Se and Cd on the formation of IP on the root surface

As shown in the right graph in Fig. 5, red layers (i.e., IPs) were apparent on the root surface following the treatments with CK and Se-only. However, IPs were rarely found on the root surface following the treatments with Cd-only, treatment with Se + Cd resulted in clear visualization of IP. This tendency is reflected more clearly in the DCB-extractable Fe concentrations, as shown in Fig. 5A. The concentrations of DCB-extractable Fe in the CK and Se-only treatments were significantly higher than those in the other treatments and no significant differences were identified between the two treatments. Compared with the CK treatment, DCB-extractable Fe was reduced by 74.5 % following the Cd-only treatment. However, compared with the Cd-only treatment, DCB-extractable Fe greatly increased by 198 % upon treatment with Se + Cd (Fig. 5A).

The minimum total amounts of Cd in IPs were found in the CK and Se-only treatments due to the absence of exogenous Cd additions in

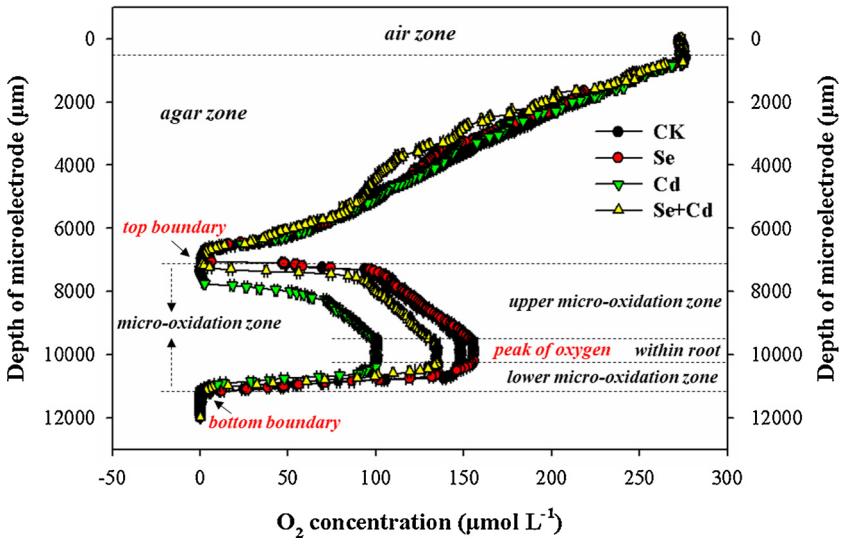

Fig. 4. Oxygen profiles of the roots of rice seedlings before treating with Fe^{2+}. CK, Se, Cd, and Se + Cd indicate the control, Se addition only, Cd addition only, and addition of both Se and Cd treatments, respectively. Data are presented as the mean ± SD (n = 3).

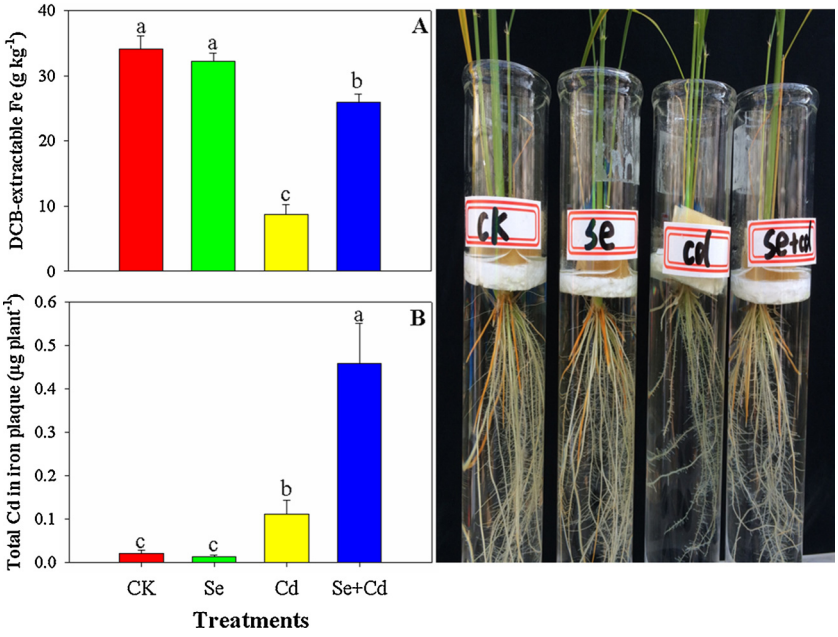

Fig. 5. Concentrations of DCB-extractable Fe (A), total amounts of Cd in iron plaque (B), and the graph of roots treated with Fe^{2+}. CK, Se, Cd, and Se + Cd indicate the control, Se addition only, Cd addition only, and addition of both Se and Cd treatments, respectively. Bars with the same lowercase letter(s) indicate no significant differences between the different treatments at $P < 0.05$.

these two treatment designs (Fig. 5B). The total amount of Cd in IPs in the Cd-only treatment was 0.11 μg $plant^{-1}$, which dramatically increased by 318 % in the treatment with Se + Cd.

3.5. Effects of Se on the accumulation of Cd in rice

Although no exogenous Cd was applied in the CK and Se-only treatments, some Cd also accumulated in rice roots and shoots as some nutrient agents contained small amounts of Cd (Fig. 6). Before the addition of Fe^{2+} to the nutrient solution, the Cd concentrations in the roots and shoots significantly decreased by 34.7 % and 33.5 %, respectively, following treatment with Se + Cd (Fig. 6A and B). However, the total Cd levels in the rice plants did not change significantly due to the higher biomass (Fig. 6C) compared with the Cd-only treatment. The Cd reductions in the roots and shoots were enhanced in seedlings treated with Fe^{2+}. The Cd concentrations in the roots and shoots were reduced by 46.3 % and 44.7 % in the Se + Cd treatment (Fig. 6D and E), respectively, compared with those in the Cd-only treatment. Furthermore, the total amount of Cd in the rice plants also significantly decreased by 23.3 % (Fig. 6F).

4. Discussion

Cd is not essential to the growth of higher plants and its overaccumulation induces physiological toxicity in plant tissues (Hsu and Kao, 2007; Ding et al., 2014). The present study found that cultivation with 5.0 μmol L^{-1} Cd greatly inhibited the growth of rice roots and shoots (Fig. 1), which is consistent with previous reports (Shao et al., 2007). This inhibition is partly attributed to the oxidative stress in rice tissues induced by Cd (Chien et al., 2001). Similarly, oxidative toxicity was clearly observed in the current results, as Cd cultivation caused overproduction of ROS (e.g., $O_2^{\cdot-}$and H_2O_2) in tissues (Fig. 3). Rice exhibits self-defense against oxidative toxicity due to its antioxidant system, such as antioxidant enzymes (Singh et al., 2016). However, the activities of SOD and CAT were depressed by Cd (Fig. 2), indicating that overaccumulation of Cd weakened the antioxidant capacity of rice; thus, the growth toxicity was further exacerbated. Nevertheless, the addition of Se can alleviate the oxidative stress induced by Cd. As a beneficial element in higher plants, Se is involved in free-radical scavenging (Zhu et al., 2009). The protective role of Se against oxidative stress can be observed for many aspects, mainly enzymatic and nonenzymatic processes. In terms of enzymatic processes, Se can act as an antioxidant by activating antioxidative enzymes, such as SOD and CAT, as shown in Fig. 2; Se may also alter the transcription of chloroplast Cu Zn SOD and glutathione peroxidase involved in quenching ROS (Seppanen et al., 2003). In addition, nonenzymatic processes include the acceleration of ROS dismutation, protection of photosynthesis and the integrity of the cell structure, and synthesis of phytochelatins (PCs) (Feng et al., 2013; Kumar et al., 2016; Chauhan et al., 2017). However, these positive roles only occur within a certain range of Se levels as excessive Se accumulation also induce growth inhibition (Figure S1).

Excluding oxidative stresses, Cd exposure also influences radial oxygen loss (ROL) from rice roots (Fig. 4). It is known that the aerenchyma channel surrounding the stele of rice roots is formed through the death and subsequent lysis of cells in the cortex (Yamauchi et al., 2013). For promoting the longitudinal diffusion of oxygen towards the root apex, a barrier is formed at the basal part of the root to minimize O_2 release from this section (Shiono et al., 2011; Nishiuchi et al., 2012). Therefore, the physical morphology of the aerenchyma and barrier is vital to the release of O_2. As shown in Fig. 1, Cd exposure shrank the root diameter, which may result in the reduction of the cross-sectional area of the aerenchyma. Subsequently, a negative influence may occur in the downward transportation of O_2 from upper part to the root apex (Pedersen et al., 2020), and the ROL would be influenced. In addition, Cd exposure might also damage the ROL barrier, which could cause a larger release of O_2 from the basal parts of rice roots but the ROL at the root apex might be reduced (Nishiuchi et al., 2012). Therefore, the ROL changes induced by Cd may be associated with physical changes of the aerenchyma and barrier, which needs further study.

ROL acts as a promoter in the formation process of IP (Wang et al., 2013); therefore, its reduction results in decreases in IP (Fig. 5). The present work innovatively found that Se enhances ROL under Cd stress, which may be the underlying mechanism of the observed positive effect of Se on the formation of IP herein (Fig. 5) and in previous works (Huang et al., 2018b). As is known, ROL is the most important factor for the formation of IP when the environmental level of Fe^{2+} is constant (Wang et al., 2013). Accordingly, we considered that the formation of IP was mainly enhanced by the elevation of ROL induced by Se application, due to the same level of Fe^{2+} in the present study. Moreover, two possible explanations may account for the effects of Se and Cd on ROL. On the one hand, the detoxification effects of Se protect the rice

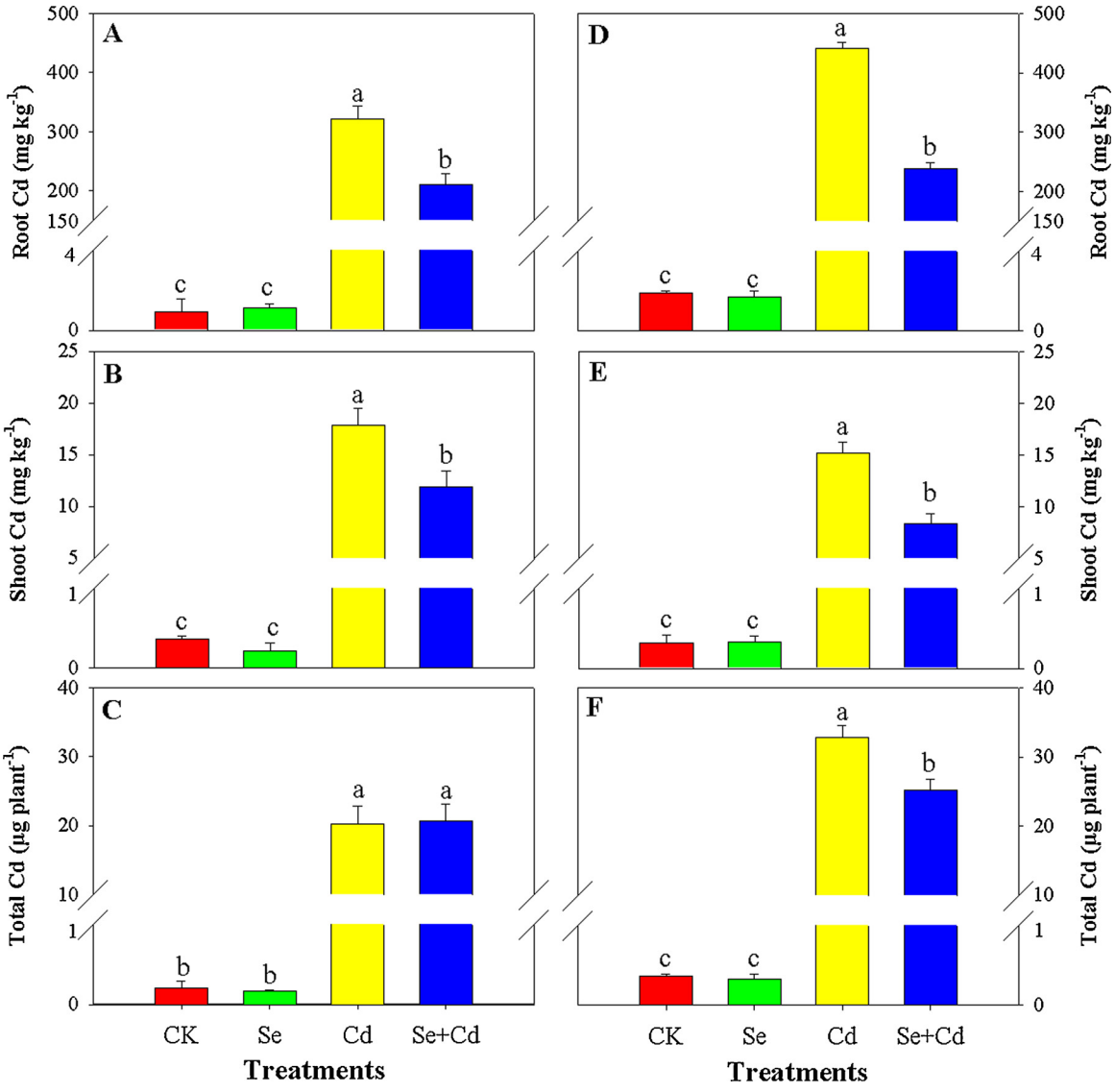

Fig. 6. Cd Concentrations in roots and shoots and the total amounts of Cd in rice seedlings before (A, B, and C) and after (D, E, and F) treating with Fe^{2+}. CK, Se, Cd, and Se + Cd indicate the control, Se addition only, Cd addition only, and addition of both Se and Cd treatments, respectively. Bars with the same lowercase letter(s) indicate no significant differences between the different treatments at $P < 0.05$.

plant from physiological injuries induced by Cd stress, thereby ensuring the microstructural integrity of the aerenchyma and barrier, which is beneficial to ROL at the newborn root sites (Shiono et al., 2011; Colmer et al., 2019). Alternatively, oxygen is the main product of the catalytic decomposition of $O_2^{\cdot-}$ and H_2O_2 (Gill and Tuteja, 2010); as a result, the interaction of Se and Cd may increase the oxygen content within roots since Se enhances the scavenging of ROS induced by Cd (Fig. 3).

The inhibitory effects of Se on Cd accumulations in rice have been widely demonstrated in previous studies (Hu et al., 2014; Wan et al., 2016; Cui et al., 2018). The current work also found that Se decreased Cd concentrations in rice tissues (Fig. 6A and B). Some potential mechanisms for the interaction of Se and Cd have been reported in numerous studies. Inhibition of the bioavailability of Cd in soil by sodium selenite has been considered to be a driving mechanism for the Cd reduction in rice (Hu et al., 2014). Se also reduces Cd uptake in rice by inducing morphological changes in rice roots, such as increasing the proportion of coarse roots (Ding et al., 2014). Recently, Cui et al.(2018) reported that Se downregulates the expression of some genes involved in the transport of Cd, such as *OsNramp5* (*Oryza sativa* Natural resistance-associated macrophage protein 5). The above mechanisms indicate that Cd absorption can be reduced by the application of Se. However, the present work demonstrates that the total amounts of Cd in rice plants did not change in response to interactive treatments with Se and Cd before the induction of IP formation (Fig. 6C). We presume that the reduced Cd concentrations in rice tissues may be associated with increases in rice biomass as it has been reported that biomass increases can dilute Cd concentrations in wheat (Perrier et al., 2016). When Fe^{2+} was added to the solution without dissolved oxygen, we found that IPs could be formed only via the ROL oxidation (Fig. 5) but that it was accordingly inhibited by the decrease in ROL induced by Cd stress. The application of Se depressed the adverse effect of Cd on the formation of IPs and also significantly reduced the total absorption of Cd in rice plants. This finding indicates that Se can effectively reduce the amount of Cd in rice plants through enhancing the formation of iron plaques in the presence of Fe^{2+} treatments; otherwise, Se mainly alleviated Cd toxicity in the absence of Fe^{2+} treatment, which has not been previously reported in other literature. As barriers to heavy metals, IPs function not only by physical resistance but also in metal sequestration by their mineral composition, e.g., goethite (α-FeOOH) (Amaral et al., 2017). The present analysis revealed that the sequestration ability of Cd was greatly enhanced by Se applications, whereas the total amounts of Cd in IPs accounted for only approximately 1 % of the total Cd (T_{Cd}) in

rice plants (Table S1). Accordingly, the 23.3 % reduction in T_{Cd} is mainly attributed to the physical resistance of IP. The absorption of Cd by the plant roots occurs primarily through epidermal cells and root hairs and thus involves the apoplast and symplast pathways (Song et al., 2017). The IPs on the root surface tightly cover the epidermal cells (Pereira et al., 2014), thereby physically restricting the symplast pathway as well as the absorption of Cd. Therefore, inhibition of Cd uptake is primarily associated with the enhanced physical resistance of IPs induced by Se.

5. Conclusions

Se alleviated the oxidative stress induced by Cd and reduced Cd concentrations in rice tissues but had no significant impact on the total amount of Cd in rice under culture conditions without Fe^{2+}. When Fe^{2+} was added to the nutrient solution, Se significantly reduced the total amount of Cd in rice due to the enhanced formation of IPs. Therefore, IPs were essential for the reduction of rice Cd induced by Se and Se enhanced the formation of IPs, primarily by increasing ROL in rice roots. The barrier capacity of IPs to Cd absorption by rice primarily depended on their physical resistance and partly on their sequestration of Cd.

CRediT authorship contribution statement

Gaoxiang Huang: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Changfeng Ding:** Data curation, Formal analysis, Project administration, Resources, Writing - review & editing. **Yushan Li:** Investigation, Validation, Visualization. **Taolin Zhang:** Formal analysis, Writing - review & editing. **Xingxiang Wang:** Conceptualization, Funding acquisition, Project administration, Resources, Writing - review & editing.

Declaration of Competing Interest

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

Acknowledgements

This work was jointly sponsored by the National Key Technology Research and Development Program of China (2015BAD05B04), Regional Soil Pollution Control Program of Ministry of Agriculture and Rural Affairs of China, and Agro-Environmental Protection Program of Jiangxi province.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2020.122860.

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