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Root iron plaque alleviates cadmium toxicity to rice (*Oryza sativa*) seedlings

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ABSTRACT

Iron plaque (IP) on root surface can enhance the tolerance of plants to environmental stresses. However, it remains unclear the impact of Fe^{2+} on cadmium (Cd) toxicity to rice (*Oryza sativa*) seedlings. In this study, the effects of different Fe^{2+} and Cd^{2+} concentration combinations on rice growth were examined hydroponically. Results indicated that Fe^{2+} concentration up to 3.2 mM did not damage rice roots while induced IP formation obviously. Cd^{2+} of 10 μM repressed rice growth significantly, while the addition of 0.2 mM Fe^{2+} to 10 μM Cd^{2+} solution (Cd + Fe) did not damage rice roots, indicating that Fe^{2+} could ameliorate Cd toxicity to rice seedlings. Microstructure analysis showed Cd + Fe treatment induced the formation of IP with dense and intricate network structure, Cd adsorption on the root surface was reduced significantly. Cd concentration of rice roots and shoots and Cd translocation from roots to shoots with Fe + Cd treatment were reduced by 34.1%, 36.0% and 20.1%, respectively, in comparison to a single Cd treatment. Noteworthy, the removal of IP resulted in a larger loss of root biomass under Cd treatment. In addition, Cd + Fe treatment increased the activities of root superoxide dismutase and catalase by 105.5% and 177.4%, and decreased H_2O_2 and $O_2^{\cdot-}$ accumulation of rice roots by 56.9% and 35.9%, and recovered Cd-triggered electrolyte leakage obviously, when compared with a single Cd treatment. The results from this experiment indicated that the formed dense IP on rice roots decreased Cd absorption and reactive oxygen species accumulation, and Fe^{2+} supply alleviated Cd toxicity to rice seedlings.

1. Introduction

Cadmium (Cd) is a transition metal element with biotoxicity. With rapid development of industry and agriculture, the discharges of "three wastes", i.e. waste gas, waste water, and waste residues, containing large amounts of Cd, lead to the pollution of farmland. According to global statistical data, about 30,000 t of Cd-containing compounds are released into the farmland (Gallego et al., 2012). In Asia, rice grains are the staple food for about 2 billion people. A daily Cd intake of 30 μg on average is reported in these regions, which poses risks of leading to the serious diseases (Sebastian and Prasad, 2014). Besides excess Cd can trigger oxidative stresses in plants, which inhibits plant growth and normal metabolism (Dai et al., 2017). Cd could induce large amounts of reactive oxygen species (ROS) in plant cells, such as H_2O_2, $O_2^{\cdot-}$, $\cdot OH$ *etc.* (Zhao et al., 2012), which resulted in a programmed cell death of rice roots (Wrzaczek et al., 2013). Therefore, it becomes an urgent problem dealing with the toxicity of Cd to rice.

Iron plaque is a layer of crystalline or amorphous iron (hydr) oxides on root surface (Fu et al., 2016), formed through the reaction of oxygen and soluble reductive Fe^{2+} (Fu et al., 2014). Previous researches have shown that wetland plants with higher radical oxygen loss (ROL) and/or more soluble reductive Fe^{2+} in the medium could form thicker IP readily (Cheng et al., 2014), and that IP formation could contribute to less Cd accumulation in plant tissues (Wang et al., 2011). Sebastian and Prasad (2016) showed that the increase of Fe content in plant protected plants from Cd-induced Fe deficiency and other metal toxicity. However, it remains unclear how Fe^{2+} and subsequent iron plaque reduce Cd toxicity to rice seedlings.

Xiao et al. (2015) found that Cd concentrations in shoots of different cultivars in flooding treatment were significantly lower than those in non-flooding treatment in pot experiment. Speculatively, lower Cd accumulation of rice shoot is associated with more Fe^{2+} under flooding conditions. Indeed, rice is a kind of wetland plant growing in flooding environment for long term, and there is high concentration of soluble reductive Fe^{2+} in rhizosphere of rice. However, the direct evidence that Fe^{2+} alleviates Cd toxicity of rice seedlings is still lacking. In this study, we investigated whether Fe^{2+} alleviates Cd toxicity, and if so, how it modulates this response.

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2. Materials and methods

2.1. Plant cultures

In the pre-test process, rice (*Oryza sativa* L.) cultivar Huahangsimiao was performed very remarkably in iron plaque formation, we used this cultivar as experimental material. Seeds were sterilized in 10% (w/w) H_2O_2 solution for 10 min, washed with deionized water and germinated at 30 °C. After germination for 3 d, uniform rice seedlings were transferred to a 6-L plastic container and grown in half-strength nutrient solution for 7 d. Rice seedlings with 3 leaves were transplanted to full strength nutrient solution for another 14 d. These 21 d-old seedlings were materials for the following experiments. The full strength nutrient solution consisted of the following macronutrients in mM: NH_4NO_3 0.429, $Ca(NO_3)_2{\cdot}4H_2O$ 1, $MgSO_4{\cdot}7H_2O$ 1.667, KH_2PO_4 1, K_2SO_4 0.513, and micronutrients in μM: Fe(III)-EDTA 50, $MnSO_4{\cdot}H_2O$ 9.1, $ZnSO_4{\cdot}7H_2O$ 0.15, $CuSO_4{\cdot}5H_2O$ 0.16, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 0.52, H_3BO_3 19, according to Yoshida et al. (1976) with minor modifications. The pH was adjusted to 5.5 with NaOH or HCl, and the nutrient solution was renewed every 3 d. In P-deficient nutrient solution, KCl was added to replace KH_2PO_4 to supplement K, and other components were consistent with full strength nutrient solution.

2.2. Experimental design

Four experiments were designed in this study, including two screening assays of Cd and Fe concentrations, one verification test of IP removed or not, one test of optimal Fe and Cd concentrations as following.

To explore the effect of Fe^{2+} on alleviating Cd toxicity to rice seedlings, screening assays with different Cd and Fe^{2+} concentrations were performed. For the screening assay of Cd concentrations, Cd at the concentrations of 0, 5, 10, or 20 μM was added into the full nutrient solution and rice seedlings were grown in it for 7 d. Since low P in nutrient solution facilitates for iron plaque formation, Fe^{2+} at the concentrations of 0, 0.2, 0.8, 1.6, 2.4 or 3.2 mM was added into P-deficient nutrient solution for similar assay of Fe^{2+} concentration and rice seedlings were grown in it for 2 d.

To verify the role of IP in ameliorating Cd toxicity, 0.2 mM Fe^{2+} was added into P-deficient nutrient solution for 2-d cultivation to obtain rice seedlings with IP presence (IPP). A portion of rice roots with IP were extracted by low concentration of DCB (Dithionite-Citrate-Bicarbonate) extraction solution (consisted of 80 mL of 0.03 M $Na_3C_6H_5O_7{\cdot}2H_2O$, 10 mL of 0.1 M $NaHCO_3$ and 0.5 g of $Na_2S_2O_4$) to remove IP for a short time (20 min) to avoid damaging rice roots when IP was removed completely (IP removed treatment, IPR). Meanwhile, another seedling continued growing in full strength nutrient solution without Fe^{2+} for 2 d (CK treatment). These three groups of rice seedlings with CK, IPP and IPR treatments were cultivated in full nutrient solution (-Cd) or full nutrient solution containing 20 μM Cd (+Cd) respectively. After 7-d treatments, IP on all rice roots were extracted by DCB extraction solution (components are shown below), and then biomasses of rice roots and shoots were weighed.

To investigate the mechanisms of Fe^{2+} enhancing tolerance of rice plants to Cd toxicity, the optimal Fe^{2+} (0.2 mM) and Cd^{2+} (10 μM) concentrations were used and rice seedlings were performed as the following treatments for 7 d: CK, P-deficient nutrient solution; Cd, supplemented with 10 μM Cd; Cd + Fe, supplemented with 10 μM Cd + 0.2 mM Fe^{2+}.

In above experiments, Fe and Cd were supplemented as $FeSO_4{\cdot}7H_2O$ and $CdCl_2{\cdot}0.5H_2O$, respectively. P-deficient nutrient solution was applied before the process of IP formation to eliminate interference residual P. All treatments were prepared in 4 replicates and the solution pH was adjusted to 5.5. Each rice seedling was grown in a 0.5-L PVC pots (8 cm in diameter; 16 cm in height).

2.3. Determination of Fe, Cd in the IP and rice tissues

IP of rice seedlings was extracted according to the method of Taylor and Crowder (1998). Briefly, rice roots were washed thoroughly and then placed in a 150-mL flask containing DCB extraction solution (40 mL of 0.03 M $Na_3C_6H_5O_7{\cdot}2H_2O$, 5 mL of 0.1 M $NaHCO_3$ and 1 g of $Na_2S_2O_4$). Then samples were shaken at 25 °C at 150 r min^{-1} for 3 h. The solution was made up to 1000 mL before determination.

Roots and shoots after DCB extraction were separated and digested with dry ashing methods for measuring Fe and Cd in the rice tissues. Fe and Cd concentrations in DCB extract solution and ashed solution were determined by atomic absorption spectrophotometer (Hitachi Z-5300, Japan).

2.4. Enzyme extraction and measurement

Briefly, root tip samples were excised, and approximate 0.2 g fresh roots were homogenized with 50 mM pH 7.8 phosphate buffer solution in chilled pestles and mortars. The mixtures were centrifugalized in 2 mL centrifuge tubes at 12,000 r min^{-1} for 15 min at 4 °C (Amako et al., 1994). The supernatant was collected for enzyme activity assays. Superoxide dismutase (SOD) activity was determined by measuring auto-oxidation inhibition of nitroblue tetrazolium (NBT) (Thounaojam et al., 2012). Peroxidase (POD) activity was determined by OD_{470} (absorbance at 470 nm wavelength, similar hereinafter) increment per minute (Cakmak and Marschner, 1992) and catalase (CAT) activity was assayed by OD_{240} decrease per minute (Kato and Shimizu, 1987).

2.5. H_2O_2, $O_2^{-}\cdot$ and electrolyte leakage assays

Root segments (2 cm from the tip) were excised and placed in a Petri dish containing 10 mL of 1 mM NBT (Sigma-Aldrich) for $O_2^{-}\cdot$ measurement or 1 g L^{-1} 3′3-diaminobenzidine (DAB, Sigma-Aldrich) solution for H_2O_2 measurement (Romero-Puertas et al., 2004). Images were captured by a microscope (Model BX43, Olympus, Japan) with cooled color CCD camera. $O_2^{-}\cdot$ quantitative assay was measured by OD_{530} with hydroxylamine chloride, sulphanilic acid and α-naphthylamine (Elstner and Heupel, 1976). H_2O_2 was measured with 0.1% (*v/v*) $TiCl_4$ dissolved in 20% (v/v) H_2SO_4 at OD_{410} (Tsai et al., 2004). Electrolyte leakage was described according to the difference between samples being expelled in autoclave at 121 °C and those at 28 °C (Dionisio-Sese and Tobita, 1998).

2.6. Evans blue staining

After treatments, the root segments (2 cm from the tip) were excised and stained with 20 g L^{-1} Evans blue for 3 min to identify dead epidermal cells (Mergemann and Saute, 2000). Subsequently, roots were rinsed thoroughly and observed by microscope with cooled color CCD camera.

2.7. Calculation of transfer factor

To estimate Cd translocation from roots to shoots, the transfer factor (TF) was calculated as follows (Hart et al., 1998): TF = Cd concentration in shoot / Cd concentration in root.

2.8. Statistical analysis

Data from experiments were subjected to one-way analysis of variance with SAS for Windows (Version 8.2, SAS Institute, Cary, NC, USA). Data were presented as means ± SE (n = 4) and multiple comparison was performed by method of least significant difference (LSD) at 95% confidential level.

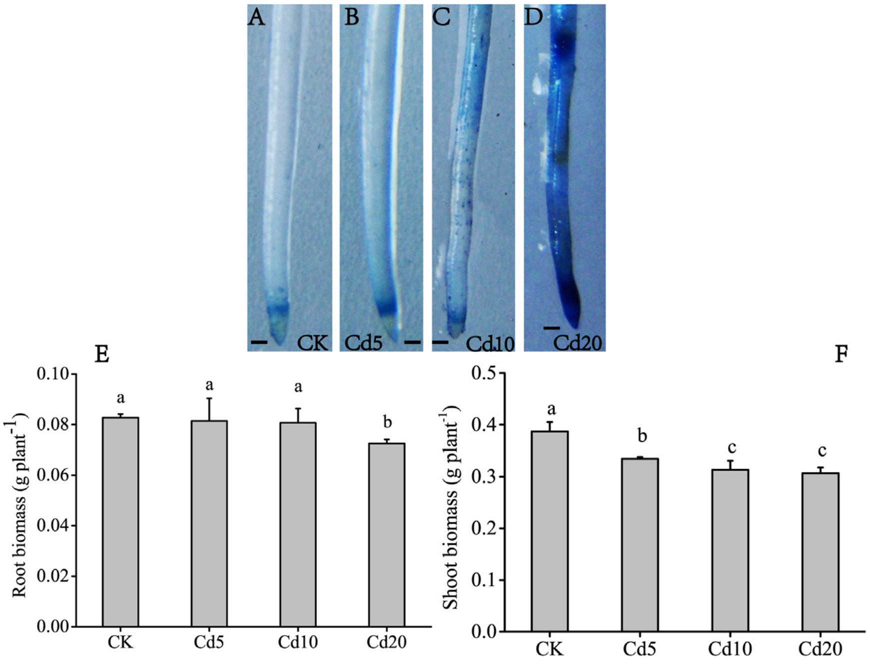

Fig. 1. Effect of different Cd treatments for 1 week on growth of rice seedlings. A-D: Evans blue staining of apical roots; E and F: roots and shoots biomass. Bars represent standard deviations of the means (n = 4). Different letters indicate significant differences among treatments at $p < 0.05$ level.

3. Results

3.1. Effect of different Cd concentrations on the growth of rice seedlings

The inhibitory effect of Cd treatment on the growth of rice seedlings increased with Cd concentrations (Fig. 1). Root biomasses were not different among the control and treatments with 5 or 10 μM of Cd treatment, but the root biomass with 20 μM of Cd was only 87% of that with the control (Figs. 1E and 1F). In the case of the shoots, biomasses with 5, 10 and 20 μM of Cd were 86.4%, 80.8% and 79.2% of those of the control, respectively. Evans blue staining can evaluate the plasma membrane integrity. Figs. 1A–1D showed that Evans blue staining became darker gradually, indicating more serious Cd toxicity occurred in rice seedlings in response to increasing Cd concentrations.

3.2. Effect of different Fe^{2+} concentrations on IP of rice roots

As shown in Fig. 2, the reddish-brown IP in basal roots and apical roots increased with increasing Fe^{2+} concentrations ranging from 0.2 mM to 3.2 mM (Figs. 2A-2B). Noteworthy, Evans blue staining was not observed widely distributing on the surface of rice root base treated with 3.2 mM of Fe^{2+} (Fig. 2C). The results suggest that IP might be a strategy to alleviate root damage due to Fe^{2+} toxicity.

3.3. Interaction effect of Fe and Cd on IP, Cd and Fe uptake

The above results suggest that Cd at 10 μM and Fe^{2+} at 0.2 mM were optimal concentrations for treatments (Figs. 1 and 2). Therefore these concentrations were applied in the following experiments. As shown in Figs. 3B-3C, reddish-brown IP was not observed on rice root surface with Cd treatment, but only with Fe^{2+} treatment. When 0.2 mM Fe^{2+} was added into Cd-containing solution, 40.7 g kg^{-1} of DCB-Fe was extracted from rice roots (Fig. 3D). Interestingly, IP was even observed on the surface of root hairs besides epidermis cell wall (Fig. 3C). Numerous root hairs with the IP intertwined each other, and dense reddish-brown IP constructed a layer of barrier (data were presented in Supplementary material at Fig. S1). Fe^{2+} addition into Cd-containing solution reduced DCB-Cd accumulation on root surface by 54.8% in comparison to only Cd treatment (Fig. 3E). Remarkably, the epidermal cells of rice roots were damaged and distorted due to exposure to Cd treatment (Fig. 3B), but the presence of Fe^{2+} in Cd-containing solution maintained the integrity of epidermal cells (Fig. 3C). The results showed that Fe^{2+} supply contributed to the amelioration of Cd toxicity in rice roots.

3.4. Growth response of rice seedlings with or without IP to Cd toxicity

To determine the effect of IP in ameliorating Cd toxicity, 20 μM Cd, which suppressed rice growth markedly, was chosen as the treatment concentration (Fig. 1). As shown in Fig. 4, compared with -Cd treatment, +Cd treatment suppressed the rice growth of CK and IPR obviously, while did not influence that of IPP (Fig. 4A). Cd treatment reduced biomasses of CK, IPP and IPR treatment by 16.7%, 9.8% and 28.9% in roots while by 15.8%, 8.9% and 28.9% in shoots, respectively (Figs. 4B-4C). In addition, both root and shoot biomasses of IPP were much higher than those of CK and IPR, which might be associated with more IP on IPP root surface (Fig. 4A). Remarkably, biomasses of rice seedlings of IPP under +Cd treatment were not significantly different from those of CK and IPR under -Cd treatment (Fig. 4).

3.5. Effect of different treatments on the activities of antioxidant enzymes

As shown in Fig. 5, Cd treatment did not significantly influence the activities of superoxide dismutase (SOD) and catalase (CAT), but increased the activity of peroxidase (POD) by 84.2% (Figs. 5A-5C). On the contrary, Fe^{2+} application into Cd-containing solution enhanced the activities of SOD, POD, and CAT in rice roots by 112.0%, 46.2%, and 163.5% respectively, in comparison to the control. Interestingly, the activity of POD was lower in the Cd + Fe treatment compared with Cd treatment (Fig. 5B).

3.6. Effect of different treatments on H_2O_2 and $O_2^{-}\cdot$ accumulation and electrolyte leakage

As shown in Fig. 6A, Cd treatment increased the accumulation of H_2O_2 in roots by 115.7% compared with the control. Fe^{2+} application into Cd-containing solution decreased H_2O_2 accumulation by 56.9% in

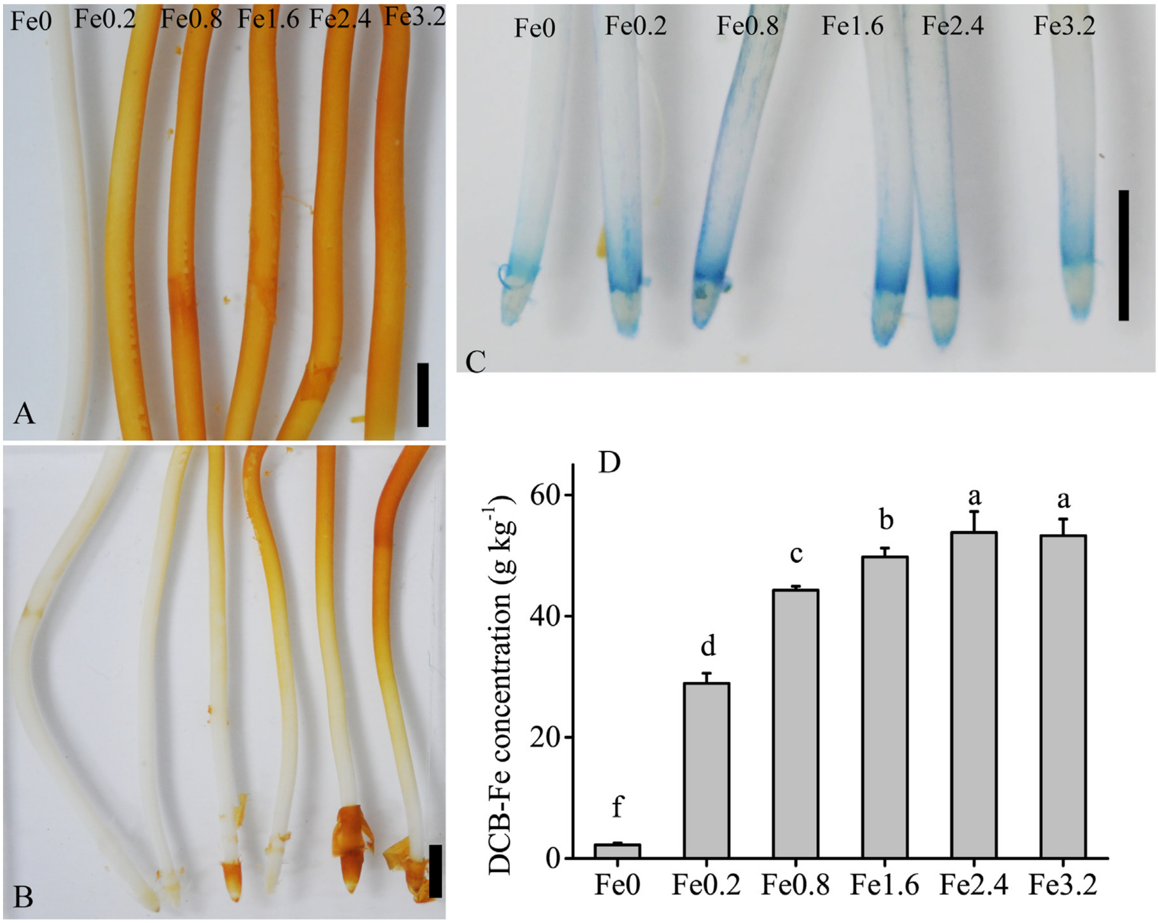

Fig. 2. Effect of different Fe^{2+} treatments for 2 d on IP formation. A: basal roots; B: apical roots; C: Evans blue staining; D: DCB-Fe concentration of the whole roots. Bars represent standard deviations of the means (n = 4). Different letters indicate significant differences among treatments at $p < 0.05$ level.

comparison to Cd-only treatment. Results from microscopic cross section further confirmed the quantitative results (data were presented in Supplementary material at Fig. S2). Noteworthy, epidermis, hypodermis and sclerenchyma cells of rice roots under Cd treatment were ruptured severely and became necrotic (Fig. S2), this phenomena might be associated with excessive ROS. The application of Fe^{2+} into Cd-containing solution reduced the concentration of $O_2^{-\cdot}$ in rice roots by 35.9% (Fig. 6B). Rice roots with Cd treatment had much deeper Evans blue staining and more electrolyte leakages of rice roots than those of the control (Figs. 6B-6C). Nevertheless, the addition of Fe^{2+} into Cd-containing solution could alleviate Cd-induced electrolyte leakage and recovered it to the level of the control (Fig. 6C).

3.7. Effect of different treatments on Fe and Cd uptake and transfer in rice seedlings

Fe concentrations in both roots and shoots were not different between the control and Cd treatment (Fig. 7B). But application of Fe^{2+} into Cd-containing solution increased Fe concentration in both roots and shoots significantly. Cd uptake in both roots and shoots were increased markedly under Cd treatment, which were 266.0 mg kg^{-1} and 43.0 mg kg^{-1} respectively (Fig. 7B). In comparison to a single Cd treatment, Cd concentrations in the roots and shoots with Cd + Fe treatment were reduced by 34.1% and 36.0%. Besides, the application of Fe^{2+} into Cd-containing solution decreased Cd transfer factor by 20.1% (Fig. 7A).

4. Discussion

4.1. Impacts of Cd on the growth of rice seedlings

Cadmium (Cd) is one of the most toxic heavy metals. Excessive Cd exhibits deleterious effects to most organisms. Its toxicological mechanism is that Cd binds the active centers of enzymes and metallothioneins, induces production of ROS and other toxic substances, as well as accelerates aging and death of plant cells (Siroka et al., 2004; Sridhar et al., 2005). Zhu et al. (2013) found that Cd inhibits root elongation and plant heightening of different genotypic plants. In cytological scale, Cd treatment suppressed mitotic index and reduced chromosome bridges significantly, thus inhibited rice growth (Singh and Shah, 2014; Wang et al., 2014a). In the present study, with increasing Cd concentration, rice biomass decreased significantly (Fig. 1E), Evans blue staining of root cells turned deeper clearly (Figs. 1C–1D). In addition, epidermis cells of the roots with Cd treatment were clearly distorted (Fig. 3B), accompanying with H_2O_2 and $O_2^{-\cdot}$ accumulation (Figs. 6A-6C) and severe Cd toxicity symptoms in the rice roots (Fig. 3B and Fig. S2). It is implied that Cd triggered elevation of reactive oxygen species production and subsequently caused direct epidermis cell necrosis and inhibited both root and shoot growth.

4.2. Blocking effect of IP and Fe^{2+} impacts on Cd adsorption and transfer in plants

It is reported that IP in the peripheral dermal tissues acts as a barrier for Pb and a buffer pool for Zn, Cu and Mn by using synchrotron X-ray fluorescence technique (Qian et al., 2015). Under soil cultivation

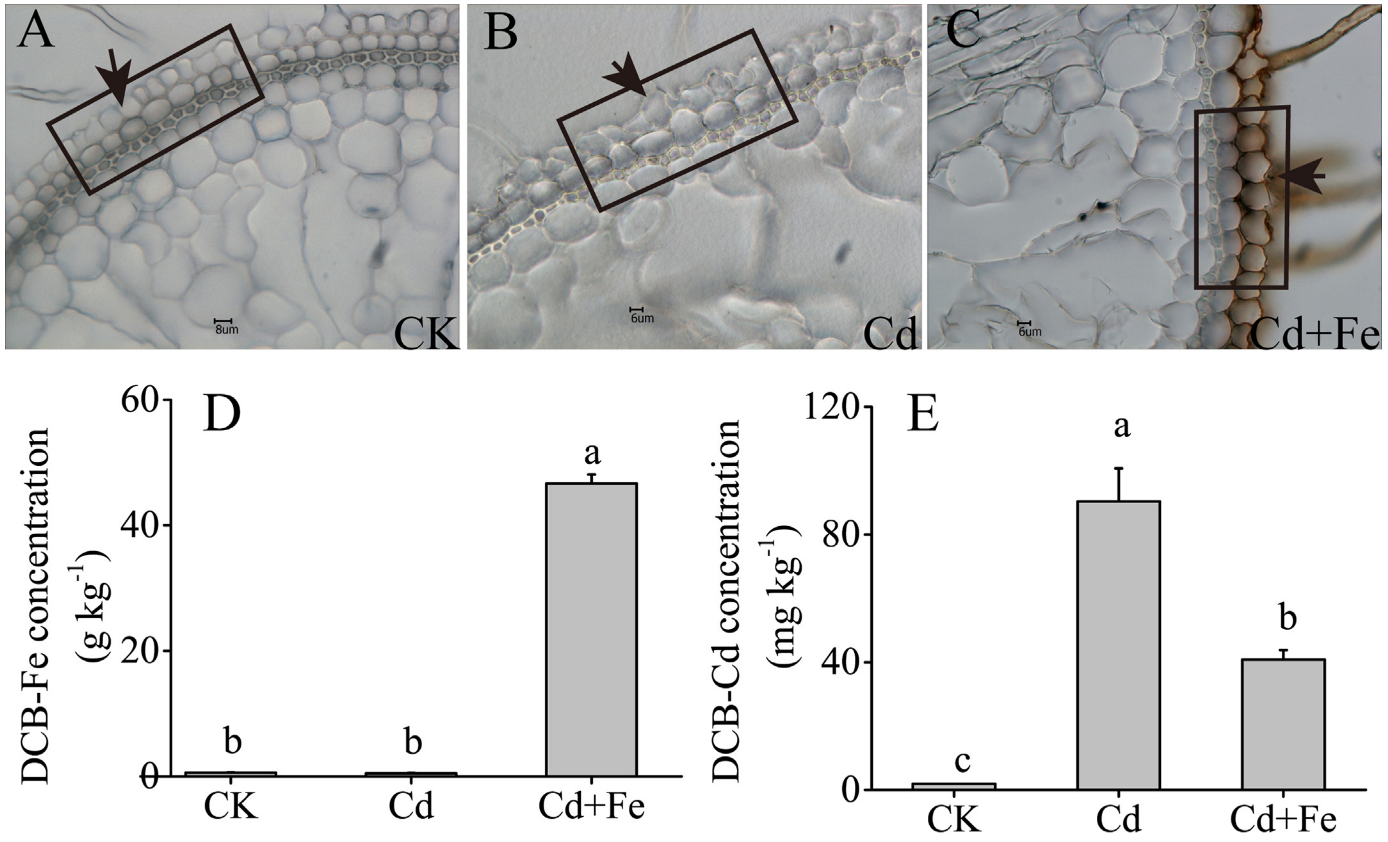

Fig. 3. Effect of different treatments for 2 d on IP formation. A-C: microscopical cross-section of basal roots; D: DCB-Fe; E: DCB-Cd. Bars represent standard deviations of the means (n = 4). Different letters indicate significant differences among treatments at $p < 0.05$ level.

condition, Liu et al. (2010) found that lesser IP was induced on the root surface of the cultivar Shanyou 63 (42.1 mg g^{-1}) than the cultivar Wuyunjing 7 (73.4 mg g^{-1}), while a higher Cd concentration appears on root surface of Shanyou 63 (390.8 μg pot^{-1}) than Wuyunjing 7 (103.9 μg pot^{-1}). In hydroponic experiments, Ye et al. (1998) revealed that seedlings of *Typha latifolia* with 33.1 mg g^{-1} of preformed IP can significantly reduce Cd accumulation on root surface compared with those without IP. These results suggest whatever in the condition of soil cultivation or nutrient solution culture, a certain thickness of IP could decrease Cd adsorption on root surface. In the present study, results from Fig. 3 supported the above view. Fe^{2+} application into Cd-containing solution supplied sufficient Fe^{2+}, which contributed to the formation of dense IP with intricate network structure on root surface (Fig. S1), and thus reduced root Cd adsorption (Fig. 3E). In addition, the

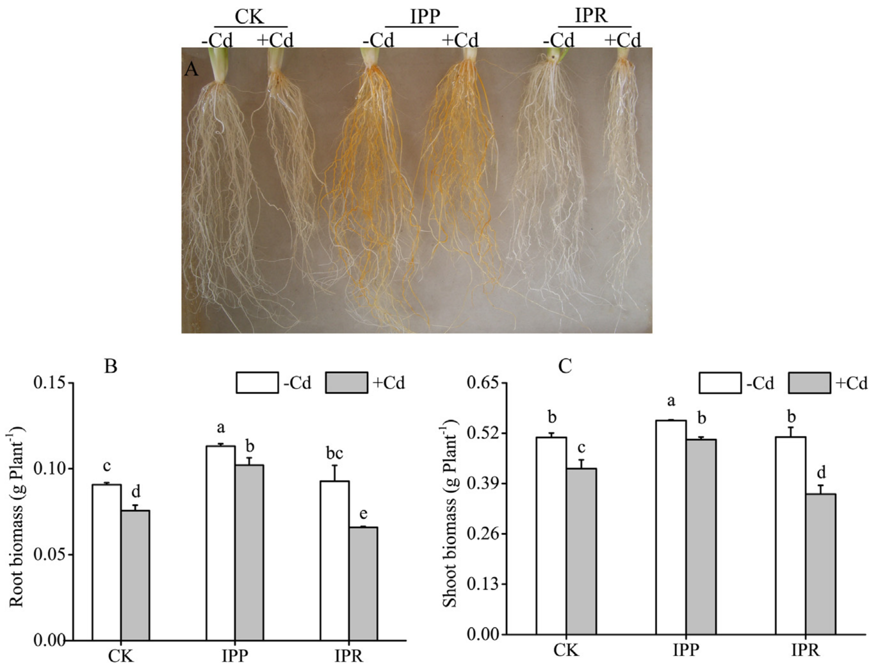

Fig. 4. Growth of rice roots without IP (CK), with IP presence (IPP) and IP removed from root surface (IPR) under -Cd and +Cd treatments for 1 week. A: whole rice roots; B: root biomass; and C: shoot biomass. Bars represent standard deviations of the means (n = 4). Different letters indicate significant differences among treatments at $p < 0.05$ level.

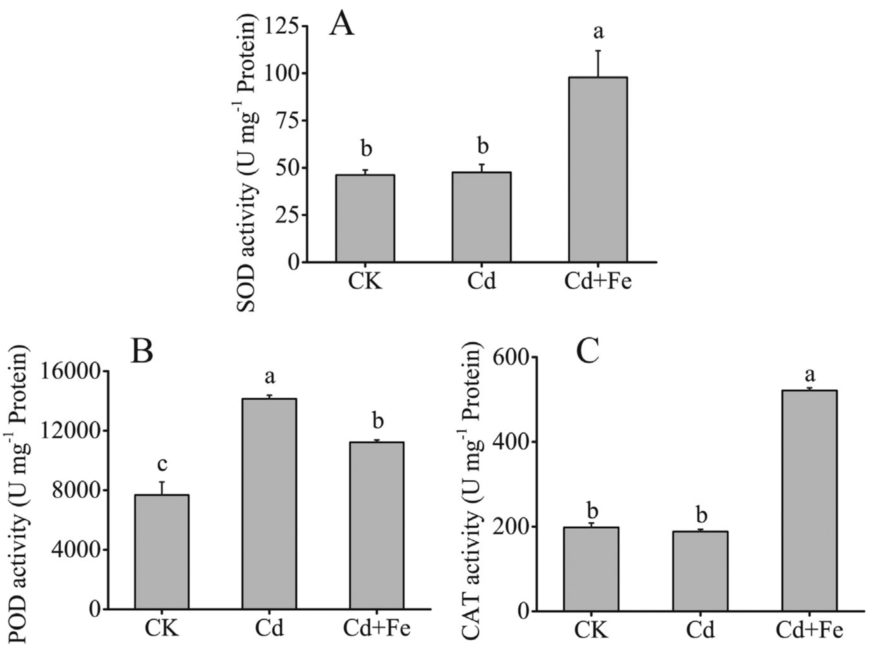

Fig. 5. Effect of different treatments for 2 d on the activities of antioxidant enzymes in rice roots. A: SOD activity; B: POD activity; C: CAT activity. Bars represent standard deviations of the means (n = 4). Different letters indicate significant differences among treatments at $p < 0.05$ level.

presence of IP in the present study could decrease Cd-induced decline of rice biomass (Fig. 4). It is implied that IP on root surface plays an important role in enhancing the tolerance of rice to Cd toxicity.

Welch et al. (1999) reported that Cd^{2+} could be taken in through Ca^{2+} and Mg^{2+}/Fe^{2+} channels, and it could compete with other divalent cations for uptake. Therefore, divalent cations, such as Zn^{2+}, Ca^{2+}, Fe^{2+} *etc*., might influence the absorption of Cd^{2+}. Besides, iron-regulated transporter 1 (OsIRT1) and natural resistance-associated macrophage protein 1(OsNramp1) played important roles in Cd uptake in plants (Chen et al., 2017; Yang et al., 2018). Meanwhile ethylene-mediated pathway positively regulated iron acquisition (Barberon et al., 2016), and lower Cd-induced oxidative stress (Asgher et al., 2014). In the present study, 0.2 mM Fe^{2+} was added into Cd-containing solution, and a large amount of Fe was absorbed and accumulated in the roots and shoots of rice seedlings, thus lowered Cd^{2+} uptake (Fig. 7). It is probably that Fe^{2+} uptake blocking Cd^{2+} entering into root tissues concerned various pathways including channels, transporters, carriers and/or ethylene-mediated iron-regulated transporters as above statements. The mechanisms underlying Fe^{2+} blocking Cd^{2+} uptake needs to be studied further.

4.3. Ameliorating effects of Fe^{2+} on Cd-induced ROS

It is well known that plant tissues suffering Cd toxicity can produce

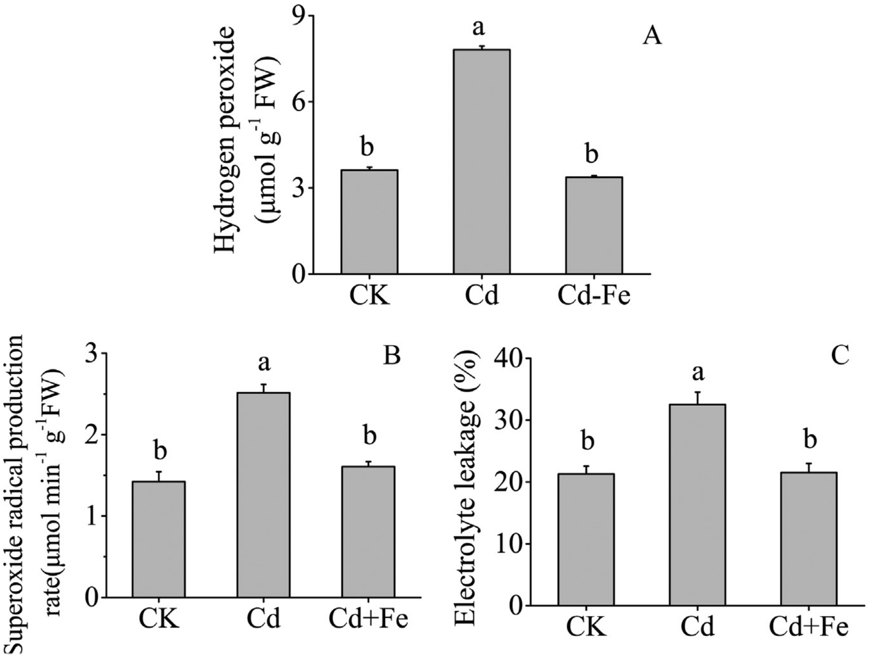

Fig. 6. Effect of different treatments for 2 d on ROS and electrolyte leakage of rice roots. A, B: H_2O_2 or $O_2^{-\cdot}$ production in root; C: electrolyte leakage. Bars represent standard deviations of the means (n = 4). Different letters indicate significant differences among treatments at $p < 0.05$ level.

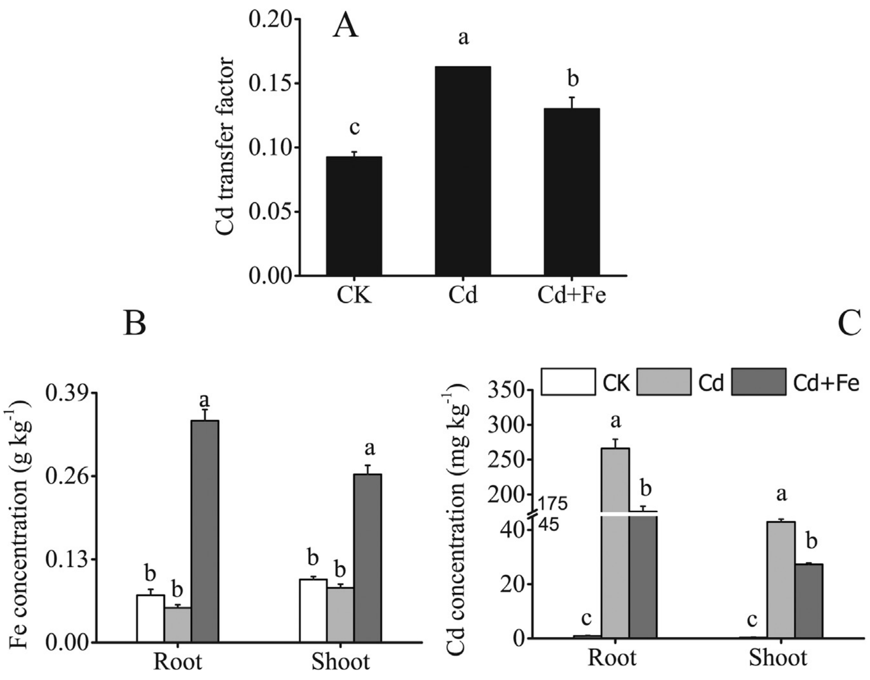

Fig. 7. Effect of different treatments for 2 d on Fe and Cd uptake and transfer in rice seedlings. A: Cd transfer; B: Fe concentration; C: Cd concentration. Bars represent standard deviations of the means (n = 4). Different letters indicate significant differences among treatments at $p < 0.05$ level.

remarkable ROS accompanying with significant decreases in antioxidant enzyme activities (Srivastava et al., 2014). On the contrary, when the Cd toxicity to plant tissues is ameliorated, ROS accumulation declines by raising activities of antioxidant enzymes, which represents the tolerance of plant tissues to Cd toxicity (Wu et al., 2015). Liu et al. (2015) reported that selenium (Se) reduced Cd-induced $O_2^{\cdot-}$, H_2O_2 and malondialdehyde accumulation, elevated Cd-inhibited activities of SOD, POD, CAT, ascorbate peroxidase and glutathione peroxidase, thereby mediated Cd toxicity. Wang et al. (2014b) also showed that lanthanum (La) increased the activities of antioxidant enzymes and consumes ROS. Agarwala and Sharma (1961) indicated that iron was one of the compositions of POD and CAT, and increasing iron supply caused an increase in the content of POD and CAT. Our previous studies have also indicated that Fe^{2+}-induced IP formation elevated the SOD, POD and CAT activities (Fu et al., 2011). The present results showed that Fe^{2+} application could raise the activities of SOD, POD and CAT (Fig. 5) and reduce Cd-induced accumulation of H_2O_2 and $O_2^{\cdot-}$ (Fig. 6). It is implied that Fe^{2+} application elevated these enzyme activities, alleviated cadmium-induced ROS burst, thus mediated the response of rice roots to Cd toxicity.

In addition, in our previous studies, mass of reddish-brown ferric oxides (i.e. iron plaque) were precipitated accompanying with the decrease of H_2O_2 consumption in low concentration H_2O_2 and Fe^{2+} titrate assay in vitro (Fu et al., 2013), indicating that large portion of Fe^{2+} were oxidized. The possible reaction equation was as follow: $2Fe^{2+} + H_2O_2 + 4H_2O = 2Fe(OH)_3 + 4H^+$. Jones et al. (2014) found that ROS such as H_2O_2, ·OH and $O_2^{\cdot-}$ could promote the oxidation of Fe^{2+} to form iron oxide precipitation (Shi et al., 2014). As a matter of fact, IP could form readily on rice root surface via secreting oxidative substances around the roots (Mei et al., 2014). It is reasonable that application of Fe^{2+} forming IP is a process consuming ROS. In the present study, Cd treatment induced large amounts of oxidative substances, including H_2O_2 and $O_2^{\cdot-}$ etc., and posed the damage to rice roots (Fig. 3B and Fig. S2), while Fe^{2+} application into Cd-containing solution reduced the Cd-induced ROS significantly and formed a layer of dense IP (Fig. S1 and Fig. S2). In other words, Fe^{2+} consumed H_2O_2 and $O_2^{\cdot-}$ directly and formed dense IP on root surface, thereby alleviated Cd toxicity. Since H_2O_2 was partially consumed by Fe^{2+}, POD activities of Cd + Fe treatment declined to the level between those of CK and Cd treatment (Fig. 5B).

5. Conclusions

Under the condition of hydroponic experiments, 20 μM of Cd could lead to excessive ROS accumulation, and consequently damage root cells, inhibit lateral root growth, and reduce the biomass of rice seedlings. The addition of 0.2 mM of Fe^{2+} could induce the formation of IP significantly, reduce the adsorption of Cd on root surface via formation of dense IP with intricate network structure, and decrease root to shoot Cd absorption and translocation in rice seedlings. In addition, Fe^{2+} application contributed to the elevation of antioxidant enzyme activities and decreased ROS accumulation. Through the above two pathways, Fe^{2+} application into Cd-containing solution ameliorated the toxicity of Cd to rice roots, and thus enhanced Cd tolerance of rice seedlings.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2018.06.015.

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