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

Youqiang Fu[a,b], Xujian Yang[b], Hong Shen[b,*]

[a] Rice Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, PR China
[b] College of Natural Resources and Environment, South China Agricultural University, Guangzhou 510642, PR China

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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, $O_2^{\cdot-}$, ·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.

* Corresponding author.
E-mail address: hshen@scau.edu.cn (H. Shen).

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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.