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Influence of iron plaque on the uptake and accumulation of chromium by rice (*Oryza sativa* L.) seedlings: Insights from hydroponic and soil cultivation

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ABSTRACT

The effects of iron plaque formation on chromium (Cr) uptake and accumulation by rice seedlings (*Oryza sativa* L.) were assessed using hydroponic and soil experiments, where each 3 levels of Fe supplementation were added to Hoagland solution (0, 30, and 100 mg Fe^{2+} L^{-1}) and a typical paddy soil (0, 1, and 2 g Fe^{2+} kg^{-1}). For each treatment, rice seedlings were exposed to different levels of Cr as chromate at 0, 0.5, 2, 5, 10, and 20 mg L^{-1} in solution or 300 mg kg^{-1} in soil. Low levels of Cr supply (0.5, 2, and 5 mg L^{-1}) promoted root biomass, while high levels (10 and 20 mg L^{-1}) decreased root and shoot biomass and undermined the density and integrity of iron plaque. Iron supply significantly increased the proportion of Cr in iron plaque, but decreased that in rice plants. The results of hydroponic experiment showed that iron plaque formed with Fe supply at 100 mg L^{-1} markedly reduced Cr accumulation in shoots of rice seedlings when exposure to 10 and 20 mg L^{-1} Cr. The soil culture experiment also demonstrated that exogenous Fe addition significantly decreased Cr concentration in leaf and stem of rice seedlings. These results suggested that iron plaque with appropriate amount was effective to reduce the uptake and accumulation of Cr in rice plants, which have strong implication for taking measures to regulate Cr accumulation in rice grains.

1. Introduction

With rapid urbanization and industrialization, chromium (Cr) contamination in agricultural ecosystem is attracting increasing concern (Gao and Xia, 2011). Industries such as electroplating and leather production exacerbate Cr spreading to biological chain (Mahmud et al., 2016). Generally, chromium has two major valence states in the soil environments (Di Palma et al., 2015), with Cr(III) being the dominant form under reducing conditions which is immobile and less toxic to plants. However, under oxidizing conditions, Cr mainly exists in the form of Cr(VI) which is very mobile and carcinogenic to humans (Ellis et al., 2002). Elevated Cr(VI) concentration in soil could be up-taken by plants, causing a serious decline in plant growth and yield, interference in photosynthesis and respiration, minerals uptake, enzymatic activities, and lead to destruction of membrane lipids and DNA damage in plants (Singh et al., 2013), directly or indirectly harming human health (Shi et al., 2014).

Since industrial revolution, elevated concentrations of heavy metals in rice grains, the staple food for more than one half of the world's population, have attracted wide attention (Zhao et al., 2015). Recently, numerous studies have observed elevated Cr level in rice around the world, posing a potential threat to human health (Giri and Singh, 2017; Shraim, 2017). For example, Giri and Singh (2017) reported that daily intakes of Cr from rice consumption exceeded the tolerable daily intakes provided by WHO for local residents living in a copper mining area. Therefore, it is particularly important to develop measures to reduce Cr accumulation and uptake by rice grown in Cr-contaminated environments.

Rice, a plant grown in agro-wetland ecosystem, shares the characteristics of most wetland plants including developed aerenchyma and efficient root metabolism (Miro and Ismail, 2013; Yamauchi et al., 2013; Kirk et al., 2014), which confer rice plant adaptability to oxygen-deficient environments. For adaptation to adverse anaerobic conditions, rice plants form iron plaque on root surface. Briefly, oxygen is transported from the upper part to root tissue to maintain normal respiration, meanwhile the Fe^{2+} around the rhizosphere is oxidized to reddish-

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brown iron coating (iron plaque) wrapping root surface due to radial oxygen loss from roots (Cheng et al., 2014). Studies have shown that iron plaque immobilized metal(loid) ions (Cd, Pb, Al and As), reducing their toxicities to and accumulation in rice plant (Chen et al., 2006; Wu et al., 2012; Cheng et al., 2014). However, the influence of iron plaque on Cr translocation and accumulation in rice under Cr(VI) stress is still unclear. Hu et al. (2014) reported that iron plaque immobilized Cr but did not affect Cr uptake and translocation in rice seedlings exposed to 1.0 mg L^{-1} Cr(III). Xu et al. (2015) also showed that iron plaque did not affect Cr uptake by yellow flag under 0.5, 5, and 50 mg L^{-1} Cr(VI) treatments. However, the above studies were based on hydroponic experiments. Compared to hydroponic conditions, soil experiments provide a more robust assessment of the effects of iron plaque on metal translocation. While iron plaque did not hinder Cd translocation to rice seedlings grown in nutrient solution with 0.1 or 1.0 mg L^{-1} Cd (Liu et al., 2007), it significantly reduced Cd concentrations in rice plants grown in soil with 2 or 10 mg kg^{-1} Cd (Liu et al., 2008). Therefore, a soil experiment is needed to accurately test the effects of iron plaque on Cr translocation in rice plant. Another factor is the amount of iron plaque. While low amount of iron plaque at 12.1 g kg^{-1} increased Zn concentrations in shoots, iron plaque reaching 24.9 g kg^{-1} decreased Zn accumulation (Zhang et al., 1998).

Based on the above considerations, the main aims of our study were to explore two fundamental questions on effects of iron plaque on uptake and accumulation of Cr(VI) in rice seedlings: (1) Could iron plaque formation with different amount decrease the toxicity and accumulation of Cr(VI) in rice plants in hydroponic culture? (2) Could enhanced formation of iron plaque reduce Cr concentrations in rice plants grown in soil? To explain these questions, a hydroponic experiment was designed to investigate Cr concentrations in rice seedlings (shoot, root, and plaque) exposed to different Cr(VI) concentrations via inducing different amounts of iron plaque. Meanwhile, another experiment was conducted to test the influence of iron plaque on Cr distribution in rice plants grown in soil spiked with Cr(VI) and Fe. Understanding the effects of iron plaque and the relationships between iron plaque and Cr (VI) is crucial for the strategy of regulating Cr accumulation in rice grains.

2. Materials and methods

2.1. Pre-culture of rice seedlings

Rice seeds (Yongyou 9, a local cultivar in Fujian Province, China), were surface-sterilized in H_2O_2 (30%, v/v) for 10 min and thoroughly washed using deionized water. Then these seeds were germinated and grown in acid-washed quartz sand for 15 d. Uniform seedlings were chose and transferred to two 10 L plastic culture boxes with 1/3 strength Hoagland solution for 15 d in greenhouse at 25–35 °C with light exposure of 12–14 h d^{-1}. The nutrient solution was changed every 3 d, and the pH was adjusted to 5.5 using 0.1 M NaOH or HCl.

2.2. Hydroponic experiment

After the 30-d pre-culture, uniform seedling was then transferred to 1 L black plastic pot containing 1 L of 1/3 strength Hoagland solution for 3 d. Then 54 seedlings were further transferred and grown in deionized water for 12 h, followed by growing in 1/3 strength Hoagland solution without P (avoiding coprecipitation of P and Fe) including 0, 30, and 100 mg L^{-1} Fe^{2+} as $FeSO_4$ for 3 d for iron plaque formation. The 54 seedlings were randomly divided to 3 groups of 0 (Fe0), 30 (Fe30), and 100 (Fe100) mg L^{-1} Fe. Afterwards, all rice seedlings were grown in normal 1/3 strength Hoagland solution for 2 d. Following iron plaque formation, rice seedlings of each group were randomly further divided to 6 subgroups and transferred to 1/3 strength Hoagland solution containing Cr(VI) as K_2CrO_4 at 0 (Cr0), 0.5 (Cr0.5), 2 (Cr2), 5 (Cr5), 10 (Cr10) and 20 (Cr20) mg L^{-1} for 6 d. There are a total of 18 treatments, each in triplicate. The treatments were Fe0Cr0, Fe0Cr0.5, Fe0Cr2, Fe0Cr5, Fe0Cr10, Fe0Cr20; Fe30Cr0, Fe30Cr0.5, Fe30Cr2, Fe30Cr5, Fe30Cr10, Fe30Cr20; Fe100Cr0, Fe100Cr0.5, Fe100Cr2, Fe100Cr5, Fe100Cr10, Fe100Cr20.

Following Cr exposure, rice seedlings were harvested, divided into shoot and root sections, and thoroughly washed with deionized water. Four samples of rice roots were selected and imaged by an Hitachi S-4800 field emission scanning electron microscope and energy dispersive X-ray spectroscopy (Tokyo, Japan): (A) root of rice seedling grown in 1/3 strength Hoagland solution; (B) root of rice seedling grown in 1/3 strength Hoagland solution containing 10 mg L^{-1} Cr(VI); (C) root of rice seedling grown in 1/3 strength Hoagland solution containing 100 mg L^{-1} Fe^{2+}; (D) root of rice seedling grown in 1/3 strength Hoagland solution containing 10 mg L^{-1} Cr(VI) and 100 mg L^{-1} Fe^{2+}.

Following harvest, each root samples of rice were scanned by STD4800 scanner (EPSON Perfection V700/V750 2.80 A) and analyzed by the WinRHIZO software (Regent Instruments Canada Inc.) to obtain root parameter including root length, volume, surface area, average diameter, and tips. After scanning, the root iron plaque was extracted using a modified dithionite–citrate–bicarbonate (DCB) method using 30 mL mixed solution of 0.03 mol L^{-1} sodium citrate ($Na_3C_6H_5O_7{\cdot}2H_2O$), 0.125 mol L^{-1} sodium bicarbonate ($NaHCO_3$), and 0.6 g sodium dithionite ($Na_2S_2O_4$) (Taylor and Crowder, 1983; Otte et al., 1989). The fresh rice root was incubated in a 100-mL beaker with 30 mL mixed DCB solution for 60 min at 25 °C. Following extraction, rice roots were washed using deionized water, and all extracts were transferred to 100 mL volumetric flask and filtered through a 0.22 μm membrane filters prior to analysis of Fe and Cr using inductively-coupled plasma mass spectrometry (ICP-MS, NexION 300 ×; Perkin Elmer, NY). Then, shoot and root samples were dried in an oven at 60 °C for 3 d and weighed. Dry plant samples were ground and digested according to Xu et al. (2017). Reagent blank and standard reference material (bush twigs and leaves, GBW07603, Chinese National Certified Reference Material) were used for quality control of the digestion procedure. The concentrations of Fe and Cr in plant digests were also determined using ICP-MS.

2.3. Soil culture experiment

A paddy soil was collected from a rice field in Lingxia town, Baicheng City, Jilin Province, and air-dried and sieved (< 3 mm). The physiochemical properties of the soil sample are shown in Table S1. Before plant cultivation, 0.2 g kg^{-1} N ($(NH_4)_2SO_4$), 0.15 g kg^{-1} P_2O_5 (KH_2PO_4) and 0.2 g kg^{-1} of K_2O (K_2SO_4) were added to the soil as base fertilizer. The pots used in the present study were 3 L cylindrical pots of polyvinyl chloride (PVC) (diameter:15 cm; height: 17 cm) filled with 3 kg air-dried soil. Chromium as K_2CrO_4 was spiked to the soil at 300 (Cr300) mg kg^{-1}. To facilitate studying the influence of iron plaque on Cr uptake by rice plants, the Cr-spiked and non-spiked soil was then divided to 3 subsamples receiving Fe supply as $FeSO_4$ at 0 (Fe0), 1 (Fe1000), and 2 (Fe2000) g kg^{-1}. The soil samples were then flooded with deionized water for 30 d and the 15-d-old seedlings grown in nutrient solution were then transferred to the pots. There are a total of 6 treatments, each in triplicate. The treatments were control (Fe0Cr0), Fe1000, Fe2000, Cr300, Fe1000Cr300, Fe2000Cr300.

After rice growth over a 40-d period, rice plant was harvested, and the seedling was divided into leaf, stem and root. The subsequent iron plaque extraction, plant digestion and determination of Fe and Cr were in accordance with the hydroponic experiment.

2.4. Statistical analyses

Data are presented as means ± SE (n = 3), and were analyzed using least significant difference (LSD) at the 5% level. ANOVA (analysis of variance) was carried out using SPSS software (19.0, SPSS, Inc.,