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Fenton reagent reduces the level of arsenic in paddy rice grain

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ABSTRACT

Hydroponic and pot experiments were conducted to examine the effects of Fenton reagent on paddy rice plant growing in arsenic-contaminated soils. Fenton reagent significantly reduced arsenic phytotoxicity, uptake by the plants and accumulation in rice grain. This is attributed to oxidation of As^{3+} to As^{5+} by hydroxyl radicals and immobilization of arsenate by reacting with precipitating Fe^{3+} to form practically insoluble compounds. Although this process enhanced the formation of Fe-enriched coatings on root surface, it appears that root plaque had limited effects on inhibiting As uptake since most of the young roots were not covered by iron plaque. It is more likely that As immobilization in the bulk soils play a major role in reducing As flux towards rhizosphere. The findings have implications for understanding As behavior in paddy field receiving rainwater-borne hydrogen peroxide and developing cost-effective techniques for reducing As level in rice grain produced from As-contaminated soils.

1. Introduction

Consumption of rice is a major pathway of human arsenic exposure, which could affect billions of people around the world (Schoof et al., 1999; Meharg, 2004; Williams et al., 2006; Zhu et al., 2008; Syu et al., 2015; Sinha and Bhattacharyya, 2015; Clemens and Ma, 2016). The anaerobic soil conditions associated with water inundation required for paddy rice farming favour reduction reactions, leading to formation of highly toxic arsenite ions (Xu et al., 2008; Li et al., 2009; Somenahally et al., 2011; Spanu et al., 2012). Arsenite tends to be predominantly present in undissociated form ($H_3AsO_3^{\circ}$) under pH conditions encountered in most paddy rice soils (Zhao et al., 2009), and therefore it may be more resistant to immobilization by soil adsorbents. In addition, under reducing conditions the arsenic-scavenging capacity of soil is weakened due to reductive dissolution of various iron compounds that play a key role in binding soluble arsenic species through either formation of practically insoluble iron arsenate minerals or adsorption of arsenate to iron oxyhydroxides (Zhao et al., 2010; Zhu et al., 2014). As such, arsenite is readily available for uptake by rice plants and accumulation in rice grain (Williams et al., 2007; Su et al., 2010; Wang et al., 2015).

Iron-enriched root plaque plays an important role in reducing the entry of As present in the soil pore water (soil solution) into rice plant roots (Lee et al., 2013; Syu et al., 2013). The formation of root plaque is believed to be mediated by oxidation of ferrous iron (Fe^{2+}) using molecular oxygen released from rice plant roots (Armstrong, 1964), and it is likely that the root-released oxygen also promotes microbially mediated oxidation of arsenite to form arsenate (Hu et al., 2015). As arsenate has the stronger affinity to Fe^{3+}, it is likely that arsenate-As tends to be intercepted more easily by the root plaque, as compared to arsenite-As (Chen et al., 2005; Liu et al., 2005).

It has been demonstrated that Fenton process involving reaction between hydrogen peroxide (H_2O_2) and ferrous iron (Fe^{2+}) resulted in enhanced oxidation of arsenite to form less toxic arsenate (Hug and Leupin, 2003). Fe^{2+} is available in flooded soils like paddy rice soils (Becker and Asch, 2005; Kögel-Knabner et al., 2010). H_2O_2 is also commonly present in rainwater (Cooper et al., 1988; Willey et al., 1996; Gonçalves et al., 2010; Guo et al., 2014). In areas with abundant rainfall, Fenton reaction may be a naturally-occurring process that can affect the biogeochemical behavior of arsenic in paddy rice soils. Where the enrichment of arsenic in rice grain becomes a significant health concern, it may be worthwhile to consider the use of Fenton reagent (a mixture of H_2O_2 and Fe^{2+}) for reducing As uptake by rice plants.

The objective of this study was to examine the effects of Fenton reagent on reducing As uptake by rice plants. The impacts of Fenton reagent on plant growth are also evaluated. In addition, the major biogeochemical mechanisms responsible for the observed phenomena are explored.

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2. Materials and methods

2.1. Materials

2.1.1. Hydroponic nutrient solution

The hydroponic nutrient solution used for the solution culture experiment consisted of the following chemical compounds: 5 mM NH_4NO_3, 2 mM K_2SO_4, 4 mM $CaCl_2$, 1.5 mM $MgSO_4{\cdot}7H_2O$, 1.3 mM KH_2PO_4, 50 μM Fe(II)-ethylenediaminetetraacetic acid (EDTA), 10 μM H_3BO_4, 1.0 μM $ZnSO_4{\cdot}7H_2O$, 1.0 μM $CuSO_4{\cdot}5H_2O$, 5.0 μM $MnSO_4{\cdot}H_2O$, 0.5 μM $Na_2MoO_4{\cdot}2H_2O$, and 0.2 μM $CoSO_4{\cdot}7H_2O$. The pH of the solution was adjusted to 5.5 using 0.1 M KOH or HCl.

2.1.2. The experimental soil

The soil sample used for the greenhouse experiment was taken from the paddy rice field of the experimental farm at the South China Agricultural University (Guangzhou, China). The soil samples were air-dried after collection and then crushed to pass a 2 mm sieve prior to the use in the experiments. The soil had a pH of 6.52 and contained 2.38% of organic matter. Total nitrogen, phosphorus and potassium were 1.06, 1.04 and 19.6 g/kg, respectively. Available nitrogen, phosphorus and potassium were 114, 77.8 and 122 mg/kg, respectively. The soil contained 15.6 mg/kg of arsenate-As and no other arsenic species were detected.

2.1.3. The rice seedlings used in the experiment

The seeds of rice (*Oryza sativa* cultivar: Tianyou 122) used in the experiment were provided by the Guangdong Academy of Agricultural Sciences. Prior to sowing, the seeds *were* surface-sterilized by soaking in 30% H_2O_2 for 15 min. The sterilized seeds were then rinsed with deionized water and placed in a container with moistened sands for germination. The pre-germinated seeds were sown into the seed bed that was covered by a plastic sheet to maintain the temperature at 28 ± 2 °C. Healthy seedlings with 4 leaves were selected for the experiment.

2.2. Experimental design

2.2.1. Solution culture experiment

The rice seedlings were grown in the hydroponic nutrient solution for 3 weeks. The seedlings were then rinsed with deionized water and transplanted into a beaker containing 500 mL of 20 mg Fe^{2+}/L solution (pH being adjusted to 5.5) for 24 h to allow the formation of iron plaques on the root surfaces of the seedlings. After this, the seedlings were rinsed to remove any soluble Fe attached to the plant surface before being used in the experiments.

Two sets of the experiments were performed aiming to collect data at the end of two different lengths of growth period: 1 day (24 h) and 30 days (720 h). For each set of the experiment, one control and one treatment were set; (a) control: plant growing in the hydroponic nutrient solution with added arsenite-As at a dose of 1 mg/L; (c) Treatment: plant growing in the hydroponic nutrient solution with added arsenite-As at a dose of 1 mg/L plus Fenton reagent (100 μM H_2O_2 and 100 μM Fe^{2+}). For the 1-day experiment, the control and treatment were labelled as C1d and T1d, respectively. For the 30-day experiment, the control and treatment were labelled as C30d and T30d, respectively.

A 500 mL plastic cup (diameter: 8 cm; height: 15 cm) was used as a hydroponic container, which was placed into a black nylon bag to avoid exposure of the plant roots to light. The lid with holes was used to support the plants. Six rice plants were grown in each hydroponic container. The plant growth units were placed randomly in a climate chamber with the daily light-dark cycle being set at 16 h:8 h. The light density during the photoperiod was fixed at 2500 lx. Temperature during the dark and light periods was set at 20 °C and 28 °C, respectively. Relative humidity was maintained at a range of 80–85%. All the experiments were performed in 4 replicates.

For the 30-day experiment, the culture solution in each hydroponic container was replenished every 3 days. This included addition of arsenite-As for the control and addition of arsenite-As plus Fenton reagent for the treatment.

At the end of the 1-day experiment, samples of the spent culture solution were taken to determine various As species. For the 30-day experiment, only the first (3 days or 72 h) spent culture solution was used for analysis of As species. These spent solution samples were labelled as CS1d and TS1d for the control and treatment of the 1-day experiment, respectively, and CS3d and TS3d for the control and the treatment of the first spent solution of the 30-day experiment, respectively.

At the end of each experiment, the plants were harvested for determinations of biomass, various As species in the plant tissues, and Fe and various As species in the root plaques. Since all the six plants growing in each hydroponic container had very similar growth performance, only three of the six plants were randomly selected: (a) the first one was used for determination of the biomass; (b) the second one was used for measurement of As in various plant organs; and (c) the third one was used to extract iron plaque.

2.2.2. Pot experiment

A greenhouse experiment was conducted to observe the growth performance of the rice plants and uptake of As by the rice plants. The experiment lasted for > 9 months, including two continuous crops with a fallow period of about 3 months. The first crop commenced on September 8, 2013 and the rice plants were harvested on January 7, 2014; the second crop commenced on April 3, 2014 and the rice plants were harvested on July 22, 2014.

The soil without added As was used as the control (Ck); Treatments 1 and 2 (T1 and T2, respectively) were the artificially contaminated soils without and with added Fenton reagent (100 μM H_2O_2:100 μM Fe^{2+}), respectively. The dose of added arsenite-As in the contaminated soils was set at 50 mg/kg. The thickness of the overlying water layer was maintained at approximately 2 cm. For T2, an appropriate amount of standardized H_2O_2 and $FeSO_4$ solution was added to the overlying water to maintain a theoretical concentration of H_2O_2 and Fe^{2+} at 100 μM each at the beginning of Fenton reagent addition for each 3-day cycle.

Two seedlings were transplanted to a soil column consisting of alternating layers (1 cm thick) of quartz sand and a relevant soil material. This design was to allow easy separation of the root materials from the soils upon harvest. The soil column was contained in a nylon mesh bag (#400 mesh; diameter: 8 cm; depth: 12 cm). Four soil columns were placed in a plastic bucket (Diameter: 22 cm; Height: 15 cm) that was filled with the same soil material. This design allowed the separation of rhizospheric soil from the bulk soil by confining the rice plant roots within the nylon mesh bag or so-called rhizo-bag.

Compound fertilizer (N:P:K = 15:15:15) was applied at a rate of 19 g per pot at the 7th day of the experiment. Additional fertilizers were added at a rate of 6.8 g/pot for compound fertilizer and 9.6 g/pot for urea in the early tillering stage of the first crop. In the second crop, 6.8 g/pot and 7 g/pot were added 7 days after transplanting of the rice seedlings and in the heading stage, respectively.

In the first crop, one of the four rhizo-bags (together with the above-ground portion) was randomly removed from each bucket in the heading stage. A second rhizo-bag was removed in the maturity stage. For the second crop, sampling was carried out in the tillering, heading and maturity stages. After collection, the soil materials in each rhizo-bag were recovered by separation from the quartz sands. One of the two rice plants from each rhizo-bag was used for measurement of biomass and another one was used for determination of various As species in the plant tissues.