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Sulfur (S)-induced enhancement of iron plaque formation in the rhizosphere reduces arsenic accumulation in rice (*Oryza sativa* L.) seedlings

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Sulfur-induced enhancement of iron plaque formation on the root surface of rice.

Abstract

The effects of two sulfur (S) sources (SO_4^{2-}, S^0), and three rates of S application (0, 30, 120 mg S/kg) on the formation of iron plaque in the rhizosphere, and on the root surface of rice, and As (arsenic) uptake into rice (*Oryza sativa* L.) were studied in a combined soil–sand culture experiment. Significant differences in As uptake into rice between +S and −S treatments were observed in relation to S sources, and rates of S application. Concentrations of As in rice shoots decreased with increasing rates of S application. The mechanism could be ascribed to sulfur, induced the formation of iron plaque, since concentrations of Fe in iron plaque on quartz sands in the rhizosphere, and on the root surface of rice increased with increasing rates of S application. The results suggest that sulfur fertilization may be important for the development approaches to reducing As accumulation in rice.

Keywords: Sulfur; Sulfate; Elemental sulfur; Arsenic; Iron plaque; Rice (*Oryza sativa* L.); Rhizosphere

1. Introduction

Anthropogenic activities, such as pesticide and herbicide application (Maclean and Langille, 1981), mining (Galbraith et al., 1995; Xie and Huang, 1998), or irrigation with contaminated groundwater (Abedin et al., 2002a,b) have significantly enhanced As levels in soil in many parts of the world (Marin et al., 1992). Rice grown on As-contaminated paddy soil can accumulate high levels of As in shoots and grains (Abedin et al., 2002b; Xie and Huang, 1998). Thus, As uptake by rice plants plays an important role in the transfer of this toxic element into food chains leading to serious health risks to humans (Meharg and Rahman, 2003).

Paddy rice oxygenates its rhizosphere, resulting in the formation of an iron (Fe) oxyhydroxide plaque (Armstrong, 1964). A recent study showed that Fe plaque is composed dominantly of ferrihydrite, goethite and siderite (Hansel et al., 2001). The Fe hydroxides in soil and solution have a very strong binding affinity for arsenate (Meng et al., 2002; Liu et al., 2004a,b), and a possible capacity to oxidize arsenite to arsenate (Otter et al., 1991). Iron plaque may be a barrier or a buffer to the uptake of As (Liu et al.,

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2004a,b). The effect of Fe plaque on plant uptake of contaminants or nutrients may depend on the amount of Fe plaque on the roots surface (Otter et al., 1989; Zhang et al., 1998). In rice plants, Fe plaque can be formed both under natural and laboratory conditions (Chen et al., 1980; Greipsson and Crowder, 1992; Greipsson, 1994, 1995). It may be important for the development of practical approaches to reducing As accumulation in rice. The formation of Fe plaque on rice roots can be affected by a number of factors, such as soil properties (Li, 1992), water management (Lu et al., 1999), iron, manganese fertilization (Shi et al., 2004; Zeng et al., 2001) and rice genotypes (Liu et al., 2001; Zhang et al., 2002).

Sulfur is one of the essential elements for all living organisms. Paddy soil sulfur concentrations show a great spatial variability (Li, 1992) due to human activities, such as anthropogenic SO_2 emission, and fertilization (Messick et al., 1993; Wang et al., 2004, 2005). Soil sulfur deficiency and sulfur excessive coexist in China (Hu and Xu, 2002). The behavior of sulfur is closely linked to redox potential of paddy soil due to the transformation of inorganic species from −2 to +6, and oxidation of organic sulfur compounds (Li, 1992; Hu and Xu, 2002).

High concentrations of Fe^{2+} usually exist at the site of SO_4^{2-} reduction by sulfate-reducing bacteria, and S^{2-} thus produced immediately reacts with Fe^{2+} to form FeS (Murase and Kimura, 1997). FeS is known to take a very long time to reduce ferric iron to form pyrite (FeS_2) (Dent, 1986). Murase and Kimura (1997) showed that monosulfide (S^{2-}) chemically reduced MnO_2 to Mn^{2+} and $Fe(OH)_3$ to Fe^{2+} ions in paddy soil. Microbes make a major contribution to the oxidation of elemental sulfur to sulfate (Wainwright, 1984) while elemental sulfur was also oxidized to sulfate coupling to the reduction of either MnO_2 or $Fe(OH)_3$ in anaerobic paddy soil cultures (Murase and Kimura, 1997). Nonetheless, there are few reports on three-way interactions between arsenate, sulfur supply and iron plaque development in arsenate uptake by rice. The aim of this study was therefore to investigate these interactions under controlled conditions.

2. Materials and methods

2.1. Soil used

The top soil layer (0–20 cm) of Typical Haplaquept (US Soil Taxonomy, 1996) from Putian city, Fujian province, China was used for the pot experiment. It was thoroughly mixed, air-dried, and ground to a particle size of <2 mm.

2.2. Experimental design

A rhizobox system with a soil–sand combination was used to collect rhizosphere and non-rhizosphere soils separately and to study the effect of Fe plaque on arsenic uptake by rice seedlings. There were 12 treatments with two rates of As (as arsenate) application (0, 20 mg As/kg) combined with three rates of S application (0, 30, 120 mg kg^{-1}), and two S sources (S^0, and SO_4^{2-}-S).

The rhizoboxes were made of Perspex, with the geometry of 7.5 cm × 6 cm × 11 cm. Each rhizobox was divided into three compartments. The size of the central compartment was 6 cm in length, 1.5 cm in width and 11 cm in height. The left and right compartments have equal size with 3 cm × 6 cm × 11 cm. The compartments were separated by nylon net with a mesh size of 40 μm. The left and right compartments were filled with 125 g soil, central compartment was filled with 90 g quartz sand.

Soil in rhizoboxes received (per kg) a basal application of 100 mg P as KH_2PO_4; 125 mg K as KH_2PO_4; 110 mg N as urea; 10 mg Mn as $MnCl_2$; 1 mg Cu as $CuCl_2 \cdot 2H_2O$; 2 mg Zn as $ZnCl_2$; 50 mg Mg as $MgCl_2 \cdot 6H_2O$; 1 mg B as H_3BO_3; 0.1 mg Mo as $Na_2MoO_4 \cdot 2H_2O$ per kg soil. These nutrients were added to the soil as solution and mixed thoroughly before potting. Elemental S was mixed thoroughly with the soil, and As as $Na_2HAsO_4 \cdot 7H_2O$, and SO_4^{2-} as Na_2SO_4 were added in liquid form.

Seeds of rice (*Oyyza sativa* L.) cultivar (No. 3 Wu-Yu-Geng) were disinfected in 30% H_2O_2 (wt:wt) solution for 15 min, followed by thoroughly washing with deionised water. The seeds were germinated in moist quartz sand. Twenty uniform germinated seeds were transplanted into the central compartment of each rhizoboxes. Soil moisture content was brought up to 100% of the water holding capacity (WHC) before seedling emergence, and was then kept submerged with deionised water for 43 days. Afterwards the pots were not watered, and at the 45th day plants were harvested.

All pots were arranged randomly inside a controlled environment growth room, with the following conditions: day/night duration 14 h/10 h, light intensity 300 μmol m^{-1} s^{-1}, day/night temperature 30 °C/20 °C and relative humidity 65%.

2.3. Harvest and sampling

Forty-five days after germination, plants were harvested by cutting shoots at the surface of the quartz sand. Roots were separated from the quartz sand. Shoots and roots were rinsed with deionised water, and all shoots and half of the roots were dried at 80 °C for 24 h and dry weights were recorded. Another half of the root samples were used for analysis of Fe plaque. The quartz sand inside the central compartment is referred to as the rhizosphere material. The soil from left and right compartments were combined and homogenized thoroughly, and is referred to as the non-rhizosphere soil.

2.4. Extraction of iron plaque

At harvest, Fe plaque on fresh root surfaces was extracted using dithionite-citrate-bicarbonate (DCB solution containing 0.03 M sodium citrate, 0.125 M sodium bicarbonate, with the addition of 0.6 g sodium dithionite) (Taylor and Crowder, 1983; Otter et al., 1991), or 1 M HCl. Roots of rice seedlings were immersed for 60 min at room temperature in 30 ml of DCB solution or 1 M HCl. They were then rinsed three times with deionised water that was added to the DCB or HCl extracts. The resulting solution was made up to 50 ml with deionised water. After extraction with DCB or HCl, roots were oven dried to constant weight at 80 °C for chemical analysis.

2.5. Chemical analysis

Dried plant materials were ground with stainless steel mill and samples weighed into clean, dry digestion tubes (100 ml). Concentrated HNO_3 (5 ml) was added and allowed to stand overnight. The tubes were placed on a digestor and the temperature was raised to 80 °C for 1 h, and then controlled at 120–130 °C for 20 h. After digestion, the solution was cooled to room temperature, diluted to 50 ml with deionised water and filtered into plastic bottles.

The concentrations of As in the acid digests of shoots were measured by atomic fluorescence spectrometer, and concentrations of Fe, Mn, S by ICP-MS. Except for S in DCB extract, the concentrations of As, Fe, Mn, S in the acid digests of roots, DCB and HCl extracts were analyzed by ICP-MS.

Total soil As was analyzed by ICP-MS after wet digestion in 4 M HCl. Soil organic C was determined by K_2CrO_7 and total N by the Kjeldahl method. Soil pH was determined by potentiometrically in a 1:2.5 suspension of soil and water. Plant-available S was extracted by 0.01 M $Ca(H_2PO_4)_2$, and determined by turbidimetry. Plant-available P was determined according to Olsen method (Lu, 1999). Soil properties are listed in Table 1.