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Influence of silicon treatment on antimony uptake and translocation in rice genotypes with different radial oxygen loss

Liping Zhang[a,1], Qianqian Yang[a,1], Shiliang Wang[a,b,*], Wanting Li[a], Shaoqing Jiang[c], Yan Liu[a]

[a] School of Geography and Tourism, Qufu Normal University, Rizhao 276826, China
[b] School of Environment, Tsinghua University, Beijing 100084, China
[c] Changshushi Middle School, Changshu 215500, China

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ABSTRACT

Antimony (Sb) pollution in soil may have a negative impact on the health of people consuming rice. This study investigated the effect of silicon (Si) application on rice biomass, iron plaque formation, and Sb uptake and speciation in rice plants with different radial oxygen loss (ROL) using pot experiments. The results demonstrated that Si addition increased the biomass of straw and grain, but had no obvious impact on the root biomass. Indica genotypes with higher ROL underwent greater iron plaque formation and exhibited more Sb sequestration in iron plaque. Silicon treatments increased iron levels in iron plaque from the different genotypes but decreased the total Sb concentration in root, straw, husk, and grain. In addition, Si treatment reduced the inorganic Sb concentrations but slightly increased the trimethylantimony (TMSb) concentrations in rice straw. Moreover, rice straw from hybrid genotypes accumulated higher concentrations of TMSb and inorganic Sb than that from indica genotypes. The conclusions from this study indicate that Sb contamination in rice can be efficiently reduced by applying Si treatment and selecting genotypes with high ROL.

1. Introduction

Antimony (Sb) is an element in group VA. Although its concentration is relatively low in the natural environment, large quantities of Sb contaminants have been released into the environment by anthropogenic activities such as smelting, mining, shooting, and burning of fossil fuels (Filella et al., 2002; Wilson et al., 2010), which measurably increases the Sb concentration in soil. The increased risk of Sb toxicity has gained attention, especially in industrial zones, near roads, and at mining sites (Filella et al., 2002; Feng et al., 2013). Antimony is toxic to most organisms and is a potential carcinogen for humans (Filella et al., 2002). Therefore, antimony and its compounds have been listed as priority controlled contaminants by the European Union (EU) and the Environmental Protection Agency of the United States.

China possesses the largest amount of Sb ore reserves in the world, and Xikuangshan in the Hunan Province is the largest Sb mine in the world. Soil around Sb smelting and mining areas has been heavily polluted by Sb (He and Yang, 1999; He, 2007). Antimony is not necessary for plants, but it can be adsorbed from soil and accumulated by numerous plants. For example, the Sb concentration reached 1367 and 1105 mg/kg in the flowers and leaves of *Achillea ageratum*, respectively, and 1150 and 1164 mg/kg in the root and stems of *Silene vulgaris*, respectively, grown near the Sb mine (Baroni et al., 2000). The Sb concentration ranged from 3.92 to 143.69 mg/kg in plants from Sb mining and smelting areas in the Hunan Province (Qi et al., 2011). Therefore, a serious risk will ensue from Sb entering the food chain through plants growing in Sb-contaminated soil (Vaculík et al., 2013). Excess Sb in soil can retard root elongation and decrease plant biomass (Feng et al., 2013). Previous studies of seed germination and pot experiments indicated that both Sb(III) and Sb(V) measurably inhibited seed germination and root development, and the grain yield and biomass of rice also decreased with increasing Sb levels in soil (He and Yang, 1999). However, information about the tolerance and accumulation of Sb in plants is very limited, and few investigations have been conducted on the effect of Sb on plant physiology and metabolism.

Silicon (Si) is the second most abundant element in the Earth's lithosphere. This element is important and beneficial for the growth and development of plants. For example, it can increase the growth and yield of plants and improve their resistance to biotic and abiotic stresses (Ma and Yamaji, 2006). Previous studies have demonstrated that Si application to soil showed positive effects on plant growth in soil contaminated by various heavy metals and toxic elements, such as lead

* Corresponding author at: School of Geography and Tourism, Qufu Normal University, Rizhao 276826, China.
E-mail address: thuswang@163.com (S. Wang).
[1] Both authors contributed equally to this work.

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(Pb), cadmium (Cd), and arsenic (As) (Vatehová et al., 2012; Fleck et al., 2013). However, little information is known about the impact of Si on Sb toxicity in rice. To our knowledge, this is the first study to investigate the effects of Si application to soil on the Sb toxicity in rice plants.

So as to acclimate the oxygen deficient environment, adventitious roots of some wetland plants such as rice (*Oryza sativa* L.) have developed plentiful aerenchyma and induction of a barrier to radial oxygen loss (ROL), defined as the oxygen transfer from aerenchyma to the rhizosphere (Armstrong, 1979). ROL is important and essential for the detoxification of phytotoxins (McDonald et al., 2001). ROL of rice roots was relevant to As tolerance and accumulation in rice (Wu et al., 2011). In addition, some studies have verified that the rhizosphere oxygenation induced by microbial activities and the plant roots oxygenation induced by ROL can convert Fe^{2+} to Fe^{3+}, which lead to the iron plaque formation on the root surface (Colmer, 2003). As the main components, ferric hydroxides, goethite, and minor concentrations of siderite accounts for 63%, 32%, and 5% of the iron plaque, respectively (Liu et al., 2004). Therefore, the structure of iron plaque is characterized as amorphous or crystalline iron (oxyhydr) oxides (Liu et al., 2004). Some studies have found that iron plaque can sequester metalloids (e.g. As and Sb), metals, and anions including silicate and carbonate on rice roots (Liu et al., 2004; Liu and Zhu, 2005). Studies have demonstrated that iron plaque has an important effect on As accumulation and toxicity in rice plants (Ultra et al., 2009; Wu et al., 2012). Iron plaque serves as a barrier to prevent As translocation from roots to shoots (Liu et al., 2014). Some previous studies investigate the impact of ROL on As tolerance and uptake (Wu et al., 2011) and the impact of Si on As uptake by rice plants (Li et al., 2009b; Seyfferth and Fendorf, 2012), but investigation on the impact of Si on Sb accumulation and speciation of rice genotypes with different ROL is very limited.

The aim of this study is to investigate the effect of Si on iron plaque formation on the root surface of different genotypes of rice, on Sb sequestration by iron plaque, and on Sb distribution and accumulation in different genotypes of rice.

2. Materials and methods

2.1. Preparation and treatment of rice seeds

Four genotypes of rice (*O. sativa* L.), including hybrid subspecies Xiangfengyou9 (HX9) and T-you207 (HT207) and indica subspecies Xiangwanxian17 (IX17) and Xiangwanxian12 (IX12), were used in our study. According to the results of a previous study, the radial oxygen loss (ROL) of HX9, HT207, IX17, and IX12 were 9.55, 15.4, 19.7, and 27.0 μmol O_2/g root dry weight/h, respectively (Wu et al., 2015). Seeds from these four rice genotypes were purchased from the Chinese Academy of Agricultural Sciences. After disinfection in a hydrogen peroxide (H_2O_2) solution (30%, w/w) for 20 min, the rice seeds were washed by deionized water, germinated in moist perlite, and then cultured for 15 days in a PVC pot (14 cm in height and 7.5 cm in diameter) with 500 mL of nutrient solution (containing macronutrients (in mM) including KH_2PO_4, 1.3; $MgSO_4{\cdot}7H_2O$, 1.5; $CaCl_2$, 4.00; K_2SO_4, 2.0; NH_4NO_3, 5.0; and micronutrients (in μM) including $Na_2MoO_4{\cdot}2H_2O$, 0.5; H_3BO_4, 10.0; Fe(II)-EDTA, 50.0; $ZnSO_4{\cdot}7H_2O$, 1.0; $CuSO_4{\cdot}H_2O$, 1.0; $CoSO_4{\cdot}7H_2O$,0.2; $MnSO_4{\cdot}H_2O$, 5.0). During the experiment, the nutrient solution was changed twice per week, and the pH of the solution was adjusted to 5.5 with KOH and HCl.

2.2. Pot experiments

The soil sample used in this study was the surface (0–20 cm depth) soils of a paddy field near the mine area of Xikuangshan, located in Lengshuijiang, Hunan Province, China. It had a pH of 6.8 and contained 13.6 mg/kg total Sb. In this study, based on the standard BCR protocol of the European Commission (Rauret et al., 1999), sequential extraction of the soil sample used in this study was carried out, and the soluble and exchangeable, reducible, oxidizable, and residual fractions of Sb in soil were sequentially and selectively extracted, and the concentrations of these four fractions were 0.92, 2.26, 3.25, and 7.17 mg/kg, respectively.

The collected soil samples were taken to the laboratory and air-dried at room temperature, then slightly ground and sieved to < 2 mm. Fertilizers (including P as $CaH_2PO_4{\cdot}H_2O$ (at 0.15 g/kg P_2O_5), K as KCl (at 0.20 g/kg K_2O), and N as CO $(NH_2)_2$ (at 0.20 g/kg N)) were thoroughly mixed with the above soil samples for the growth of rice seedlings. Antimony solution (potassium hexahydroxoantimonate (V): $KSb(OH)_6$) were applied at 50 mg/kg to all treatments with exception of the control. Silicon was then added as a SiO_2 gel. All treatments with Sb and Si addition in this study were as follows: Control, no Si and no Sb addition; Treatment A, Sb only (Si0); Treatment B, Sb and 10 mg Si/kg (Si10); Treatment C, Sb and 20 mg Si/kg (Si20); Treatment D, Sb and 50 mg Si/kg (Si50).

All treatments were mixed thoroughly and equilibrated for 20 days. A series of polyethylene pots (20 cm in height, 20 cm in diameter) were filled with 5.0 kg of soil. Three rice seedlings were planted per pot. All treatments were conducted in triplicate. Under the waterlogged conditions of 2 cm water depth above the soil surface, the rice seedlings in the pots were grown in a greenhouse (with a photoperiod of 12 h light (25 °C)/12 h night (20 °C) and a relative humidity of 70%), and a sodium lamp (1200 PAR) supplied natural light. Rice plants were not harvested until the mature stage (90 days after rice seedings transplantation).

2.3. Extraction analysis of iron plaque

Iron plaque on the surface of the rice root was examined by dithionite-citrate-bicarbonate (DCB) extraction, as detailed by Liu et al. (2004). The DCB solution contained sodium citrate ($Na_3C_6H_5O_7{\cdot}2H_2O$, 0.03 M), sodium bicarbonate ($NaHCO_3$, 0.125 M), and sodium dithionite (NaS_2O_4, 0.06 M). Fresh root from the rice plants (1 g) was soaked in the DCB solution (30 mL) for 1 h at room temperature (25 °C). After that, the roots were rinsed three times with deionized water, and the rinsed water was added to the above DCB extracts. The final extraction solution was increased to 100 mL by the addition of deionized water prior to analysis. The Fe and Sb concentrations in the above DCB-extraction solutions were analyzed. The Fe concentration in the extract was determined by atomic absorption spectroscopy (AAS, TAS-990, Beijing Puxi Instruments Co., P.R. China). The antimony concentration was determined by hydride generation atomic fluorescence spectrometry (HG-AFS, AFS-8230, Beijing Haiguang Instruments Co., China).

2.4. Analysis of the total Sb in rice plants

After being harvested, the rice plants were washed cleanly and divided into root, straw, husk, and grain. These four sections were dried and crushed using a mechanical mill. Then, the milled sample (0.5 g) was weighed and put into a conical flask (100 mL) with 10 mL of concentrated nitric acid. The samples were digested using an electric hot plate (120 °C). After digestion, the solution was increased to 20 mL with ultrapure water. The Sb concentration in the digested solutions from the root, straw, husk, and grain was measured by hydride generation atomic fluorescence spectrometry (HG-AFS, afs-8230, Beijing Haiguang Instruments Co., China). A certified reference material (CRM) (bush twigs and leaves) was used for quality control, and the recovery ratios of Sb were in the range of 85–103%.

2.5. Analysis of Sb speciation

Cultivars (HX9 and IX12) with the highest and lowest ROL rates were selected to determine the Sb speciation. Milled samples (1.0 g) of straw were put into 50 mL centrifuge tubes and digested with nitric acid