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Influence of sulphur on arsenic accumulation and metabolism in rice seedlings

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ABSTRACT

The influence of sulphur on the accumulation and metabolism of arsenic in rice was investigated. Rice seedlings were grown in nutrient solutions with low sulphate (1.8 μM SO_4^{2-}) or high sulphate (0.7 mM SO_4^{2-}) for 12 or 14 d, before being exposed to 10 μM arsenite or arsenate for 2 or 1 d, respectively. In the arsenite exposure treatment, low sulphate-pretreated rice accumulated less arsenite than high sulphate pretreated plants, but the arsenite concentrations in shoots of low sulphate pretreated rice were higher than those of high sulphate pretreated. In the arsenate exposure treatment, the low sulphate pre-treatments also resulted in less arsenite accumulation in rice roots. Sulphur deprivation in nutrient solution decreased the concentrations of non-protein thiols in rice roots exposed to either arsenite or arsenate. The low sulphate-pretreated plants had a higher arsenic transfer factor than the high sulphate-pretreated plants. The results suggest that rice sulphate nutrition plays an important role in regulating arsenic translocation from roots to shoots, possibly through the complexation of arsenite-phytochelatins.

1. Introduction

Arsenic is a toxic environmental metalloid originating from anthropogenic activities and geogenic sources. Arsenic is not only phytotoxic but also carcinogenic to humans (Smith et al., 2002). Arsenic intake by humans mainly comes from drinking water and foods; the level of intake is elevated in the areas where water and/or soil are contaminated (Meharg and Rahman, 2003; Zhu et al., 2008b). For example, in Southeast Asia and China, where rice is the staple food, buildup of arsenic in paddy soil and irrigation water has led to elevated arsenic accumulation in rice grain, which may pose a potential risk to human health (Meharg and Rahman, 2003; Meharg, 2004; Liao et al., 2005; Zhu et al., 2008a,b). Even at background levels of arsenic, rice grain and straw still accumulate relatively high levels of arsenic compared with other cereal crops (Williams et al., 2007). Therefore, it is urgent to understand the mechanism of arsenic accumulation and translocation in rice in order to counteract the problem of arsenic contamination in rice.

Arsenate enters root cells via phosphate transporters and arsenite via aquaporins (Meharg and Hartley-Whitaker, 2002; Meharg and Jardine, 2003; Ma et al., 2008; Zhao et al., 2009). Plant roots reduce arsenate to arsenite rapidly by an arsenate reductase (Bleeker et al., 2006; Dhankher et al., 2006; Duan et al., 2007). Arsenite in roots may be detoxified through efflux to the external medium (Xu et al., 2007) or by chelation with thiol (SH)-containing compounds (Raab et al., 2005). The principal chelators for arsenite in plants are phytochelatins, which are synthesized from reduced glutathione by the enzyme phytochelatin synthase (Clemens et al., 1999) under the stress of arsenic or heavy metals such as Cd and Pb. Reduced glutathione is not only a substrate for PCs synthesis but is also a reductant for enzymatic or nonenzymatic reduction of arsenate to arsenite (Duan et al., 2005; Bleeker et al., 2006; Dhankher et al., 2006).

Upon exposure to arsenate, a number of genes involved in sulphate transport and sulphur metabolism were up-regulated in rice (Norton et al., 2008). The concentrations of reduced glutathione and phytochelatins in wheat dropped dramatically under the conditions of sulphur deprivation (McMahon and Anderson, 1998). It is therefore hypothesized that the sulphate supply influences arsenic accumulation and metabolism in rice.

2. Materials and methods

2.1. Plant culture

Seeds of rice (*Oryza sativa* L. cv Jiahua 1) were surface sterilized in 10% H_2O_2 (w/w) for 15 min, washed with tap water then deionized water thoroughly, and germinated in moist perlite. After germination, uniform seedlings were transferred to a 7-l container with 1/6th strength macronutrients and 1/4th strength micronu-

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trients (Hewitt, 1966; Liu et al., 2004). The composition of the full nutrient solution was 5.0 mM NH_4NO_3, 2.0 mM K_2SO_4, 4.0 mM $CaCl_2$, 1.5 mM $MgSO_4$, 1.3 mM KH_2PO_4, 50 μM $FeNa_2$-EDTA, 10 μM H_3BO_3, 1.0 μM $ZnSO_4$, 1.0 μM $CuSO_4$, 5.0 μM $MnSO_4$, 0.2 μM $CoSO_4$ and 0.5 μM Na_2MoO_4 (pH adjusted to 5.5 with KOH or HCl solutions). Nutrient solution was renewed every 3 d. Plants were grown in a growth room with a 14 h light period (260–350 $\mu E\ m^{-2}\ s^{-1}$), a relative humidity of 60–70% and 28 °C/20 °C day/night temperatures.

Rice seedlings (14-d old) were transferred to PVC pots (7.5 cm diameter and 14 cm height, one plant per pot) containing 1/5th strength macronutrients and 1/3th strength micronutrients with either low sulphate (1.8 μM SO_4^{2-}) or high sulphate (0.7 mM SO_4^{2-}). In the solution with low sulphate, sulphate salts used for major nutrients were replaced with chloride salts.

2.2. Plant treatments

The first set of experiments was designed to investigate the effects of sulphate supply and arsenic speciation on: (1) arsenic speciation and concentrations in roots and shoots and (2) the concentrations of non-protein thiols, phytochelatins and reduced glutathione in roots. After 14 d of pretreated with low sulphate or high sulphate, rice plants were conducted with eight treatments according to sulphate pre-treatments (low sulphate, high sulphate), species of arsenic exposure (arsenate, arsenite) and sulphate supply during arsenic exposure (low sulphate, high sulphate). Each treatment was replicated in four pots. Arsenate and arsenite were added to the solution at 10 μM as $Na_3AsO_4{\cdot}12H_2O$ or $NaAsO_2$, respectively, and the exposure lasted 24 h. Roots and shoots were washed with de-ionized water, blotted dry and weighed. Plant samples were frozen in liquid nitrogen and stored at −80 °C.

In the second set of experiments, arsenic speciation in roots, shoots and efflux solution as well as the concentrations of non-protein thiols in roots were investigated. After 12 d of pretreatment with low sulphate or high sulphate in nutrient solution, 24 rice plants were exposed to either 10 μM arsenite or arsenate, in the low sulphate or high sulphate nutrient solutions for 48 h (low sulphate pre-treatment: low sulphate + arsenite and low sulphate + arsenate; high sulphate pre-treatment: high sulphate + arsenite and high sulphate + arsenate). Each treatment was replicated in six pots. Arsenate and arsenite were added to the solution at 10 μM as $Na_3AsO_4{\cdot}12H_2O$ or $NaAsO_2$, respectively. The nutrient solutions with arsenic were renewed every 24 h to maintain the concentrations and speciation of arsenite or arsenate. After 48 h arsenic addition, 12 rice plants were placed in a vessel containing 25 ml ice-cold phosphate buffer (0.5 mM $Ca(NO_3)_2$, 5 mM MES, and 1 mM K_2HPO_4, pH 6.0) for 10 min, and then transferred to 25 ml normal nutrient solution for efflux experiment. Accumulative efflux time was 0.5, 1, 10, 60 and 180 min. The solution was filtered though 0.45 μm filters and kept in the dark on ice for arsenic species analysis. After efflux, the roots were washed, blotted dry and weighed.

The other twelve rice plants were harvested for the determination of arsenic speciation and concentrations, and non-protein thiols concentrations. Plant roots were washed with de-ionized water, then immersed in 25 ml ice-cold phosphate buffer (0.5 mM $Ca(NO_3)_2$, 5 mM MES, and 1 mM K_2HPO_4, pH 6.0) for 10 min. Tissues were quickly frozen in liquid nitrogen and lyophilized.

2.3. Analysis of thiol compounds

Roots and shoots were ground to a fine powder in a mortar and pestle with liquid nitrogen. Non-protein thiols were extracted by homogenization of the roots material (about 10 mg DW or 100 mg FW) in 2 ml of ice-cold 0.1% trifluoroacetic acid (v/v) containing 6.3 mM diethylenetriaminepentaacetic acid (pH < 1) with a mortar pestle and quartz sand and centrifuged at 10,000 × *g* for 10 min at 4 °C. An aliquot of the supernatant (200 μl) was mixed with 200 μl 1.8 mM 5,5′-dithio-2-nitrobenzoic acid containing 6.3 mM diethylenetriaminepentaacetic acid and 200 mM 4-(2-hydroxyethyl)piperazine-1-propanesulfonic acid (pH 7.8). After mixing the solutions imminently, the absorbance at 412 nm was read in Multiskan Spectrum (Spectra 190, Dynex Technologies, USA). Concentration of non-protein thiols is expressed as reduced glutathione equivalents (Sneller et al., 2000; Gasic and Korban, 2007).

Total glutathione, reduced glutathione and oxidized glutathione were determined with the reduced glutathione and oxidized glutathione Assay Kit (Beyotime, PR China). Colorimetric determination was conducted using a Multiskan Spectrum (Spectra 190, Dynex Technologies, USA). The concentration of total phytochelatins was calculated as phytochelatins = non-protein thiols – total GSH (Hartley-Whitaker et al., 2001).

2.4. Determination of arsenic speciation, total arsenic and phosphorus

Samples of the ground root (about 10 mg DW or 100 mg FW) and shoot (about 50 mg or 200 mg FW) material were extracted with 5 ml of 1% HNO_3 in a microwave accelerated reaction system (CEM Microwave Technology, USA). The extracts were filtered though 0.45 μm filters and kept in the dark on ice. Arsenic speciation in the efflux solutions and plant extracts were determined by high performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP–MS) (7500a, Agilent Technologies) (Zhu et al., 2008a).

Ground plant materials (root and shoot) were digested in 5 ml of $HNO_3/HClO_4$ (85/15, v/v) on a heating block (Digestion Systems of AIM500, A. I. Scientific, Australia). The concentrations of arsenic were measured by an atomic fluorescence spectrometry (AF-610A, Beijing Ruili Analytical Instrument Co., Beijing, China). The phosphorus concentrations were determined on an inductively coupled plasma-optical emission spectrometer (ICP-OES, Optima 2000 DV, PerkinElmer, USA). A reagent blank and standard reference plant material (GBW10016 from the National Research Center for Standard Materials in China) were included to verify the accuracy and precision of the digestion and subsequent analytical procedures.

2.5. Data analysis

Transfer factor was calculated as the ratio of shoot arsenic concentration to root arsenic concentration. One-way or two-way analysis of variance (ANOVA) followed by Duncan's multiple comparisons ($P < 0.05$) was performed to test the significance of treatment effects using SPSS16.0 for windows.

3. Results

3.1. Influence of sulphur on plant growth and phosphate accumulation

There were no significant differences in the fresh weights of roots between the two sulphate pre-treatments, whereas the shoot biomass in the pre-treatment with low sulphate was significantly lower than that in the high sulphate pre-treatment (Table 1). The phosphorus concentrations in rice roots exposed to arsenate were statistically lower than those exposed to arsenite. The low sulphate pre-treatment increased phosphorus concentration in both roots and shoots. No significant interactions between arsenic species and