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Is the effect of silicon on rice uptake of arsenate (As^V) related to internal silicon concentrations, iron plaque and phosphate nutrition?

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Arsenate uptake by rice seedlings is affected by both Si (internal and external) and iron plaque on root surface.

Abstract

Solution culture experiments were conducted to investigate the effects of silicon (Si) on arsenate (As^V) uptake by rice. The addition of Si to the pretreatment or uptake solution significantly decreased shoot and root As concentrations ($P < 0.001$ and $P < 0.05$). The presence of Si in the pretreatment or uptake solution also significantly decreased shoot P concentrations ($P < 0.001$). The data demonstrated that both internal and external Si inhibited the uptake of As and P. Results of As uptake kinetics showed that the mechanism of the effect of Si on arsenate uptake is not caused by direct competition for active sites of transporters with As. The effect of Si on As uptake was not entirely mediated through the effect of Si on P uptake. Although the addition of Si to pretreatment solutions still significantly decreased shoot and root As concentrations, the extent of reduction became smaller when rice roots were coated with iron plaque.

Keywords: Arsenate; Iron plaque; Kinetics uptake; Phosphate; Silicate

1. Introduction

Arsenic (As) is a widespread environmental contaminant posing serious health risks to humans and animals, and As-contaminated soils, sediments and water bodies are major sources of As contamination in food chains. Groundwater contamination with As is reported from many regions of the world with the most severe problems occurring in South-East Asia, such as Bangladesh, India and China (Wang and Jiang, 1994; Chen et al., 1995; Mandal et al., 1997; Nickson et al., 1998; Chowdhury et al., 1999). Contaminated groundwater is not only used for drinking water but also extensively used for irrigation. Long-term use of As-contaminated groundwater for irrigation has resulted in elevated soil As levels in agricultural soils (Ullah, 1998; Alam and Satter, 2000). Paddy rice is the staple food crop in South-East Asia and As concentration as high as 1.8 mg kg^{-1} has been recorded in rice grain produced in the As-affected areas in Bangladesh (Meharg and Rahman, 2003). A recent risk analysis suggests that consumption of rice with elevated arsenic may cause health problems (Williams et al., 2005). Unfortunately, there are currently no effective countermeasures to reduce the risks associated with soil As contamination.

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Although silicon (Si) has not been listed among the essential elements for higher plants, it is well known that Si is beneficial for healthy growth and development of many plant species, especially graminaceous plants such as rice (*Oryza sativa* L.) (Lian, 1976; Liang et al., 1994; Epstein, 1994, 1999). Si is also well known to be effective in alleviating abiotic stresses in higher plants (Hodson and Evans, 1995; Rogalla and Römheld, 2002; Liang et al., 2003, 2005). Our previous study demonstrated that addition of Si to the nutrient solution can decrease As uptake by rice seedlings (Guo et al., 2005). The mechanisms involved in the effect of Si on uptake of As are so far not clear. It has been reported that effects of Si on P uptake are somehow dependent on the external P concentrations in the nutrient solution, and are also influenced by the Si already present in the plants (Ma and Takahashi, 1989, 1990). Arsenic and phosphorus are both Group V_A elements, and have similar electron configurations and chemical properties (Asher and Reay, 1979; Meharg and Macnair, 1992), but it is not clear if changes in internal Si and P concentrations in uptake solution can also affect arsenate uptake by rice.

An additional complication with rice is that, in the field, roots are usually coated with iron plaque due to the release of oxygen or oxidants into the rhizosphere, which promotes the oxidation of ferrous to ferric iron, the latter being precipitated on the root surface (Bacha and Hossner, 1977; Chen et al., 1980; Liu et al., 2004, 2005). It has been shown that the presence of iron plaque interferes with the conventional P–As interactions in soil-plant systems (Chen et al., 2005). We therefore hypothesize that the presence of iron plaque may also interfere with Si–As interactions.

Application of Si- and P-fertilizers is a common practice in rice production systems in Southeast Asia, so for the purpose of developing countermeasures to reduce As transfer to rice plants, it seems essential to understand the interactions between Si, P and iron plaque with regard to As uptake by rice plants. Therefore, the objectives of the present study were to investigate: 1) the effect of Si pretreatments on As uptake by rice seedlings; 2) the effects of Si and the pretreatment of rice with Si on the kinetics of As uptake by rice roots; 3) the interactive effect of Si and P on As; and 4) the effect of iron plaque on the interaction between Si and As.

2. Materials and methods

2.1. Experiment I: effect of Si pretreatments on arsenic uptake

Seeds of rice (*Oryza sativa* L. cv. Weiyou 77) obtained from the Agricultural Department of Hunan province were sterilized in 10% (v/v) H_2O_2 for 10 min followed by thoroughly washing in deionized water, and then germinated on moist perlite. After three weeks the seedlings were removed from the perlite and washed carefully under tap water to remove any adhering particles. Uniform seedlings were selected and transplanted in PVC pots (7.5 cm diameter × 14 cm high, one seedlings per pot) containing 500 ml modified Hoagland's nutrient solution containing (in mM): NH_4NO_3, 1.68; K_2SO_4, 0.67; $MgSO_4$, 0.50; $CaCl_2$, 1.33; KH_2PO_4, 0.44; and (in μM), Fe (II)-EDTA, 25; $CuSO_4$, 0.5; $ZnSO_4$, 0.5; $MnSO_4$, 2.5; H_3BO_3, 5; Na_2MoO_4, 0.25; $CoSO_4$, 0.1; NaCl, 50. In addition to a control with no added Si, Si was added as $K_2SiO_3 \cdot nH_2O$ to the growth medium to yield final concentrations of 0.5 and 2 mM Si. These solutions were referred to as pretreatment solutions. After two weeks, rice seedlings were exposed to arsenic ($Na_3AsO_4 \cdot 12H_2O$, 13.3 μM As) with the same three silicon levels. Thus, treatments were in the following combination of pretreatment and Si concentration in uptake solution: 0 (pretreatment) to 0, 0.5 or 2 mM Si (uptake solution); 0.5 to 0 or 0.5 mM Si; and 2 to 0 or 2 mM Si, giving seven treatments all together. Potassium (as 0.4 M KCl solution) was added differentially to give all treatments the same K concentration introduced by potassium metasilicate in the medium. The rice seedlings were grown in a controlled environment greenhouse with ambient light (roughly 13 h day/11 h night, 500–1100 μmol m^{-1} s^{-1}). Temperature fluctuation in the greenhouse was between 20 and 35 °C at day and night. The relative humidity was 70%. Nutrient solutions were renewed twice a week, and the pH was adjusted to 5.5 using 0.1 M KOH or HCl. There were three replicates in a fully randomized design and the pots were re-randomized every day during the growth period. The rice seedlings were harvested after three more weeks.

2.2. Experiment II: effects of Si treatments on kinetics of Arsenate uptake by rice roots

The cultivation method of rice seedlings was the same as described above. After growing three weeks in perlite, uniform seedlings were selected and transplanted to PVC pots (one seedling each pot) containing 500 ml modified Hoagland's nutrient solution. The seedlings were allowed to grow in a growth chamber with 14/10 h light/dark cycle. The light intensity was 280 μmol m^{-2} s^{-1}. Three weeks after transplanting, rice seedlings were grown with or without silicon ($K_2SiO_3 \cdot nH_2O$, 1 mM Si) in the same nutrition solution as the pretreatment. Potassium (as 2 M KCl solution) was added to give all treatments the same K concentration. After a further two weeks, roots were excised at the basal node and used to measure kinetics of As uptake. Care was taken to ensure the roots were exposed to air for a minimal period.

Roots were excised and incubated in deionized water for 20 min at room temperature and were then transferred to conical flasks containing 1000 ml test solution, and incubated for further 30 min according to Meharg and Jardine (2003) and Chen et al. (2005). The test solutions with different concentrations of As were 1.5, 3.0, 7.5, 15.0, 30.0 and 75.0 μM. The test solution contained an additional 1 mM Si ($K_2SiO_3 \cdot nH_2O$) or no Si. A stock solution of As was prepared from sodium arsenate ($Na_3AsO_4 \cdot 12H_2O$). All test solutions contained 5.0 mM 2-(*N*-morpholin) ethansulfonic acid (MES) and 0.5 mM $Ca(NO_3)_2$ with the pH adjusted to 6.5 using KOH or HCl. Finally, the roots were thoroughly rinsed with an ice-cold solution containing 1 mM K_2HPO_4, 5 mM MES and 0.5 mM $Ca(NO_3)_2$ for 20 min to remove the As species in the free space of the roots. The roots were oven-dried at 70 °C for 72 h, weighed, and analyzed.

2.3. Experiment III: effect of silicon on As uptake with different P concentrations in the nutrient solution

Seedlings (three weeks old) prepared as described above were transferred to PVC pots (one seedling per pot) containing 500 ml modified Hoagland's nutrient solution (pH 5.5). After 20 days, rice seedlings were exposed to 13.3 μM As as $Na_3AsO_4 \cdot 12H_2O$ with two levels of Si (0, 1 mM Si, as $K_2SiO_3 \cdot nH_2O$) and 4 levels of P (6.5, 13, 130 and 260 μM). Potassium (as 2 M KCl solution) was added differentially to give all treatments the same K concentration in the medium. The seedlings were grown in a growth chamber with 14/10 h light/dark cycle. The light intensity was 280 μmol m^{-2} s^{-1}. The rice seedlings were harvested after further 18 days. The As and P concentrations in shoots and roots were determined as described in Section 2.5.

2.4. Experiment IV: a short-term As uptake (up to 24 h) by rice roots with or without iron plaque

Seedlings (three weeks old) prepared as described above were transferred to PVC pots (one seedling per pot) containing 500 ml modified Hoagland nutrient solution (pH 5.5). The solution culture experiment was performed in four stages. At stage I, all seedlings were grown in normal nutrient solution (−Si) for 20 days. At stage II, half of the seedlings were transferred to nutrient