Effect of external and internal phosphate status on arsenic toxicity and accumulation in rice seedlings

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Abstract

Phosphorus (P) deficiency is thought to exacerbate the arsenic (As) phytotoxicity in paddy rice. The experiments were conducted to investigate the effects of external phosphate supply on As accumulation in rice and its toxicity under phosphate deficiency conditions. Rice seedlings pretreated with a phosphorus deficient nutrient solution (–P) for 14 d accumulated more As than those pretreated with a normal phosphorus supply nutrient solution (+P). Rice pretreated with –P showed As toxicity symptoms after being exposed to 50 $\mu\text{mol/L}$ arsenate for 4 h, while +P rice did not show any toxicity symptoms. Arsenic toxicity symptoms can be alleviated by increasing external P concentrations. The arsenate uptake rate and accumulation corresponded with the As toxicity in rice plants. Arsenic concentrations in rice roots decreased with increasing external phosphate concentrations. The lowest As accumulation and the highest P accumulation were found when the external P concentration reached 100 $\mu\text{mol/L}$. In short, P deficiency increased the sensitivity of rice to arsenate and increasing of external phosphate supply could alleviate As toxicity.

Key words: phosphorus; arsenate; arsenic toxicity; arsenic accumulation; rice seedlings

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Introduction

Arsenic (As) is one of the worst cancer-causing chemicals which widely distributes in the environment from both natural and anthropogenic sources. Human beings can be exposed to As through their occupation, drinking water, and diet (ATSDR, 1993). The highest concentration of As was found in the seafood, followed by rice and rice products (Tao and Bolger, 1998). Rice is the staple food for the majority of population in South East Asia where has been found to have high levels of As in groundwater and soils. In large areas of Bangladesh, West Bengal and India, As-contaminated groundwater is usually used for irrigation of paddy soils (Abedin *et al.*, 2002; Chen *et al.*, 2004). In a typical Bangladeshi diet, it is estimated that rice can be a significant dietary source of inorganic As (Williams *et al.*, 2005, 2006). Therefore, rice As contamination is a serious problem, and it is urgent to develop strategies for minimizing As accumulation and toxicity in rice plants.

In contrast, as the chemical analogue of As, phosphorus (P) is one of 17 essential elements required for plant growth. However, P is frequently the most limiting element for plant growth and development. Crop yield on 30%–40% of the world's arable land is limited by P availability (Runge-Metzger, 1995; von Uexküll and Mutert, 1995). And, about 67% of the farm land in China was shown to be serious P deficient for crop growth. In addition, P is

low bioavailable because it rapidly transforms to insoluble complexes with cations, particularly with aluminum and iron under acid conditions (Vance *et al.*, 2003). Thus, P fertilization is the common agriculture strategy to improve crop yield, especially for rice. At present, 12 Asian countries have reported high As levels in part of their groundwater resources. Often these resources are used to irrigate paddy soil (Heikens, 2006). Therefore, it is important to understand As uptake and accumulation by rice plants when P fertilizer is supplied after P starvation.

Arsenate, the oxidized inorganic form of As, is taken up and translocated via the phosphate transporter systems (Asher and Reay, 1979; Lee, 1982; Ullrich-Eberius *et al.*, 1989; Meharg and Macnair, 1992). The competition of uptake and translocation between phosphate and arsenate was shown in lots of plants (Chen *et al.*, 2004; Liu *et al.*, 2004; Rosa *et al.*, 2006). Over-accumulation of shoot P is one of the mechanisms of plants to increase arsenate resistance. Kobayashi *et al.* (2005) used arsenate-sensitive and arsenate resistance mutants of the green alga to investigate the relationship between P accumulation and As resistance. They found that arsenate-resistance mutants had higher P contents than wild type and arsenate-sensitive mutants (Kobayashi *et al.*, 2005). The similar result was found using the *Arabidopsis ars1* mutant (Lee *et al.*, 2003). Furthermore, maintaining a greater ratio of P/As in the roots may also be one of the reasons for the high As tolerance of the hyperaccumulator *Pteris vittata* (Singh

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and Ma, 2006). These results confirmed that plant internal P status affect arsenate resistance. Fayiga *et al.* (2008) showed that it was possible to optimize plant As removal by adjusting nutrients in the growth medium. Phosphorus-fertilizer increased As uptake in *P. calomelanos* and *P. vittata*, which can be used as a cost-effective amendment for As polluted soils (Jankong *et al.*, 2007; Fayiga and Ma, 2006). However, so far, no experimental data have shown the effects of external fertilization on As uptake and accumulation by rice plants after P starvation.

In this study, experiments were conducted to investigate the effects of internal and external P on As resistance and As accumulation in rice seedlings. These results would provide some insights into the P-modulated As accumulation and toxicity in rice plants, and would be helpful in exploring P fertilization strategies for rice yield under the condition of both P deficiency and As contamination.

1 Materials and methods

1.1 Plant materials

Rice (*Oryza sativa* L. cv Jiahua 1) seeds were sterilized in 10% H₂O₂ solution for 15 min, then washed with de-ionized water. The seeds were germinated in moist perlite. After 3 weeks, uniform seedlings were transplanted to PVC pots (7.5 cm diameter and a height of 14 cm, one plant per pot) containing 500 mL nutrient solution, modified from Hewitt (1966). At the first week, 1/6 strength macronutrient and 1/4 strength micronutrient solution was used, then, 1/3 strength macronutrient and 1/2 strength micronutrient solution was used. Full-strength macronutrient composition was: 5.0 mmol/L NH₄NO₃, 2.0 mmol/L K₂SO₄, 4.0 mmol/L CaCl₂, 1.5 mmol/L MgSO₄, 1.3 mmol/L KH₂PO₄, and micronutrient composition was: 50 mmol/L Fe(II)-EDTA, 10 mmol/L H₃BO₄, 1.0 μmol/L ZnSO₄·7H₂O, 1.0 μmol/L CuSO₄·5H₂O, 5.0 μmol/L MnSO₄·H₂O, 0.5 μmol/L Na₂MoO₄·2H₂O, 0.2 μmol/L CoSO₄·7H₂O. The nutrient solution was renewed every 2 d and the pH was adjusted to 5.6 with KOH or HCl. The plants were grown in a growth room at a 14-h light period (260–350 μE/(m²·s); 28°C/20°C of day/night; 60% relative humidity). All the experiments were carried out with three replicates.

1.2 Effect of internal P status on As accumulation in rice seedlings

For this experiment, 6 pots of 7-week-old rice plants were selected. To get different internal P status, 3 pots were grown in normal nutrient solution, 3 pots were pretreated with P-free (–P) nutrient solution (KCl was added instead of KH₂PO₄ to compensate for the reduction in potassium concentration). After 14 d growing, all plants were exposed to 50 μmol/L arsenate in –P nutrient solution for 24 h. When the plants were harvested, they were thoroughly washed with tap water, and then with de-ionized water, adhering water was then removed with filter paper. Root and shoot of each plant was separated and oven-dried at 70°C for 2 d, and dry weight was determined.

1.3 Effect of external P concentrations on As toxicity in rice seedlings

In this experiment, 12 pots of 7-week-old rice seedlings were selected. Rice seedlings were exposed to P-free (–P) nutrient solution for 14 d, and then were treated with 50 μmol/L arsenate and re-supplied with different phosphate concentrations (0, 25, 50, 100 μmol/L) at the same time. After 24 h, the plants were harvested as described above.

1.4 As and P uptake curves under different external P concentrations

Rice seedlings were pre-treated with –P nutrient solution for 14 d, and then were exposed to 50 μmol/L arsenate with 0, 25, 50, 100 μmol/L phosphate re-supplying, respectively, for 24 h. The plant growth medium 1 mL was sampled for P and As analysis at time intervals of 0, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h. Water losses through transpiration were compensated by the addition of de-ionized water at hourly intervals according to Zhao *et al.* (2002).

1.5 Total As and P concentrations analysis

Oven-dried plant materials were digested in nitric acid on a heating block (Digestion Systems of AIM500, A. I. Scientific, Australia), and the temperature was at 100°C for 1 h, then at 120°C for 60 h. Reagent blank and standard reference (GBW07605, from the National Research Center for Standard Materials in China) were used to verify the precision of analytical procedures. The concentrations of As were measured by an atomic fluorescence spectrometry (AF-610A, Beijing Ruili Analytical Instrument Co., Beijing, China). The concentrations of P were measured by inductively coupled plasma optical emission spectrometer (ICP-OES, Optima 2000 DV, Perkin-Elmer, USA).

1.6 Statistical analysis

Analysis of variance (ANOVA) on concentrations of nutrients was performed using SPSS 11.5 for windows. Curve fitting was performed by using Sigma Plot 9.0 (Jandel Scientific, Erkrath, Germany).

2 Results and discussion

2.1 Effect of internal P status on As toxicity in rice seedlings

The pre-experiment has shown that P deficiency for 14 d could decrease root and shoot P concentrations to 2.4 and 3.2 mg/g, closed to P deficiency critical level (1.8–2.6 mg/g) determined by Fageria (1976) for rice. Therefore, the rice seedlings were pre-treated with –P and +P nutrient solutions for 14 d, and then were exposed to 50 μmol/L arsenate in –P nutrient solutions for 24 h.

As shown in Fig. 1a, –P pretreated rice plants accumulated more As than +P ones when exposed to 50 μmol/L arsenate in –P nutrient solution for 24 h. Furthermore, –P pretreated rice plants were more sensitive to arsenate than +P ones. The –P rice showed acute As toxicity symptoms after exposing to 50 μmol/L arsenate 4 h, while +P pretreated rice did not show any As toxicity symptoms

Fig. 1 Arsenic accumulation in rice seedlings (a) and arsenic toxicity symptoms of rice (b) after being pre-treated with -P and +P nutrient solution for 14 d, and then exposed to 50 $\mu\text{mol/L}$ arsenate for 24 h in -P nutrient solution. Data are means $\pm$ SE ($n = 3$).

after exposure 24 h (Fig. 1b). Geng *et al.* (2006) also reported that 9 d P-deprived rice showed acute As toxicity symptoms after exposed to 26.7 $\mu\text{mol/L}$ arsenate 4 h, while +P pre-treatment rice had no As toxicity symptoms. Arsenate is taken up via the phosphate transporter (Meharg and Macnair, 1992) and high-affinity phosphate transporter will be induced under phosphate deficiency conditions (Vance *et al.*, 2003; Schachtman and Shin, 2007). Therefore, more arsenate was taken up through high-affinity phosphate transporter by -P pre-treated rice plants under phosphate deficiency conditions, and then showed acute As toxicity symptoms.

2.2 External P addition alleviated As toxicity of rice seedlings

In order to find out whether increasing of external phosphate concentrations can alleviate As toxicity symptoms caused by As exposure under low internal P status, rice seedlings grew in -P nutrient solution for 14 d were exposed to 50 $\mu\text{mol/L}$ arsenate with different phosphate concentrations in the nutrient solutions. Table 1 shows that there was no difference in dry weight of rice roots and shoots under different phosphate concentrations, while there was difference in fresh weight. Fresh weight of rice roots and shoots without phosphate supply was lower than the other treatments and the treatment with 100 $\mu\text{mol/L}$ phosphate supply had the highest fresh weight in shoots (Table 1). These results corresponded with As toxicity symptoms in rice seedlings. Rice seedlings with 0, 25, and 50 $\mu\text{mol/L}$ phosphate in nutrient solution showed As

toxicity symptoms (leaves curled and losing water), while rice with 100 $\mu\text{mol/L}$ phosphate supply did not show any toxicity symptoms (Fig. 2).

Arsenic concentrations were determined in rice plants with different external phosphate supply. Figure 3a shows that As concentrations in rice roots decreased significantly with increasing external P concentrations in nutrient solution, while As concentrations in rice shoots kept unchanged until external P concentration reached 100 $\mu\text{mol/L}$. Furthermore, As accumulation decreased significantly when external phosphate concentration reached 50 $\mu\text{mol/L}$ and P accumulation increased significantly when

Fig. 2 Arsenic toxicity symptoms of rice which were pre-treated with -P nutrient solution for 14 d, and then exposed to 50 $\mu\text{mol/L}$ arsenate with 0, 25, 50, and 100 $\mu\text{mol/L}$ phosphate re-supplying, respectively.

Table 1 Dry and fresh weight of rice roots and shoots pre-treated with -P nutrient solution

		Treatment			
		+P 0 $\mu\text{mol/L}$	+P 25 $\mu\text{mol/L}$	+P 50 $\mu\text{mol/L}$	+P 100 $\mu\text{mol/L}$
Dry weight	Root (g)	0.22 $\pm$ 0.03 a	0.26 $\pm$ 0.01 a	0.23 $\pm$ 0.01 a	0.24 $\pm$ 0.03 a
	Shoot (g)	0.72 $\pm$ 0.02 a	0.78 $\pm$ 0.03 a	0.81 $\pm$ 0.06 a	0.81 $\pm$ 0.09 a
Fresh weight	Root (g)	2.13 $\pm$ 0.10 a	2.72 $\pm$ 0.16 a	2.48 $\pm$ 0.16 a	2.71 $\pm$ 0.31 a
	Shoot (g)	2.40 $\pm$ 0.12 b	3.12 $\pm$ 0.10 ab	3.54 $\pm$ 0.32 ab	4.21 $\pm$ 0.57 a

The rice roots and shoots were pre-treated with -P nutrient solution for 14 d, and then exposed to 50 $\mu\text{mol/L}$ arsenate with 0, 25, 50, and 100 $\mu\text{mol/L}$ phosphate re-supplying, respectively.

Data are means $\pm$ SE ($n = 3$), treatments with the different letter (a, b) are significantly different ($P < 0.05$).

external phosphate concentration reached 100 $\mu\text{mol/L}$ (Fig. 3b). These results support the observations that arsenate competes uptake transporter with phosphate and increasing of external phosphate can alleviate As toxicity (Abedin *et al.*, 2002; Wang *et al.*, 2002; Geng *et al.*, 2005). However, P-fertilizer increased As uptake in *P. calomelanos* and *P. vittata* (Jankong *et al.*, 2007; Fayiga and Ma, 2006). This result possibly caused by the enhanced mobility of As in the soil which occurred by the replacement of As by P from the soil binding site as a competitive anion exchange (Smith *et al.*, 2002). Fitz and Wenzel (2002) have reported that the effects of P on the uptake and toxicity of As in plants depended on plant species, chemical speciation of As, growth medium and the experimental conditions. Therefore, the difference between our result and Jankong's may be caused by different plant species and growth medium.

2.3 Effect of external P concentrations on As and P uptake curves by rice seedlings

Rice seedlings showed As toxicity symptoms at different time with different phosphate re-supplying when exposed to 50 $\mu\text{mol/L}$ arsenate. In $-P$ nutrient solutions, acute As toxicity symptoms (shoot curled) appeared after 4 h

exposure; in $+P$ 25 and $+P$ 50 $\mu\text{mol/L}$ treatments, the toxicity symptoms were found after As exposure for 12 h; and in $+P$ 100 $\mu\text{mol/L}$ treatment, no toxicity symptom was found after 24 h As exposure. These results indicate that external phosphate may affect As uptake speed as well as accumulation in rice plants. In order to identify this hypothesis, time-dependent As and P uptake rate was measured.

Figure 4a shows the cumulative uptake of arsenate, expressed on a basis of rice roots fresh weight. The rate of arsenate accumulation by roots decreased significantly with increasing of phosphate concentrations in the nutrient solution. In $+P$ 0 $\mu\text{mol/L}$ treatment, rice seedlings accumulated arsenate rapidly, at 4 h, the curve reached the saturated point (0.12 mg/g root fw). In $+P$ 25 $\mu\text{mol/L}$ and $+P$ 50 $\mu\text{mol/L}$ treatments, rice seedlings accumulated arsenate slowly, at 12 h, the curves reached the saturated point (0.16 and 0.12 mg/g root fw, respectively). In $+P$ 100 $\mu\text{mol/L}$ treatment, arsenate accumulation rate was similar to $+P$ 25 and $+P$ 50 $\mu\text{mol/L}$ treatments, but 4 h, the curve reached the saturated point (0.05 mg/g root fw). The As uptake rate had no significant difference between $+P$ 0, 25, and 50 $\mu\text{mol/L}$ treatments, while decreased significantly in $+P$ 100 $\mu\text{mol/L}$ treatment after 24 h arsenate exposure

Fig. 3 Arsenic concentrations in rice roots and shoots (a), and arsenic and phosphorus accumulation in the whole rice plants (b). The rice roots and shoots were pretreated with the same as in Table 1. Data are means $\pm$ SE ($n = 3$).

Fig. 4 Time-dependent arsenic (a), and phosphate (b) uptake curves of rice. The rice roots and shoots were pre-treated with the same as in Table 1. Data are means $\pm$ SE ($n = 3$).

(Fig. 4a). These results were confirmed by As content in the whole rice plants (Fig. 3b). Arsenic content in +P 0 $\mu\text{mol/L}$ and +P 25 $\mu\text{mol/L}$ rice had no difference, while +P 100 $\mu\text{mol/L}$ rice got the lowest As content. These results also corresponded with As toxicity symptoms (Fig. 2).

Figure 4b shows the accumulation curves of phosphate in rice roots with different concentrations of phosphate supply. The rate of phosphate accumulation increased with increasing of external phosphate concentrations and was approximately linear ($R = 0.991, 0.995$, and 0.994 for +P 25, 50, and 100 $\mu\text{mol/L}$ treatments, respectively) in the first 12 h (Fig. 4b). The uptake rate of P and As was compared when external P and As concentration was the same (50 $\mu\text{mol/L}$). The result showed that phosphate uptake rate was higher than As uptake rate. This result suggests that P possibly is preferentially taken up by P/As transporters compared to its toxic analogue (Meharg and Macnair, 1994). Wang *et al.* (2002) also reported that phosphate had a higher affinity to the uptake system in *P. vittata* root than arsenate.

3 Conclusions

Rice seedlings with lower internal P status were more sensitive to arsenate. Increasing of external phosphate concentrations can alleviate As toxicity symptoms through decreasing uptake of arsenate and over-accumulation of internal P.

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