Environmental and Experimental Botany xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Influence of sulphur on arsenic accumulation and metabolism in rice seedlings

Jing Zhang[a,b], Quan-Zhi Zhao[a], Gui-Lan Duan[b,*], Yan-Chao Huang[b]

[a] Henan Key Laboratory of Regulation and Control of Crop Growth and Development, Henan Agricultural University, Zhengzhou 450002, China
[b] Department of Soil Environmental Sciences, Research Center for Eco-environmental Sciences, Chinese Academy of Sciences, 18 Shuangqing Road, Haidian District, Beijing 100085, China

ARTICLE INFO

Article history:
Received 27 November 2009
Received in revised form 11 May 2010
Accepted 13 May 2010

Keywords:
Sulphur
Arsenic speciation
Arsenite
Arsenate
Rice (*Oryza sativa*)

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-

* Corresponding author. Tel.: +86 10 62849158; fax: +86 10 62923563.
E-mail address: duangl@rcees.ac.cn (G.-L. Duan).

0098-8472/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.envexpbot.2010.05.007

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 μE 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

Please cite this article in press as: Zhang, J., et al., Influence of sulphur on arsenic accumulation and metabolism in rice seedlings. Environ. Exp. Bot. (2010), doi:10.1016/j.envexpbot.2010.05.007

Table 1
Biomass and phosphorus concentrations in rice roots and shoots which were exposed to 10 μM arsenite or 10 μM arsenate in low sulphate or high sulphate nutrient solutions for 24 h after pre-treatment with low sulphate or high sulphate nutrient solutions for 14 d. S: sulphur, LS: low sulphate, HS: high sulphate, AsIII: arsenite, AsV: arsenate, P: phosphorus. Data are means ± SE (n = 4). Different letters indicate significant differences within the same column (P < 0.05).

S pre-treatment	Treatment	Biomass (g plant^{-1} FW)		P concentration (mg g^{-1} FW)	
		Root	Shoot	Root	Shoot
LS	LS + AsIII	0.66 ± 0.05 a	0.94 ± 0.07 b	0.62 ± 0.02 abc	1.42 ± 0.06 a
	HS + AsIII	0.60 ± 0.02 a	0.89 ± 0.01 b	0.63 ± 0.01 a	1.37 ± 0.02 ab
	LS + AsV	0.71 ± 0.02 a	1.01 ± 0.04 ab	0.55 ± 0.01 d	1.42 ± 0.05 a
	HS + AsV	0.65 ± 0.02 a	0.96 ± 0.02 b	0.63 ± 0.02 ab	1.34 ± 0.05 abc
HS	LS + AsIII	0.69 ± 0.02 a	1.41 ± 0.04 a	0.58 ± 0.01 cd	1.29 ± 0.01 bc
	HS + AsIII	0.66 ± 0.06 a	1.20 ± 0.14 ab	0.59 ± 0.02 bcd	1.35 ± 0.02 abc
	LS + AsV	0.73 ± 0.08 a	1.60 ± 0.23 a	0.57 ± 0.01 d	1.24 ± 0.02 c
	HS + AsV	0.65 ± 0.05 a	1.33 ± 0.14 a	0.57 ± 0.01 d	1.25 ± 0.01 bc
Analysis of variance					
Arsenic species		0.382	0.286	0.028	0.126
Sulphate		0.485	0.001	0.001	0.006
Arsenic × sulphate		0.767	0.981	0.314	0.288

sulphate treatment on the biomass and phosphorus concentrations of roots and shoots were detected.

3.2. Influence of sulphur on arsenic accumulation

Fig. 1 shows arsenic speciation and concentrations in roots and shoots of rice plants pretreated with low sulphate or high sulphate for 14 d, and then exposed to arsenite or arsenate with low sulphate or high sulphate supply for 24 h. No methylated arsenic species were detected in the rice plants. The low sulphate pre-treatment had a significant effect on the concentrations of rice arsenite accumulation in either arsenite or arsenate treatment (Fig. 1A; Table 4). After low sulphate pre-treatments, the high sulphate treatments had higher root arsenite concentrations than the low sulphate treatments, either expose to arsenite or arsenate. No significant root arsenate concentrations differences were found among the four +arsenate treatments, as well as among the +arsenite treatments (Fig. 1A). After high sulphate pre-treatments, No significant root arsenite or arsenate concentrations differences were found high or low sulphate treatments. After the low sulphate pre-treatments, arsenate concentrations in roots were higher than arsenite concentrations, both in the low sulphate + arsenite and the high sulphate + arsenite treatments, in other treatments, arsenate concentrations in roots were lower than arsenite concentrations (Fig. 1A). In shoots, low sulphate pre-treatment plants had higher arsenite concentrations than those of high sulphate pre-treatment, either in low sulphate + arsenite or in high sulphate + arsenite treatments (Fig. 1B). After low sulphate pre-treatments, shoot arsenite concentrations of low sulphate + arsenate treatments were higher than those of high sulphate + arsenate. In the plants exposed to arsenate, the low sulphate pre-treatments decreased the proportion of arsenite in the roots significantly but had no significant effect on arsenic speciation in the shoots.

3.3. Influence of sulphur on thiols accumulation

Sulphur supply showed a significant effect on the concentrations of non-protein thiols, phytochelatins and reduced glutathione in roots (Fig. 2; Table 4). Reduced glutathione concentrations in roots also showed significant differences in response to arsenic species and arsenic species × sulphate supply interactions (Table 4). After high sulphate pre-treatment, the treatments of high sulphate + arsenite had the highest root reduced glutathione concentrations, and there were no significant root reduced glutathione concentrations difference among the other treatments (Fig. 2B).

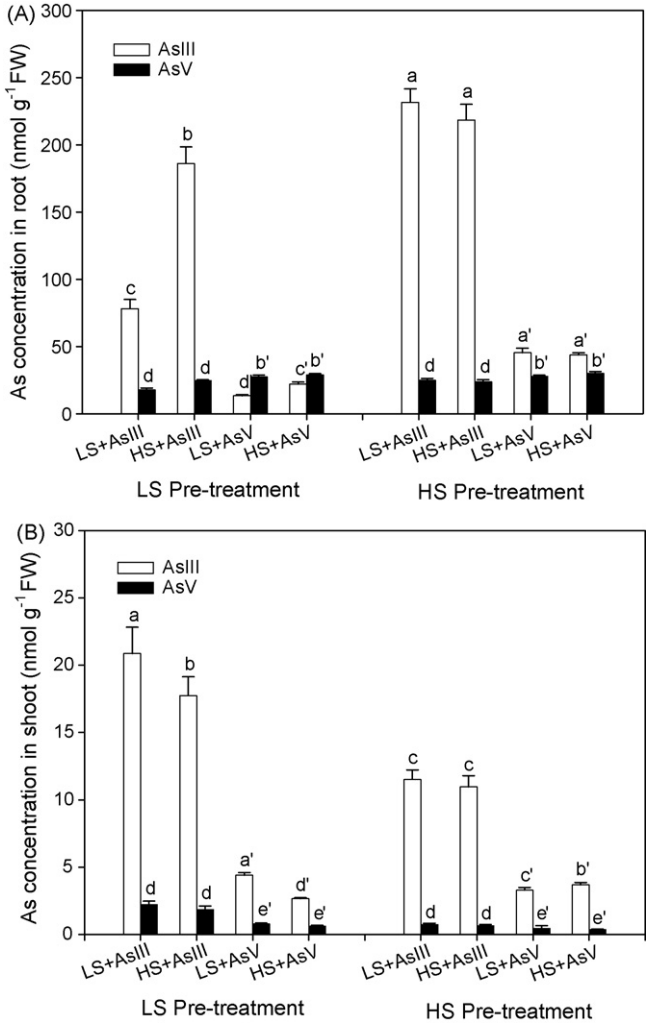

Fig. 1. Concentrations of arsenite and arsenate in rice roots (A) and shoots (B) which were exposed to 10 μM arsenite or 10 μM arsenate in low sulphate or high sulphate nutrient solutions for 24 h after pre-treatment with low sulphate or high sulphate nutrient solutions for 14 d. LS: low sulphate, HS: high sulphate, AsIII: arsenite, AsV: arsenate. Data are means ± SE (n = 4). Bars of arsenite and arsenate with the same letter are not significantly different (P < 0.05); small letters: +AsIII, small letters′: +AsV.

Please cite this article in press as: Zhang, J., et al., Influence of sulphur on arsenic accumulation and metabolism in rice seedlings. Environ. Exp. Bot. (2010), doi:10.1016/j.envexpbot.2010.05.007

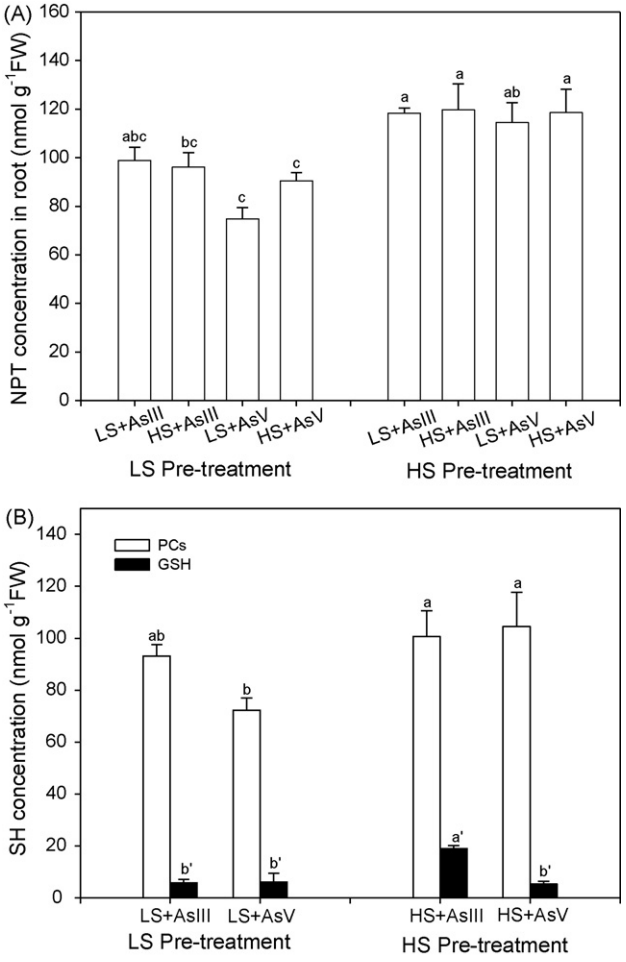

Fig. 2. Concentrations of non-protein thiols (A), phytochelatins and reduced glutathione (B) in rice roots which were exposed to 10 μM arsenite or 10 μM arsenate in low sulphate or high sulphate nutrient solutions for 24 h after pre-treatment with low sulphate or high sulphate nutrient solutions for 14 d. LS: low sulphate, HS: high sulphate, AsIII: arsenite, AsV: arsenate, NPT: non-protein thiols, PCs: phytochelatins, GSH: reduces glutathione. Data are means ± SE ($n = 3$). Bars of thiols with the same letter are not significantly different ($P < 0.05$); small letters: PCs, small letters′: GSH.

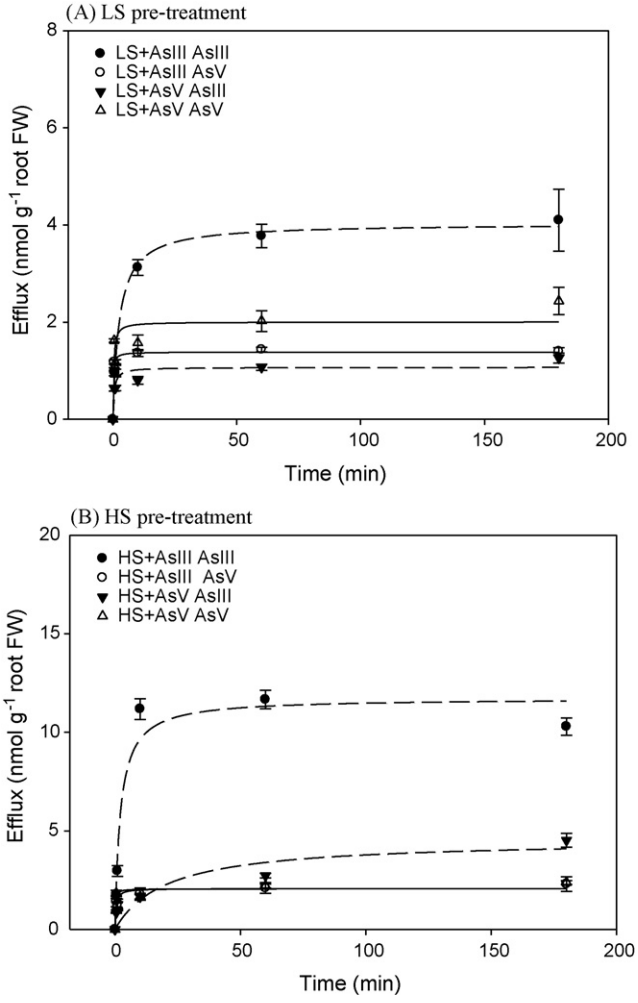

Fig. 3. Efflux of arsenite and arsenate to high sulphate nutrient solution of rice roots which were exposed to 10 μM arsenite (A) or 10 μM arsenate (B) in low sulphate and high sulphate nutrient solutions for 48 h after pre-treatment with low sulphate and high sulphate nutrient solutions for 12 d, respectively. LS: low sulphate, HS: high sulphate, AsIII: arsenite, AsV: arsenate. Data are means ± SE ($n = 3$).

3.4. Influence of sulphur on arsenic efflux

After 12 d low sulphate pre-treatments, a higher percentage (75%) of arsenite was found in the solution after 180 min efflux in the low sulphate + arsenite treatments (Fig. 3A), and the percentage (66%) of arsenate was higher than arsenite in the low sulphate + arsenate treatments (Fig. 3B). After 12 d of high sulphate pre-treatments, arsenite in the efflux solution was the dominant arsenic species, with 82% in the high sulphate + arsenite treatments and 66% in the high sulphate + arsenate treatments (Fig. 3B). The arsenic species in the efflux solutions were in good agreement with arsenic species in plant roots (Fig. 4A).

3.5. Influence of sulphur and arsenic species on arsenic translocation from roots to shoots

After 48 h exposure to arsenite, total arsenic concentrations in rice roots and shoots showed statistically significant differences between sulphate treatments, arsenic species and interaction of arsenic species × sulphate treatments (Table 2). Total arsenic concentrations in roots in high sulphate + arsenite after high sulphate pre-treatments were higher than those in low sulphate + arsenite after low sulphate pre-treatments. By contrast, the total arsenic concentrations of the shoots in the treatments of low sulphate + arsenite after low sulphate pre-treatments were higher than those in the high sulphate + arsenite after high sulphate pre-treatments. In the +arsenite treatments and +arsenate treatments with high sulphate supply, total arsenic amounts in the roots were 85.49% and 78.80% the total arsenic in the whole plants, while those were 56.33% and 61.82% in the +arsenite treatments and the +arsenate treatments with deficient sulphate supply. Regardless the species of arsenic (arsenite or arsenate) supplied to the rice plants, the translocation factor of arsenic transported from roots to shoots was higher in the low sulphate pre-treatments than in the high sulphate pre-treatments (Table 2).

3.6. Influence of sulphur on the ratios of non-protein thiols concentrations to arsenite concentrations in roots

The concentrations of non-protein thiols in the high sulphate pre-treatment were significantly higher than those in the low sulphate pre-treatments (Table 3). The ratios of non-protein thiols concentrations to arsenite concentrations in roots from the high sulphate + arsenite treatments after high sulphate pretreated were the lowest among the four arsenic and S treatments (Table 3). No significant differences of the ratio in the pre-treatments of low sulphate and high sulphate were detected (Table 3).

Please cite this article in press as: Zhang, J., et al., Influence of sulphur on arsenic accumulation and metabolism in rice seedlings. Environ. Exp. Bot. (2010), doi:10.1016/j.envexpbot.2010.05.007

Table 2
Total arsenic concentrations in roots and shoots and transfer factor of total arsenic from roots to shoots in rice which were exposed to 10 μM arsenite (AsIII) or 10 μM arsenate (AsV) in LS or high sulphate nutrient solutions for 48 h after pre-treatment with low sulphate or high sulphate nutrient solutions for 12 d, respectively. S: sulphur, LS: low sulphate, HS: high sulphate, AsIII: arsenite, AsV: arsenate. Data are means ± SE ($n = 3$). Different letters indicate significant differences within the same column ($P < 0.05$).

S pre-treatment	Treatment	Total arsenic (nmol g^{-1} DW)		Transfer factor
		Root	Shoot	
LS	LS + AsIII	1825.99 ± 156.02 b	554.79 ± 50.53 a	0.31 ± 0.05 a
	LS + AsV	736.42 ± 95.94 b	195.97 ± 3.34 c	0.27 ± 0.03 a
HS	HS + AsIII	7374.41 ± 980.72 a	348.34 ± 34.02 b	0.05 ± 0.00 b
	HS + AsV	2120.05 ± 153.02 b	147.15 ± 20.69c	0.07 ± 0.01 b
Analysis of variance				
Arsenic species		<0.001	<0.001	0.871
Sulphate		<0.001	0.004	<0.001
Arsenic × sulphate		0.003	0.040	0.370

Table 3
Concentrations of non-protein thiols and comparison of non-protein thiols and arsenite in rice roots which were exposed to 10 μM arsenite or 10 μM arsenate in low sulphate or high sulphate nutrient solutions for 48 h after pre-treatment with low sulphate or high sulphate nutrient solutions for 12 d, respectively. S: sulphur, LS: low sulphate, HS: high sulphate, AsIII: arsenite, AsV: arsenate. Data are means ± SE ($n = 3$). Different letters indicate significant differences within the same column ($P < 0.05$).

S pre-treatment	Treatment	Root non-protein thiol concentration (μmol SH g^{-1} DW)	Ratio non-protein thiol/arsenite
LS	LS + control	5.45 ± 0.70 c	–
	LS + AsIII	8.06 ± 0.80 c	5.20 ± 0.01 bc
	LS + AsV	5.16 ± 0.74 c	20.52 ± 5.51 a
HS	HS + control	11.11 ± 0.31 b	–
	HS + AsIII	14.95 ± 0.40 a	2.44 ± 0.32 c
	HS + AsV	16.00 ± 1.57 a	13.84 ± 1.16 ab
Analysis of variance			
Arsenic species		0.370	0.001
Sulphate		<0.001	0.133
Arsenic × sulphate		0.077	0.506

For all samples in experiments 2, compared to the total arsenic in roots and shoots by $HNO_3/HClO_4$ (85/15, v/v) acid digestion and AFS determination, the average extraction efficiency by 1% nitric acid followed by HPLC-ICP–MS speciation analysis was 88.6 ± 6.5% (data not shown).

4. Discussion

In this study, we show that arsenite is the main form of arsenic in rice roots when plants are exposed to arsenate pretreated with the high sulphate nutrient solution and arsenite pretreated either low sulphate or high sulphate. Arsenite is taken up into plant roots via aquaporins such as NIP-type aquaporins (Ma et al., 2008), and arsenate is taken up via the phosphate transport systems (Meharg and Hartley-Whitaker, 2002). In shoots, arsenite was the predominant species both in the +arsenite and +arsenate treatments with different sulphur supplies (Figs. 1 and 4). Similar results were reported in rice plants (Wang et al., 2008) and *Pteris vittata* (Su et al., 2008) grown in normal nutrient solutions.

Regardless of whether plants were treated with arsenite or arsenate, the results of the present study demonstrated that arsenic accumulation and translocation in rice plants were significantly influenced by sulphur (Figs. 1 and 4; Table 2). In the +arsenite treatments, although arsenite concentrations of roots in the high sulphate pre-treatments were higher than those in the low sulphate pre-treatments, arsenite concentrations of shoots in the high sulphate pre-treatments were lower than those in the low sulphate pre-treatments (Fig. 1). Moreover, the higher transfer factor of total arsenic in the plants with low sulphate treatments indicates that arsenic translocation from roots to shoots was enhanced by sulphur deprivation; this was the case in both arsenite and arsenate exposure. Sulphur deprivation in the growth medium also resulted in lower accumulation of arsenite than arsenate in roots exposed to arsenate. The decreased arsenate reduction capacity in sulphur-deficient rice plants may be due to a lower level of reduced glutathione, which is required for arsenate reduction catalyzed by arsenate reductase such as OsACR2s (Duan et al., 2007). In the arsenic-hyperaccumulator *P. vittata*, decreased reduced glutathione synthesis in BSO-treated plants was also associated with a decrease in arsenate reduction (Zhao et al., 2003).

Although different sulphur supplies did not significantly influence the ratio of root concentrations of non-protein thiols to

Table 4
Significance of treatment effects (ANOVA *F* values) in Figs. 1, 2 and 4. AsIII: arsenite, AsV: arsenate, NPT: non-protein thiol, SH: thiol, PC: phytochelatin, GSH: reduced glutathione.

Treatment	Expt 1							Expt 2			
	Root arsenic concentration		Shoot arsenic concentration		Root SH concentration			Root arsenic concentration		Shoot arsenic concentration	
	AsIII	AsV	AsIII	AsV	NPT	PCs	GSH	AsIII	AsV	AsIII	AsV
Arsenic species	< 0.001	<0.001	<0.001	<0.001	0.129	0.693	0.010	< 0.001	<0.001	<0.001	0.004
Sulphate	< 0.001	0.005	<0.001	<0.001	0.001	0.017	0.014	< 0.001	<0.001	0.052	<0.001
Arsenic × sulphate	< 0.001	0.059	<0.001	0.001	0.609	0.078	0.008	0.001	0.043	0.587	0.038

Please cite this article in press as: Zhang, J., et al., Influence of sulphur on arsenic accumulation and metabolism in rice seedlings. Environ. Exp. Bot. (2010), doi:10.1016/j.envexpbot.2010.05.007

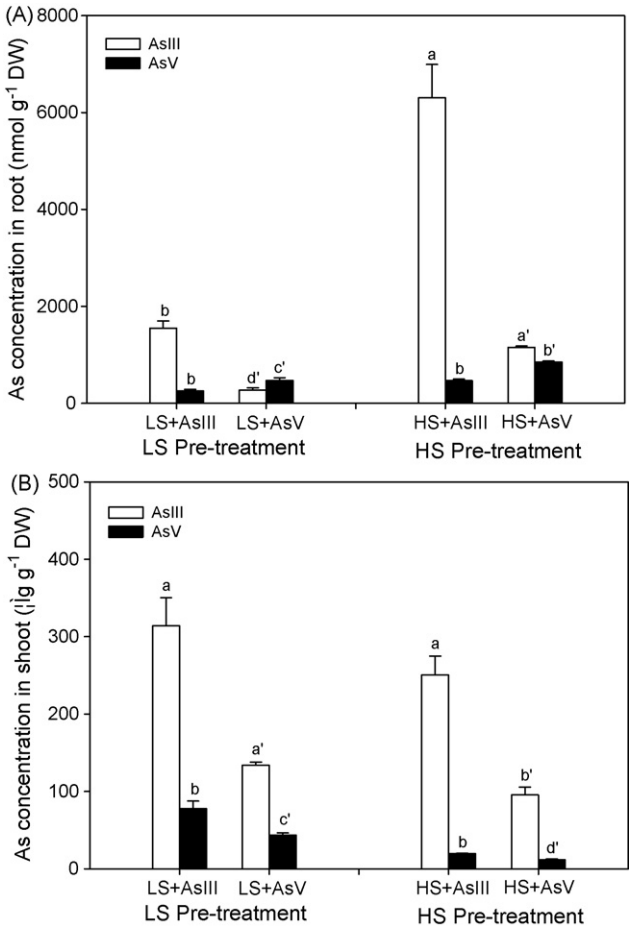

Fig. 4. Concentrations of arsenite (AsIII) and arsenate (AsV) in rice roots (A) and shoots (B) which were exposed to 10 μM arsenite or 10 μM arsenate in low sulphate and high sulphate nutrient solutions for 48 h after pre-treatment with low sulphate and high sulphate nutrient solutions for 12 d, respectively. LS: low sulphate, HS: high sulphate, AsIII: arsenite, AsV: arsenate. Data are means ± SE ($n = 3$). Bars of arsenite and arsenate with the same letter are not significantly different ($P < 0.05$); small letters: +AsIII, small letters′: +AsV.

arsenite, sulphate deprivation in the nutrient solution decreased roots non-protein thiols concentrations. Synthesis of thiol compounds results in a larger demand for sulphur (McMahon and Anderson, 1998). Pickering et al. (2000) found that nearly 100% of the arsenic was bound to thiols in *Brassica juncea* roots and shoots by X-ray absorption spectroscopy. Up to 40% of total arsenic was found in arsenic-phytochelatin compounds in sunflower (*Helianthus annuus*) (Raab et al., 2005). The ratio of root non-protein thiols/arsenite in the low sulphate treatments (5.20 ± 0.01) and the high sulphate treatments (2.44 ± 0.32) were consistent with the results of Bleeker et al. (2003) that ratios of phytochelatin-thiols to arsenic varied from 2 to 5 in *Cytisus striatus*. Phytochelatins have higher affinity for arsenite than does reduced glutathione (Raab et al., 2004, 2007). Moreover, more phytochelatins than reduced glutathione accumulated in roots indicated that phytochelatins were likely to be the major arsenite chelators, as described in *Isatis capadocia* (Karimi et al., 2009). Arsenite has been found to be the dominant species of arsenic in xylem saps of various plant species (Zhao et al., 2009). To date, there is no evidence of arsenite-phytochelatins in the xylem sap (Raab et al., 2005). The role of thiols in arsenite translocation remains to be investigated.

Xu et al. (2007) and Zhang et al. (2008) reported that a portion of accumulated arsenite and arsenate would efflux from plants, and arsenic speciation and concentration of efflux in the external medium are consistent with the arsenic speciation and percentage in rice roots (Figs. 3 and 4). Therefore, it is reasonable to conclude that the differences of arsenic concentrations and speciation of efflux solution in the high sulphate and low sulphate treatments could be caused by the effect of sulphur on arsenic metabolism and translocation in rice.

In conclusion, this study highlights the important role of sulphur on arsenic accumulation and metabolism in rice. Sulphur deprivation in rice increased the translocation of arsenic from roots to shoots. Further research is needed to identify the amounts and types of arsenic complexed with thiol groups and the species of arsenic transported from roots to shoots.

Acknowledgements

We thank Dr Yong-Guan Zhu, Fang-Jie Zhao and Barry Rosen for critical reading of the manuscript. This work was supported by the National Natural Science Foundation of China (20720102042 and 20777083), Bureau of International Cooperation, The Chinese Academy of Sciences (GJHZ200828), the Knowledge Innovation Program of the Chinese Academy of Sciences (No. RCEES-QN-200702) and the Special Funds for Young Scholars of RCEES, CAS. Jing Zhang is thankful for grant-aided support from Henan Agricultural University (No. 30200330).

References

Bleeker, P.M., Schat, H., Jos, R.V., Verkleij, V.A.C., Ernst, W.H.O., 2003. Mechanisms of arsenate tolerance in *Cytisus striatus*. New Phytologist 157, 33–38.
Bleeker, P.M., Hakvoort, H.W.J., Bliek, M., Souer, E., Schat, H., 2006. Enhanced arsenate reduction by a CDC25-like tyrosine phosphatase explains increased phytochelatin accumulation in arsenate-tolerance *Holcus lanatus*. The Plant Journal 45, 917–929.
Clemens, S., Kim, E., Neumann, D., Schroeder, J., 1999. Tolerance to toxic metals by a gene family of phytochelatin synthases from plants and yeast. The EMBO Journal 18, 3325–3333.
Dhankher, O.P., Rosen, B.P., McKinney, E.C., Meagher, R.B., 2006. Hyperaccumulation of arsenic in the shoots of Arabidopsis silenced for arsenate reductase (ACR2). Proceedings of the National Academy of Sciences, USA 103, 5413–5418.
Duan, G.L., Zhou, Y., Tong, Y.P., Mukhopadhyay, R., Rosen, B.P., Zhu, Y.G., 2007. A CDC25 homologue from rice functions as an arsenate reductase. New Phytologist 174, 311–321.
Duan, G.L., Zhu, Y.G., Tong, Y.P., Cai, C., Kneer, R., 2005. Characterization of arsenate reductase in the extract of roots and fronds of Chinese brake fern, an arsenic hyperaccumulator. Plant Physiology 138, 461–469.
Gasic, K., Korban, S.S., 2007. Expression of Arabidopsis phytochelatin synthase in Indian mustard (*Brassica juncea*) plants enhances tolerance for Cd and Zn. Planta 225, 1277–1285.
Hartley-Whitaker, J., Ainsworth, G., Vooijs, R., Ten Bookum, W., Schat, H., Meharg, A.A., 2001. Phytochelatins are involved in differential arsenate tolerance in Holcus lanatus. Plant Physiol. 126 (1), 299–306.
Hewitt, E.J., 1966. Sand and water culture methods used in the study of plant nutrition, 2nd ed. Technical Communication No. 22. Commonwealth Bureau of Horticulture and Plantation Crops. The Eastern Press.
Karimi, N., Ghaderian, S.M., Raab, A., Feldmann, J., Meharg, A.A., 2009. An arsenic-accumulating, hypertolerant brassica, *Isatis capadocia*. New Phytologist 184, 41–47.
Liao, X.Y., Chen, T.B., Xie, H., Liu, Y.R., 2005. Soil as contamination and its risk assessment in areas near the industrial districts of Chenzhou City, Southern China. Environment International 31, 791–798.
Liu, W.J., Zhu, Y.G., Smith, F.A., Smith, S.E., 2004. Do iron plaque and genotypes affect arsenate uptake and translocation by rice seedlings (*Oryza sativa* L.) grown in solution culture? Journal of Experimental Botany 55, 1703–1713.
Ma, J.F., Yamaji, N., Mitani, N., Xu, X.Y., Su, Y.H., McGrath, S., Zhao, F.J., 2008. Transporters of arsenite in rice and their role in arsenic accumulation in rice grain. Proceedings of the National Academy of Sciences, USA 105 (29), 9931–9935.
McMahon, P., Anderson, J., 1998. Preferential allocation of sulphur to γ-glutamylcysteinyl peptides in wheat plants grown at low sulphur nutrition in the presence of cadmium. Physiologia Plantarum 104, 440–448.
Meharg, A.A., Hartley-Whitaker, J., 2002. Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. New Phytologist 154, 29–43.
Meharg, A.A., Jardine, L., 2003. Arsenite transport into paddy rice (*Oryza sativa*) roots. New Phytologist 157, 39–44.
Meharg, A.A., Rahman, M., 2003. Arsenic contamination of Bangladesh paddy field soils: Implications for rice contribution to arsenic consumption. Environmental Science and Technology 37, 229–234.
Meharg, A.A., 2004. Arsenic in rice-understanding a new disaster for South-East Asia. Trends in Plant Science 9, 415–417.

Norton, G.J., Lou-Hing, D.E., Meharg, A.A., Price, A., 2008. Rice–arsenate interactions in hydroponics: whole genome transcriptional analysis. Journal of Experimental Botany 59 (8), 2267–2276.
Pickering, I.J., Prince, R.C., George, M.J., Smith, R.D., George, G.N., Salt, D.E., 2000. Reduction and coordination of arsenic in Indian mustard. Plant Physiology 122, 1171–1177.
Raab, A., Feldmann, J., Meharg, A.A., 2004. The nature of arsenic-phytochelatin complexes in *Holcus lanatus* and *Pteris cretica*. Plant Physiology 134, 1113–1122.
Raab, A., Ferreira, K., Meharg, A.A., Feldmann, J., 2007. Can arsenic-phytochelatin complex formation be used as an indicator for toxicity in *Helianthus annuus*? Journal of Experimental Botany 58, 1333–1338.
Raab, A., Schat, H., Meharg, A.A., Feldmann, J., 2005. Uptake, translocation and transformation of arsenate and arsenite in sunflower (*Helianthus annuus*): formation of arsenic-phytochelatin complexes during exposure to high arsenic concentrations. New Phytologist 168, 551–558.
Smith, A.H., Lopipero, P.A., Bates, M.N., Steinmaus, C.M., 2002. Public health-arsenic epidemiology and drinking water standards. Science 296, 2145–2146.
Sneller, F.E.C., van Heerwaarden, L.M., Koevoets, P.L.M., Vooijs, R., Schat, H., Verkleij, J.A.C., 2000. Derivatization of phytochelatins from Silene vulgaris, induced upon exposure to arsenate and cadmium: comparison of derivatization with Ellman's reagent and monobromobimane. Journal of Agricultural and Food Chemistry 48, 4014–4019.
Su, Y.H., McGrath, S.P., Zhu, Y.G., Zhao, F.J., 2008. Highly efficient xylem transport of arsenite in the arsenic hyperaccumulator *Pteris vittata*. New Phytologist 108, 434–441.
Wang, L.H., Duan, G.L., Williams, P.N., Zhu, Y.G., 2008. Influences of phosphorus starvation on OsACR2.1 expression and arsenic metabolism in rice seedlings. Plant and Soil 313, 129–139.
Williams, P.N., Villada, A., Deacon, C., Raab, A., Figuerola, J., Green, A.J., Feldmann, J., Meharg, A.A., 2007. Greatly enhanced arsenic shoot assimilation in rice leads to elevated grain levels compared to wheat and barley. Environmental Science and Technology 41, 6854–6859.
Xu, X.Y., McGrath, S.P., Zhao, F.J., 2007. Rapid reduction of arsenate in the medium mediated by plant roots. New Phytologist 176, 590–599.
Zhang, X., Lin, A.J., Zhao, F.J., Xu, G.Z., Duan, G.L., Zhu, Y.G., 2008. Arsenic accumulation by the aquatic fern Azolla: comparison of arsenate uptake, speciation and efflux by *A. caroliana* and *A. filiculoides*. Environmental Pollution 156, 1149–1155.
Zhao, F.J., Wang, J.R., Barker, J.H.A., Schat, H., Bleeker, P.M., McGrath, S.P., 2003. The role of phytochelatins in arsenic tolerance in the hyperaccumulator *Pteris vittata*. New Phytologist 159, 403–410.
Zhao, F.J., Ma, J.F., Meharg, A.A., McGrath, S.P., 2009. Arsenic uptake and metabolism in plants. New Phytologist 181, 777–794.
Zhu, Y.G., Sun, G.X., Lei, M., Teng, M., Liu, Y.X., Chen, N.C., Wang, L.H., Carey, A.M., Deacon, C., Raab, A., Meharg, A.A., Williams, P.N., 2008a. High percentage inorganic arsenic content of mining impacted and non-impacted Chinese rice. Environmental Science and Technology 42 (13), 5008–5013.
Zhu, Y.G., Williams, P.N., Meharg, A.A., 2008b. Exposure to inorganic arsenic from rice: a global health issue? Environmental Pollution 154, 169–171.

Please cite this article in press as: Zhang, J., et al., Influence of sulphur on arsenic accumulation and metabolism in rice seedlings. Environ. Exp. Bot. (2010), doi:10.1016/j.envexpbot.2010.05.007