Excessive sulfur supply reduces arsenic accumulation in brown rice

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ABSTRACT

The objective of this study was to investigate the influence of excessive sulfur (S) supply on iron plaque formation and arsenic (As) accumulation in rice plants. A combined soil-sand pot experiment was conducted by using two As levels (0, 20 mg/kg) combined with three S concentrations (0, 60, 120 mg/kg). The results showed that excessive S supply significantly decreased As concentration in brown rice, but As concentration in root increased with increasing rate of S supply. Moreover, bioconcentration factors for leaves and stems were 8–35 fold of that for brown rice, indicating that As was mainly accumulated in rice leaves and stems instead of brown rice. Furthermore, excessive S supply significantly decreased translocation factor of As compared to treatment without S supply. These results indicated that excessive S may reduce As translocation from soils and roots to grain. The mechanism could be ascribed to excessive S that induced the decrease of As availability, the increase of iron plaque formation under As stress, and the increase of glutathione in rice leaves and roots. Therefore, excessive S can reduce As accumulation in brown rice exposed to As contaminated soils though it may result in loss of rice yield.

Keywords: arsenic toxicity; glutathione; iron plaque; *Oryza sativa* L.; plant uptake

Arsenic (As), an extremely toxic metalloid pollutant, is distributed in soil through anthropogenic activities, such as pesticide and herbicide application, mining, or irrigation with contaminated groundwater (Hu et al. 2007). Rice grown on As-contaminated paddy soil can accumulate high levels of As, approximately 10-fold larger than other cereals (Lombi et al. 2009). Thus, As uptake by rice plants is recognized as a major route of As transfer into food chain which has the potential to become a new disaster for population in Southeast Asia (Heikens et al. 2007). Therefore, it is crucial to understand the mechanism of As uptake by rice and to find strategies for reducing As accumulation in grain for enhanced food safety.

Paddy rice (*Oryza sativa* L.) oxygenates its rhizosphere, resulting in the formation of an iron oxyhydroxide plaque (Hansel et al. 2001). The effect of iron

plaque on plant uptake of heavy metals or nutrients may depend on the amount of iron plaque on the root surface (Hu et al. 2007). The Fe hydroxides in soil and solution have a very strong binding affinity for arsenate (AsO_4^{3-}) and a possible capacity to oxidize arsenite (AsO_3^{3-}) to arsenate (Liu et al. 2004). The iron plaque may act as a barrier or a buffer to As uptake due to the adsorption and immobilization of metals by iron plaque (Liu et al. 2004).

Reduced glutathione (GSH), a low molecular weight tri-peptide, is the most abundant source of non-protein thiol in plant cells (Khan et al. 2008). GSH has a function in the detoxification of heavy metals through the formation of phytochelatins (PCs) (Hassan et al. 2005), which can be transported to the tonoplast, taken up by active transport systems, and deposited in the vacuole, thus decreasing the toxicity of heavy metals (Sharma and Dietz 2006). As in paddy soils predominantly occurs in its reduced form, arsenite, which can react with GSH and/or PCs forming As-PCs or As-GS complex (Tuli et al. 2010). Once formed in roots and leaves, they could be trapped as peptide-thiol complexes such as γ-glutamylcysteine, a precursor of GSH (Dhankher et al. 2002). Nonetheless, there are no reports on the interactions between excessive S, iron plaque development, GSH and As accumulation in rice. The objective of this study was to investigate these interactions under greenhouse culture.

MATERIAL AND METHODS

Experimental design. The topsoil (0–20 cm) from Putian city, Fujian province, China was used for the pot experiment. The soil classified as Typical Haplaquept contained 797 mg/kg total S, 35.1 mg/kg $Ca(H_2PO_4)_2$ extractable S, 3.96 mg/kg total As, 0.36 mg/kg HCl extractable As.

There were six treatments with two levels of As ($Na_2HAsO_4 \cdot 7 H_2O$) (0, 20 mg As/kg) combined with three S levels (Na_2SO_4) (0, 60, 120 mg S/kg). Each treatment had four replicates. A rhizobag system with a soil-sand combination was used to collect rhizosphere and non-rhizosphere soils separately. The rhizobags were made of nylon net with a mesh size of 40 μm and filled with 500 g acid-washed quartz sand. In total 2 kg dried soil was placed in each pot, all of which received a based dressing of nutrients as Fan et al. (2010).

Rice (*Oryza sativa* L.) seeds were sterilized in 10% H_2O_2 (v/v) solution for 30 min, followed by thor-

oughly washing with deionized water. The seeds were germinated in moist filter paper at 25°C. In each pot, two uniform germinated seeds were transplanted and grown for 160 days. Soil was kept submerged with deionized water for the whole growth period.

Sampling and analysis. Before harvesting, two pieces of fresh penultimate leaves of each plant were sampled and GSH content was analyzed according to DTNB [5,5'-dithilbis-(2-nitrovenzoic acid)] method (Griffith 1980). Rice plants were harvested and divided into roots, shoots and seeds. Half of the fresh roots were used for the extraction of iron plaque by ascorbic citrate acetic (ACA) method (Gao et al. 2007). Quartz sand collected from rhizosphere was extracted by 0.1 mol/L HCl (Hu et al. 2007). In ACA extracts and HCl solution, As concentration was determined by atomic fluorescence spectrometry, and Fe, Mn and S concentrations were determined by inductively-coupled plasma optical emission spectroscopy. Soil total S was digested by $Mg(NO_3)_2$ (Butters and Chenery 1959).

Dried rice plants were digested according to Fan et al. (2010). A reagent blank and a standard reference material (Shrub, GBW 07602, National certified reference materials) were included to verify the accuracy and precision of analysis. The recovery is 95.8%.

Data analysis. In order to study the translocation pattern of As in rice plants, bioconcentration factor (BCF) and translocation factor (TF) was calculated according to Singh et al. (2010). Analysis of variance (ANOVA) followed by the least significant difference (*LSD*) test and correlation analysis were performed using the Windows-based SPPS 13.0 (SPSS Inc., Chicago, USA).

RESULTS

Effect of excessive S on rice biomass and As uptake. In –S treatments, As supply decreased dry weight of root ($P < 0.01$) and grain ($P < 0.05$). And in +S treatments, As addition also showed similar decreasing trend on dry weight of root, shoot and grain compared to –As treatments. Furthermore, dry weight of root, shoot and grain tended to decrease with the increase of S levels (Figure 1).

The average As concentrations in roots were 7.5, 11.3, 111.5 and 66.9 fold those in leaves, stems, brown rice and husk in +As treatments, respectively, while the corresponding values in –As treatments were 3.2, 4.9, 77.3 and 38.8 respectively (Table 1). Furthermore, S addition significantly
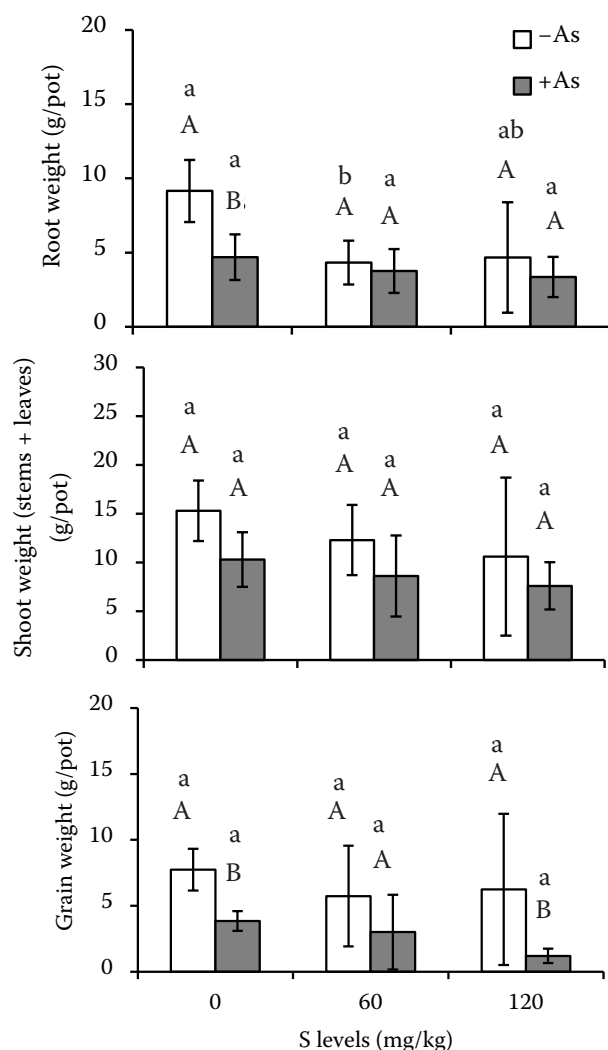
Figure 1. Effect of S application on biomass of rice (Different lower- and upper-case letters indicate significant difference at $P < 0.05$ (*LSD* test) for S levels and As levels)

($P < 0.05$) decreased As concentrations in brown rice, leaves, stems (not significant in +As treatments) and husk in both +As and –As treatments.

Furthermore, BCF for leaves and stems were 8–35 fold that for brown rice and 5–20 fold that for husk (Table 2), indicating that As was mainly accumulated in rice leaves and stems. Moreover, S supply significantly decreased BCF of As for leaves, stems, brown rice and husk compared to –S treatments. The TF shows movement of heavy metals from root to brown rice. In the present study, TF of As was significantly lower with S supply than without S supply (Table 2), suggesting that S application may inhibit As transfer from rice root to grain.

Effect of excessive S on GSH in leaves and iron plaque on the root surface. GSH concentra-

tions in rice leaves were related to S and As levels (Figure 2). In –As treatments, GSH content tended to increased with S levels although their difference was not statistically significant ($P > 0.05$). In +As treatments, 120 mg S/kg supply significantly increase GSH in rice leaves compared to 0 and 60 mg S/kg treatments (Figure 2).

The application of S significantly decreased ACA-extractable Fe and Mn in iron plaque on the root surface of rice in –As treatments (Table 3). In +As treatments, S supply showed higher Fe, Mn ($P < 0.05$) and As ($P < 0.05$) than treatments without S. As addition significantly increased ACA-extractable As in iron plaque ($P < 0.01$). Compared to –As treatments, As addition had higher ACA-extractable S but the difference was significant only in 120 mg S/kg addition treatment.

Elements in rhizosphere quartz sand and non-rhizosphere soil. In –As treatments, HCl-extractable Fe in the rhizosphere was significantly higher with 120 mg S/kg addition than with 0 and 60 mg S/kg (Figure 3a). Compared to –As treatments, As addition significantly increased HCl-extractable As in the rhizosphere in all S levels treatments (Figure 3a).

The concentrations of SO_4^{2-} were significantly higher ($P < 0.01$) in the non-rhizosphere soils with S addition than without S addition (Figure 3b). In +As treatments, 120 mg S/kg addition had significantly higher soil pH than treatments with 0 and 60 mg S/kg addition (Figure 3b). In treatments with 0 and 60 mg S/kg addition, soil pH in +As treatment was significantly lower ($P < 0.05$) than in –As treatment. But in 120 mg S/kg addition treatment, soil pH in +As treatment was significantly higher ($P < 0.01$) than in –As treatment.
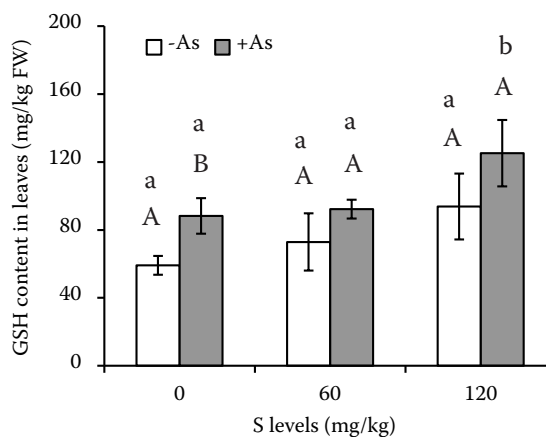

Figure 2. Effect of S application on glutathione (GSH) content in the penultimate leaves of rice. Different lower- and upper-case letters indicate significant difference at $P < 0.05$ (*LSD* test) for S levels and As levels

Table 1. Effect of sulfur application on concentrations (mg/kg dry weight) of As in rice

S levels (mg/kg)	Roots		Leaves		Stems		Brown rice		Husk	
	−As	+As	−As	+As	−As	+As	−As	+As	−As	+As
0	19.4 ± 2.8	98.9 ± 15.5[c]	7.0 ± 0.3[a]	23.2 ± 0.9[a]	4.7 ± 0.4[a]	12.8 ± 2.2	0.40 ± 0.06[a]	1.57 ± 0.33[a]	0.79 ± 0.13[a]	2.58 ± 0.47[a]
60	17.6 ± 1.3	129.1 ± 15.5[b]	5.6 ± 0.8[b]	17.4 ± 1.3[b]	3.6 ± 0.3[b]	11.2 ± 1.3	0.17 ± 0.03[b]	0.90 ± 0.06[c]	0.42 ± 0.06[ab]	1.51 ± 0.54[c]
120	21.4 ± 2.2	183.7 ± 25.6[a]	5.4 ± 0.8[b]	14.5 ± 2.2[c]	3.6 ± 0.5[b]	12.5 ± 1.3	0.19 ± 0.04[ab]	1.23 ± 0.21[b]	0.29 ± 0.01[b]	2.07 ± 0.41[b]
	analysis of variance for As levels									
0	$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$	
60	$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$	
120	$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$	
S levels × As	$P < 0.05$		$P < 0.01$		ns		$P < 0.05$		$P < 0.05$	

Values are means ± standard deviations; ns – not significant. Values followed by a different letter within a column indicate significance at $P < 0.05$ (*LSD* test) for S levels

DISCUSSION

The results from the present study strongly suggested that excessive S application could significantly decrease As uptake by brown rice, which could be ascribed to three principal mechanisms. Firstly, excessive S supply increased the formation of iron plaque on the root surface under As stress (Table 3). Iron plaque may be a barrier or a buffer to the uptake of As (Liu et al. 2004). A significant, positive correlation between As in roots and iron plaque was found (Table 4), indicating that iron plaque may act as a buffer to the uptake of As by rice roots. However, no significant relationship was found between As content in rice leaves, grain, husk and As in iron plaque (Table 4). Moreover, the lower BCF and TF of As in brown rice with S supply (Table 2) indicates that As translocation from soils and roots to brown rice was inhibited by S. All these results suggested that excessive S induced the transformation of iron plaque may induce As uptake and be trapped in rice roots but can reduce As translocation from roots to shoots and grain. This may be ascribed to S induced expression of genes controlling As accumulation and transport in rice under As stress (Rai et al. 2011). Secondly, S supply induced GSH formation in rice (Figure 2), which can react with As forming As-PCs or As-GS complex (Tuli et al. 2010). And As-PCs or As-GS complex can then be taken up into the

Table 2. Effect of sulfur application on bioconcentration factor (BCF) and translocation factor of As in rice

S levels (mg/kg)	BCF-leaves		BCF-stems		BCF-brown rice		BCF-husk		Translocation factor	
	−As	+As	−As	+As	−As	+As	−As	+As	−As	+As
0	38.3 ± 6.9[a]	15.8 ± 3.4[a]	26.0 ± 2.1[a]	8.9 ± 3.1	2.10 ± 0.50[a]	1.10 ± 0.47	4.21 ± 0.46[a]	1.77 ± 0.59	0.022 ± 0.005[a]	0.016 ± 0.001[a]
60	20.0 ± 3.9[b]	12.7 ± 2.1[ab]	11.6 ± 2.1[b]	8.0 ± 2.4	0.57 ± 0.15[b]	0.63 ± 0.14	1.39 ± 0.13[b]	1.03 ± 0.23	0.010 ± 0.002[b]	0.007 ± 0.001[b]
120	25.3 ± 6.0[b]	11.1 ± 1.9[b]	15.1 ± 0.1[b]	9.7 ± 1.6	0.82 ± 0.34[b]	0.96 ± 0.13	1.24 ± 0.19[b]	1.64 ± 0.38	0.009 ± 0.003[b]	0.007 ± 0.003[b]
	analysis of variance for As levels									
0	$P < 0.01$		$P < 0.01$		$P < 0.05$		$P < 0.01$		ns	
60	ns		ns		ns		ns		ns	
120	ns		$P < 0.05$		ns		ns		ns	
S levels × As	$P < 0.05$		$P < 0.01$		$P < 0.05$		$P < 0.01$		ns	

Values are means ± standard deviations; ns – not significant. Values followed by a different letter within a column indicate significance at $P < 0.05$ (*LSD* test) for S levels

Table 3. Effect of sulfur application on concentrations of ascorbic citrate acetic (ACA)-extractable Fe, Mn, As and S in iron plaque on root surface of rice

S levels (mg/kg)	Fe (g/kg)		Mn (mg/kg)		As (mg/kg)		S (g/kg)	
	−As	+As	−As	+As	−As	+As	−As	+As
0	15.6 ± 4.1[a]	12.8 ± 3.4	119.6 ± 69.6[a]	86.6 ± 35.2[ab]	61.1 ± 3.2	168.0 ± 4.0[c]	0.6 ± 0.4[c]	0.8 ± 0.3[c]
60	9.3 ± 1.0[b]	13.7 ± 0.8	52.0 ± 9.1[b]	110.6 ± 28.9[a]	49.6 ± 7.2	251.6 ± 48.3[a]	1.3 ± 0.2[b]	1.3 ± 0.2[b]
120	10.8 ± 2.9[b]	11.1 ± 1.9	60.8 ± 14.1[b]	50.5 ± 6.1[b]	65.6 ± 16.0	201.9 ± 24.4[b]	2.3 ± 0.1[a]	1.8 ± 0.1[a]
	analysis of variance for As levels							
0	ns		ns		$P < 0.01$		ns	
60	ns		ns		$P < 0.01$		ns	
120	ns		ns		$P < 0.01$		$P < 0.01$	
S levels × As	$P < 0.05$		$P < 0.05$		$P < 0.01$		$P = 0.054$	

Values are means ± standard deviations; ns – not significant. Values followed by different letter with a column indicate significance at $P < 0.05$ (*LSD* test) for S levels
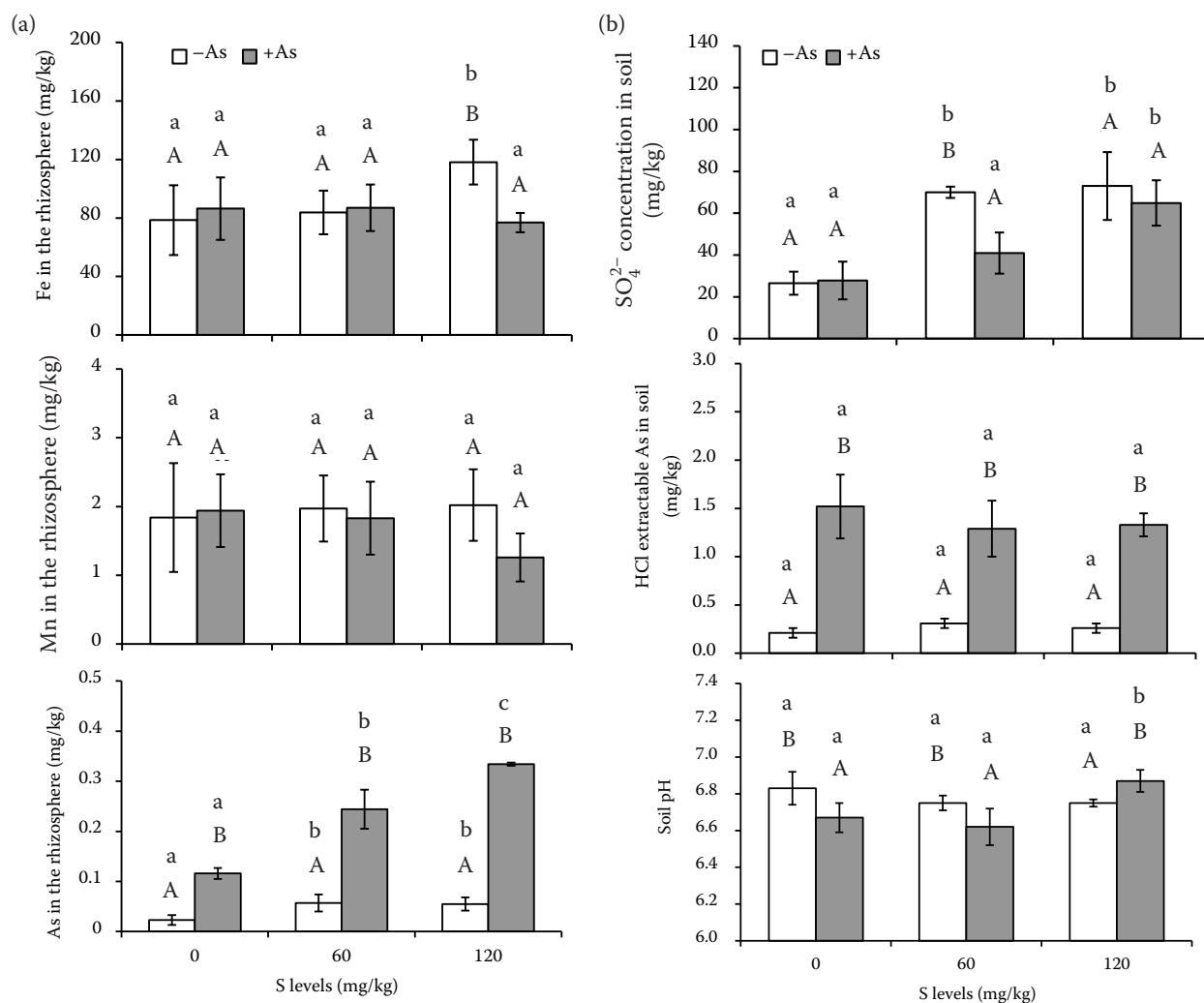

Figure 3. (a) Effect of S application on 0.1 mol/L HCl-extractable Fe, Mn and As concentrations in the rhizosphere of rice; (b) effect of S application on $Ca(H_2PO_4)^{2-}$-extractable SO_4^{2-}, HCl-extractable As concentrations and pH in the non-rhizosphere of rice. Different lower- and upper-case letters indicate significant difference at $P < 0.05$ (*LSD* test) for S levels and As levels

vacuole, subsequently decreasing As transportation from rice roots and leaves to brown rice (Zhao et al. 2010). Thirdly, excessive S supply may decrease As availability by forming indiscerptible As_2S_3 or FeAsS. Bacterium, such as *Desulfotomaculum auripigmentum*, was reported to induce precipitation of As_2S_3 by its reduction of As(V) to As(III) and S(VI) to S(–II) under anoxic conditions with excessive S (Figure 3b) (Newman et al. 1997). And in the environment with high Fe (II) or FeS, FeAsS may be formed by coprecipitation of As with Fe and S (Farquhar et al. 2002). Furthermore, higher soil pH, resulted by As addition and excessive S supply (Figure 3b), may promote the formation of these indiscerptible compounds. Therefore, due to the integration of these mechanisms, excessive S supply could significantly inhibit As uptake and accumulation in brown rice exposed to As contaminated soils.

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