Sulfate and glutathione enhanced arsenic accumulation by arsenic hyperaccumulator *Pteris vittata* L.

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**Abstract**

This experiment examined the effects of sulfate (S) and reduced glutathione (GSH) on arsenic uptake by arsenic hyperaccumulator *Pteris vittata* after exposing to arsenate (0, 15 or 30 mg As L⁻¹) with sulfate (6.4, 12.8 or 25.6 mg S L⁻¹) or GSH (0, 0.4 or 0.8 mM) for 2-wk. Total arsenic, S and GSH concentrations in plant biomass and arsenic speciation in the growth media and plant biomass were determined. While both S (18–85%) and GSH (77–89%) significantly increased arsenic uptake in *P. vittata*, GSH also increased arsenic translocation by 61–85% at 0.4 mM (p < 0.05). Sulfate and GSH did not impact plant biomass or arsenic speciation in the media and biomass. The S-induced arsenic accumulation by *P. vittata* was partially attributed to increased plant GSH (21–31%), an important non-enzymatic antioxidant counteracting oxidative stress. This experiment demonstrated that S and GSH can effectively enhance arsenic uptake and translocation by *P. vittata*.

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1. Introduction

Arsenic-contaminated soils and waters are of great concern worldwide due to arsenic’s toxicity as a carcinogen (Tripathi et al., 2007). Phytoextraction has the potential to clean up those contaminated sites. It uses hyperaccumulating plants to concentrate arsenic in the aboveground biomass, which can be harvested and removed. Identification of arsenic hyperaccumulators, such as fern species in the *Pteris* genus (Ma et al., 2001; Srivastava et al., 2005) and *P. vittata* (Cao et al., 2004), makes phytoextraction of arsenic-contaminated sites a viable technology.

As the first-known arsenic hyperaccumulator, *Pteris vittata* L. (Chinese brake fern) has received much attention due to its exceptional ability to tolerate and hyperaccumulate arsenic. When cultivated in an arsenic-contaminated soil containing 1500 mg kg⁻¹ arsenic, it accumulated 22,630 mg kg⁻¹ arsenic in its fronds (Ma et al., 2001). In a hydroponic system containing 150 mg L⁻¹ arsenic, the plant accumulated up to 27,000 mg kg⁻¹ arsenic (Wang et al., 2002).

As an efficient arsenic hyperaccumulator, *P. vittata* must possess mechanisms to efficiently detoxify the accumulated arsenic in the biomass. Several mechanisms have been proposed including chelation, compartmentalization, biotransformation and cellular repair (Gonzaga et al., 2006). The roles of non-enzymatic antioxidant glutathione (GSH) in arsenic accumulation by *P. vittata* remain unclear. With the increase of arsenic levels in the growth media, GSH concentrations in the fronds of *P. vittata* significantly increased (Cao et al., 2004). For example, GSH concentration in the mature fronds of *P. vittata* growing in soil with 200 mg As kg⁻¹ for 12 weeks was 2.8 times more than that with 20 mg As kg⁻¹. Similar trends were observed by Srivastava et al. (2005) and Singh et al. (2006) in hydroponic systems. However, this was not observed by Zhao et al. (2003). GSH concentrations in the fronds of *P. vittata* remained unchanged when *P. vittata* was exposed to 0.5 mM arsenic for 5 d or to 50 μM arsenic for 1–7 d in a hydroponic system (Zhao et al., 2003). The shorter exposure time and/or the lower arsenic concentrations may account for the different results.

One of the arsenic detoxification mechanisms in *P. vittata* is via reduction of arsenate (AsV) to arsenite (AsIII) in the fronds, which is then possibly transported to vacuoles for storage (Lombi et al., 2002). This has been confirmed in yeast where vacuolar...
accumulation of AsIII–GSH complexes is mediated by an ABC-type transporter (Tripathi et al., 2007). There is considerable evidence that at least part of the arsenic accumulated by plants is coordinated with GSH (Meharg and Hartley-Whitaker, 2002). In plants, in vitro transport of GSH–AsIII across tonoplast vesicles of Arabidopsis thaliana has been demonstrated and was also postulated to be carried out by an ABC transporter (Petrà et al., 2006). However, no transport processes that mediate the vacuolar deposition of inorganic arsenic have been identified in plants.

Sulfur is an essential nutrient in plants, which is required for synthesis of amino acids and protein, and is taken up as sulfate by plants (Marschner, 1995). In addition, it is also a precursor for formation of GSH in plants. While Tu and Ma (2005) investigated the effects of arsenic on plant macro (P, K, Ca, and Mg) and micro (Fe, Mn, Cu, Zn, B and Mo) nutrient status, Fayiga et al. (2008) examined the effects of macronutrients Ca, K, N and P on plant growth and arsenic uptake by P. vittata. However, little information is available on the effects of S on plant growth and arsenic uptake by P. vittata. Such knowledge may lead to more effective phytoremediation strategies to remove arsenic from contaminated water and soil.

The objectives of this hydroponic study were to 1) examine the effect of sulfate and GSH on plant growth and arsenic uptake by P. vittata, and 2) determine the relationship between S and GSH in P. vittata under arsenic stress. The results from this study shed light on the feasibility of using S and GSH to enhance arsenic accumulation by P. vittata.

2. Materials and methods

2.1. Plants and experiment setup

Five-month-old P. vittata were obtained from Mid-Florida Research & Education Center, University of Florida. To acclimatize the plants, they were grown hydroponically for two weeks in a controlled environment, with temperature of 20–25 °C and humidity of 65–70%. A 14-h photoperiod with a daily photosynthetic photon flux of 350 μmol m⁻² s⁻¹ was supplied by cool-white fluorescent lamps. Hoagland nutrition medium at 0.2-strength with vigorous aeration was used to maintain plant growth (Srivastava et al., 2005). The nutrition solution was replenished twice a week. After acclimatization for 2 weeks, they were transferred into 0.2-strength Hoagland nutrient solution containing different concentrations of arsenic, and different concentrations of S or GSH.

This experiment was setup as completely randomized design in a 3 by 3 factorial scheme consisting of three arsenic levels (0, 15 and 30 mg L⁻¹) as Na₂H₂AsO₄·7H₂O and three S levels (6.4, 12.8, or 25.6 mg L⁻¹ as Na₂SO₄) (S experiment), or three reduced glutathione levels (0, 0.4 and 0.8 mM) (GSH experiment) (Sigma chemical Company). Each treatment was replicated 4 times with one plant per pot. Concentration of S was 50, 100 and 200% of that in 0.2-strength Hoagland solution. The GSH concentrations were within the concentrations in plants, which are usually at millimolar levels (Jain et al., 1996) and up to 1 mM of GSH has been used for plant uptake in the literature (Herschbach and Rempenberg, 1994).

Upon harvest, the plants were separated into fronds and roots including rhizomes. Concentrations of GSH and arsenic speciation were determined using samples that were flash-frozen in liquid nitrogen and stored at -80 °C, while biomass and total As and S analysis were done on oven-dried (65 °C for 2 d) samples.

2.2. Total As and S concentration determination

Oven-dried frond and root samples were digested with nitric acid and hydrogen peroxide for arsenic examination, or nitric acid, hydrochloric acid and hydrogen peroxide for S determination on a hot block digester (Environmental Express, Mt. Pleasant, S.C.) using USEPA Method 3050B. Total As and S concentrations in digested solution were analyzed by graphite furnace atomic absorption spectrophotometry (GFAAS; AA240Z, Varian Inc., CA) and multi-channel inductively coupled plasma spectrometry (Thermo Jarrell Ash 1W 61-E, Franklin, MA). Quality control of arsenic analysis was included using standard reference material 1547 (peach leaves; US National DMD) with recoveries of 100 ± 20%.

2.3. Arsenic speciation

Arsenic speciation was performed by extracting plant samples ultrasonically in 10 ml of methanol/water mixture (1:1 v/v) two times for 4 h at 60 °C (Zhang et al., 2002). The two extracts were decanted into a 100-ml volumetric flask and diluted to 100 ml with water. Arsenate and arsenite were separated using an arsenic-speciation cartridge (Waters Corporation, MC), which retains arsenate. Total arsenic (AsV and AsIII) and arsenite (AsIII) were determined by GFAAS. The growth media was diluted as required and specified using arsenic-speciation cartridge. To check the reliability of this method, arsenic speciation of P. vittata samples was analyzed using both arsenic-speciation GFAAS method and HPLC-ICP-MS method (Ma et al., unpublished). The sum of AsV and AsIII concentrations determined by HPLC-ICP-MS and the total As concentration determined by arsenic-speciation GFAAS were in good agreement.

2.4. GSH and GSSG concentration determination

Oxidized glutathione (GSSG) and reduced glutathione (GSH) were determined following Madhava and Sresty (2000). The procedure utilizes the enzymatic recycling method to quantify GSH by using glutathione reductase. The procedure was as follows. 0.5 g fresh frond samples were homogenized in 3 ml of 2% (v/v) metaphosphoric acid containing 2 mM EDTA and 4% (w/v) polyvinylpolypyrrolidone by using a precooled mortar and pestle and then centrifuged at 14,000 g for 10 min. The pH of the extract was brought to about 5.5 with 10% sodium citrate. Total glutathione content was estimated by mixing 700 μl of 0.3 mM NADPH and 100 μl of 6 mM DTNB (5,5′-dithiobis-2-nitrobenzoic acid) and the sample extract of 200 μl to give a volume of 1.0 ml, directly in a cuvette with 1 cm light path. To this solution, 10 μl of 100 units of glutathione reductase was added and then the absorbance was monitored at 412 nm. Total glutathione concentrations were calculated from the standard curves prepared by using GSH. To 0.5 ml of the extract, 4 μl of 2-× inosipyrrodine was added. The solution was shaken vigorously for 1 min and incubated at room temperature for 1 h. Oxidized glutathione concentration was calculated from the prepared standard curves using oxidized glutathione. The GSH concentration was estimated as the difference between total glutathione and oxidized glutathione.

2.5. Statistical analysis

Fern biomass and concentrations of As, S and GSH were calculated as the means of at least 3 replications. All data were expressed as mean ± S.D. Duncan’s Multiple Rang Test was employed to compare the changes among different treatments at p < 0.05 level.

3. Result and discussion

This experiment determined the effects of S and GSH on the plant growth and arsenic uptake by P. vittata under different arsenic stress. P. vittata plants were grown in 0.2-strength Hoagland solution containing different levels of arsenate (0, 15 or 30 mg L⁻¹) and sulfate (6.4, 12.8, or 25.6 mg L⁻¹), or GSH (0, 0.4 or 0.8 mM) for two weeks.

3.1. Addition of S did not change P. vittata biomass

After two weeks of growth, no visible toxicity symptom in P. vittata was observed in all plants (data not shown). This indicated that P. vittata tolerated arsenic concentration as high as 30 mg L⁻¹. Since S is an essential plant nutrient, it is expected that addition of S would increase its biomass. However, S had no effect on the frond or root biomass of P. vittata even under arsenic stress (Table 1; p < 0.05). Our result may imply that S level even at 50% of that in 0.2-strength Hoagland solution was adequate to support plant growth of P. vittata. This is consistent with the observation that P. vittata is moderate in their demand for nutrients as they often grow in a nutrient-poor limestone substrate (Bondada and Ma, 2002).

While increase of S from 6.4 to 25.6 mg L⁻¹ in the growth media had no effect on the biomass of P. vittata, this was not the case with barley seedling. Chen and Huerta (1997) evaluated the effect of S (3.2 and 32 mg L⁻¹) on the growth of barley seedling under Cd-stress (28 μg L⁻¹). Though plant biomass is reduced in both S treatments under Cd-stress, the reduction is less with the high S treatment. They postulated that S is a critical nutritional factor in plants to counter Cd toxicity in barley seedling. Intracellular GSH has been suggested as a “first line defense” against Cd toxicity in A. thaliana (Howden et al., 1995).
3.2. Addition of S increased S concentrations in P. vittata

Sulfur requirement for optimal plant growth varies from 1.0 to 5.0 g S kg\(^{-1}\) in plants (Marschner, 1995). The S concentrations in P. vittata fronds were within the range reported for other plants. In the absence of arsenic, S concentrations ranged from 2.0 to 2.7 g S kg\(^{-1}\) in the fronds and 0.86–1.1 g S kg\(^{-1}\) in the roots of P. vittata, with twice of the S being allocated to the fronds than to the roots (Fig. 1).

As expected, addition of S significantly increased S concentrations in the fronds and roots of P. vittata regardless of the arsenic levels in the growth media (Table 1; Fig. 1; \(p < 0.05\)). For example, as S concentrations in the growth media increased from 6.4 to 25.6 mg L\(^{-1}\), the S concentrations increased by 35% from 2.0 to 2.7 g kg\(^{-1}\) in the fronds and increased by 22% from 0.86 to 1.05 g kg\(^{-1}\) in the roots.

In a hydroponic experiment, Luongo and Ma (2005) reported that, after exposure to 10 mg L\(^{-1}\). As for 2-week, phosphate concentrations in P. vittata increased by 92% in the fronds and 455% in the roots compared to the controls. However, for a given S level, arsenic had no effect on S concentrations in the fronds and roots of P. vittata (Table 1; Fig. 1; \(p < 0.05\)). This means that arsenic concentrations were increased only in the presence of arsenic (15 or 30 mg L\(^{-1}\) As), arsenic concentrations in the fronds and roots were significantly enhanced (\(p < 0.05\)) with increasing S levels. For example, with S concentrations increased from 6.4 to 12.8 and to 25.6 mg L\(^{-1}\) in the growth media, arsenic concentrations increased by 52% and 33% in the fronds, and by 18% and 85% in the roots (Table 1; Fig. 1).

We demonstrated for the first time that addition of S to the growth media greatly enhanced arsenic accumulation in P. vittata. When S concentrations were increased from 6.4 to 12.8 mg L\(^{-1}\), the arsenic translocation factor (TF), which is defined as the ratio of arsenic concentration in the fronds to the roots, remained at ~3.0 (Table 1). This means that arsenic concentrations were increased equally in the fronds and roots. The results indicated that addition of S helped both arsenic uptake and translocation by P. vittata. Thus, doubling the S concentration in 0.2-strength Hoagland solution from 12.8 to 25.6 mg L\(^{-1}\) helps arsenic accumulation by P. vittata.

As expected, for a given S level, the arsenic concentrations in the fronds and roots of P. vittata were greater with greater arsenic concentrations in solution (Table 1; Fig. 2). For example, the highest arsenic concentrations in P. vittata were observed in the treatment with the highest S concentration together with arsenic and S in the fronds. Due to the lack of data on S uptake and metabolism by P. vittata, they indicated the need for a detailed investigation on S nutritional status and As metabolism in P. vittata, which was an objective of this study.

Though S had no effect on P. vittata biomass, it significantly increased its arsenic uptake. In the absence of arsenic, the differences among arsenic concentrations in the fronds and roots under different S treatments were insignificant (\(p > 0.05\)) (Table 1; Fig. 2). However, in the presence of arsenic (15 or 30 mg L\(^{-1}\) As), arsenic concentrations in the fronds and roots were significantly enhanced (\(p < 0.05\)) with increasing S levels. For example, with S concentrations increased from 6.4 to 12.8 and to 25.6 mg L\(^{-1}\) in the growth media, arsenic concentrations increased by 52% and 33% in the fronds, and by 18% and 85% in the roots (Table 1; Fig. 2).

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Table 1

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Dry biomass (g plant(^{-1}))</th>
<th>As TFa</th>
<th>GSHa</th>
<th>GSSGa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frond As</td>
<td>Root As</td>
<td>Frond S</td>
<td>Root S</td>
</tr>
<tr>
<td><strong>ANOVA F values</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>As rate</td>
<td>0.003b</td>
<td>0.372</td>
<td>0.387</td>
<td>13.9*</td>
</tr>
<tr>
<td>S rate</td>
<td>0.015</td>
<td>2.45a</td>
<td>0.95a</td>
<td>1.15c</td>
</tr>
<tr>
<td>As × S rate</td>
<td>1.28</td>
<td>2.53a</td>
<td>0.99a</td>
<td>1.23b</td>
</tr>
<tr>
<td><strong>Duncan multiple range test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>As rate (mg L(^{-1}))</td>
<td>0.003b</td>
<td>0.372</td>
<td>0.387</td>
<td>13.9*</td>
</tr>
<tr>
<td>15</td>
<td>0.86a</td>
<td>2.45a</td>
<td>0.95a</td>
<td>1.15c</td>
</tr>
<tr>
<td>30</td>
<td>0.83a</td>
<td>2.52a</td>
<td>0.94a</td>
<td>1.37a</td>
</tr>
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<td><strong>S rate (mg L(^{-1}))</strong></td>
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<td>0.90c</td>
<td>1.07c</td>
</tr>
<tr>
<td>12.8</td>
<td>0.88a</td>
<td>2.57b</td>
<td>0.96b</td>
<td>1.22b</td>
</tr>
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<td>25.6</td>
<td>0.87a</td>
<td>2.85a</td>
<td>1.12a</td>
<td>1.46a</td>
</tr>
</tbody>
</table>

a TF: Translocation factor: concentration ratio in the fronds to roots.

b Significant at \(p < 0.05\).

c Values followed by different letters for a given treatment are significantly different at \(p < 0.05\).

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3.3. Addition of S enhanced arsenic accumulation by P. vittata

In a study on the effect of S on arsenic uptake by P. vittata, Vetterlein et al. (2009) observed a close relationship between arsenic and S uptake by P. vittata. They indicated that the need for a detailed investigation on S nutritional status and As metabolism in P. vittata, which was an objective of this study.

Though S had no effect on P. vittata biomass, it significantly increased its arsenic uptake. In the absence of arsenic, the differences among arsenic concentrations in the fronds and roots under different S treatments were insignificant (\(p > 0.05\)) (Table 1; Fig. 2). However, in the presence of arsenic (15 or 30 mg L\(^{-1}\) As), arsenic concentrations in the fronds and roots were significantly enhanced (\(p < 0.05\)) with increasing S levels. For example, with S concentrations increased from 6.4 to 12.8 and to 25.6 mg L\(^{-1}\) in the growth media, arsenic concentrations increased by 52% and 33% in the fronds, and by 18% and 85% in the roots (Table 1; Fig. 2).

We demonstrated for the first time that addition of S to the growth media greatly enhanced arsenic accumulation in P. vittata. When S concentrations were increased from 6.4 to 12.8 mg L\(^{-1}\), the arsenic translocation factor (TF), which is defined as the ratio of arsenic concentration in the fronds to the roots, remained at ~3.0 (Table 1). This means that arsenic concentrations were increased equally in the fronds and roots. The results indicated that addition of S helped both arsenic uptake and translocation by P. vittata. Thus, doubling the S concentration in 0.2-strength Hoagland solution from 12.8 to 25.6 mg L\(^{-1}\) helps arsenic accumulation by P. vittata.

As expected, for a given S level, the arsenic concentrations in the fronds and roots of P. vittata were greater with greater arsenic concentrations in solution (Table 1; Fig. 2). For example, the highest arsenic concentrations in P. vittata were observed in the treatment

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Fig. 1. Effects of different S and As levels on the sulfate concentrations in the fronds and roots of P. vittata. The levels of added S were 6.4, 12.8, 25.6 mg L\(^{-1}\) and added arsenic were 0, 15, and 30 mg L\(^{-1}\). Bars represent standard deviations of at least three replications.
3.4. Addition of S enhanced the GSH concentrations in *P. vittata*

In addition to its role as an essential macronutrient, sulfur also serves as a precursor for GSH formation in plants (Marschner, 1995; Ernst, 1998). There is significant evidence that arsenic exposure generates reactive oxygen species (ROS) through the conversion of arsenate to arsenite, a process that readily occurs in plants (Singh et al., 2006). As the most prominent non-enzymatic antioxidant in plant, GSH plays a central role in its defense against oxidative stress by contributing to a number of processes, such as free radical scavenging, reduction of peroxides, and modulation of the cellular redox status and thiol-disulphide status of proteins (Cnubben et al., 2001). Glutathione levels are constitutively higher in plants adapted to stress conditions. The increased GSH pool apparently renders them substantially more resistant to different stresses.

As expected, the GSH concentrations in *P. vittata* fronds were significantly increased (\( r^2 = 0.98; p < 0.05 \)) with increasing S in the growth media regardless of the arsenic levels, with the increase being more prominent at the highest As and S level (Table 1; Fig. 2). For example, as S increased from 12.8 to 25.6 mg L\(^{-1} \), the GSH concentrations in the fronds increased from 1.2 to 1.31 μmol g\(^{-1} \) fw (\( \sim 8.3\% \) increase) in the absence of arsenic, and from 1.28 to 1.38 μmol g\(^{-1} \) fw (\( \sim 33\% \) increase) at 30 mg As L\(^{-1} \) (Fig. 3). However, the concentrations of oxidized glutathione (GSSG) remained unchanged (Table 1). The GSH concentrations observed in this experiment were similar to those reported by Singh et al. (2006).

At each S level, increased GSH concentrations were observed with increasing arsenic concentration, i.e., arsenic clearly induced GSH formation in *P. vittata* (\( r^2 = 0.99; p < 0.05 \)), which is consistent with the observation of Singh et al. (2006). In the presence of 25.6 mg L\(^{-1} \) S, the GSH concentration increased from 1.31 to 1.38 and to 1.70 μmol g\(^{-1} \) as arsenic concentrations were increased from 0 to 15 and to 30 mg L\(^{-1} \) (Fig. 3). Though the specific role played by GSH in arsenic accumulation by *P. vittata* is still unclear, it is possible that S-enhanced arsenic uptake by *P. vittata* may be related to GSH.

3.5. Effect of GSH on plant arsenic accumulation in *P. vittata*

Though GSH concentrations are increased upon arsenic exposure in *P. vittata*, GSH has not been shown to play a significant role in arsenic accumulation by *P. vittata* (Zhao et al., 2003). However, arsenic-induced increase of GSH in *P. vittata* may represent a defense system, which is not as direct as the primary defense response such as vacuole compartmentalization (Singh et al., 2006). Since GSH is capable of reducing As\(^{V} \) to As\(^{III} \) in solution (Scott et al., 1993), one possible role GSH may play is to enhance As\(^{V} \) reduction to As\(^{III} \) in the growth media and in plant biomass.

To test this hypothesis, we determined the effect of exogenous GSH in enhancing plant arsenic uptake, and in arsenic speciation in the growth media and *P. vittata*. Similar to S, addition of GSH at low concentration (0.4 mM) increased plant arsenic uptake by *P. vittata*. However, GSH addition of higher concentration at 0.8 mM had no effect on plant arsenic uptake (Table 2; Fig. 4). At 15 mg L\(^{-1} \) arsenic, addition of 0.4 mM GSH increased arsenic concentration in the fronds by 77% from 550 to 974 mg kg\(^{-1} \); the increase was more pronounced at 30 mg kg\(^{-1} \) arsenic, which was by 89% from 1297 to 2449 mg kg\(^{-1} \) (Fig. 4a). The impact of GSH on root arsenic was limited.

Addition of GSH had no effect on arsenic reduction in the growth media at both arsenic concentrations (Table 3). This was also the case with arsenic speciation in the fronds of *P. vittata*. Regardless of

<table>
<thead>
<tr>
<th>Table 2</th>
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<tbody>
<tr>
<td><strong>Effects of As and GSH on the As concentrations in <em>P. vittata</em> fronds (mg kg(^{-1} ).</strong></td>
</tr>
</tbody>
</table>

\[
\begin{array}{|c|c|c|c|}
\hline
\text{As rate (mg L}^{-1}\text{)} & \text{Frond As} & \text{GSH rate (mM)} & \text{Frond As} \\
\hline
0 & 4.80c & 0 & 618b \\
15 & 781b & 0.4 & 1142a \\
30 & 1678a & 0.8 & 703b \\
\hline
\end{array}
\]

Values followed by different letters in a column are significantly different at \( p < 0.05 \) based on Duncan tests.
the amount of GSH added, ~72–79% of the arsenic was present as AsIII at 15 mg As L⁻¹ and ~80–86% at 30 mg As L⁻¹. Though in an in vitro experiment, Scott et al. (1993) demonstrated GSH-induced reduction of AsV to AsIII and complexation of AsIII with GSH in solution under N-environment, our data indicated that GSH did not play a role in arsenic reduction in the growth media or in P. vittata.

Plants have been shown to take up GSH. In a hydroponic experiment, GSH concentration in the roots of Brassica napus is increased from 0.5 to 2.5 μmol g⁻¹ fw after being fed with 1 mM GSH for 18 h (Anne et al., 1999). Similarly, GSH concentration in A. thaliana is increased from 1.2 to 3.5 μmol g⁻¹ fw. We expect that the GSH concentration in P. vittata would be increased after being fed with 0.4 or 0.8 mM GSH for 2 weeks. The increased GSH in P. vittata likely helped its arsenic uptake and translocation.

Though both S and GSH helped arsenic uptake in P. vittata, they played slightly different roles, which was apparent when looking at arsenic TF (Table 4). Arsenic TF did not change much with increasing S in the S experiment, indicating that S enhanced both arsenic uptake and translocation in P. vittata. In other words, the arsenic taken up by P. vittata was translocated to the fronds, resulting in similar TFs under different S treatments. However, this was not the case in the GSH experiment. Addition of GSH at 0.4 mM almost doubled arsenic TF compared to the control, i.e., 10 vs. 6.2 at 15 mg As L⁻¹ and 8.5 vs. 4.6 at 30 mg As L⁻¹ (Table 4). This indicated that addition of GSH helped arsenic translocation from the roots to the fronds without changing arsenic speciation. However, this effect was not observed at 0.8 mM GSH, indicating too much GSH was not beneficial for plant arsenic uptake or translocation.

Similar effects were observed with exogenous histine, a common amino acid in proteins. Addition of 0.4 mM histine increased arsenic concentrations from 656 to 1292 mg kg⁻¹ in the fronds and from 88.6 to 178 mg kg⁻¹ in the roots, with arsenic TF remaining 7.2 to 7.4 at 15 mg As L⁻¹ (data not shown). At 0.8 mM histine, arsenic concentrations in both fronds and roots were reduced. How GSH and histine enhance arsenic uptake and translocation in P. vittata warrants further examination.

Singh et al. (2006) compared the metabolic adaptations between P. vittata and Pteris ensiformis (a non-arsenic hyperaccumulator) to arsenic-induced oxidative stress and found that GSH concentration increased upon arsenic exposure in P. vittata, whereas it significantly decreased in P. ensiformis. There is also considerable evidence that at least part of the arsenic accumulated by plants is coordinated with GSH, which may help plant in arsenic detoxification (Meharg and Hartley-Whitaker, 2002). However, the precise mechanisms of S-enhanced and GSH-enhanced plant arsenic accumulation by P. vittata need further investigation.

Acknowledgements

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Table 4

<table>
<thead>
<tr>
<th>As rate (mg L⁻¹)</th>
<th>S experiment</th>
<th>GSH experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>S rate (mg L⁻¹)</td>
<td>TF (mM)</td>
<td>GSH rate (mM)</td>
</tr>
<tr>
<td>15</td>
<td>6.4</td>
<td>3.2</td>
</tr>
<tr>
<td>15</td>
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TF = translocation factor, which is defined as arsenic concentrations in the fronds to the roots.

References


