



Effects of selenium on arsenic uptake in arsenic hyperaccumulator *Pteris vittata* L.

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ABSTRACT

Selenium (Se) is a non-metallic element, which has the capability to increase the antioxidative capacity and stress tolerance of plants to heavy metals. Plants vary considerably in their physiological response to Se. The reported research investigated the effects of Se on arsenic (As) uptake by As hyperaccumulator *Pteris vittata* L. and determined possible mechanisms of interaction. *Pteris vittata* plants were exposed hydroponically to 0, 150 or 300 μM of Na_2HAsO_4 in the presence of 0, 5 or 10 μM of Na_2SeO_4 for 5 or 10 d. Application of 5 μM Se enhanced As concentration by *P. vittata* fronds by 7–45%. At 5 μM , Se acted as an antioxidant, inhibiting lipid peroxidation (reduced by 26–42% in the fronds) via increased levels of thiols and glutathione (increased by 24% in the fronds). The results suggest that Se is either an antioxidant or it activates plant protective mechanisms, thereby alleviating oxidative stress and improving arsenic uptake in *P. vittata*.

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1. Introduction

Selenium (Se) is an essential nutrient for humans and animals (Schwartz and Foltz, 1957), though it is not required by plants. Selenium is a component in the antioxygenic enzyme glutathione peroxidase (Rotruck et al., 1973). The harmful effects and toxicity mechanisms of Se have been discussed extensively (Lauchli, 1993); however, some reports indicate that Se may positively affect plant growth at low concentrations (Xue et al., 2001). For example, Se affects plant tolerance to metal toxicity (Whanger, 1981). Hartikainen et al. (2000) demonstrated that depending on dosage, Se exerts a dual effect on ryegrass. At low concentrations it acts as an antioxidant and stimulates plant growth, whereas at higher concentrations it acts as a pro-oxidant, reducing plant growth.

Drotar et al. (1985) were the first to show glutathione peroxidase activity in tissue-cultured plant cells. Thereafter, Se-dependent glutathione peroxidase has also been found in marine diatom (Price and Harrison, 1988) and green alga (Yokota et al., 1988). Glutathione peroxidase purified from *Chlamydomonas* by Shigeoka et al. (1991) is similar to that occurring in mammalian tissue. Takeda et al. (1997) reported that without Se, 40% of external hydrogen peroxide (H_2O_2) in *Chlamydomonas* cells was scavenged by ascorbate peroxidase and the residual H_2O_2 by catalase,

while in Se-containing medium external H_2O_2 is primarily removed by glutathione peroxidase.

In recent years, arsenic (As) pollution has become a major public health and environmental concern in many countries (Smith et al., 2002). Remediation of As-contaminated soil and water is necessary for protecting both human life and agricultural production. Phytoremediation of As-contaminated soils has been considered a cost-effective and environment-friendly technique. The effectiveness of phytoremediation is determined by two factors, i.e., identification of plants with high As hyperaccumulating potential and knowledge of factors to maximize As accumulation. The first stipulation was likely validated by the discovery of the As hyper accumulator *Pteris vittata* L. (Ma et al., 2001a), commonly known as Chinese brake fern, and subsequently other fern species *Pityrogramma calomelanos* (Francesconi et al., 2002), and *P. longifolia* and *P. umbrosa* (Zhao et al., 2002; Meharg, 2003). A total of seven cultivars of *P. cretica* were identified as As hyperaccumulators (Ma et al., 2001b; Meharg, 2003; Zhao et al., 2002). Recently, Srivastava et al. (2006) reported *P. biaurita* L., *P. quadriaurita* Retz and *P. ryukyuensis* Tagawa as As hyperaccumulators. These As hyperaccumulators are all ferns and can be potentially used for phytoremediation of As-contaminated sites. Among these ferns, *P. vittata* has received the most attention due to its ability to accumulate large quantities of As (up to 2.3% of dry weight) in its aboveground biomass (Srivastava et al., 2006).

One approach to improve plant tolerance to environmental stress is to increase their antioxidant capacity (Foyer et al., 1994; Srivastava et al., 2005b). Studies on maize have shown that elevated antioxidant levels can protect the photosynthetic apparatus

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of plants from oxidative damage (Van Breusegem et al., 1999). Owing to the antioxidant role of Se, it is hypothesized that Se can counteract the detrimental effects of As stress in plants. The objective of this study was to test this hypothesis and investigate the antioxidant and stress effects of Se on As uptake by *P. vittata*.

2. Methods

Spores of *P. vittata* L. were collected from the plants growing along the poles of the trail in Alfred A. Ring Park, Gainesville, Florida and propagated in a seedbed. Spores were collected from a mature fertile frond by placing the fronds in a paper bag. The spores were transferred into a small plastic container and stored in a warm dry atmosphere. The spores were germinated by sowing on a mixture of moist soil (50% sand, 25% peat and 25% garden soil) in seed trays. Spores were covered with a plastic film to maintain moisture. After six weeks when the spores were germinated and prothalli developed, Osmocote® extended time-release fertilizer (N–P–K = 18–6–12) was added to the soil as base fertilizer with a ratio of 1:1000 (Tu and Ma, 2002). After sporophytes grew to two to three fronds, they were transplanted individually into 2-in. plastic pots containing Miracle-Gro® potting soil (The Scotts Miracle-Gro® Co., Marysville, Ohio). After one month, these plants were transferred to 500 ml pots (one plant per pot) containing 0.2-strength Hoagland nutrient solution (HNS; Hoagland and Arnon, 1938). Plants were acclimatized in a hydroponic system for two weeks. Experiment was carried under the simplest hydroponic system, i.e., water culture system with slight modification (Gibeaut et al., 1997). The platform that holds the plants was made of Styrofoam and floats directly on the nutrient solution. An air pump supplies air to the air stone that bubbles the nutrient solution and supplies oxygen to the roots of the plants.

The experimental design was set up as a completely randomized design with seven treatments and three replications. The acclimated plants were transferred into 0.2-strength HNS, which was spiked with 0, 150, or 300 μM of As ($\text{Na}_2\text{HAsO}_4 \cdot 7 \text{H}_2\text{O}$), and 0, 5 or 10 μM selenate (Na_2SeO_4). The solution was aerated continuously and replaced twice per week during the acclimatization. After addition of arsenic to the nutrient medium, losses of water via transpiration were replenished by frequent additions of deionized water by maintaining a constant volume.

The plants were kept within a growth chamber with an 8 h light period at a light intensity of $350 \mu\text{mol m}^{-2} \text{s}^{-1}$, $25^\circ\text{C}/20^\circ\text{C}$ day/night temperature and 60–70% relative humidity. Plants were harvested 5 d and 10 d after As treatment. Upon harvest, each fern was separated into fronds and roots. Tissue samples were either air-dried for total As, phosphorus (P), Se and plant biomass analysis or frozen in liquid nitrogen and stored at -80°C for biochemical analyzes.

2.1. As, Se and P analyzes

Air-dried frond or root samples (0.5 g) were digested with concentrated nitric acid on a temperature-controlled digestion block (Environmental Express, Mt. Pleasant, SC) using USEPA Method 3050B (U.S. EPA, 1994). Arsenic and Se analysis was performed with a graphite furnace atomic absorption spectrophotometer (Perkin–Elmer SIMAA 6000, Norwalk, CT). A NIST standard reference material (SRM) was used for quality control. Calibration work with certified As and Se standard solution was included. Matrix spikes were carried out in 10% samples, with an average recovery of $94 \pm 6\%$. Analytical SRM recovery was within 10% of the true value.

Phosphorus was determined by a modified molybdenum blue method (Carvalho et al., 1998). Briefly, the pH of the digestion solution was adjusted to around seven with NaOH and H_2SO_4 . Ten

micro litre of the solution was pipetted into a 20 ml glass test tube, to which 0.5 ml of L-cysteine (5% w/v in 0.6 M HCl) was added. The test tube was capped tightly and incubated for 5 min at 80°C to allow complete reduction of arsenate into arsenite. The solution was cooled to room temperature and P was determined by the molybdenum blue method (Murphy and Riley, 1962).

2.2. Lipid peroxidation

Lipid peroxidation was measured as the amount of thiobarbituric acid reacting substances (TBARs) determined by thiobarbituric acid (TBA) reaction, following the method of Heath and Packer (1968) with slight modification (Ohkawa et al., 1979). Approximately 0.5 g of frozen-tissues was cut into small pieces and homogenized with 2.5 ml of 5% (wt/v) trichloroacetic acid (TCA) in a glass homogeniser using a cold mortar and pestle over ice. The homogenates were transferred into 50 ml Nalgene® centrifuge tubes and centrifuged at 10,000g for 15 min at room temperature ($20\text{--}22^\circ\text{C}$). The concentrations of lipid peroxides, together with the oxidatively modified proteins of plants, were quantified and expressed as total TBARS in terms of $\mu\text{mol g}^{-1}$ fresh weight using an extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$.

2.3. Reduced glutathione (GSH)

Reduced glutathione was measured by the method using glutathione reductase (GR) (Gossett et al., 1994). Over ice, 1 g of frozen-fronds was ground with inert sand and 5 ml of ice-cold 6% (v/v) *m*-phosphoric acid (pH 2.8) containing 1 mM EDTA in a cold mortar and pestle. The homogenate was centrifuged at 22,000g for 15 min and the supernatant removed and then filtered through a 0.45 μm ultrafilter. Two solutions were then prepared. Solution A consisted of 110 mM $\text{Na}_2\text{PO}_4 \cdot 7\text{H}_2\text{O}$, 40 mM $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 15 mM EDTA, 0.3 mM 5, 5'-dithiobis-(2-nitrobenzoic acid), and 0.4 ml L^{-1} BSA. Solution B consisted of 1 mM EDTA, 50 mM imidazole, 0.2 ml L^{-1} BSA, and an equivalent of 1.5 units GR activity (baker's yeast, Sigma Chemical Company). Total glutathione was measured in a reaction mixture consisting of 400 μL of solution A, 320 μL of solution B, 400 μL of a 1:50 dilution of the extract in 5% (w/v) Na_2HPO_4 (pH 7.5) and 80 μL of NADPH. The reaction rate was measured using a spectrophotometer by following the change in absorbance at 412 nm for 4 min.

2.4. Total non-protein thiol

Frozen-fronds were ground in liquid nitrogen and 100 mg of ground plant material was extracted with 300 μL solution containing 1 M NaOH and 1 g L^{-1} NaBH_4 . After thorough mixing and 10 min incubation at room temperature, the mixture was centrifuged for 3 min at 14,000g and 300 μL of the supernatant fluid was added to 50 μL of concentrated HCl (37%). After thorough mixing, the samples were again centrifuged for 3 min at 14,000g and 20 μL of the supernatant fluid was added to 1 mL of a solution containing 300 μM 5,5-dithiobis(2-nitrobenzoic acid) in 100 mM potassium phosphate buffer, pH 7.5 (Ellman's reagent). After 15 min incubation at room temperature, the optical density was read at 412 nm in a spectrophotometer. A set of samples containing a range of GSH concentrations (0–100 mmol per 20 μL) was prepared at the same time and used to make a standard curve ($r^2 > 0.99$). This method measures free non-protein thiols and is not a representation of total or conjugated thiols.

2.5. Data analyzes

All results were expressed as an average of three replications. Treatment effects were determined by analyzes of variance using

the general linear model procedure of the Statistical Analysis System (SAS Institute Inc., 1996). Duncan's test at a 5% probability was used for post hoc comparisons to separate treatment differences. All results were expressed as means followed by standard deviations.

3. Results

3.1. Plant biomass

Biomass of *P. vittata* varied with exposure time as well as As and Se concentration (Table 1). Plant biomass in the control medium nearly doubled over a 5 d period. For example, biomass of the control plants after 5 d and 10 d of growth was 3.0 and 5.5 g (fw). On average, frond biomass increased 8.8% whereas root biomass increased 75% when incubation period increased from 5 d to 10 d. Greater biomass was measured in roots than the fronds.

Addition of As increased plant biomass with higher concentrations producing greater biomass. Biomass after 5 d exposure increased from 5.5 to 6.6 g as As application rates increased from 150 to 300 μM (Table 1). However, addition of higher concentrations of Se generally reduced plant biomass. The reduction was more apparent with root biomass and higher As concentration. For the 300 μM As treatment after 5 d and 10 d exposures, addition of 10 μM Se reduced frond biomass by 30% (6.6–4.6 g) and 25% (6.9–5.2 g), and root biomass by 73% (5.1–1.4 g) and 44% (7.8–4.4 g) (Table 1).

3.2. Arsenic concentrations and distribution

Increasing As exposure time and concentration resulted in greater As accumulation in the fronds and roots of *P. vittata* (Fig. 1). Frond As contents increased by 2–4 fold as exposure time (5–10 d) or application rate (150–300 μM) doubled. The fronds accumulated the highest As at 1587 mg As kg^{-1} after exposure to 300 μM As for 10 d. Addition of Se significantly enhanced As concentrations in both fronds and roots except those treated with 300 μM As and 10 μM Se for 10 d (Fig. 1), which were lower than those exposed to 300 μM As and 5 μM Se for 10 d. The 5 μM Se rate was more effective than 10 μM in increasing plant As concentrations.

In addition to As concentrations, As distribution in *P. vittata* was also affected by As exposure time and application rate (Fig. 1). Translocation factor, which is defined as the As concentration ratio in the fronds to the roots, describes the ability of a plant in translocating As from roots to fronds. The TF of *P. vittata* increased from an average of 1.2–3.1 as exposure time increased from 5 d to 10 d (data not shown), which is typical of hyperaccumulators (Ma et al.,

2001a,b). In other words, as more As was taken up by *P. vittata*, more As was translocated from roots to fronds.

3.3. Total P and Se

Total P concentrations in the fronds ranged from 2.5 to 6.1 g kg^{-1} and roots from 1.4 to 4.4 g kg^{-1} (Table 2). Longer exposure time resulted in greater P concentrations in fronds and roots, with the corresponding average being 49% and 16% greater as exposure time increased from 5 d to 10 d. In other words, the increase in P concentrations in the fronds was substantially greater than that in the roots. However, no trend was observed with changes in either As or Se concentrations.

Though *P. vittata* was efficient in As accumulation, its ability in Se uptake was limited. Average Se concentrations increased from 4.1 to 9.5 mg kg^{-1} in fronds and 6.3 to 7.1 mg kg^{-1} in roots as exposure time increased from 5 d to 10 d (Table 3). Higher Se application rate resulted in greater Se accumulation in the plants; average Se concentrations increased by 72% (5.0 vs. 8.6 mg kg^{-1}) as Se concentrations increased from 5 to 10 μM .

3.4. Lipid peroxidation

Lipid peroxidation is one of the expected consequences of stress-induced cellular buildup of reactive oxygen species (ROS). Lipid peroxidation is measured by determining the contents of thiobarbituric acid reactive substances (TBARS) in plants. Increasing As concentrations increased TBARS levels in fronds (Fig. 2). The ameliorative role of exogenously supplied Se was confirmed by increased As uptake (Fig. 1) while TBARS levels remained low (Fig. 2).

Increasing As concentrations significantly increased lipid peroxidation in the fronds as indicated by a rapid increase in TBARS (Fig. 2). For example, TBARS concentrations in the fronds increased from 13 to 29 to 63 $\mu\text{M/g}$ fw after the 5 d exposure as As concentrations increased from 0 to 150 to 300 μM . The added Se significantly inhibited lipid peroxidation in the fronds. Compared to the controls, addition of 5 and 10 μM Se reduced the TBARS concentrations in the fronds by 26–42% and 27–35%. There were no significant differences in TBARS between two application rates of Se (5 and 10 μM) or two exposure times (5 and 10 d).

3.5. Reduced glutathione (GSH)

Glutathione exists in both reduced and oxidized states. Glutathione maintains the cellular redox status and serves as substrate for phytocheletin synthesis. The effect of Se on GSH concentration was both dose- and time- dependent (Fig. 3a). The concentration of GSH in the fronds increased with increasing As concentrations, with those exposed for 10 d being significantly greater than those exposed for 5 d. For example, GSH concentration in the fronds after 5 d exposure increased from 0.48 to 0.93 to 1.4 mmol/kg dw as arsenic concentrations increased from 0 to 150 to 300 μM . The GSH concentration after 10 d exposure increased from 0.63 to 1.3 to 2.2 mmol/kg dw.

Addition of Se increased GSH concentrations in the fronds, which was more pronounced with longer exposure time (10 vs. 5 d) for both As concentrations. For example, in the presence of 150 μM As, addition of 5 μM Se increased the GSH concentration from 1.4 to 1.7 mmol/kg dw as exposure time increased from 5 to 10 d. Though addition of 10 μM Se increased GSH concentrations compared to the control, increasing Se concentrations from 5 to 10 μM resulted in a mixed effect on GSH concentrations. The GSH concentrations in the fronds were greater in the presence of Se than those in the absence of Se.

Table 1

Plant biomass after exposure to different concentrations of As and Se for 5 d or 10 d (g fw)

Solution concentration (μM)		5 d exposure		10 d exposure	
Arsenic	Selenium	Fronds	Roots	Fronds	Roots
0	0	3.00 \pm 0.27b ^a	1.70 \pm 0.44b	5.47 \pm 1.35a	4.57 \pm 0.55bc
150	0	5.53 \pm 0.21ab	5.03 \pm 1.30a	6.67 \pm 2.20a	6.43 \pm 3.01ab
150	5	6.53 \pm 1.32a	5.00 \pm 0.89a	7.17 \pm 2.4a	7.97 \pm 0.38a
150	10	6.53 \pm 0.92a	3.20 \pm 0.66ab	5.17 \pm 0.66a	8.10 \pm 1.50a
300	0	6.60 \pm 1.39a	5.13 \pm 1.3a	6.93 \pm 1.3a	7.77 \pm 1.27a
300	5	7.07 \pm 2.82a	2.83 \pm 1.20b	6.70 \pm 0.45a	2.27 \pm 0.84c
300	10	4.60 \pm 0.66ab	1.41 \pm 0.47b	5.17 \pm 1.18a	4.37 \pm 2.03bc

^a Values refer to the mean followed by standard deviation. Means followed by the same letter in a column were not significantly different at $P < 0.05$.

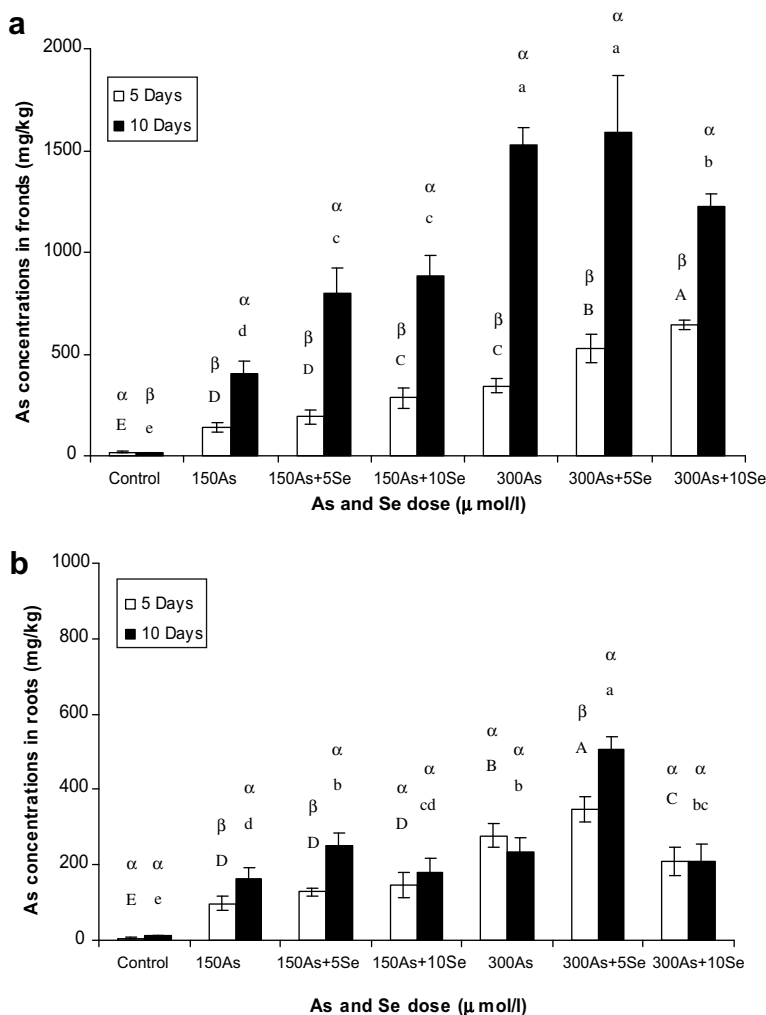


Fig. 1. Arsenic concentrations (mg kg^{-1} dry mass) in the fronds (a) and roots (b) of *P. vittata* after exposure to different concentrations of As and Se for 5 or 10 d. Vertical bars indicate standard deviation of three separate experiments. Means followed by the same capital letter were not significantly different at $P < 0.05$ for 5 d exposure; means followed by the same lower case letter were not significantly different at $P < 0.05$ for 10 d exposure; means followed by the same Greek letter were not significantly different at $P < 0.05$ for plants between 5 d and 10 d exposure.

Table 2

Total P concentrations in the fronds and roots of *P. vittata* after exposure to different concentrations of As and Se for 5 d or 10 d (g kg^{-1})

Solution concentration (μM)		P concentrations (g kg^{-1})			
Arsenic	Selenium	5 d		10 d	
		Fronds	Roots	Fronds	Roots
0	0	$2.19 \pm 0.85\text{a}^{\text{a}}$	$3.22 \pm 0.73\text{ab}$	$2.51 \pm 0.25\text{c}$	$1.43 \pm 0.80\text{b}$
150	0	$3.45 \pm 0.97\text{a}$	$1.96 \pm 0.54\text{bc}$	$4.31 \pm 0.64\text{b}$	$3.03 \pm 1.1\text{ab}$
150	5	$3.08 \pm 0.75\text{a}$	$2.28 \pm 0.32\text{bc}$	$4.46 \pm 0.44\text{b}$	$4.64 \pm 0.15\text{ab}$
150	10	$3.54 \pm 0.50\text{a}$	$2.23 \pm 0.78\text{bc}$	$6.05 \pm 0.82\text{a}$	$3.28 \pm 0.88\text{ab}$
300	0	$2.07 \pm 0.62\text{a}$	$2.69 \pm 0.98\text{bc}$	$3.25 \pm 0.89\text{bc}$	$3.40 \pm 0.17\text{ab}$
300	5	$2.57 \pm 0.51\text{a}$	$1.51 \pm 0.33\text{c}$	$5.12 \pm 0.52\text{ab}$	$3.02 \pm 1.5\text{ab}$
300	10	$2.64 \pm 0.14\text{a}$	$4.16 \pm 0.14\text{a}$	$4.67 \pm 0.73\text{b}$	$4.35 \pm 2.0\text{a}$

^a Values refer to the mean followed by standard deviation. Means followed by the same letter in a column were not significantly different at $P < 0.05$.

The addition of $5 \mu\text{M}$ selenate to plants after 5 d As exposure had significantly more GSH than the plants treated with $10 \mu\text{M}$.

3.6. Total non-protein thiol

Concentrations of acid-soluble thiol (non-protein) in fronds were significantly elevated with increasing As concentrations

Table 3

Total Se concentrations in the fronds and roots of *P. vittata* after exposure to different concentrations of As and Se for 5 d or 10 d (g kg^{-1})

Solution concentration (μM)		Se concentrations (mg kg^{-1})			
Arsenic	Selenium	5 d		10 d	
		Fronds	Roots	Fronds	Roots
0	0	<1	<1	<1	<1
150	5	$2.4 \pm 0.78\text{bc}^{\text{a}}$	$3.8 \pm 0.75\text{b}$	$6.40 \pm 2.55\text{b}$	$5.3 \pm 1.74\text{b}$
150	10	$4.3 \pm 0.98\text{b}$	$8.4 \pm 2.12\text{a}$	$8.40 \pm 2.09\text{ab}$	$9.0 \pm 2.29\text{a}$
300	5	$1.7 \pm 0.56\text{c}$	$3.17 \pm 0.68\text{b}$	$11.65 \pm 2.05\text{a}$	$5.7 \pm 2.55\text{b}$
300	10	$8.0 \pm 2.8\text{a}$	$10 \pm 3.18\text{a}$	$11.63 \pm 3.45\text{a}$	$5.3 \pm 1.79\text{Ab}$

^a Values refer to the mean followed by standard deviation. Means followed by the same letter in a column were not significantly different at $P < 0.05$.

(Fig. 3b). Addition of Se at $5 \mu\text{M}$ increased the thiol concentration, but it decreased at $10 \mu\text{M}$ Se. Total non-protein thiol concentrations were much greater than were GSH concentrations, which accounted for 32–47% of total non-protein thiols in the fronds (Fig. 3b). The two were highly correlated with a correlation coefficient of 0.96. Significant increase in thiol concentrations was observed with an increase in exposure time across all As and Se levels.

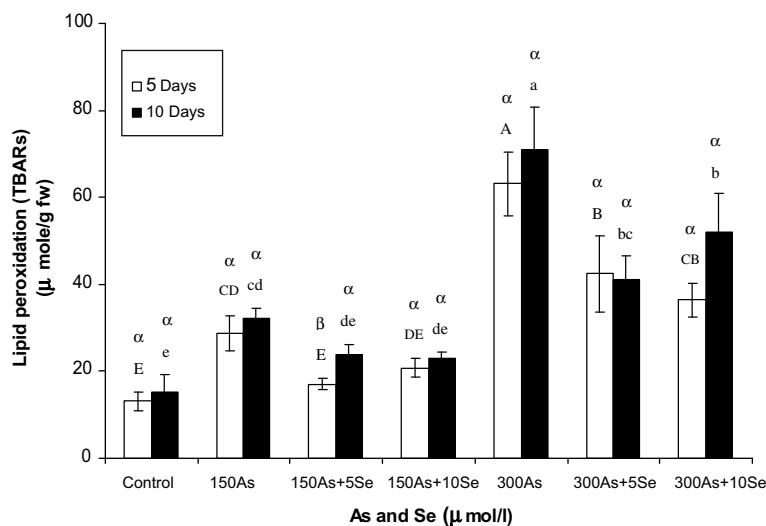


Fig. 2. Lipid peroxidation ($\mu\text{mol malondialdehyde g}^{-1} \text{fw}$) in fronds of *P. vittata* after exposure to different concentrations of As and Se for 5 or 10 d. Vertical bars indicate standard deviation of three separate experiments. Means followed by the same capital letter were not significantly different at $P < 0.05$ for 5 d exposure; means followed by the same lower case letter were not significantly different at $P < 0.05$ for 10 d exposure; means followed by the same Greek letter were not significantly different at $P < 0.05$ for plants between 5 d and 10 d exposure.

4. Discussion

Arsenic is known to induce oxidative stress in plants by generating various ROS (Meharg and Hartley-Whitaker, 2002), resulting in a range of responses in plants, including readjustment of transport and metabolic processes, and growth inhibition (Tu and Ma, 2003). Like other environmental stresses, arsenic may create conditions in the thylakoids where the energy level exceeds the amount that can be dissipated by the metabolic pathways of the chloroplast (Dat et al., 2000). As a consequence, the electron transport processes in the thylakoid membranes are impeded and toxic symptoms may develop. Toxic intermediates are generated in the cell wall region as well as inside the cell during this process. This affects membrane permeability, enzyme activity, the pool of metabolites, photosynthetic activity, and leaf chlorosis and necrosis (Karabal et al., 2003; Nguyen et al., 2000).

Plants have several mechanisms to defend themselves against the toxic effects of arsenic. Compartmentation and translocation of As are reported to be the basic mechanisms involved in As tolerance (Marschner, 1995; Tu and Ma, 2003). However, addition of Se had no impact on arsenic translocation in *P. vittata* (Fig. 1). When these mechanisms become insufficient, biochemical processes can become involved in the detoxification of imposed stress (Dixit et al., 2001; Srivastava et al., 2005b). There are, in theory, three mechanisms that a tolerant plant might use to survive or even grow in a stress situation (Steffen, 1991): (a) It may minimize the production of ROS; (b) protect itself from the deleterious degradative reactions of ROS by efficiently scavenging ROS; (c) or repair the injury after the degradation has occurred.

When exposed to 20 mg L^{-1} Se, *P. vittata* accumulated large amounts of Se without visible toxicity symptoms, with Se concentrations in the fronds being as high as 1028 mg kg^{-1} (Srivastava et al., 2005a). However, it accumulated only $1.7\text{--}12 \text{ mg kg}^{-1}$ Se in the fronds when exposed to $0.3\text{--}0.7 \text{ mg L}^{-1}$ Se (Table 3). Even at these low Se concentrations, its impacts on plant As uptake were observed (Fig. 1). The protective role of Se in *P. vittata* was probably related to its antioxidative functions via production of reduced glutathione and non-protein thiols (Fig. 3). Compared to the control, As accumulation in *P. vittata* fronds was significantly higher when subjected to Se application (Fig. 1). Though greater As concentra-

tions in plants cause greater oxidative stress (Fig. 2), greater As concentrations also promoted plant growth. Increasing As application rate from 150 to 300 μM resulted in 11–12% increase in biomass of *P. vittata* (Table 1). Tu and Ma (2002) reported that adding arsenic up to 100 mg kg^{-1} to a soil significantly increased biomass of *P. vittata* in a greenhouse experiment. The biomass increase is attributed to increased plant P uptake, which was not, however, the case in this study (Table 2).

Despite a marked increase in TBARS (Fig. 2), *P. vittata* seemed to defend itself from the detrimental effects of the high As by increasing the activity of GSH and non-protein thiol (Fig. 3; Singh et al., 2006). The unique role of Se in *P. vittata* was apparent at exposure to $5 \mu\text{M}$ Se. Addition of Se not only enhanced As uptake by *P. vittata* but also promoted the induction of GSH and non-protein thiol to reduce the adverse effect of As toxicity. This was supported by inhibition of TBARS induction (Fig. 2), which is similar to those data by Hartikainen et al. (2000) in ryegrass.

The positive interaction between Se and As might be attributable also to Se-induced increase in reducing power needed in plant photosynthesis because Se is known to exhibit a photovoltaic action (Hartikainen and Xue, 1999). The role of Se in plant tolerance to oxidative stress suggests that Se induces a mechanism that protects photosynthetic machinery from damage by slightly altering the susceptibility of their cell membranes. In general, the antioxidative effect of Se is associated with improved GSH-peroxidase (GSH-Px) activity (Xue et al., 2001) and thus enhanced scavenging of hydrogen peroxide. The added Se contributed to the maintenance of antioxidative capacity by inducing more GSH and non-protein thiol, which is demonstrated by reduction in TBARS (Figs. 2 and 3). However, unlike As (Table 1), addition of Se failed to promote plant growth.

These results call for further studies of the role of Se in As accumulation by *P. vittata*. However, they clearly indicated that Se may exert a beneficial role in plants under As stress, supporting the finding of Hartikainen and Xue (1999) that Se defends plants subjected to UV exposure. However, it is unclear why addition of Se reduced the biomass of *P. vittata* (Table 1) even though it reduced oxidative stress measured by TBARS (Fig. 2). Even if the present study supports the mechanism of Se-dependent GSH increase in *P. vittata*, the Se-induced increase

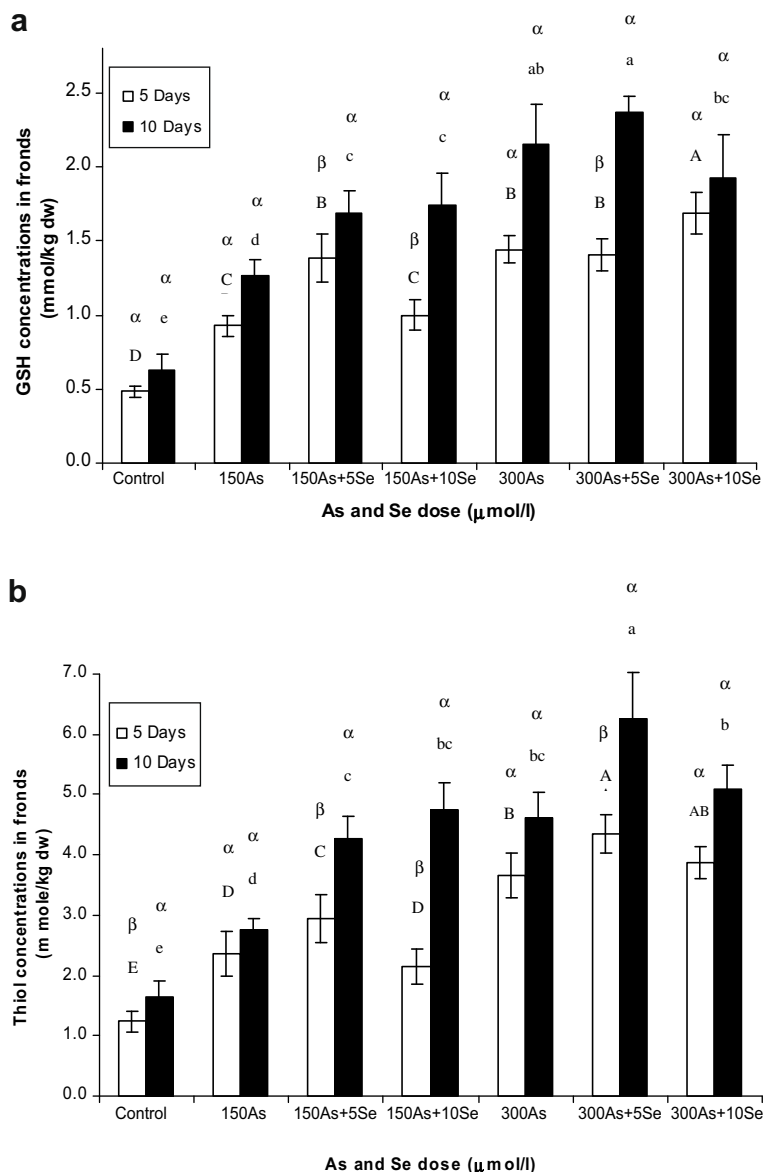


Fig. 3. Changes in reduced glutathione (a) and non-protein thiol (b) concentrations (mmol kg^{-1} dry mass) in fronds of *P. vittata* after exposure to different concentrations of As and Se for 5 and 10 d. Vertical bars indicate standard deviation of three separate experiments. Means followed by the same capital letter were not significantly different at $P < 0.05$ for 5 d exposure; means followed by the same lower case letter were not significantly different at $P < 0.05$ for 10 d exposure; means followed by the same Greek letter were not significantly different at $P < 0.05$ for plants between 5 d and 10 d of exposure.

in antioxidative substances may not be marked enough to explain the enhanced antioxidative capacity and stimulated As uptake in *P. vittata*. It could be possible that Se at low concentrations acted as an elicitor, up-regulating defense genes and down-regulating growth-related genes.

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