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A comparison of arsenic tolerance, uptake and accumulation between arsenic hyperaccumulator, *Pteris vittata* L. and non-accumulator, *P. semipinnata* L.—A hydroponic study

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ABSTRACT

The differences in arsenic (As) tolerance, uptake and accumulation between *Pteris vittata* (an As hyperaccumulator) and *P. semipinnata* (nonaccumulator) were investigated under hydroponic conditions. The results showed that As uptake by *P. vittata* was significantly higher ($p < 0.05$) than that of *P. semipinnata*. Significantly higher concentrations of As accumulated in the fronds of *P. vittata*, while in the roots of *P. semipinnata*. The short-term (<24 h) uptake kinetics were fitted a hyperbolic equation which could be divided into linear and saturable components (described by Michaelis–Menten kinetics/model). The increase in hydrogen peroxide (H_2O_2) content in both plant species significantly correlated ($p < 0.05$) with increasing As content in the plants and As exposure time, especially for midrib of *P. semipinnata*. *P. semipinnata* showed higher concentrations of H_2O_2 than those of *P. vittata*. The relative electrical conductivity (REC, %) values in the root and pinnae followed a similar trend as plant H_2O_2 contents, increasing with As exposure, especially for *P. semipinnata*. Significantly higher REC (%) values ($p < 0.05$) were observed in the root than that in pinnae of *P. semipinnata*. The results indicated that high doses of As produced oxidative damages in both plant species.

1. Introduction

Arsenic contamination in the environment is wide-spread due to both natural and anthropogenic reasons such as mining, smelting, landfilling of industrial wastes and agricultural activities [1,2,3]. Contamination in excess of 1000 mg As kg^{-1} has been recorded at many sites throughout Australia, the United States, Africa, and other parts of the world [4,5,6,7]. Worldwide, tens of thousands of As contaminated sites have been found, with the As concentrations as high as 30,000 mg kg^{-1} soil [8]. Heavy metal/metalloids have been reported to be autoxidable and can cause cellular injury in plant by production of reactive oxygen species (ROS) [9]. There is significant evidence that exposure of *Holcus lanatus* to inorganic arsenic species results in the generation of ROS [10]. Another toxicity of As to plant is the damage of chloroplast membrane and cell membrane by peroxidation of membrane lipids [11,12].

Leaching of As from contaminated soils could pose a potential risk to groundwater. In some areas of Bangladesh and India, As concentrations exceeding 2000 μg l^{-1} have been found in groundwater [7]; about 400 million people are at risk of As poisoning [13]. Meharg and Rahman [14] reported that As concentration in rice grain was up to 1.7 mg kg^{-1} in some areas in Bangladesh due to irrigation with As contaminated groundwater. Arsenic transportation in soil-water-plant systems is the major pathway of drinking water and food chain contamination [15]. Remediation of metal contaminated soils has become an urgent issue.

Pteris vittata is an As hyperaccumulating plant which can accumulate up to 22,600 mg kg^{-1} As in its aboveground tissues, while As concentration in the roots was very low [16]. These As accumulation patterns make it possible to be used for phytoextraction of As contaminated soils [17]. However, the detailed mechanisms of As toxicity and As hyperaccumulation in *P. vittata* are still largely unknown. *Pteris semipinnata* does not hyperaccumulate As; concentrations measured in field-collected fronds ranged from 1 to 18 mg kg^{-1} [18]. This makes it feasible to compare the behaviour of hyperaccumulator and non-accumulator of the same genus when exposed to As, which enable us to better understand the mechanisms of As tolerance and accumulation in *P. vittata*. Hence, the main objectives of this study were to: (a) investigate the effects of

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different As concentrations and exposure time on As accumulation in the two fern species; (b) build an As uptake model and As desorption by Pi from the roots of *P. vittata*; (c) study As toxicity to both fern species.

2. Materials and methods

2.1. Cultivation of ferns

Two plant species belonging to Pteridaceae family were chosen for this study. Spores of *P. vittata* and *P. semipinnata* were collected from an As mining site in Shaoguan, Guangdong Province, China.

Plants of both species were propagated from spores. After germination, young ferns were grown in seedbeds containing a mixture of soil (2/3, with As concentrations of 2.3 mg kg^{-1}) and peat moss (1/3) until they achieved three or four fronds and a height 6 to 8 cm (about 5 months after sowing).

2.2. Experimental

2.2.1. High and low dose response exposure experiment

Hydroponic cultures were used in this experiment. The young plants of *P. vittata* and *P. semipinnata* with about 3–4 fronds and a height of 6–8 cm were transplanted to a 1500 ml plastic pot containing 0.25-strength Hoagland nutrient solution (with pH adjusted to 6.3 using NaOH or HCl) for 50 days. The nutrient solution, which was continuously vigorous aerated, was changed once every 5 days. The plants were grown in the greenhouse at room temperature (25–30 °C) and natural sunlight (13 h/11 h, day/night) under a complete random block design. They were then exposed to 0, 50, 100, 300, 500 and 1000 (for *P. semipinnata*, this concentration was not used) μmol L^{-1} As (supplied as Na_2HAsO_4) to study high dose response. For low dose response, As with concentrations of 0, 1, 3, 5, 10, 15, 25 μmol L^{-1} were selected. There were four replicates for each treatment. Plant materials were harvested after 10 days. Plant samples were separated into aboveground and underground tissues, dried at 60 °C for 2 days, weighed and ground to powder for analysis of As.

2.2.2. Short-term absorption of As by the roots of P. vittata

The young plants were grown in 0.25-strength Hoagland nutrient solution for 50 days. The roots with 7–8 cm from root tip were cut and then immersed in 100 μmol L^{-1} As (supplied with Na_2HAsO_4). Arsenic concentrations in the roots immersed for 0, 10, 20, 30, 60, 120, 180, 360, 720 and 1080 min were determined. There were four replicates for each treatment.

2.2.3. Arsenic desorption from the roots of P. vittata by Pi

The young plants were grown in 0.25-strength Hoagland nutrient solution for 50 days. Seven to eight cm root tip was cut and then immersed in 100 μmol L^{-1} As (supplied with Na_2HAsO_4) for 1080 min. The roots were then rinsed carefully and treated with 0, 10, 100 and 1000 μmol L^{-1} phosphate buffer solution (PBS, supplied with K_2HPO_4 and KH_2PO_4, pH 6.30) for As desorption. The roots were washed, dried and digested after desorbed for 0, 15, 30, 60, 120, 180, 360, 720 and 1440 min and As were determined. Four replicates were used for each treatment.

2.2.4. Effects of As on H_2O_2 content in P. vittata and P. semipinnata

The young plants were grown in 0.25-strength Hoagland nutrient solution for 50 days. The growing condition of plants was the same as described in Section 2.2.1. For dose-dependant test, the plants were exposed to 0, 100, 500 and 1000 μmol L^{-1} As (supplied with Na_2HAsO_4) for 3 days. For time-dependant effects, the plants were treated with 1000 μmol L^{-1} As for 0, 0.5, 1, 3, 6 days. There were four replicates for each treatment. H_2O_2 concentrations in the pinnae, midribs and roots were determined (based on fresh weight).

2.2.5. Effects of As on REC in P. vittata and P. semipinnata

This experiment was carried out under the same conditions as described above (Section 2.2.4). Relative electrical conductivity (REC, %) of pinnae and root was determined using a conductivity meter (DDS-11A, Orion, USA).

2.3. Chemical analysis

Total As in plants was determined according to the method described by Zhao et al. [19] with some modification: Ground plant tissues (0.2 g) were digested with HNO_3 and $HClO_4$ (85/15, v/v) with a relatively lower final temperature of 165 °C. Arsenic was determined using Graphite Furnace Atomic Absorption Spectrometry (GF-AAS, AAnalyst 800, PerkinElmer, Norwalk, CT). A standard reference plant material (1573a, tomato leaves) from the US Department of Commerce was used for quality assurance. Recovery rates of As were within 100 ± 15%.

The content of H_2O_2 was assayed by monitoring the A_{415} of the titanium-peroxide complex, based on the procedures described in Brennan and Frenkel [20]. Electrical conductivity (EC) of plant was determined according to the method described by Zhou and Leul [21].

2.4. Statistical analysis

Data were analyzed using SPSS 13.0 and SigmaPlot 9.01. All the values reported in this paper were the means of four replicates. Significant differences among treatments were determined by Duncan's multiple range test, at $p < 0.05$.

3. Results and discussion

3.1. Arsenic uptake and accumulation in P. vittata and P. semipinnata

One of the important factors affecting the success of phytoextraction of metal-contaminated soils is the availability of high biomass plants with the ability to accumulate large quantities of the specific metal in their shoot tissues [22]. The present 10-day exposure experiment showed that the two species differed markedly in As accumulation pattern in the fronds. Significantly higher concentrations of As in *P. vittata* were observed than those in *P. semipinnata* in the present study. The As accumulated in root and frond reached 1800 and 2800 mg kg^{-1} dry weight respectively, when treated with 1000 μmol L^{-1} for 10 days, which were 9–18 times higher than those of *P. semipinnata* under the same exposure level (Fig. 1). Dose-dependent As accumulation in roots of *P. vittata* was almost linear when As concentrations in nutrient solution ranged from 0 to 300 μmol L^{-1}. Frond As accumulation increased rapidly with increasing As concentration in nutrient solution ranging from 0 to 500 μmol L^{-1}. The plants accumulated increasing amounts of As in roots and fronds of both plant species with increasing As exposure levels, and the increase was more pronounced in the fronds than that in the roots of *P. vittata*. Arsenic appeared to accumulate preferentially in the fronds of *P. vittata* in all As treatments (Fig. 1). However, in *P. semipinnata*, As concentrations in the fronds had a slow increase, with much lower concentrations of As when compared with those of roots (Fig. 1). Root As concentrations in treatments of 300 and 500 μmol L^{-1} for 10 days were 6.80 and 6.75 times higher than those of fronds, respectively (Fig. 1). Wang et al. [18] also observed that *P. semipinnata* accumulated very low concentrations of As in its fronds (1–18 mg kg^{-1}). This may be explained

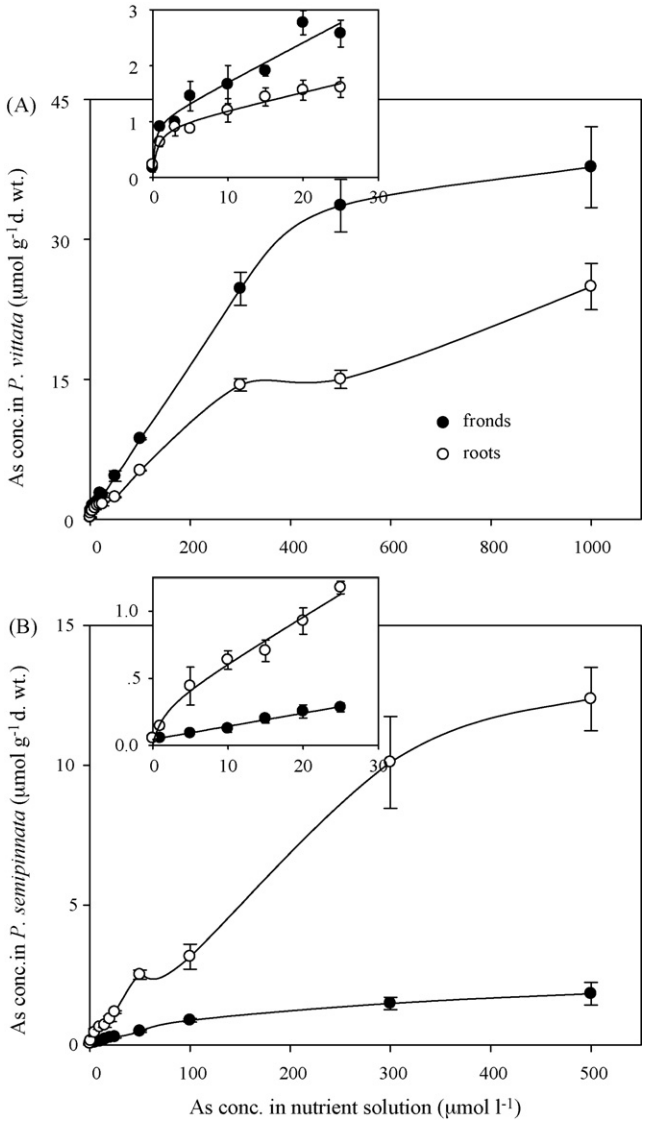

Fig. 1. Arsenic concentrations in fronds and roots of *P. vittata* (A) and *P. semipinnata* (B) when treated with 0 to 1000 μmol L^{-1} arsenate(0 to 500 μmol L-1 arsenate for *P. semipinnata)*. The insert depicts As concentrations in *P. vittata* and *P. semipinnata* at very low As exposure (0 – 25 μmol L^{-1}). Each point is the average of four replicates and error bars mean ± SD.

by the higher rates of As uptake (reflected by higher V_{max} and lower K_m values for *P. vittata*) and translocation to the frond in *P. vittata* than *P. semipinnata*.

The difference in uptake pattern between these two fern species was also observed in low dose response experiment (Fig. 1 and Table 1). About 10 times higher As concentrations in the fronds of *P. vittata* were noted compared to *P. semipinnata*, when As concentrations were below 25 μmol L^{-1} in the nutrient solution (Fig. 1). The concentrations of As in the roots of *P. vittata* increased rapidly under 0–3 μmol L^{-1} As treatments, followed by a relatively slow increase until As concentration in the nutrient solution reached 25 μmol L^{-1}. Significantly lower concentrations of As in roots of *P. semipinnata* were observed. *P. vittata* possesses higher V_{max} value and lower K_m value when compared to *P. semipinnata* (Table 1). The ability to transfer large amounts of As to the frond in the As hyperaccumulator species is the major difference from the non-accumulator species [23]. In both low- and high-dose exposure experiments, *P. vittata* accumulated more As in the fronds than those in roots. Singh and Ma [24] also reported that As was concentrated primarily in the fronds of *P. vittata*, whereas it was concentrated in the roots of *P. ensiformis* (a non-hyperaccumulator).

Table 1
Kinetic parameters for arsenate uptake by roots of *P. vittata* and *P. semipinnata* grown in 25% Hoagland solution with arsenate concentrations of 0-25 μmol L^{-1} for 10 days, obtained by fitting a hyperbolic curve [33].

Plant species	V_{max} (μmol g^{-1} d. wt.)	K_m (μmol L^{-1})	R^2
P. vittata	0.9211	0.5617	0.9518
P. semipinnata	0.3011	1.346	0.9851

Internal detoxification of As must exist in *P. vittata* since a large amount of As was accumulated in its fronds. Some tolerant plants detoxify heavy metals by binding them to their cell walls [25]; chelating metals in the cytosol by peptides [26,27], or storing metals in vacuoles to avoid cell damage [28]. A strong correlation between PCs production and arsenate influx was demonstrated in arsenate-tolerant *Holcus lanatus* [29]. Though PC synthesis can be induced by As in *P. vittata*, PCs may only play a minor role in the detoxification of As since only a small proportion (1–3%) of As in *P. vittata* can be complexed with PCs [30,31]. Compartmentalization of As in *P. vittata* played a significant role in minimizing arsenic impact on plant growth and metabolism [24]. Lombi et al. [32] indicated that As in the fronds of *P. vittata* was mainly distributed in the upper and lower epidermal cells of the pinnae, especially in the vacuoles.

3.2. Short-term As uptake kinetics of *P. vittata*

Short-term uptake of As was determined by incubating excised roots of *P. vittata* in 100 μmol L^{-1} arsenate. A better fit of arsenate uptake to a hyperbolic curve with dissection of Michealis–Menten model and linear part during 1080 min experiment ($R^2 = 0.9969$, $p < 0.001$) has been observed (Fig. 2a). The data presented in Fig. 2a were applied to a hyperbolic equation which is commonly used to analyze uptake of metals [33]. It commonly expressed as:

$$V = \frac{V_{max}[S]}{K_m + [S]} + k[S]$$

where V represents As uptake velocity; V_{max} represents the maximum uptake velocity; K_m refers to Michaelis–Menten constant which is related to the root permeability; [S] represents As concentrations in nutrient solution; and k is linear constant which is related to the ability As adsorption by the surface of root cells. The smooth, non-saturating curve could be dissected into linear and saturable components with V_{max} and K_m values at 74.56 nmol g^{-1} d. wt. and 948.8 nmol L^{-1}, respectively (Fig. 2a). The similar nonsaturating kinetics has been reported in studies of Cd uptake in wheat [33] and Zn uptake in *Thlaspi caerulescens* and *T. arvense* [34].

The linear-uptake component represents As in the free space or bound to surface of root cell that can be desorbed by PBS, where the saturable component was the result of carrier-mediated transport across root-cell plasma membrane [33]. The initial stage in As uptake was the diffusion of As from nutrient solution to root free space. Then exchange adsorption with HCO_3^- occurred at the surface of cell cytoplasm and cell wall before As was transported into internal cytoplasm. Therefore, apoplastic binding is a requisite step in the absorption of a ion. The Michealis-Menten model suggests that transport of As is an active process. The saturable curve can be explained by saturation of As binding sites in plasma membrane and limitation of energy supply which is a driving force [35].

The maximum absorption took place within the first 60 min. Arsenic absorption rate decreased very sharply during 360 min experimental period with increasing As exposure time ($R^2 = 0.9906$, $p < 0.001$) (Fig. 2b).

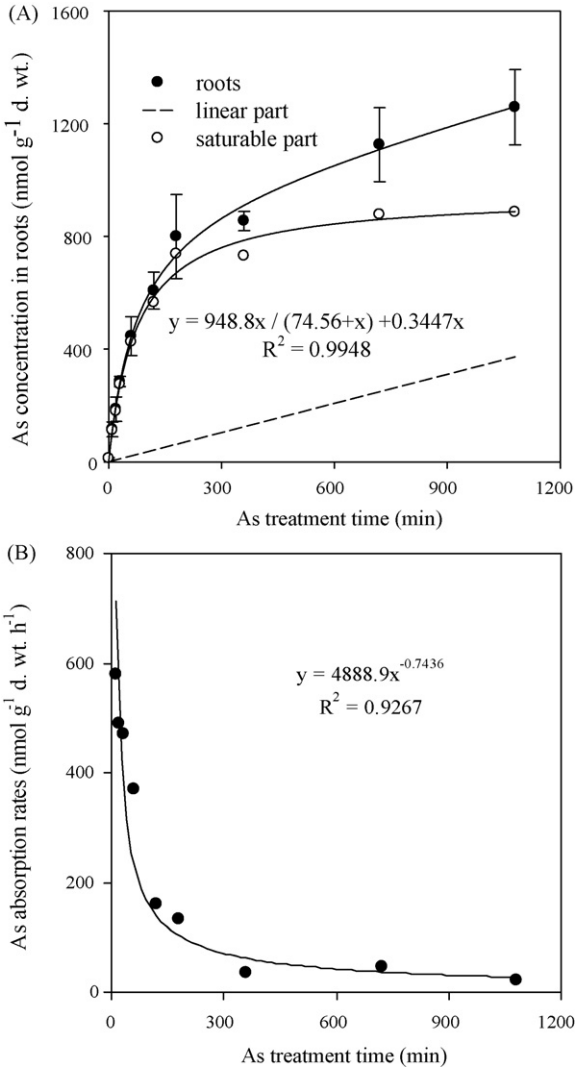

Fig. 2. Short-term absorption of As by the roots of *P. vittata* exposed to 100 μmol L^{-1} As. (A): Kinetics of As absorption; (B): Arsenic absorption rate. Data symbols and error bars represent means ($n = 4$) and SD, respectively.

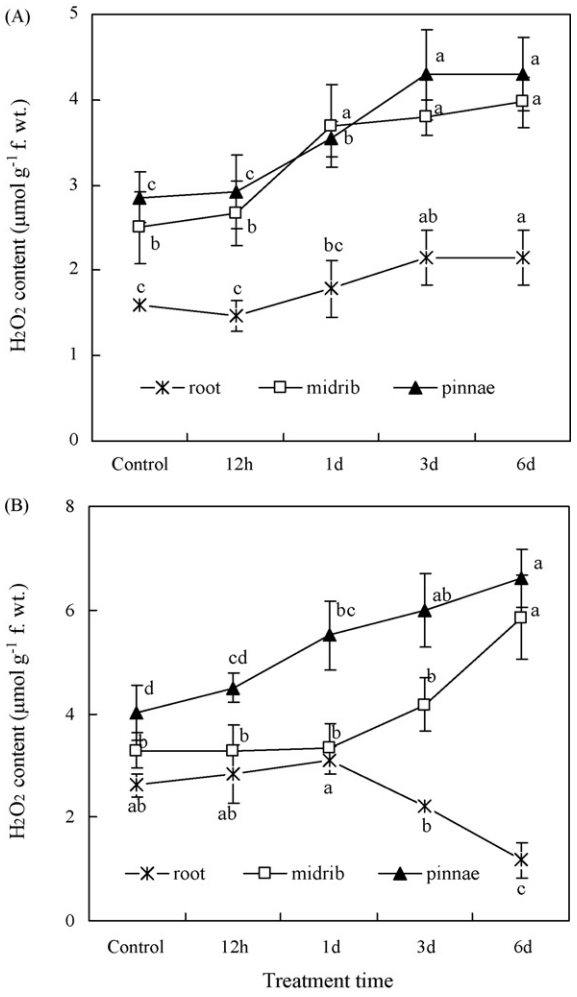

Fig. 3. Time course of As action on levels of H_2O_2 in *P. vittata* (A) and *P. semipinnata* (B) exposed to 1000 μmol L^{-1} As. Data represent means ± SD of four replicates.

3.3. Arsenic desorption from the roots of P. vittata by Pi

Previous studies on As uptake by plants have shown that As may be taken up via Pi uptake system since they have similar electron configuration and chemical properties [36,37]. The desorption results presented in Table 2 indicated that PBS (pH 6.25) with concentrations of 10, 100 and 1000 μmol L^{-1} were more effective than water at rapidly removing As from the root surface of *P. vittata* which was pretreated with 100 μmol L^{-1} As for 24 h. This may be due to the competition between As and Pi for the binding sites at the surface of root cells. In general, the phosphate/arsenate plasma membrane carriers possess a much higher affinity for phosphate than arsenate [38]. The As desorption rate increased with the increase of PBS concentrations. Most of the removed As from the root surface of the plant occurred during the first 15 min of desorption when treated with 1000 μmol L^{-1} PBS (pH 6.25), while 30 min and 60 min were needed for 100 and 10 μmol L^{-1} PBS (pH 6.25) treatments. There

Table 2
As concentrations in the roots of *P. vittata* after desorption with 10, 100 and 1000 μmol L^{-1} phosphate buffer solution (PBS, pH 6.25) from 0 to 1440 min.

Desorption time (min)	As conc. in the roots (μmol g^{-1} d. wt.)			
	0 Pi	10 Pi	100 Pi	1000 Pi
0	1.53 ± 0.13a	1.53 ± 0.13a	1.53 ± 0.13a	1.53 ± 0.13a
15		1.50 ± 0.09a	1.33 ± 0.12b	1.16 ± 0.04b
30	1.51 ± 0.08a	1.35 ± 124b	1.17 ± 0.11bc	1.04 ± 0.11b
60		1.08 ± 0.05c	1.04 ± 0.05c	1.02 ± 0.05b
180	1.49 ± 0.11a	1.04 ± 0.11c	1.04 ± 0.11c	1.02 ± 0.09b
360		1.02 ± 0.06c	1.02 ± 0.08c	1.02 ± 0.06b
720		1.00 ± 0.08c	1.01 ± 0.06c	1.02 ± 0.07b
1440	1.49 ± 0.10a	1.04 ± 0.07c	1.01 ± 0.11c	1.02 ± 0.10b

Values are mean ($n = 4$) ± SD; different letters in the same column denote significant differences ($p < 0.05$) according to the Duncan's multiple range test.

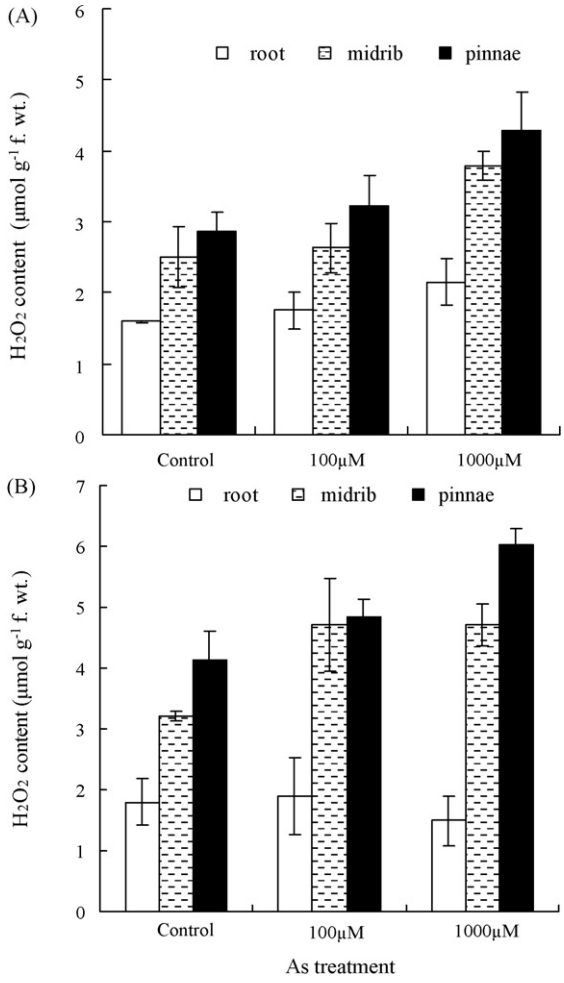

Fig. 4. Effect of As on H_2O_2 content in *P. vittata* (A) and *P. semipinnata* (B) exposed to different concentrations for 3 days. Data represent means ± SD of four replicates.

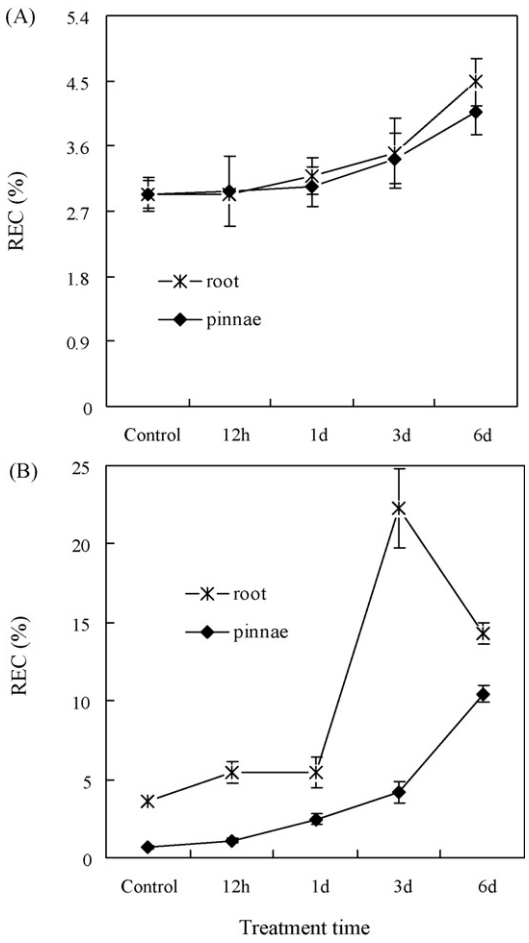

Fig. 5. Effect of As on REC (%) values of *P. vittata* (A) and *P. semipinnata* (B) exposed to 1000 μmol L^{-1} arsenate. Data symbols and error bars represent means ($n = 4$) and SD, respectively.

was no significant difference ($p > 0.05$) in total As desorbed between different PBS concentrations.

3.4. As toxicity on ferns

It has been noted that heavy metals/metalloids caused an increased in production of H_2O_2 in plants [39,40]. Evidence for the induction of ROS by As has been reported in both plants and animals [10,41]. The present results indicated that As treatments resulted in generation of H_2O_2 in both As hyperaccumulating and non-hyperaccumulating ferns (Figs. 3 and 4). Fig. 3 shows the time-dependant effects of As on levels of H_2O_2 in root, midrib and pinna of *P. vittata* and *P. semipinnata*. At 1000 μmol L^{-1}, As induced H_2O_2 production in both plant species. A continuous increase in H_2O_2 was observed during the first 6 days in the midrib and pinna of both species and in the root of *P. vittata*, but was not observed in the root of *P. semipinnata*. Levels of H_2O_2 in midrib of *P. vittata* remained almost unchanged after treatment for 1 day, whereas the H_2O_2 levels in the root and pinna remained unchanged for 3 days. At the end of 3 days, the levels of H_2O_2 increased by 35%, 47% and 50% for root, midrib and pinna, respectively when compared with the control. Hydrogen peroxide content in midrib and pinna of *P. semipinnata* increased significantly ($p < 0.05$) with increasing treatment time. The highest concentrations of H_2O_2 were observed at day 6, with increase of 78% and 64%, respectively. At 3- and 6-day, levels of H_2O_2 in roots of *P. semipinnata* decreased significantly ($p < 0.05$). This may be because the root plasma membrane of *P. semipinnata* was seriously destroyed by As toxicity (Fig. 5). ROS are generally believed as the by-products of various cellular oxygen consuming processes, such as photosynthetic or respiratory electron transport [42].

Effects of different concentrations of As on levels of H_2O_2 in ferns are illustrated in Fig. 4. Hydrogen peroxide contents in root, midrib and pinna of *P. vittata* increased significantly ($p < 0.05$) under 1000 μmol L^{-1} As treatment. For *P. semipinnata*, exposure to As (100 and 1000 μmol L^{-1}) resulted in a significant ($p < 0.05$) increase in H_2O_2 content in both midrib and pinna. However, the concentration of H_2O_2 in root decreased slightly ($p > 0.05$) under 1000 μmol L^{-1} As. In addition, higher concentrations ($p < 0.05$) of H_2O_2 in midrib and pinna of *P. semipinnata* were noted, when compared with *P. vittata*. Cho and Seo [43] also reported that H_2O_2 concentrations in Cd-tolerant *Arabidopsis thaliana* seedlings were much lower than those of Cd-sensitive type. *P. vittata* can develop the antioxidative systems efficiently to defend itself against oxidative stress caused by As [44]. It is evident that uptake of As induced a strong antioxidative response with increasing superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) activities, and increasing glutathione (GSH), and ascorbate acid (AsA) in *P. vittata* [44,45].

Although ROS may act as secondary messengers to regulate gene expression and protein biosynthesis involved in the stress defences, they are cytotoxic at higher concentrations [46,47]. They may cause damages to DNA, lipids, chlorophyll and almost every other organic constituent of living cells [42]. Plant cell membranes

are generally considered to be primary sites of metal injury due to lipid peroxidation caused by enhanced toxic oxygen free radicals and protein denaturalization caused by excess metal concentrations [48,49,50]. In the present study, REC (%) was used to measure the degree of cell membrane breakage. Fig. 5 shows that membranes of both plant species especially *P. semipinnata* are destroyed since REC (%) increased significantly after exposure to As. In the presence of 1000 μmol L^{-1} As, REC (%) values increased in both fern species with treatment time, except for root of *P. semipinnata* at day 6. Significant ($p < 0.05$) increases in REC (%) were observed in midrib of *P. vittata* and root of both fern species. In the pinna of *P. semipinnata*, treatment of 1000 μmol L^{-1} caused an obvious ($p < 0.05$) increase of REC (%) after treatment for 1 day. *P. semipinnata* showed more serious root and pinna damage than *P. vittata* after 1 day, with maximum REC (%) values of 22% and 10.4% in root and pinna of *P. semipinnata*, compared with 4.5% and 4.1% in *P. vittata*, respectively. Previous studies indicated that tolerant plants showed a lower level of H_2O_2 and lower level of lipid peroxidation, compared to sensitive plants when exposing to heavy metals (such as Cu, Cd and As) [10,43]. A comparison of responses to As stress in the present study showed that *P. vittata* is more tolerant to As than *P. semipinnata* since the latter possesses higher H_2O_2 production and REC (%) value when exposed to As (Figs. 3–5). The results revealed the better adaptation of *P. vittata* to As stress.

4. Conclusion

Uptake of As by *P. vittata* was much higher than that of *P. semipinnata*. Most of the As (82–90%) accumulated in the fronds of *P. vittata*, while only 29–41% in the fronds of *P. semipinnata*, except for the control treatment (about 71% As was distributed in the fronds).

The short-term (<18 h) and low concentration (<25 μmol L^{-1}) uptake kinetics in *P. vittata* was nonsaturated and could be described by a hyperbolic curve with a disection of Michealis-Menten model. PBS can desorb As from surface and free space of the root cell rapidly. The function of Pi transporter during As uptake by plants should be studied in further work.

Concentrations of H_2O_2 and REC (%) values of both fern species increased with the increase of As concentrations in nutrient solution and exposure time. The level of H_2O_2 and REC (%) value were lower in *P. vittata* than *P. semipinnata* in all treatments. This indicated its ability to transport As to aboveground tissues, a more efficient strategy to protect itself from oxidative damage caused by high levels of As. *P. vittata* is therefore more suitable for phytoextraction of As contaminated environment.

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