Role of transpiration in arsenic accumulation of hyperaccumulator *Pteris vittata* L.

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Abstract Mechanisms of *Pteris vittata* L. to hyperaccumulate arsenic (As), especially the efficient translocation of As from rhizoids to fronds, are not clear yet. The present study aims to investigate the role of transpiration in the accumulation of As from the aspects of transpiration regulation and ecotypic difference. Results showed that As accumulation of *P. vittata* increased proportionally with an increase in the As exposure concentration. Lowering the transpiration rate by 28~67 % decreased the shoot As concentration by 19~56 %. Comparison of As distribution under normal treatment and shade treatment indicated that transpiration determines the distribution pattern of As in pinnae. In terms of the ecotypic difference, the *P. vittata* ecotype from moister and warmer habitat had 40 % higher transpiration and correspondingly 40 % higher shoot As concentration than the ecotype from drier and cooler habitat. Results disclosed that transpiration is the main driver for *P. vittata* to accumulate and redistribute As in pinnae.

Keywords Arsenic · Ecotype · Phytoextraction · *Pteris vittata* L. · Transpiration

Introduction

Phytoremediation is an attractive technology treating arsenic (As)-contaminated soils because it is low cost, easy to operate,

and favorable to landscape. *Pteris vittata* L. is a known hyperaccumulator with high As concentration and large biomass (Chen et al. 2002; Ma et al. 2001), which has been successfully applied to ~200 ha of contaminated soil in China. Recently, the possibility of using *P. vittata* to remediate As-contaminated water has been proposed and verified (Guo et al. 2012; Zhao and Guo 2013). A better understanding of the ecophysiological mechanisms of *P. vittata* to accumulate As would favor the efficient utilization of this species for environment remediation.

It has been found that the cytoplasmic supernatant of *P. vittata*'s pinnae is the major place storing As taken up from growth media, reaching up to 60 % of total As in the whole seedling (Chen et al. 2005). The particularly efficient translocation is one of the most important processes in the accumulation of As by *P. vittata* (Poynton et al. 2004; Su et al. 2008). Mineral ions enter into the xylem of plant roots by apoplastic method or symplastic method and then are generally transported upward driven by transpiration (Hinsinger 1998; Russell and Shorrocks 1959). Whether hyperaccumulators use the same way to largely transport trace elements is not clear yet.

Root-to-shoot translocation of cadmium (Cd) generally occurred via the xylem and is driven by transpiration from the leaves; however, for the Cd hyperaccumulator *Sedum alfredii*, transpiration played a very limited role (Lu et al. 2009). Similar phenomenon was found for the nickel (Ni) hyperaccumulator *Leptoplax emarginata* (Bartoli et al. 2012). In contrast, a significantly positive relationship is observed between the shoot Cd concentration and the transpiration of *Phytolacca americana* (Liu et al. 2010). And, in several hyperaccumulators, transpiration was found to be the main translocation way of aluminum (Al) and lead (Pb). Lowering the transpiration rate of the Al hyperaccumulator *Fagopyrum esculentum* Moench by 75 % significantly decreased the aboveground Al concentration (Shen and Ma

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2001). Increasing transpiration enabled Indian mustard to take up twofold more Pb (Salt et al. 1995). Such difference in the soil-plant transfer may result both from the variation in elements and the difference in plant species (Basta et al. 2005).

Until now, there has been no direct study on the interaction between As accumulation and transpiration in As hyperaccumulators, whereas several studies have implied the potential relationship between As accumulation and transpiration. In contrast to plants with low tolerance to As that often reduce water uptake when confronting heavy metal stress (Kholodova et al. 2011; Vernay et al. 2007), the As hyperaccumulator *P. cretica* has been reported to increase water uptake under As stress (Lee and Lee 2011). A recent study found that the translocation of As in *P. vittata* involves three steps: (1) the movement of As(V) (prevailing species in the rhizosphere) using the phosphorus pathway from the epidermis to the endodermis, (2) the reduction of As(V) to As(III) in the endodermis, and (3) the later transport of As(III) via a passive and more efficient process, likely related to water uptake (Lei et al. 2012).

We propose that, unlike the Ni hyperaccumulator *L. emarginata* (Bartoli et al. 2012) or Cd hyperaccumulator *S. alfredii*, for the As hyperaccumulator *P. vittata* with its large biomass (~ 9 t dry weight $\text{h m}^{-2} \text{ year}^{-1}$), transpiration is the main upward translocation process for As after the reduction of As(V) to As(III). This study aims to verify this hypothesis by investigating the role of transpiration in the As hyperaccumulation. The As accumulation pattern in *P. vittata*'s fronds under different As exposure concentrations and transpiration-altering treatments was studied. In addition, *P. vittata* has a wide distribution in China, which enables the evolution of ecotypes under diverse habitats. These ecotypes may have different water use efficiency values (Wan et al. 2013), presenting appropriate materials for research on the relationship between transpiration and As accumulation. The responses to transpiration regulation methods of two *P. vittata* ecotypes from habitats with different climatic conditions were further investigated, disclosing the relationship between water metabolism and As accumulation in the hyperaccumulator *P. vittata*.

Materials and methods

Plant culture

Spores of *P. vittata* were sown on clean and humid soils, covered by cling film, and placed in a greenhouse with 16 h of light at an intensity of $300 \text{ mE m}^{-2} \text{ s}^{-1}$, day/night temperatures of 26/15 °C, and average relative humidity of 50 %. Seedlings with the uniform height of ~ 20 cm were transplanted to a modified Hoagland nutrient solution containing $7.5 \times 10^{-4} \text{ M K}_2\text{SO}_4$, $6.5 \times 10^{-4} \text{ M MgSO}_4$, $0.25 \times 10^{-4} \text{ M KCl}$, $0.5 \times 10^{-3} \text{ M Ca(NO}_3)_2$, $0.625 \times 10^{-4} \text{ M KH}_2\text{PO}_4$, $0.25 \times$

$10^{-5} \text{ M H}_3\text{BO}_3$, $0.25 \times 10^{-6} \text{ M MnSO}_4$, $1 \times 10^{-7} \text{ M CuSO}_4$, $1 \times 10^{-6} \text{ M ZnSO}_4$, $1.25 \times 10^{-9} \text{ M (NH}_4)_6\text{MoO}_4$, and $0.25 \times 10^{-4} \text{ M Fe-EDTA}$ (pH adjusted to ~ 6.2). Chemicals used in the nutrient solution were of analytical grade (Sinopharm Chemical Reagent, Beijing, China).

Experiment 1: effects of As concentrations and shade on the transpiration and As accumulation

Young seedlings that propagated from spores collected from a greenhouse in Beijing were exposed for 7 days to 0, 5, 10, 20, or 40 mg L^{-1} As in form of $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$. The control was provided with a daily photoperiod of 16 h. Shade treatment was provided with a daily photoperiod of 2 h, with other conditions kept the same as the control.

Water loss was recorded by weighing hydroponic bottles and plants every day. Control bottles without plants were treated identically to account for water loss due to evaporation. The transpiration rate was calculated by dividing the daily water loss due to plant transpiration by fresh weight of plant shoots.

At the end of experiment, plants were harvested, washed by running tap water followed by deionized water and oven-dried at 60 °C for the later analysis of As concentration.

Experiment 2: micro-distribution of As in *Pteris vittata* under transpiration adjustments

Precultured *P. vittata* seedlings, as described in “Experiment 1: effects of As concentrations and shade on the transpiration and As accumulation”, were transplanted to culture solution containing 5 mg L^{-1} As in the form of $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$. Pinnae in the middle of mature fronds which no longer sprouted new pinna were chosen for the experiment. One side of the selected pinna was covered by aluminum foil with some perforations underneath to allow respiration (designated as the shade treatment), whereas pinna on the other side was maintained at normal light period (designated as the control).

One day later, *P. vittata* was removed from the culture solution, rinsed with deionized water, and then freeze-dried for synchrotron radiation X-ray fluorescence spectroscopy (SRXRF) analysis. SRXRF scanning was performed at an X-ray micro-beam station at Beamline 4W1B of the Beijing Synchrotron Radiation Facility. The electron storage ring was operated at 2.2 GeV with the current ranging between 59 and 114 mA. Detailed procedures for the measurement and analysis are given by Lei et al. (2012).

Experiment 3: As accumulation of two ecotypes under transpiration regulation

Spores of two *P. vittata* ecotypes were collected from Hunan Province (HN) and Guangdong Province (GD). The climates of the sampling sites are described in Table 1. Young seedlings

Table 1 Climates of the original habitats of two *P. vittata* ecotypes

	GD	HN
Annual average temperature (°C)	22	17
Annual precipitation (mm)	1736	1426
Annual sunshine duration (h)	1906	1174
Annual evaporation (mm)	1526	1124

GD and HN indicate the Guangdong and Hunan ecotype of *P. vittata*

that propagated from these two spores were transferred to transpiration inhibition or enhancement treatments.

The transpiration inhibition treatment was mimicked by alternative split-root osmotic stress (Dodd et al. 2007; Hu et al. 2006), which can decrease transpiration but keep photosynthetic efficiency as our preliminary experiments showed. Half of the roots were immersed in a normal nutrient solution, while the other half were exposed to a culture solution containing 10 and 20 % polyethylene glycol (PEG) 6000, designated as PEG10 and PEG20 hereafter, respectively. The osmotic potentials of PEG10 and PEG20-added solution were calculated as -0.148 and -0.491 MPa, respectively (Michel and Kaufmann 1973; Money 1989). Twelve hours later, roots in the PEG-added solution were washed with deionized water and transferred to the normal nutrient solution and roots in the normal solution were transferred to the PEG-added solution. This cycle was repeated every 12 h. The transpiration enhancement treatment was created by adding 1–4 mM KNO_3 to the culture solution, designated as K1 to K4 hereafter. Plants of control were kept in the normal nutrient solution. Each treatment has four replicates. As concentration in the culture solution was kept at 1 mg L^{-1} .

Transpiration measurement During the experimental period, three pinnae in the middle of mature fronds which no longer sprouted new pinna were chosen for the measurement of transpiration rate. Li-6400 portable photosynthesis system (LI-COR, USA) was used to measure the transpiration rate at maximum sunlight conditions from 11:00 to 13:00. The daily water loss due to plant transpiration was also recorded.

Ten days after the treatment, fresh pinnae were collected and immediately measured with an environmental scanning electron microscope fitted with energy dispersive X-ray for the stomatal size, stomatal aperture, and relative potassium (K) content of guard cells. Details of the analysis method are given by Li et al. (2005). Each measurement had 10 replicates. The rest of the plant samples were collected, washed, and oven-dried at 60°C for the later analysis of As concentration.

Chemical analysis and data process

Chemical analysis Oven-dried plant samples were finely ground and digested with a mixture of HNO_3 and HClO_4 (v/v

$v=10:1$) and analyzed for As concentration (Hydride Generation Atomic Fluorescence Spectrometer, Haiguang AFS-2002). The quality control of the digestion and analysis was verified using certified reference material (GSV-2) from the National Standard Materials Center. The recovery rate of As was 90.9–109.1 %.

Data process The translocation index was defined as the ratio of the root-to-shoot As concentration. A two-factor ANOVA, ecotype $\times$ transpiration-altering treatments, was performed to analyze differences in transpiration rates and As concentrations of HN and GD ecotypes. ANOVA and linear regression were performed using SPSS statistical program package (SPSS, Inc., Chicago, USA., Release 13.0). The significance level was set at an error probability of 0.05.

Results

Effects of As concentrations and shade on the transpiration and As accumulation

Experiment 1 showed that the supplied As concentrations and shade treatment significantly influenced the transpiration and As accumulation of *P. vittata*.

Under normal light condition, the transpiration rate of *P. vittata* increased when exposed to low concentrations of As (5 and 10 mg L^{-1}) in comparison to the As-free solution ($P<0.05$, Fig. 1a). When exposed to high concentrations of As (20 and 40 mg L^{-1}), the transpiration rate of *P. vittata* decreased, indicating that high As concentration in the nutrient solution may injure the normal physiological activities of *P. vittata*. Shade decreased the transpiration rate of *P. vittata* by 56, 67, 32, and 28 % in the 5, 10, 20, and 40 mg L^{-1} As treatment, respectively.

Under both normal light condition and shade treatment, the shoot As concentrations of *P. vittata* increased with an increase in the exposure As concentration (Fig. 1b). There was a linear relationship between As in nutrient solution and As in *P. vittata* shoots when As in the nutrient solution was lower than 10 mg L^{-1} As. Shade treatment decreased shoot As concentrations of *P. vittata* by 47, 56, 51, and 45 % in the 5, 10, 20, and 40 mg L^{-1} As treatment, respectively.

Distribution of As in fronds in light and dark

Using SRXRF, experiment 2 studied the micro-distribution patterns of As under transpiration adjustments. Provided with normal light, As contents decreased from vein to edge (Fig. 2). As concentration at pinna edge was 1/2 of that at the pinna vein, posing a positive As gradient, which may favor the transfer of As from vein to edge. Shade treatment, which decreased the water loss of *P. vittata* by ~ 50 % (data not

Fig. 1 Effects of As concentrations and shade on transpiration (a) and As accumulation (b) of *P. vittata*

provided), noticeably decreased As content at all the six scanning sites, with the largest decrease occurring at the edge (~89 %). The positive gradient of As from vein to edge was less obvious in the shaded treatment. Results indicated that inhibiting transpiration partly blocked the loading of As from vein to edge of *P. vittata*'s pinnae, implying the role of transpiration in determining the distribution of As in pinnae.

Fig. 2 Effect of shade on As distribution in pinnae. As count indicates the normalized fluorescence intensity, indicating the As concentration

Relationship between transpiration and As accumulation in two *P. vittata* ecotypes

Morphological characteristics The rhizoid/frond biomass ratio of the GD ecotype from moister and warmer habitat was significantly lower than that of the HN ecotype from dryer and cooler habitat ($P < 0.05$, Table 2). The GD ecotype had higher pinnae density and larger leaf area per unit dry weight than the HN ecotype. The GD ecotype also had 9.4 % higher stomatal density and 55 % larger average stomatal size than the HN ecotype. Differences in morphological characteristics between the GD and HN ecotype indicated that under varied climatic conditions, *P. vittata* can develop into ecotypes with different pinnae and rhizoid characteristics, contributing to their difference in transpiration.

Transpiration rate Both ecotype and transpiration-altering treatments led to significant differences in the transpiration of *P. vittata*, and the interaction between ecotype and transpiration treatments also contributed to the difference in transpiration rates (two-way ANOVA analysis, $P < 0.05$).

The alternative split-root osmotic stress caused by PEG decreased the transpiration of both ecotypes (Fig. 3). PEG10 led to a 20 and 18 % decrease in transpiration rates of the GD and HN ecotype, respectively. PEG20 caused more obvious impacts: 64 and 42 % decrease in transpiration rates of the GD and HN ecotype, respectively.

Results indicated that the transpiration enhancement method, adding K, could increase the transpiration rate. With an increase in the added K concentration, transpiration displayed a slight increase. The K4 treatment increased the transpiration rates of the GD and HN ecotype by 23 and 56 %, respectively. In agreement with the change in transpiration rate, the addition of K obviously increased K content in the guard cells of both ecotypes ($P < 0.05$, Fig. 4). There existed significantly positive correlation between K concentrations of guard cells and

Table 2 The morphological characteristics of two ecotypes

Ecotype	GD	HN
Rhizoid/frond biomass ratio	0.18±0.01b	0.22±0.03a
Pinna density	1.52±0.13a	1.33±0.08a
Pinna area/unit dry weight (m ² g ⁻¹)	0.33±0.24a	0.22±0.03a
Stoma density (mm ⁻²)	58.4±11.8a	53.4±4.7a
Average stoma size(μm ²)	46.5±7.7a	30.0±5.9b

GD and HN indicate the Guangdong and Hunan ecotype of *P. vittata*. Pinna density=number of pinnae/length of frond. Values are means±SD. Values with different letters within rows indicate significant differences between two ecotypes ($P<0.05$, $n=4$ for the rhizoid/frond biomass ratio and for the rest $P<0.05$, $n=10$).

transpiration rates ($P<0.05$), indicating that K increased transpiration rate by improving the K concentration of guard cells.

The GD ecotype exhibited higher transpiration rates than the HN ecotype in all these treatments except PEG20, in accordance with the habitat climates and the morphological characteristics of these two ecotypes (Tables 1 and 2). The difference between two ecotypes was significant in all these treatments except K4 ($P<0.05$). Comparing the responses of two ecotypes to transpiration regulation methods, it was found that split-root osmotic stress reduced the transpiration of the GD ecotype to a larger extent.

Fig. 3 Transpiration rates of two *P. vittata* ecotypes under transpiration regulation. GD and HN indicate the Guangdong and Hunan ecotype of *P. vittata*. Different capital letters beside the error bar indicate significant difference among treatments for the GD ecotype. Different lowercase letters beside the error bar indicate significant difference among treatments for the HN ecotype. Asterisks indicate significant differences between ecotypes ($P<0.05$, $n=4$). CK is the control with *P. vittata* growing in the normal culture solution. PEG10 and PEG20 indicate the transpiration-decreasing treatments, created by adding 10 and 20 % polyethylene glycol 6000 to the culture solution, respectively. K1–K4 indicate the transpiration enhancement treatments, created by adding 1–4 mM KNO₃ to the culture solution

Fig. 4 Relative content of potassium in guard cells of two *P. vittata* ecotypes. GD and HN indicate the Guangdong and Hunan ecotype of *P. vittata*. Different capital letters beside the error bar indicate significant difference among treatments for the GD ecotype. Different lowercase letters beside the error bar indicate significant difference among treatments for the HN ecotype ($P<0.05$, $n=4$). CK is the control with *P. vittata* growing in the normal culture solution. K1–K4 indicate the transpiration enhancement treatments, created by adding 1–4 mM KNO₃ to the culture solution

As accumulation Arsenic accumulation in both ecotypes obviously decreased under split-root osmotic stress and increased with the addition of K (Table 3). Both ecotypic variation and transpiration regulation led to a significant difference in As accumulation of *P. vittata*, and the interaction effect of ecotype and transpiration regulation methods also contributed ($P<0.05$).

In accordance with the decreased transpiration rates, shoot As concentrations of the GD ecotype under PEG10 and PEG20 treatments decreased by ~68 and ~80 % in comparison to the control, respectively. The HN ecotype displayed a less obvious decrease in shoot As concentration: 55 and 60 % under the treatment of PEG10 and PEG20, respectively. PEG treatments displayed no obvious impacts on the root As concentration of the GD ecotype but decreased the root As concentration of the HN ecotype. PEG treatments greatly decreased the translocation index of the GD ecotype, being ~1/4 of the control. The decrease in translocation index of the HN ecotype under split-root osmotic stress was less obvious.

The enhancement of transpiration by adding K slightly increased the shoot As concentration of the GD ecotype, with a similar trend with transpiration rates. The K4 treatment of the GD ecotype had a 72.5 % higher translocation index than the control ($P<0.05$). The HN ecotype displayed a more obvious increase. The K4 treatment led to an 85 % increase in As concentration and 210 % increase in As translocation index ($P<0.05$). Potassium had no obvious effect on the root As concentration of the GD ecotype but slightly increased the root As concentrations of the HN ecotype (Table 3).

Table 3 As accumulation of two *P. vittata* ecotypes under transpiration alteration treatments

Ecotype	Treatment	As in shoot (mg kg ⁻¹)	As in root (mg kg ⁻¹)	Translocation index
GD	PEG20	558.8±174.5b	683.9±85.9a	0.81±0.17c
	PEG10	887.6±257.1b	779.4±51.9a*	1.13±0.27c
	CK	2768±258a*	703±99a	4.0±0.6b*
	K1	3327±409a*	922±218a*	3.8±1.1b
	K2	2953±192a*	699±56a*	4.3±0.6b
	K4	3054±276a	527±276a	6.9±3.0a
HN	PEG20	756.3±261.2c	610.9±70.6b	1.22±0.3b
	PEG10	836.3±229.1c	470.9±189.6b	1.86±0.44b
	CK	1952±148b	842±359a	1.9±0.7b
	K1	1974±98b	435±78b	4.4±0.5a
	K2	2064±160b	431±20b	4.8±0.2a
	K4	3615±1858a	608±282ab	5.9±0.9a

GD and HN indicate the Guangdong and Hunan ecotype of *P. vittata*. CK is the control with *P. vittata* growing in the normal culture solution. PEG10 and PEG20 indicate the transpiration-decreasing treatments, created by adding 10 and 20 % polyethylene glycol 6000 to the culture solution, respectively. K1–K4 indicate the transpiration enhancement treatments, created by adding 1–4 mM KNO₃ to the culture solution. Values are means±SD. Values with different letters within columns indicate significant differences between various treatments at $P<0.05$ ($n=4$). Asterisks indicate significant difference between two ecotypes at $P<0.05$ ($n=4$)

Comparing these two ecotypes, the shoot As concentrations of the GD ecotype were higher than those of the HN ecotype in most treatments except PEG10 and K4 treatment. The GD ecotype displayed a greater decrease in As accumulation under the inhibition of transpiration but a smaller increase in the transpiration enhancement treatments than the HN ecotype.

Relationship between transpiration and As accumulation To better evaluate the contribution of transpiration to As accumulation in *P. vittata*, a comparison between the measured As amount and the implied As amount calculated from transpiration (obtained by multiplying the water loss and the As concentration in the culture solution) was conducted. The predicated As accounted for more than half of the measured As amount (Fig. 5). Transpiration accounted for ~74 and ~50 % of the As accumulated by the GD and HN ecotype, respectively.

K obviously increased the contribution of transpiration to As accumulation in the HN ecotype. Transpiration accounted for ~80 % of As accumulated by the HN ecotype in the K1 treatment. The GD ecotype showed a less obvious increase in the contribution of transpiration in the K treatment, in accordance with the change in the shoot As concentration.

Discussions

Contribution of transpiration to As accumulation of *P. vittata* The current study indicates that transpiration made a notable contribution to As accumulation by *P. vittata*, based on the observation that (a) with an increase in the exposure

concentration of As, the shoot As concentration of *P. vittata* increased proportionally, (b) inhibition of transpiration decreased As concentrations in shoots, while improving transpiration increased As concentrations in shoots, and (c) the *P. vittata* ecotype with higher transpiration rate accumulated more As in the aboveground parts than that with lower transpiration rate.

Lei et al. (2012) suggested that before entering into the endodermis of *P. vittata* rhizoids, As utilizes the phosphate pathway, while after that, phosphate pathway no longer works for As, which means that the transport of As after the endodermis requires another carrier. Based on results from the present study, this carrier may be closely related to water uptake. The earlier view is that water molecules enter into cells through passive diffusion, but recently, it has been suggested that some specialized water transport carriers like aquaglyceroporins play an important role (Luu and Maurel 2005; Preston et al. 1992). The aquaglyceroporins, a class of water channel not only permeable to water molecules but also permeable to glycerol and small solutes (Thomas et al. 2002), have been identified as carriers of As in bacteria, yeast, humans, and other vertebrates (Mukhopadhyay et al. 2014) but not found in the As hyperaccumulator yet (Mathews et al. 2011). Either through passive diffusion or aquaglyceroporin-aided transport, the As translocation in *P. vittata* is found to be closely related to water uptake.

In addition, suppression of transpiration obviously decreased As concentration at the edge of *P. vittata* pinnae, implying the role of transpiration in As accumulation and distribution in *P. vittata*. Inhibiting transpiration blocked the movement of As from vein to epidermis, indicating that the lack of enough transpiration as a driving force disabled the transfer of

Fig. 5 The measured and implied As amount. Measured As amount=shoot As concentration of *P. vittata*×shoot biomass. Implied As amount=As concentration of the culture solution×water loss. GD and HN indicate the Guangdong and Hunan ecotype of *P. vittata*. CK is the control with *P. vittata* growing in the normal culture solution. PEG10 and PEG20 indicate the transpiration-decreasing treatments, created by adding 10 and 20 % polyethylene glycol 6000 to the culture solution, respectively. K1–K4 indicate the transpiration enhancement treatments, created by adding 1–4 mM KNO_3 to the culture solution. Different capital letters beside the error bar indicate significant difference among treatments for the measured As amount. Different lowercase letters beside the error bar indicate significant difference among treatments for the implied As amount. Asterisks indicate significant differences between measured As and calculated As in *P. vittata* ($P<0.05$, $n=4$)

As from vein to epidermis, which is one of the main places where As accumulates in *P. vittata*.

Evolutionary theory behind the relationship between transpiration and As accumulation The *P. vittata* ecotype with higher transpiration rate displayed correspondingly morphological characteristics, including higher pinna density, larger leaves, and more stomata, indicating that the difference in transpiration rates has become a heritable trait of this ecotype.

Studies have found that plants growing under different climatic conditions can adapt to local climate and evolve genetically stable variation in morphological and physiological

characteristics (Abd El Rahman and Krzywinski 2008; Zhang et al. 2005). An extreme example is certain plant species in arid regions showing correspondingly small and thick leaves and extremely low transpiration rates (Ma et al. 2008). Such morphological and physiological adaptation of plant ecotypes to local environment has been also found in hyperaccumulators, such as Zn hyperaccumulator *S. alfredii* (Yang et al. 2010) and Pb/Cd/thallium (Tl) co-hyperaccumulator *Biscutella laevigata* (Wierzbińska and Pielichowska 2004) s.

In this study, the GD ecotype from a habitat with higher temperature, precipitation, and evaporation exhibited higher transpiration rates and the corresponding morphological characteristics. The adaptation of leaf structure, morphologically or histologically, often reflects clearly the effect of environmental stress and therefore is often used as an index evaluating the impacts of environmental conditions on plants (Dinis et al. 2011; Souto et al. 2009). The significant interactive effect of ecotype and transpiration regulation ($P<0.05$) indicated that different ecotypes had varying responses to transpiration-altering measures. To adapt to local climatic conditions, *P. vittata* can evolve constitutive and hereditary traits in these ecotypes, resulting in different transpiration rates, which led to a difference in As accumulation.

From the evolutionary perspective, it is considered that the appearance of heavy metal tolerance is the result of long-term exposure to high metal concentration in the growth media (Lombi et al. 2000). But, how did a small group of plants evolve the heavy metal hyperaccumulation is still unclear. In this study, it is found that under different climatic conditions, *P. vittata* formed varied water uptake mechanisms, which contributed to different As accumulation capacities.

The hyperaccumulation of certain metals may be important in the osmotic adjustment of plants under water stress, based on the discovery that the nickel (Ni) hyperaccumulation in *Stackhousia tryonii* is a trait protecting the plant from drought in serpentine soils, with Ni acting as an osmoregulatory agent (Bhatia et al. 2005). Arsenic is closely related to the water consumption in hyperaccumulators. In the current study, As accumulation and transpiration displayed strong correlation. Higher transpiration due to transpiration regulation or ecotypic difference always led to higher As accumulation by *P. vittata*. And, on the other hand, low As exposure greatly increased the transpiration of *P. vittata*, similar to another As hyperaccumulator *Pteris cretica* (Lee and Lee 2011). It requires further investigation in that the evolution of As accumulation is related to water stress, similar to the inadvertent uptake theory of hyperaccumulation, which is that hyperaccumulation resulted from inadvertent uptake if the accumulated elements are chemically similar to essential nutrients and plant populations evolve more efficient uptake and translocation systems for these nutrients (Cappa and Pilon-Smits 2014; Pollard et al. 2014; Verbruggen et al. 2009).

However, studies on different but related physiological processes in different ecotypes of one species may provide essential information for the further dissection of genetics study of these traits.

Conclusion

This study disclosed the positive correlation between transpiration and As accumulation in As hyperaccumulator *P. vittata* by comparing As concentration in two *P. vittata* ecotypes from habitats with diverse climatic conditions. Transpiration is the main driver for *P. vittata* to accumulate and re-distribute As in pinnae. It is suggested that *P. vittata* ecotypes may evolve different As accumulation abilities, together with the formation of different water use efficiency, as a result of living in habitats with diverse climatic conditions.

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