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A comparison of arsenic accumulation and tolerance among four populations of *Pteris vittata* from habitats with a gradient of arsenic concentration

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HIGHLIGHTS

- As accumulation and As tolerance of *P. vittata* were considered to be two independent processes.
- *P. vittata* from low-As habitats exhibited higher As accumulation than that from high-As habitats.
- As transporters on *P. vittata* root contribute to the intraspecific difference in As accumulation.
- *P. vittata* populations with strong As tolerance adopted an avoidance strategy.

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ABSTRACT

Arsenic (As) contamination poses a high risk to human health. Phytoremediation based on As hyperaccumulator *Pteris vittata* has been utilized on large areas of contaminated farmland in southern China. However, the reason for the observed differences in As removal among *P. vittata* populations remains unclear. In this study, spores of four *P. vittata* populations were collected from four neighboring sites with varying soil As concentration (from 108 $mg \cdot kg^{-1}$ to 7527 $mg \cdot kg^{-1}$) and then cultured in a controlled environment to analyze their differing abilities in terms of As accumulation and tolerance. The results indicate that populations from low-As habitats exhibited 80% greater shoot As concentrations compared with those from high-As habitats. On the other hand, populations from high-As habitats exhibited approximately five times greater biomass compared with those from low-As habitats when exposed to the same As stress. Thus, the As accumulation and tolerance of *P. vittata* were suggested to be two independent processes. Further investigations reveal that the As absorption and As species conversion occurring in roots are two essential activities that bridge the soil As concentration and the responses of *P. vittata* to As. Depending on the As concentration of the target soil, the selection of different *P. vittata* populations can result in approximately an eight-fold difference in terms of remediation efficiency.

1. Introduction

Pteris vittata is an arsenic (As) hyperaccumulating fern that accumulates high As concentrations and is widely distributed in China (Chen et al., 2002; Ma et al., 2001). In situ phytoremediation projects that use *P. vittata* have been established on farmlands and residential areas, achieving high As removal rates (Chen et al., 2007; Ebbs et al., 2009; Kertulis-Tartar et al., 2006). As accumulation has been found to vary among *P. vittata* populations (Wu et al., 2009), but the reason for this variation remains unclear. A better understanding of As accumulation variation in different fern populations is of practical importance in screening for the appropriate phytoremediation plant varieties. Furthermore, an elaboration of such variation can also aid the focused investigation on the genes responsible for As tolerance or hyperaccumulation (Kim, 2009; Sundaram et al., 2009).

Hyperaccumulators often grow in soils that contain high metal(loid) concentrations, suggesting that such high concentrations may exert a selection pressure on hyperaccumulator evolution. An adaptive evolutionary process caused by the stress of excessive metals is generally accepted to result in heavy metal tolerance (Krämer, 2010), whereas the relationship between plant hyperaccumulation and habitat metal concentration remains unclear. Hyperaccumulation often coexists with hypertolerance (Baker et al., 1988), and thus, accumulation is considered as a hyperaccumulator strategy for tolerating the presence of heavy metals (Singh and Ma, 2007). However, hyperaccumulation has recently been reported as a possible constitutive trait independent from tolerance (Maestri et al., 2010). Maestri et al. (2010) stated that one mechanism is unlikely to function on a large number of hyperaccumulator species. Heavy metal accumulation and tolerance in

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hyperaccumulators *Thlaspi caerulescens* and *Thlaspi goesingense* display opposite trends with increasing soil metal concentration (Lombi et al., 2000; Meerts and Van Isacker, 1997).

The role of habitat As concentrations in creating variations in the As accumulating ability of As hyperaccumulators remains open for discussions. Some studies found that nonmetallicolous *P. vittata* populations accumulate more As compared with metallicolous populations (Wang et al., 2002; Wu et al., 2009), whereas other studies did not find obvious differences in the accumulation abilities of various *P. vittata* populations (Gumaelius et al., 2004; Zhao et al., 2002). Such dissimilar conclusions may be attributed to the interference from certain influencing factors, such as soil properties and climatic conditions (Lefèbvre and Vernet, 1990; Lombi et al., 2000). Discriminating the influence of heavy metal concentrations in soil from other factors is difficult because of the lack of appropriate plant varieties. *P. vittata* is widely distributed in China, including some old nonferrous mining areas that have produced populations from neighboring but separate habitats with large differences in soil As concentration. These populations can serve as appropriate plant sources for studies on the lone effect of habitat As stress on As accumulation in *P. vittata* populations.

This study aims to provide relevant information for understanding the evolutionary process of As hyperaccumulation and for selecting the appropriate plant varieties for phytoremediation practices by investigating the differences in the As accumulation and tolerance of four *P. vittata* populations. In addition, physiological variations among *P. vittata* populations are examined, with focus on root uptake kinetics and As species transformation, which are regarded as essential physiological activities associated with the As accumulation in *P. vittata* populations (Duan et al., 2005; Huang et al., 2008; Luongo and Ma, 2005; Poynton et al., 2004).

2. Materials and methods

2.1. Field survey

The sampling sites are located in an area with a 600-year mining history in the Hunan province, southern China, and the *P. vittata* populations were collected from four neighboring sites with almost the same habitat conditions except for varying soil As concentrations. The locations, soil properties, plant communities, growth conditions, and soil As concentrations of the sampling sites are described in Table 1. In each sampling site (3 m × 3 m), one soil sample (mixture of four sampling points on the diagonal, with depths of 0 cm to 15 cm) and three plant samples as replicates were collected for analysis.

2.2. Spore collection and plant culture

Spores from the four *P. vittata* populations were collected from the study sites and were then germinated to produce progeny via spore-initiated sexual propagation. The germination soils, which were collected from a farmland in Beijing, contained 18.5 $g \cdot kg^{-1}$ organic matter, 0.88 $g \cdot kg^{-1}$ total phosphorus (P), 1.04 $g \cdot kg^{-1}$ total nitrogen, 142 $mmol \cdot kg^{-1}$ of cation-exchange capacity, 26% of clay (<0.002 mm), and 8 $mg \cdot kg^{-1}$ total As. Experiments were conducted in a greenhouse with day/night periods of 16 h/8 h, lighting intensity of 300 $mE \cdot m^{-2} \cdot s^{-1}$ provided by fluorescent and incandescent lamps, day/night temperatures of 23 °C to 25 °C/20 °C to 22 °C, and 60% relative humidity.

2.3. Hydroponic experiments on As accumulation in four *P. vittata* populations

Precultured seedlings with heights of approximately 10 cm were transferred to a modified Hoagland nutrient solution containing the following in $mol \cdot L^{-1}$: K_2SO_4 1.875×10^{-4}, $MgSO_4$ 1.625×10^{-4}, KCl 0.25×10^{-4}, $Ca(NO_3)_2$ 0.5×10^{-3}, KH_2PO_4 0.625×10^{-4}, H_3BO_3 0.25×10^{-5}, $MnSO_4$ 0.25×10^{-6}, $CuSO_4$ 0.25×10^{-7}, $ZnSO_4$ 0.25×10^{-6}, $(NH_4)_6MoO_4$ 1.25×10^{-9}, and Fe-EDTA 0.25×10^{-4}. The pH level was adjusted to 6.2, and disodium arsenate heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$) was added to a concentration of 1 $mg \cdot As \cdot L^{-1}$. Nothing was added to the control.

After growing in the nutrient solutions above for 30 d, the *P. vittata* populations were harvested, washed with tap water, and then rinsed thrice with deionized water. Each plant was divided into shoot (including frond and petiole) and root (rhizoid), the fresh weights (FW, g) were determined, and the plant height (distance from the top of the plant to the upper surface of the rhizome), pinna density (number of pinnas/length of frond), root length (length of the longest root), and root surface area were measured using the methylene blue dye method as described by Mu et al. (2006). The plant materials were freeze-dried under vacuum (<20 Pa) at −50 °C for 48 h and then stored in a −30 °C freezer prior to X-ray absorption near-edge structure (XANES) analyses. Then, the dry weights (DW, g) of all samples were determined for further analysis on As concentrations. Each treatment was performed in four replicates, each containing two plants.

Arsenic uptake kinetics were investigated using a modified general ion depletion method according to Wang et al. (2002). Plants with heights of approximately 10 cm were chosen from precultured seedlings, rinsed with deionized water, and then soaked in a pretreatment solution containing 0.5 mM $CaCl_2$ and 5 mM MES at pH level of 6.0 for 20 h. Then, the plants were removed, rinsed with deionized water, and then transferred to an As-uptake incubation solution containing 1 μM As (as Na_2HAsO_4), 0.5 mM $CaCl_2$, and 5 mM MES at pH level of 6.0 at 25 °C. At 0, 0.25, 0.5, and 1 h, and then hourly to 20 h of incubation, 0.5 mL of incubation solution was removed for As concentration determination. The incubation volume was replenished with deionized water. Deionized water was used at hourly intervals to compensate for water losses through transpiration. After 20 h, the roots were separated from the shoots, rinsed with deionized water, dried at 60 °C, and then

Table 1
Arsenic concentration of four *P. vittata* populations and their associated soils sampled in Hunan Province, China.

Sample site	Geographic coordinates	Altitude (m)	Dominant species	Soil			*P. vittata*		TF	AF
				pH	Total As ($mg \cdot kg^{-1}$)	Soluble As ($mg \cdot kg^{-1}$)	As in shoot ($mg \cdot kg^{-1}$)	As in root ($mg \cdot kg^{-1}$)		
HN1	E113°09′59″ N25°43′15″	555	*Miscanthus floridulus*, *P. vittata*	7.6	108	n.d. *	465 ± 104b	328 ± 162b	1.8 ± 1.1ab	4.3 ± 0.9a
HN2	E113°09′05″ N25°48′04″	460	*P. vittata*, *Equsetum ramosissimum*, *Chrysopogon aciculatus*	7.9	1159	0.05	1003 ± 624b	682 ± 359b	1.5 ± 1.0ab	0.9 ± 0.5b
HN3	E113°09′39″ N25°43′25″	605	*M. floridulus*	7.2	3579	0.05	402 ± 109b	506 ± 242b	1.0 ± 0.8b	0.1 ± 0.0b
HN4	E113°09′53″ N25°43′23″	499	*M. floridulus*, *P. vittata*, *Patrinia monandra* C.B. Clarke	6.3	7527	21.7	4207 ± 1426a	146 3 ± 549a	2.9 ± 0.6a	0.6 ± 0.2b

* n.d. means not detected. Data for plant samples is indicated in mean ± SD ($n = 3$). Different letters within a column indicate significant differences among populations ($p < 0.05$). TF is the translocation factor, which is the quotient of As in the shoot divided by the As in the root. AF is the accumulation factor, which is the quotient of As in the shoot divided by the total As in soil.