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Arsenic speciation, and arsenic and phosphate distribution in arsenic hyperaccumulator *Pteris vittata* L. and non-hyperaccumulator *Pteris ensiformis* L.

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Pteris vittata may effectively reduce arsenite in its fronds.

Abstract

This study examined the roles of arsenic translocation and reduction, and P distribution in arsenic detoxification of *Pteris vittata* L. (Chinese Brake fern), an arsenic hyperaccumulator and *Pteris ensiformis* L. (Slender Brake fern), a non-arsenic hyperaccumulator. After growing in 20% Hoagland solution containing 0, 133 or 267 μM of sodium arsenate for 1, 5 or 10 d, the plants were separated into fronds, rhizomes, and roots. They were analyzed for biomass, and concentrations of arsenate (AsV), arsenite (AsIII) and phosphorus. Arsenic in the fronds of *P. vittata* was up to 20 times greater than that of *P. ensiformis*, yet showing no toxicity symptoms as did in *P. ensiformis*. While arsenic was concentrated primarily in the fronds of *P. vittata* as arsenite it was mainly concentrated in the roots of *P. ensiformis* as arsenate. Arsenic reduction in the plants took longer than 1-d. *P. vittata* maintained greater P in the roots while *P. ensiformis* in the fronds. The high arsenic tolerance of the hyperaccumulator *P. vittata* may be attributed to its ability to effectively reduce arsenate to arsenite in the fronds, translocate arsenic from the roots to fronds, and maintain a greater ratio of P/As in the roots.

Keywords: *Pteris vittata*; *Pteris ensiformis*; Arsenic; Uptake; Speciation; Translocation; Phosphate

1. Introduction

In recent years, arsenic has received great attention as a global problem mostly stemming from the crisis in South-East Asia. Arsenic-contaminated soil constitutes one of the major sources of arsenic in drinking water (Welch et al., 2000). Remediation of As-contaminated soil and water is necessary to protect both human life and agricultural production. Recently, use of phytoremediation technique to clean up As-polluted sites including groundwater is gaining attention due to the environmental concerns and remediation costs associated with conventional remediation processes.

Recent discovery of several arsenic hyperaccumulators makes it possible to phytoremediate arsenic-contaminated soils (Komar et al., 1998; Ma et al., 2001; Francesconi et al., 2002; Zhao et al., 2002; Meharg, 2003). Among the known arsenic hyperaccumulators, *Pteris vittata* L. (Chinese brake fern) is one of the most efficient and most studied. It can accumulate up to 22,630 mg As kg^{-1} in its aboveground biomass (fronds), indicating its high tolerance to arsenic and efficient mechanisms of arsenic detoxification (Ma et al., 2001). Rapid translocation of arsenic from roots to fronds coupled with arsenic reduction from arsenate (AsV) to arsenite (AsIII) in the fronds has been proposed as one of the mechanisms for arsenic detoxification in *P. vittata* (Ma et al., 2001; Zhao et al., 2003; Webb et al.,

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2003; Tu et al., 2004a). Other mechanism may involve sequestration of arsenic away from sites of metabolism in the cytoplasm, most probably vacuoles (Lombi et al., 2002; Wang et al., 2002; Webb et al., 2003).

Plants vary greatly in their sensitivity and resistance to arsenic (Meharg and Hartley-Whitaker, 2002), with arsenic resistance having been identified in a range of plant species (Sharples et al., 2000; Meharg and Hartley-Whitaker, 2002). Plant resistance to arsenic is generally achieved by restricted uptake of arsenic by the roots or limited translocation of arsenic from the roots to the shoots and further by phytotoxicity at relatively low arsenic concentrations in plant tissues (Meharg and Macnair, 1992).

The phosphate/arsenate uptake system plays a role in arsenate influx in plants. It has been shown that arsenate and phosphate are taken up by the same plasma membrane transport system in plants (Ullrich-Eberius et al., 1989). Phosphate has little effect on *P. vittata* when growing for 20-week in a soil spiked with 200 mg As kg^{-1}; however, phosphate significantly improved plant growth when the arsenic concentration increased to 400 mg kg^{-1} (Tu and Ma, 2003). They further proposed that a minimum ratio of P/As of 1:2 in the plant is required for normal growth of *P. vittata*. Large genotypic differences in arsenic tolerance have been reported among different fern species (Meharg, 2003). A study comparing two fern species of different genus, *P. vittata* (an arsenic hyperaccumulator) and *Nephrolepis exaltata* (Boston fern, a non As-hyperaccumulator), demonstrated that *P. vittata* displayed greater P/As ratios in the roots than *N. exaltata* after arsenic exposure (Tu and Ma, 2004). They suggested the ability of *P. vittata* in maintaining high P/As ratio in the roots may have played a role in detoxifying arsenic.

Though arsenic distribution and reduction in *P. vittata* have been examined previously (Ma et al., 2001; Webb et al., 2003; Tu et al., 2004a), the impacts of exposure time on arsenic distribution and reduction in different tissues of *P. vittata*, especially rhizomes, have not been reported. The study by Tu and Ma (2004) used two fern species from two genus, making the comparison less convincing. Recently, a *Pteris* species — *Pteris ensiformis* (Slender brake fern) has been shown to be a non-arsenic hyperaccumulator and sensitive to arsenic (Ma et al., unpublished). Hence, it is possible to compare arsenic accumulation characteristics in two fern species of the same genus for the first time.

To better understand the mechanisms of plant arsenic detoxification, we chose two fern species of *Pteris* genus, i.e. an arsenic hyperaccumulator *P. vittata* and a non-As-hyperaccumulator *P. ensiformis*, to study the roles of arsenic distribution and reduction as well as P distribution in different plant tissues. The main objectives of this study were to (1) compare arsenic distribution in roots, rhizomes and fronds between the two ferns, (2) determine arsenic reduction in different plant parts, and (3) examine P distribution in different plant parts. Information obtained from this study should enhance our understanding of the arsenic detoxification mechanisms by hyperaccumulator *P. vittata*.

2. Materials and methods

2.1. Experiment setup

Two fern species, *P. vittata* L. and *P. ensiformis* L., were used in this study. Four-month-old plants were procured from a nearby nursery. To acclimatize the plants to hydroponic environment, they were grown hydroponically for 2 weeks in a controlled environment, with temperatures ranging from 23 to 28 °C and humidity of ~70%. A 14-h photoperiod with a daily photosynthetic photon flux of 350 μmol m^{-2} s^{-1} was supplied by cool-white fluorescent lamps. Hoagland–Arnon nutrition medium (Hoagland and Arnon, 1938) at 0.2-strength with vigorous aeration was used to maintain plant growth. The nutrient solution was replenished twice a week. After acclimatization for 2 weeks, they were transferred into 0.2-strength Hoagland nutrient solution containing 0, 133, or 267 μM of arsenate ($Na_2HAsO_4 \cdot 7H_2O$) (SIGMA, St. Louis, MO). Plants were harvested at three intervals, i.e. 1, 5 and 10 d, after arsenic treatment. Each treatment had three replicates. All plants used in the experiments were of similar size, with approximate fresh weights of 5.0 g. Upon harvest, the plants were separated into fronds, rhizomes, and roots. Arsenic speciation was performed using samples that were flash-frozen in liquid nitrogen and stored at −80 °C, while total P and As analyses were done on air-dried (65 °C for 2 d) samples.

2.2. Arsenic determination

Plant samples were digested using USEPA Method 3050A. Briefly, 0.1–0.5 g of ground plant material was weighed directly into a polypro propylene digestion vessel, mixed with 10 ml of 1:1 (v/v) nitric acid (trace metal grade) and covered with a clean polypropylene ribbed watch glass. The samples were placed into a temperature-controlled digestion block (Environmental Express, Mt. Pleasant, SC) and heated for 16 h at 45 °C. The temperature was then raised to 105 °C for 3 h, with more 1:1 nitric acid added as needed to maintain a minimum of 5 ml in the vessel. The samples were then removed from the block and two 0.5-ml aliquots of 30% H_2O_2 were added. After having been returned to the block for another 20 min, the samples were cooled, brought to a final volume of 50 ml, filtered and capped for arsenic analysis.

Arsenic analysis was performed with a transversely heated, Zeeman background correction equipped graphite furnace atomic absorption spectrophotometer (GFAAS, Perkin–Elmer SIMAA 6000, Norwalk, CT). Palladium nitrate (1000 mg L^{-1}) was used as the modifier. Pyrolysis temperature was at 1200 °C and atomization was carried out at 2100 °C. The standard reference material was carried through the digestion and analyzed as a part of the quality control protocol. Reagent blanks and internal standards were used where appropriate to ensure accuracy and precision in arsenic analysis.

2.3. Phosphate determination

Plant samples were digested using the H_2SO_4/H_2O_2 method (Jones et al., 1991). Because arsenate interferes with P determination using the molybdenum blue method (Murphy and Riley, 1962), P was determined using a modified method of Carvalho et al. (1998). Briefly, the pH of the digestion solution was adjusted to approximately 5 with NaOH and HCl. Ten milliliter of the solution was pipetted into a 20-ml glass test tube, and to this 0.5 ml of L-cysteine (5% w/v in 0.6 M HCl) was added. The test tube was capped tightly to allow arsenate reduction for 5 min at 80 °C. The solution was cooled to room temperature, and P was determined by the molybdenum blue method.

2.4. Arsenic speciation

Arsenic speciation was performed by extracting plant samples ultrasonically in 10 ml of methanol/water mixture (1:1 v/v) two times for 4 h at 60 °C (Zhang et al., 2002). The two extracts were decanted into a 100-ml volumetric flask and diluted to 100 ml with water. Arsenate and arsenite were separated using an As speciation cartridge (Metal Soft Center, Highland Park, NJ), which retains arsenate (Meng et al., 2001). Total arsenic (AsV and AsIII) and arsenite (AsIII) were determined by a GFAAS (Perkin–Elmer SIMMA 6000, Norwalk, CT). To check