

Three new arsenic hyperaccumulating ferns

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Abstract

Phytoremediation, an emerging, plant-based technology for the removal of toxic contaminants from soil and water, has been receiving increased attention. The prerequisite for successful phytoremediation is the existence of hyperaccumulator plants. Designed to search for new arsenic (As) hyperaccumulators, an experiment was conducted under greenhouse conditions in a completely randomized design with four replications. This experiment identified *Pteris biaurita* L., *P. quadriaurita* Retz and *P. ryukyuensis* Tagawa as new hyperaccumulators of As and re-confirmed *Pteris cretica* as a hyperaccumulator. The average As concentration ranged from 1770 to 3650 mg kg⁻¹ DW in the fronds and 182 to 507 mg kg⁻¹ DW in the roots of *P. cretica*, *P. biaurita*, *P. quadriaurita* and *P. ryukyuensis* after having been grown in 100 mg As kg⁻¹ soil. There was a greater percentage of As (III) as compared to As (V) in the fronds of these plants. Based on our study, *P. ryukyuensis* is the most promising candidate to phytoremediate As contaminated soils compared to the other three species. The nutrient requirements or distributions within the *Pteris* species were altered distinctly when the plants were exposed to As.

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1. Introduction

Arsenic (As) is one of the most toxic elements present in soils and water. Over the years, arsenic has been widely used in agriculture and industrial practices such as pesticides, fertilizers, wood preservatives, smelter wastes and coal combustion ash, which are of great environmental concern (Smith et al., 1998; Nriagu, 2002). Arsenic contamination affects biological activities as a teratogen, carcinogen and mutagen as well as having detrimental effects on the immune system (Squibb and Fowler, 1983). The evidence of health risks from As contamination is so compelling that in

2002 the Environmental Protection Agency lowered the maximum contaminant level of As in drinking water from 50 to 10 µg l⁻¹, making remediation of As contaminated water an increasingly important and potentially expensive issue (Smith et al., 2002).

Phytoextraction, a promising new method that uses green plants to detoxify metals, is an alternative to the conventional means to remediate As contaminated sites (Salt et al., 1998). This technology is a relatively inexpensive form of ecological engineering that has proven effective in some cases (Raskin et al., 1994). Obvious prerequisites for successful phytoextraction are the existence of metal hyperaccumulators with the ability to accumulate large amounts of the metal contaminant in their aboveground tissues with a high biomass. The term metal hyperaccumulator was first used to describe a plant species that hyperaccumulates nickel (Ni). The

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term was later broadened to characterize plants achieving metal concentrations $>1000 \text{ mg kg}^{-1}$ (Reeves and Baker, 2000).

Many plants have been reported to accumulate more than 1000 mg kg^{-1} arsenic in their tissues (Porter and Peterson, 1975), however, they cannot be classified as hyperaccumulators since arsenic accumulation in these plants occurs very slowly over an extended period of time. In addition, a large portion of the arsenic is sequestered in the roots. Most importantly, a lack of rapid growth, large biomass production and high uptake capacity render these plants unsuitable for phytoremediation.

Although the definition of arsenic hyperaccumulators is not clearly defined and can be considered arbitrary, the working definition is a plant that accumulates a minimum arsenic concentration of 1000 mg kg^{-1} in the aboveground biomass, and has a higher concentration in the aboveground biomass than in both the roots and the soil (Bondada and Ma, 2003; Meharg, 2003).

The first known arsenic hyperaccumulating plant, *Pteris vittata* L., also known as Chinese brake fern, was discovered by Komar et al. (1998) from an arsenic-contaminated site that was contaminated from pressure-treating lumber using chromated-copper-arsenate (CCA). However, the hyperaccumulator was not well publicized until after 2001 when several other scientists made a similar discovery (Ma et al., 2001a; Chen et al., 2002; Visoottiviseth et al., 2002). Among the 14 plant species collected from the CCA site, this fern is the only one that hyperaccumulates arsenic, with arsenic concentrations in the fronds (aboveground biomass) being as high as 4360 mg kg^{-1} , as compared to 184 mg kg^{-1} in the soil (Komar et al., 1998). In a subsequent screening study of 17 fern species, three cultivars of *Pteris cretica*, i.e. *albo-lineata*, *mayii* and *parkerii*, were classified as arsenic hyperaccumulators (Ma et al., 2001b). Arsenic concentrations in their fronds ranged from 1114 to 2046 mg kg^{-1} after growing in an arsenic-contaminated soil containing 245 mg kg^{-1} arsenic for 8 weeks. The other 14 species are not arsenic hyperaccumulators, all being non-*Pteris* ferns and having arsenic concentrations less than 46.6 mg kg^{-1} in the fronds.

In addition to *P. vittata* and *P. cretica*, several other arsenic hyperaccumulating plants have been reported recently including *Pityrogramma calomelanos* (Francesconi et al., 2001) and *Pteris longifolia* and *Pteris umbrosa* (Meharg, 2003; Zhao et al., 2002). Besides the three cultivars of *P. cretica* identified by Ma et al. (2001b), four additional cultivars of *P. cretica* have also been identified as arsenic hyperaccumulators, i.e. *chilsii*, *crista* and *rowerii* (Meharg, 2003) and *wismsetti*

(Zhao et al., 2002). Except for *P. calomelanos*, all known arsenic hyperaccumulator species are ferns and belong to the *Pteris* genus. However, not all *Pteris* species hyperaccumulate arsenic (Meharg, 2003; Zhao et al., 2002).

In this study, we hypothesized that screening ferns in the *Pteris* genus could identify more arsenic hyperaccumulators. We tested the hypothesis by growing different *Pteris* species under controlled greenhouse conditions. The biomass and nutrient [phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg)] accumulation in these ferns and their relations to As were also studied.

2. Materials and methods

2.1. Arsenic accumulation by *Pteris* species grown from spores

Spores of *P. cretica* L. (a known As hyperaccumulator), *Pteris biaurita* L., *P. quadriaurita* Retz. and *P. ryukyuensis* Tagawa were collected from the natural habitat of each species. Spores were sprinkled onto a moist soil (50% sand, 25% peat and 25% garden soil) in a seed tray. The trays were covered with a plastic film to maintain moisture. After spore germination and the prothalli development, fertilizer was applied. Once sporelings grew to having two to three fronds, they were transplanted individually into 2-in. plastic pots containing just potting soil. After 1 month, these plants were transferred to pots containing 2.5 kg of soil.

The experiment was conducted in controlled environmental conditions with an 8-h light period at 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. The study was set up as a completely randomized design in a 4×2 factorial. Four fern species *P. cretica*, *P. biaurita*, *P. quadriaurita* and *P. ryukyuensis* (one plant in each pot) were grown in a clean soil (control) and a soil spiked with $100 \text{ mg As kg}^{-1}$, added as $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$. Each treatment was replicated four times. Forty-five days after transplanting, the plants were harvested and washed with deionized water. The plants were then separated into fronds (aboveground biomass) and roots (underground biomass). Plant parts were dried at 60°C for 48 h and dry weights recorded.

2.2. Chemical analysis

Ground plant material (0.5 g) was digested with nitric acid and hydrogen peroxide on a temperature-controlled digestion block (Environmental Express, Mt. Pleasant, S.C.) using USEPA Method 3050A in

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