Rapid report

Arsenic hyperaccumulation by different fern species

Author for correspondence:
S. P. McGrath
Tel: +44 (0)1582 763133
Fax: +44 (0)1582 760981
Email: steve.mcgrath@bbsrc.ac.uk

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F. J. Zhao, S. J. Dunham and S. P. McGrath

Agriculture and Environment Division, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK

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Summary

• *Pteris vittata* was the first identified arsenic (As) hyperaccumulator. Our aim was to test whether As hyperaccumulation occurs in other fern species, and whether *P. vittata* collected from both contaminated and uncontaminated environments accumulates As similarly.

• Three accessions of *P. vittata*, two cultivars of *Pteris cretica*, *Pteris longifolia* and *Pteris umbrosa* were grown with 0–500 mg As kg^{-1} added to the substrate. A second experiment compared As uptake by five common ferns obtained from commercial suppliers.

• The results show that, in addition to *P. vittata*, *P. cretica*, *P. longifolia* and *P. umbrosa* also hyperaccumulate As to a similar extent. There was little difference between different *Pteris* species, or between different accessions of *P. vittata*. By contrast, *Asplenium nidus*, *Davallia canarensis*, *Polypodium aureum*, *Polystichum tsussimense* do not hyperaccumulate As.

• This study identified three new species of As hyperaccumulators in the *Pteris* genus and suggests that As hyperaccumulation is a constitutive property in *P. vittata*.

Introduction

Hyperaccumulation of heavy metals or metalloids is a rare phenomenon in terrestrial higher plants. To date, some 400 taxa of hyperaccumulator species have been identified, with about three-quarters of them being nickel (Ni) hyperaccumulators (Baker *et al.*, 2000). Although a wide range of plant species have been identified as being arsenic (As) resistant (Meharg & Hartley-Whitaker, 2002), As hyperaccumulation was discovered only recently, initially in the brake fern *Pteris vittata* (Ma *et al.*, 2001). This fern can accumulate up to 22 630 mg As kg^{-1} in the shoot (frond) dry weight (d. wt). Furthermore, the bioconcentration factor, defined as the ratio of shoot As concentration to soil As concentration, was greater than 10. This fern also possesses another character typical of metal/metalloid hyperaccumulators (McGrath *et al.*, 2002): a highly efficient root to shoot transport of the metal/metalloid, leading to a large ratio of shoot to root As concentrations (Tu & Ma, 2002). These characters are in direct contrast to those of many other As-resistant plants, which achieve As tolerance mainly through reduced uptake of As by suppression of a high-affinity phosphate/arsenate uptake system (Meharg & Hartley-Whitaker, 2002).

A recent survey in Thailand has identified another As hyperaccumulator fern, *Pityrogramma calomelanos*

(Visoottiviseth *et al.*, 2002). Both *Pteris vittata* and *Pityrogramma calomelanos* have relatively large biomass and, together with their remarkable ability to extract As from soil, offer the possibility of phytoremediation of As-contaminated soils (Ma *et al.*, 2001; Tu & Ma, 2002; Visoottiviseth *et al.*, 2002).

Both As hyperaccumulating ferns were discovered from a survey of vegetation of As-contaminated land (Ma *et al.*, 2001; Tu & Ma, 2002; Visoottiviseth *et al.*, 2002). From these studies, we hypothesize that As hyperaccumulation may occur in other fern species. This can be tested by more surveys of vegetation growing on high As soils, or more quickly by growing different fern species under controlled conditions with elevated As supply. The latter approach was used in this study. In addition, we also compared As accumulation by different accessions of *Pteris vittata*, collected from both contaminated and uncontaminated environments. The aim was to evaluate whether As hyperaccumulation in *Pteris vittata* is a constitutive property.

Materials and Methods

Arsenic accumulation by *Pteris* species raised from spores

Spores of *P. vittata* L. were kindly provided by Dr Lena Ma (University of Florida, Gainesville, USA) and were also obtained from Oxford Botanical Garden (Oxford, UK) and B & T World Seeds (Olonzac, France). The Florida accession was from an As-contaminated site (containing 153 mg As kg^{-1} soil), whereas the Oxford and B & T accessions were from uncontaminated sites. Spores of *Pteris cretica* L. Albo-lineata, *Pteris cretica* L. Wimsetti, *Pteris longifolia* L and *Pteris umbrosa* R. Br. were obtained from B & T World Seeds. Spores were sprinkled on to moist general purpose compost in a seed tray. The trays were covered with a plastic cling film to maintain moisture. After spore germination, the prothalli developed and were fertilized, and grew into sporelings with true leaves (fronds). At the two to three true-leaf stage (76 d after sowing), sporelings were transplanted individually into 10-cm pots each containing 250 g general purpose compost. There were four treatments for each accession of *P. vittata* and other *Pteris* species: 0, 50, 100 and 500 mg As kg^{-1} compost. Each treatment was replicated four times. Arsenic had been added in a solution containing Na_2HAsO_4, and mixed thoroughly with the compost. Deionized water was used throughout. After 30 d, fronds were cut and washed with deionized water. Roots were washed with tap water, and then with deionized water. Plant samples were dried at 60°C for 48 h and dry weights recorded. The experiment was conducted inside a controlled environment growth chamber under the following conditions: 16 h light period with a light intensity of 350 μmol m^{-2} s^{-1}, 25°C/20°C day/night temperature, and 60–70% relative humidity.

Arsenic accumulation by pregrown ferns

Young sporelings (4–6 fronds) of *Asplenium nidus* L., *Davallia canarensis* (L.) Smith, *Polypodium aureum* (L.) J. Sm., *Polystichum tsus-simense* (Hook) J. Sm., *P. cretica* Albo-lineata and *P. cretica* Alexandrae were obtained from a local garden centre. The plants were transferred individually to a 10-cm pot, each containing 250 g compost. There were two treatments: 0 and 100 mg As kg^{-1} compost. Arsenic was added as Na_2HAsO_4 and mixed thoroughly with the compost before transplanting. Because of the limited availability of the sporelings, one pot per treatment was included for *Asplenium nidus*, *Polypodium aureum*, *Polystichum tsus-simense* and *Pteris cretica* Alexandrae, and two pots per treatment for *Davallia canarensis* and *Pteris cretica* Albo-lineata. Plants were grown inside a controlled environment chamber (conditions as described above) for 30 d. Fronds were harvested and washed with deionized water, and dried at 60°C for 48 h.

Chemical analysis

Ground plant materials were digested with a mixture of HNO_3 and $HClO_4$ (85 : 15, v : v), and the concentrations of As, phosphorus (P) and sulphur (S) were determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES; Fisons-ARL Accuris, Ecublens, Switzerland). The detection limit of the instrument for As in solution was 0.05 mg l^{-1}. Blanks and internal standards were included for quality assurance.

Results

Arsenic accumulation by different *Pteris* species

There were slight symptoms of As phytotoxicity in the 500 mg As kg^{-1} treatment, with the edge and tips of some pinnae showing necrosis. However, growth was not significantly inhibited by the As additions (data not shown).

All species of *Pteris* tested in this study, including three accessions of *P. vittata*, two cultivars of *P. cretica* (Albo-lineata and Wimsetti), and *P. longifolia* and *P. umbrosa*, hyperaccumulated As in the fronds (Fig. 1a). The concentration of As in the fronds increased linearly with increasing concentration of As added to the substrate, reaching 6200–7600 mg As kg^{-1} d. wt in the 500 mg As kg^{-1} treatment. There were no significant differences between different species or accessions in As hyperaccumulation. In the +As treatments, the ratio of As concentration in fronds to that in the substrate (the bioconcentration factor) ranged from 11.7 to 21.6 (mean 14.9), and there were no significant differences between different *Pteris* species or accessions of *P. vittata*.

The concentration of As in the roots also increased linearly with As addition (Fig. 1b). However, the concentrations of As were much smaller in the roots than in the fronds. On

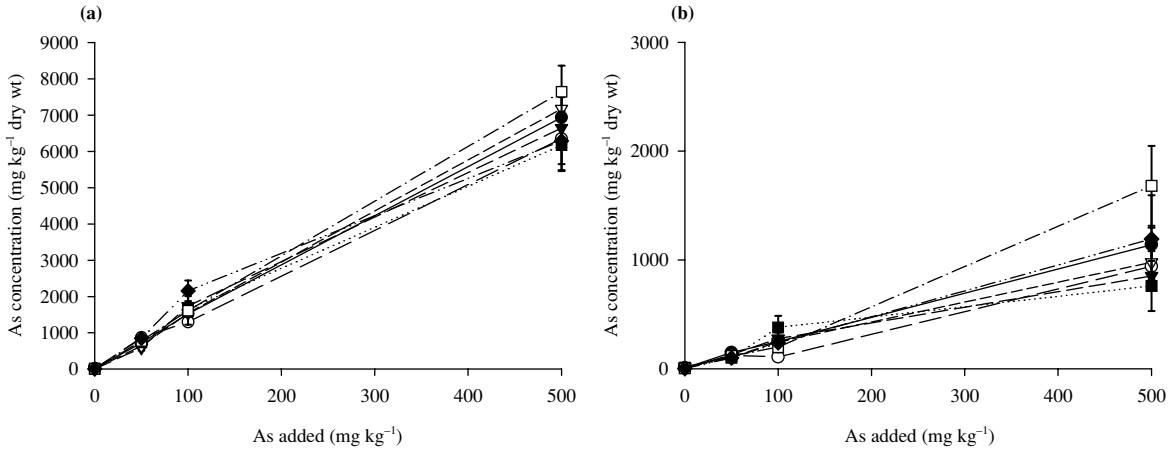

Fig. 1 Concentrations of As in fronds (a) and roots (b) of different *Pteris* species. Closed circle, *P. vittata* (Florida); open circle, *P. vittata* (B & T); closed triangle, *P. vittata* (Oxford); open triangle, *P. cretica* Albo-lineata; closed square, *P. cretica* Wimsetti; open square, *P. longifolia*; diamond, *P. umbrosa*. Vertical bars represent ± SE (n = 4).

Table 1 Concentrations of phosphorus (P) and sulphur (S) in fronds and roots of different *Pteris* species and accessions

	P (mg kg^{-1} d. wt)		S (mg kg^{-1} d. wt)	
Species	Frond	Root	Frond	Root
P. vittata (Florida)	3591 ± 167	2375 ± 151	2774 ± 78	2134 ± 56
P. vittata (B & T)	3347 ± 253	2110 ± 266	2800 ± 66	2328 ± 95
P. vittata (Oxford)	3624 ± 232	2450 ± 145	3638 ± 420	2138 ± 52
P. cretica Albo-lineata	4329 ± 234	2670 ± 223	2995 ± 248	2347 ± 107
P. cretica Wimsetti	3453 ± 245	2210 ± 164	2408 ± 154	2289 ± 112
P. longifolia	4308 ± 135	2206 ± 196	2987 ± 163	2287 ± 61
P. umbrosa	3735 ± 174	2315 ± 141	2816 ± 111	2235 ± 83

Values are means ± SE of different As treatments.

average, the concentration of As in the fronds was 6.8 times higher than in the roots. There were no significant differences in root As between the species and the accessions tested.

Table 1 shows the mean concentrations of P and S in the fronds and roots for each species or accession. The As treatments had no significant effect on the concentrations of either P or S in the fronds and roots. There were significant ($P < 0.01$) differences between species or accessions in the P and S concentrations. However, neither P nor S concentration correlated with the concentration of As in either roots or fronds. By contrast to As, the frond to root concentration ratios for P and S were 1.5–1.9 and 1.1–1.7, respectively.

Arsenic accumulation by different pregrown ferns

Six pregrown ferns were tested in this experiment, including two cultivars of *P. cretica* (Albo-lineata and Alexandrae). The concentrations of As in fronds were below the detection limit in the control treatment (no As). In the 100 mg As kg^{-1} treatment, the two cultivars of *P. cretica* hyperaccumulated As in the fronds to 2200–3030 mg kg^{-1} d. wt, whereas *A. nidus*, *D. canarensis*, *P. aureum*, and *P. tsus-simense* did not hyperaccumulate As, with the concentration of As varying between 60 and 168 mg kg^{-1} d. wt (Fig. 2). Although statistical analysis could not be performed for this experiment, the difference between *P. cretica* and other ferns (13- to 50-fold) in As accumulation was striking.

Discussion

In the present study, we have identified three new As hyperaccumulator species in the genus *Pteris*, including *P. cretica*, *P. longifolia* and *P. umbrosa*. These species and the first identified hyperaccumulator of As, *P. vittata* (Ma *et al.*, 2001), hyperaccumulated As to a similar extent, characterized by a high bioconcentration factor (> 10) and a high ratio of frond to root As concentration (*c.* 7). By contrast, four species of common house ferns, *A. nidus*, *D. canarensis*, *P. aureum* and *Polystichum tsus-simense*, did not hyperaccumulate As. The *Pteris* species and the other newly identified As hyperaccumulator, *Polypodium calomelanos* (Francesconi *et al.*, 2002; Visoottiviseth *et al.*, 2002), belong to the order Pteridales (Jones, 1987). By

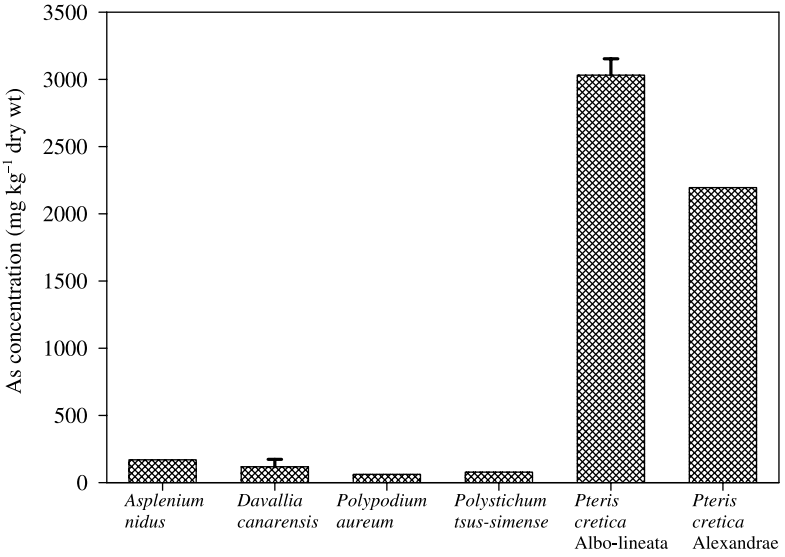

Fig. 2 Concentrations of As in fronds of different pregrown ferns: $n = 1$ for *Asplenium nidus*, *Polypodium aureum, Polystichum tsus-simense* and *Pteris cretical* Alexandrae, and $n = 2$ for *Davallia canarensis* and *Pteris cretica* Albo-lineata.

contrast, the four nonhyperaccumulator fern species tested in this study and the nonhyperaccumulator species shown by Visoottiviseth *et al.* (2002) are all outside the order Pteridales. Pteridales includes many other genera, and it would be interesting to examine how widespread the As hyperaccumulation trait occurs within this order. This is of interest not only to taxonomy and phylogeny, but also to the development of phytoremediation.

The Florida accession of *P. vittata* was from an As contaminated soil (Ma *et al.*, 2001), whereas other accessions of *P. vittata*, as well as other *Pteris* species tested, were from uncontaminated soils. The fact that they all hyperaccumulate As similarly indicates that As hyperaccumulation is a constitutive trait. It is not clear how this trait evolved and what its evolutionary benefits are. Meharg & Hartley-Whitaker (2002) postulated that terrestrial plants appear to have evolved around subaerial hot springs, which could have high levels of As. Consequently, early terrestrial plant forms may have had to cope with high levels of As in their environment, with this capability conferred on modern plants.

It has been shown that As nonhyperaccumulating plants take up arsenate via the phosphate transport systems (Asher & Reay, 1979; Lee, 1982; Ullrich-Eberius *et al.*, 1989; Meharg & Hartley-Whitaker, 2002). Our recent study demonstrated that this is also the case for the As hyperaccumulator *P. vittata* (Wang *et al.*, 2002). In this context, it is interesting to see that the concentrations of P in both roots and fronds of different *Pteris* species were not affected significantly by the additions of arsenate to the substrate. Furthermore, the concentrations of P in the roots and fronds of the *Pteris* species were comparable to those of As nonaccumulating plants (Marschner, 1995), thus there is no indication that *Pteris* hyperaccumulated P. The lack of interactions between As and P in this study may have several possible explanations: (1) addition of arsenate may increase P availability in the substrate through exchange with adsorbed P; (2) the phosphate systems in the plant roots may have a higher affinity for phosphate than for arsenate (Meharg & Macnair, 1990), thus enabling phosphate to out-compete with arsenate; (3) P uptake by plants is tightly regulated, and influenced more by the internal P status than by competition with arsenate; and (4) arsenate is likely to be reduced to arsenite in roots, and transported to fronds as either uncomplexed or complexed arsenite, resulting in a lack of competition with phosphate transport from roots to fronds.

Phytochelatins (PCs) have been shown to be involved in the detoxification of As in As nonhyperaccumulating plants (Pickering *et al.*, 2000; Schmöger *et al.*, 2000; Hartley-Whitaker *et al.*, 2001). Schmöger *et al.* (2000) showed that three thiol groups provided by two PC_2 molecules coordinate with one As molecule, giving rise to a 1 : 3 stoichiometry for As to S. PCs were not determined in this study. However, it is clear that the As treatments had no significant effect on the concentrations of S in both roots and fronds of *Pteris*. If all As in the fronds was to be complexed with PCs in a 1 : 3 stoichiometry, the concentration of S required for the synthesis of PCs alone would have to be about three times higher than the observed values of total S found in plants from the 500 mg As kg^{-1} treatment. In the 100 mg As kg^{-1} treatment, between 66% and 100% of the observed total S in the fronds would be required for the synthesis of PCs. This is highly unlikely, considering the major requirements of S for the synthesis of proteins and enzymes. These calculations assume that PCs–As complexes are sequestered and that PCs are not recycled. If PCs were recycled (e.g. after shuttling As to the vacuoles) then the absolute amount of PCs required for detoxifying As would be smaller. Analysis of frond extracts using high-pressure liquid chromatography (HPLC)–inductively coupled plasma mass spectrometry revealed that the majority of As was present as inorganic arsenite (Ma *et al.*,

2001; Francesconi *et al.*, 2002; Wang *et al.*, 2002), showing no evidence of the presence of PCs–As complexes. However, PCs–As complexes may not be stable during extraction or subsequent HPLC separation (Schmöger *et al.*, 2000). Further studies are needed to elucidate the mechanisms for As detoxification in *Pteris* and other related species that hyperaccumulate As.

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