Critical Review

The Arsenic Hyperaccumulator Fern *Pteris vittata* L.

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Arsenic (As) contaminated soils and waters are becoming major global environmental and human health risks. The identification of natural hyperaccumulators of As opens the door for phytoremediation of the arsenic contaminant. *Pteris vittata* is the first identified naturally evolving As hyperaccumulator. More than a decade after its discovery, we have made great progress in understanding the uptake, transport, and detoxification of As in the fern. The molecular mechanisms controlling As accumulation in *P. vittata* are now beginning to be recognized. In this review, we will try to summarize what we have learned about this As accumulator, with particular emphasis on the current knowledge of the physiological and molecular mechanisms of arsenic phytoremediation. We also discuss the potential strategies to further enhance phytoextraction abilities of *P. vittata*.

1. Introduction

Arsenic (As), which is a common element in the earth's crust, is a metalloid that is harmful for organisms living in the environment. Environmental As contamination results from natural processes, such as rock weathering and volcanic emissions. Human activities can enhance the As contamination in groundwater and soil (*1, 2*). For example, serious soil contamination with As due to the extensive use of arsenical pesticides, herbicides, etc., has been reported at numerous sites worldwide. Arsenic diffused in soils and groundwater can enter the food chain through drinking water and contaminated vegetables/agricultural products (*3*). Arsenic contamination in soil and water is a global problem (*2*). Millions of people all over the world are at risk from exposure to arsenic directly or indirectly, which has various acute and chronic effects on human health (*4*).

Extensive efforts have been made to reduce the negative effects of As contamination on the environment and human health. Among them, phytoremediation has been proved as a promising new technology for environmental cleanup. The term "phytoremediation" consists of the Greek prefix *phyto* (plant) and the Latin root *remedium* (to correct or remove an evil) (*5*). Therefore, phytoremediation is a technology that removes contaminants or pollutants by growing particularly selected plants. Compared with physical and chemical technologies, phytoremediation is environmentally friendly because it uses plants' natural ability to absorb and degrade toxic chemicals and pollutants from soil or water, and the contaminants can then be extracted from the harvested plants and processed appropriately. This technology tends also to be more cost-effective than conventional strategies because at least 3 or more times less expense is needed according to the previous analyses (*6*) (Table 1). In the case of phytoremediation of heavy metal pollutants (e.g., As, Cd, Pb), naturally occurring and genetically engineered plants which hyperaccumulate heavy metals are required (*7*). Phytoremediation processes include growing the selected plants in a contaminated field for a period of time to remove contaminants from the site, harvesting these accumulators, and processing and disposing of the contaminated materials (*8*).

For As phytoremediation to succeed, the phytoremediating plants must fulfill three criteria: the plant roots must be able to take up and deplete the soil As; the plants must be capable of translocating and accumulating As in the shoots that can then be harvested and processed; and the plant must have mechanisms to protect itself from the toxicity of high concentrations of As in its body (*9*). Recently, many plant As hyperaccumulators have been found, and interestingly, these naturally evolved As accumulators all belong to the fern family, and the majority of them are members of the *Pteris* genus (Table 2). However, not all members of the *Pteris* genus are able to hyperaccumulate arsenic (*10*). Because other plant species including the primitive ferns or their allies do not accumulate as much arsenic as the ferns in *Pteridaceae*, the natural As hyperaccumulators have been proposed as model systems to study the evolution of arsenic tolerance and metabolism (*10*). Recent research progresses on the arsenate reductases from yeast and fern *P. vittata* have shed light on the evolution of arsenic tolerance in As hyperaccumulating plants (*11, 12*).

P. vittata, the first identified As hyperaccumulator, has received extensive attention since its discovery in 2001 (*13*). Recent advances in understanding the mechanisms of As absorption, translocation, and compartmentalization within the vacuoles of *P. vittata* cells provided novel insights into plant physiology and molecular biology of phytoremediation of As. Because many excellent reviews have discussed the overall strategies for reducing arsenic hazards (*3, 14, 15*), this review focuses on *P. vittata* and how they remediate As from contaminated soil. It will pay particular attention to recent physiological and molecular developments in the study

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TABLE 1. Estimates of Phytoremediation Costs versus Costs of Established Technologies (*6*)

contaminant	phytoremediation costs	estimated cost using other technologies	source
metals	$80 per cubic yard	$250 per cubic yard	Black (*90*)
site contaminated with petroleum hydrocarbons (site size not disclosed)	$70,000	$850,000	Jipson (*91*)
10 acres lead contaminated land	$500,000	$12 million	Plummer (*92*)
radionuclides in surface water	$2 to $6 per thousand gallons treated	none listed	Richman (*93*)
1 ha to a 15 cm depth (various contaminants)	$2,500 to $15,000	none listed	Cunningham et al. (*94*)

of *P. vittata* that highlight the mechanisms of phytoextraction and challenges for future As risk reduction.

2. *P. vittata* Is an Arsenic Hyperaccumulating Fern

P. vittata belongs to the *Pteris* genus. Like other ferns, the life history of *P. vittata* consists of a cycle between a diploid sporophyte generation and a gametophyte generation. During the sporophyte stage, spore-forming organs known as sori form on the fronds. Each sorus contains sporangia, inside which individual spore mother cells produce four haploid spores via meiosis. After maturity, the spores scatter to locate favorable conditions (humidity). A spore germinates and grows into a young green plant known as a prothallus, which is the gametophyte of the fern. This produces the archegonia and antheridia, which produce a single egg and swimming sperm by mitosis, respectively. The prothallus is able to support gamete synthesis by the presence of rhizoids (root-like stems), which absorb vital nutrients and sufficient water from the surrounding environment. As the male and female sex organs have differing maturation times, the sperm seeks a separate individual on which the archegonia are already established so fertilization can occur. This restores the haploid gametophyte into a diploid sporophyte, producing a new independent generation.

P. vittata became well-known after being the first natural As accumulator in 2001 to be identified (*13*). It meets all the required criteria to qualify for being a natural phytoextractor. When *P. vittata* sporophytes are grown in the presence of As, their root systems can uptake As which is transported to the above-ground part of the plants through the xylem, with the highest levels of As in fronds (*13, 16*−*18*). The amounts of As accumulated in fronds can be up to 93% of the total As content in the plants (*13, 16, 17*), and 25 times more than that in the roots (*18*). Old fronds can accumulate 13,800 mg As kg^{-1} dry biomass, which is about 142 times higher than the As levels in the soil in which the plants were grown (*19*). Recent results showed that trichome on the fronds contained the highest levels of As compared with the other frond tissues including epidermal and mesophyllous cells (*20*). In a trichome, the basal and stalk cells accumulate more As than the cap cell. The results suggest an important role for the trichome of *P. vittata* in As accumulation. Further study on hyperaccumulation of As in *P. vittata* trichomes will help to clarify the mechanisms underlying hyperaccumulation and detoxification of As in the heavy metal hyperaccumulators and to further improve their ability to remove As.

In addition to sporophytes, gametophytes of *P. vittata* are also tolerant to high doses of As in the growth medium and can accumulate As (*9, 21*). For example, gametophytes of *P. vittata* grow normally in a medium containing 20 mM arsenate, and the amount of As accumulated was greater than 2.5% of their dry weight (*9*). When gametophytes were grown on a medium supplemented with 2 mM arsenate, they exhibited a greater ability to accumulate As (*21*). Furthermore, Yang et al. (*21*) found that the callus of *P. vittata* induced from gametophytes also displayed similar characteristics in As accumulation. When 7.5 g of *P. vittata* callus was grown in 150 mL of half-strength MS liquid culture containing 450 μg of arsenate for 2 days, the total As in the medium was reduced by over 60%. A *P. vittata* callus accumulated similar amounts of As as its sporophytes if they were exposed to 2 mM arsenate for 15 or 30 days. Three patterns of *P. vittata* are shown in Figure 1. All of them are highly tolerant to As, which suggest that accumulation of As is a common cellular mechanism for *P. vittata*.

3. *P. vittata* Is Hypertolerant to As

Compelling evidence has shown that all the callus, sporophytes, and gametohytes of *P. vittata* have strong As resistance that enable them to grow normally in growth media containing high levels of As that kill non-As-accumulators (*9, 21*). For example, the gametophytes of non-As-accumulator *Ceratopteris richardii* died at 0.1 mM of arsenate; in sharp contrast, the gametophytes of *P. vittata* did not show any stress phenotypes in response to 20 mM arsenate in the growth medium (*9*). Exposure to 0.2 mM of arsenate killed the callus of *Arabidopsis thaliana*, whereas no cell death was detected in *P. vittata* callus in the presence of a 10 times higher concentration of arsenate. It is fairly interesting that low levels of As in growth media or soils promote *P. vittata* growth (*13, 22*). Ma et al. (*13*) reported that 100 ppm arsenic markedly stimulated fern growth, resulting in a 40% increase in biomass compared with the control. Most recently, Srivastava et al. (*22*) also showed that certain concentrations of As (150−300 μM arsenate) increased biomass of *P. vittata* by 11−12%. These observations have raised the question of whether As serves as a nutrient component during plant growth and development. Some studies favor the hypothesis: arsenic hyperaccumulation-induced P and K enhancements in the fronds at low As levels is directly related to the As-induced growth stimulation (*23*); arsenic can reduce Cu phytotoxicity in the As hyperaccumulator *P. vittata* (*24*); and As accumulates preferentially in young fronds and translocates out of senesescing fronds to the young tissues (*25*). Arsenic maybe has dual effects on *P. vittata* growth, depending on its concentrations. Beyond the threshold stress level, growth of *P. vittata* plants or cells is also inhibited (*26, 27*). It is clear that *P. vittata* has a higher tolerance to As than other plants.

Plants have evolved a variety of tolerance mechanisms to adapt to an environment polluted with heavy metals. These include avoidance, exclusion, and intracellular detoxification processes (e.g., chelation, compartmentation, and/or biotransformation). For example, As-tolerant *Holcus lanatus* activates an altered phosphate uptake system to limit As uptake (*28, 29*). However, based on the current evidence, limitation of As uptake is apparently not the way for *P. vittata* to tolerate high levels of As in the growth conditions because no altered

TABLE 2. Arsenic Hyperaccumulators in the World

As hyperaccumulator	location	references
Pteris vittata	America	Ma et al. (*13*)
	China	Chen et al. (*16*)
Cretan Brake	China	Wei et al. (*95*)
Pityrogramma calomelanos	Southern Thailand	Francesconi et al. (*96*)
		Visoottiviseth et al. (*17*)
Pteris cretica		
Pteris longifolia		
Pteris umbrosa	Rothamsted	Zhao et al. (*43*)
Pteris multifida Poir	China	Du et al. (*97*)
Pteris cretica chilsii		
Pteris cretica crista		
Pteris cretica rowerii		
Pteris cretica mayii		
Pteris cretica parkerii	Aberdeen	Meharg (*10*)
Pteris biaurita		
Pteris quadriaurita		
Pteris ryukyuensis	America	Srivastava et al. (*98*)
Pteris multifida		
Pteris oshimensis	China	Wang et al. (*99*)
Pteris aspericaulis		
Pteris cretica var. Nervosa		
Pteris fauriei		
Pteris multifida		
Pteris multifida f. Serrulata		
Pteris oshimensis	China	Wang et al. (*100*)
Pteris umbrosa R. Br	Australia	Koller et al. (*101*)

arsenate/phosphate uptake kinetics was detected in the stressed *P. vittata* (*26*). Arsenite extrusion as in *E. coli* (*30*) is also unlikely a tolerance mechanism for *P. vittata*. Compartmentation of toxic As into vacuoles of the target cells, such as fronds (*27, 31, 32*) and trochome cells (*20*), after its rapid uptake and translocation should be a key mechanism of As tolerance in this fern although the detailed mechanisms underlying As tolerance in *P. vittata* are largely unknown.

4. Arsenic Uptake, Transport, and Detoxification in *P. vittata*

Arsenate [As (V)] and arsenite [As (III)] are the most common forms of arsenic in the environment. Arsenate and arsenite are interconvertible depending on the redox status of the environment, and arsenate is the predominant form of As in aerobic soils (*4*). The mechanisms for arsenate or arsenite uptake are also different. In *Saccharomyces cerevisiae*, arsenate gets into the cell by phosphate transporters due to the chemical similarity between As (V) and Pi (*33*), and is reduced to arsenite by an arsenate reductase (*34*). However, arsenite is taken up via aquaglyceroporins (*35*) or hexose permeases (*36*). Finally, arsenite was sequestered in the vacuole via the MRP1 homologue Ycf1p in its glutathionated form (*37*).

Efficient uptake in root system, rapid transport to shoots, and sequestration into vacuoles of As are unique and important characteristics for As hyperaccumulators, such as *P. vittata* (*38*−*41*) (Figure 2). For example, more than 75% of arsenate taken up by roots was transported to the fronds within 8 h after exposure (*41*). This complicated regulatory mechanism ensures the plants can efficiently extract As from the environment, and protect themselves from As toxicity as well. How *P. vittata* regulates As uptake, translocation, and vacuolar sequestration has drawn extensive research attention.

The first question that needs answering is whether arsenate is taken up by root cells directly, or if it needs to be converted to arsenite first before getting into the plant root cells. Extensive research results show that arsenate is the major form absorbed by root cells, because the dominant As species detected in root systems by using various assay methods (e.g., speciation analysis, HPLC-IPC-MS, and X-ray absorption spectrometry) is arsenate, accounting for 60−70% of the total As in roots (*40, 42*−*45*), whereas arsenite is dominant in the fronds whose content is 70−90% of the total As in plants (*26, 31, 40, 42*−*46*).

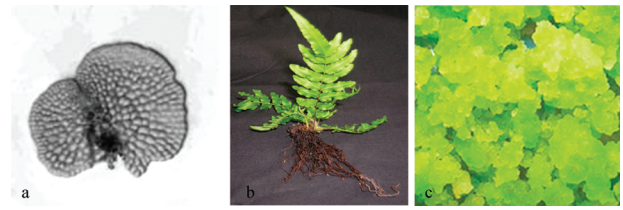

FIGURE 1. (a) Gametophytes (modified from Gumaelius et al. (*8*)), (b) sporophytes, and (c) callus (modified from Yang et al. (*18*)) of *Pteris vittata*.

The next question is how arsenate is taken up by root cells. Recent studies showed that arsenate is taken up by the phosphate transport system in *P. vittata* because arsenate is a phosphate chemical analogue (*26, 40*). In an 18-d hydroponic experiment, Wang et al. (*26*) found that increased phosphate supply decreased As uptake markedly. Increasing arsenate supply decreased the P concentration in the roots, but not in the fronds. Presence of phosphate in the uptake solution decreased arsenate influx markedly, whereas P starvation for 8 d increased the maximum net influx by 2.5-fold. The rate of arsenite uptake was 10% of that for arsenate in the absence of phosphate. Neither P starvation nor the presence of phosphate affected arsenite uptake. Competitive studies (*40*) showed that the arsenate influx could be described by Michaelis−Menten (indicating higher affinity of the transport protein for arsenate). Quantitative analysis of kinetic parameters showed that phosphate inhibited arsenate influx in a directly competitive manner. Most recently, Su et al. (*45*) reported that the arsenate depletion by *P. vittata* in the growth solution is directly related to the amount of phosphate in it. In the absence of phosphate, about 95% of the arsenate added in the solution was depleted over a 24 h period. In sharp contrast, only 35% of original arsenate was depleted in the presence of phosphate, indicating an inhibitory role of phosphate on arsenate uptake by *P. vittata*. The results strongly support the hypothesis that *P. vittata* root cells take up arsenate by the phosphate transport pathway.

There is still a controversy about where arsenate is converted to arsenite. Kertulis et al. (*47*) and Pickering et al. (*44*) reported that *P. vittata* transports mainly arsenate from roots to fronds, and that fronds are the main location of arsenate reduction. However, some studies provided strong evidence against this hypothesis (*45, 48*). For example, the activity of glutathione-dependent arsenate reductase, which is responsible for reduction of arsenate to arsenite, was only detected in roots of *P. vittata* suggesting that arsenate reduction occurs mainly in roots (*48*). Another line of evidence comes from the latest study by Su et al. (*45*) which showed that 93−98% of As in the xylem sap of *P. vittata* is arsenite regardless of whether the plants were treated with arsenate or arsenite. Addition of L-buthionine-sulphpximine (BSO) substantially inhibited glutathione biosynthesis and subsequent arsenate reduction in roots of *P. vittata* (*45, 49*). The results strongly suggest that arsenate is converted to arsenite in the roots after uptake and that arsenite is the predominate As form to be transported to the shoots.

Translocation of arsenite through xylem from root to shoots appears to be a common feature in both As hyperaccumulators and non-hyperaccumulators (*50, 51*). However, *P. vittata* translocates arsenite extremely efficiently (*45*).

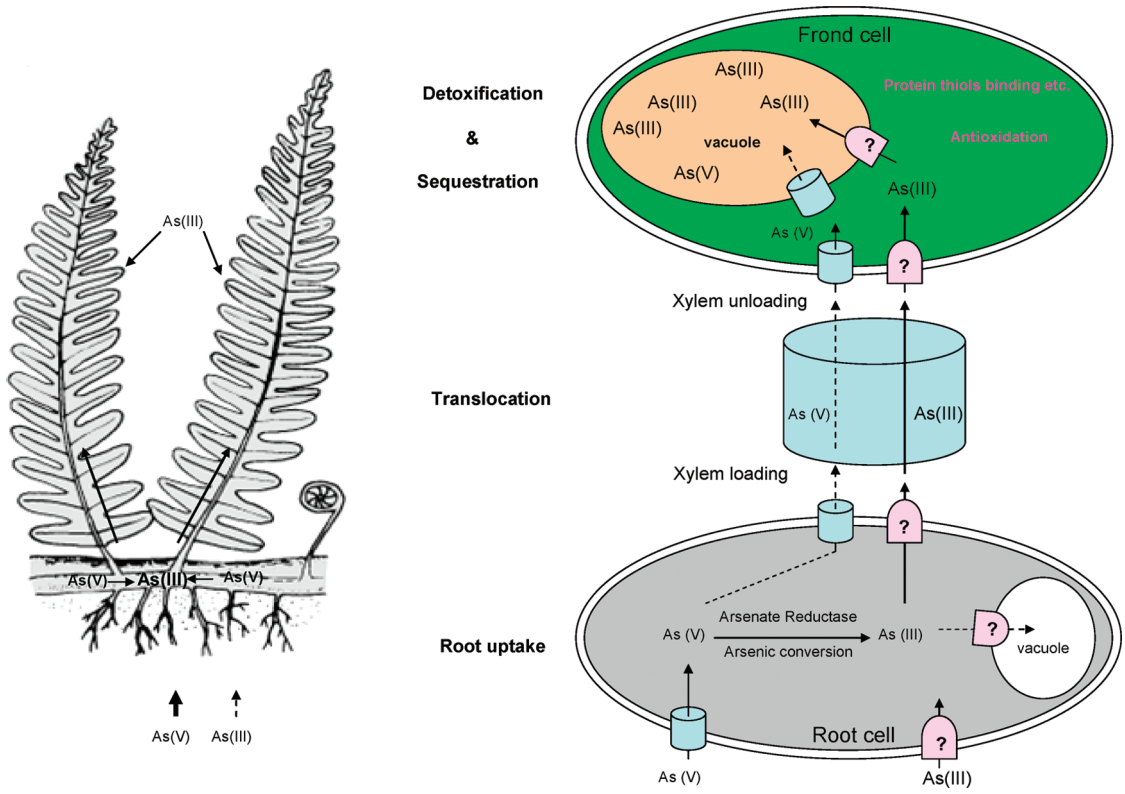

FIGURE 2. Schematic diagram of arsenic uptake, translocation, detoxification, and sequestration in *P. vittata*. Thirty to forty percent of arsenate taken up by roots was reduced to arsenite rapidly; arsenite was the predominant species in the xylem sap when *P. vittata* was subjected to arsenate, accounting for 93−98% of the total As and 80% of As in fronds; arsenite is sequestered into vacuoles of the fronds and trichome cells.

Currently, the mechanisms of long distance transport of arsenite are still unclear. Based on the available results, there are three possibilities to contribute to efficient translocation of As from roots to shoots. The first one is efficient loading of arsenite to the xylem. Arsenic loading is also considered as a key step for As phytoremediation in *P. vittata*. The second factor affecting translocation efficiency of As may be the low degree of complexation of arsenite by thiol-containing compounds (*49, 52*), which plays an important role in As detoxification in non-As-hyperaccumulating plants (*51, 53, 54*). Finally, the lack of strong efflux of arsenite from the *P. vittata* cells to the environment may also contribute to high efficiency of arsenite translocation from the roots to the shoots. Rapid extrusion of arsenite from the root cells of non-As-hyperaccumulating plants, such as tomato and rice, has been observed (*45, 55*). Clearly, *P. vittata* has greater ability for internal arsenite transport than non-As-hyperaccumulating plants.

How arsenite is sequestered into vacuoles of the fronds and trichome cells and how *P. vittata* cells detoxify As remains largely unknown. What is known from the previous results is that metabolic detoxification of As via methylation and biotransformation of As to organo-arsenic compounds is not a major mechanism for protection against As toxicity in the *P. vittata* cells (*43*). As mentioned earlier, phytochelatin dependent pathway also seems not to be a critical mechanism for As detoxification and tolerance in *P. vittata* (*27*). Further studies and new approaches are needed to uncover the mechanisms of arsenite vacuolar sequesteration and detoxification, which will provide novel insights into As tolerance and improvement of phytoremediation of As in our environment.

5. *P. vittata* As Model to Study the Molecular Mechanisms of As Hyperaccumulation

P. vittata has been used predominantly in physiological and biochemical mechanisms of As phytoremediation since it was recognized as the first natural plant As accumulator. The significance of *P. vittata* in study of plant As hyperaccumulation becomes more and more evident by the increasing number of publications that have reported using it in the recent past. With the increasing understanding of the physiology of As uptake, transport, and detoxification, elucidation of the molecular mechanisms underlying these processes is becoming more urgent. During the last two decades, identification of genes and the regulatory networks of plant development and stress tolerance has heavily relied on the model plant *Arabidopsis thaliana* (*56*), and/or crop models such as rice (*57*). However, *Arabidopsis* and the crop models are clearly not suitable for molecular genetics study of heavy metal phytoremediation because they are not natural heavy metal hyperaccumulators. Therefore, development of functional cloning and analysis methods using *P. vittata* is one of the most important steps in understanding As hyperaccumulation in plants.

However, the sporophyte of *P. vittata* is a perennial plant, which has a large genome size of approximately 4834 Mb (J. Banks, unpublished data) (*9*), which is 40, 11, and 1.6 times those of *Arabidopsis*, rice, and humans, respectively. The fern is actually reported as a "species complex" in India and includes five cytotypes, viz. diploid, triploid, tetraploid, pentaploid, and hexaploid with the basic number of 29 chromosomes (*58*). In the past, only several genes were isolated although extensive efforts have been made. For example, a cDNA encoding a phytochelatin synthase (PvPCS1) has been characterized (*59*). Expression of *PvPCS1* in *Saccharomyces cerevisa* increased their Cd tolerance, sug-

gesting that *PvPCS1* may mediate arsenic phytoremediation by increasing As chelation. Ellis et al. (*12*) isolated an arsenate reductase gene (*PvACR2*) from gametophytes, which plays an important role in reduction of AsV to AsIII. *PV4-8* encodes a cytosolic triosephosphate isomerase (cTPI) (*60*). *E. coli* expressing *PV4-8* displayed increased arsenate resistance. Recently, a cDNA encoding a glutaredoxin (Grx) Pv5-6 was isolated from a frond expression cDNA library based on the ability of the cDNA to increase arsenic resistance in *E. coli* (*61*). The results indicated that PvGrx5 has a role in regulating intracellular arsenite levels by either directly or indirectly modulating the aquaglyceroporin. Lack of the related mutants, genome sequence information, and genetic transformation methods in this extraordinary As hyperaccumulator has seriously limited further development of the understanding and genetic improvement of As phytoremediation in plants.

Fortunately, this fern has two independent generations: sporophyte and gametophyte. Recently, Gumaelius et al. (*9*) have studied the morphological, anatomical, and physiological characteristics of the gametophyte of *P. vittta*, with particular attention on As responses and accumulation in the body. They found that the gametophytes of *P. vittata* have great potential to be used as a model system for analysis of As hyperaccumulation. First, the gametophytes of *P. vittata* are propagated by haploid spores from the parent sporophyte plant fronds and have a fast life cycle (about two months). Second, the autotrophic haploid gametophyte can be cultured and generated on the in vitro medium and soil. The mature gametophyte is approximately 2 mm and consists of a small single-layered sheet of cells. A simple and efficient experimental system of a *P. vittata* callus suspension culture was established from the gametophytes (*21*). This not only increases ease of use for the gametophyte in experimental analysis, but also greatly reduces the cost and requirements for growth space. Third, these gametophytes behave similarly to their perennial sporophytes in As tolerance and accumulation (*9, 21*). Finally, mutagenesis is likely to be used to create mutants which will greatly promote identification of genes in regulating As hyperaccumulation. There are still challenges for future research using gametophyte of *P. vittata* as a model to study the genetic basis of As hyperaccumulation. These include (1) establishment of an efficient genetic transformation system; (2) identification of suitable molecular markers for gene cloning; (3) development of tests that can be used for functional study of the regulatory network; and (4) development of characterization systems/techniques for sporophytes for verification of gene functions. It is believed that with the fast developing technologies we will discover the molecular mechanisms of As hyperaccumulation in *P. vittata*, providing novel insights into the mechanisms of phytoremediation and promoting the development of superior plants for the phytoremediation of metals.

6. Approaches to Maximize As Accumulation in *P. vittata*

We still have long way to go before we could create a super As cleaner of *P. vittata*. Based on what we have learned about the physiological and biochemical features of *P. vittata*, some current approaches can be used to enhance the ability of *P. vittata* in As uptake, translocation, and accumulation leading to ultimate As phytoremediation in the contaminated environment (Figure 2).

6.1. Modification of As Uptake and Transport through the Aid of Other Nutrients. As we mentioned earlier, As is likely taken up by P uptake systems because both of them belong to the same chemical group (VA elements) and have similar geochemical behavior (*62*−*64*). Compelling and convincing evidence has demonstrated the effects of P in the growth environment on As uptake of *P. vittata* using various experimental systems (*26, 39, 65*). High concentrations of phosphate inhibit arsenic accumulation in *P. vittata*. Phosphate amendments significantly enhanced plant As uptake from the two tested soils (chromated-copper-arsenate contaminated soil and As-spiked contaminated soil) with frond As concentrations increasing up to 265% relative to the control (*66*). Recently, Santos et al. (*67*) evaluated the effects of timing in phosphate application on plant growth and arsenic removal by *P. vittata* at different developmental ages. They found that the use of young ferns, coupled with feeding of low initial P or split-P application, increased the efficiency of arsenic removal by *P. vittata*. These research results provide us important strategic insights for developing cultivation approaches for efficient phytoremediation of As contamination.

Other nutrient elements that can be adjusted for increasing As accumulation of *P. vittata* include calcium (Ca^{2+}) and nitrogen (N). For example, Liao et al. (*68*) observed that excessive Ca^{2+} in the growth media negatively affects As translocation in *P. vittata*. Li et al. (*69*) investigated the impact of As on chloroplast ultrastructure and Ca^{2+} distribution in *P. vittata* using histochemical methods. They found that the Ca^{2+} level in mature pinnae was markedly increased after addition of As, consistent with As toxicity although the Ca^{2+} concentration in fronds was not significant (*69*). These findings indicate that there is a close relationship between Ca^{2+} and As toxicity in *P. vittata*. Fertilization of N is essential for promoting plant growth and increasing plant yield. The application of N also increased biomass, As accumulation, and ultimate phytoremediation efficiency in *P. vittata* (*70*). Potassium (K) also influences As accumulation by *P. vittata* in the field (*71*). It is apparent that adjustment of compositions of nutrients could be a way to increase the As removal from the contaminated environment. However, detailed studies need to be conducted because this approach might be environment-dependent.

6.2. Increasing the Antioxidant Capacity. In plants, stress from reactive oxygen species (ROS) can lead to cell membrane damage and cell death (*72, 73*). Arsenic is known to induce oxidative stress in plants by generating various ROS (*3*), resulting in a range of responses in plants, including readjustment of transport and metabolic processes, and growth inhibition (*74*). The establishment of an antioxidative system has been considered as an important mechanism for plants to respond to heavy metal stress including As (*75, 76*).

In *P. vittata*, Cao et al. (*77*) reported that both enzymatic and nonenzymatic antioxidants played significant roles in As detoxification and hyperaccumulation. Enzymatic antioxidants are more important when plants are exposed to low As exposure, whereas nonenzymatic antioxidants are more critical at high As exposure. Srivastava et al. (*78*) showed that activity of antioxidative enzymes and thiobarbituric acid-reacting substances in arsenic-treated *P. vittata* were correlated with arsenic hyperaccumulation and symptoms of toxicity. Selenium (Se) alleviated oxidative stress and improved arsenic uptake in *P. vittata* by functioning as an antioxidant or activating plant protective mechanisms (*22*). Superoxide dismutase (SOD) may play important roles in accumulation and detoxification of As in both As-accumulating (*Pteris vittata* and *P. multifida*) and nonaccumulating (*P. ensiformis* and *P. semipinnata*) species (*79*). Therefore, genetic or other modifications of redox status of plants could be another option to increase the As accumulation in *P. vittata*.

6.3. Management of Plant−Microbe Symbiosis Aids an Effective Cleanup of As in Soil. Extensive literature exists on the role of rhizospheric microorganisms on degradation of organic pollutants and biocontrol of soil-borne pathogens and biofertilization (*80*−*82*). Recently, it has been found that microbe−*P. vittata* symbiosis within the plant rhizosphere

can significantly increase the efficiency of As phytoremediation (*83*−*87*).

When *P. vittata* roots were inoculated by *Glomus mosseae* and *Gigaspora margarita* (arbuscular mycorrhizal (AM) fungus), As removal ability and plant biomass were significantly higher in mycorrhizal than in non-mycorrhizal *P. vittata* (*83, 85*). At high soil As level (300 mg/kg), 43% and 125% increases in frond As content and frond dry weight were detected in the mycorrhizal *P. vittata* (*83*). Further study showed that the effects of various fungi on plant growth and As and P distribution in ferns are different, therefore appropriate *P. vittata*−AM fungi combination would be an alternative approach in bioremediation of contaminated environments (*86*). Currently, the species that are the most common in the rhizosphere of *P. vittata* include *Glomus microaggregatum*, *Glomus mosseae*, *Glomus brohultii*, and *Glomus geosporum* (*88*). Moreover, low to moderate levels of AM colonization in *P. vittata* (4.2−12.8%) were observed at uncontaminated and metal-contaminated sites.

Conclusion and Perspectives

Environmental cleanup of polluted land is an increasingly important issue that we need to face. Compelling evidence has demonstrated that phytoremediation is a promising technology for the clean up of toxic metals such as As in the environment. In recent years, great progress has been made in identification of pollutant hyperaccumulators and characterization of the mechanisms of phytoremediation, but many challenges still lie ahead for future phytoremediation researchers and in the global application of the technology. We must understand at the molecular level how phytoremediation process is regulated in *planta*. Advanced technologies and high-throughput sequencing of the entire genome of the gametophytes of Chinese brake fern *P. vittata* will facilitate integrative research in elucidating molecular mechanisms of phytoremediation, including As uptake, As transformation, translocation, and detoxification. To increase the efficiency of phytoremediation, we must explore combinatorial strategies, such as *P. vittata*−microbe symbiosis and adjustment of other nutrients as described above. Further study on the mechanisms underlying promotion of phytoremediation efficiency by plant−microbe symbiosis and nutrients will provide insights into novel strategies for cleaning up As contamination in soil and groundwater.

In reality, soil or groundwater contaminated with a single heavy metal is rare, and therefore multipollutant removal is the goal of phytoremediation. Recently, it has been noted that *P. vittata* L. may have potential for phytoremediation of multiple toxic metals (*13, 16*). Future analysis on multimetal hyperaccumulation in *P. vittata* should be conducted, and combinatorial methods for removing toxic heavy metals need to be developed. Also, safe processing of harvested hyperaccumulators containing high contents of heavy metals to avoid secondary environmental pollution, and development of the appropriate technology to detoxify the toxic form of As(III) are problems to which we must pay attention (*89*). Furthermore, we must address the problem of whether use of *P. vittata* will disrupt local ecosystems, which has been a concern about the application of phytoremediation technology. Finally, we need to develop integrative approaches for enhancing the social acceptability of phytoremediation, for boosting the transformation of green technology in environmental cleanup, and for promoting global application of this technology in cleaning up our environment.

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