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Mechanisms of Efficient Arsenite Uptake by Arsenic Hyperaccumulator *Pteris vittata*

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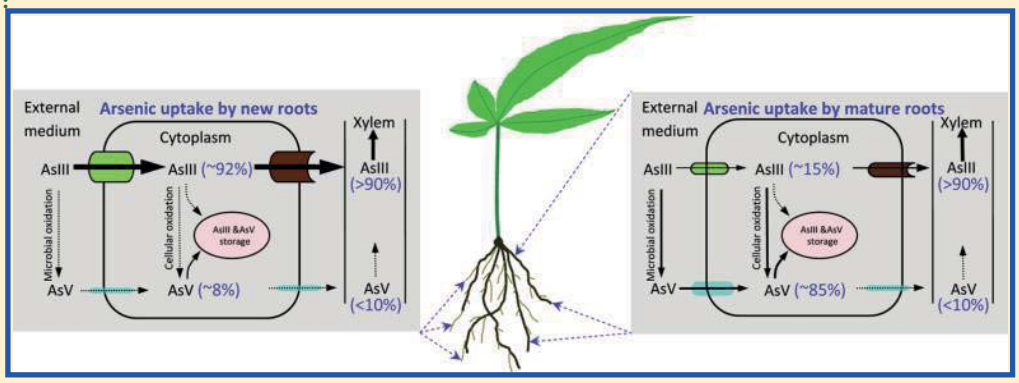
ABSTRACT:



Arsenate (AsV) and arsenite (AsIII) are two dominant arsenic species in the environment. While arsenate uptake is via phosphate transporter in plants, including arsenic hyperaccumulator *Pteris vittata*, AsIII uptake mechanisms by *P. vittata* are unclear. In this study, we investigated AsIII uptake by *P. vittata* involving root radial transport from external medium to cortical cells and xylem loading. In the root symplastic solution, AsIII was the predominant species (90−94%) and its concentrations were 1.6−21 times those in the medium. AsIII influx into root symplast followed Michaelis−Menten kinetics with K_m of 77.7 μM at external AsIII concentrations of 2.6−650 μM. In the presence of metabolic inhibitor 2,4-dinitrophenol (DNP), arsenic concentrations in the root symplast were reduced to the levels lower than in the medium, indicating that a transporter-mediated active process was mainly responsible for AsIII influx into *P. vittata* roots. Unlike radial transport, AsIII loading into xylem involved both high- and low-affinity systems with K_m of 8.8 μM and 70.4 μM, respectively. As indicated by the effect of 2,4-DNP, passive diffusion became more important in arsenic loading into xylem at higher external AsIII. The unique AsIII uptake system in *P. vittata* makes it a valuable model to understand the mechanisms of arsenic hyperaccumulation in the plant kingdom.

INTRODUCTION

Arsenic is considered a class I human carcinogen.[1] Worldwide arsenic contamination in the environment from both natural and anthropogenic sources is of increasing global concern.[2,3] Being highly toxic to most forms of life, arsenic contamination in soil−water−plant systems has posed serious risks to food safety and public health.

Arsenic occurs predominantly in inorganic form as arsenate (AsV) and arsenite (AsIII) in most ecosystems. Though AsIII is predominant in anaerobic conditions, it is also present in aerobic environments where AsV typically dominates. For example, as a result of biochemical transformation by plant roots[4] and microbially mediated AsV reduction,[5] AsIII accounts for an important part of arsenic pools in plant rhizosphere and aerobic soils. For example, due to microbial reduction of AsV, AsIII was the predominant species eluted from oxic contaminated mine tailings.[6] Both AsV and AsIII are present in surface water and groundwater.[7] In South Asia, where the most extensive arsenic contamination in groundwater is observed, AsIII accounts for the dominant species (67−99%) in most groundwater samples.[8,9] Even in oxygen-rich conditions, AsIII in water can exist as a metastable species with slow oxidation kinetics from AsIII to

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AsV.[7,10] However, the rate can be much faster due to the presence of AsIII-oxidizing microbes and catalytic metal oxides.

Compared to AsV, AsIII is far more toxic to most organisms due to its high affinity to bind with sulfhydryl groups, thus disrupting protein structure and functions involved in cellular metabolism.[11] In addition, under circumneutral pH, AsIII exists as a neutral molecular species (pK_a = 9.2) with relatively low chemical reactivity, leading to much more difficult removal of AsIII from water by traditional treatment technologies.[12]

The arsenic hyperaccumulator *Pteris vittata* is efficient in taking up both AsV and AsIII.[13] On the basis of depletion techniques and radiotracer ^{73}AsV uptake studies, it is established that AsV is taken up by *P. vittata* by phosphate transporters following Michaelis−Menten kinetics, with P inhibiting AsV uptake in a directly competitive manner.[14−16] Since AsIII is predominantly present as a neutral species in the environment, it is hypothesized that AsIII is taken up by plants via aquaglyceroporins,[17] which have been shown to transport small uncharged solutes such as glycerol, silicic acid, and AsIII.[18,19] Competitive inhibition study of AsIII uptake by glycerol (0.1 mM AsIII and 0.1−100 mM glycerol) in excised rice roots suggests that AsIII transport into rice is via aquaglyceroporins.[18] A recent study[10] where glycerol addition had no effect on AsIII uptake by *P. vittata* indicate that the AsIII uptake system in *P. vittata* is different from rice.

Since AsIII is an analogue of silicic acid, it is understandable that silicic acid inhibits AsIII uptake by rice roots, consistent with the hypothesis that both silicic acid and AsIII are taken up by rice via aquaglyceroporins.[20] However, addition of 0.5 mM silicic acid had no effect on AsIII uptake by *P. vittata* roots at 15 μM AsIII during 1 day uptake study,[21] again indicating that the AsIII uptake system by *P. vittata* is different from that by rice. The fact that neither glycerol nor silicic acid inhibited AsIII uptake by *P. vittata* suggests that aquaglyceroporins may not be a major channel for AsIII transport into *P. vittata* roots.

As a useful model to examine transporter-mediated uptake processes, a Michaelis−Menten saturation curve can indicate either active uptake (against a concentration gradient by use of energy) or passive uptake (facilitated diffusion down a concentration gradient). Aquaglyceroporin-mediated transport of small neutral molecules such as AsIII, silicic acid, and glycerol across cellular membranes is via facilitated diffusion. When the solute is transported against a concentration gradient from external medium to living cells, the transport is active and energy-dependent. To further distinguish active transport from facilitated diffusion, a metabolic inhibitor, such as 2,4-dinitrophenol (uncoupler of oxidative phosphorylation), can be employed to inhibit active uptake by disabling cellular energy production.[19]

Previous study has shown the ability of *P. vittata* to accumulate AsV from contaminated drinking water.[22,23] However, little is known about AsIII uptake kinetics by *P. vittata* and the underlying mechanisms. In this study, *P. vittata* were exposed to AsIII at low and high concentrations for 6 h in aerated hydroponic solutions. Our objectives were to (1) investigate the kinetics of AsIII uptake systems in *P. vittata* by use of a Michaelis−Menten kinetic model, (2) determine arsenic speciation in root symplastic solution and xylem sap after AsIII exposure, and (3) determine the impact of metabolic inhibition on AsIII uptake in *P. vittata*. Results from the present study should provide important information on the mechanisms of AsIII uptake by *P. vittata*, which may serve as a model plant to better understand arsenic hyperaccumulation mechanisms in plants.

MATERIALS AND METHODS

Plant Growth. Healthy *P. vittata* plants with 4−5 fronds (4 months old) from Milestone Agriculture Inc. (Apopka, FL) were transferred to 0.2× strength Hoagland solution for acclimation. Plants were grown in a controlled room with 8-h photoperiod at light intensity of 350 μmol·m^{-2}·s^{-1}, 28/23 °C day/night temperature, and 70% relative humidity. The nutrient solutions were aerated continuously and changed weekly. After 2 weeks of acclimation, ferns of similar size with sufficient new root tips were transferred to 0.1 mM $CaCl_2$ for 12 h before the experiment.

AsIII Uptake in Low and High Arsenite Concentration Ranges. Short-term AsIII uptake by *P. vittata* was tested with four replicates over both low (0−32.5 μM) and high (65−650 μM) AsIII concentration ranges. The ferns were transferred to 3.5 L of 0.1 mM $CaCl_2$, which was spiked with 0, 2.6, 5.2, 7.8, 13, 32.5, 65, 130, 260, or 650 μM AsIII (as $NaAsO_2$) (Sigma, St. Louis, MO). At the end of 6 h treatment under aeration, the growth medium was speciated by use of an As-speciation cartridge (Waters Corp., Milford, MA). Fern roots were thoroughly rinsed with deionized water, followed by 10 min of desorption in 1 mM K_2HPO_4.

Metabolic Inhibitor Experiment. To determine the effects of metabolic inhibitor on AsIII uptake by *P. vittata*, 2 mM 2,4-dinitrophenol (DNP) was employed. 2,4-DNP was dissolved in ethanol to make a stock solution before its addition to the uptake medium, with a final ethanol concentration of 0.3% (v/v). No effect at this concentration of ethanol on AsIII uptake was observed in our preliminary experiment. *P. vittata* plants were exposed to low and high AsIII concentrations (5.2, 65 and 650 μM AsIII) with and without 2 mM 2,4-DNP. After 6 h of treatment, root-cell symplastic solutions and xylem saps were extracted. Each treatment was repeated four times.

Symplastic Solution and Xylem Sap Collection. Symplastic solution was extracted from the new root tips by centrifugation.[19] Following 6 h of exposure to AsIII, plant roots were rinsed briefly with tap water and immersed in 1 mM K_2HPO_4 for ∼15 min to remove the adsorbed arsenic species. After that, ∼1.5 cm root tips were excised from *P. vittata*. After a quick wash with distilled water, root tips were blotted dry and placed into 0.22 μM filter unit (Ultrafreer-MC, Millipore Corp., Billerica, MA) with the cut ends facing down. By centrifugation at 2000*g* for 15 min at 4 °C, apoplastic solutions were obtained and root segments were frozen at −80 °C for 2 h, followed by thawing at room temperature for 10 min. After centrifugation at 2000*g* for 15 min at 4 °C, symplastic solutions were collected from the frozen−thawed root tips and stored at −80 °C until analysis.

To check the validity of the extraction method for both apoplastic and symplastic solution as well as the purity of each fraction, the activity of malic dehydrogenase (MDH) in the apoplastic and symplastic saps was determined in our preliminary experiment. The activity of MDH in the symplastic sap from root tips of *P. vittata* was ∼0.4 unit·mL^{-1} (1 unit of enzyme catalyzes the conversion of 1 μmol of substrate to product per minute). In contrast, the activity of MDH in the apoplastic solution was <2.5% that of the symplastic solution, indicating high purity of the symplastic solution from the method.

For xylem sap, the roots were cut at ∼1 cm above the rhizomes and placed into a Scholander pressure chamber for 30 min to collect xylem sap flowing from the rhizomes to the fronds. Samples were preserved in a −80 °C freezer immediately.

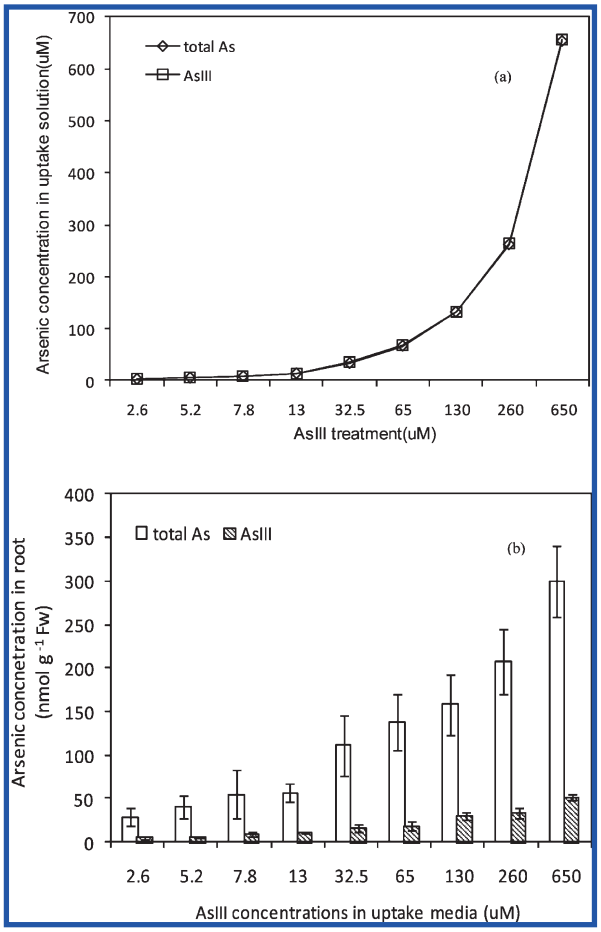

Figure 1. Concentrations of total As and AsIII in (a) uptake solution and (b) root biomass after exposure of *P. vittata* to 2.6−650 μM AsIII for 6 h. Total As = AsIII + AsV. Little organic arsenic species were detected in root biomass. Data are means and standard errors of four replicates.

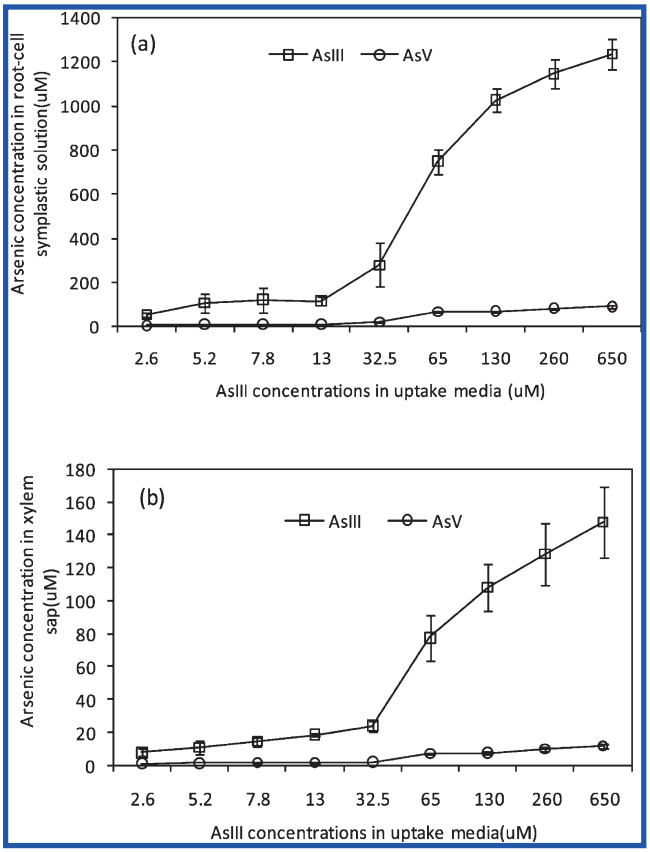

Figure 2. Concentrations of AsIII and AsV in (a) root symplastic solution and (b) xylem sap of *P. vittata* after exposure to 2.6−650 μM AsIII for 6 h. Total As = AsIII + AsV. Little organic arsenic species were detected in symplastic solution or xylem sap. Data are means and standard errors of four replicates.

Arsenic Analysis and Speciation. Arsenic concentration and speciation in the root symplastic solutions and xylem saps were determined by coupling high-performance liquid chromatography (HPLC) to inductively coupled plasma mass spectrometry (ICP-MS). Speciation of arsenic species (arsenite, arsenate, methylarsonic acid, and dimethylarsinic acid) was conducted on a Perkin-Elmer series 200 HPLC system hyphenated with a Perkin-Elmer Elan DRC-e ICP-MS for As detection. A Hamilton PRPX-200 cation-exchange column (250 × 4.1 mm in dimension and 10 μm particle size) was employed for the separation of arsenic species. The mobile phase was 0.1% formic acid with a flow rate of 1 mL/min. A nebulizer feed valve was used to direct the flow from HPLC into the ICP-MS, and an internal standard valve was used for postcolumn injection of internal standards. The m/z signal 75 was monitored for As. Data were collected and treated by use of Chromera software (Perkin-Elmer). The instrumental parameters were optimized against a tuning solution recommended by the manufacturer, with the nebulizer gas flow being optimized daily for maximum sensitivity. Quality assurance was obtained through the use of blanks, standard curves, standard check solutions and spiked samples, which were run during sample analysis. More detailed information for this cation-exchange HPLC-ICP-MS arsenic speciation method can be found elsewhere.[24]

For arsenic speciation in the root biomass, the bulk of root samples of *P. vittata* (stored at −80 °C overnight) were cut into pieces, ground with liquid nitrogen in mortars, and extracted ultrasonically with a 1:1 methanol/water solution for 2 h.[21] Prior experiment showed that arsenic species in organic forms in *P. vittata* biomass were negligible;[25] therefore, only inorganic arsenic including AsIII and AsV was determined. AsV and AsIII in the plant extracts were separated by use of As-speciation cartridges (Waters Corp., Milford, MA). Total arsenic and AsIII concentrations in the extracts and uptake medium were determined by a graphite furnace atomic absorption spectrophotometer (GFAAS; AA240Z, Walnut Creek, CA). Preliminary experiment with control species of *P. vittata* showed that little arsenic was detected in root symplastic solution or xylem sap, and negligible arsenic was found in root biomass.

Data Analysis. All values were expressed as means ± SE (n = 4). Two-parameter Michaelis−Menten kinetic model was fitted to AsIII uptake data, and the kinetic parameters were calculated in SigmaPlot 11.0.

RESULTS

Arsenic Speciation in the Growth Media, Root Symplastic Solutions, and Xylem Sap. During the 6 h of uptake, AsIII accounted for 100% of total As in the growth medium (Figure 1a). Since a large volume of growth medium was used (3.5 L per plant),

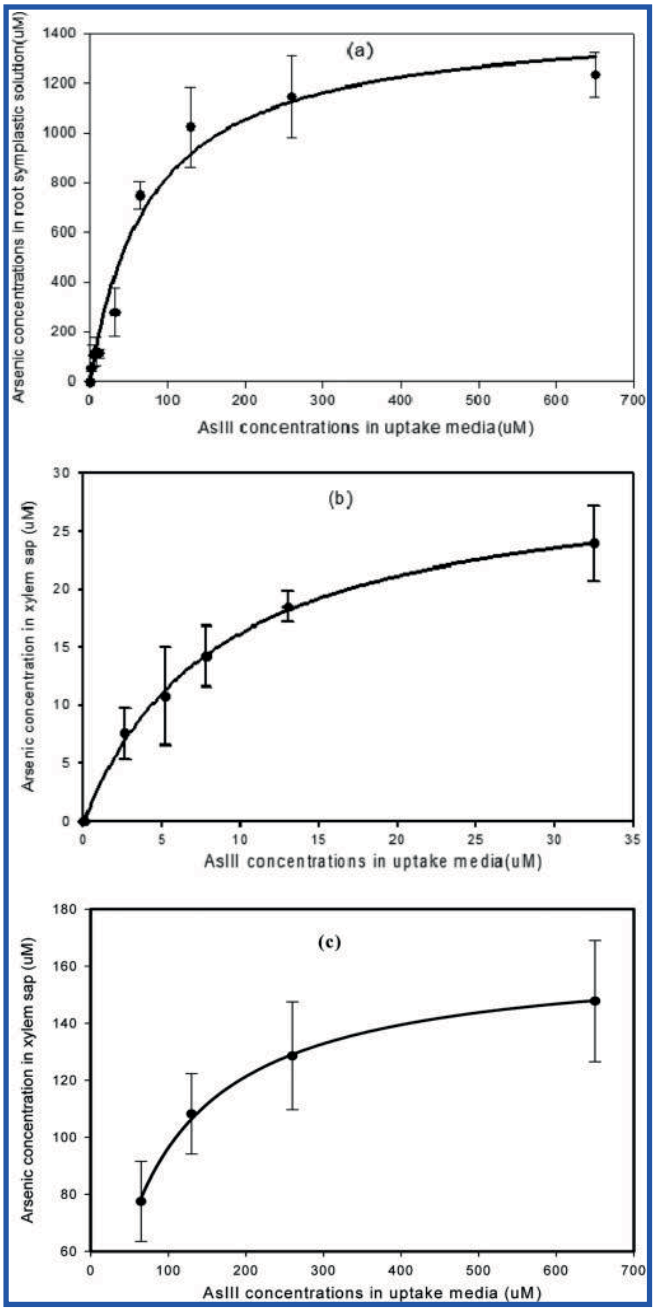

Figure 3. Arsenic concentrations in (a) root symplastic solution (0−650 μM AsIII) and (b, c) xylem sap (0−32.5 and 65−650 μM AsIII) of *P. vittata* after exposure to AsIII for 6 h. The data were fitted to the Michaelis−Menten kinetic model. Data are means ± standard errors of four replicates.

AsIII concentrations in the medium changed little after 6 h of uptake (Figure 1a), which is an important prerequisite for AsIII uptake kinetic study. In the root biomass (including new root tips and mature roots), AsV was the predominant species, accounting for 82−87% of the extracted arsenic in all treatments (Figure 1b). By contrast, the symplastic solutions extracted from the new root tips and xylem saps were dominated by AsIII (90−94%) in all treatments (Figure 2). No methylated arsenic species were detected in root biomass, root symplastic solutions, or xylem saps (data not shown).

AsIII Radial Transport from Media to the Root Symplast and DNP Inhibition. Arsenic concentrations in the root symplastic solutions increased with increasing AsIII concentrations in the growth medium and were significantly greater (8−21-fold) than those in the growth medium (Figure 3a). During the 6 h uptake, AsIII influx into the root symplast saturated gradually over external AsIII concentrations from 2.6 to 650 μM, and the uptake data can be well described by a Michaelis−Menten model with an apparent K_m of 77.7 μM and V_{max} of 1.46 μmol of $As \cdot mL^{-1} \cdot (6\ h)^{-1}$ (Table 1). Significantly higher arsenic concentrations in the root symplastic solutions than those in the uptake medium over the whole concentration range (Figure 3a) indicated that AsIII was transported from the external medium to the cortical cells against the external AsIII concentration gradient, which is an important characteristic of active uptake.

To verify this, metabolic inhibitor 2,4-DNP was employed to investigate its effect on AsIII transport into the roots of *P. vittata*. With 2 mM 2,4-DNP spiked into the growth medium containing 5.2, 65, or 650 μM AsIII, arsenic concentrations (with AsIII accounting for 90−94% in the root symplastic solutions) were decreased to levels lower than those in the growth medium, being only ~4% of those in the controls without 2,4-DNP (Figure 4a). These results indicated that transporter-mediated active uptake played a dominant role in AsIII radial transport, with passive influx being a minor component.

Arsenic Loading to Xylem Sap via Dual Affinity Systems. With external AsIII concentrations at 2.6−32.5 μM and 65−650 μM, dual affinity systems exhibiting saturation kinetics were present for xylem loading of arsenic in *P. vittata* (Figure 3b,c). On the basis of the Michaelis−Menten kinetic model, K_m values of the high- and low-affinity systems were 8.78 μM and 70.4 μM, respectively (Table 1). In the presence of 2,4-DNP, arsenic loading to xylem sap was effectively inhibited with AsIII concentration being 13% of the controls at 5.2 μM AsIII (Figure 4b). However, the inhibitory effect of 2,4-DNP on arsenic loading to xylem sap was gradually decreased at high AsIII concentration range. As shown in Figure 4b, arsenic concentrations in xylem sap accounted for 27% and 46% of the corresponding controls at 65 μM and 650 μM, respectively, suggesting a more important role of passive diffusion in low-affinity loading system. Over the whole concentration range, arsenic concentrations in the xylem sap (dominated by AsIII) accounted for 10−12% of those in the root symplastic solutions (Figure 3).

DISCUSSION

Our data indicate that, at external AsIII of 2.5−650 μM, radial transport of AsIII from growth medium into *P. vittata* roots was mainly via an active process, with passive diffusion being a minor component. This is in contrast to AsIII influx into rice roots, which is via aquaglyceroporin channels.[20] For xylem loading of arsenic, both high- and low-affinity systems were involved in *P. vittata*. Specifically, the role of passive diffusion in xylem loading becomes more important at higher external AsIII concentrations (65−650 μM) in the growth medium.

AsIII Dominated in the Growth Medium, Root Symplastic Solution, and Xylem Sap. During the 6 h uptake experiment, little AsIII oxidation was observed in the growth medium, as only AsIII was detectable in the medium regardless of AsIII concentrations (Figure 1a), which is a critical prerequisite for the AsIII uptake kinetic study.

Table 1. Michaelis–Menten Kinetic Parameters with External AsIII Supply to *P. vittata*[a]

external AsIII concentration range (μM)	K_m (μM)	V_{max} [nmol of As·mL^{-1}· (6 h)$^{-1}$]	R^2	p
	Radial Transport from External Media into Root Symplastic Solution			
0–650	77.7	1463	0.9746	<0.0001
	Loading to Xylem Sap			
0–32.5	8.78	30.5	0.9978	<0.0001
65–650	70.4	164	0.9981	0.0009

[a] Kinetic parameters were calculated by fitting the influx data in Figure 3 to a two-parameter hyperbola via nonlinear regression. Curve-fitting was achieved by use of the fitting regimes in SigmaPlot 11.0.

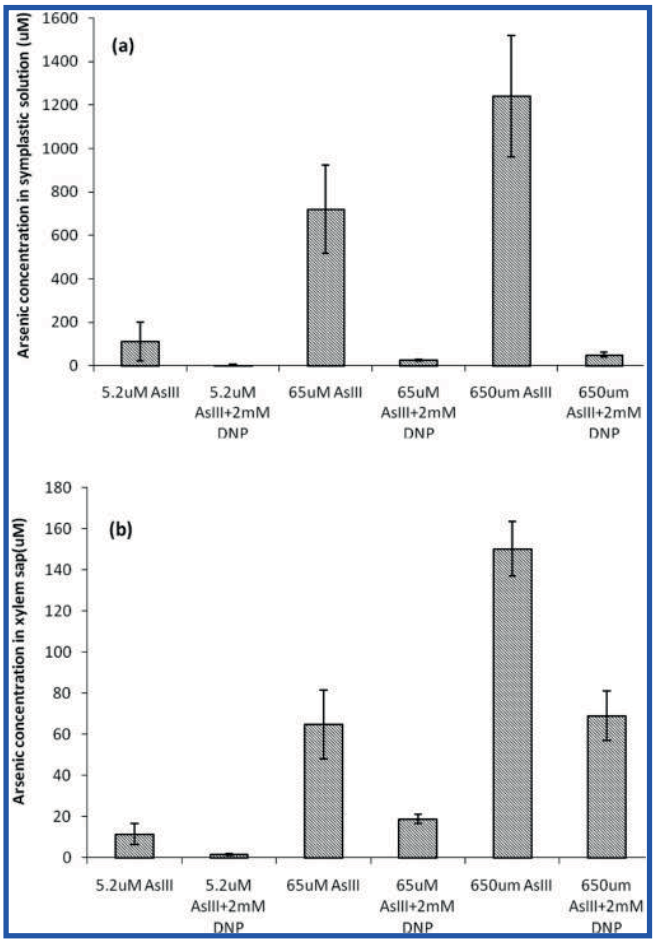

Figure 4. Effects of 2,4-DNP on arsenic concentrations in (a) root symplast and (b) xylem sap in *P. vittata* after exposure to 5.2, 65, and 650 μM AsIII for 6 h. Data are means and standard errors of four replicates.

In addition to the growth medium, AsIII was also the predominant species in the root symplastic solution, accounting for 90–94% of total arsenic (Figure 2a), which was documented for the first time. The root symplastic solutions in the present study were extracted from the new root tips, which represent the actively transporting regions of the roots. Though no AsV was detected in the growth medium, a small portion of AsV (6–10%) was present in the root symplastic solution. This limited oxidation of AsIII to AsV may have occurred inside the roots. This is supported by data from Mathews et al.,[26] who observed 35% AsV in *P. vittata* roots after exposure to 0.10 mM AsIII for 1 day under sterile conditions where no AsIII oxidation occurred in the growth medium. However, oxidation of AsIII to AsV may have also occurred on the surface of roots, where microbes were present.[26]

Similar to root symplastic solution, AsIII also dominated in the xylem sap, accounting for 90–94% of the total As (Figure 2b), which is similar to that reported by Su et al.[27] They observed that AsIII accounted for 93–98% of the total As in the xylem sap of *P. vittata* after exposure to 5 μM AsIII for 1–24 h. However, AsIII concentrations in the xylem sap in the present study were much less than those detected by Su et al.[27] For example, the AsIII concentration in the xylem sap was 10.7 μM after exposure to 5.2 μM AsIII for 6 h in our study, compared to ~220 μM after exposure to 5 μM AsIII for 8 h by Su et al.[27] This difference can be attributed to different AsIII concentrations in the root biomass, which seems to be the limiting factor for As translocation from the roots to the fronds, as AsIII is preferentially loaded into xylem sap rather than AsV.[27] In their study,[27] ~75% of the total extractable As was present as AsIII in the roots, compared to 13–18% AsIII in this study (Figure 1b), which led to a significantly reduced As concentrations (mainly as AsIII) in the xylem sap (Figure 3).

The predominance of AsV in the root biomass (Figure 1b) may result from three processes: (1) microbial oxidation of AsIII to AsV on the root surface, especially mature root surface, before being taken up,[21] (2) AsIII oxidation to AsV inside the roots,[26] and (3) preferential translocation of AsIII from the roots to the fronds, leaving AsV in the roots.[27] AsIII oxidation in the growth medium in the presence of *P. vittata* has been previously observed[21] and is most likely microbially mediated.[26] Since the AsIII oxidation most likely occurred on the root surface, the oxidized AsV was probably immediately taken up by *P. vittata* roots due to its high affinity for AsV (K_m value of 0.52–1.1 μM),[14,16] making AsV undetectable in the uptake medium.

AsIII Influx into *P. vittata* Roots Was a Transporter-Mediated Active Process. Kinetic data in this study (Table 1) showed that AsIII radial transport from external medium into the root symplastic solutions followed saturation kinetics and the uptake data can be well-fitted to a Michaelis–Menten function. The significantly greater arsenic concentrations in the root symplastic solutions than in the external growth medium strongly suggested that AsIII transport into *P. vittata* roots was against concentration gradient, that is, active transport, which was further confirmed by the effective inhibition of AsIII influx into the roots by 2,4-DNP over the whole concentration range (Figure 4a). Suppressed AsIII uptake by metabolic inhibitor suggests that AsIII transport into the roots of *P. vittata* was dominated by the transporter-mediated active uptake, with the passive diffusion being a minor component, which is different from the AsIII uptake system in rice[18,20] as suggested by a recent study.[21]

Table 2. Comparison of AsIII and AsV Uptake by the Roots of Rice and *P. vittata*

plant species	As species	external arsenic concn (μM)	experiment method	uptake time and medium	K_m (μM)	ref
excised rice roots	AsIII	50, 100, 200, 300, 400, and 500	concentration-dependent uptake	0.33 h, distilled water, pH 7.0	180	18
intact *P. vittata* with 4−5 fronds	AsV	5.0	depletion technique	8 h, 0.5 mM $CaCl_2$+ 5 mM MES, pH 6.0	0.52	14
intact *P. vittata* with 5−6 fronds	^{73}AsV	0.1, 0.5, 1.5, 2.5, 5.0, and 7.5	^{73}AsV radioactive tracer	0.5 h, 0.1 mM $CaCl_2$, pH 6.5	1.1	16
intact *P. vittata* with 5−6 fronds	AsIII	2.6, 5.2, 7.8, 13, 32.5, 65, 130, 260, and 650	concentration-dependent uptake	6 h, 0.1 mM $CaCl_2$, pH 6.0	77.7	this work

Compared to the K_m of AsIII transporter in rice roots (180 μM)[18] a 2.3 times lower K_m value (77.7 μM) was observed for AsIII transporters in *P. vittata* roots (Table 1), indicating much higher affinity of the AsIII transporters in *P. vittata* roots than those in rice. This is consistent with the extraordinary uptake ability of *P. vittata* for arsenic even in uncontaminated environments with low arsenic bioavailability.[13] Compared to AsV uptake kinetics by *P. vittata* (K_m = 0.52−1.1 μM; Table 2), the much higher K_m value for AsIII uptake indicates higher affinity of AsV transporter than AsIII transporter for its substrate during root uptake. This is consistent with the observations of Wang et al.,[14] where AsIII uptake rate was 10% of that for AsV uptake in the absence of phosphate, although no kinetic data were provided for AsIII uptake in that study.

For most nonhyperaccumulators, arsenic tolerance is generally achieved by reduced arsenic uptake with the absence of a high-affinity uptake system or the development of a low-affinity system at higher arsenic concentrations, which are considered as important mechanisms for plants to survive arsenic toxicity in the environment.[28,29] However, regarding *P. vittata*, a transporter-mediated active uptake with relatively low K_m value was responsible for AsIII influx into root symplast over a wide concentration range of external AsIII (Figure 3a), which makes an important contributing factor to the efficient AsIII uptake by *P. vittata*.

Considering AsIII concentrations in most contaminated water[8,9] are within the concentration range in the present study (2.6−650 μM), efficient root uptake of AsIII by *P. vittata* may provide an ecofriendly and cost-effective cleanup technology for water treatment, especially for developing countries such as Bangladesh where extensive arsenic contamination occurs in drinking water.

Dual-Affinity Systems Were Involved in Xylem Loading of Arsenic in *P. vittata*. As indicated by the kinetic data (Table 1), dual-affinity systems were involved in the xylem loading process in *P. vittata* (Figure 3b,c), with a high-affinity system being dominant at low AsIII concentrations (K_m = 8.78 μM with 0−32.5 μM AsIII) and a low-affinity system operating at high substrate concentrations (K_m = 70.4 μM with 65−650 μM AsIII). Furthermore, as indicated by the decreased effect of 2,4-DNP on arsenic concentrations in xylem sap (Figure 4b), the passive component played a more important role (27−46%) in xylem loading at higher external AsIII concentrations (65−650 μM). However, we are uncertain whether the passive loading of AsIII into xylem sap was mediated by aquaglyceroporins, which warrants further investigation at the molecular level.

The unique AsIII transport system in *P. vittata* is not only important from a scientific standpoint to advance our understanding of AsIII hyperaccumulation mechanisms in *P. vittata* but also has potential implications to better understand AsIII uptake in other plants. By modifying the genes responsible for AsIII uptake system, genetic engineering may enhance arsenic uptake for phytoremediation application and hence mitigate arsenic contamination in waters and soils.

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