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Phytate promoted arsenic uptake and growth in arsenic-hyperaccumulator *Pteris vittata* by upregulating phosphorus transporters☆

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ABSTRACT

While phosphate (P) inhibits arsenic (As) uptake by plants, phytate increases As uptake by As-hyperaccumulator *Pteris vittata*. Here we tried to understand the underling mechanisms by investigating the roles of phytate in soil As desorption, P transport in *P. vittata*, short-term As uptake, and plant growth and As accumulation from soils. Sterile soil was used to exclude microbial degradation on phytate. Results showed that inorganic P released 3.3-fold more As than that of phytate from soil. However, *P. vittata* accumulated 2–2.5 fold more As from soils with phytate than that in control and P treatment. In addition, different from P suppression on As uptake, solution uptake experiment showed that As uptake in phytate treatment was comparable to that of control under 0.1–7.5 μM As after 1–24 h. Moreover, responding to phytate, *P. vittata* P transporter *PvPht1;3* increased by 3-fold while *PvPht1;1* decreased by 65%. The data suggested that phytate upregulated *PvPht1;3*, thereby contributing to As uptake in *P. vittata*. Our results showed that, though with lower As release from soil compared to P, phytate induced more As uptake and better growth in *P. vittata* by upregulating P transporters.

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1. Introduction

Arsenic (As) is of environmental concern due to its toxicity in the environment. *Pteris vittata* (Chinese Brake fern) is an efficient As-hyperaccumulator, which has potential for phytoremediation of As-contaminated soil (Ma et al., 2001; da Silva et al., 2018). In contaminated soils, it can accumulate as much as 23 g kg^{-1} As in the fronds.

In aerobic soils, arsenate (AsV) is the prevalent species, the primary form for plant uptake (Isayenkov and Maathuis, 2008). As a phosphate (P) analog, AsV has similar properties as P and is taken up by plants via P transporters (DiTusa et al., 2016). Unlike toxic As, P is a major nutrient for plant growth. Plants generally meet their P requirement by taking up inorganic P from soils (Marschner and Rimmington, 1995). However, majority of soil P (~50–80%) exists as organic P, which are unavailable to plants (Turner et al., 2002).

Among organic P, phytate (inositol hexakisphosphate), a stable compound resistant to degradation, is dominant in soils, constituting 25–50% of organic P (Lung and Lim, 2006; Turner et al., 2006). Cultivation of *P. vittata* in growth media showed that phytate increased its P acquisition and As accumulation (Liu et al., 2017a,b), however, its impacts in soils are unknown. The interactions of phytate with soils via sorption and precipitation govern its bioavailability (Yan et al., 2014). Therefore, it is important to evaluate its effect on plant As uptake in soils. In addition, the ability of *P. vittata* in phytate-P acquisition from soils may be restricted by microbes as they compete with plant uptake (George et al., 2005). Moreover, it was reported that P starvation enhances As uptake (Chen et al., 2016, 2017), but limited information is available on phytate.

Studies showed that As is taken up by P transporter 1 (*Pht1*) (Wu

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et al., 2011; Narayanan et al., 2011; Cao et al., 2017). Specifically, *P. vittata Pht1* family members *PvPht1;1* and *PvPht1;3* can transport both P and As (Cao et al., 2018; DiTusa et al., 2016). In *P. vittata* gametophytes, while *PvPht1;1* increased responding to P starvation, *PvPht1;3* responded to both P deficiency and As exposure. Whereas *PvPht1;1* has similar affinity for P and As, the affinity of *PvPht1;3* for As is much greater (DiTusa et al., 2016). Therefore, it is important to know how they respond to phytate and how phytate affects As influx into *P. vittata* roots.

Therefore, this work was to: (1) examine the effects of P and phytate on As release from soils and on As uptake by *P. vittata* in soils; (2) evaluate the responses of *P. vittata* P transporters (*PvPht1;1* and *PvPht1;3*) to phytate, P or As; and (3) examine the effect of phytate on As influx into *P. vittata* roots. Information obtained from this study helps to better understand the mechanism of phytate-enhanced As uptake and develop strategies for more efficient phytoremediation using *P. vittata*.

2. Materials and methods

2.1. Plant propagation and soil preparation

Spores of *P. vittata* were surface-sterilized by immersing in 75% ethanol for 2 min and in 10% sodium hypochlorite for 12 min, followed by rinses in sterile Milli-Q water (Lessl et al., 2013). Sterilized spores were suspended in 2 mL sterile Milli-Q water, which were uniformly dispersed. The plants germinated on Petri dishes (100 mm × 13 mm; 500 μL per plate) with modified Murashige and Skoog solid medium. The modified media were autoclaved, which contained (mg L^{-1}): KNO_3, 1900; NH_4NO_3, 1650; $CaCl_2 \cdot 2H_2O$, 440; $MgSO_4 \cdot 7H_2O$, 370; KH_2PO_4, 170; myo-inositol, 100; $Na_2EDTA \cdot 2H_2O$, 37.3; $FeSO_4 \cdot 7H_2O$, 27.8; $MnSO_4 \cdot 4H_2O$, 22.3; $ZnSO_4 \cdot 7H_2O$, 8.6; H_3BO_3, 6.2; glycin, 2; KI, 0.83; pyridoxine·HCl, 0.5; nicotinic acid, 0.5; $Na_2MoO_4 \cdot 2H_2O$, 0.25; thiamine·HCl, 0.1; $CuSO_4 \cdot 5H_2O$, 0.025; $CoCl_2 \cdot 6H_2O$, 0.025; sucrose, 30000; and agar, 7000 at pH 6.0 (Mathews et al., 2010).

Petri dishes were placed in a growth chamber under warm fluorescent lamps with 14 h photoperiod, a light intensity of 180 μmol m^{-2} s^{-1}, 60% humidity and ~26 °C/20 °C day/night. After ~20 d of growth, spores were germinated and gametophytes were subcultured into fresh media monthly (Chen et al., 2016). After 2–3 months of cultivation, sporophytes were emerged and were then subcultured into fresh media bimonthly (Liu et al., 2017b). After three transfers, uniform sporelings with 6–7 fronds and 3–4 cm size stage were used for experiments.

Surface soil (0–15 cm) was collected from Nanjing, China. Soil was air-dried, well mixed and passed through a 2 mm sieve. Soil property including pH, total organic carbon (TOC), and total elements were determined. Briefly, pH was measured in 1:5 soil to 0.01 M $CaCl_2$ solution after 1 h shaking and TOC was analyzed with a TOC analyzer (Element arvario TOC cube, Germany) after removing carbonate with HCl. Total element concentrations were analyzed with flame atomic absorption spectrophotometry (FAAS; PinAAcle 900T, PerkinElmer; detection limit = 20 μg L^{-1}) and inductively coupled plasma mass spectrometry (ICP–MS; PerkinElmer NexION 300X; detection limit = 70 ng L^{-1}) after digestion using USEPA Method 3050B (Liu et al., 2017a). They were: pH 7.02, 1.02% TOC, 7.12 mg kg^{-1} As, 548 mg kg^{-1} P, 24.7 g kg^{-1} Fe, 0.8 g kg^{-1} Ca, and 609 mg kg^{-1} Mn.

The soil was mixed with 80 mg kg^{-1} As ($Na_2HAsO_4 \cdot 7H_2O$; Sigma-Aldrich, St. Louis, MO, USA), which was brought up to 80% field capacity and incubated for 2 months, which is referred to as As-soil. The soil was set at ~50% field capacity for one week (Xu et al., 2014), which was transferred to containers and sterilized by autoclaving at 121 °C and 1.2 MPa for 20 min before growing plants.

2.2. Effect of phytate on As release from soils and As uptake by P. vittata in sterile soils

Effect of P and phytate on As release from soils was determined by incubating 10 mL of phytate (phytic acid) or P ($HNa_2PO_4 \cdot 12H_2O$) solutions with 1 g of soil samples at pH 6 with 10 or 100 mg L^{-1} inorganic P or phytate-P. The no P solution was used as a control. The mixture was shaken at 150 rpm and 25 °C for 6 h to reach equilibrium (Lung and Lim, 2006). The suspension pH was maintained at ~6 with HCl or NaOH. After 6 h, the samples were centrifuged at 6000 g for 10 min, filtered through 0.45-μm filters and diluted before analysis. The P concentrations were determined after persulfate digestion and solution As concentration was analyzed with ICP–MS (Lung and Lim, 2006).

The effect of phytate on As uptake and the ability of *P. vittata* to use phytate as a P source from soil was determined by growing *P. vittata* in sterile As-soil to exclude microbial interferes (George et al., 2005). In this experiment, uniform sporelings were transferred to containers containing 50 g As-soil at 50% field capacity at soil depth of ~60 mm including following treatments with 4 replicates: 10 or 100 mg kg^{-1} inorganic P (P_{10} and P_{100}) or phytate-P ($phytate_{10}$ and $phytate_{100}$). The solution pH values were adjusted to ~6 using NaOH or HCl and the solutions were filter-sterilized (0.22 μm) before use. Phytate (HPLC purity ≥ 90%; Aladdin) contains ≤ 0.6% soluble P. While containers without plant and phytate were included to examine changes in soil property, phytate-amended soil without plant was used to monitor its stability during experiment. Plants were grown at 50% field capacity in a growth chamber, which were rearranged randomly biweekly (Lessl et al., 2013).

Plants were harvested after 90 d of growth and the roots were washed with ice-cold phosphate buffer (1 mM Na_2HPO_4, 10 mM MES and 0.5 mM $Ca(NO_3)_2$, pH 5.7) and then Milli-Q water to remove surface adsorbed elements. Plant fresh biomass was recorded after blotting dry, which was separated into the roots and fronds, and lyophilized at −65 °C (FreezZone 12, LABCONCO). After recording dry weight, plant materials were cut with stainless steel scissors and ground with liquid N_2 to obtain homogeneous samples, which were stored at −80 °C before analyses. Besides fresh biomass, dry weight was used to calculate elemental contents (Liu et al., 2017b). Plant available P in soil was determined using 0.05 M $(NH4)_2SO_4$ solution (1:25 soil to solution ratio for 4 h; Wenzel et al., 2001).

2.3. Effect of phytate on P transporters in P. vittata

A hydroponic experiment was used to examine the effect of phytate on P transporter expression in *P. vittata* since it is difficult to extract root RNA from soil. *P. vittata* of uniform height of ~25 cm with 7–8 fronds were acclimatized for 4 wk in 450 mL 0.2-strength Hoagland solution (0.2X HS) under constant aeration. The solutions were replenished with Milli-Q water daily and renewed weekly. The plants were grown in a greenhouse under 14 h photoperiod, 180 μmol m^{-2} s^{-1} light intensity, 26 °C/20 °C day/night temperature, and 75% relative humidity until white new roots were developed (Liu et al., 2017a). After 4 wk of preculture, the plants were transferred to 0.2X HS solution containing 50 μM As or 50 μM phytate. Antibiotic solution at 30 mg L^{-1} chloramphenicol (Sigma-Aldrich) was added to inhibit microbial growth (Tu et al., 2004). After 7 d, intact roots were rinsed with Milli-Q water, and transferred to a solution containing 0.5 mM $CaCl_2$ and 5 mM MES at pH 6.0, which were quickly frozen in liquid N_2 and stored at −80 °C (DiTusa et al., 2016).

Total RNA extraction and qRT-PCR analysis of *P. vittata* roots followed Cao et al. (2018). Briefly, total RNA was extracted from the

roots with a Plant Total RNA Kit (Sigma-Aldrich). Manufacturers' instructions were followed using a HiScript II One Step RT-PCR Kit (Vazyme Biotech, Nanjing, China). The qRT-PCR analysis was performed with a SYBR Green PCR Master Mix (Vazyme Biotech, Nanjing, China) and a CFX Connect Real-Time PCR Detection System (BIO-RAD). First-strand cDNA was used as a template for semi-quantitative PCR amplification after 30 cycles with gene-specific primers (Cao et al., 2018). Band intensities were quantified using an IMAGEJ software (DiTusa et al., 2016).

2.4. Effect of phytate on As uptake by P. vittata from growth media

The effect of phytate on As uptake by *P. vittata* was studied in a solution containing 0.1, 0.5, 2.5, 5.0 or 7.5 μM AsV ($Na_2HAsO_4 \cdot 7H_2O$) and 0 or 50 μM phytate, after growing for 0, 1, 6, 12, and 24 h with 3 replicates. After 3 wk of preculture, plants were transferred to solution after being rinsed in 0.5 mM $CaCl_2$ and 5 mM MES solution (pH = 6.0). At different times, 1 mL of solution was taken to determine As concentration, with 1 mL Milli-Q water being added. Water loss was compensated by Milli-Q water, with temperature at 25 ± 0.5 °C. After 24 h, *P. vittata* roots were separated, rinsed with Milli-Q water, blotted dry and weighed.

2.5. Chemical and statistical analysis

Total As and Fe contents in plants and soils were determined using ICP–MS and FAAS following acid digestion using USEPA Method 3050B. Standard soil and plant reference materials D056-540 and Hunan Rice GBW10045 were included for quality control. The As concentrations for D056-540 and GBW10045 were 267 ± 2.17 mg kg^{-1} and 0.112 ± 0.002 μg kg^{-1} (mean ± SD, n = 3), which were comparable to certified values at 264 ± 30 mg kg^{-1} and 0.110 ± 0.002 μg kg^{-1}. In addition, internal standards were included for quality assurance. Standard solutions at 1 μg L^{-1} As and 1 mg L^{-1} Fe were measured every 10 samples to monitor instrument stability. Standards and samples were acidified in 0.1 M HNO_3 before analysis (Suprapur; Merck, Darmstadt, Germany) (Reichard et al., 2005).

Plant and soil P was analyzed using a modified molybdenum blue method after removing AsV interference using cysteine reduction (Ghosh et al., 2011). Briefly, the solution pH was adjusted to ~5 with NaOH and HCl. To 10 mL of the solution, ~0.5 mL of L-cysteine (5% w/v in 0.6 M HCl) was added. The test tube was capped to allow AsV reduction for 5 min at 80 °C. The solution was then cooled at room temperature, with P being determined spectrophotometrically (UV–2550, Shimadzu, Japan) at 880 nm (Liu et al., 2017b). Plant As, Fe and P concentrations were expressed on a dry weight (dw) basis.

Data are presented as mean of all replicates with standard error. Differences were determined by a one-way analysis of variance based on ranks followed by the Duncan's multiple range tests at $p < 0.05$ (SPSS 10.0, SPSS Corporation).

3. Results and discussion

3.1. Phytate increased plant growth and As uptake in P. vittata in sterile soil

It is known that *P. vittata* accumulates more As with than without phytate in growth media (Liu et al., 2017a,b). To further examine its effects on As uptake, we extended it to sterile soil to exclude microbes. Similar to growth media, 10 mg kg^{-1} phytate ($phytate_{10}$) in sterile soils greatly enhanced *P. vittata* biomass (Fig. 1A). Its gain in fresh biomass after 90 d growth in control soil was 0.29 g $plant^{-1}$, which increased to 1.52 g $plant^{-1}$ in $phytate_{10}$,

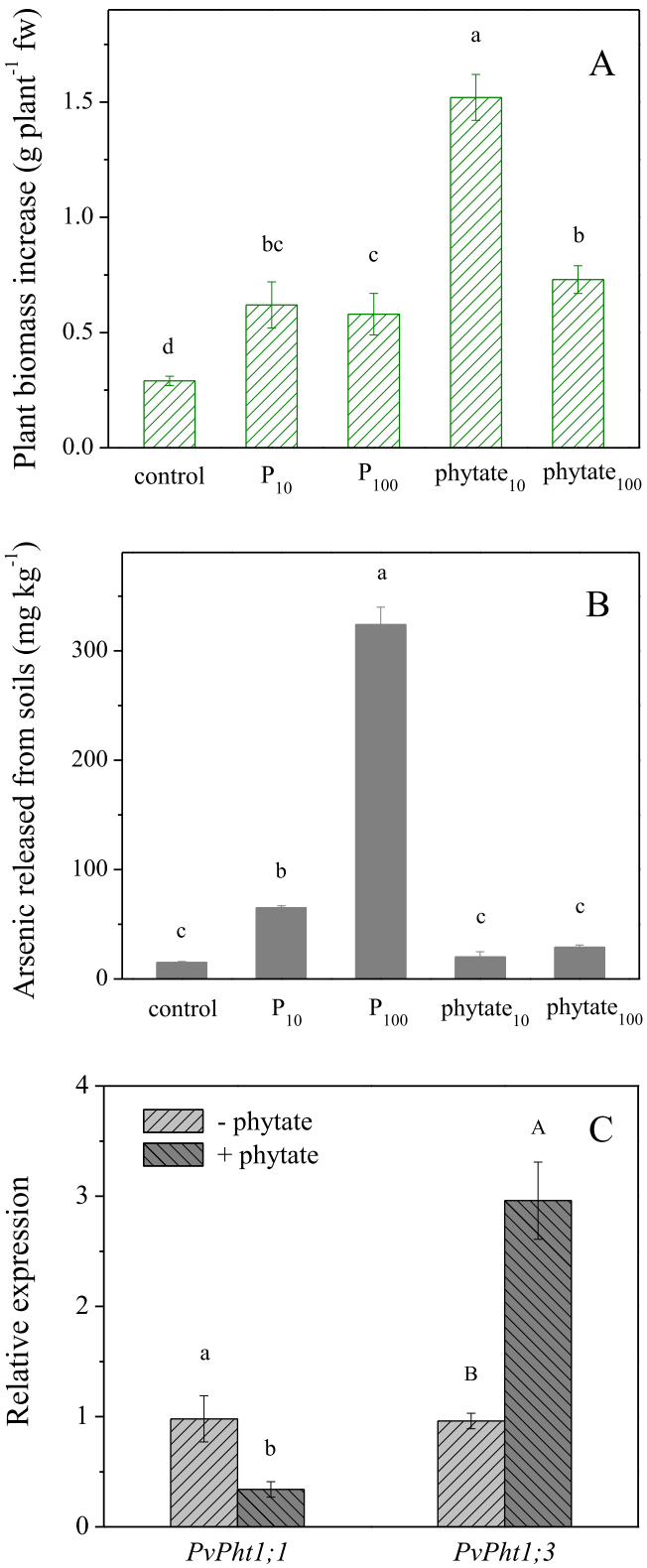

Fig. 1. Effect of P and phytate on As release from As-spiked soils (A), increase in plant biomass in *P. vittata* after growing for 90 d in sterile As-spiked soil (P_{10} and P_{100} = 10 and 100 mg L^{-1} P, $phytate_{10}$ and $phytate_{100}$ = 10 and 100 mg L^{-1} phytate-P) (B), and reverse transcription-PCR detection of transcript abundance for *P. vittata* phosphate transporters (*PvPht1;1* and *PvPht1;3*) (C). *P. vittata* were pre-cultured in 0.2-strength Hoagland nutrient solution for 3 w, and grown in fresh solution supplemented with 50 μM As with or without 50 μM phytate for 1 w. The bars indicate the standard error of four replicates and means marked with different letters indicate significant differences ($p < 0.05$).

2.1–2.5 fold that of phytate$_{100}$, P_{10} and P_{100} treatments at 0.58–0.73 g plant^{-1}. The results suggested that phytate at 10 mg kg^{-1} benefited *P. vittata* growth in soils, which was consistent with agar experiment, showing phytate at 50 μM promoted *P. vittata* growth (Liu et al., 2017b).

Compared to P treatment, *P. vittata* with phytate generally exhibited lower P accumulation but greater As accumulation in the fronds (Fig. 2AB). The frond As in the control was 3551 mg kg^{-1}, which increased to 5174 mg kg^{-1} with phytate$_{10}$ (Fig. 2A). By contrast, frond As decreased to 2100–2647 mg kg^{-1} in P_{10} and P_{100} treatment. The inhibition of P on As uptake was due to their competitive uptake by P transporters (Meharg and Hartley–Whitaker, 2002). To better compare plant As uptake, both plant biomass and total As accumulation are presented (Table 1). The highest biomass and As accumulation in *P. vittata* was observed with phytate$_{10}$ treatment. Though with lower As concentration than the control, *P. vittata* in phytate$_{100}$ treatment gained larger biomass (Fig. 1A), thus accumulating higher As than control (721 vs. 529 μg plant^{-1} in fronds) (Table 1). In contrary, P_{100} treatment decreased frond As to 492 μg plant^{-1}, consistent with P inhibition on As uptake (Tu and Ma, 2003; Rahman et al., 2007).

The data showed phytate enhanced As accumulation by *P. vittata*, different from P (Fig. 2A; Table 1). To determine if the effect was due to increased As release by phytate, we compared their As release from soils. Though both P and phytate increased As release, P was much more efficient than phytate (Fig. 1B). Soluble As was increased with increasing concentrations of P but not phytate. At 10 and 100 mg L^{-1} P, 65 and 324 μg kg^{-1} As was released, accounting for 0.1–0.4% of soil As. In contrast, there was little As release (20–29 μg kg^{-1}) with 10 and 100 mg L^{-1} phytate, showing little difference with the control. The data suggested that enhanced As accumulation by *P. vittata* with phytate was not from As release from soils.

In soils, phytate often binds with cations such as Fe and Ca to form poorly soluble complexes. Therefore, it is necessary to examine the effect of phytate on plant Fe and Ca uptake by *P. vittata* from soil. Results showed that phytate did not affect Fe or Ca concentrations in plant tissues, consistent with soil desorption experiment (Figs. 1B and 2CD). In the control, Fe concentration in the fronds and roots were 7.45 and 13.9 mg g^{-1}, similar to phytate treatments but higher than P treatments (4.58–8.17 mg g^{-1}; Fig. 2C). Similarly, frond and root Ca concentrations were 2.37 and 4.16 mg g^{-1} in the control, and 1.96–2.13 and 3.84–4.42 mg g^{-1} in phytate treatment, both higher than P treatment at 1.41–1.63 and 3.29–3.55 mg g^{-1} (Fig. 2D). Compared to the control, phytate showed little effect on Fe and Ca uptake by *P. vittata*, but compared to P treatment, Fe and Ca concentrations were greater in phytate treatment (Fig. 2CD), which may have helped *P. vittata* growth (Fig. 1A).

3.2. Effect of phytate on P transporter expression in *P. vittata*

To gain insight into the effects of phytate on As uptake by *P. vittata*, we compared As uptake with or without 50 μM phytate using solution-depletion method by varying times and concentrations. Phytate-induced difference in As uptake by *P. vittata* under hydroponic conditions would apply to soil only at high As levels. In soils with low As, plant As uptake is likely limited by ion diffusion to the roots (Caille et al., 2005). In addition, to avoid plant As toxicity, a short term (24 h) and low As concentrations (0.1–7.5 μM) were used.

Generally, P competes with As for plant uptake as they are

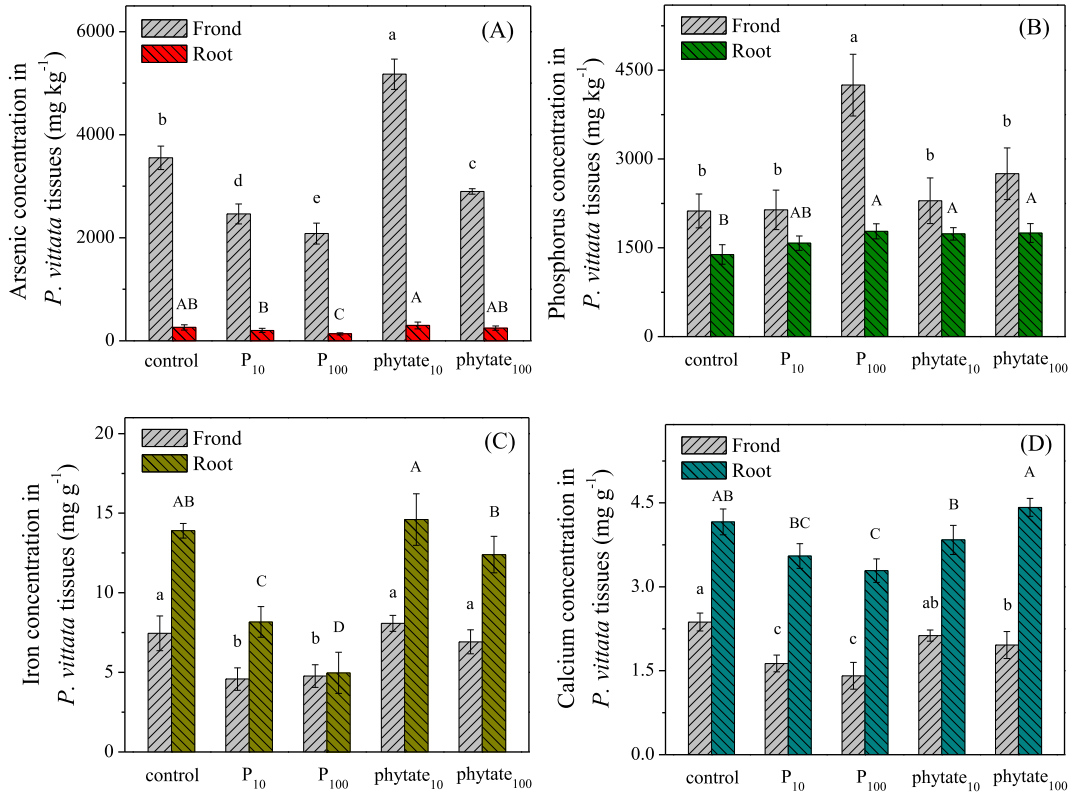

Fig. 2. Arsenic (A), P (B), Fe (C) and Ca (D) concentrations in *P. vittata* tissues after growing for 90 d in sterile As-spiked soil containing: (1) control; (2) 10 mg kg^{-1} P (P_{10}); (3) 100 mg kg^{-1} P (P_{100}); (4) 10 mg kg^{-1} phytate (phytate$_{10}$); and (5) 100 mg kg^{-1} phytate (phytate$_{100}$). The bars indicate the standard error of four replicates and means marked with different letters indicate significant differences ($p < 0.05$).

Table 1
Plant biomass (dw) and total As, P, Fe, and Ca accumulation in As-hyperaccumulator *P. vittata* after 90 d of growth on sterile As-spiked soil with 10 or 100 mg kg^{-1} P or phytate-P.

Treatment	Frond g $plant^{-1}$	Root g $plant^{-1}$	Frond As μg $plant^{-1}$	Root As μg $plant^{-1}$	Frond P mg $plant^{-1}$	Root P mg $plant^{-1}$	Frond Fe mg $plant^{-1}$	Root Fe mg $plant^{-1}$	Frond Ca mg $plant^{-1}$	Root Ca mg $plant^{-1}$
Control	0.15 ± 0.01 c*	0.12 ± 0.02 ab	529 ± 61 b	30.1 ± 7.52 b	0.32 ± 0.06 c	0.16 ± 0.03 b	1.11 ± 0.20 c	1.61 ± 0.20 b	0.35 ± 0.02 cd	0.48 ± 0.09 ab
P_{10}	0.25 ± 0.02 ab	0.13 ± 0.03 ab	669 ± 53 c	26.4 ± 2.17 c	0.54 ± 0.06 b	0.21 ± 0.03 ab	1.16 ± 0.19 c	1.08 ± 0.17 c	0.41 ± 0.06 bc	0.47 ± 0.07 ab
P_{100}	0.24 ± 0.02 b	0.11 ± 0.02 b	492 ± 42 d	14.2 ± 1.53 d	1.00 ± 0.17 a	0.19 ± 0.03 b	1.12 ± 0.22 c	0.52 ± 0.13 d	0.33 ± 0.06 d	0.35 ± 0.07 b
$phytate_{10}$	0.27 ± 0.01 a	0.14 ± 0.02 a	1372 ± 123 a	42.9 ± 11.9 a	0.61 ± 0.12 b	0.25 ± 0.05 a	2.14 ± 0.15 a	2.12 ± 0.59 a	0.56 ± 0.02 a	0.55 ± 0.13 a
$phytate_{100}$	0.25 ± 0.00 ab	0.12 ± 0.01 ab	721 ± 18 c	30.7 ± 8.00 b	0.68 ± 0.10 b	0.22 ± 0.02 ab	1.72 ± 0.20 b	1.53 ± 0.21 bc	0.49 ± 0.06 ab	0.55 ± 0.06 a

* Letters indicate the standard error of four replicates and means marked with different letters indicate significant differences at $p < 0.05$.

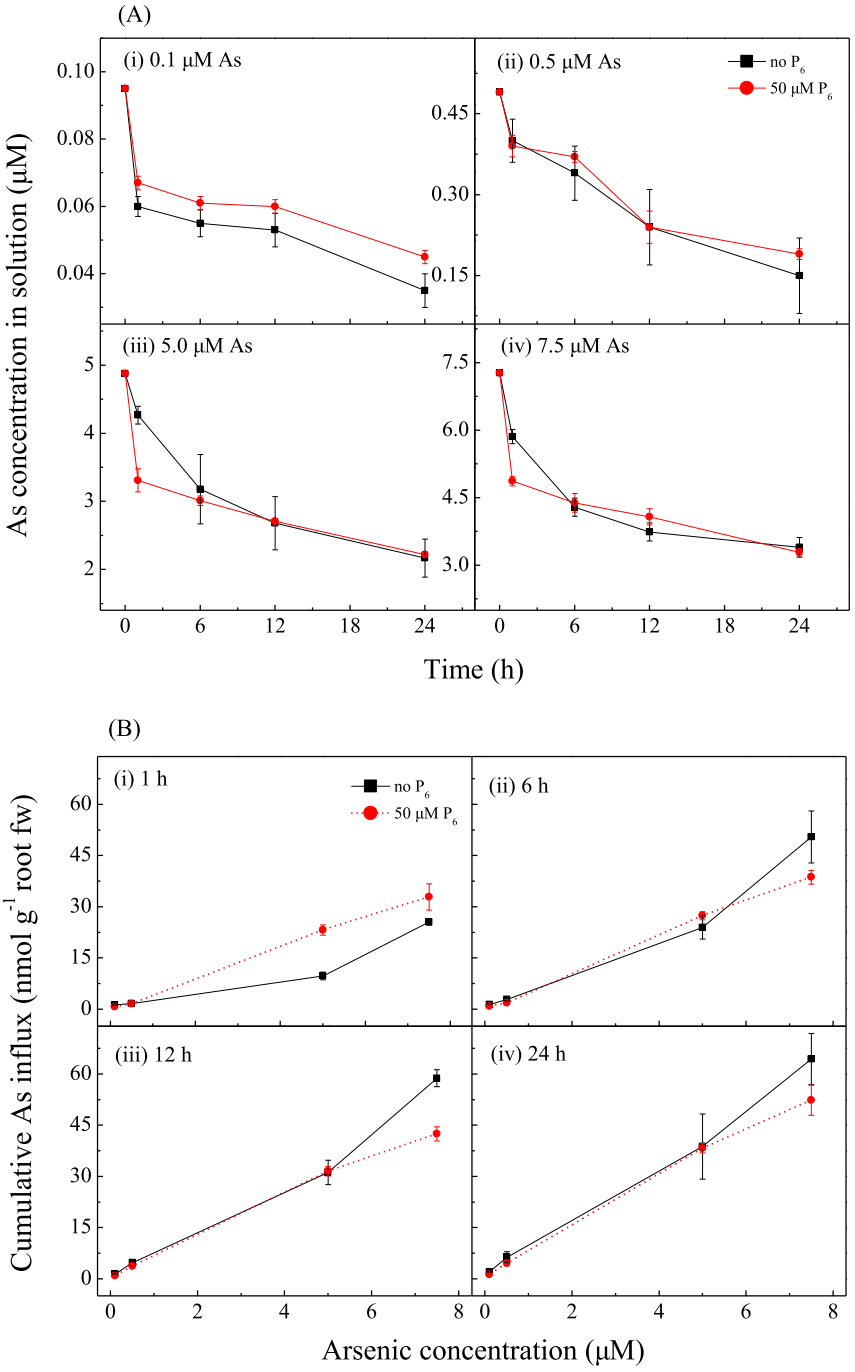

Fig. 3. Arsenic depletion from growth media (A) and cumulative As uptake (B) by *P. vittata* at 0.1–7.5 μM As with and without 50 μM phytate after 1–24 h. The bars indicate the standard error of three replicates.

chemical analogues. However, this was not the case for phytate based on either As depletion or cumulative As uptake by *P. vittata* (Fig. 3). Solution As concentration decreased sharply after 1 h in all treatments, suggesting fast As uptake by *P. vittata* (Fig. 3A). After that, As depletion was similar with or without phytate except for 0.1 μM As. In addition, based on cumulative As uptake by *P. vittata*, it was similar except at 7.5 μM As (Fig. 3B).

Since As is taken up by plants via P transporters, As uptake may relate to P transporters (Poynton et al., 2004; de Oliveira et al., 2015). Thus, the effects of phytate on P transporters of *P. vittata* were investigated. It is known that both P deficiency and As exposure up-regulate P transporters in *P. vittata*, specifically *Pht1* family members *PvPht1;1* and *PvPht1;3* (DiTusa et al., 2016; Cao et al., 2018). Therefore, to exclude the influence of P deficiency and As exposure on P transporters, nutrient media containing P and As were used as control. As shown in Fig. 1C, transcript abundance for *PvPht1;3* increased 3-fold in presence of phytate, whereas *PvPht1;1* transcript decreased by 65%. The results revealed different phytate-induced responses of *PvPht1* genes, showing up-regulation of *PvPht1;3*, which may have contributed to As uptake by *P. vittata* (Fig. 2A; Table 1).

Research showed that *Pht1* transporters have different affinity for As. Shin et al. (2004) found that mutants of *Arabidopsis* transporters *AtPht1;1* and *AtPht1;4* showed different As uptake rates. Also wheat (*T. aestivum*) showed different P and As uptake rates, suggesting different affinity of P transporters (Zhu et al., 2006; DiTusa et al., 2016). As such, it is possible that *PvPht1;3* may have high affinity for As and its up-regulation by phytate may have enhanced As uptake by *P. vittata*. Indeed, among plant *Pht1* transporters, *PvPht1;3* exhibits unique ability to take up As (DiTusa et al., 2016). Overexpressing *PvPht1;3* caused PAM2 yeast cells to accumulate 2-fold more As than cells expressing Arabidopsis *Pht1* transporter *AtPht1;5* (DiTusa et al., 2016). In addition, inhibition of As on P uptake by *PvPht1;3* indicated its high affinity for As, comparable to that for P (DiTusa et al., 2016).

4. Conclusions and environmental implication

As a P analog, AsV is taken up via P transporters by plants including *P. vittata*. As a result, P inhibits As plant uptake directly, which is unfavorable for phytoremediation of As-contaminated soils. However, phytate enhanced As uptake by *P. vittata* in growth media. To further evaluate its effects on As uptake, the ability of phytate to promote As uptake in *P. vittata* was demonstrated in sterile soils. The results showed that while P suppressed As plant uptake, phytate increased it. *P. vittata* in soil with phytate accumulated 2.5-fold more As than the control. The qRT-PCR results showed that phytate increased the abundance of *P. vittata* P transporter *PvPht1;3*, which has a high affinity for As. As such, the up-regulation of P transporter may have enhanced As uptake, contributing to phytate-promoted As uptake in *P. vittata*. Better understanding the possible mechanisms of phytate-promoted As uptake by *P. vittata* and improving phytate-P assimilation by plants may help efficient phytoremediation of As-contaminated soils.

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