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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