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Phytate induced arsenic uptake and plant growth in arsenic-hyperaccumulator *Pteris vittata*☆

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ABSTRACT

Phytate is abundant in soils, which is stable and unavailable for plant uptake. However, it occurs in root exudates of As-hyperaccumulator *Pteris vittata* (PV). To elucidate its effect on As uptake and growth, *P. vittata* were grown on agar media (63 μM P) containing 50 μM As and/or 50 or 500 μM phytate with non As-hyperaccumulator *Pteris ensiformis* (PE) as a congeneric control for 60 d. Phytate induced efficient As and P uptake, and enhanced growth in PV, but had little effects on PE. The As concentrations in PV fronds and roots were 157 and 31 mg kg^{-1} in As_{50}+$phytate_{50}$, 2.2- and 3.1-fold that of As_{50} treatment. Phosphorus uptake by PV was reduced by 27% in As treatment than the control (P vs. P+As) but increased by 73% comparing $phytate_{500}$ to $phytate_{500}$+As, indicating that PV effectively took up P from phytate. Neither As nor phytate affected Fe accumulation in PV, but phytate reduced root Fe concentration in PE (46–56%). As such, the increased As and P and the unsuppressed Fe uptake in PV probably promoted PV growth. Thus, supplying phytate to As-contaminated soils may promote As uptake and growth in PV and its phytoremediation ability.

1. Introduction

Arsenic (As) is of environmental concern due to its toxicity and ubiquity in the environment. *Pteris vittata* (PV; Chinese Brake fern) is the first-known As-hyperaccumulator (Ma et al., 2001). In contaminated soils, it can accumulate up to 23 g kg^{-1} As in the fronds. In uncontaminated soils, it can take up 744 mg As kg^{-1}, which is much greater than typical plants at ≤ 10 mg kg^{-1} (Ma et al., 2001; Matschullat, 2000).

In soils, As is often present in its oxidized form arsenate (AsV), which is a phosphate (P) analog, sharing similar chemical properties and behavior (Meharg and Hartley–Whitaker, 2002). During plant uptake, AsV and P compete for root P transporters (de Oliveira et al., 2015; Ditusa et al., 2016). For instance, As accumulation in duckweed (*Spirodela polyrhiza*) is negatively correlated with P uptake when they co-exist (Rahman et al., 2007). Similarly, a negative correlation between AsV and P uptake by *P. vittata* has also been observed (Lou et al., 2010; Tu and Ma, 2003). Moreover, Esteban et al. (2003) suggested that plant P uptake system has a much higher affinity for P than for AsV. Therefore, the inhibition of P on AsV uptake is more effective than that of P uptake by AsV (Lou et al., 2010). Obviously, such inhibition is unfavorable for phytoremediation of As-contaminated soils.

P. vittata is native to nutrient-poor soils, which contain mostly unavailable P including both organic and inorganic P (Ramaekers et al., 2010). Organic P consists of a large pool of unavailable P in soils, accounting for 30–80% of total soil P, predominantly as phytate (inositol hexakisphosphate) (Richardson et al., 2005; Turner et al., 2006). Within the soil fraction, phytate generally makes up ~50% organic P and ~25% total P (Lessl et al., 2013; Richardson et al., 2005). However, phytate is a stable compound resistant to biochemical degradation, rendering it unavailable for plant uptake (Turner et al., 2002). Besides, phytate is also the predominant form of P storage in plant seeds and grains (Park et al., 2006). Furthermore, its production as low molecular weight organic acid (LMWOA) has been detected in PV root exudates, which contributes to its efficient Fe and As acquisition from insoluble Fe-As minerals

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(Liu et al., 2016; Tu et al., 2004). Unlike typical plant LMWOAs (e.g., citrate and oxalate), as an organic P and the dominant LMWOA in PV rhizosphere microniche, phytate may affect As uptake by PV thus its phytoremediation efficiency of As-contaminated soils. Therefore, it is important to understand how phytate influences As and/or P uptake and plant growth in the hyperaccumulator. However, limited information is available regarding this aspect.

Arsenic is a non-essential element for plants and its uptake depends on plant species and rhizosphere properties. The presence of P or phytate in rhizosphere may affect As uptake due to competition between P and AsV (Liu et al., 2004). While there has been substantial research on effects of P on As uptake by plants, less attention has been paid to the influence of phytate on plant As uptake, especially in As-hyperaccumulators. Arsenic uptake is linked to P nutrition, with decreased inorganic P leading to increased As uptake in plants (Meharg and Hartley–Whitaker, 2002). As an important organic P source in both soils and PV root exudates, sparingly-available phytate may also have a significant impact on As uptake in PV. In addition, plant biomass is an important factor for successful application of phytoremediation (Singh and Ma, 2006). Iron is an essential nutrient for plant growth and health. Liu et al. (2015, 2016) observed that PV biomass was closely correlated with plant Fe content (R^2 = ~0.80), illustrating the important role of Fe in PV growth.

Therefore, the objective of this study was to determine the influence of phytate on As and P uptake in *P. vittata* (PV) and non As-hyperaccumulator *P. ensiformis* (PE). The specific objectives were to (1) examine the effect of phytate on plant As uptake and transport, and (2) investigate the effect of As/P/Fe accumulation on plant growth. Information obtained from this study may expand our knowledge on effect of phytate on plant As uptake, as well as the effect of As/P/Fe metabolism on plant growth in As-hyperaccumulator, which may be of great importance to better understand the mechanisms and garner fundamental clues to develop strategies for more efficient phytoremediation of As-contaminated soils using *P. vittata*.

2. Materials and methods

2.1. Spores sterilization and gametophyte culture

Two ferns, As-hyperaccumulator *P. vittata* and non As-hyperaccumulator *P. ensiformis*, were used in this study. Their spores were first sterilized with 75% alcohol for 2 min and rinsed three times with sterile deionized (DI) water, then surface sterilized in a 10% sodium hypochlorite for 12 min and rinsed thoroughly with sterile DI water before being sown in Petri dishes with solid medium (Chen et al., 2016; Lessl et al., 2013). Half-strength modified Murashige and Skoog (MS) medium containing 0.8% (w/v) agar was autoclaved. It contained (mg L^{-1}): KNO_3, 950; NH_4NO_3, 825; KH_2PO_4, 8.5; $MgSO_4 \cdot 7H_2O$, 185; $CaCl_2 \cdot 2H_2O$, 220; KI, 0.415; H_3BO_3, 3.1; $MnSO_4 \cdot 4H_2O$, 11.2; $ZnSO_4 \cdot 7H_2O$, 4.3; $Na_2MoO_4 \cdot 2H_2O$, 0.125; $CuSO_4 \cdot 5H_2O$, 0.0125; $CoCl_2 \cdot 6H_2O$, 0.0125; $Na_2EDTA \cdot 2H_2O$, 18.6; $FeSO_4 \cdot 7H_2O$, 13.9; myo-inositol, 50; glycin, 1; thiamine·HCl, 0.05; pyridoxine·HCl, 0.25; nicotinic acid, 0.25; sucrose, 15 000; and pH 6.0 (Mathews et al., 2010). Spores were suspended in 2 mL sterile DI water and uniformly dispersed on agar in sterile Petri dishes (100 mm × 13 mm; 500 μL per plate). Petri dishes were placed in a growth chamber under warm fluorescent lamps with 14 h photoperiod and a light intensity of 180 mol m^{-2} s^{-1}, at ~26°C/20°C day/night, and a humidity of 60%. After ~20 d of growth, spores germinated and the gametophytes were subcultured into fresh media monthly (Chen et al., 2016). After additional 2–3 months of cultivation, sporophytes emerged and were subcultured into fresh media bimonthly.

2.2. Experimental setup

After five transfers, uniform sporophytes with 3–4 cm in size were cultured in sterile media including following treatments of three replicates each: (1) 0.5-strength MS medium control; (2) 50 μM As (As_{50}); (3) 50 μM phytate ($phytate_{50}$); (4) As_{50}+$phytate_{50}$; (5) 500 μM phytate ($phytate_{500}$); and (6) As_{50}+$phytate_{500}$. The pH was adjusted to ~6 after addition of As and/or phytate using 0.01 M NaOH or HCl. Phytate and AsV solutions were filter-sterilized (0.22 μm) before use. Arsenic was added as AsV ($Na_2HAsO_4 \cdot 7H_2O$; Sigma-Aldrich, St. Louis, USA). Phytate (HPLC purity ≥ 90%; Aladdin) contains ≤ 0.6% soluble P. The plant-free 0.5-strength MS control was used to examine the recoveries of P, As, and Fe, which were 101%, 98.6%, and 99.2%, respectively. A control phytate-amended media without plant was used to monitor its stability during treatment. No evidence of phytate degradation was found in plant-free media controls.

After 60 d of growth, plant fresh biomass was recorded after washing the roots 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 Milli-Q water to remove surface adsorbed elements. Plant biomass increases after 60 d of growth were calculated based on difference between the initial and final fresh biomass. Plants were separated into the roots and fronds, blotted dry and lyophilized at −65°C (FreezZone 12, LABCONCO). Their dry weights were recorded and plant materials were ground with liquid nitrogen and stored at −80°C for further analysis. While fresh weight was used to express plant biomass, dry weight was used to calculate elemental concentrations.

2.3. Chemical analysis

Freeze-dried plant materials were digested with HNO_3/H_2O_2 using USEPA Method 3050B for total elemental concentrations (de Oliveira et al., 2015). The growth media were centrifuged at 10 000 *g* and 4°C for 10 min. Total As concentration in the digests and media was analyzed with inductively coupled plasma mass spectrometry (ICP–MS; NexION300X, PerkinElmer; detection limit = 70 ng L^{-1}). Blank and certified reference material for rice samples (GSB-23, Chinese geological reference materials) were used for quality control. The As mean ± standard error was 0.110 ± 0.002 μg kg^{-1}, which was comparable to the certified values (0.116 ± 0.002 μg kg^{-1}). The internal standards were carried to ensure accuracy and precision. Iron concentrations were measured by flame atomic absorption spectrophotometry (FAAS; PinAAcle 900 T, PerkinElmer; detection limit = 20 g L^{-1}). Standard solutions of 1 μg L^{-1} As and 1 mg L^{-1} Fe were measured every 20 samples to monitor the stability of the ICP–MS and FAAS. Standards and samples were prepared and acidified in 0.1 M HNO_3 (Suprapur; Merck) (Chen et al., 2016; Richardson et al., 2005).

Plant and medium P was analyzed using a modified molybdenum blue method after removing AsV interference via cysteine reduction (Singh and Ma, 2006). Briefly, the pH of the digestion solution 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 tightly to allow AsV reduction for 5 min at 80°C. The solution was cooled to room temperature and P was determined by the molybdenum blue method (Singh and Ma, 2006). Plant As, Fe and P concentrations were expressed on a dry weight (dw) basis.

2.4. Statistical analysis

Data are presented as the mean of three replicates with standard error. Analysis of variance (ANOVA) and Tukey's mean grouping were used to determine the significance of the interactions

between the treatment means. All statistical analyses were performed with SAS statistical software (SAS Inst. Cary, North Carolina, USA).

3. Results and discussion

3.1. P. vittata grew better on media amended with arsenic and phytate

After growing on half-strength MS medium amended with As and/or phytate for 60 d, *P. vittata* (PV) and *P. ensiformis* (PE) showed no toxicity symptom. Overall, PV grew better than PE in all treatments. Enhanced growth of PV was observed in the presence of As and/or phytate, with the most being in As+phytate treatment. In contrast, the growth of PE showed no significant difference among treatments (Fig. 1).

Specifically, the increase in fresh PV biomass after 60 d of growth was 9.5 g plant^{-1} in the control and it increased to 11.8 and 13.8 g plant^{-1} in As_{50} and $phytate_{50}$ treatment, showing that both As and phytate enhanced plant growth (Fig. 1). Arsenic has been reported to promote PV growth. For example, Singh and Ma (2006) found that at 133 μM As, the growth of PV increased by 23% after 5 d of exposure compared to the control. Moreover, Srivastava et al. (2009) found that PV biomass increased from 5.5 to 6.6 g when As increased from 150 to 300 μM after 5 d exposure. Chen et al. (2016) reported that PV sporophyte biomass increased by ~14% at 10–500 mM AsV after 28 d of growth, higher than the 9% increase in the no As control. In soils, it has also been reported that As induced substantial plant growth in PV (Lessl et al., 2014; Tu and Ma, 2002). Xu et al. (2014) attributed PV biomass increase to As-induced P uptake. However, the direct evidence and mechanistic basis for the beneficial role of As on PV growth is unknown. Besides, Lessl et al. (2013) found that PV can effectively utilize phytate as a sole source of P for growth. They attributed PV's ability to grow directly with phytate to an adaptive trait of phytase exudation (Lessl et al., 2013).

With both As and phytate enhancing PV growth, their co-presence induced even more significant growth in PV ($p < 0.05$).

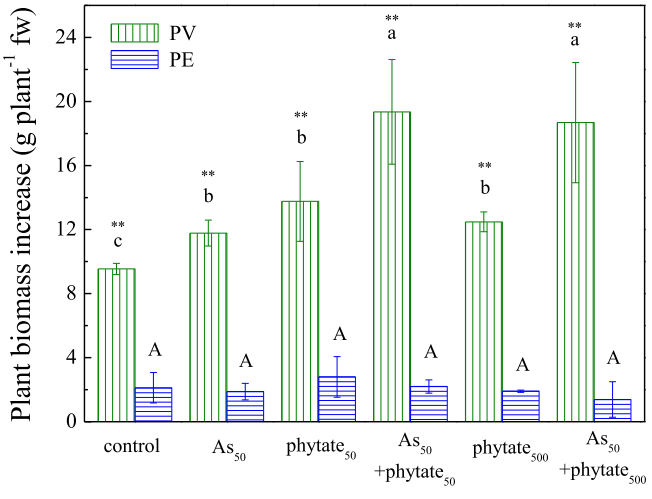

Fig. 1. Increase in plant biomass of *P. vittata* and *P. ensiformis* after growing for 60 d in 0.5-strength MS agar containing: (1) control; (2) 50 μM As (As_{50}); (3) 50 μM phytate ($phytate_{50}$); (4) As_{50}+$phytate_{50}$; (5) 500 μM phytate ($phytate_{500}$); and (6) As_{50}+$phytate_{500}$. The bars indicate the standard error of triplicates and means marked with different letters indicate significant differences ($p < 0.05$). ** represents significant difference between the two ferns at $p < 0.01$.

For example, the increase in PV biomass in As_{50}+$phytate_{50}$ treatment was 19.4 g plant^{-1}, which was 1.6- and 1.4-fold that of As_{50} and $phytate_{50}$ treatment. A similar biomass increase was obtained in As_{50}+$phytate_{500}$ treatment at 18.7 g plant^{-1}, increased by 59% and 50% compared to As_{50} and $phytate_{500}$ treatment (Fig. 1). Unlike PV, As or phytate had little effect on PE biomass, with the biomass increase at 1.39–2.12 g plant^{-1} for all treatments (Fig. 1). The results indicated that As and phytate promoted PV growth but not PE growth. Enhanced growth of PV may result from enhanced As, P and/or Fe uptake (Liu et al., 2015). Therefore, plant As, P and Fe concentrations were determined.

3.2. Phytate promoted As uptake in P. vittata

The As concentrations in dry PV fronds and roots were 69.8 and 10.1 mg kg^{-1} in As_{50} treatment (Fig. 2A), which increased by 2.2- and 3.1-fold to 157 and 31 mg kg^{-1} in the As_{50}+$phytate_{50}$ treatment, indicating phytate promoted As uptake in PV. However, the As uptake promotion was not correlated with phytate concentrations. For example, As concentrations in PV fronds and roots decreased to 121 and 29 mg kg^{-1} in the As_{50}+$phytate_{500}$ treatment.

Generally, inorganic P competes with As for plant uptake since they are chemical analogues. However, this was not the case for phytate-P. It is reported that PV roots secrete phytase, which is the only enzyme that hydrolyzes phytate to inorganic P (Lessl et al., 2013). Moreover, enzyme-mediated hydrolysis of phytate in PV root extracts is not inhibited by 0.5–5 mM AsV (Lessl et al., 2013). As such, it might be expected that some P would have been released into the media due to phytate application and phytase exudation. However, with soluble P at 21–41 μM in phytate-spiked media (Table 2), As uptake by PV was not suppressed. In fact, it increased by 4.1–4.7 and 4.8–5.6 fold in PV fronds and roots compared to the As_{50} treatment (106–121 vs. 26 and 25–29 vs. 5.2 μg plant^{-1}) (Table 1).

Compared to PV, PE accumulated much lower As and the difference was more pronounced when As was coupled with phytate. Arsenic concentrations in PE fronds were 6.5 and 7.8 mg kg^{-1} in As_{50} and As_{50}+$phytate_{50}$ treatments, which were 11 and 20 times lower than that of PV (Fig. 2AB). While phytate promoted As uptake in PV, As concentrations in PE fronds and roots changed slightly from 6.5 and 5.4 mg kg^{-1} in As treatment to 6.2–7.8 and 4.2–5.5 mg kg^{-1} in As+phytate treatment, indicating that phytate had limited effect on As uptake by PE. In addition, based on total As, PE accumulated 9.1 and 34–46 times less As than PV (3.4 vs. 31 and 2.9–4.4 vs. 132–150 μg plant^{-1}) (Table 1) in As and As+phytate treatments. Besides, as an As-hyperaccumulator, PV transported most of As to the fronds (81–83%) whereas the non-hyperaccumulator PE concentrated 38–48% of As in the roots (Table 1). The difference in As accumulation between PV and PE implied the different roles of phytate played in As uptake by the two plants.

Both plant biomass and elemental concentrations are important factors for successful application of phytoremediation (Singh and Ma, 2006). The significantly-enhanced plant growth (Fig. 1) and As accumulation (Fig. 2A) in the presence of phytate implied that phytate may help efficient phytoremediation of As-contaminated soils.

3.3. Arsenic coupled with phytate did not inhibit P uptake in P. vittata

In addition to phytate-induced As uptake, phytate also induced more P uptake by PV, with higher phytate induced higher P accumulation. For example, P concentrations in the fronds and roots in the As_{50} treatment were 2223 and 1721 mg kg^{-1}, which decreased

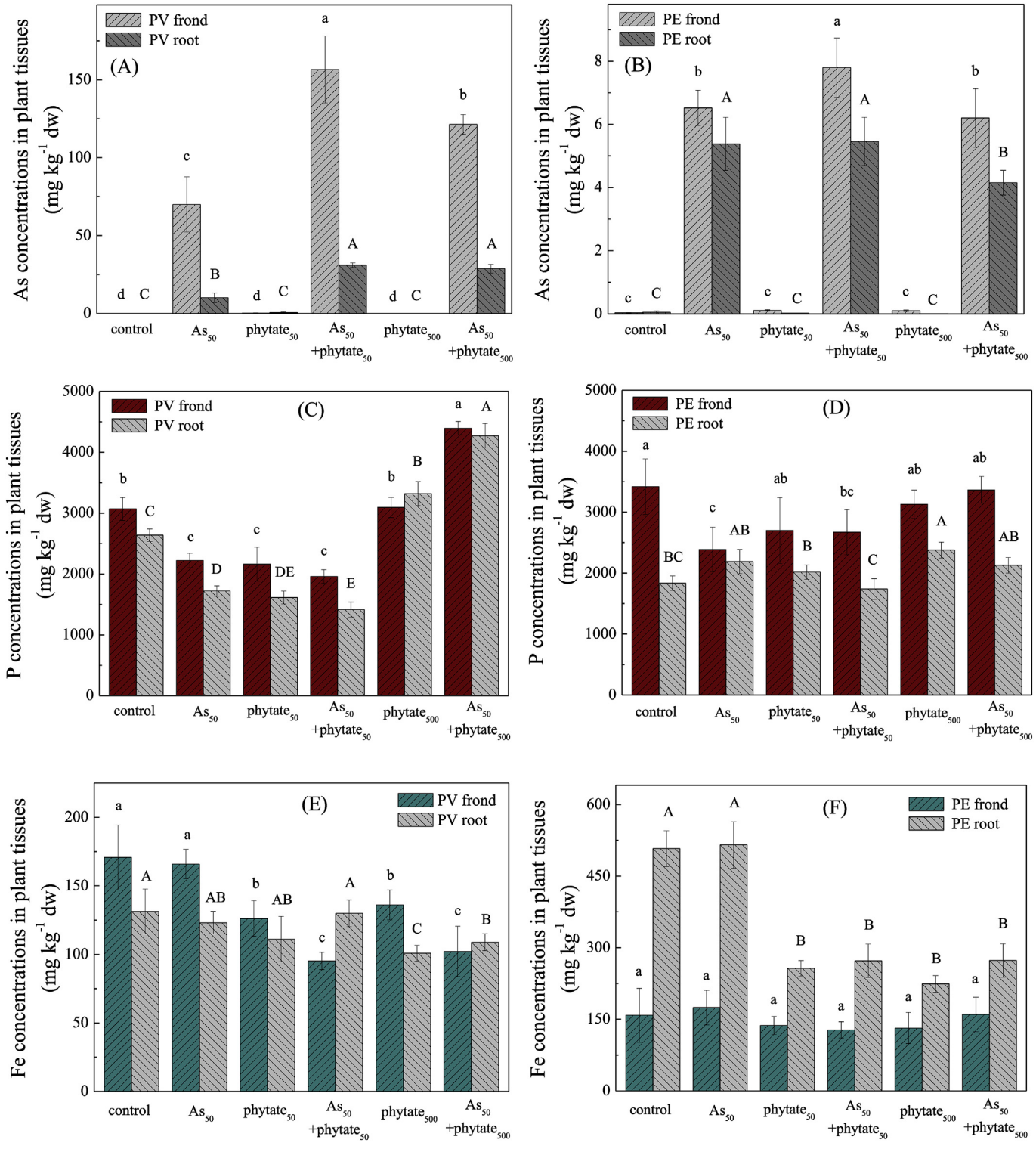

Fig. 2. Arsenic (A, B), P (C, D), and Fe (E, F) concentrations in *P. vittata* (PV) and *P. ensiformis* (PE) tissues after growing for 60 d in 0.5-strength MS agar, which was served as control containing 63 μM P and was amended with 50 μM As (As_{50}) and/or 50 or 500 μM phytate ($phytate_{50}$ or $phytate_{500}$). The bars indicate the standard error of triplicates and means marked with different letters indicate significant differences ($p < 0.05$).

Table 1
Plant biomass and total As, P and Fe accumulation after 60 d of growth in As-hyperaccumulator *P. vittata* (PV) and non-hyperaccumulator *P. ensiformis* (PE) on 0.5-strength MS media amended with 50 μM As and/or 50 or 500 μM phytate. The 0.5-strength MS medium was used as a control which contains 63 μM P and 50 μM Fe.

Treatment	Frond (dw) g plant^{-1}	Root (dw) 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}
PV								
control	0.35 ± 0.01 d*	0.49 ± 0.02 b	–	–	1.07 ± 0.10 c	1.28 ± 0.01 c	0.07 ± 0.007 b	0.06 ± 0.004 c
As_{50}	0.37 ± 0.01 d	0.51 ± 0.02 b	25.7 ± 8.4 b	5.2 ± 1.6 c	0.82 ± 0.07 c	0.88 ± 0.02 c	0.06 ± 0.002 b	0.06 ± 0.006 c
$phytate_{50}$	0.71 ± 0.10 bc	0.77 ± 0.19 a	0.13 ± 0.01 c	0.36 ± 0.2 d	1.52 ± 0.22 b	1.23 ± 0.23 bc	0.09 ± 0.012 ab	0.08 ± 0.013 b
As_{50}+$phytate_{50}$	0.77 ± 0.02 b	0.93 ± 0.03 a	121 ± 19.3 a	28.9 ± 1.6 a	1.51 ± 0.08 b	1.32 ± 0.15 bc	0.07 ± 0.003 b	0.12 ± 0.010 a
$phytate_{500}$	0.65 ± 0.09 c	0.70 ± 0.17 ab	0.06 ± 0.01 c	0.01 ± 0.00 d	2.00 ± 0.34 b	2.35 ± 0.66 b	0.09 ± 0.007 a	0.07 ± 0.020 c
As_{50}+$phytate_{500}$	0.88 ± 0.03 a	0.89 ± 0.19 a	106 ± 7.2 a	25.2 ± 3.5 b	3.85 ± 0.22 a	3.78 ± 0.69 a	0.09 ± 0.014 ab	0.10 ± 0.023 ab
PE								
control	0.24 ± 0.03 c	0.29 ± 0.07 a	–	–	0.81 ± 0.02 bc	0.53 ± 0.10 b	0.04 ± 0.018 a	0.15 ± 0.026 a
As_{50}	0.27 ± 0.03 bc	0.30 ± 0.04 a	1.8 ± 0.05 b	1.6 ± 0.08 a	0.65 ± 0.04 c	0.65 ± 0.12 a	0.05 ± 0.010 a	0.15 ± 0.008 a
$phytate_{50}$	0.31 ± 0.02 ab	0.33 ± 0.10 a	0.03 ± 0.01 c	0.01 ± 0.00 b	0.82 ± 0.12 bc	0.66 ± 0.19 a	0.04 ± 0.007 a	0.08 ± 0.032 b
As_{50}+$phytate_{50}$	0.36 ± 0.36 a	0.29 ± 0.04 a	2.8 ± 0.27 a	1.6 ± 0.25 a	0.95 ± 0.10 a	0.51 ± 0.09 a	0.05 ± 0.005 a	0.08 ± 0.005 b
$phytate_{500}$	0.25 ± 0.00 c	0.30 ± 0.01 a	0.03 ± 0.00 c	–	0.77 ± 0.05 c	0.71 ± 0.03 a	0.03 ± 0.009 a	0.07 ± 0.006 b
As_{50}+$phytate_{500}$	0.24 ± 0.24 c	0.33 ± 0.05 a	1.5 ± 0.57 b	1.4 ± 0.30 a	0.81 ± 0.17 ab	0.70 ± 0.10 a	0.04 ± 0.016 a	0.09 ± 0.024 b

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

Table 2
Soluble As, P and Fe concentrations in the growth media after 60 d of growth of *P. vittata* and *P. ensiformis* in 0.5-strength MS medium, which was used as a control containing 63 μM P and 50 μM Fe and amended with 50 μM As and/or 50 or 500 μM phytate.

Treatment	*P. vittata*			*P. ensiformis*		
	Soluble As (μM)	Soluble P (μM)	Soluble Fe (μM)	Soluble As (μM)	Soluble P (μM)	Soluble Fe (μM)
control	–	6.10 ± 0.53 f	17.1 ± 1.76 b	–	11.5 ± 1.18 e	20.3 ± 1.86 b
As_{50}	29.2 ± 1.87 a	18.9 ± 2.96 e	31.1 ± 1.87 a	44.2 ± 1.38 a	32.1 ± 5.49 b	15.8 ± 1.17 c
$phytate_{50}$	–	28.4 ± 4.57 c	17.6 ± 1.88 b	–	17.5 ± 2.69 d	29.2 ± 0.23 a
As_{50}+$phytate_{50}$	8.07 ± 1.26 c	21.5 ± 3.45 d	10.3 ± 1.24 d	44.4 ± 2.36 a	37.6 ± 5.68 a	28.9 ± 0.69 a
$phytate_{500}$	–	35.3 ± 2.21 b	14.5 ± 0.91 c	–	27.7 ± 1.27 c	20.3 ± 0.44 b
As_{50}+$phytate_{500}$	11.9 ± 0.95 b	41.6 ± 6.88 a	9.56 ± 0.80 e	45.0 ± 3.35 a	33.9 ± 0.81 b	15.9 ± 0.33 c

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

to 1960 and 1416 mg kg^{-1} in As_{50}+$phytate_{50}$ and increased to 4394 and 4272 mg kg^{-1} in As_{50}+$phytate_{500}$ treatment (Fig. 2C). Though lower in P concentration, PV gained larger biomass in As_{50}+$phytate_{50}$ than As_{50} treatment (Fig. 1), thus having higher total P content (2.8 vs. 1.7 mg plant^{-1}) (Table 1). The data suggested that PV readily accessed P from phytate in presence of As.

Phytate is a prevalent form of organic P in soils, contributing >25% of total soil P (Turner et al., 2002). However, the phytase-mediated hydrolysis of phytate in most plants is inhibited by As (Hayes et al., 2000; Paivoke and Simola, 2001), but this is not the case with PV as its phytase is resistant to As (Lessl et al., 2013), allowing for sufficient acquisition of P from phytate. This was supported by the results based on total P calculation, showing that PV accumulated greater P from media containing As+phytate than 0.5-strength MS control containing 63 μM P (2.8–7.6 vs. 2.3 mg plant^{-1}) (Table 1).

In addition, total P contents were 2.3 and 1.7 mg plant^{-1} for PV in the control and As_{50} treatment, and increased to 4.4 and 7.6 mg plant^{-1} in $phytate_{500}$ and As_{50}+$phytate_{500}$ treatment, showing that As inhibited P uptake by 26% when P was supplied while it increased P uptake by 73% when phytate was provided. It was possible that As may have competed with uptake of P by PV, but not uptake of phytate-P. Both P limitation and presence of As induce P deficiency in plants. Arsenic can substitute for P in plants, but it is unable to carry out P's role in energy transfer, thus as plant As increases it reacts as if there were P deficiency (Tu et al., 2004). Besides, As competes with P for uptake and disrupts processes involving phosphorylation and P signaling pathways, inducing P-deficiency symptoms in plants (Abercrombie et al., 2008). During the 60 d growth, the low-availability P environment may elicit P deficiency responses in PV (6.10 μM soluble P in the 0.5-strength MS control media) (Table 2). During P deficiency, plant reacts by increasing P uptake (Singh and Ma, 2006). Since P and As are analogues, PV may not be able to differentiate between them and mistakenly uptake As, thereby increasing plant As uptake.

As for PE, frond P concentration and total P content in As_{50} treatment decreased by 30% and 20% compared to that of the control (2388 vs. 3418 mg kg^{-1}; 0.65 vs. 0.81 mg plant^{-1}) (Fig. 2D; Table 1), which is consistent with previous findings that As competes with P for plant uptake (Rahman et al., 2007; Tu and Ma, 2003). Similar to PV, P content in PE increased in phytate and As+phytate treatments, but the increase was more dramatic in PV than PE. For example, total P increase was 0.4–5.3 mg plant^{-1} in PV, which was 3–32 fold that of PE (Table 1). The results indicated a higher efficiency in phytate utilization by PV than PE in presence of As. Lessl et al. (2013) found that both PV and PE exudated phytase, while phytase activity in PE exudates decreased ~50% at 0.5 mM As, PV exudates were unaffected. The greater phytase activity in PV in presence of As may have contributed to its higher efficiency in P acquisition from phytate, illustrating an inherent difference between the two species.

As such, the impact of As on PV biomass and P concentration depends on the forms of P supplied. Despite having improved biomass, PV in As treatment had the lowest P accumulation, showing that As stimulated PV growth and probably competed with its P uptake. However, As did not show inhibition on P uptake

when P was supplied as phytate, with higher phytate inducing higher P accumulation in PV. In addition to growth promotion, the As_{50}+$phytate_{500}$ treatment showed significantly higher P and As uptake than As_{50} treatment ($p < 0.05$). Thus, growing on As-contaminated soils with phytate may enhance As and P plant uptake and plant growth in PV.

3.4. Iron accumulation and distribution in P. vittata

Neither As nor phytate had significant effect on Fe uptake and distribution in PV, with Fe being evenly distributed in the fronds and roots (Table 1). Total frond and root Fe contents in PV in the control were 0.07 and 0.06 mg $plant^{-1}$, similar to those in As and/or phytate treatments (Table 1). While As did not affect Fe uptake by PV, phytate significantly reduced root Fe contents in PE (from 0.15 to 0.07–0.08 mg $plant^{-1}$) (Table 1).

Generally, phytate chelates Fe to form Fe-phytate complex, which can be soluble or insoluble depending on the number of Fe^{3+} ions in the complex (Nielsen et al., 2013). Monoferric phytate is water soluble, but tetraferric phytate is not, indicating the solubility of Fe-phytate decreases with increasing Fe in the complex. Liu et al. (2016) found that 96% and 87% of Fe is sequestered as insoluble Fe-phytate at phytate:Fe ratios of 3:1 and 8:1. The 0.5-strength MS medium contains 50 μM Fe. In the $phytate_{50}$ and $phytate_{500}$ treatments with phytate:Fe molar ratios at 1:1 and 10:1, phytate probably bound Fe^{3+} to form insoluble Fe-phytate. This was supported by Trela (2010) who observed >90% reduction in Fe concentration at phytate:Fe ratio of 1:1. The poorly soluble Fe-phytate was probably responsible for the low Fe concentrations in PE roots in phytate treatments (Fig. 2F). In contrary, PV probably effectively hydrolyzed Fe-phytate to P and Fe, which did not block its Fe uptake. The results implied that PV was more efficient in Fe acquisition in presence of phytate than PE.

4. Conclusions and environmental implication

Arsenate and P are chemical analogues and taken up by P transporters in plants, with higher affinity for P than AsV. As a result, P is an efficient inhibitor for As uptake by plants. Our results showed that inorganic P suppressed As uptake by PV, which is unfavorable for phytoremediation of As-contaminated soils. In contrary, phytate coupled with As promoted both P and As uptake by PV, indicating a beneficial role of phytate in enhancing As uptake.

Phytate accounts for a large pool of unavailable P in soils. It is also the predominant form of P storage in plant seeds and grains. Furthermore, its production as root exudates has been detected in PV, which contributes to its efficient Fe and As solubilization from insoluble Fe-As minerals. However, phytate is a stable compound resistant to biochemical degradation, rendering it unavailable for plant root uptake. The fact that increased P uptake by PV in phytate and/or As treatments implied that PV effectively used phytate as a P source with or without As.

Iron uptake was unaffected in PV but decreased in PE possibly due to the formation of poorly soluble Fe-phytate in media, indicating the greater efficiency of PV in Fe utilization in presence of phytate than PE. As such, it was possible that the enhanced As and/or P uptake together with the unsuppressed Fe uptake in presence of phytate induced greater plant growth in PV than PE. The enhanced As accumulation and growth promotion may help efficient phytoremediation of As-contaminated soils. Grown on As-contaminated soils with phytate also has the benefit of no need for P fertilizers during phytoremediation.

Information obtained from this study may enhance our understanding of the As hyperaccumulation mechanisms in PV, which may be of importance since the ubiquity of phytate in PV rhizosphere microniche and its potential effects on plant As uptake and plant growth. This may help to develop strategies for more efficient phytoremediation of As-contaminated soils using PV.

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