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Arsenic-induced nutrient uptake in As-hyperaccumulator *Pteris vittata* and their potential role to enhance plant growth

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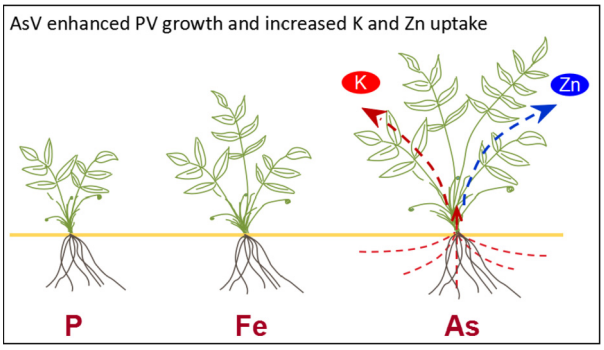
HIGHLIGHTS

- Studied As on growth and nutrient uptake of three *Pteris* ferns under sterile media.
- As-hyperaccumulators (*P. vittata* and *P. multifida*) were included.
- Non-hyperaccumulator (*P. ensiformis*) was used as control.
- Arsenic, Fe and P increased hyperaccumulator biomass.
- As-induced K and Zn uptake may help promote growth of hyperaccumulators.

GRAPHICAL ABSTRACT



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ABSTRACT

It is known that arsenic (As) promotes growth of As-hyperaccumulator *Pteris vittata* (PV), however, the associated mechanisms are unclear. Here we examined As-induced nutrient uptake in *P. vittata* and their potential role to enhance plant growth in sterile agar by excluding microbial effects. As-hyperaccumulator *P. multifida* (PM) and non-hyperaccumulator *P. ensiformis* (PE) belonging to the *Pteris* genus were used as comparisons. The results showed that, after 40 d of growth, As induced biomass increase in hyperaccumulators PV and PM by 5.2–9.4 fold whereas it caused 63% decline in PE. The data suggested that As played a beneficial role in promoting hyperaccumulator growth. In addition, hyperaccumulators PV and PM accumulated 7.5–13, 1.4–3.6, and 1.8–4.4 fold more As, Fe, and P than the non-hyperaccumulator PE. In addition, nutrient contents such as K and Zn were also increased while Ca, Mg, and Mn decreased or unaffected under As treatment. This study demonstrated that As promoted growth in hyperaccumulators and enhanced Fe, P, K, and Zn uptake. Different plant growth responses to As among hyperaccumulators PV and PM and non-hyperaccumulator PE may help to better understand why hyperaccumulators grow better under As-stress.

1. Introduction

Arsenic (As) is of environmental concern due to its toxicity and

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carcinogenicity (Mass et al., 2001). In soils, As is often present in its oxidized form arsenate (AsV), which is a chemical analogue for phosphate (P) (Meharg and Hartley–Whitaker, 2002). *Pteris vittata* (PV; Chinese Brake fern) is the first-known As-hyperaccumulator (Ma et al., 2001). It tolerates soil As concentrations up to 1500 mg kg^{-1} and accumulates up to 23 g kg^{-1} As in the fronds, making it useful in phytoremediation of As-contaminated soils (Tu et al., 2002; Kertulis-Tartar et al., 2006).

The success of phytoremediation depends on many factors including plant biomass, and soil As concentration and availability to plants (Fitz and Wenzel, 2002; Cattani et al., 2009). However, high biomass production of hyperaccumulators is a key factor (Shelmerdine et al., 2009). Similar to other hyperaccumulators, the yield of *P. vittata* at 1.03–1.3 t ha^{-1} yr^{-1} is lower than crop maize at 2.4–5.2 t ha^{-1} yr^{-1} (Kertulis-Tartar et al., 2006; Shelmerdine et al., 2009; Ray et al., 2013). Therefore, it is important to explore ways to increase plant biomass to improve its phytoremediation efficiency.

Our previous studies observed that As, Fe, and P promoted PV growth (Liu et al., 2015, 2016). It is understandable that Fe and P promote plant growth since they are essential elements, however, it is unclear how As promotes plant growth. Arsenic, a main ingredient of pesticide, is toxic to plants. Literature showed that As is positively correlated to K, Na, La, and Sm, but negatively correlated to Ca, suggesting that As affects element accumulation by PV (Wei et al., 2006; Wei and Zhang, 2007). Thus, it would be interesting to study the effects of toxicant As and nutrients Fe and P on plant growth of hyperaccumulator and non-hyperaccumulator plants.

In this study, besides *P. vittata*, hyperaccumulator *P. multifida* (PM) and non-hyperaccumulator *P. ensiformis* (PE) were included to examine the impacts of As, Fe, and P on plant growth in sterile system to exclude microbial effects. In addition, we emphasized the effects of As on nutrient uptake to better understand the mechanisms of As-induced plant growth in hyperaccumulators. The specific objectives were to: 1) compare the growth responses of three plants to As, Fe, and P; 2) examine the accumulation of As, Fe, and P; and 3) assess the influence of As on plant uptake of other nutrients (Ca, K, Mg, Mn, Zn, and Ni). Information obtained from the study helps to remove more As from contaminated sites by optimizing nutrient status to enhance plant growth.

2. Materials and methods

2.1. Spore sterilization and gametophyte culture

Spores of three ferns were surface-sterilized by immersion in 10% sodium hypochlorite and 75% ethanol for 30 min, followed by several rinses in sterile DI water (Zhu et al., 2011; Lessl et al., 2013). Sterilized spores were grown in Petri dishes with Murashige and Skoog (MS) solid media. The MS media were autoclaved, containing (mg L^{-1}): KNO_3, 1900; NH_4NO_3, 1650; KH_2PO_4, 170; $MgSO_4 \cdot 7H_2O$, 370; $CaCl_2 \cdot 2H_2O$, 440; KI, 0.83; H_3BO_3, 6.2; $MnSO_4 \cdot 4H_2O$, 22.3; $ZnSO_4 \cdot 7H_2O$, 8.6; $Na_2MoO_4 \cdot 2H_2O$, 0.25; $CuSO_4 \cdot 5H_2O$, 0.025; $CoCl_2 \cdot 6H_2O$, 0.025; $Na_2EDTA \cdot 2H_2O$, 37.3; $FeSO_4 \cdot 7H_2O$, 27.8; myo-inositol, 100; glycin, 2; thiamine·HCl, 0.1; pyridoxine·HCl, 0.5; nicotinic acid, 0.5; sucrose, 30000; agar, 7000; pH 6.0 (Mathews et al., 2010).

Spores were suspended in 2 mL sterile DI water and uniformly dispersed on agar (500 μL per plate) in sterile Petri dishes (100 mm × 13 mm) for germination. Petri dishes were placed in a growth chamber under warm fluorescent lamps with 14 h photoperiod and a light intensity of 350 μmol m^{-2} s^{-1}, at ca. 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 (Mathews et al., 2010).

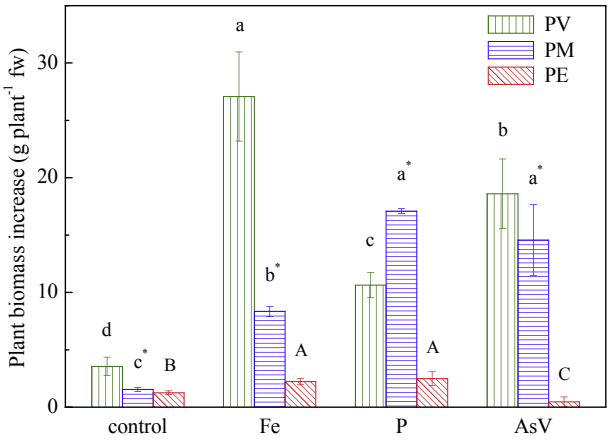

Fig. 1. The increase in plant fresh biomass after 40 d of growth in *P. vittata* (PV), *P. multifida* (PM) and *P. ensiformis* (PE) on low-Fe and low-P (0.014 mM Fe and 0.13 mM P) MS media with following treatments: (1) control; (2) 0.1 mM Fe; (3) 1.25 mM P; and (4) 0.2 mM AsV. Standard error of triplicates and means marked with different letters indicate significant differences ($p < 0.05$).

2.2. Utilization of nutrients and plant growth analysis

In this study, a modified MS medium was used, with Fe and P concentrations being lowered to 0.014 mM Fe and 0.13 mM P to ensure plant needs. This medium was mixed thoroughly with As, Fe or P ($Na_2HAsO_4 \cdot 7H_2O$, KH_2PO_4, or $FeSO_4 \cdot 7H_2O$; Sigma-Aldrich, St. Louis, USA; Aladdin Industrial Inc., Shanghai, China). Iron and P concentrations were spiked to 0.1 and 1.25 mM, the original levels in MS medium. Arsenic concentration at 0.2 mM was based on previous study showing 0.13–0.17 mM As in the PV rhizosphere porewater, which was similar to 0.13–0.65 mM in previous hydroponic experiments (Fayiga et al., 2008; Xu et al., 2014). More importantly, 0.2 mM As is below 0.27 mM, which caused toxicity symptom in three plants (Liu et al., 2016).

After 14 months of preculture, plants with 10–12 leaves and 3–4 cm in size were used for experiment. The plant-free media were used to examine the recoveries of As, Fe or P, which were 98.5–100%. Each treatment was performed in triplicate and all plants were grown in an incubator with the same conditions described above. During treatment, no renewing of the media was performed and no evidence of bacterial growth was found.

2.3. Chemical analysis

Plants were allowed to grow in the media for 40 d before harvest. Fresh weight was recorded before and after the experiment to monitor the increase in plant biomass. Plant roots were washed in ice-cold phosphate buffer (1 mM Na_2HPO_4, 10 mM MES and 0.5 mM $Ca(NO_3)_2$, pH 5.7) and DI water to remove surface adsorbed elements. Plant samples were then separated into the fronds and roots, immediately lyophilized (FreezZone 12, LABCONCO) and stored at −80 °C before further analysis. Media samples of different treatments were analyzed for As and nutrients concentrations.

Growth media and freeze-dried plant materials were digested with HNO_3/H_2O_2 (USEPA method 3050B) (Tu et al., 2004). Total As, Zn, Ni, Mn, K, and Mg concentrations in plant and media digest were determined by inductively coupled plasma mass spectrometry (ICP–MS; NexION300X, PerkinElmer). Standard reference material Hunan rice GBW10045 was included for concentration

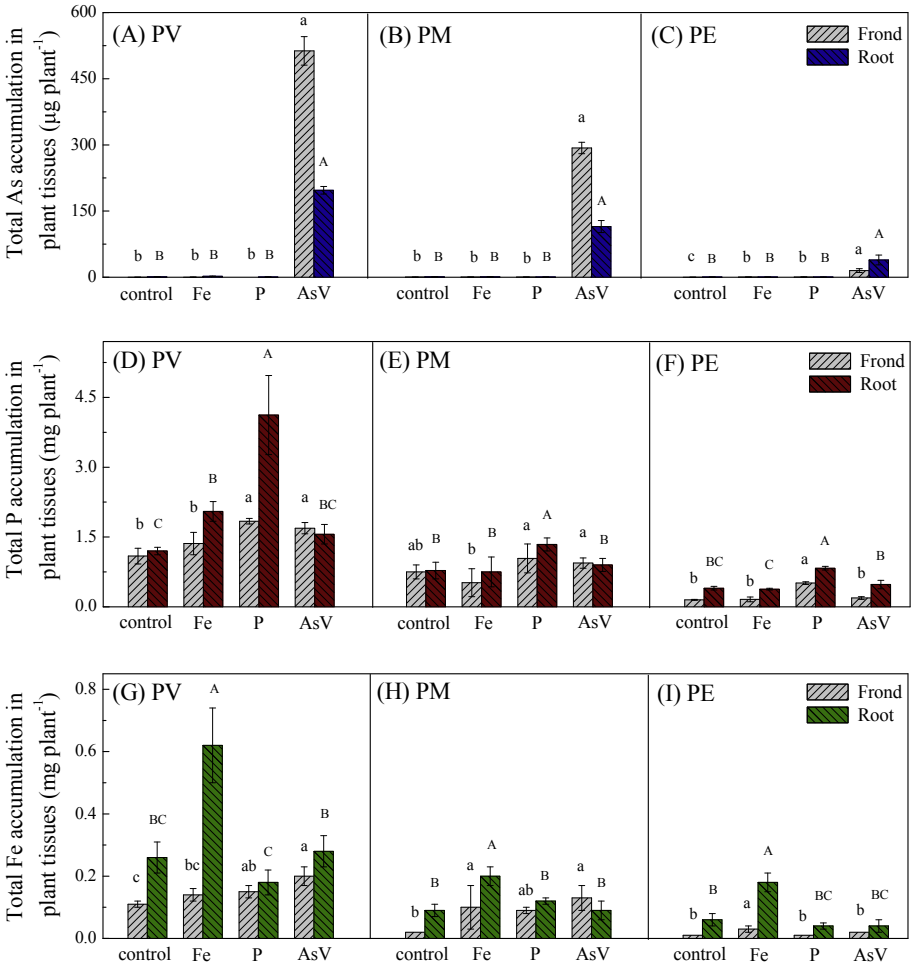

Fig. 2. Plant total As (A–C), P (D–F), and Fe (G–I) accumulation in As-hyperaccumulators *P. vittata* (PV) and *P. multifida* (PM) and non-hyperaccumulator *P. ensiformis* (PE) after 40 d of growth on low-Fe and low-P MS media amended with 0.1 mM Fe, 1.25 mM P, or 0.2 mM AsV. The low-Fe and low-P (0.014 mM Fe and 0.13 mM P) MS medium was used as a control. Standard error of triplicates and means marked with different letters indicate significant differences ($p < 0.05$).

Table 1
Soluble As, P, and Fe concentrations in the growth media after 40 d of growth in As-hyperaccumulators *P. vittata* and *P. multifida* and non-hyperaccumulator *P. ensiformis* on low-Fe and low-P MS media amended with 0.1 mM Fe, 1.25 mM P, or 0.2 mM As. The low-Fe and low-P (0.014 mM Fe and 0.13 mM P) MS medium was used as a control.

	P. vittata			*P. multifida*			*P. ensiformis*		
Treatment	Soluble As (μM)	Soluble P (μM)	Soluble Fe (μM)	Soluble As (μM)	Soluble P (μM)	Soluble Fe (μM)	Soluble As (μM)	Soluble P (μM)	Soluble Fe (μM)
control	0.01 ± 0.00 a[a]	3.03 ± 0.26 c	BDL c	0.01 ± 0.00 b	4.68 ± 0.21 b	4.94 ± 0.74 b	0.01 ± 0.00 b	9.35 ± 0.32 b	2.19 ± 0.53 b
Fe	0.01 ± 0.00 a	2.10 ± 0.33 c	4.48 ± 0.75 a	BDL b	2.46 ± 0.15 b	59.2 ± 16.1 a	BDL b	9.35 ± 0.56 b	35.5 ± 3.96 a
P	0.01 ± 0.00 a	68.4 ± 11.1 a	3.88 ± 0.48 a	BDL b	577 ± 38.4 a	2.61 ± 0.35 b	BDL b	706 ± 87 a	3.98 ± 0.47 b
As	5.79 ± 0.89 a	8.98 ± 1.05 b	1.48 ± 0.37 b	83 ± 15 a	4.29 ± 0.37 b	2.30 ± 0.48 b	176 ± 7.4 a	10.0 ± 0.56 b	2.44 ± 1.19 b

[a] Standard error of three replicates and means marked with different letters indicate significant differences at $p < 0.05$; BDL = below detection limit.

assays for quality assurance and quality control. The As, Zn, Ni, Mn, K, and Mg concentrations obtained for GBW10045 were 0.116 ± 0.002, 14.2 ± 1.0, 0.317 ± 0.05, 9.12 ± 0.38, 718 ± 86, 252 ± 22 mg kg^{-1} (mean ± SD, n = 3), which were in good agreement with the certified values of 0.11 ± 0.002, 14.4 ± 0.8, 0.31 ± 0.04, 9.0 ± 0.4, 700 ± 100, and 250 ± 10 mg kg^{-1}. The internal standards were carried to ensure accuracy and precision. Standard solutions were measured every 20 samples to monitor the stability of ICP–MS. The average recoveries were 102–108%. Standards and samples were prepared and acidified in 0.1 M HNO_3 (Chen et al., 2016).

Phosphorus concentration in the same digest was analyzed with a modified molybdenum blue method after removing As 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 85 °C. The solution was cooled to room temperature and P was determined by the molybdenum blue method. Iron and Ca concentrations were analyzed with a flame atomic absorption spectrophotometer (FAAS; PinAAcle 900T, PerkinElmer). While plant biomass was expressed on a fresh weight basis, plant elemental concentrations were expressed on a dry weight basis.

2.4. Statistical analysis

Data are presented as the mean of three replicates with standard error. Analysis of variance and Duncan's multiple range tests were

Table 2
Plant dry biomass and As, P, and Fe concentrations in As-hyperaccumulators *P. vittata* and *P. multifida* and non-hyperaccumulator *P. ensiformis* after 40 d of growth on low-Fe and low-P (0.014 mM Fe and 0.13 mM P) MS media amended with 0.1 mM Fe, 1.25 mM P, or 0.2 mM AsV. The low-Fe and low-P MS medium was used as a control.

Treatment	Frond (dw) g plant^{-1}	Root (dw) g plant^{-1}	Frond As mg kg^{-1} dw	Root As mg kg^{-1} dw	Frond P mg kg^{-1} dw	Root P mg kg^{-1} dw	Frond Fe mg kg^{-1} dw	Root Fe mg kg^{-1} dw
P. vittata								
control	0.85 ± 0.10 b[a]	0.74 ± 0.05 d	0.15 ± 0.01 b	1.01 ± 0.18 b	1287 ± 128 c	1626 ± 148 b	135 ± 12 b	348 ± 50 a
Fe	1.34 ± 0.16 a	2.81 ± 0.21 a	0.13 ± 0.00 b	0.72 ± 0.08 b	1012 ± 65 c	731 ± 44 d	104 ± 15 b	219 ± 27 b
P	0.65 ± 0.06 c	1.06 ± 0.17 c	– b	0.50 ± 0.08 b	2850 ± 264 a	3857 ± 209 a	238 ± 49 a	167 ± 29 b
AsV	0.94 ± 0.05 b	1.42 ± 0.11 b	545 ± 16 a	139 ± 14 a	1797 ± 130 b	1093 ± 91 c	211 ± 38 a	199 ± 29 b
P. multifida								
control	0.56 ± 0.10 b	0.82 ± 0.13 b	0.45 ± 0.09 b	0.73 ± 0.08 b	1341 ± 116 b	940 ± 75 b	41 ± 10 c	104 ± 11 bc
Fe	0.46 ± 0.19 b	0.86 ± 0.19 b	0.54 ± 0.15 b	1.04 ± 0.07 b	1086 ± 178 c	844 ± 170 bc	213 ± 50 a	242 ± 41 a
P	0.55 ± 0.14 b	1.03 ± 0.05 b	0.65 ± 0.07 b	0.45 ± 0.07 b	1873 ± 68 a	1302 ± 97 a	163 ± 37 ab	116 ± 15 b
AsV	0.97 ± 0.06 a	1.37 ± 0.06 a	303 ± 33 a	84 ± 6.7 a	960 ± 59 c	656 ± 72 c	134 ± 39 b	67 ± 17 c
P. ensiformis								
control	0.12 ± 0.01 b	0.41 ± 0.03 a	1.19 ± 0.18 b	0.99 ± 0.14 b	1265 ± 61 b	985 ± 77 b	83 ± 14 b	136 ± 27 b
Fe	0.19 ± 0.04 a	0.38 ± 0.02 a	1.69 ± 1.63 b	0.72 ± 0.09 b	803 ± 88 c	987 ± 83 b	148 ± 26 a	461 ± 65 a
P	0.15 ± 0.02 b	0.34 ± 0.05 a	2.59 ± 0.81 b	0.61 ± 0.13 b	3485 ± 322 a	2471 ± 339 a	101 ± 12 b	113 ± 8 b
AsV	0.16 ± 0.02 ab	0.38 ± 0.06 a	97 ± 35 a	102 ± 15 a	1205 ± 23 b	1265 ± 48 b	131 ± 16 a	111 ± 27 b

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

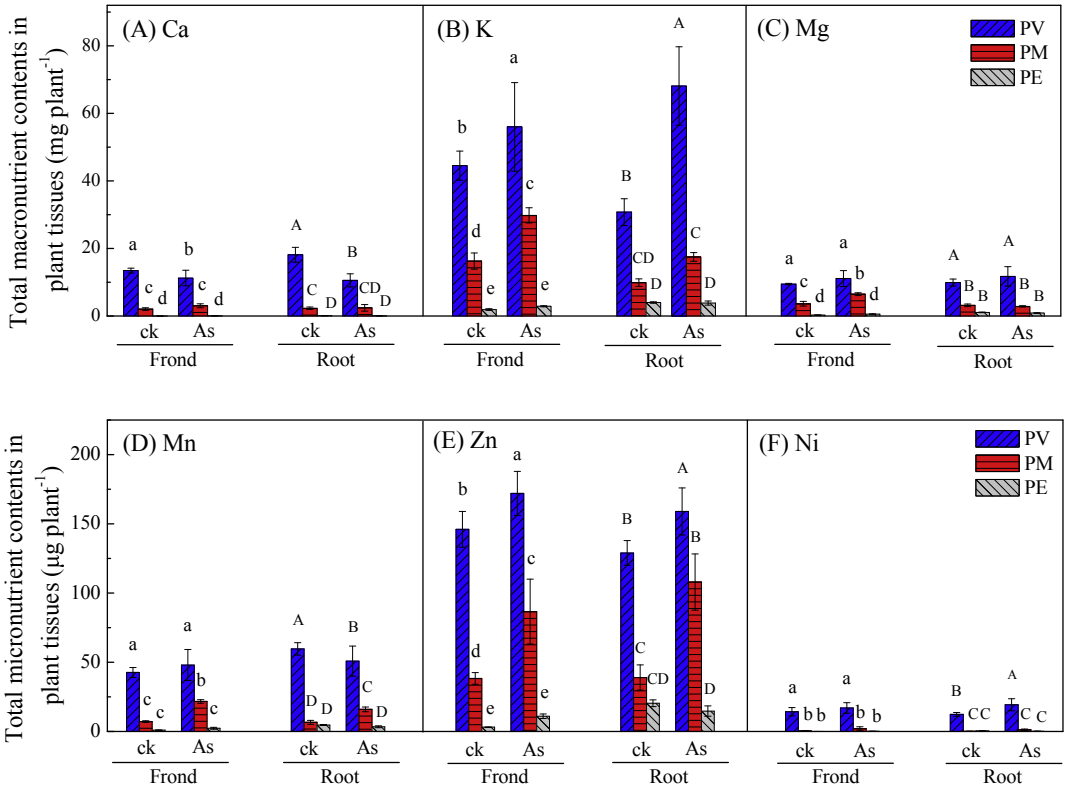

Fig. 3. Effect of As on total Ca (A), K (B), Mg (C), Mn (D), Zn (E), and Ni (F) accumulation in biomass of two hyperaccumulators (*P. vittata* and *P. multifida*) and non-hyperaccumulator (*P. ensiformis*) after 40 d of growth on low-Fe and low-P (0.014 mM Fe and 0.13 mM P) MS with or without 0.2 mM As. Standard error of triplicates and means marked with different letters indicate significant differences ($p < 0.05$).

used to determine significance of the interactions between treatment means. All statistical analyses were performed with SAS statistical software (SAS Inst., Cary, North Carolina, USA) and all figures were drawn using Origin (version 8.0).

3. Results and discussion

3.1. Effects of As, Fe, and P on plant growth

After growing in sterile media for 40 d, all three plants showed little toxicity symptom. Compared to the control, addition of As, Fe or P induced greater plant growth in hyperaccumulators (PV and PM), but As decreased plant growth in non-hyperaccumulator PE (Fig. 1; $p < 0.05$). In addition, hyperaccumulators PV and PM gained much more growth than PE in all treatments.

Among treatments, the best plant growth was observed in PV in Fe-treatment, 27.1 compared to 3.56 g plant^{-1} in the control (Fig. 1). Though less than Fe-treatment, plant biomass increase at 18.6 g plant^{-1} in As-treatment was greater than that of P-treatment at 10.6 g plant^{-1}, suggesting As was more effective in enhancing PV growth than P. Different from PV, hyperaccumulator PM gained best growth in P-treatment at 17.1 g plant^{-1}, but it was lower than those of PV. Similarly, As enhanced PM growth (14.3 vs. 1.55 g plant^{-1}), but it decreased PE growth from 1.3 to 0.46 g plant^{-1}. The

Table 3
Effect of arsenate on macronutrient concentrations (mg g^{-1}) and micronutrient concentrations (mg kg^{-1}) in plant tissues of two hyperaccumulators (*P. vittata* and *P. multifida*) and the non-hyperaccumulator (*P. ensiformis*).

	Ca		K		Mg		Mn		Zn		Ni	
	Frond	Root	Frond	Root	Frond	Root	Frond	Root	Frond	Root	Frond	Root
P. vittata												
control	5.72 ± 0.71 a[a]	8.11 ± 0.93 a	18.8 ± 1.45 c	13.8 ± 1.46 b	4.02 ± 0.36 c	4.44 ± 0.43 a	18.1 ± 2.88 ab	26.7 ± 1.48 a	61.7 ± 2.26 b	57.5 ± 2.38 bc	6.05 ± 1.13 a	5.51 ± 0.46 b
As	4.93 ± 0.66 a	4.05 ± 0.55 b	24.5 ± 4.00 b	26.1 ± 3.46 a	4.86 ± 0.68 b	4.49 ± 0.91 a	21.0 ± 3.47 a	19.4 ± 2.82 b	75.8 ± 1.77 ab	61.2 ± 4.43 b	7.51 ± 1.74 a	7.37 ± 1.18 a
P. multifida												
control	3.62 ± 0.13 b	2.80 ± 0.12 c	29.1 ± 2.39 a	12.1 ± 1.11 bc	6.47 ± 0.53 a	3.89 ± 0.15 a	12.9 ± 1.22 c	7.99 ± 0.58 e	69.0 ± 8.85 ab	47.0 ± 3.72 cd	0.77 ± 0.07 b	0.17 ± 0.05 c
As	3.14 ± 0.78 b	1.76 ± 0.65 d	30.7 ± 1.80 a	12.9 ± 1.40 bc	6.67 ± 0.44 a	2.08 ± 0.14 b	22.3 ± 1.59 a	11.8 ± 1.62 c	88.7 ± 21.4 a	79.0 ± 13.0 a	2.10 ± 1.26 b	0.88 ± 0.39 c
P. ensiformis												
control	0.11 ± 0.01 c	0.23 ± 0.01 e	15.8 ± 0.24 c	9.87 ± 0.21 c	2.28 ± 0.18 d	2.66 ± 0.13 b	7.81 ± 0.57 d	11.5 ± 0.53 cd	26.0 ± 3.54 c	50.0 ± 2.11 bc	0.40 ± 0.06 b	0.94 ± 0.14 c
As	0.29 ± 0.04 c	0.11 ± 0.02 e	18.7 ± 1.71 c	10.0 ± 0.53 c	3.41 ± 0.45 c	2.31 ± 0.14 b	14.6 ± 2.95 bc	8.87 ± 0.43 de	70.5 ± 10.2 ab	38.9 ± 9.27 d	0.86 ± 0.12 b	0.40 ± 0.23 c

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

data showed that both Fe and P promoted PV and PM growth, with As showing similar impacts as Fe and P. The data indicated a beneficial role of As to enhance growth of As-hyperaccumulators.

3.2. Arsenic, Fe, and P concentration and distribution in plants

Plant biomass is an important factor for successful application of phytoremediation (Singh and Ma, 2006). To better understand the relation between elemental uptake and plant growth, Fe, P, and As concentrations in plants were determined. Table 2 compares As, Fe, and P concentrations in PV, PM, and PE tissues and Fig. 2 shows their total accumulation in three plants.

Arsenic at 0.2 mM or 15 mg L^{-1} increased its plant uptake, with As concentrations in PV and PM being significantly higher than that in PE (Table 2). The average As concentrations in the fronds and roots were 545 and 139 mg kg^{-1} for PV, and 303 and 84 mg kg^{-1} for PM, which were 3.1–5.6 and 1.4 fold that of PE (Table 2). The As accumulated in plants corresponded to the As depletion in media (Table 1). The initial As in media was 0.75 mg, with 0.71 mg being accumulated in PV (Fig. 2A) and 0.02 mg in media (Table 1), showing 95% of As was taken up by PV. The results showed that hyperaccumulators PV and PM with higher As accumulation had greater growth than non-hyperaccumulator PE, implying a correlation between As uptake and plant growth in hyperaccumulators. Besides, typical of As-hyperaccumulators, more As was accumulated in the fronds than the roots for PV and PM. For example, compared to 72% in PV and PM, PE translocated 27% of As from the roots to fronds (Fig. 2A–C).

Similar to As-treatment, plants in P-treatment accumulated more P than other treatments. Among three plants, PE frond P concentration was the highest at 3485 mg kg^{-1}, 1.2- and 1.9-fold that of PV and PM (Table 2). Though with higher P concentration, PE gained lower biomass increase than PV and PM (Fig. 1; Table 2), therefore lower total P content in plant (1.34 vs. 5.96 and 2.38 mg $plant^{-1}$) (Fig. 2D–F) and higher P in the growth media (706 vs. 68.4 and 577 μM) (Table 1).

Different from As and P, Fe concentration in PV in Fe-treatment was lower than the control (104 vs. 135 mg kg^{-1} for fronds and 219 vs. 348 mg kg^{-1} for roots) (Table 2). However, PV gained a greater biomass with Fe than the control (Fig. 1; Table 2), thus accumulating higher total Fe (Fig. 2G). In addition, different from efficient As translocation in hyperaccumulators (Fig. 2AB), most of Fe was stored in the roots (82% for PV and 67% for PM) (Fig. 2GH). Generally, Fe is transported to plant shoots for its role in chlorophyll synthesis (Marschner and Rimmington, 1996). Apparently, hyperaccumulator was inefficient in translocating Fe, which was consistent with 385 and 20 mg kg^{-1} Fe in PV roots and fronds observed by Ghosh et al. (2011). High root Fe concentrations in the tens of mg kg^{-1} range have also been found in other plants (Kosegarten and Koyro, 2001; Gramlich et al., 2013).

In soils, it has been reported that As induced growth in PV (Tu et al., 2002; Xu et al., 2014). Xu et al. (2014) attributed it to As-induced P uptake by PV. This is possible due to As-induced P release from soils, leading to more P uptake by plants (Gao and Mucci, 2001; Cao et al., 2004). However, this may not be the case in the present study since MS agar with low-Fe and low-P was used as the media. Besides, in a sterile system, rhizosphere bacteria don't play a role either. One possible explanation for As-induced growth of hyperaccumulators is that it may serve as a nutrient, which seems unlikely. Another possibility is that As induces nutrient uptake by the plant. In the medium with low-Fe and low-P, PV growth was also increased in As-treatment, suggesting factors beyond Fe and P. Therefore, elements (Ca, K, Mg, Mn, Zn, and Ni) were determined in plants to better understand elemental uptake and As-induced plant growth.

3.3. Arsenic enhanced K and Zn uptake by hyperaccumulators

Among nutrients, K and Zn uptake in hyperaccumulators PV and PM were enhanced in As-treatment while Ca uptake was decreased and Mg, Zn, and Ni uptake were not affected (Fig. 3; Table 3). Unlike PV and PM, K and Zn uptake by PE decreased in As-treatment. The different responses between hyperaccumulators PV and PM and non-hyperaccumulator PE to As exposure indicated that As-induced K and Zn uptake may play a role in As-induced plant growth. The important role of K in plant growth was also noticed by Komar (1999), relating the increased plant biomass with increases in K concentration in PV. While investigating As distribution in PV, Lombi et al. (2002) found that As and K were positively correlated ($R = 0.87$). Potassium is known to serve as a dominant cation to counterbalance anions in plants (Kumar et al., 2015). Thus, under As exposure, more K was taken up to counterbalance anions resulting from excessive As uptake. Besides, Cao et al. (2004) found that As uptake by PV enhanced Zn uptake. As such, it is possible that the increased K and Zn uptake under As exposure may be related to As-promoted plant growth in hyperaccumulators.

However, such an increase in plant nutrient uptake under As stress is different from typical plants. For example, Duan et al. (2013) reported that As limits Zn accumulation in rice grains. Norton et al. (2010) found that the decrease in grain Zn concentration is associated with the increase in grain As. Thus, enhanced uptake of plant nutrients by hyperaccumulators under As stress may imply its ability to copy with As stress, favorable for plant As accumulation.

4. Conclusions

Previous studies observed that As, Fe, and P enhanced growth of As-hyperaccumulator PV, however, the associated mechanisms are unclear. Therefore, in present study, a sterile system was introduced to investigate their effects on plant growth of two hyperaccumulators PV and PM and non-hyperaccumulator PE. The results showed that their growth responded differently to As, Fe, and P treatment. Hyperaccumulators PV and PM gained the largest biomass in Fe and P treatment (27.1 and 17.1 g $plant^{-1}$), with As being the second best (18.6 and 14.6 g $plant^{-1}$). Unlike PV and PM, PE showed lower biomass increase at 1.26 g $plant^{-1}$. As, Fe, and P increased their plant uptake by hyperaccumulators, which helped better plant growth compared to PE. In addition, As-induced K and Zn uptake may be related to As-induced plant growth in hyperaccumulators. Different plant growth responses to As among hyperaccumulators PV and PM and non-hyperaccumulator PE may help to better understand why hyperaccumulators grow better under As-stress.

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