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Do arsenate reductase activities and oxalate exudation contribute to variations of arsenic accumulation in populations of *Pteris vittata*?

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Abstract

Purpose Although arsenic (As) hyperaccumulation is a constitutive property for *Pteris vittata*, there is intraspecific variation in As accumulation among metallicolous (from As-contaminated soils) and nonmetallicolous populations (from uncontaminated soils) and the related mechanisms is still not clear.

Materials and methods Pot trials, hydroponic culture, and manual simulation were conducted to investigate the roles of arsenate reductase and root exudates in accumulating As in *P. vittata*, which were collected from two uncontaminated sites including Sun Yat-sen University campus, Guangdong Province (ZD), and a botanical garden in Guangxi Academy of Forestry Sciences, Nanning City, Guangxi Province (NN), and two As and Pb/Zn mining and/or smelting sites located in Shaoguan of Guangdong Province (SG) and Guiyang of Hunan Province (GY).

Results and discussion The nonmetallicolous populations (ZD and NN) possessed more efficient uptake of arsenate and arsenite than the metallicolous populations (SG and GY). There were significant ($p < 0.05$) difference in arsenate reductase activities in roots among the four populations of *P. vittata* and that the higher arsenate reductase activities were recorded in the nonmetallicolous populations (110 nkat mg^{-1} protein for ZD, 160 nkat mg^{-1} protein for NN) compared with the metallicolous populations (62.9 nkat mg^{-1} protein for SG, 78.1 nkat mg^{-1} protein for GY). Root exudates from the nonmetallicolous population (NN) and the metallicolous population (GY) of *P. vittata* contained similar compositions of organic acids including oxalic, malic, and succinic acids, of which oxalate were dominant (> 67%). The NN population exuded 4.23 times more oxalate than the SG population. Root exudates from the NN population mobilized significantly ($p < 0.05$) more As from As-contaminated soils than those from the SG population, of which oxalate had the most effective in As mobilization.

Conclusions The present study suggests that higher arsenate reductase activities and oxalate exudation in the nonmetallicolous populations may play an important role in increasing their efficiency in phytoremediation of As-contaminated soils.

Keywords Arsenate reductase · Arsenic species · Chinese brake fern · Intraspecific variation · Oxalate secretion

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1 Introduction

Arsenic (As) is not only ubiquitous in the natural condition but also an environmental and food chain contaminant (Zhao et al. 2010). The first As hyperaccumulator identified, *Pteris vittata*, has offered hope that As-contaminated soils widely present could be coped with using efficient, environmentally friendly, cost-effective phytoextraction technology (Ma et al. 2001). However, in spite of important progress made in recent years, the complexity of hyperaccumulation is far from being understood (Rascio and Navari-Izzo 2011) and potential use of hyperaccumulators in phytoremediation is limited.

P. vittata has received considerable attention for years. Arsenic is mainly distributed (78–96%) in fronds of *P. vittata* (Ma et al. 2001), and is mainly stored in the vacuoles (Lombi et al. 2002; Pickering et al. 2006). Over 85% As in fronds are in the form of arsenite [As(III)], and the remaining mostly as arsenate [As(V)], minimum as dimethylarsinic acid (DMAA) and monomethylarsonic acid (MMAA) (Ma et al. 2001; Wang et al. 2002). *P. vittata* has effective reduction of As(V) to As(III) to detoxify As once taken into the plant (Poynton et al. 2004; Su et al. 2008). After 1 h of exposure to As(V), As(III) could be determined in *P. vittata* (Su et al. 2008). Arsenate reductase in roots plays an important role in the detoxification of As in the fern (Duan et al. 2005; Liu et al. 2009). Recently, two arsenate reductase proteins including PvACR2 and Pv2.5–8 have been identified from sporophytes and gametophytes of *P. vittata*, respectively (Cesaro et al. 2015). *P. vittata* could be present in many countries and regions (Ma et al. 2001; Chen et al. 2002; Zhao et al. 2002; Visoottiviseth et al. 2002; Wu et al. 2007). There is intraspecific variation in As accumulation among populations of *P. vittata*, although As hyperaccumulation is a constitutive property (Wu et al. 2009; Wan et al. 2013). Our previous work indicated that nonmetallicolous populations (from uncontaminated soils) of *P. vittata* possessed more effective As accumulation than metallicolous populations (from As-contaminated soils) (Wu et al. 2015). However, it is not clear whether the metallicolous and nonmetallicolous populations of *P. vittata* from contrasting conditions possess similar or different mechanisms of arsenate reduction.

Plants generally release root exudates to response to nutrient deficiencies, inorganic ion stresses, or interact with neighbors in rhizosphere soil (Ryan et al. 2001). Root exudation represents a significant carbon cost to the plant, with young seedlings typically exuding about 30–40% of their fixed carbon as root exudates (Whipps 1990). Among root exudates, organic acids are the most common and important due to their ability to mobilize nutrients and heavy metals (Bais et al. 2006). It has been suggested that the organic acids secreted from the roots of *P. vittata* were highly efficient in solubilize As in soils and apparently enhanced As accumulation by the fern (Tu et al. 2004; Liu et al. 2016). However, the role of organic acids in metal accumulation in hyperaccumulators remains controversial. Zhao et al. (2001) found that root exudates of *Noccaea caerulescens* (a Zn/Cd hyperaccumulator) were not involved in Zn and Cd hyperaccumulation. Therefore, the contribution of root exudates to As accumulation in *P. vittata* need further research.

The main objectives of the present study were to (1) investigate the variation of As accumulation and arsenate reductase activities among four populations of *P. vittata*, (2) determine the differences in organic acids secreted from roots of the nonmetallicolous and metallicolous populations of *P. vittata*, and (3) quantify the ability of root exudates to As mobilization in soils.

2 Materials and methods

2.1 Spores of *P. vittata* collection

From November to December 2014, spores of *P. vittata* and corresponding rhizosphere soils were collected from two uncontaminated sites including Sun Yat-sen University campus, Guangdong Province (ZD), and a botanical garden in Guangxi Academy of Forestry Sciences, Nanning City, Guangxi Province (NN), and two As and Pb/Zn mining and/or smelting sites located in Shaoguan of Guangdong Province (SG) and Guiyang of Hunan Province (GY). The spores and soil samples at each site were collected from at least four plots (5 × 5 m). Each plot was sampled from three random points. The spores were air-dried and reserved for the following plant culture. Both SG and GY were heavily contaminated by As (24,618 and 28,933 mg kg^{-1}), Pb (6339 and 3273 mg kg^{-1}), and Zn (5791 and 10,424 mg kg^{-1}), while the corresponding metal concentrations at ZD and NN were below the class II standards of Chinese soil environmental quality (GB15618–1995) (Table S1, Electronic Supplementary Material).

2.2 Plant culture

The spores of each population of *P. vittata* were sprinkled on arable land soils in a plastic tray, respectively. The tray was covered with plastic cling film to maintain moisture. After germination, the sporelings were fertilized biweekly with 20% Hoagland's nutrient solution (Hoagland and Arnon 1938): 0.2 mM KH_2PO_4, 1.0 mM KNO_3, 1.0 mM $Ca(NO_3)_2{\cdot}4H_2O$, 0.4 mM $MgSO_4{\cdot}7H_2O$, 9 μM H_3BO_3, 1.8 μM $MnCl_2{\cdot}4H_2O$, 0.15 μM $ZnSO_4{\cdot}7H_2O$, 0.07 μM $CuSO_4{\cdot}5H_2O$, 0.03 μM $H_2MoO_4{\cdot}H_2O$, and 4 μM Fe-EDTA. Two-month-old sporelings (with two to three fronds) of *P. vittata* were transplanted to another plastic tray for further growth and the following experiments. Both spore germination and plant culture were performed in a greenhouse with temperature control (28/23 °C, day/

night). In addition to natural sunlight, a 12-h photon flux density of 380 μmol m^{-2} s^{-1} was supplied via an assembly of cool-white fluorescent lamps.

2.3 Pot trial

As-contaminated soils were collected from abandoned rice fields located in Shantou City, Guangdong Province. Their selected physical and chemical properties were as follows: pH = 7.49 (soil:deionized water = 1:5) and 251 mg As kg^{-1}, 125 mg Pb kg^{-1}, and 144 mg Zn kg^{-1}.The soils were air-dried and sieved through a 2-mm mesh. Two three-month-old sporelings of each of the four populations (NN, ZD, SG, and GY) of *P. vittata* were transferred to each pot, each of which being filled with 2.0 kg of the soils. The fern were watered with deionized water every 2 days to maintain 70–80% water-holding capacity (WHC) and fertilized with 20% Hoagland's nutrient solution at weekly intervals. After 4 months, fronds and roots of *P. vittata* were harvested separately, washed thoroughly with tap water, and rinsed with deionized water to remove any soil/substrate particles attached. Part of samples was homogenized to powder with liquid nitrogen. The frozen powder was stored at − 80 °C for analysis of arsenate reductase activity and As species. The rest of the samples was oven-dried at 70 °C for 3 days for the analysis of total As.

2.4 Root exudate collection

Three-month-old sporelings (with four to six fronds) of each of the two populations (NN and GY) previously cultured were transplanted to polyethylene pots filled with 2.0 L 20% modified Hoagland's nutrient solution (Hoagland and Arnon 1938). The plants were mounted on floating polyfoam boards with their roots suspended in the solution. To ensure plant uniformity, only sporelings with similar fresh weight (1–2 g) and root length (8–12 cm) were selected for the experiments. The pH of the nutrient solution was adjusted to 6.0 (using dilute HCl or NaOH), aerated vigorously, and renewed twice per week to prevent depletion of metals and nutrients. After growing in hydroponic systems for 6 weeks, the sporelings of each population of *P. vittata* were exposed to 20% Hoagland's nutrient solution subjected to 0, 200, or 2000 μM As(V) ($Na_2HAsO_4{\cdot}7H_2O$) or As(III) ($NaAsO_2$). There were two sporelings in each pot and four replicates for each treatment. After 2 days of exposure, root exudates were collected according to methods described by Tu et al. (2004). Briefly, plant roots were soaked in 30 mg L^{-1}chloramphenicol for 2 h to minimize microbial growth and washed with sterile Milli-Q water. The sporelings were subsequently transferred to 500 mL of sterile Milli-Q water to collect root exudates for 6 h. The root exudate was immediately filtered, lyophilized to 25 mL, and stored at − 80 °C for further analysis.

After collection of root exudates, the fern were divided into fronds and roots and rinsed with tap water. The roots were then incubated in an ice-cold phosphate buffer [containing 1 mM Na_2HPO_4, 10 mM2-(N-morpholino) ethanesulfonic acid (MES) and 0.5 mM $Ca(NO_3)_2$] for 10 min to ensure desorption of As from surface and free space of the roots. Plant tissues were oven-dried at 70 °C for 72 h to constant weight.

2.5 Arsenic mobilization from As-contaminated soils by the root exudates

The As-contaminated soils used in the As mobilization were same as previously used as pot trials. Four milliliters of previously reserved root exudates collected from the fern exposed to 0 As was added to a centrifuge tube with 100 mg of the As-contaminated soil. Four milliliters of deionized water was used as a control. Fifty microliters of chloroform was added to prevent microbial decomposition of root exudates. The solutions were mechanically shaken at 25 °C for 24 h and centrifuged at 10,000*g* for 15 min. The supernatant was acidified using concentrated HNO_3 (50 μL) for total As determination. The net As mobilization from As-contaminated soil was calculated as follows:

$$\text{Net As-mobilization}\left(\text{mg kg}^{-1}\text{g}^{-1}\right) = \frac{\text{As}_S - \text{As}_{RE} - \text{As}_C}{\text{M}_{Soil} \times \text{Root}} \quad (1)$$

where As_S is the amount of As in the supernatant, As_{RE} is the amount of As contained in root exudates, As_C is the amount of As solubilized by control, M_{Soil} is the soil weight, and Root is the dry weight of roots.

2.6 Arsenic mobilization from As-contaminated soil by organic acids

Based on organic acids identified in the root exudates of the fern, the role of oxalic acid, malic acid, and succinic acid on As mobilization in the As-contaminated soils was investigated. Arsenic dissolution from the As-contaminated soil was carried out in a batch experiment. Into a 10-mL centrifuge tube, 100 mg of the soil was weighed. This was followed by adding 4 mL of oxalic, malic, or succinic acid solution with concentrations ranging from 0 to 10 mM and 50 μL of chloroform to inhibit microbial activity. Deionized water was used as a control. The centrifuge tube was capped, shaken mechanically at room temperature for 24 h, and centrifuged at 10,000*g* for 15 min. The supernatant was removed and acidified with 10 μL of concentrated HNO_3 for total As determination.

2.7 Chemical analysis

2.7.1 As determination in the plant

Analyses of total As in the plant were conducted by digesting the plant tissues with an acid mixture [concentrated HNO_3 + concentrated $HClO_4$ (5:1, *v*/*v*)] and determined with anatomic fluorescence spectrometer (AFS-9780, Beijing Haiguang Instruments Company, China). A standard reference plant material [GBW10023 (GSB-14)] from the National Research Center for Standards, China, was used for quality assurance. Recovery rate of the standard reference materials was within 85 ± 5%. For As(III) and As(V) in the plant, the samples were ground under liquid nitrogen and extracted with methanol:water (1:1 *v*/*v*) under sonication for repeated three times, and the three extracts were combined (Zhang et al. 2002). Total As in the extracts were determined with anatomic fluorescence spectrometer.

2.7.2 Determination of arsenate reductase activity in roots of *P. vittata*

The roots reserved were ground and homogenized with quartz sand in 6 mL buffer solution containing 50 mM3-(N-morpholino) propanesulfonic acid (MOPS) and 50 mM MES, which was adjusted to pH of 6.5 with NaOH. After the homogenate centrifuged at 10,000*g* for 30 min at 4 °C, the supernatant was filtered through filter paper. The filtrate was passed through Sephadex PD-10 desalting columns. Arsenate reductase activity in the roots was assayed using the methods described by Duan et al. (2005) and Liu et al. (2009).

2.7.3 Determination of organic acids in root exudates

Organic acids in root exudates reserved were analyzed by high-performance liquid chromatography (Shimadzu, LC-20A) equipped with a reversed-phase C_{18} anion -exchange analytical column and a multiwavelength UV detector at 210 nm. The mobile phase was 25 mM KH_2PO_4 (adjusted to pH 2.5 with concentrated ortho-phosphoric acid) at a flow rate of 1 mL min^{-1}. Individual organic acids in the root exudates were identified and calibrated by comparing retention times with those of standards prepared with known concentrations of oxalic, malic, succinic, and citric acids. The organic acid concentrations were expressed on a dry root weight (dwt) basis.

2.8 Statistical analysis

The differences in plant biomass, As concentrations, As accumulation, organic acid concentrations, and arsenate reductase activities among populations of *P. vittata* were assessed using Fisher's protected least significant difference after ANOVA. All statistical procedures were carried out using SAS 8.1 software. Unless indicated otherwise, all treatment means were tested for significant difference at $p < 0.05$.

3 Results

3.1 Variations of As accumulation in four populations of *P. vittata*

There was insignificant difference in frond biomass among four populations of *P. vittata* after growing As-contaminated soils for 4 months (Fig. 1a). The metallicolous populations (SG and GY) had significantly ($p < 0.05$) higher root biomass (0.33 and 0.54 g) than the nonmetallicolous populations (ZD and NN) (0.10 and 0.01 g). According to Figs. 1 and 2, there was significant ($p < 0.01$) difference in concentrations of total As, As(III), and As(V) in fronds of the four populations. The present study further showed that the nonmetallicolous populations exhibited a significantly ($p < 0.01$) higher As accumulation in fronds than the metallicolous populations in As-contaminated soils, which was contributed to higher As concentrations in fronds (Fig. 1). Generally, the highest concentrations of As(III) and As(V) in fronds were observed in the nonmetallicolous populations, while the lowest were recorded in the metallicolous populations (Fig. 2). Concentrations of As(III) and As(V) in the fronds of NN population were about 13.7- and 17.2-fold for those of SG population, respectively.

3.2 Arsenate reductase activities in roots of four populations of *P. vittata*

The present study indicated that there were significant ($p < 0.05$) difference in arsenate reductase activities in roots among the four populations of *P. vittata* (Fig. 3). Apparently higher arsenate reductase activities were recorded in the nonmetallicolous populations (110 nkat mg^{-1} protein for ZD, 160 nkat mg^{-1} protein for NN) compared with the metallicolous populations (62.9 nkat mg^{-1} protein for SG, 78.1 nkat mg^{-1} protein for GY). This is consistent with the results that the nonmetallicolous populations possessed higher As(III) concentrations in fronds than those in the metallicolous populations (Fig. 2a).

3.3 Arsenic accumulation in the two populations of *P. vittata* exposed to As(V) and As(III)

Based on the results of previous pot trials, sporelings of *P. vittata* were exposed to 0, 200, or 2000 μM As(V) or As(III) for 2 days in hydroponic culture for further investigating variations of As accumulation in the two populations (NN and SG). Table 1 shows that the nonmetallicolous

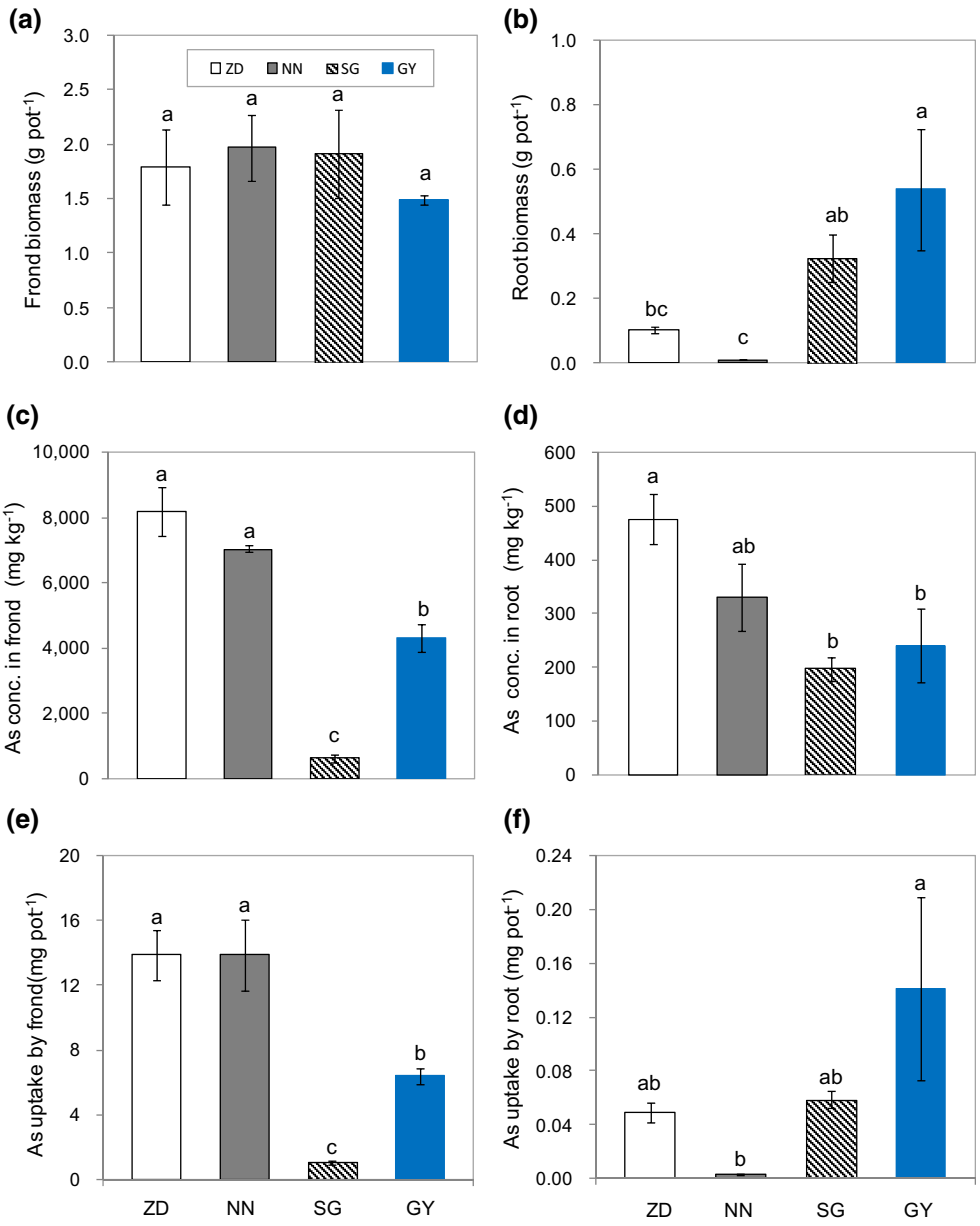

Fig. 1 Frond (**a**) and root (**b**) dry biomass, arsenic concentrations (**c**, **d**), and accumulation (**e**, **f**) in four populations (ZD, NN, SG, and GY) of *Pteris vittata* growing on As-contaminated soils for 4 months. Bars marked with different letters are significantly different among four populations according to least significant difference (LSD) test ($p < 0.05$) (mean ± SE, $n = 4$)

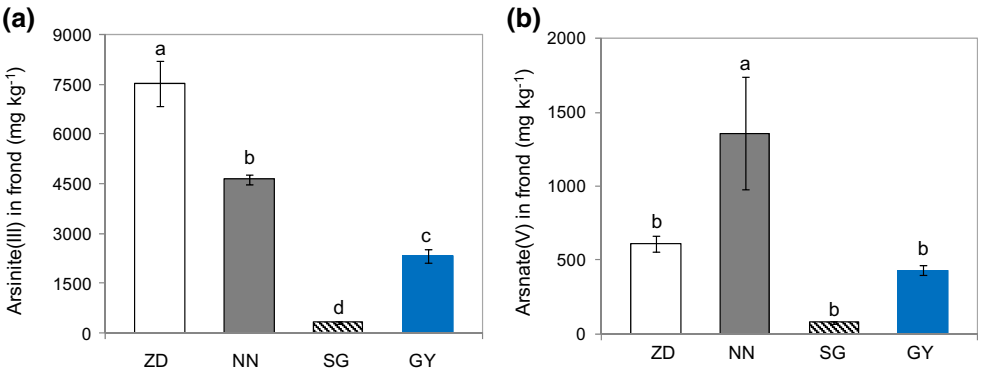

Fig. 2 Arsenite (**a**) and arsenate (**b**) concentrations in fronds of four populations (ZD, NN, SG, and GY) of *Pteris vittata* growing on As-contaminated soils for 4 months. Bars marked with different letters are significantly different among four populations according to least significant difference (LSD) test ($p < 0.05$) (mean ± SE, $n = 4$)

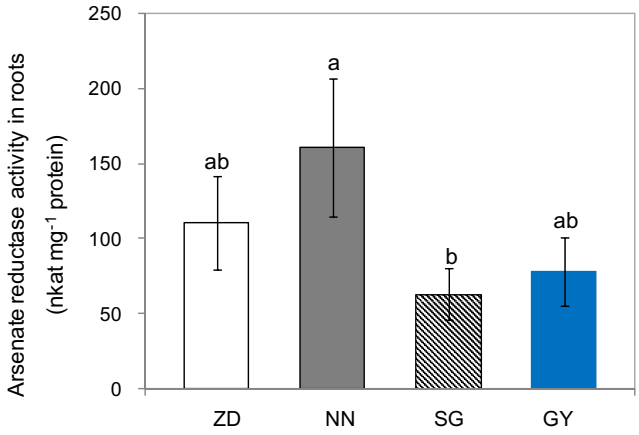

Fig. 3 Arsenate reductase activity in the roots of four populations (ZD, NN, SG, and GY) of *Pteris vittata* growing on As-contaminated soils for 4 months. Bars marked with different letters are significantly different among four populations according to least significant difference (LSD) test ($p < 0.05$) (mean ± SE, $n = 4$)

population (NN) possessed apparently higher frond As concentrations than the metallicolous population (SG) under any of As treatment. After exposure to 2000 μM As(V) or As(III), frond As concentrations in the NN population were significantly ($p < 0.01$) higher than those in the SG population. In addition, frond As concentrations under As(III) treatments (707–2258 mg kg^{-1}) were comparable with those under As(V) treatments (751–1366 mg kg^{-1}). These results further demonstrated that the nonmetallicolous population of *P. vittata* had a apparently higher accumulation capacity for As than the metallicolous populations, regardless of exposed to As(V) or As(III).

3.4 Pattern of root exudates

As shown in Table 1, root exudates from the two populations of *P. vittata* contained similar compositions of organic acids including oxalic, malic, and succinic acids. Oxalate was the predominant organic acid in the root exudates of the two populations of *P. vittata*, accounted for 67.2–97.7% of total organic acid secreted (Table 1). As expected, oxalate concentrations increased with increasing As concentrations in nutrient solution. For example, oxalate concentrations in root exudates of NN were 68.7 mg kg^{-1} under the control, which increased to 90.4 mg kg^{-1} under 2000-μM As(V) treatment (Table 1). Although As(III) is more toxic than As(V),the species of As seemed to have insignificant effects on oxalate secretion from fern plants (Table 1). The oxalate concentrations in root exudates of the NN population were significantly ($p < 0.05$) higher than those in the GY population, except those exposed to 2000-μM As(III) treatment. The oxalate concentrations in root exudates of the NN population (90.4 mg kg^{-1}) were 5.23 times of those in the SG population (17.3 mg kg^{-1}) under 2000-μM As(V) treatment, suggesting that oxalate exudation was related to efficiency of As accumulation in the former populations.

3.5 Arsenic mobilization from As-contaminated soils by root exudates and exogenous supply of organic acids

Root exudates from the NN population mobilized significantly ($p < 0.01$) more As from As-contaminated soils than those from the GY population (Fig. 4a), suggesting that the former

Table 1 Arsenic concentrations in fronds, oxalic acid, malic acid, and succinic acid concentrations in root exudates of the two populations (NN and GY) of *Pteris vittata* after growing for 2 days in 0.2-strength Hoagland solution containing 0, 200, or 2000 μM arsenate ($Na_2HAsO_4 \cdot 7H_2O$) or arsenite ($NaAsO_2$) (mean ± SE, $n = 4$)

Level (μM)	Population	As concentration in frond (mg kg^{-1})	Oxalic acid concentration (mg kg^{-1} d.wt)	Malic acid concentration (mg kg^{-1} d.wt)	Succinic acid concentration (mg kg^{-1} d.wt)
Arsenate					
0	NN	15.5 ± 3.27 a	68.7 ± 10.0 a	2.93 ± 1.21 a	0.66 ± 0.05 b
	GY	10.4 ± 3.14 ab	26.5 ± 4.96 b	3.83 ± 1.31 a	1.74 ± 0.16 a
200	NN	775 ± 44.8 a	86.6 ± 8.13 a	2.04 ± 0.00 ab	n.d.
	GY	751 ± 80.7 a	32.3 ± 5.99 b	4.19 ± 1.65 a	2.49 ± 0.48
2000	NN	1724 ± 118 a	90.4 ± 7.71 a	4.00 ± 0.09 a	1.33 ± 0.49 ab
	GY	1366 ± 73.1 b	17.3 ± 1.69 b	5.40 ± 1.66 a	3.04 ± 1.06 a
Arsenite					
200	NN	1005 ± 93.0 a	96.9 ± 3.08 a	4.12 ± 0.36 b	0.53 ± 0.03 b
	GY	707 ± 160 a	33.0 ± 10.4 b	7.15 ± 0.86 a	2.34 ± 0.43 a
2000	GY	2258 ± 15.4 a	59.5 ± 14.1 a	3.58 ± 0.56 a	0.41 ± 0.02
	NN	941 ± 91.1 b	34.8 ± 1.17 a	3.27 ± 0.65 a	n.d.

Different letters within the same column indicate significant difference between inoculated treatments at the level of $p < 0.05$

n.d. not detectable

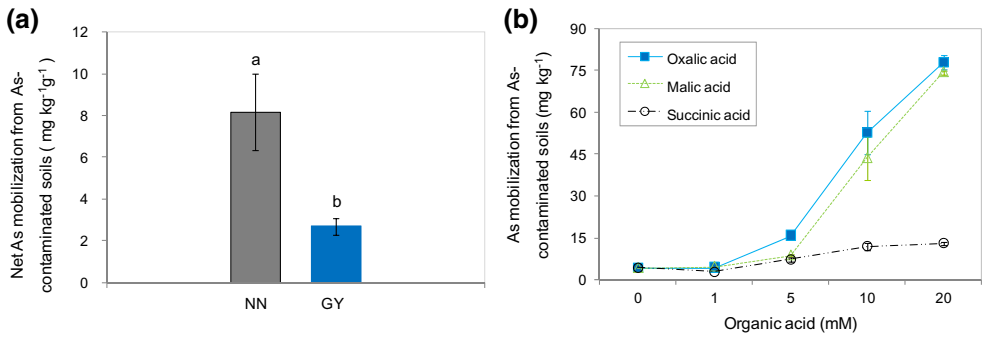

Fig. 4 Arsenic mobilization from As-contaminated soils by root exudates (**a**) collected from the two populations (NN and GY) of *Pteris vittata* growing in 0.2-strength Hoagland solution for 2 days and exogenous supply of organic acids (**b**) including oxalic acid, malic acid, and succinic acid. Bars marked with different letters are significantly different among four populations according to least significant difference (LSD) test ($p < 0.05$) (mean ± SE, $n = 4$)

possesses greater ability to solubilize As than the later. Oxalic, malic, and succinic acids were the three organic acids identified in the root exudates of *P. vittata* (Table 1). Therefore, the role of the three organic acids on As mobilization in soils was examined. As expected, net As mobilization from As-contaminated soils increased with increasing concentrations of organic acids (Fig. 4b). Among the three organic acids, oxalic acid was the most effective in As mobilization and succinic acid was the least.

4 Discussion

Based on the results of pot trials (Fig. 1) and hydroponic culture (Table 1), the present study showed that the nonmetallicolous populations of *P. vittata* possessed significantly higher As accumulation in the fronds than the metallicolous populations. This is in line with Wu et al. (2015), who showed that the nonmetallicolous populations of *P. vittata* possessed more effective As accumulation than the metallicolous populations. The nonmetallicolous populations exhibit apparent advantage for enhancing phytoextration of As-contaminated soils and the advantage need to investigate under field conditions. Furthermore, the present study indicated that the nonmetallicolous populations of *P. vittata* could uptake more As(III) compared to the nonmetallicolous populations. The As(III) influx fitted well with Michaelis–Menten kinetics in both nonmetallicolous and metallicolous populations of *P. vittata*, and higher value of V_{max} for As(III) was recorded in the nonmetallicolous populations (Wu et al. 2015). An aquapor in PvTIP4;1 mainly expressed in roots may be involved in As(III) uptake by *P. vittata* (He et al. 2016). Halimaa et al. (2014) found that expression of genes with possible contribution to Zn, Cd, and Ni hyperaccumulation and hypertolerance was difference between *Noccaea caerulescens* ecotypes. Molecular mechanisms of intravariations in As accumulation of *P. vittata* need further investigation.

The majority (> 67%) of As species in fronds was As(III) either in the nonmetallicolous or in the metallicolous populations, in line with the previous works (Wang et al. 2002; Su et al. 2008). Zhang et al. (2002) showed that conversion of As(V) to As(III) is an essential process for As detoxification in *P. vittata* because As(III) may be stored and sequestered in vacuoles to inhibit its toxicity to the plant (Lombi et al. 2002). In addition, the rate of As(III) to total As in fronds of the nonmetallicolous populations were apparently higher than that in the metallicolous populations (data not shown). Higher arsenate reductase activities were recorded in the nonmetallicolous populations compared with the metallicolous populations, which indicated that the former populations may be possess more effective capacity for conversion of As(V) to As(III) (Fig. 3). Su et al. (2008) found that roots are the main location of As(V) reduction in *P. vittata*. As(V) can be directly reduced to As(III) by arsenate reductase in roots of *P. vittata* (Duan et al. 2005). The present study demonstrated that metallicolous and nonmetallicolous populations of *P. vittata* from contrasting conditions (As-contaminated soils vs clean soils) possess similar mechanisms for As(V) reduction.

The composition of root exudates of *P. vittata* in the present study was consistent with Lou et al. (2010). Oxalate was the predominant organic acid in the root exudates of nonmetallicolous or metallicolous populations of *P. vittata*. Oxalate is typical organic acid in plant root exudates (Li et al. 2003; Zhu et al. 2011). Tao et al. (2016) also found that oxalate was the dominant organic acid in the root exudates of a Cd hyperaccumulator, *Sedum alfredii*. Phytic acid was not detected in root exudates of *P. vittata* in the present study, which was in line with Lou et al. (2010) and Das et al. (2017). However, Tu et al. (2004) and Liu et al. (2016) found that phytic acid was the predominant organic acid in the root exudates of *P. vittata*. The inconsistent composition of root exudates of *P. vittata* may be due to difference in the age of an individual plant and external factors like biotic and abiotic stressors. Valentinuzzi et al. (2015) found that the growth

stage of the plant and different trap solutions to collect root exudates markedly influenced the composition and concentration of organic acids in root exudates.

The present study showed that significant ($p < 0.05$) higher oxalate concentrations were recorded in the NN population, except 2000-μM As(III) treatment (Table 1). Similarly, Tao et al. (2016) observed variations in oxalate secretion between genotypes of the same plant species. They found that hyperaccumulating ecotype of *S. alfredii* had nearly 2-fold higher oxalate secretion than nonhyperaccumulating ecotype. Garcia et al. (2001) showed that root exudation is positively correlated with root growth of two Lupinus cultivars, which means that actively growing root systems secrete more exudates. Our previous study found that the nonmetallicolous populations of *P. vittata* possessed higher biomass and more actively growing roots than the metallicolous populations (Wu et al. 2015), which seemed to be resulted in higher oxalate secretion in the nonmetallicolous populations. Moreover, manual simulation indicated that root exudates from the NN population mobilized apparent more As from As-contaminated soils than that from the GY population, of which oxalic acid possessed the most effective in As mobilization (Fig. 4). It has been reported that oxalate could mobilize Cd and Al (Dytrtova et al. 2011; Morita et al. 2011). As discussed formerly, there were higher frond As concentrations and more amounts of oxalic acid recorded in the nonmetallicolous population, which suggested that As-stimulated oxalate secretion increased As uptake and accumulation in the fern and that exogenous oxalate supply maybe promoted As accumulation efficiently. The information obtained in the present study will be very valuable in revealing and explaining the physiology mechanisms of As accumulation and tolerance in *P. vittata*.

5 Conclusions

The present study indicated that the nonmetallicolous populations of *P. vittata* possess more effective capacity for conversion of As(V) to As(III) than the metallicolous populations. The present study identified oxalate as dominant organic acid from both nonmetallicolous and metallicolous populations of *P. vittata*. The nonmetallicolous populations with more As accumulation excreted higher concentrations of oxalate than the metallicolous populations. Our data also clearly showed the role of root exudates in releasing As from As-contaminated. The nonmetallicolous populations of *P. vittata* possessed more effective As accumulation than the metallicolous populations, which was contributed to higher arsenate reductase activities and higher concentrations of oxalate in the roots. Further investigating of intravariations of accumulating As in *P. vittata* and the associated molecular mechanisms may be useful to increase plant uptake of As, which has implication for more efficient phytoextration of As-contaminated soils.

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