Phosphorus- and Iron-Deficiency Stresses Affect Arsenic Accumulation and Root Exudates in *Pteris vittata*

Chongyang Yang, Mei-Fang Chien, Ying-Ning Ho, and Chihiro Inoue

***Abstract*—Phosphorus (P) and iron (Fe) are important elements for arsenic (As) transportation between plant and As contaminated soil. In the case of As phytoremediation, a better understanding of the roles to the P and Fe in regulating As bioavailability and plant growth will be beneficial to optimize the phytoremediation process. In this study, we demonstrated that both As absorption and translocation from root to shoot of *Pteris vittata* were enhanced under phosphorus deficiency. In high arsenic concentration treatments, *P. vittata* assimilated more As under nutrient deficiency. Moreover, As persuaded plants to secrete oxalic acid in sufficient nutrients. P- and Fe-deficiency stimulate more oxalic acid exuded by plants.**

***Index Terms*—Arsenic, *Pteris vittata*, Phosphate deficiency, Iron deficiency, Root exudates.**

I. Introduction

Arsenic (As) contamination has been a major issue worldwide, which commonly exists in natural water and soils threatening the human health [1]. With the expansion of crops to desert regions, groundwater is used for irrigation schemes increasing the As transmission risk and its accumulation in crops [2]. Arsenic exists in the environment in both inorganic and organic forms, and inorganic arsenicals are more toxic than organic forms [3]. Both arsenite (As(III)) and arsenate (As(V)) are often found in both anaerobic and aerobic soil environments [4]. The toxicity of As in soil is depended on complex metabolism of As which comprises reduction to a trivalent state and oxidative to a pentavalent state [5]. As(V) is a molecular analog of phosphate, which interferes with essential cellular processes such as oxidative phosphorylation and ATP synthesis [6]. As(III) is even more broadly toxic because its propensity to bind to sulfhydryl groups, impairing the function of general proteins [7]. It is critical to lessen its environmental impact.

Recently, phytoremediation has been established as a promising technology in remediation of heavy metal contaminated soil and water since it is cost-effective and eco-friendly [8]. *Pteris vittata* (fern) is the first discovered As hyperaccumulator, which can accumulate up to 22,630 mg As kg^{-1} in the shoot [9]. Considering its conspicuous ability of As detoxification and hyperaccumulation, the uptake and metabolism of As by *P. vittata* have drawn much attention.

P. vittata prefers to grow in calcareous soil and assimilate As(V) more effectively. However, As(V) has very strong binding affinity for Fe-(hydro)oxide, thereby presents an insoluble form and reduces the availability of both Fe and As [10]. In addition, As(V) is an analog of phosphate, competing for the same sorption sites and for the same uptake carriers in the root plasmalemma [4]. Hence, to establish the systems that the interaction effects between arsenate, phosphate and iron on As behavior may shed light on the uptake and metabolism of As by *P. vittata*.

Root exudates are often released to the root surface or into the rhizosphere, which is the important plant metabolites to enhance the mobilization of sparingly soluble nutrients in the rhizosphere [11], [12]. Under the nutrient deficient conditions, plants often secrete organic acids seeking for the nutrients to maintain the growth, which could release available As simultaneously to be absorbed by plants more easily. In this study, we focused on As accumulation and metabolite exudation of *P. vittata* induced by P- and Fe-deficiency to elucidate the interactions between As, P, and Fe and their effects on As uptake by *P. vittata*.

II. Materials and Methods

A. Hydroponic Plant Cultivation

Arsenic hyperaccumulator *P. vittata* was cultivated in hydroponic system. Six months old plug seedlings of ferns were supported by Fujita Co., Ltd (Tokyo, Japan). Plants were acclimatized in 0.2X Hoagland solution (HS) for 4-6 weeks, which were renewed weekly. The plants were grown under 16 h photoperiod in plant grow lights and at 25 ℃. Prior to As exposure, plants were treated in 0.2X HS, 0.2X modified HS with no phosphorus (KH_2PO_4), 0.2X modified HS with no iron (NaFe-EDTA) for 7 days.

B. Root Exudates and Arsenic Concentration Analysis

Acclimated plants were rinsed with Milli-Q water and transferred to 250 mL 0.2X HS, 0.2X modified HS with no P, 0.2X modified HS with no Fe and spiked with 0, 50, 500, or 5000 μg L^{-1} of As(V) with three replicates, respectively ($Na_2HAsO_4 \cdot 7H_2O$; Wako, Osaka, Japan). After 7 days of treatments, the plants were treated by washing the roots with Milli-Q water to remove surface adsorbed As. Root exudates were collected according Liu *et al.* [13]. Briefly, in order to restrain microbial growth of rhizosphere, plant roots were soaked in 30 mg L^{-1} antibiotic solution, chloramphenicol (Sigma-Aldrich, St. Louis, USA) for 2 h. And afterward, plants were transferred into the shading plastic flask contained 20 mL Milli-Q water, subsequently used to collect root exudates for 12 h. The collected solution was instantly filtrated by 0.45 μm filters and stored at 4°C. Organic acids in root exudates were measured by high performance liquid

Manuscript received April 20, 2019; revised September 12, 2019. This research was supported by the scholarship from China Scholarship Council (CSC, No. 201606630018). Part of this study was financially supported by Japan Society for the Promotion of Science (JSPS, No. 18J11625).

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chromatography (HPLC; PerkinElmer, Waltham, MA, USA). Plants were washed and separated into the fronds and roots, and then oven-dried at 50 ℃ for 1 week after air-dried at room temperature for 3 days. Plant dry biomass was recorded and then 0.1 g plant materials were digested with HNO_3 (Wako, Osaka, Japan) at 130 ℃ for 2 h. The As concentrations in plants were analyzed by NexION 2000 inductively coupled plasma mass spectrometer (ICP-MS; PerkinElmer).

C. Data Processing and Statistical Analysis

Translocation factor (TF) of As from roots to shoots and enrichment coefficient (EC) of As in a plant were calculated as follows [14]:

$$TF=\frac{[As]_{shoot}}{[As]_{root}} \quad (1)$$

$$EC=\frac{[As]_{plant}}{[As]_{background}} \quad (2)$$

All treatments were replicated three times in the experiments. The means and standard error (SE) were calculated. One-way analysis of variance (ANOVA) was carried out with SPSS 22.0. When a significant ($P < 0.05$) difference was observed between treatments, multiple comparisons were made by the LSD test.

III. Results and Discussions

A. Arsenic Concentration and Accumulation in Plants

The concentration of As in plants is shown in Table I. With the increasing the concentration of As in solution, *P. vittata* showed the much more uptake of As(V). The phosphorus (P) starvation treatment substantially increased the As concentration of shoots in *P. vittata* reaching up to 1539.63 mg kg^{-1} dry weight (Table 1). The concentration of As in the shoots of *P. vittata* is significantly high while spiked with high concentration of As (5000 μg L^{-1}) under the Fe-deficient condition. In all of the As treatments, the concentrations of As in the shoots were always greater than that in the roots, with the translocation factor (TF) varying from 1.57 to 9.40. Ferns with deficiency of P in nutrient solution showed the higher TF than that in sufficient nutrient solution and Fe deficiency except for the treatment with high As concentration (5000 μg L^{-1}) treatment. Considering the TF values, *P. vittata* was suitable for extracting As from contaminated sites. Similarly, the values of extraction coefficient (EC) that ranged from 156.13 to 3589.42 has also exhibited potential hyperaccumulation ability. The P starvation treatment showed a significantly higher extraction efficiency than other two treatments. The results indicated that *P. vittata* was more resistant to arsenic and can accumulate it with more As being translocated from the roots to the fronds. The P starvation can increase more As accumulation in ferns.

TABLE I: Arsenic Behavior in *P. vittata*

Conditions	[As] in solution (μg L^{-1})	[As] in shoots (mg kg^{-1})	[As] in roots (mg kg^{-1})	TF	EC
0.2X HS	0	0.49 ±0.05	0.31 ±0.03	1.57	NG
	50	9.17 ±1.03	3.18 ±0.13	2.88	435.05
	500	60.91 ±11.11	31.06 ±7.35	1.96	198.80
	5000	523.51 ±181.44	326.61 ±79.06	1.60	156.13
P deficiency	0	0.59 ±0.08	0.34 ±0.05	1.75	NG
	50	20.84 ±7.12	3.51 ±1.33	5.94	3234.90
	500	209.85 ±92.47	22.32 ±10.10	9.40	3589.42
	5000	1539.63 ±392.32	568.34 ±94.34	2.71	3345.58
Fe deficiency	0	0.72 ±0.22	0.43 ±0.02	1.68	NG
	50	7.33 ±1.40	2.62 ±0.10	2.80	250.01
	500	61.40 ±7.27	38.58 ±5.73	1.59	213.32
	5000	1247.05 ±398.43	253.09 ±40.04	4.93	524.85

On the basis of plants dry weight biomass, cumulative uptake of As(V) is shown in Fig.1. *P. vittata* exhibited the ability of As accumulation with the intensification of the As supplement. As accumulation in plants shoots was enhanced up to 828.32 μg $plant^{-1}$ in the P-deficient treatments, especially in solution with high As concentration (5000 μg L^{-1}) (Fig. 1a). No significance difference was observed in case of As accumulation in shoots for Fe-deficient treatments in solution spiked with low As concentration (0, 50, 500 μg L^{-1}). Nevertheless, As accumulation in shoots was higher in Fe-deficient treatments than that of in Fe-sufficient treatments spiked with high As concentration (5000 μg L^{-1}), up to 710.13 μg $plant^{-1}$. The As accumulation in plants roots is shown in Fig. 1b. It does no matter whether the treatment was nutrient sufficient, P-deficient, or Fe-deficient, As accumulation in roots showed nearly no significant difference in solution with As treatments since root is not the target site for contaminants' storage in hyperaccumulators, which is different from non-hyperaccumulators. However, with the treatments of high As concentration (5000 μg L^{-1}), As accumulation in roots displayed higher in P-deficient treatments than that of in nutrient sufficient and Fe-deficient treatments.

Phosphorus (P) and iron (Fe) are essential nutrient elements for plants, which affect plant growth and metabolism. The P is a major component of nucleic acids, phospholipids, and intermediary metabolites [15]. It has been reported that As(V), as a P analog, is taken up by plants via P transporter systems and both easily form a competitive relationship as a substrate for the phosphate uptake system

[16], [17]. In a hydroponic system, limitation of P can enhance As(V) uptake and accumulation by plants. Although As can substitute for P in plants, it cannot reveal the function of P in energy transfer [17]. During the pre-culture with P starvation, the low-availability P environment may evoke P deficiency responses in *P. vittata*. Once As(V), as P analog, is furnished, *P. vittata* may not be able to distinguish between them and mistakenly uptake As, thereby increasing the accumulation of As in plants [18]. Our results of inhibitory effect of P on plant As accumulation may support this hypothesis.

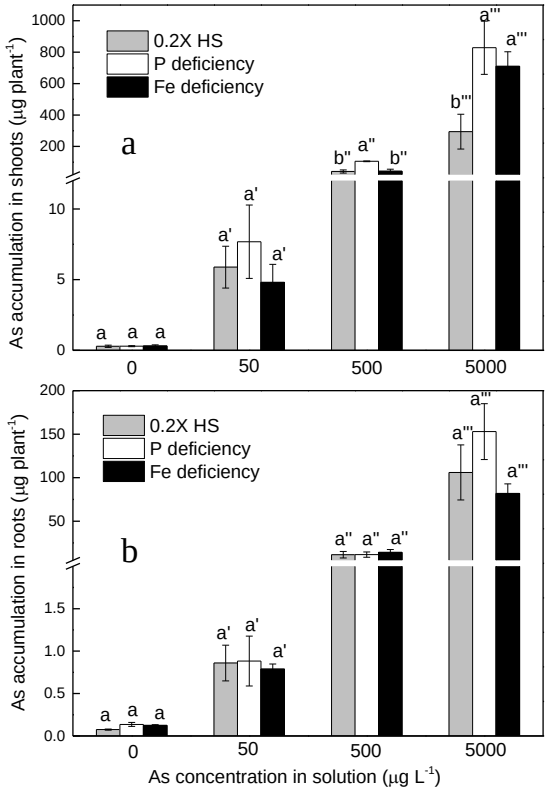

Fig. 1. As accumulation in shoots (a) and in roots (b) of *P. vittata* under the 0.2X HS, P-deficiency and Fe-deficiency conditions. Different letters on the bar indicate significant difference at p<0.05 levels.

The Fe is a constituent of several components of the cellular electron transport system (ETS) [19]. Deficiency of Fe may generate superoxide radical and other reactive oxygen species (ROS), bringing about oxidative damage [19]. However, a large amount of evidence in the literature suggests that Fe-deficient plants are better protected against oxidative damages, which may since catalytic Fe is favorable to generation of oxygen free radicals [20], [21]. That might be the reason behind the fact that no damage to ferns was happened, and As accumulation ability was not influenced by Fe-deficient treatments. Moreover, phytosiderophores, the chelating substances with specific affinity for Fe(III) in plants, exists in an unstable state under Fe-deficiency condition, which may cause disorder phenomenon in As accumulation by plants. Previous reports also found the plant accumulation of divalent-cation metals can be enhanced under Fe deficiency, which owing to that Fe-deficiency induced the expression of a Fe transporter gene, facilitating the transport of heavy-metal divalent cations [22]. As for trivalent-cation metalloids such as arsenic, it has not been reported.

B. Oxalic Acid Is Exuded from Plant Roots

Oxalic acid was determined as the most root exudates of *P. vittata* in different treatments, which is shown in Fig. 2. Oxalic acid showed a significant increase in treatments with adequate nutrition (0.2X HS) and As augmentation, varying from 3.45 to 13.80 mg g^{-1} root dry weight (DW). High concentration of oxalic acid was observed in treatments spiked with no and slight As under P- and Fe-deficient conditions, with maximum values of 47.72 and 38.77 mg g^{-1} root DW, respectively. However, considerable decline was found with double stress from As augmentation and nutrition shortage.

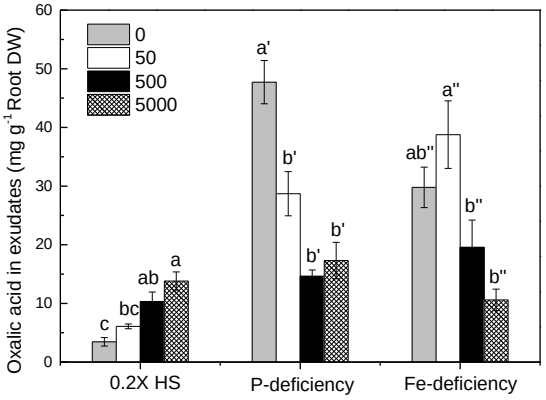

Fig. 2. Oxalic acid exuded from *P. vittata* under the 0.2X HS, P-deficiency and Fe-deficiency conditions. Different letters on the bar indicate significant difference at $p<0.05$ levels.

Root exudates are the important plant metabolites to improve nutrition acquisition [11], [12]. In our study, oxalic acid was the dominant low molecular weight organic acids (LMWOAs) in root exudates of *P. vittata*, which is similar to the research of Fu *et al*. [23]. Nevertheless, phytic acid which is specific in the root exudates of *P. vittata* was not detected in this study. The composition of LMWOAs in root exudates may be greatly influenced by the growth stage of plants.

Organic acids secreted by plants root in response to P deficiency to converting insoluble P to soluble forms in the environment, subsequently acquiring much more P to be assimilated by plants and then supporting their growth [24]. To maximize P acquisition and utilization efficiency, plants often carry on own adjustment to develop stress reaction withstanding extreme P-limiting conditions. Similar to the P limiting conditions, many physiological changes occur in plants to response to Fe deficiency, in order to enhance the absorption of Fe from the soil [25]. The extrusion of organic acid in response to Fe deficiency is assumed to occur in the rhizosphere to enhance the solubilization of iron oxide in soil via their chelation ability [26]. Our results of that oxalic acid increment in P- and Fe-deficiency are coincident with these positive effects of organic acids in exudation has been demonstrated in many investigations involving P- and Fe-deficiency conditions.

Organic acids in root exudates are also assumed to act as important roles in many plant processes, such as metal detoxification and nutrient acquisition [27]. In response to As stress, plants excrete the root exudates to confront with instant variation in rhizosphere. Besides, organic acids in root exudates are considered to engage microbial dissolution of

As-bearing minerals and thus release As into the soil with soluble forms, thereby enhancing As uptake by *P. vittata* [28]. Further, it has been demonstrated that As-induced increment in root exudates could elicit As solubilization and then uptake by *P. vittata* [11]. In our study, the increases in oxalic acids secreted by *P. vittata* with increasing As under adequate nutrition may testify this assumption. It is, however, difficult to use above hypothesis making clear the phenomena that lower oxalic acid extrusion with no regular variation in responses to double stress from As increasing and nutrition deficiency. To precisely identify the relationship of root exudation spiked with As in various concentrations under any of the investigated nutrient deficiencies, further experimental evidence need to be proposed.

IV. Conclusion

This study introduced the As uptake by *P. vittata* and plant metabolite influenced by phosphate and iron nutrients, which may enhance our understanding of the As hyperaccumulation mechanisms in *P. vittata* and to develop strategies for more efficient phytoremediation of As-contaminated soil by *P. vittata*.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Chongyang Yang conducted the research and wrote the paper; Mei-fang Chein, Ying-Ning Ho and Chihiro Inoue analyzed the data and revised the paper; all authors had approved the final version.

Acknowledgment

We express our special thanks to Fujita Co., Ltd (Tokyo, Japan) for providing seedlings of *P. vittata* and to Dr. Kitajima for his valuable technical support in culturing *P. vittata*.

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