Phytate exudation by the roots of *Pteris vittata* can dissolve colloidal FePO₄

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**Abstract**

Phosphorus (P) is a limiting nutrient in many soils, and P availability may often depend on iron (Fe) speciation. Colloidal iron phosphate (FePO₄_{coll}) is potentially present in soils, and we tested the hypothesis that phytate exudation by *Pteris vittata* might dissolve FePO₄_{coll} by growing the plant in nutrient solution to which FePO₄_{coll} was added. The omission of P and Fe increased phytate exudation by *P. vittata* from 434 to 2136 mg kg⁻¹ as the FePO₄_{coll} concentration increased from 0 to 300 mM. The total P in *P. vittata* tissue increased from 2880 to 8280 mg kg⁻¹, and the corresponding increases in the trichloroacetic acid (TCA) extractable P fractions were inorganic P (860–5100 mg kg⁻¹), soluble organic P (250–870 mg kg⁻¹), and insoluble organic P (160–2030 mg kg⁻¹). That is, FePO₄-solubilizing activity was positively correlated with TP, TCA P fractions in *P. vittata*, TP in growth media, and root exudates. This study shows that phytate exudation dissolved FePO₄_{coll} due to the chelation effect of phytic acid on Fe; however, the wider question of whether phytic acid excretion was prompted by deprivation of P, Fe, or both remains to be answered.

**Keywords** Phosphorus · Iron phosphate · Iron phosphate solubilizing activity · Phytate · *Pteris vittata*

**Introduction**

Phosphorus (P) is an important nutrient for plant growth (Meyer et al. (Misson et al. 2018)) and world food production (Fresne et al. (Fresne et al. 2021)), playing a major role in plant metabolic processes (He et al. (He 2020)). Since the 1960s, the extensive use of P-fertilizers (Lin et al. (Liu et al. 2016)), and many other P products has caused excessive P levels to more frequently disturb water bodies and aquatic systems (Lei et al. (Lessl and Ma 2013)) and cause many environmental problems such as eutrophication (Liu et al. (Liu et al. 2017)) and also threat to human health (Xu et al. (Zhang et al. 2007)).

In soil solutions, the colloids carrying P govern the mobility and availability of P in the aqueous phase of soil (Gottselig et al. (Gottselig 2017)); however, the availability of P also depends on the P concentration and its speciation (Montalvo et al. (Moradi 2020)). The colloids (diameter of 1–1000 nm) are rich in Al/Fe oxyhydroxides (Wang et al. (Wang et al. 2021); Eltohamy et al. (Eltohamy et al. 2021); Wang et al. (Xu et al. 2020)) and strongly sorb P in solution decreasing its availability (Mentalboe et al. (Violante and Caporale 2015)). Also, high concentrations of Fe/Al oxyhydroxides in the Ah soil horizon may promote P sorption in the colloidal phase and thereby promote P loss via leaching (Misson et al. (Montalvo et al. 2015)). The association of colloidal P with Fe oxyhydroxides showed the important role of Fe in catchment areas, the P mobility may be increased by the Fe rich colloids, but decreased the P availability (Baken et al. (Baken et al. 2016)). Jiang et al. ((Jones 1998)) claimed that the majority of P bind to Fe oxides, and after the dissolution of Fe oxides, this P may be released and available for plants and microbes, while the previous study of Baken et al. ((Baken et al. 2014)) showed that Fe rich colloids can decrease the P bioavailability to algae and promote...
eutrophication of natural water. Thus, it is very important to reuse the FePO$_4$$_{coll}$ from soil solution before going to water bodies.

P deficiency affects a plant’s metabolism processes, causing the synthesis and release of organic acids into the rhizosphere (Ryan et al. 2001; Wang et al. (Wang et al. 2016); Chen et al. (Chen et al. 2017)). Different plants produce different root exudates which promote the uptake of nutrients (Han et al. (Han et al. 2017)) and P utilization (Wang and Lambers (Wang et al. 2013)). For example, P deficiency induces citric acid exudation by white lupin (Lupinus albus) in response to P deficits (Cheng et al. (Cheng et al. 2011)), and oats (Avena sativa) appear to do likewise (Wang et al. (Wang et al. 2020)). In contrast, Chinese brake fern (Pteris vittata) may respond by releasing phytic acid (Fu et al. (Fu et al. 2017)). In the rhizosphere, organic acids may act as metal chelators and affect the dissolution and release of P from mineral phases (Jones (Lambers and Plaxton 2015)).

Chinese brake fern (Pteris vittata) is a widely distributed plant in Asia, Europe, Africa, and Australia, can be grow in different environments (Wan et al. (Wang and Lambers 2019)), with a high yield of about 36 ton per hectare (Song et al. (Subbarao et al. 1997)). Besides, Pteris vittata (P. vittata) is also native to soils (tropical) of low nutrient status that contain mostly unavailable organic and inorganic P (Liu et al. (Mathews et al. 2010)). This association suggests that P. vittata may access the P and Fe from insoluble phases such as those described in the preceding paragraph and/or mineralize organic P (Fu et al. (Fu et al. 2017)). Unlike the more typical organic acids (citrate and oxalate), phytic acid has been detected in root exudates of P. vittata (Tu et al. (Tu et al. 2011)) where it may be the main source of P storage, as it is in cereal grains and their products (Thavarajah et al. (Thavarajah et al. 2010)). Phytic acid forms strong chelates with Fe ions at a wide pH range (De Stefano et al. (De Stefano et al. 2003); Trela (Tu et al. 2004)) and consequently also adsorbs to Fe/Al soil minerals (Chen and Arai (Chen and Arai 2019)).

Colloidal P and Fe are frequently associated (Niyungeko et al. 2018; Fresne et al. (Fresne et al. 2021), Jiang et al. (Jones 1998)), and affect the uptake of P from colloids (Montalvo et al. (Moradi 2020); Zhang et al. (Ryan et al. 2001)). In addition, Soga et al. (Song et al. 2019) claimed that organic acid exudation may dissolve nanoFePO$_4$ and release P for plant uptake; however, they did not identify which organic acids may be involved. Moreover, these earlier studies did not mention the potential role of phytate exudation in dissolution of FePO$_4$$_{coll}$, possibly because phytic acid contains a high constituent concentration of P. There is however evidence that phytate exudation from P. vittata releases arsenic (an analog to P) from contaminated soil (Tu et al. (Tu et al. 2011)); however, the mechanism remains obscure due to the complexities of working in a soil system. To avoid these complexities in our study of P. vittata and the effect of phytate exudation on FePO$_4$$_{coll}$ dissolution, we used a hydroponic system.

Materials and methods

Plant conditioning

P. vittata plants (height 5–10 cm) were purchased from Guangdong province, and seedlings of pea (Pisum sativum) and lettuce (Lactuca sativa) from a local nursery in Hangzhou, Zhejiang Province, China. The P. vittata, pea, and lettuce were first conditioned in 0.2-strength aerated Hoagland (Table S1) solution at pH 6.5. The solution was buffered with 1 mM KOH-MES (2-(N-morpholino) ethane sulfonic acid) (Mathews et al. (Meyer et al. 2020)). Water losses due to evapotranspiration were replaced daily with Milli-Q water and replaced every 2 weeks. The plants were raised in a growth chamber at ~70% relative humidity, 25 °C day/night temperature, and a 16 h light and 8 h darkness (Wan et al. (Wan 2020)). When the new fronds and leaves were emerging, the conditioned plants were used in experiments 2.2–2.4.

During conditioning, the plants were raised in a growth chamber at ~70% relative humidity, 25 °C day/night temperature, with 16 h of light per day (Wan et al. (Wan 2020)).

Phosphorus accumulation experiment

There were three plant species P. vittata, pea, and lettuce. Each pot contained three conditioned plants of the one species and contained 500 mL of half-strength of aerated Hoagland nutrient solution. The composition of the solution was modified to contain 0, 50, 100, and 150 mg P. L$^{-1}$ as potassium dihydrogen phosphate (KH$_2$PO$_4$). Solutions were topped up daily with pure water to replace loss due to evapotranspiration, and solutions were replaced twice a week. Plants were harvested after 15 days, and the roots were separated from the leaves and dried at 80 °C.

Subsamples of dried leaves/fronds roots were ground and digested in concentrated nitric acid (HNO$_3$). Diluted digests were analyzed for P using an inductively coupled plasma-atomic emission spectrometer (ICP-OES) according to the manufacturer’s instructions (iCAP 6000 series, Thermo Fisher scientific, USA).

Organic acid excretion by P. vittata, pea, and lettuce

Three conditioned P. vittata, pea and lettuce plants (the “Plant conditioning” section) were transferred to separate pots containing 500 mL of P and Fe free, half strength Hoagland nutrient solution, buffered at pH 6.0 with 0.5 M MES buffer for 3 days (Fayiga et al. (Fayiga et al. 2008)). The organic acid concentrations were determined as described in section S 2.2.
Effect of *P. vittata* on FePO$_4$coll dissolution

The experiment was conducted in Fe- and P-free 0.2-strength Hoagland media. Five conditioned *P. vittata* plants (the “Plant conditioning” section) were grown in 500 mL per pot of sterile, Fe and P free, MES (0.5 M) buffered half strength Hoagland solution to investigate the role of phytic acid excretion on the dissolution of FePO$_4$coll. The FePO$_4$coll was prepared and characterized as described in the supplementary material (S2.2, Fig. 1). The hydroponic solutions contained solid phase FePO$_4$coll at mass/volume concentrations of (i) 0 mM FePO$_4$coll (control), (ii) 100 mM FePO$_4$coll, (iii) 200 mM FePO$_4$coll, and (iv) 300 mM FePO$_4$coll. Chloramphenicol at 30 mg L$^{-1}$ was added to minimize microbial activity. Evapotranspiration losses were replaced with pure water daily, and the solution was replaced every 2 weeks. Analysis for P and Fe, in the growth media and *P. vittata*, was conducted as in section S 2.2. The trichloroacetic acid (TCA)

![Fig. 1 Characterization of FePO$_4$coll by SEM, DLS and XRD pattern.](image)

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Fig. 3  TCA P fractions in *P. vittata* and linear regression between Fe-P-solubilizing activity and TP, inorganic P, soluble organic P, insoluble organic P, in *P. vittata* tissue, TP in root exudates and growth media.

Error bars indicate the SE (n=3), values followed by the same letter are not significantly different (P<0.05).
P fractions in *P. vittata* samples were analyzed as explained in section S 2.3. In addition, the FePO$_4$-solubilizing activity in root exudates grown in P and Fe half strength Hoagland solutions containing different concentration (0–300

![Graph](https://via.placeholder.com/150)

**Fig. 4** *Pteris vittata* biomass, P and Fe contents in growth media, phytase and Fe-P-solubilizing activity in root exudates grown in P and Fe half strength Hoagland solutions containing different concentration (0–300

mM) of FePO$_4$coll. Error bars indicate the SE (*n*=3), values followed by the same letter are not significantly different (*P*<0.05)

**Effect of oxalic acid, citric acid, and phytic acid on FePO$_4$coll dissolution**

Solid FePO$_4$coll was prepared and characterized after Liu et al. (2017b) as detailed in supplementary material (S 2.2). The in vitro experiment was performed in 10 mM NaClO$_4$. The 100 μM FePO$_4$coll was mixed with phytic acid, oxalic
and citric acid at organic acid: FePO$_4^{\text{coll}}$ molar ratios of 1:1, 2:1, 3:1, 10:1, 30:1, and 60:1 in a centrifuge tube with final volume 50 mL. The pH was adjusted to 6.0 after the addition of organic acids using 0.1 M NaOH and HCl, and the redox potential of the solution were maintained at 67.2 mV. To minimize microbial activity, chloramphenicol at 30 mg L$^{-1}$ was also added (Subbarao et al. (Thavarajah et al. 2009)). Samples were shaken at 200 rpm for 24 h at 28 $^\circ$C then centrifuged at 5000 rpm for 10 min. The supernatant was analyzed for P and Fe using ICP-MS calibrated and operated as per the manufacturer’s instructions (NexION300X, PerkinElmer, USA).

**Statistical analysis**

Results were compared using analysis of variance and a 5% significance level with the least significant difference (Tukey). Linear regression modeling was used to predict the effect of the Fe-P-solubilizing activity and TCA fractions in fern, TP in root exudates and growth media and effect of TP in $P. \text{vittata}$, growth media, root exudates, and Fe in growth media on the Fe-P-solubilizing activity and TCA fractions in fern, TP in $P. \text{vittata}$ root exudates and growth media and effect of TP in the Fe-P-solubilizing activity and TCA fractions in fern, TP in $P. \text{vittata}$, growth media, root exudates, and Fe in growth media.

**Results**

**FePO$_4^{\text{coll}}$ characterization**

The SEM images of the white FePO$_4^{\text{coll}}$ powder showed that the particle diameters were 273–435 nm and that there were aggregates of FePO$_4^{\text{coll}}$ at 2 $\mu$m and 500 nm resolution (Fig. 1A, B, C, D). The XRD pattern identified FePO$_4^{\text{coll}}$ at 27$^\circ$, 38$^\circ$, 41$^\circ$, and 44$^\circ$ and dynamic light scattering (DLS) analysis showed the particle size ranged from 200 to 550 nm. The EDS analysis showed the high elemental weight percentage of O (74.84%), P (24.46%), and Fe (0.70%) (Fig S1). The FTIR (Fig. S2) showed the O–H and H–OH vibration at 3400 and 1635 cm$^{-1}$ and the Fe–O–P, O–P–O absorbance were recorded at 1049 and 590 cm$^{-1}$.

**P accumulation and organic acid exudation**

When exposed to 0–150 mg P L$^{-1}$, the concentration of P in $P. \text{vittata}$ was 2200–9680 mg P kg$^{-1}$, while in lettuce and pea the concentrations were 3200–7600 and 3300–6500 mg P kg$^{-1}$ respectively (Fig. 2). Limiting the supply of P- and Fe-induced $P. \text{vittata}$ to excrete phytic acid (155 mg k$^{-1}$g and citric acid 310 mg k$^{-1}$g of fresh weight) (Fig. 2A). Pea and lettuce excreted only citric acid, and the concentrations were about half that excreted by $P. \text{vittata}$ (Fig. 2B). Increasing the FePO$_4^{\text{coll}}$ concentration caused progressive increases in the excreted concentration of phytic acid (Fig. 2C). The $P. \text{vittata}$ exposed to 300 mM FePO$_4^{\text{coll}}$ released significantly more phytic acid ($P <0.05$) (2136 mg kg$^{-1}$) than the plants exposed to 200 mM (749 mg kg$^{-1}$), 100 mM (444 mg kg$^{-1}$), and 0 mM FePO$_4^{\text{coll}}$ (434 mg kg$^{-1}$).

**Role of phytate exudation in FePO$_4^{\text{coll}}$ dissolution**

In $P. \text{vittata}$, TP, TCA fractions, and Fe contents also increased with increasing FePO$_4^{\text{coll}}$ concentration. The total P concentration increased from 2880 to 8280 mg kg$^{-1}$ and the TCA fractions were inorganic P (860–3100 mg kg$^{-1}$), soluble organic P (250–870 mg kg$^{-1}$), and insoluble organic P (160–2030 mg kg$^{-1}$) (Fig. 3). There was also a dose response relationship between the external concentration of FePO$_4^{\text{coll}}$ and the internal concentrations of Fe, e.g., with 300 mM FePO$_4^{\text{coll}}$ the plants contained the highest Fe concentration ($P <0.05$, 3560 mg kg$^{-1}$ fresh weight) (Fig. 3). The total P in root exudates also increased as the treatments concentration increases (Fig. 3).

| Phytic acids: FePO$_4^{\text{coll}}$ | Predicted P (μM) | Predicted Fe-phytate (μM) | Predicted Fe(μM) |
|-----------------------------------|-------------------|--------------------------|------------------|
| 1:1                               | 5.1±0.52$^a$      | 92±1.09$^a$              | 2.2±0.65$^f$     |
| 2:1                               | 8.0±0.12$^a$      | 83±0.68$^b$              | 3.2±0.88$^e$     |
| 3:1                               | 8.6±1.02$^a$      | 84±1.1$^b$               | 5.8±0.97$^d$     |
| 10:1                              | 22±1.09$^a$       | 69±0.68$^c$              | 19±1.0$^c$       |
| 30:1                              | 64±1.2$^b$        | 44±0.94$^c$              | 35±0.25$^b$      |
| 60:1                              | 95±0.78$^a$       | 2±0.25$^c$               | 55±0.68$^a$      |

Means with SE ($n$ =3), values followed by the same letter are not significantly different ($P<0.05$)
Biomass Fe, P content in growth media, phytase, and Fe-P-solubilizing activity

The biomass of *P. vittata* also increased as the FePO$_4$ concentration increased from 0 to 300 mM (Fig. 4). The concentrations of P and Fe in the hydroponic media followed dose response relationship with the FePO$_4$ addition. The P and Fe were higher (80.65 and 51.06 mM) ($P<0.05$) in 300 mM followed by 200 mM (72.32 and 36.1 mM) while 100 mM have less P and Fe concentration (Fig. 4). In root exudates, the phytase and Fe-P-solubilizing activity increased as the concentration of FePO$_4$ increased from 0 to 300 mM. The
phytase activity was greater in 300 mM FePO₄₉coll (8.3 h⁻¹ g⁻¹ root FW), followed by 200 and 100 mM, while the control had the least phytase activity (3.2 h⁻¹ g⁻¹ root FW). The same trend was observed in the Fe-P-solubilizing activity which also increased from 0.86 to 6.85 (μg P mL⁻¹) as FePO₄₉coll concentration increased from 0 to 300 mM (Fig. 4).

**Linear regression between Fe-P-solubilizing activity and P fractions**

Linear regression analysis showed that FePO₄₉-solubilizing activity have strong and significantly (P<0.05) correlate with P. vittata TP (R² = 0.93), inorganic P (R² = 0.89), soluble organic P (R² = 0.86), but a weak correlation with insoluble organic P (R² = 0.21) in P. vittata tissue (Fig. 5). The analogous effects (P<0.05) in the growth media were on TP (R² = 0.86), and on TP in root exudates (R² = 0.99) (Fig. 5). We also find a strong and significant correlation between biomass and P (R²=0.80). Fe (R² = 0.91) in growth media, P in root exudates (R² = 0.80) and TP in P. vittata tissue (R² = 0.91) (Fig. 5).

**Solubilizing effects of organic acids on FePO₄₉coll in vitro**

The dissolution of FePO₄₉coll increased as the organic acids: FePO₄₉coll molar ratio increased (Fig. 6). In the case of phytic acid, a molar ratio 1:1 (phytic acid: FePO₄₉coll) released 32.5 μM P and 3.12 μM Fe, while the same molar ratios of citric and oxalic acids released 14.65 and 16.3 μM P and 4.5 and 5.4 μM Fe. At the higher molar ratio of 60:1 (organic acid: FePO₄₉coll), the phytic, citric, and oxalic acid released 69.8, 45.2, and 54.3 μM P respectively and the corresponding Fe concentrations were 36.82, and 25.6 μM (Fig. 5). Equilibrium speciation modeling (Visual MINTEQ) predicted that at lower ratios of (phytic acid: FePO₄₉coll) more P and less Fe would be released into solution as a consequence of the predominance of the insoluble tetraFe-phytate complex, while at higher molar ratios, such as 60:1, the more soluble monoFe phytate complex would predominate (Table 1).

**Discussion**

**FePO₄₉coll characterization**

The diffraction peaks of FePO₄ at 27°, 34°, 41°, and 44° matched with a crystalline phase of FePO₄·2H₂O (JCPDS no. 002-0250) (Liu et al. (Mathews et al. 2010)). The diffraction peak at 41° showed the hexagonal FePO₄ morphology (JCPDS no. 29-0175) (Liu et al. (Liu et al. 2017)). According to the reference of MDI Jade software, the diffraction peak at 27° showed hexagonal structure (α = 90, β = 90, γ = 120), 34° and 41° showed orthorhombic (α = 90, β = 90, γ = 90) (PDF:50-1634); however, the diffraction peak at 41° has less density than that at 34°. The diffraction peak at 44° showed the monoclinic structure (α = 90, β = 107, γ = 90) (PDF:30-0661). The FTIR peaks between 1000 and1200 cm⁻¹ showed a basic structural units of PO₄ into structure network, and it is investigated that this structure has a strong connection with FePO₄ (Prokúpková et al. (Richardson et al. 2000)). The O–H and H–O–H peaks showed the water molecule and the Fe–O–P and O–P–O confirmed our XRD results. Our FTIR results are in agreement with the Segà et al. ((Song et al. 2019)) who also observed the Fe–O–P and O–P–O at 1049 and 590 cm⁻¹.

**P accumulation by P. vittata**

P. vittata accumulated a large amount of P (Fig. 2A) and released phytic acid (Fig. 2B) while lettuce and pea plants accumulated less P and did not release phytic acid. Our results are in agreement with Lessl and Ma ((Li et al. 2020)), who suggested P. vittata as a model plant for P uptake, and Fu et al. ((Fu et al. 2017)) observed that under P limitation, P. vittata can release phytic acid. On this basis only, P. vittata as tested for FePO₄₉coll dissolution.

**Phytic acid exudation**

P. vittata released phytic acid under P and Fe limited conditions (FePO₄₉coll 0–300 mM) (Fig. 2C). Phytic acid is a major source of P in plants, and it is not usually secreted under environmental stress, e.g., Arabidopsis thaliana, Triticum aestivum, and Brassica species release malic and citric acids. Similarly, Oryza sativa releases only citric acid, and P. esinoformis releases oxalic and citric acids (Fu et al. (Fu et al. 2017)). Plants commonly release organic acids because they release P through the desorption of P from metal oxide...
via ligand exchange, and the release of $H^+$ plays an important role in the dissolution of P from metal oxides (Jones (Lambers and Plaxton2015)). The rhizosphere pH depends upon the $HCO_3^-$, $OH^-$, and $H^+$ (secreted by roots) which is determined by the complement of ions taken up by roots (Hinsinger et al. (Hinsinger et al.2003)). There is also a possibility that \textit{P. vittata} may release phytate due to Fe stress by analogy with the secretion of phytosiderophores (Ahmed and Holmström (Ahmed and Holmström2014)) that complex Fe (Li et al. (Lin et al.2019)), facilitating transport to the roots (Ahmed and Holmström (Ahmed and Holmström2014)). That is, our study does not discriminate between deprivation of P or of Fe as the cause of phytate release by the roots.

**Role of phytate in FePO$_4_{coll}$ dissolution**

The secreted phytate in root exudates may dissolve FePO$_4_{coll}$ in the following ways (1) lower down pH or forming the insoluble complex with metals at the mineral surface which further change the mineral electric surface potential negative and affect the P binding to mineral and (2) by the effect of phosphorylated inositol rings on Fe (Wang and Lambers (Wang et al.2013); Lambers and Plaxton (Lei et al.2020); Violante and Caporale (Wan et al.2010)). At low molar concentration of phytic acid and Fe, water insoluble, tetraferric phytate complex formed, but at higher phytate concentrations, this was replaced by the water soluble monoferric phytate complex (Trela (Tu et al.2004); Nielsen et al. (Prokůpová et al.1996)).

In our experiment, the uptake of P (Fig. 3) shows that the released phytic acid dissolved the FePO$_4_{coll}$, which is consistent with the results of in vitro chelating experiments at 1:1 molar ratio (phytic acid: FePO$_4_{coll}$) (Table 2 and Fig. 6). The data showed that Fe was precipitated by phytic acid as Fe-phytate at a low concentration of phytic acid (Table 2). These data were confirmed by visual MINTEQ software (Table 1). At low concentration of phytic acid, the strong chelation may be due to the insoluble Fe-phytate, and as concentration increased, the Fe-phytate became water soluble. The Fe uptake may be because of plant-secreted siderophores phytosiderophores that make complex with Fe, then transported to plant across the root plasma membrane (Ahmed and Holmström (Ahmed and Holmström2014)). The transported Fe-siderophore complex reduced to Fe$^{2+}$ in the plant cell membrane and then uptake by cells. However, there is a need for more investigation for \textit{P. vittata} and phytosiderophores’ role in FePO$_4_{coll}$ dissolution. Our results agreed with Sega et al. ((Song et al.2019)) who claimed that root exudation may dissolve the FePO$_4$ nanoparticles which release P and Fe. However, they did not mention which organic acid could dissolve FePO$_4$. However, in addition the molar ratio (P/Fe) FePO$_4_{coll}$(product) was of 1:0.5, but according to Fig. 4, the molar ratio of P/Fe in solution was 1:2.6, 1:2.03, and 1:1.5 for treatments that initially contained 100, 200, and 300 mM of FePO$_4_{coll}$. That is, at the highest FePO$_4$ concentration, the high P/Fe ratio in solution at the end of the experiment ratio showed that the phytate exudation dissolved the FePO$_4_{coll}$.

**Role of phytase and Fe-P-solubilizing activity on FePO$_4_{coll}$**

The phytase enzyme also plays the dominant role in P and Fe released in our experiment. Under P starvation, plant cells promote the activity of phytases (Tu et al. (Vendelboe et al.2012)), and Fe adsorption from Fe-phytate is improved by adding phytase enzymes to oat-based beverages (Zhang et al. (Zhang et al.2021)). The phytase secretion in our experiment is independent of microbial activity under limiting P and Fe conditions, which is consistent with our finding of high concentrations of Fe and P in growth media and also in \textit{P. vittata} tissue (Fig. 4). We also claimed that Fe-P-solubilizing activity in root exudates of \textit{P. vittata} played an important role in FePO$_4_{coll}$ dissolution as evidenced by positive correlations between increased Fe-P-solubilizing activity and increased uptake of Fe and P by \textit{P. vittata}(Fig. 5), which extends the findings of Subbarao et al. ((Thavarajah et al.2009)). This may explain the apparently conflicted environmental adaptation of excreting a P-rich substance (phytate) in response to a P-limitation; however, our results do not exclude the possibility that this response was elicited by an Fe-limitation, or a joint P and Fe limitation. Nonetheless, several studies have shown that \textit{P. vittata} exudes phytic acid under stress caused by root exposure to minerals such as phosphate rock and FeAsO$_4$ (Fu et al. (Fu et al.2017); Liu et al. (Liu et al.2017)). Moreover, the hypothetical cost/benefit ration of such a response would be multiplied in soil where the released phytate would likely be constrained to the rhizosphere rather than diluted in a hydroponic medium.

**Conclusion**

The combined deprivation of P and Fe caused \textit{P. vittata} to release phytic, which efficiently dissolved FePO$_4_{coll}$ releasing P and Fe that were taken up by the plant. However, whether deprivation of one or both of P and Fe is sufficient to cause phytate exudation remains unknown. The phytase activity in root exudates may have facilitated the uptake of the dissolved Fe and P, since FePO$_4_{coll}$ increased both plant biomass and the internal concentrations of P and Fe. Fe-P-solubilizing activity is not unique to the root exudates of \textit{P. vittata}; however, the release of phytic acid as a stress response merits further investigation.
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