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

**Supporting information**

Table S 1. Chemical composition of the P and Fe free, half-strength Hoagland hydroponic solution

S2.2Preparation of colloidal iron phosphate

S 2.3. Organic acid analysis

S 2.4 Chemical analysis in *Pteris vittata* and growth media

S 2.5 Fe–P solubilizing and phytase activity analysis

References

Table S 1. Chemical composition of the P and Fe free, half-strength Hoagland hydroponic solution

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| **Component** | **Concentration** |
| NH4NO3 | 0.6 mmol NO-3 L−1 |
| KNO3 | 1.0 mmol K L−1 |
| Ca (NO3)2 | 1.2 mmol Ca L−1 |
| MgSO4 | 0.12 mmol M L−1 |
| MnCl2 | 1.1 × 10−3 mmol Mn L−1 |
| H3BO4  ZnSO4  CuSO4 | 0.6 × 10−2 mmol B L−1  1.0 × 10−4 mmol Zn L−1  4 × 10−5 mmol Cu L−1 |

**S2. Material and Methods**

S2.1Preparation of colloidal iron phosphate

The iron phosphate was prepared by thermal heating of ferric chloride (FeCl3) and KH2PO4 (Liu et al. 2017). The reagents were mixed at 2:3 molar ratio in 60 mL ethanol solution (50 wt %). All the reagents were bought from Aladdin Industrial Inc. Shanghai, China with purity > 99%. The mixture was stirred for 3 h at room temperature, and then autoclaved at 170 °C for 12 h. The mixture was allowed to cool at room temperature, centrifuged, washed with deionized water and dried at 60 °C for 10 h, then calcined with NaCl at 500 °C for 3 h. The final solution was dialyzed against demineralized water, the water was replaced three times per day (Bollyn et al. 2017). The sample were freeze dried and white powder was obtain as final product. The powder was stored in desiccator for further use. The product was characterized using XRD (D8 Advance X-ray Powder Diffractometer, Bruker, Country), dynamic light scattering (DLS) using a Zetasizer (Nano ZS90, Malverb, UK) were used to determine the hydrodynamic size distribution and Scanning electronic microscope (Gemini SEM 300; ZEISS, Germany).

S 2.2. Organic acid analysis

First the roots were washed with pure water and dipped in a 30 mg L−1 chloramphenico solution to inhibit microbial growth (Subbarao et al., 1997). Then the ferns were grown in a 500 mL plastic beaker that was covered with a black plastic sheet to minimize the entry of light. For the collection of root exudates, each plant was placed in 300 mL of sterilized pure water for 24 h and there were four replications. The collected exudates were freeze dried and kept at −80 °C then dissolved in 1.5 mL of 20 mM NaH2PO4 (pH 2.7), passed through a polyether sulfone filter (0.22 μm), and degassed. The solution was analysed for the three acids using high-performance liquid chromatography equipped with a reverse-phase dC18 anion-exchange analytical column (Agilent Zorbax-Aq 4.6 × 250 mm, 5-m, Germany) and a multi-wavelength detector at 215 nm (Waters, Milford, USA). The sample injection volume was 10 μL with a 20 mM NaH2PO4 (pH 2.7) flowing at 0.5 mL min−1, and a column temperature of 30 °C. Phytic and citric acids of 99% purity (Sigma-Aldrich, St. Louis, U.S.A.) were used as standards. The organic acids were identified by retention time, and their concentration was calculated from the peak area and expressed on a fresh weight basis (Arnetoli et al., 2008).

S 2.3 Chemical analysis in *Pteris vittata* and growth media

Dried P. vittata samples (200 g) were powdered using a mortar and pestle and ground with 15 mL of trichloroacetic acid (TCA) (10% w: v). The suspension was held at 0–5 °C for 8 h then centrifuged at 3000 g for 10 min. The pellet was extracted by following the same procedure in 15 mL of 5% w:v trichloroacetic acid (TCA) and the supernatants were bulked (Ae et al., 1990). The TCA soluble, molybdate reactive (inorganic P fraction) in the supernatants was measured by the molybdenum blue method of Murphy and Riley (1962). Total P in the TCA supernatants (inorganic plus ester P fraction) was measured after Kjeldahl digestion by the same method. The solid residue was digested and the TCA-insoluble P fraction was also measured using the same method. Total P and Fe content of the samples was determined independently after digestion with a mixture of nitric, perchloric and sulfuric acids (3:1:1 v/v) by ICP-OES. In the growth media the concentration of P and Fe was measured by inductively coupled plasma mass spectrometry (NexION300X, PerkinElmer, USA).

S 2.4 Fe–P solubilizing and phytase activity analysis

The FePO4 (Fe–P) solubilization capacity of root exudates was measured by placing 0.5 mL of root exudates collected in sec 2.4, plus 5 mg of FePO4coll (Fe–P) and 0.5 mL sodium acetate buffer (1 M, pH 5.6) into a 5 mL flask ( Subbarao et al., 1997). The Fe–P had been washed 3–4 times with distilled water to remove water-soluble P. Chloroform (0.1 mL) was added to the flask to minimize microbial activity and the suspensions were shaken for 24 h at 22 °C at 160 rpm. The suspensions were centrifuged at 16,000 g for 10 min and P was measured in the supernatant using the molybdenum blue method of Murphy and Riley (1962). We took pure water as the control. The difference between the P content in the control and supernatants was expressed as the Fe–P solubilizing activity of the root exudates.

Phytase activity was assayed using the method of Richardson et al., (2000). Briefly, 4 mL of MES/Ca buffer was added to 200 mL of the nutrient solution containing the roots of the intact P. vittata plants and incubated for 4 h at 25 °C. The plants were then removed and pNPP or IHP substrate was added. Phytase activity was expressed in units of h−1 g−1 root FW.

**References**

Ae, N., Arihara, J., Okada, K., Yoshihara, T., Johansen, C., 1990. Phosphorus uptake by pigeon pea and its role in cropping systems of the Indian subcontinent. Science (80-. ). 248, 477–480. <https://doi.org/10.1126/science.248.4954.477>

Arnetoli, M., Montegrossi, G., Buccianti, A., Gonnelli, C., 2008. Determination of organic acids in plants of silene paradoxa L. by HPLC. J. Agric. Food Chem. 56, 789–795. https://doi.org/10.1021/jf072203d

Bollyn, J., Faes, J., Fritzsche, A., Smolders, E., 2017. Colloidal-Bound Polyphosphates and Organic Phosphates Are Bioavailable: A Nutrient Solution Study. J. Agric. Food Chem. 65, 6762–6770. <https://doi.org/10.1021/acs.jafc.7b01483>

Liu, Y., Li, Z., You, Y., Zheng, X., Wen, J., 2017. Synthesis of different structured FePO4 for the enhanced conversion of methyl cellulose to 5-hydroxymethylfurfural. RSC Adv. 7, 51281–51289. <https://doi.org/10.1039/c7ra09186a>

Richardson, A.E., Hadobas, P.A., Hayes, J.E., 2000. Acid phosphomonoesterase and phytase activities of wheat (Triticum aestivum L.) roots and utilization of organic phosphorus substrates by seedlings grown in sterile culture. Plant, Cell Environ. 23, 397–405.

Subbarao, G.V., Ae, N., Otani, T., 1997. Genotypic variation in iron-, and aluminum-phosphate solubilizing activity of pigeonpea root exudates under p deficient conditions. Soil Sci. Plant Nutr. 43, 295–305. https://doi.org/10.1080/00380768.1997.10414754