

A new type of plant metalloreductase maintains root growth under low phosphorus

Gatersleben, 11.01.2024 Phosphorus is essential for undisturbed plant growth and development. However, in many soils, phosphorus is only poorly available. One mechanism used by plants to increase phosphorus availability is the release of malate, an organic acid, which can form complexes with iron or aluminium in the soil, thereby liberating sorbed phosphate. However, this response can also result in iron overaccumulation, which can inhibit root growth. An international research team led by the IPK Leibniz Institute found out that the protein HYP1, a member of a yet poorly characterized family of electron transporters, helps to protect roots from increased iron reactivity induced in response to phosphorus deficiency. Today, the results were published in the journal "Nature Communications".

The release of low-molecular weight carboxylates, such as malate, is used by many plant species to mine poorly available phosphorus (P) from the soil. Malate can increase the availability of phosphate, the P form taken up by plants, by chelating trivalent aluminium or iron (Fe). Work in the last years has demonstrated that the accumulation of Fe(III)-malate complexes in the apoplast of root tips attenuates root growth under low-P conditions because the now soluble Fe(III) (ferric Fe) participates in reactions leading to oxidative stress. However, how root tips cope with this increased ferric Fe availability has remained elusive as all currently known ferric reductases, which are required for Fe uptake, are repressed in response to P deficiency or not expressed in root tips.

"By searching for other potential ferric reductases among P deficiency-induced genes, we found a previously uncharacterized gene encoding a domain predicted to participate in ferric reduction", explains Dr. Ricardo Giehl, co-leader of IPK's research group "Molecular Plant Nutrition". "When we disrupted this gene, Fe over accumulated in root tips and root growth under low P was even more severely inhibited".

The gene was, therefore, named *HYPERSENSITIVE TO LOW P1 (HYP1)*. HYP1 is a member of the CYBDOM family and possesses a cytochrome *b561* domain, which is common in several ferric reductases active in animal cells, such as the duodenal cytochrome *b* (Dcytb) involved with dietary Fe uptake in humans. "Although CYBDOMs are ubiquitous in several organisms and come typically as large families in plants, their function in any organism is still poorly understood", explains Rodolfo Maniero, first author of the study.

Using AlphaFold-supported modeling and site-directed mutagenesis, the researchers found that HYP1 can coordinate three *b*-hemes, which are critical for the protein's activity. Electrophysiological characterization of HYP1 in oocytes revealed that the protein can

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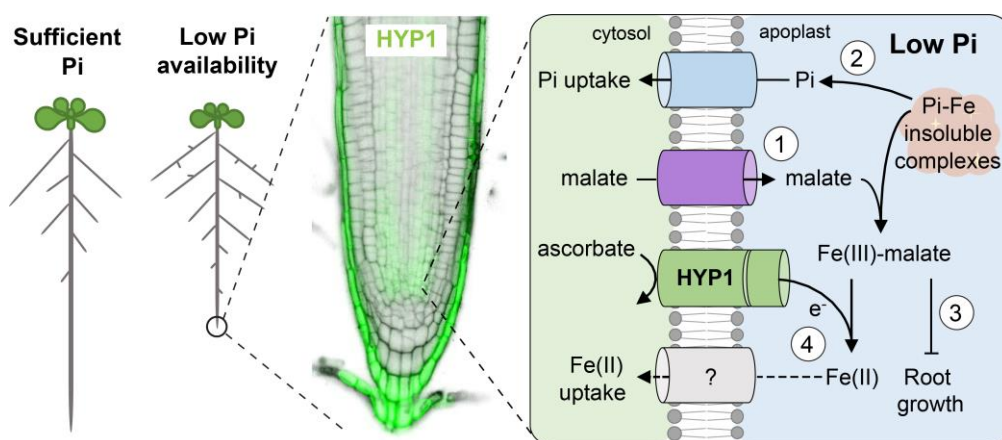
transport electrons derived from ascorbate (vitamin C) across the plasma membrane. Acceptors of these electrons are Fe(III) and Cu(II) (cupric ion), indicating that the protein is a metalloreductase.

“Regarding the ferric reductase activity of HYP1, our results demonstrate that this function is critical to prevent malate-induced Fe overaccumulation in the apoplast and to maintain cell elongation and meristem integrity in root tips exposed to low P”, explains Dr. Ricardo Giehl. “Our results also suggested that an Fe-uptake mechanism is active in root tips, where the well-known ferric reductase FRO2 and the Fe(II) transporter IRT1 are not present”, says the IPK scientist. “Our study not only shed light on the physiological roles of CYBDOMs but also identified a new target to improve root growth under limiting-P conditions”.

Original publication:

Maneiro *et al.* (2024): Ferric reduction by a CYBDOM protein counteracts increased iron availability in root meristems induced by phosphorus deficiency. *Nature Communications*. DOI: [10.1038/s41467-023-43912-w](https://doi.org/10.1038/s41467-023-43912-w)

Graphic:



The new study shows that HYP1 is an ascorbate-dependent metalloreductase induced under low phosphorus (P) conditions to counteract malate-induced iron (Fe) overaccumulation in root tips.

In response to P deficiency, plant roots release malate (1) to solubilize phosphate (Pi) sorbed onto Fe oxides or hydroxides for further uptake (2). As a result, also high amounts of soluble Fe(III)-malate complexes are formed, which can generate oxidative stress in the apoplast (3), thereby inhibiting root growth. Fe(III) reduction by HYP1 prevents Fe overaccumulation and maintains root growth (4).