Phosphate acquisition and metabolism in plants

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Abstract

Plants need at least 13 different nutrients to maintain optimal growth. Nitrogen and phosphorus, from the Greek "phôs" (meaning "light") and "phoros" (meaning "bearer"), are the main nutrients limiting plant growth in both agricultural and natural ecosystems. Agriculture has relied heavily since the mid 1950s on the use of synthetic ammonium- and phosphorus-based fertilizers to increase crop productivity. While industrial synthesis of ammonium relies on the chemical conversion of atmospheric nitrogen, phosphorus is mined from finite reserves concentrated in a few countries. Considering our current dependence of phosphorus fertilizers for food production and the geopolitical aspects associated with current resources, it will be important to develop technologies enabling the maintenance of high crop yield with reduced fertilizer input. This will require an in-depth knowledge on the various pathways that enables plants to acquire phosphorus from the soil and maximize its economical use for growth and reproduction. In this primer we give an overview of the factors limiting phosphorus acquisition by plants and highlight various pathways and strategies plants have evolved at the level of development, metabolism and signal transduction to adapt to phosphorus deficiency.

Plants, phosphorus, and agriculture

Phosphorus (P) occurs in oxidized forms *in natura*, most commonly as orthophosphate (PO_4^{3-}). Plants acquire P almost exclusively by transporting soluble inorganic $H_2PO_4^-$ (Pi) from the soil into their roots. The Pi concentration in most soil solutions ranges between 0.5 and 10 μM, whereas the optimal intracellular Pi concentration is 5–20 mM. Thus, to enter the plant, Pi must travel against a steep extracellular–intracellular concentration gradient. Furthermore, as cells are negatively charged, with an electrical potential of approximately –120 mV, Pi must also

move against a charge gradient. Thus, acquisition of extracellular Pi by cells is a process that requires energy. Proton pump ATPases (H^+-ATPases) located in the plasma membrane use adenosine triphosphate (ATP), the main energy currency of the cell, to export H^+ to the apoplastic space. The energy stored in the transmembrane H^+ electrochemical gradient is then used to drive Pi uptake via Pi-H^+ co-transporters.

Once inside the cell, Pi is incorporated into organic phosphate (Po), where the phosphate group is attached to a carbon atom via one of its oxygen atoms. Cellular Po includes nucleic acids (DNA and RNA), phospholipids, phosphoproteins, and numerous sugar phosphates. Whereas Pi is incorporated into various organic molecules primarily via the generation of ATP during photosynthesis in plants, this process is dependent on mitochondrial oxidative phosphorylation in animals.

The soluble Pi available to plant roots typically represents only a small fraction of the total P present in the soil. Most Pi is immobile, being strongly sorbed onto soil particles and forming insoluble complexes with calcium, as well as oxides and hydroxides of iron or aluminum, processes that are strongly influenced by soil composition and pH. P is one of the least mobile plant macronutrients in soil, being much less mobile than nitrate and potassium. A substantial portion of P in the soil is also present as Po, such as nucleic acids and inositol phosphates, which are obtained from digested and decaying plant, animal, and microbial biomass. Although Po is more abundant than Pi in many soils, it remains unavailable to plants until the Pi moiety is released into the soil solution via hydrolytic processes.

Since ancient times, humans have recognized the need to add fertilizers to the soil to increase crop yield. The rapid growth-promoting effects of fertilizers come both from its readily available soluble Pi, as well as its slow-release Pi from organic sources. However, only a fraction of the soluble Pi added to the soil is readily available to plants; much becomes unavailable through a combination of precipitation and sorption onto soil particles, percolation into deeper layers of soil, and surface leaching. Plant roots directly acquire as little as 20% of the Pi contained in commercial fertilizers. Despite this apparent inefficiency, fertilizers have played an essential role in boosting crop productivity over the last century.

Animal manure was the first readily available form of fertilizer, containing Pi and Po derived from the digestion of plants. Locally produced manure remains a substantial source of P in

agricultural soils, particularly in developing nations (Figure 1A). Trading for phosphate on an international scale began in the 19th century, with the exploitation of guano deposits from Peru (Figure 1B). The word guano originates from the Quechua (an indigenous language of Peru) word "huanu," meaning "dung." Guano is essentially derived from the accumulation of excrements at the nesting sites of large fish-eating seabird colonies or in the roosting caves of bats. Bird guano typically contains 8–15% of N and a similar amount of P, with P occurring mainly as soluble ammonium phosphate or poorly soluble calcium phosphates (e.g., hydroxyapatite [$Ca_5(PO_4)_3OH$], the main component of bones and teeth). Andean communities exploited guano from small coastal islands as fertilizer for well over 1,500 years. In the mid-19th century, guano became a major export product from Peru to Europe. Several other guano-rich deposits were discovered worldwide (e.g., on islands along the coast of Namibia and uninhabited islands in the Pacific and Caribbean) and exploited. The guano trade declined steeply in the late-19th century with the introduction of large-scale ammonia synthesis from atmospheric N_2 using the Haber-Bosch process, in conjunction with the exploitation of inland phosphate rock mining. Currently, bird and bat guano fertilizers are largely locally sourced and used for small-scale subsistence farming.

Although P can be extracted from igneous rocks, commercially extracted P is principally derived from sedimentary deposits of apatite generated in marine environments over geological time. Nearly 90% of the P derived from mining is used in fertilizer production. The use of extracted P has increased considerably since the mid-1950s, largely due to the reliance on synthetic fertilizers to maintain or increase crop yield (Figure 1A). Since 2000, P use in fertilizers has exceeded 20 million tons per year. Analogies can be drawn between P mining and the exploitation of petroleum; both resources accumulated over millions of years but could be depleted in centuries. The world's largest P-rich reserves are in Morocco (including Western Sahara) and China, with both countries accounting for approximately 70% of the total reserves (Figure 1C). Current economically exploitable P-rich deposits could be exhausted within 50–100 years, with the mining of sub-optimal phosphate rock potentially extending production for an additional 200–300 years. Regardless of the various estimates, P-rich deposits are finite resources, and their limited availability will eventually become a key issue for long-term food security. Avoiding a P scarcity will necessitate multi-pronged approaches that aim to recover and/or recycle P, as well as reduce demand by improving plant genetic resources and agricultural practices to maintain high crop productivity while minimizing soil fertilizer input.

Pi concentration is a limiting factor for the growth of both terrestrial and aquatic plants. Thus, an increased input of Pi (along with nitrate) into waterways from agricultural runoff, as well as leaching associated with fertilizer application, contribute to eutrophication (from the Greek "eutrophus," meaning "well-nourished"). Water eutrophication typically leads to massive algal blooms that sometimes include toxin-producing algae (e.g., Dinophyceae) and cyanobacteria (Figure 1D). Eutrophication results in ecological imbalances and water deoxygenation, the latter of which is caused by the heterotrophic mineralization of excess algal biomass, which can result in the death of aquatic animals.

How do plants optimize Pi acquisition from the soil?

Pi uptake by plants rapidly creates a zone depleted of the nutrient around the roots because of its low concentration and limited mobility in soil. Plants have thus evolved numerous developmental and metabolic adaptations to increase Pi acquisition efficiency (PAE) (Figure 2A). These adaptations primarily increase the ability of the root system to explore larger volumes of soil and improve its capacity to extract poorly available forms of P, including from the Po pool.

The Pi supply can strongly influence root architecture. Plants invest proportionally more carbon in root growth than shoot growth in response to Pi deficiency. Typically, the upper soil layers have a higher Pi content than the lower layers. Under Pi deficiency, primary root growth is inhibited, secondary root growth is stimulated, and gravitropism is reduced, resulting in a denser root system in the upper soil layers. The root system is highly plastic and can adapt to heterogeneous P distribution, enhancing its density in soil patches with higher P content. An increase in root hair length and density is also typically observed under Pi-deficient conditions, resulting in a large extension of the root surface area amenable to Pi acquisition. Root growth and root hair induction are controlled by a complex interplay between various phytohormones. Changes in the quantity and/or distribution of auxin, ethylene, cytokinin, and strigolactones, as well as the sensitivity of the signal transduction pathways that respond to these hormones, have a major effect on the root system architecture under Pi deficiency. Furthermore, the response of roots to Pi deficiency is affected by the concentration of other ions (e.g., iron and nitrate) as well as by the light intensity and its wavelength (e.g., blue light promotes primary root growth

inhibition). Therefore, the pathways involved in root growth adaptation to Pi deficiency integrate multiple environmental parameters.

The roots of 90% of terrestrial plant species establish a symbiotic relationship with mycorrhizal fungi. The plant contributes organic carbon (e.g., hexose) to support fungal growth, while the mycorrhizal hyphae acquire Pi and nitrate from the soil and transfer these nutrients to the plant. Whereas root hairs extend a few millimeters from the root surface, the fungal hyphal network of arbuscular mycorrhizal fungi (the predominant mycorrhizae in temperate grasslands and rainforests) extends up to 25 cm from the root and that of ectomycorrhizal fungi (typically associated with trees in northern forests) up to a few meters. Pi deficiency can stimulate mycorrhizal root colonization, improving the plant's access to Pi by increasing the volume of soil explored for nutrients. The increased colonization is partly mediated by strigolactones secreted from Pi-deficient roots. Recent studies have revealed that the plant's P status can influence the root microbiome, partly through suppressing the plant's immunity. In some instances, such changes in the microbiome can be associated with improved P nutrition, as shown in the non-mycorrhizal crucifer *Arabidopsis thaliana*: a Pi deficiency enabled the endemic fungal endophyte *Colletotrichum tofieldiae* to colonize the roots and enhance Pi acquisition and transfer to the plant.

Under Pi deficiency, roots can also promote the release of Pi into the soil solution from insoluble (bound) pools of Pi and from Po. Roots can release bound Pi by secreting carboxylic acids (e.g., malate, citrate, and oxalate) into the rhizosphere. This process can liberate soluble Pi via ligand exchange on P-sorption particles and through interactions with Ca, Al, and Fe cations that form insoluble complexes with Pi. Carboxylic acid secretion can also be associated with proton release, resulting in rhizosphere acidification, which consequently increases the release of Pi from the bound Pi pool in certain types of soil (e.g., calcareous soils). Pi-deficient roots increase the mineralization of Po in the soil by secreting various hydrolytic enzymes, including nucleases, phosphodiesterases, phosphatases, and phytases, into the rhizosphere. Although arbuscular mycorrhizal fungi do not substantially enhance the retrieval of Pi from insoluble Pi and Po pools, ectomycorrhizal fungi are able to acquire Pi from these sources.

The absorption of soluble Pi into the root is mediated by high- and low-affinity Pi-H^+ co-transporters encoded by the *PHT1* gene family. PHT1 proteins are located at the plasma membrane, with strong expression in the epidermal cells and root tip and more moderate

expression in other root tissues, such as cortical cells. Considering the crucial role of PHT1 in Pi uptake and PAE, it is not surprising that their activity is controlled at both the transcriptional and post-transcriptional levels. The transcription of several *PHT1* genes is strongly increased under Pi deficiency, and several transcription factors involved in this process have been identified. Furthermore, red light and the phytochrome pathway also regulate *PHT1* transcript levels. The stability and plasma membrane localization of PHT1 proteins are regulated post-translationally by the Pi supply via ubiquitination and phosphorylation, respectively. Pi acquired by the root must be loaded into the xylem vessel for transfer to the shoot. As the xylem is a conduit composed of dead cells, it is equivalent to the apoplastic space. Thus, Pi export is required to load the root intracellular Pi into the extracellular xylem space. This step is mediated by the expression of the PHO1 Pi exporter in the xylem parenchyma cells. Transcription factors involved in regulating *PHO1* expression have been identified, and PHO1 stability is influenced by the Pi status via ubiquitination.

Although the adaptations of roots to enhance PAE are shared by a wide spectrum of plants, some species growing in the world's most P-deprived soils have pushed some of these adaptations one step further. This is the case for many plants belonging to the Proteaceae family that grow in south-western Australia (e.g., Hakeas and Banksias), the Cape Floristic region of South Africa (e.g., Proteas), and South America (e.g., Orites) (Figure 3A). Proteaceae are typically non-mycorrhizal and produce cluster roots (also known as proteoid roots) when grown under Pi-deprived conditions. Cluster roots represent a mass of small rootlets that can reach a density of a few hundred per centimeter of the main root axis. These rootlets are determinate, meaning that the meristems are lost with complete differentiation of the vascular strand to the tip. Cluster roots typically last a few weeks from initiation to senescence before a new cycle emerges upon further growth of the main root axis (Figure 3C). The effect of cluster roots on increasing Pi acquisition is mainly derived from very large bursts of carboxylates released from these ephemeral structures, which can represent over 50% of the carbon fixed by the plant through photosynthesis. These adaptive features make Proteaceae highly sensitive to phosphate toxicity and thus unfit for growth in soils rich in P. Cluster roots are not limited to Proteaceae and also occur in a few other families, including Leguminosae. Incidentally, the legume white lupine (*Lupinus albus*) is the best-studied model for cluster root biology (Figure 3B).

How do plants optimize Pi use?

Plants can adapt to Pi-deficient conditions by improving PAE and optimizing the internal use of Pi and Pi-derived metabolites to maintain growth, i.e., increase the ratio of biomass or yield generated per molecule of acquired Pi (P use efficiency [PUE]; Figure 2B). Phospholipids constitute one of the major pools of Po inside cells. Several plant species, including the high-PUE members of the Proteaceae family, respond to Pi deficiency by replacing phospholipids with non-phosphorylated galactolipids and sulfolipids, consequently liberating Pi for other metabolic processes. Such a shift in lipid composition involves activating numerous phospholipases and lipid biosynthetic enzymes, whose corresponding genes are typically strongly upregulated at the transcriptional level under Pi deficiency.

Long-term Pi deficiency leads to decreased Pi and ATP levels in the cell. Surprisingly, however, the inorganic pyrophosphate (PPi) levels remain relatively stable. This characteristic is exploited to increase PUE by replacing the enzymes that catalyze enzymatic steps that are negatively affected by the decreased availability of ATP and Pi with enzymes that catalyze similar reactions using PPi. For example, in glycolysis, the conversion of fructose-6-phosphate to fructose-2,6-bisphosphate mediated by the ATP-dependent phosphofructokinase is replaced with a PPi-dependent phosphofructokinase under Pi deficiency.

Plants not only produce extracellular nucleases and phosphatases that recycle soil Po under Pi deprivation but also a suite of similar intracellular hydrolytic enzymes that scavenge and recycle intracellular Po. This increased scavenging activity is often associated with senescence and ensures that the maximum amount of Pi is recovered within the plants to sustain the growth of sink tissues and ensure that Pi is transferred to storage organs (e.g., seeds and tubers) instead of being (temporarily) lost in the soil litter. Transcriptomic studies have revealed considerable overlap in the gene expression network between leaf senescence and the Pi deficiency response. Nucleic acids represent approximately 40% of the Po pool in plants, with a large fraction incorporated in ribosomal RNA. Ribosome degradation via autophagy involves a range of nucleases and phosphatases induced by both Pi deficiency and senescence. Interestingly, highly P-efficient Proteaceae contain extremely low levels of cytosolic and plastidial ribosomal RNA. While this feature likely reduces protein translation capacity, it appears to fit a strategy in this plant family whereby low growth rates are traded for high resilience to P-deficient soil. However, even in fast-growing model plants such as *A. thaliana*, the degradation of both mitochondrial and plastidial DNA during leaf senescence contributes to PUE under Pi deficiency. Plants typically contain numerous genes encoding purple acid phosphatases (29

genes in *A. thaliana*) that can liberate Pi from a broad spectrum of substrates. Many of these phosphatases are strongly upregulated under Pi-deficient conditions and during senescence.

Numerous Pi-H^+ co-transporters belonging to the *PHT1* gene family are expressed in aboveground tissues, including seeds and reproductive tissues. Several *PHT1* members partition Pi between the source (e.g., old leaves) and sink (young expanding leaves, roots, and seeds) tissues. Similarly, the gene encoding the Pi exporter PHO1 is expressed in the chalazal seed coat and is involved in transferring Pi from the maternal seed coat to the filial embryo. Understanding how PUE can be improved requires a deeper knowledge of how Pi transport between tissues (e.g., from senescing tissues to sink tissues) is controlled and coordinated. For example, the high photosynthetic efficiency of Proteaceae under very low leaf Pi concentration is thought to be partially caused by the preferential partitioning of Pi into the photosynthetic mesophyll cells, with minimal transfer to the non-photosynthetic epidermal cells.

The main storage organelle of plant cells is the vacuole, representing up to 90% of the cell volume in leaves. Plant cell vacuoles contain up to 80% of the total cellular Pi under Pi-sufficient conditions. By contrast, the vacuolar Pi concentration gradually decreases to maintain a near-constant level of cytoplasmic Pi concentration under Pi-deficient conditions. Moreover, excess Pi is stored in the vacuole after Pi replenishment. The movement of Pi across the tonoplast (i.e., the membrane of the vacuole) thus plays a crucial role in buffering the effects of Pi deficiency on cellular metabolism. The transporters involved in the import (PHT5) and export (VPE) of Pi across the tonoplast have only been identified recently. Although *VPE* genes are transcriptionally induced by Pi deficiency, *PHT5* genes are not, suggesting that PHT5 regulation may occur at the post-translational level.

The movement of Pi across the plastid is achieved by a range of transporters. These include a group of Pi translocators that exchange Pi for phosphorylated C3, C5, and C6 compounds (e.g., triose phosphate, phosphoenoylpyruvate, and glucose-6-phosphate, respectively), resulting in the net transport of phosphorylated carbon across the plastid envelope, while conserving the Pi balance. Other plastidial Pi transporters belonging to the PHT2 and PHT4 families are located in the plastid envelope or thylakoids; however, their precise regulation and function in Pi homeostasis remain unknown. Similar uncertainties exist for the mitochondrial Pi transporters belonging to the PHT3 family and the Golgi-localized PHT4;6. Although PUE and internal Pi cycling rely on coordinated Pi transport across various tissues and organelles, our current

knowledge of the contribution and regulation of the Pi transporters involved in these processes remains fragmentary.

How do plants sense and signal Pi deficiency?

The numerous developmental and metabolic changes in Pi-deficient plants are coordinated by several signal transduction pathways that modulate gene and protein expression (Figure 4). Recent studies have identified the binding of inositol pyrophosphates (PP-InsP), in particular bisdiphosphoinositol tetrakisphosphate ($InsP_8$), to proteins harboring the SPX domain as central to cell Pi sensing and the activation of numerous P deficiency adaptive responses.

PP-InsP is synthesized from the stepwise phosphorylation of inositol-*kis*-hexaphosphate ($InsP_6$ or phytate) to diphosphoinositol pentakisphosphate ($InsP_7$) and $InsP_8$, which contain one and two pyrophosphate groups, respectively (Figure 4). The enzymes involved in PP-InsP synthesis (ITPK and VIH) are bi-functional, having both kinase and phosphatase activities regulated by the cell ATP/ADP ratio, which depends on the cell's Pi status. Therefore, under Pi-sufficient conditions, the high ATP/ADP ratio stimulates the kinase reaction, leading to high $InsP_8$ levels. However, the low ATP/ADP ratio favors the phosphatase reaction under Pi-deficient conditions, leading to low $InsP_7$ and $InsP_8$.

The SPX domain is present in yeast, plant, and metazoan proteins involved in various aspects of Pi metabolism. In plants, the SPX domain occurs as a stand-alone domain in some proteins (thus named SPX proteins). It is also associated with other domains in proteins involved in Pi transport (e.g., PHO1 and PHT5) or protein ubiquitination (e.g., NLA). SPX binds PP-InsP at high affinity via a series of conserved lysine residues. In one well-described signaling module, PP-InsP bound to the SPX domain allows the SPX proteins to interact with PHR, a key transcription factor that regulates the expression of most Pi starvation-responsive (PSR) genes. Under Pi-sufficient conditions, the high PP-InsP level promotes SPX–PHR interaction and blocks the binding of PHR to its target promoter motif sequence, P1BS. Under Pi-deficient conditions, the SPX protein cannot bind PHR because of low PP-InsP levels, enabling PHR to activate genes involved in PAE and PUE at the transcriptional level. PP-InsP levels may also regulate the activity of other SPX-containing proteins, such as NLA, a ubiquitin E3 ligase involved in the ubiquitination and degradation of the Pi transporter PHT1. The impact of SPX-containing proteins on the Pi deficiency response may occur via the interaction of the SPX

domain with other proteins, as in the case of PHR, or via a more direct effect of PP-InsP binding on the activity of the proteins. In animals, $InsP_7$ mediates protein-independent pyrophosphorylation of key enzymes involved in metabolism. Although not yet observed, the possibility of $InsP_7$-mediated pyrophosphorylation of proteins in plants further suggests that modulation of PP-InsP levels by Pi status may influence PAE and PUE via various distinct pathways.

SPX-containing proteins can also bind and regulate proteins involved in nitrate metabolism, such as the NRT1 nitrate transporter and NLP transcription factor, as well as the transcription factor ORE1, which functions in senescence. SPX and PP-InsP are thus likely key components of the observed synergy between P and N status, as well as senescence.

The plant's response to Pi deficiency involves long-distance systemic signaling between the shoot and root. microRNA 399 (miR399) is a key systemic signaling molecule in the Pi deficiency response. Shoot miR399 synthesis is upregulated under Pi deficiency—a response controlled by the PHR1 transcription factor. miR399 is transported from the shoot to the root via the phloem, where it modulates the mRNA level of *PHO2*, encoding a component of the protein ubiquitination pathway involved in PHO1 and PHT1 protein degradation in roots. Several other microRNAs, as well as mRNA, have been identified in the phloem of Pi-deprived plants. Some of these molecules might also contribute directly to the plant's systemic response to Pi deficiency.

Conclusion

Remarkable progress has been made over the last decade in identifying the key pathways and components involved in the plant's adaptation to Pi deficiency and their broader integration with other aspects of plant biology. While our understanding of these fundamental aspects will surely progress further, we can start applying such knowledge to improve crops to maintain high yields under reduced P fertilizer input. Various key genes can be introduced, modified, or modulated using molecular tools (e.g., transgenesis, CRISPR-mediated genome editing, and more traditional mutagenesis techniques) to achieve this outcome. Interestingly, decades of rice (*Oryza sativa*) breeding under high P input have led to the exclusion, in most current rice cultivars, of the *PSTOL* gene identified 10 years ago as a key determinant of root growth and rice resistance to Pi deficiency. This observation highlights that, in the context of societal resistance to the use of transgenesis—and to a lesser extent of CRISPR gene editing—in crop

improvement, modern breeding methods and plant selection under low P input could have a substantial beneficial impact.

Declaration of interests

The author declares no competing interests.

Further reading

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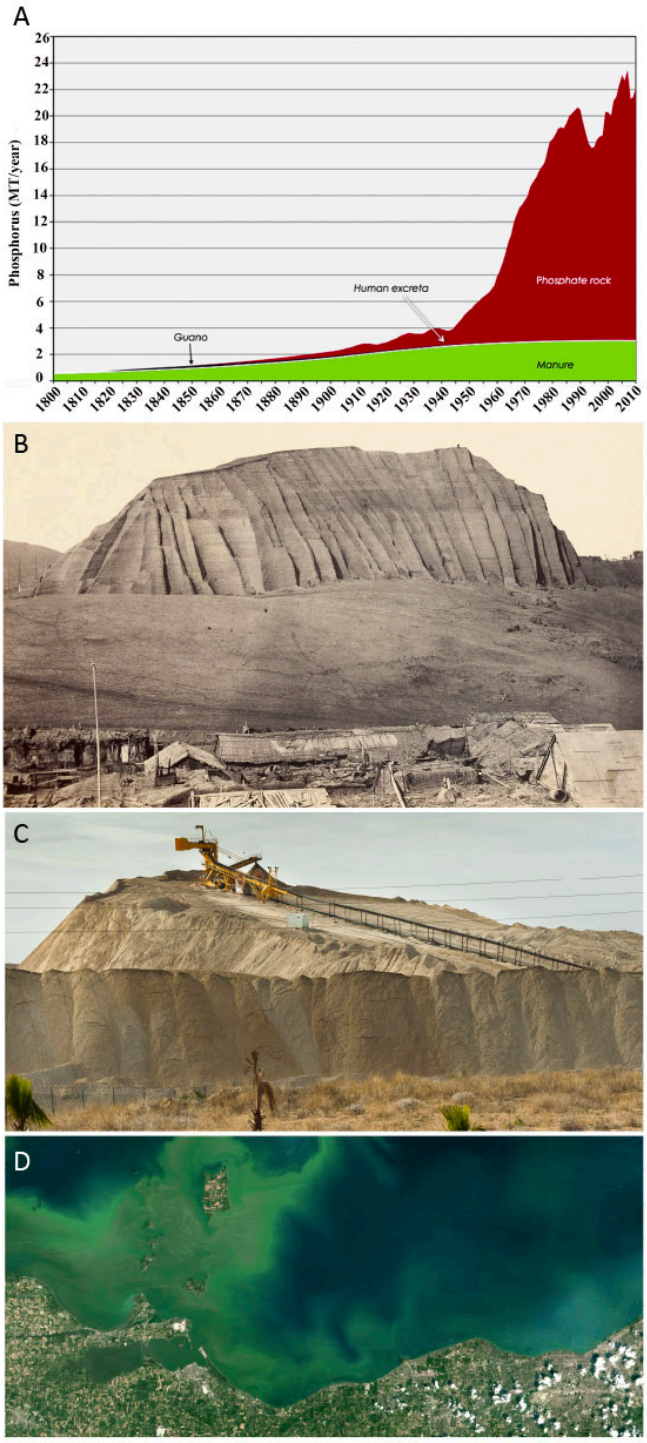
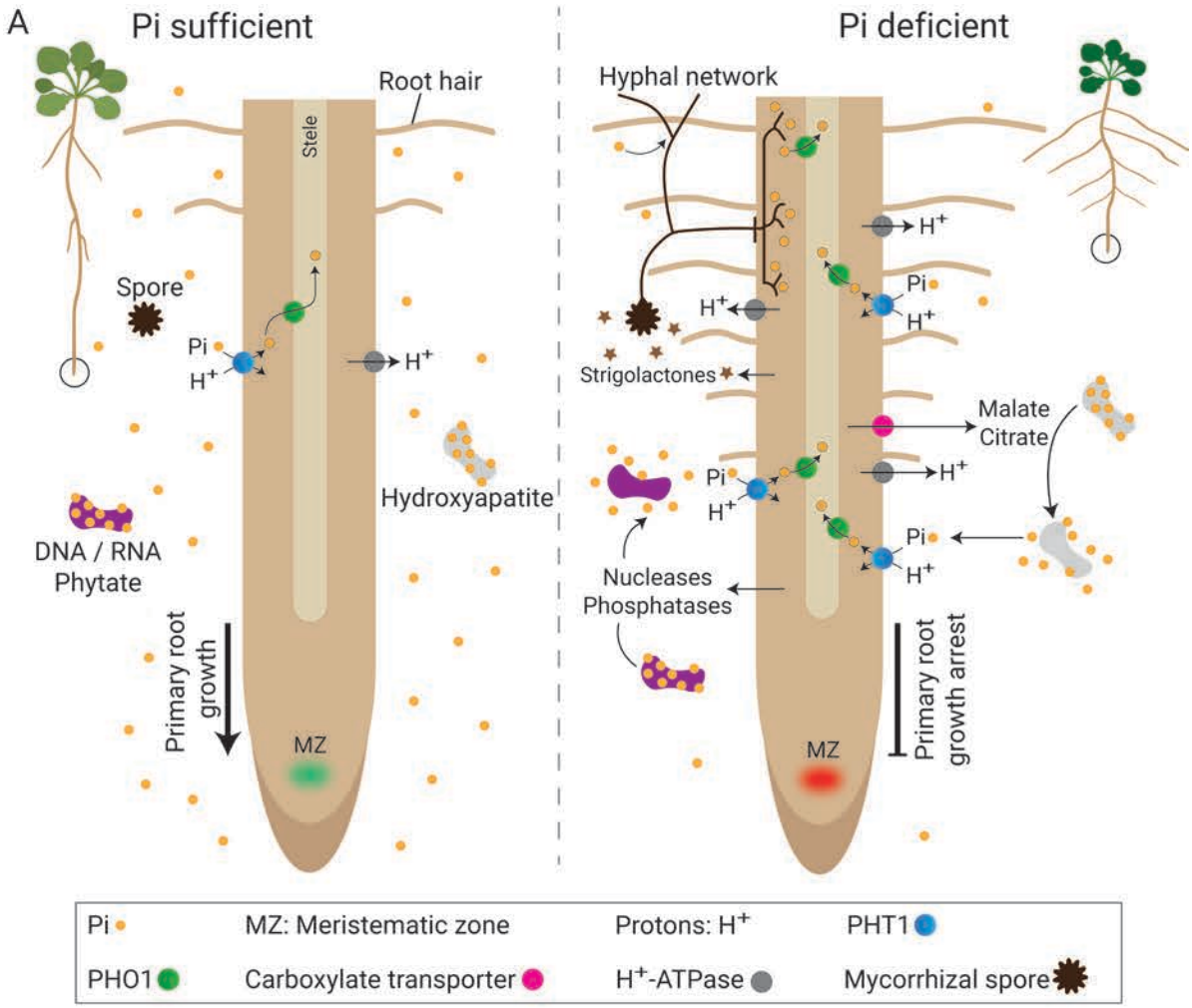


Figure 1. Phosphorus mining and its connection to fertilizer production and eutrophication. **A,** Global sources of agricultural P fertilizers from 1800 to 2010 (adapted from Ashley et al. (2011), doi: 10.1016/j.chemosphere.2011.03.001). **B,** A 2,000,000-ton guano hill in Chincha Islands, Peru, photographed in 1865. These deposits were mostly exhausted by 1874, following an export boom to Europe. **C,** Phosphate mine in Khouribga, Morocco. **D,** Satellite image of an algal bloom (green) in Lake Erie, Ohio, USA on 28th July 2015. The algal bloom was caused by agricultural fertilizer runoff into the lake.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Pi sufficient
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Root hair
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Spore
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Strigolactones
Malate
Citrate
Nucleases
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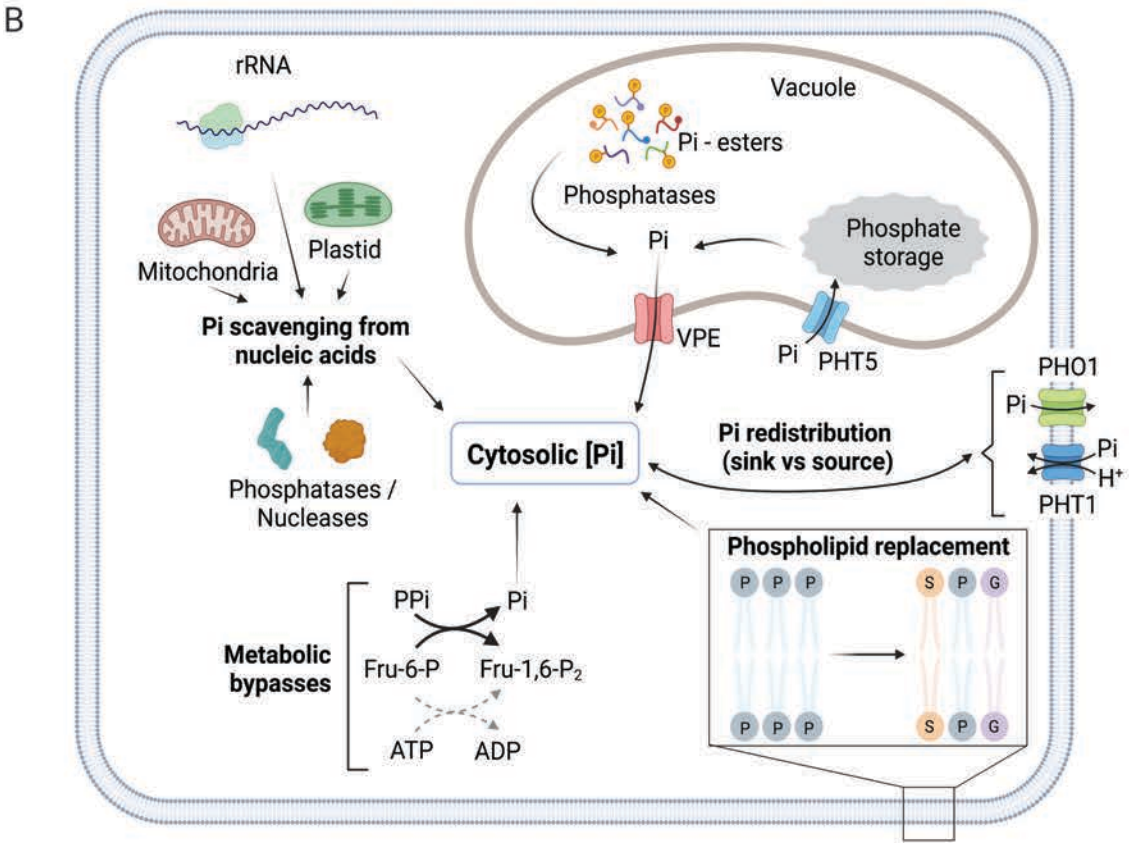
PHT1
PHO1
Carboxylate transporter
H+-ATPase
Mycorrhizal spore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rRNA
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Pi scavenging from
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PHT5
PHO1
Pi
Pi
H+
PHT1
Cytosolic [Pi]
Pi redistribution
(sink vs source)
Phosphatases /
Nucleases
Phospholipid replacement
P
S
G
Metabolic
bypasses
PPi
Pi
Fru-6-P
Fru-1,6-P2
ATP
ADP

Figure 2. Major developmental and metabolic adaptations of Pi-deficient plants. A. Increase in PAE involves changes in several aspects of root biology. Pi-deficient plants (right) have a shorter primary root, denser and longer secondary roots with reduced gravitropy, and denser and longer root hairs than Pi-sufficient plants (left). Pi deficiency stimulates the release of strigolactone, which enhances root mycorrhization (spore and hyphae are shown in black). Pi-deficient roots mobilize Pi (yellow dots) from soil Po (e.g., DNA, RNA, and phytate) via the release of nucleases and phosphatases, and from poorly soluble P forms (e.g., hydroxyapatite) by the release of carboxylates (e.g., malate and citrate) via transporters (pink spheres) and protons via H^+-ATPases (grey spheres). The expression of the PHT1 Pi-H^+ co-transporter (blue spheres) and vascular PHO1 Pi exporter (green spheres) are also enhanced under Pi deficiency. **B.** An increase in PUE involves phospholipid replacement by galacto- and sulfolipids, Pi scavenging from Po (including organellar DNA and ribosomal RNA) by nucleases and phosphatase, Pi redistribution between tissues (sink versus source) and within cell organelles (e.g., vacuole) by various Pi transporters, as well as the activation of metabolic bypasses that do not use ATP.

Figure 3. Plants that produce cluster roots under Pi deficiency. **A,** *Hakea prostrata*, a shrub that grows in the nutrient-poor sandy soil of Western Australia. **B,** The legume *Lupinus alba* (white lupin). **C,** Time course of the development and senescence of cluster roots from *H. prostrata* from day 1 (far left) to day 20 (far right); Source: Laliberté et al. (2014) doi.org/10.1111/nph.13203.

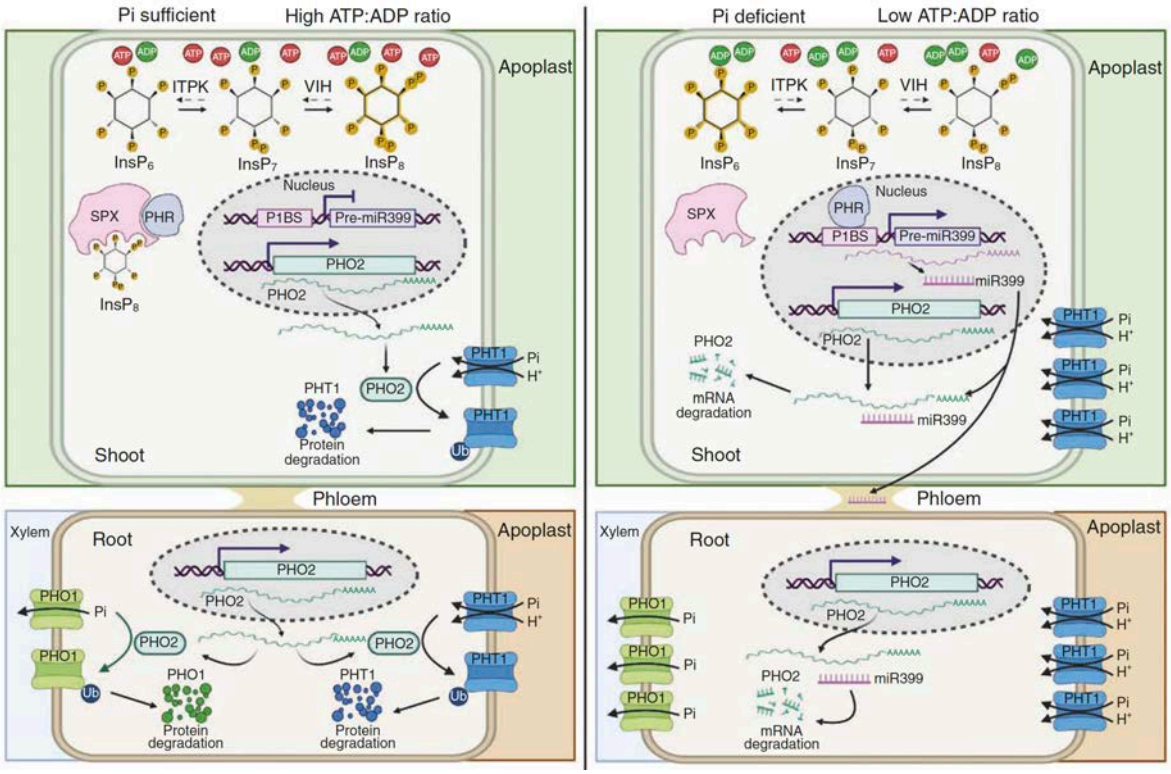

Figure 4. Major elements of the central Pi signal transduction pathway involving PP-InsP. Under Pi-sufficient conditions (left), the high ATP/ADP ratio favors $InsP_8$ synthesis by inducing the kinase activity of ITPK and VIH enzymes. SPX can bind PHR1 in the presence of $InsP_8$, preventing PHR1 from activating the transcription of numerous *PSR* genes (including the pre-miR399 locus) in the nucleus by binding to the *P1BS* sequence in the promoter. *PHO2* expression leads to the ubiquitination and degradation of the Pi importer PHT1 and Pi exporter PHO1. Under Pi-deficient conditions (right), the low ATP/ADP ratio triggers the ADP-transferase activity of ITPK, as well as the phosphatase activity of VIH, resulting in low $InsP_8$ levels. Under these conditions, PHR1 is free to activate the transcription of *PSR* genes. The mature miR399 microRNA binds to and inactivates *PHO2* transcripts, leading to increased PHT1 and PHO1 protein levels. Furthermore, miR399 moves from the shoots to the roots via the phloem, where it regulates *PHO2* turnover.