Phosphate Nutrition: Improving Low-Phosphate Tolerance in Crops

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Abstract

Phosphorus is an essential nutrient that is required for all major developmental processes and reproduction in plants. It is also a major constituent of the fertilizers required to sustain high-yield agriculture. Levels of phosphate—the only form of phosphorus that can be assimilated by plants—are suboptimal in most natural and agricultural ecosystems, and when phosphate is applied as fertilizer in soils, it is rapidly immobilized owing to fixation and microbial activity. Thus, cultivated plants use only approximately 20–30% of the applied phosphate, and the rest is lost, eventually causing water eutrophication. Recent advances in the understanding of mechanisms by which wild and cultivated species adapt to low-phosphate stress and the implementation of alternative bacterial pathways for phosphorus metabolism have started to allow the design of more effective breeding and genetic engineering strategies to produce highly phosphate-efficient crops, optimize fertilizer use, and reach agricultural sustainability with a lower environmental cost. In this review, we outline the current advances in research on the complex network of plant responses to low-phosphorus stress and discuss some strategies used to manipulate genes involved in phosphate uptake, remobilization, and metabolism to develop low-phosphate-tolerant crops, which could help in designing more efficient crops.

Contents

Phosphorus (P): a chemical element indispensable for plant nutrition

Inorganic phosphate (P_i): chemical forms of P ($H_2PO_4^-$ or HPO_4^{2-}) available in the soil solution and directly assimilated by plants into metabolites

Arbuscular mycorrhiza (AM): a symbiotic interaction between a fungus and a plant in which the hyphae of the fungus penetrate the cortical cells of the plant's roots and form branching invaginations (arbuscules)

INTRODUCTION

Phosphorus (P) is an essential nutrient that is required for plant development and reproduction, and it is one of the main components of the fertilizers required to sustain modern agriculture. Because of precipitation and mineralization processes, levels of inorganic phosphate (orthophosphate; P_i)—the only form of P that can be assimilated by plants—in many different types of soils are commonly suboptimal for vegetative growth and crop productivity. Approximately 70% of global cultivated land, including acidic and alkaline calcareous soils, suffers from P_i deficiency, making P_i nutrition a research area of great priority (12, 57, 68). When P_i is supplied as fertilizer, it is rapidly immobilized in the soil owing to its high reactivity with cations such as calcium and magnesium in calcareous soils or aluminum and iron in acidic soils. The abundance of soil microorganisms also affects P_i nutrition in a complex manner: Although arbuscular mycorrhizal (AM) fungi and certain plant growth–promoting rhizobacteria may enhance plant P_i acquisition, many other microbial species have the opposite effect because of their competition with roots for P_i uptake and because their activities convert P_i into organic forms that are not readily available for plant uptake (57, 162). Even under a well-designed P_i fertilization scheme, plant roots acquire no more than 30% of the applied P_i, and the rest is lost owing to fixation in the soil and microbial activity. This situation has led to the excessive application of fertilizers, contributing to the enrichment of water bodies with nutrients that cause eutrophication and toxic algal blooms (19, 147).

Global consumption of P_i fertilizer is currently approximately 50 million tons (62, 63), with a projected annual increase of 20 million tons by 2030 (12, 162). Recent estimates indicate that rock phosphate, apatite, and other raw materials used in the manufacture of P_i fertilizers, which occur in finite deposits mainly in China, the United States, and Morocco, are becoming increasingly

limited (63, 159). P_i fertilizer use is expected to increase worldwide, particularly in tropical and subtropical regions, such as the Cerrado region in Brazil, where land and water are still available for agricultural expansion but where there is a prevalence of acidic soils with low P_i availability (46). Therefore, improvements in crop nutrition to maximize phosphate acquisition efficiency (PAE) and phosphate utilization efficiency (PUE) are urgently needed to secure food production while protecting soil and water resources. In the past few years, considerable advances have been made toward understanding how plants adapt to low-P_i stress and the mechanisms that increase P_i uptake, transport, and utilization.

In this review, we summarize and discuss the relevant information on these topics and highlight some strategies that could be used to develop low-P_i-tolerant crops. We apologize to the many authors whose important contributions could not be cited because of space limitations.

Phosphate acquisition efficiency (PAE): the ratio between P_i shoot content under P_i-deficient conditions and P_i shoot content under a normal P_i supply

Phosphate utilization efficiency (PUE): the ratio of the total plant P, grain P, biomass yield, or grain yield to the total P, soil P, or P_i fertilizer applied

Root system architecture: root pattern distribution characterized by strong plasticity involving changes in the length of the primary root; the number, angle, and size of lateral roots; and the number and size of root hairs

PHOSPHATE ACQUISITION

Plant uptake of available P_i occurs through the concerted action of several P_i transporters and is greatly influenced by the root exploration capacity; the P_i uptake and scavenging capacity, which is determined mainly by the root system architecture; and the potential association of the root with soil microbes, such as AM fungi, that assist the plant in scavenging P_i from the soil (**Figure 1**). PAE is affected by other root traits that increase P_i availability in the soil solution, including the type and rate of efflux of organic acids (OAs) and phosphatases from the root into the rhizosphere.

Phosphate Transporters

Plants have low-P_i-inducible high-affinity and constitutive low-affinity P_i uptake systems. The high-affinity system functional at low P_i concentrations has an apparent K_m ranging from 3 to 10 μM, whereas the low-affinity system that operates at high P_i availability has a K_m ranging from 50 to 300 μM (125). The *Pht* (*Phosphate Transporter*) genes that encode P_i transporters have been characterized in detail in *Arabidopsis* and grouped into four families: *Pht1*, *Pht2*, *Pht3*, and *Pht4* (for a review, see 125). Remarkable progress has also been made in characterizing the P_i transporters in several economically important plant species, including tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*), soybean (*Glycine max*), rice (*Oryza sativa*), barley (*Hordeum vulgare*), and maize (*Zea mays*) (1, 35, 45, 106, 107, 116, 122–124, 150, 153, 163, 176).

P_i transporters encoded by members of the *Pht1* gene family, which are predominantly expressed in epidermal cells and in the outer cortex of the root, have been identified as mediators of P_i uptake at the root–soil interface when P_i is limited (102, 142). These proteins are part of the so-called direct P_i uptake pathway and transport P as P_i anions, mainly $H_2PO_4^-$ and HPO_4^{2-}, against a concentration gradient between the soil solution (which typically contains 0.1–10 μM P_i) and the cytoplasm of the root epidermal cell (which typically contains 5–10 mM P_i) (125) (**Figure 1**). Although the majority of *Pht1* transcripts have been located in root epidermal cells and root hairs, many of them have also been detected in leaves, stems, cotyledons, pollen grains, seeds, flowers, and potato tubers, suggesting their involvement not only in P_i uptake by roots but also in internal root-to-shoot distribution (1, 18, 23, 35, 107, 123). In soybean, 14 members of the *Pht1* family encode high-affinity P_i transporters predominantly expressed in roots under low-P_i conditions (35). In *Medicago truncatula*, 5 transporters encoded by *Pht1* genes are inducible by low P_i, of which MtPT1 (*Medicago truncatula* PHOSPHATE TRANSPORTER 1) and MtPT2 play an important role in P_i uptake; MtPT4 plays a role in P_i translocation from mycorrhizal fungi into the roots (54, 83, 178). In rice, the *Pht1* gene family is composed of 13 members (*OsPT1–13*); 4 of

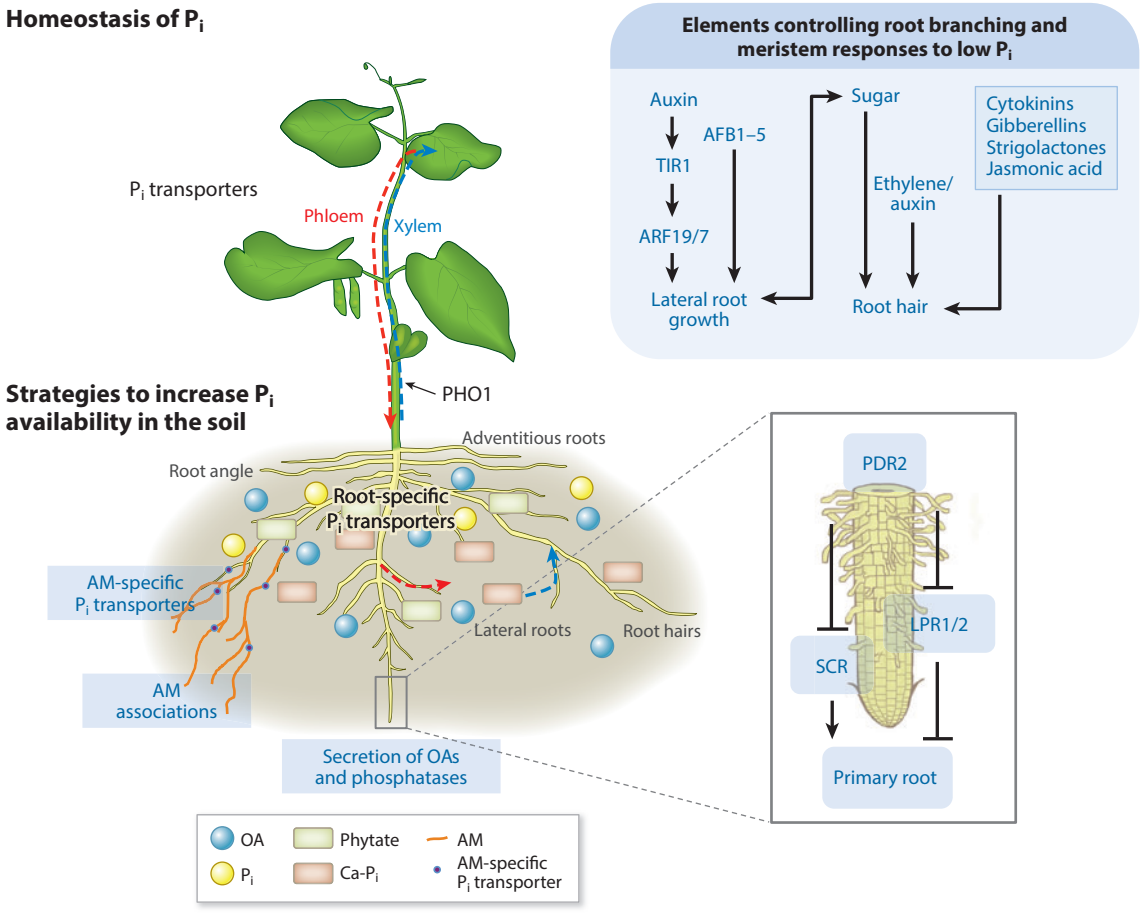

Figure 1

Plant strategies to enhance phosphate (P_i) availability in the soil. P_i is fixed in the soil owing to reactions with cations and converted by microbial activity into organic forms that are not readily available for plant uptake. The production and secretion of organic acids (OAs) and phosphatases as well as the arbuscular mycorrhizal (AM) associations with the root system of bean, for example, enhance P_i availability in the soil and P uptake by roots. In addition, the development of a highly branched root system, controlled by several genes (*TIR1*, *PDR2*, *SCR*, *LPR1/2*), sugar, and hormones, enhances these root traits. The concerted action of P_i transporters in the root–soil and AM interfaces, along with those present in the aboveground tissues, facilitates the acquisition and translocation of P_i into different plant organs. PHO1 plays a key role in P_i homeostasis. Red and blue dashed arrows along the stem denote phloem and xylem transport, respectively. Blue and red dashed lines in the rhizosphere indicate P_i uptake and OA secretion, respectively, in the roots.

these (*OsPT2*, *OsPT3*, *OsPT6*, and *OsPT7*) are highly expressed in P_i-deprived roots, but several (such as *OsPT6*) apparently play a dual role in P_i uptake from the soil and P_i translocation inside the plant (1, 47, 116). In maize, five *Pht1* genes (*ZmPht1;1–5*) are induced by P_i limitation not only in roots but also in other tissues, such as young and old leaves, anthers, pollen, and seeds (107). Therefore, at least some members of the *Pht1* gene family play an important role in P_i uptake from the soil solution, particularly when this nutrient is present in limiting amounts.

In contrast to *Pht1* genes, members of the *Pht2*, *Pht3*, and *Pht4* gene families have been associated mainly with P_i distribution within subcellular compartments, and their gene products are specifically located in the plastid inner membrane, mitochondrial inner membrane, and Golgi

compartment, respectively (21, 48, 163). In *Arabidopsis*, the low-affinity transporter PHT2;1, encoded by a member of the *Pht2* family, is located in chloroplasts, and a *pht2;1* mutation reduces P_i transport into the chloroplast and decreases P_i allocation throughout the whole plant. However, the *pht2;1* mutant is still viable, suggesting alternative mechanisms for P_i import into chloroplasts (163). Although the *Pht2* family of transporter genes is also present in *Medicago truncatula* and potato, compelling evidence of its function in these plants is still lacking. Mitochondrial P_i transporter genes have also been identified in soybean, maize, and rice (153); however, as is the case for chloroplast transporters, further investigation is needed to determine their physiological function and to evaluate their potential as biotechnological tools.

Auxin: a plant hormone implicated in the plasticity of the root system architecture, including the lateral root development triggered by low-P_i conditions

The concerted action of P_i transporters ensures P_i distribution to specific tissues, cells, and organelles. From an agronomic point of view, the ubiquity, diversity, and tissue distribution of P_i transporters ensure that photosynthesis and respiration operate normally even under high-stress conditions to sustain growth and reproduction (**Figure 1**). Therefore, naturally occurring or engineered alterations of the expression of different P_i transporters represent an opportunity to optimize uptake and proper distribution of P_i within a plant to improve yield.

Root System Architecture Modifications in Response to Low Phosphate Availability

Because of the low mobility of P_i in soil and the deposition of plant residues over time, surface soil layers generally have higher P_i content than subsoil layers. Root architecture modifications induced in response to low-P_i stress enhance topsoil foraging and allow the exploitation of these P_i reserves (**Figure 1**).

Regulation of root architecture. Significant progress has been made in dissecting the mechanisms underlying changes in root system architecture in response to P_i deficiency, particularly in *Arabidopsis*. In wild and cultivated plants, including *Arabidopsis*, maize, rice, and tomato, P_i availability alters root traits by modulating the developmental programs that control lateral root primordium initiation and emergence, primary and lateral root growth, the angle of lateral root growth, and the density and elongation rate of root hairs (87, 88, 118, 174). The effect of P_i availability on root development is complex, species specific, and genotype dependent and involves crosstalk between different hormone-signaling pathways (**Figure 1**). Signaling pathways—activated by auxins, ethylene, cytokinins, gibberellins, strigolactones, jasmonic acid, nitric oxide, sugars, or the redox status of the root meristem—play an important role in the root system architecture responses to low P_i availability (for reviews, see 17 and 51).

The first visible event in *Arabidopsis* plants experiencing P_i deficiency is a strong reduction of primary root growth, which occurs rapidly after transfer to a low-P_i medium and is followed by an arrest of cell division and loss of the quiescent center identity (139, 140, 152). Concomitantly, the formation of abundant lateral roots and root hairs that express high levels of P_i transporters and phosphatases is enhanced by P_i limitation (140) (**Figure 1**).

Different mechanisms have been proposed to modulate primary root growth and the enhanced lateral root and root hair formation in response to low-P_i conditions, including changes in auxin transport and an increase in sensitivity to auxin (88, 89, 104, 118). An increase in sensitivity to auxin in pericycle cells has been proposed to be responsible for increased lateral root initiation and emergence in plants grown under P_i deficiency (88, 118). This response was directly related to the findings that the expression of the *Arabidopsis* auxin receptor TIR1 (Transport Inhibitor Response 1) is higher in P_i-deficient plants than in plants grown under P_i-sufficient conditions and that TIR1 knockout mutants are impaired in the lateral root response to low P_i (118). The

increased lateral root formation mediated by TIR1 in response to P_i deficiency requires the presence of the ARF7 (Auxin Response Factor 7) and ARF19 transcription factors, which transduce this enhanced auxin response into the formation of new lateral roots (118) (**Figure 1**). However, it has also been observed that differential accumulation of auxin plays an important role in increasing the emergence of preformed lateral root primordia (104). Guo et al. (49) recently reported that expansins—cell wall proteins that influence cell division and expansion rates—may also play an important role in the root architecture responses to P_i deficiency. Overexpression of *GmEXB2*, which encodes a β-expansin in soybean, caused an increase of 69% and 53% in root cell division and elongation, respectively; 170% more growth; and 20% higher P_i uptake at both low and high external P levels in *Arabidopsis* (49).

Lateral root growth for enhanced phosphate acquisition. Root architecture responses to P_i availability vary significantly among and within species. For instance, in white lupin (*Lupinus albus*), the formation of clusters of lateral roots known as proteoid roots is the most evident response to low P_i, whereas in common bean (*Phaseolus vulgaris*), changes in the angle of lateral root emergence are the typical response, with perpendicular lateral root growth predominating over downward growth and promoting topsoil foraging (71, 91). Root architecture traits that promote topsoil foraging in common bean are root shallowness, adventitious root formation, and increased dispersion of lateral branching from the basal roots (5, 58, 78, 100, 126) (**Figure 1**). These characteristics imply that plants grown in soils with low P_i availability have a shallower and broader root system that increases the capacity of the plant to explore the upper layers of the soil in which organic and inorganic P_i-rich patches are most commonly present.

Common bean plants with P_i-efficient genotypes develop root systems with differential growth angles of lateral roots and/or increased adventitious root formation (58, 92) (**Figure 1**). The formation of greater root biomass through adventitious roots is less expensive for the plant because adventitious roots are metabolically less demanding per unit of P_i (92, 100, 109). These root traits are controlled by a low-P_i-sensing system and are influenced by ethylene, permitting the plant to explore a larger volume of the upper layers of the soil (5, 100) (**Figure 1**).

Field trials and greenhouse experiments with bean, maize, and soybean suggest that genotypes that develop shallow root systems are better adapted to low-P_i soils (9, 78, 170, 189–192). However, the response to P_i deficiency in terms of lateral root formation and elongation shows species and genotypic variations. In maize, some genotypes respond by increasing the number and length of lateral roots, whereas others show the opposite effect. The mature root system in maize is composed mainly of nodal roots, so the maintenance of nodal root formation could result in an increased proportion of root length in the adventitious root system when the overall growth is inhibited by P_i deficiency (9).

A novel root phenotype in common bean associated with the efficiency of P_i acquisition is the basal root whorl number, defined as the number of distinct tiers of basal roots that emerge in a tetrarch fashion along the base of the hypocotyl (99). In low-P_i soils, genotypes with three whorls produced almost twice the shoot biomass, greater total root length, and greater leaf area compared with related genotypes with two whorls (98, 99). Therefore, a larger basal root whorl number is beneficial for P_i acquisition in low-P_i soils and may be a useful trait for the selection of genotypes with better performance in these soils (98). Additionally, although there is no extensive information about the role of aerenchyma formation in roots under P_i deficiency, this root trait has also been observed to contribute to low-P_i adaptation in common bean and maize (36, 121).

The role of root hairs in phosphate acquisition. Under low-P_i conditions, root hairs can contribute almost 70% of the total surface area of the roots and can be responsible for up to 90%

of the P_i acquired by plants (6–8) (**Figure 1**). The role of root hairs as an important element of the network of root traits involved in P_i uptake efficiency was determined in barley and *Arabidopsis* mutants lacking root hairs, in which P_i uptake is drastically reduced (7, 8, 39). Field trials with two barley cultivars—Zita, which has short root hairs, and Salka, which has long root hairs—showed that the Salka cultivar is more effective in exploring the soil, acquires more P_i, and produces more shoot biomass than the Zita cultivar (40). Using a novel mathematical model and experimental data available in the literature, Brown et al. (11) evaluated the costs and benefits of the length, density, and longevity of root hairs with respect to P_i acquisition. Two interesting predictions emerged: (*a*) that the greatest gains in P_i uptake efficiency are likely to derive from increasing root hair length and longevity rather than their density, and (*b*) that a highly efficient root system for P_i scavenging and uptake should be composed mainly of roots that are able to develop and maintain long root hairs capable of releasing more P_i-mobilizing exudates in the upper layers of the soil, where P_i-rich patches are found. Plants with these root traits could be engineered to produce and release larger amounts of OAs and phosphatases that can efficiently hydrolyze phytate and other organic P_i esters.

Organic Acid Exudation

The production and root exudation of OAs, such as citrate, malate, and oxalate, enhance P_i availability in highly P_i-fixing soils (**Figure 1**). OAs release P_i bound to Al^{3+}, Fe^{3+}, and Ca^{2+}, which are common in the upper layers of the soil, where P_i-rich soil patches are most frequently found. OA exudation is controlled at the transcriptional level in monocots and dicots and is highly induced under P_i deficiency in wild species naturally adapted to P_i-deficient soils, such as white lupin and several other species of the Proteaceae family. In crops such as rapeseed (*Brassica napus*), rice, alfalfa (*Medicago sativa*), chickpea (*Cicer arietinum*), maize, wheat (*Triticum aestivum*), soybean, triticale, and rye (*Secale cereale*), OA exudation increases in response to both P_i deficiency and aluminum toxicity (137). OA exudation is a major trait in breeding crops with improved PAE, and this trait also contributes to a high-PUE phenotype in some soybean varieties (32).

OA exudation occurs mainly at the root tip, but, interestingly, in white lupin and species of the Proteaceae family it is restricted to the low-P_i-inducible proteoid roots. These structures not only increase the root surface area by more than 100-fold to aid in exploration for P_i but also secrete OAs in millimolar concentrations into the rhizosphere, mainly as citrate and malate (66, 70, 162). The metabolic investment in root OA exudation can reach 20% of the total carbon fixed by photosynthesis, highlighting the importance of OA to enhancing P_i uptake (92).

In white lupin, OA exudation from proteoid roots has been associated with enhanced activity of some metabolic enzymes, such as phosphoenolpyruvate carboxylase, malate dehydrogenase, and citrate synthase (114, 158). However, although wheat and tomato plants have increased concentrations of OAs in roots and shoots under low-P_i conditions, apparently no exudation of OAs occurs, suggesting that particular OA transporters may be required for OA exudation. Three classes of transporters for malate and citrate have been identified so far. Malate excretion at the plasma membrane occurs through ALMT (aluminum-activated malate transporter) channels (59, 141), whereas citrate excretion is catalyzed by members of the MATE (multidrug and toxic compound extrusion) family of transporters. In *Arabidopsis*, the MATE family contains 56 plasma membrane–located members, of which 2 have been shown to excrete citrate out of the cytosol. AtMATE/AtDTX42 (*Arabidopsis thaliana* DETOXIFICATION 42; At1g51340) and its closest sorghum homolog (SbMATE) confer Al^{3+} tolerance by mediating the excretion of citrate into the rhizosphere in response to Al^{3+} (81, 95). In contrast, AtFRD3 (*Arabidopsis thaliana* FERRIC REDUCTASE DEFECTIVE 3), a close homolog, is implicated in the excretion of citrate into

the xylem and is required for long-distance iron transport and uptake into leaf cells (34, 132). The function of most plant MATE proteins and their possible role in adaptation to low-P_i stress have not been established.

The quantity and type of OAs secreted by roots determine the level of P_i solubilization in the rhizosphere. The synthesis and exudation of piscidic acid by the roots of some plants, such as pigeon pea (*Cajanus cajan*), have been reported to be highly effective in releasing P_i from iron- and aluminum-P_i complexes (64). Pigeon pea has been used as an intercrop because of its capacity to enhance P_i availability. Piscidic acid exudation has not been reported in crops, and the mechanisms of its synthesis and exudation have not been elucidated. Therefore, elucidating the pathway of piscidic acid biosynthesis represents an opportunity to engineer a novel trait for improving PAE in crops.

Production and Secretion of Phosphatases

Between 30% and 70% of the total P in agricultural and natural soils is present in organic forms. Therefore, the production and secretion of different types of enzymes, such as acid phosphatases and nucleases, into the rhizosphere contribute to the release of P_i from these organic sources (10). For instance, purple acid phosphatases can act on a wide range of organic molecules, cleaving P_i from ester linkage sites, and are relatively stable over wide intervals of pH (4.0–7.6) and temperature (22–60°F) (**Figure 1**). The importance of purple acid phosphatases to plant P_i nutrition has been clearly shown through the study of the *pup* (*phosphatase under-producer*) and *atpap10* (*Arabidopsis thaliana purple acid phosphatase 10*) mutants of *Arabidopsis*, which have reduced purple acid phosphatase activity and display a decreased ability to grow when supplied with organic P_i sources (157, 167), and through analyses of transgenic plants that overproduce acid phosphatases, which have improved use of organic P sources (74, 94, 167, 177).

Purple acid phosphatase synthesis and secretion have been important to developing low-P_i tolerance in soybean and common bean (77, 169). For example, the level of PvPAP3 expression in common bean was identified as an important trait for low-P_i adaptation, given that its expression is induced at a higher level in the P_i-efficient genotype G19833 than in the P_i-inefficient genotype DOR364 (77).

The Role of Mycorrhizae in Phosphate Acquisition in Agricultural Soils

The mycorrhizal state is considered the norm for most plants in natural ecosystems. As the AM fungi colonize plant root cortical cells, extraradical hyphae operate as an extension of the root system and acquire P_i from regions of the soil beyond the P_i-depletion zone close to the root. AM symbiosis has been associated with changes in the composition and concentration of plant hormones such as cytokinins, auxins, ethylene, and strigolactones in roots, which in turn are involved in regulation of the root architecture both before and after root colonization by mycorrhizal fungi (50).

The P_i contribution of AM to the plant varies widely among the different plant–fungus species combinations, ranging from the total amount of P_i assimilated by the plant down to a negligible contribution (146, 164). *Pht1* P_i transporter genes have been identified in tomato, potato, *Medicago*, and rice that are specifically expressed in mycorrhizal roots and mediate P_i transport across the periarbuscular membrane (45, 54, 106, 127). Some of these transporters, such as OsPT11 and OsPT13, are indispensable for establishing this association and contribute 70% of the symbiotically acquired P_i (182).

A field of research is being opened by studies of common molecular regulatory mechanisms in symbiotic associations of legumes with *Rhizobium* bacteria for nitrogen fixation and AM

associations. However, further investigation is necessary to determine whether it will be possible to simultaneously improve P_i and nitrogen assimilation. Although the role of AM in plant nutrition under agricultural conditions remains controversial because P_i fertilization reduces their capacity to colonize roots, AM fungi are potentially useful for creating sustainable agricultural systems using low-P_i-input cropping (37).

PHOSPHATE SENSING AND MOLECULAR RESPONSES TO PHOSPHATE DEFICIENCY

To coordinate the molecular and morphological responses to P_i limitation, plants require monitoring systems to perceive and integrate information on the local and whole-plant P_i status (17, 31, 161, 183). Although the nature of the local and systemic P_i sensor(s) in plants is still unknown, many components of the P_i-deficiency-signaling pathway have been identified during the past decade (17). Changes in root system architecture in response to low P_i are regulated mainly by sensing the local concentration of P_i in the soil solution (8, 152). However, long-distance signaling also affects root responses to P_i status because the assimilation and subsequent partitioning of carbon between shoots and roots is influenced by P_i deficiency.

Local Phosphate Sensing

The *PDR2* (*Phosphate Deficiency Response 2*), *LPR1* (*Low Phosphate Root 1*), and *LPR2* genes, which encode an endoplasmic reticulum-resident protein (156) and multicopper oxidases (152), respectively, have been identified as important regulators of meristem responses to low P_i availability in the *Arabidopsis* primary root (**Figure 1**). In P_i-deficient conditions, *pdr2* mutants have exaggerated reduction in rates of cell division and elongation in primary root growth, whereas *lpr1* and *lpr2* mutants show the opposite phenotype, sustaining normal primary root growth (152, 155). These phenotypes suggest that mutations in the *PDR2* and *LPR* genes affect local P_i sensing. Application of phosphite, a nonmetabolizable analog of P_i, reverts the *pdr2* phenotype, indicating that this mutant is affected in the local response to P_i availability but is not a component of the local P_i-sensing mechanism (155). PDR2 has been proposed to function together with LPR1 in regulating root meristem activity through an endoplasmic reticulum–located pathway that regulates SCR (Scarecrow) levels by restricting the movement of SHR (Short-Root) from the endodermis to adjacent cell layers under P_i-deficient conditions (108, 156) (**Figure 1**). Transcriptomic analyses have revealed that PDR2, SCR, and SHR orthologs in maize, wheat, common bean, white lupin, and rice exhibit altered expression patterns under P_i deficiency (13, 56, 112–114), suggesting that although changes in the root architecture may differ among these plant species, similar pathways are involved in determining root patterning and root meristem activity in response to P_i availability.

Long-Distance Phosphate Signaling

Plant metabolic acclimation to P_i deficiency involves sensing the nutrient status in the shoot and is dependent on photosynthesis, the transport of sugars, and their further distribution between source and sink tissues (82). Many P_i-deficiency-induced genes require sugars for maximal expression (53, 67, 82). For example, in white lupin, the P_i transporter gene *LaPT1* and the secreted acid phosphatase *LaSAP1* gene are upregulated by exogenous sucrose in P_i-sufficient seedlings grown in darkness and are repressed in cluster roots in dark-adapted plants grown in P_i-deficient conditions (82). The importance of crosstalk between low-P_i signaling and

sugar levels is also highlighted by the following two important findings: (*a*) Expression of root P_i-deficiency-induced genes is impaired in the *Arabidopsis pho3* (*phosphate 3*) mutant, which is defective in sucrose loading into the phloem, and (*b*) an increase in shoot and root sucrose content caused by elevated expression of *SUC2* (*Sucrose Transporter 2*) in the *hps1* (*hypersensitive to phosphate starvation 1*) *Arabidopsis* mutant leads to overexpression of 73% of the low-P_i-responsive genes under P_i-sufficient conditions (72, 85).

Together with sugars, long-distance movement of microRNAs (miRNAs) has been proposed to act in systemic P_i sensing (72, 115, 161). The best-characterized example of systemically transported miRNAs involved in the regulation of P_i responses is miR399. miR399 directs the cleavage of the *PHO2* mRNA, which encodes the low-P_i-responsive UBC24 (ubiquitin-conjugating E2 24) enzyme (3, 16, 115). *pho2* mutants of rice and *Arabidopsis* overaccumulate P_i in their leaves when grown in P_i-sufficient soil, resulting in symptoms of P_i toxicity (26, 165). Therefore, miR399 participates in P_i homeostasis in the plant by modulating PHO2 mRNA levels. The presence of miR399 in response to P_i deficiency in various plant species, including rice, tobacco (*Nicotiana tabacum*), pumpkin (*Cucurbita maxima*), common bean, soybean, and oil rapeseed (*Brassica napus*) (79, 80, 115, 151, 179), suggests that the systemic P_i-signaling pathway is well conserved among angiosperms.

Some of the major components involved in transducing low-P_i systemic signals have been described in the past 10 years. PHR1 (PHOSPHATE STARVATION RESPONSE 1) and PHL1 (PHR1-LIKE 1) regulate the expression of a large subset of P_i-deficiency-responsive genes. Both PHR1 and PHL1 encode members of the MYB family of DNA-binding proteins that have a predicted coiled-coil domain involved in protein dimerization and that regulate the expression of P_i-deficiency-responsive genes, including miR399, by binding to the GNATATNC P1BS (PHR1-specific binding sequence) DNA motif (3, 134). PHR1 has two predicted SUMOylation sites, and its SUMOylation is mediated by the nuclear-located SIZ1 [SUMO (small ubiquitin-like modifier) E3 ligase 1)] (101). PHR1 orthologs have been identified in several plant species, including maize, rice, common bean, and wheat (129, 160, 166, 168, 188). In addition to PHR1, other P_i-responsive transcription factors that play important roles in different aspects of the metabolic and morphological responses to P_i limitation have been identified in several plant species (22, 28–30, 188) (**Table 1**).

miR399: a microRNA upregulated by low-P_i conditions that mediates the cleavage of the *PHO2* mRNA

PHO2 (PHOSPHATE 2): a ubiquitin-conjugating E2 enzyme that controls PHO1 activity and whose transcript levels are finely tuned by miR399

PHR1 (PHOSPHATE STARVATION RESPONSE 1): a MYB-type transcription factor that plays a central role in transcriptional regulation under low-P_i conditions

PHO1 (PHOSPHATE 1): a phosphate exporter involved in P_i transport from root to shoot by loading P_i into the xylem vessels; it is regulated through the SPX and EXS domains

PHOSPHATE TRANSLOCATION AND REMOBILIZATION INSIDE THE PLANT

One of the most important pathways for controlling P_i homeostasis was initially defined by the identification of *PHO1*, a gene implicated in the translocation of P_i from roots to shoots (52, 171) (**Figure 1**). *pho1* null mutants have a low P_i level in shoots and show the typical phenotype associated with P_i deficiency, including severely reduced shoot growth and seed yield as well as anthocyanin accumulation (120). PHO1 specifically facilitates P_i efflux out of the cells and into the xylem vessel (149) and is located primarily in the early endosomes/*trans*-Golgi network, although under high-P_i conditions it can be located partially at the plasma membrane (2). Thus, PHO1 may mediate P_i efflux by loading trafficking vesicles with P_i before they reach the plasma membrane. *Brassica rapa* has the largest *PHO1* gene family, represented by 23 genes, whereas soybean has 14 putative *PHO1* homologs, and *Brachypodium distachyon* and maize have only 2 *PHO1* homologs each (55). Rice has 3 *PHO1* homologs, which cluster with *Arabidopsis AtPHO1* and *AtPHO1;H1* (143). The study of insertion mutants of the rice *OsPHO1;2* gene confirmed its importance in plant growth; *ospho1;2* mutants have a strong reduction in P_i transfer from root to shoot, as indicated by high P_i accumulation in roots and a P_i-deficient phenotype in leaves (143).

Table 1 Major transcription factors involved in phosphate (P_i) sensing and molecular responses to phosphate deficiency in different plant species

Transcription factor	Description	Differential expression under low-P_i conditions					Main effects	Reference(s)
		Rice[a]	Maize[b]	Wheat[c]	Common bean[d]	White lupin[e]		
PHR1 (OsPHR2, PvPHR1, ZmPHR1, TaPHR1)	MYB TF	NC	NC	NC	NC	NC	Overexpression in rice improved root architecture in hydroponic and soil pot experiments showing P_i overaccumulation and toxicity Overexpression in wheat stimulated lateral branching, improved P_i uptake in hydroponic and soil pot experiments, and increased grain yield in field trials	160, 166, 188
PTF1 (OsPTF1, ZmPTF1)	bHLH TF	Up	Up	ND	ND	Up	Overexpression in rice increased the number of tillers, biomass, P_i content, and P_i uptake Overexpression in maize improved root development, increased the number of tassel branches, increased kernel size, and increased levels of soluble sugars in roots	75, 184
MYB2P-1 (OsMYB2P1)	R2R3 MYB TF that is involved in low-P_i signaling in rice	Up	Up	Up	ND	Up	Overexpression in rice enhanced tolerance to P_i deficiency, whereas suppression by RNAi in rice increased sensitivity to P_i deficiency	22
WRKY75	WRKY TF that positively regulates low-P_i responses but negatively regulates lateral root and root hair growth in *Arabidopsis*	Up	ND	ND	Up[f]	Up[f]	NO	28

(*Continued*)

Table 1 (*Continued*)

Transcription factor	Description	Differential expression under low-P_i conditions					Main effects	Reference(s)
		Rice[a]	Maize[b]	Wheat[c]	Common bean[d]	White lupin[e]		
WRKY6	WRKY TF	Up[f]	ND	ND	Up[f]	Up[f]	NO	14
ZAT6	C2H2 zinc-finger TF involved in root architecture, P_i uptake, and P_i accumulation in *Arabidopsis*	Up[f]	Up[f]	Up[f]	Up[f]	ND	NO	30
BHLH32	bHLH TF that negatively regulates low-P_i responses	Up[f]	Up[f]	ND	ND	Up[f]	NO	15
MYB62	MYB family member	Up[f]	Up[f]	ND	ND	Up[f]	NO	29

Abbreviations: bHLH, basic helix-loop-helix; RNAi, RNA interference; TF, transcription factor. Differential-expression and effect abbreviations: NC, no change; ND, no data; NO, not overexpressed; Up, upregulated.

[a]Data reported by Li et al. (73).

[b]Data reported by Calderón-Vázquez et al. (13).

[c]Data reported by Oono et al. (113).

[d]Data reported by Hernández et al. (56).

[e]Data reported by O'Rourke et al. (114).

[f]In reference to the transcription factor family.

Interestingly, *Arabidopsis* lines expressing low levels of the *PHO1* transcript because of gene silencing have normal shoot size but a low shoot P_i content, similar to that of the *pho1* mutant (133). Expression of the rice *PHO1* ortholog in the *Arabidopsis pho1* null mutant also leads to plants able to maintain normal growth despite having low shoot P_i (133). These results suggest that plants with reduced levels of PHO1 can have normal growth with a limited amount of P_i without suffering the drastic metabolic and morphological effects normally caused by P_i deprivation. The finding that low levels of PHO1 are still capable of allowing P_i transport to the shoot but eliminate many of the symptoms of P_i deficiency suggests that PHO1 either is part of the systemic P_i sensory system or contributes to the transport of a systemic signal (either a miRNA or a metabolite) that regulates the responses to P_i limitation; it also suggests that when PHO1 is expressed at low levels, it is incapable of functioning as a sensor or transporting the systemic signal (**Figure 2**). Because several miRNAs and mRNAs are transported by the plant vascular system, it will be interesting to examine the effect of low-P_i stress on RNA trafficking in the vascular system. Comparison of both miRNA and mRNA content in the phloem and xylem saps of wild-type, *pho1*, and *PHO1*-underexpressing lines grown in contrasting P_i conditions will probably allow the identification of RNAs that are important in the systemic signaling to P_i deprivation.

The PHO1 protein contains an SPX (SYG/Pho81/XPR1) tripartite domain in its N terminus and an ERD1/XPR1/SYG1 domain in the C-terminal region (171). PHO1 function is tightly controlled by PHO2 through its N-terminal SPX domain (84). The SPX domain is present in several low-P_i-responsive genes, such as those encoding P_i transporters and signaling proteins in yeast and plants (33, 143, 173, 148). *Arabidopsis* and rice have four and six low-P_i-responsive genes, respectively, which contain a conserved SPX domain (33, 173). In rice, the OsSPX1–6 proteins localize to different organelles, suggesting that they could have different roles in P_i homeostasis. Specifically, the expression of *OsSPX1* is induced by P_i deficiency via the PHR1-PHO2 signal pathway (173). In RNA-interference (RNAi) *OsSPX1* rice lines, P_i-deficiency-responsive genes (*OsPT2* and *OsPT8*) are highly induced, which causes an increase in the P_i content in leaves (165).

P_i transporters in plants are ubiquitous, suggesting that they play different and complementary functions in maintaining P_i homeostasis. *OsPT1*, *OsPT2*, and *OsPT6* are known to actively participate in P_i homeostasis because suppression of these genes leads to a decrease in total P_i transport from roots to shoots (1, 150). Phylogenetic analysis showed that TaPHT1;2 is closely related to OsPT2, suggesting that TaPHT1;2 may have a similar role in wheat (97). In soybean, two low-affinity P_i transporters encoded by members of the *Pht1* gene family, GmPT1 and GmPT2, participate in P_i translocation (176). Interestingly, almost all GmPTs are upregulated not only by P_i deficiency but also by nitrogen, potassium, and iron deficiency, suggesting crosstalk among different nutrient-signaling pathways (122).

METABOLIC RESPONSES

The metabolic effects of P_i deficiency on global gene expression in plants include enhanced expression of genes encoding alternative glycolytic enzymes, mitochondrial electron transport proteins that do not require P_i or adenylates as cosubstrates, and enzymes that allow the scavenging of internal sources of P_i, such as nucleases, phosphatases, and phospholipases, which remobilize P_i from different P_i-containing substrates (4, 20, 42, 186) (**Figure 1**). However, despite the importance of these metabolic traits to optimize P_i usage, their direct importance to develop or select low-P_i-tolerant crops has not been established; we therefore do not discuss in detail recent advances in this area and direct readers to a recent review on this topic (119). Part of the problem with studying the contribution of metabolic alterations to optimizing P_i usage during P_i deprivation is that most of these enzymes are encoded by multigene families. Therefore, because most of these

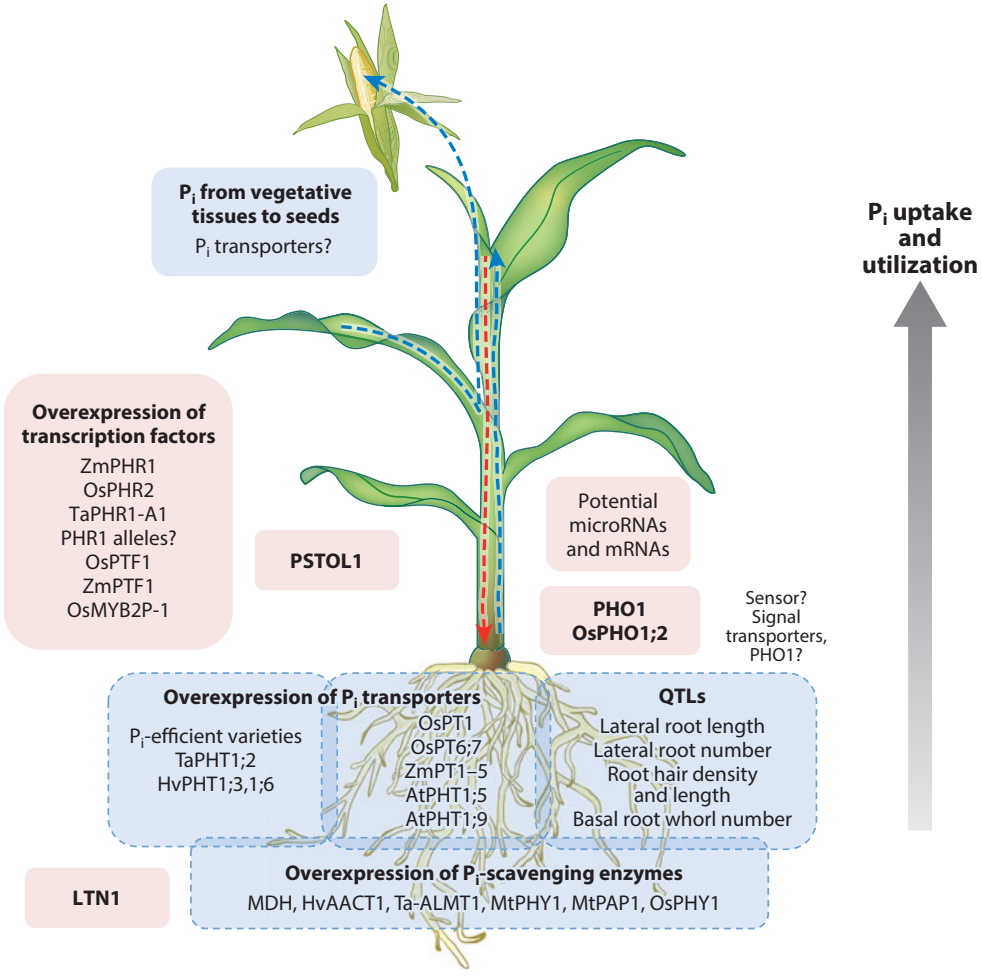

Figure 2

Development of a crop tolerant to low phosphate (P_i). Manipulation of the expression of transcription factors, P_i transporters, and P_i-scavenging enzymes as well as the identification of quantitative trait loci (QTLs) associated with P_i acquisition and utilization efficiencies (involved in root traits) must be considered to develop low-P_i tolerance in crops. PSTOL1, PHO1, and potential microRNAs and mRNAs are central elements in this process. In addition, strategies to avoid P_i toxicity must be considered. Red and blue dashed arrows along the stem denote phloem and xylem transport, respectively. Blue boxes with dashed edges denote the integration of genes and QTLs controlling root traits that are relevant for high P_i uptake efficiency.

pathways are well conserved in many organisms, a potential alternative is to study the effect of mutations on some of these genes in simpler models, such as yeast or microalgae, using robotic systems that allow the simultaneous analysis of hundreds of mutants that can be directly compared with the wild-type strain in different media compositions.

IMPROVING TOLERANCE TO LOW-PHOSPHATE STRESS

Genetic diversity among species provides an opportunity to improve low-P_i tolerance. Several studies have started to dissect the components of complex traits such as those determining root

system architecture and PAE. In this section, we describe some of the most relevant efforts in this regard.

Quantitative trait locus (QTL): a portion of DNA that contains or is linked to genes related to a quantitative attribute

Quantitative Trait Loci Associated with Low-Phosphate Tolerance in Crops

As discussed above, modifications to the root system architecture can be crucial for a plant to adapt to low-P_i stress. Quantitative trait loci (QTLs) that associate root traits such as lateral root length and lateral root number with PAE and PUE have been identified in rice, common bean, and maize. In the low-P_i-tolerant maize genotype Mo17, QTLs associated with lateral root branching and length and root hair length have been reported (189, 190) (**Figure 2**). In rice, *qREP-6* (*Root Elongation Under Phosphorus Deficiency 6*), a QTL associated with lateral root length, produces a positive correlation between P_i shoot content and tiller number in low-P_i soils (145). Through the use of P_i-inefficient and P_i-efficient *Brassica napus* genotypes, two low-P_i-specific QTLs that respectively accounted for 9.4% and 16.8% of the phenotypic variations in plant dry weight, P_i uptake, root system architecture, and root volume in low-P_i soils were detected (181). In common bean, QTL analysis showed the importance of basal roots and adventitious roots for P_i acquisition (78, 111) and also the importance of root hair density and length for P_i efficiency in the field.

In addition, some QTLs associated with other root traits of P_i-efficient genotypes, such as basal root whorl number, adventitious root formation, and root hair length, have been identified. The basal root whorl number is associated with three major QTLs, explaining 58% of the phenotypic variation in the acquisition of P_i efficiency in common bean (91, 98). The production of greater root biomass at a lower metabolic cost was related to two major QTLs accounting for 19–61% of the phenotypic variation under low-P_i field conditions (111). Root hair length and density and root exudation correlated with higher yield in P_i-limited soils, and 19 and 5 QTLs associated with these traits were identified in bean and maize, respectively (180, 189), suggesting that all of these root traits should be considered targets to improve P_i efficiency in crops.

Results in rice suggest that the low-P_i tolerance naturally present in wild cultivars can be used to improve PAE and PUE in modern varieties (41, 175). The low-P_i-tolerance QTL *Pup1* was identified by crossing the *indica* landrace Kasalath (tolerant of P_i deficiency) with the *japonica* cultivar Nipponbare (intolerant of P_i deficiency). Introgression of *Pup1* into modern rice varieties increased P_i uptake by 170% and grain yield by 250% in low-P_i soils (41, 175). Recently, Gamuyao et al. (41) sequenced the gene responsible for the *Pup1* QTL and identified it as *PSTOL1* (*Phosphorus Starvation Tolerance 1*), encoding the Pup1 protein kinase. Expression of *PSTOL1* in low-P_i-intolerant varieties demonstrated that it acts as an enhancer of early root growth, thereby enabling plants to acquire more P_i and other nutrients (**Figure 2**).

Genetic Engineering Approaches to Enhance Phosphate Acquisition and Utilization Efficiencies

Several key components of the low-P_i response in plants have been identified, including transcription factors, transporters and hydrolitic enzymes. Several groups have attempted to determine whether overexpression or suppression of some of these genes has a positive effect on PUE and/or PAE. We describe some of the most promising approaches to engineering low-P_i tolerance in transgenic plants.

Manipulation of phosphate transporters. As discussed above, the Pht1 family of P_i transporters is pivotal for P_i uptake from the soil and the transport of P_i from the root to the shoot. This notion is supported by the finding that in different plant species the expression of at least one member

Table 2 Phosphate (P_i) transporters that could potentially be used to improve phosphate acquisition and utilization efficiencies

Overexpressed P_i transporter	Species transformed	Main effects	Reference(s)
HvPht1;1 and HvPht1;6	Rice (suspension cells)	Improved P_i uptake	124
OsPT1 (OsPht1;1)	Rice	Twofold increase in shoot P_i content, a larger number of tillers, and 20% more panicles at harvesting per plant compared with wild-type plants when grown exclusively under high-P_i conditions Enhanced P_i transport to shoot at the beginning of the grain-filling stage Increased production of root hairs compared with wild-type plants when grown under low-P_i conditions	144, 150
OsPT8 (OsPht1;8)	Rice	Stunted growth and 50% less shoot and root biomass compared with wild-type plants when grown under in vitro P_i-sufficient conditions P_i toxicity in greenhouse, with 2.3–3.2-fold-higher total P_i concentration in culm and panicle axis Decreased productivity (number of tillers, number of seed-setting tillers, and seed setting)	65
AtPht1;5	*Arabidopsis*	Increased shoot biomass and total leaf area as well as lower P_i and higher P_i in shoot and root, respectively, compared with wild-type plants Twofold increase of P_i in siliques, premature senescence, and increased transcripts of low-P_i-induced genes	105
AtPHT1;9	*Arabidopsis*	20–30% higher shoot fresh weight compared with wild-type plants when grown under low-P_i conditions	128

of the *Pht1* family correlates with better performance in low-P_i soils. For example, *TaPHT1;2* transcript levels are higher in the roots of a low-P_i-tolerant wheat variety than in those of a low-P_i-sensitive wheat variety under contrasting P_i supplies (23). In barley, *HvPHT1;3* and *HvPHT1;6* are also highly expressed in P_i-efficient genotypes (61) (**Figure 2**). Therefore, searching within the genetic diversity of major crops for alleles of high-affinity transporters that improve P_i uptake efficiency is a potentially interesting approach for breeding programs that aim to improve PAE.

The effect of overexpressing P_i transporters on P_i uptake has also been extensively addressed in both model and economically important plant species. For instance, overexpression of *AtPht1;5* in *Arabidopsis* resulted in an increase in shoot biomass, total leaf area, and both the number and length of root hairs, in addition to a twofold increase in P_i accumulation in siliques, premature senescence, and an increase in the transcript levels of genes involved in P_i scavenging (105). *Arabidopsis AtPHT1;9*-overexpressing lines also exhibit a higher shoot fresh weight than did wild-type plants under P_i-limited conditions (128) (**Figure 2**, **Table 2**). It would be interesting to test whether the control of PHT1;5 by a promoter strongly controlled by early-senescence events enhances P_i remobilization from senescent organs and produces a convenient P_i distribution in seed crops.

Overexpression of *OsPT1* (*OsPht1;1*) in rice results in higher P_i content in shoots and higher grain yield compared with wild-type plants (144). This effect is observed exclusively in plants grown with a high P_i supply; under P_i deficiency, no differences are observed. However,

OsPT1-overexpressing rice lines show higher production of root hairs under low-P_i conditions (150). Although the expression of *OsPT8* is not responsive to P_i availability, *OsPT8*-overexpressing rice plants displayed severe P_i toxicity symptoms, and *OsPT8* suppression by RNAi causes a 40–50% decrease in plant growth under both high- and low-P_i conditions (65). More important, suppression of *OsPT8* reduces P_i transport from the panicle axis to grains, producing very few filled grains in *OsPT8* RNAi lines (65) (**Table 2**).

In general, overexpression of P_i transporters in different plant species has yielded challenging and often controversial results because a high transcript level does not always correlate with efficient P_i uptake. On the contrary, in some cases high transcript levels have resulted in P_i toxicity symptoms (65) (**Table 2**). It would be interesting to test whether redirecting P_i from vegetative tissues to seeds by manipulating key P_i transporters involved in homeostasis avoids P_i toxicity symptoms and allows normal plant development in these P_i overaccumulators (**Figure 2**, **Table 2**). As occurs in some Proteaceae family members, such high P_i reserves in seeds would allow seedlings of the next progeny to grow with less external P_i (71).

Manipulation of key transcription factors and other phosphate-regulatory proteins. Manipulation of the expression of P_i-regulatory proteins that participate in the responses to P_i deficiency to improve PAE and PUE has proven to be a promising approach, particularly by modulating the root system architecture (**Table 1**). Similar to the results observed by overexpressing PHR1 in *Arabidopsis* (110), overexpression of the maize, rice, and wheat orthologs of *PHR1* (*ZmPHR1*, *OsPHR2*, and *TaPHR1-A1*, respectively) in transgenic plants leads to enhanced root elongation, enhanced root hair growth, upregulation of high-affinity P_i transporter genes, and improved P_i uptake (166, 168, 188) (**Table 1**). Data from field trials showed that overexpressing *TaPHR1* in transgenic wheat improved grain yield by increasing the number of grains per spike (166). However, overexpression of the *Brassica napus PHR1* gene causes retarded growth and lower biomass accumulation even though it generates a threefold increase in P_i content and higher expression of PHT1;4 (129). Although these results suggest that manipulation of the level of PHR1 expression is a promising approach to increase PUE in crop plants, we still require a better understanding of the mode of action of PHR1 and its interactors to more precisely modulate the PUE (**Figure 2**). In rice, overexpression of OsSIZ1, a regulator of PHR1 activity, suggests that SUMOylation is involved in the mechanisms of tolerance to P_i deficiency, given that the OsSIZ1-overexpressor lines show less anthocyanin accumulation and higher uptake of P_i in low-P_i soils (76). It will be interesting to determine whether the observed natural genetic variation in the PUE of *Arabidopsis* or other plants, such as maize and wheat, is related to the presence of specific alleles of PHR1 or other components of the local or systemic P_i-signaling pathways. It is clear that, at least in *Arabidopsis*, PHR1 plays a more important role than the closely related PHR-like 1 transcription factor in the regulation of low-P_i responses, so it is possible that variants of these genes exist within the genetic diversity of important crops (**Figure 2**).

The rice OsPTF1 (*Oryza sativa* Phosphate Starvation–Induced Transcription Factor 1) protein, a basic helix-loop-helix transcription factor, confers low-P_i tolerance when overexpressed in rice (184) (**Table 1**). In this study, which used hydroponic culture conditions, the tiller number, root and shoot biomass, and P_i content of transgenic plants were approximately 30% higher than those of control plants (**Table 1**). Moreover, when plants were grown in pots and field conditions in low-P_i soils, the tiller number, shoot biomass, panicle weight, and P_i contents of *OsPTF1*-overexpressing plants were 20–40% higher than those of wild-type plants, which was attributed to enhanced P_i uptake due to a 20% increase in root surface (184) (**Table 1**). The maize genome contains at least two sequences that are highly homologous to OsPTF1. Transgenic maize lines overexpressing ZmPTF1 exhibit improved tolerance to low-P_i stress and

increased expression of genes involved in sucrose synthesis and carbon assimilation in leaves (75) (**Table 1**). Overexpression of the OsMYB2P-1 transcription factor in rice confers low-P_i tolerance, with a concomitant increase in shoot and root biomass, a larger number of tillers, and relatively higher root P_i, by inducing a more robust root system, including longer primary and adventitious roots and a larger number of root hairs (22). In *OsMYB2P-1* overexpressors, P_i-responsive genes such as *OsIPS1* (*Oryza sativa INDUCED BY PHOSPHATE STARVATION 1*), *OsPAP10*, and *OsmiR399* are upregulated under P_i-deficient conditions (22) (**Table 1**). Rice mutants in the LTN1 transcription factor present enhanced elongation of the primary and adventitious roots as well as enhanced P_i uptake and translocation, leading to P_i overaccumulation in shoots under low-P_i conditions (60) (**Table 1**). In contrast, similar to results in *Arabidopsis*, the manipulation of several other P_i-regulatory elements in rice—such as *OsmiR399*, *OsPHR2*, and *OsSPX1*—led to P_i toxicity symptoms and a strong inhibition of plant growth irrespective of the P_i status (60, 165, 188). The finding that P_i toxicity is produced by alterations in the expression of some of the key components of the low-P_i-signaling pathway in several plant species suggests that it should be possible to optimize P_i uptake or transport inside the plant by modulating some of these P_i-regulatory components to a specific level in the right tissues or cell types (**Figure 2**).

Manipulation of organic acid biosynthesis and transport. The goal of enhancing P_i availability in the soil through OA exudation has been addressed in two ways: (*a*) improving OA synthesis and accumulation and (*b*) facilitating OA efflux. Exudation of OAs—citrate and malate in particular—plays an important role in ameliorating Al^{3+} toxicity and enhancing the capacity of plant roots to scavenge P_i from soil (137, 162). Overexpression of citrate synthase and malate dehydrogenase from both microbial and plant origins enhances OA exudation and improves P_i acquisition and plant biomass production in low-P_i soil (24, 69, 154). Although the initial results obtained using this approach were controversial (24, 25), recent reports confirm that overexpression of genes encoding enzymes involved in OA biosynthesis does improve aluminum tolerance and P_i uptake capacity (90, 130, 172). In one study, transgenic tobacco lines expressing a mitochondrial malate dehydrogenase gene from the mycorrhizal fungi *Penicillium oxalicum* showed a more than 200% increase in P_i content and a more than 100% increase in biomass when grown in aluminum phosphate, iron phosphate, or calcium phosphate (90). These reports indicate that manipulation of OA biosynthesis may facilitate the use of sparingly soluble P_i sources by plants (**Figure 2**).

A limiting step for OA efflux could be their transport across the plasma membrane or within the plant for root exudation. Therefore, facilitating OA efflux could enhance the effectiveness of OAs in optimizing P_i use in the field. In sorghum, barley, maize, and wheat, root-specific MATE transporters mediate citrate efflux, which may protect against P_i deficiency or the presence of toxic concentrations of Al^{3+} (38, 95, 96, 138). Overexpression of the barley *HvAACT1* MATE gene in wheat and barley produces an increase in OA efflux and Al^{3+} tolerance in acidic soil, although the possible improvement in P_i acquisition has not been assessed (187). In one study, overexpression of a wheat malate transporter (Ta-ALMT1) enhanced the P_i uptake and grain yield of barley plants grown in acidic soil (27). Therefore, combining OA overproduction with enhanced release may be an interesting avenue to improve P_i solubilization. Moreover, a biased rhizosphere that attracts mycorrhizal fungi or P_i-solubilizing bacteria may be a further advantage of OA exudation because these compounds represent a readily available carbon source for microbial nutrition (**Figure 2**).

Manipulation of phosphatase secretion. Wang et al. (169) explored the potential of the *Arabidopsis AtPAP15* gene to improve P_i availability from organic P_i by overexpressing the gene in soybean. Transgenic plants showed improved P_i efficiency and accumulated more dry weight and P_i content than wild-type plants did. Importantly, these transgenic soybean plants produced a

larger number of seeds per plant compared with nontransformed controls when grown in acidic soil amended with phytate as a P_i source (169).

Many attempts have been made to exploit the potential of phytase enzymes to enhance P_i availability in phytate-rich soil. Phytase genes from *Aspergillus* sp. and *Bacillus subtilis* have been overexpressed in subterranean clover (*Trifolium subterraneum*), *Arabidopsis*, and tobacco (43, 44, 103, 131, 185). These plants can grow in media supplied with phytate as the only P_i source, and they produce more biomass and accumulate more total P. Similarly, other studies showed that expression of two *Medicago truncatula* genes, *MtPHY1* (*Medicago truncatula PHYTASE 1*) and *MtPAP1*, in white clover (*Trifolium repens*) and alfalfa and overexpression of *OsPHY1* in tobacco (74, 93, 94) resulted in improved growth when using phytate as a P_i source in comparison with control plants. However, in *phytase A*–overexpressing tobacco plants, positive results have been observed in vitro, but their capacity to use naturally occurring phytate as a P_i source is significantly reduced in natural low-P_i soils (44). Therefore, although the overexpression of phosphatase/phytase genes in many plant species has generally been fruitful in vitro, their effectiveness in natural soils remains to be determined.

Naturally or transgenically expressed phytases are only able to hydrolyze soluble phytate, which represents just a small fraction of the total phytate present in the soil. Phytin, a mixture of phytate with cations (K^+, Ca^{2+}, Mg^{2+}, or Zn^{2+}), is more abundant and largely insoluble. Therefore, the combined expression of phytases with increased OA exudation potentially represents a more effective strategy to increase phytate solubility from phytin and indicates its potential use as a P_i source to sustain plant growth (**Figure 2**).

CONCLUDING REMARKS

The central importance of P_i in plant nutrition and agricultural sustainability has long been recognized. Given the low diffusion rate and chemical immobilization of P_i in soils, root architecture is a key trait for optimizing P_i acquisition in crops. Only approximately 20% of the topsoil is explored by roots during plant growth; therefore, enhancing topsoil foraging is essential to improving P_i usage. Information regarding the mechanisms by which low P_i availability modulates important root traits such as root hair formation and root hair growth has been obtained from *Arabidopsis*, white lupin, and several crop plants. However, because evaluating the phenotypes of roots in soil is a major challenge, to enable more effective identification of QTLs and genes responsible for root traits that can improve PAE, we urgently need novel imaging systems for effective and efficient assessment of root system architecture phenotypes in plants grown in natural soils. Possible solutions include the use of in vitro systems that can enable prediction of root system phenotypes under different soil conditions or the use of nondestructive imaging technologies, such as ground-penetrating radar imaging. Although it is not the topic of this review, it is important to note that the role of soil microbes (either present in the rhizosphere or associated with plants as endophytes) in plant responses to biotic and abiotic factors, particularly in plant nutrition, is becoming increasingly important and has begun to be uncovered through the recent use of genomics to characterize the microbiome associated with plants (117).

The accumulation of metals that affect P_i solubility resulting from anthropogenic activities and interactions of P_i with aluminum in acidic soils deserves further attention (135, 136). It is also important to consider a redesign of the fertilizers currently utilized in the field. López-Arredondo & Herrera-Estrella (86) reported an alternative weed control and fertilization system based on producing transgenic plants capable of using phosphite as a P_i source and then using phosphites instead of phosphates as fertilizers. This system has the potential to provide cultivated plants with a competitive advantage over weeds and soil microorganisms that are unable to metabolize phosphite, and the application of a single compound could achieve both P_i fertilization and weed

control at a lower cost. The use of this novel system could be considered as an integrative approach to be implemented in the low-P_i-tolerant varieties that have already been developed.

A few regulatory elements and a myriad of signaling components have been identified as mediators of the adaptation of plants to low-P_i stress. The challenge now is to understand how these regulatory and signaling components mediate the many responses that plants, as sessile multicellular organisms, require to survive and reproduce in an adverse environment. As illustrated by the case of PHO1, in which manipulation of its expression level allows plants to sustain better growth with lower P_i content, a better understanding of the local and systemic signals and the mechanisms that transduce these signals into metabolic and morphologic responses promises to provide novel avenues to improve crop yield using lower P_i inputs.

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