



Tansley review

Functional biology of plant phosphate uptake at root and mycorrhiza interfaces

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Summary

Key words: arbuscular–mycorrhizal symbiosis, functional genomics of phosphate transport, gene expression, nutrient uptake, phosphate transporters, root–soil interface.

Phosphorus (P) is an essential plant nutrient and one of the most limiting in natural habitats as well as in agricultural production world-wide. The control of P acquisition efficiency and its subsequent uptake and translocation in vascular plants is complex. The physiological role of key cellular structures in plant P uptake and underlying molecular mechanisms are discussed in this review, with emphasis on phosphate transport across the cellular membrane at the root and arbuscular–mycorrhizal (AM) interfaces. The tools of molecular genetics have facilitated novel approaches and provided one of the major driving forces in the investigation of the basic transport mechanisms underlying plant P nutrition. Genetic engineering holds the potential to modify the system in a targeted way at the root–soil or AM symbiotic interface. Such approaches should assist in the breeding of crop plants that exhibit improved P acquisition efficiency and thus require lower inputs of P fertilizer for optimal growth. Whether engineering of P transport systems can contribute to enhanced P uptake will be discussed.

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I. Introduction

Phosphorus (P) serves various basic cellular functions in bioenergetics (coupled to the biosynthesis of adenosine

triphosphate (ATP)) and in the activation of metabolic intermediates, as a component in signal transduction cascades and the post-translational regulation of enzymes and as a structural element in nucleic acids and phospholipids. P is a

major limiting nutrient for plant productivity, mainly because of its low mobility in soil. Like all other mineral nutrients, P enters the biosphere predominantly via the pedosphere through the root system of plants, where it is absorbed as inorganic orthophosphate (Pi), which is the preferred form taken up by plants. Following its uptake, Pi is distributed to various sink tissues such as growing roots, developing leaves, flowers and seeds. It circulates through the vascular network allowing complex control mechanisms to co-ordinate the distribution of Pi *in planta*. Important parameters in determining P acquisition efficiency (i.e. unit P absorbed per unit of root length) are related to root morphology, biochemistry and physiology (Marschner, 1995). Root development is remarkably sensitive to variations in the supply and distribution of P in the soil (Wiersum, 1958; Forde & Clarkson, 1999; Forde & Lorenzo, 2001) and roots respond in many ways to altering P availability, for example by changes in root architecture (López-Bucio *et al.*, 2003), enhanced root secretory activities (Neumann *et al.*, 2000), modification of Pi transport systems enhancing P uptake at low ambient concentrations, and establishment of arbuscular–mycorrhizal (AM) symbioses allowing capture of nutrients well beyond the rhizosphere (Smith & Gianinazzi-Pearson, 1988; Marschner, 1995). Considerable progress towards understanding the molecular basis of these responses has been made (reviewed in Schachtman *et al.*, 1998; Raghothama, 2000; Rausch & Bucher, 2002; Franco-Zorrilla *et al.*, 2004; Karandashov & Bucher, 2005). The advances in plant genomics research have provided many useful tools to help unravel the complexity of the regulatory pathways associated with the responses of the plant to variation in P availability (including the development of the mycorrhizal symbiosis). Recent findings in plant P nutrition have brought the interdependence of cellular structures in roots and the biochemical and molecular mechanisms involved in Pi uptake to the forefront of plant science. This review focuses on the role of root hair cells and mycorrhizal cortical cells, which are both located at interfaces mediating Pi uptake, and explores the molecular and biochemical mechanisms involved in Pi transport at these interfaces.

While research over many years has broadened our understanding of the multilayered processes directing plant–pathogen interactions (Schenk *et al.*, 2000; Dangl & Jones, 2001; Hahlbrock *et al.*, 2003; Farmer & Schulze-Lefert, 2005; Chisholm *et al.*, 2006), our knowledge of the mechanisms governing the establishment and functioning of the AM symbiosis has only recently experienced such an impressive expansion. This discrepancy is in contrast to the world-wide distribution of the AM symbiosis and its beneficial effects for plant nutrition and fitness. For example, in addition to improving nutrient supply (see section III), colonization of roots by AM fungi has been shown to protect plants against pathogens (Cordier *et al.*, 1998), salt stress in arid and semiarid areas (Al-Karaki, 2000), and moderate drought stress (Subramanian *et al.*, 1995; Auge, 2001); however, the

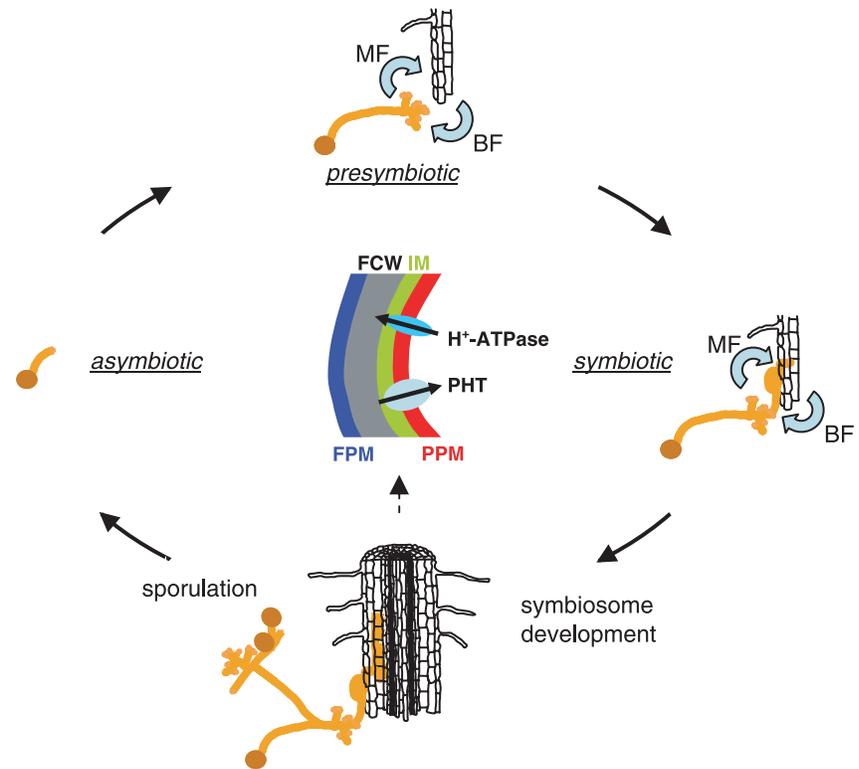
mycorrhizal symbiosis has been largely neglected in crop breeding. Modern agricultural soils are almost universally maintained at high fertility and the selection of new cultivars is usually made under these conditions. Selection will thus not normally distinguish between plants varying in nutrient efficiency (Stevens & Rick, 1986). Possibly as a consequence, modern crop cultivars that do not exhibit high nutrient efficiency are highly responsive to colonization by AM fungi in low-P conditions. However, Toth *et al.* (1990) suggested that present breeding programmes for disease resistance in some crop plants negatively affect the ability of plants to form mycorrhizas which, presumably, negatively affects the nutrient acquisition efficiency of these varieties. Thus, mycorrhizas may have potential for improving crop nutrition (see sections III and V).

1. Mycorrhiza development

The term ‘mycorrhiza’ literally means ‘fungus root’ and was first used in 1885 (Frank, 1885) to describe the intimate association between biotrophic mycorrhizal fungi and plant roots. The AM symbiosis is the most common nonpathogenic and soil-based symbiosis, being formed in the roots of 80% of vascular plants (Smith & Read, 1997). Mycorrhizal associations have evolved to improve the fitness of both plant and fungal symbionts. In systems managed by humans, mycorrhizal associations often improve plant productivity, but this is not always the case and the symbiosis can span a wide range of species interactions from mutualism to parasitism under different environmental conditions (Johnson *et al.*, 1997). Mycorrhizal fungi can even be considered to be parasitic on plants when the net cost of the symbiosis exceeds the net benefits, for example in well-fertilized substrate with high soluble Pi content. Because of the complexity of mycorrhizal associations, an understanding of the several parameters affecting mycorrhizal functioning, such as the morphology and physiology of both symbionts, and biotic and abiotic factors at the rhizosphere, community and ecosystem levels, is required to construct predictive models of mycorrhizal functioning (Johnson *et al.*, 1997). In addition to this, an appreciation of how mycorrhizas function in complex natural systems is necessary for their management in agriculture, forestry and restoration systems.

During mycorrhiza formation, the AM fungus undergoes several developmental stages. In the asymbiotic stage, spores germinate and AM fungi show limited hyphal development in the absence of a host plant. However, in the presence of root exudates, they switch to the presymbiotic stage which is characterized by extensive hyphal branching (Fig. 1; Buee *et al.*, 2000). Subsequent to hyphal branching, the fungus contacts the root surface via appressorium formation before hyphal penetration of the root epidermis. This is followed by symbiotic colonization of the root cortex tissue, which involves formation of intracellular arbuscules (tree-like heavily branched structures) or hyphal coils, and, concomitantly, the production of a

Fig. 1 Development of a functional arbuscular–mycorrhizal (AM) symbiosis. AM symbiosis development is dependent on the life cycle of the microbial symbiont, which is a biotrophic AM fungus belonging to the phylum Glomeromycota, and ranges from asymbiotic (absence of plant effects) to presymbiotic (plant–fungus cross-talk before physical contact) to symbiotic growth (root colonization). Fungal colonization of a root cortical cell leads to development of the symbiosome which is characterized by the root–fungus interface, the site at which nutrient exchange occurs. Chemical cross-talk between the symbionts includes release and perception of plant-derived branching (BF) and fungus-derived mycorrhiza factors (MF), respectively (blue arrows). Fungal spores and hyphae are depicted in brown and orange, respectively. Membrane integral proteins are involved in plant inorganic orthophosphate (Pi) uptake (e.g. PHT for phosphate transporter, and the P-type H⁺-ATPase). Fungal (FPM) and plant (PPM) plasma membranes demarcate the symbiotic interface. FCW, fungal cell wall; IM, interfacial matrix.



sporulative extraradical mycelium (Smith & Read, 1997). Genre *et al.* (2005) presented a detailed intracellular view of the structural changes in *Medicago truncatula* root epidermal cells during early stages of root colonization by AM fungi. This included the formation of a tunnel-like structure in a root epidermal cell destined to be colonized by the fungus before physical contact of the two symbionts. This clearly showed that the host plant plays a key role in orchestrating the AM infection process (Eckardt, 2005), and it is tempting to speculate that similar changes occur during colonization of cortical cells. Overall, these developmental processes require molecular communication between the AM fungus and the plant, including exchange and perception of signals by the symbiotic partners. A major step forward in deciphering the molecular cross-talk in the AM symbiosis was the identification of a branching factor present in root exudates as a strigolactone, (i.e. 5-deoxy-strigol) (Akiyama *et al.*, 2005). Strigolactones are a group of sesquiterpene lactones, which have an important ecological role as seed-germination stimulants for the parasitic weeds *Striga* and *Orobancha*. It is noteworthy that two different groups of species, i.e. parasitic plants and symbiotic fungi, perceive 'positional information' by sensing the same secreted molecule which initiates such different responses as seed germination and hyphal branching.

In root symbioses, the symbiosome is the cellular environment hosting the microbial symbiont where the mutual exchange of nutrients and metabolites occurs. In AM this is

the cortical cell lumen harbouring hyphal coils or arbuscules surrounded by the perihyphal or periarbuscular plasma membrane, respectively. Thus, despite intracellular accommodation of the microsymbiont, the cytoplasm of the symbiotic partners never mix and are always separated by the plant and microbial plasma membranes, thus demarcating the symbiotic interface. Both the microbe- and the plant-derived symbiosome membranes tightly regulate the exchange of compounds, which is generally facilitated by membrane-integral transport proteins. A single arbuscule has a short life span of only a few days (Smith & Read, 1997). It is subsequently degraded by the cortical cell which continues to live and can eventually be infected by other hyphae. Collectively, these results demonstrate that multiple processes are involved in the AM fungus–plant interaction; however, the molecular complexity of these processes is still far from being fully understood.

Extensive forward genetic approaches were used to dissect the components of the signal perception and transduction pathway(s) in the root nodule and the AM symbioses in legume species. These approaches led to the identification of several symbiosis genes, the corresponding plant mutants of which are generally unable to support infection by both rhizobial bacteria and AM fungi. This is clear evidence for the existence of a common symbiotic pathway in legume species (Kistner & Parniske, 2002). While rhizobial nodulation (Nod) factors were shown to induce in their legume hosts the expression of many genes and set in motion developmental processes leading

to root nodule formation (Oldroyd & Downie, 2004), the nature and function of mycorrhization (Myc) factors are still an enigma. Myc factors are likely to be soluble, fungus-derived compounds that trigger expression of mycorrhiza-responsive genes (Kosuta *et al.*, 2003) and structural changes in host roots (Olah *et al.*, 2005).

2. Mycorrhiza-specific gene expression at the symbiotic interface

Mycorrhiza formation results from a complex interaction between the AM fungus and the host plant in which gene expression of the root cells destined to be colonized by the fungus is changed as a result of cell-to-cell contact and/or diffusion of (presumably more than one) signal molecules. The molecular basis underlying mycorrhizal symbiosome formation and function has just begun to be elucidated. Extensive transcript profiling has revealed numerous genes that are reported to be up-regulated or repressed in mycorrhizas (Journet *et al.*, 2002; Liu *et al.*, 2003; Wulf *et al.*, 2003; Brechenmacher *et al.*, 2004; Guimil *et al.*, 2005; Hohnjec *et al.*, 2005; Kistner *et al.*, 2005). Experimental evidence also exists for cell-specific localization of either transcripts (as revealed by *in situ* hybridization of RNA) or promoter activity (or both) of several genes involved in arbuscule function (i.e. in P nutrition) and development. The encoded proteins are likely to be operating at the root–fungus interface in cortical cells. These include genes encoding P-type H⁺-ATPases (Moriau *et al.*, 1999; Krajinski *et al.*, 2002) and Pi transporter genes from potato (*Solanum tuberosum*) and tomato (*Lycopersicon esculentum*) (Rausch *et al.*, 2001; Nagy *et al.*, 2005), *M. truncatula* (Harrison *et al.*, 2002), *Lotus japonicus* (Maeda *et al.*, 2006), and the cereals barley (*Hordeum vulgare*), wheat (*Triticum aestivum*) and maize (*Zea mays*) (Glassop *et al.*, 2005). *Medicago truncatula* serine carboxypeptidase (*MtSCPI*), a gene sharing identity with Ser carboxypeptidase II proteins from barley, wheat and *Arabidopsis thaliana*, and *MtCell1*, a gene encoding a membrane-anchored endo-1,4-β-D-glucanase-like protein, have been shown to be up-regulated in the root cortex upon colonization of *M. truncatula* with *Glomus versiforme* (Liu *et al.*, 2003). A mycorrhiza-specific class III chitinase gene is similarly regulated in cells containing developing or mature arbuscules (van Buuren *et al.*, 1999; Salzer *et al.*, 2000; Bonanomi *et al.*, 2001). Interestingly, several genes have been identified that are induced both in mycorrhized cortical cells and during rhizobial root colonization in legume species, for example the early nodulin genes *ENOD2*, *ENOD40* (van Rhijn *et al.*, 1997) and *ENOD11* (Chabaud *et al.*, 2002), the *Vicia faba* leghaemoglobin gene *VFLb29* (Vieweg *et al.*, 2004), and the gene calcium-binding protein 1 (*Cbp1*), which encodes a protein sharing similarities with calcium-binding proteins (Kistner *et al.*, 2005). This supports the model already outlined for the common symbiotic pathway in AM and rhizobium-induced nodule development using signal

transduction based on conserved mechanisms. It also provides good support for the hypothesis that during evolution of terrestrial plants nature has recruited components of an ancient signalling pathway to optimize P and nitrogen (N) nutrition, in the symbioses between roots and two different microorganisms, i.e. AM fungi and rhizobial bacteria. It has recently been reported that expression of *ENOD11* is induced by a diffusible factor secreted from AM fungal hyphae in *M. truncatula* (Kosuta *et al.*, 2003). This work is a cornerstone in the identification of fungal signals involved in early steps of the AM symbiosis development (Olah *et al.*, 2005).

II. Phosphate uptake and its regulation

1. Phosphate uptake mechanisms at the root–soil interface

Mathematical models of nutrient uptake from the soil indicate that root growth and extension into unexploited volumes of soil are of great significance in acquiring nutrients that diffuse slowly in the soil, such as P, zinc (Zn), and to a certain extent potassium (K) (Clarkson, 1985). Competition between roots depends on their density in the soil (root length per unit volume of soil) and the pattern of their distribution. Root architecture is determined by an interplay between the genetically inherited developmental programme and external biotic and abiotic stimuli (Schiefelbein & Benfey, 1991; Aeschbacher, 1994; Lynch, 1995; Zobel, 1996). Typical root structures that occur in most vascular plants are the root hair cells (trichoblasts), which are tubular in shape and extend from root epidermal cells via tip growth (Gilroy & Jones, 2000). The isolation of several *A. thaliana* mutants with altered root hair elongation has demonstrated that hair elongation is under genetic control (Schiefelbein & Somerville, 1990; Grierson *et al.*, 1997). A genetic model for root hair morphogenesis that defines the roles of several genes and includes functional relationships between genes was proposed by Parker *et al.* (2000). The formation of root hairs considerably increases the root diameter of a plant with relatively little dry matter investment. These tubular structures make up between 70 and 90% of the total root surface area (Bates & Lynch, 1996), and play a dominant role in a number of root functions. Root hairs represent the most distant outpost of the root symplast, and are among the first cells that come into contact with the soil solution. Numerous studies have reported the importance of these structures in nutrient acquisition processes (Gilroy & Jones, 2000). In addition, root hairs are instrumental in the anchorage of plants in the soil (Bailey *et al.*, 2002), in water uptake, and in the establishment of *Rhizobium* symbiosis in legumes (Kalsi & Etzler, 2000; Cullimore *et al.*, 2001). Both root hair length and density increase in response to iron (Fe) and P deficiencies, affecting, for example, the Pi acquisition efficiency of the plant by enhancing nutrient uptake from the soil into the root at the level of the root epidermis (Bates &

Lynch, 1996; Ma *et al.*, 2001; Schmidt & Schikora, 2001; Zimmermann *et al.*, 2003). In the model plant *A. thaliana*, low P availability stimulates root hair elongation by a factor of about 3 as a result of increased growth duration and rate (Bates & Lynch, 1996) and root hair length and density correlate with Pi acquisition efficiency in different *A. thaliana* accessions (Narang *et al.*, 2000). Similarly, root hairs are abundant in rape (*Brassica napus*), spinach (*Spinacia oleracea*) and tomato at low P concentrations (< 10 μM), but are absent or rudimentary at high P (> 100 μM) (Föhse & Jungk, 1983). When the Pi or K⁺ uptake rates of different plant species are compared, a close positive correlation can be demonstrated between the uptake rate per unit root length and the volume of the root hair cylinder (Itoh & Barber, 1983; Marschner, 1995). The study of root-hairless mutants has revealed an important role of root hairs in Pi uptake from the soil solution; in a low-P environment, the hairs are important in P acquisition and plant survival, but they might be dispensable under high-P conditions (Bates & Lynch, 2000; Gahoonia *et al.*, 2001). A study including barley genotypes differing in root hair length showed that long-hair genotypes are better adapted to low-P soils and express high yield potentials in both low- and high-P soils (Gahoonia & Nielsen, 2004). Complementary to these observations, the use of molecular biological tools allowed the localization of nutrient transport systems for N, P, K, sulphur (S) and Fe to the root epidermal layer, including the root hair cells (Mimura *et al.*, 1990; Lagarde *et al.*, 1996; Lauter *et al.*, 1996; Daram *et al.*, 1998; Hartje *et al.*, 2000; Takahashi *et al.*, 2000; Chiou *et al.*, 2001; Vert *et al.*, 2002; Zimmermann *et al.*, 2003).

The development of a barrier such as the cellular plasma membrane and later the endomembrane systems was a prerequisite for the development of life, and enabled single cells to support metabolic, reproductive and developmental activities under stable physicochemical conditions (Buchanan *et al.*, 2000). The hydrophobic nature of the plasmalemma and the endomembranes allows accumulation of hydrophilic compounds, such as most nutrients and metabolites, on either side of the membrane. Maintenance or establishment of these biochemical gradients requires a selective transport of compounds across the membrane barriers. Inward currents of several ions (i.e. K⁺, Cl⁻ and Ca²⁺) have been measured in root hairs (Schieffelbein *et al.*, 1992; Felle, 1994; Gassmann & Schroeder, 1994; Grabov & Böttger, 1994; Kochian *et al.*, 1994), which indicates the presence of transport systems for plant nutrients at the root–soil interface. Plants and fungi use a P-type H⁺-ATPase pump to generate an electrochemical gradient across the plasma membrane at the expense of ATP. The driving force for Pi influx is the proton gradient generated by this H⁺-ATPase (Ullrich-Eberius *et al.*, 1984; Thibaud *et al.*, 1988; Daram *et al.*, 1998; Sze *et al.*, 1999; Karandashov & Bucher, 2005). Consequently, the large membrane potential difference with a negative potential of the cytoplasm (–150 to –200 mV)

allows cotransport of Pi and other anions with protons in a secondary transport process. Weisenseel *et al.* (1979) measured H⁺ currents entering the root hair tip of barley, whereas Kochian *et al.* (1994) measured H⁺ influx near the tip and efflux at the basal region of root hairs from *Limnobium stoloniferum*. The findings of these studies on the localization of H⁺-ATPase activity in root hairs are supported by immunolocalization of the protein in rhizodermal cells (Parets-Soler *et al.*, 1990) and histochemical localization of H⁺-ATPase gene expression in root hairs (Moriau *et al.*, 1999).

2. The phosphate transporter (*Pht1*) gene family

It was the pioneering work of Emmanuel Epstein (Epstein & Hagen, 1952; Epstein *et al.*, 1963) that demonstrated that ion uptake processes across the plasma membrane follow Michaelis–Menten kinetics comparable to those of enzymatic processes. This concept allowed the calculation of the functional parameters of nutrient uptake systems such as pH optima, the Michaelis constant K_m , the uptake velocity V_{max} , and the minimal concentration of the ion at which transport occurs (i.e. C_{min}). Analysis of the kinetics of nutrient uptake into plant roots using a radiotracer medium-depletion method (Cogliatti & Clarkson, 1983; Drew *et al.*, 1984) revealed that Pi uptake kinetics in plants are generally hyperbolic and monophasic at low Pi concentrations (μM range) in the medium and biphasic at high Pi concentrations (mM range). Most interpretations concerning the kinetics of Pi uptake into plant cells under varying Pi concentrations propose the presence of two independent Michaelis–Menten-type systems, a high- and a low-affinity transport mechanism, although the presence of up to seven transport kinetics has been suggested, for example for Pi uptake into maize roots (Ullrich-Eberius *et al.*, 1984; Nandi *et al.*, 1987; Furihata *et al.*, 1992). However, in suspension-cultured tobacco (*Nicotiana tabacum*) cells, only one Michaelis–Menten-type Pi transport system exhibiting a high affinity for Pi has been described, with no evidence for low-affinity Pi transport (Shimogawara & Usuda, 1995). Moreover, concentration-dependent Pi influx of barley mesophyll protoplasts shows a combination of Michaelis–Menten-type kinetics at low Pi concentrations and a linear increase at higher Pi concentrations (Mimura *et al.*, 1990). Taken together, the diverse biochemical data on Pi uptake in whole plants and in cells have revealed the necessity for the identification and detailed molecular and biochemical characterization of the respective Pi transport systems to establish an overall picture of plant Pi acquisition and its regulation.

With the growing number of Pi transporter sequences from plants, a new nomenclature was introduced classifying plant Pi transporters into three families: Pht1, Pht2 and Pht3 (Bucher *et al.*, 2001; Mudge *et al.*, 2002; Poirier & Bucher, 2002; Rausch & Bucher, 2002). The following sections explore the molecular physiology of Pi uptake and the regulation of the *Pht1* genes involved and their encoded proteins.

The first cDNA clones and genes encoding vascular plant Pi transporters of the Pht1 family were isolated based on sequence information from an *A. thaliana* expressed sequence tag clone (Muchhal *et al.*, 1996; Leggewie *et al.*, 1997; Mitsu-kawa *et al.*, 1997; Smith *et al.*, 1997; Daram *et al.*, 1998; Liu *et al.*, 1998b), which became available in 1995 from the Arabidopsis Biological Resource Center (Columbus, OH, USA) and exhibited homology to the yeast high-affinity Pi transporter PHO84 (Bun-Ya *et al.*, 1991). A BLAST search of the databases available on the National Center for Biotechnology Information server at <http://www.ncbi.nih.gov> performed in August 2006 revealed that the Pht1 family presently consists of > 100 proteins from both monocot and eudicot species. Plant Pht1 transporters are secondary transporters belonging to the phosphate:H⁺ symporter (PHS) family within the major facilitator superfamily (MFS). The MFS consists of at least 17 distinct families present in bacteria, *archaea*, and eukarya, each of which generally transports a single class of compounds. Work by Pao *et al.* (1998) supported the hypothesis that an internal tandem gene duplication event gave rise to a primordial MFS protein before divergence of the family members. The three-dimensional structures of oxalate (OxIT), lactose (LacY) and glycerol-3-phosphate/inorganic phosphate (GlpT) transporters from *Oxalobacter formigens* and *Escherichia coli*, respectively, have been elucidated; these demonstrate the presence of 12 transmembrane domains, substantiating the structural model of MFS proteins (Hirai *et al.*, 2002; Abramson *et al.*, 2003; Huang *et al.*, 2003). It can be assumed that Pht1 proteins share structural similarities with these three well-characterized MFS proteins; however, experimental evidence clarifying the computational Pht1 protein topology is still lacking. Multiple alignments of Pht1 and homologous nonplant transporters have revealed the presence of several highly conserved sites for post-translational modification, as well as a highly conserved region, the Pht1 signature GGDYPLSATIxSE, in the fourth putative transmembrane domain (Karandashov & Bucher, 2005); however, the function of these sequences is presently unknown. Functional complementation of different yeast mutants defective in their own Pi transport activities and overexpression of a Pht1 protein in tobacco cells have demonstrated great variability in affinity for Pi, ranging from 3 to ~700 M. This also supports the presence of both high- and low-affinity Pi transporters within the Pht1 family in plants (Harrison *et al.*, 2002; Rausch & Bucher, 2002; Rae *et al.*, 2003; Nagy *et al.*, 2005).

Expression analysis and suggestions on the proposed function of *Pht1* genes in different plant species have been published and extensively discussed (Schachtman *et al.*, 1998; Raghothama, 1999, 2000; Smith *et al.*, 2000; Bucher *et al.*, 2001; Poirier & Bucher, 2002; Rausch & Bucher, 2002; Karandashov & Bucher, 2005) and therefore, to prevent reiteration, the next section focuses on the most recent findings which are increasing our understanding of the molecular regulation of Pi transport.

3. Regulation of Pi uptake

The haploid *A. thaliana* genome contains nine genes which form the *A. thaliana Pht1* gene family, and this in turn belongs to the inorganic solute cotransporter gene families which consist of 84 members. Comparison of the haploid *A. thaliana* genome with those of various other species shows differing numbers of *Pht1* genes. For example, the haploid rice (*Oryza sativa*) genome contains 13 *Pht1* genes (Paszukowski *et al.*, 2002) and to date eight *Pht1* genes have been cloned in barley (Schunmann *et al.*, 2004a), whereas maize has been shown to contain at least five homologous genes (Nagy *et al.*, 2006). At least five *Pht1* genes are expressed in potato and tomato (Nagy *et al.*, 2005), four in tobacco (Kai *et al.*, 2002), and three in *M. truncatula* and *L. japonicus* (Liu *et al.*, 1998b; Harrison *et al.*, 2002; Maeda *et al.*, 2006). Recently, the phylogenetic relationships among *Pht1* genes from different families have been reviewed (Karandashov & Bucher, 2005). Most *Pht1* genes are strongly expressed in roots, especially in rhizodermal cells including root hair cells, in the root cap, and in the outer cortex (Daram *et al.*, 1998; Liu *et al.*, 1998a; Chiou *et al.*, 2001; Karthikeyan *et al.*, 2002; Mudge *et al.*, 2002; Schunmann *et al.*, 2004a). Expression has also been observed in other organs such as leaves and pollen, suggesting roles for Pht1 proteins in addition to Pi uptake at the root-soil interface, for example remobilization of stored Pi from leaves via the phloem (Rae *et al.*, 2003), or Pi uptake in the elongating pollen tube (Mudge *et al.*, 2002; Nagy *et al.*, 2006). The rather diverse pattern of *Pht1* gene expression indicates that these genes must be regulated by distinct mechanisms conferring organ- or tissue-specific expression dependent on environmental or internal cues. This view was substantiated by a detailed analysis of promoter regions of the barley *Pht1;1* gene which revealed that distinct regulatory domains serve different functions, i.e. expression in root epidermal cells including root hair cells, expression in the root tip, and induction of expression in response to low P (Schunmann *et al.*, 2004b). A regulatory element, such as the P1BS element, for which there is evidence of a functional role in plants, has been identified in six barley Pht1 promoters (Schunmann *et al.*, 2004a). A P-responsive P1BS element (GnATATnC) was first identified by Rubio *et al.* (2001) in *A. thaliana*. A MYB transcription factor, phosphate starvation response 1 (PHR1), was found to bind to the P1BS element, indicating that this element is associated with the P-starvation response (Rubio *et al.*, 2001). In the same work, *A. thaliana* plants carrying a mutant *phr1* allele were shown to exhibit impaired P-regulated expression of a range of Pi-starvation responsive genes with little effect on *Pht1;1* expression. Therefore, the presence of P1BS-like elements in the promoters of *Pht1* genes suggests that the motif carries out regulatory functions in Pi transport, allowing finely tuned expression of the transporters in response to the internal and external P conditions. Detailed elucidation of the regulatory

network, including the *cis*- and *trans*-acting factors involved in the regulation of Pi transport across membranes, would be useful. A set of 111 transcription factors from *A. thaliana* have been shown to be up- or down-regulated by Pi starvation, including PHR1 (Wu *et al.*, 2003). Interestingly, leaves and roots have largely nonoverlapping sets of transcription factors, implying distinct regulatory changes in leaves and roots during Pi deprivation stress.

Spatial and temporal patterns of *Pht1* gene promoter activities, *Pht1* transcript abundances and encoded protein accumulation in the plasma membrane indicated primarily transcriptional control of Pi transport in plants (Muchhal & Raghothama, 1999; Chiou *et al.*, 2001; Misson *et al.*, 2004; Shin *et al.*, 2004). AtSIZ1 is a plant small ubiquitin-like modifier (SUMO) E3 ligase involved in proteolysis in *A. thaliana* and was shown to be involved in the control of Pi starvation-dependent responses (Miura *et al.*, 2005). Sumoylation is a novel post-translational modification system biochemically analogous to, but functionally distinct from, ubiquitinylation. Sumoylation involves the covalent attachment of a SUMO to substrate proteins. This modification system plays crucial roles in many different biological processes, including protein localization and stability, transcriptional activities, nucleocytoplasmic signalling and transport, and genome replication, as well as the regulation of gene expression (Wilson, 2004). AtSIZ1 was localized to the nucleus and loss of AtSIZ1 function resulted in exaggerated Pi starvation responses, including a moderate increase in *Pht1;4* transcript abundances at the onset of Pi starvation, and increased shoot Pi concentrations in conditions of high P. The MYB transcriptional activator PHR1 of Pi starvation response genes is an AtSIZ1 sumoylation target. These results indicated that sumoylation is a control mechanism that acts on different Pi deficiency responses. However, it is noteworthy that, in the *phr1* mutant, expression of *Arabidopsis Pht1;1* was not significantly different from that in the wild-type control, in contrast to the reduced expression of two other Pi starvation-induced genes, *AtIPS1* and *At4* (Rubio *et al.*, 2001). Shin *et al.* (2006) have shown that a knock-out in the *A. thaliana* gene *At4*, which is characterized by the absence of a single long open-reading frame in its transcripts, resulted in a slightly higher Pi content in the shoot in low-P conditions, concomitant with weak up-regulation of several *Pht1* genes. In addition, it was speculated that *At4* transcript abundances in the wild type might be adjusted at the post-transcriptional level by the activity of a micro-RNA (miRNA; Shin *et al.*, 2006). miRNA is typically 21–23 nucleotides long, and is thought to regulate the expression of target genes. For that purpose, a miRNA is complementary to part of one or more mRNAs to which it can anneal. The primary mode of action of plant miRNAs is to facilitate cleavage of the mRNA. In addition, miRNAs may also inhibit protein translation, or target methylation of genomic sites that correspond to targeted mRNAs. *miRNA399* (*miR399*)-dependent post-transcriptional regulation of a gene encoding a putative

ubiquitin-conjugating enzyme (UBC) was shown to be involved in the regulation of *Pht1;1* gene expression by Pi availability (Fujii *et al.*, 2005). Overexpression of *miR399* in *A. thaliana* resulted in down-regulation of UBC and exaggerated accumulation of Pi in the shoot (Chiou *et al.*, 2006). Subsequently, Aung *et al.* (2006) and Bari *et al.* (2006) showed that a mutation in a *miR399* target gene is responsible for the Pi overaccumulator phenotype in the *pho2* mutant (Delhaize & Randall, 1995). Taken together, these data indicate that regulation of plant Pi transporters, although mainly transcriptional, also involves post-transcriptional and post-translational modification of regulatory components.

4. Subcellular targeting of phosphate transport proteins

In the yeast *Saccharomyces cerevisiae*, transcription of a secreted acid phosphatase, PHO5, is repressed in response to high concentrations of extracellular inorganic phosphate (Lau *et al.*, 1998). To investigate the signal transduction pathway leading to transcriptional regulation of PHO5, a genetic selection for mutants that express PHO5 constitutively was carried out. Within five complementation groups, mutations were found in genes required for the *in vivo* function of the Pi transport system (PHO84, a Pi transporter and PHO86), the respective mutants being defective in high-affinity phosphate uptake. It has now been shown that PHO86 is an endoplasmic reticulum (ER) resident protein which is required for packaging of PHO84 into coat protein II (COPII) vesicles, allowing ER exit and subsequent localization in the plasma membrane (Lau *et al.*, 2000). Pht1 proteins in plants share relatively high homology to PHO84, suggesting that a similar system for Pht1 protein targeting and therefore a functional PHO86 homologue could exist in plants. However, comparison with sequences deposited in GenBank did not reveal homology of PHO86 with plant proteins, indicating that the gene encoding the plant protein cannot be identified via heterologous screening of plant cDNA libraries. This obstacle was overcome by screening the mutant population that also yielded the identification of *PHR1* (see section II. 3). Mutation of the *PHF1* gene resulted in ER retention and reduced accumulation of the plasma membrane Pht1;1 transporter in *A. thaliana*. By contrast, other membrane proteins involved in the Pi starvation response were not mislocalized, indicating that plants have accessory proteins specific for selected plasma membrane proteins, allowing their exit from the ER. *PHF1* encodes a plant-specific protein structurally related to the SEC12 proteins of the early secretory pathway, thus representing the PHO86 analogue (Gonzalez *et al.*, 2005). An intriguing aspect of the cell biology of Pi transport is the asymmetric distribution of Pht1 proteins in the plasma membrane of root hair cells, with increasing protein abundance being found towards the tip of the root hair (Chiou *et al.*, 2001; Gordon-Weeks *et al.*, 2003). This raises important questions with respect to the subcellular targeting mechanism of transport

proteins, a topic that is presently being intensively studied, for example in work on the proteins involved in short- and long-distance auxin distribution (Blakeslee *et al.*, 2005).

5. Functional genomics of direct phosphate uptake in plants

In addition to resolving gene regulation mechanisms in response to internal and external cues, the elucidation of gene function is of prime scientific interest. The individual roles and contribution to direct Pi uptake of Pht1 proteins in the plant have just started to be elucidated in the model plant *A. thaliana*. Eight out of the nine *Pht1* genes in *A. thaliana* are expressed in roots, including the strongly expressed *Pht1;1* and *Pht1;4* genes, the expression patterns of which show extensive overlap (Muchhal *et al.*, 1996; Karthikeyan *et al.*, 2002; Mudge *et al.*, 2002; Misson *et al.*, 2004; Shin *et al.*, 2004). Pi-starved *pht1;4* mutant plantlets exhibit a strongly reduced (40%) Pi uptake capacity without apparent changes in the expression of the other *Pht1* genes (Misson *et al.*, 2004). However, the Pi content of mutant seedlings grown on Pi-deficient or -sufficient medium was not significantly different from that of the corresponding wild-type controls. Furthermore, no obvious growth defects or visible phenotypes were associated with the mutants when plants were grown at low Pi concentration. Intriguingly, analysis of a *Pht1;1-Pht1;4* loss-of-function mutant clearly revealed that *Pht1;1* and *Pht1;4* are responsible for a significant proportion of the total Pi uptake capacity of *A. thaliana* roots under both low- and high-Pi concentrations (Shin *et al.*, 2004). Measurements of Pi uptake from a low- and a high-Pi solution revealed significant differences between wild-type and double knock-out plants in their capacity to exploit a high-Pi source and to rapidly accumulate Pi in both roots and shoots following a period of starvation. Rates of Pi uptake were always higher in the wild type than in the double knock-out, indicating that both transporters contributed to Pi uptake at low and high Pi concentrations. Loss of *Pht1;1* and *Pht1;4* activity also led to increased elongation of root hairs and lateral roots, reduced Pi content in shoots, and anthocyanin accumulation. This is unequivocal evidence for a key role of Pht1 proteins in plant P uptake and thus the regulation of the Pi starvation response pathway via the control of plant P homeostasis.

III. Phosphate uptake assisted by the AM symbiosis

1. The route of symbiotic Pi uptake

In the ecologically and agriculturally important AM associations, the host plant mainly derives P and other minerals (including K, S, N, Zn and copper (Cu)) from the fungus, which in turn benefits from plant-based photosynthetic assimilates, namely glucose (Marschner, 1995; Smith & Read, 1997). Strongly

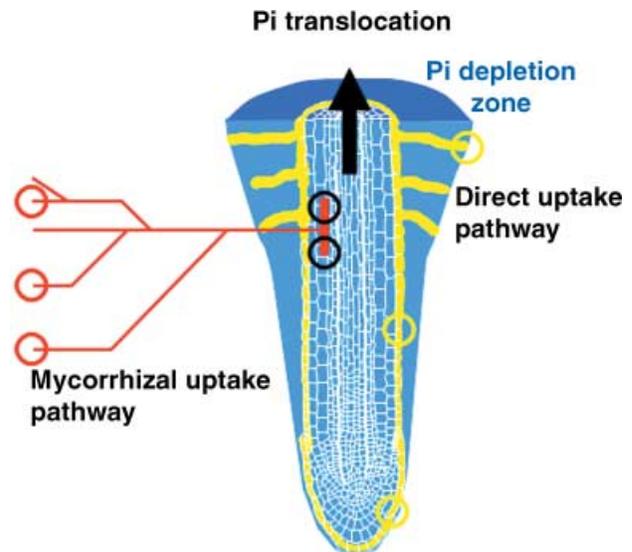


Fig. 2 Schematic representation of the two inorganic orthophosphate (Pi) uptake pathways into a mycorrhiza. In the direct Pi uptake pathway, Pht1 transporters (yellow circles) located at the epidermis, located in root hairs and other parts of the epidermis, are involved in Pi uptake from the soil solution directly into root cells. The rate of uptake usually exceeds the rate of diffusion of Pi, which supports rapid formation of a depletion zone (blue zone) close to the root surface, thus limiting the rate of direct Pi uptake. The mycorrhizal uptake pathway involves uptake of Pi from the soil solution by arbuscular–mycorrhizal (AM) fungal transporters (red circles) located in extraradical hyphae. Pi is subsequently translocated towards the root and eventually to the symbiotic interface in the root cortex. Pht1 transporters located at this interface (black circles) are involved in absorption of Pi in root cortical cells.

reduced mobility of Pi in the soil and rapid direct Pi uptake into the root lead to the development of a Pi depletion zone around the root hair cylinder and a rapid decline of Pi acquisition over time (Marschner, 1995; Roose & Fowler, 2004). Whereas in nonmycorrhizal roots the extension of the Pi depletion zone is closely related to root hair length (Marschner & Dell, 1994), in mycorrhizal roots the depletion zone of Pi greatly exceeds the root hair cylinder (Jungk & Claassen, 1989). This indicates that Pi, which is not directly available to the plant, is being delivered by the fungal hyphae. Thus, the presence of the Pi depletion zone in the rhizosphere is a major factor contributing to the advantage of plants forming mycorrhizal associations. Strictly speaking, a mycorrhizal plant does not constitute a rhizosphere, but rather a mycorrhizosphere, composed of the rhizosphere and the hyphosphere. In this symbiotic system, the fungus bridges the mycorrhizosphere and Pi is transported (mainly in the form of polyphosphates) from the AM fungus–soil interface to the intraradical symbiotic interface (Fig. 2).

Only a few reports indicate that AM fungi produce ectoenzymes, which provide host plants with the potential to access organic P forms that are normally unavailable to

nonmycorrhizal roots. Extraradical hyphae of carrot (*Daucus carota*) mycorrhizas with *Glomus intraradices* in monoxenic cultures in the absence of other soil microorganisms transferred significantly more P to roots when they had access to phytate as the organic P source than when they did not (Koide & Kabir, 2000). This showed unequivocally that extraradical hyphae of *G. intraradices* can hydrolyse organic P, and can transport the resultant inorganic P to host roots. Moreover, a study on *Tagetes patula* in symbiosis with *Glomus etunicatum* proposed a new strategy for acquisition of P in AM associations in which the fungal partner activates components of the low-P adaptation system of the host plant, namely phosphatase secretion, and thus improves the overall efficiency of P uptake (Ezawa *et al.*, 2005).

Research including the quantification of Pi uptake and transport by fungal hyphae in the soil to the plant has improved our understanding of a functional mycorrhiza (Smith *et al.*, 2003). Differences among plant species in the benefit derived from AM colonization have often been attributed to differences in root physical properties, especially in root hair development. Analysis of the growth response to P in five pasture species that differed significantly in the length of their root hairs demonstrated a beneficial effect of AM symbiosis on P acquisition. This was inversely related to the root hair length of the host plant, but was not closely correlated to root diameter, root length per plant or root:shoot ratio. It has been suggested that root hairs and the external hyphae of the AM symbiosis act as alternative but similar ways of shortening the distance for the diffusion of P in soils (Schweiger *et al.*, 1995). Accordingly, the root-hairless barley mutant, *bald root barley (brb)*, was clearly more responsive to mycorrhization than the corresponding wild type (Jakobsen *et al.*, 2005). Root hairs thus represent important cellular structures involved in the 'direct uptake pathway' of nutrients as opposed to the 'mycorrhizal uptake pathway' expanding from extraradical fungal hyphae to the symbiosomes (Figs 1, 2). It was demonstrated that the mycorrhizal Pi uptake pathway could dominate Pi supply to plants irrespective of whether colonized plants exhibited improved growth and/or total P uptake (Smith *et al.*, 2003, 2004). The fact that the AM fungal contribution to plant Pi uptake is greater in a root-hairless mutant than in its wild type indicates that fine tuning of both uptake pathways is required to meet the needs of the plant for this important nutrient (Jakobsen *et al.*, 2005).

2. *Pht1* genes involved in the AM symbiosis and evolutionary conservation of their regulation

Arabidopsis thaliana, the model plant widely used in forward and reverse genetics studies, does not form mycorrhizal associations and therefore is not amenable to the analysis of AM fungus–root interactions. In contrast to *A. thaliana* and other Brassicaceae, legumes such as *M. truncatula* and *L. japonicus* (Young *et al.*, 2003) and solanaceous plants

establish mutualistic AM symbioses under natural conditions (Bhattarai & Mishra, 1984; Barker *et al.*, 1998) and can thus be used as experimental systems for molecular-genetic work in mycorrhizas. Interestingly, an H⁺-ATPase gene exhibited arbuscule-specific expression in mycorrhizal tissue of *M. truncatula* (Krajinski *et al.*, 2002), and a H⁺-ATPase protein was localized in the plant membrane around arbuscule hyphae in a tobacco mycorrhiza, which corroborated the existence of nutrient transport activities at the interface between the two symbiotic organisms (Gianinazzi-Pearson *et al.*, 2000). The identification of the potato Pi transporter gene *StPT3*, which is expressed in cortical cells colonized by AM fungi, represented a starting point for a detailed analysis of Pi transport at the AM symbiotic interface in solanaceous species (Rausch *et al.*, 2001). The *StPT3* promoter directs mycorrhiza-specific gene expression similarly in transgenic roots of distantly related plant species carrying a chimeric *StPT3* promoter-reporter gene including potato, petunia (*Putunia hybrida*), carrot, *M. truncatula* and *L. japonicus*, indicating a high degree of conservation of the signal recognition and transduction pathways in AM symbiotic Pi transport (Karandashov *et al.*, 2004). The identification of mycorrhiza-specific rice OsPT11 (Paszkowski *et al.*, 2002) and *M. truncatula* MtPT4 (Harrison *et al.*, 2002) Pi transporters, both nonorthologous to *StPT3*, recently indicated the presence of a second mycorrhiza-specific Pi uptake system in vascular plants. This was subsequently substantiated by the cloning of four mycorrhiza-specific Pi transporters nonorthologous to *StPT3* from both potato (*StPT4* and 5) and tomato (*LePT4* and 5) (Nagy *et al.*, 2005). In summary, the group of orthologous Pi transporters including OsPT11, MtPT4, and the solanaceous orthologues comprise subfamily I within the Pht1 family, thus including members from both dicot and monocot species. Several new members of this subfamily were added, namely Pht1;8 from barley and Pht1;6 from maize (Glassop *et al.*, 2005; Nagy *et al.*, 2006). By contrast, potato *StPT3*, tomato *LePT3* (accession number AY804011) and the recently identified *LjPT3* from *L. japonicus* (Maeda *et al.*, 2006) cluster in Pht1 subfamily III which is evolutionarily younger than subfamily I, because it contains proteins solely from dicotyledonous species, including nonmycorrhizal *A. thaliana* (Karandashov *et al.*, 2004; Nagy *et al.*, 2005). While *StPT3* was clearly defined as a high-affinity transporter (Rausch *et al.*, 2001), it was demonstrated that subfamily I transporters exhibit low affinity for Pi (Harrison *et al.*, 2002). However, the latter should be handled with some care as the currently available yeast mutants used for functional complementation with *Pht1* genes do not seem to be particularly suitable for the analysis of low-affinity Pi transporters (Harrison *et al.*, 2002; Paszkowski *et al.*, 2002; Nagy *et al.*, 2005, 2006).

With respect to the specificity of the symbiotic interaction and Pi transport, it has been demonstrated that the regulatory mechanism(s) controlling expression of *StPT3* and subfamily

I genes becomes operative when cortical cells are colonized by fungal species from the phylum Glomeromycota but not from other phyla (Karandashov *et al.*, 2004; Paszkowski *et al.*, 2002). Overall, *StPT3* promoter activation attributes a functional parameter with predictive value to the taxonomy of AM fungi and the phylum Glomeromycota with respect to Pi transport. Phylogenetic footprinting has led to the identification of candidate regulatory elements in promoter sequences of *StPT3*, *StPT4*, *LePT4* and *MtPT4* (Karandashov *et al.*, 2004). It can therefore be hypothesized that evolutionarily conserved regulatory mechanisms, including perception of signal molecules, form the basis of mycorrhiza-specific Pi transport within at least the dicotyledonous species.

3. Functional genomics of mycorrhizal Pi transport

Despite the large number of mycorrhiza-inducible Pi transporters identified to date, the functional genomics of Pi transport at the symbiotic interface between AM fungi and host plants is less well understood. The MtPT4 protein has been shown to accumulate in the membrane fraction of mycorrhizas in *M. truncatula*. Immunolocalization studies were consistent with a location of the protein on the periarbuscular membrane, i.e. in the host plant plasma membrane around fine branches of an arbuscule (Harrison *et al.*, 2002), reflecting (as discussed in section II. 4) the asymmetric distribution of Pht1 proteins. This finding regarding subcellular distribution is consistent with a function of MtPT4 in symbiotic Pi uptake.

The first functional study in the literature of a mycorrhiza-inducible Pi transporter, LePT4 from tomato, revealed considerable redundancy between Pht1 proteins at this interface in a solanaceous species, and showed that under the chosen experimental conditions LePT4 was dispensable (Nagy *et al.*, 2005). Thus, the situation at the symbiotic interface of tomato and potato is comparable to that at the root–soil interface of *A. thaliana* in terms of redundancy of Pi transport systems, and can be explained by a genome duplication giving rise to two highly similar paralogues, LePT4/LePT5 and StPT4/StPT5, respectively. In a study investigating the tomato *reduced mycorrhizal colonisation* (*rmc*) mutant, which is resistant to colonization by most AM fungi except one *G. intraradices* isolate (Barker *et al.*, 1998), development of arbuscules and vesicles in the *rmc* cortex coincided with greatly increased transcript abundance of the mycorrhiza-inducible Pi transporters *LePT3* and *LePT4* and concomitant symbiotic P transfer (Poulsen *et al.*, 2005). Thus, in colonized roots of tomato, high abundance of *LePT3* and *LePT4* transcripts is a reliable marker for a functional mycorrhizal uptake pathway in *rmc*. Similarly, AM-specific *MtPT4* expression in *M. truncatula* correlated with arbuscule formation rather than with fungal colonization (Isayenkov *et al.*, 2004), indicating that the arbuscule is an important site of symbiotic Pi uptake. By contrast, *StPT3* promoter studies in potato revealed the

expression of this Pi transporter gene in cells harbouring arbuscules and also in cells containing branched or coiled hyphae (Karandashov *et al.*, 2004). Branched or coiled hyphae are characteristic of the *Paris* morphological type of AM, which has been reported to be more common than the *Arum* type, which carries mainly arbuscules (Cavagnaro *et al.*, 2003). Thus *StPT3* induction is triggered by fungal colonization of cortical cells independent of the type of fungal structures formed, indicating that, in both types of AM symbiosis, symbiotic Pi transport occurs.

To date, the most intriguing study on the functional role of mycorrhiza-specific Pi transporters originates from work on StPT3-like LjPT3 from *L. japonicus* (Maeda *et al.*, 2006). In this work, clear experimental evidence was provided for a mycorrhizal Pi transport function of the transporter. Knock-down of the *LjPT3* gene resulted in reduced growth of plants carrying transformed roots which were colonized by a mycorrhizal fungus, reduced allocation of radiotracer Pi in the shoot, and decreased fungal colonization of mycorrhizas. Additionally, when *Mesorhizobium loti* was inoculated in combination with the mycorrhizal fungus *Glomus mosseae*, necrotic root nodules were observed in roots of knock-down plants. This is strong evidence for an important role of *LjPT3* expression, and probably its corresponding transport activity, in the development and functioning of the two root symbioses. Moreover, it is tempting to speculate that mycorrhizal Pi transport is involved in self–nonself recognition in mycorrhizas and nodules.

4. Functional diversity in mycorrhizal phosphate uptake

Different crop cultivars exhibit different Pi uptake efficiencies depending on the AM fungal species associated with them (Bryla & Koide, 1998; Olsen *et al.*, 1999). This functional diversity is also reflected in the observation that the contribution of the direct Pi uptake pathway and the mycorrhizal uptake pathway to total P uptake can vary dramatically, depending on the associated plant and fungal species (Smith *et al.*, 2003, 2004). The observed differences in Pi transport between AM fungi and their various host plants may be caused by numerous factors. Evidence was provided for the concurrent operation of the direct and the mycorrhizal Pi uptake pathways based on Pi delivery via both pathways and simultaneous expression of the respective Pi transporter genes involved (Poulsen *et al.*, 2005). It is of great interest to establish whether plant Pi transporters, their expression pattern, and/or biochemical properties play an important role in determining functional diversity. Certainly, many other parameters should be considered, including the acquisition efficiency of the host plant (which influences the establishment of the Pi depletion zone), root architecture, root colonization and AM fungal proliferation into the soil, the activity of fungal nutrient transporters in the extraradicle hyphae (i.e. the fungus–soil interface), the P translocation

processes within the hyphae, transfer of Pi across the AM interfacial matrix, and the influence of plant processes including root exudation in relation to metabolism and Pi starvation (Plaxton, 1998; Raghothama, 1999).

IV. Agricultural potential

In recent years, knowledge of the molecular and biochemical mechanisms of Pi uptake in plants has greatly increased. Have these scientific achievements created sufficient capacity to exploit the potential of biotechnology for the improvement of Pi efficiency in sustainable agriculture?

Overexpression of the *A. thaliana Pht1;1* high-affinity Pi transporter gene in cultured tobacco cells resulted in enhanced cell growth under Pi-limited conditions (Mitsukawa *et al.*, 1997). By contrast, overexpression of a Pht1 transporter in barley did not enhance the Pi uptake rate in transgenic plants under any conditions tested (Rae *et al.*, 2004). This suggests either that Pi transport activity is regulated by post-translational mechanisms or, alternatively, that Pi availability at the root–soil interface is usually the rate-limiting step in Pi acquisition in nonmycorrhizal plants, rather than the Pi transport rate across the plasma membrane of rhizodermal cells. The latter hypothesis does not contradict the data of Mitsukawa and colleagues, and is supported by studies on the engineering of root secretory processes where enhanced secretion of acid phosphatases (phytases) and organic acids was shown to improve plant P nutrition in low-P conditions via an increase in soil Pi availability (Koyama *et al.*, 2000; López-Bucio *et al.*, 2000; Richardson *et al.*, 2001; Mudge *et al.*, 2003; Zimmermann *et al.*, 2003; Xiao *et al.*, 2005).

Alternatively, the generation and subsequent ectopic expression of mutated genes encoding engineered Pi transporters with optimized biochemical parameters could benefit plant growth under low-P conditions. These parameters could include affinity for Pi or the minimal concentration of Pi at which a transporter is still active (C_{\min}). However, because of the rapid establishment of a Pi depletion zone in the rhizosphere, it seems likely that changes at the level of Pi transport activity need to be accompanied by optimization of root traits related to soil Pi availability. Moreover, it could be fruitful to investigate how to improve the efficiency of both the direct and the mycorrhizal Pi uptake pathways. To this end, a better understanding of the mechanisms underlying functional diversity in AM symbiosis is certainly required. The answer to the question posed in the first paragraph of this section is thus a definite ‘not yet’, as further basic research is clearly needed before Pi acquisition efficiency can be engineered in crop plants.

V. Conclusions and perspectives

During growth under conditions of Pi limitation, plants undergo radical adaptive changes at the cell, organ and plant

system levels to guarantee reproductive success. Root cell types involved in Pi uptake from the root environment include epidermal cells, such as root hair cells, root cap cells at the tip, and cortical cells in mycorrhizas. These cell types are the prominent sites of Pi transporter gene expression in roots, and are therefore the sites at which Pi transport across the plasma membrane occurs. Physiological, molecular, cell biological and genetic work on Pi uptake in vascular plants allows us to conclude that soil Pi availability and the formation of Pi depletion zones around roots and mycorrhizas are the major physical parameters determining plant Pi acquisition efficiency. The efficiency of a plant in mining the soil for soluble Pi at low cost (i.e. low investment of carbon) largely depends on the root (or mycorrhiza) absorptive surface area, which is strongly influenced by root architecture and the ability to establish a functional mycorrhizal symbiosis. Another important factor, which has only briefly been mentioned in this review, is the production of root exudates which are involved in mobilizing otherwise immobile phosphates. In fundamental research, our knowledge of the molecular regulation of the Pi starvation response and the expression of Pi transporters provides molecular tools with which to elucidate how plants sense Pi depletion or colonization by AM fungal hyphae, which eventually trigger respective response pathways. These tools will be instrumental for the establishment and screening of suitable plant populations to identify regulatory mutants. Continued investigations into how activation of *Pht1* gene promoters occurs in response to the biotic and abiotic environment of the root will help to identify and understand gene regulatory networks including *cis*- and *trans*-acting elements, and the gene module of coregulated genes in a single cell. The post-transcriptional regulation of Pi transport, including the control of protein translation, cellular targeting, transport activity and proteolysis, is presently not well understood and offers great potential for innovative research.

Functional genomics studies of Pi uptake need to be directed to all *Pht1* genes involved in either the direct or the mycorrhizal Pi uptake pathway, or in Pi allocation in the plant. In this work, suitable single and multiple gene loss-of-function mutants will be extremely helpful. Moreover, functional diversity in mycorrhizal Pi uptake in different plant–AM fungus combinations and the control mechanisms involved are likely to be dependent on the molecular cross-talk between plant and fungal symbiont. To improve our knowledge of the role of fungal and plant metabolic status, elucidation of the chemical signals that orchestrate Pi transporter gene expression will possibly be the critical step towards a systems view of Pi uptake dynamics. Understanding of the ecology and functioning of the AM symbiosis in the natural or agricultural ecosystem is essential for developing sound strategies for improvement of plant growth and productivity via controlled activity of the symbiotic partners. We are obviously just beginning to unravel the complexity of the biology of plant Pi uptake.

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References

- Abramson J, Smirnova I, Kasho V, Verner G, Kaback HR, Iwata S. 2003. Structure and mechanism of the lactose permease of *Escherichia coli*. *Science* 301: 610–615.
- Aeschbacher R. 1994. The genetic and molecular basis of root development. *Annual Review of Plant Physiology and Plant Molecular Biology* 45: 25–45.
- Akiyama K, Matsuzaki K, Hayashi H. 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* 435: 824–827.
- Al-Karaki G. 2000. Growth of mycorrhizal tomato and mineral acquisition under salt stress. *Mycorrhiza* 10: 51–54.
- Auge RM. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* 11: 3–42.
- Aung K, Lin S-I, Wu C-C, Huang Y-T, Su C-L, Chiou T-J. 2006. *pho2*, a phosphate overaccumulator, is caused by a nonsense mutation in a microRNA399 target gene. *Plant Physiology* 141: 1000–1011.
- Bailey PHJ, Currey JD, Fitter AH. 2002. The role of root system architecture and root hairs in promoting anchorage against uprooting forces in *Allium cepa* and root mutants of *Arabidopsis thaliana*. *Journal of Experimental Botany* 53: 333–340.
- Bari R, Datt Pant B, Stitt M, Scheible W-R. 2006. PHO2, MicroRNA399, and PHR1 define a phosphate-signaling pathway in plants. *Plant Physiology* 141: 988–999.
- Barker S, Stummer B, Gao L, Dispain I, O'Connor P, Smith S. 1998. A mutant in *Lycopersicon esculentum* Mill. with highly reduced VA mycorrhizal colonization: isolation and preliminary characterisation. *The Plant Journal* 15: 791–797.
- Bates TR, Lynch JP. 1996. Stimulation of root hair elongation in *Arabidopsis thaliana* by low phosphorus availability. *Plant, Cell & Environment* 19: 529–538.
- Bates TR, Lynch JP. 2000. The efficiency of *Arabidopsis thaliana* (Brassicaceae) root hairs in phosphorus acquisition. *American Journal of Botany* 87: 964–970.
- Bhattacharai I, Mishra R. 1984. Study on the vesicular-arbuscular mycorrhiza of three cultivars of potato (*Solanum tuberosum* L.). *Plant and Soil* 79: 299–303.
- Blakeslee JJ, Peer WA, Murphy AS. 2005. Auxin transport. *Current Opinion in Plant Biology: Cell Signalling and Gene Regulation* 8: 494–500.
- Bonanomi A, Wiemken A, Boller T, Salzer P. 2001. Local induction of a mycorrhiza-specific class III chitinase gene in cortical root cells of *Medicago truncatula* containing developing or mature arbuscules. *Plant Biology* 3: 194–199.
- Brechenmacher L, Weidmann S, van Tuinen D, Chatagnier O, Gianinazzi S, Franken P, Gianinazzi-Pearson V. 2004. Expression profiling of up-regulated plant and fungal genes in early and late stages of *Medicago truncatula*–*Glomus mosseae* interactions. *Mycorrhiza* 14: 253–262.
- Bryla DR, Koide RT. 1998. Mycorrhizal response of two tomato genotypes relates to their ability to acquire and utilize phosphorus. *Annals of Botany* 82: 849–857.
- Buchanan BB, Gruissem W, Russel LJ. 2000. *Biochemistry and molecular biology of plants*. Rockville, MA, USA: American Society of Plant Physiologists.
- Bucher M, Rausch C, Daram P. 2001. Molecular and biochemical mechanisms of phosphorus uptake into plants. *Journal of Plant Nutrition and Soil Science* 164: 209–217.
- Buee M, Rossignol M, Jauneau A, Ranjeva R, Becard G. 2000. The pre-symbiotic growth of arbuscular mycorrhizal fungi is induced by a branching factor partially purified from plant root exudates. *Molecular Plant–Microbe Interactions* 13: 693–698.
- Bun-Ya M, Nishimura M, Harashima S, Oshima Y. 1991. The PHO84 gene of *Saccharomyces cerevisiae* encodes an inorganic phosphate transporter. *Molecular and Cellular Biology* 11: 3229–3238.
- van Buuren ML, Maldonado-Mendoza IE, Trieu AT, Blaylock LA, Harrison MJ. 1999. Novel genes induced during an arbuscular mycorrhizal (AM) symbiosis formed between *Medicago truncatula* and *Glomus versiforme*. *Molecular Plant–Microbe Interactions* 12: 171–181.
- Cavagnaro TR, Smith FA, Ayling SM, Smith SE. 2003. Growth and phosphorus nutrition of a Paris-type arbuscular mycorrhizal symbiosis. *New Phytologist* 157: 127–134.
- Chabaud M, Venard C, Defaux-Petras A, Becard G, Barker DG. 2002. Targeted inoculation of *Medicago truncatula* *in vitro* root cultures reveals *MtENOD11* expression during early stages of infection by arbuscular mycorrhizal fungi. *New Phytologist* 156: 265–273.
- Chiou TJ, Aung K, Lin SI, Wu CC, Chiang SF, Su CL. 2006. Regulation of phosphate homeostasis by MicroRNA in *Arabidopsis*. *The Plant Cell* 18: 412–421.
- Chiou TJ, Liu H, Harrison MJ. 2001. The spatial expression patterns of a phosphate transporter (MtPT1) from *Medicago truncatula* indicate a role in phosphate transport at the root/soil interface. *The Plant Journal* 25: 281–293.
- Chisholm ST, Coaker G, Day B, Staskawicz BJ. 2006. Host–microbe interactions: shaping the evolution of the plant immune response. *Cell* 124: 803–814.
- Clarkson DT. 1985. Factors affecting mineral nutrient acquisition by plants. *Annual Review of Plant Physiology* 36: 77–115.
- Cogliatti DH, Clarkson DT. 1983. Physiological changes in, and phosphate uptake by potato plants during development of, and recovery from phosphate deficiency. *Physiologia Plantarum* 58: 287–294.
- Cordier C, Pozo MJ, Barea JM, Gianinazzi S, Gianinazzi-Pearson V. 1998. Cell defense responses associated with localized and systemic resistance to *Phytophthora parasitica* induced in tomato by an arbuscular mycorrhizal fungus. *Molecular Plant–Microbe Interactions* 11: 1017–1028.
- Cullimore JV, Ranjeva R, Bono JJ. 2001. Perception of lipo-chitoooligosaccharidic Nod factors in legumes. *Trends in Plant Science* 6: 24–30.
- Dangl JL, Jones JDG. 2001. Plant pathogens and integrated defence responses to infection. *Nature* 411: 826–833.
- Daram P, Brunner S, Persson BL, Amrhein N, Bucher M. 1998. Functional analysis and cell-specific expression of a phosphate transporter from tomato. *Planta* 206: 225–233.
- Delhaize E, Randall PJ. 1995. Characterization of a phosphate-accumulator mutant of *Arabidopsis thaliana*. *Plant Physiology* 107: 207–213.
- Drew MC, Saker LR, Barber SA, Jenkins W. 1984. Changes in the kinetics of phosphate and potassium absorption in nutrient-deficient barley roots measured by a solution-depletion technique. *Planta* 160: 490–499.
- Eckardt NA. 2005. Insights into plant cellular mechanisms: of phosphate transporters and arbuscular mycorrhizal infection. *The Plant Cell* 17: 3213–3216.
- Epstein E, Hagen CE. 1952. A kinetic study of the absorption of alkali cations by barley roots. *Plant Physiology* 27: 457–474.
- Epstein E, Rains DW, Elzam OE. 1963. Resolution of dual mechanisms of potassium absorption by barley roots. *Proceedings of the National Academy of Sciences, USA* 49: 684–692.

- Ezawa T, Hayatsu M, Saito M. 2005. A new hypothesis on the strategy for acquisition of phosphorus in arbuscular mycorrhiza: up-regulation of secreted acid phosphatase gene in the host plant. *Molecular Plant-Microbe Interactions* 18: 1046–1053.
- Farmer E, Schulze-Lefert P. 2005. Biotic interactions: From molecular networks to inter-organismal communities. *Current Opinion in Plant Biology* 8: 343–345.
- Felle H. 1994. The H⁺/Cl⁻ symporter in root-hair cells of *Sinapis alba*. *Plant Physiology* 106: 1131–1136.
- Föhse D, Jungk A. 1983. Influence of phosphate and nitrate supply on root hair formation of rape, spinach and tomato plants. *Plant and Soil* 74: 359–368.
- Forde BG, Clarkson DT. 1999. Nitrate and ammonium nutrition of plants: physiological and molecular perspectives. *Advances in Botanical Research* 30: 1–90.
- Forde BG, Lorenzo H. 2001. The nutritional control of root development. *Plant and Soil* 232: 51–68.
- Franco-Zorrilla JM, Gonzalez E, Bustos R, Linhares F, Leyva A, Paz-Ares J. 2004. The transcriptional control of plant responses to phosphate limitation. *Journal of Experimental Botany* 55: 285–293.
- Frank B. 1885. Über die auf Wurzelsymbiosen beruhende Ernährung gewisser Bäume durch unterirdische Pilze. *Berichte der Deutschen Botanischen Gesellschaft* 3: 128–145.
- Fujii H, Chiou TJ, Lin SI, Aung K, Zhu JK. 2005. A miRNA involved in phosphate-starvation response in *Arabidopsis*. *Current Biology* 15: 2038–2043.
- Furihata T, Suzuki M, Sakurai H. 1992. Kinetic characterization of two phosphate uptake systems with different affinities in suspension-cultured *Catharanthus roseus* protoplasts. *Plant and Cell Physiology* 33: 1151–1157.
- Gahoonia TS, Nielsen NE. 2004. Barley genotypes with long root hairs sustain high grain yields in low-P field. *Plant and Soil* 262: 55–62.
- Gahoonia TS, Nielsen NE, Joshi PA, Jahoor A. 2001. A root hairless barley mutant for elucidating genetics of root hairs and phosphorus uptake. *Plant and Soil* 235: 211–219.
- Gassmann W, Schroeder JI. 1994. Inward-rectifying K⁺ channels in root hairs of wheat. *Plant Physiology* 105: 1399–1408.
- Genre A, Chabaud M, Timmers T, Bonfante P, Barker DG. 2005. Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. *The Plant Cell* 17: 3489–3499.
- Gianinazzi-Pearson V, Arnould C, Oufattole M, Arango M, Gianinazzi S. 2000. Differential activation of H⁺-ATPase genes by an arbuscular mycorrhizal fungus in root cells of transgenic tobacco. *Planta* 211: 609–613.
- Gilroy I, Jones DL. 2000. Through form to function: root hair development and nutrient uptake. *Trends in Plant Science* 5: 56–60.
- Glassop D, Smith SE, Smith FW. 2005. Cereal phosphate transporters associated with the mycorrhizal pathway of phosphate uptake into roots. *Planta* 222: 688–698.
- Gonzalez E, Solano R, Rubio V, Leyva A, Paz-Ares J. 2005. PHOSPHATE TRANSPORTER TRAFFIC FACILITATOR1 is a plant-specific SEC12-related protein that enables the endoplasmic reticulum exit of a high-affinity phosphate transporter in *Arabidopsis*. *The Plant Cell* 17: 3500–3512.
- Gordon-Weeks R, Tong Y, Davies TGE, Leggewie G. 2003. Restricted spatial expression of a high-affinity phosphate transporter in potato roots. *Journal of Cell Science* 116: 3135–3144.
- Grabov A, Böttger M. 1994. Are redox reactions involved in regulation of K⁺ channels in the plasma membrane of *Limnium stoloniferum* root hairs? *Plant Physiology* 105: 927–935.
- Grierson C, Roberts K, Feldmann K, Dolan L. 1997. The COW1 locus of *Arabidopsis* acts after RHD2, and in parallel with RHD3 and TIP1, to determine the shape, rate of elongation, and number of root hairs produced from each site of hair formation. *Plant Physiology* 115: 981–990.
- Guimil S, Chang H-S, Zhu T, Sesma A, Osbourn A, Roux C, Ioannidis V, Oakeley EJ, Docquier M, Descombes P, Briggs SP, Paszkowski U. 2005. Comparative transcriptomics of rice reveals an ancient pattern of response to microbial colonization. *Proceedings of the National Academy of Sciences, USA* 102: 8066–8070.
- Hahlbrock K, Bednarek P, Ciolkowski I, Hamberger B, Heise A, Liedgens H, Logemann E, Nurnberger T, Schmelzer E, Somssich IE, Tan J. 2003. Non-self recognition, transcriptional reprogramming, and secondary metabolite accumulation during plant/pathogen interactions. *Proceedings of the National Academy of Sciences, USA* 100: 14569–14576.
- Harrison MJ, Dewbre GR, Liu JY. 2002. A phosphate transporter from *Medicago truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. *The Plant Cell* 14: 2413–2429.
- Hartje S, Zimmermann S, Klonus D, Müller-Röber B. 2000. Functional characterisation of LKT1, a K⁺ uptake channel from tomato root hairs, and comparison with the closely related potato inwardly rectifying K⁺ channel SKT1 after expression in *Xenopus* oocytes. *Planta* 210: 723–731.
- Hirai T, Heymann JA, Shi D, Sarker R, Maloney PC, Subramaniam S. 2002. Three-dimensional structure of a bacterial oxalate transporter. *Nature Structural Biology* 9: 597–600.
- Hohnjec N, Vieweg MF, Puhler A, Becker A, Kuster H. 2005. Overlaps in the transcriptional profiles of *Medicago truncatula* roots inoculated with two different *Glomus* fungi provide insights into the genetic program activated during arbuscular mycorrhiza. *Plant Physiology* 137: 1283–1301.
- Huang Y, Lemieux MJ, Song J, Auer M, Wang D-N. 2003. Structure and mechanism of the glycerol-3-phosphate transporter from *Escherichia coli*. *Science* 301: 616–620.
- Isayenkov S, Fester T, Hause B. 2004. Rapid determination of fungal colonization and arbuscule formation in roots of *Medicago truncatula* using real-time (RT) PCR. *Journal of Plant Physiology* 161: 1379–1383.
- Itoh S, Barber SA. 1983. Phosphorus uptake by six plant species as related to root hairs. *Agronomy Journal* 75: 457–461.
- Jakobsen I, Chen BD, Munkvold L, Lundsgaard T, Zhu Y-G. 2005. Contrasting phosphate acquisition of mycorrhizal fungi with that of root hairs using the root hairless barley mutant. *Plant, Cell & Environment* 28: 928–938.
- Johnson NC, Graham JH, Smith FA. 1997. Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytologist* 135: 575–585.
- Journet EP, van Tuinen D, Gouzy J, Crespeau H, Carreau V, Farmer MJ, Niebel A, Schiex T, Jaillon O, Chatagnier O, Godiard L, Micheli F, Kahn D, Gianinazzi-Pearson V, Gamas P. 2002. Exploring root symbiotic programs in the model legume *Medicago truncatula* using EST analysis. *Nucleic Acids Research* 30: 5579–5592.
- Jungk A, Claassen N. 1989. Availability in soil and acquisition by plants as the basis for phosphorus and potassium supply to plants. *Zeitschrift für Pflanzenernährung und Bodenkunde* 152: 151–157.
- Kai M, Takazumi K, Adachi H, Wasaki J, Shinano T, Osaki M. 2002. Cloning and characterization of four phosphate transporter cDNAs in tobacco. *Plant Science* 163: 837–846.
- Kalsi G, Etzler ME. 2000. Localization of a nod factor-binding protein in legume roots and factors influencing its distribution and expression. *Plant Physiology* 124: 1039–1048.
- Karandashov V, Bucher M. 2005. Symbiotic phosphate transport in arbuscular mycorrhizas. *Trends in Plant Science* 10: 22–29.
- Karandashov V, Nagy R, Wegmüller S, Amrhein N, Bucher M. 2004. Evolutionary conservation of phosphate transport in the arbuscular mycorrhizal symbiosis. *Proceedings of the National Academy of Sciences, USA* 101: 6285–6290.
- Karthikeyan AS, Varadarajan DK, Mukatira UT, D'Urzo MP, Damsz B, Raghobarama KG. 2002. Regulated expression of *Arabidopsis* phosphate transporters. *Plant Physiology* 130: 221–233.
- Kistner C, Parniske M. 2002. Evolution of signal transduction in intracellular symbiosis. *Trends in Plant Science* 7: 511–518.

- Kistner C, Winzer T, Pitzschke A, Mulder L, Sato S, Kaneko T, Tabata S, Sandal N, Stougaard J, Webb KJ, Szczygłowski K, Parniske M. 2005. Seven *Lotus japonicus* genes required for transcriptional reprogramming of the root during fungal and bacterial symbiosis. *The Plant Cell* 17: 2217–2229.
- Kochian LV, Jones DL, Shaff JE. 1994. The role of ion transport processes in root hair tip growth in *Limnium stoloniferum*. *Current Topics in Plant Physiology* 12: 150–160.
- Koide RT, Kabir Z. 2000. Extraradical hyphae of the mycorrhizal fungus *Glomus intraradices* can hydrolyse organic phosphate. *New Phytologist* 148: 511–517.
- Kosuta S, Chabaud M, Loughon G, Gough C, Denarie J, Barker DG, Becard G. 2003. A diffusible factor from arbuscular mycorrhizal fungi induces symbiosis-specific *MtENOD11* expression in roots of *Medicago truncatula*. *Plant Physiology* 131: 952–962.
- Koyama H, Kawamura A, Kihara T, Hara T, Takita E, Shibata D. 2000. Overexpression of mitochondrial citrate synthase in *Arabidopsis thaliana* improved growth on a phosphorus-limited soil. *Plant Cell Physiology* 41: 1030–1037.
- Krajinski F, Hause B, Gianinazzi-Pearson V, Franken P. 2002. *Mtha1*, a plasma membrane H⁺-ATPase gene from *Medicago truncatula*, shows arbuscule-specific induced expression in mycorrhizal tissue. *Plant Biology* 4: 754–761.
- Lagarde D, Basset M, Lepetit M, Conejero G, Gaymard F, Astruc S, Grignon C. 1996. Tissue-specific expression of *Arabidopsis AKT1* gene is consistent with a role in K⁺ nutrition. *The Plant Journal* 9: 195–203.
- Lau WT, Howson RW, Malkus P, Schekman R, O'Shea EK. 2000. Pho86p, an endoplasmic reticulum (ER) resident protein in *Saccharomyces cerevisiae*, is required for ER exit of the high-affinity phosphate transporter Pho84p. *Proceedings of the National Academy of Sciences, USA* 97: 1107–1112.
- Lau WW, Schneider KR, O'Shea EK. 1998. A genetic study of signaling processes for repression of PHO5 transcription in *Saccharomyces cerevisiae*. *Genetics* 150: 1349–1359.
- Lauter F-R, Ninnemann O, Bucher M, Riesmeier JW, Frommer WB. 1996. Preferential expression of an ammonium transporter and of two putative nitrate transporters in root hairs of tomato. *Proceedings of the National Academy of Sciences, USA* 93: 8139–8144.
- Leggiewe G, Willmitzer L, Riesmeier JW. 1997. Two cDNAs from potato are able to complement a phosphate uptake-deficient yeast mutant: Identification of phosphate transporters from higher plants. *The Plant Cell* 9: 381–392.
- Liu J, Blaylock LA, Endre G, Cho J, Town CD, VandenBosch KA, Harrison MJ. 2003. Transcript profiling coupled with spatial expression analyses reveals genes involved in distinct developmental stages of an arbuscular mycorrhizal symbiosis. *The Plant Cell* 15: 2106–2123.
- Liu C, Muchhal US, Uthappa M, Kononowicz AK, Raghothama KG. 1998a. Tomato phosphate transporter genes are differentially regulated in plant tissues by phosphorus. *Plant Physiology* 116: 91–99.
- Liu H, Trieu AT, Blaylock LA, Harrison MJ. 1998b. Cloning and characterization of two phosphate transporters from *Medicago truncatula* roots: Regulation in response to phosphate and to colonization by arbuscular mycorrhizal (AM) fungi. *Molecular Plant-Microbe Interactions* 11: 14–22.
- López-Bucio J, Cruz-Ramírez A, Herrera-Estrella L. 2003. The role of nutrient availability in regulating root architecture. *Current Opinion in Plant Biology* 6: 280–287.
- López-Bucio J, de La Vega OM, Guevara-García A, Herrera-Estrella L. 2000. Enhanced phosphorus uptake in transgenic tobacco plants that overproduce citrate. *Nature Biotechnology* 18: 450–453.
- Lynch J. 1995. Root architecture and plant productivity. *Plant Physiology* 109: 7–13.
- Ma Z, Bielenberg DG, Brown KM, Lynch JP. 2001. Regulation of root hair density by phosphorus availability in *Arabidopsis thaliana*. *Plant, Cell & Environment* 24: 459–467.
- Maeda D, Ashida K, Iguchi K, Chechetka SA, Hijikata A, Okusako Y, Deguchi Y, Izui K, Hata S. 2006. Knockdown of an arbuscular mycorrhiza-inducible phosphate transporter gene of *Lotus japonicus* suppresses mutualistic symbiosis. *Plant and Cell Physiology* 47: 807–817.
- Marschner H. 1995. *Mineral nutrition of higher plants*. London, UK: Academic Press.
- Marschner H, Dell B. 1994. Nutrient uptake in mycorrhizal symbiosis. *Plant and Soil* 159: 89–102.
- Mimura T, Dietz K, Kaiser W, Schramm M, Kaiser G, Heber U. 1990. Phosphate transport across biomembranes and cytosolic phosphate homeostasis in barley leaves. *Planta* 180: 139–146.
- Misson J, Thibaud MC, Bechtold N, Raghothama K, Nussaume L. 2004. Transcriptional regulation and functional properties of *Arabidopsis* Pht1;4, a high affinity transporter contributing greatly to phosphate uptake in phosphate deprived plants. *Plant Molecular Biology* 55: 727–741.
- Mitsukawa N, Okumura S, Shirano Y, Sato S, Kato T, Harashima S, Shibata D. 1997. Overexpression of an *Arabidopsis thaliana* high-affinity phosphate transporter gene in tobacco cultured cells enhances cell growth under phosphate-limited conditions. *Proceedings of the National Academy of Sciences, USA* 94: 7098–7102.
- Miura K, Rus A, Sharkhuu A, Yokoi S, Karthikeyan AS, Raghothama KG, Baek D, Koo YD, Jin JB, Bressan RA, Yun D-J, Hasegawa PM. 2005. The *Arabidopsis* SUMO E3 ligase SIZ1 controls phosphate deficiency responses. *Proceedings of the National Academy of Sciences, USA* 102: 7760–7765.
- Morieux L, Michelet B, Bogaerts P, Lambert L, Michel A, Oufattole M, Boutry M. 1999. Expression analysis of two gene subfamilies encoding the plasma membrane H⁺-ATPase in *Nicotiana glauca* reveals the major transport functions of this enzyme. *The Plant Journal* 19: 31–41.
- Muchhal US, Pardo JM, Raghothama KG. 1996. Phosphate transporters from the higher plant *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* 93: 10519–10523.
- Muchhal US, Raghothama KG. 1999. Transcriptional regulation of plant phosphate transporters. *Proceedings of the National Academy of Sciences, USA* 96: 5868–5872.
- Mudge SR, Rae AL, Diatloff E, Smith FW. 2002. Expression analysis suggests novel roles for members of the Pht1 family of phosphate transporters in *Arabidopsis*. *The Plant Journal* 31: 341–353.
- Mudge SR, Smith FW, Richardson AE. 2003. Root-specific and phosphate-regulated expression of phytase under the control of a phosphate transporter promoter enables *Arabidopsis* to grow on phytate as a sole P source. *Plant Science* 165: 871–878.
- Nagy R, Karandashov V, Chague V, Kalinkevich K, Tamasloukht M, Xu G, Jakobsen I, Levy AA, Amrhein N, Bucher M. 2005. The characterization of novel mycorrhiza-specific phosphate transporters from *Lycopersicon esculentum* and *Solanum tuberosum* uncovers functional redundancy in symbiotic phosphate transport in solanaceous species. *The Plant Journal* 42: 236–250.
- Nagy R, Vasconcelos MJ, Zhao S, McElver J, Bruce W, Amrhein N, Raghothama KG, Bucher M. 2006. Differential regulation of five Pht1 phosphate transporters from maize (*Zea mays* L.). *Plant Biology* 8: 186–197.
- Nandi S, Pant R, Nissen P. 1987. Multiphasic uptake of phosphate by corn roots. *Plant, Cell & Environment* 10: 463–474.
- Narang RA, Bruene A, Altmann T. 2000. Analysis of phosphate acquisition efficiency in different *Arabidopsis* accessions. *Plant Physiology* 124: 1786–1799.
- Neumann G, Massonneau A, Langlade N, Dinkelaker B, Hengeler C, Römheld V, Martinoia E. 2000. Physiological aspects of cluster root function and development in phosphorus-deficient white lupine (*Lupinus albus* L.). *Annals of Botany* 85: 909–919.
- Olah B, Briere C, Becard G, Denarie J, Gough C. 2005. Nod factors and a diffusible factor from arbuscular mycorrhizal fungi stimulate lateral root formation in *Medicago truncatula* via the DMI1/DMI2 signalling pathway. *The Plant Journal* 44: 195–207.

- Oldroyd GE, Downie JA. 2004. Calcium, kinases and nodulation signalling in legumes. *Nature Reviews Molecular Cell Biology* 5: 566–576.
- Olsen J, Schaefer J, Edwards D, Hunter M, Galea V, Muller L. 1999. Effects of mycorrhizae, established from an existing intact hyphal network, on the growth response of capsicum (*Capsicum annuum* L.) and tomato (*Lycopersicon esculentum* Mill.) to five rates of applied phosphorus. *Australian Journal of Agricultural Research* 50: 223–237.
- Pao SS, Paulsen IT, Saier MH Jr. 1998. Major facilitator superfamily. *Microbiology and Molecular Biology Reviews* 62: 1–34.
- Parets-Soler A, Pardo J, Serrano R. 1990. Immunocytolocalization of plasma membrane H⁺-ATPase. *Plant Physiology* 93: 1654–1658.
- Parker JS, Cavell AC, Dolan L, Roberts K, Grierson CS. 2000. Genetic interactions during root hair morphogenesis in *Arabidopsis*. *The Plant Cell* 12: 1961–1974.
- Paszkowski U, Kroken S, Roux C, Briggs SP. 2002. Rice phosphate transporters include an evolutionarily divergent gene specifically activated in arbuscular mycorrhizal symbiosis. *Proceedings of the National Academy of Sciences, USA* 99: 13324–13329.
- Plaxton W. 1998. Metabolic aspects of phosphate starvation in plants. In: Lynch J, Deikman J, eds. *Phosphorus in plant biology: regulatory roles in molecular, cellular, organismic, and ecosystem processes*. Rockville, MD, USA: American Society of Plant Physiologists, 229.
- Poirier Y, Bucher M. 2002. Phosphate transport and homeostasis in *Arabidopsis*. In: Somerville C, Meyerowitz EM, eds. *The Arabidopsis book*. Rockville, MD, USA: American Society of Plant Biologists, 1–35.
- Poulsen KH, Nagy R, Gao LL, Smith SE, Bucher M, Smith FA, Jakobsen I. 2005. Physiological and molecular evidence for Pi uptake via the symbiotic pathway in a reduced mycorrhizal colonization mutant in tomato associated with a compatible fungus. *New Phytologist* 168: 445–454.
- Rae AL, Cybinski DH, Jarmey JM, Smith FW. 2003. Characterization of two phosphate transporters from barley; evidence for diverse function and kinetic properties among members of the Pht1 family. *Plant Molecular Biology* 53: 27–36.
- Rae AL, Jarmey JM, Mudge SR, Smith FW. 2004. Over-expression of a high-affinity phosphate transporter in transgenic barley plants does not enhance phosphate uptake rates. *Functional Plant Biology* 31: 141–148.
- Raghothama KG. 1999. Phosphate acquisition. *Annual Review of Plant Physiology and Plant Molecular Biology* 50: 665–693.
- Raghothama KG. 2000. Phosphate transport and signaling. *Current Opinion in Plant Biology* 3: 182–187.
- Rausch C, Bucher M. 2002. Molecular mechanisms of phosphate transport in plants. *Planta* 216: 23–37.
- Rausch C, Daram P, Brunner S, Jansa J, Laloi M, Leggewie G, Amrhein N, Bucher M. 2001. A phosphate transporter expressed in arbuscule-containing cells in potato. *Nature* 414: 462–470.
- van Rhijn P, Fang Y, Galili S, Shaul O, Atzmon N, Wininger S, Eshed Y, Lum M, Li Y, To VV, Fujishige N, Kapulnik Y, Hirsch AM. 1997. Expression of early nodulin genes in alfalfa mycorrhizae indicates that signal transduction pathways used in forming arbuscular mycorrhizae and rhizobium-induced nodules may be conserved. *Proceedings of the National Academy of Sciences, USA* 94: 5467–5472.
- Richardson AE, Hadobas PA, Hayes JE. 2001. Extracellular secretion of *Aspergillus* phytase from *Arabidopsis* roots enables plants to obtain phosphorus from phytate. *Plant Journal* 25: 641–649.
- Roose T, Fowler AC. 2004. A mathematical model for water and nutrient uptake by plant root systems. *Journal of Theoretical Biology* 228: 173–184.
- Rubio V, Linhares F, Solano R, Martin AC, Iglesias J, Leyva A, Paz-Ares J. 2001. A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. *Genes and Development* 15: 2122–2133.
- Salzer P, Bonanomi A, Beyer K, Vögeli-Lange R, Aeschbacher RA, Lange J, Wiemken A, Kim D, Cook DR, Boller T. 2000. Differential expression of eight chitinase genes in *Medicago truncatula* roots during mycorrhiza formation, nodulation, and pathogen infection. *Molecular Plant–Microbe Interactions* 13: 763–777.
- Schachtman DP, Reid RJ, Ayling SM. 1998. Phosphorus uptake by plants: From soil to cell. *Plant Physiology* 116: 447–453.
- Schenk PM, Kazan K, Wilson I, Anderson JP, Richmond T, Somerville SC, Manners JM. 2000. Coordinated plant defense responses in *Arabidopsis* revealed by microarray analysis. *Proceedings of the National Academy of Sciences, USA* 97: 11655–11660.
- Schieffelbein JW, Benfey PN. 1991. The development of plant roots: new approaches to underground problems. *The Plant Cell* 3: 1147–1154.
- Schieffelbein JW, Shipley A, Rowse P. 1992. Calcium influx at the tip of growing root-hair cells of *Arabidopsis thaliana*. *Planta* 187: 455–459.
- Schieffelbein JW, Somerville CR. 1990. Genetic control of root hair development in *Arabidopsis thaliana*. *The Plant Cell* 2: 235–243.
- Schmidt W, Schikora A. 2001. Different pathways are involved in phosphate and iron stress-induced alterations of root epidermal cell development. *Plant Physiology* 125: 2078–2084.
- Schunmann PH, Richardson AE, Smith FW, Delhaize E. 2004a. Characterization of promoter expression patterns derived from the Pht1 phosphate transporter genes of barley (*Hordeum vulgare* L.). *Journal of Experimental Botany* 55: 855–865.
- Schunmann PHD, Richardson AE, Vickers CE, Delhaize E. 2004b. Promoter analysis of the barley Pht1;1 phosphate transporter gene identifies regions controlling root expression and responsiveness to phosphate deprivation. *Plant Physiology* 136: 4205–4214.
- Schweiger PF, Robson AD, Barrow NJ. 1995. Root hair length determines beneficial effect of a *Glomus* species on shoot growth of some pasture species. *New Phytologist* 131: 247–254.
- Shimogawara K, Usuda H. 1995. Uptake of inorganic phosphate by suspension-cultured tobacco cells: kinetics and regulation by Pi starvation. *Plant and Cell Physiology* 36: 341–351.
- Shin H, Shin H-S, Chen R, Harrison MJ. 2006. Loss of At4 function impacts phosphate distribution between the roots and the shoots during phosphate starvation. *The Plant Journal* 45: 712–726.
- Shin H, Shin H-S, Dewbre GR, Harrison MJ. 2004. Phosphate transport in *Arabidopsis*: Pht1;1 and Pht1;4 play a major role in phosphate acquisition from both low- and high-phosphate environments. *The Plant Journal* 39: 629–642.
- Smith FW, Ealing PM, Dong B, Delhaize E. 1997. The cloning of two *Arabidopsis* genes belonging to a phosphate transporter family. *The Plant Journal* 11: 83–92.
- Smith S, Gianinazzi-Pearson V. 1988. Physiological interactions between symbionts in vesicular-arbuscular mycorrhizal plants. *Annual Review of Plant Physiology and Plant Molecular Biology* 39: 221–244.
- Smith FW, Rae AL, Hawkesford MJ. 2000. Molecular mechanisms of phosphate and sulphate transport in plants. *Biochimica et Biophysica Acta* 1465: 236–245.
- Smith SE, Read DJ. 1997. *Mycorrhizal symbiosis*. San Diego, CA, USA: Academic Press.
- Smith SE, Smith FA, Jakobsen I. 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiology* 133: 16–20.
- Smith SE, Smith FA, Jakobsen I. 2004. Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake. *New Phytologist* 162: 511–524.
- Stevens MA, Rick CM. 1986. Genetics and breeding. In: *The tomato crop: a scientific basis for improvement*. Atherton JG, Rudich J, eds. London, UK: Chapman & Hall, 35–109.
- Subramanian KS, Charest C, Dwyer LM, Hamilton RI. 1995. Arbuscular mycorrhizas and water relations in maize under drought stress at tasselling. *New Phytologist* 129: 643–650.
- Sze H, Li X, Palmgren MG. 1999. Energization of plant cell membranes by H⁺-pumping ATPases. Regulation and biosynthesis. *The Plant Cell* 11: 677–690.

- Takahashi H, Watanabe-Takahashi A, Smith FW, Blake-Kalff M, Hawkesford MJ, Saito K. 2000. The roles of three functional sulphate transporters involved in uptake and translocation of sulphate in *Arabidopsis thaliana*. *The Plant Journal* **23**: 171–182.
- Thibaud J-B, Davidian J-C, Sentenac H, Soler A, Grignon C. 1988. H⁺ cotransports in roots as related to the surface pH shift induced by H⁺ excretion. *Plant Physiology* **88**: 1469–1473.
- Toth R, Toth D, Starke D, Smith DR. 1990. Vesicular-arbuscular mycorrhizal colonization in *Zea mays* affected by breeding for resistance to fungal pathogens. *Canadian Journal of Botany* **68**: 1039–1044.
- Ullrich-Eberius CI, Novacky A, van Bel AJE. 1984. Phosphate uptake in *Lemna gibba* G1: energetics and kinetics. *Planta* **161**: 46–52.
- Vert G, Grotz N, Dedaldechamp F, Gaymard F, Guerinot ML, Briata JF, Curie C. 2002. IRT1, an *Arabidopsis* transporter essential for iron uptake from the soil and for plant growth. *The Plant Cell* **14**: 1223–1233.
- Vieweg MF, Fruhling M, Quandt HJ, Heim U, Baumlein H, Puhler A, Kuster H, Andreas MP. 2004. The promoter of the *Vicia faba* L. leghemoglobin gene *VfLb29* is specifically activated in the infected cells of root nodules and in the arbuscule-containing cells of mycorrhizal roots from different legume and nonlegume plants. *Molecular Plant–Microbe Interactions* **17**: 62–69.
- Weisenseel M, Dorn A, Jaffe L. 1979. Natural H⁺ currents traverse growing roots and root hairs of barley (*Hordeum vulgare* L.). *Plant Physiology* **64**: 512–518.
- Wiersum LK. 1958. Density of root branching as affected by substrate and separations. *Acta Botanica Neerlandica* **7**: 174–190.
- Wilson VG. 2004. *Sumoylation, molecular biology and biochemistry*. Norwich, UK: Horizon Bioscience.
- Wu P, Ma L, Hou X, Wang M, Wu Y, Liu F, Deng XW. 2003. Phosphate starvation triggers distinct alterations of genome expression in *Arabidopsis* roots and leaves. *Plant Physiology* **132**: 1260–1271.
- Wulf A, Manthey K, Doll J, Perlick AM, Linke B, Bekel T, Meyer F, Franken P, Kuster H, Krajinski F. 2003. Transcriptional changes in response to arbuscular mycorrhiza development in the model plant *Medicago truncatula*. *Molecular Plant–Microbe Interactions* **16**: 306–314.
- Xiao K, Harrison MJ, Wang ZY. 2005. Transgenic expression of a novel *M. truncatula* phytase gene results in improved acquisition of organic phosphorus by *Arabidopsis*. *Planta* **222**: 27–36.
- Young ND, Mudge J, Ellis TH. 2003. Legume genomes: more than peas in a pod. *Current Opinion in Plant Biology* **6**: 199–204.
- Zimmermann P, Zardi G, Lehmann M, Zeder C, Amrhein N, Frossard E, Bucher M. 2003. Engineering the root–soil interface via targeted expression of a synthetic phytase gene in trichoblasts. *Plant Biotechnology Journal* **1**: 353–360.
- Zobel R. 1996. Genetic control of root systems. In: Waisel Y, Eshel A, Kafkafi U, eds. *Plant roots: the hidden half, 2nd edn*. New York, NY, USA: Marcel Dekker, 21–30.



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