Roles of Arbuscular Mycorrhizas in Plant Phosphorus Nutrition: Interactions between Pathways of Phosphorus Uptake in Arbuscular Mycorrhizal Roots Have Important Implications for Understanding and Manipulating Plant Phosphorus Acquisition[1]

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In this Update, we review new findings about the roles of the arbuscular mycorrhizas (mycorrhiza = fungus plus root) in plant growth and phosphorus (P) nutrition. We focus particularly on the function of arbuscular mycorrhizal (AM) symbioses with different outcomes for plant growth (from positive to negative) and especially on the interplay between direct P uptake via root epidermis (including root hairs when present) and uptake via the AM fungal pathway. The results are highly relevant to many aspects of AM symbiosis, ranging from signaling involved in the development of colonization and the regulation of P acquisition to the roles of AM fungi in determining the composition of natural plant assemblages in ecological settings and their changes with time.

P is critical for plant growth and makes up about 0.2% of dry weight, but it is one of the most difficult nutrients for plants to acquire. In soil, it may be present in relatively large amounts, but much of it is poorly available because of the very low solubility of phosphates of iron, aluminum, and calcium, leading to soil solution concentrations of 10 μM or less and very low mobility (Schachtman et al., 1998). In consequence, uptake of orthophosphate (Pi) by root epidermal cells including root hairs (the direct pathway) leads to lowering of Pi concentrations in the rhizosphere (so-called depletion zones), because replacement does not keep pace with uptake (Fig. 1). Plants and fungi take up P as negatively charged Pi ions ($H_2PO_4^-$), which poses additional problems, because the concentration in cells is about 1,000-fold higher than in the soil solution and the cell membrane has an inside-negative electric potential. Pi uptake, therefore, requires metabolic energy and involves high-affinity transporter proteins in the Pht1 family (Bucher, 2007). Accordingly, plants have evolved a range of strategies that increase either Pi uptake capacity or availability of Pi in soil (Marschner, 1995). The most common of these strategies worldwide is AM symbiosis. Scattered through the plant kingdom are other strategies that enhance Pi availability or uptake, such as the formation of dense "cluster roots" that produce organic anions that release Pi from poorly available inorganic forms, but these are much less common (Cheng et al., 2011; Lambers et al., 2011). In many cases, it appears that cluster root formation may be an alternative strategy to AM formation, as in most Proteaceae and also white lupin (*Lupinus albus*), but some plants, such as *Casuarina*, develop both cluster roots and AM (Lambers et al., 2008).

The majority (probably 70%–80%) of terrestrial plant species are capable of interacting with AM fungi in nature, and the activities of AM fungi and plant roots are closely integrated as a result of coevolution over at least 450 million years (Smith and Read, 2008). The major advantage of the AM symbiosis for plants in acquiring P is that AM fungi provide a very effective pathway (the AM pathway) by which P is scavenged from large volumes of soil and rapidly delivered to cortical cells within the root, bypassing direct uptake. The AM pathway can reduce the impact of Pi depletion in the rhizosphere and so improve plant P nutrition and growth (Fig. 1). The extent to which an AM plant grows better than a nonmycorrhizal (NM) counterpart (as in a pot experiment) depends in part on the size of its root system, including numbers and extent of root hairs. In general, plants with low root-shoot biomass ratios, slow root growth rates, and/or poor development of root hairs show relatively larger growth increases when AM. Conversely, growth differences between AM and NM plants tend to disappear as available soil P is increased, because of lower P depletion in rhizospheres (Smith and Read, 2008). Nevertheless, growth increases of AM plants over NM counterparts are maintained if high total soil P is poorly available (Bolan, 1991).

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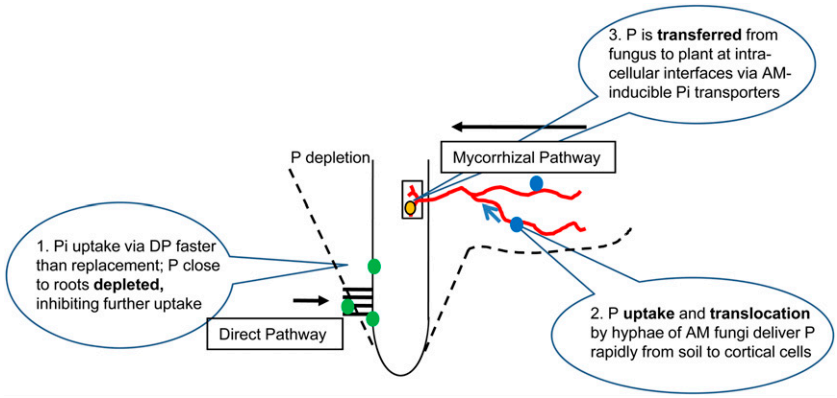

Figure 1. The two pathways of P uptake in an AM root involve different regions of the root, different cell types, and different Pi transporters. In the direct pathway (DP), Pi is absorbed from the rhizosphere by plant Pi transporters in epidermis and root hairs (green circles) close to the root surface. Uptake is normally faster than replacement by diffusion from the bulk soil, resulting in reduced Pi concentrations (depletion) close to the roots (callout 1). In the mycorrhizal pathway, Pi is taken up into AM fungal hyphae by fungal Pi transporters (blue circles) several centimeters from the root and translocated to intracellular fungal structures (arbuscules and hyphal coils) in root cortical cells (callout 2). Plant Pi transporters, induced in colonized cells (yellow circle), transfer Pi from the interfacial apoplast to plant cortical cells (callout 3).

Recent physiological and molecular research has revealed clearly that the AM pathway plays a major role in P uptake, regardless of the extent to which an AM plant benefits in terms of increased growth or P uptake. At the same time, plants provide all the organic carbon (C) requirements of the fungi, so that AM symbioses are mutualistic, based on an exchange of plant C for soil P and other nutrients that we do not consider here, including nitrogen (Smith and Read, 2008; Smith and Smith, 2011). Use of C by AM fungi can be offset at least partly by higher rates of photosynthesis and/or savings in C costs of root production in AM plants (lower root-shoot ratios than NM counterparts). Furthermore, costs of the AM fungi will not be deleterious if plant growth is not C limited.

In the context of P nutrition, AM symbioses should not be regarded (as they sometimes are) as optional strategies that are "implemented" by plants when soil P supplies are low and are ineffective or even eliminated when P supplies are high. For plants capable of forming AM, the NM condition is nearly always "unnatural." In other words, it is an artifact of growth in sterilized soil as in experiments or in horticulture or agriculture if soil is fumigated. The NM state can be regarded as "natural" only where potentially AM plants can survive and reproduce in soils lacking AM fungi or in nonhost species (such as members of the Brassicaceae) that never become mycorrhizal. High-P fertilizer application can greatly lower the percentage of root length colonized. The lower percentage of root length colonized at high P availability does not necessarily imply plant suppression or control of fungal activity, because high P increases root growth and hence reduces the ratio of colonized to noncolonized root length; there may be no effects of P on the fungus per se (Smith et al., 1992; Marschner, 1995). However, very high P application can certainly alter characteristics of root colonization (particularly reducing arbuscule development) and markedly decrease AM fungal biomass per plant, including both biomass in roots and in soil (Smith and Read, 2008). Bruce et al. (1994) showed that early (up to 15 d) reduction in colonization in cucumber (*Cucumis sativus*) with additional P was mediated by slower growth of fungal infection units within roots, but later there was also a reduction in the rate of formation of new entry points. The latter observation has been significantly extended by Balzergue et al. (2011), who showed marked reductions in appressorium formation on pea (*Pisum sativum*) roots at high P (750 μM; i.e. about 2 orders of magnitude higher than soil solution), which, importantly, was mediated by internal plant-derived signals.

Agronomic practices may lower inoculum in soil and subsequent colonization as a result of frequent use of P fertilizer, long fallow periods, cultivation of nonhost crops (especially members of the Brassicaceae), or frequent soil tillage that disrupts networks of AM fungal hyphae in soil (Jansa et al., 2006). Alteration of the mycorrhizal status of a plant (by soil sterilization) from the AM condition, receiving P via both the direct and AM pathways, to an unnatural NM condition, receiving P only via the direct pathway, may induce physiological changes that can be classed as stress responses. Much of the research reviewed here has been done since the acceptance that plant responses to AM colonization vary from highly positive to negative (Johnson et al., 1997; Smith and Smith, 2011). Previously, it was assumed that responses should normally be positive (or zero), resulting in the neglect of mechanisms underlying the negative responses (or "growth depressions"). The use of plants that can grow better when AM symbiosis is eliminated (i.e. that show conventional AM growth depressions) is revealing unexpected aspects of the

integration of plant and fungal processes. These plants include not only wild species but crops such as wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), upland rice (*Oryza sativa*), and tomato (*Solanum lycopersicum*), and the findings have important consequences for understanding and potentially improving P uptake efficiency in agricultural systems.

FUNCTIONAL DIVERSITY IN AM SYMBIOSES

Plants that develop AM symbioses can in most cases be colonized by AM fungi from different taxa. In other words, there seems to be very little specificity in the symbiosis. However, plant species can have preferences for individual AM fungi, resulting in different densities of colonization, and in some cases colonization can be very low (Smith et al., 2009). These effects have been mostly demonstrated with fungi from culture collections and with plants that show positive growth responses. The variation in extent of colonization by different fungi is also applicable to natural ecosystems, where the symbionts may have coevolved over millennia, or in agricultural systems, where individual AM fungi may have been preferentially selected by particular crop management strategies.

Colonization by different AM fungi does not result in the same growth responses in a single AM plant species (Klironomos, 2003; Munkvold et al., 2004; Smith et al., 2004), and colonization by the same AM fungus does not necessarily result in the same growth responses in different plant species (or even varieties). This diversity of responses was nicely shown with naturally co-occurring plants and AM fungi from the same site (Klironomos, 2003). In this case, the growth responses in individual plant species ranged from positive to negative. It is clear, therefore, that there is considerable functional diversity among plant-AM fungal symbioses in terms of benefits (P supply to the plant, in this context) and costs (C supply to the fungus). Individual plants in the field will be colonized by many AM fungal taxa, and the sum total of benefits and costs contributes to success, in terms of growth and reproduction. The outcomes of the symbioses are determined by interactions between plant and AM fungal genomes as well as environmental conditions (e.g. soil pH and P chemistry).

AM PLANTS HAVE TWO PATHWAYS FOR P UPTAKE FROM SOIL

The two pathways by which AM plants absorb P involve different cell types, different Pi transporters, and P access from different regions and volumes of soil (Fig. 1). Direct uptake, by root epidermis, including root hairs when they are formed, accesses Pi in the soil solution close to the roots. Expression of genes encoding high-affinity Pi transporters (PiTs) in these cells is maximal in the root apex and root hairs (Gordon-Weeks et al., 2003) and declines in more mature regions. Expression is often reduced with high P supply and by AM colonization (Javot et al., 2007). These reductions will lead to lower direct uptake in older regions of the root, but their relative importance is not clear.

AM colonization, and hence the potential operation of the AM pathway, occurs behind the root apex. AM fungi grow extensively in soil to form a well-developed hyphal network that absorbs Pi (via fungal high-affinity PiTs) from up to several centimeters from the root surface and can markedly extend the depletion zone (Fig. 1). P is translocated rapidly to the roots (probably as polyphosphate), overcoming the slow diffusion that occurs in the soil solution. The individual fungal hyphae have much smaller diameters than roots, allowing access to narrower soil pores and hence increasing the soil volume explored (Drew et al., 2003; Smith and Read, 2008; Schnepf et al., 2011). These factors are the major cause of increased P uptake and positive AM growth responses. Specialized AM fungus-plant interfaces develop within root cortical cells, associated with complex fungal structures known as arbuscules and also with coiled hyphae (Smith and Read, 2008). These structures are completely enveloped by the plant plasma membrane, so that the interfaces are bound by specialized membranes of plant and fungus with an apoplastic region between them. This organization is important with respect to the control of nutrient transfers between the symbionts (Smith and Smith, 1990). Mechanisms of Pi release from fungus to the interfacial apoplast are obscure, but uptake into the plant is increasingly well understood. AM-inducible plant PiT genes, which are different from those in the direct pathway, are expressed, sometimes exclusively, in the colonized cortical cells (Bucher, 2007; Javot et al., 2007). These PiTs are involved in the uptake of Pi released by the fungi and have been shown to occur in all potentially AM plants investigated, regardless of their responsiveness to AM fungal colonization. Additionally, H^+-ATPases energize the plant plasma membrane surrounding the intracellular fungal structures, facilitating active Pi uptake (Smith and Read, 2008).

The direct and AM pathways are potentially independent, and it used to be assumed that direct uptake made a constant contribution to total plant P uptake, with the AM pathway providing an "extra" contribution in those plants that responded positively to colonization (Smith and Smith, 2011). New research, using a combination of molecular and physiological approaches, shows that this assumption is incorrect and that there is a complex interplay that results in highly variable contributions of the two pathways.

The addition of radioisotopes ^{32}P and ^{33}P and their subsequent exchange with soil P facilitates the quantification of hyphal Pi uptake via the AM pathway from a defined soil compartment (Jakobsen, 1994). If radioactive P is mixed with soil in a compartment enclosed by a 25- to 35-μm nylon mesh, which allows in-growth of hyphae but not roots, P uptake from that hyphal com-

partment (HC) can be calculated as radioactive P_{Plant}/SA_{HC}, where SA_{HC} is the specific activity (i.e. radioactive P/plant available P per unit soil weight). The specific activity becomes relatively constant 4 to 5 weeks after the addition of a P isotope (Morel and Plenchette, 1994), and this corresponds with the time needed for the proliferation of AM fungal mycelium in soil during the establishment of AM plants. These approaches allow calculation of the contribution of the AM pathway to total plant P uptake and hence (by difference) also the contribution of direct uptake (Smith et al., 2004). Relative contributions of AM and direct uptake can also be measured using dual isotope labeling, where one P radioisotope is added to an HC and the other is added to a compartment accessible to both hyphae and roots (Pearson and Jakobsen, 1993). Both approaches have shown that uptake via the AM pathway does occur and may dominate total P uptake, even in plants that do not grow better when colonized by AM fungi. Therefore, the contributions of the AM pathway can be "hidden"; they cannot be determined simply from the total P content of AM and NM plants grown under the same conditions (Jakobsen, 1999). The apparent deactivation of the direct pathway in AM plants might be caused by down-regulation of the plant PiTs in root epidermis plus root hairs (directly via the presence of AM fungi or indirectly via increased plant P status) and/or by competition for P between roots and hyphae in the depletion cylinder around the root (Schnepf et al., 2008). Both will be considered in more detail below.

INTERPLAY BETWEEN DIRECT AND AM UPTAKE OF P

A growing AM root system is one in which direct and AM pathways potentially operate at the same time but not necessarily in the same regions of the root or to the same extent. The direct pathway will be most effective immediately behind the apex, where the root epidermis (including root hairs), armed with high-affinity PiTs, grows into undepleted soil. Farther back, direct uptake almost certainly declines, due to reduced activity of PiTs in the epidermis, loss of root hairs, and depletion of Pi in the rhizosphere. However, the AM pathway will come into play and rapidly contribute to plant P uptake (Fig. 1). The temporal aspects of this change in activity of the pathways is supported by a modeling approach that showed that P influx (μmol P cm^{-2} s^{-1}) into a single root (direct uptake) would be higher than that into AM fungal hyphae for a few days only and that hyphal influx (AM pathway) soon exceeds root influx by 1 order of magnitude (Schnepf et al., 2011).

Measurements of P delivery via AM and direct pathways, using radioactively labeled Pi (see above), also show that the two pathways make variable contributions to whole plant P uptake even in the same species, because different AM fungi deliver different amounts of P. Also, the same AM fungus does not deliver the same proportion of total P to different plant species (Munkvold et al., 2004; Smith et al., 2004). The AM contribution decreases with increasing soil P supply, as direct uptake increases (Nagy et al., 2009). Unsurprisingly, this is associated with a decreasing percentage of root length colonized. The finding that wheat, barley, and tomato can receive a large proportion of total P as hidden P uptake via the AM pathway, even though they generally do not take up more total P when AM, shows that the contribution of direct uptake must be lower than in NM plants. In an extreme case, the direct pathway was completely inoperative in tomato when colonized by the AM fungus *Glomus intraradices* (Smith et al., 2004).

Several investigations have shown higher expression of PiTs in the direct pathway in NM compared with AM plants, which was not necessarily associated with lower plant P status and hence a "P-starvation response" (Javot et al., 2007). We suggest that up-regulation may be part of a battery of "stress responses" also including increased root-shoot ratio and length of root hairs (Marschner, 1995), which are deployed if a normally AM plant is prevented from forming mycorrhizas at low P. It has also been suggested that if lower direct uptake is not compensated by a large contribution of the AM pathway, the plants will become P deficient, providing one explanation for the reduced growth of "nonresponsive" AM plants, especially when growth depressions occur in plants with very low colonization (Grace et al., 2009). Very low colonization will inevitably result in very low P uptake via the AM pathway (Smith et al., 2009). There is also some evidence for low contributions of direct uptake in plants that respond positively to AM. This can be shown for positively responsive medic (*Medicago truncatula*) by using data from Smith et al. (2004) to make new estimates of amounts of P taken up by the direct pathway in AM plants. Values are about 1.1 and 1.4 mg P g^{-1} root dry weight with the two fungi tested, compared with about 3.3 mg P g^{-1} root dry weight for the small NM plants. However, in AM linseed, (*Linum usitatissimum*), also positively responsive, direct P uptake was only decreased by one of the two AM fungi. The outstanding questions include whether the decreased P delivery by the direct pathway is mediated by reduced expression of PiT genes in the direct pathway and/or by competition for uptake of Pi between direct and AM pathways. The latter could certainly occur at high root and hyphal length densities in soil (Schnepf et al., 2008) and is an explanation for the lack of positive mycorrhizal growth responses. However, competition seems unlikely to have significant impact at low length densities (low plant growth and percentage of colonization). If reduced PiT expression is involved, why this occurs and by what signaling pathways are key questions to be addressed.

EXPRESSION OF GENES INVOLVED IN DIRECT AND AM PATHWAYS: IS THERE SIGNALING BETWEEN FUNGUS AND PLANT THAT INFLUENCES GENE EXPRESSION AND DELIVERY OF Pi?

Recognition between symbiotic partners and the establishment of a functional AM symbiosis involve

the exchange of many signals, and the molecular basis of early steps in the interaction includes "common symbiosis genes" that are shared with the legume-*Rhizobium* symbiosis (Hata et al., 2010). However, events farther downstream are separate for the individual AM fungal or bacterial symbioses. While this is an extremely active area of research, it is beyond the scope of this Update. Examples of AM-induced genes relevant to P nutrition include the AM-specific PiTs (Javot et al., 2007). The underlying signaling for AM-specific induction of genes is largely unknown, but lysophosphatidylcholine has been identified as a key for the activation of AM-specific PiT genes in potato (*Solanum tuberosum*) and tomato (Drissner et al., 2007).

Almost all of the work carried out so far on signaling involved in P-starvation responses has been carried out in AM nonhost species, particularly Arabidopsis (*Arabidopsis thaliana*) and also white lupin (Cheng et al., 2011). Such plants are believed to have evolved from AM ancestors, rejecting AM (and other mycorrhizal) symbioses as a strategy to enhance the acquisition of P, but they constitute only about 10% of terrestrial plants (Smith and Read, 2008). The absence of AM P uptake could potentially result in a higher likelihood of P starvation and more marked deployment of P-starvation responses when growing in very-low-P soils. This point needs to be borne in mind when using data from non-AM hosts to infer likely responses of normally AM plants. Increased attention to responses in AM host species in the presence and absence of AM fungal symbionts is needed. In the AM nonhost Arabidopsis, induction and regulation of genes involved in the direct pathway are controlled by a complex network of gene signaling in response to Pi starvation (very low external Pi), with coupling to phytohormonal and sugar responses (Rouached et al., 2010; Chiou and Lin, 2011; Lei et al., 2011). Under Pi starvation, a range of genes is induced by the transcription factor PHR1. PHR1 binds the key integrating cis-regulatory motif P1BS found in the promoter of most PHR1-dependent Pi starvation-induced genes in nonhost plants. The microRNA (miR) miR399 is induced by PHR1 and negatively regulates the enzyme PHO2 that normally suppresses the expression of PiT genes. Hence, accumulation of miR399 in Pi-starved plants leads to derepression of PiT genes and to a potential increase in Pi uptake via the direct pathway. The PHO2/miR399 pathway is fine-tuned by noncoding RNA transcripts, including *IPS1* (Lin et al., 2009). Components of the Pi-starvation signaling pathway are conserved in plants that can become AM. Recently, cis-acting elements have been identified in promoters of several AM-induced PiTs, where one of the motifs is P1BS. P1BS is located near an AM-specific motif, MYCS, and both are required for activation of the AM-induced PiT promoters (Chen et al., 2011). The requirement for the P1BS motif in AM-induced PiT expression is thus linked directly to Pi-starvation signaling and could explain the lack of induction of PiTs in the AM pathway under high external Pi 2009 (1 mM) in AM plants with 10% to 20% colonization (Chen et al., 2011). High levels of miR399 in AM roots coincided with low expression of *PHO2* (Branscheid et al., 2010). The authors speculated that PHO2 activity must be kept low to sustain AM symbiosis, despite a high local root P status in AM plants (Fig. 2). Shared components between non-AM Pi-starvation signaling and AM signaling can also be differentially regulated in the AM and NM situations. In tomato, miR395 is induced by AM colonization only, whereas miR172 is induced both at high P supply and by AM colonization (Gu et al., 2010). The function of these miRs is unknown at present, but their expression patterns suggest a possible role in AM-related Pi signaling and interplay between direct and AM pathways. In medic, a Pi starvation-induced acid phosphatase gene (Liu et al., 1998) and *Mt4* (a noncoding RNA, homologous to *IPS1*) are rapidly down-regulated in the AM symbiosis (Burleigh and Harrison, 1998). Finding the regulatory elements responsible for these differential expression patterns would increase the understanding of the interplay between direct and AM pathways.

Phytohormones play a role in Pi-starvation responses, both through involvement in root development and in sugar signaling (Rouached et al., 2010). In Arabidopsis roots, Pi-starvation genes are repressed by exogenous cytokinins, and cytokinin as well as GA_3 contents are decreased in Pi-starved Arabidopsis plants. Auxin and ethylene are key phytohormones in regulating lateral root and root hair development, which is affected by Pi starvation (Rubio et al., 2009) as well as by AM colonization. Strigolactone has recently been shown to act in concert with auxin to differentially regulate lateral root development and shoot branching in Arabidopsis, depending on the Pi level in the growth medium (Ruyter-Spira et al., 2011). Strigolactones also act as early signals between the partners in AM symbioses. When secreted by plant roots, they induce branching of hyphae growing from germinating AM fungal spores (Akiyama et al., 2005) and possibly play a similar role in arbuscule formation in cortical cells (Zhang et al., 2010), with consequent significance for the operation of the AM pathway. Furthermore, lower strigolactone synthesis in high-P plants is implicated as one factor contributing to reduced numbers of fungal appressoria and lower colonization at high P (Balzergue et al., 2011). MYC factors involved in partner recognition and the development of AM colonization appear to play an additional role in lateral root production (Maillet et al., 2011). How these signals and developmental processes interact with P nutrition has not yet been fully revealed.

Pi-starvation responses in plants are also interconnected with sugar signaling. Suc is the main photosynthate translocated between shoot and root via the phloem, and a high root-shoot ratio of Suc is required for Pi-starvation responses as well as some phytohormone changes, such as cytokinin (Hammond and White, 2008; Lei et al., 2011). Again in Arabidopsis, it has recently been proposed that Suc is a global regu-

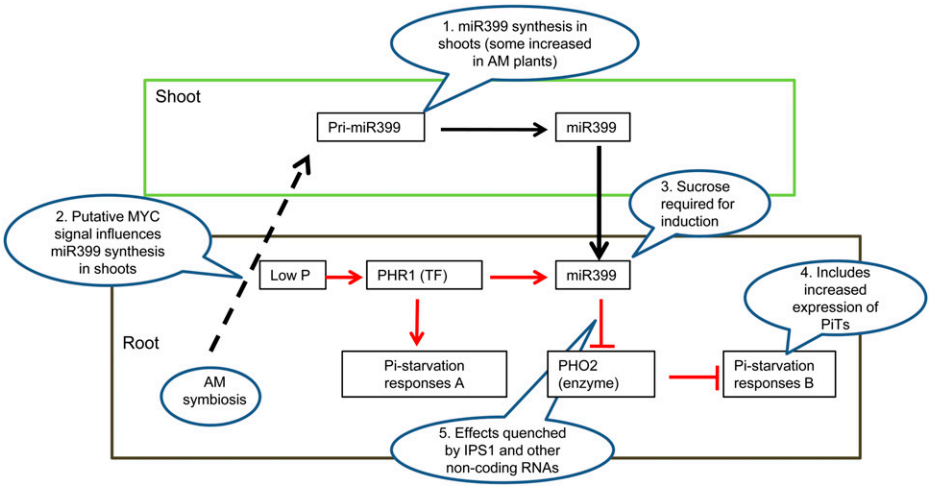

Figure 2. Possible signaling events in AM roots based on studies of Pi starvation in nonmycorrhizal plants and miR399 expression in AM medic. In NM plants, low P increases the activity of the transcription factor (TF) PHR1, which binds to the P1BS element in promoters of several Pi starvation-induced genes (A) and increases their expression. PHR1 also increases the expression of miR399s. miR399s are probably largely synthesized in shoots, where they accumulate more in AM than in NM plants (callout 1); this implies the transfer of (unknown) MYC signals from root to shoot in AM plants (callout 2). miR399s are transferred from shoots to roots. Accumulation in roots is influenced by PHR1 and by Suc transport from shoots (callout 3). High miR399 levels under low P reduce the activity of the enzyme encoded by *PHO2* and hence increase PHO2-dependent Pi-starvation responses, including increased expression of PiTs (callout 4). Effects of miR399s in reducing PHO2 activity can be quenched by noncoding RNAs such as IPS1 (callout 5). PHO2 might then inhibit Pi-starvation responses and reduce the expression of PiTs. (Modified from Branscheid et al. [2010].)

lator of a whole suite of P-starvation responses (Lei et al., 2011). Supply of Suc to the root can induce some PiTs, and high photosynthate levels are required for miR399 induction in roots at the onset of Pi deficiency in common bean (*Phaseolus vulgaris*), which is a potentially AM plant but was not colonized in this research (Liu et al., 2010). In AM plants, sugar transport to roots is increased; this might cause changes in the root-shoot ratio of Suc and could modify the outcomes of the signaling pathways.

In summary, there are many shared components between AM symbiosis and Pi-starvation signaling pathways that are interconnected with sugar and phytohormone signaling. This offers a great potential for cross talk between the direct and AM pathways, but specific regulatory elements responsible for such cross talk have not yet been identified.

CONSEQUENCES OF "HIDDEN P UPTAKE" VIA THE AM PATHWAY AND REDUCTIONS IN DIRECT UPTAKE IN WIDER CONTEXTS

Demonstration that the AM pathway plays a dominant role in plant P uptake and that the contribution of direct uptake may be reduced over the full range of plant growth and P uptake responses needs to be considered in many contexts; here, we present some examples. The "mutualism-parasitism continuum" of AM plant responses (Johnson et al., 1997), which has gained very wide acceptance, must be reevaluated. The concept was mainly based on the assumptions (now shown to be in error) that a lack of positive mycorrhizal growth response was caused by net costs of the symbiosis outweighing net benefits (i.e. at least partial failure of the delivery of P via the AM pathway) coupled with high fungal C use (fungal parasitism). The fungi can no longer be regarded simply as parasites, because the AM pathway delivers P (although in varying amounts with individual fungi), regardless of the whole plant response. Realization of their normally mutualistic rather than parasitic status should change perspectives on the function of AM symbioses and their evolutionary advantages (Smith and Smith, 2011).

High fungal C use associated with a lack of P "benefit" as the primary cause of AM growth depression should also be questioned, because reduced growth may occur with very low AM fungal biomass (Grace et al., 2009; Smith et al., 2009). We have presented an alternative scenario in which poor growth of AM plants is the result of a reduction in P delivery via the direct pathway, which is not compensated for by uptake via the AM pathway, leading to reduced total plant P uptake and P deficiency (Smith et al., 2009; Smith and Smith, 2011). This hypothesis has opened up new possibilities involving the interplay between fungal and plant processes at the levels of signaling and molecular control as well as in competition for resources in soil.

A few experiments have shed new light on the importance of the operation of the AM pathway in plant competition. Using tomato, it was shown that

AM wild-type plants had a competitive advantage over non-AM mutant plants when they were grown together with an AM fungal symbiont, even though they had similar biomass when grown singly regardless of inoculation with AM fungi (Cavagnaro et al., 2004). Subsequently, Facelli et al. (2010) used ^{32}P in a hyphal compartment to show that P uptake from soil and delivery via the AM pathway (formed by *Gigaspora margarita*) allowed the AM wild-type tomato to preempt P uptake by the non-AM mutant, despite reductions in direct uptake in the wild-type. This uptake led to a positive growth response in competition, which was not observed when the genotypes were grown alone. These results indicate that AM symbioses may have ecological benefits that cannot be predicted from AM growth responses determined for plants grown singly in pots with single AM fungal genotypes.

Another novel outcome of the interplay between the AM and direct uptake pathways is shown by the effects of AM colonization on arsenate uptake by plants. Arsenate and Pi are taken up by the same plant PiTs in the direct pathway, so that reduced uptake capacity of the direct pathway decreases arsenate as well as Pi uptake. Most evidence indicates that the AM pathway transfers little arsenic to plants, so the combination of these two effects helps to explain decreased arsenic-P ratios and increased arsenate tolerance of AM compared with NM plants (Christophersen et al., 2009), effects that are significant for crops growing on soils contaminated with arsenic.

BACK TO BASICS IN SOIL

The influence of different forms of P present in soils on the availability and uptake of P from natural and fertilizer sources remains the subject of active research (Bünemann et al., 2011). It is generally accepted that AM and NM plants access the same forms of inorganic soil P, including P that is reversibly adsorbed to various soil minerals and exchanges with the soil solution (Marschner, 1995; Frossard et al., 2011). Many AM plants can acquire more total P than NM plants from the same soil, which is thought to involve increased spatial exploitation by hyphae in soil (Marschner, 1995). The competition for P in soil between AM fungal hyphae and roots has already been raised as a possible explanation for reduced uptake via the direct pathway, but it is hard to accept in situations where plants are poorly colonized and root and hyphal length densities are low. Positive mycorrhizal growth responses can increase if poorly available P is applied to soil, even for plants that show little or no positive response at low P. This finding shows that AM plants can access poorly available P more effectively than NM plants, but the mechanisms by which they do so are not well understood (Bolan, 1991). There is some evidence that AM fungi can exploit sources of organic P in soils, but the quantitative contribution of this process to the supply of P to plants is probably small (Joner et al., 2000). Higher exploitation of poorly available soil P by AM plants is increasingly important in the contexts of understanding AM responsiveness and the utilization of poor-quality fertilizer sources. All these uncertainties require investigation if we are to understand the soil-AM plant continuum relating to P uptake.

CONCLUSION: THE "BIG PICTURE"

Phosphate rock reserves are a finite resource on which crop productivity in huge areas of P-deficient arable land now depend, globally. Phosphate fertilizer is becoming increasingly expensive, and availability is subject to political and industrial pressures in a global environment where increasing food production will be critical (Cordell et al., 2009). The vast majority of terrestrial plant species, including crops, are normally AM in field situations and therefore possess a strategy that has effectively supported plant productivity and influenced plant-plant interactions on P-deficient soils for many millions of years. Modifying the processes involved in AM function could underpin the development of crops with optimal P-uptake efficiencies. The key to identifying useful approaches is to understand the processes that operate in AM plants and not to rely on entrenched paradigms that are now shown to be inadequate. New information on the contributions of and interplay between the two pathways of P uptake in AM plants (direct and AM), gained by a combination of plant physiological and molecular approaches, has opened up new perspectives on symbiotic AM function, including the potentially deleterious reductions in P delivery via the direct pathway and hence the possibility of modification to increase agricultural plant productivity.

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