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Regulation of plants' phosphate uptake in common mycorrhizal networks: Role of intraradical fungal phosphate transporters

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ABSTRACT

We have recently identified two genes coding for inorganic phosphate transporters (Pht) in sorghum (*Sorghum bicolor*) and flax (*Linum usitatissimum*) that were induced in roots colonized by arbuscular mycorrhizal (AM) fungi. Mycorrhizal acquisition of inorganic phosphorus (Pi) was strongly affected by the combination of plant and AM fungal species, but the expression level of these genes coding for AM-inducible Pi transporters did not explain differences in plant phosphorus acquisition where flax and sorghum are sharing a common mycorrhizal network. In the present study, we investigated the possible role of fungal Pi transporters in the regulation of mycorrhizal Pi acquisition by measuring their expression in roots of flax and sorghum. One Pi transporter of *Rhizophagus irregularis* (*RiPT5*) showed a positive correlation with mycorrhizal Pi acquisition of sorghum. This indicates that a possible involvement in the regulation of mycorrhizal Pi acquisition. In general, expression of AMF Pi transporters was more related to mycorrhizal Pi acquisition of sorghum than of flax, indicating plant species-specific differences in the regulation of mycorrhizal Pi acquisition.

KEYWORDS

Arbuscular mycorrhizal fungi; common mycorrhizal networks; fungal inorganic phosphate transporters; phosphate uptake; resource exchange

The majority of plants is associated with arbuscular mycorrhizal fungi (AMF) with which they trade substantial amounts of photosynthetically-fixed carbon in exchange for mineral soil nutrients.[1] AMF colonize simultaneously several plants from the same or different species,[2] and form thereby far-reaching common mycorrhizal networks (CMNs). We are particularly interested in resource exchange within such complex CMNs. Therefore, we established a model system consisting of 2 different host plant species, flax and sorghum, sharing a common fungal symbiont to investigate experimentally the nutrient exchange in mycorrhizal networks.[3] With the help this model system, we revealed that resources are exchanged under unequal terms of trade in CMNs. Depending on the fungal symbiont, flax was able to acquire the lion's share of nutrients delivered by a CMN, while sorghum received only marginal nutritional benefits, although contributing most of the carbon allocated to the common fungal partner.[3] More recently, we tried to shed light on the different abilities of flax and sorghum to acquire nutrients from CMNs depending on the identity of the fungal symbiont by focusing on the model plant's phosphate acquisition pathways.[4]

Plants associated with AMF exhibit a specific symbiotic phosphate uptake pathway, which begins at the extraradical hyphae in the soil, from where inorganic phosphate (Pi) is translocated toward the roots and released from the arbuscule into the periarbuscular space.[5] There, it is taken up across the plant's periarbuscular membrane by specifically induced phosphate transporters belonging to the Pht1 family of plant Pi transporter.[6] Remarkably, these mycorrhiza-induced phosphate transporters have been found to be crucial for mycorrhizal Pi acquisition in several studies using mutants with reduced or inhibited mycorrhiza-inducible transporter gene expression.[7,8] In order to study the mycorrhiza-induced *Pht1* genes in flax and sorghum, we characterized and analyzed the expression of Pi transporters of the Pht1 family in both plant species, and identified a set of mycorrhiza-inducible Pi transporters in both plants.[4] Although mycorrhizal Pi acquisition was strongly affected by AMF species in our model system, a corresponding change in the expression of mycorrhiza-inducible Pht1 transporters was only marginally detected. Besides Pi uptake across the plant's periarbuscular membrane, the release of Pi from AMF into the periarbuscular space is another crucial step of mycorrhizal Pi pathway. However, mechanisms of Pi release from fungus to the periarbuscular space are less-well studied and remain obscure up to now.[5]

In order to reveal the role of fungal Pi transporter in mycorrhizal Pi pathway, expression of several fungal Pi transporters genes of *Rhizophagus irregularis* (strain TERI commercial) and *Funeliformis mosseae* (strain ISCB 22, both kept in our fungal strain collection[9]) were investigated in roots of flax and sorghum of the model system used in previously published studies[3,4] (Table S1 and S2). We designed gene expression assays for different Pi transporters of *F. mosseae* (*FmPT1, FmPT3, FmPT7*) and *R. irregularis* (*RiPT1, RiPT3, RiPT5, RiPT7*). The

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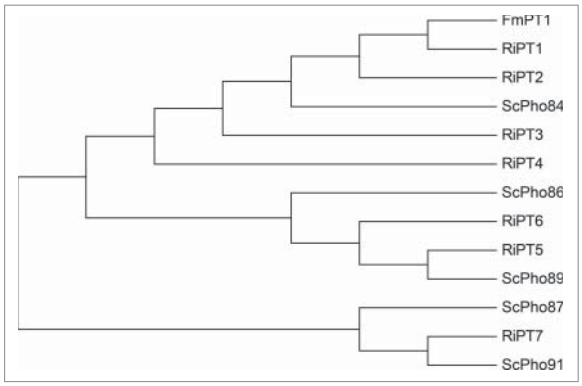

Figure 1. Neighbor joining tree of the *Rhizophagus irregularis* phosphate transporter (PT) family, based on the amino acid sequences of their full open reading frames. Sequence names consist of species code (first letter of genus and first letter of species name) and the PT number. Species codes; Fm: *Funelliformis mosseae*; Ri: *Rhizophagus irregularis*; Sc: *Saccharomyces cerevisiae*. Sequence names of *R. irregularis* correspond to PT characterized in Table S1. Sequence names of *F. mosseae* and *S. cerevisiae* were obtained from NCBI GenBank: ScPho84 (NP_013583), ScPho86 (NP_012418), ScPho87 (NP_009966), ScPho89 (NP_009855), ScPho91 (NP_014410) and FmPT1 (AAZ22389). We also provide GenBank accession numbers for sequences from *R. irregularis*: RiPT1 (KU219928), RiPT2 (KU219929), RiPT3 (KU219930), RiPT4 (KU219931), RiPT5 (KU219932), RiPT6 (KU219933) and RiPT7 (KU219934). For phylogenetic analysis, the PT amino acid sequences were aligned with ClustalW (http://www.ebi.ac.uk/Tools/msa/clustalw2/) using the following multiple alignment parameters: gap opening penalty 15, gap extension penalty 0.3, and delay divergent sequences set to 25%; and the Gonnet series was selected as the protein weight matrix. Neighbor joining trees were constructed using Poisson correction model for distance computation in MEGA4.[17]

targeted genes were homologs of transporters of *Saccharomyces cerevisiae* encoding for high- (*ScPHO84, PHO89*) and low- (*ScPHO87, PHO91*) affinity Pi transporters located in the plasma membrane[10] (Fig. 1).

Expression of fungal Pi transporters in roots of flax and sorghum were affected by different plant species and different culture systems (Fig. 2). The two Pi transporters of *R. irregularis RiPT3* and *RiPT7* were significantly more expressed in sorghum roots than in flax roots over both culture treatments (Fig. 2a). In contrast, *RiPT1* and *RiPT5* were more expressed in flax roots. Contradictory behaved the expression of Pi transporter genes of *F. mosseae*; the three transporters investigated were significantly stronger expressed in flax roots over both cultural treatments (Fig. 2b). Culture treatments mainly affected the expression of Pi transporters of *R. irregularis* in sorghum roots. *RiPT1, RiPT3 and RiPT7* were significantly more expressed in sorghum roots of mixed culture compared to the monoculture treatment (Fig. 2a). On the contrary, the expression of *RiPT5* in roots of mixed culture was dramatically reduced compared to the monoculture treatment. The expression of 4 fungal Pi transporters showed a high interrelation with mycorrhizal Pi acquisition in association with sorghum (Fig. 3). Remarkably, *RiPT5* was the only transporter showing a clear tendency for a positive correlation between mycorrhizal Pi acquisition and expression level (Fig. 3; Table S3). *FmPT3* and *FmPT7* showed a significant negative correlation with mycorrhizal Pi

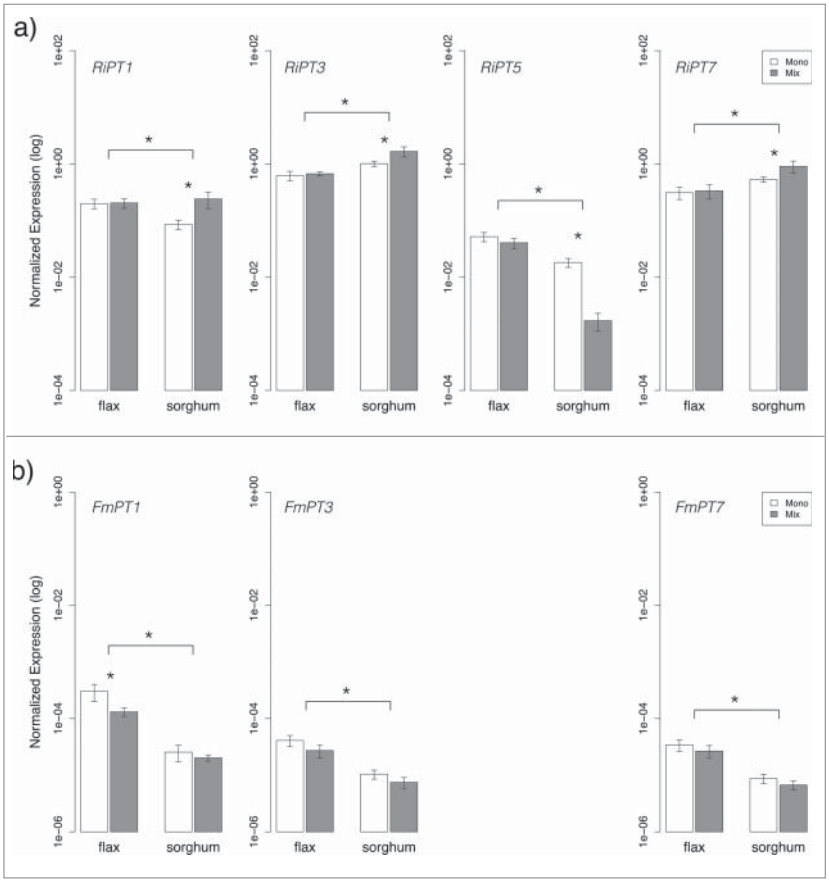

Figure 2. Normalized expression of phosphate (Pi) transporters of *Rhizophagus irregularis* (a) and *Funelliformis mosseae* (b) in roots of flax or sorghum. Plants were cultivated either in monoculture accompanied by a plant individual of the same species (white bars) or in mixed culture accompanied by a plant individual of the other species (gray bars). Translation elongation factor from the fungus (TEF-1 α) was used as the reference transcript. Sequences of primers are given in table S2. Error bars show standard errors (N = 4). Stars indicate significant differences between plant species over both culture treatments, or among culture treatments within plants according to 2-way t-test ($p < 0.05$).

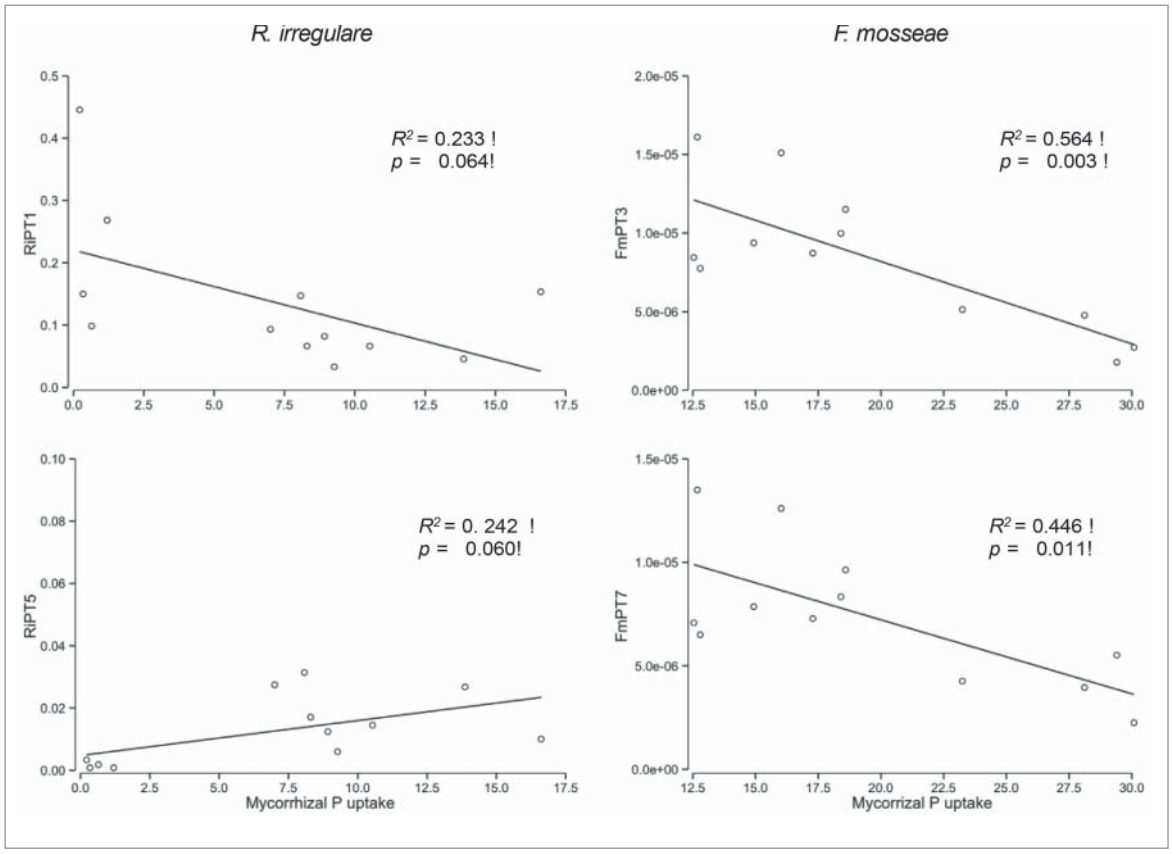

Figure 3. Relation between fungal Pi transporter expression in sorghum roots and mycorrhizal Pi acquisition. Normalized expression of 4 different Pi transporter of *Rhizophagus irregularis* and *Funnelliformis mossea* is shown as function of mycorrhizal Pi acquisition. Translation elongation factor from the fungus (TEF-1 α) was used as the reference transcript. Mycorrhizal acquisition of inorganic phosphate of sorghum was estimated based on uptake of labeled phosphorus only available for AM fungi.[3,4] Twelve data points were used for calculation. Coefficient of determination (R^2) and p-value of linear regression model are displayed for each interaction.

acquisition of sorghum. Generally speaking, gene expression of fungal transporters in sorghum roots was much stronger related to mycorrhizal Pi acquisition (mean $R^2 = 0.259$), while in flax roots no such relation, whether positive nor negative, could be detected (mean $R^2 = -0.03$; Table S1).

RiPT1, RiPT2, RiPT3 and FmPT1 cluster with ScPHO84, a high affinity Pi:H^+ symporter from *S. cerevisiae* (Fig. 1). RiPT1 and RiPT3 were suggested to have different functional characteristics (i.e. different affinities for Pi and/or to different regulation patterns of gene expression with Pi availability; Fig. 1).[11] RiPT7 clusters with ScPHO87 from *S. cerevisiae*, a low-affinity Pi:H^+ transporter.[10,11] The most promising candidate of AMF Pi transporters involved in intraradical Pi transfer into periarbuscular space is *RiPT5*, which clusters with the high affinity Pi:Na^+ transporter ScPHO89 of *S. cerevisieae* (Fig. 1).[11] The exact role of the putative Pi:Na^+ transporters in AMF is not yet clarified. But AMF possess an extensive tubular vacuoles system involved in the translocation of polyphosphate along hyphae.[12] The transporter system operating the Pi exchange between the tubular vacuoles system and the cytoplasm is not known so far, but the Pi:Na^+ transporter system could be involved and release Pi depending on the plant demand at the plant fungal interface. Nevertheless, its expression was strongly reduced in roots of sorghum cultivated in mixed culture with flax. In this treatment, the mycorrhizal Pi acquisition of sorghum was much lower compared to the other treatments (for details see[3,4]). Additionally, *RiPT5* was the only fungal transporter exhibiting a positive correlation with mycorrhizal Pi acquisition of sorghum (Fig. 3; Table S3). Hence, there is room for speculation that *RiPT5* could be a significant transporter for intraradical Pi transfer from the AMF to the plant at the plant-fungal interface. It is unfortunate that the homologous transporter of *F. mosseae* (*FmPT5*) was not surveyed in this study. A similar correlation between mycorrhizal Pi acquisition and expression of *FmPT5* in sorghum roots could strengthen the importance of these homologous transporters for the intraradical mycorrhizal Pi transfer from AMF to plants. However, expression of *RiPT5* was not affected in flax roots, although flax increased its mycorrhizal Pi acquisition twofold from mono- to mixed culture (for details see[3,4]). Similarly, expression of fungal Pi transporters was generally more related to mycorrhizal Pi acquisition of sorghum compared to flax (Table S3). We could speculate that dependent on plant species, mycorrhizal Pi acquisition could be either more regulated by the fungus or by the plant.

Fungal regulation of mycorrhizal Pi acquisition may take place by a suppressed expression of such Pi transporters responsible for intraradical Pi transfer. This would reduce the amount of Pi in peri-arbuscular space and consequently reduce the amount of Pi available for sorghum *via* the mycorrhizal pathway. But why would AMF reduce Pi transfer to the periarbuscular space? It is generally accepted that some plants and AMF are able to reduce resource transfer to symbiotic partners, when not receiving a "satisfying" benefit in return.[13,14] In the

present study sorghum was the main carbon provider to AMF and consequently Pi transfer from AMF to sorghum should therefore rather be increase under the assumption of such market dynamics.[15] However, transcriptional regulation of fungal Pi transporters could also inhibit the re-uptake of Pi by the fungal cells, leaving Pi in the periarbuscular space and consequently remaining available for the plant. A rather speculative explanation could be that the expression of plant and fungal Pi transporters at the arbuscule level could be mediated by complex interactions between the 3 partners (sorghum, flax, AM fungal species), independently of the availability of Pi.[16] A detailed analysis of the *R. irregularis* and *F. mosseae* phosphate transporters expression would be suitable to clarify this incomplete, but first picture.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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