Review

Take a Trip Through the Plant and Fungal Transportome of Mycorrhiza

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Soil nutrient acquisition and exchanges through symbiotic plant–fungus interactions in the rhizosphere are key features for the current agricultural and environmental challenges. Improved crop yield and plant mineral nutrition through a fungal symbiont has been widely described. In return, the host plant supplies carbon substrates to its fungal partner. We review here recent progress on molecular players of membrane transport involved in nutritional exchanges between mycorrhizal plants and fungi. We cover the transportome, from the transport proteins involved in sugar fluxes from plants towards fungi, to the uptake from the soil and exchange of nitrogen, phosphate, potassium, sulfate, and water. Together, these advances in the comprehension of the mycorrhizal transportome will help in developing the future engineering of new agro-ecological systems.

The Mycorrhizal Trade of Nutrients

Mycorrhizal systems are characterized by nutritional exchanges between plant and fungal partners. The fungus supplies the autotrophic host with mineral nutrients and water, thereby promoting plant growth. In return, the host plant provides sugar photosynthates to its fungal partner. We review here current knowledge on the systems involved in carbon (C), nitrogen (N), phosphate (P), potassium (K), and sulfur (S) transport, as well as water acquisition and exchange in the two major mycorrhizal associations of the rhizosphere: arbuscular mycorrhiza (AM, see Glossary) (Figure 1) and ectomycorrhiza (ECM) (Figure 2). These mutual exchanges, ensuring benefits for both partners, primarily take place at the symbiotic interface between the plant and the fungus, termed arbuscules and the Hartig net in AM and ECM symbioses, respectively (Figures 1B and 2B).

We also report the recent progress in mycorrhizal ‘omics’ programs of the plant–fungal transportome (Box 1). Finally, we discuss the engineering of mycorrhizal systems through key transporter candidates as a potential improvement of plant fertilization for optimized nutrient use efficiency, yielding significant benefits to agriculture, energy, and the environment (Box 2).

Trends

Plant growth and development are highly dependent on rhizosphere nutrient availability which is often a limiting factor. This constraint has forced land plants to evolve various strategies, including beneficial interactions with soil microorganisms.

The symbiotic interactions between plant roots and fungi, termed mycorrhizal symbioses, provide reciprocal benefits for both partners, as for instance for the plant partner the acquisition of nitrogen (N), phosphate (P), potassium (K), and sulfate (S), the primary macronutrients used in plant fertilizer.

Plant and fungal transport systems display ‘mycorrhiza-specific’ and ‘fine-tuning’ regulation to control nutrient fluxes towards the symbiotic interface, delimiting the site of reciprocal nutrient exchanges between the partners.

The selection and engineering of mycorrhizal partners based on the plant and fungal transportome, targeting the key transporters resulting from the massive generation and analysis of ‘omics’ data, will ensure agro-ecological improvement of crop nutrition.

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Carbon Flux and Transport in Mycorrhizal Associations
From Source Leaves to Mycorrhizal Roots

In mycorrhizal associations, the plant provides the heterotrophic symbiont with sugar from photosynthetic reactions. These sugar fluxes towards the rhizosphere are finely regulated by both plant and fungal transport components [3].

Sucrose is synthesized in the mesophyll, loaded into the phloem, unloaded towards roots, and cleaved into glucose and fructose to supply underground tissues. Mycorrhizal colonization of plant roots increases the sink strength and creates an additional sugar demand for the plant. Thus, a large proportion of photoassimilates are redirected towards the colonized rhizosphere. In addition, the C supplied towards the symbiont may also come from sugar reserves stored in cellular compartments. Fungal colonization thus affects root starch dynamics, but recent studies suggest that the fungus is primarily fed by soluble sugars stemming from sucrose transported towards the root [4]. These sugar fluxes are coordinated by transport systems, comprising sucrose transporters (SUTs), monosaccharide transporters (MSTs), and the SWEET (sugars will eventually be exported transporter) family [3].

All plants have a small family of SUTs, classified into four types [5], that represent key players for long-distance transport of sugar from shoot to root. Sucrose export towards colonized roots involves fine and specific regulation of sucrose transporters. For instance, the expression of all SUT types is regulated in leaves and colonized roots of Medicago truncatula and tomato when inoculated with AM fungi [6,7]. Until now, a single sut1 knockdown mutant with impaired sucrose export from potato leaves has been investigated in AM plants, and this had no effect on fungal colonization rates [8]. This result suggests that sucrose export from source leaves by SUT is not the bottleneck of the mycorrhiza-driven sink. Nevertheless, once sucrose reaches the AM root, some specific SUT2 type II transporters seem to retrieve sugars back towards the plant cells [9], suggesting a mechanism of reverse transport towards cortical cells to control symbiotic fungal growth. However, this mechanism controlling the diversion of plant sugar resources by other microorganisms seems to be bypassed through the recently identified SWEET transporters (see below).

In sink organs, transport of monosaccharides resulting from the sucrose cleavage by plant invertases and starch degradation is mediated by a large family of MSTs, classified into seven clades [10,11]. Because the C source mainly taken up by the fungus seems to be glucose, most studies have focused on the expression of plant MSTs to pinpoint differentially regulated candidates depending on fungal species and plant varieties [12–14]. Interestingly, such MSTs (MtSt1 and MtHext1) can also be differentially regulated in non-colonized cells and neighboring arbuscular cells, suggesting that sugar flow from non-colonized adjacent cells might feed the arbuscular cells through plasmodesmata [13,15]. As opposed to the phosphate transport components (see below), no mycorrhiza-specific activation of a candidate MST/SUT has been discovered from genome-wide array studies.

Thus, the newly identified SWEET sugars will eventually be exported transporters family, recently characterized as exporters of both sucrose and monosaccharides using nanosensors [16], may be the key components regulating the diversion of plant sugar resources in symbiotic and pathogenic interactions [3,17–19]. This discovery of sugar efflux transporters is a major breakthrough in plant biology, and a SWEET candidate may regulate the export towards the plant–fungus interface (Figures 1C and 2C). Recently, the SWEET family of potato was characterized and several candidates showed differential regulation of their expressions in response to AM fungi [20]. Thus, engineering the SWEET export components represent a very promising tool in agronomy to optimize carbon export towards the rhizosphere (Box 2).
Sugar Uptake by Fungal Partners at the Symbiotic Interface

It is now commonly accepted that glucose is the major form of C taken up at the plant–fungus interface [21,22]. However, the detailed transport proteins involved in the symbiotic efflux from plant cells towards the interface are still unknown. Here again, SWEETs may be interesting candidates because the putative exporter genes, StSWEET2c, StSWEET7a, and StSWEET12a, showed localized transcriptional induction in arbuscule-containing cells [20]. In addition, it was suggested that the fungus might also be able to take up residues from the plant cell wall (e.g., xyllose) that are present both at the arbuscular interface and when the intercellular hyphae colonize the roots [22,23]. These recent views also suggest that cell-wall monosaccharides may represent a secondary source of C, and suggest new avenues for engineering axenic culture of the fungus (without the host plant).

On the fungal side, monosaccharide transporters were recently identified from ECM [21,24–27] and AM [22,28] fungi (Table 1). Within each species, distinct MST proteins seem to be responsible for sugar uptake at the symbiotic interfaces, from the soil in the growing mycelium and for sugar partitioning within internal fungal structures (Figures 1C and 2C). In AM, RtMST2 from *Rhizophagus irregularis* is primarily expressed in symbiotic intra-radical structures, and its silencing resulted in impaired formation, malformed arbuscules, and reduced phosphate transporter (MtPT4) expression [22]. RtMST2 is so far the only transporter shown to directly mediate sugar uptake at mycorrhizal interfaces. Finally, a putative sucrose transporter (RiSUC1) has been identified in AMF [22], but no clear evidence of sucrose transfer has yet been observed in AM- and ECM.

Recent progress in ‘omics’ technologies has enabled the identification of complete sugar transporter families, particularly in model ECM fungi [26,27,29] (Box 1). Here again, the identification of the key candidates regulating C allocation at symbiotic interfaces for the concurrent development of the mycorrhiza represents a timely agro-ecological challenge for optimizing crop yield through microbial interactions in the rhizosphere.

Fungal Nitrogen Absorption and Transport in Mycorrhizal Associations

Nitrate Uptake and Allocation in Arbuscular Mycorrhiza and Ectomycorrhiza

Nitrate (NO₃⁻) is the most important source of N in soil solutions, and is more mobile than ammonium (NH₄⁺). However, owing to the high energy demand imposed by the reduction of NO₃⁻ to NH₄⁺, microbes often prefer the direct assimilation of NH₄⁺ [30].

Nitrate passes the plasma membranes through specific and highly regulated transporters via an energy-dependent uptake process. In plants, large families of nitrate transporters (NRT) and peptide transporters (PTR) have been described that belong to the NPF (NRT1/PTR) [31], NRT2, and NRT3 families [32]. Some of the plant NRTs are induced by the presence of the fungus in both AM- and ECM-colonized roots [33–35], but are also induced in response to high phosphate or low nitrate concentrations [35,36]. This complex gene expression modulation suggests a mechanism of NO₃⁻ acquisition depending on the plant and fungal nutritional status as well as crosstalk with other nutrients. However, the NRT1/PTR families in land plants comprise more than 50 members and contain members with distinct activities (i.e., gluconolactone and phytohormone transport [37–41]) that could be regulated under mycorrhizal conditions.

Mycorrhizal fungi only possess a limited number of high-affinity transporters belonging to the NRT2 family (Figures 1C and 2C). One or two NRT2 genes were identified in the genomes of *Laccaria bicolor* [42,43], *Hebeloma cylindrosporum* [44,45], and *R. irregularis* [46,47]. Interestingly, NRT2 from *Tuber borchii* was found to be strongly expressed in the Hartig net and mantles, but weakly expressed in free-living mycelia [48]. In *R. irregularis*, NRT2 is expressed both in the intra- (IRM) and extra-radical mycelium (ERM), but is only regulated by the N source in the ERM.
Thereby, such NRT candidates indicated that the plant and the fungus compete for the nitrate present in the symbiotic interface, and represent interesting tools for optimizing fertilization of mycorrhizal crops. It is also possible that fungal NRTs might be involved in bidirectional transfers, as described for several plant NRTs [31].

**Plant and Fungal Ammonium Transporters in Mycorrhizal Systems**

Phylogenetic analyses on plant ammonium transporters (AMT) have revealed four clades, AMT1–4 [49]. Some AMTs are induced during mycorrhizal colonization in AM plants: for instance...
in Lotus japonicus [33], Glycine max [50], Oryza sativa [51], M. truncatula [52], or Sorghum bicolor [53], and also in ECM trees: Populus tremula x tremuloides [54] and Pinus pinaster [55] (Table 1 and Figures 1C and 2C). AM-inducible AMTs are present in all clades, and some were detected on the branch domain of periarbuscular membranes, indicating that active NH$_4^+$ transfer occurs around arbuscular branches by recruiting NH$_4^+$ in the acidic periarbuscular space and releasing the uncharged NH$_3$ into the cytoplasm of the arbusculated cells [33,50,53]. Thus, protons coming from the deprotonation process remain at the symbiotic interface, and could facilitate the transport of other nutrients through H$^+$-dependent processes. It is worthwhile

Figure 2. Plant and Fungal Transportome in the Ectomycorrhiza (ECM). (A) A 2 month old ECM between Pinus pinaster and Hebeloma cylindrosporum. (B) Cross-section of Pinus pinaster–Hebeloma cylindrosporum ECM showing extra-radical hyphae, the fungal mantle, and the Hartig net where nutrient exchanges take place. (C) To acquire nutrients and water from the soil, ECM fungi express a diverse set of transport systems in extra-radical hyphae: AMT, NTR, PTR, OPT, and AAT families are involved in nitrogen (N) uptake; H$^+$/Pi and Na$^+$/Pi phosphotransporters (PTs) in phosphate (P) uptake; MIP in water ($H_2$O) uptake; and Trk and possibly HAK in potassium (K) uptake. Carbon (C), provided mainly by the host plant, is taken up by ECM fungi not only at the symbiotic interface but also from the soil by MST proteins. Nutrient release towards the host plant involves AQP1 aquaporins for water. One NRT and some AMTs are expressed in the Hartig net, suggesting that they might be involved in N release or retrieval at the symbiotic interface, respectively. However, no P, K, or S transport proteins have been identified in fungal Hartig net so far, although some candidates are hypothesized. On the plant side, AMT members participate in fungal N uptake, Pht1 in P uptake, and PIP in water uptake. Expression revealed that plant SKOR and SULTR proteins are possibly involved in K and S uptake from the fungus, respectively. The transport of C from plant to fungal cells within the Hartig net involves plant SUT and possibly MST and SWEET proteins. Unbroken lines indicate that the expression, localization, and/or function of at least one member of the family has been described in symbiotic context. Broken lines indicate that the expression, localization, and function of all members of the family are still unknown in a symbiotic context, even if candidates are hypothesized in some cases. Abbreviations: e.c., epidermal cells; c.c., cortical cells; c.cyl., central cylinder.
To note that the AM-inducible MtAMT2;3 (unable to complement a yeast transport mutant) may have a specific sensing/signaling function, as opposed to the other AM-inducible transporters [50,52,53].

**Low- and high-affinity** AMTs have been characterized and were found to be regulated by N sources in *H. cylindrosporum* and *T. borchii* [56,57]. Differential expression in the Hartig net or mantle compared to free-living mycelia was observed in *Amanita muscaria* and *Tuber melanosporum* [58,59] (Figure 2C). Concerning AM fungi, their ability and efficiency to take up $\text{NH}_4^+$ and to transfer N to their host plants seem to depend on the AM fungal species [60]. Three high-affinity AMTs were identified in *R. irregularis* (GintAMT1 [61], GintAMT2 [62], GintAMT3 [63]). GintAMT1 is expressed in the extra-radical mycelium, suggesting a role in $\text{NH}_4^+$ acquisition from the soil, while GintAMT2 might be involved in retrieving leaked $\text{NH}_4^+$, as observed in yeast.

The mechanism underlying $\text{NH}_4^+$ export from the fungal cell into the apoplastic interface is still unknown (Figures 1C and 2C). A fungal aquaporin in the ECM fungus *L. bicolor* [64], and homologs of a putative $\text{NH}_4^+$ export protein of *Saccharomyces cerevisiae* (*Ato3*) identified in ECM symbiosis (*A. muscaria* [54] and *L. bicolor* [42]) and AM symbiosis (two genes in *R. irregularis* genome; JGI protein ID: 347857, 28309), represent potential candidates for nutrient export at the symbiotic interface.
The improvement of amino acid transfer in ECM roots may be due to the transport of glycine, glutamate, and allantoin from the fungal symbionts [67]. Some fungal amino acid transporters (AAT) with high affinity for basic amino acids and lower affinity for neutral and acidic amino acids were functionally characterized in A. muscaria [68] and H. cylindrosporum [69]. Some AATs, identified in the L. bicolor genome, appear to be expressed in colonized root tips [42]. One AA
permease was characterized in *Funneliformis mosseae* [70], and many putative AA transporter genes were found to be highly expressed in the ERM and IIRM of *R. irregularis* [71]. Although the transport of N has been well studied in both AM and ECM associations, several key elements are still missing (e.g., characterization of oligopeptide transporters), particularly at symbiotic interfaces (Figures 1C and 2C).

**Fungal Phosphate Absorption and Transport in Mycorrhizal Association**

Phosphorus is primarily present in soil as inorganic P (Pi) derived from bedrock and is predominantly chemically bound to the surface of clay minerals. The high immobility of soluble Pi present as free orthophosphate, in combination with rapid absorption, results in the development of a depletion zone around plant roots [72]. Among strategies developed by plants, the extent of rhizosphere coverage through mycorrhizal fungal hyphae increases the soil volume explored and can overcome the limited P availability [73].

**Regulation of Plant Phosphate Transporters in Mycorrhiza**

Uptake of the negatively charged orthophosphate requires an energy-driven transport process mediated by phosphate transporters and energized by H⁺-ATPases [74,75]. The plant Pi transporters (Pht) are classified into three families, Pht1, Pht2, and Pht3 [76]. Only Pht1 low- and high-affinity H⁺/Pi symporters were described to be involved in the mycorrhizal pathway so far [77]. Most Pht1 genes are strongly expressed in the root epidermal cells under P deficiency, including root hairs and cortical cells, suggesting a role in Pi uptake [78,79]. The mycorrhiza-specific Pi transporters all belong to the family of Pht1 transporters, but cluster in two different subgroups, respectively named subfamilies I and III [80]. Most members of subfamily I are only expressed in arbuscule-containing cortical cells during AM symbiosis [77,81]. AM-induced Pht1 genes of subfamily III are more broadly expressed in plant roots, but are specifically induced in cortical cells during AM symbiosis [82–90] (see detailed list in Table 1). Interestingly, the mycorrhiza-specific induction of Pht1 transporter genes is conserved between perennial woody and herbaceous plant species [91] (Figures 1 and 2). Remarkably, these Pi transporters are not only specifically induced in mycorrhiza but have also been found, in several studies using mutants with reduced [83] or inhibited AM-inducible Pht1 gene expression, to be crucial for mycorrhizal Pi uptake because the development of symbiosis was affected in these mutants (*OsPT11* or *OsPT13* in rice [92]; PT4 in *M. truncatula* [81]). Two or three mycorrhiza-specific Pht1 transporters have been described in plants, indicating the presence of two or three Pi transport systems, suggesting functional redundancy. They probably also differ in affinity for Pi. In rice, where two mycorrhiza-specific Pht1 transporters were described, symbiotic Pi uptake is mediated by a single functional Pi transporter, PT11 [92]. In *M. truncatula*, where one AM-inducible transporter is reported, the development of symbiosis affected by mutation of MtPT4 could be restored depending on the presence of the ammonium transporter MtAMT2;3 and N status, showing an interdependence of plant N and P status [52].

The upregulation of AM-inducible plant Pi transporters is often accompanied by the down-regulation of other Pi transporters, in particular those thought to be involved in direct Pi uptake [87]. This interplay between Pht1 transporters may reflect the balance between direct and mycorrhizal pathways for Pi uptake [76]. However, it is still not clear whether the downregulation results indirectly from the improvement of Pi acquisition or from a direct plant response to symbiosis. The regulation of the numerous Pi transporters is probably dependent on still unknown post-transcriptional and post-translational regulations [93].

**Phosphate Transporters in Mycorrhizal Fungi**

In AM fungi, proton- and sodium-coupled (H⁺/Pi and Na⁺/Pi) transporters have been described that enable Pi uptake in a wide soil pH range [94] (Figure 1Q). For instance, homologs of
S. cerevisiae PHO84 were described in extra-radical hyphae of *Glomus versiforme* and *R. irregularis* [95,96], and putative Na⁺/Pi symporters have also been identified *in silico* in *R. irregularis*, *F. mosseae*, and *Rhizophagus clarus* transcriptomes [71,97,98]. RIPT5, which clusters with the high-affinity Na⁺/Pi transporter, was the only fungal transporter exhibiting a positive correlation with mycorrhizal Pi acquisition of sorghum. One could speculate that RIPT5 could be a significant transporter (exporter) for intra-radical Pi transfer from the AMF to the plant at the plant–fungus interface. Unfortunately, the lack of tools for genetic manipulation of AM fungi constitutes a bottleneck for the full understanding of fungal Pi transporter function in mycorrhizal systems.

In ECM fungi, several genes putatively encoding Pi transporters have been identified [1,99]. Most of these transporters are H⁺/Pi transporters, suggesting that the efficiency of fungal Pi uptake strongly relies on external pH values (Figure 1C). The two H⁺/Pi transporters, found initially in extra-radical hyphae of *H. cylindrosporum*, could mediate Pi uptake when soil P availability was low (HcPT1.1) or high (HcPT2) [100,101]. Similarly to HcPT1.1, upregulation by low Pi has been found for other H⁺/Pi transporters in *Tricholoma* spp. [102], *Boletus edulis* [103], *Rhizopogon luteolus* [104], and *Leucocortinarius bulbiger* [104]. The Na⁺/Pi transporter TmPT3 from *T. melanosporum* might be related to alkaline soils requiring Pi uptake independent of proton gradients [1]. However, the molecular mechanisms underlying fungal Pi secretion towards the plant root cells remain uncertain [105].

### Fungal Potassium Absorption and Transport in Mycorrhizal Association

Although an improvement of K content in plants colonized by AM and ECM fungi was recognized, the effective involvement of mycorrhizal symbioses in plant K nutrition is still under debate owing to the limited availability of data [106,107]. Recent progress suggests that mycorrhizal fungi have a positive effect on plant K acquisition, mainly under K-limited and stress conditions [108–110], and this may also be the case in most natural ecosystems.

### Potassium Acquisition in Mycorrhizal Fungi

In fungi, two highly conserved families of K transporters have been identified, the Trk (transporter of K) and HAK (high-affinity K uptake) transporters [111] (Figures 1C and 2C). In addition to their function in K acquisition, some Trks can also transport sodium cations, suggesting their involvement in either sodium nutrition or fungal cell detoxification [112,113]. Interestingly, although at least one Trk is present in all sequenced ECM fungi, none can be found in the genomic database of the AM fungus *R. irregularis* [107]. However, one HAK transporter was detected in *R. irregularis*, suggesting that HAKs are probably the main players in K uptake by AM fungi. Both the sequencing of other Glomeromycota species and further characterization of fungal HAKs will bring valuable information on the K acquisition strategies used by AM fungi [107]. In the ECM fungus *H. cylindrosporum*, overexpression of one Trk member resulted in a reduction of both K and P contents in shoots at low K, indicating a less-cooperative behavior of the fungus for plant K nutrition and providing further evidence of P–K crosstalk in mycorrhizal systems [109].

### Potassium Translocation from Mycorrhizal Fungi to Host Plants–A Missing Link

Because it is strongly inferred that K transfer from the fungus to the host plant needs polarized expression of transport systems [1], we can assume that fungal K exporters are specifically expressed, localized, and regulated at the mycorrhizal interface. Two types of ion channels, known as SKC (shaker-like K channel) and TOK (tandem-pore outward K channel), are good candidates to play this role in mycorrhizal fungi [107] (Figures 1C and 2C). Most species belonging to the Agaricomycotina and basal fungi possess at least one SKC gene. Surprisingly, two putative SKC genes were detected in *R. irregularis* but none in Ascomycota. The specific loss of SKC genes in Ascomycota, together with the lack of functional activity of SKC candidates
from ectomycorrhizal fungi in heterologous systems, raises questions on their effective role in K transport in fungi (K. Garcia, unpublished results). Therefore, TOK channels are promising candidates for fungal K release into the plant during ECM association, even if they were not identified in *R. irregularis* [107]. A major effort will now be necessary to decipher the complete molecular basis of K mobilization, translocation, and release to the host in mycorrhizal fungi.

Plant K transport systems are well described but no molecular data are available concerning their role at the plant–fungus interface. Interestingly, a putative K uptake (KUP) transporter was 44-fold upregulated in AM-colonized roots of *L. japonicus* [33]. The lack of data on the possible role of plant K transport systems in AM and ECM associations reveals the need for further studies to unravel the molecular players in such plant–microbe interactions, especially under stress conditions.

**Fungal Sulfate Absorption and Transport in Mycorrhizal Association**

S is usually taken up by plants as sulfate to synthesize various molecules essential to sustain cell growth and viability [114]. Nutritional benefits of symbiotic interactions might help the plant in case of S deficiency: S as sulfate and as organic S-containing compounds (cysteine, methionine, and glutathione) can be transported via the mycorrhizal pathway from the fungus to the plant [115]. As an example, we identified three and five sulfate permeases in *R. irregularis* and *L. bicolor* genomic databases, respectively (JGI protein ID *R. irregularis*: 349405, 5057, 21574; *L. bicolor*: 186401, 232756, 698697, 447269, 308283), and two and three putative methionine permeases, respectively (JGI protein ID *R. irregularis*: 27643, 11950; *L. bicolor*: 250926, 166470, 298666). S affects AM fungal community structures in roots of different soybean cultivars [116]. However, even if S is a key component of plant metabolic pathways, data are scarce about the physiological impact of S transfer during AM symbiosis because S deficit is less frequent in agricultural soils. Under S starvation, four putative sulfate transporters (SULTRs) of *M. truncatula* are downregulated in roots and leaves of mycorrhizal plants [1,117]. One SULTR member of *L. japonicus* (*LjSultr1;2*) was induced by AM symbiosis in S-sufficient roots, whereas its homolog in *M. truncatula* (*MtSultr1;2*) was strongly upregulated in mycorrhizal roots of S-starved but not S-replete plants [115,117,118]. Moreover, a single *Sultr* gene, *LjSultr1;2*, seems to mediate both direct and symbiotic pathways of S uptake in *L. japonicus*. These differences suggest not only that *Sultr* expression may be mediated by both soil S concentrations and mycorrhizal colonization but also that *Sultr* may have specific functions in different plants. In contrast to phosphate transporters, no SULTR transporter showed mycorrhiza-specific induction of its gene expression (Figure 1C).

**Fungal Water Absorption and Transport in Mycorrhizal Association**

**Water Channels**

Root water uptake from the soil and its distribution within the plant is important for all physiological processes. Water movement occurs by gradient-driven flow through membranes, a process which is mediated and regulated by water channels termed aquaporins (AQPs) [64,119]. AQPs are a family of pore-forming integral membrane proteins belonging to the family of MIPs (major intrinsic proteins) present in all living organisms and forming large families in plants. Based on amino acid sequences, AQPs can be divided into five subfamilies: plasma membrane-intrinsic proteins (PIPs), tonoplast-intrinsic proteins (TIPs), and NOD26-like intrinsic proteins (NIPs) that were first identified in the symbiosomes of legumes but are also present in the plasma membrane and endoplasmic reticulum (ER), as well as small basic intrinsic proteins (SIPs) found in the ER only in dicots, and uncharacterized intrinsic proteins (XIPs) localized in the plasma membrane [120].

**Expression and Regulation of Aquaporins During Mycorrhizal Symbiosis**

The role of plant AQPs in mycorrhizal symbioses has been described in both AM and ECM symbioses [121,122]. Recently, the relevance of fungal AQPs for the functioning of
ECM symbiosis was demonstrated for an AQP of *L. bicolor* by an overexpression approach [123]. In *R. irregularis*, two AQP genes were functionally characterized and were expressed in both arbuscule-enriched cortical cells and ERM [124]. In addition, the first aquaporin from *R. clarus* was described using virus-induced gene silencing in tobacco [125]. The authors proposed a crucial role for this channel in water transport at both uptake and release sites of the mycorrhiza, as well as in polyP translocation from the outer to the inner hyphae.

Some plant genes encoding AQPs were induced by AM colonization, as shown for TIPs in parsley and *M. truncatula* [126,127] (Figure 1C). In ECM poplar plants, an increase in the water transport capacity of mycorrhizal roots correlated with upregulation of several PIP-encoding genes [128] (Figure 2C). Interestingly, opposite results were observed in ECM birch roots because two PIP genes related to water-stress response were downregulated [129]. Differential regulation of AQP encoding genes by AM symbiosis and drought conditions has been observed for some PIPs in *R. irregularis*-inoculated *Phaseolus vulgaris*, *G. max*, and *Lactuca sativa* roots [122,130]. Thus, AM and ECM symbioses lead to both increased or decreased expression of AQP genes, but the functionality of AQP in mycorrhizal systems is still poorly characterized.

The facilitated water transport in AM and ECM symbiotic interactions, respectively, might also be linked to increased membrane water permeability, requiring upregulation of AQPs under drought stress [131]. Some particular patterns of AQP regulation were detected in colonized roots, and these were related to an overall improvement of drought tolerance, as shown by improved growth and water status of mycorrhizal plants [132].

### Concluding Remarks and Future Perspectives

Improved nutrient availability for plants and fungi is one of the main features of the mycorrhiza. Nutrient uptake at the soil–fungus interface, as well as coordinated exchanges between both partners at the plant–fungus interfaces formed by mycorrhiza, include differentiated expression and/or concerted regulation of a large number of specialized membrane transport systems of the fungal and plant partners. So far, molecular players have been identified and characterized more on the plant side in AM and more on the fungal side in ECM (Table 1). The rapid progress in ‘omics’ technologies for symbiotic fungi and their host plants, combined with reverse genetic approaches, will surely enable uncovering the whole transportome of mycorrhizal systems (Box 1). This will consequently enable the analysis of the function and regulation of transport at symbiotic interfaces. Further approaches based on engineering the mycorrhizal transportome will certainly improve crop nutrition and yields (Box 2). Together, these will constitute major steps towards a better understanding and management of the agro-ecological outcome of plant–fungus interactions (see Outstanding Questions).

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### Outstanding Questions

Would it be possible to improve plant nutrition by the selection and engineering of mycorrhizal associations through a transport system-based strategy? The unraveling of plant and fungal transportomes involved in mycorrhizal nutrient fluxes could constitute the basis for plant breeding by selecting the most efficient mycorrhizal communities and individual organisms.

Are nutrients themselves one of the most important regulators of mycorrhizal symbioses? High P contents in soil inhibit root colonization by AM fungi, but this can be partially restored by deficiency of other nutrients. It can be hypothesized that nutrients are key signaling molecules of mycorrhizal partners through molecular systems sensing their availability.

Do host plants act on signaling and regulatory events to trigger the expression of fungal transport systems at symbiotic interfaces, and does this operate reciprocally? Signal cascades leading to the expression of transport systems for the release of nutrients in arbuscules and Hartig nets are unknown. It is possible that plant molecules (e.g., small RNAs) may regulate the expression of fungal transporters, and that fungal compounds (e.g., effectors) activate the expression of plant transporters. Again, nutrients themselves may take part in this regulatory mechanism.

Are plant transport systems shared by both AM and ECM associations? Some trees, such as poplar, apple tree, and eucalyptus, are able to interact with both AM and ECM fungi. For instance, some poplar P transporters are expressed in both associations. It is tempting to speculate that a common ‘mycorrhizal’ trophic pathway is conserved, but so far there is no molecular evidence that mycorrhizal pathways for nutrient exchange are shared by ECM and AM fungi.
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