Plant Aquaporins and Mycorrhizae: Their Regulation and Involvement in Plant Physiology and Performance

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Abstract The establishment of a mycorrhizal symbiosis can change plant aquaporin gene expression and protein accumulation. However, the regulation of plant aquaporins seems to be dependent on the plant and fungal species involved in the symbiosis. The implications of such regulation on plant water relations, plant physiology and plant performance under optimal or stressful conditions have been the subject of intensive investigation in the last years. Results from different studies suggest that mycorrhizal symbioses act on host plant aquaporins and alter both plant water relations and plant physiology in order to cope better with stressful environmental conditions such as drought. The fungal aquaporins have been related to water transport in the fungal mycelium and in the internal exchange membranes at the symbiotic interface. Indeed, it is generally observed that mycorrhizal plants exhibit higher osmotic and hydrostatic root hydraulic conductance under drought stress conditions. Moreover, mycorrhizal plants also grow to a greater extent than non-mycorrhizal plants under drought conditions, indicating that the changes induced by the symbiosis on plant aquaporins contribute to enhance the plant tolerance to drought. These effects are likely to be the result of the combined action of different aquaporins regulated by the mycorrhizal symbiosis (including PIPs, TIPs, NIPs and SIPs), influencing the transport of water and, most probably, also of other solutes of physiological importance for the plant under drought stress conditions.

1 Introduction

The term mycorrhiza applies to a mutualistic symbiosis between roots of most higher plants and a group of soil fungi belonging to the phyla Glomeromycota, Basidiomycota or Ascomycota (Varma 2008). There are several types of mycorrhizal symbiosis, according to the plant and fungus involved and the morphological

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characteristics of the structures formed during the association. The most abundant are the arbuscular mycorrhizal (AM) symbiosis, which are formed by almost 80 % of terrestrial plants, including many important agricultural species. The fungi involved develop specialized structures called arbuscules inside the root cells, where there is an exchange of nutrients between both symbionts (Genre et al. 2005). Another type of abundant mycorrhiza is the ectomycorrhizal symbiosis, involving an important number of tree species (Wang and Ding 2013). In ectomycorrhizae, the fungi form a Hartig net, where resources are exchanged with the host plant root (Agerer 2001). By the mycorrhizal association, the plant receives soil nutrients (especially phosphorus) and water, while the fungus receives a protected ecological niche and plant-derived carbon compounds for its nutrition (Varma 2008).

During the establishment of a mycorrhizal symbioses (especially during the AM symbiosis), plant root cells undergo extensive morphological alterations in order to accommodate the presence of an endophytic symbiont, with most of these changes concerning vacuolar or cytoplasmic membrane systems. In fact, during the formation of the AM symbiosis, the plant plasma membrane extends to form a novel periarbuscular membrane, which closely surrounds the fungal hyphae resulting in an estimated three- to tenfold increase in the outer plant cell surface area (Genre et al. 2005, 2008). In addition, both AM and ectomycorrhizal symbioses have been shown to alter root hydraulic properties (Muhsin and Zwiazek 2002b; Khalvati et al. 2005; Lehto and Zwiazek 2011; Bárzana et al. 2012). Thus, Krajinski et al. (2000) already hypothesized a variation of expression affecting genes that encode membrane-associated proteins, and it is not surprising that the establishment of a mycorrhiza can change plant aquaporin gene expression and protein accumulation. The implications that such regulation has on plant water relations, plant physiology and plant performance under optimal or stressful conditions have been the subject of intensive research.

2 Arbuscular Mycorrhizal Symbiosis and Plant Aquaporins

Research on regulation of host plant aquaporins by AM symbiosis is relatively recent. The first report on the modulation of aquaporin genes by the AM symbiosis was provided by Roussel et al. (1997) followed by Krajinski et al. (2000), who found mycorrhiza-induced expression of TIP (tonoplast intrinsic protein) aquaporins in parsley and *Medicago truncatula*, respectively. Downregulation of host plant aquaporins was described by Ouziad et al. (2006), who showed a decrease in the expression of PIP (plasma membrane intrinsic protein) and TIP aquaporins by mycorrhizal colonization in tomato plants. Uehlein et al. (2007) found PIP and NIP (nodulin26-like intrinsic protein) aquaporin genes from *Medicago truncatula* that were also induced by mycorrhization. These authors related the mycorrhiza-induced change in expression of the two genes with physiological changes in the plant roots, i.e. the symbiotic exchange processes located at the periarbuscular membrane (Uehlein et al. 2007). In any case, in the studies mentioned above, plants were

cultivated under optimal conditions, and the question of host plant aquaporin regulation under water deficit conditions remained unresolved. Thus, Porcel et al. (2006) cloned genes encoding PIPs from soybean and lettuce, and their expression pattern was studied in AM and non-AM plants cultivated under well-watered or drought stress conditions. The starting hypothesis in this study was that if AM fungi can transfer water to the root of the host plants, the plant should increase its permeability for water and aquaporin genes should be upregulated in order to allow a higher rate of transcellular water flow. However, the results obtained showed that the PIP genes studied were downregulated in both plant species under drought stress and that such downregulation was even more severe in plants colonized by *G. mosseae* than in non-AM plants (Porcel et al. 2006). The expression of *GmPIP2* gene from soybean was analysed in a time course, and it was found that in AM plants the downregulation of *GmPIP2* occurred before than in non-AM plants. It was proposed that such an effect of the AM symbiosis advancing the downregulation of *GmPIP2* gene may have physiological importance to cope with drought stress. In fact, according to Aharon et al. (2003) and Jang et al. (2007), the overexpression of PIP aquaporins in transgenic tobacco and *Arabidopsis* improves plant vigour under favourable growth conditions, but the overexpression of such PIP genes had a negative effect during drought stress, causing fast wilting. Hence, the decreased expression of plasma membrane aquaporin genes during drought stress in roots of AM plants can be a regulatory mechanism to limit water loss from cells (Barrieu et al. 1999; Porcel et al. 2006).

The expression of four PIP aquaporin genes in roots from *Phaseolus vulgaris* was analysed in mycorrhizal and non-mycorrhizal plants subjected to drought, cold or salinity in order to illustrate the complexity of the response of aquaporin genes to AM fungi (Aroca et al. 2007). Three of these PIP genes showed differential regulation by AM symbiosis under the specific conditions of each stress applied. In fact, *PvPIP1;1* expression was slightly inhibited by *G. intraradices* inoculation under drought stress conditions, while non-mycorrhizal plants did not change its expression pattern. Cold stress inhibited *PvPIP1;1* expression similarly in AM and non-AM plants. Finally, salinity raised expression of *PvPIP1;1* in both groups of plants, but the enhancement was considerably higher in AM plants. The expression of *PvPIP1;2* was inhibited by the three stresses in the same way in AM and non-AM plants. In contrast, *PvPIP1;3* expression showed important differences in AM and non-AM plants according to the stress imposed. This gene was clearly induced in non-AM plants under drought stress but inhibited in AM plants. Under salinity the expression of this gene was also induced in both groups of plants, especially in AM. Under cold stress the behaviour was the opposite since it was inhibited in non-AM plants and induced in AM. The expression of *PvPIP2;1* was induced in non-AM plants under drought stress but was downregulated in AM plants. The response of *PvPIP2;1* expression to cold stress was not significant for any of the two plant groups and, again, the gene was considerably upregulated under salinity, especially in AM plants. Thus, the expression of each PIP gene analysed responded differently to each stress, and this response also depended on the AM fungal presence. These results point to the possibility that each PIP gene analysed could have a different

function and regulation by AM symbiosis under the specific conditions of each stress studied (Aroca et al. 2007).

When root hydraulic conductivity (*Lp*) was measured in *P. vulgaris* plants, it was found that the regulation of root hydraulic properties by AM symbiosis was strongly correlated with the amount of PIP2 protein and its phosphorylation state, resulting in enhanced *Lp* values under drought, cold and salinity stresses in AM plants (Aroca et al. 2007).

Giovannetti et al. (2012) focused on two putative aquaporin genes, *LjNIP1* and *LjXIP1*, which were found to be upregulated in a transcriptomic analysis performed on roots of *Lotus japonicus* colonized by the AM fungus *Gigaspora margarita*. Using a laser microdissection approach, they demonstrated that *LjNIP1* was specifically expressed in arbuscule-containing cells, whereas *LjXIP1* transcripts were present in both non-colonized cortical cells from mycorrhizal roots and in cortical cells from non-mycorrhizal roots. The potential role of *LjXIP1* remains to be elucidated. In contrast, functional experiments with yeast protoplasts demonstrated that *LjNIP1* can transport water. On the basis of these functional results for *LjNIP1*, of its localization in the inner membrane system of arbusculated cells and of expression timing, it was proposed that LjNIP1 protein was potentially involved, directly or indirectly, in cell turgor regulation, in facilitating colonized cell adaptation to osmotic stresses and/or in the actual transfer of water from the fungus to the plant (Giovannetti et al. 2012).

Recently, Liu et al. (2014) conducted an experiment in which AM and non-AM rice plants were subjected to different temperature and exogenous trehalose treatments. Trehalose was used since it has been shown to act as important abiotic stress protectant and as a signalling molecule. Thus, authors of this study wanted to elucidate if trehalose might stimulate the expression of fungal and plant aquaporin genes and if trehalose might also stimulate AM fungal and rice root water uptake. The results showed that AM fungal inoculation enhanced rice root water uptake at both normal and low temperatures. At low temperature, AM rice plants showed higher expression levels for several plant PIPs than non-AM rice plants. Application of exogenous trehalose demonstrated that trehalose could regulate AM fungal and rice water absorption by inducing the expression of several *OsPIPs* and a fungal aquaporin gene. It was concluded that one of the mechanisms by which AM fungi improve plant resistance to low temperature was a fungal-enhanced trehalose accumulation in rice, which could act as a signal inducing fungal and host plant aquaporins expression that then maintained better water relations in mycorrhizal plants at low temperatures (Liu et al. 2014).

2.1 Arbuscular Mycorrhizal Fungal Aquaporins

The AM fungi also have aquaporin genes. Aroca et al. (2009) cloned the first aquaporin from an AM fungus (*GintAQP1*). Although the functionality of this aquaporin could not be demonstrated, authors found evidence supporting the idea that fungal

aquaporins could compensate the downregulation of host plant aquaporins caused by drought. Also, they found that *GintAQP1* expression was upregulated in parts of the mycelium that were not osmotically stressed by NaCl, while other parts of the mycelium were stressed. This suggests possible communication between unstressed and stressed mycelium. More recently, Li et al. (2013) have described two functional aquaporins (*GintAQPF1* and *GintAQPF2*) from the same AM fungus (*Rhizophagus intraradices*). GintAQPF1 was localized in the plasma membrane, whereas GintAQPF2 was localized both in plasma and intracellular membranes. Both aquaporins could transport water, as shown by heterologous expression in yeast protoplasts, and the expression of the two genes in arbuscule-enriched cortical cells and extraradical mycelia of maize roots was enhanced significantly under drought stress. Thus, the two AM fungal aquaporins were related to water transport in the extraradical mycelium and in the periarbuscular membrane (Li et al. 2013). In any case, future research is needed to understand the role of AM fungal aquaporins for the fungus or for the symbiosis, under optimal and water deficit conditions.

2.2 *Regulation of Aquaporins by the AM Symbiosis Under Drought Stress Conditions and Influence on the Transport of Water and Other Solutes of Physiological Importance*

Although many aquaporins are highly selective for water, the selectivity filters of plant aquaporins show a high divergence (Sui et al. 2001), suggesting wide functional diversity for these proteins (Bansal and Sankararamakrishnan 2007) (See chapter "Structural Basis of the Permeation Function of Plant Aquaporins"). Thus, it has become increasingly clear that some aquaporins do not exhibit a strict specificity for water and can transport other small neutral molecules such as urea (Liu et al. 2003), ammonia (Loque et al. 2005), carbon dioxide (CO_2) (Uehlein et al. 2003), boric acid (Mitani et al. 2008), hydrogen peroxide (H_2O_2) (Bienert et al. 2007), silicic acid (Ma and Yamaji 2006) and some other molecules with physiological significance (Bienert et al. 2008), highlighting the paramount relevance of aquaporins for plant physiology.

The function and regulation of aquaporins have been intensively integrated to explain the remarkable hydraulic properties of plants. However, the identification of aquaporin substrates other than water, as mentioned above, has opened the possibilities for the involvement of aquaporins in many other processes of physiological significance for plants (Chaumont and Tyerman 2014; Li et al. 2014). In the mycorrhizal symbiosis, the importance of aquaporins for both water and nutrient exchange was recognized by Maurel and Plassard (2011) and supported recently by results obtained with AM maize plants (Bárzana et al. 2014), elaborated upon below.

The first report of involvement of a host plant aquaporin in the functioning of AM symbiosis under drought stress by a mechanism unrelated to water transport

was provided by Porcel et al. (2005). They studied the effects of reduced expression of the PIP aquaporin-encoding gene NtAQP1 in mycorrhizal NtAQP1-antisense tobacco plants under well-watered and drought stress conditions. The study aimed at elucidating whether or not the impairment in NtAQP1 gene expression affected the AM fungal colonization pattern, as well as to find out if such impairment had any effect on the symbiotic efficiency of two AM fungi. The reduction of NtAQP1 expression had no effect on root colonization, suggesting that either NtAQP1 function is irrelevant for the process of root colonization or that the impairment in NtAQP1 gene expression had been compensated for by changes in the abundance or the activity of other aquaporin isoforms. In contrast, when Porcel et al. (2005) measured the symbiotic efficiency of the two AM fungi (in terms of plant biomass production), they observed that under drought stress, mycorrhizal wild-type plants grew faster than mycorrhizal NtAQP1 antisense plants. This indicates that the symbiotic efficiency of both AM fungi was greater in wild-type than in antisense plants and that the transport meditated by NtAQP1 seems to be important for the efficiency of the symbiosis under drought stress conditions (Porcel et al. 2005). This was related to the fact that NtAQP1 allows CO_2 passage and is involved in plant growth promotion (Uehlein et al. 2003).

Recently, Bárzana et al. (2014) conducted an investigation aimed at elucidating in which way the AM symbiosis modulates the expression of the whole set of aquaporin genes present in maize, both under optimal water conditions and under different drought stress scenarios. An additional objective was to characterize those aquaporins showing regulation by the AM symbiosis, in order to shed further light on the molecules that could be involved in the mycorrhizal responses to drought. The AM symbiosis regulated the expression of a wide number of aquaporin genes in the host plant (16 genes out of 36 existing in maize), comprising members of the different aquaporin subfamilies (Bárzana et al. 2014). Several of these AM-regulated aquaporins (ZmPIP1;3, ZmPIP2;2, ZmTIP1;1, ZmTIP1;2, ZmNIP1;1, ZmNIP2;1 and ZmNIP2;2) were functionally characterized in heterologous expression systems with *Xenopus laevis* oocytes and by yeast complementation. It was shown that they can transport water, but also other molecules of physiological importance for plant performance under both normal and stress conditions (glycerol, urea, ammonia, boric acid, silicon or hydrogen peroxide). The regulation of these genes depended on the watering conditions and on the severity of the drought stress imposed. The different aquaporin regulation patterns suggest that under short-term drought conditions, the AM symbiosis may stimulate further the physiological processes in which these aquaporins are involved, but when the drought becomes sustained, the AM symbiosis restricts most of the processes in which these aquaporins participate (Fig. 1).

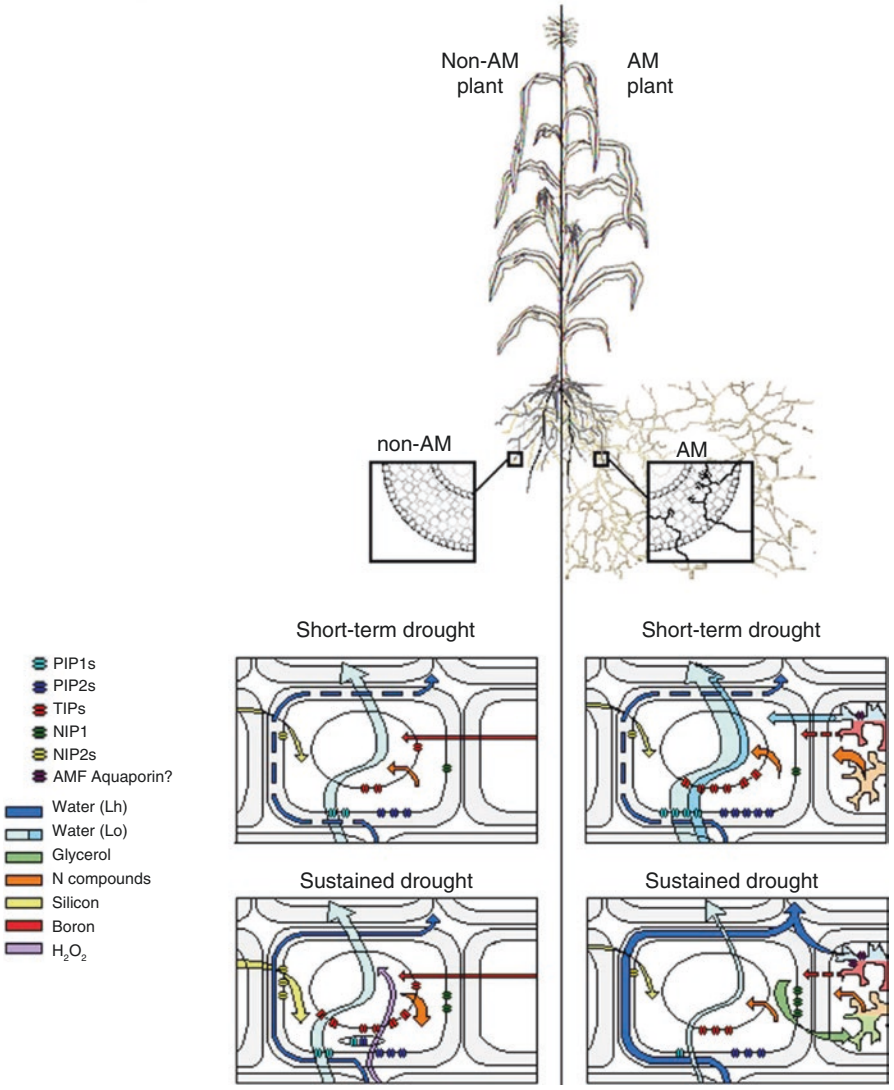
AM maize plants maintained higher values of root water flux than non-AM plants. These effects have been related to the increased absorbing surface caused by fungal hyphae growing in the soil, combined with the ability of the fungus to take up water from soil pores inaccessible to roots (Marulanda et al. 2003; Allen 2009; Ruth et al. 2011). Thus, under such conditions, mycelial water uptake from soil pores inaccessible to roots and its transference from AM fungal hyphae to plant

cells can be critical to improve water supply to the plant, potentially increasing flow via cell-to-cell and apoplastic pathways (Bárzana et al. 2012). In this sense, all the maize PIP2s regulated by the AM symbiosis showed capacity to transport water, especially ZmPIP2;2, which had a particularly high intrinsic water transport capacity. It has also been proposed that TIPs may provide a quick way for cellular osmotic balance by controlling the exchange of water between vacuole and cytosol (Forrest and Bhave 2007), playing an important role under osmotic stress conditions (Katsuhara et al. 2008). Thus, since TIPs regulate the exchange of water between vacuole and cytosol, they may also have an influence on root water flux by affecting exchange of water between transcellular and symplastic water pathways. In the study with maize, *ZmTIP1;1* and *ZmTIP1;2* were highly expressed in all treatments and in the oocyte system both exhibited a high capacity for water transport. Therefore, regulation of PIP and TIP aquaporins was proposed to be a key factor in regulation of plant water transport by AM symbiosis (Fig. 1, blue arrows).

Some maize aquaporins, including ZmNIP1;1, ZmTIP4;1 and ZmTIP4;2, were shown to have the capacity to transport glycerol. The physiological function of glycerol in plants remains unclear, while its utilization is well known in fungi and bacteria (Dietz et al. 2011). However, a study has shown a transfer of glycerol from host plants to pathogenic fungi (Wei et al. 2004), and Gustavsson et al. (2005) suggested that export of plant-derived glycerol may be also important for symbiotic fungi. Thus, ZmNIP1;1, ZmTIP4;1 and ZmTIP4;2 may be important for the AM symbiosis or for the plant-fungus interaction under sustained drought stress (Fig. 1, green arrows). This would explain why these aquaporins were so finely regulated by the AM symbiosis (Bárzana et al. 2014).

Nitrogen is one of the most important nutrients for all living organisms, being needed for the synthesis of compounds essential for growth. The ammonium ion (NH_4^+) and its conjugated base ammonia (NH_3) are the potential primary sources of N (besides NO_3^-) in plant nitrogen nutrition. Transport of urea and NH_3/NH_4^+ into the vacuole would avoid their toxicity in the cytoplasm and/or allow storage of N (Wang et al. 2008), and whenever required as an N-source, these compounds could be remobilized by a passive, low-affinity transport pathway, such as that provided by TIPs (Liu et al. 2003). In maize several TIPs have been shown to transport these compounds (Liu et al. 2003; Loque et al. 2005), including ZmTIP1;1 and ZmTIP1;2, which were regulated by the AM symbiosis (Bárzana et al. 2014). In the AM symbiosis, ammonium is suggested to be the major nitrogen compound transferred to the host plant, with urea playing a role as an intermediate solute (Govindarajulu et al. 2005; Tian et al. 2010; Perez-Tienda et al. 2011). This suggests that these aquaporins could be involved in the fungus-based nitrogen nutrition of the host plants (Fig. 1, orange arrows), as was also proposed for ectomycorrhizal fungi (Dietz et al. 2011).

Boron and silicon are metalloids with key structural functions in plant cells (see chapter "Plant Aquaporins and Metalloids"): boron cross-links the pectin fraction of cell walls and polymers of hydrated silica-gel are important for the physical strength of plant cells, especially in monocots like maize (Miwa et al. 2009). Most of the aquaporins regulated by the AM symbiosis have the capacity to transport boron, and



ZmNIP1;1 and ZmNIP2;2 could transport silicon (Bárzana et al. 2014). Thus, the regulation of these aquaporins by the AM symbiosis could have structural functions in maize plants under abiotic stress conditions, including drought (Fig. 1, red and yellow arrows).

Hydrogen peroxide (H_2O_2) is one of the most abundant reactive oxygen species (ROS) continuously produced in the metabolism of aerobic organisms. At low concentrations, it acts as a signal molecule controlling different essential processes in plants during normal growth and development, but it also functions as a defensive signal molecule against various abiotic and biotic stresses (Miller et al.

Fig. 1 Hypothetical model presenting the role of maize aquaporins in the regulation by the AM symbiosis of plant physiology and performance under different growing conditions. Under short-term drought, the expression of most PIPs in AM plants was kept high or even increased. Also, AM fungal hyphae increased the absorbing surface in soil. This, combined with the fungal ability to take up water from soil pores inaccessible to roots, allowed AM plants to maintain higher *Lp* values than non-AM plants (as shown in *blue arrows*). AM fungal aquaporins could be involved in the release of water from hyphae to plant. TIPs may also have an influence on *Lp* by affecting exchanges of water between transcellular and symplastic water pathways. The aquaporins regulated by the AM symbiosis can transport a variety of compounds of physiological importance for the plant, including glycerol (as shown in *green arrows*), that may be important for the plant-fungus interaction under sustained drought stress conditions. Moreover, nitrogen compounds (shown in *orange arrows*) provided by the root and the AMF may be translocated through ZmTIP1;1 and ZmTIP1;2 into the vacuole and stored. Under sustained drought, a remobilization of N stored in the vacuole may be necessary in non-AM plants, which upregulated ZmTIP1;1 and ZmTIP1;2. The B requirements (shown in *red arrows*) in non-AM plants may be guaranteed by the aquaporins that can transport B. In AM plants, the AM fungus may provide directly B to the host plant (shown in *dashed red arrows*), and plant aquaporins involved in B transport are downregulated in order to avoid toxicity due to an excess of B. In plants, Si can mechanically impede penetration by fungi and, thereby, a diminution of Si uptake (shown in *yellow arrows*) in mycorrhizal plants can be expected. Thus, mycorrhization reduced the expression of ZmNIP2;1 and ZmNIP2;2 (transporting Si). In non-AM plants, ZmNIP2;2 increased its expression under sustained drought conditions, probably to avoid plant lodging. TIP1s (for instance, ZmTIP1;1) could play a key role in the detoxification of excess H_2O_2 generated under stress conditions. Additionally, the mobilization of H_2O_2 via aquaporins could serve as a regulatory mechanism for membrane internalization of plant PIPs (as shown in non-AM roots under sustained drought), with subsequent decrease of water transport

2010). Several aquaporins regulated by the AM symbiosis could transport H_2O_2, especially ZmTIP1;1 (Bárzana et al. 2014). Bienert et al. (2007) proposed that TIP1s could play a key role as an additional mechanism for the detoxification of excess H_2O_2 generated under stress conditions (Fig. 1, purple arrows). This idea fits with the high gene expression and protein content maintained for ZmTIP1;1 under drought stress conditions. Additionally, the transport and mobilization of H_2O_2 via aquaporins could serve as a regulatory mechanism for membrane internalization of plant PIPs (Boursiac et al. 2008), with subsequent effects on water transport in AM plants.

3 Ectomycorrhizal Symbiosis and Aquaporins

Most land trees and shrubs establish a symbiosis with ectomycorrhizal (EM) fungi. In the EM symbiosis, the fungal hyphae do not penetrate living cells of the root, but instead they form a mantle around the roots and a net between epidermal and cortical cells called the Hartig net (Barea et al. 2011). Similar to AM fungi, EM fungi also receive carbon compounds from the host plant and they provide to the host plant mineral nutrients and water (Guehl and Garbaye 1990; García et al. 2011). However, EM fungi are able to grow without the presence of host roots, since they

are capable of obtaining carbon compounds from external sources due to hydrolytic enzyme activities (Tedersoo et al. 2012; Moore et al. 2015).

As for AM fungi, EM fungi also increase abiotic stress tolerance of the host plant, including higher tolerance to drought and salt stress (Yi et al. 2008; Kipfer et al. 2012), both of which can cause dehydration of plant tissues (Aroca et al. 2012). In this part of the chapter, it will be first described how EM symbiosis modifies plant water relations and more precisely root water uptake. Later, the effects of EM symbiosis on plant aquaporin regulation will be summarized, and finally the regulation of the EM fungal aquaporins under different environmental conditions will be mentioned. Moreover, the regulation of aquaporins and water relations in the ectendomycorrhizal (EDM) symbiosis will be briefly described. In this symbiosis, the fungus forms a mantle and a Hartig net but also forms intracellular structures, although the cell walls of both partners remain unaltered (Dexheimer and Pargney 1991).

3.1 Water Relations in EM Plants

EM symbioses enhance drought and salinity tolerance of the host plants (Yi et al. 2008; Kipfer et al. 2012), and also regulate differentially leaf transpiration rate (E) and root water uptake, changing leaf water status. Under non-stressful conditions, Xu et al. (2015) found that white spruce (*Picea glauca*) trees inoculated with the EM fungus *Laccaria bicolor* had higher E and leaf water potential than non-inoculated trees. The inoculated plants also presented higher root water uptake capacity in terms of root hydraulic conductance normalized on a root volume basis. However, Calvo-Polanco and Zwiazek (2011) did not find any differences in E or root hydraulic conductance between trees (jack pine or white spruce) inoculated or not with the EM fungus *Suillus tomentosus* under optimal growth conditions. So, the response of E and root hydraulic conductance to EM symbiosis may be dependent on the combination of tree-fungus species. In fact Muhsin and Zwiazek (2002a) found previously the same results as Xu et al. (2015) in white spruce trees but using *Hebeloma crustuliniforme* as the EM fungus. Similarly, the fungus *L. bicolor* only increased root hydraulic conductance and E in white spruce trees, but not in *Ulmus americana*, *Populus tremuloides* or *Betula papyrifera* trees (Table 1). From Table 1, it can be inferred that under optimal growth conditions, EM symbioses rarely modify E (2 cases out of 11), although E was never decreased. On the other hand, increased root hydraulic conductance is not always matched with increased E. Unfortunately, in most cases, leaf water status was not determined, so it is difficult to correlate changes in root hydraulic conductance and E by EM symbiosis with changes in leaf water status.

Under osmotic stress (drought or salinity), the behaviour of root hydraulic conductance and E in EM plants is also species dependent. Thus, the EM fungus *H. crustuliniforme* increased root hydraulic conductance in white spruce and aspen (*P. tremuloides*) trees, but not in *B. papyrifera* trees under salt stress (Table 2). Most

Table 1 Effects of EM symbiosis on leaf transpiration rate (E), root hydraulic conductivity (*Lp*), plant growth and leaf water status (LWS) of trees growing under optimal growth conditions

Plant species	Fungal species	E	Lp	Growth	LWS	Source
Picea glauca	*Laccaria bicolor*	↑	↑	→	↑	Xu et al. (2015)
P. glauca	*Suillus tomentosus*	→	→	→	?	Calvo-Polanco and Zwiazek (2011)
Pinus banksiana	*S. tomentosus*	→	→	↑	?	Calvo-Polanco and Zwiazek (2011)
Ulmus americana	*Hebeloma crustuliniforme*	→	↑	→	?	Calvo-Polanco et al. (2009)
U. americana	*L. bicolor*	→	→	→	?	Calvo-Polanco et al. (2009)
Populus balsamífera	*H. crustuliniforme*	→	↑	→	→	Siemens and Zwiazek (2008)
P. tremuloides	*L. bicolor*	→	→	↑	?	Yi et al. (2008)
P. tremuloides	*H. crustuliniforme*	→	→	→	?	Yi et al. (2008)
Betula papyrifera	*L. bicolor*	→	→	→	?	Yi et al. (2008)
B. papyrifera	*H. crustuliniforme*	→	→	→	?	Yi et al. (2008)
P. glauca	*H. crustuliniforme*	↑	↑	↑	?	Muhsin and Zwiazek (2002a)

The direction of the arrows indicates the direction of the change. ↑, increase; ↓, decrease; →, no change; ?, non-recorded

interesting is that the same EM fungus *H. crustuliniforme* had no effect on root hydraulic conductance in aspen trees under optimal growth conditions, but increased it under salt stress (Yi et al. 2008; Tables 1 and 2). Again, there is no relationship between changes in root hydraulic conductance and E caused by EM symbiosis under osmotic stress (Table 2). Also, the growth promotion caused by EM symbiosis under osmotic stress was not always related to changes in root hydraulic conductance or E (Table 2), indicating that other mechanisms are behind the better performance of EM plants under osmotic stress, such as better antioxidative mechanisms (Alvarez et al. 2009), synthesis of specific proteins linked to EM symbiosis (Kraj and Grad 2013) or better nutritional status (Danielsen and Polle 2014).

3.2 Root Water Uptake in EM Plants

Although root hydraulic conductance is one factor that determines root water uptake (U), root hydraulic conductance and U do not always correlate (Aroca et al. 2001; Doussan et al. 2006), potentially because root hydraulic conductance is typically determined in detached root systems (Li and Liu 2010) and the influence of leaf transpiration is lost. Vandeleur et al. (2014) found that shoot topping reduced root hydraulic conductance from 30 to 60 % depending on the plant species. Moreover, root hydraulic conductance is frequently measured in roots removed from their surrounded soil, and the soil hydraulic conductance factor is missing as well as the

Table 2 Effects of EM symbiosis on leaf transpiration rate (E), root hydraulic conductivity (*Lp*), plant growth and leaf water status (LWS) of trees growing under stressful conditions

Plant species	Fungal species	Stress	E	Lp	Growth	LWS	Source
Picea glauca	*S. tomentosus*	10 % Polyethylene glycol	→	→	→	?	Calvo-Polanco and Zwiazek (2011)
P. glauca	*S. tomentosus*	60 mM NaCl	→	→	→	?	Calvo-Polanco and Zwiazek (2011)
Pinus banksiana	*S. tomentosus*	10 % Polyethylene glycol	↓	→	→	?	Calvo-Polanco and Zwiazek (2011)
P. banksiana	*S. tomentosus*	60 mM NaCl	→	→	↑	?	Calvo-Polanco and Zwiazek (2011)
Populus x canescens	*Paxillus involutus*	Stopping watering	→	↑	↑	↑	Beniwal et al. (2010)
Populus tremuloides	*Laccaria bicolor*	25 mM NaCl	→	→	↑	?	Yi et al. (2008)
P. tremuloides	*Hebeloma crustuliniforme*	25 mM NaCl	→	↑	→	?	Yi et al. (2008)
Betula papyrifera	*L. bicolor*	25 mM NaCl	→	→	→	?	Yi et al. (2008)
B. papyrifera	*H. crustuliniforme*	25 mM NaCl	→	→	→	?	Yi et al. (2008)
P. glauca	*H. crustuliniforme*	25 mM NaCl	↑	↑	↑	?	Muhsin and Zwiazek (2002a)

The direction of the arrows indicates the direction of the change. ↑, increase; ↓, decrease; →, no change; ?, non-recorded

potential hydraulic lift mechanism (the water that is absorbed by deeper roots and released into upper soil layers) (Doussan et al. 2006). Therefore, besides root hydraulic conductance, U determinations are essential to really ascertain if EM symbiosis actually enhances capacity of host roots to take up water. Bogeat-Triboulot et al. (2004) found an increase of U in *Pinus pinaster* trees inoculated with the EM fungus *Hebeloma cylindrosporum*. At the same time, EM trees had also higher root hydraulic conductance normalized on a root area basis and had an elevated amount of soil adhering to the roots, perhaps facilitating root water absorption. In this case, the root hydraulic conductance was determined by the high pressure flow meter (HPFM) technique, in which the roots remain in the soil and not disturbed. However, in a previous study, Colpaert and Van Assche (1993) found a negative effect on U by EM inoculation in *Pinus sylvestris* trees, but accompanied by a reduction in plant growth, which indirectly may decrease U, because aerial

parts demanded less water. Measuring U in intact trees is necessary to understand the effects of EM on plant water relations as a whole.

The transport of water from root epidermal cells to root xylem vessels can follow three paths, namely, apoplastic, symplastic and transcellular (see chapter "Aquaporins and Root Water Uptake"). Since symplastic and transcellular paths cannot be discriminated empirically, the sum of both is called the cell-to-cell path. The apoplastic path corresponds to water circulating through cell walls, and cell-to-cell path corresponds to water flowing by the plasmodesmata and crossing cell membranes (plasma membrane and/or tonoplast) (Steudle and Peterson 1998). Since EM fungal mycelia penetrate root apoplastic spaces, EM symbiosis may modify the proportion of water flowing through each root pathway.

In earlier studies, Behrmann and Heyser (1992) found no evidence supporting that EM symbiosis enhanced the transport capacity of the apoplastic path using different apoplastic tracers. However their results could be limited to the EM association studied (*Pinus sylvestris-Suillus bovinus* association). In contrast, Muhsin and Zwiazek (2002b) found evidence supporting that EM symbiosis enhanced root hydraulic conductance by increasing the amount of water flowing via the apoplastic path. This conclusion was based on studies of the inhibition of root hydraulic conductance by $HgCl_2$, since root hydraulic conductance of EM roots presented less inhibition than that of non-mycorrhizal plants. Anyway, these results could change depending on the pH of the soil solution, since the differences in the proportion of apoplastic water flow between EM and non-EM plants depend on the root medium pH, being more pronounced at extreme pH (Siemens and Zwiazek 2011). Vesk et al. (2000) found that EM sheaths could be very impermeable to water, so under these circumstances the cell-to-cell pathway should predominate, since apoplastic access to water will be limited by fungal structures. In this sense, Lee et al. (2010) and Xu et al. (2015) found that hydraulic conductivity increased for root cortical cells of EM trees compared to non-EM trees, indicating that the cell-to-cell path could be enhanced by the EM symbiosis. All the above results should be taken with caution, since most probably it depends on the species involved in the EM symbiosis.

3.3 Aquaporin Regulation by EM Symbioses

Since root hydraulic conductance is governed in part by aquaporins, and more precisely the cell-to-cell path (Maurel et al. 2008), EM symbioses may regulate aquaporin expression and activity of the host trees. The activity of aquaporins has been estimated by using different chemicals that inhibit aquaporins, which could also affect fungal aquaporin activity. As mentioned above, Muhsin and Zwiazek (2002b) found that aquaporin activity was downregulated by the symbiosis between *Ulmus americana* and *Hebeloma crustuliniforme* partners using $HgCl_2$ as an inhibitor of aquaporin activity, indicating that flow via the apoplastic path may have increased. However, using the same aquaporin inhibitor, no enhancement of aquaporin activity in the symbiosis between *Pinus banksiana* and *Suillus tomentosus* partners was

observed (Lee et al. 2010). However the use of $HgCl_2$ could have side effects that can interfere with the root hydraulic conductance measurements. Aquaporin activity is enhanced by phosphorylation of different serine residues (Johansson et al. 1998; Azad et al. 2008) (see also chapter "Plant Aquaporin Posttranslational Regulation"). However, the phosphorylation state of aquaporins in EM plants has not been assayed yet, although it has been determined in AM plants (Aroca et al. 2007; Calvo-Polanco et al. 2014; Bárzana et al. 2015).

The influence of EM symbioses on the expression of specific aquaporins of the host plant has been studied. Downregulation of the expression of two PIP genes out of eight by the symbiosis of the EM fungus *Laccaria bicolor* in *Picea glauca* roots has been reported (Xu et al. 2015). However, in this study EM symbiosis enhanced root hydraulic conductance (at both whole root and cell levels), so it is possible that such enhanced root hydraulic conductance could be mediated by aquaporins of the EM fungus or by an increase in water flow through the apoplastic path. In addition, post-translational modification could take place (see chapter "Plant Aquaporin Posttranslational Regulation") and enhance aquaporin activity in EM roots. Two other studies reported an upregulation in the expression of several PIP genes in EM roots. Tarkka et al. (2013) found an increase in the expression of five PIPs and one SIP (small and basic intrinsic protein) aquaporin genes in *Quercus robur* roots inoculated with the EM fungus *Piloderma croceum* using RNA-seq technique, but no measurement of root hydraulic conductance was performed. Marjanovic et al. (2005) found also an increase in the expression of one PIP (*PttPIP2;5*) gene in poplar (*Populus tremula* x *tremuloides*) roots inoculated with the EM fungus *Amanita muscaria*. Interestingly, this PIP gene had high capacity of transporting water when expressing in *Xenopus laevis* oocytes, and their expression correlated with higher root hydraulic conductance in EM trees. Obviously, more work is needed in order to establish the function of plant aquaporins in the regulation of root hydraulic conductance by EM symbiosis. These studies should include the use of plants with altered levels in the expression of specific aquaporins.

As commented above, EM fungi have their own aquaporins as do AM fungi. Dietz et al. (2011) found seven aquaporin genes in the *L. bicolor* genome, three of them having a high water and ammonia transport capacity when expressed in *Xenopus laevis* oocytes. Most recently, Nehls and Dietz (2014), analysing the genome of 480 fungal species, found around 50 putative orthodox aquaporin genes accounting for several EM fungal species. These findings point to the potential role of EM fungal aquaporins in the water relations of host plants. Hence, when white spruce trees were inoculated with *L. bicolor* strains overexpressing JQ585595 *L. bicolor* aquaporin, they showed enhanced root hydraulic conductance under optimal growth temperatures, but lower under suboptimal temperatures (Xu et al. 2015). Moreover, it has been found that one *L. bicolor* aquaporin (*LbAQP1*) is essential in the formation of the Hartig net in trembling aspen trees (Navarro-Ródenas et al. 2015) and this was related to the capacity of this aquaporin to transport NO, H_2O_2 and CO_2 that could act as signalling molecules. Obviously, more studies are

necessary in other EM fungal species to understand the possible role of fungal aquaporins in EM symbiosis.

3.4 Aquaporins and Ectendomycorrhizal (EDM) Symbiosis

Ectendomycorrhizal (EDM) symbiosis also may increase plant host tolerance to environmental stresses, although this symbiosis has been less studied. Morte et al. (2010) found that symbiosis with the fungus *Terfezia claveryi* (desert truffle) was essential for the survival of *Helianthemum almeriense* plants under arid conditions. In a previous greenhouse study, *T. claveryi*-inoculated plants showed higher leaf water potential, transpiration rate and net photosynthesis rate than non-inoculated plants (Morte et al. 2000). Similar results were found by Turgeman et al. (2011) in the symbiosis between *Helianthemum sessiliflorum* and *Terfezia boudieri*.

Regarding root hydraulic properties modified by EDM symbiosis, Siemens and Zwiazek (2008) found no effect of *Wilcoxina mikolae* inoculation on root hydraulic conductance of balsam poplar (*Populus balsamifera*) trees. Unfortunately no other studies examining the effect of EDM symbiosis on root hydraulic properties have been published to date. Nevertheless, Navarro-Ródenas et al. (2013) found that *T. claveryi* inoculation modified the expression of *H. almeriensis* aquaporins, although a defined trend was not observed, since each aquaporin responded differently. As for the other mycorrhizal fungi, EDM fungi also possess their own aquaporins. Navarro-Ródenas et al. (2012) cloned one aquaporin from the EDM fungus *T. claveryi*. This aquaporin was able to transport water and CO_2 when expressed in *Saccharomyces cerevisiae*, and its expression responded to an osmotic stress applied *in vitro*.

4 Conclusions

Based on the reviewed literature, we propose that AM symbioses act on host plant aquaporins in a concerted manner to alter both plant water relations and physiology and allowing the plant to cope better with stressful conditions (Fig. 1). In support of this idea, it is generally observed that AM plants exhibit higher root hydraulic conductance under drought stress conditions. Moreover, AM plants also grow more than non-AM plants under drought conditions, indicating that, apart from the improved P nutrition, all these changes induced by the AM symbiosis on plant aquaporins contributed to the enhanced plant tolerance to drought (Bárzana et al. 2014). These effects are likely the result of the combined action of the different aquaporins regulated by the AM symbioses (including PIPs, TIPs, NIPs and SIPs), influencing the transport of water and, most probably, also of signalling molecules and other solutes of physiological importance for the plant under drought stress conditions. In

EM symbioses the regulation of plant aquaporins seems to be highly dependent on the plant and fungal species involved in the symbiosis. Moreover, the regulation of root hydraulic conductivity has been related also to the activity of the EM fungal aquaporins and with increases in water flow via the apoplastic pathway.

5 Perspectives

There is a broad consensus that it is necessary to analyse the diversity of plant aquaporin isoforms, of their substrates and their cellular localizations in order to understand their physiological functions with respect to whole plant hydraulics, plant development, nutrient acquisition and plant responses to various environmental stresses (Gomes et al. 2009; Li et al. 2014). The main objective of future research should be to identify those aquaporin isoforms regulated by the AM symbiosis having a key influence on the capacity of roots to transport water or the capacity for the transport *in planta* of other solutes. Moreover, the role of fungal aquaporins during formation and functioning of AM symbioses requires future investigation. For that, the localization of these aquaporins in the fungal mycelium is of great interest.

More integrative physiological studies are also needed to understand the role of aquaporins in EM and EDM symbioses. These studies should include measurements of the rate of whole root water uptake normalized to root surface area and plant water status. Also, the phosphorylation state of aquaporins in roots of EM and EDM plants should be determined, as well as the use of plants with altered expression levels of specific aquaporins. Although the study of *L. bicolor* aquaporins has increased our knowledge about the role of fungal aquaporins in the EM symbiosis, the study of aquaporins from other EM (EDM) fungi is necessary.

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