



Root hydraulic conductance, aquaporins and plant growth promoting microorganisms: A revision

María Daniela Groppa, María Patricia Benavides, Myriam Sara Zawoznik*

Cátedra de Química Biológica Vegetal, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, (1113) Ciudad Autónoma de Buenos Aires, Argentina

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ABSTRACT

A considerable number of soil and rhizospheric fungi and bacteria collectively known as plant growth promoting microorganisms (PGPM) have demonstrated ability to colonize plant roots and to provide benefits to their hosts. Among these benefits, many authors documented improved root hydraulic conductance and alleviation of abiotic stresses such as drought and salinity. Today, it is accepted that movement through aquaporins represents a quite faster pathway of water movement across biological membranes. This review is intended to reflect the state of art in the knowledge of PGPM effects on plant water status and root hydraulic conductance, with special emphasis on the experimental data that prove or suggest an impact of PGPM on root aquaporins under both normal and water limiting conditions.

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1. Introduction

Terrestrial plants have coevolved with a broad spectrum of soil inhabitants which associate to their roots. A considerable number of microorganisms have demonstrated to benefit plants by directly promoting their growth and/or by lessening or preventing the deleterious effects of plant pathogens (Glick, 1995). These microorganisms include both fungi and bacteria and are usually referred to as plant growth promoting microorganisms (PGPM) or plant growth promoting rhizobacteria (PGPR). When the reduction of plant pathogen damage is the predominant effect, the microbial partner often receives the denomination of biocontrol agent (BCA).

Considering the kingdom of fungi, arbuscular mycorrhizal fungi (AMF) stand out as the most significant and widespread group of PGPM. Ectomycorrhizal fungi (EMF) are also important symbionts of particular relevance for many woody plants, while several members of the genus *Trichoderma* (Phylum *Ascomycota*) are well-recognized BCA. Considering the kingdom of bacteria, a wide range of microorganisms including different species and strains of *Bacillus*, *Burkholderia*, *Pseudomonas*, and the well-known nitrogen fixing organisms *Rhizobium*, *Bradyrhizobium*, *Azotobacter*, *Azospirillum* and *Herbaspirillum*, are classically regarded as important PGPR.

Since the first description of PGPR made by Kloepper and Schroth (1978), great insight has been gained regarding this heterogeneous group of microorganisms. Today it is widely accepted that

AMF, EMF and PGPR promote plant growth, at least in part, because they facilitate water and nutrient uptake and distribution, and this ability has been attributed to various mechanisms. In the case of mycorrhizal symbioses, it should be mentioned the contribution of extramatrical mycelium in exploring the soil (Rousseau et al., 1994) and the direct uptake and transfer of water and dissolved solutes through fungal hyphae, which can penetrate soil pores inaccessible to root hairs due to their smaller size (Allen, 1982; Marulanda et al., 2003). In the case of PGPR, the bacterial synthesis of bioactive substances like auxins, gibberellins and cytokinins, and the regulation of ethylene levels through the action of the enzyme ACC deaminase have been linked to greater and/or more branched root systems and higher growth rates (Patten and Glick, 1996; Dobbelaere et al., 2003; Glick, 2005), in addition to improved nitrogen contents under rhizobial symbiosis and other plant-microbe interactions. These mechanisms, however, do not rule out the occurrence of specific molecular events that may contribute to the improved water status often observed in plants living in association with these microorganisms. In line with these concepts, several reports document that plants colonized by different PGPM showed higher root hydraulic conductance and/or increased tolerance against drought and salinity, a condition that also imposes water stress (Sarig et al., 1992; Augé, 2001; Marjanović et al., 2005b; Aroca et al., 2007; Dimkpa et al., 2009).

The purpose of this review is to reflect the state of art in the knowledge of PGPM effects on plant water status and root hydraulic conductance, with special emphasis on the experimental data that prove or suggest an impact of PGPM on root aquaporins under both normal and water limiting conditions.

* Corresponding author.

E-mail address: myriamz@ffyba.uba.ar (M.S. Zawoznik).

2. The role of aquaporins in plant water transport

Mesophytic plants must take up significant quantities of water to sustain growth and development, especially under high evapotranspiration demands. Water is extracted from soil by roots and root hairs and transported radially to the xylem by two main paths: the apoplastic route, formed by the continuum of cell walls, middle lamellae and intercellular spaces, and the symplastic route, represented by the cytoplasmic network interconnected by plasmodesmata. Once inside xylem vessels, water movement is explained on the basis of transpiration driving force and cohesion-tension mechanism (Steudle, 2001).

Water may also flow from one cell to another following a transmembrane route, by simple diffusion through the cell membrane or by passing through protein structures embedded in the cell membrane matrix known as aquaporins. Aquaporins belong to the large major intrinsic protein (MIP) family of transmembrane channels, represented in all kingdoms (Chrispeels and Agre, 1994). They transport water and other small solutes following osmotic or hydrostatic pressure gradients (Forrest and Bhave, 2007; Maurel et al., 2008), and are usually blocked by mercury derivatives, which target on cysteine residues of these and other cellular proteins (Maurel and Chrispeels, 2001).

Based on the similarity of the amino acid sequence, five groups or subfamilies of plant aquaporins have been described up to now: tonoplast intrinsic proteins (TIP), nodulin-like intrinsic proteins (NIP), plasma membrane intrinsic proteins (PIP), small and basic intrinsic proteins (SIP), and a previously unrecognized group of proteins found in the bryophyte *Physcomitrella patens* and designated as X intrinsic proteins (XIP) to emphasize the lack of information about these proteins, that are present in many plant species but not in monocots (Danielson and Johanson, 2008).

Aquaporins have been shown to control the extensive water transport from the roots to the leaves during the transpiration stream and also to regulate other processes, such as the transport of assimilates through sieve elements in the phloem, the closure or aperture of stomata in leaves, the movement of leaves and the control of cytoplasmic homeostasis (Chaumont et al., 2005). They are regulated both at transcriptional and activity levels. Phosphorylation and dephosphorylation have been reported to be involved in the molecular gating of aquaporins (Maurel et al., 1995; Johansson et al., 1996), as well as both cytoplasmic pH and Ca^{2+} (Tournaire-Roux et al., 2003; Alleva et al., 2006; Martínez-Ballesta et al., 2006). Regulation of aquaporins activity seems to be the typical short-term response to environmental stresses. In the longer run, water transport by aquaporins can be down-regulated by the reduction of transcript formation (Ouziad et al., 2006).

It is accepted that movement through aquaporins represents a quite faster pathway of water (and small solutes) movement across membranes, something that may be particularly important in situations where adjustment of water flow is physiologically critical (Martínez-Ballesta et al., 2006). However, experimental data concerning the expression and activity of these proteins in plants exposed to water deficits are controversial. According to Smart et al. (2001), two opposite descriptions of the role of aquaporins in response to dehydration stress were outlined, with many authors reporting induction of some aquaporins under dehydration stress, which is expected to result in greater membrane water permeability and facilitated water transport; and many others communicating down-regulation, a response that was interpreted as a way to allow cellular water conservation by means of a decreased membrane water permeability. On the other hand, a biphasic response has been suggested under saline stress, with down-regulation of PIPs to avoid cell dehydration at short term, followed by a further increase of expression to gain cell turgor after

solute accumulation took place inside cells (Aroca and Ruiz-Lozano, 2009b).

Among plant aquaporins, PIPs are considered the main determinant in regulating root hydraulic conductivity (L_p) and root water uptake (Siefritz et al., 2002; Javot et al., 2003; Postaire et al., 2010). For this reason, considerable attention has been given to the regulation and functioning of PIPs and to the impact that abiotic stresses such as drought and salinity may have on them.

3. Abscisic acid, aquaporins and rhizospheric microorganisms

Abscisic acid (ABA) is widely recognized as a plant hormone especially involved in plant responses to water deficits (Bray, 2002; Taiz and Zeiger, 2006). It was reported that ABA induces transcription factors that regulate expression of aquaporins of the PIP subfamily (Kaldenhoff et al., 1996; Shinozaki et al., 1998) and many studies found increases in PIPs mRNA after ABA treatment, even when these were frequently transient and dependent on ABA concentration (Zhu et al., 2005; Beaudette et al., 2007), and not always coupled to increases in PIP protein content (Aroca et al., 2006). The effects of ABA on PIP aquaporin gene expression are not fully understood; besides, it should be reminded that ABA affects plant metabolism and water transfers via multiple mechanisms, with a wide spectrum of important actions at different scales of plant organization involving cell, organ and whole plant levels (Tardieu et al., 2010).

Based on experiments using transformed maize lines, Parent et al. (2009) demonstrated that under water deficit, overproduction of ABA caused an increase in the mRNA expression of most aquaporin PIP genes, while the opposite was observed in under-producing lines, with a similar pattern in the protein contents of 4 PIPs. This resulted in more than sixfold differences in root hydraulic conductivity on comparing sense and antisense lines, and these differences translated into differences in whole-plant hydraulic conductance. Nevertheless, an ABA-independent pathway involved in aquaporin genes regulation was also reported (Jang et al., 2004).

It is well known that PGPM are usually active plant hormone producers (Antoun and Prévost, 2005; Dodd et al., 2010). Although indolacetic acid is certainly the plant hormone that received more attention in this field, ABA production in defined liquid media was demonstrated for several strains of rhizospheric microorganisms belonging to the genera *Rhizobium* (Dangar and Basu, 1991), *Pseudomonas* (Cohen et al., 2011), *Bacillus* (Karadeniz et al., 2006; Forchetti et al., 2007) and *Azospirillum* (Perrig et al., 2007; Cohen et al., 2008). Based on experimental data, it was also suggested that rhizospheric microorganisms can affect plant ABA flows by the release or consumption of other plant growth regulators or their precursors (Jiang and Hartung, 2008; Yang et al., 2009), as well as by the release of volatile compounds involved in ABA sensing (Zhang et al., 2008). Fig. 1 illustrates the complexity of these hormonal interactions.

In view of this complex scenario, the role of rhizospheric microorganisms on these proteins and on root hydraulic properties has become a focus of particular interest in recent years.

4. Plant growth promoting microorganisms and root aquaporins

4.1. Arbuscular mycorrhiza (AM)

It is accepted that AM symbiosis protects host plants against the deleterious effects of water deficit as a result of physical, nutritional and cellular mechanisms (Ruiz-Lozano, 2003). The first report in English language describing the involvement of a PGPM in

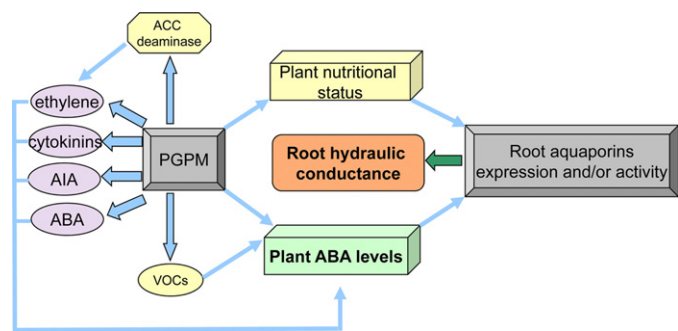


Fig. 1. Plant growth promoting microorganisms (PGPM) may affect root hydraulic conductance through a complex network of biochemical interactions. Microbial biosynthesis of plant hormones and microbial-mediated regulation of endogenous plant hormone levels, particularly of abscisic acid, which in turn is involved in aquaporin gene expression, seems to be of capital relevance in these processes. Additionally, changes in the nutritional status of colonized plants and volatile organic compounds (VOCs) released by certain microorganisms may directly or indirectly impact on root aquaporins expression and/or activity, and therefore, on root hydraulic conductance.

aquaporin biosynthesis was published by Roussel et al. (1997). In this article, *pcrb7* mRNA, a gene encoding a member of the widespread family of membrane intrinsic proteins, accumulated in parsley roots colonized by the symbiotic AM fungus *Glomus fasciculatum*. Interestingly, this accumulation took also place in suspension-cultured cells of parsley following treatment with an elicitor from the phytopathogenic fungus *Phytophthora sojae*.

Northern blot analyses performed by Krajinski et al. (2000) found induced expression of *Mtrb7-2* in *Medicago truncatula* roots upon inoculation with the AMF *Glomus mosseae*. This gene codifies a tonoplast aquaporin (*Mtaqp1*), whose *in vivo* expression in *Xenopus* oocytes demonstrated high selectivity for water (while did not facilitate glycerol uptake) and insensitivity to mercury, a rather unusual feature for aquaporins (Daniels et al., 1994; Biela et al., 1999).

These initial works were performed under well watered conditions. Siefert et al. (2002) provided evidence that tobacco plants impaired in NtAQP1 expression, a plasma membrane aquaporin preferentially expressed in roots, had reduced root hydraulic conductivity and lower water stress resistance under drought stress. Going ahead in the study of this aquaporin and its involvement in plant water flow, Porcel et al. (2005) investigated the behavior of NtAQP1 antisense tobacco plants uninoculated or inoculated with two AMF (*G. mosseae* and *G. intraradices*) under both, drought stress and well-watered conditions. Under drought stress, the increase in shoot dry weight due to inoculation was more pronounced in wild type plants (8-fold) than in antisense plants (4-fold), for which they concluded that water transport mediated by NtAQP1 may be important for the efficiency of this symbiosis under drought.

Though plant aquaporins were extensively characterized, the physiological relevance of these water channels in the response of mycorrhized and non-mycorrhized plants to water restrictions remains elusive. For instance, Porcel et al. (2006) found that lettuce and soybean plants mycorrhized by *G. mosseae* and *G. intraradices* responded to drought stress by down-regulating the expression of some PIP genes more severely and faster than non-mycorrhized plants, achieving less negative water potential and higher relative water contents. However, Alguacil et al. (2009) reported that the increase in leaf relative water content observed in lettuce plants inoculated with *G. intraradices* grown under drought and high carbon dioxide concentrations was coupled to an enhanced expression of *PIP2*.

In an earlier work, it was proposed that decreased expression of plasma membrane aquaporin genes during drought stress can

be a regulatory mechanism to limit water loss from cells (Barriue et al., 1999). On the other hand, Ouziad et al. (2006) found reduced transcript levels of both a tonoplast and a plasmalemma aquaporin gene in the roots of *Lycopersicon esculentum* colonized by a mixture of *Glomus geosporum* and *Glomus intraradices*, with this reduction being even greater in plants living under sustained salt stress (0.8% NaCl). Interestingly, AMF colonization resulted in drastic increases of transcript levels of the three aquaporin genes analyzed (*LePIP1*, *LePIP2* and *LeTIP*) in tomato leaves upon salt stress, while genes encoding two Na^+/H^+ transporters were unaffected.

In line with the results of Ouziad and coworkers, Aroca et al. (2007) found that Lp of *Phaseolus vulgaris* mycorrhized plants under control conditions was about half that of non-AM plants, and that this parameter decreased as a result of drought, cold or salinity in non-AM plants, while it remained almost unchanged in AM plants. Linked to these results they found differential expression of various PIP genes, suggesting specific functions and regulation mechanisms for each PIP gene under different environmental stresses. In agreement with such uneven responses, earlier findings obtained by quantitative real-time reverse transcription-PCR analysis in *Arabidopsis thaliana* demonstrated that only one of a set of 13 PIP genes was up-regulated by cold treatment (most of them were down-regulated by this stress), while marked up- or down-regulation of PIPs were observed after drought stress and a less severely modulation was detected under high salinity (Jang et al., 2004). It should also be mentioned that overexpression of a plasma membrane aquaporin in transgenic tobacco improved plant vigor under favorable conditions, but not under drought or salt stress (Aharon et al., 2003).

It has been reported that under drought conditions AM symbiosis regulates ABA content (Goicoechea et al., 1997; Ludwig-Müller, 2000; Estrada-Luna and Davies, 2003) and expression of some host plant aquaporin genes (Porcel et al., 2006; Ruiz-Lozano et al., 2006; Aroca et al., 2007, 2008a,b). At the same time, abscisic acid has been found to be necessary for arbuscular development (Herrera-Medina et al., 2007).

According to results obtained by Aroca et al. (2008b), AM plants regulate better and faster their ABA levels than non-AM plants, allowing a more adequate balance between leaf transpiration and root water movement during drought and recovery. Ruiz-Lozano et al. (2009) found that application of exogenous ABA to maize plants enhanced Lp regardless of water conditions, but plants colonized by *Glomus intraradices* showed lower Lp values than non-mycorrhized plants; and these values were even lower when mycorrhized plants were supplied with exogenous ABA. This effect clearly correlated with the accumulation pattern of PIPs, most of which reduced their expression and protein level in AM plants fed with ABA.

Far less known, though not less important, is the possible contribution of plant aquaporins to ammonia transfer in plants living in mycorrhizal symbiosis. As significant nitrogen transfers from the fungus to the plant occur during this interaction (He et al., 2003), it was proposed that aquaporins could be at least a component of the low affinity ammonium transport system (Uehlein et al., 2007). Ammonia permeability of tonoplast intrinsic aquaporins from *Triticum aestivum* and *Arabidopsis thaliana* was communicated some years ago (Jahn et al., 2004; Loque et al., 2005). On the other hand, the work performed by Niemietz and Tyerman (2000) indicates that ammonia might potentially permeate through nodulin 26, a major NIP protein of the peribacteroid membrane, a membrane type that was functionally linked to the periarbuscular membrane (Uehlein et al., 2007).

The finding of Dynowski et al. (2008) that plasma membrane water channels may facilitate the diffusion of H_2O_2 , a key signalling molecule under both biotic and abiotic stresses, has opened interesting new viewpoints. Of note is the recent discovery that

exogenous application of H₂O₂ led to a significant enhancement of root hydraulic conductance in *Phaseolus vulgaris*; and that the patterns of PIP gene expression in AM and non-AM roots exposed to H₂O₂ changed in opposite directions, observation that was related to the well recognized antioxidant capacity of AM roots (Benabdellah et al., 2009).

4.2. Ectomycorrhiza (EM)

The role of ectomycorrhiza in plant water uptake and distribution is not clear. Although it is generally accepted that EM symbiosis increases growth and plant survival under salinity and water stress, some studies found little or no effect of ectomycorrhiza on root water transport and/or plant water relations (Diebolt and Mudge, 1987; Coleman et al., 1990). Negative effects on root hydraulic conductivity and water uptake under ectomycorrhizal symbioses have also been reported (Nardini et al., 2000; Calvo-Polanco et al., 2008).

It has been postulated that mycorrhiza can affect the cell-to-cell pathway at least through effects on plant aquaporin expression (Letho and Zwiazek, 2011); however, only a few reports specifically dealing with ectomycorrhiza, plant hydraulic conductance and water channels could be identified for this revision.

In order to assess to what extent water channel transport could account for the increased root water flows in plants living in symbiosis with ectomycorrhizal fungi, Muhsin and Zwiazek (2002) undertook an indirect approach and analyzed the effect of different temperatures and HgCl₂ addition on several hydraulic parameters of American elm (*Ulmus americana*) roots colonized or non colonized by the ectomycorrhizal fungus *Hebeloma crustuliniforme*. While decreasing temperatures led to reductions of root hydraulic conductance in both mycorrhized and non-mycorrhized seedlings, this parameter exhibited higher values in mycorrhized roots at all temperatures. On the other hand, HgCl₂ had greater impact on root hydraulic conductance of non-mycorrhized as compared to mycorrhized roots, for which these authors suggested that EM increased hydraulic conductance of roots by decreasing water flow resistance of the apoplast, rather than by water channel-mediated transport. It should be mentioned, however, that the use of mercury-derivatives to get evidence of aquaporins involvement has been objected, since these compounds target cysteine residues in aquaporins but, unfortunately, in other cellular proteins as well (Maurel and Chrispeels, 2001).

Some years later, Marjanović et al. (2005a) reported increased root transcript levels of three poplar putative water channels belonging to the PIP2 group in the hybrid poplar *Populus tremula* × *tremuloides* inoculated with the ectomycorrhizal fungus *Amanita muscaria*, and found about 57% larger water transport capacity in mycorrhized plants than in non-mycorrhized plants. Interestingly, these authors analyzed separately main roots and fine roots, and observed different patterns of aquaporin expression between these categories, with distinct responses to mycorrhizal inoculation. In a later study (Marjanović et al., 2005b), the influence of drought on the expression of five genes encoding putative aquaporins was surveyed. In fine roots of drought-stressed plants, mycorrhization resulted in increased transcript amounts of two of those genes (*PttPIP2.2* and *PttPIP2.4*), and again, it was concluded that there are specific patterns of aquaporin gene expression in main and fine roots, with a more stable aquaporin gene expression in fine roots under drought stress.

Water transport properties in whole roots and root cells of jack pine (*Pinus banksiana* Lamb.) seedlings inoculated with the ectomycorrhizal fungus *Suillus tomentosus* was recently investigated by Lee et al. (2010). These authors reported twofold to fourfold increases in hydraulic conductivity of root cortical cells and whole root systems in mycorrhized seedlings as compared to non-mycorrhized plants, with HgCl₂ having greater inhibitory

effect on the first group. This observation led them to propose that aquaporin-mediated water transport was largely responsible for the differences observed. Hydraulic conductivity of root cortical cells was significantly reduced by 50 mM NaCl and, whereas this decline was followed by a quick recovery to the pre-treatment level in mycorrhized seedlings, reduction progressed over time in non-mycorrhized seedlings.

4.3. Trichoderma

Trichoderma species are saprophytic inhabitants of soil, but some may thrive as opportunistic, avirulent plant symbionts (Harman et al., 2004). Many *Trichoderma* isolates behave as effective BCA in different plant species. Only one research group working on the effects of *Trichoderma* on *Theobroma cacao* seedlings published results suggesting a possible link between aquaporin regulation and plant water status in plants inoculated with different *Trichoderma* species (Bailey et al., 2006). Using quantitative real-time reverse transcription PCR, Bae et al. (2009) analyzed changes in expressed sequence tags (ESTs) in cacao seedlings inoculated with an isolate of *Trichoderma hamatum* (DIS 219b). This analysis revealed altered expression of 19 ESTs after water stress, 7 in leaves and 17 in roots. DIS 219b colonization delayed the drought-altered expression of all seven ESTs responsive to drought in leaves by at least 3 days, but had less influence on the expression pattern of the drought-responsive ESTs in roots. One of those drought-responsive ESTs obtained from roots, designated as *TcTIP* and putatively encoding a tonoplast intrinsic protein, was repressed in cacao roots after imposing water stress both in uninoculated and inoculated plants. This repression was interpreted as a way to reduce membrane water permeability and encourage water conservation during periods of drought, as earlier suggested (Luu and Maurel, 2005; Secchi et al., 2007).

Other mechanisms of drought and salinity alleviation, which were explained by the abilities of these PGPM to reprogram plant gene expression, probably through activation of a limited number of general plant pathways, have been ascribed to *Trichoderma* and other biocontrol fungi (Shoresh et al., 2010). Among them, it was reported enhanced synthesis of osmotin-like proteins and up-regulation of drought stress-related genes, including marker genes involved in drought stress tolerance, like RD29A and ERD1 (Alfano et al., 2007; Sherameti et al., 2008).

4.4. Plant growth promoting rhizobacteria

Contribution of rhizospheric bacteria to plant tolerance against drought and salinity has been largely documented, and most of the mechanisms that may account for these effects were enumerated and described in recent revisions (Yang et al., 2009; Aroca and Ruiz-Lozano, 2009a; Dimkpa et al., 2009). However, direct relationships between colonization of roots by PGPR and plant water channels have been far less explored, with only four papers published to date and just two exclusively dealing with rhizobacteria.

Although rhizobia constitute one of the best characterized PGPR groups, and despite the fact that nodulin-26 – a water channel isolated from the peribacteroid membrane of soybean nodules – was the first plant aquaporin to be identified (Maurel et al., 2008), only one report provides some experimental data suggesting an effect of rhizobia on PIP expression (Porcel et al., 2006). In this research, the expression profile of two soybean aquaporins, *GmPIP1* and *GmPIP2*, was investigated in the roots of *Glycine max*, considering as variable factors water availability and mycorrhization. Drought stress reduced the expression of both genes, while inoculation with *Bradyrhizobium japonicum* had little effect, except for *GmPIP2*, whose transcript levels were significantly reduced in well watered non-mycorrhized soybean plants. The meaning of PIPs in

Table 1
Aquaporin expression in plants colonized by plant growth promoting microorganisms.

PGPM	Microorganism	Plant species	Gene	Experimental approach	Stress	Main observation	Reference
AMF	<i>Glomus fasciculatum</i> <i>Glomus mosseae</i> <i>G. mosseae</i> <i>Glomus intraradices</i>	<i>Petroselinum crispum</i> (parsley)	<i>pcrb7</i> (TIP)	RT-PCR. Northern blot		Accumulation of transcripts	Roussel et al. (1997)
		<i>Medicago truncatula</i> <i>Nicotiana tabacum</i> (tobacco)	<i>Mtrb7-2</i> (TIP) <i>NtAQP1</i> (PIP)	Northern blot Response of antisense plants	Drought	Accumulation of transcripts Antisense plants more affected than WT under drought	Krajinski et al. (2000) Porcel et al. (2005)
		<i>Glycine max</i> (soybean)	<i>GmPIP2</i> <i>GmPIP1</i>	Northern blot. Real-time PCR	Drought	Earlier and more pronounced downregulation with <i>G. mosseae</i> under drought	Porcel et al. (2006)
		<i>Lactuca sativa</i> (lettuce)	<i>LsPIP1</i> <i>LsPIP2</i>				
	<i>Glomus geosporum</i> <i>G. intraradices</i> (in mixture)	<i>Lycopersicon esculentum</i> (tomato)	<i>LePIP1</i> <i>LePIP2</i> <i>LeTIP</i>	Northern blot. Real-time PCR	Salinity	<i>LePIP1</i> and <i>LeTIP</i> downregulated in roots and upregulated in leaves under salinity. <i>LePIP2</i> : unaffected in roots	Ouziad et al. (2006)
	<i>G. intraradices</i>	<i>Phaseolus vulgaris</i> (common bean)	Several PIPs	Northern blot	Drought salinity cold	Lp decreased upon inoculation or stresses; almost unchanged by stresses in mycorrhized plants. PIPs affected with different patterns	Aroca et al. (2007)
		<i>Lycopersicon esculentum</i> (tomato)	Several PIPs	Response of an ABA-defective mutant cultivar (<i>sitiens</i>). Northern blot	Drought	PIPs affected with different patterns	Aroca et al. (2008a)
		<i>Lactuca sativa</i> (lettuce)	<i>LsPIP2</i>	Response to exogenous ABA addition. Northern blot Real-time PCR	Drought	Higher Lp in mycorrhized plants; faster recovery than uninoculated plants; downregulation of <i>LsPIP2</i> by mycorrhization and by ABA Upregulation upon inoculation or/and under drought	Aroca et al. (2008b) Alguacil et al. (2009)
		<i>Zea mays</i> (maize)	3 PIP1 3 PIP2	Response to exogenous ABA addition. Real-time PCR	Drought	Lp increased after ABA addition and decreased upon inoculation or drought. Most PIPs were downregulated in mycorrhized plants	Ruiz-Lozano et al. (2009)
	EMF	<i>Hebeloma crustuliniforme</i>	<i>Ulmus americana</i> (american elm)		Different temperatures and addition of HgCl ₂		Greater Lp in mycorrhized plants. AQP apparently most involved
<i>Amanita muscaria</i>		<i>Populus tremula x tremuloides</i> (poplar)	Several PIPs	Semiquantitative RT-PCR		Upregulation of some PIPs Increased Lp in mycorrhized roots	Marjanović et al. (2005a)
Not specified					Drought	Upregulation of some PIPs in mycorrhized plants; more stable aquaporin gene expression in fine roots under drought	Marjanović et al. (2005b)
<i>Suillus tomentosus</i>		<i>Pinus banksiana</i> (jack pine)		Addition of HgCl ₂		Greater Lp in root cortical cells of mycorrhized plants. AQP-mediated water transport may be important	Lee et al. (2010)
<i>Trichoderma</i>	<i>Trichoderma hamatum</i> (strain DIS 219b)	<i>Theobroma cacao</i> (cacao)		Real-time PCR. Analysis of DR-ESTs including <i>TcTIP</i> , a putative tonoplastic AQP	Drought	Inoculation delayed altered expression of several DR-ESTs; little effect on DR-ESTs in roots	Bae et al. (2009)
PGPR	<i>Bradyrhizobium japonicum</i>	<i>Glycine max</i> (soybean)	<i>GmPIP2</i> <i>GmPIP1</i>	Northern blot. Real-time PCR	Drought	<i>GmPIP2</i> expression reduced under WW conditions; <i>GmPIP1</i> expression unaffected	Porcel et al. (2006)
	<i>Pseudomonas mendocina</i> <i>Bacillus megaterium</i>	<i>Lactuca sativa</i> (lettuce) <i>Zea mays</i> (maize)	<i>LsPIP2</i> 3 PIP1 3 PIP2	Real-time PCR Real-time PCR	Drought Salinity	Upregulation only under WW conditions Lp increased in inoculated plants. Salinity upregulated most PIPs. Inoculation upregulated all PIP2. <i>ZmPIP2;1</i> extremely downregulated in inoculated plants exposed to salt	Alguacil et al. (2009) Marulanda et al. (2010)
	<i>Azospirillum brasilense</i> (strain Az39)	<i>Hordeum vulgare</i> (barley)	<i>HvPIP2,1</i>	Semiquantitative RT-PCR	Salinity	Upregulation of <i>HvPIP2,1</i> by inoculation and salt	Zawoznik et al. (2011)

AQP: aquaporin; DR-ESTs: drought-responsive expressed sequence tags; Lp: root hydraulic conductance; WT: wild type; WW: well watered.

terms of biological nitrogen fixation, if any, has not been addressed. However, since aquaporins may also facilitate the passage of small neutral molecules and gases including urea, ammonia or CO₂ (Luu and Maurel, 2005), it has been suggested that regulation of PIP genes might also be operating in the rhizobial symbiosis under well-watered conditions (Porcel et al., 2006).

The effect of the PGPR *Pseudomonas mendocina* on lettuce plants growing under drought and elevated CO₂ concentrations was compared to that of the AM *Glomus intraradices* (Alguacil et al., 2009). Drought decreased expression of *PIP2* (a gene particularly active in water transport across plasma membranes, according to previous works) in all treatments. Inoculation with *P. mendocina* improved relative water content and contributed to alleviation of drought stress, though this was not related to an increase of *PIP2* gene expression, as it happened in the case of *Glomus intraradices* inoculated plants. Conversely, *PIP2* expression was significantly enhanced by *P. mendocina* only under non-limiting water conditions. In an earlier study, this rhizobacterium had shown positive effects on the metabolism of reactive oxygen species in lettuce plants exposed to severe drought stress, stimulating the activities of antioxidant enzymes and increasing proline accumulation in leaves (Kohler et al., 2008).

Recently, Marulanda et al. (2010) focused on the influence of a *Bacillus megaterium* strain on root hydraulic properties and aquaporin regulation in maize plants growing under salt stress (150 mM NaCl). The strain used had previously been shown to promote clover growth under drought conditions (Marulanda et al., 2009). A set of six genes encoding plasma membrane aquaporins highly expressed in roots were analyzed by quantitative real-time PCR. Inoculated plants had higher root hydraulic conductance and displayed greater relative water contents and fewer necrotic areas in their leaves after salt treatment than uninoculated plants. On the other hand, *B. megaterium* inoculation resulted in up-regulation of *ZmPIP2;1*, *ZmPIP2;5*, and *ZmPIP2;6* genes in roots, while *ZmPIP1;1* and *ZmPIP1;5* were mostly up-regulated by salt treatment. Increments in PIP protein amount could be verified only in salt treated plants. Of note is that *ZmPIP2;1* was up-regulated by salt addition in uninoculated roots and extremely down-regulated (undetected) in inoculated roots. These authors concluded that differential changes in PIP expression patterns and abundance might partly explain the differences observed regarding salt stress tolerance and inoculation.

The ability of the PGPR *Azospirillum* to alleviate drought, salinity and osmotic stress was extensively investigated and documented, and several mechanisms (apart from the well recognized feature of more developed root systems in inoculated plants) have been proposed to explain this behavior. In 1992, Sarig et al. informed a 25–40% increase in hydraulic conductivity of inoculated *Sorghum bicolor* root systems over non-inoculated controls. Changes in ion selectivity at root level leading to higher K⁺/Na⁺ ratios, changes in the saturation patterns of membrane phospholipids, bacterial-induced changes in the elasticity of root cell membranes, and osmotic adjustments involving higher levels of compatible solutes are some of the mechanisms documented in connection to these mitigating effects under drought and salinity (Bashan et al., 1992; Creus et al., 2004; Hamdia et al., 2004).

Plant performance and aquaporin expression of *HvPIP2,1* gene in the roots of *Azospirillum*-inoculated barley seedlings growing under non-stressed conditions or under salt stress (200 mM NaCl) was recently assessed (Zawoznik et al., 2011). *HvPIP2,1* codifies *HvPIP2,1* protein, the root aquaporin most expressed at RNA level and apparently the most involved in root hydraulic conductance in barley seedlings (Katsuhara and Shibasaki, 2007). Seedlings were inoculated with *Azospirillum brasilense* Az39, a strain for which *in vitro* ABA production has been demonstrated (Perrig et al., 2007). The growth rate of uninoculated plants was more adversely affected

by saline treatment than that of inoculated seedlings; this was associated to higher putrescine contents and lower levels of *HvPIP2;1* transcripts in the roots. Of note is that *Azospirillum* inoculation itself triggered the transcription of this PIP aquaporin. Table 1 summarizes the experimental data provided along item 4.

5. Final remarks

The availability of powerful molecular tools generated by recent advances in molecular biology in conjunction with the new “-omics” approaches have permitted considerable progress in the knowledge of the mechanisms underlying the phenomenon of alleviation of abiotic stresses by PGPM, which Yang et al. (2009) proposed to designate as ‘induced systemic tolerance’, in analogy to the well-known concept of induced systemic resistance. The number of plant–microbe associations in which this protective effect was documented has been growing over time, and caught the attention of several research groups worldwide.

Many PGPM-induced physical and chemical changes that lead to increased root hydraulic conductivity and enhanced drought and salinity tolerance have already been reported. Many others, like those related to the regulation of root aquaporins, an effect that is probably mediated by complex hormonal mechanisms in which plant ABA levels may play a central role, are just beginning to emerge. In the last decade, considerable advances in this field have been achieved for arbuscular mycorrhizal symbioses; we hope that in the near future this encouraging trend could reach other plant–microbe interactions of relevance in terms of plant productivity.

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