Evaluation of the Possible Participation of Drought-induced Genes in the Enhanced Tolerance of Arbuscular Mycorrhizal Plants to Water Deficit

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1 Introduction

Drought stress is considered one of the most important abiotic factors that interferes with normal development and growth of plants, having a major adverse effect on plant survival and productivity (Kramer and Boyer 1997; Bray 2004). However, plants can respond to drought stress at morphological, anatomical and cellular levels with modifications that allow the plant to avoid the stress or to increase its tolerance (Bray 1997). The morphological and anatomical adaptations can be of vital importance for some plant species, but they are not a general response of all plant species. In contrast, the cellular responses to water deficit seem to be conserved in the plant kingdom. Plants can adapt to water deficit by the induction of specific genes such as genes encoding proteins involved in the biosynthesis of osmoregulatory compounds, genes encoding late embryogenesis abundant (LEA) proteins, genes encoding proteins with chaperone activity such as proteins 14-3-3 or binding proteins (BiPs), as well as modulating the expression of genes encoding aquaporins (AQP) (Zhu et al. 1997; Alvim et al. 2001; Zhu 2002; Wang et al. 2003; Bray 2004; Luu and Maurel 2005).

In addition to the intrinsic protective systems of plants against water deficit, most terrestrial plants can also establish a symbiotic association with arbuscular mycorrhizal (AM) fungi. The AM symbiosis is present in all natural ecosystems, even in those affected by adverse environmental conditions (Smith and Read 1997). A number of studies have demonstrated that the AM symbiosis can protect the host plants against the detrimental effects of drought stress (Augé 2001, 2004; Ruiz-Lozano 2003). These studies have suggested several mechanisms by which the AM symbiosis can alleviate drought stress in host plants. The most important are: direct uptake and transfer of water through the fungal hyphae to the host plant (Hardie 1985; Ruiz-Lozano and Azcón 1995; Marulanda et al. 2003), better osmotic adjustment of AM plants (Augé et al. 1992; Ruiz-Lozano et al. 1995; Kubikova et al.

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2001), enhancement of plant gas exchange (Augé et al. 1992; Ruiz-Lozano et al. 1995; Goicoechea at al. 1997; Green et al. 1998), changes in soil water retention properties (Augé et al. 2001), and protection against the oxidative damage generated by drought (Ruiz-Lozano et al. 1996, 2001; Porcel et al. 2003; Porcel and Ruiz-Lozano 2004).

The understanding of the water relations of AM plants and the physiological processes involved in the enhanced tolerance of mycorrhizal plants to water limitation has increased in recent years. However, there are still many unknown aspects which must be elucidated, mainly at the molecular level (Ruiz-Lozano 2003). For that reason, our research group has initiated an investigation aimed at evaluating the possible participation of drought-induced genes in the enhanced tolerance of AM plants to drought stress. The following sections present a discussion on the most important results obtained.

2 Late Embryogenesis Abundant Proteins

These are a group of proteins that accumulate in plant seeds during their maturation phase, when tolerance to desiccation is required (Close 1996). It has been demonstrated that late embryogenesis abundant (LEA) proteins also accumulate in vegetative plant tissues during periods of water deficit, which reinforced a role for these proteins as desiccation protectant (Moons et al. 1997). It seems that during cellular dehydration LEA proteins play an important role in maintenance of the structure of other proteins, vesicles or endomembrane structures in the sequestration of ions such as calcium, in binding or replacement of water, and functioning as molecular chaperones (Close 1996; Koag et al. 2003). The overexpression of LEA proteins in plants and yeast confers tolerance to osmotic stresses (Imai et al. 1996; Xu et al. 1996; Babu et al. 2004). Dehydrins are an important group of LEA proteins (LEA group 2). They represent the most conspicuous soluble proteins induced by a dehydration stress and have been observed in over 100 independent studies of drought stress, cold acclimation, salinity stress, embryo development and responses to ABA. Among the water-stress-induced proteins so far identified, dehydrins are the most frequently observed (Close 1997; Cellier et al. 1998). Dehydrins could play a fundamental role in the dehydration response of plants to a range of environmental and developmental stimuli (Close 1996). However, the existence of multiple targets for dehydrins (euchromatin, cytosol, cytoskeleton) suggest that the direct consequences of dehydrin activity are biochemically diverse.

As these proteins seems to be part of the universal plants responses against dehydration, it is of interest to determine whether the AM symbiosis is able to alter the pattern of dehydrin accumulation under drought stress and whether such possible alteration functions in the protection of the host plants against drought. As a first approach, we cloned two dehydrin-encoding genes from *Glycine max* (*gmlea 8* and *gmlea 10*) and one from *Lactuca sativa* (*lslea 1*) and analyzed their contribution to the response against drought in mycorrhizal soybean and lettuce plants.

The genes *gmlea* and *lslea* were only expressed in drought stressed treatments (Figs. 1 and 2), corroborating that these dehydrins are important for the plant response against drought stress (Cellier et al. 1998; Giordani et al. 1999). In lettuce plants, *lslea 1* gene was also induced by drought stress in the three treatments

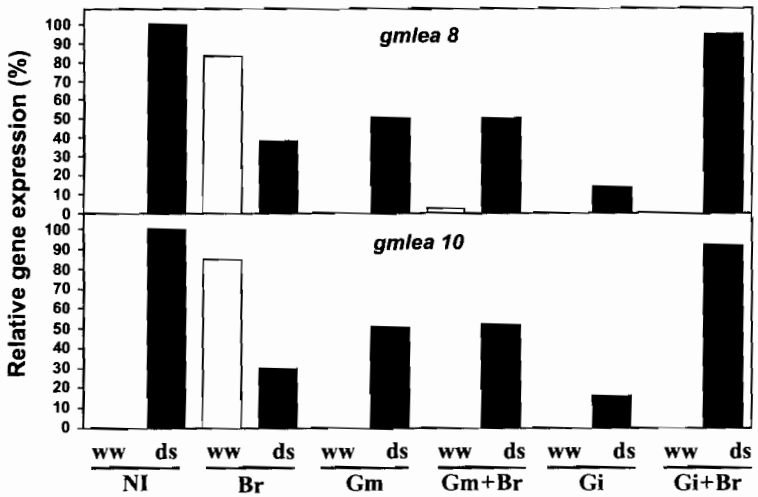

Fig. 1 Relative expression in soybean roots of *gmlea8* and *gmlea10* genes. Values were obtained after normalization of northern blots to the ribosomal RNA loaded into each membrane. Treatments are designed as *NI* noninoculated controls, *Br Bradyrhizobium japonicum*, *Gm Glomus moosseae*, *Gm+Br G. mosseae* plus *B. japonicum*, *Gi Glomus intraradices*, *Gi+Br G. intraradices* plus *B. japonicum*. Plants were either well-watered (*ww*) or drought stressed (*ds*) for 10 days. Reproduced from Porcel et al. (2005b), with permission from Oxford University Press

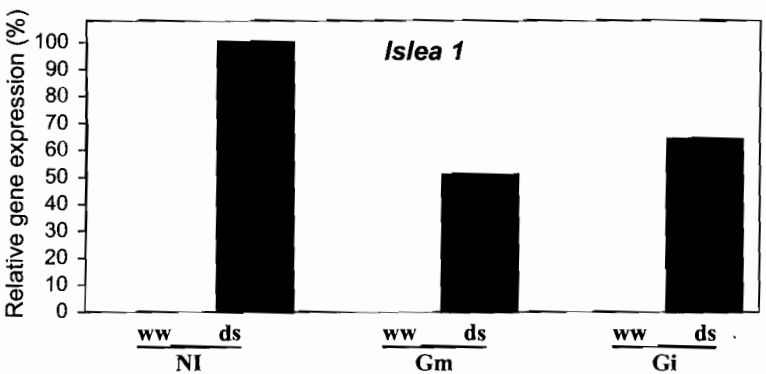

Fig. 2 Relative expression in lettuce roots of *lslea 1* gene. Values were obtained after normalization of northern blots to the ribosomal RNA loaded into the membrane. Treatments are designed as *NI* noninoculated controls, *Gm Glomus moosseae*, *Gi Glomus intraradices*. Plants were either well-watered (*ww*) or drought stressed (*ds*) for 10 days. Reproduced from Porcel et al. (2005b), with permission from Oxford University Press

assayed (Fig. 2). However, the level of induction was clearly higher in roots from noninoculated plants than in roots from both AM treatments.

Another effect observed in the case of soybean (but not of lettuce) was the lower *gmlea* gene expression in roots from plants colonized by *G. intraradices* alone compared to that of plants colonized by *G. mosseae* alone (Fig. 1). However, functional diversity among different AM fungi has been widely observed in several aspects of the symbiosis (Burleigh et al. 2002).

In any case, the expression of *gmlea* and *lslea* genes in soybean and lettuce plants, respectively, was lower in drought stressed AM plants than in noninoculated plants. To understand this effect, it must be considered that abscisic acid (ABA) induces the expression of water deficit-responsive genes such as *lea* (Giordani et al. 1999). It has been proposed that mycorrhization can alter the levels of ABA in the host plant and that under drought stress the levels of ABA are lower in AM than in non-AM plants (Goicoechea et al. 1997; Estrada-Luna and Davis 2003); thus, the level of *lea* gene expression can be lower in these plants. Additionally, AM plants can be less strained by drought stress than non-AM plants and, for that reason, the expression of the *lea* genes studied is lower. In previous studies in which other authors and ourselves have found physiological or biochemical mechanisms involved in the enhanced tolerance to drought stress in AM plants, it has been proposed that primary drought-avoidance mechanisms (i.e., direct water uptake by hyphae) or increased water uptake related to mycorrhizal changes in root morphology (Kothari et al. 1990) or soil structure (Augé et al. 2001; Augé 2004) might have contributed to the AM protection of host plants against drought (Porcel et al. 2003). This hypothesis was supported by data on relative water content (RWC), which were significantly higher in AM plants than in non-AM plants. Also, previous studies with soybean plants subjected to a similar drought stress level have shown that AM plants exhibit higher leaf water potential (ψ) than non-AM plants (Porcel and Ruiz-Lozano 2004).

Concluding, results demonstrate that the levels of *lea* transcript accumulation in soybean and lettuce plants colonized by either *G. mosseae* or *G. intraradices* were considerably lower than those of the corresponding nonmycorrhizal plants, suggesting that the accumulation of LEA proteins is not a mechanism by which the AM symbiosis protects their host plant (Porcel et al. 2005b). This rather suggests that mycorrhizal plants were less strained by drought due to primary drought-avoidance mechanisms.

3 Δ^1-Pyrroline-5-Carboxylate Synthetase (P_5CS)

As a soil dries out and its water potential becomes more negative, plants must decrease their water potential to maintain a favorable water flow gradient from soil into roots. The most important mechanism to achieve such an effect, known as osmotic adjustment or osmoregulation, is to decrease the plant osmotic potential by active accumulation of organic ions or solutes (Morgan 1984). Of these metabolites, proline is probably the most widespread in plants (Yoshiba et al. 1995;

Armengaud et al. 2004). It has been shown that proline accumulates under conditions of water shortage, high salinity, chilling, heat, and heavy metal exposure. It plays a major role in osmoregulation and osmotolerance (Demir 2000). However, proline performs also an important function as a protective compatible osmolyte in scavenging of free radicals and facilitating a correction of altered redox potential by replenishment of the NADP+ supply (Hasegawa et al. 2000; Hare et al. 2003).

Accumulation of proline is mainly due to de novo synthesis, although a reduced rate of catabolism has also been observed (Kishor et al. 1995). The first two steps of proline biosynthesis are catalyzed by Δ^1-pyrroline-5-carboxylate synthetase (P5CS) by means of its γ-glutamil kinase and glutamic-γ-semialdehyde dehydrogenase activities. Subsequently, the Δ^1-pyrroline-5-carboxylate (P5C) formed is reduced by P5C reductase (P5CR) to proline (Hu et al. 1992). The rate-limiting step in this pathway is represented by the γ-glutamil kinase activity of P5CS, which is sensitive to feedback inhibition by relatively low levels of proline (Smith et al. 1984). In addition, in *Arabidopsis*, the P5CS-encoding gene is induced by drought stress, salinity and ABA, but P5CR is not (Yoshiba et al. 1995). The overexpression of the P5CS-encoding gene in transgenic tobacco plants has been shown to increase proline production and to confer tolerance of such plants to osmotic stress (Kishor et al. 1995). Hence, the P5CS-encoding gene is of key importance for the biosynthesis of proline in plants (Ábrahám et al. 2003).

Investigations carried out so far on osmoregulation in the AM symbiosis are scarce and somewhat contradictory. While some studies have shown an increase in proline accumulation in mycorrhizal plants subjected to drought (Ruiz-Lozano et al. 1995; Azcón et al. 1996; Goicoechea et al. 1998), the same studies also demonstrated that the increase in proline accumulation was quite variable depending on the AM fungus involved. For instance, while plants colonized by *G. deserticola* accumulated 120 nmol of proline per g fresh weight, plants colonized by *G. intraradices* only accumulated 41 nmol proline per g fresh weight (Ruiz-Lozano et al. 1995). It has also been shown that under low Ca in the medium AM plants accumulated more proline than nonAM plants when subjected to PEG-induced drought stress, while under high Ca in the medium this was not so (Ruiz-Lozano and Azcón 1997). On the contrary, other studies regarding drought (Ramakrishnan et al. 1988) or salt stress (Ruiz-Lozano et al. 1996) have shown a lower proline accumulation in AM plants than in nonAM ones.

Determining the expression pattern of genes such as *p5cs* in AM plants under osmotic stress conditions should provide an insight into the role of the AM symbiosis in the process of osmotic adjustment during drought stress. We cloned a P5CS-encoding gene from *Glycine max* (*gmp5cs*) and another from *Lactuca sativa* (*lsp5cs*) and analyzed their contribution to the response against drought in mycorrhizal soybean and lettuce plants. In fact, several investigations on the relationship between the expression of the key gene involved in the synthesis of proline (*p5cs*) and the accumulation of proline under water stress indicate that the level of proline in plants is mainly regulated at the transcriptional level during water stress (Hu et al. 1992; Yoshiba et al. 1995; Ábrahám et al. 2003; Armengaud et al. 2004).

Results showed that *gmp5cs* and *lsp5cs* (Figs. 3a and b, 4) genes responded to drought and were upregulated in drought-stressed treatments, suggesting that they

are important for the plant response against water deficit (Kishor et al. 1995; Hare et al. 2003; Parvanova et al. 2004). A contrasting result was obtained, however, in soybean plants singly inoculated with *B. japonicum*, where the *gmp5cs* gene showed little up-regulation in roots under drought stressed conditions (Fig. 3a). To explain this result, it must be considered that the expression of *p5cs* genes has two regulatory pathways, an ABA-dependent and an ABA-independent pathway, and that both can act simultaneously and with cumulative effects (Savoure et al. 1997; Ábrahám et al. 2003). Hence, it may be possible that nodulation itself can be affecting one of these regulatory pathways, avoiding the accumulation of *p5cs* transcripts. In contrast, the mycorrhization of nodulated plants restore, at least in part, the normal *p5cs* transcripts accumulation pattern by compensating in some way such ABA-dependent and ABA-independent pathways.

In contrast, in nodules of plants singly inoculated with *B. japonicum*, the pattern of *gmp5cs* gene expression was the expected one (Fig. 3b), namely upregulation under drought stress conditions. An elevated rate of proline biosynthesis in nodules has been suggested to stimulate ureide synthesis in legumes and to help transfer redox potential from the nodule cytoplasm to the bacteroids (Kohl et al. 1988). Proline may also act as a carbon and nitrogen source for the bacteroids. An additional an

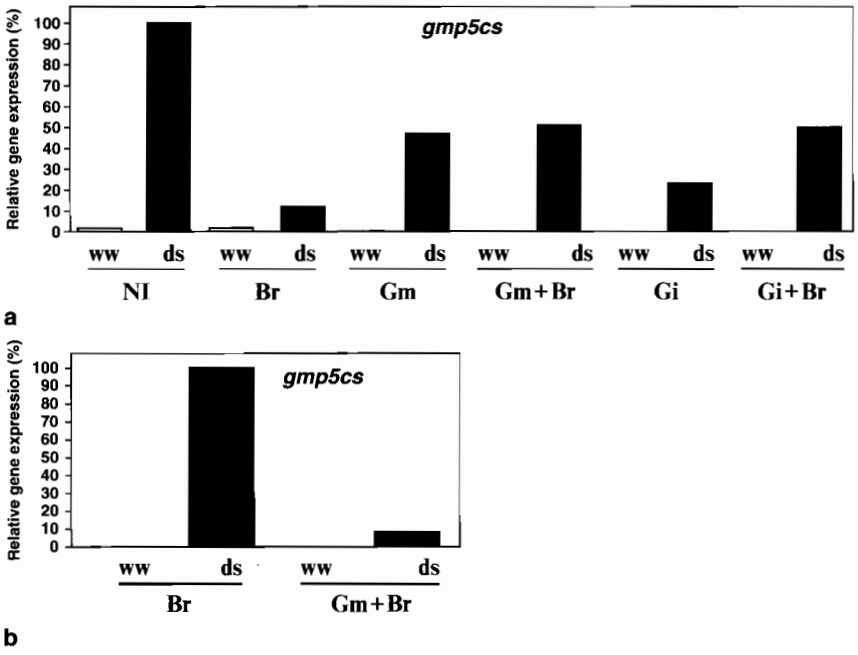

Fig. 3 Relative expression in soybean roots (**a**) and nodules (**b**) of *gmp5cs* gene. Values were obtained after normalization of northern blots to the ribosomal RNA loaded into each membrane. Treatments are designed as in Fig. 1. Reproduced from Porcel et al. (2004), with permission from Elsevier

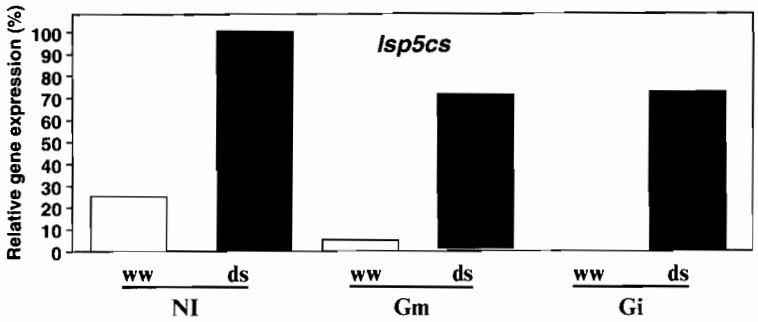

Fig. 4 Relative expression in lettuce roots of *lsp5cs* gene. Values were obtained after normalization of northern blots to the ribosomal RNA loaded into each membrane. Treatments are designed as in Fig. 2. Reproduced from Porcel et al. (2004), with permission from Elsevier

important role of proline in nodules may be its involvement in osmoregulation (Delauney and Verma 1993). In fact, the osmoticum in infected nodule cells is known to be 4- to 5-fold higher than in root cells (Verma et al. 1978). Hence, the upregulation of *gmp5cs* in nodules of droughted plants may represent an osmoregulatory adaptation to increased concentration of solutes. What is not explained by that hypothesis is why the expression of the *gmp5cs* gene in nodules from soybean plants dually inoculated with the *G. mosseae* and with *B. japonicum* was considerably lower than in the corresponding nonmycorrhizal plants. However, under drought stress, AM plants normally present lower levels of ABA (Goicoechea et al. 1997; Estrada-Luna and Davies 2003). Hence, an ABA-dependent regulation pathway could explain the decrease in *gmp5cs gene* expression in nodules of these double inoculated plants as compared to those of single nodulated soybean plants. This mechanism may explain, in the same way, why the levels of *gmp5cs* and *lsp5cs* gene expression are lower in roots from droughted soybean and lettuce AM plants than in roots from droughted soybean and lettuce noninoculated plants.

Nevertheless, as also happened with *lea* genes, the expression of *gmp5cs* and *lslp5cs* genes decreased in drought stressed AM plants as compared to noninoculated plants (Figs. 3a and b, 4). This was probably due to a decrease in ABA level in AM plants and to the fact that AM plants were less strained by drought stress than nonAM plants by primary drought-avoidance mechanisms. The results demonstrate that the induction of *p5cs* gene do not seems to be a mechanism by which the AM symbiosis protects their host plant (Porcel et al. 2004).

4 Genes Encoding 14-3-3 Proteins and Binding Proteins

14-3-3 proteins are ubiquitous eukaryotic proteins that have wide-ranging regulatory functions by acting as phosphoserine/phosphothreonine-binding proteins (Roberts and de Bruxelles 2002). These proteins function in the regulation of signal

transduction pathways, generally acting as adapters, chaperones, activators or repressors (Palmgren et al. 1998), and they regulate the activities of a wide array of targets via direct protein–protein interactions. Binding of 14-3-3 proteins to a target serves either to directly regulate the activity of that protein, to affect its interactions with other protein or to modify the intracellular localization of the target (Roberts 2003). Targets for 14-3-3 include proteins involved in metabolism, signal transduction, chromatin function, ion transport, and vesicle trafficking (Roberts 2003).

14-3-3 protein family plays a central role in stress resistance, disease and growth control during the cell life-cycle (Chung et al. 1999). In the case of stress responses, support for such roles comes from the observation of changes in 14-3-3 gene expression during stress responses and from the detection of interactions between 14-3-3s and proteins with signaling or protective functions (Roberts et al. 2002; Wang et al. 2003).

The luminal binding protein (BiP) is a molecular chaperone of endoplasmic reticulum (ER) present in all kingdoms. The role of BiP in the ER is to transiently bind to unfolded proteins and to prevent intramolecular and intermolecular interactions that can result in permanent misfolding or aggregation, with the subsequent loss of their function (Gething and Sambrook 1992; Hendershot et al. 1996). Thus, both the increase of secretory activity and accumulation of unfolded proteins within the ER, as usually happens under abiotic stresses, result in the induction of BiP (Galili et al. 1998).

Some studies have demonstrated that overexpression of BiP genes in cultured mammalian cells and tobacco leaf protoplast attenuates ER stress caused by ionophore or tunicamycin (Laitusis et al. 1999). It is also well known that overexpression of BiP in mammalian cultured cells (Laitusis et al. 1999) prevents the induction of unfolded protein response (UPR)-induced genes and increases cell tolerance to stress, suggesting that BiP directly alleviates the ER stress. Plant BiP expression has been shown to respond to a variety of abiotic and biotic stress conditions, such as water stress, fungus infestation, insect attack, nutritional stress, cold acclimation and elicitors of the plant-pathogenesis response (Anderson et al. 1994; Kalinski et al. 1995; Figueiredo et al. 1997; Fontes et al. 1999). Furthermore, it has been demonstrated that constitutive overexpression of BiP in tobacco is enough to confer tolerance to water stress (Alvim et al. 2001).

Many studies on 14-3-3 proteins or BiPs have been carried out in plants, animals and yeasts. In contrast, there is no information about these proteins with chaperone activity in AM fungi or in the AM symbiosis. A 14-3-3 protein- and a BiP-encoding genes from *Glomus intraradices* were identified after differential hybridization of a cDNA library constructed from the fungus growing in vitro and subjected to drought stress by addition of 25% PEG 6000. Subsequently, their expression patterns were studied under drought stress in vitro and also when forming natural symbioses with different host plants.

The up-regulation of *Gi14-3-3* (Fig. 5) and *GiBiP* (Fig. 6) genes under conditions of water deficit (induced in vitro by PEG addition or in vivo by withholding plant irrigation) indicates that these fungal genes have a role in the answer of the fungus against drought. These genes are, probably, involved in the protection of

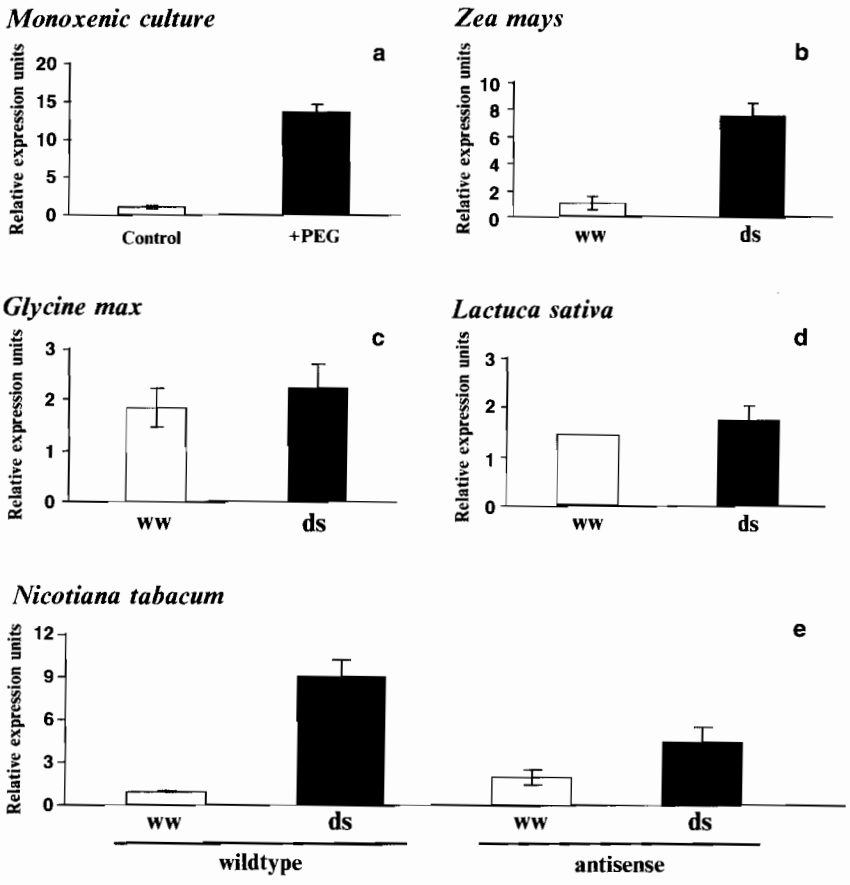

Fig. 5 Analysis of *Gi14-3-3* gene expression by real time quantitative RT-PCR in the AM fungus *G. intraradices* grown (**a**) in vitro and subjected to drought by addition of PEG (25%) to the growing medium or maintained under control conditions without PEG, or in the AM fungus *G. intraradices* during natural symbioses with maize plants (**b**), soybean plants (**c**), lettuce plants (**d**) or two tobacco plant lines (**e**), an aquaporin antisense mutant and the corresponding wild type. Plants were either well-watered (*ww*) or drought stressed (*ds*). Reproduced from Porcel et al. (2006b), with permission from Springer

the fungus itself (induction of the gene in vitro) and may be also involved in the protection of the host plant (induction of gene expression when forming natural symbiosis with plants).

It has been demonstrated that one of the effects of 14-3-3 proteins against osmotic stresses is carried out through the activation of the plasma membrane proton ATPase (Chelysheva et al. 1999; Babakov et al. 2000; Kerkeb et al. 2002). The activity of plasma membrane H^+-ATPase is highly regulated by factors that affect the cell physiology, including stress conditions (Palmgren 1998), and enhanced ATPase

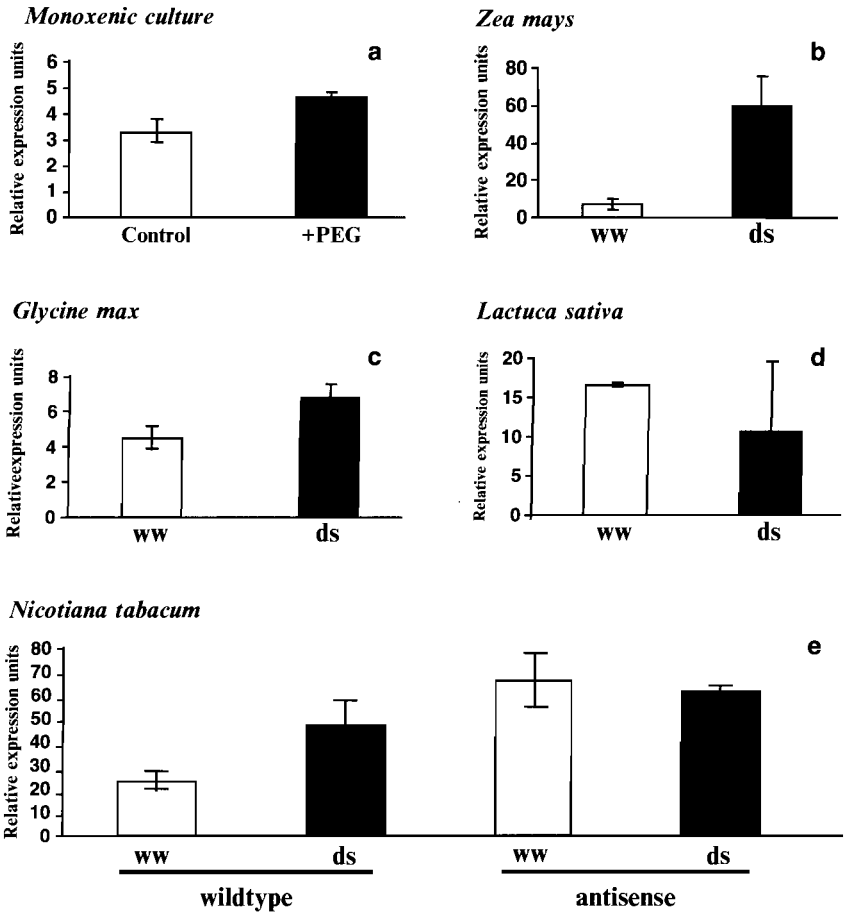

Fig. 6 Analysis of *GiBiP* gene expression by real time quantitative RT-PCR in the AM fungus *G. intraradices* grown (**a**) in vitro and subjected to drought by addition of PEG (25%) to the growing medium or maintained under control conditions without PEG, or in the AM fungus *G. intraradices* during natural symbioses with maize plants (**b**), soybean plants (**c**), lettuce plants (**d**) or two tobacco plant lines (**e**), an aquaporin antisense mutant and the corresponding wild type. Plants were either well-watered (*ww*) or drought stressed (*ds*). Reproduced from Porcel et al. (2007), with permission from Elsevier

activity is crucial for the protective system that different organisms have developed against external adverse influences (Serrano 1989). In the case of plants, the effects of 14-3-3 proteins are important for tolerance to water limitation since it has been shown that the plasma membrane H^+-ATPase plays an essential role in the regulation of plant cell turgor. In fact, it exports protons to create an electrochemical gradient across the plasma membrane, which is then used by cell as the driving force for nutrient uptake, phloem loading, water movement, stomatal closure and opening (Comparot et al. 2003). There is clear evidence for 14-3-3 mediated activation of the

H^+-ATPase in response to osmotic stresses. It has been demonstrated that osmotic stress induces a redistribution of 14-3-3 proteins between the cytoplasm and the plasma membrane of sugar beet cells. This effect is accompanied by an increase in H^+-pump activity (Chelysheva et al. 1999; Babakov et al. 2000; Kerkeb et al. 2002). Increased H^+ transport through phosphorylation and 14-3-3 binding to the proton ATPase are part of the early responses of cells to perturbation in growth conditions such as osmotic stress (Finnie et al. 2002; Kerkeb et al. 2002).

The induction of BiP mRNA by osmotic stress may represent a primary response to water stress that is activated as soon as the stress is sensed and may accommodate a regulatory function. In fact, BiP has been shown to associate with water-stress-induced proteins (Cascardo et al. 2000). The protective role of BiP against water stress may be the preservation of protein structure and of high secretory activity mediated by the water stress adaptive cellular response (Ingram and Bartels 1996).

Curiously, a non-significant effect of drought stress on *Gi14-3-3* gene expression was observed in this study when the fungus was associated to soybean plants (Fig. 5c). This result may be related to the fact that the AM colonization in these plants was considerably lower than in the rest of treatments. In any case, the induction of *Gi14-3-3* or *GiBiP* genes was more or less intense depending on the host plant (Figs. 5 and 6). This was quite evident when the fungus was associated to the two plants that are more sensitive to drought (lettuce and antisense tobacco plants) (Ruiz-Lozano et al. 1995; Porcel et al. 2005a). The varying results obtained with the different plants assayed indicate that the importance of *Gi14-3-3* and *GiBiP* when coping with drought stress may depend on the intrinsic physiological characteristics of the host plant. Hence, results suggest that Gi14-3-3 and GiBiP proteins take part of the mechanisms by which the AM symbiosis enhances the tolerance of the host plant against drought, although the real implication of these proteins may depend on the sensitivity of the host plant against water deficit (Porcel et al. 2006b, 2007).

Our findings provide new evidence that the contribution of AM fungi to the enhanced drought tolerance of the host plant can be mediated by proteins with chaperone-like activity, such as 14-3-3 or BiP proteins. However, as 14-3-3 and BiP proteins have multiple targets in the cell, the precise mechanism of Gi14-3-3- or GiBiP-mediated drought stress tolerance remains unknown. It is likely that Gi14-3-3 protein can regulate the activity of plasma membrane H+-ATPases of either the fungus or the host plant, in order to activate its pumping activity, which is essential to cope with osmotic stress (Serrano 1989; Palmgren 1998; Comparot et al. 2003), while GiBiP protein may facilitate the proper folding and maturation of water stress-induced proteins involved in the osmotic response mechanism (Cascardo et al. 2000).

5 Modulation of Aquaporins

Aquaporins are water channel proteins that facilitate and regulate the passive movement of water molecules down a water potential gradient across membranes of living cells. These proteins belong to the large major intrinsic protein (MIP) family of

transmembrane proteins and are represented in all kingdoms (Maurel 1997). Two major classes of plant aquaporins, located in the plasma membrane (PIPs) or tonoplast (TIPs), respectively, have been identified so far. Another two classes of plant aquaporins are the homologues to the soybean Nodulin-26 aquaporin (NIPs) and the small basic intrinsic proteins (SIPs) (Johanson et al. 2001). The localization and function of SIPs are unknown at the moment (Luu and Maurel 2005), although the membrane of endoplasmic reticulum seems to contain SIPs (Ishikawa et al. 2005).

The discovery of aquaporins in plants has caused a significant change in the understanding of plant–water relations. In recent years, much effort has been concentrated on investigating the function and regulation of PIP aquaporins. These aquaporins seem to play a specifically important role in controlling transcellular water transport. For instance, they are abundantly expressed in roots where they mediate most of soil water uptake (Javot and Maurel 2002), and transgenic plants downregulating one or more PIP genes had lower root water uptake capacity (Siefritz et al. 2002; Javot et al. 2003). However, the relationship that exists between aquaporins and plant responses to drought still remains elusive and with contradictory results (Aharon et al. 2003; Lian et al. 2004). Moreover, the contribution of aquaporin genes to the enhanced tolerance to drought in AM plants had never been investigated. Krajinski et al. (2000) proposed that the upregulation of aquaporins by the AM symbiosis probably optimizes nutrient and water exchange between both symbiotic partners. They may also permit efficient osmoregulation of the highly compartmented root cells (Maurel et al. 2002). However, the studies by Krajinski et al. (2000) were carried out under well-watered conditions and they did not test the expression of the aquaporin gene in AM plants under drought stress conditions. Several aquaporin-encoding genes have been shown to be upregulated in ectomycorrhizal poplar plants, and this was correlated with an increased water transport capacity of mycorrhizal poplar roots (Marjanovic et al. 2005). Finally, Porcel et al. (2005a) have shown that the impairment of a PIP gene in an antisense tobacco mutant reduced the symbiotic efficiency of two AM fungi under drought stress conditions.

Tolerance to drought stress in plants is a complex phenomenon and involves many changes at both biochemical and physiological levels (Ingram and Bartels 1996). Osmotic adjustment and modulation of tissue hydraulic conductivity are both required to maintain tissue water potential (Bohnert et al. 1995). Such mechanisms, which regulate water flux, are likely to be mediated, in part, by aquaporins (Maurel 1997). Since aquaporins are regulated both at transcriptional and activity levels (Martre et al. 2002), we have considered it of interest to study whether the expression of aquaporin-encoding genes in roots is altered by the AM symbiosis as a mechanisms to enhance host plant tolerance to water deficit. To achieve this, genes encoding plasma membrane aquaporins (PIPs) from soybean and lettuce were cloned and their expression pattern studied, in AM and non-AM plants cultivated under well-watered or drought stress conditions. If AM fungi can transfer water to the root of the host plants, it is expected that the plant must increase its permeability for water and that aquaporin genes should be upregulated in order to allow a higher rate of transcellular water flow (Javot and Maurel 2002).

Results showed that, in contrast to the above hypothesis, the PIP genes studied were downregulated both in soybean (Fig. 7) and lettuce (Fig. 8) under drought stress and that such downregulation was even more severe in plants colonized by *G. mosseae* than in non-AM plants. A similar result was obtained very recently by Ouziad et al. (2006) regarding the expression of PIP and TIP genes in roots of AM tomato plants subjected to salt stress. Furthermore, when the expression of *gmPIP2* was analyzed in a time-course, we observed that AM plants downregulated that gene significantly at 5 and 12 days after inoculation, while both non-AM control plants maintained *gmPIP2* gene expression almost unaltered until 20 days after inoculation (data not shown). This effect of the AM symbiosis anticipating the downregulation of *gmPIP2* gene may have a physiological importance to help AM plants to cope with drought stress (Porcel et al. 2006a). In fact, according to Aharon et al. (2003), the overexpression of a PIP aquaporin in transgenic tobacco improves plant vigour under favorable growth conditions, but the overexpression of such PIP gene has no beneficial effect under salt stress, and even has a negative effect during drought stress, causing fast wilting. Hence, the decreased expression of plasma

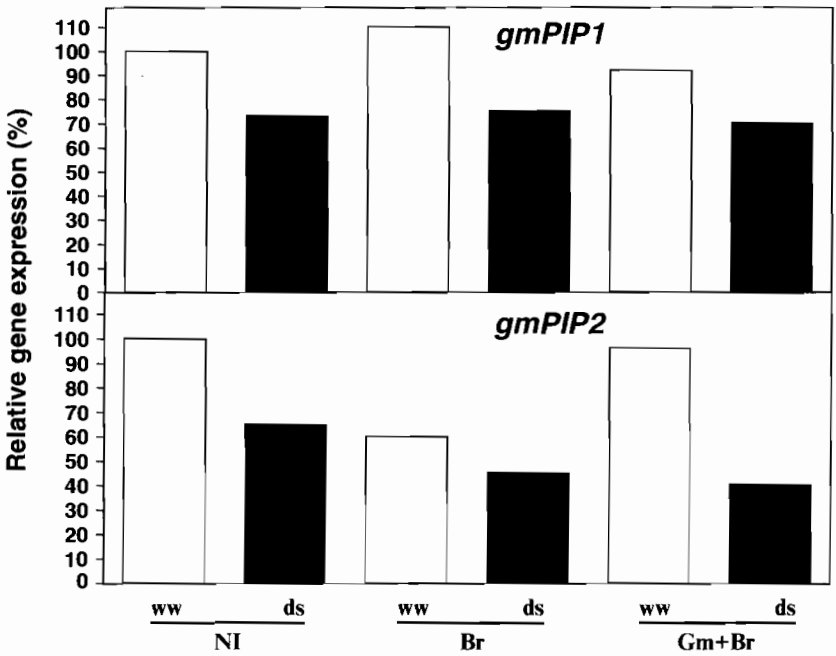

Fig. 7 Relative expression in soybean roots of *gmPIP1* and *gmPIP2* genes. Values were obtained after normalization of northern blots to the ribosomal RNA loaded into each membrane. Treatments are designed as in Fig. 1. Reproduced from Porcel et al. (2006a), with permission from Springer

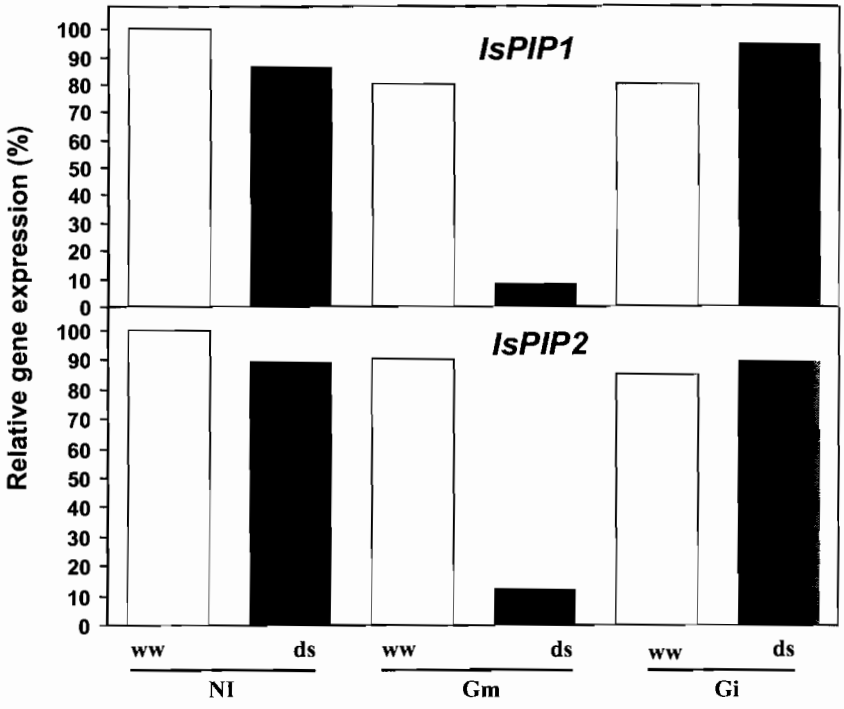

Fig. 8 Relative expression in lettuce roots of *lsPIP1* and *lsPIP2* genes. Values were obtained after normalization of northern blots to the ribosomal RNA loaded into each membrane. Treatments are designed as in Fig. 2. Reproduced from Porcel et al. (2006a), with permission from Springer

membrane aquaporin genes during drought stress in AM plants can be a regulatory mechanism to limit the water lost from the cells (Barrieu et al. 1999). In support of this hypothesis data on leaf ψ and RWC show that AM plants (soybean and lettuce) had higher leaf ψ and water content than non-AM plants.

The up- or downregulation by drought stress of mRNAs encoding aquaporins homologues has been described in the roots of many plant species (Javot and Maurel 2002). There are currently two opposite descriptions of the role of aquaporins in response to dehydration stress (Smart et al. 2001). The first is based on evidence that expression of some aquaporins is induced under dehydration stress (Barrieu et al. 1999; Jang et al. 2004), which is predicted to result in greater membrane water permeability and facilitated water transport. The second is based on the fact that aquaporin activity is downregulated under dehydration stress, which should result in decreased membrane water permeability and may allow cellular water conservation (Yamada et al. 1995; Smart et al. 2001) during periods of dehydration stress.

Results from lettuce plants also colonized by *G. mosseae* point in the same direction (Fig. 8), namely that under drought stress conditions there is a higher downregulation of the PIP genes studied (and also at the protein level, as revealed

by western blot) in AM plants than in non-AM plants. In contrast to *G. mosseae*, plants colonized by *G. intraradices* do not exhibit such downregulation of PIP gene expression or protein accumulation. The expression of PIP genes under drought stress in these plants is similar to control non-AM plants. The exact reason for the different influence of *G. mosseae* and *G. intraradices* on lettuce *PIP* gene expression is not known. However, in a previous study, also with lettuce, we evaluated the ability of six AM fungal species, including *G. mosseae* and *G. intraradices*, to enhance the amount of soil water uptake by these plants (Marulanda et al. 2003). The study demonstrated that there were substantial differences among the six AM fungi used. One of the most efficient fungi stimulating water uptake by plants was *G. intraradices*, while *G. mosseae* showed a reduced ability to improve plant water uptake. This may suggest that the strategy of both fungi to protect the host plant against water deficit is different. *G. intraradices* seems to have an important capacity to enhance the rate of water uptake by lettuce roots. This means that the water movement in these roots must be enhanced and, thus, the root water permeability must also increase, maybe by maintaining high levels of PIP aquaporin gene expression as we observe in this study. Contrarily, *G. mosseae* seems to direct its strategy for plant protection against water deficit toward the conservation of the water existing in the plant and by that reason downregulates the expression of PIP genes. Such downregulation of PIP genes has been interpreted as a mechanism to decrease membrane water permeability and to allow cellular water conservation (Yamada et al. 1995; Smart et al. 2001). In any case, both strategies seems to protect the host plant in a similar way since lettuce plants had similar RWC and leaf ψ regardless of the fungus colonizing their roots.

Concluding, results suggest that AM plants respond to drought stress by downregulating the expression of the two *PIP* genes studied and anticipating its downregulation as compared to non-AM plants, rather than by maintaining high levels of these PIP genes expression (Porcel et al. 2006a). This downregulation of *PIP* genes is likely to be a mechanism to decrease membrane water permeability and to allow cellular water conservation. It must be considered, however, that as *PIP* are members of a multi-gene family, other *PIP* isoforms in soybean and lettuce plants may be regulated differently and that depending on the AM fungus implicated in the symbiosis the patter of aquaporin gene expression can also be different.

6 Conclusions

Considering the overall results presented in this review, it is evident that there are some aspects that are more promising than others for future research on that topic. It seems that there is no sense in investigating more in deep the possible role of *lea* and *p5cs* genes. This must be considered, however, with caution since we only analyzed a few of the *lea* genes belonging to the dehydrin group, while other *lea* genes existing in plants still remains to be checked. In contrast, the evidence obtained with these two genes suggest that it is of interest to study the possible role

of ABA in the modulation of the host plant answer to water deficit by AM symbiosis. In the same way, results obtained with aquaporin genes suggest the interest in studying the correlation between up- or downregulation of aquaporin gene expression and root hydraulic conductivity and plant water status. The number of aquaporin genes analyzed should also be enhanced since aquaporins constitute a multigenic family in plants and it is likely that some of the genes not analyzed so far can be regulated by the AM symbiosis in relation to the alleviation of drought stress in the host plant. Finally, the interesting results obtained in relation to *Gi14-3-3* and *GiBiP* genes, whose function can be exerted over a wide range of targets proteins, open an interesting perspective to identify final effectors proteins responsible of the enhanced tolerance against water deficit mediated by these 14-3-3 and BiP proteins.

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