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REVIEW

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Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies

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Abstract Water deficit is considered one of the most important abiotic factors limiting plant growth and yield in many areas on earth. Several eco-physiological studies have demonstrated that the arbuscular mycorrhizal (AM) symbiosis often results in altered rates of water movement into, through and out of the host plants, with consequent effects on tissue hydration and plant physiology. It is now accepted that the contribution of AM symbiosis to plant drought tolerance is the result of accumulative physical, nutritional, physiological and cellular effects. This review considers several aspects that should be investigated at a molecular level in order to gain a whole understanding of the different mechanisms by which the AM symbiosis protects the host plants against the detrimental effects of water deficit.

Keywords Arbuscular mycorrhiza · Dehydrin · Osmotic adjustment · Osmotic stress · Oxidative stress

Introduction

In nature, plants are frequently exposed to adverse environmental conditions that have a negative effect on plant survival, development and productivity. Drought and salinity are considered the most important abiotic factors limiting plant growth and yield in many areas (Kramer and Boyer 1997). Drought and salinity share a common osmotic component (in terms of water limitation for plants) as the main factor (although not the only one in the case of salinity) responsible for their negative effects on plant development. Thus, osmotic stress is broadly used to refer to situations where insufficient water availability limits plant growth and development (Zhu et al. 1997).

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Although different plant species can vary in their sensitivity and response to the decreased water potential caused by water deficit, it may be assumed that all plants have an encoded capability for stress perception, signalling and response (Bohnert et al. 1995). Plants can respond to osmotic 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 osmotic stress seem to be conserved in the plant kingdom.

In addition to the intrinsic protective systems of plants against stress, plants grow in association with a number of soil microorganisms that can alleviate the stress symptoms. Arbuscular mycorrhizal (AM) fungi are widespread microorganisms able to establish a symbiotic association with the roots of most terrestrial plants. AM plants have an improved ability for nutrient uptake and tolerance to biotic and abiotic stresses while the fungus acquires a protected ecological niche and plant photosynthates (Smith and Read 1997). The AM symbiosis is present in all natural ecosystems, even in those affected by adverse environmental conditions (Barea and Jeffries 1995), and it can be defined as a specialized system for nutrient uptake and transfer, more efficient than roots alone (Varma and Hock 1998). Nevertheless, the physiological role of the AM symbiosis is not limited to uptake and transfer of nutrients to the host plant. Many other beneficial effects for the host plant and for ecosystems have been described (Smith and Read 1997), including enhancement of tolerance to drought or soil-borne pathogens (Sánchez-Díaz and Honrubia 1994; Ruiz-Lozano et al. 1995a; Azcón-Aguilar and Barea 1996).

Augé (2001) recently compiled the existing literature on plant water relations, drought and AM symbiosis. The aim of the present review is to open new perspectives for molecular studies that can contribute to gaining a global

understanding of the different mechanisms by which the AM symbiosis protects host plants against water deficit.

AM symbiosis and osmotic stress alleviation

Several eco-physiological studies investigating the role of AM symbiosis in protection against osmotic stresses have demonstrated that the symbiosis often results in altered rates of water movement into, through and out of host plants, with consequent effects on tissue hydration and plant physiology (Augé 2001). In the earliest work on the subject, Safir et al. (1971, 1972) concluded that the AM symbiosis probably affected the water relations of plants indirectly through improved P nutrition. The notion that AM effects on water relations had mainly a nutritional basis was prevalent for several years. Nevertheless, considering the reports during the 1990s presenting more than one time measurement of stomatal conductance or water potential, it now seems obvious that AM fungi can modify host water relations in a way entirely unrelated to improved P acquisition (Augé 2001). Moreover, it is likely that the contribution of the AM symbiosis to plant drought tolerance results from a combination of physical, nutritional, physiological and cellular effects. Studies carried out so far have suggested several mechanisms by which AM symbiosis can alleviate drought stress in host plants. The most important are discussed in the following sections.

Uptake and transfer of water through the fungal hyphae to the host plant

The pioneer studies of Allen (1982) and Hardie (1985) indicated a possible role of AM fungal hyphae in water uptake and transfer to the host plant. Hyphae with a diameter of 2–5 μm can penetrate soil pores inaccessible to root hairs (10–20 μm diameter) and so absorb water that is not available to non-mycorrhizal plants. Allen (1991) estimated that the rate of water transport by extraradical hyphae to the root was 0.28 ng s^{-1} per entry point, a level sufficient to modify plant water relations. Faber et al. (1991) measured rates of water transport by hyphae ranging from 375 to 760 nl H_2O h^{-1}. In contrast, predicted rates of water uptake by hyphae on the basis of hyphal entry points per unit of root length, hyphal cross-sectional area or water potential gradients suggest that hyphal water transport rates are negligible (Fitter 1985; George et al. 1992; Koide 1993). Since no clear conclusion could be drawn from previous studies, Ruiz-Lozano and Azcón (1995) designed an experiment with lettuce plants grown in containers that had three vertical compartments (Fig. 1). The upper compartment (root compartment) contained the mycorrhizal plants and was separated from the next compartment by a 50-μm nylon screen that allowed penetration by AM hyphae but not by roots. In the lower compartment (compartment II, located at 10 cm from the root compartment), water was applied by injection to attain –0.05 MPa. Addition of water to the lowermost compartment increased plant fresh weight by 150% in plants colonized by *Glomus fasciculatum* (Thaxter) Gerd. & Trappe emend. Walker & Koske, and by 215% in those colonized by *G. deserticola* Trappe, Bloss & Menge, as compared to a non-inoculated P-fertilized treatment (Fig. 2).

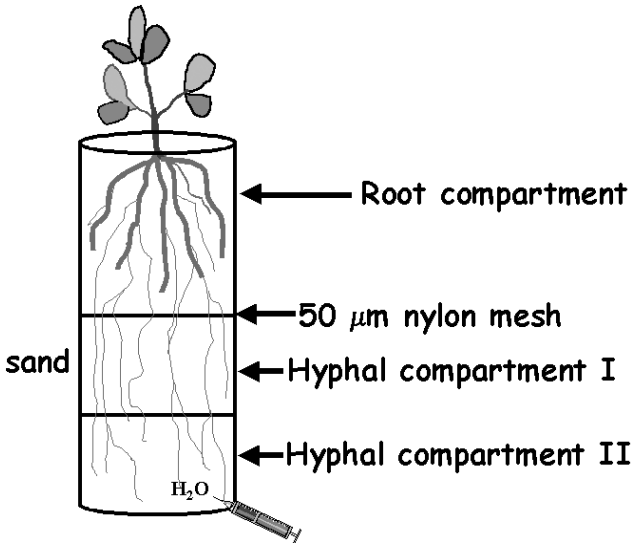

Fig. 1 Experimental system used in studying hyphae-to-root water transfer (Ruiz-Lozano and Azcón 1995). With the permission of Physiologia Plantarum

Similarly, leaf water content (Fig. 2) and gas exchange (data not shown) increased in mycorrhizal plants with water applied to the hyphal compartment II. The positive effects of the AM fungi on plant growth and water uptake were enhanced by water addition to the hyphal compartment (Ruiz-Lozano and Azcón 1995).

Osmotic adjustment

As soil dries out and soil water potential becomes more negative, plants must decrease their water potential to maintain a favourable gradient for water flow from soil into roots. The most important mechanism which achieves such an effect, known as osmotic adjustment or osmoregulation, is a decrease in the plant osmotic potential by active accumulation of organic ions or solutes (Morgan 1984; Sánchez-Díaz and Aguirreolea 1993; Hoekstra et al. 2001). Osmotic adjustment is common to all cellular organisms (Csonka and Hanson 1991). It allows cells to maintain turgor and the processes that depend on it, such as cellular expansion and growth, stomatal opening and photosynthesis, as well as keeping a gradient of water potential favourable to water entrance into the plant. The solutes which participate in osmotic adjustment are inorganic ions (mainly K^+ and Cl^-) or uncharged organic compounds like proline or glycine betaine, as well as carbohydrates like sucrose, pinitol or mannitol.

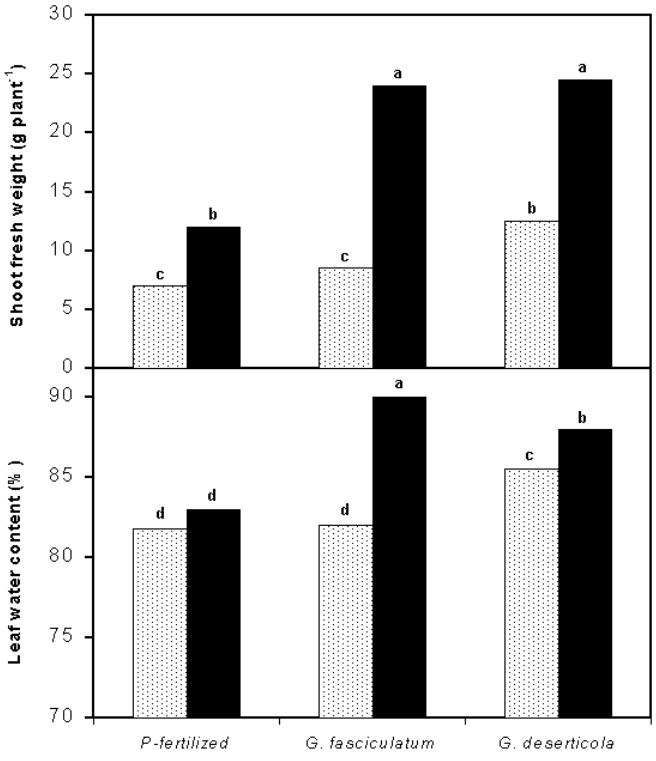

Fig. 2 Shoot fresh weight and leaf water content in P-fertilized or mycorrhizal (*Glomus fasciculatum* and *G. deserticola*) lettuce plants grown with two levels of soil water content in the hyphal compartment II: dry soil (*dotted bars*) or water added to reach –0.05 MPa (*filled bars*), different letters indicate significant differences ($P<0.05$) (Ruiz-Lozano and Azcón 1995). With the permission of Physiologia Plantarum

Proline is a non-protein amino acid that forms in most tissues subjected to water stress and, together with sugar, it is readily metabolized upon recovery from drought (Kameli and Losel 1993; Singh et al. 2000). The accumulation of proline in plant tissues during periods of drought is due primarily to de novo synthesis, although a reduced rate of catabolism has also been observed (Rhodes et al. 1986). In addition to acting as an osmoprotectant, proline also serves as a sink for energy to regulate redox potentials, as a hydroxyl radical scavenger, as a solute that protects macromolecules against denaturation, and as a means of reducing acidity in the cell (Kishor et al. 1995).

To date, studies carried out on osmoregulation in AM symbiosis are scarce, although an increase in proline accumulation has been observed in mycorrhizal plants subjected to drought (Ruiz-Lozano et al. 1995a; Azcón et al. 1996; Goioechea et al. 1998). It has also been shown that mycorrhizal colonization and drought interact in modifying free amino acid and sugar pools in roots (Augé et al. 1992b). Finally, a greater osmotic adjustment has recently been reported in leaves of mycorrhizal basil plants than in non-mycorrhizal ones during a period of lethal drought (Kubikova et al. 2001).

Enhancement of plant gas exchange

A number of studies have demonstrated that, during soil drying, mycorrhizal plants often maintain higher gas exchange rates than non-mycorrhizal plants of similar size and nutrient status (Augé et al. 1987; Bethlenfalvay et al. 1987; Augé 1989; Augé et al. 1992a; Sánchez-Díaz and Honrubia 1994; Ruiz-Lozano et al. 1995a, 1995b; Goicoechea et al. 1997). Mycorrhizal and non-mycorrhizal plants also often show different critical points or thresholds of stomatal behaviour during drought periods. For example, leaf water potential was about 0.2 MPa lower in *Glomus fasciculatum*-colonized wheat plants than in similar-sized non-mycorrhizal plants when stomata began to close (Allen and Boosalis 1983). In a similar way, leaf water potential at stomatal closure was 0.7 MPa lower in rose plants colonized by *G. deserticola* or *G. intraradices* Schenck & Smith than in similar-sized non-mycorrhizal plants (Augé et al. 1986). Duan et al. (1996) reported that mycorrhizal plants maintained higher stomatal conductance, transpiration rate and shoot water than non-mycorrhizal plants. Higher foliar water status was associated with lower xylem-sap abscisic acid (ABA) concentrations and lower ABA fluxes to leaves in mycorrhizal plants under low soil water conditions. The mechanism by which the AM symbiosis achieves such an effect is not clear, but Duan et al. (1996) have suggested that AM fungi probably increase the ability of the root system to scavenge water in dried soil, resulting in less strain on foliage, and hence higher stomatal conductance and shoot water content at a particular soil water potential. On the other hand, evidence has come from other studies that arbuscular mycorrhizas may influence leaf transpiration, even after leaves have been detached (Green et al. 1998).

Changes in soil water retention properties

In the absence of a clear plant-based explanation for mycorrhizal influence on stomatal conductance and other leaf water relations, Augé et al. (2001) proposed that AM and non-AM plants may behave differently during drought because the symbiosis affects soil water retention properties. The physical, chemical and biological actions of AM fungal hyphae and hyphal exudates on soils affect soil structure (Jastrow and Miller 1991; Oades and Waters 1991), which in turn affects soil moisture retention properties (Hamblin 1985). Consequently, Augé et al. (2001) investigated whether the AM symbiosis influences soil moisture retention properties and found that 7 months of mycorrhization by *G. intraradices* in a Sequatchie loam altered the characteristic soil moisture curve relative to "non-mycorrhizal" soils with similar rooting densities. Once the soil matric potential began to decline, changes in soil matric potential per unit change in soil water content were smaller in "mycorrhizal" than in the "non-mycorrhizal" soil. Within the range of about –1 to –5 MPa, the "mycorrhizal" soil had to dry out more than

Table 1 Total nitrate reductase (*NR*) activity (μmol NO_2^- g^{-1} fresh weight h^{-1}) and specific NR activity (μmol NO_2^- mg^{-1} protein) in mycorrhizal [*Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe, *G. fasciculatum*, *G. deserticola*] or non-inoculated (non-fertilized and P-fertilized) lettuce plants grown under well-watered or drought-stressed conditions. Means followed by the *same letter* are not significantly different (n=5) (Ruiz-Lozano and Azcón 1996). With the permission of Agriculture, Ecosystems and Environment

Treatments	Total NR	Specific NR
Well watered		
Control	18.2d	10.1cd
P fertilized	19.2d	11.3c
G. mosseae	36.8b	21.6b
G. fasciculatum	25.6c	15.1c
G. deserticola	42.2a	24.8a
Drought stressed		
Control	7.8f	4.6d
P fertilized	11.6e	7.3d
G. mosseae	21.4d	12.6c
G. fasciculatum	21.0d	12.4c
G. deserticola	36.6b	21.5b

the "non-mycorrhizal" soil to reach the same soil matric potential. The study revealed that the "mycorrhizal" soil had significantly more water-stable aggregates and substantially higher extraradical hyphal densities than the "non-mycorrhizal" soil which correlated well with the improved moisture retention properties of the "mycorrhizal" soil.

Stimulation of assimilative activities essential for plant growth

NO_3^- ions are the main N source for most higher plants (Schmidt 1982; Marschner 1986), and in most soils the major part of the inorganic N pool consists of NO_3^- because of rapid nitrification of NH_4^+ mineralized from organic material or applied to the soil as fertilizer (Schmidt 1982). Nitrate reductase (NR) is the first enzyme in the NO_3^- assimilation pathway and probably represents the rate-limiting step in this process (Campbell 1988). However, NR levels are drastically decreased by adverse environmental conditions such as drought stress (Sánchez-Díaz and Aguirreolea 1993). This is mainly due to a lower flux of NO_3^- from soil to roots since NR is a typical substrate-induced plant enzyme (Hoff et al. 1992). AM fungi have the gene set for assimilatory NO_3^- reduction (Ho and Trappe1975; Kaldorf et al. 1994), and NR activity has been located in arbuscule-containing cells (Kaldorf et al. 1998). In addition, levels of NR activity are higher in AM plants subjected to drought stress than in the non-mycorrhizal ones (Table 1), and such enhanced NR activity correlates with a higher tolerance of mycorrhizal plants to drought in terms of plant biomass production (Tobar et al. 1994; Ruiz-Lozano and Azcón 1996).

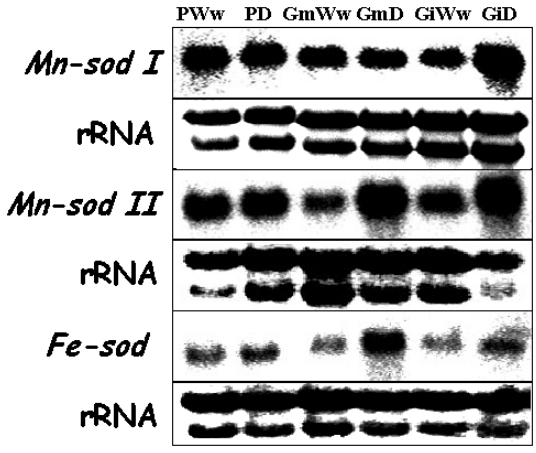

Fig. 3 Northern blot of root total RNA (20 μg) from P-fertilized (*P*) or mycorrhizal [*Glomus mosseae* (*Gm*) or *G. intraradices* (*Gi*)] lettuce plants cultivated under well-watered (*Ww*) or drought-stressed (*D*) conditions. Blots were hybridized with probes for *Mn-sodI*, *Mn-sodII* or *Fe-sod* genes. The panel under each Northern analysis shows the amount of rRNA loaded for each treatment (Ruiz-Lozano et al. 2001a). With the permission of Journal of Experimental Botany

Protection against oxidative damage generated by drought

Drought causes an oxidative stress in plants, and many of the degenerative reactions associated with abiotic stresses are mediated by reduced oxygen species such as superoxide radicals or hydrogen peroxide (Smirnoff 1993). Information about the activity of antioxidant enzymes in the AM symbiosis is scarce and has focused mainly on the role of superoxide dismutases (SODs). Little attention has been paid to the role of other important antioxidant enzymes such as catalase (CAT), ascorbate peroxidase (APX) or glutathione reductase (GR). Pioneer studies on this subject have shown that the AM fungus *G. mosseae* possesses CuZn-SOD activity and that mycorrhizal clover roots exhibit two additional SOD isoforms as compared to non-mycorrhizal roots: a mycCuZn-SOD and a Mn-SOD (Palma et al. 1993). Mycorrhizal lettuce plants subjected to drought have increased SOD activity compared to non-mycorrhizal controls (Ruiz-Lozano et al. 1996) and molecular analyses have confirmed this response at the transcriptional level (Ruiz-Lozano et al. 2001a). Three cDNAs putatively encoding two Mn-SODs and one Fe-SOD were used to follow gene expression in lettuce roots. The most interesting results were obtained in relation to the *Mn-sodII* gene (Fig. 3). The expression of this gene in P-fertilized plants was unaffected by drought stress. In contrast, changes in transcript accumulation occurred in mycorrhizal plants both as a consequence of fungal presence and of drought stress. Under well-watered conditions, fungal presence greatly decreased *Mn-sodII* gene expression (52% in the case of *G. mosseae* and 29% in the case of *G. intraradices*) relative to non-mycorrhizal plants. In contrast, when the plants were subjected to drought stress, both mycorrhizal treatments induced a significant increase in *Mn-sodII* transcript accumulation.

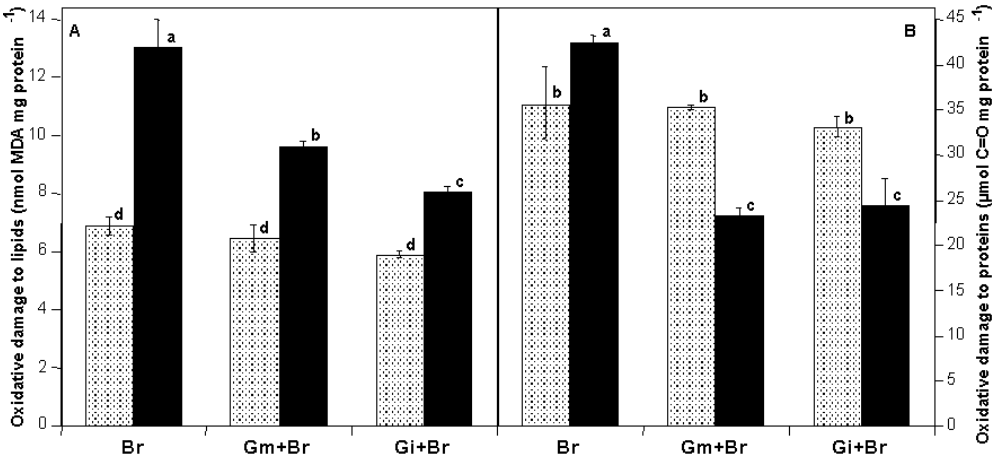

Fig. 4 Effect of drought stress and microbial treatment on oxidative damage to lipids (**A**) or proteins (**B**) in nodules of soybean plants. Treatments are *Bradyrhizobium japonicum* (Maier and Brill) (*Br*), *G. mosseae* plus *B. japonicum* (*Gm+Br*), *G. intraradices* plus *B. japonicum* (*Gi+Br*). Plants were either well-watered (*dotted bars*) or drought stressed for 10 days (*filled bars*), different letters indicate significant differences according to Duncan's multiple-range test. Error bars represent the SE within each treatment (n=5) (Ruiz-Lozano et al. 2001b). With the permission of The New Phytologist

This increase was 50% in *G. mosseae*-colonized plants and 138% in *G. intraradices*-colonized roots relative to non-mycorrhizal plants. Both the increase in SOD activity (Ruiz-Lozano et al. 1996) and the increase in *Mn-sodII* gene expression (Ruiz-Lozano et al. 2001a) were related to enhanced tolerance to drought, in terms of plant growth maintenance, by both mycorrhizal treatments (decrease in plant growth as consequence of drought by only 10–16% in mycorrhizal plants as compared to 30% in P-fertilized plants).

In addition, other studies have shown that the AM symbiosis can alleviate drought-induced nodule senescence in legume plants (Ruiz-Lozano et al. 2001b). The most remarkable observation was the substantial reduction in oxidative damage to lipids and proteins in nodules of mycorrhizal plants subjected to drought as compared to the nodules of non-mycorrhizal plants (Fig. 4). Such a reduction in oxidative damage to biomolecules may be the main mechanism by which the AM symbiosis protects root nodules in legume plants against premature nodule senescence induced by drought stress. Moreover, AM symbiosis can considerably increase the GR activity both in roots and nodules of soybean plants subjected to drought stress (Porcel et al. 2003). GR is an important component of the ascorbate-glutathione cycle since it is the enzyme that regenerates oxidized glutathione into its reduced form (Noctor and Foyer 1998).

All these results suggest that mycorrhizal protection against oxidative stress caused by drought may be one of the most important mechanisms by which the AM symbiosis increases the tolerance of host plants to drought. These observations agree with the proposal by Bartels (2001) that both the prevention of oxidative stress and the elimination of reactive oxygen species are the most effective approaches used by plants to gain tolerance against several abiotic stresses, including drought.

Perspectives for molecular studies

Although in recent years there has been an increase in the understanding of the water relations of AM plants and the physiological processes involved in enhanced tolerance of mycorrhizal plants to water limitation, there are still many unknown aspects which must be elucidated. It is likely that molecular techniques will shed further light on the different mechanisms that play a role in the protection of host plants against water deficit. As a first approach, and considering the actual knowledge on the topic summarized previously, it is proposed that research should focus on the following aspects.

Osmotic adjustment

The typical first response of living organisms to water deficit is osmotic adjustment. In plants, proline is an important organic compound that participates in the osmotic adjustment (Morgan 1984; Kishor et al. 1995). The first two steps of proline biosynthesis are catalysed 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). 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). Evidence for the existence of a gene encoding a P5CS in AM fungi, as well as in the host plant, and the establishment of the expression pattern of such genes 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 osmotic stress. Such an approach based on degenerate primers designed on conserved regions of P5CS proteins in a variety of organisms has been undertaken in order to identify the corresponding gene in AM fungi or host plant (Ruiz-Lozano et al., unpublished).

Aquaporins

If water deficit persists and the osmotic adjustment is insufficient to avoid plant damage, there are other mechanisms by which plants cope with water limitation. First, plants can increase water permeability in their tissues. Aquaporins are water channel proteins that facilitate the passive movement of water molecules down a water potential gradient. These proteins belong to the large major intrinsic protein (MIP) family of transmembrane channels that are represented in all kingdoms (Chrispeels and Agre 1994). Two classes of plant aquaporins, located in the plasma membrane and tonoplast respectively, have been identified so far (Johnson et al. 1990; Kammerloher et al. 1994).

Transcripts of MIP-encoding genes which accumulate under osmotic stress have been isolated from root cDNA libraries of ice plants (Bohnert et al. 1995). The encoded proteins are homologous to plant and animal aquaporins (Chrispeels and Agre 1994). It has been suggested that vacuolar and plasma membrane aquaporins, acting together, are responsible for the cytosolic osmoregulation that is necessary for maintaining normal metabolic processes (Kjelbom et al 1999). However, inhibition studies of aquaporins in vivo and antisense mutant studies have indicated that aquaporins are also important for the bulk flow of water in plants (Kjelbom et al. 1999). The high expression of genes encoding aquaporins in tissues involved in water transport suggests a role in transcellular water flow through living cells (Barrieu et al. 1998).

Some studies have demonstrated that AM symbiosis development induces the expression of genes encoding aquaporins (Roussel et al. 1997; Krajinski et al. 2000). However, these studies were carried out under well-watered conditions. Since other studies have suggested that aquaporins contribute significantly to the hydraulic conductivity of cells (Tazawa et al. 1996) and that they have a role in cellular osmoregulation (Kjelbom et al. 1999), it could be of interest to study at a molecular level whether such over-expression of MIPs induced by the AM symbiosis increases the water permeability of the host under drought stress conditions plant and whether this has an effect on the water relations of mycorrhizal plants.

Late embryogenesis abundant proteins

Another important mechanism involved in drought tolerance by plants is the induction of a group of proteins called late embryogenesis abundant (LEA) proteins, which accumulate in seeds during their maturation phase, when tolerance to desiccation is required (Close 1996). A number of studies have demonstrated that LEA proteins also accumulate in vegetative plant organs during periods of water deficit, and it has been proposed that LEA proteins play an important role in maintenance of the structure of other proteins and membranes, in sequestration of ions, in binding of water, and in functioning as molecular chaperones (Close 1996). Currently, it is known that over-expression of LEA proteins in plants and yeast confers tolerance to osmotic stresses (Xu et al. 1996; Imai et al. 1996). Dehydrins are an important group of LEA proteins (LEA D-11 family). 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 (Close 1996). Dehydrins have specific conserved domains such as the S, Y and K segments (Dure 1993). While the S segment is a tract of serine residues, the Y (VDYGNP) and the K (EKKGIMDKIKEKLPG) segments are useful for degenerate primer design for molecular investigation. No studies have been so far published on dehydrins in mycorrhizal plants. It would be of interest to determine whether the AM symbiosis alters the pattern of LEA protein accumulation under osmotic stress and whether such alteration functions in the protection of the host plants against drought.

Antioxidant proteins

As mentioned above, water deficit in plants also induces an increased concentration of free radicals in cells thus generating oxidative stress (Moran et al. 1994; Sgherri and Navari-Izzo 1995; Iturbe-Ormaextxe et al. 1998). Gamble and Burke (1984), who appear to have been the first to consider the relationship between antioxidant systems and water deficit, showed that GR activity in wheat leaves was higher in droughted than in well-watered plants. Since then, several reports have shown a positive correlation between tolerance to water deficit and increased antioxidant activities (for a review see Smirnoff 1993; Noctor and Foyer 1998).

Plants possess a number of antioxidant mechanisms that protect them from the production of reactive oxygen species (ROS). The term ROS is generic, embracing not only free radicals such as superoxide and hydroxyl radicals, but also H_2O_2 and singlet oxygen. These radicals are among the most reactive species known to chemistry, capable of reacting indiscriminately to cause oxidative damage to biomolecules such as lipid peroxidation, denaturation of proteins and mutation of DNA (Halliwell and Gutteridge 1989; Bowler et al. 1992).

The efficient destruction of O_2^- and H_2O_2 requires the action of several antioxidant enzymes acting in synchrony. Superoxide is rapidly converted to H_2O_2 by the action of SOD (Bowler et al. 1992). However, since H_2O_2 is a strong oxidant that rapidly oxidizes thiol groups, it

cannot be allowed to accumulate to excess (Noctor and Foyer 1998). CATs convert H_2O_2 to water and molecular oxygen in peroxisomes. An alternative mode of H_2O_2 destruction is via peroxidases, which are found throughout the cell and which have a much higher affinity for H_2O_2 than CAT (Jiménez et al. 1997). Plants also show high activities for the enzymes of the ascorbate-glutathione cycle in which H_2O_2 is scavenged. In the first step of this pathway APX, which is the most important peroxidase in H_2O_2 detoxification (Noctor and Foyer 1998), catalyses the reduction of H_2O_2 to water by ascorbate, and the resultant monodehydroascorbate and dehydroascorbate are reduced back to ascorbate by monodehydroascorbate reductase and by dehydroascorbate reductase plus GR, respectively (Iturbe-Ormaetxe et al. 2001).

As previously mentioned, mycorrhizal protection against the oxidative stress caused by drought is perhaps one of the most important mechanisms by which the AM symbiosis increases the tolerance of host plants against drought (Ruiz-Lozano et al 2001b). Evidence is available that AM symbiosis alters the activity of some plant antioxidant enzymes, that the AM fungi themselves possess CuZn-SOD activity and that increased SOD activity in AM plants under drought stress is associated with enhanced plant tolerance to drought (Palma et al. 1993; Ruiz-Lozano et al. 1996; Ruiz-Lozano et al. 2001a). Hence, the possible existence of genes encoding for antioxidant enzymes other than SODs (e.g. APX, GR or CAT) in AM fungi, as well as their expression patterns, should be examined in the AM symbiosis under osmotic stress.

Concluding remarks

Future studies of enzymes involved in osmotic adjustment such as P5CS, of proteins that affect cellular and plant water movement such as aquaporins, of proteins with chaperone activity such as LEA proteins and of antioxidant enzymes other than SODs, should increase our knowledge about mechanisms by which the AM symbiosis protects host plants against drought. In addition, untargeted approaches based on screening of cDNA libraries from AM fungi or AM plants grown under osmotic stress conditions could also be used to isolate osmotic stress-regulated genes which play a role in the response of the AM symbiosis to water deficit.

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References

Allen MF (1982) Influence of vesicular-arbuscular mycorrhizae on water movement through *Bouteloua gracilis* Lag ex Steud. New Phytol 91:191–196

Allen MF (1991) The ecology of mycorrhizae. Cambridge University Press, Cambridge

Allen MF, Boosalis MG (1983) Effects of two species of VA mycorrhizal fungi on drought tolerance of winter wheat. New Phytol 93:67–76

Augé RM (1989) Do VA mycorrhiza enhance transpiration by influencing host phosphorus status? J Plant Nutr 12:743–753

Augé RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3-42

Augé RM, Scheckel KA, Wample RL (1986) Greater leaf conductance of well-watered VA mycorrhizal rose plants is not related to phosphorus nutrition. New Phytol 103:107–116

Augé RM, Scheckel KA, Wample RL (1987) Leaf water and carohydrate status of VA mycorrhizal rose exposed to water deficit stress. Plant Soil 99:291–302

Augé RM, Stodola AJW, Brown MS, Bethlenfalvay GJ (1992a) Stomatal responses of mycorrhizal cowpea and soybean to short-term osmotic stress. New Phytol 120:117–125

Augé RM, Foster JG, Loescher WH, Stodola AW (1992b) Symplastic sugar and free amino acid molality of *Rosa* roots with regard to mycorrhizal colonization and drought. Symbiosis 12:1-17

Augé RM, Stodola AJW, Tims JE, Saxton AM (2001) Moisture retention properties of a mycorrhizal soil. Plant Soil 230:87–97

Azcón R, Gómez M, Tobar RM (1996) Physiological and nutritional responses by *Lactuca sativa* to nitrogen sources and mycorrhizal fungi under drought. Biol Fertil Soils 22:156–161

Azcón-Aguilar C, Barea JM (1996) Arbuscular mycorrhizas and biological control of soil-borne plant pathogens — an overview of the mechanisms involved. Mycorrhiza 6:457–464

Barea JM, Jeffries P (1995) Arbuscular mycorrhizas in sustainable soil-plant systems. In: Varma A, Hock B (eds) Mycorrhiza: structure, function, molecular biology and biotechnology. Springer, Berlin Heidelberg New York, pp 521–561

Barrieu F, Chaumont F, Chrispeels MJ (1998) High expression of the tonoplast aquaporin ZmTIP1 in epidermal and conducting tissues of maize. Plant Physiol 117:1153–1163

Bartels D (2001) Targeting detoxification pathways: an efficient approach to obtain plants with multiple stress tolerance. Trends Plant Sci 6:284–286

Bethlenfalvay GJ, Brown MS, Mihara KL, Stafford AE (1987) The *Glycine-Glomus-Bradyrhizobium* simbiosis. V. Effects of mycorrhizal on nodule activity and transpiration in soybean under drought stress. Plant Physiol 85:115–119

Bohnert HJ, Nelson DE, Jensen RG (1995) Adaptations to environmental stresses. Plant Cell 7:1099–1111

Bowler C, Van Montagu M, Inzé D (1992) Superoxide dismutase and stress tolerance. Annu Rev Plant Physiol Plant Mol Biol 43:83–116

Bray EA (1997) Plant responses to water deficit. Trends Plant Sci 2:48–54

Campbell WJ (1988) Nitrate reductase and its role in nitrate assimilation in plants. Physiol Plant 74:214–219

Chrispeels MJ, Agre P (1994) Aquaporins: water channel proteins of plant and animal cells. Trends Biochem Sci 19:421–425

Close T (1996) Dehydrins: Emergence of a biochemical role of a family of plant dehydration proteins. Physiol Plant 97:795–803.

Csonka LN, Hanson AD (1991) Prokaryotic osmoregulation: genetics and physiology. Annu Rev Microbiol 45:569–606

Duan X, Newman DS, Reiber JM, Green CD, Saxton AM, Augé RM (1996) Mycorrhizal influence on hydraulic and hormonal factors implicated in the control of stomatal conductance during drought. J Exp Bot 47:1541–1550

Dure L (1993) Structural motifs in LEA proteins of higher plants. In: Close TJ, Bray EA (eds) Response of plants to cellular dehydration during environmental stress. American Society of Plant Physiologists, Rockville, Md., pp 91–103

Faber BA, Zasoski RJ, Munns DN (1991) A method for measuring hyphal nutrient and water uptake in mycorrhizal plants. Can J Bot 69:87–94

Fitter AH (1985) Functioning of vesicular-arbuscular mycorrhizas under field conditions. New Phytol 99:257–265
Gamble PE, Burke JJ (1984) Effect of water deficit on the chloroplast antioxidant system. I. Alterations in glutathione reductase activity. Plant Physiol 76:615–621
George E, Häuser KU, Vetterlein D, Gorgus E, Marschner H (1992) Water and nutrient translocation by hyphae of *Glomus mosseae*. Can J Bot 70:2130–2137
Goicoechea N, Antolin MC, Sánchez-Díaz M (1997) Gas exchange is related to the hormone balance in mycorrhizal or nitrogen-fixing alfalfa subjected to drought. Physiol Plant 100:989–997
Goicoechea N, Szalai G, Antolín MC, Sánchez-Díaz M, Paldi E (1998) Influence of arbuscular mycorrhizae and *Rhizobium* on free polyamines and proline levels in water-stressed alfalfa. J Plant Physiol 153:706–711
Green CD, Stodola A, Augé RM (1998) Transpiration of detached leaves from mycorrhizal and nonmycorrhizal cowpea and rose plants given varying abscisic acid, pH, calcium and phosphorus. Mycorrhiza 8:93–99
Halliwell B, Gutteridge JMC (1989) Free radicals in biology and medicine. Clarendom Press, Oxford
Hamblin AP (1985) The influence of soil structure on water movement, crop root growth, and water uptake. Adv Agron 38:95–158
Hardie K (1985) The effect of removal of extraradical hyphae on water uptake by vesicular-arbuscular mycorrhizal plants. New Phytol 101:677–684
Ho I, Trappe JM (1975) Nitrate reducing capacity of two vesicular-arbuscular mycorrhizal fungi. Mycologia 67: 886–888
Hoekstra FA, Golovina EA, Buitink J (2001) Mechanisms of plant desiccation tolerance. Trends Plant Sci 6:431–438
Hoff T, Stummann BM, Henningsen KW (1992) Structure, function and regulation of nitrate reductase in higher plants. Physiol Plant 84: 616–624
Hu CA, Delaunew AJ, Verma DPS (1992) A bifunctional enzyme (Δ^1-pyrroline-5-carboxylate synthetase) catalyzes the first two steps in proline biosynthesis in plants. Proc Natl Acad Sci USA 89:9354–9358
Imai R (1996) A *lea*-class gene of tomato confers salt and freezing tolerance when overexpressed in *Saccharomyces cerevisisae*. Gene 170:243–248
Iturbe-Ormaetxe I, Escuredo PR, Arrese-Igor C, Becana M (1998) Oxidative damage in pea plants exposed to water deficit or paraquat. Plant Physiol 116:173–181
Iturbe-Ormaetxe I, Matamoros MA, Rubio MC, Dalton DA, Becana M (2001) The antioxidants of legume nodule mitochondria. Mol Plant-Microbe Interact 14:1189–1196
Jastrow JD, Miller RM (1991) Methods for assessing the effects of biota on soil structure. Agric Ecosyst Environ 34:279–303
Jiménez A, Hernández JA, del Río LA, Sevilla F (1997) Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea leaves. Plant Physiol 114:275–284
Johnson KD, Höfte H, Chrispeels MJ (1990) An intrinsic tonoplast protein of proteins storage vacuoles in seeds is structurally related to a bacterial solute transporter (GlpF). Plant Cell 2:525–532
Kaldorf M, Zimmer W, Bothe H (1994) Genetic evidence for the occurrence of assimilatory nitrate reductase in arbuscular mycorrhizal fungi. Mycorrhiza 5:23–28
Kaldorf M, Schemelzer E, Bothe H (1998) Expression of maize and fungal nitrate reductase in arbuscular mycorhiza. Mol Plant-Microbe Interact 11:439–448
Kameli A, Losel DM (1993) Carbohydrates and water status in wheat plants under water stress. New Phytol 125:609–614
Kammerloher W, Fischer U, Pienchottka GP, Schäffner AR (1994) Water channels in the plant plasma membrane cloned by immunoselection from a mammalian expression system. Plant J 6:187–199
Kishor PB, Hong Z, Miao GH, Hu CA, Verma DPS (1995) Overexpression of Δ^1-pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. Plant Physiol 108:1387–1394
Kjellbom P, Larsson C, Johansson I, Karlsson M, Johanson U (1999) Aquaporins and water homeostasis in plants. Trends Plant Sci 4:308–314
Koide R (1993) Physiology of the mycorrhizal plant. Adv Plant Pathol 9:33–54
Krajinski F, Biela A, Schubert D, Gianinazzi-Pearson V, Kaldenhoff R, Franken P (2000) Arbuscular mycorrhiza development regulates the mRNA abundance of *Mtaqp1* encoding a mercury-insensitive aquaporin of *Medicago truncatula*. Planta 211:85–90
Kramer PJ, Boyer JS (1997) Water relations of plants and soils. Academic Press, San Diego, Calif.
Kubikova E, Moore JL, Ownlew BH, Mullen MD, Augé RM (2001) Mycorrhizal impact on osmotic adjustment in *Ocimum basilicum* during a lethal drying episode. J Plant Physiol 158:1227–1230
Marschner H (1986) Mineral nutrition of higher plants. Academic Press, London
Moran JF, Becana M, Iturbe-Ormaetxe I, Frechilla S, Klucas RV, Aparicio-Tejo P (1994) Drought induces oxidative stress in pea plants. Planta 194:346–352
Morgan JM (1984) Osmoregulation and water stress in higher plants. Annu Rev Plant Physiol 33:299–319
Noctor G, Foyer CH (1998) Ascorbate and glutathione: keeping active oxygen under control. Annu Rev Plant Physiol Plant Mol Biol 49:249–279
Oades JM, Waters AG (1991) Aggregate hierarchy in soils. Aust J Soil Res 29:815–828
Palma JM, Longa MA, del Rio LA, Arines J (1993) Superoxide dismutase in vesicular-arbuscular red clover plants. Physiol Plant 87:77–83
Porcel R, Barea JM, Ruiz-Lozano JM (2003) Antioxidant activities in mycorrhizal soybean plants under drought stress and their possible relationship to the process of nodule senescence. New Phytol 157:135–143
Rhodes D, Handa S, Bressan RA (1986) Metabolic changes associated with adaptation of plant cells to water stress. Plant Physiol 82:890–903
Roussel H, Bruns S, Gianinazzi-Pearson V, Hahlbrock K, Franken P (1997) Induction of a membrane intrinsic protein-encoding mRNA in arbuscular mycorrhiza and elicitor-stimulated cell suspension cultures of parsley. Plant Sci 126:203–210
Ruiz-Lozano JM, Azcón R (1995) Hyphal contribution to water uptake in mycorrhizal plants as affected by the fungal species and water status. Physiol Plant 95:472–478
Ruiz-Lozano JM, Azcón R (1996) Mycorrhizal colonization and drought stress exposition as factors affecting nitrate reductase activity in lettuce plants. Agric Ecosyst Environ 60:175–181
Ruiz-Lozano JM, Azcón R, Gómez M (1995a) Effects of arbuscular mycorrhizal *Glomus* species on drought tolerance: physiological and nutritional plant responses. Appl Environ Microbiol 61:456–460
Ruiz-Lozano JM, Gómez M, Azcón R (1995b) Influence of different *Glomus* species on the time-course of physiological plant responses of lettuce to progressive drought stress periods. Plant Sci 110: 37–44
Ruiz-Lozano J, Azcón R, Palma JM (1996) Superoxide dismutase activity in arbuscular-mycorrhizal *Lactuca sativa* L. plants subjected to drought stress. New Phytol 134:327–333
Ruiz-Lozano JM, Collados C, Barea JM, Azcón R (2001a) Clonig of cDNAs encoding SODs from lettuce plants which show differential regulation by arbuscular mycorhizal symbiosis and by drought stress. J Exp Bot 52:2241–2242
Ruiz-Lozano JM, Collados C, Barea JM, Azcón R (2001b) Arbuscular mycorrhizal symbiosis can alleviate drought-induced nodule senescence in soybean plants. New Phytol 151:493–502
Safir GR, Boyer JS, Gerdemann JW (1971) Mycorrhizal enhancement of water transport in soybean. Science 172:581–583

Safir GR, Boyer JS, Gerdemann JW (1972) Nutrient status and mycorrhizal enhancement of water transport in soybean. Plant Physiol 49:700–703

Sánchez-Díaz M, Aguirreolea J (1993) Efectos fisiológicos que causa la falta persistente de agua en los cultivos. Phytoma 51:26–36

Sánchez-Díaz M, Honrubia M (1994) Water relations and alleviation of drought stress in mycorrhizal plants. In: Gianinazzi S, Schüepp H (eds) Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems. Birkäuser, Basel, pp 167–178

Schmidt E (1982) Nitrification in soil. In: Stevenson FJ (ed) Nitrogen in agricultural soils. American Society of Agronomy, Madison, Wis., pp 253–287

Sgherri CLM, Navari-Izzo F (1995) Sunflower seedlings subjected to increasing water deficit stress: oxidative stress and defence mechanisms. Physiol Plant 93:25–30

Singh DK, Sale PWG, Pallaghy CK, Singh V (2000) Role of proline and leaf expansion rate in the recovery of stressed white clover leaves with increased phosphorus concentration. New Phytol 146:261–269

Smirnoff N (1993) The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol 125:27–58

Smith SE, Read DJ (1997) Mycorrhizal symbiosis. Academic Press, San Diego, Calif.

Tazawa M, Asai K, Wasaki N (1996) Characteristics of Hg- and Zn-sensitive water channels in the plasma membrane of chara cells. Bot Acta 109:388–396

Tobar RM, Azcón R, Barea JM (1994) Improved nitrogen uptake and transport from ^{15}N-labeled nitrate by external hyphae of arbuscular mycorrhiza under water-stressed conditions. New Phytol 126:119–122

Varma A, Hock B (eds) (1998) Mycorrhiza: structure, function, molecular biology and biotechnology. Springer, Berlin Heidelberg New York

Xu DP, Duan X, Wang B, Hong B, Ho TD, Wu R (1996) Expression of a late embryogenesis abundant protein gene, *HVA1*, from barley confers tolerance to water deficit and salt stress in transgenic rice. Plant Physiol 110:249–257

Yoshiba Y, Kiyosue T, Katagiri T, Ueda H, Mizoguchi T, Yamaguchi-Shinozaki K, Wada K, Harada Y, Shinozaki K (1995) Correlation between the induction of a gene for Δ^1-pyrroline-5-carboxylate synthetase and the accumulation of proline in *Arabidopsis thalina* under osmotic stress. Plant J 7:751–760

Zhu JK, Hasegawa PM, Bressan R (1997) Molecular aspects of osmotic stress in plants. Crit Rev Plant Sci 16:253–277