Chapter 13
Contribution of Arbuscular Mycorrhizal Symbiosis to Plant Drought Tolerance: State of the Art

**JM Ruiz-Lozano, R. Porcel, G. Bárzana, R. Azcón and R. Aroca**

Abstract Water deficit is considered one of the most important abiotic constraints limiting plant growth and yield in many areas on the Earth. Indeed, it accounts for over 70 % of yield losses during crop production. Several eco-physiological studies have demonstrated that the arbuscular mycorrhizal (AM) symbiosis improves plant tissue hydration and physiology under drought stress conditions. It is currently accepted that the contribution of AM symbiosis to plant drought tolerance is the result of accumulative physical, nutritional, physiological, and cellular effects. This chapter summarizes, from physiological and molecular points of view, the current knowledge about the mechanisms by which the AM symbiosis is thought to protect host plants against the detrimental effects of water deficit. Thus, the role of fungal hyphae in water uptake and transfer to plant tissues and in modification of soil water retention properties is updated. At the same time, the implication of plant aquaporins in regulation of root hydraulic properties is discussed. The influence of the AM symbiosis on host plant processes such as osmotic adjustment, gas exchange, hormonal balance, and antioxidant capacity is also reviewed.

13.1 Introduction

In nature, plants are constantly exposed to adverse environmental conditions that have a negative effect on plant survival, development, and productivity. Drought is considered the most important abiotic factor limiting plant growth and yield in

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Fig. 13.1 Common plant responses to water deprivation
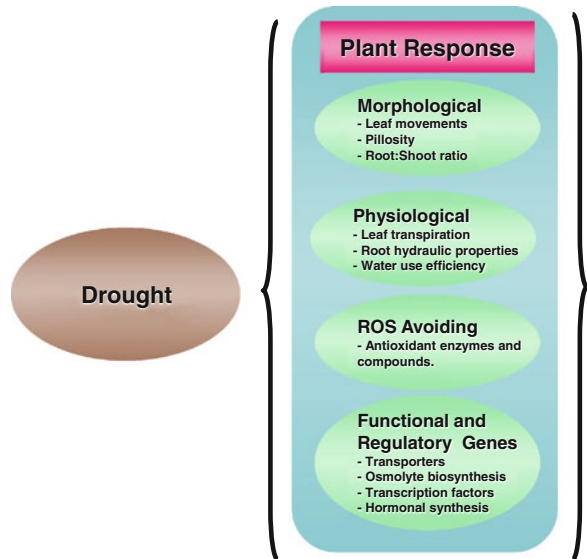
many areas (Bray 2004). The severity of drought is unpredictable as it depends on many factors such as occurrence and distribution of rainfall, evaporative demands, and moisture storing capacity of soils (Farooq et al. 2009). In addition, the global climate change is contributing to spread the problems of water deficit to regions where drought was negligible in the past. These difficulties will be particularly pronounced in currently semi-arid agricultural zones and/or under conditions of irrigation that often exacerbate soil salinization (Denby and Gehring 2005).

Although different plant species can vary in their sensitivity and response to soil water deficit, it may be assumed that all plants have encoded capability for stress perception, signaling, and response (Bohnert et al. 1995). Plants have developed several mechanisms in order to cope with drought stress (Fig. 13.1), including morphological adaptations, osmotic adjustment, optimization of water resources, antioxidant systems able to diminish the harmful effects of reactive oxygen species (ROS) linked to drought, and induction of a variety of stress-responsive genes and proteins (Farooq et al. 2009). Indeed, under drought conditions, plants trend to increase their root: shoot ratio in order to provide sufficient water to their aerial parts (Turner et al. 2001). At the same time, when plants perceive a decrease of the soil water availability, they close their stomata in order to prevent leaf water lost (Loreto and Centritto 2008). Plants can also accumulate compatible solutes in their tissues, which are involved in osmotic adjustment in order to maintain water content in shoot cells, as well as, a water potential gradient favorable to the entrance of water into the roots (Armengaud et al. 2004). The molecular responses may be initiated through hormonal signals which induce the accumulation of several stress-responsive proteins (Bray 2004).

Apart from to the intrinsic protective systems of plants against stress, plants grow associated 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 improved ability for nutrient uptake and tolerance to biotic and abiotic stresses while the fungus acquires a protected ecological niche and plant photosynthates (Varma 2008). 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 2008). Nevertheless, the physiological role of the AM symbiosis is not limited to uptake and transfer of nutrients to the host plant. Indeed, it is thought that the transition of plants from aquatic environment to land was possible through the association of plants with mycorrhizal fungi (Wang et al. 2010). In most cases studied, the association between an AM fungi (AMF) and a plant makes the host plant more tolerant to drought (Aroca et al. 2011). The mechanisms involved in such protective effect are reviewed in the following sections.

13.2 Uptake of Water Through the Fungal Hyphae and Transfer to the Host Plant: Implications of Aquaporins

One of the most common explanations for the improved water status and physiology in mycorrhizal plants is the strong increased absorbing surface caused by soil-growing hyphae combined with the fungal capability to take up water from soils with low water potential (Augé 2001; Ruiz-Lozano 2003; Lehto and Zwiazek 2011). Hyphal length density associated with AM root has been estimated to vary from 1 to more than 100 m per gram of soil (Smith et al. 2010). Average diameters of hyphae of AM fungi are in the range of 2–20 μm, which are one or two orders of magnitude narrower than roots. This size difference has important implications for access to water-filled pores, because hyphae may be able to penetrate a much higher proportion of pores than roots. In addition, AM hyphae have been suggested to maintain liquid continuity in the soil and limit the loss of soil hydraulic conductivity caused by air gaps (Allen 2007; Smith et al. 2010).

Studies by Allen (1982) and Hardie (1985) indicated a possible role of AM fungal hyphae in water uptake and transfer to the host plant. AM hyphae can penetrate soil pores inaccessible to root hairs 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 0.1 μl h^{-1} per hyphal entry point, a level sufficient to modify plant water relations. Faber et al. (1991) measured rates of water transport by hyphae to range from 0.37 to 0.76 μl h^{-1} per hypha. In contrast, negligible rates of water uptake by hyphae were also predicted (George et al. 1992; Koide 1993). Ruiz-Lozano and Azcón (1995) designed an experiment with lettuce plants grown in containers that had three compartments arranged
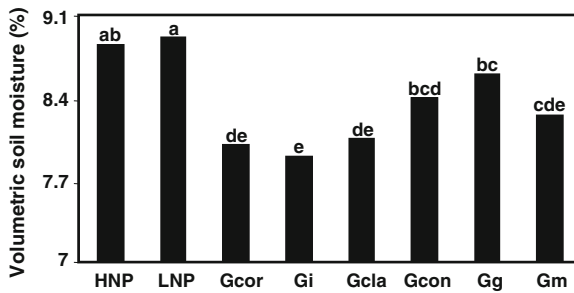
Fig. 13.2 Volumetric soil moisture (%) in lettuce plants fertilized with two rates of N and P (4 mM N 1 mM P, HNP and 2 mM N 0.5 mM P, LNP) or mycorrhizal lettuce plants colonized by *G. coronatum* (*Gcor*), *G. intraradices* (*Gi*), *G. claroideum* (*Gcla*), *G. constrictum* (*Gcon*), *G. geosporum* (*Gg*) or *G. mosseae* (*Gm*). Reproduced from Marulanda et al. (2003) with kind permission from John Wiley and Sons

vertically. The upper compartment (root compartment) was separated from the next compartment by a 50-μm nylon screen that allowed penetration by AM hyphae but not by roots. Addition of water to the lowermost compartment (located at 10 cm from the root compartment) increased plant fresh weight by 150 % in plants colonized by *Glomus fasciculatum*, and by 215 % in those colonized by *G. deserticola*, as compared to a non-inoculated P-fertilized treatment. Similarly, leaf water content and gas exchange increased in mycorrhizal plants with water application to the hyphal compartment. The study demonstrated a fungal activity in water uptake and translocation to the host plant (Ruiz-Lozano and Azcón 1995).

In a subsequent study, Marulanda et al. (2003) evaluated and compared the ability of six AM fungi to alter rates of root water uptake under drought stress conditions. Lettuce plants colonized by *Glomus coronatum*, *G. intraradices*, *G. claroideum*, and *G. mosseae* depleted soil water to a higher extent than comparably sized uninoculated control plants or plants colonized by *G. constrictum* or *G. geosporum*. The differences ranged from 0.6 % volumetric soil moisture for *G. mosseae*-colonized plants to 0.95 % volumetric soil moisture for *G. intraradices*-colonized plants (Fig. 13.2).

These differences in soil moisture were equivalent to 3– 4.75 ml plant^{-1} day^{-1}, respectively, and could not be ascribed to differences in plant size, but to the activity of AM fungi. The AM fungi tested in this study differed in their effectiveness to enhance plant water uptake from soil and this ability seemed to be related to the amount of external mycelium produced by each AM fungus (Fig. 13.3) (Marulanda et al. 2003).

More recently, Khalvati et al. (2005) designed a new split-root hyphae system to quantify the contribution of the hyphae to plant water uptake in mycorrhizal barley plants grown under well-watered and drought conditions. Compared to the non-mycorrhizal treatment, 4 % of water in the hyphal compartment was transferred to the root compartment through the arbuscular mycorrhizal hyphae under drought conditions. However, the much higher hyphal density found in the root

Fig. 13.3 Extraradical mycelium production (cm g soil^{-1}) by mycorrhizal lettuce plants colonized by six different AM fungi. See legend for Fig. 13.2. Reproduced from Marulanda et al. (2003) with kind permission from John Wiley and Sons
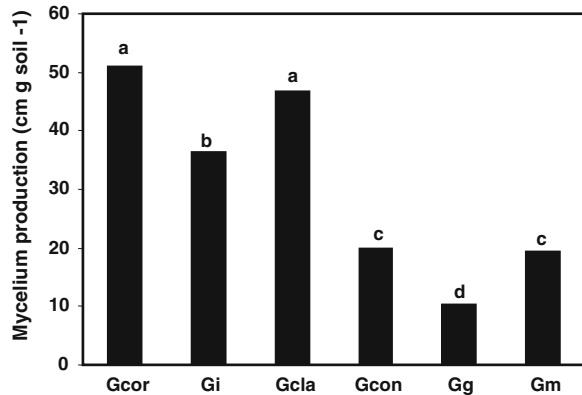
compartment than in the hyphal compartment suggested that a larger amount of water uptake by the arbuscular mycorrhizal hyphae could occur in the root compartment. Indeed, the same authors have quantified very recently the hyphal contribution to the total plant water uptake by means of high-resolution online water content sensors (Ruth et al. 2011). The direct and indirect hyphal contribution to the total plant water uptake was estimated to be at least 20 %, which is considerably higher than the 4 % estimated previously (Ruth et al. 2011).

The studies mentioned above demonstrated that increased water uptake by hyphae may be less important when the soil is near saturation and large pores are filled with water as the root surfaces are also in contact with water. However, as the soil dries and water is retained only in smaller pores where fungal hyphae can grow, but roots cannot, the water uptake function of hyphae becomes more significant for survival (Allen 2007; Lehto and Zwiazek 2011).

13.3 Implications of aquaporins in water relations of AM plants

Since 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). In fact, in the last years it has been demonstrated that AM symbiosis regulates root hydraulic properties, including root hydraulic conductivity (Lo), and these effects have been linked to regulation of plant aquaporins (Ruiz-Lozano and Aroca 2010a). Aquaporins are water channel proteins that facilitate and regulate the passive movement of water molecules down a water potential gradient (Kruse et al. 2006). These proteins belong to the large major intrinsic protein (MIP) family of transmembrane proteins and are represented in all kingdoms (Maurel 2007). In plants, aquaporins are subdivided into five evolutionarily distinct subfamilies, the plasma membrane intrinsic proteins (PIPs), the tonoplast intrinsic

proteins (TIPs), the small basic intrinsic proteins (SIPs), the nodulin26-like intrinsic proteins (NIPs) (Chaumont et al. 2001; Johanson et al. 2001), and the uncharacterized X intrinsic proteins (XIPs) (Gupta and Sankararamakrishnan 2009), which have been recently shown to transport a variety of uncharged substrates (Bienert et al. 2011).

The discovery of aquaporins in plants has caused a significant change in the understanding of plant water relations and much effort has been concentrated on investigating the function and regulation of aquaporins. High levels of aquaporin expression were shown not only in tissues with high water fluxes across membranes, e.g., in fast growing regions, in shoots, and leaves, but also in roots where water uptake occurs (Otto and Kaldenhoff 2000). Hence, aquaporins seem to play a specifically important role in controlling transcellular water transport in plant tissues (Javot and Maurel 2002; Zhao et al. 2008).

The literature on aquaporins modulation by AM symbiosis has risen significantly in the last decade as reviewed by Uehlein et al. (2007) and Ruiz-Lozano and Aroca (2010a). The first report on the modulation of aquaporin genes by AM symbiosis was provided by Roussel et al. (1997) followed by Krajinski et al. (2000), who found mycorrhiza-induced expression of TIP aquaporins in parsley and *Medicado truncatula*, respectively. 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). PIP and NIP aquaporin genes from *Medicago truncatula* were also shown to be induced by mycorrhization, while other four aquaporin genes analyzed did not change their expression pattern as consequence of mycorrhization (Uehlein et al. 2007). Authors of this work related the mycorrhiza-induced change in expression of the two genes with physiological changes in the plant roots, i.e., the symbiotic exchange processes located at the pariarbuscular membrane (Uehlein et al. 2007).

However, the studies mentioned above were carried out under well-watered conditions and they did not test the expression of the aquaporin gene in AM plants under water deficit conditions. Thus, 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 (Porcel et al. 2006). As mentioned previously, the starting hypothesis was that 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. In contrast, to this hypothesis, results obtained showed that the PIP genes studied were downregulated both in soybean (Fig. 13.4a) and in lettuce (Fig. 13.5) under drought stress and that such downregulation was even more severe in plants colonized by *G. mosseae* than in non-AM plants (Porcel et al. 2006).

When the expression of *GmPIP2* gene from soybean was analyzed in a time course (Fig. 13.4b), it was clearly visible that AM plants already downregulated that gene significantly at 5 days after inoculation (dai) and 12 dai, while both non-AM control plants still maintained *GmPIP2* gene expression almost unaltered. At

Fig. 13.4 **a** Northern blot of total RNA from soybean roots using *GmPIP1* and *GmPIP2* gene probes. **b** Northern blot of total RNA from soybean roots harvested 5, 12, 20, or 35 days after inoculation (dai) using *GmPIP2* gene probe. Treatments are designed as NI, non-inoculated controls; Br, *Bradyrhizobium japonicum*; Br + Gm, *B. japonicum* plus *G. mosseae*. Plants were either well watered (*ww*) or drought stressed (*ds*). The percentage of gene expression is indicated by numbers close to each northern. Reproduced from Porcel et al. (2006), with kind permission of Springer Science and Business Media
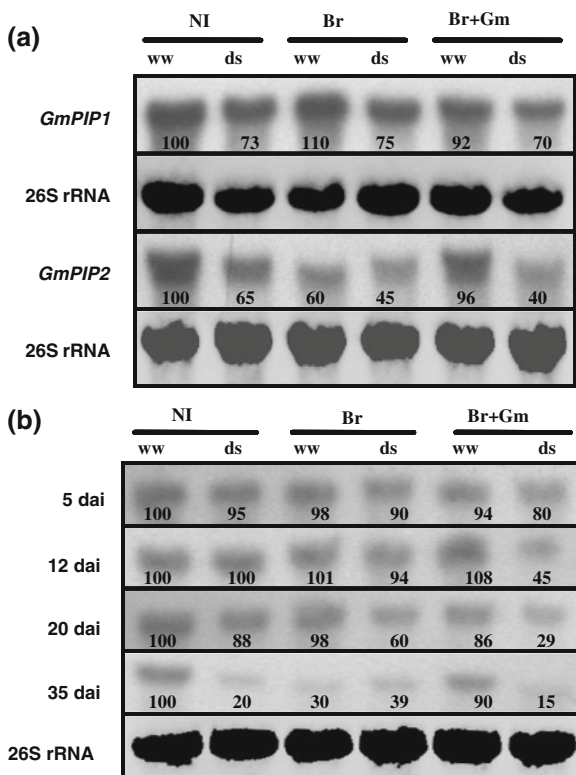
20 dai, the more intense downregulation of that gene in AM plants than in both non-AM plants was still clearly visible. Finally, at 35 dai all treatments had the same level of *GmPIP2* gene expression.

The 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. In fact, according to Aharon et al. (2003) and Jang et al. (2007), the overexpression of PIP aquaporins in transgenic tobacco and Arabidopsis improves plant vigor under favorable growth conditions, but the overexpression of such PIP genes was negative during drought stress, causing fast wilting. Hence, the decreased expression of plasma 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; Porcel et al. 2006).

To further illustrate the complexity of the response of aquaporin genes to AM fungi, the expression of four PIP aquaporin genes from *Phaseolus vulgaris* (Aroca et al. 2007) was analyzed in mycorrhizal and non-mycorhizal plants subjected to three different osmotic stresses: drought, cold, or salinity. Three of these PIP genes showed differential regulation by AM symbiosis under the specific conditions of each stress applied (Fig. 13.6). In fact, *PvPIP1;1* was slightly inhibited by *G. intraradices* under drought stress conditions, while non-mycorrhizal plants did not
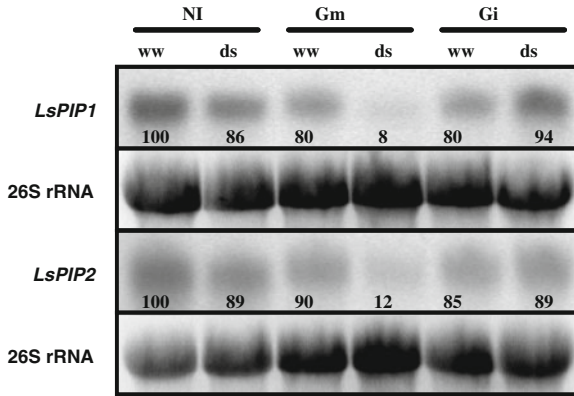
Fig. 13.5 Northern blot of total RNA from lettuce roots, using *lsPIP1* and *lsPIP2* gene probes. Treatments are designed as NI, non-inoculated controls; *Gm, Glomus mosseae* and *Gi, Glomus intraradices*. Plants were either wellwatered (*ww*) or drought stressed (*ds*). The percentage of gene expression is indicated by numbers close to each northern. Reproduced from Porcel et al. (2006), with kind permission of Springer Science and Business Media

change its expression pattern. Cold stress inhibited its expression similarly in AM and non-AM plants. Finally, salinity raised the gene expression in both groups of plants, but the enhancement was considerably higher in AM plants. The gene *PvPIP1;2* was inhibited by the three stresses in the same way in AM and non-AM plants. In contrast, *PvPIP1;3* showed important differences in AM and non-AM plants according to the stress imposed. This gene was clearly induced in non-AM plants under drought stress but inhibited in AM plants.

Under cold stress the behavior was the opposite since it was inhibited in non-AM plants and induced in AM ones. Finally, under salinity it was also induced in both groups of plants, especially in AM ones. The gene *PvPIP2;1* was induced in non-AM plants under drought stress but resulted downregulated in AM plants. The response of this gene to cold stress was not significant for any of the two plant groups and, again, the gene was considerably upregulated under salinity, especially in AM plants.

When *Lo* was measured, authors found that AM symbiosis regulated root hydraulic properties and enhanced *Lo* tolerance to drought, cold, and salinity stresses. The regulation of root hydraulic properties by AM symbiosis was strongly correlated with the regulation of PIP2 protein amount and phosphorylation state.

Thus, the results obtained so far on regulation of PIP aquaporins by the AM symbiosis show that the effects of the symbiosis on PIP genes depend on the own intrinsic properties of the osmotic stress, on the plant species studied, and on the specific aquaporin gene analyzed. In any case, the induction or inhibition of particular aquaporins by AM symbiosis should result in a better regulation of plant water status and contribute to the global plant resistance to the stressful conditions (Barrieu et al. 1999; Jang et al. 2004) as evidenced by their better growth and water status under conditions of water deficit. In addition, the results obtained

Fig. 13.6 Northern blots analysis using 3′ UTR as probes of *PvPIP1;1* (**a**), *PvPIP1;3* (**b**), and *PvPIP2;1* (**c**) in total RNA of *Phaseolus vulgaris* roots not inoculated (non-AM) or inoculated (AM) with *Glomus intararadices*. Plants were either kept as control or subjected to drought, cold, or salinity stresses. Control value of NI roots was referred as 100. Reproduced from Aroca et al. (2007), with permission from John Wiley and Sons
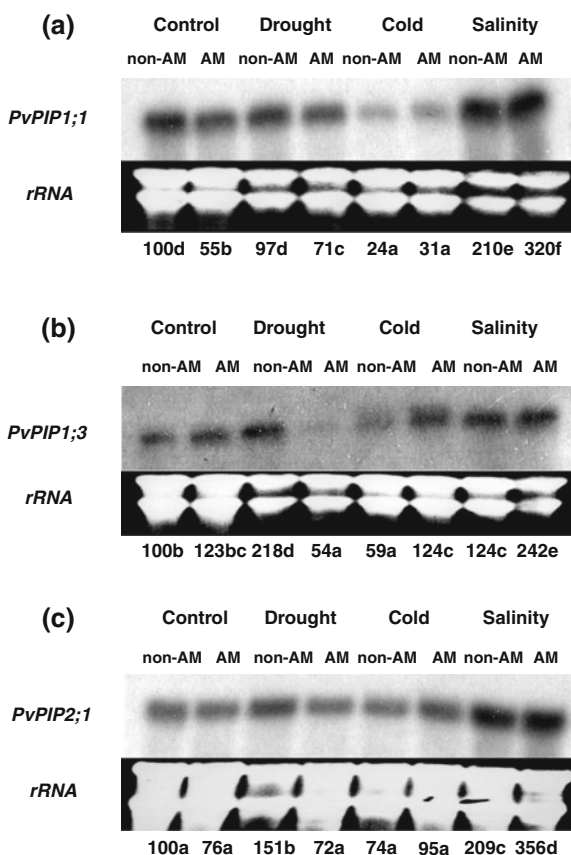
by Uehlein et al. (2007) suggest that the role of aquaporins in the AM symbiosis could be more complex than simply regulating plant water status. In fact, they described the induction by the AM symbiosis of specific PIP and NIP aquaporin isoforms exhibiting permeability to water and to ammonia, respectively. Authors suggested that these aquaporins could be involved in the symbiotic exchange processes between the fungus and the plant, which opens new perspectives in the study of aquaporins in the AM symbiosis.

To finish this topic, it is noticeable that Aroca et al. (2009) cloned the first aquaporin from an AM fungus (*GintAQP1*). Authors found some evidences supporting the idea that fungal aquaporins could compensate the downregulation of host plant aquaporins caused by drought. Also, they found that *GintAQP1* expression was upregulated in the mycelium part not osmotically stressed when the other mycelium part was stressed by NaCl. So, a possible communication between unstressed and stressed mycelium could be taken place. Obviously, the study of more fungal aquaporins is needed to completely understand this possible communication and the role of AM fungal aquaporins under conditions of water deficit.

13.4 Osmotic Adjustment

As soil dries out and soil water potential becomes more negative, plants must decrease their water potential to avoid cell dehydration and to maintain a favorable gradient for water flow 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; Hoekstra et al. 2001). Osmotic adjustment 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 favorable to water entrance into the plant. The solutes which participate in osmotic adjustment are inorganic ions (mainly K^+ and Cl^-) or uncharged organic compounds such as proline or glycine betaine, as well as carbohydrates such as sucrose, pinitol or mannitol.

Proline is a non-protein amino acid that accumulates in most tissues subjected to water stress and, together with sugars, it is readily metabolized upon recovery from drought (Singh et al. 2000). 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).

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; 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 non-AM plants when subjected to PEG-induced drought stress, while under high Ca in the medium this was not so (Ruiz-Lozano and Azcón 1997). Recently, Bheemareddy and Lakshman (2011) have examined the accumulation of metabolites in AM and non-AM *Triticum aestivum* plants and found that proline, carbohydrates, and proteins accumulated more in droughted AM plants than in the corresponding non-AM ones. In contrast, other studies regarding drought (Porcel et al. 2004; He et al. 2011) or salt stress (Ruiz-Lozano et al. 1996a; Jahromi et al. 2008; Sheng et al. 2011) have shown a lower proline accumulation in AM plants than in non-AM ones.

To further illustrate the complex response of AM plants in terms of solute accumulation, it is noticeable that in a recent study with lettuce plants (Ruiz-Lozano et al. (2011) found that under drought stress non-AM plants accumulated more proline in shoots than AM plants. In contrast, in roots, AM plants subjected to drought accumulated more proline than non-AM plants. This suggest that in root tissues AM plants accumulate more proline in order to cope with the low water

potential of drying soil and to keep a water potential gradient favorable to water entrance into the roots, as was observed in soybean plants (Porcel and Ruiz-Lozano 2004). In this way, AM plants would have a better water status than non-AM plants and their shoots would be less strained by drought stress. By that reason shoots of AM plants would need to accumulate less proline (Ruiz-Lozano et al. 2011). Moreover, Sheng et al. (2011) found enhanced reducing sugar accumulation in AM maize plants subjected to salt stress, while the content of proline was lower than in non-AM plants. Authors proposed that the high levels of sugars in AM plants may be the result of an increase in photosynthetic capacity of plants and that these sugars contributed to the osmotic adjustment of the plants (Sheng et al. 2011).

It has also been shown that mycorrhizal colonization and drought interact in modifying free amino acid, starch, and sugar pools in roots (Augé et al. 1992; Schellembaum et al. 1998). Similarly, a greater osmotic adjustment has been reported in leaves of mycorrhizal basil plants than in nonmycorrhizal ones during a lethal drought period (Kubikova et al. 2001). More recently, Wu et al. (2007) also described in citrus plants that AM seedlings accumulated more soluble sugars, soluble starch, and total non-structural carbohydrates in leaves and roots than corresponding non-AM seedlings in response to drought stress, thus providing better osmotic adjustment in AM seedlings, which correlated with glucose, fructose, and sucrose accumulation.

The regulation of proline biosynthesis by the AM symbiosis has also been studied at the molecular level, by studying the expression pattern of Δ^1-pyrroline-5-carboxylate synthetase (P5CS) genes in lettuce and soybean plants subjected to drought stress (Porcel et al. 2004). 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 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. In addition, in *Arabidopsis*, the P5CS-encoding gene is induced by drought stress, salinity, and ABA, but P5CR is not (Yoshiba et al. 1995). Hence, the P5CS-encoding gene is of key importance for the biosynthesis of proline in plants (Ábrahám et al. 2003).

A P5CS-encoding gene from *Glycine max* (*gmp5cs*) and another from *Lactuca sativa* (*lsp5cs*) were cloned and their contribution to the response against drought in mycorrhizal soybean and lettuce plants was analyzed. Results showed that *gmp5cs* and *lsp5cs* genes responded to drought and were upregulated in drought-stressed treatments, suggesting that they are important for the plant response against water deficit. Nevertheless, the expression of *gmp5cs* and *lslp5cs* genes decreased in drought-stressed AM plants as compared to non-inoculated plants. A similar result was obtained more recently with *lsp5cs* gene (Ruiz-Lozano et al. 2011). 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 non-AM plants by

primary drought-avoidance mechanisms. The results suggest that the induction of *p5cs* gene does not seem to be a mechanism by which the AM symbiosis protects their host plant (Porcel et al. 2004).

13.5 Enhancement of Plant Gas Exchange

An important number of studies have demonstrated that, during soil drying, mycorrhizal plants often maintain higher gas exchange rates than nonmycorrhizal plants of similar size and nutrient status (reviewed by Augé 2001; Ruiz-Lozano and Aroca 2010b). Currently, it is not clear how AM effects on stomatal behavior vary among AM fungi or host plants. Indeed, the literature shows that *gs* of some Citrus species are little affected by AM colonization, while soybean, sunflower, lettuce, or rose plants show frequent alteration of stomatal behavior by AM symbiosis (Augé 2001). Stomatal conductance rates are usually higher in AM than in non-AM plants, which implies that AM plants have a lower resistance to vapor transfer from inside the leaves to the atmosphere when exposed to the same water conditions. These higher *gs* rates in AM plants have been associated with lower xylem-sap abscisic acid (ABA) and lower ABA fluxes to leaves in AM plants (Ebel et al. 1997). AM and non-AM root systems exposed to the same stress and constrained to similar soil volumes may not necessarily suffer similar strain (see topic 2 above) that would explain the higher *gs* rates in AM plants (Duan et al. 1996).

Recently, Augé et al. (2008) have studied if the changes in *gs* induced by the AM symbiosis are accompanied by changes in leaf hydraulic conductance and in gradients of leaf water potential. They observed that under amply watered conditions the fungus *Glomus intraradices* enhanced by 27 % *gs* of squash plants, but leaf hydraulic conductance did not increase in these plants and, consequently, the water potential gradient across the leaf was higher in AM leaves. Authors concluded that the higher gradients of water potential in leaves of AM plants were consistent with the higher rates of gas exchange found in AM plants and that, presumably, are necessary to supply the carbon needs of the fungal symbiont.

It is also noticeable that AM and non-AM plants have shown different critical points or thresholds of stomatal behavior during drought episodes (Augé et al. 1986). Stomatal conductance in AM plants has also remained unaffected by declines in available soil moisture longer than in non-AM plants (Osundina 1995; Duan et al. 1996). Furthermore, AM plants showed higher *gs* than non-AM plants when only a portion of a root system was dried and before drying affected leaf water status and these effects occurred independently of plant size or phosphorus nutrition through non-hydraulic signals (Augé et al. 1992, 1994, 1995). Thus, it has been proposed that root dehydration alters root metabolism leading to the production of a non-hydraulic, chemical signal that moves to leaves where it induces stomatal closure (Augé 2000). Root-to-shoot regulation of stomatal behavior during soil drying may occur via a multiple chemical signal, including cytokinins and ABA (Druge and Schonbeck 1992; Goicoechea et al. 1997). In fact,

Goicoechea et al. (1997) noted that higher *gs* and transpiration of mycorrhizal alfalfa plants was associated with altered ABA/cytokinins ratios in their leaves.

It has been also shown that the influence of AM fungi on *gs* can be mediated by the host plant life style and the above-mentioned results can vary when the host plant shows a water conservative strategy as is the case of *Rosmarinus officinalis*. *R. officinalis* is a perennial Mediterranean plant well adapted to dry conditions that avoids drought stress by reducing its transpiration rate, as well as its *gs* (Munné-Bosch et al. 1998). The behavior of these plants against drought stress was studied by Sánchez-Blanco et al. (2004). Authors showed that, both under well-watered and under drought stress conditions, AM symbiosis enhanced root hydraulic conductivity and plant water status, but these effects did not correlate with *gs* since no significant differences in *gs* between AM and non-AM plants were found. On the contrary, significant increases of *gs* were found in two arid land woody plants such as *Olea europaea* and *Rhamnus lycioides* inoculated with the AM fungus *Glomus intraradices* (Caravaca et al. 2003; Querejeta et al. 2003). In spite of the common enhancement of *gs* in both plants, WUE was significantly enhanced only in *Olea europaea*, but not in *Rhamnus lycioides* (Caravaca et al. 2003; Querejeta et al. 2003). Authors related this differential behavior with the own plant lifestyle. *Olea* is a long-lived, slow-growing evergreen tree with a conservative water use strategy, while *Rhamnus* is a drought-deciduous shrub with a shorter lifespan.

Reduced *gs* under drought stress conditions in AM *Anthyllis cytisoides* plants has been observed by Goicoechea et al. (2004). *A. cytisoides* is a deciduous shrub plant that exhibits a slow growth rate and behaves as a drought avoider. It exhibits a resting period and one of its strategies to withstand prolonged water deficit is defoliation. When these plants were recovered from drought, young leaves from previously stressed mycorrhizal plants continued showing lower *gs* values than those from non-AM plants, concomitantly with a higher rate of leaf abscission. Leaf shedding can be a beneficial adaptation that reduces water loss and redistributes resources in order to favor plant survival under drought stress (Munné-Bosch and Alegre 2004). Thus, authors considered that this behavior could minimize both water loss by transpiration and whole plant respiration, preventing mycorrhizal plants from suffering desiccation. They concluded that, in this way, AM symbiosis conferred a greater responsiveness of *A. cytisoides* to drought.

The question whether native, drought-adapted AMF improve host plant performance to a greater extent than non-native AMF was addressed by Querejeta et al. (2006). They conducted a field experiment in which seedlings of two native wild shrub species were inoculated with either native or non-native *Glomus* species before outplanting in an agricultural land. The results obtained showed that native AMF enhanced *gs* values to a greater extent than non-native AMF in both shrub species.

AM symbiosis not only modifies *gs* values. There are also several reports in the literature showing an increase of plant photosynthetic activity and of water use efficiency (WUE) by the AM symbiosis both under well-watered and osmotic stress conditions (Ruiz-Lozano et al. 1995a; Wu and Xia 2006; Khan et al. 2008).

13.6 Regulation of Plant Hormonal Balance

The alteration in the homeostasis of plant hormones has been implicated in the enhanced drought tolerance exhibited by AM plants (Augé 2001; Ruiz-Lozano 2003). There are a large number of publications showing that the levels of plant hormones such as cytokinins, jasmonates, auxins, and abscisic acid (ABA) actually change upon the establishment of AM symbiosis (Hause et al. 2007). The knowledge of how plant hormones control and regulate the establishment and functioning of AM symbiosis has been recently summarized by López-Raez et al. (2011) as follows: (1) they redirect assimilates toward the root tissue colonized by the fungus and regulate mycorrhizal plant growth; (2) they are involved in the formation of fungal structures and the establishment of functional symbiosis; and (3) they regulate defence mechanisms and stress alleviation in mycorrhizal plants. However, there are still huge gaps in our knowledge on how both partners in the symbiosis can contribute to the regulation of hormone production (Ludwig-Müller 2010). Moreover, results by Grunwald et al. (2009) indicate that hormonal changes could be highly dependent on the symbiotic fungus used for inoculation.

ABA has been the most studied phytohormone in the field of the AM symbiosis. In fact, ABA is considered the most important signal transduction pathway among all the plant responses to stresses (Zhang et al. 2006; Hirayama and Shinozaki 2007). Some studies have suggested that an enhancement of ABA production in mycorrhizal roots could contribute to the suppression of the salicylic acid-dependent defence mechanisms activated during AMF colonization of roots (Herrera-Medina et al. 2007). These studies have also demonstrated that ABA participates in the susceptibility of tomato to infection by AM fungi, and that it seems to play an important role in the development of the complete arbuscule and its functionality (Herrera-Medina et al. 2007). More recently, an antagonistic interaction between ABA and ethylene, and different roles of each of the two hormones during AM formation has been found (Martín-Rodriguez et al. 2011).

Apart from its role during the formation of the AM symbiosis, ABA must be also considered in relation to plant responses to drought stress. Indeed, ABA modulates plant water status through regulation of important plant processes such as root hydraulic conductivity (L) (Schraut et al. 2005; Aroca 2006) and transpiration rate (Wilkinson and Davies 2002; Zhang et al. 2006), as well as, by induction of genes that encode enzymes and other proteins involved in cellular dehydration tolerance (Zhang et al. 2006; Hirayama and Shinozaki 2007). Several reports have shown that mycorrhization can alter ABA levels in the host plant, although contradictory results were also obtained. Studies of plants growing under normal conditions showed increased ABA levels in AM as compared to non-AM maize (Danneberg et al. 1992), soybeans (Murakami-Mizukami et al. 1991; Meixner et al. 2005), as well as *Medicago truncatula* and *Tropaeolum majus* (Ludwig-Müller 2010). On the contrary, Allen et al. (1982) reported decreased ABA levels in leaves from AM *Bouteloua gracilis* plants and an unchanged ABA content in their roots. ABA has also been detected in fungal hyphae at higher

levels than in roots (Esch et al. 1994) and it was suggested that ABA in AM fungi may control the flow of water and mineral salts from the soil to the hyphae or from the fungus to the root cells. On the other hand, Duan et al. (1996) tested whether AM symbiosis changes the movement of ABA from roots to shoots at a particular degree of drought or, if contrarily, the stomata of leaves of AM and non-AM plants differ in their sensitivity to ABA. They found that AM symbiosis did not alter stomatal sensitivity to ABA, but that the amount of ABA moving in xylem was lower in AM than in similar-sized non-AM plants in dry soil (Duan et al. 1996). Other studies have also shown that when plants are subjected to drought stress, the levels of ABA are lower in AM than in non-AM plants (Goicoechea et al. 1997; Estrada-Luna and Davies 2003). Similarly, ectomycorrhizal larch plants subjected to osmotic stress due to the addition of polyethylene glycol had lower ABA levels than their non-mycorrhizal counterparts (Rincon et al. 2005). Thus, the combined influence of AM symbiosis and exogenous ABA application on the responses of lettuce plants during drought was studied (Aroca et al. 2008b). The results showed that the application of exogenous ABA had contrasting effects on the physiological responses of AM and non-AM plants and on the expression of several stress-related genes (*lea*, *p5cs* or *nced*). The results obtained in that study suggested that 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 (Aroca et al. 2008b). The changes in stress and ABA related genes have also been attributed to the different ABA levels in AM and non-AM plants (Ruiz-Lozano et al. 2006).The fact that a key gene for ABA biosynthesis is also differentially expressed in AM roots (Jahromi et al. 2008; Aroca et al. 2008b) corroborates the importance of ABA during AM symbiosis.

Other investigations aimed at elucidating whether the effects of the AM symbiosis on plant tolerance in relation to water deficit are mediated by the endogenous ABA content of the host plant and whether the exogenous application of ABA modifies such effects (Aroca et al. 2008a). The mycorrhization of two tomato plant lines (wild-type and *sitiens*) with *G. intraradices* differently regulated the expression of several drought-induced genes in wild-type and in *sitiens* plants, which suggested that the effects of the AM symbiosis on plant responses to water deficit were also mediated by the plant ABA phenotype (endogenous plant ABA content).

More recently, another study has evaluated the combined influence of AM symbiosis and exogenous ABA application on plant root hydraulic properties and on PIP aquaporin gene expression and protein levels after both a drought and a recovery period (Ruiz-Lozano et al. 2009). Results showed first that the application of exogenous ABA enhanced Lo in all plant, regardless of water conditions, and second that AM plants showed lower Lo values than non-AM plants, especially when plants were supplied with exogenous ABA, which accentuated the differences in Lo between AM and non-AM plants. This effect was clearly correlated with the accumulation pattern of the different PIPs analyzed, since most of them reduced their expression and protein levels in AM plants fed with ABA as compared to their non-AM counterparts. As a whole, results suggested that the

combination of exogenous ABA and AM symbiosis inhibits the expression of PIP aquaporins as a strategy of water conservation in the host plant, which allowed these plants to maintain higher shoot RWC (Ruiz-Lozano et al. 2009). Thus, it can be concluded that ABA is necessary for sustained root colonization by AM fungi (Fester and Hause 2007) and to improve the symbiotic efficiency under drought stress conditions (Aroca et al. 2008a, b; Ruiz-Lozano et al. 2009).

13.7 Changes in Soil Water Retention Properties and Soil Hyphal Density

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. Authors found that 7 months of mycorrhization by *G. intraradices* in a Sequatchie loam altered the soil moisture characteristic curve relative to "nonmycorrhizal" soils with similar rooting densities. The study revealed that the "mycorrhizal" soil had significantly more water stable aggregates and substantially higher extraradical hyphal densities than the "nonmycorrhizal" soil which correlated well with the improved moisture retention properties of the "mycorrhizal" soil. The effect of AM hyphae in stabilizing soil structure by enmeshing soil particles and stabilizing aggregates has also been shown more recently (Hallet et al. 2009). In addition, the secretion by AM fungi of hydrophobic proteinaceous substances, referred to as glomalin (Rillig et al. 2002), also contributes to soil stability and water retention (Bedini et al. 2009).

Augé and co-workers tested also whether the effects of AM fungi occurred mostly via mycorrhization of roots, mycorrhization of soil or both. They used wild-type (myc+) and noncolonizing (myc−) bean mutants planted into soils previously produced using AM or non-AM sorghum plants in order to partition mycorrhizal influence on *gs* and drought resistance into soil and root components (Augé 2004; Augé et al. 2004). The studies conducted showed that about half of the considerable promotion of *gs* by two AM fungi was attributable to soil colonization and about half to root colonization. Moreover, merely growing in a mycorrhizal soil resulted in promotion of *gs* of non-AM plants under both amply watered and droughted conditions. That means that AM soil can affect the physiology of non-AM plants when grown in that soil. To explain this surprising result, authors suggested a nonhydraulic basis for the AM influence on the non-AM plant and proposed that the biochemical effects of the different soil organisms that interact with AM fungi in the rhizosphere (including P solubilizers, N_2 fixer, producers of hormones or siderophores, etc.) may contribute to the effects of AM soil on *gs* of host and nonhost plants (Augé et al. 2004).

In order to better understand why merely growing in a mycorrhizal soil resulted in promotion of *gs* of non-AM plants, Augé et al. (2007) further studied a possible

relationship between the degree of soil mycorrhization (estimated as soil hyphal density) and variations in plant *gs* before and during drought. The objectives of the study were to learn if the amount of soil hyphae would also contribute to variation in stomatal behavior and soil drying and to test if soil colonization would make stronger contributions than root colonization to these variables. Results obtained showed that both hypotheses were true. Firstly, less hyphae in soil were related to higher *gs* values. Secondly, soil colonization had more influence on *gs* values than did root colonization. Thus, authors concluded a role for mycorrhization of soil itself in contributing to the regulation of stomatal behavior of host plants (Augé et al. 2007).

13.8 Protection Against the Oxidative Damage Generated by Drought

During drought stress, different metabolic pathways are uncoupled and electrons, that have a high-energy state, are transferred to molecular oxygen to form ROS. At saturating CO_2 concentration, the leakage rate to oxygen is very low [3.5 mmol/mg chlorophyll/h, (Steiger and Beck 1981)], but under shortage of carbon dioxide up to 50 % of the entire photosynthetic electron flow may end up as O_2^- (Biehler and Fock 1996). ROS, such as $^1O_2$, H_2O_2, O_2^- and $HO^{\cdot}$, are toxic molecules capable of causing oxidative damage to proteins, DNA, and lipids (Miller et al. 2010). On the other hand, ROS can act as signaling molecules for stress responses and generation of ROS is an early event in plant response to stress (Singh et al. 2011). Plants have evolved several antioxidants mechanisms to avoid oxidative damage linked to stressful conditions. These mechanisms can be divided as enzymatic or nonenzymatic. Enzymatic antioxidants include superoxide dismutase (SOD), glutathione reductase (GR), catalase (CAT), ascorbate- or thiol-dependent peroxidases, and the enzymes of the ascorbate–glutathione pathway. Non-enzymatic mechanisms include compounds able to scavenge directly several ROS as are ascorbic acid (AsA), glutathione (GSH), or α-tocopherol (for a recent review of antioxidant systems in plants see Scheibe and Beck 2011). Briefly, 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. However, since H_2O_2 is a strong oxidant that rapidly oxidizes thiol groups, it cannot be allowed to accumulate to excess. 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. Plants also contain high activities for the enzymes of the ascorbate–glutathione cycle in which H_2O_2 is scavenged. In the first step of this pathway ascorbate peroxidase (APX), which is the most important peroxidase in H_2O_2 detoxification, catalyzes the reduction of H_2O_2 to water by ascorbate, and the resulting monodehydroascorbate and dehydroascorbate are reduced back to

ascorbate by monodehydroascorbate reductase (MR) and by dehydroascorbate reductase (DR) plus GR, respectively (Noctor and Foyer 1998; Miller et al. 2010; Scheibe and Beck 2011).

Information about the activity of antioxidant enzymes in the AM symbiosis has focused mainly on the role of superoxide dismutases (SODs). 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. 1996b) 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-sod II* gene. 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 decreased *Mn-sod II* 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-sod II* transcript accumulation. 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. 1996b) and the increase in *Mn-sod II* gene expression (Ruiz-Lozano et al. 2001a) were related to enhanced tolerance to drought, in terms of plant growth maintenance, by both mycorrhizal treatments.

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. Such reduction in oxidative damage to biomolecules was considered the main mechanism by which the AM symbiosis protected 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). The 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).

More recently there are few reports showing regulation of host antioxidant compounds by the AM symbiosis under drought stress. Indeed, Wu et al. (2006a, b); Wu and Zou (2009) and Ruiz-Sánchez et al. (2011) found an increase of AsA content in AM plants. Ascorbic acid is an important non-enzymatic antioxidant compound since it is involved in the removal of H_2O_2 by ascorbate peroxidases, which use AsA as electron donor, and is closely related to GSH in the ascorbate–glutathione cycle (Noctor and Foyer 1998). An increase in GSH content in AM
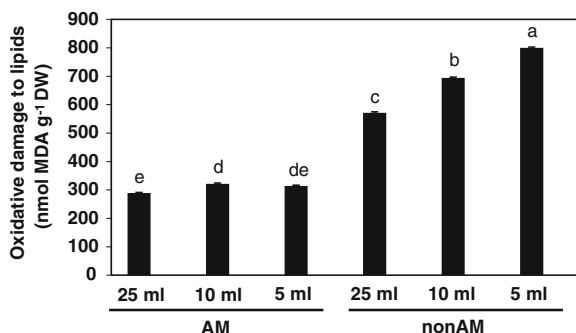
Fig. 13.7 Oxidative damage to lipids of rice plants inoculated or not with the AM fungus *G. intraradices*. Plants were subjected to drought stress by irrigation with 10 or 5 mL of aqueous nutrient solution or remained as well-watered control receiving 25 mL of aqueous nutrient solution. Adapted from Ruiz-Sánchez et al. (2010) with kind permission from Elsevier

plants has also been found (Wu et al. 2006a; Wu and Zou 2009; Ruiz-Sánchez et al. 2010), concomitantly with a reduced oxidative damage to lipids (Fig. 13.7). GSH has not only the functions of scavenging peroxides or regenerating AsA pool, but it also regulates the expression of photosynthetic genes and may keep the cell pools of reducing power (NADPH) under necessary conditions for plant living cells. No data about other antioxidant compounds regulated by AM symbiosis like α-tocopherol are available in the literature. Interesting results were found by Subramanian et al. (2006), who showed an increase in the ascorbic acid pool content in tomato fruits of AM-colonized plants under several water regimes (from well watered plants to severe droughted ones), without almost affecting plant yield. These results are really interesting in order to improve tomato food quality due to the high importance of ascorbic acid in human nutrition (Ginter 2007). Interestingly, and pointing to the same direction, Baslam and Goicoechea (2012) have recently described positive combined effects of water deficit and AM symbiosis on the accumulation of several antioxidant compounds in lettuce plants. Such compounds include carotenoids, anthocyanins, and phenolics which can improve the nutritional properties of lettuce as they are also beneficial for human health.

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. In any case, Porcel and Ruiz-Lozano (2004) also proposed that since AM symbiosis prevented leaf dehydration through drought avoidance mechanisms; it also diminished the production of ROS, and therefore the host plant may not need to enhance the activity of the antioxidant enzymes. The same hypothesis was also claimed by other authors (Caravaca et al. 2005; Alguacil et al. 2006). Moreover, Marulanda et al.

(2007) compared the effectiveness of four AM fungal isolates (two autochthonous drought-tolerant *Glomus* spp and two allochthonous drought-sensitive spp) on a drought-adapted plant (*Lavandula spica*) growing under drought conditions. Non-significant differences between antioxidant enzyme activities such as GR, CAT, and SOD in colonized plants were found. The two autochthonous strains of *Glomus intraradices* and *Glomus mosseae* were more effective in terms of shoot and root growth, water content, and nutrients uptake by lavender plants in the drought-stressed soil than similar allochthonous strains. These effects were concomitant with an increase in water content and a decline in H_2O_2 accumulation and antioxidant compounds such as glutathione and ascorbate.

Additional studies have shown that the antioxidant responses of AM plants can be modified by the soil conditions and composition. Thus, Ruiz-Lozano et al. (2011) cultivated AM and non-AM lettuce plants on soil amended or not with a treated SB waste under drought stress conditions. Accumulation of hydrogen peroxide, the oxidative damage to lipids, and the activity of four antioxidant enzymes were measured. Results showed that these parameters varied considerably in AM and non-AM plants depending on the presence or absence of SB waste. Authors related this variation in antioxidant enzyme activities with the availability of micronutrients in the growing substrate, e.g., CAT, APX, and SOD are metalloenzymes and their activity can be determined by the availability of the metals they utilize (Alguacil et al. 2003). Thus, both excess and deficiency of micronutrients can modulate the activity of these metalloenzymes. Taken together, these results pointed out the multiple factors involved in the drought tolerance enhancement by AM symbiosis.

13.9 Conclusions

There are clear evidences that the AM symbiosis enhances host plant tolerance to drought stress. This effect is achieved by alteration of several physiological or ecological processes, which are summarized in Fig. 13.8. These include the improvement of soil water retention properties and the ability of AMF hyphae to take up water from sources inaccessible to roots and the transfer of such water to the host plant. The contribution of hyphae to total plant water uptake has been estimated to be up to 20 %. In this process, the regulation of host plant aquaporins and root hydraulic properties is also involved. The AM symbiosis may also improve the plant osmotic adjustment by accumulation of different compounds such as proline, sugars, free amino acids, etc., although this effect may differ according to the plant tissue considered. Mycorrhizal plants exhibit enhanced gas exchange parameters and, usually, improved WUE, which also contributes to maintain plant growth and productivity under drought stress conditions. This effect has been closely related to the regulation of plant hormonal balance, being ABA the plant hormone most commonly modulated by the AM symbiosis under drought conditions. Finally, it is becoming clear that the AM symbiosis protects the host
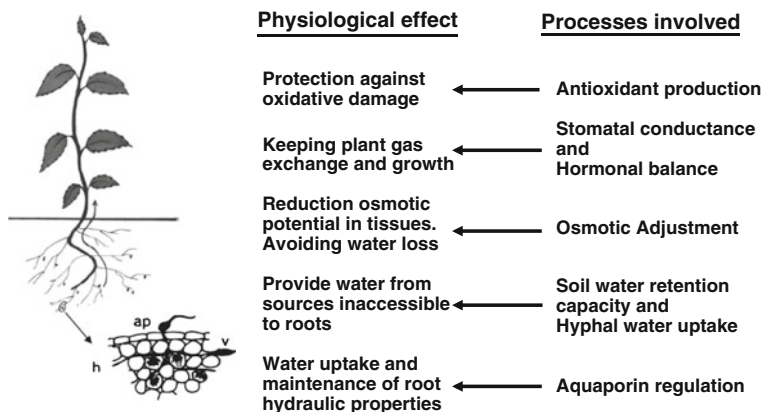
Physiological effect   **Processes involved**

Protection against   Antioxidant production
oxidative damage

Keeping plant gas   Stomatal conductance
exchange and growth   and
                           Hormonal balance

Reduction osmotic   Osmotic Adjustment
potential in tissues.
Avoiding water loss

Provide water from   Soil water retention
sources inaccessible   capacity and
to roots   Hyphal water uptake

Water uptake and   Aquaporin regulation
maintenance of root
hydraulic properties

Fig. 13.8 Schematic representation of the integral physiological processes by which AM fungi can improve the tolerance of the host plant to drought. Adapted from Evelin et al. (2009), with kind permission from Oxford University Press

plant against the detrimental effects of ROS generated by drought. Thus, improved antioxidant enzymatic activities and/or accumulation of non-enzymatic antioxidant compounds result in reduced oxidative damage to AM plants.

13.10 Perspectives

Although in recent years there has been an increase in the understanding of the water relations of AM plants and the processes involved in enhanced tolerance of mycorrhizal plants to water limitation, there are still many unknown aspects which must be elucidated. Indeed, the role of plant hormones such as jasmonates or ethylene in such a protective effect, as well as, the signaling pathways involved should be elucidated. For that, the use of plant mutants altered in the production of these hormones could be an important tool. The relationship between aquaporin regulation and root hydraulic properties also needs further attention. Moreover, the possible role of the own fungal aquaporins should be the subject of future studies. Photosynthetic efficiency and WUE are dependent on the activity of carbon assimilatory enzymes such as Rubisco. Thus, studies dealing with the effects of AM symbiosis on carbon assimilatory enzymes during drought stress are also needed. Finally, the participation of other antioxidant compounds such as α-tocopherol, carotenoids, or anthocyanins in the reduction of oxidative damage by AM symbiosis should be investigated, with special emphasis on the role of AMF on the accumulation of these compounds and the improvement of nutritional quality of host plant species of interest for human nutrition.

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