

Chapter 2

Mycorrhizal Fungi to Alleviate Drought Stress on Plant Growth

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Introduction

Plants are frequently subjected to different abiotic environmental stresses that determine geographic distribution and adversely affect growth, development, and agronomic yield. Drought is one of the major constraints on plant productivity worldwide and is expected to increase with climatic changes (IPCC 2007 and EEA 2011). The symbiotic relationship between arbuscular mycorrhizal (AM) fungi and the roots of higher plants is widespread in nature, and several ecophysiological studies have demonstrated that AM symbiosis is a key component in helping plants to cope with water stress and in increasing drought resistance, as demonstrated in a number of host plant and fungal species (Augé 2001; Ruiz-Lozano 2003; Smith and Read 2008; Ruiz-Lozano and Aroca 2010).

The alleviating effect of AM symbiosis in response to drought generally relies on the positive effects of AM fungi on the uptake and transport of water and on an improved uptake of nutrients, especially of available soil phosphorus (P) and other immobile mineral nutrients, resulting in the hydration of plant tissues, a sustainable physiology and a clear promotion of growth (Fig. 2.1; Augé 2001). AM

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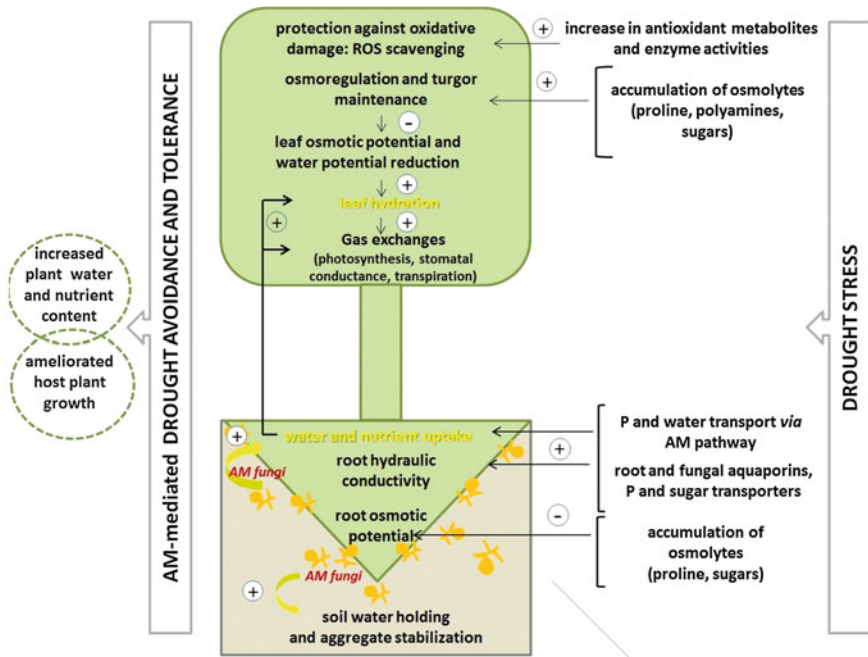


Fig. 2.1 Arbuscular mycorrhizal (AM) symbiosis can help plants to cope with the detrimental effects of soil water deficit acting, directly or indirectly, on plant functionality both above- and belowground. At the levels of both leaves and roots, the osmotic stress usually caused by drought is counteracted by mycorrhizal plants through biochemical changes that mostly include increased biosynthesis of metabolites (mainly proline and sugars) that act as osmolytes. These compounds contribute to the lowering of the osmotic potential, and in turn, of the leaf water potential. These lower potentials allow the plants to maintain high organ hydration and turgor that sustain overall cell physiological activity, mainly related to the photosynthetic machinery. AM plants withstand drought-induced oxidative stress by the increased production of antioxidant compounds that scavenge ROS and enhance the activities of antioxidant enzymes. AM root colonization can enhance root growth, architecture, and hydraulic properties and can thus induce the formation of a highly functional root system for nutrient/water uptake. At the same time, AM fungal hyphae in the soil provide an efficient pathway for nutrient/water uptake and transport, allowing a more efficient exploitation of the water and nutrient reservoirs in the soil where only fungal hyphae can grow, thereby bypassing the zones of water and nutrient depletion around the roots. Molecular mechanisms activated by AM symbiosis to counteract drought include gene activation of functional proteins, such as the membrane transporter aquaporins and, potentially, ion and sugar transporters, in both roots and fungi. Improved nutrient/water uptake and transport in roots translates into enhanced hydration of the aboveground organs that in turn affects physiological and biochemical processes. In addition, AM symbiosis can increase the resistance of plants to drought through secondary actions such as the improvement of soil structural stability that in turn increases the retention of soil water

symbiosis has a variety of effects on the defensive responses of host plants, depending on the species of host plant and the AM fungus involved (Bezemer and van Dam 2005).

In addition, the numerous confounding influences and system feedbacks inherent to the nature of AM symbiosis must be differentiated when describing the effects of AM on water balance. The AM-mediated response of many physiological and biochemical traits to changes in water availability may be confounded by concurrent changes in plant growth and nutrient availability (Smith and Read 2008) and can cause some drawbacks that limit our ability to clearly understand how AM fungi enable drought resistance in plants.

The comparison of plants of similar size and nutritional status is thus recognized as a fair requirement when evaluating the function of AM fungi during drought stress. Extensive study has demonstrated AM-mediated plant resistance to drought conditions, but the underlying mechanisms have not yet been clearly elucidated. Our incomplete understanding of how AM symbiosis affects the ability of plants to withstand conditions of limited water represents an important challenge to meeting the goal of improved plant productivity under the projected critical global scenarios.

AM-Mediated Plant Strategies to Cope with Drought: Avoidance Versus Tolerance

Despite the large variability in the effects of water stress on plants, they are able to respond to drought through two major strategies: avoidance of water stress and drought tolerance (Bray 1997). According to accepted terminology (Levitt 1980; Ludlow 1989; Turner 1997), plants can be classified as drought avoiders or as drought tolerant based on the absolute value of leaf water potential: drought avoidance allows the plant to withstand water-limiting conditions by maintaining a higher water status, mainly through enhanced water uptake and/or minimized water loss; tolerance to dehydration is associated with survival and sustained physiological activity when the leaf water potential is low, resulting in the ability of leaves to endure dehydration.

AM symbiosis protects host plants against the detrimental effects of drought stress through mechanisms of drought avoidance (Augé 2001; Ruiz-Sanchez et al. 2010). Strategies of drought avoidance in mycorrhizal plants rely on the ability to maintain an adequate hydration status on the level of whole plants as characterized by relative water content, although a thorough review of the literature indicates that leaf water potential was not measured in some experiments (Augé 2001; Augé and Moore 2005).

The improved capability of drought avoidance mediated by AM colonization has often been associated with the AM promotion of plant growth through enhanced nutrition. However, when not considering shoot size and nutritional effects, the influence of AM symbiosis on leaf hydration, mainly via the increased water uptake characteristic of mycorrhizal plants, may be the basis for their improved drought resistance. On top of being drought avoiders, mycorrhizal plants

have also been characterized as drought tolerant, mainly because of more improved osmotic adjustment, which allows the hydration and turgor of leaves to be sustained when leaf water potentials are low.

This distinction between drought strategies related to the AM-mediated responses of plants is fundamental for a better comprehension of the ecological and agricultural consequences for a plant species, because the AM-mediated response to drought is a complex process involving numerous metabolites and metabolic pathways. Studies to date investigating the role of AM symbiosis in ameliorating plant responses to drought stress have suggested the up-regulation and down-regulation of several physiological and biochemical processes. (1) The direct uptake and transfer of water and nutrients by AM fungi, (2) increased osmotic adjustment, (3) enhanced gas exchange and water use efficiency, and (4) better protection against oxidative damage when water is limiting may ameliorate, mitigate, and compensate the negative impacts of water stress in mycorrhizal plants.

Augé (2001) compiled a comprehensive review of the literature covering subjects such as plant strategies for controlling water status under drought and the metabolic processes underlying responses of mycorrhizal plants to oxidative stress. Ruiz-Lozano (2003) reviewed several aspects in need of investigation at the molecular level for understanding the different mechanisms by which AM symbiosis protects host plants from the detrimental effects of water deficit in terms of osmotic stress. These authors have provided new perspectives for molecular studies that could contribute to a global understanding of the different mechanisms by which AM symbiosis protects host plants against water deficit. Progress has also been made on the interpretation of the relationships between the different pathways regulated by the host plant or by the AM symbiotic relationship (Smith et al. 2010).

The aim of the present review is to outline the recent advances in the study of drought resistance by AM symbiosis with a particular focus on nutrient and water uptake/transport and on the lesser-known protective metabolites.

Biochemical–Metabolomic Responses of AM Plants to Drought

Role of Metabolic Changes in Osmoregulation

When water is limiting, decreased stomatal conductance and increased diffusive resistance to CO₂ could lead to increased plant water potential. To maintain water uptake from the soil, though, the water potential must be reduced. To achieve such an effect, plants can rely on mechanisms of ‘osmotic adjustment’ or ‘osmoregulation’ that decrease the osmotic potential resulting from the accumulation of compatible solutes or osmolytes (Munns 1988; Serraj and Sinclair 2002).

Osmolytic accumulation in plant cells can act as a mechanism of osmotic adjustment for decreasing the cellular osmotic potential and thus for maintaining water absorption and turgor. Osmolytic accumulation can also protect cellular components, such as cell membranes and proteins, and sustain the physiological activity of plants (Serraj and Sinclair 2002).

The accumulation of metabolites alone, however, may not always be sufficient to account for their effect on osmotic adjustment under drought stress. An alternative role for osmolytes as scavengers of reactive oxygen species (ROS) has been suggested (Hoekstra et al. 2001). Typical metabolites that can prevent the negative effects of drought include amino acids such as proline, other nitrogenous compounds such as polyamines, and a wide range of sugars and alcohol sugars. AM-mediated biochemical changes under conditions of drought stress principally involve the accumulation of protective metabolites such as osmolytes.

The colonization of roots by AM fungi in various plant species induces proline accumulation when water is limiting (Ruiz-Lozano et al. 1995; Azcón et al. 1996; Goicoechea et al. 1998; Yooyongwech et al. 2013). The enhanced accumulation of proline in these studies was linked to AM-induced drought resistance with proline acting as osmoprotectant. Conversely, in several studies, while proline content increased in response to water deficit, a lower accumulation of proline has been observed in mycorrhizal plants relative to nonmycorrhizal counterparts (Ruiz-Lozano and Azcón 1997; Wu and Xia 2006; Aroca et al. 2008; Ruiz-Sánchez et al. 2010; Abbaspour et al. 2012; Fan and Liu 2011; Asrar et al. 2012; Doubková et al. 2013), suggesting that AM symbiosis enhanced host plant resistance to drought.

In fact, proline could also be considered as a marker of the potential injury caused by water deficit, indicating that mycorrhizal plants, characterized by lower proline accumulation, were less stressed than the nonmycorrhizal plants. Furthermore, proline can act as an effective scavenger of ROS in the protection against denaturation and in the stabilization of membranes and subcellular structures (Kishor et al. 2005). The levels of free polyamines, other soluble nitrogenous compounds, increased in the leaves of drought-stressed mycorrhizal plants, and this increase was interpreted as indicating that free polyamines could serve as osmoprotectants under drought conditions, conferring drought resistance to mycorrhizal plants (Goicoechea et al. 1998).

AM symbiosis can increase the drought tolerance of plants if the commonly observed higher rates of photosynthesis lead to an increased accumulation of nonstructural carbohydrates that, acting as osmoprotectants, can lower the osmotic potential (Augé 2001; Porcel and Ruiz-Lozano 2004; Khalvati et al. 2005). Several studies have reported the accumulation of carbohydrates when plants are subjected to water stress in both woody species such as *Citrus* (Wu and Xia 2006) and *Macadamia* cultivars (Yooyongwech et al. 2013) and in herbaceous species such as lettuce cultivars (Baslam and Goicoechea 2012) and pistachio (Abbaspour et al. 2012). Carbohydrate accumulation in these studies was correlated with improved plant performance under drought stress, but the leaf osmotic potential was not evaluated; so correlating changes in carbohydrate levels with differential capacities of osmoregulation in mycorrhizal plants was thus not possible.

In addition, other reports have observed an AM-mediated decrease in soluble sugars in *Erythrina variegata* (Monoharan et al. 2010) and *Casuarina equisetifolia* (Zhang et al. 2010) exposed to drought stress, and this pattern was correlated with lower amounts of drought injury in the host plant. Furthermore, considering the positive mycorrhizal effect on plant growth, transport to sink organs and higher turnover rates of carbohydrates are likely to occur and suggest that the increased net assimilation rates do not necessarily indicate the accumulation of carbohydrates. In addition to the dynamic balance between the demands of growth and osmotic adjustment, another significantly relevant and competitive sink for carbohydrates is represented by the AM fungi themselves, which commonly drain photosynthate from the plants.

New insights are emerging into the regulation of uptake, exchange, and competition for carbohydrates by membrane transporters at the cellular plant–fungus (symbiotic) interface (see review by Doidy et al. 2012). Genes encoding transport proteins specific to the uptake of sucrose and hexoses have been identified by transcriptomic and genomic analyses at the AM symbiotic interface, in both plants and fungi (Doidy et al. 2012). The future application of combined physiological and molecular genetic approaches will open promising perspectives for a better understanding of the regulatory role of sugar transporters in the partitioning and allocation of carbohydrates between plants and AM fungi and hence of the effects of AM symbiosis in response to environmental limiting conditions, such as drought.

The above findings support the assessment that the direct measurement of physiological parameters such as soil and/or leaf water potential and turgor are fundamental for clearly interpreting the significance of AM-induced biochemical changes and for unambiguously interpreting the data (Augé 2001). Changes in metabolite levels could then be more confidently attributed to the strategies of drought tolerance or avoidance. Decreases in osmolytes have previously been interpreted as a mechanism of drought avoidance, while accumulation of osmoprotectants has been associated with drought tolerance (Augé and Moore 2005, 2010; Ruiz-Sánchez et al. 2010). Metabolic profiling of plants exposed to stress conditions is an important tool for studying stress-induced changes in metabolites, including osmolytes, but we lack knowledge of the regulation of the genes encoding enzymes of the osmolytic biosynthetic pathway.

Protection Against Oxidative Stress: Antioxidant Metabolites

Protection against oxidative damage by various antioxidants is another fundamental mechanism that can enhance drought resistance in mycorrhizal plants (Ruiz-Lozano 2003). Drought involves the production of excess ROS, such as singlet oxygen, superoxides, hydrogen peroxide, and hydroxyl radicals, leading to cell damage or death (Smirnoff 1993). Plants are characterized by a complex response network of antioxidant compounds and enzymes that defend plant cells

against excess ROS. Direct reactions can quench ROS activity, as can indirect responses such as hormone-mediated signaling to up-regulate primary and activate secondary defense genes (see review Apel and Hirt 2004; Kwak et al. 2006).

Oxidative stress occurs when the antioxidant defense system is overloaded and is unable to maintain an adequate cellular redox balance. The antioxidant system includes both enzymatic (e.g., superoxide dismutases, ascorbate peroxidases, and catalases) and nonenzymatic molecules (e.g., ascorbate, glutathione, flavonoids, carotenoids, and tocopherols; Mittler 2002). Antioxidants act not only as direct ROS scavengers but also as key sensors of the cellular redox status, so they trigger a number of signaling events for tightly controlling cellular ROS levels.

The amelioration of stress resistance by AM symbiosis is often related to the enhancement of antioxidant levels or activities in plants (Wu et al. 2006a, b; Wu and Zou 2009; Ruiz-Sánchez et al. 2010; Baslam and Goicoechea 2012). Ruiz-Sánchez et al. (2010) found that AM symbiosis ameliorated the response of plants to drought by improving photosynthetic performance but mainly through the accumulation of the antioxidant compound glutathione, which was concomitant with a reduction in oxidative damage to membrane lipids and to low cellular levels of hydrogen peroxide. In the same study, while glutathione levels increased, ascorbate levels decreased in mycorrhizal plants compared to nonmycorrhizal counterparts. This comprehensive study further supports the premise that mycorrhizal protection against drought-induced oxidative stress may be a crucial mechanism by which AM symbiosis increases the resistance of host plants to drought (Ruiz-Lozano 2003). In addition, it suggests differential up-regulation of the various antioxidant systems, with preferential activation of the systems that are more effective in protecting plants against drought. As already discussed in the case of changes in proline levels in response to drought, these antioxidant compounds can also be viewed as markers of drought stress: low accumulations of both glutathione and ascorbate in mycorrhizal plants of lavender under drought conditions were correlated with a high level of resistance to plant drought (Marulanda et al. 2007).

Among other potential ROS scavengers, flavonoids might also play a role in protecting mycorrhizal plants against oxidative damage: AM-mediated increases in the amounts of these compounds were sometimes found when plants were exposed to drought conditions (Abbaspour et al. 2012). Several studies suggest that AM symbiosis helps plants to alleviate drought stress by enhancing the activities of antioxidant enzymes, such as superoxide dismutase, guaiacol peroxidase, peroxidase, and catalase (Ruiz-Lozano et al. 1996; Wu and Zou 2009). Increased activity of superoxide dismutase was confirmed by transcriptomic analysis of the genes encoding this enzyme (Ruiz-Lozano et al. 2001). Protection against drought stress in soybean plants may originate from an enhanced activity of glutathione reductase concomitant with lower glutathione levels and decreased oxidative damage to biomolecules (Porcel et al. 2003; Ruiz-Lozano et al. 2001). Increases in the activities of several antioxidant enzymes induced by mycorrhizae have been associated with an AM-mediated enhancement in photosynthetic activity, plant biomass, and nutrient status (Alguacil et al. 2003; Roldán et al.

2008). The response of the specific antioxidant compound or enzyme, however, may depend on the host plant and the fungal species.

Even though most research on plant antioxidants has focused on nonvolatile compounds, volatile organic compounds emitted by leaves may contribute to an additional protective system against abiotic stresses (Kesselmeier and Staudt 1999; Peñuelas and Munné-Bosch 2005). Accumulating evidence supports the hypothesized role of volatile isoprenoids, in particular isoprene, in the protection against oxidative stress by mediating the oxidative status of plants through direct ROS scavenging, indirect alteration of ROS signaling, and/or membrane stabilization during abiotic stress, including drought (Peñuelas and Munné-Bosch 2005; Vickers et al. 2009).

Many plants that form AM mycorrhizae emit isoprenoids involved in the protection against several stresses, but the contribution of the mycorrhizae to the production of isoprenoids by plants has been scarcely investigated, especially under drought stress (Rapparini et al. 2008; Asensio et al. 2012). Moreover, the roots of mycorrhizal plants produce high amounts of specific isoprenoid-derived apocarotenoids (Walter and Strack 2011) and strigolactones (Lopez-Ráez et al. 2008).

We recently tested whether AM symbiosis affected the allocation of carbon resources to different classes of isoprenoids such as the volatile nonessential isoprenoids (monoterpenes and sesquiterpenes) and the nonvolatile essential isoprenoids (abscisic acid (ABA), chlorophylls, and carotenoids; Asensio et al. 2012). By subjecting tomato plants to stressors such as drought and to an exogenous application of jasmonic acid, we examined the AM symbiotic interaction in conditions where isoprenoids usually play a role in resistance to stress and in plant defense. Our results suggested that mycorrhizal plants use complex feedback responses associated with the activation of different pathways of isoprenoid production. Root colonization favored the production of essential rather than nonessential isoprenoids, especially under conditions of drought stress or after the application of jasmonic acid. In an overall view of the mycorrhizal plant system, carotenoids are both a sink of the universal precursors of isoprenoids and a concomitant source of important growth regulators such as apocarotenoids, ABA, and strigolactones, which are specifically produced when plant roots are colonized by AM fungi (Bouwmeester et al. 2007; Cazzonelli and Pogson 2010). We, accordingly, proposed that a more important demand of carotenoid-derived compounds and pigments is expected to increase in AM plants, especially under stress conditions where these isoprenoid compounds might play a role in plant protection and defense. The accumulation of carotenoids has also been found in lettuce plants (Baslam and Goichoechea 2012). Increased production of strigolactones in host plants during nutrient deficiency and salt stress (Yoneyama et al. 2007; Lopez-Ráez et al. 2008; Aroca et al. 2013) promoted AM fungal development and symbiotic establishment, suggesting a potential function of these compounds in enabling plants to overcome these abiotic constraints.

Physiological Responses of AM Plants to Drought

Aboveground Processes Affecting Plant–Water Relations

The physiological effects of AM symbiosis include aboveground modifications of water relations and physiological status in terms of leaf water potential, relative water content, stomatal conductance, CO₂ assimilation, and efficiency of photosystem II as compared to nonmycorrhizal plants (Augé 2001; Barzana et al. 2012). Many studies have shown an enhancement of the rates of gas exchange (stomatal conductance, transpiration, and photosynthetic rates) in mycorrhizal plants over nonmycorrhizal counterparts under water-limited conditions, independently of growth- or nutrition-mediated effects (see review Augé 2001; Ruiz-Lozano 2003; Sanchez-Blanco et al. 2004; Khalvati et al. 2005; Lee et al. 2012).

The mechanism by which AM symbiosis affects these physiological parameters is still unclear. The role played by ABA has been suggested as one of the nonnutritional mediated mechanisms by which AM symbiosis influence stomatal conductance and other physiological traits when plants are drought stressed (Ludwig-Müller 2010). In support of this hypothesis, recent studies have shown that ABA levels increased in response to water deficit and increased more in nonmycorrhizal plants than in mycorrhizal plants, suggesting that AM plants experience less intense drought stress (Doubková et al. 2013). Furthermore, these physiological processes may vary depending on host plant and especially on fungal species. Both stomatal conductance and photosynthesis varied widely during drought depending on the AM fungal species, even when comparing plants of similar size.

Several studies have reported that gas exchange in host plants is often related to the effect of AM symbiosis on the hydration of leaves (Augé 2001). Despite the numerous findings showing the positive effects of AM symbiosis on foliar gas exchange, the influence of these processes on leaf water potential in mycorrhizal plants subjected to drought is still unclear. In several studies, leaf water potential did not differ between mycorrhizal and nonmycorrhizal plants under drought stress (Augé 2001). Nevertheless, recent studies have demonstrated a higher (less negative) leaf water potential in mycorrhizal plants in water-limited conditions, which was interpreted as an AM-mediated mechanism of avoidance to mitigate the negative impact of drought on plant growth (Porcel and Ruiz-Lozano 2004; Asrar et al. 2012).

Leaf water potential is recognized as an index of the water status of an entire plant and hence represents a fundamental trait revealing a potentially improved resistance of plants to drought through better hydration. Hence, measurements of water use efficiency (WUE) provide an integrated measure of plant water use and thus allow a further dissection of the plant–water relations of mycorrhizal plants when water is limiting.

The extensive survey of the literature by Augé (2001) covered repeated attempts to examine the impacts of AM symbiosis on WUE. At the time these studies were conducted, however, the response in WUE was highly variable under

water stress: increases or decreases in WUE with AM symbiosis were observed. A sampling of the recent literature confirmed this variable response, showing an increase in WUE in *Antirrhinum majus* L. (Asrar et al. 2012) and the lack of a positive AM effect on this trait in *Knautia arvensis* during drought (Doubkóva et al. 2012).

A large part of plant resistance to drought is the ability to manage excess radiation resulting from limitation of photosynthesis by drought (for a review see Chaves et al. 2003) and reduced CO₂ availability leading to an inefficient use of incident light and to an increased susceptibility to photodamage (Powles 1984). Photoprotective mechanisms regulate the excitation energy that reaches the reaction centers of the photosystem by the dissipation of thermal energy (Demmig-Adams and Demmig 2006); the mechanisms also scavenge oxidative molecules and repair oxidative damage (Fernandez-Marín et al. 2009).

Recent reports have indicated that AM symbiosis under drought conditions enhances the photochemical efficiency of photosystem II, given by Fv/Fm, assessed by chlorophyll fluorescence in rice plants (Ruiz-Sánchez et al. 2011) and in woody tree nut species (Yooyongwech et al. 2013). Such results indicate the improved performance of the photosynthetic machinery and the absence of photoinhibition when mycorrhizal plants were exposed to water deficit. These findings are consistent with those of another recent study investigating the effect of root inoculation of different tree species with a combination of both AM and ectomycorrhizal fungi (Fini et al. 2011). The dynamics of photosystemic function and the potential forms of thermal dissipation, including those regulated by xanthophylls, however, have not yet been studied in detail.

Belowground Role of Root Systems and AM Fungi

Drought resistance in plants is strongly affected by their nutritional status. Soil-water deficit is tightly linked to low nutrient availability and to poor soil structure, so various hypotheses have been formulated to explain the underlying plant nutrition mechanisms involved in AM-induced resistance to drought. Improved nutrient uptake by AM fungi is a fundamental mechanism that can alleviate the adverse effects of water stress on plant growth.

One of the most common explanations for the improved nutrient status in mycorrhizal plants is the enhanced absorbing surface provided by the hyphae in the soil together with the ability of fungi to take up water from soil with low water potential (Augé 2001; Ruiz-Lozano 2003). The diameter size of hyphae (2–5 µm) is one or two times smaller than the diameter size of roots (10–20 µm), a trait conferring the ability to access very small soil pores that retain water and nutrients as soil dries. This allows to bypass the zones of water and nutrient depletion around the roots and, thus, a more extensive exploration of the soil (Miransari et al. 2007; Smith et al. 2010, 2011) that in turn may induce dense growth of roots (Miransari et al. 2007; Subramanian et al. 2006).

AM symbiosis is considered the most common strategy for enhancing P availability in the soil or P uptake capacity (Smith et al. 2011). Recent findings have provided new evidence for the contribution of the two well-recognized pathways (roots and fungal hyphae) by which P can be absorbed in mycorrhizal plants. These results suggest a pivotal role of a 'hidden P uptake' into plants via the AM fungal pathway (AM fungal hyphae; Smith et al. 2011), including when mycorrhizal plants experience conditions of drought stress (Smith et al. 2010). The authors suggested that the AM pathway may be active in P uptake even in plants that do not grow during drought conditions.

The relative contribution of the AM pathway to P uptake by plants and hence the contribution of direct uptake by roots under water stress has not yet been estimated. New molecular genetic studies investigating the expression of genes encoding high-affinity P transporters in the root cells of mycorrhizal plants will provide further information on the functional relevance of the direct pathway in P uptake and on the interplay of these two pathways of P uptake in AM plants when exposed to environmental conditions of stress (Smith et al. 2009; Smith and Smith 2011).

The fundamental contribution of P nutrition in the promotion of plant growth by AM symbiosis is well documented, but little information is available on the role of nitrogen (N) nutrition in the AM-mediated responses of plants to environmental limiting conditions, including drought. Even though few studies have investigated N uptake, an increased uptake of ammonium by fungal hyphae and the significant transfer of N from the fungus to the roots have been demonstrated (He et al. 2003), especially under drought conditions (Subramanian and Charest 1999). This increase was concomitant with increased activities of the main N-assimilating enzymes (Ruiz-Lozano and Azcón 1996).

Improved N uptake and assimilation have been associated with enhanced P nutritional status or is independent of P nutrition (Ruiz-Lozano and Azcón 1996). A recent review (Smith and Smith 2011) suggested that mycorrhizal plants could benefit from N uptake and transfer to the roots via the AM fungal pathway when exposed to water-limited conditions. Lee et al. (2012) recently investigated the role of N uptake and assimilation in the promotion of AM-mediated growth of perennial ryegrass using an N-labeled tracing technique. They found that AM symbiosis improved plant fitness under drought mainly by improving the plant water status and N uptake that, together with an enhancement of the activities of N-assimilating enzymes, resulted in increased amounts of proteins and amino acids.

The role of AM fungal hyphae in water uptake when water is limiting, as with P uptake, is still a matter of debate (Augé 2001; Smith et al. 2010). Difficulties in clearly interpreting the physiological and biochemical outcomes of AM symbiosis under drought conditions are due to the nature of AM symbiosis, because differentiating the effects of roots alone or of AM fungi alone from their combined effects is difficult (Ruth et al. 2011). This distinction becomes crucial when investigating the plant–fungus water relations where isolating the direct effect of AM symbiosis and understanding the real contribution of the AM fungi to the water balance of entire plants are also difficult. Specialized compartmented pot

systems have been designed for separating whole plants, including the root system, from the hyphal structure, but only a few attempts have been made to estimate the relative contribution of AM fungi to the total water uptake of the plant and the bulk flow velocity within the hyphae (Faber et al. 1991; Ruiz-Lozano and Azcón 1995).

In a recent study on barley plants inoculated with *Glomus intraradices*, Ruth et al. (2011) used a compartmented ‘split plant-hyphal’ chamber together with a specifically adapted online system for monitoring the soil water content to provide an accurate estimate of the water content of the two compartments and thus to derive the hyphal water flow. They monitored the presence of the water flow in the fungal hyphae and estimated the hyphal water flow at approximately 20 % of the total water uptake of the plant. These findings are consistent with earlier results that suggested a direct uptake and transfer to the host plants via the AM hyphae (Ruiz-Lozano and Azcón 1995; Marulanda et al. 2003; Khalvati et al. 2005), confirm previous estimates of the hyphal water flow (Faber et al. 1991; Cui and Nobel 1992) and support the premise of a significant contribution of fungal hyphae to plant water uptake (Allen 1982; Ruiz-Lozano and Azcón 1995). Discrepancies with other studies that found a low (Khalvati et al. 2005) or negligible contribution of the hyphae to the water balance of the plant (Cooper and Tinker 1981; Fitter 1985; George et al. 1992; Koide 1993) may be due to functional differences in the experimental designs of the compartmented systems.

In light of the enhanced water uptake by AM symbiosis during drought from improved P nutrition or growth, both of these mechanisms may also affect root hydraulic conductivity (Koide 1993). The hydration of leaves is indeed caused by the balance between the transpiration stream and water uptake by roots. AM symbiosis improves the plant water content by regulating the properties of plant hydraulics, including root hydraulic conductivity, although some authors have reported an enhanced (Sanchez-Blanco et al. 2004; Aroca et al. 2008) or reduced (Aroca et al. 2007; Ruiz-Lozano et al. 2009) effect of AM fungi on this trait. The role of the membrane transporter aquaporins in root hydraulic conductivity at the cellular level and their contributions to the transpiration stream have been investigated (Conner et al. 2013; Maurel et al. 2008) and will be discussed in the next section.

In addition to the effects of AM symbiosis on plant–water relations where AM fungi act independently and directly on nutrient and water uptake, AM symbiosis could increase drought resistance in plants through secondary actions such as the improvement of soil structural stability that in turn increases the retention of soil water (Augé 2001; Ruiz-Lozano 2003). AM fungal hyphae can enhance soil structure through the entanglement of soil particles to form aggregates and through the production of the glycoprotein glomalin (Rillig and Mummey 2006; Singh et al. 2011). AM fungi, in part due to their filamentous structure, also influence the development of soil structure both in the rhizosphere and in bulk soil (Miransari et al. 2007).

Augé et al. (2001) reported that the soil in which mycorrhizal plants were grown was characterized by more water-stable aggregates and substantially higher extraradical hyphal densities than the soils of nonmycorrhizal plants, and this

pattern correlated well with the improved retention of moisture of the mycorrhizal soil. By binding roots to the soil, fungal hyphae may even maintain liquid continuity and limit the loss of hydraulic conductivity caused by air gaps (Augé 2001; Augé et al. 2001).

New Insights into the Molecular Genetic Basis of Water Relations in AM Symbiosis Under Drought: Membrane-Protein Water Transporters

The physiological responses of mycorrhizal plants to drought stress can be regulated by the expression of drought-related plant genes, e.g., those involved in signaling and regulatory pathways or those encoding enzymes that synthesize functional or structural metabolites. Emerging insights are provided by studies on the regulation of important genes that encode significant components of the cellular water transport system, such as the aquaporins. These components are membrane proteins that channel water, uncharged molecules, across cell membranes in both roots and leaves (Conner et al. 2013; Maurel et al. 2008). These proteins may even increase root hydraulic conductivity and leaf water potential and decrease the transpiration rate in the leaves of mycorrhizal plants (Ruiz-Lozano et al. 2006, 2009; Aroca et al. 2008).

Both regulation and activity of aquaporin genes are modulated by conditions of water stress and thus have a potential role in the symbiotic exchange of water and nutrients between AM partners. Aquaporins are generally considered to be involved in the processes of symbiotic exchange at the plant–fungus interface, suggesting a fine regulation of water relations and the determination of the transport properties of the two partners (Maurel and Plassard 2011).

AM regulation of plant aquaporin genes under drought stress generally improves plant water status and drought tolerance (Aroca et al. 2007; Aroca and Ruiz-Lozano 2009; Li et al. 2012). In particular, the expression of genes encoding aquaporins has been demonstrated (Uehlein et al. 2007), and an aquaporin has been identified in AM fungal structures, both in the periarbuscular membrane and the extraradical mycelia (Aroca et al. 2009; Li et al. 2012). Both plant and fungal aquaporins are affected by stresses, including drought (Uehlein et al. 2007; Aroca et al. 2009; Li et al. 2012).

A relevant decrease in the expression of aquaporin genes in mycorrhizal plants compared to nonmycorrhizal plants has been observed under conditions of drought stress (Porcel et al. 2006 and Aroca et al. 2007), but other properties of these membrane proteins may also play a relevant role in the overall water relations of AM plants when water is limiting. An earlier study in *Phaseolus vulgaris* inoculated with *G. intraradices* found the commonly observed positive AM-mediated effect on plant water content but also found different effects of AM plant responses to drought on the regulation of aquaporins (Aroca et al. 2007). The authors

observed a lower expression of aquaporin genes in roots of mycorrhizal plants compared to nonmycorrhizal plants under drought conditions, suggesting that a mechanism of water conservation was employed by the AM plants. In the same experiment, AM symbiosis did not affect the phosphorylation state and amount of aquaporins and in particular the abundance of those proteins more functionally active in water transport, and this pattern was associated with a concomitant decrease in root hydraulic conductivity and foliar transpiration rates. The regulation of root hydraulic properties by AM symbiosis was strongly correlated with the regulation of aquaporin levels and phosphorylation state, and the authors suggested that down-regulating the activity of these proteins might provide a better explanation for these changes during water deficit. The drought-induced decrease in the transpiration stream observed in *Phaseolus* mycorrhizal plants, however, was concomitant with an increased free exuded sap flow, suggesting a higher water uptake from the soil in mycorrhizal plants compared to nonmycorrhizal plants under water-limited conditions and explaining the overall AM-improved water status (Aroca et al. 2007).

Li et al. (2012), however, recently reported an enhanced expression of two functional genes encoding aquaporins in both the roots of maize plants and in AM fungi when plants were subjected to drought stress. Since this pattern was concomitant with protein accumulation and a significant increase in root water content, the authors suggested that AM fungi improved plant water status by regulating the expression and activity of aquaporins in both plants and fungi. These studies provide molecular support for potential water transport via AM fungi to the host plant, suggesting that the simultaneous regulation of both expression and activity of aquaporins in host plants and fungi might represent a mechanism for enhancing plant tolerance to drought.

Another recent study used an appropriate inhibitor of aquaporin activity and an apoplastic tracer dye to separately measure the flow of water through the apoplastic pathway and via the root aquaporins ('cell-to-cell' pathway; Bárzana et al. 2012). The authors found an enhanced apoplastic water flow in the mycorrhizal roots that was competitive to the 'cell-to-cell' pathway during drought stress. The ability of AM plants to switch between the two transport pathways has thus been hypothesized as a mechanism that confers a higher flexibility in drought responses compared to nonmycorrhizal plants.

The mechanisms of nutrient exchange between the symbionts are not well defined, so the study of these membrane proteins should also provide a better understanding of the preferred mechanism of nutrient exchange in this symbiotic association. Recent findings suggest a potential involvement of the aquaporins themselves. Uehelin et al. (2007) identified various transmembrane aquaporins in the periarbuscular membranes of *Medicago truncatula* and found an AM-induced expression of specific aquaporin genes. They also suggested that aquaporins could act as low-affinity transporters of ammonia and/or ammonium. Further research is evidently necessary to fully understand the contribution of aquaporin genes to the enhanced drought resistance of AM plants.

Ecological Effects of AM Symbiosis: Ecosystem Services

Plants in ecosystems perform a series of functions (defined as ‘ecosystem services’) that are beneficial to the well-being of humans, providing multiple resources and processes (Daily 1997). Trade-offs and links between plants and soil microbial communities can act as drivers of a wide range of processes in ecosystems (Lavorel 2013 and Grigulis et al. 2013). Given the beneficial functions of AM fungi on plant fitness, resilience against environmental stresses, nutrient cycling, and soil quality, AM symbiosis is now recognized to play a fundamental role as a provider of ecosystem services.

Various ecosystem services delivered by AM have been identified: biofertilization from the AM promotion of plant growth, which in turn reduces fertilizer requirements, stabilization of soil structure, and bioregulation consequent to the plant metabolic modifications by AM fungi (Gianinazzi et al. 2010). Linking functional traits of plants and soil microbes, such as AM fungi, with their delivery of multiple ecosystem services is currently considered a rational mean for assessing the functioning of a given ecosystem (De Bello et al. 2010).

Less attention, however, has been given to beneficial soil organisms in general and AM in particular and their influence on the processes of ecosystems that contribute to the ecosystem services in agroecology. In this context, Gianinazzi et al. (2010) recently examined several aspects of plant–AM combinations that should be investigated further for appropriately managing the contribution of mycorrhizal fungi to ecosystem services and thus for optimizing the impact of these beneficial organisms while guaranteeing plant productivity and quality in agrosystems. The positive effect of AM on the ability of plants to counteract the conditions of drought confers to AM a pivotal role as a valuable technology not only for the sustainability of agricultural systems, but also for the restoration of degraded natural arid and semi-arid areas, where multiple environmental stresses, including drought, occur (Gianinazzi et al. 2010; Barea et al. 2011).

In light of the assessment of the multiple ecosystem services provided by AM, critical advances are required for elucidating the functional importance and value of plant and mycorrhizal diversity that are necessary for the functioning of ecosystems. These are also required for clarify the links among plant traits and their associated AM fungal characteristics to quantify the contribution of plant–AM fungi associations to ecosystem services under various environmental constraints (Barea et al. 2011; Grigulis et al. 2013; Lavorel 2013).

The role of AM symbiosis in the functional traits of both plants and microbes that could characterize above- and belowground ecosystem services has not yet been explored. Despite the recent advance in knowledge on mycorrhizal functioning, further research is necessary to better understand the significance and value of AM symbiosis in delivering ecosystem services in both agrosystems and natural environments.

An appropriate assessment of plant–AM feedbacks is therefore essential for predicting the effects of environmental constraints such as drought on ecosystem processes and, thus, for the provision of ecosystem services. Various advanced approaches can provide new insight to this field. The application of a trait-based approach to both plant and AM fungal communities represents a promising opportunity to understand how functional AM feedbacks between plant and AM fungi translate into interactions between ecosystem services (Lavorel 2013).

The new field of system biology that investigates plants at an ecological level, including all relationships and networks of plant communities, benefits from the different ‘omic’ technologies, from transcriptomics to proteomics, functional genomics, and metabolomics. New advances are represented by the emerging ‘ecometabolomic’ approach that aims to dissect the global metabolomic response of an organism to environmental changes (Sardans et al. 2011; Peñuelas et al. 2013). In particular, this new ‘omic’ system will allow the detection of the main metabolic pathways responsible for organismic responses and could provide improved knowledge of plant and mycorrhizal genes and their regulatory networks involved in the responses.

These integrated studies should provide the possibility of extrapolating plant responses from individual components to the level of ecosystems and of taking a step forward in our knowledge of the mechanisms and processes underlying the changes in resource use under future global change (Peñuelas et al. 2013). These pioneering approaches provide interesting perspectives and a very valuable framework for further studies focusing on integrated analyses of the effects of AM symbiosis under abiotic constraints for better quantifying the ecosystem services delivered by symbiosis, which has important implications for ecosystems in water-limited environments under future climatic changes.

Concluding Remarks

To summarize, mycorrhizal plants employ various protective mechanisms to counteract drought stress. Considerable progress has been made in understanding the role of AM symbiosis in conferring drought resistance to plants, but different aspects still require attention for unraveling novel metabolites and hidden metabolic pathways. The accumulated physiological, biochemical, and molecular data based on classical approaches will benefit from the various ‘omic’ techniques and their combinations. An in-depth investigation using the advanced methodologies could help to elucidate the mechanisms of drought avoidance and/or tolerance induced by AM symbiosis and to discriminate the drought-induced processes of the protective mechanisms regulated by AM symbiosis.

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