

Induction of Plant Tolerance to Semi-arid Environments by Beneficial Soil Microorganisms – A Review

R. Aroca and J.M. Ruiz-Lozano

Abstract Currently arid or semi-arid land areas are increasing worldwide due to global warming and the soil is becoming saline because of the use of intensive irrigation in the crop fields. Consequently, the proportion of plants living under water shortage conditions is increasing. This phenomenon is limiting seriously crop production in such areas. In many cases, the fields are being abandoned and become uncultivable again in a period of time due to erosion. Although plants have their own mechanisms to cope with drought stress, they become more tolerant to drought when associated with different soil microorganisms. Among these soil microorganisms, the most abundant and effective are rhizobia, plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF). Rhizobia fix atmospheric nitrogen and transport this fixed nitrogen to the legume host plant. PGPR promote plant growth by means of several mechanisms. They are present in almost all ecosystems across the world. AMF were vital to plants when starting to colonize dry land surface, and hence for improving plant mineral nutrition, especially uptake of phosphorous, among other factors. Here we detail the most recent advances about how these microorganisms enhance plant drought tolerance at physiological and molecular levels, including decreased oxidative stress, improved water status or regulation of aquaporins. It has been found that legume plants inoculated with rhizobia grow faster under drought conditions than non-inoculated ones. However, how rhizobial symbiosis affects root water transport has not been addressed yet. At the same time, it seems that there is a correlation between drought tolerance in rhizobial bacteria and rhizobia-induced plant drought tolerance, at least in terms of reducing plant oxidative stress. Under drought conditions, PGPR regulate the levels of stress-related hormones, i.e. abscisic acid and ethylene. The regulation of these hormones could be the cause of an enhancement of plant drought tolerance mediated by PGPR. However, a more detailed molecular approach is still needed to fully understand this process. Arbuscular mycorrhizal symbiosis improves almost every

R. Aroca (✉)

Departamento de Microbiología del Suelo y Sistemas Simbióticos, Estación Experimental del Zaidín (CSIC), Profesor Albareda, 1, 18008, Granada, Spain
e-mail: raroa@eez.csic.es

physiological parameter of the host plant under drought stress, i.e. water status, leaf transpiration, photosynthesis or root water uptake. However, the molecular basis for this improvement is far to be understood. At the same time, AMF in combination with rhizobia or PGPR usually have an accumulative beneficial effect on plant drought tolerance, although this depends on the specific pair of strains inoculated. Therefore, although there are many studies in order to understand at the physiological level how beneficial soil microorganisms induce plant drought tolerance, there is still a lack of knowledge about the molecular basis behind this improvement.

Keywords Aquaporins · Arbuscular mycorrhizal fungi · Drought · Oxidative stress · Plant growth promoting rhizobacteria · Rhizobia

1 Introduction

In the last decades the dry land surface becoming arid or semi-arid is rising progressively, increasing concomitantly, vegetative surface areas subjected to drought (Herrmann and Hutchinson, 2005). Plants have developed several mechanisms to cope with drought episodes during their evolution, ranging from morphological characteristics to molecular functions (Fig. 1). Morphological mechanisms

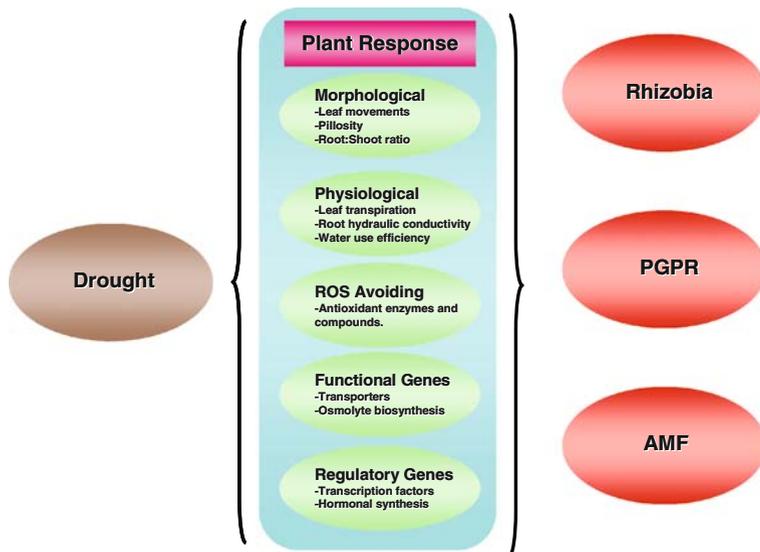


Fig. 1 Scheme summarizing the modulation of several plant processes by some beneficial soil microorganisms (rhizobia, plant growth promoting rhizobacteria and arbuscular mycorrhizal fungi) in response to drought stress. ROS: reactive oxygen species, PGPR: plant growth promoting rhizobacteria, AMF: arbuscular mycorrhizal fungi

include, among others, leaf movements and abscission, increased leaf pillosity or greater root:shoot ratio (Gu et al., 2007; Susiluoto and Berninger, 2007). The above-cited mechanisms are aimed to avoid water loss from the leaves and to increase the area of soil explored by the roots in order to take up more water and nutrients.

The most common physiological mechanisms plants have to cope with drought episodes are changes in leaf transpiration and root hydraulic properties. Thus, leaf transpiration and leaf conductance decrease under water stress conditions, and therefore, in some circumstances the water-use efficiency rises (Tambussi et al., 2007). As the water-use efficiency rises, the amount of water needed to produce the same amount of plant biomass decreases. This mechanism is important to keep plant growth under water-limited environments (Tambussi et al., 2007). At the same time, plant root hydraulic conductivity (L) usually decreases under drought conditions, although the contrary has also been reported (Siemens and Zwiazek, 2004). Under severe drought conditions L decreases in order to avoid water lost from root tissues. However, when the water deficit is moderate, plants can increase their L in order to take up more water from soil (Siemens and Zwiazek, 2004). These changes in L are related to changes in the abundance of aquaporins (Aroca et al., 2006). Aquaporins are proteinaceous channels present in the membranes of all living organisms that facilitate the passage of water following an osmotic gradient (Maurel, 2007).

Under water-deficit conditions the production of reactive oxygen species (ROS) increases in plant tissues, but plants have several compounds and enzyme systems capable of removing ROS efficiently (Shvaleva et al., 2006) (ROS: reactive oxygen species). Although there are several antioxidant compounds in plants, the most important and better studied are ascorbate and glutathione (Noctor, 2006). Plants have several antioxidant enzymes that, acting in synchrony, are able to remove ROS generated during drought. These enzymes include those from the ascorbate-glutathione cycle, catalases and superoxide dismutases (Wu et al., 2006a).

The above-cited mechanisms that allow plants to cope with drought stress are regulated by changes in gene expression. Drought-regulated genes can be divided into two groups: functional or regulatory. Functional genes include those encoding for transporters, detoxification enzymes, chaperones or enzymes involved in osmolyte biosynthesis. On the other hand, regulatory genes encode for transcription factors, protein kinases or phosphatases, or enzymes involved in hormone biosynthesis (Shinozaki and Yamaguchi-Shinozaki, 2007).

It must be considered, however, that in nature, plants usually interact with several soil microorganisms that make the plants more efficient to cope with environmental stresses like drought. The most important soil microorganisms that associate with plants are nitrogen-fixing bacteria in the case of legumes (rhizobia), plant growth promoting rhizobacteria (PGPR) and mycorrhizal fungi (Barea et al., 2005). In the present review we intend to describe the most recent physiological and molecular advances about how the above-cited soil microorganisms allow plants to be more tolerant to drought stress.

2 Rhizobial Symbiosis

2.1 Background

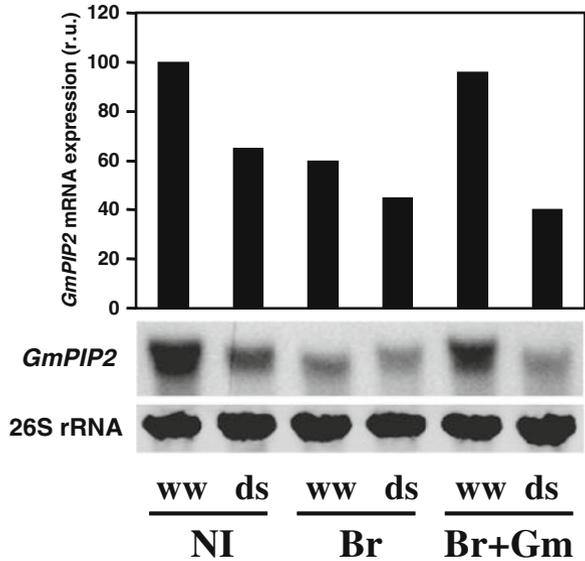
The term rhizobia include collectively all nitrogen-fixing bacteria that form nodules in legume plants, including several genera (reviewed by Willems, 2006). It has been estimated that approximately 53 million tonnes of nitrogen are harvested globally in food crops each year, and most of this nitrogen is coming from inorganic fertilizers (Garg and Geetanjali, 2007). Thus, the biological nitrogen fixation carried out by rhizobia offers an opportunity for reducing nitrogen inputs, and also diminishes nitrogen loss to the atmosphere. In the past recent years, a substantial progress of how rhizobial bacteria and legume plants start the symbiosis and which signals and genes are involved has been made (Kinkema et al., 2006; Garg and Geetanjali, 2007). Here, we focus on how this symbiosis improves legume plant tolerance to drought stress.

2.2 Rhizobia-Induced Plant Drought Tolerance

A beneficial effect by nodulation on plant growth under drought stress conditions has been usually observed in legume plants (Goicoechea et al., 1997; Figueiredo et al., 1999, Shisanya, 2002; Mnasri et al., 2007). However, there are few examples in the literature where it has been proven that nodulated plants had better water status than non-nodulated ones as evidenced by their leaf water potential (Ψ_w ; Mrema et al., 1997). Plant water status depends on the balance between water loss from the leaves and water uptake by roots. In fact, no differences in leaf transpiration between nodulated and non-nodulated plants under drought conditions have been observed so far (Goicoechea et al., 1997; Figueiredo et al., 1999). Surprisingly, there is no information about how rhizobial symbiosis may affect root water uptake or root hydraulic properties. This point should be checked in the near future. The only insight about the possible regulation of root hydraulic properties by rhizobial symbiosis was found by Porcel et al. (2006). In this work, Porcel and co-workers found a diminution of the expression of a plasma membrane aquaporin gene in the roots of soybean plants inoculated with the nitrogen-fixing bacteria *Bradyrhizobium japonicum* under both control and water-deprived conditions (Fig. 2). However, since aquaporin family in plants is composed of about 30 different genes, a more comprehensive study is needed to elucidate the significance of these changes. At the same time, it is known that some aquaporins may transport ammonia or ammonium, and hence they could be involved in the transport of nitrogen compounds between the nodules and the host plant (Tyerman et al., 2002; Uehlein et al., 2007). In fact, some plant aquaporins have been detected by immunogold labelling on different nodule tissues of soybean plants (Fleurat-Lessard et al., 2005).

It is known that plant nitrogen status regulates root hydraulic properties. In fact, when plants are deprived of nitrogen, root hydraulic conductivity is lower

Fig. 2 Expression analysis of the *Glycine max* aquaporin *GmPIP2* gene in roots of non-inoculated plants (NI), inoculated with *Bradyrhizobium japonicum* (Br) or double-inoculated with Br and the arbuscular mycorrhizal fungi *Glomus mosseae* (Br+Gm), under well-watered (ww) or drought stress (ds) conditions. r.u. means relative units. Data adapted from Porcel et al. (2006)



than when enough amount of nitrogen is available (Gloser et al., 2007). Therefore, since nodulated plants have usually more nitrogen content than non-nodulated ones (Figueiredo et al., 1999; Shisanya, 2002), it is possible that this difference in nitrogen content also accounts for differences in root hydraulic properties. However, this hypothesis needs to be checked empirically.

The beneficial effects of rhizobial symbiosis on plant growth and yield under water stress conditions are bacterial-genotype specific. Thus, Mnasri et al. (2007) found that *Phaseolus vulgaris* plants inoculated with a salt-tolerant nitrogen-fixing bacterial strain (*Ensifer meliloti*) were more tolerant to drought than those inoculated with a salt-sensitive bacterial strain (*Rhizobium tropici*). These data clearly illustrate the relationship between the osmotic stress tolerance of the bacterial strain and the efficiency of the symbiosis. Similar results were reported by Swaine et al. (2007), since they found that a strain of *Bradyrhizobium elkanii* isolated from a drought environment was more tolerant to an in vitro osmotic stress than strains isolated from wet environments.

It is known that drought stress causes an oxidative stress in nodules (Porcel et al., 2003), and it is possible that the different efficiency of the symbiosis under drought conditions among different bacterial strains could be related to their different tolerance to drought-induced oxidative stress. However, again, this hypothesis needs to be checked empirically.

As summary, nodulation confers plant drought tolerance in terms of growth, improvement of water status and diminution of oxidative stress. However, this beneficial effect depends on the origin of the rhizobial strain, with the strains more tolerant to osmotic stresses also conferring more tolerance to such stresses to plants.

2.3 Perspectives

There is increasing information about how the nitrogen-fixing bacteria and the plant recognize each other (Kinkema et al., 2006; Garg and Geetanjali, 2007) and how the nodule per se suffers an oxidative stress under drought conditions (Ruiz-Lozano et al., 2001b; Porcel et al., 2003; Gunther et al., 2007). However, there is still lack of information about how rhizobial symbiosis confers plant drought tolerance. Experiments dealing with the effects of nodulation on root hydraulic properties and aquaporin expression and with the different antioxidant capacities among different nitrogen-fixing bacterial strains are needed.

3 Plant Growth Promoting Rhizobacteria (PGPR)

3.1 Background

The term plant growth promoting rhizobacteria (PGPR) refers to soil bacteria which are able to colonize root systems and promote plant growth. In the broadest sense PGPR include rhizobia, but the term PGPR is usually referred to free-living bacteria present either in the rhizosphere, in the root surface, or inhabiting spaces between cortical cells (Gray and Smith, 2005). The mechanisms by which the PGPR enhance plant growth are diverse and include non-symbiotic nitrogen fixation, phosphate solubilization, counteraction of plant pathogen microorganisms, or regulation of different plant hormone levels (Gray and Smith, 2005; Tilak et al., 2005; Cohen et al., 2008). Here, we intend to highlight the mechanism by which PGPR induce plant drought tolerance.

3.2 PGPR-Induced Plant Drought Tolerance

Several free-living soil bacteria induce plant drought tolerance in terms of plant growth promotion (Arkhipova et al., 2007; Jaleel et al., 2007; Sziderics et al., 2007). However, the mechanism involved could be very different. For example, Arkhipova et al. (2007) found that the beneficial effect on lettuce plant growth under water-limited conditions caused by the inoculation with a *Bacillus* sp. strain was related to an increased level of cytokinins. Another mechanism involved in plant drought tolerance induction by PGPR is the diminution of ethylene production. Thus, PGPR containing 1-aminocyclopropane-1-carboxylate deaminase enzyme, which catabolizes the ethylene precursor, are able to diminish the ethylene contents in plant tissues under drought stress, favouring the plant growth and a better water status (Mayak et al., 2004). Abscisic acid (ABA) could also be involved in the enhancement of plant drought tolerance by PGPR. Arkhipova et al. (2007) found that *Bacillus* sp.-inoculated lettuce plants had also increased amounts of ABA when compared to non-inoculated plants. Since ABA is necessary

for plant drought tolerance (Davies et al., 2005), alterations of ABA levels could be another mechanism for tolerance enhancement. Also, Cohen et al. (2008) recently found that the PGPR *Azospirillum brasilense* is able to synthesize ABA in vitro and to increase its production in presence of NaCl. *Arabidopsis* plants inoculated with *Azospirillum* had more ABA content than non-inoculated ones (Cohen et al., 2008).

One of the mechanisms by which ABA enhances plant drought tolerance is via regulation of leaf transpiration and root hydraulic conductivity (Aroca et al., 2006). However, although Arkhipova et al. (2007) found an increase of ABA levels in lettuce plants inoculated with *Bacillus* sp., authors did not find any difference in stomatal aperture between inoculated and non-inoculated plants. This behaviour could be caused by the counterbalance of the higher levels of cytokinins, since the ratio between ABA and cytokinins determines stomatal aperture (Goicoechea et al., 1997; Dodd, 2003; Davies et al., 2005; Arkhipova et al., 2007). Unfortunately, there is only one report in the literature showing a positive effect of a PGPR (*Azospirillum brasilense*) inoculation on root hydraulic conductivity under control and osmotic stress conditions (Sarig et al., 1992). It is possible that this positive effect described by Sarig et al. (1992) could be caused by an up-regulation of aquaporins. Marulanda et al. (2006) found that under drought conditions, *Retama sphaerocarpa* plants inoculated with *Bacillus thuringiensis* took up more water than non-inoculated plants. However, in this experiment, inoculated plants also had more root biomass than non-inoculated ones, and the water uptake calculated on the basis of root biomass was lower in inoculated plants. From these experiments we can see the necessity of studying how PGPR modify aquaporin expression.

In 1999, Timmusk and Wagner (1999) found that prior to drought stress *Arabidopsis* plants inoculated with the PGPR *Paenibacillus polymyxa* had an elevation of copies of mRNA encoding for ABA-related genes (*ERD15* and *RAB18*), and moreover, these plants were more tolerant to drought than non-inoculated ones. The authors said that the inoculation with the bacteria could be causing a mild biotic stress and preparing the plants to cope with subsequent drought stress. More recently, Sziderics et al. (2007) have found that pepper plants inoculated with several PGPRs showed, after a mild osmotic stress, lower expression of genes related with abiotic stresses than non-inoculated plants. Authors explained this different behaviour considering that inoculated plants suffered less from the stress and also had less expression of stress-related genes. Although here we report some of the experiments where a gene expression analysis has been made in order to clarify which mechanisms could be involved in the induction of plant drought tolerance by PGPRs, a more wide and comprehensive approach is necessary. In this way, we could understand which genes (regulatory or functional) are behind this drought tolerance enhancement.

Taken together, it seems that PGPR are able to modify the hormonal contents of plants and, in this way, improve their drought tolerance (Fig. 3). At the same time, this growth promotion increases the capacity of the plants to take up more water during drought episodes.

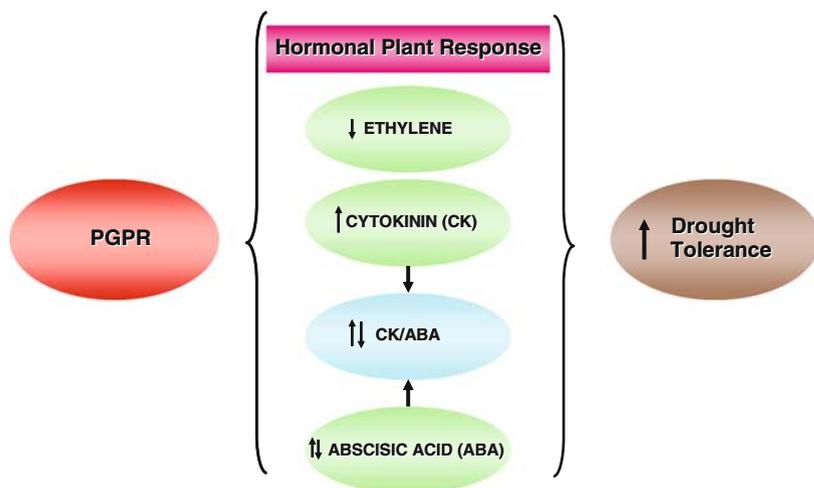


Fig. 3 Scheme showing how plant growth promoting rhizobacteria (PGPR) modify plant hormonal contents in order to increase their drought tolerance

4 Mycorrhizal Fungi

4.1 Background

The transition of plants from aquatic environment to land was possible through the association of plants with mycorrhizal fungi (Strullu-Derrien and Strullu, 2007). By the symbiosis between plants and mycorrhizal fungi, the plants get more nutrients and water resources from the soil, and the fungi get a protected niche and carbon skeletons from the plant, since the fungi are obligate symbionts (Entry et al., 2002). Among the different kinds of mycorrhizal symbiosis, arbuscular mycorrhizal one has the widest spread. About 90% of vascular plants establish a symbiosis with arbuscular mycorrhizal fungi (AMF; Gai et al., 2006). At the same time, by the symbiosis plants become more tolerant to environmental stresses with an osmotic component like drought (Augé, 2001; Ruiz-Lozano, 2003). Here we will describe the most recent advances on how this drought tolerance improvement is achieved from physiological and molecular points of view.

4.2 Arbuscular Mycorrhizal Symbiosis-Induced Plant Drought Tolerance

In most of the cases studied, the association between an AMF and a plant makes the host plant more tolerant to drought in terms of plant growth (Wu and Xia, 2006; Bolandnazar et al., 2007). However, this positive effect on plant growth depends on the AMF species involved (Marulanda et al., 2003, 2007; Wu et al., 2007)

and on the drought intensity (Aliasgharzad et al., 2006). One of the mechanisms involved in this induction of drought tolerance is the regulation of plant transpiration. In general, under both well-watered and drought conditions, AMF-colonized plants have higher transpiration rate than non-inoculated plants (Wu and Xia, 2006; Bolandnazar et al., 2007; Wu et al., 2007). By this mechanism, AMF-colonized plants are able to fix more CO₂ than non-inoculated plants and hence their growth is improved (Querejeta et al., 2007). In addition, in some cases, also their water-use efficiency is stimulated independent of changes in transpiration rate (Bolandnazar et al., 2007). These changes described in transpiration rate by AM symbiosis are correlated with changes in the ABA:cytokinins ratio (Goicoechea et al., 1997).

At the same time, AMF-colonized plants, by the action of the fungal hyphae, are able to explore more soil and therefore to take up more water from it than non-inoculated plants (Marulanda et al., 2003; Khalvati et al., 2005; Bolandnazar et al., 2007). AMF colonization induces an increase in root hydraulic conductivity of the host plants under osmotic stress conditions (Sánchez-Blanco et al., 2004; Aroca et al., 2007). However, when aquaporin expression was analyzed in AM and non-AM roots under drought stress, a lower expression has been found in AM ones (Porcel et al., 2006; Aroca et al., 2007). These results could indicate a water conservative mechanism in AM plants, decreasing the amount of water lost from the roots to the soil, following a water potential gradient. The above-cited works did not cover the expression of the full aquaporin gene family of the host plants, and this kind of work is absolutely needed. Also, the possible involvement of the AMF aquaporins on the greatest water uptake capacity of the host plants has not been addressed because no aquaporins from any AMF have been described yet.

The beneficial effects of the AM symbiosis on plant drought tolerance have not been only assayed by measuring plant growth or plant water status. At the same time, these beneficial effects have been addressed by measuring the expression of some genes related to drought stress. These genes include late embryogenesis abundant proteins (LEA) or Δ^1 -pyrroline-5-carboxylate synthetase (P5CS) enzyme. LEA proteins are involved in acquiring desiccation tolerance (Tunnacliffe and Wise, 2007), and P5CS enzyme catalyzes the limiting step in the synthesis of the osmolyte proline (Aral and Kamoun, 1997). Porcel et al. (2004, 2005) found that both kinds of genes increased their expression under drought conditions more in non-AM plants than in AM ones. Thus, the use of these two genes as stress markers can be used in studies involving AM symbiosis and osmotic stresses (Aroca et al., 2008; Jahromi et al., 2008).

Other beneficial effect of the AM symbiosis on plant drought tolerance is the diminution of the oxidative stress generated during drought periods (Porcel and Ruiz-Lozano, 2004; Wu et al., 2006a,b). In general, AMF-colonized plants had highest activities of several antioxidant enzymes than non-colonized ones (Wu et al., 2006a), but it depends on the enzyme activity, plant organ and the AMF genotype involved (Lambais et al., 2003; Wu et al., 2006b). At the same time, some superoxide dismutase isoforms from lettuce are specifically up-regulated by drought conditions in AMF-colonized plants (Ruiz-Lozano et al., 2001a). However, Porcel and Ruiz-Lozano (2004) found some evidence supporting the idea that the lower

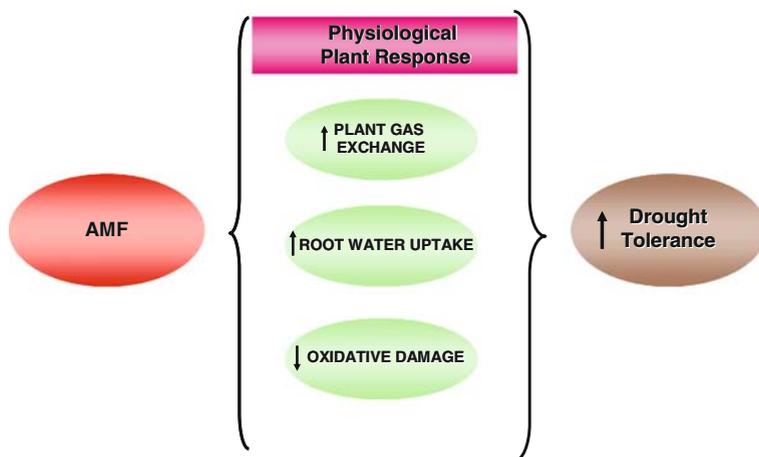


Fig. 4 Scheme showing how arbuscular mycorrhizal fungi (AMF) modify plant physiology in order to increase their drought tolerance

oxidative damage found in AMF-colonized plants under drought stress could be a consequence of their better water status. These results, as commented before, highlight the importance of studying at molecular level how AM symbiosis improves the water status of the host plants under drought conditions.

To summarize, AM symbiosis improves almost all the physiological aspects of plants during drought, like growth rate, leaf transpiration, root water uptake or diminishing the oxidative damage associated to drought (Fig. 4). However, the molecular basis behind this improvement is still far from being known.

4.3 Interaction Between Arbuscular Mycorrhizal Fungi and Other Beneficial Soil Microorganisms

In the literature there are several reports showing a positive effect on plant drought tolerance when AMF and either PGPR or rhizobia are inoculated together (Tarafdar and Rao, 2007; Valdenegro et al., 2001; Behl et al., 2007). Thus, Valdenegro et al. (2001) found that the positive effects of three different AMF isolates on plant growth in *Medicago arborea* plants under drought conditions were stimulated by the co-inoculation with the PGPR *Enterobacter* sp., depending on the rhizobial strain inoculated and on the AMF isolate. The same authors also found that some combinations of AMF, PGPR and rhizobia increased the nodule number present in the roots. This dependency on the pair of symbionts co-inoculated was also found for lettuce plants inoculated with the PGPR *Bacillus megaterium* and three different isolates of AMF, being one of the combinations harmful in terms of plant growth (Marulanda-Aguirre et al., 2008; Fig. 5). It would be very interesting to elucidate which molecular signals are involved in this different behaviour of plant growth depending on the counterpart microorganisms inoculated.

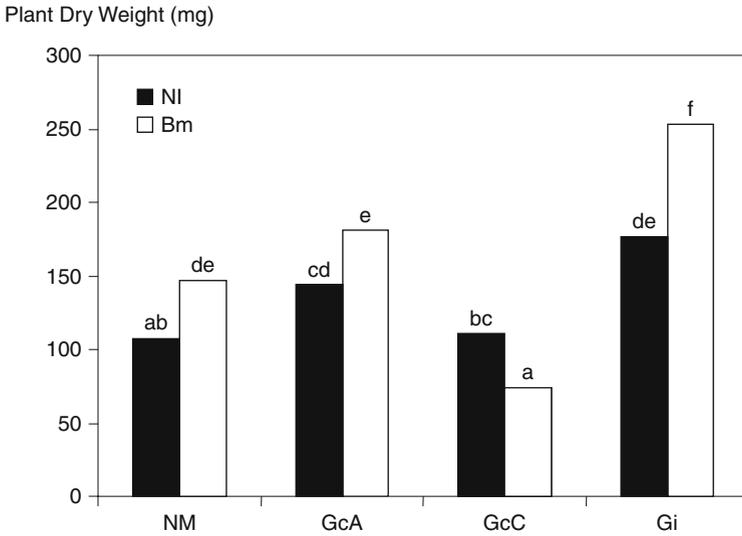


Fig. 5 Total dry weight of lettuce non-inoculated plants (NI) or inoculated with a *Bacillus megaterium* strain (Bm), alone (NM) or with different arbuscular mycorrhizal fungal isolates. GcA: *Glomus constrictum* autochthonous; GcC: *Glomus constrictum* from collection; Gi: *Glomus intraradices*. Different letters mean significant differences ($p < 0.05$) after ANOVA and LSD tests. Data adapted from Marulanda-Aguirre et al. (2008)

One of the most common beneficial effects of AMF is the diminution of the oxidative stress occurring in the nodules of the legumes under drought stress (Ruiz-Lozano et al., 2001b; Porcel et al., 2003). This diminution is caused in part by a higher activity of the antioxidant enzyme glutathione reductase in the nodules of the AMF-colonized roots (Porcel et al., 2003). However, this beneficial effect could also be related to the lower water deficit suffered by the nodules of the AMF-colonized plants (Porcel et al., 2003). Specific experiments to resolve this question are needed in the near future. On the other hand, carbon metabolism of nodules from roots of *Anthyllis cytisoides* is improved under drought conditions by AM symbiosis (Goicoechea et al., 2005). How this improvement on nodule carbon metabolism is taking place is still unknown.

Therefore, although in general there is a positive interaction between AMF and other beneficial soil microorganisms, sometimes a negative effect is found. Which molecular signals are behind this beneficial or negative interactions need to be elucidated.

5 Conclusion

It is clear that the symbiosis between the microorganisms cited here and plants confers an enhancement of plant drought tolerance. However, in the case of rhizobial symbiosis, there are still basic physiological studies to be done. These studies would

include analysis of the behaviour of root hydraulic conductivity under drought conditions in nodulated and non-nodulated plants. Also, studies on how nodulation affects plant aquaporin gene expression would complement the above physiological studies. Regarding PGPR and AMF symbiosis, studies at molecular level focusing on which signals are involved in both the drought tolerance enhancement per se and in the different interrelationships between these two kinds of symbionts are needed.

As conclusion we can summarize that rhizobia improve plant drought tolerance in terms of growth, water status and diminution of oxidative damage, but these beneficial effects depend on the origin of the strain inoculated. At the same time, it seems from the literature that the beneficial effect of PGPR on plant drought tolerance is caused by changes in hormonal contents, mainly that of ABA, ethylene and cytokinins. Moreover, PGPR could also improve the capacity of plants to take up more water under drought conditions. Finally, at physiological level it is clear that AM symbiosis makes the plants more tolerant to drought stress, and that this is enhanced by the combination of other soil beneficial microorganisms. However, this interaction is adverse to the plant in some circumstances.

References

- Aliasgharзад N., Neyshabouri M.R., Salimi G. (2006) Effects of arbuscular mycorrhizal fungi and *Bradyrhizobium japonicum* on drought stress of soybean. *Biologia* 61, 324–328.
- Aral B., Kamoun P. (1997) The proline biosynthesis in living organisms. *Amino Acids* 13, 189–217.
- Arkhipova T.N., Prinsen E., Veselov S.U., Martineko E.V., Melentiev A.I., Kudoyarova G.R. (2007) Cytokinin producing bacteria enhances plant growth in drying soil. *Plant Soil* 292, 305–315.
- Aroca R., Ferrante A., Vernieri P., Chrispeels M.J. (2006) Drought, abscisic acid and transpiration rate effects on the regulation of PIP aquaporin gene expression and abundance in *Phaseolus vulgaris* plants. *Ann. Bot.* 98, 1301–1310.
- Aroca R., Porcel R., Ruiz-Lozano J.M. (2007) How does arbuscular mycorrhizal symbiosis regulate root hydraulic properties and plasma membrane aquaporins in *Phaseolus vulgaris* under drought, cold or salinity stresses? *New Phytol.* 173, 808–816.
- Aroca R., Vernieri P., Ruiz-Lozano J.M. (2008) Mycorrhizal and non-mycorrhizal *Lactuca sativa* plants exhibit contrasting responses to exogenous ABA during drought stress and recovery. *J. Exp. Bot.* 59, 2029–2041.
- Augé R.M. (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* 11, 3–42.
- Barea J.M., Pozo M.J., Azcón R., Azcón-Aguilar C. (2005) Microbial co-operation in the rhizosphere. *J. Exp. Bot.* 56, 1761–1778.
- Behl R.K., Ruppel S., Kothe E., Narula N. (2007) Wheat \times *Azotobacter* \times VA mycorrhiza interactions towards plant nutrition and growth – A review. *J. Appl. Bot. Food Qual.-Angew. Bot.* 81, 95–109.
- Bolandnazar S., Aliasgharзад N., Neishabury M.R., Chaparzadeh N. (2007) Mycorrhizal colonization improves onion (*Allium cepa* L.) yield and water use efficiency under water deficit condition. *Sci. Hortic.* 114, 11–15.
- Cohen A.C., Bottini R., Piccoli P.N. (2008) *Azospirillum brasilense* Sp 245 produces ABA in chemically-defined culture medium and increases ABA content in arabidopsis plants. *Plant Growth Regul.* 54, 97–103.

- Davies W.J., Kudoyarova G., Hartung W. (2005) Long-distance ABA signaling and its relation to other signaling pathways in the detection of soil drying and the mediation of the plant's response to drought. *J. Plant Growth Regul.* 24, 285–295.
- Dodd I.C. (2003) Hormonal interactions and stomatal responses. *J. Plant Growth Regul.* 22, 32–46.
- Entry J.A., Rygiewicz P.T., Watrud L.S., Donnelly P.K. (2002) Influence of adverse soil conditions on the formation and function of arbuscular mycorrhizas. *Adv. Environ. Res.* 7, 123–138.
- Figueiredo M.V.B., Vilar J.J., Burity H.A., de França F.P. (1999) Alleviation of water stress effects in cowpea by *Bradyrhizobium* spp. Inoculation. *Plant Soil* 207, 67–75.
- Fleurat-Lessard P., Michonneau P., Maeshima M., Drevon J.J., Serraj R. (2005) The distribution of aquaporins subtypes (PIP1, PIP2 and gamma-TIP) is tissue dependent in soybean (*Glycine max*) root nodules. *Ann. Bot.* 96, 457–460.
- Gai J.P., Christie P., Feng G., Li X.L. (2006) Twenty years of research on community composition and species distribution of arbuscular mycorrhizal fungi in China: a review. *Mycorrhiza* 16, 229–239.
- Garg N., Geetanjali (2007) Symbiotic nitrogen fixation in legume nodules: process and signalling. A review. *Agron. Sustain. Dev.* 27, 59–68.
- Gloser V., Zwieniecki M.A., Orians C.M., Holbrook N.M. (2007) Dynamic changes in root hydraulic properties in response to nitrate availability. *J. Exp. Bot.* 58, 2409–2415.
- Goicoechea N., Antolín M.C., Sánchez-Díaz M. (1997) Gas exchange is related to the hormone balance in mycorrhizal or nitrogen-fixing alfalfa subjected to drought. *Physiol. Plant.* 100, 989–997.
- Goicoechea N., Merino S., Sánchez-Díaz M. (2005) Arbuscular mycorrhizal fungi can contribute to maintain antioxidant and carbon metabolism in nodules of *Anthyllis cytisoides* L. subjected to drought. *J. Plant Physiol.* 162, 27–35.
- Gray E.J., Smith D.L. (2005) Intracellular and extracellular PGPR: commonalities and distinctions in the plant-bacterium signalling processes. *Soil Biol. Biochem.* 37, 395–412.
- Gu M.M., Rom C.R., Robbins J.A., Oosterhuis D.M. (2007) Effect of water deficit on gas exchange, osmotic solutes, leaf abscission, and growth of four birch genotypes (*Betula* L.) under a controlled environment. *Hortscience* 42, 1383–1391.
- Gunther C., Schlereth A., Udvardi M., Ott T. (2007) Metabolism of reactive oxygen species is attenuated in leghemoglobin-deficient nodules of *Lotus japonicus*. *Mol. Plant-Microbe Interact.* 20, 1596–1603.
- Herrmann S.M., Hutchinson C.F. (2005) The changing contexts of the desertification debate. *J. Arid Environ.* 63, 538–555.
- Jaleel C.A., Manivannan P., Sankar B., Kishorekumar A., Gopi R., Somasundaram R., Panneerselvam R. (2007) *Pseudomonas fluorescens* enhances biomass yield and ajmalicine production in *Catharanthus roseus* under water deficit stress. *Colloid Surf. B-Biointerfaces* 60, 7–11.
- Jahromi F., Aroca R., Porcel R., Ruiz-Lozano J.M. (2008) Influence of salinity on the in vitro development of *Glomus intraradices* and on the in vivo physiological and molecular responses of mycorrhizal lettuce plants. *Microb. Ecol.* 55, 45–53.
- Khalvati M.A., Hu Y., Mozafar A., Schmidhalter U. (2005) Quantification of water uptake by arbuscular mycorrhizal hyphae and its significance for leaf growth, water relations, and gas exchange of barley subjected to drought stress. *Plant Biol.* 7, 706–712.
- Kinkema M., Scott P.T., Gresshoff P.M. (2006) Legume nodulation: successful symbiosis through short- and long-distance signaling. *Funct. Plant Biol.* 33, 707–721.
- Lambais M.R., Rios-Ruiz W.F., Andrade R.M. (2003) Antioxidant responses in bean (*Phaseolus vulgaris*) roots colonized by arbuscular mycorrhizal fungi. *New Phytol.* 160, 421–428.
- Marulanda A., Azcón R., Ruiz-Lozano J.M. (2003) Contribution of six arbuscular mycorrhizal fungal isolates to water uptake by *Lactuca sativa* L. plants under drought stress. *Physiol. Plant.* 119, 526–533.
- Marulanda A., Barea J.M., Azcón R. (2006) An indigenous drought-tolerant strain of *Glomus intraradices* associated with a native bacterium improves water transport and root development in *Retama sphaerocarpa*. *Microb. Ecol.* 52, 670–678.

- Marulanda A., Porcel R., Barea J.M., Azcón R. (2007) Drought tolerance and antioxidant activities in lavender plants colonized by native drought-tolerant or drought-sensitive *Glomus* species. *Microb. Ecol.* 54, 543–552.
- Marulanda-Aguirre A., Azcón R., Ruiz-Lozano J.M., Aroca R. (2008) Differential effects of a *Bacillus megaterium* strain on *Lactuca sativa* plant growth depending on the origin of the arbuscular mycorrhizal fungus coinoculated: physiologic and biochemical traits. *J. Plant Growth Regul.* 27, 10–18.
- Maurel C. (2007) Plant aquaporins: novel functions and regulation properties. *FEBS Lett.* 581, 2227–2236.
- Mayak S., Tirosh T., Glick B.R. (2004) Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. *Plant Sci.* 166, 525–530.
- Mnasri B., Aouani M.E., Mhamdi R. (2007) Nodulation and growth of common bean (*Phaseolus vulgaris*) under water deficiency. *Soil Biol. Biochem.* 39, 1744–1750.
- Mrema A.F., Granhall U., Sennerby-Forsse L. (1997) Plant growth, leaf water potential, nitrogenase activity and nodule anatomy in *Leucaena leucocephala* as affected by water stress and nitrogen availability. *Trees-Struct. Funct.* 12, 42–48.
- Noctor G. (2006) Metabolic signaling in defence and stress: the central roles of soluble redox couples. *Plant Cell Environ.* 29, 409–425.
- Porcel R., Aroca R., Azcón R., Ruiz-Lozano J.M. (2006) PIP aquaporin gene expression in arbuscular mycorrhizal *Glycine max* and *Lactuca sativa* plants in relation to drought stress tolerance. *Plant Mol. Biol.* 60, 389–404.
- Porcel R., Azcón R., Ruiz-Lozano J.M. (2004) Evaluation of the role of genes encoding for Δ^1 -pyrroline-5-carboxylate synthetase (P5CS) during drought stress in arbuscular mycorrhizal *Glycine max* and *Lactuca sativa* plants. *Physiol. Mol. Plant Pathol.* 65, 211–221.
- Porcel R., Azcón R., Ruiz-Lozano J.M. (2005) Evaluation of the role of genes encoding for dehydrin proteins (LEA D-11) during drought stress in arbuscular mycorrhizal *Glycine max* and *Lactuca sativa* plants. *J. Exp. Bot.* 56, 1933–1942.
- Porcel R., Barea J.M., Ruiz-Lozano J.M. (2003) Antioxidant activities in mycorrhizal soybean plants under drought stress and their possible relationship to the process of nodule senescence. *New Phytol.* 157, 135–143.
- Porcel R., Ruiz-Lozano J.M. (2004) Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. *J. Exp. Bot.* 55, 1743–1750.
- Querejeta J.I., Allen M.F., Alguacil M.M., Roldán A. (2007) Plant isotopic composition provides insight into mechanisms underlying growth stimulation by AM fungi in a semiarid environment. *Funct. Plant Biol.* 34, 683–691.
- Ruiz-Lozano J.M. (2003) Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. *New perspectives for molecular studies, Mycorrhiza* 13, 309–317.
- Ruiz-Lozano J.M., Collados C., Barea J.M., Azcón R. (2001a) Cloning of cDNAs encoding SODs from lettuce plants which show differential regulation by arbuscular mycorrhizal symbiosis and by drought stress. *J. Exp. Bot.* 52, 2241–2242.
- Ruiz-Lozano J.M., Collados C., Barea J.M., Azcón R. (2001b) Arbuscular mycorrhizal symbiosis can alleviate drought-induced nodule senescence in soybean plants. *New Phytol.* 151, 493–502.
- Sánchez-Blanco M.J., Ferrández T., Morales M.A., Morte A., Alarcón J.J. (2004) Variations in water status, gas exchange, and growth in *Rosmarinus officinalis* plants infected with *Glomus deserticola* under drought conditions. *J. Plant Physiol.* 161, 675–682.
- Sarig S., Okon Y., Blum A. (1992) Effect of *Azospirillum brasilense* inoculation on growth dynamics and hydraulic conductivity of *Sorghum bicolor* roots. *J. Plant Nutr.* 15, 805–819.
- Shinozaki K., Yamaguchi-Shinozaki K. (2007) Gene networks involved in drought stress response and tolerance. *J. Exp. Bot.* 58, 221–227.
- Shisanya C.A. (2002) Improvement of drought adapted tepary bean (*Phaseolus acutifolius* A. Gray var. *latifolius*) yield through biological nitrogen fixation in semi-arid SE-Kenya. *Eur. J. Agron.* 16, 13–24.

- Shvaleva A.L., Silva F.C.E., Breia E., Jouve L., Hausman J.F., Maroco J.P., Rodrigues M.L., Pereira J.S., Chaves M. (2006) Metabolic responses to water deficit in two *Eucalyptus globus* clones with contrasting drought sensitivity. *Tree Physiol.* 26, 239–248.
- Siemens J.A., Zwiazek J.J. (2004) Changes in root water flow properties of solution culture-grown trembling aspen (*Populus tremuloides*) seedlings under different intensities of water-deficit stress. *Physiol. Plant.* 121, 44–49.
- Strullu-Derrien C., Strullu D.G. (2007) Mycorrhization of fossil and living plants. *C. R. Palevol* 6, 483–494.
- Susiluoto S., Berninger F. (2007) Interactions between morphological and physiological drought responses in *Eucalyptus microtheca*. *Silva Fenn.* 41, 221–233.
- Swaine E.K., Swaine M.D., Killham K. (2007) Effects of drought on isolates of *Bradyrhizobium elkanii* cultured from *Albizia adianthifolia* seedlings of different provenances. *Agrofor. Syst.* 69, 135–145.
- Sziderics A.H., Rasche F., Trognitz F., Sessitsch A., Whilhelm E. (2007) Bacterial endophytes contribute to abiotic stress adaptation in pepper plants (*Capsicum annuum* L.). *Can. J. Microbiol.* 53, 1195–1202.
- Tambussi E.A., Bort J., Araus J.L. (2007) Water use efficiency in C-3 cereals under Mediterranean conditions: a review of physiological aspects. *Ann. Appl. Biol.* 150, 307–321.
- Tarafdar J.C., Rao A.V. (2007) Response of arid legumes to VAM fungal inoculation. *Symbiosis* 22, 265–274.
- Tilak K.V.B.R., Ranganayaki N., Pal K.K., De R., Saxena A.K., Nautiyal C.S., Mittal S., Tripathi A.K., Johri B.N. (2005) Diversity of plant growth and soil health supporting bacteria. *Curr. Sci.* 89, 136–150.
- Timmusk S., Wagner E.G.H. (1999) The plant-growth-promoting rhizobacterium *Paenibacillus polymyxa* induces changes in *Arabidopsis thaliana* gene expression: A possible connection between biotic and abiotic stress responses. *Mol. Plant-Microbe Interact.* 12, 951–959.
- Tunnacliffe A., Wise M.J. (2007) The continuing conundrum of the LEA proteins. *Naturwissenschaften* 94, 791–812.
- Tyerman S.D., Niemietz C.M., Bramley H. (2002) Plant aquaporins: multifunctional water and solute channels with expanding roles. *Plant Cell Environ.* 25, 173–174.
- Uehlein N., Fileschi K., Eckert M., Bienert G. P., Bertl A., Kaldenhoff R. (2007) Arbuscular mycorrhizal symbiosis and plant aquaporin expression. *Phytochemistry* 68, 122–129.
- Valdenegro M, Barea J.M., Azcón R. (2001) Influence of arbuscular-mycorrhizal fungi, *Rhizobium meliloti* strains and PGPR inoculation on the growth of *Medicago arborea* used as model legume for re-vegetation and biological reactivation in a semi-arid mediterranean area. *Plant Growth Regul.* 34, 233–240.
- Willems A. (2006) The taxonomy of rhizobia: an overview. *Plant Soil* 287, 3–14.
- Wu Q.S., Xia R.X. (2006) Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. *J. Plant Physiol.* 163, 417–425.
- Wu Q. S., Zou Y.N., Xia R.X. (2006a) Effects of water stress and arbuscular mycorrhizal fungi on reactive oxygen metabolism and antioxidant production by citrus (*Citrus tangerine*) roots. *Eur. J. Soil Biol.* 42, 166–172.
- Wu Q. S., Zou Y.N., Xia R.X. (2006b) Reactive oxygen metabolism in mycorrhizal and non-mycorrhizal citrus (*Poncirus trifoliata*) seedlings subjected to water stress. *J. Plant Physiol.* 163, 1101–1110.
- Wu Q. S., Zou Y.N., Xia R.X., Wang M.Y. (2007) Five *Glomus* species affect water relations of *Citrus tangerine* during drought stress. *Bot. Stud.* 48, 147–154.