Mycorrhiza (2012) 22:555–564
DOI 10.1007/s00572-012-0433-3

ORIGINAL PAPER

Plant potassium content modifies the effects of arbuscular mycorrhizal symbiosis on root hydraulic properties in maize plants

Mohamed Najib El-Mesbahi · Rosario Azcón · Juan Manuel Ruiz-Lozano · Ricardo Aroca

Received: 23 November 2011 / Accepted: 14 February 2012 / Published online: 28 February 2012

Abstract It is well known that the arbuscular mycorrhizal (AM) symbiosis helps the host plant to overcome several abiotic stresses including drought. One of the mechanisms for this drought tolerance enhancement is the higher water uptake capacity of the mycorrhizal plants. However, the effects of the AM symbiosis on processes regulating root hydraulic properties of the host plant, such as root hydraulic conductivity and plasma membrane aquaporin gene expression, and protein abundance, are not well defined. Since it is known that K^+ status is modified by AM and that it regulates root hydraulic properties, it has been tested how plant K^+ status could modify the effects of the symbiosis on root hydraulic conductivity and plasma membrane aquaporin gene expression and protein abundance, using maize (*Zea mays* L.) plants and *Glomus intraradices* as a model. It was observed that the supply of extra K^+ increased root hydraulic conductivity only in AM plants. Also, the different pattern of plasma membrane aquaporin gene expression and protein abundance between AM and non-AM plants changed with the application of extra K^+. Thus, plant K^+ status could be one of the causes of the different observed effects of the AM symbiosis on root hydraulic properties. The present study also highlights the critical importance of AM fungal aquaporins in regulating root hydraulic properties of the host plant.

Keywords Arbuscular mycorrhizal symbiosis · Drought · Plasma membrane aquaporins · Potassium · Root hydraulic properties

M. N. El-Mesbahi · R. Azcón · J. M. Ruiz-Lozano · R. Aroca (✉)
Departamento de Microbiología del Suelo y Sistemas Simbióticos,
Estación Experimental del Zaidín (CSIC),
Profesor Albareda 1,
18008 Granada, Spain
e-mail: raroca@eez.csic.es

Introduction

Plants in nature are exposed to several environmental constraints which depress their growth and development. However, most plants establish a symbiosis with arbuscular mycorrhizal (AM) fungi which increases their tolerance to several stressful conditions (Ruiz-Lozano et al. 2006; Evelin et al. 2009). In return, AM fungi find a niche to complete their life cycle and acquire carbon from the plant (Bago et al. 2002). It is well documented that AM affect the response of plants to drought and that water regimes may affect the development of the symbiosis (Ruiz-Lozano et al. 2006; Aroca et al. 2008b). Most research on how AM symbiosis improves plant host tolerance to drought stress has focused on single or combined anatomical, physiological, biochemical, and molecular approaches (Ruiz-Lozano et al. 1995, 1996; Miller et al. 1997; Goicoechea et al. 2004; Porcel et al. 2004, 2006; Krishna et al. 2005). Results conclude that AM plants are able to take up more water from the soil than non-AM plants under drought conditions (Ruiz-Lozano and Azcón 1995; Marulanda et al. 2003; Khalvati et al. 2005; Allen 2007) due to the direct transfer of water from the fungal hyphae to the host roots (Ruth et al. 2011). This higher water uptake capacity of AM plants could be one of the causes of their higher drought tolerance.

In the last years, evidence has pointed to the critical role of plant aquaporins in regulating water uptake by roots. Thus, Postaire et al. (2010) found that the *Arabidopsis* AtPIP1;2 aquaporin knockout line had lower root hydraulic conductivity (*L*) than wild-type plants. Similar results were previously reported for another *Arabidopsis* aquaporin knockout line (Javot et al. 2003) and using aquaporin-silenced tobacco plants (Siefritz et al. 2002). Plant aquaporins constitute a large and diverse gene family composed of 30 to 70 different proteins,

classified into five groups based on amino acid sequence similarities, each of which is subdivided in several subgroups (Maurel et al. 2008; Park et al. 2010).The five main groups are: plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins, nodulin-like intrinsic proteins, small and basic intrinsic proteins, and the most recently discovered uncharacterized intrinsic proteins.

Several publications have reported on how the AM symbiosis regulates host root aquaporin expression and abundance. Under non-stressed conditions, some studies found an upregulation of aquaporin gene expression by the AM symbiosis (Roussel et al. 1997; Uehlein et al. 2007; Aroca et al. 2008a, b; Alguacil et al. 2009), while others reported their downregulation (Ouziad et al. 2006; Porcel et al. 2006; Ruiz-Lozano et al. 2009). The different results are related to the specific genes analyzed (Ouziad et al. 2006; Aroca et al. 2007, 2008a; Ruiz-Lozano et al. 2009). However, a consistent downregulation of PIP protein abundance in the AM symbiosis has been reported under non-stressed conditions (Aroca et al. 2007; Benabdellah et al. 2009; Ruiz-Lozano et al. 2009). Under drought conditions, Ruiz-Lozano et al. (2009) found an upregulation of two out of seven PIP genes in AM maize roots and confirmed this upregulation at the protein level, but a downregulation of PIP gene expression by the AM symbiosis under drought conditions, as well as a lower protein levels, has also been observed (Porcel et al. 2006; Aroca et al. 2008b). This phenomenon depends on the AM fungal species involved and the aquaporin isoform studied (Porcel et al. 2006). These apparent discrepancies in the modification of aquaporin expression by the AM symbiosis fit with the idea that each plant aquaporin gene should have a specific function in each plant tissue and under specific environmental conditions (Jang et al. 2007; Peng et al. 2007; Ruiz-Lozano and Aroca, 2010).

Potassium (K^+) tissue concentration is an important physiological parameter which regulates root water uptake capacity and that is also altered by the AM symbiosis (Porras-Soriano et al. 2009; Benlloch-González et al. 2010). Drought influences K^+ uptake and K^+ tissue concentration resulting from K^+ availability also modifies plant response to drought (Egilla et al. 2001; Cakmak 2005). Although there is much information about the effects of K^+ starvation on root hydraulic properties, to our knowledge, there is only one study associating analysis of PIP gene expression. Thus, Liu et al. (2006) found that an increase in root hydraulic conductivity and an upregulation of several PIP genes was caused by K^+ starvation in rice plants. Also, a decrease in root water transport capacity caused by K^+ channel inhibitors has been observed (Tazawa et al. 2001; Liu et al. 2006). These results indicate a tight co-regulation between water and K^+ uptake processes.

While it is well-documented that water regime, AM symbiosis, and plant K^+ status affect root hydraulic properties (Liu et al. 2006; Aroca et al. 2008b), how the interaction between these three factors modifies root hydraulic properties has not yet been explored. In this context, the present research was aimed at evaluating the combined effects of plant K^+ contents, drought, and AM symbiosis on root hydraulic conductivity and aquaporin gene expression and protein abundance. To achieve this goal, maize plants were grown in the presence or absence of the AM fungus *Glomus intrardices*, under drought or well-watered conditions and at three levels of potassium. Shoot nutrient concentration, leaf stomatal conductance, root hydraulic conductivity, and root PIP aquaporin expression and abundance were analyzed. Moreover, since an aquaporin from the AM fungus used here has been cloned (Aroca et al. 2009), its gene expression was also analyzed.

Material and methods

Biological material and experimental design

Maize (*Zea mays* L.) seeds were surface-disinfected by incubating them for 10 min in 0.1% (*w/v*) NaClO and rinsed three times in distilled water. Seeds were sown in 1-L pots filled with a mixture of sterilized sand and soil (1:1). Soil was collected from Estación Experimental del Zaidín (Granada, Spain), sieved (2 mm), and then sterilized by steaming (1 h at 100°C for three consecutive days). The soil had a pH of 8.1 (water); 1.81% organic matter, nutrient concentrations (milligrams per kilogram): N, 2.5; P 6.2 ($NaHCO_3$-extractable P); K, 132.0. The soil texture was made up of 35.8% sand, 43.6% silt, and 20.5% clay. Plants were grown in a glasshouse with temperature ranging from 22°C to 27°C, RH from 40% to 60%, and a photoperiod of 16:8 (light/dark) supplemented with fluorescent lamps.

At sowing, 10 g of AM fungal inoculum (*Glomus intraradices* Schenck and Smith, isolate BEG 121) was added to half of the pots. Inoculum was bulked in an open-pot culture of *Trifolium repens* L. mixed with *Sorghum vulgare* Pers. X *Sorghum*×*drummondii* (Steud.) Millsp. & Chase plants and consisted of substrate (vermiculite/sepiolite, 1:1), spores, mycelia, and infected root fragments. Uninoculated control plants received the same amount of autoclaved mycorrhizal inoculum together with a 3-ml aliquot of a filtrate (<20 μm) of the inoculum in order to provide a general microbial population free of AM fungal propagules. All pots received per week 10 mL nutrient solution composed of 3 mM KNO_3, 4.4 mM $Ca(NO_3)_2$, 0.4 mM NaH_2PO_4, 1.5 mM $MgSO_4$, 67 μM EDTA–Fe, 5 μM $MnSO_4$, 1 μM $CuSO_4$, 1 μM $ZnSO_4$, 30 μM H_3BO_3, and 0.15 μM Na_2MoO_4. At the same time, one third of the pots received an extra 10 mL 10 mM K_2SO_4 solution once a week (level 1 of K^+), while another third of the pots received an extra 10 mL 20 mM

K_2SO_4 solution per week (level 2 of K^+). Finally, the remaining one third of the pots received 10 mL water, as controls.

Simultaneously, 6 weeks after sowing, half of the pots from each treatment were subjected to drought stress during an additional week. Water was supplied daily to maintain soil at 100% of field capacity during the first 6 weeks after sowing, then half of the plants were allowed to dry for 2 days until soil water content reached 75% of field capacity, while the other half were maintained at field capacity. The 100% soil water holding capacity corresponded to 20% volumetric soil moisture, and the 75% soil water holding capacity corresponded to 12% volumetric soil moisture, both measured with a ThetaProbe (AT Delta-T Devices Ltd., Cambridge, UK) and determined experimentally in a previous experiment using a pressure plate apparatus. The soil water content was measured daily (morning) with the ThetaProbe ML2 before rewatering (at the end of the afternoon), and the amount of water lost was added to each pot in order to keep the soil water content at the desired level of 75% field capacity (Porcel and Ruiz-Lozano 2004). During the 24-h period between each rewatering, the soil water content progressively decreased, reaching a minimum value of approximately 85% and 60% of field capacity for well watered and water stressed plants, respectively. Plants were maintained under such conditions for 1 week before harvesting. These percentages of soil moisture were selected based on previous studies carried out also in maize (Bárzana et al. 2012).

Mycorrhizal development and growth analyses

Five plants per treatment were harvested. The percentage of root length colonized by *G. intraradices* was estimated microscopically after clearing washed roots in 10% KOH and staining with 0.05% trypan blue in lactic acid (*v/v*). The extent of mycorrhizal colonization (percent and total root length) was calculated according to the gridline intersect method (Giovannetti and Mosse 1980). Shoot and root dry weights were measured after incubating plant material at 75° C in an oven during 2 days.

Root hydraulic conductivity (*L*)

Root hydraulic conductivity was measured 2 h after dawn in plants exuding under atmospheric pressure as previously described by Ruiz-Lozano et al. (2009) and Marulanda et al. (2010). Briefly, at harvest, shoots of five plants per treatment were removed 1 cm above the root insertion. A silicon tube was attached to the stem; the exuded sap of the first 15 min was discarded to avoid phloem contaminations, and the exuded sap in the following 2 h was collected and weighed. Osmotic potential of the collected sap and the nutrient solution was measured using a cryoscopic osmometer (Osmomat 030, Gonotec Gmbh, Berlin, Germany). *L* was calculated as the ratio between root water flux (grams water per gram root dry weight per hour) and the osmotic potential gradient (in megapascals) between the exuded sap and the nutrient solution.

Stomatal conductance

Stomatal conductance was recorded 2 h after dawn by a porometer system (Porometer AP4, Delta-T Devices Ltd., Cambridge, UK) in the last fully expanded leaf of five plants per treatment. Measurements were done the same day that *L* was measured in five different plants in order to elucidate any correlation between the two parameters. Each measurement was repeated three times in each leaf, and the mean of the three measurements was considered in order to diminish variability in this parameter.

Plant mineral analyses

Mineral analyses were carried out in 0.05 g of ground leaf material of five plants per treatment using an ICP plasma analyzer, as previously described by Marulanda et al. (2010).

PIP aquaporin expression and protein abundance

Three different samples of RNA and microsomes coming from three different plant roots were analyzed. RNA extraction and expression analyses of six maize PIP aquaporin genes (*ZmPIP1;1*, *ZmPIP1;2*, *ZmPIP1;5*, *ZmPIP2;1*, *ZmPIP2;5*, and *ZmPIP2;6*) by quantitative real-time PCR were performed as previously described by Marulanda et al. (2010). These genes were selected based on their reported high expression levels in root tissues (Hachez et al. 2006). At the same time, the expression of the aquaporin of *G. intraradices* (*GintAQP1*) was quantified (Aroca et al. 2009). PIP1 and PIP2 protein abundance in root tissues was analyzed by Western blots using specific antibodies, as described by Hachez et al. (2006) and Marulanda et al. (2010). The anti-PIP1 antibody used recognizes all the PIP1 proteins present in maize except ZmPIP1;6, while the anti-PIP2 antibody recognizes ZmPIP2;1, 2;2, and 2;7, and most probably ZmPIP2;3, 2;4 and 2;6. At the same time, specific antibodies against ZmPIP1;2, 2;1, 2;5 and 2;6 were used.

Statistical analyses

Means of each parameter from different treatments were compared using one way-ANOVA and Fisher least significant difference (LSD) tests at significant level of $p<0.05$.

The sources of variation were water regime, AM fungal inoculation,, and potassium added, giving 12 different treatments: plants with or without AM fungal inoculation, plants well watered or subjected to drought, and plants treated with three levels of K^+.

Results

Growth and mycorrhizal and physiological parameters

At harvest, no significant differences ($p>0.05$) in total and shoot dry weights were observed among the 12 treatments (data not shown). Overall total and shoot dry weights were 0.91 and 0.44 g, respectively. However, level 2 of K+ increased root dry weight by 30% under well-watered conditions (Fig. 1). Also, root dry weight of plants from level 2 of K^+ and subjected to drought was lower (by 28%) than root dry weight of the corresponding plants from level 1 of K^+ (Fig. 1).

No mycorrhizal colonization was detected in non-inoculated plants. Percent root length colonization ranged from 64% in water-stressed plants under control K^+ conditions to 82% in well-watered plants under level 2 of K^+. However, no significant differences ($p>0.05$) were found among the different treatments (data not shown). Total root length colonized was calculated since differences in root development were observed (Fig. 1). Drought stress diminished root length colonized under conditions where no extra K^+ added (15%) and even more under level 2 of extra K^+ (48%) (Table 1). Under well-watered conditions, extra K^+ addition at level 2 increased total root length colonized by about 30% (Table 1).

In the absence of extra added K^+, root hydraulic conductivity (L) was not affected by AM fungal inoculation (Fig. 2a). Drought treatment decreased L by 75% in non-AM plants but had no effect in mycorrhizal ones (Fig. 2a).

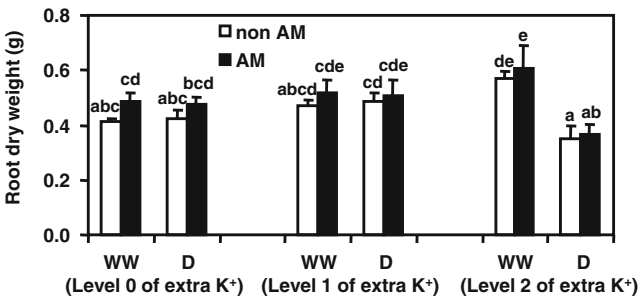

Fig. 1 Root dry weight of AM (*black bars*) or non-AM (*white bars*) maize plants, grown well-watered (*WW*) or subjected to drought (*D*) under conditions of no extra potassium added, or levels 1 and 2 of extra K^+. *Bars* represent mean±SE ($n=5$). Different *letters* indicate significant differences among treatments ($p<0.05$) after ANOVA and LSD tests

Table 1 Total root length (centimeters) colonized by the AM fungus *G. intraradices* in maize plants well-watered (WW) or subjected to drought (D) under conditions of no added potassium (level 0), or levels 1 or 2 of extra K^+

	Level 0 of extra K^+	Level 1 of extra K^+	Level 2 of extra K^+
WW	326±20 b	364±14 b	456±14 c
D	278±12 a	365±8 b	240±22 a

Means±SE are shown ($n=4$). Different letters indicate significant differences among treatments ($p<0.05$) after ANOVA and LSD tests

Addition of both extra K^+ levels almost doubled L values, but only in AM plants (except in those subjected to water stress and treated with level 1 of K^+; Fig. 2a). Thus, under added K^+ conditions, AM plants always showed L values higher than non-AM, regardless of water regime or K^+ dose (Fig. 2a). Plants treated with level 1 or 2 of K^+ had the same L values within each water regime or within each inoculation treatment conditions (Fig. 2a).

Under well-watered conditions in the absence of K^+ applications, stomatal conductance was not affected by the AM symbiosis (Fig. 2b). However, drought treatment reduced stomatal conductance more abruptly in non-AM plants (by 77%) than in mycorrhizal ones (by 35%) (Fig. 2b). Application of the two levels of K^+ did not cause any significant ($p>0.05$) change in stomatal conductance in plants subjected to drought, but they reduced stomatal conductance in well watered plants by around 44% (Fig. 2b), which could indicate a possible osmotic stress.

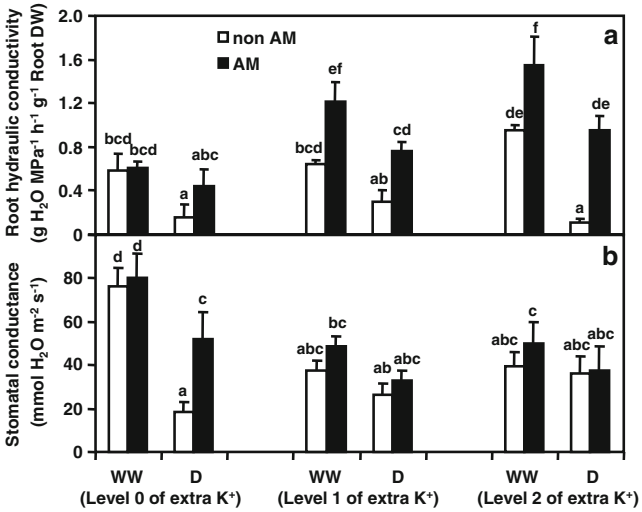

Fig. 2 Root hydraulic conductivity (**a**) and stomatal conductance (**b**) of AM (*black bars*) or non-AM (*white bars*) maize plants, grown well-watered (*WW*) or subjected to drought (*D*) under conditions of no extra potassium added, or levels 1 and 2 of extra K^+. *Bars* represent mean±SE ($n=5$). Different *letters* indicate significant differences among treatments ($p<0.05$) after ANOVA and LSD tests

Shoot K^+ content was increased almost three-fold by both added K^+ levels, and level 2 of K^+ application further increased shoot K^+ content in water-stressed AM plants (Fig. 3a). The effect of the application of K_2SO_4 on plant sulfur content was also analyzed, but no significant ($p>0.05$) differences were found among all the treatments (data not shown). The application of K^+ modified the contents of other nutrients involved in *L* regulation (Cabañero and Carvajal 2007; Li et al. 2009). Added K^+ had an effect on phosphorous content in AM plants subjected to drought under level 2 of K^+ application, where a two-fold increase was observed (Fig. 3b). On the contrary, both levels of applied K^+ decreased shoot Mg^{2+} content by half in all plant treatments (Fig. 3c). No significant differences ($p>0.05$) in shoot calcium content were found among treatments (data not shown).

Since level 2 of K^+ changed root growth, total root length colonized by *G. intraradices* under well-watered conditions, shoot P contents of AM plants, and increased the differences in K^+ contents between AM and non-AM plants under drought conditions, this level of extra K^+ was not considered for further analyses (PIP expression and protein abundance).

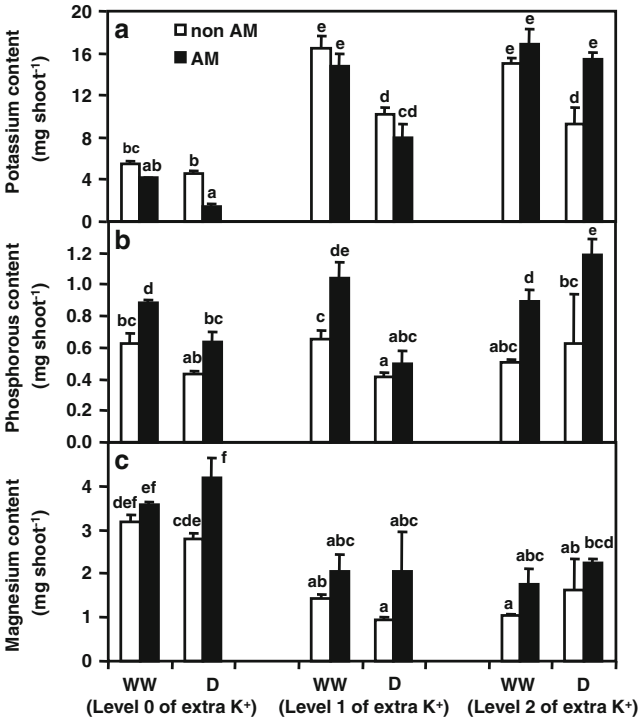

Fig. 3 Shoot total K^+ (**a**), phosphorous (**b**), and magnesium (**c**) of AM (*black bars*) or non-AM (*white bars*) maize plants, grown well-watered (*WW*) or subjected to drought (*D*) under conditions of no extra potassium added, or levels 1 and 2 of extra K^+. *Bars* represent mean±SE ($n=5$). Different *letters* indicate significant differences among treatments ($p<0.05$) after ANOVA and LSD tests

PIP aquaporin expression and abundance

Root expression of *ZmPIP1;5* was not significantly altered ($p>0.05$) by any treatment (Fig. 4c). AM inoculation did not change the expression of any of the *PIP* genes analyzed in the absence of level 1 of K^+ added under well-watered conditions, except that of *ZmPIP2;1* which showed a slight upregulation (34%; Fig. 4d). In the absence of added K^+, drought treatment almost doubled the expression of *ZmPIP1;1* and *ZmPIP2;5* in both AM and non-AM roots and that of *ZmPIP1;2* in AM roots (Fig. 4). Application of level 1 of K^+ did not change the expression of any of the analyzed *PIP* genes under well-watered conditions, although differences in *ZmPIP2;1* gene expression were observed between AM and non-AM roots (Fig. 4d). In contrast, under drought conditions, level 1 of K^+ caused a downregulation of five of the six PIP genes in AM and non-AM roots, although the decrease was more pronounced in AM roots (Fig. 4).

Expression of the fungal *GintAQP1* gene was downregulated (around 90%) by drought stress independently of the K^+ treatment. However, level 1 of K^+ increased slightly the expression of *GintAQP1* by 30% in AM roots under well-watered conditions (Fig. 5).

Six different antibodies were used to determine the regulation of PIP protein abundance in root membranes by the AM symbiosis, drought stress, and K^+ supply (Fig. 6). PIP protein abundance was reduced by the AM symbiosis under well-watered conditions in the absence of added K^+. Drought treatment reduced ZmPIP2;6 protein abundance in non-AM roots in the absence of extra K^+, while no other changes in PIP protein amounts was observed in these roots. However, drought treatment increased the amount of PIP protein in AM roots in the absence of added K^+, especially that of PIP1s and ZmPIP1;2.

Application of extra K^+ at level 1 did not change the amount of any of the PIP proteins in non-AM roots under well-watered conditions (Fig. 6). Conversely, applied K^+ caused an increase in the amount of all analyzed PIP proteins in AM roots under well-watered conditions, being highest for PIP1s and PIP1;2 proteins. Application of level 1 of K^+ decreased ZmPIP2;6 protein abundance under drought conditions and, to a greater extent, in AM roots than in non-mycorrhizal ones.

Discussion

Previous reports on the effect of AM inoculation on root hydraulic conductivity (*L*) under both well-watered or water stress conditions are not consistent. The results range from positive effects under both water conditions (Dell'Amico et al. 2002; Sánchez-Blanco et al. 2004), or only under

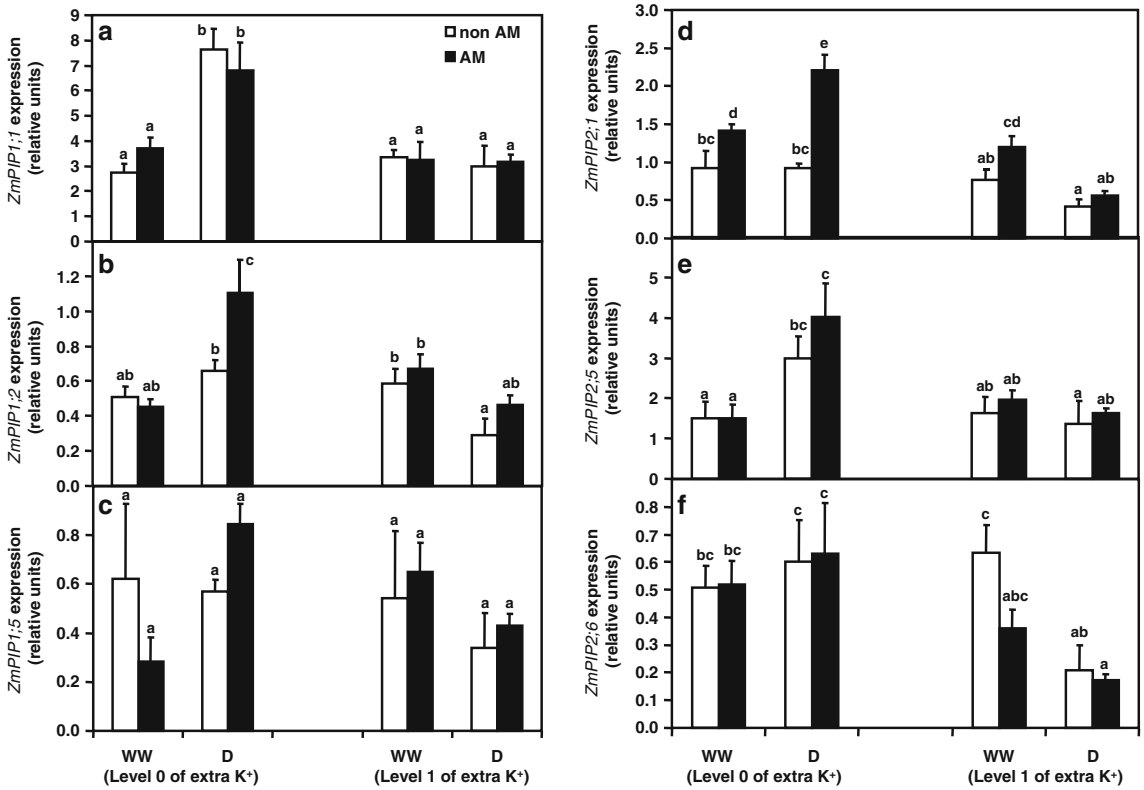

Fig. 4 Root expression of *ZmPIP1;1* (**a**), *ZmPIP1;2* (**b**), *ZmPIP1;5* (**c**); *ZmPIP2;1* (**d**), *ZmPIP2;5* (**e**) and *ZmPIP2;6* genes of AM (*black bars*) or non-AM (*white bars*) maize plants, grown well-watered (WW) or subjected to drought (*D*) under conditions of no extra potassium added, or levels 1 and 2 of extra K^+. *Bars* represent mean±SE (n=5). Different *letters* indicate significant differences among treatments (p< 0.05) after ANOVA and LSD tests

well-watered conditions (Graham and Syvertsen 1984; Aroca et al. 2008b), to negative effects under both water conditions (Levy et al. 1983) or only under well-watered conditions (Aroca et al. 2007). There are also reports of no AM effect under either water condition (Ruiz-Lozano et al. 2009) or only under water stress conditions (Aroca et al. 2007, 2008b). In the present study, no effect of the AM symbiosis on *L* values in maize plants was observed in absence of extra K^+ doses, independently of the water regime. These results confirm those of Ruiz-Lozano et al. (2009) using the same maize cultivar and *G. intraradices*

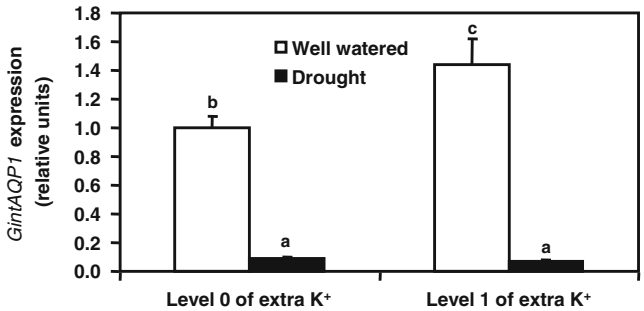

Fig. 5 GintAQP1 gene expression of well-watered (*white bars)* or droughted (*black bars*) AM maize plants grown with or without added K^+. *Bars* represent mean±SE (n=5). Different *letters* indicate significant differences among treatments (p<0.05) after ANOVA and LSD tests

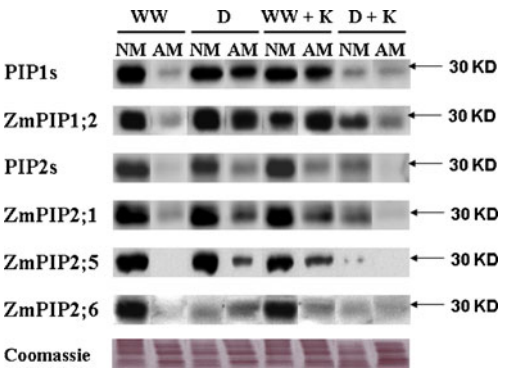

Fig. 6 Western blots of microsomes from roots of AM (*AM*) or non-AM (*NM*) maize plants grown under well-watered conditions (*WW*) or subjected to drought (*D*) with (+K) or without level 1 of extra K^+. The antibodies used are indicated on the right. *PIP1s*: an antibody raised against several PIP1 proteins; *ZmPIP1*;2: an antibody raised only against ZmPIP1;2 protein; *PIP2s*: an antibody raised against several PIP2 proteins; *ZmPIP2*;1: an antibody raised against ZmPIP2;1 and ZmPIP2;2 proteins; *ZmPIP2*;5: an antibody raised only against ZmPIP2;5 protein; *ZmPIP2*;6: an antibody raised against only ZmPIP2;6 protein. The *bottom panel* shows Coomassie-Blue-stained gels

isolate. The use of different plant and fungal species could explain previously observed discrepancies.

Plant K^+ concentration has not been taken into account in previous studies of AM effects on *L*, although the AM symbiosis can modify the amount of K^+ in the host plant tissues, either enhancing K^+ contents (Querejeta et al. 2007; Porras-Soriano et al. 2009) or reducing them (Berreck and Haselwandter 2001; present study). On the other hand, it is well documented that K^+ starvation usually increases root hydraulic conductivity (Liu et al. 2006; Benlloch-González et al. 2010). In the present study, it was found that different K^+ content of plant tissues influences the regulation of *L* by the AM symbiosis under both well-watered and drought stress conditions. The discrepancies about how mycorrhiza regulates *L* could therefore be related to different K^+ contents of the host plant studied.

Curiously, added K^+ only increased root hydraulic conductivity in AM maize plants, although these plants had the same shoot K^+ content as non-mycorrhizal ones. The lack of an *L* response to K^+ in non-AM plants could be caused by a different stomatal conductance behavior (Steudle 2000), but this seems unlikely as no differences in stomatal conductance between AM and non-AM plants were observed upon K^+ application. Dell'Amico et al. (2002) found a strong correlation between stomatal conductance and *L* in tomato plants, but AM plants always showed higher *L* for a given stomatal conductance value. This behavior of mycorrhizal plants could result from the ability of the external mycelium to take up water (Ruth et al. 2011), and the same mechanism could be acting under the conditions of added K^+ in the present study. Recently, Oddo et al. (2011) reported the enhancement of whole plant and shoot hydraulic conductances in *Laurus nobilis* trees after short-term application of K^+ fertilization. These findings corroborate with the current data for AM maize plants at the root level.

It is known that P deficiency may cause reductions in root hydraulic conductivity (Fan et al. 2007; Li et al. 2009). Since AM plants are able to take up more P from the soil, they could explain their higher *L* values as compared with non-mycorrhizal plants. However, the AM maize plants always had a higher leaf concentration of P than non-AM plants, but it was only in the presence of added K^+ that AM plants showed higher *L* values than non-mycorrhizal ones, without any further increase in leaf P concentrations. So, different P concentrations were not related to differences in *L* values observed in the present study between AM and non-AM maize plants.

A decrease in magnesium contents was also observed in the shoots of extra K^+-treated maize plants. Cabañero and Carvajal (2007) found that Mg^{2+} deprivation could cause a transitory *L* elevation. This mechanism could explain why *L* values increased in AM plants upon their exposure to added K^+ but not why non-mycorrhizal plants failed to respond to K^+. Analyses of PIP gene expression and protein abundance were performed in an attempt to answer to this question.

The differences observed in root hydraulic conductivity values between AM and non-AM maize plants under extra K^+ conditions are difficult to explain in relation to the *PIP* gene expression data. In fact, no differences in the expression of the *PIP* genes analyzed were found between mycorrhizal and non-mycorrhizal roots under extra K^+ conditions. At the same time, the increase in *L* values observed in AM plants after K^+ addition was accompanied by a downregulation of all the *PIP* genes analyzed under drought conditions, while such an effect was not observed under well-watered conditions. So, the *PIP* gene expression data does not correlate with *L* values. This could be caused by a lack of correlation between *PIP* gene expression and PIP protein abundance (Aroca et al. 2005; Marulanda et al. 2010; Muries et al. 2011). However, application of K^+ changed the *PIP* expression pattern under drought conditions when comparing AM and non-AM plants, and differences for *ZmPIP1;5* and *ZmPIP2;1* gene expression were eliminated. So, although *PIP* gene expression cannot account for the increase in *L* values observed in AM plants after K^+ application, they could partially explain the different *PIP* expression patterns reported previously between AM and non-AM plants (Porcel et al. 2006; Aroca et al. 2008b; Ruiz-Lozano et al. 2009).

When studying aquaporins in mycorrhizal plants, not only root but also fungal ones should be taken into account. It is known that extraradical hyphae of AM fungi are able to take water up directly from the soil and transport it to the host roots (Ruth et al. 2011). In this context, the *GintAQP1* aquaporin expression data could partly explain why AM maize plants increased their root hydraulic conductivity in presence of added K^+ under well-watered conditions, since the fungal gene expression was increased slightly by such K^+ addition. However, drought stress caused a downregulation of *GintAQP1* expression for both K^+-supplied and non-supplied plants. Aroca et al. (2009) reported that *GintAQP1* did not show any water transport activity when it was expressed in *Xenopus laevis* oocytes. Other aquaporin genes, apart from *GintAQP1*, must exist in *G. intraradices*. In fact, recently, Tisserant et al. (2012) found two new aquaporin genes in *G. intraradices*. Also, seven different aquaporin genes have been reported for the ectomycorrhizal fungus *Laccaria bicolor* (Dietz et al. 2011). Hence, it cannot be ruled out that other aquaporins from the AM fungal partner could be involved in the K^+-dependent increment of *L* values observed in AM plants.

Since aquaporin gene expression and protein abundance do not always match (Aroca et al. 2005; Marulanda et al. 2010; Muries et al. 2011), it is crucial to look at PIP protein abundance to have a complete picture of the root water uptake properties. In accordance with previous results (Aroca et al. 2007; Benabdellah et al. 2009; Ruiz-Lozano

et al. 2009), the AM symbiosis diminished the amount of PIP protein in maize roots under well-watered conditions, when extra K^+ was absent. However, root hydraulic conductivity and stomatal conductance values were the same in both mycorrhizal and non-mycorrhizal maize plants. Again, *G. intraradices* aquaporins could be involved in the overall *L* values observed in the AM plants.

Drought stress increased the amount of all PIP proteins in the roots of AM maize plants, while the only effect observed in non-AM roots was a reduction in the amount of ZmPIP2;6 protein. The higher amount of protein in AM roots could explain why these roots kept their *L* at the same level as well-watered roots, even though the expression of *GintAQP1* was downregulated. Aroca et al. (2009) reported that, when the expression of host plant *PIP* aquaporins was downregulated, the expression of *GintAQP1* increased. Here, the opposite effect was observed in that, when the amount of maize PIP protein increased, the expression of *GintAQP1* decreased. This behavior could be caused by a compensatory mechanism between plant and fungal aquaporins, as was previously suggested (Aroca et al. 2009). However, this pattern could not be confirmed in the maize–*G. intraradices* mycorrhiza under conditions of added K^+.

The observed reduction in root hydraulic conductivity caused by drought treatment in non-mycorrhizal maize plants without K^+ addition was not matched by a diminution in PIP root protein abundance, except for the ZmPIP2;6 protein. Data about the capacity of the ZmPIP2;6 protein to transport water or any other solute are not currently available. Also, no correlation was found between *L* values and ZmPIP2;6 protein amounts in the roots of different maize lines with different concentrations of abscisic acid (Parent et al. 2009). However, the possibility that this specific PIP protein plays a role during drought periods needs to be further tested.

The lack of a response of root hydraulic conductivity to a K^+ application in non-AM maize plants under well-watered conditions was associated with a lack also in the variation in amounts of root PIP protein. At the same time, the increase in *L* values in the AM plants following the K^+ application under well-watered conditions was accompanied by an increase in root PIP protein. However, AM maize plants had lower amounts of several PIP proteins (ZmPIP2s, ZmPIP2;5, and ZmPIP2;6) in their root tissues than non-AM plants, while they maintained higher *L* values. Not only different sub-cellular localization (Sorieul et al. 2011) or phosphorylation state (Maurel et al. 1995) of PIP proteins between AM and non-AM roots but also the activity of fungal aquaporins could contribute to such *L* differences between AM and non-AM plants. Also, the decrease in *L* values caused by drought treatment in both AM and non-AM maize plants exposed to extra K^+ was accompanied by a diminution in the amount of PIP proteins in roots from either kind of plants. Again, the reduction in protein amount was more abrupt in AM maize roots, although *L* decreased more in non-mycorrhizal plants. These results highlight, once again, the crucial role that fungal aquaporins may play in regulating overall *L* values in AM plants.

In conclusion, it has been demonstrated that differences observed between AM and non-AM maize plants in terms of root hydraulic properties and PIP aquaporin expression and abundance depend on the K^+ status of the plant. Also, the results presented here point to a potential role of AM fungal aquaporins in regulating the *L* behavior of the host plant. So, the possible involvement of the new aquaporin genes cloned from *G. intraradices* should be addressed (Tisserant et al. 2012). However, why AM plants increase their *L* values under extra K^+ conditions and not non-mycorrhizal plants remains an unanswered question. Since K^+ uptake capacity could control *L* values (Tazawa et al. 2001; Liu et al. 2006), it is possible that the higher capacity of AM roots to take up K^+ (Querejeta et al. 2007; Porras-Soriano et al. 2009) or the putative direct transport of K^+ by the AM fungus to the host roots (Dupré de Boulois et al. 2006) could also influence root and hyphal water uptake capacity. Future studies using water and potassium channel inhibitors could contribute to answering this question.

References

Alguacil MM, Kohler J, Caravaca F, Roldán A (2009) Differential effects of *Pseudomonas mendoica* and *Glomus intraradices* on lettuce plants. Physiological response and aquaporin PIP2 gene expression under elevated atmospheric CO_2 and drought. Microb Ecol 58:942–951

Allen MF (2007) Mycorrhizal fungi: highways for water and nutrients in arid soils. Vadose Zone J 6:291–297

Aroca R, Amodeo G, Fernández-Illescas S, Herman EM, Chaumont F, Chrispeels MJ (2005) The role of aquaporins and mmebrane damage in chilling and hydrogen peroxide indiced changes in the hydraulic conductance of maize roots. Plant Physiol 137:341–353

Aroca R, Porcel R, Ruiz-Lozano JM (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, Alguacil MM, Vernieri P, Ruiz-Lozano JM (2008a) Plant responses to drought stress and exogenous ABA application is modulated differently by mycorrhization in tomato and an ABA-deficient mutant. Microb Ecol 56:704–719

Aroca R, Vernieri P, Ruiz-Lozano JM (2008b) Mycorrhizal and non-mycorrhizal *Lactuca sativa* plants exhibit contrasting responses to exogenous ABA during drought stress and recovery. J Exp Bot 59:2029–2041

Aroca R, Bago A, Sutka M, Paz JA, Cano C, Amodeo G, Ruiz-Lozano JM (2009) Expression analysis of the first arbuscular mycorrhizal fungi aquaporin described reveals concerted gene expression between salt-stressed and nonstressed mycelium. Mol Plant Microb Interact 22:1169–1178

Bago B, Pfeffer PE, Zipfel W, Lammers P, Shachar-Hill Y (2002) Tracking metabolism and imaging transport in arbuscular mycorrhizal fungi. Metabolism and transport in AM fungi. Plant Soil 244:189–197

Bárzana G, Aroca R, Paz JA, Chaumont F, Martinez-Ballesta MC, Carvajal M, Ruiz-Lozano JM (2012) Arbuscular mycorrhizal symbiosis increases relative apoplastic water flow in roots of the host plant under both well-watered and drought stress conditions. Ann Bot (on-line first) doi:10.1093/aob/mcs007

Benabdellah K, Ruiz-Lozano JM, Aroca R (2009) Hydrogen peroxide effects on root hydraulic properties and plasma membrane aquaporin regulation in *Phaseolus vulgaris*. Plant Mol Biol 70:647–661

Benlloch-González M, Fournier JM, Benlloch M (2010) K^+ deprivation induces xylem water and K^+ transport in sunflower: evidence for a co-ordinated control. J Exp Bot 61:157–164

Berreck M, Haselwandter K (2001) Effect of the arbuscular mycorrhizal symbiosis upon uptake of cesium and other cations by plants. Mycorrhiza 10:275–280

Cabañero FJ, Carvajal M (2007) Different cation stresses affect specifically osmotic root hydraulic conductance, involving aquaporins, ATPase and xylem loading of ions in *Capsicum annum* L. plants. J Plant Physiol 164:1300–1310

Cakmak I (2005) The role of potassium in alleviating detrimental effects of abiotic stresses in plants. J Plant Nutr Soil Sci 168:521–530

Dell'Amico J, Torrecillas A, Rodríguez P, Morte A, Sánchez-Blanco MJ (2002) Responses of tomato plants associated with the arbuscular mycorrhizal fungus *Glomus clarum* during drought and recovery. J Agric Sci 138:387–393

Dietz S, von Bülow J, Beitz E, Nehls U (2011) The aquaporin gene family of the ectomycorrhizal fungus *Laccaria bicolor*: Lessons for symbiotic functions. New Phytol 190:927–940

Dupré de Boulois H, Voets L, Delvaux B, Jakobsen I, Declerck S (2006) Transport of radiocaesium by arbuscular mycorrhizal fungi to *Medicago truncatula* under in vitr conditions. Environ Microbiol 8:1926–1934

Egilla JN, Davies FT Jr, Drew MC (2001) Effect of potassium on drought resistance of *Hibiscus rosa-sinensis* cv. Leprechaun: Plant growth, leaf macro- and micronutrient content and root longevity. Plant Soil 229:213–224

Evelin H, Kapoor R, Giri B (2009) Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Ann Bot 104:1263–1280

Fan MS, Bai RQ, Zhao XF, Zhnag JH (2007) Aerenchyma formed under phosphorus deficiency contributes to the reduced root hydraulic conductivity in maize roots. J Integr Plant Biol 49:598–604

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular-arbuscular infection in roots. New Phytol 84:489–500

Goicoechea N, Merino S, Sánchez-Díaz M (2004) Contribution of arbuscular mycorrhizal fungi (AMF) to the adaptations exhibited by the deciduous shrub *Anthyllis cytisoides* under water deficit. Physiol Plant 122:453–464

Graham JH, Syvertsen JP (1984) Influence of vesicular-arbuscular mycorrhiza on the hydraulic conductivity of roots of two citrus rootstocks. New Phytol 97:277–284

Hachez C, Moshelion M, Zelazny E, Cavez D, Chaumont F (2006) Localization and quatification of plasma membrane aquaporin expression in maize primary root: a clue to understanding their role as cellular plumbers. Plant Mol Biol 62:305–323

Jang JY, Rhee JY, Kim DG, Chung GC, Lee JH, Kang H (2007) Ectopic expression of a foreign aquaporin disrupts the natural expression pattern of endogenous aquaporin genes and alters plant responses to different stress conditions. Plant Cell Physiol 48:1331–1339

Javot H, Lauvergeat V, Santoni V, Martin-Laurent F, Guclu J, Vinh J, Heyes J, Franck KI, Schäffner AR, Bouchez D, Maurel C (2003) Role of a single aquaporin isoform in root water uptake. Plant Cell 15:713–725

Khalvati MA, 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

Krishna H, Singh SK, Sharma RR, Khawale RN, Grover M, Patel VB (2005) Biochemical changes in micropropagated grape (*Vitis vinifera* L.) plantlets due to arbuscular-mycorrhizal fungi (AMF) inoculation during *ex vitro* acclimatization. Sci Hortic 106:554–567

Levy Y, Syvertsen JP, Nemec S (1983) Effect of drought stress and vesicular-arbuscular mycorrhiza on *Citrus* transpiration and hydraulic conductivity of roots. New Phytol 93:61–66

Li YS, Mao XT, Tian QY, Li LH, Zhang WH (2009) Phosphorous deficiency-induced reduction in root hydraulic conductivity in *Medicago falcata* is associated with ethylene production. Environ Exp Bot 67:172–177

Liu HY, Sun WN, Su WA, Tang ZC (2006) Co-regulation of water channels and potassium channels in rice. Physiol Plant 128:58–69

Marulanda A, Azcón R, Ruiz-Lozano JM (2003) Contribution of six arbuscular mycorrhizal fungal isolates to water uptake by *Lactuca sativa* plants under drought stress. Physiol Plant 119:526–533

Marulanda A, Azcón R, Chaumont F, Ruiz-Lozano JM, Aroca R (2010) Regulation of plasma membrane aquaporins by inoculation with a *Bacillus megaterium* strain in maize (*Zea mays* L.) plants under unstressed and salt-stressed conditions. Planta 232:533–543

Maurel C, Kado RT, Guern J, Chrispeels MJ (1995) Phosphorylation regulates the water channel activity of the seed-specific aquaporin alpha-TIP. EMBO J 14:3028–3035

Maurel C, Verdoucq L, Luu DT, Santoni V (2008) Plant aquaporins: membrane channels with multiple integrated functions. Annu Rev Plant Biol 59:595–624

Miller RM, Hetrick BAD, Wilson GWT (1997) Mycorrhizal fungi affect root stele tissue in grasses. Can J Bot 75:1778–1784

Muries B, Faize M, Carvajal M, Martínez-Ballesta MC (2011) Identification and differential induction of the expression of aquaporins by salinity in broccoli plants. Mol Biosyst 7:1322–1335

Oddo E, Inzerillo S, La Bella F, Grisafi F, Salleo S, Nardini A (2011) Short-term effects of potassium fertilization on the hydraulic conductance of *Laurus nobilis* L. Tree Physiol 31:131–138

Ouziad F, Wilde P, Schmelzer E, Hildebrandt U, Bothe H (2006) Analysis of expression of aquaporins and Na^+/H^+ transporters in tomato colonized by arbuscular mycorrhizal fungi and affected by salt stress. Environ Exp Bot 57:177–186

Parent B, Hachez C, Redondo E, Simonneau T, Chaumont F, Tardieu F (2009) Drought and abscisic acid effects on aquaporin content translate into changes in hydraulic conductivity and leaf growth rate: a trans-scale approach. Plant Physiol 149:2000–2012

Park W, Scheffler BE, Bauer PJ, Campbell BT (2010) Identification of the family of aquaporin genes and their expression in upland cotton (*Gossypium hirsutum* L.). BMC Plant Biol 10:142

Peng YH, Lin WL, Cai WM, Arora R (2007) Overexpression of a *Panax ginseng* tonoplast aquaporin alters salt tolerance, drought tolerance and cold acclimation ability in transgenic *Arabidopsis* plants. Planta 226:729–740

Porcel R, Ruiz-Lozano JM (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

Porcel R, Azcón R, Ruiz-Lozano JM (2004) Evaluation of the role of genes encoding for D-(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, Aroca R, Azcón R, Ruiz-Lozano JM (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

Porras-Soriano A, Soriano-Martín ML, Porras-Piedra A, Azcón R (2009) Arbuscular mycorrhizal fungi increased growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions. J Plant Physiol 166:1350–1359

Postaire O, Tournaire-Roux C, Grondin A, Boursiac Y, Morillon R, Schäffner AR, Maurel C (2010) A PIP1 aquaporin contributes to hydrostatic pressure-induced water transport in both the root and rosette of *Arabidopsis*. Plant Physiol 152:1418–1430

Querejeta JI, Allen MF, Alguacil MM, Roldán A (2007) Plant isotopic composition provides insight mechanisms underlying growth stimulation by AM fungi in a semiarid environment. Funct Plant Biol 34:683–691

Roussel H, Bruns S, Gianinazzi-Pearson V, Hahlbrock K, Franken P (1997) Induction of a membrane intrinsic protein-encoding mRNA in arbuscular mycorrhiza and elicitor-stimulated cell suspension culture of parsley. Plant Sci 126:203–210

Ruiz-Lozano JM, Aroca R (2010) Modulation of aquaporin genes by the arbuscular mycorrhizal symbiosis in relation to osmotic stress tolerance. In: Symbioses and stress: joint ventures in biology, cellular origin, life in extreme habitats. Edited by Seckbach J, Grube M. Springer Science + Business Media; pp 359–374

Ruiz-Lozano JM, Azcón R (1995) Hyphal contribution to water uptake in mycorrhizal plants as affected by the fungal species and water status. Physiol Plant 95:472–478

Ruiz-Lozano JM, Gómez M, Azcón R (1995) Influence of different *Glomus* species on the time-course of physiological plant-responses of lettuce to progressive drought stress periods. Plant Sci 110:37–44

Ruiz-Lozano JM, Azcón R, Palma JM (1996) Superoxide dismutase activity in arbuscular mycorrhizal *Lactuca sativa* plants subjected to drought stress. New Phytol 134:327–333

Ruiz-Lozano JM, Porcel R, Aroca R (2006) Does the enhanced tolerance of arbuscular mycorrhizal plants to water déficit involve modulation of drought-induced plant genes? New Phytol 171:693–698

Ruiz-Lozano JM, Alguacil MM, Bárzana G, Vernieri P, Aroca R (2009) Exogenous ABA accentuates the differences in root hydraulic properties between mycorrhizal and non mycorrhizal maize plants through regulation of PIP aquaporins. Plant Mol Biol 70:565–579

Ruth B, Khalvati M, Schmidhalter U (2011) Quantification of mycorrhizal water uptake via high-resolution on-line water content sensors. Plant Soil 342:459–468

Sánchez-Blanco MJ, Ferrández T, Morales MA, Morte A, Alarcón JJ (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

Siefritz F, Tyree MT, Lovisolo C, Schubert A, Kaldenhoff R (2002) PIP1 plasma membrane aquaporins in tobacco: from cellular effects to function in plants. Plant Cell 14:869–876

Sorieul M, Santoni V, Maurel C, Luu DT (2011) Mechanisms and effects of retention of over-expressed aquaporin AtPIP2;1 in the endoplasmatic reticulum. Traffic 12:473–482

Steudle E (2000) Water uptake by roots: effects of water deficit. J Exp Bot 51:1531–1542

Tazawa M, Sutou E, Shibasaka M (2001) Onion root water transport sensitive to water channel and K^+ channel inhibitors. Plant Cell Physiol 42:28–36

Tisserant E, Kohler A, Dozolme-Seddas P, Balestrini R, Benabdellah K, Colard A, Croll D, Da Silva C, Gomez SK, Koul R, Ferrol N, Fiorilli V, Formey D, Franken Ph, Helber N, Hijri M, Lanfranco L, Lindquist E, Liu Y, Malbreil M, Morin E, Poulain J, Shapiro H, van Tuinen D, Waschke A, Azcón-Aguilar C, Bécard G, Bonfante P, Harrison MJ, Küster H, Lammers P, Paszkowski U, Requena N, Rensing SA, Roux C, Sanders IR, Shachar-Hill Y, Tuskan G, Young JPW, Gianinazzi-Pearson V, Martin F (2012) The transcriptome of the arbuscular mycorrhizal fungus *Glomus intraradices* (DAOM 197198) reveals functional tradeoffs in an obligate symbiont. New Phytol 193:755–769

Uehlein N, Fileschi K, Eckert M, Bienert GP, Bertl A, Kaldenhoff R (2007) Arbuscular mycorrhizal symbiosis and plant aquaporins expression. Phytochemistry 68:122–129