Contribution of the arbuscular mycorrhizal symbiosis to the regulation of radial root water transport in maize plants under water deficit

Gabriela Quiroga[1], Gorka Erice[1,2], Ricardo Aroca[1], François Chaumont[3], Juan Manuel Ruiz-Lozano[1*]

[1]Departamento de Microbiología del Suelo y Sistemas Simbióticos. Estación Experimental del Zaidín (CSIC). Profesor Albareda nº 1, 18008 Granada, Spain.

[2]Present address: Atens, Agrotecnologias Naturales S.L., La Riera de Gaia, Tarragona, Spain.

[3]*Louvain Institute of Biomolecular Science and Technology, UC Louvain, Croix du Sud 4-L7.07.14. B-1348, Louvain-la-Neuve, Belgium.*

*Correspondance: Dr. Juan Manuel Ruiz-Lozano

Telph. + 34 958181600

e-mail: juanmanuel.ruiz@eez.csic.es

Abstract

In roots, water flows radially through three parallel pathways: apoplastic, symplastic and transcellular (the last two referred as the cell-to-cell), with a different contribution depending on the environmental conditions. Thus, during drought, the cell-to-cell pathway, which is largely regulated by aquaporins, dominates. While it is accepted that water can flow across roots following the apoplastic, symplastic and transcellular pathways, the relative contribution of these pathways to whole root hydraulic conductivity is not well stablished. In addition, the symbiosis with arbuscular mycorrhizal (AM) fungi was reported to modify root water transport in host plants. This study aims to understand if the AM symbiosis alters radial root water transport in the host plant and whether this modification is due to alteration of plant aquaporins activity or amounts and/or changes in apoplastic barriers. Hence, the combined effect of mycorrhizal fungus, water deficit and application of the aquaporin inhibitor sodium azide (NaN_3) on radial root water transport of maize plants was analyzed. The development of Casparian bands in these roots was also assessed. NaN_3 clearly inhibited osmotic root hydraulic conductivity (Lo). However, the inhibitory effect of sodium azide on Lo was lower in AM plants than in non-AM plants, which together with their higher relative apoplastic water flow values suggests a compensatory mechanism for aquaporin activity inhibition in AM plants, leading to a higher hydrostatic root hydraulic conductivity (Lpr) compared to non-AM plants. This effect seems to be related to the mycorrhizal regulation of aquaporins activity through posttranslational modifications. The development of Casparian bands increased with drought and AM colonization, although this did not decrease water flow values in AM plants. The work provides new clues on the differential mycorrhizal regulation of root water transport.

***Keywords*:** arbuscular mycorrhizal symbiosis; aquaporins; root hydraulic conductivity; sodium azide

1. Introduction

In plants, the water status of the shoot is determined by the root resistance to water flow, which is the highest within the soil-plant-atmosphere continuum (SPAC) (Steudle and Peterson, 1998). Within the SPAC, the balance between water uptake and water loss is finely tuned between root hydraulic properties and stomatal control in leaves. Thus, according to the demands of the shoot, root water supply can be adjusted (Kim et al., 2018). Roots are the first organs to sense drought in soil, as it starts with a decrease in soil water potential. Thus, they play a crucial role in the response to dehydration (Zingaretti et al., 2013). Drought is a major constraint in crop production at global scale, and expected to increase in coming years (Lesk et al., 2016). Therefore, studies on the effect of water deprivation in plant roots are extremely important. In the case of maize, a staple crop worldwide whose yield is heavily affected by this constraint (Daryanto et al., 2016), understanding the mechanisms of drought tolerance seems essential.

Water must flow radially across a series of concentric cell layers in the root to move from soil into the vascular tissues. These layers are the epidermis, the exodermis (not always present), one or several layers of cortex cells, the endodermis, the pericycle, the xylem parenchyma cells, and, finally, the vessels (Hachez et al., 2006a). In this radial movement, water and nutrients obtained from soil are translocated to the vascular tissues by three major routes, apoplastic, symplastic and transcellular (the last two referred to as cell-to-cell), following a hydrostatic (bulk) or osmotic gradient. This radial transport was best described by the composite transport model (Steudle and Peterson, 1998), although subsequent studies have shown new aspects to be considered, such as the contribution of the serial radial pathways (cortex, endodermis) alongside to the parallel components and the development and composition of apoplastic barriers in root tissues (Schreiber et al.,

2005; Meyer et al., 2011; Ranathunge et al., 2017; Kreszies et al., 2019; Wang et al., 2019). Depending on the environmental conditions, the relative contribution of each pathway to overall water uptake or hydraulic conductivity may change substantially (Steudle, 2000, 2001; Hachez et al., 2006a; Vandeleur et al., 2009). Moreover, under drought conditions, root hydraulics is adjusted by switching between the cell-to-cell and apoplastic pathways, depending on the driving forces (Ranathunge et al., 2004; Barberon, 2017). According to this, under transpiring conditions (i.e. in the day with normal water supply), the hydrostatic pressure gradient would dominate the transport of water and solutes, increasing the contribution of the apoplastic pathway. Apoplastic barriers in endodermal and exodermal cell walls can block this water transport pathway (Kreszies et al., 2018). In the absence of transpiration (i.e. in the case of drought stress), the osmotic gradient would govern water and solutes transport following the cell-to-cell pathway (Kim et al., 2018). It is currently known that all these pathways are interconnected and operate in combination along plant tissues, producing a system with series and parallel resistances, so that water moves by a combination of hydraulic and osmotic forces that explain the deviations from the original model of root water movement (Steudle and Peterson, 1998; Knipfer and Fricke, 2010; Fritz and Ehwald, 2011).

The water transport capacity of the root system (root hydraulic conductivity; Lpr) is regulated in a large proportion by aquaporins (Tournaire-Roux et al., 2003; Vadez, 2014) that contribute to the transcellular water flux. These proteins are small channels that allow the passage of water and small molecules through the membranes of most living organisms. In vascular plants they constitute a large family (>30 members) subdivided in the following subfamilies: PIPs (plasma membrane intrinsic proteins), TIPs (tonoplast intrinsic proteins), NIPs (nodulin 26-like intrinsic proteins) and SIPs (small basic intrinsic proteins) (Chaumont et al., 2001; Maurel et al., 2015). Some plants contains also the

uncharacterized XIPs (X intrinsic proteins) (Gupta and Sankararamakrishnan, 2009), which are not present in maize. Under adverse environmental conditions, aquaporins appear to have a key role in the regulation of plant water balance (Kapilan et al., 2018), affecting important parameters such as the root hydraulic conductivity (Hachez et al., 2006b). The use of aquaporin inhibitors may provide, thus, information on the relative participation of cell-to-cell and apoplastic paths in the whole-root water uptake. Several inhibitors of aquaporin activity have been used to this purpose, being mercurials the most widely used. Hg causes conformational changes in the protein leading to aquaporin pore blockage and inhibition of water transport (Niemietz and Tyerman, 2002). Nevertheless, Hg has been reported to have a large number of collateral effects that causes indirect inhibition of cellular metabolism (Kamaluddin and Zwiazek, 2001; Niemietz and Tyerman, 2002; Maurel et al., 2008). Sodium azide has also been commonly used to inhibit aquaporin activity and Fitzpatrick and Reid (2009) compared sodium azide (0.5 mM) and butyric acid (10 mM) and observed that sodium azide was more efficient than butyric acid inhibiting the activity of aquaporins. Such an effect was related to the fact that azide has a dual effect: it causes acidification of cytoplasm but also inhibits the phosphorylation process, both effects contributing to close the aquaporin channel (Tournaire-Roux et al., 2003).

Arbuscular mycorrhizal (AM) symbiosis occurring between soil fungi from the subphylum Glomeromycotina and most plant roots enhance water and nutrient uptake from soil due to a vast mycelial network that can access further than the root depletion zone in the rhizosphere (Smith and Read, 2008). Inside the plant, they provide numerous benefits to plant physiology, the most evident being the stimulation of plant growth and improved mineral nutrition (Azcón-Aguilar and Barea, 2015). In addition, they have the ability to improve plant performance under different abiotic stresses such as drought,

119 salinity, waterlogging or pollution (Lenoir et al., 2016). In the case of water deficit, the
120 enhancement of drought tolerance was reported in different plant species (Ortiz et al.,
121 2015; Chitarra et al., 2016; Ruiz-Lozano et al., 2016; Quiroga et al., 2017). Under these
122 conditions, but also when the plant is well irrigated, AM symbiosis was found to differently
123 regulate root water transport, generally inducing a rise in Lpr (Aroca et al., 2007; Bárzana
124 et al., 2012, 2014, 2015; Quiroga et al., 2017).

125 While it is accepted that water can flow across roots following the apoplastic,
126 symplastic and transcellular pathways, the relative contribution of these pathways to whole
127 root hydraulic conductivity is not well stablished. Moreover, results by Knipfer and Fricke
128 (2010) in barley emphasize that membranes (and aquaporins) are control points for radial
129 water transport in roots and question the well accepted idea that low-resistance apoplastic
130 pathway of water movement driven by hydrostatic gradients is required in roots to meet the
131 transpirational water demand of the shoot. Furthermore, AM fungi were suggested to
132 modulate the switching between water transport pathways in roots (Bárzana et al., 2012),
133 which would provide higher flexibility to these plants to cope with water stress. This
134 mycorrhizal water regulation could be in part mediated by the regulation of aquaporins, as
135 it was found in several species, including maize (Bárzana et al., 2014; Quiroga et al.,
136 2017). However, this aspect requires a more in deep investigation to elucidate the
137 mechanisms and the conditions under which this could occur.

138 Therefore, the aim of this investigation was to determine if the AM symbiosis alters
139 the routes of radial water movement in the root of the host plant. We hypothesize that this
140 may be achieved by the regulation of the direct water supply to the plant via fungal hyphae
141 and that this effect may be mediated by changes in the host plant aquaporins activity or
142 amounts, as well as, by changes in apoplastic barriers. Hence, the combined effect of the
143 presence of a mycorrhizal fungus, water deficit and application of the aquaporin inhibitor

144 sodium azide (NaN_3) on radial root water transport and aquaporins accumulation and
145 phosphorylation was studied in maize plants. We also analyzed the development of
146 Casparian bands in these roots. Unravelling the mechanisms by which the mycorrhizal
147 symbiosis governs water movements in roots is a step forward in the understanding of the
148 AM-induced drought tolerance.

149

150 2. Materials and methods

151 2.1. Experimental design

152 The experiment consisted of a factorial design with three factors: (1) watering
153 treatment, so that half of the plants were grown under well-watered (WW) conditions
154 throughout the entire experiment and the other half was subjected to drought stress (DS)
155 for 15 days before harvest; (2) inoculation treatment, with non-inoculated control plants
156 (Non-AM) and plants inoculated with the AM fungus *Rhizophagus irregularis*, strain EEZ
157 58 (AM); (3) chemical treatment, so that sodium azide (NaN_3) was added 30 minutes
158 before harvest to half of the plants, resulting in eight different treatments with fifteen
159 replicates per treatment (n=15), giving a total of 120 plants.

160

161 2.2. Soil and biological materials

162 The growing substrate consisted of a mixture of soil and sand (v/v 1:9). Soil was
163 collected at the grounds of IFAPA (Granada, Spain), sieved (2 mm), diluted with quartz-
164 sand (<1 mm) and sterilized by steaming (100°C for 1 h on 3 consecutive days). The
165 original soil had a pH of 8.1 (water); 0.85% organic matter, nutrient concentrations (mg kg^-

166 [1]): N, 1; P, 10 ($NaHCO_3$-extractable P); K, 110. The soil texture was made of 38.3% sand,
167 47.1% silt and 14.6% clay.

168 Seeds of *Zea mays* L. from the drought-sensitive cultivar PR34B39 were provided
169 by Pioneer Hi-Bred (Spain) and used in previous studies (Quiroga et al., 2017; 2018). Two
170 seeds were sown in 1.5 L pots containing 1250 g of the substrate described above and
171 thinned to one seedling per pot after emergence. At the time of planting, half of the plants
172 were inoculated with ten grams of AM inoculum. *Rhizophagus irregularis* (Schenck and
173 Smith), strain EEZ 58 was used as AM fungal inoculum. The inoculum consisted of soil,
174 spores, mycelia and infected root fragments. Non inoculated plants received a 10 mL
175 aliquot of a filtrate (<20 μm) of the AM inoculum in order to provide the natural microbial
176 population free of AM propagules.

177

178 **2.3. Growing conditions**

179 Plants were grown for eight weeks under greenhouse conditions (25/20ºC, 16/8 light
180 dark period, 50-60% RH and average photosynthetic photon flux density 800 μmol m^{-2} s^{-1}).
181 They were irrigated three times per week with 50 mL of Hoagland nutrient solution
182 (Hoagland and Arnon, 1950) modified to contain 25% P in order to avoid AM symbiosis
183 inhibition. The same amount of water was applied on alternate days. A drought stress
184 treatment was applied for the last 2 weeks, by irrigating plants with half the
185 water/Hoagland volume of well-watered ones (25 mL vs. 50 mL). To avoid a combination
186 of drought stress plus nutrient deficiency, for droughted treatments a 2X Hoagland nutrient
187 solution was used, so that 25 mL provided the same nutrient levels as 50 mL of a 1X
188 Hoagland nutrient solution used to irrigate well-watered plants. This water stress was
189 similar to previous studies (Quiroga et al., 2017; 2018) and is considered as a severe

stress. Sodium azide (NaN_3) 2 mM was added to the nutrient solution and applied to half of the plants 30 minutes before harvesting. The exposure time and the concentration of the compound were established in preliminary tests.

2.4. Parameters measured

2.4.1. Biomass production

At harvest (8 weeks after sowing) the shoot and root system of eight replicates per treatment were separated and the dry weight (DW) measured after drying in a forced hot-air oven at 70 ºC for 2 days.

2.4.2. Symbiotic development

Roots of maize were stained according to Phillips and Hayman (1970), in order to differentiate fungal structures. The extent of mycorrhizal colonization was calculated according to the gridline intersect method (Giovannetti and Mosse, 1980) in five replicates per treatment.

2.4.3. Stomatal conductance

Stomatal conductance (*gs*) was measured two hours after the onset of photoperiod in the second fully expanded youngest leaf from at least seven plants per treatment with a porometer system (Porometer AP4, Delta-T Devices Ltd, Cambridge, UK) following the manufacter's recommendations. Measurements were taken one day before harvest, thus, before the NaN_3 treatment.

2.4.4. Leaf chlorophyll content

Leaf chlorophyll content was estimated four hours after sunrise using a Chlorophyll Content Measurement System CL-01 (SPAD, Hansatech Instruments ltd., Norfolk, UK) on the second fully expanded youngest leaf for each plant. This device determines relative chlorophyll content using dual wavelength optical absorbance (620 and 940 nm wavelengths) measurements from leaves samples. Relative chlorophyll content was measured in 10 different plants per treatment after 8 weeks of growth and before the NaN_3 treatment.

2.4.5. Photosynthetic efficiency

The efficiency of photosystem II of light adapted maize leaves was measured with Fluor-Pen FP100 (Photon Systems Instruments, Brno, Czech Republic) as previously described in Quiroga et al. (2017, 2018) in the second fully expanded youngest leaf of 10 different plants of each treatment after 8 weeks of growth and before the NaN_3 treatment.

2.4.6. Hydrostatic root hydraulic conductivity (Lpr)

The Lpr was determined at noon in seven plants per treatment with a Scholander pressure chamber, 30 minutes after NaN_3 application and following the method described by Bárzana et al. (2012). A gradual increase of pressure (0.2, 0.3 and 0.4 MPa) was applied at 2-minutes intervals to the detached roots. Sap was collected at the three pressure points. Sap flow was plotted against pressure, with the slope being the root hydraulic conductance (L) value. Lpr was determined by dividing L by root dry weight (RDW) and expressed as mg H_2O g RDW^{-1} MPa^{-1} h^{-1}.

2.4.7. Osmotic root hydraulic conductivity (Lo)

Lo was measured at noon on detached roots exuding under atmospheric pressure by the free exudation method (Benabdellah et al., 2009) and using eight plants per treatment. Under these conditions, water is only moving following an osmotic gradient. Therefore, the water would be moving through the cell-to-cell path (Steudle and Peterson, 1998). The exuded sap was collected after 2 hours and weighed. The osmolarity of the exuded sap and the nutrient solution was determined using a cryoscopic osmometer (Osmomat 030, Gonotec Gmbh, Berlin, Germany) and used for *Lo* calculation, according to Aroca et al. (2007). Lo was calculated as $Lo = Jv/\Delta\Psi$, where Jv is the exuded sap flow rate and $\Delta\Psi$ the osmotic potential difference between the exuded sap and the nutrient solution where the pots were immersed. Measurements were carried out 30 minutes after applying NaN_3.

2.4.8. Relative apoplastic water flow

Relative changes in apoplastic water flux among treatments were estimated using light green dye (light green SF yellowish; Sigma-Aldrich Chemical, Gillingham, Dorset; colour index 42095, molecular weight 792.85 g mol^{-1}), which has the ability to move apoplastically but not symplastically (López-Pérez et al., 2007). Fluorescent dyes may not precisely measure the total apoplastic water flux (Zimmerman and Steudle, 1998). However, they can be used to determine relative changes in the apoplastic water transport of plants from different treatments (Voicu and Zwiazek, 2004; Bárzana et al., 2012; Quiroga et al., 2018). Thus, the relative apoplastic water flow was calculated as explained in Quiroga et al. (2018), using eight plants per treatment. Briefly, 30 min after NaN_3 application, detopped root systems were immersed in 250 μM light green solution

inside the pressure chamber and kept in this solution during measurement. Sap was collected after 2 min at 0.2, 0.3 and 0.4 MPa in a Scholander pressure chamber. At the end, the absorbance of the whole collected sap was determined immediately at 630 nm. The average baseline absorbance value in the nutrient solution before addition of the dye was subtracted to the values obtained after adding the dye and in the collected sap. The percentage of apoplastic pathway was calculated from the ratio between the absorbance in the sap flow and in the nutrient solution. The concentration of dye in the nutrient solution of each treatment was considered to be 100%.

2.4.9. Apoplastic barriers

To detect the development of Casparian bands, hand-cut sections from fresh root tissue were taken at 50 mm of root tips and stained for 1 h with 0.1% (w/v) berberine hemisulfate and for 45 min with 0.5% toluidine blue (w/v) (Brundrett et al., 1988; Hachez et al., 2006a; Kreszies et al., 2019), then mounted in 0.1% $FeCl_3$ in 50% glycerol. Sections were immediately examined under an epifluorescence microscope with A filter (excitation at 340-380 nm, emission at 425 nm). The same sections were also used to detect autofluorescence of lignified tissues, using the filter setup (UV illumination) as employed for berberine hemisulphate-stained sections.

2.4.10. Aquaporins abundance and PIP2s phosphorylation status

Sub-cellular fractionation was performed according to Hachez et al. (2006a) with slight modifications. Pieces of intact roots were grinded with 6 mL of a protein extraction buffer containing 250 mM Sorbitol, 50 mM Tris-HCl (pH 8), 2 mM EDTA and protease inhibitors. All steps were performed at 4ºC. The homogenate was centrifuged during 10 min at 770 *g* and the supernatant obtained was centrifuged 10 min at 10000g. The

284 resulted supernatant was finally centrifuged during 30 min at 100000g and the final pellet
285 (corresponding to the microsomal fraction) was resuspended in 20 µL of suspension buffer
286 (5 mM KH_2PO_4, 330 mM sucrose, 3 mM KCl, pH 7.8) and sonicated twice for 5 s. Total
287 protein amounts were quantified by Bradford analysis and abundance of specific proteins
288 was measured by ELISA. A 2 µg aliquot of microsomal fraction was incubated at 4ºC
289 overnight in carbonate/bicarbonate coating buffer at pH 9.6. The next day, proteins were
290 cleaned by 3x 10 min washes with Tween Tris-buffered saline solution (TTBS), and
291 blocked with 1% Bovine serum albumin (BSA) on TTBS 1 hour at room temperature. After
292 three more washes with TTBS, proteins were incubated with 100 µL of the primary
293 antibody (1:1000 in TTBS v/v) for 1 hour at room temperature.

294 We used ten different primary antibodies, two antibodies that recognize several
295 PIP1s and PIP2s, three antibodies that recognize the phosphorylation of PIP2 proteins in
296 the C-terminal region: PIP2A (Ser-280), PIP2B (Ser-283) and PIP2C (Ser-280/Ser-283)
297 (Calvo-Polanco *et al.* 2014), as well as antibodies recognizing ZmPIP2;1/2;2, ZmPIP2;4,
298 ZmPIP2;5, ZmPIP2;6 and ZmTIP1;1 (Hachez et al., 2006a). A goat anti-rabbit IgG coupled
299 to horseradish peroxidase (Sigma-Aldrich Co.) was used as secondary antibody at 1:
300 10000.

301

302 **2.5. Statistical analysis**

303 Statistical analyses were performed in SPSS Statistics (Version 23, IBM Analytics).
304 Data were analyzed by one-way ANOVA. Duncan's or T-Test were used to find out
305 differences between means at α=0.05.

306

307

3. Results

3.1. Plant biomass and symbiotic development

The application of sodium azide did not affect plant biomass and symbiotic development due to its short time of application (only 30 minutes). In contrast, AM colonization enhanced plant dry weight both under well-watered conditions (by 50%) and under drought stress (by 18%) (Figure 1). Drought stress reduced plant dry weight over 30% both in AM and non-AM plants.

The AM root colonization was about 60% of mycorrhizal root length, with no significant differences under well-watered and drought stress conditions (Table 1).

3.2. Stomatal conductance (gs)

The water stress imposed significantly decreased stomatal conductance in both non-mycorrhizal and mycorrhizal plants (in both cases more than 65% drop). However, inoculation with the mycorrhizal fungus caused a 1.8 and 1.6 fold increase respectively in stomatal conductance compared to non-inoculated plants regardless of the water treatment (Table 1).

3.3. Chlorophyll content and efficiency of photosystem II

Chlorophyll content was measured by SPAD and was reduced by drought stress in both AM and non-AM plants (Table 1). However, under drought stress conditions AM plants maintained higher values of chlorophyll content than non-AM plants (an increase of 24%).

330 The efficiency of photosystem II was significantly reduced by drought stress in non-
331 AM plants only, while AM plants maintained similar values than under well-watered
332 conditions (Table 1).

333

334 **3.4. Hydrostatic root hydraulic conductivity (Lpr)**

335 The AM symbiosis increased Lpr in maize plants, although the increase was
336 statistically significant only under well-watered conditions (Figure 2). Moreover, under well-
337 watered conditions, the application of sodium azide increased Lpr by 42% in *R. irregularis*-
338 inoculated plants, but not in non-AM plants. Drought stress reduced Lpr in both treatments,
339 regardless of sodium azide application. Under drought stress, the mycorrhization of maize
340 roots tended to enhance Lpr, but the differences were not statistically significant.

341

342 **3.5. Osmotic root hydraulic conductivity (Lo)**

343 The mycorrhization increased considerably Lo values under well-watered
344 conditions, both in absence and in presence of sodium azide (Figure 2). Under drought
345 stress conditions the increase was statistically significant only in presence of sodium
346 azide. Indeed, both under drought stress and under well-watered conditions, the
347 application of sodium azide reduced significantly Lo in non-AM plants (t-student), but
348 maintained similar values in AM plants. Thus, AM plants treated with sodium azide showed
349 4 to 5 fold higher Lo values compared to non-AM plants, regardless of the watering
350 conditions.

351 The application of sodium azide affected not only the Lo values but also the number
352 of plants exuding under these conditions. Indeed, under well-watered conditions, 100% of

353 the plants exuded spontaneously (both AM and non-AM), while sodium azide application
354 reduced this percentage to 75% in non-AM plants but unaltered the percentage in AM
355 plants. Under drought stress conditions, 38% of non-AM plants exuded spontaneously,
356 while after sodium azide application only 13% of the plants got free exudation. In the case
357 of AM plants, 100% of them exuded spontaneously under drought stress and this
358 percentage was only reduced to 88% after application of sodium azide (data not shown).

359

360 **3.6. Relative apoplastic water flow**

361 Interestingly, the application of sodium azide increased the percentage of relative
362 apoplastic water flow in AM and non-AM plants cultivated under well-watered conditions
363 (Figure 2). However, under drought stress no significant differences were found. The
364 mycorrhization itself also increased the apoplastic water flow both under well-watered
365 (21% of increase) and under drought stress conditions (86% of increase). Drought stress
366 reduced considerably this parameter in AM and non-AM plants, regardless of sodium
367 azide application.

368

369 **3.7. Apoplastic barriers**

370 Structural changes in plants need longer time than other physiological or
371 biochemical changes. As sodium azide was applied only for 30 min, the development of
372 apoplastic barrier was unaffected by the chemical treatment. Figure 3 shows the
373 development of Casparian bands in the exo- and endodermis of root sections taken at 50
374 mm from tips. Under well-watered conditions a weak signal was detected in the
375 endodermis of maize roots, which was more intense in AM plants than in non-AM ones. In

376 the exodermis the formation of Casparian bands was visible, but weaker and more diffuse
377 than in endodermis. Drought stress increased the development of Casparian bands of both
378 exo- and endodermis. Again the intensity of the signal in endodermis was stronger in AM
379 plants than in non-AM ones. In exodermis the signal was more diffuse in non-AM plants
380 and more localized in AM plants.

381

382 **3.8. Aquaporin protein accumulation and PIP2s phosphorylation status**

383 In general, a drop in aquaporin protein levels was observed when plants were
384 inoculated with the AM fungus, regardless of the watering conditions (Figure 4, 5).
385 However, the decrease was not significant for all the analyzed aquaporins or treatments,
386 being more evident when using specific antibodies for different PIP2 isoforms than when
387 using the general antibodies for PIP1 or PIP2.

388 The application of NaN_3 positively regulated protein accumulation in non-AM plants
389 under well-watered conditions in the case of ZmPIP2;4, ZmPIP2;5, ZmTIP1;1; PIP2A and
390 PIP2C (Figure 4, 5). Interestingly, under drought stress NaN_3 had the opposite effect in
391 non-AM plants in the case of ZmPIP2;5, PIP2A, PIP2B and PIP2C, which decreased their
392 accumulation. In AM plants, the application of NaN_3 only affected negatively the root
393 accumulation of PIP2B (PIP2 phosphorylated at Ser283) proteins.

394 In the absence of NaN_3, drought stress increased the accumulation of PIP1 and
395 PIP2 proteins in non-AM plants (Figure 4). In AM plants no effect was observed. In
396 presence of NaN_3, drought stress decreased the accumulation of ZmPIP2;4, ZmPIP2;5
397 and ZmTIP1;1 in non-AM plants and, again, no effect was observed in AM plants.

398

4. Discussion

When a mycorrhizal fungus colonizes plant roots, structural changes are produced in cells, affecting root water uptake and transport. The improvement of plant physiology by the AM symbiosis during water-limiting conditions has been extensively studied in different crop species (Ruiz-Lozano et al., 2012; Augé et al., 2015), although studies of their differential effect on root water transport are still elusive. In previous studies, the AM symbiosis was suggested to modulate the switching between apoplastic and cell-to-cell water transport in roots when the water conditions were limiting (Bárzana et al., 2012). The focus of the present study was to better understand how the arbuscular mycorrhizal symbiosis regulates radial root water transport in maize plants. For that we measured osmotic (Lo) and hydrostatic (Lpr) root hydraulic conductivities and we used sodium azide as inhibitor of aquaporins activity and of cell-to-cell water transport (Tournaire-Roux et al., 2003; Grondin et al., 2016). The development of Casparian bands was also assessed.

The AM symbiosis positively affected plant development and physiology under drought stress

The beneficial effect of mycorrhizal inoculation during drought stress was confirmed by plant growth and physiological data. AM plants exhibited higher dry weight under the two watering conditions. Additionally, AM plants enhanced stomatal conductance (gs) both under well-watered or drought stress conditions and the efficiency of photosystem II and chlorophyll content were also significantly higher during water deprivation. The maintenance of a high stomatal conductance allows the plant a better CO_2 uptake for photosynthesis (Sheng et al., 2008). This combined with the improved chlorophyll content and efficiency of photosystem II are surely related with the improved plant growth. These

423 changes have been linked to a higher capacity for CO_2 fixation in AM plants. For instance,
424 a higher RuBisCo activity was found in droughted AM grapevine plants (Valentine et al.,
425 2006) or in AM rice plants subjected to salinity (Porcel et al., 2015). More recently, Chen et
426 al. (2017) found a higher activity of key Calvin cycle enzymes in AM cucumber plants and
427 Quiroga et al. (2019) have found that the maximum carboxylation capacity of PEPc
428 (Vpmax) and CO_2-saturated photosynthetic rate (Vmax) were higher in AM maize plants
429 subjected to drought, revealing the higher photosynthetic capacity of AM maize plants,
430 which translated into improved growth of mycorrhizal droughted plants.

431

432 **The inhibition of aquaporins activity negatively affected root water transport**

433 NaN_3 is a metabolic inhibitor that mimics O_2-deficient conditions by blocking the
434 cytochrome pathway respiration, and consequently induces intracellular acidosis. Although
435 not all aquaporins were found to be regulated by pH, the group of PIPs was commonly
436 characterized as pH-dependent (Vitali et al., 2019). In consequence, NaN_3 application
437 leads to H^+-dependent gating of PIPs, due to a conserved structural basis for cytosolic pH
438 sensing, where a histidine residue (His193 in spinach PIP2;1) of cytosolic loop D is
439 involved (Tournaire-Roux et al., 2003; Törnroth-Horsefield et al., 2006; Fischer and
440 Kaldenhoff, 2008; Frick et al., 2013b). Protonation of this residue would interact with the N-
441 terminal divalent cation-binding site, stabilizing the closed conformation of the aquaporin
442 (Frick et al., 2013b). Recently, it was found that the pH-dependence is also regulated by
443 tetramer stoichiometry in PIPs (Jozefkowicz et al., 2016). Besides, sodium azide is more
444 efficient than other aquaporin inhibitors since it has a dual effect, causing cytoplasm
445 acidification and inhibition of phosphorylation (Tournaire-Roux et al., 2003; Fitzpatrick and
446 Reid, 2009).

447 The effect of sodium azide on root water flow rate (*Jv*) was previously studied
448 (Kamaluddin and Zwiazek, 2001; Tournaire-Roux et al., 2003; Postaire et al., 2010; Sutka
449 et al., 2011). A decrease in *Jv* similar to that of $HgCl_2$ (another aquaporin inhibitor) after
450 treatment with sodium azide was observed in Arabidopsis plants and it was reversed upon
451 washout of the inhibitor (Postaire et al., 2010). Kamaluddin and Zwiazek (2001) also
452 observed an increase in apoplastic flow of water with the NaN_3-induced decrease in root
453 water flow rates. These results are in agreement with the present study. Indeed, NaN_3
454 application increased the apoplastic water flow both in AM and in non-AM plants. For
455 instance, Lo was decreased by azide in non-AM plants, suggesting an inhibition of root
456 aquaporins activity, but compensated by the increase of apoplastic water flow in these
457 plants. In AM plants the apoplastic water flow and Lo values were already higher than in
458 non-AM plants. Thus, in AM plants the inhibitory effect of sodium azide on Lo was lower
459 than in non-AM plants, which together with the higher apoplastic water flow values
460 suggests a compensatory mechanism for aquaporin activity inhibition in these plants,
461 leading to a higher Lpr compared to non-AM plants. The general decrease of Lpr with
462 water stress, that was slightly compensated with the mycorrhizal presence, could be
463 related to the minimization of water loss from roots (Kaneko et al., 2015). Some
464 differences in Lpr and Lo values between AM and non-AM plants in response to sodium
465 azide or drought were not statistically significant when all treatments were analyzed jointly,
466 but the use of a higher number of plants (here, n= 7-8 biological replicates) may have led
467 to significant differences. In fact, pairwise comparison with t-student test showed indeed
468 significant differences.

469

470

Apoplastic barriers developed under drought stress conditions

The root system has a high plasticity for modulating the development of apoplastic barriers in order to adapt to different environmental stresses (Pauluzzi and Bailey-Serres, 2016). In our study the development of Casparian bands increased due to drought stress, as evidenced by other authors with different plant species (Shen et al., 2014; Kreszies et al., 2018; 2019). This was in parallel with a significant decrease of Lpr, as well as, of the percentage of apoplastic water flow. Lo was not significantly decreased by drought stress, except in AM plants untreated with sodium azide. In general, apoplastic barriers decrease Lpr although this effect may vary, and there are reports of no correlation between increased suberin deposition and decreased water permeability (Ranathunge and Schreiber, 2011). In any case, it has been shown that apoplastic barriers have a more pronounced effect on Lpr and lower or no effect on Lo. This has been proposed as a mechanism to avoid water loss toward soil via the nonselective apoplastic pathway, while favouring the water passage through the highly regulated cell-to-cell pathway (Kreszies et al., 2019). In this study, AM plants exhibited enhanced development of Casparian bands compared to non-AM plants. In contrast, AM plants maintained enhanced Lpr and Lo values and percentage of apoplastic water flow as compared to non-AM plants. An explanation for these contradictory effects may be related with a different composition of Casparian bands and suberin deposits in AM and non-AM plants. Indeed, it has been proposed that the effect of apoplastic barriers on radial water movement may depend on their composition and of the microstructure of their deposits (Schreiber et al., 2005). Thus, a different composition of the apoplastic barriers in AM and non-AM plants may explain also the different effect on root water flow in these plants, even if Casparian bands seem more developed in AM plants. More studies are required to analyze the composition of these barriers and to elucidate this hypothesis.

Aquaporins accumulation and posttranslational modifications are affected by sodium azide application and mycorrhization

The time of application and concentration of the sodium azide (30 minutes, 2 mM) was in the same range of previous studies (Tournaire-Roux et al., 2003; Postaire et al., 2010; Grondin et al., 2016) and enough for inhibiting osmotic root water conductivity, although no clear effect was observed on protein abundance of aquaporins. It should be taken into account that the effect of NaN_3 is mainly at posttranslational level affecting the water transport activity, not necessarily affecting gene expression or protein abundance. On the other hand, aquaporin regulation may be cell-specific, being masked in whole-organ extractions. Furthermore, many aspects of the aquaporin regulation have to be considered, as gating, cycling or internalization due to environmental stresses (Chu et al., 2018). Indeed, the activity of aquaporins must be controlled by regulation mechanisms allowing a rapid response to the frequent environmental changes that plants undergo. Posttranslational modifications are key to achieve such a rapid and reversible regulation (Chaumont and Tyerman, 2014; Vandeleur et al., 2014), and they control protein catalytic activity, stability, subcellular localization and interaction with other proteins (Prak et al., 2008).

Phosphorylation is the most widespread protein modification, affecting basic cellular processes. Phosphorylation/de-phosphorylation of specific serine residues in plant aquaporins generates conformational changes controlling the aquaporin gating (Santoni, 2017) or modifying its subcellular localization under stress conditions (Luu and Maurel, 2013). For instance, the phosphorylation of Ser283 is required for targeting AtPIP2;1 to the plasma membrane (Prak et al., 2008). Maize PIP1s and PIP2s aquaporins were also shown to phosphorylate *in vivo* (Van Wilder et al., 2008). In addition, the phosphorylation of Ser274 in the C-terminal region or of Ser115 in loop B of a PIP2 in spinach open the

521 pore and enhances the water transport (Törnroth-Horsefield et al., 2006). Interestingly, we
522 found that NaN_3 treatment significantly decreased phosphorylation levels (PIP2A, PIP2B
523 and PIP2C) under drought stress in non-AM plants, while in AM plants it was significant
524 only in the case of PIP2s phosphorylated at Ser283 (PIP2B). This result suggests a more
525 intense closing of these channels in non-AM plants in response to the sodium azide
526 application. Aquaporins gating by pH is another common phenomenon in plants that
527 naturally occurs in response to flooding (Tournaire-Roux et al., 2003; Frick et al., 2013b)
528 and that is also a consequence of NaN_3 application, as explained above. A conserved
529 histidine residue in loop D is considered a major pH sensor regulating channel gating. The
530 drop in cytosolic pH can be accompanied with an increase in cytosolic Ca^{+2} concentration,
531 being possible its interaction with the divalent cation binding site, and maintaining loop D in
532 a closed conformation at low pH (Frick *et al.* 2013b). However, divalent cations can directly
533 inhibit aquaporins in some cases (Verdoucq et al., 2008). Altogether, this reveals how
534 complex the gating of aquaporin proteins is, and the interplay among phosphorylation, pH
535 and cation binding in the regulation of the pore opening.

536 Surprisingly, in this study we found that some aquaporins increased their protein
537 levels when treated with NaN_3 (ZmPIP2;4, ZmPIP2;5, ZmTIP1;1) under normal irrigation.
538 These aquaporins may be insensitive to the inhibitor and the protein increase could be a
539 compensation mechanism to the decrease in root water conductivity. SoPIP2;1 was
540 demonstrated to increase its water permeability when treated with mercury (Frick et al.,
541 2013a). It is noteworthy that protein levels in membranes do not give information about
542 their location, as they can be located in the secretory pathway such as endoplasmic
543 reticulum, Golgi, or different vesicles, apart from plasma membrane, which affect their
544 functionality as water channels (Chevalier and Chaumont, 2015). On the other hand, we
545 observed a general drop in aquaporin protein levels when plants were inoculated with the

AM fungus, a result that is consistent with previous studies (Bárzana et al., 2014; Quiroga et al., 2019), being that more evident when using specific antibodies for different PIP2 isoforms than when using the general antibodies for PIP1s or PIP2s. This may suggest that some specific aquaporins may decrease in presence of the AM fungus, while other PIP isoforms not tested here should be more abundant in AM plants. This should be checked in future studies with a larger set of isoform-specific antibodies.

5. Conclusion

This study provides some clues on the differential mycorrhizal regulation of root water transport. Indeed, the presence of a mycorrhizal fungus significantly modified the radial transport of water within the root system. Thus, in AM plants without sodium azide application, Lpr, Lo and the percentage of apoplastic water flow raised as compared to non-AM plants. When sodium azide was applied, there was a clear inhibition of Lo in non-AM plants, both under well-watered conditions and under drought stress. In AM plants the inhibition was weaker and not significant. This was particularly important under drought stress, since 88% of AM plants treated with sodium azide got free sap exudation and had 4 fold higher Lo values than non-AM plants, where only 13% of the plants got free sap exudation. This seems to be related to the regulation of aquaporins activity through posttranslational mechanisms rather than with the regulation of aquaporin protein accumulation, probably due to the short time of sodium azide exposure. However, this should be addressed in future studies in order to understand the specific mechanisms involved.

Author statement

JMR-L conceived the study and participated in the analysis of data and manuscript writing. GQ and GE performed the experiments and the statistical analysis. RA and FC participated in the design of the experiments and interpretation of data. All authors read and approved the final manuscript.

Acknowledgements

This work was financed by Ministerio de Economía y Competitividad (Project AGL2017-88341-R) (JMRL and RAA) and by grants from the Belgian National Fund for Scientific Research (FNRS) and the Belgian French community (Project ARC16/21-075) (FC). GQ is a FPI grant holder. We would like to thank Sonia Molina and Mª Carmen Perálvarez for technical assistance. We thank DuPont Pioneer Corporation for providing us the maize seeds.

References

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 and salinity stresses? New Phytol. 173, 808–816.

Augé R.M., Toler H.D., Saxton A.M. 2015. Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis. Mycorrhiza 25, 13–24.

Azcón-Aguilar C., Barea J.M. 2015. Nutrient cycling in the mycorrhizosphere. J. Soil Sci.

591 Plant Nutr. 25, 372–396.

592 Barberon M. 2017. The endodermis as a checkpoint for nutrients. New Phytol. 213, 1604–
593 1610.

594 Bárzana G., Aroca R., Bienert G.P., Chaumont F., Ruiz-Lozano J.M. 2014. New insights
595 into the regulation of aquaporins by the arbuscular mycorrhizal symbiosis in maize
596 plants under drought stress and possible implications for plant performance. Mol.
597 Plant-Microbe Interact. 27, 349–363.

598 Bárzana G., Aroca R., Paz J.A., Chaumont F., Martinez-Ballesta M.C., Carvajal M., Ruiz-
599 Lozano J.M. 2012. Arbuscular mycorrhizal symbiosis increases relative apoplastic
600 water flow in roots of the host plant under both well-watered and drought stress
601 conditions. Ann. Bot. 109, 1009–1017.

602 Bárzana G., Aroca R., Ruiz-Lozano J.M. 2015. Localized and non-localized effects of
603 arbuscular mycorrhizal symbiosis on accumulation of osmolytes and aquaporins and
604 on antioxidant systems in maize plants subjected to total or partial root drying. Plant,
605 Cell Enviror. 38, 1613–1627.

606 Benabdellah K., Ruiz-Lozano J.M., Aroca R. 2009. Hydrogen peroxide effects on root
607 hydraulic properties and plasma membrane aquaporin regulation in *Phaseolus*
608 *vulgaris*. Plant Mol. Biol. 70, 647–661.

609 Brundrett M.C., Enstone D.E., Peterson C. A. 1988. A berberine-aniline blue fluorescent
610 staining procedure for suberin, lignin, and callose in plant tissue. Protoplasma 146,
611 133–142.

612 Calvo-Polanco M., Molina S., Zamarreño A.M., García-Mina J.M., Aroca R. 2014. The
613 symbiosis with the arbuscular mycorrhizal fungus *Rhizophagus irregularis* drives root

water transport in flooded tomato plants. Plant Cell Physiol. 55, 1017–1029.

Chaumont F., Barrieu F., Wojcik E., Chrispeels M.J., Jung R. 2001. Aquaporins constitute a large and highly divergent protein family in maize. Plant Physiol. 125, 1206-1215.

Chaumont F., Tyerman S.D. 2014. Aquaporins: Highly regulated channels controlling plant water relations. Plant Physiol. 164, 1600-1618.

Chen S., Zhao H., Zou C., Li Y., Chen Y., Wang Z. Jiang Y., Liu A., Zhao P., Wang M., Ahammed G.J. (2017) Combined inoculation with multiple arbuscular mycorrhizal fungi improves growth, nutrient uptake and photosynthesis in cucumber seedlings. Front. Microbiol. 8, 1–11.

Chevalier A.S., Chaumont, F. 2015. Trafficking of plant plasma membrane aquaporins: multiple regulation levels and complex sorting signals. Plant Cell Physiol. 56, 819-829.

Chitarra W., Pagliarani C., Maserti B., Lumini E., Siciliano I., Cascone P., Schubert A., Gambino G., Balestrini R., Guerrieri E. 2016. Insights on the impact of arbuscular mycorrhizal symbiosis on tomato tolerance to water stress. Plant Physiol. 171, 1009–1023.

Chu T.T.H., Hoang T.G., Trinh D.C., Bureau C., Meynard D., Vernet A., Ingouff M., Vinh Do N., Périn C., Guiderdoni E., Gantet P., Maurel C., Luu D.T. 2018. Sub-cellular markers highlight intracellular dynamics of membrane proteins in response to abiotic treatments in rice. Rice 11, 23.

Daryanto S., Wang L., Jacinthe P.A. 2016. Global synthesis of drought effects on maize and wheat production. PLoS ONE 11, 1–15.

Fischer M., Kaldenhoff R. 2008. On the pH regulation of plant aquaporins. J. Biol. Chem.

283, 33889–33892.

Fitzpatrick K.L., Reid R.J. 2009 The involvement of aquaglyceroporins in transport of boron in barley roots. Plant, Cell Environ. 32, 1357–1365.

Frick A., Järvå M., Ekvall M., Uzdavinys P., Nyblom M., Törnroth-Horsefield S. 2013a. Mercury increases water permeability of a plant aquaporin through a non-cysteine-related mechanism. Biochem. J. 454, 491–499.

Frick A., Järvå M., Törnroth-Horsefield S. 2013b. Structural basis for pH gating of plant aquaporins. FEBS Lett. 587, 989–993.

Fritz M., Ehwald R. 2011. Mannitol permeation and radial flow of water in maize roots. New Phytol. 189, 210–217.

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

Grondin A., Mauleon R., Vadez V., Henry A. 2016. Root aquaporins contribute to whole plant water fluxes under drought stress in rice (*Oryza sativa* L.). Plant, Cell Environ. 39, 347–365.

Gupta A.B., Sankararamakrishnan R. 2009. Genome-wide analysis of major intrinsic proteins in the tree plant *Populus trichocarpa*: Characterization of XIP subfamily of aquaporins from evolutionary perspective. BMC Plant Biol. 9, 134.

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

Hachez C., Zelazny E., Chaumont F. 2006b. Modulating the expression of aquaporin

genes in planta: A key to understand their physiological functions? Biochim. Biophys.Acta - Biomembr. **1758**, 1142–1156.

Hoagland D.R., Arnon D.I. 1950. The water-culture method for growing plants without soil. Calif. Agric. Experiment. Stat. Circ. 347, 1–32.

Jozefkowicz C., Sigaut L., Scochera F., Soto G., Ayub N., Pietrasanta L.I., Amodeo G., Gonzalez-Flecha F.L., Alleva K. 2016. PIP water transport and its ph dependence are regulated by tetramer stoichiometry. Biophys. J. 110, 1312–1321.

Kamaluddin M., Zwiazek J.J. 2001. Metabolic inhibition of root water flow in red-osier dogwood (*Cornus stolonifera*) seedlings. J. Exp. Bot. 52, 739–745.

Kaneko T., Horie T., Nakahara Y., Tsuji N., Shibasaka M., Katsuhara M. 2015. Dynamic regulation of the root hydraulic conductivity of barley plants in response to salinity/osmotic stress. Plant Cell Physiol. 56, 875–882.

Kapilan R., Vaziri M., Zwiazek J.J. 2018. Regulation of aquaporins in plants under stress. Biol. Res. 51, 4.

Kim Y.X., Ranathunge K., Lee S., Lee Y., Lee D., Sung J. 2018. Composite transport model and water and solute transport across plant roots: an update. Front. Plant Sci. 9, 193.

Knipfer T., Fricke W. 2010. Root pressure and a solute reflection coefficient close to unity exclude a purely apoplastic pathway of radial water transport in barley (*Hordeum vulgare*). New Phytol. 187, 159–170.

Kreszies T., Schreiber L., Ranathunge K. 2018. Suberized transport barriers in Arabidopsis, barley and rice roots: From the model plant to crop species. J. Plant

680 Physiol. 227, 75–83.

681 Kreszies T., Shellakkutti N., Osthoff A., Yu P., Baldauf J.A., Zeisler-Diehl V.V.,
682 Ranathunge K., Hochholdinger F., Schreiber L. 2019. Osmotic stress enhances
683 suberization of apoplastic barriers in barley seminal roots: Analysis of chemical,
684 transcriptomic and physiological responses. New Phytol. 221, 180–194.

685 Lenoir I., Fontaine J., Lounès-Hadj Sahraoui A. 2016. Arbuscular mycorrhizal fungal
686 responses to abiotic stresses: A review. Phytochemistry 123, 4–15.

687 Lesk C., Rowhani P., Ramankutty N. 2016. Influence of extreme weather disasters on
688 global crop production. Nature 529, 84–87.

689 López-Pérez L., Fernández-García N., Olmos E., Carvajal M. 2007. The Phi thickening in
690 roots of broccoli plants: An acclimation mechanism to salinity? Internat. J. Plant Sci.
691 168, 1141–1149.

692 Luu D.T., Maurel C. 2013. Aquaporin trafficking in plant cells: an emerging membrane-
693 protein model. Traffic 14, 629–635.

694 Maurel C., Boursiac Y., Luu D.-T., Santoni V., Shahzad Z., Verdoucq L. 2015. Aquaporins
695 in plants. Physiol. Rev. 95, 1321–58.

696 Maurel C., Verdoucq L., Luu D.-T., Santoni V. 2008. Plant aquaporins: membrane
697 channels with multiple integrated functions. Annu.Rev. Plant Biol. 59, 595–624.

698 Meyer C.J., Peterson C.A., Steudle E. 2011. Permeability of Iris germanica's multiseriate
699 exodermis to water, NaCl, and ethanol. J. Exp. Bot. 62, 1911–1926.

700 Niemietz C.M., Tyerman S.D. 2002. New potent inhibitors of aquaporins: Silver and gold
701 compounds inhibit aquaporins of plant and human origin. FEBS Lett. 531, 443–447.

Ortiz N., Armada E., Duque E., Roldán A., Azcón R. 2015. Contribution of arbuscular mycorrhizal fungi and/or bacteria to enhancing plant drought tolerance under natural soil conditions: Effectiveness of autochthonous or allochthonous strains. J. Plant Physiol. 174, 87–96.

Pauluzzi G., Bailey-Serres J. 2016. Flexible ion barrier. Cell 164, 345–346.

Phillips J.M., Hayman D.S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. British Mycol. Soc. 55, 158–161.

Porcel R., Redondo-Gómez S., Mateos-Naranjo E., Aroca R., Garcia R., Ruiz-Lozano J.M. 2015. Arbuscular mycorrhizal symbiosis ameliorates the optimum quantum yield of photosystem II and reduces non-photochemical quenching in rice plants subjected to salt stress. J. Plant Physiol. 185, 75–83.

Postaire O., Tournaire-Roux C., Grondin A., Boursiac Y., Morillon R., Schaffner A.R., 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.

Prak S., Hem S., Boudet J., Viennois G., Sommerer N., Rossignol M., Maurel C., Santoni V. (2008) Multiple phosphorylations in the c-terminal tail of plant plasma membrane aquaporins: role in subcellular trafficking of AtPIP2;1 in response to salt stress. Mol. Cel. Proteom. **7**, 1019–1030.

Quiroga G., Erice G., Aroca R., Chaumont F., Ruiz-Lozano J.M. 2017. Enhanced drought stress tolerance by the arbuscular mycorrhizal symbiosis in a drought-sensitive maize cultivar is related to a broader and differential regulation of host plant aquaporins than in a drought-tolerant cultivar. Front. Plant Sci. 8, 1056.

725 Quiroga G., Erice G., Aroca R., Zamarreño Á.M., García-Mina J.M., Ruiz-Lozano J.M.
726 2018. Arbuscular mycorrhizal symbiosis and salicylic acid regulate aquaporins and
727 root hydraulic properties in maize plants subjected to drought. Agric. Water Manag.
728 202, 271–284.

729 Quiroga G., Erice G., Ding L., Chaumont F., Aroca R., Ruiz-Lozano J.M. 2019 The
730 arbuscular mycorrhizal symbiosis regulates aquaporins activity and improves root cell
731 water permeability in maize plants subjected to water stress. Plant, Cell Environ. (In
732 press) doi: 10.1111/pce.13551.

733 Ranathunge K., Kim Y.X., Wassmann F., Kreszies T., Zeisler V., Schreiber L. 2017. The
734 composite water and solute transport of barley (*Hordeum vulgare*) roots: effect of
735 suberized barriers. Ann. Bot. 119, 629–643.

736 Ranathunge K., Kotula L., Steudle E., Lafitte R. 2004. Water permeability and reflection
737 coefficient of the outer part of young rice roots are differently affected by closure of
738 water channels (aquaporins) or blockage of apoplastic pores. J. Exp. Bot. 55, 433–
739 447.

740 Ranathunge K., Schreiber L. 2011. Water and solute permeabilities of Arabidopsis roots in
741 relation to the amount and composition of aliphatic suberin. J. Exp. Bot. **62**, 1961–
742 1974.

743 Ruiz-Lozano J.M., Aroca R., Zamarreño Á.M., Molina S., Andreo-Jiménez B., Porcel R.,
744 García-Mina J.M., Ruyter-Spira C., López-Ráez J.A. 2016. Arbuscular mycorrhizal
745 symbiosis induces strigolactone biosynthesis under drought and improves drought
746 tolerance in lettuce and tomato. Plant, Cell Environ. 39, 441–452.

747 Ruiz-Lozano J.M., Porcel R., Azcón R., Aroca R. 2012. Regulation by arbuscular

748 mycorrhizae of the integrated physiological response to salinity in plants: new
749 challenges in physiological and molecular studies. J. Exp. Bot. 63, 695–709.

750 Santoni V. 2017. Plant aquaporin posttranslational regulation. In: *Plant Aquaporins,*
751 *Signaling and Communication in Plants*. (eds F. Chaumont and S.D. Tyerman),
752 Springer International Publishing, pp. 83–105.

753 Schreiber L., Franke R., Hartmann K.D., Ranathunge K., Steudle E. 2005. The chemical
754 composition of suberin in apoplastic barriers affects radial hydraulic conductivity
755 differently in the roots of rice (*Oryza sativa* L. cv. IR64) and corn (*Zea mays* L. cv.
756 Helix). J. Exp. Bot. 56, 1427–1436.

757 Shen J., Xu G., Zheng H.Q. 2014. Apoplastic barrier development and water transport in
758 *Zea mays* seedling roots under salt and osmotic stresses. Protoplasma 252, 173–180.

759 Sheng M., Tang M., Chen H., Yang B., Zhang F., Huang Y. 2008. Influence of arbuscular
760 mycorrhizae on photosynthesis and water status of maize plants under salt stress.
761 Mycorrhiza 18, 287–296.

762 Smith S., Read D. 2008. *Mycorrhizal symbiosis*, 3rd Ed. Academic Press, London, UK.

763 Steudle E. 2000. Water uptake by roots: effects of water deficit. J. Exp. Bot. 51, 1531–
764 1542.

765 Steudle E. 2001. The cohesion-tension mechanism and the acquisition of water by plant
766 roots. Annu. Rev. Plant Physiol. Plant Mol. Biol. 52, 847–75.

767 Steudle E., Peterson C.A. 1998. How does water get through roots ? J. Exp. Bot. 49, 775–
768 788.

769 Sutka M., Li G., Boudet J., Boursiac Y., Doumas P., Maurel C. 2011. Natural variation of

770 root hydraulics in arabidopsis grown in normal and salt-stressed conditions. Plant
771 Physiol. 155, 1264–1276.

772 Törnroth-Horsefield S., Wang Y., Hedfalk K., Johanson U., Karlsson M., Tajkhorshid E.,
773 Neutze R., Kjellbom P. 2006. Structural mechanism of plant aquaporin gating. Nature
774 439, 688–694.

775 Tournaire-Roux C., Sutka M., Javot H., Gout E., Gerbeau P., Luu D.T., Bligny R., Maurel
776 C. 2003. Cytosolic pH regulates root water transport during anoxic stress through
777 gating of aquaporins. Nature 425, 393–397.

778 Vadez V. 2014 Root hydraulics: The forgotten side of roots in drought adaptation. Field
779 Crops Res. 165, 15–24.

780 Valentine A.J., Mortimer P.E., Lintnaar M., Borgo R. 2006. Drought responses of
781 arbuscular mycorrhizal grapevines. Symbiosis 41, 127–133.

782 Vandeleur R.K., Mayo G., Shelden M.C., Gilliham M., Kaiser B.N., Tyerman S.D. 2009.
783 The role of plasma membrane intrinsic protein aquaporins in water transport through
784 roots: diurnal and drought stress responses reveal different strategies between
785 isohydric and anisohydric cultivars of grapevine. Plant Physiol. 149, 445–460.

786 Vandeleur R.K., Sullivan W., Athman A., Jordans C., Gilliham M., Kaiser B.N., Tyerman
787 S.D. 2014 Rapid shoot-to-root signalling regulates root hydraulic conductance via
788 aquaporins. Plant, Cell Environ. 37, 520–538.

789 Van Wilder V., Miecielica U., Degand H., Derua R., Waelkens E., Chaumont F. 2008.
790 Maize plasma membrane aquaporins belonging to PIP1 and PIP2 subgroups are *in*
791 *vivo* phosphorylated. Plant Cell Physiol. 49, 1364-1377.

792 Verdoucq L., Grondin A., Maurel C. 2008. Structure–function analysis of plant aquaporin
793 *At* PIP2;1 gating by divalent cations and protons. Biochem. J. 415, 409–416.

794 Vitali V., Jozefkowicz C., Canessa Fortuna A., Soto G., González Flecha F.L., Alleva K.
795 2019. Cooperativity in proton sensing by PIP aquaporins. FEBS J. 286, 991-1002.

796 Voicu MC, Zwiazek JJ. 2004. Cycloheximide inhibits root water flow and stomatal
797 conductance in aspen (*Populus tremuloides*) seedlings. Plant, Cell Environ. 27, 199-
798 208.

799 Wang P., Calvo-Polanco M., Reyt G., Barberon M., Champeyroux C., Santoni V., Maurel
800 C., Franke R.B., Ljung K., Novak O., Geldener N., Boursiac Y., Salt D.E. 2019.
801 Surveillance of cell wall diffusion barrier integrity modulates water and solute transport
802 in plants. Scientific Reports 9, 4227.

803 Zingaretti S.M., Inácio M.C., Lívia M.P., Tiago A.P., Suzelei C.F. .2013. Water Stress and
804 Agriculture. In *Responses of organisms to water stress*. (ed S. Akinci), pp. 151–179.
805 InTechOpen, London, UK.

806 Zimmermann HM, Steudle E. 1998. Apoplastic transport across young maize roots: effects
807 of the exodermis. Planta 206, 7-19.

808

809

Table 1. Percentage of mycorrhizal root length, stomatal conductance (gs), SPAD values and photosystem II efficiency in the light-adapted state (ΔFv/Fm') of maize plants inoculated or not with the AM fungus *Rhizophagus irregularis* and submitted to two water regimes (well-watered-WW- or drought stress DS).

	Mycorrhization (%)	gs (mmol H_2O m^{-2} s^{-1})	SPAD	ΔFv/Fm'
WW non-AM	**n.d.**	**49.8 ± 7.6 b**	**12.1 ± 0.7 a**	**0.65 ± 0.01 a**
WW AM	**64.5 ± 6.7**	**138.8 ± 10.4 a**	**11.5 ± 0.5 a**	**0.63 ± 0.01 a**
DS non-AM	**n.d.**	**16.0 ± 1.7 c**	**7.8 ± 0.4 c**	**0.58 ± 0.02 b**
DS AM	**57.8 ± 4.0**	**42.1 ± 4.1 b**	**9.7 ± 0.3 b**	**0.62 ± 0.01 a**

Data represent the means of five values ± SE for mycorrhization, seven values ± SE for gs and ten values for SPAD and ΔFv/Fm'. Different letter indicates significant differences between treatments ($p < 0.05$) based on t-test for mycorrhization and on Duncan's test for the other parameters. n.d. non detected.

826 **Figure legends**

827

828 **Figure 1.** Total dry weight of maize plants inoculated or not with the AM fungus
829 *Rhizophagus irregularis* and submitted to two water regimes (well-watered, WW or drought
830 stress, DS). Data show the mean ± SE for fifteen plants per treatment. Different letter
831 indicates significant differences between treatments ($p < 0.05$) based on Duncan's test.

832

833 **Figure 2.** Hydrostatic root hydraulic conductivity (*Lpr*), osmotic root hydraulic conductivity
834 (*Lo*) and relative apoplastic water flow of maize plants inoculated or not with the AM
835 fungus *Rhizophagus irregularis* and submitted to two water regimes (well-watered, WW or
836 drought stress, DS). A group of plants from each treatment was treated with NaN_3 for 30
837 min before measurements or kept untreated (-). Data show the mean ± SE for seven
838 plants per treatment for Lpr and apoplastic water flow and for eight plants for Lo. Different
839 letter indicates significant differences between treatments ($p < 0.05$) based on Duncan's
840 test. Asterisk denotes significant differences between AM and non-AM plants based on t-
841 test.

842

843 **Figure 3.** Development of Casparian bands in root sections taken at 50 mm from the root
844 tip. **A-B,** transverse control root sections not stained with berberine hemisulphate. **C-F,**
845 transverse root sections stained with berberine hemisulphate and toluidine blue as
846 described in the materials and methods. **(C)**, Non-AM plants under well-watered
847 conditions. **(D)**, AM plants under well-watered conditions. **(E)**, Non-AM plants under
848 drought stress. **(F)**, AM plants under drought stress. The presence of Casparian bands

849 was indicated by green-yellow fluorescence (see white arrows in C, D, E, F).

850

851 **Figure 4.** Relative protein abundance in the microsomal fraction of roots from plants
852 inoculated or not with the AM fungus *Rhizophagus irregularis* and submitted to two water
853 regimes (well-watered, WW or drought stress, DS). A group of plants from each treatment
854 was treated with NaN_3 for 30 min before harvest or kept untreated (-). Data indicate the
855 mean ± SE for three biological replicates per treatment. Different letter indicates significant
856 differences between treatments ($p<0.05$) based on Duncan's test.

857

858 **Figure 5.** PIP2A (Ph-Ser280), PIP2B (Ph-Ser283) and PIP2C (Ph-Ser280/Ser283) relative
859 protein abundance in the microsomal fraction of roots from plants inoculated or not with
860 the AM fungus *Rhizophagus irregularis* and submitted to two water regimes (well-watered,
861 WW or drought stress, DS). A group of plants from each treatment was treated with NaN_3
862 for 30 min before harvest or kept untreated (-). Data indicate the mean ± SE for three
863 biological replicates per treatment. Different letter indicates significant differences between
864 treatments ($p<0.05$) based on Duncan's test.

865