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Differential root and cell regulation of maize aquaporins by the arbuscular mycorrhizal symbiosis highlights its role in plant water relations

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Abstract

This study aims to elucidate if the regulation of plant aquaporins by the arbuscular mycorrhizal (AM) symbiosis occurs only in roots or cells colonized by the fungus or at whole root system. Maize plants were cultivated in a split-root system, with half of the root system inoculated with the AM fungus and the other half uninoculated. Plant growth and hydraulic parameters were measured and aquaporin gene expression was determined in each root fraction and in microdissected cells. Under well-watered conditions, the non-colonized root fractions of AM plants grew more than the colonized root fraction. Total osmotic and hydrostatic root hydraulic conductivities (Lo and Lpr) were higher in AM plants than in non-mycorrhizal plants. The expression of most maize aquaporin genes analysed was different in the mycorrhizal root fraction than in the non-mycorrhizal root fraction of AM plants. At the cellular level, differential aquaporin expression in AM-colonized cells and in uncolonized cells was also observed. Results indicate the existence of both, local and systemic regulation of plant aquaporins by the AM symbiosis and suggest that such regulation is related to the availability of water taken up by fungal hyphae in each root fraction and to the plant need of water mobilization.

KEYWORDS
arbusculated cell, colonized root fraction, drought, laser microdissection, mycorrhiza, uncolonized root fraction

1 | INTRODUCTION

Aquaporins are membrane intrinsic proteins that are located in different cell membranes. They constitute a highly diverse protein family, with over 30 isoforms in most higher plants. Plant aquaporins are classified within the Major Intrinsic Proteins (MIPs) superfamily. Based on subcellular localization and on sequence homology, MIPs constitute five subfamilies, namely, plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), nodulin-26 like intrinsic proteins (NIPs), small and basic intrinsic proteins (SIPs) and X-intrinsic proteins (XIPs) (Luang & Hrmova, 2017). Aquaporins transport water, but some of them can also transport other relevant molecules for the plant, such as CO_2, metalloids, urea, ammonia, H_2O_2, oxygen or even ions (Fox et al., 2017; Singh et al., 2020;

Zwiazek et al., 2017). It is already reported that aquaporin isoforms contribute to several plant physiological functions (Afzal et al., 2016; Chaumont & Tyerman, 2014; Li et al., 2014; Ortiz-Delvasto et al., 2024). Particularly, their contribution to the whole plant water transport and in the stress responses has been well established (Chaumont & Tyerman, 2014; Domec et al., 2021; Singh et al., 2020).

The arbuscular mycorrhizal (AM) symbiosis involves a group of microscopic soil fungi and the roots of almost 80% of terrestrial plants, including many important agricultural species such as maize. After root colonization, the fungi develop specialized structures called arbuscules inside the root cells, where there is an exchange of nutrients and water between both symbionts (Ezawa & Saito, 2018; Genre et al., 2005; Püschel et al., 2020). Moreover, when the symbiosis is established, the AM fungus produces a massive extension of mycelium outside the roots that explores the surrounding soil. Such mycelium facilitates the connection between the roots and the soil moisture and assists in the uptake of water and nutrients. Thus, it plays an essential role in taking up water and nutrients during drought conditions (Allen, 2009; Kakouridis et al., 2022).

The AM symbiosis has been reported in the literature as beneficial for improving the resilience of the majority of crops to water stress (Augé, 2001; Augé et al., 2015; Bahadur et al., 2019; Cheng et al., 2021; Santander et al., 2017). Indeed, AM symbiosis is essential for plants to acquire nutrients and water from the soil and increases their resistance to environmental stresses (Das & Sarkar, 2024; Varma, 2008). During the establishment of the AM symbiosis, root cells suffer important morphological alterations at vacuolar and cytoplasmatic membrane systems to accommodate the presence of the fungal symbiont (Genre et al., 2005; Krajinski et al., 2000). This affects the expression of genes encoding membrane-associated proteins such as aquaporins. The implications that such regulation has on plant physiology, plant water relations and plant performance under optimal or stressful conditions have been the subject of intensive research in the last years (Ruiz-Lozano & Aroca, 2017; Singh et al., 2020). Indeed, the role of aquaporins for both nutrient and water exchanges during mycorrhizal symbiosis was proposed by Maurel and Plassard (2011) and subsequently supported by other studies that have shown how the AM symbiosis regulates the expression of a high number of aquaporins in colonized roots (Balestrini et al., 2019; Giovannetti et al., 2012; Hogekamp et al., 2011). Particularly, several studies have been focused on maize, showing an AM-mediated regulation of members from the different aquaporin subfamilies with a variety of substrates that can be transported across them (Bárzana et al., 2014; Quiroga et al., 2017, 2020).

Previous results have also shown that AM symbiosis has the capacity to alter root hydraulic conductivity (Lpr), enhancing it mostly under stress conditions (Aroca et al., 2007; Bárzana et al., 2012, 2014; Quiroga et al., 2017, 2018, 2020; Quiroga, Erice, Aroca, et al., 2019; Quiroga, Erice, Ding, et al., 2019; Sánchez-Romera et al., 2016). The involvement of plant aquaporins in these processes has been proved by a number of studies (reviewed by Ruiz-Lozano & Aroca, 2017), and recently, it has been shown that the presence of the AM fungus in the root increases the water permeability of root cells. This phenomenon has been correlated with the induction of some aquaporin genes and the increase of the phosphorylation status of PIP2s, which implies a higher activity of their water channels (Quiroga, Erice, Ding, et al., 2019). Moreover, the presence of a mycorrhizal fungus significantly modified the radial transport of water within the root system (Quiroga, Erice, Aroca, et al., 2019), and it has been demonstrated an enhancement of the membrane water permeability of both intact root cortex cells and protoplasts from AM plants as compared to those of non-AM plants (Quiroga, Erice, Ding, et al., 2019). Droughted AM maize plants maintained membrane water permeability (Pf) levels observed in non-stressed plants, while these levels declined drastically in the absence of the AM fungus. Interestingly, Pf values higher than 12 $\mu m\, s^{-1}$ were found only in protoplasts extracted from AM plants, revealing the higher water permeability of AM root cells as compared to non-AM ones (Quiroga, Erice, Ding, et al., 2019). Under these conditions, the AM symbiosis differentially regulated plant aquaporins, inducing *ZmPIP2;2*, *ZmPIP2;6* and the fungal aquaporin *GintAQPF2* genes.

In any case, it must be taken into account that in the above-mentioned study on cell hydraulic conductivity (Lpc) and Pf values were measured on isolated maize cortical cells. Some of these cells may be colonized by the AM fungus, and others may not be colonized. Since it is not already known if the mycorrhizal effect on cell water transport is local or systemic, it can be hypothesized that if the effect of the AM symbiosis is not systemic, it may be diluted in the whole root system and this aspect needs to be the object of a study specifically devoted to elucidate if the regulation of plant aquaporins occurs only in the cells colonized by the AM fungus or if it is extended to the whole root system.

The present study is based on previous studies on AM regulation of host plant aquaporins. All these studies were conducted on maize since it is one of the most important cereals both for animal and human consumption. Worldwide, maize is cultivated on more than 142 million ha, and it is estimated to account for one-third of the total global grain production (Daryanto et al., 2016). Thus, the impact of drought on the productivity of this cereal will become of primary importance due to its role as a source of staple food around the world (Lobell et al., 2008), and the search for sustainable methods to maintain their productivity is an important challenge in modern agriculture (Shi et al., 2017). The aquaporins selected for this study were those previously shown to have a key role in the AM-mediated response of maize to drought stress (Quiroga et al., 2017). Indeed, Bárzana et al. (2014) showed that from the 36 aquaporin genes present in maize, up to 16 genes were regulated by the AM symbiosis, depending on the degree of drought stress imposed (Bárzana et al., 2014). Subsequently, Quiroga et al. (2017) compared the regulation of these 16 aquaporin genes in a drought-resistant and drought-sensitive maize variety and identified the aquaporin genes that were key in the responses of AM plants to drought stress. In addition, the three AM fungal aquaporin genes described so far in *Rhizophagus intraradices* (Aroca et al., 2009; Li et al., 2013) were also included in this study.

The aim of the present study was to determine, both at tissue and cell levels, if the regulation of plant aquaporins by the AM symbiosis occurs only in cells colonized by the AM fungus or if it extends systemically to the whole root system. For that, maize plants were cultivated in a split-root system, as described by Bárzana et al. (2015). By this method, the root system is divided into two halves so that one-half is inoculated with the AM fungus and the other half remains uninoculated. This system allows obtaining mycorrhizal plants with a mycorrhizal root fraction and a non-mycorrhizal root fraction (Bárzana et al., 2015; Khaosaad et al., 2010). In this way, we can compare Lo and Lpr values and aquaporin gene expression patterns at the tissue level in the mycorrhizal and the non-mycorrhizal root fractions of an AM plant. This will provide the first evidence of whether the effect of the AM symbiosis on root hydraulic properties and aquaporin expression is localized only in the root fraction colonized by the AM fungus or is systemic, appearing in the non-mycorrhizal root fraction. Furthermore, the laser microdissection technique (Balestrini et al., 2007) was used to isolate maize cortical cells colonized by the AM fungus (arbusculated cells) from the mycorrhizal root fraction, as well as, maize uncolonized cortical cells from the non-mycorrhizal root fraction, for subsequent analysis of aquaporins gene expression at the cell level.

We hypothesized that the presence of the AM fungus in the root system could regulate plant aquaporins locally by direct effects of the AM fungus but also systemically due to indirect effects such as water uptake by the fungal hyphae in soil pores inaccessible to roots, hormonal changes in the root system or changes in the soil water availability.

2 | MATERIALS AND METHODS

2.1 | Experimental design and statistical analysis

The experiment consisted of a factorial design with two factors: (1) inoculation treatment, including plants inoculated with the AM fungus *R. intraradices*, (Ri) and non-inoculated control plants (C); (2) water regime so that one-half of the plants were cultivated under well-watered conditions (WW) throughout the entire experiment and the other half of the plants were subjected to water deficit (WD) for 2 weeks just before harvest. Each treatment had 20 replicates, giving a total of 80 pots. The experiment was repeated twice.

In this study, maize plants were cultivated into a split-root system (Figure 1) in containers prepared ad hoc for this split-root assay. These containers were constructed from two 1 L plastic pots fastened together, side by side, with adhesive tape, as described by Neumann et al. (2009) and Bárzana et al. (2015). Thus, each maize plant had its root system divided into two portions, the left one and the right one (see Figure 1). In the AM treatments, the AM inoculum was applied only to a single root compartment (always to the left root compartment, see Figure 1). Thus, we got control non-AM plants where none of the root portions was colonized by the AM fungus and AM plants with only a root portion colonized (the left portion), while the right root portion remained uncolonized, although it took part of an AM plant.

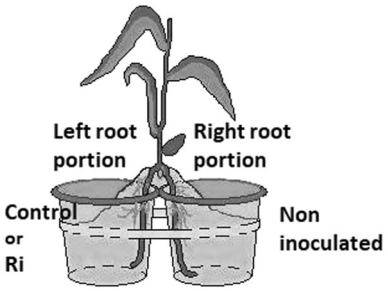

FIGURE 1 Split root system used to cultivate maize inoculated or not (Control) with the AM fungus *Rhizophagus intraradices* (Ri). Plants were cultivated with the root system divided into two fractions (left and right). For control plants, both root fractions were uninoculated. For AM plants, the AM inoculum was applied only to the left root fraction, while the right fraction remained always uncolonized by the AM fungus, although belonging to a mycorrhizal root system. AM, arbuscular mycorrhizal.

Statistical analyses were performed with SPSS Statistics (Version 27; IBM Analytics) using two-way analysis of variance, with AM inoculation (M), water regime (W) and their interaction (M X W) as sources of variation. Duncan's was used as a post hoc test to find out differences between means at $\alpha = 0.05$.

2.2 | Biological material and growth conditions

The growing substrate consisted of a mixture of soil and sand (v/v 1:1). Soil was collected at the grounds of IFAPA, sieved (2 mm), diluted with quartz-sand (<1 mm) and sterilized by steaming (100°C for 1 h on 3 consecutive days). The soil had a pH of 8.1 (water); 0.85% organic matter, nutrient concentrations (mg kg^{-1}): N, 1; P, 10 ($NaHCO_3$-extractable P); K, 110. The soil texture was made of 38.3% sand, 47.1% silt and 14.6% clay.

Seeds of *Zea mays* L. (cv PR34B39; Pioneer Hi-Bred) were pre-germinated on sand for 10 days and then transferred to the containers prepared ad hoc for this split-root assay. Each root compartment was filled with 1300 g of the soil/sand mixture described above and harboured half root system from maize seedlings.

Mycorrhizal inoculum consisted of soil, spores and mycelia. It was a commercial inoculum provided by MycAgro Lab. The AM fungus was *R. intraradices* (Schenck and Smith). Twenty grams of inoculum were added to appropriate compartments at sowing time. Non-inoculated control plants received the same amount of autoclaved mycorrhizal inoculum together with a 10 mL aliquot of a filtrate (<20 µm) of the AM inoculum to provide a general microbial population free of AM propagules.

Maize plants were grown under greenhouse conditions (25/20°C, 16/8 light-dark period, 50%–60% RH and average photosynthetic photon flux density 800 µmol m^{-2} s^{-1}) for a total of 8 weeks. Four weeks after sowing, all plants started receiving 10 mL per pot and per week of Hoagland nutrient solution (Hoagland & Arnon, 1950) containing only 25% of P to provide basic nutrients, but avoiding inhibition of AM symbiosis due to a high P application. Soil

moisture was measured with the ML2 ThetaProbe (AT Delta-T Devices Ltd.) as described previously (Quiroga et al., 2017). Water was supplied daily to maintain soil in both root fractions at 100% of field capacity during the first 6 weeks after sowing. Then, half of the plants were allowed to dry (DS treatments) until soil water content reached 60% of field capacity (2 days needed), while the other half of plants were maintained at field capacity (WW treatments). This level of drought stress has been applied in our previous studies with maize plants and reduces significantly plant physiological performance (Quiroga et al., 2017; Quiroga, Erice, Ding, et al., 2019). Plants were maintained under such conditions for 14 additional days. The same watering treatment was applied to both root compartments so that plants were either WW or subjected to physiological drought stress in both root compartments.

2.3 | Parameters measured

2.3.1 | Biomass production and symbiotic development

At harvest (8 weeks after sowing), the shoot and root system were separated and weighed to determine fresh weights. For each treatment, eight replicates were used to determine shoot and root dry weight (SDW and RDW) after drying in a forced hot-air oven at 70°C for 2 days.

To visualize and quantify AM fungal structures, roots (aliquots of both root compartments) were stained with trypan blue according to Phillips and Hayman (1970). The percentage of mycorrhizal colonization was calculated by the gridline intersect method according to Giovannetti and Mosse (1980) in six replicates per treatment.

2.3.2 | Stomatal conductance

Stomatal conductance was measured 2 h after the onset of the photoperiod with a porometer system (Leaf porometer; model SC-1; Decagon Devices) following the user manual instructions. Measurements were taken 1 day before harvest in the second youngest leaf from 10 plants per treatment.

2.3.3 | Efficiency of photosystem II

The efficiency of photosystem II was measured with a FluorPen FP100 (Photon Systems Instruments), which allows a noninvasive assessment of plant photosynthetic performance by measuring chlorophyll fluorescence. FluorPen quantifies the quantum yield of photosystem II as the ratio between the actual fluorescence yield in the light-adapted state (FV′) and the maximum fluorescence yield in the light-adapted state (FM′), according to Oxborough and Baker (1997). Measurements were taken in the second youngest leaf of 10 different plants of each treatment.

2.3.4 | Osmotic root hydraulic conductance (L) and conductivity (Lo)

At harvest, the osmotic root hydraulic conductance (L) and conductivity (Lo) were measured on detached roots exuding under atmospheric pressure for 2 h (Aroca et al., 2007). Under these conditions, water is only moving following an osmotic gradient. Therefore, the water would be moving through the cell-to-cell path (Steudle & Peterson, 1998). L was calculated as $L = Jv/\Delta\Psi$, where Jv is the exuded sap flow rate and $\Delta\Psi$ is the osmotic potential difference between the exuded sap and the nutrient solution where the pots were immersed. Data were expressed as mg H_2O MPa^{-1} h^{-1}. These measurements were carried out 3 h after the onset of light. The osmotic root hydraulic conductivity (Lo) was calculated from L values by dividing L by the RDW and expressed as mg H_2O g RDW^{-1} MPa^{-1} h^{-1}.

These parameters were measured in both root compartments on five replicates per treatment and per root compartment. Thus, to measure in the left root compartment, the pot containing the right root fraction was covered with a double plastic bag to avoid the hydration of such a compartment. To measure these parameters in the right root compartment the pot containing the left root fraction was covered with a double plastic bag to avoid hydration of such compartment. Total L and Lo for each treatment was also calculated as the sum of L or Lo values in each root fraction.

2.3.5 | Hydrostatic root hydraulic conductivity (Lpr)

Lpr was determined at noon with a Scholander pressure chamber as described by Bárzana et al. (2012). A gradual increase of pressure (0.3, 0.4 and 0.5 MPa) was applied at 2 min 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 RDW and expressed as mg H_2O g RDW^{-1} MPa^{-1} h^{-1}.

Lpr was measured in both root fractions on five replicates per treatment and per root compartment. Thus, to measure in the left root fraction, the root fraction contained in the right compartment was detached from the system. To measure Lpr in the right root fraction, the root fraction contained in the left compartment was detached from the system. The total Lpr for each treatment was also calculated as the sum of Lpr values in each root portion.

2.3.6 | Root hormonal content

Abscisic acid (ABA), indole-acetic acid (IAA), jasmonic acid (JA) and jasmonate isoleucine (JA-Ile) were analysed according to Albacete et al. (2008) with some modifications. Briefly, the hormone extracts were filtered through 13 mm diameter Millex filters with 0.22 µm pore size nylon membrane (Millipore). Ten microlitres of filtrated extract were injected in a U-HPLC-MS system consisting of an Accela

Series U-HPLC (ThermoFisher Scientific) coupled to an Exactive mass spectrometer (ThermoFisher Scientific) using a heated electrospray ionization (HESI) interface. Mass spectra were obtained using Xcalibur software version 2.2 (ThermoFisher Scientific). For quantification of the plant hormones, calibration curves were constructed for each analysed component (1, 10, 50 and 100 $\mu g\ L^{-1}$). The total hormonal content for each treatment was also calculated as the sum of hormonal values obtained in each root fraction.

2.3.7 | Quantitative real-time RT-PCR at root tissue level

Three biological replicates of maize roots were used to extract total RNA, as described in Quiroga et al. (2017). First-strand cDNA was synthesized using 1 µg of purified RNA with the Maxima H Minus first-strand cDNA synthesis kit (Thermo Scientific TM), following the manufacturers' instructions.

The expression of seven previously selected maize aquaporins (*ZmPIP1;1*, *ZmPIP1;3*, *ZmPIP2;2*, *ZmPIP2;4*, *ZmTIP1;1*, *ZmTIP2;3* and *ZmTIP4;1* (Quiroga et al., 2017) was measured by qRT-PCR using 1 µL of diluted cDNA (1:9) with PowerUpTM SYBRTM Green Master Mix in a QuantStudioTM 3 system (Thermo Fisher Scientific). The reaction was repeated for 40 cycles at an annealing temperature of 58°C for all primers. Four reference genes were measured in all the treatments for the normalization of gene expression values. These genes were poliubiquitin (gi:248338), tubulin (gi:450292), GAPDH (gi:22237) and elongation factor 1 (gi:2282583) (Bárzana et al., 2014). Standardization was carried out based on the expression of the best-performing reference gene under our specific conditions, which were chosen by using the 'NormFinder' algorithm (Andersen et al., 2004) (https://moma.dk/normfinder-software). Thus, expression levels were normalized according to the *ZmGAPDH* gene. Fungal aquaporins (*GintAQP1*, *GintAQPF1* and *GintAQPF2*) were analysed as previously described (Aroca et al., 2009; Li et al., 2013) using the fungal elongation factor 1α (Accession No. DQ282611) as a reference gene for standardization. The relative abundance of transcripts was calculated using the $2^{-\Delta\Delta C_t}$ method (Livak & Schmittgen, 2001). The threshold cycle (C_t) of each biological sample was determined in duplicate. Negative controls without cDNA were used in all PCR reactions.

2.3.8 | Laser microdissection and quantitative real-time RT-PCR at cell level

Tissue preparation for laser microdissection

Maize roots were cut into sections of about 5 mm using a razor blade and fixed in freshly prepared Farmer's fixative (absolute ethanol/glacial acetic acid 3:1) overnight at 4°C for paraffin embedding. Samples were then dehydrated in a series of ethanol concentrations (70%, 90% in sterile water and 100% twice on ice), followed by two steps in Neoclear (Merck), with each incubation on ice for 30 min. Neoclear was then progressively replaced with paraffin (Paraplast Plus), and the samples were embedded in paraffin within Petri dishes, as described by Fochi et al. (2017). Twelve micrometres thick sections were cut with a rotary microtome, placed and stretched out on Leica RNase-free PEN slides (Leica Microsystems) with ddH_2O (sterilized and filtered with a 0.2 µm filter). Paraffin sections were air-dried on a warming plate set at 40°C, stored at 4°C, and utilized within 48 h.

Laser microdissection

A Leica LMD6 Laser microdissection system (Leica Microsystems Inc.) was employed to separate the diverse cell types from the tissue sections. Immediately before to use, the slides containing the sections were subjected to deparaffinization with Neoclear for 8–10 min, followed by a 1 min wash in 100% ethanol, and subsequently air-dried.

The deparaffinised slides were placed face-down on the microscope, and two different cell types were selected from maize roots, microdissected and collected separately: (i) cells containing visible fungal arbuscules (A), mainly selecting those in which the arbuscule occupied the whole cell and (ii) non-colonized cells (NM). Approximately 1500 cells for each cell-type population were collected for each replicate, and the pools were brought to a final volume of 50 µL with Pico Pure extraction buffer and processed for RNA extraction following manufacturer's instructions (Life Technologies). At least three independent biological replicates of each cell type were collected for downstream gene expression analyses.

Quantitative real-time RT-PCR at cell-type level

To extract RNA from collected cells, the Pico Pure kit (Life Technologies) was used. The RNA was eluted in 25 µL of elution buffer and treated with RNase-free DNAse (TURBO™ DNase; Ambion), following the manufacturer's instructions. RNA quantification was determined using a NanoDrop One (ThermoFisher) spectrophotometer.

A One-Step RT-PCR protocol (Balestrini et al., 2007; Fochi et al., 2017) was applied on RNA extracted from the different LMD samples for checking DNA contamination, using specific primers for plant and fungal elongation factor genes. For gene expression quantification, One-Step RT-qPCR was performed using the Connect™ Real-Time PCR Detection System (Bio-Rad Laboratories) apparatus. In detail, the reactions were carried out in a final volume of 20 µL with 10 µL of QuantiFast SYBR Green RT-PCR Master Mix, 0.2 µL of QuantiFast RT Mix, 1 µL of a mix of forward and reverse primers 0.5 µM each, 6.8 µL of RNA free water and 2 µL of RNA diluted (1:3). The RT-qPCR cycling programme consisted of a 10 min/50°C RT step, 2 min/95°C holding step followed by 40 cycles of two steps (5 s/95°C and 10 s/60°C). In this experiment, the same specific primers for the different considered aquaporin genes described above for root tissues were used.

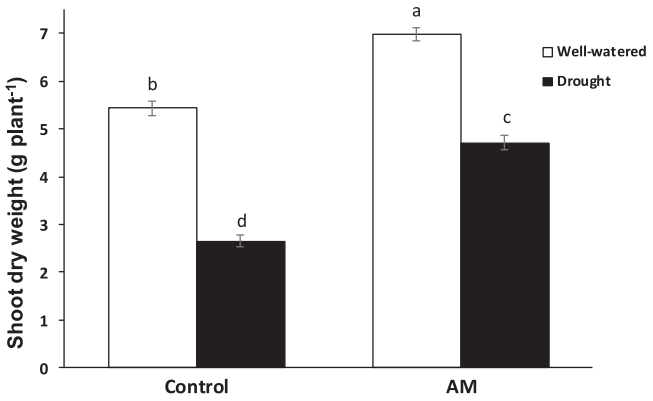

FIGURE 2 Shoot dry weight of maize plants inoculated or not (Control) with the AM fungus *Rhizophagus intraradices* (AM). Plants were cultivated either under well-watered conditions (white columns) or subjected to drought stress for 15 days before harvest (black columns). Data represents the means of 20 values ± SE. The different letter indicates significant differences between treatments ($p < 0.05$) based on Duncan's test. AM, arbuscular mycorrhizal.

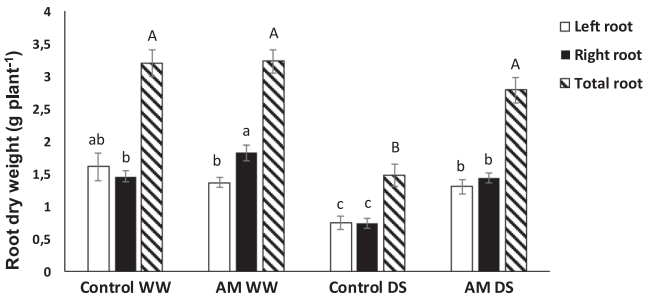

FIGURE 3 Root dry weight in the different root fractions or in the whole root of maize plants inoculated or not (Control) with the AM fungus *Rhizophagus intraradices* (AM). Plants were cultivated with the root system divided into two fractions (left and right). For AM plants, AM inoculum was applied only to the left root fraction, while the right fraction remained always uncolonized by the AM fungus, although belonging to a mycorrhizal root system. See Figure 1 for understanding AM presence or absence in the left and the right root fractions. Plants were cultivated either under well-watered conditions (WW) or subjected to drought stress for 15 days before harvest (DS). Data represents the means of 20 values ± SE. The different letter indicates significant differences between treatments ($p < 0.05$) based on Duncan's test. AM, arbuscular mycorrhizal.

3 | RESULTS

3.1 | Plant biomass production and symbiotic development

The SDW (Figure 2) was enhanced by the AM symbiosis both under WW conditions (by 30%) and even more under drought stress conditions (by 78%). Drought stress affected negatively the SDW, reducing it by 51% in non-AM plants and by 32% in AM plants. The total RDW (Figure 3) was similar in AM and non-AM plants under WW conditions. Under drought stress conditions, AM plants maintained a total RDW similar to the values under WW conditions, while non-AM plants considerably decreased their total RDW (by 54% as compared to the WW plants). Regarding the root development in each root fraction, it was similar in both fractions for all treatments except in AM plants under WW conditions, which had a higher root development (an increase of about 30%) in the non-mycorrhizal root fraction (the right one) than in the mycorrhizal root fraction (the left one).

The percentage of root colonization by *R. intraradices* in the left root fraction of AM plants was over 80% in both, WW and drought stressed plants (data not shown). No AM fungal colonization was observed in control uninoculated plants or in the right root fraction of AM plants.

3.2 | Stomatal conductance and efficiency of photosystem II

The stomatal conductance was clearly affected by the drought stress imposed and decreased by 76% in control uninoculated plants and by 44% in AM plants (Figure 4a). The stomatal conductance was lower in AM plants than in non-AM ones under WW conditions. However, under drought stress, AM plants maintained a higher stomatal conductance than non-AM plants.

The efficiency of photosystem II (Figure 4b) was decreased by drought stress in non-AM plants only (decrease by 18%). No differences were found in AM plants as a consequence of drought stress.

3.3 | Osmotic root hydraulic conductance (L) and root hydraulic conductivity (Lo)

Total L values (Figure 5a) were enhanced by the AM symbiosis both under WW conditions (64% of the increase) and under drought stress conditions (60% of the increase). In any case, drought stress decreased L values as compared to WW conditions by 80% in non-AM plants and by 54% in AM plants. Regarding the L values in each root fraction, no significant differences were found between the left and the right root fraction.

Total Lo values (Figure 5b) were lower under drought stress conditions than under WW conditions. Moreover, total Lo values were similar in AM and non-AM plants both under WW conditions and under drought stress, although a slight increase was observed in AM plants, but such increase was not statistically significant. Regarding Lo values in each root fractions, also no significant differences were found between the left and the right root fraction. The only exception was found in AM plants cultivated under WW conditions, where the Lo values were higher in the left root fraction (inoculated root portion) than in the right root fraction (uninoculated root portion). This effect was due to higher root development in the right root fraction since, as can be seen in

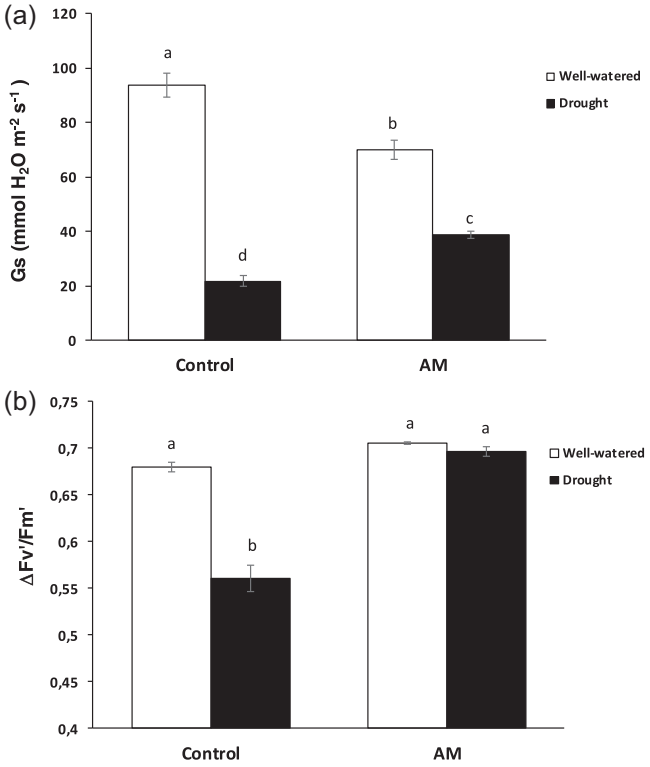

FIGURE 4 Stomatal conductance (a) and efficiency of photosystem II (b) of maize plants inoculated or not (Control) with the AM fungus *Rhizophagus intraradices* (AM). Plants were cultivated either under well-watered conditions (white columns) or subjected to drought stress for 15 days before harvest (black columns). Data represents the means of 20 values ± SE. Different letter indicates significant differences between treatments ($p < 0.05$) based on Duncan's test. AM, arbuscular mycorrhizal.

L values, when the RDW is omitted from the calculation, no significant differences are observed between the two root fractions (see L values, Figure 5a).

3.4 | Hydrostatic root hydraulic conductivity (Lpr)

The total Lpr values were higher in the AM plants than in the non-AM plants (Figure 5c), both under WW conditions (130% of increase) and under drought stress conditions (45% of the increase). Regarding the Lpr values in each root fraction, these values were similar in the left root fraction and in the right root fraction for each treatment. However, under WW conditions AM plants also exhibited higher Lpr values in each root fraction as compared to the non-AM plants. Under drought stress conditions, no differences were observed.

3.5 | Root hormonal content

The total root ABA content (Figure 6a) was considerably enhanced by the drought stress imposed, both in AM plants and in non-AM plants. In any case, the values of ABA content were similar for AM and non-AM plants. The only significant result was obtained in relation to the ABA content in the non-AM root fraction (right root) of AM plants subjected to drought stress, which was significantly higher (by 45%) than in the AM root fraction (left root).

Under WW conditions, the total root IAA content decreased by 29% in AM plants as compared to non-AM ones (Figure 6b). In contrast, under drought stress conditions, no significant differences were found. For the different treatments, the IAA content was similar in the two root fractions, regardless of the watering conditions.

The total root JA content decreased by 30% in non-AM plants as a consequence of drought stress, while no decrease was observed in AM plants (Figure 6c). For the different treatments, the JA content was similar in the two root fractions, regardless of the watering conditions.

The total root JA-Ile content was enhanced by AM symbiosis, especially under drought stress conditions where the increase was by 93% (Figure 6d). Under WW conditions the JA-Ile content in the two root fractions was similar for all treatments. In contrast, under drought stress conditions, the levels of JA-Ile were significantly higher in both AM root fractions than in both non-AM root fractions.

3.6 | Expression of maize and AM fungal aquaporins at root tissue level

In control uninoculated plants, the expression of most aquaporins was not significantly affected by the drought stress imposed, except

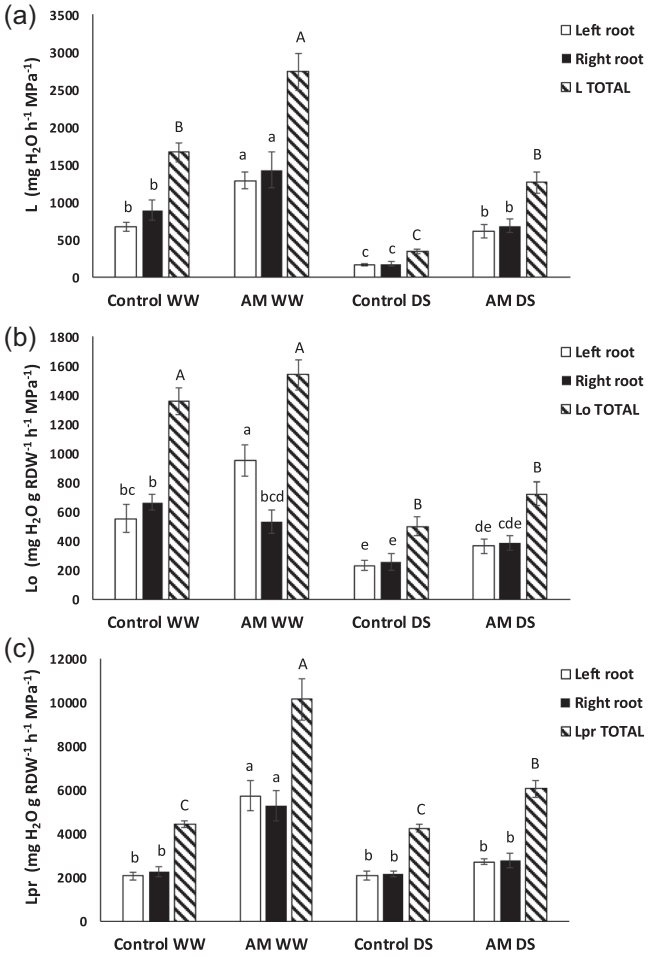

FIGURE 5 (a) Osmotic root hydraulic conductance (L), (b) osmotic root hydraulic conductivity (Lo) and (c) hydrostatic root hydraulic conductivity (Lpr) in the different root fractions or in the whole root of maize plants inoculated or not (Control) with the AM fungus *Rhizophagus intraradices* (AM). Data represents the means of 10 values ± SE. See the legend for Figures 3 and 1 for understanding AM presence or absence in the left and the right root fractions. AM, arbuscular mycorrhizal.

in the case of *ZmTIP1;1* and *ZmTIP2;3* that increased their expression under drought stress (Figure 7e,f, entire arrows). Regarding AM plants, we first compared the gene expression in the two root fractions (the left one containing the AM fungus and the right one uninoculated with the fungus). The expression of four out of the seven maize genes showed a similar pattern. Thus, under WW conditions, *ZmPIP1;1*, *ZmPIP1;3*, *ZmPIP2;4* and *ZmTIP2;3* decreased their expression in the uninoculated right root fraction as compared to the AM-inoculated left root fraction (see dashed arrows). In contrast, under drought stress conditions, these genes increased their expression in the uninoculated right root fraction as compared to the AM-inoculated left root fraction (Figure 7a,b,d,f). *ZmPIP2;2* decreased its expression in the uninoculated right root fraction as compared to the AM-inoculated left root fraction both, under WW and under drought stress conditions (Figure 7c). In contrast, *ZmTIP4;1* increased its expression in the uninoculated right root fraction as compared to the AM-inoculated left root fraction both, under WW and under drought stress conditions (Figure 7g). When analysing the effect of the drought stress on the expression of each aquaporin gene in the root fractions (see entire arrows), we observed that *ZmPIP1;1*, *ZmPIP2;4* and *ZmTIP2;3* decreased their expression as a consequence of drought in the inoculated left root fraction, but increased their expression in the uninoculated right root fraction (Figure 7a,d,f). *ZmPIP1;3* increased its expression by drought stress in the uninoculated right root fraction but it did not change in the inoculated left root fraction (Figure 7b). On the contrary, *ZmTIP1;1* decreased its expression by drought in the inoculated left root fraction, but it did not change in the uninoculated right root fraction (Figure 7e). Finally, as expected, the AM fungal aquaporin *GintAQPF2* was only detected in the inoculated AM left fraction (Figure 7h) and decreased its expression by drought stress. The other two fungal aquaporins were not detected under our experimental conditions.

3.7 | Expression of maize and AM fungal aquaporins at root cell level

In control uninoculated plants, the expression of *ZmPIP1;1* and *ZmTIP1;1* was up-regulated by drought stress (Figure 8a,e, entire arrows). The rest of the aquaporin genes analysed did not show

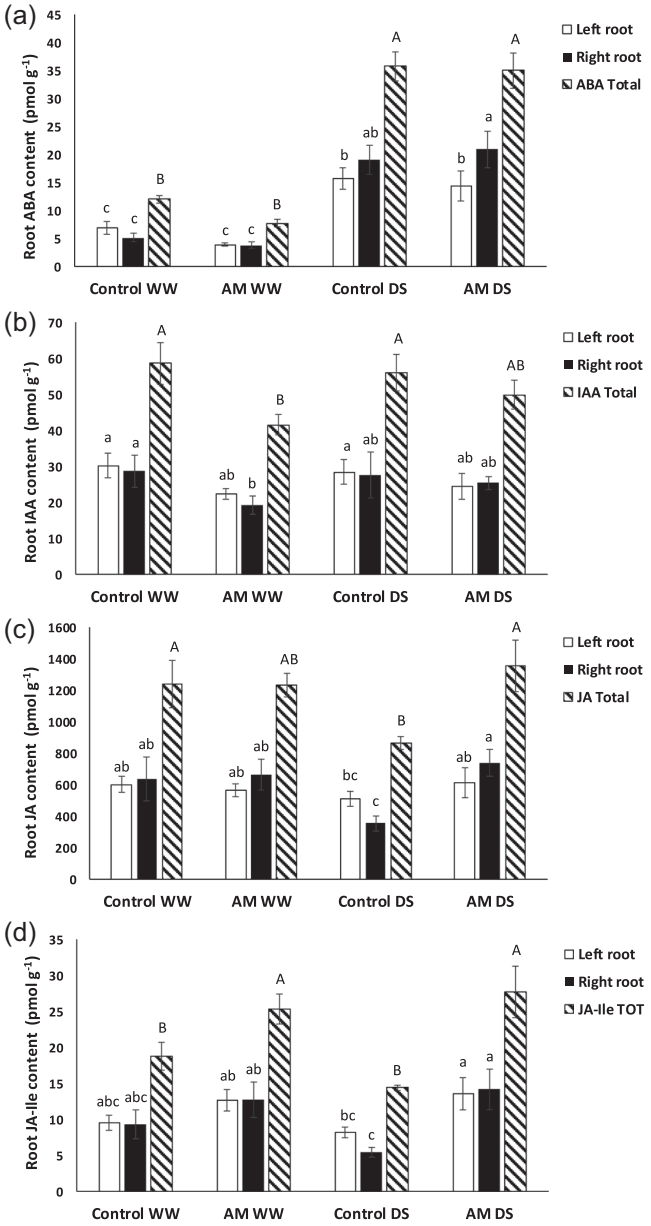

FIGURE 6 (a) ABA, (b) IAA, (c) JA and (d) JA-Ile contents in the different root fractions or in the whole root of maize plants inoculated or not (Control) with the AM fungus *Rhizophagus intraradices* (AM). Data represents the means of 10 values ± SE. See legend for Figures 3 and 1 for understanding AM presence or absence in the left and the right root fractions. ABA, abscisic acid; AM, arbuscular mycorrhizal; IAA, indole-acetic acid; JA, jasmonic acid; JA-Ile, jasmonate isoleucine.

significant changes in these plants as a consequence of the water treatment. In AM plants, we measured the gene expression in cells colonized by the AM fungus (cells containing arbuscules), collected in the inoculated left root fraction and in cells without the AM fungus collected in the uninoculated right root fraction (see Figure 1). In these AM plants, the expression of most of the aquaporin analysed showed a significant up-regulation in the cells uncolonized by the AM fungus when plants were cultivated under WW conditions (Figure 8a,b,d–f, see dashed arrows). In contrast, under drought stress, this up-regulation was only found for *ZmPIP1;1* (Figure 8a), but not for the rest of aquaporin genes. *ZmTIP4;1* showed up-regulation of gene expression in uncolonized cells when cultivated under WW conditions only (Figure 8g). When analysing the effect of the drought stress on the expression of each aquaporin gene in each type of cell (arbusculated or uncolonized), we observed that drought stress did not affect aquaporin gene expression in the arbusculated cells (Figure 8a,b,d–g). In contrast, in the uncolonized cells of AM plants, the expression of *ZmPIP1;3*, *ZmPIP2;4*, *ZmTIP1;1*, *ZmTIP2;3* and *ZmTIP4;1* was considerably down-regulated by drought stress (see entire arrows, Figure 8b,d–g). Finally, the AM fungal aquaporin *GintAQPF2* was only detected in the inoculated AM fraction (Figure 8h) and enhanced its expression by drought stress. The other

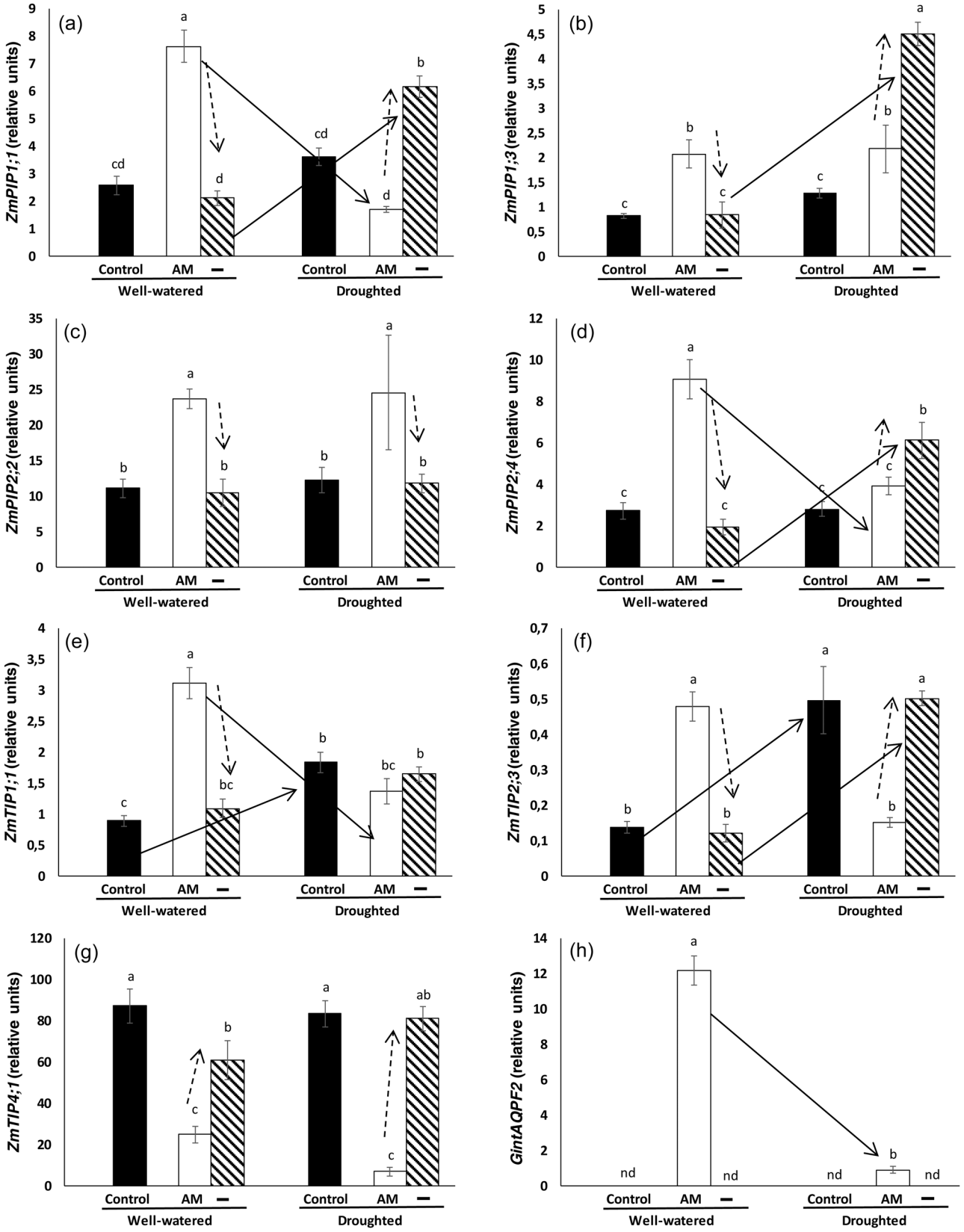

FIGURE 7 (See caption on next page).

two fungal aquaporins and the maize *ZmPIP2;2* genes (Figure 8c) were not detected under our experimental conditions.

4 | DISCUSSION

Worldwide, maize is one of the major cereal crops, providing up to 30% of food calories to 4.5 billion people in the world, with annual production of over one trillion tons (FAOSTAT, 2019) and it is expected to double its demand by 2050 (Lobell et al., 2008). However, drought stress has a negative impact on plant growth and development, considerably reducing the productivity of crops such as maize (Daryanto et al., 2016; Gupta et al., 2020; Lesk et al., 2016). Thus, to guarantee food production in the near future for an ever-increasing world population, it is necessary to find sustainable approaches to keep maize crop productivity under stressful conditions (Gupta et al., 2020; Trenberth et al., 2014).

The AM symbiosis has been widely reported as a suitable tool for improving the resilience of the majority of crops to drought stress (Augé, 2001; Augé et al., 2015; Cheng et al., 2021). The symbiosis produces changes in the plant and its own soil properties (Augé, 2004; Bedini et al., 2009), resulting in better plant performance under adverse environmental conditions (Cheng et al., 2021; Das & Sarkar, 2024; Ruiz-Lozano et al., 2012). A number of studies have shown in the last years that the AM symbiosis regulates root hydraulic conductivity in different plant species, including maize (Aroca et al., 2008; Bárzana et al., 2012, 2014; Quiroga et al., 2017, 2018; Quiroga, Erice, Aroca, et al., 2019; Quiroga, Erice, Ding, et al., 2019; Ruiz-Lozano et al., 2009; Sánchez-Blanco et al., 2004). It has been also demonstrated that the AM symbiosis alters several aquaporin isoforms in the host plant (Bárzana et al., 2014; Giovannetti et al., 2012; Hogekamp et al., 2011; Quiroga et al., 2017; Quiroga, Erice, Aroca, et al., 2019; Quiroga, Erice, Ding, et al., 2019; Ruiz-Lozano et al., 2022). The osmotic root hydraulic conductivity (Lo) is highly related to the activity or density of aquaporins in the plasma membrane. It is, thus, considered as an estimation of water flow via the cell-to-cell pathway (Chaumont & Tyerman, 2014; Fox et al., 2017). However, the intimate mechanisms of such an effect are not well understood yet.

The main objective of this study was to determine, at both tissue and cell levels, if the regulation of maize aquaporins by the AM symbiosis occurs only in cells colonized by the AM fungus or if it extends systemically to the whole root system. For that, plants were cultivated in a split root system so that we can have a mycorrhizal root fraction (the left one) where the AM fungus is present and an uninoculated root fraction (the right one) belonging to the same root system but without the fungus (Bárzana et al., 2015). This growing system did not affect plant growth, and the beneficial effects of AM symbiosis on this parameter were clearly visible here. Indeed, under drought stress, AM plants maintained higher stomatal conductance and efficiency of photosystem II, which sustained CO_2 fixation and biomass production in these plants. Thus, plant growth was improved by AM fungal inoculation, especially under drought stress conditions, where AM symbiosis alleviated the detrimental effects of the drought stress imposed. This has been widely observed in previous studies (Cheng et al., 2021; Das & Sarkar, 2024; Santander et al., 2017) and confirmed here.

Along the soil–plant–atmosphere continuum, the highest resistance to water movement is found in roots (Steudle et al., 1987). Hence, to keep the stomata open and sustain plant growth, the root hydraulic conductivity must be maintained high enough, even under drought-stress conditions (Sack & Holbrook, 2006). Once water is transferred to the host root, it may follow an apoplastic path or penetrate inside cells via the symplastic pathway (Kakouridis et al., 2022). The apoplastic pathway is favoured under WW conditions and is faster because the water travels extracellularly through the cell wall and matrix and moves directly and continuously via the transpiration stream, facing little resistance. In contrast, the symplastic pathway is favoured under limiting water availability and is slower because water has to cross plasma membranes or plasmodesmata, following an osmotic gradient (Kakouridis et al., 2022; Steudle & Peterson, 1998). Thus, the osmotic root hydraulic conductance (L) and the osmotic root hydraulic conductivity (Lo) presented in Figure 5 estimate water movement along the symplastic pathway. Our data confirm that both L and Lo were enhanced by the AM symbiosis, especially under drought stress conditions, as previously observed (Aroca et al., 2007; Bárzana et al., 2012, 2014, 2015; Quiroga, Erice, Aroca, et al., 2019; Quiroga, Erice, Ding, et al., 2019; Sánchez-Romera et al., 2016). The water movement through cell membranes (symplastic pathway) is thought to be regulated by aquaporins (Maurel et al., 2015). These proteins allow a quick modification of membrane water permeability, helping the plant to maintain the water balance during drought episodes and affecting root hydraulic conductivity (Chaumont & Tyerman, 2014; Fox et al., 2017; Maurel et al., 2008, 2015). In higher plants, PIPs and TIPs have been highlighted for their involvement in the control of

FIGURE 7 Aquaporin gene expression in roots of uninoculated maize plants (Control, black bars) or in the two root fractions of maize plants inoculated with the AM fungus *Rhizophagus intraradices* (AM). Plants were cultivated with the root system divided into two fractions (left and right). For AM plants, the mycorrhizal inoculum was applied only to the left root fraction (AM, white bars), while the right fraction remained always uncolonized by the AM fungus (dashed bars), although belonging to a mycorrhizal root. See Figure 1 for understanding AM presence or absence in the left and the right root fractions. Plants were cultivated either under well-watered conditions or subjected to drought stress for 15 days before harvest. Data represents the means of 3 values ± SE. The different letter indicates significant differences between treatments ($p < 0.05$) based on Duncan's test. Dashed arrows indicate the increases or decreases of gene expression when comparing between both root fractions. Solid arrows indicate the increases or decreases of gene expression when comparing well-watered plants with those subjected to drought stress. AM, arbuscular mycorrhizal.

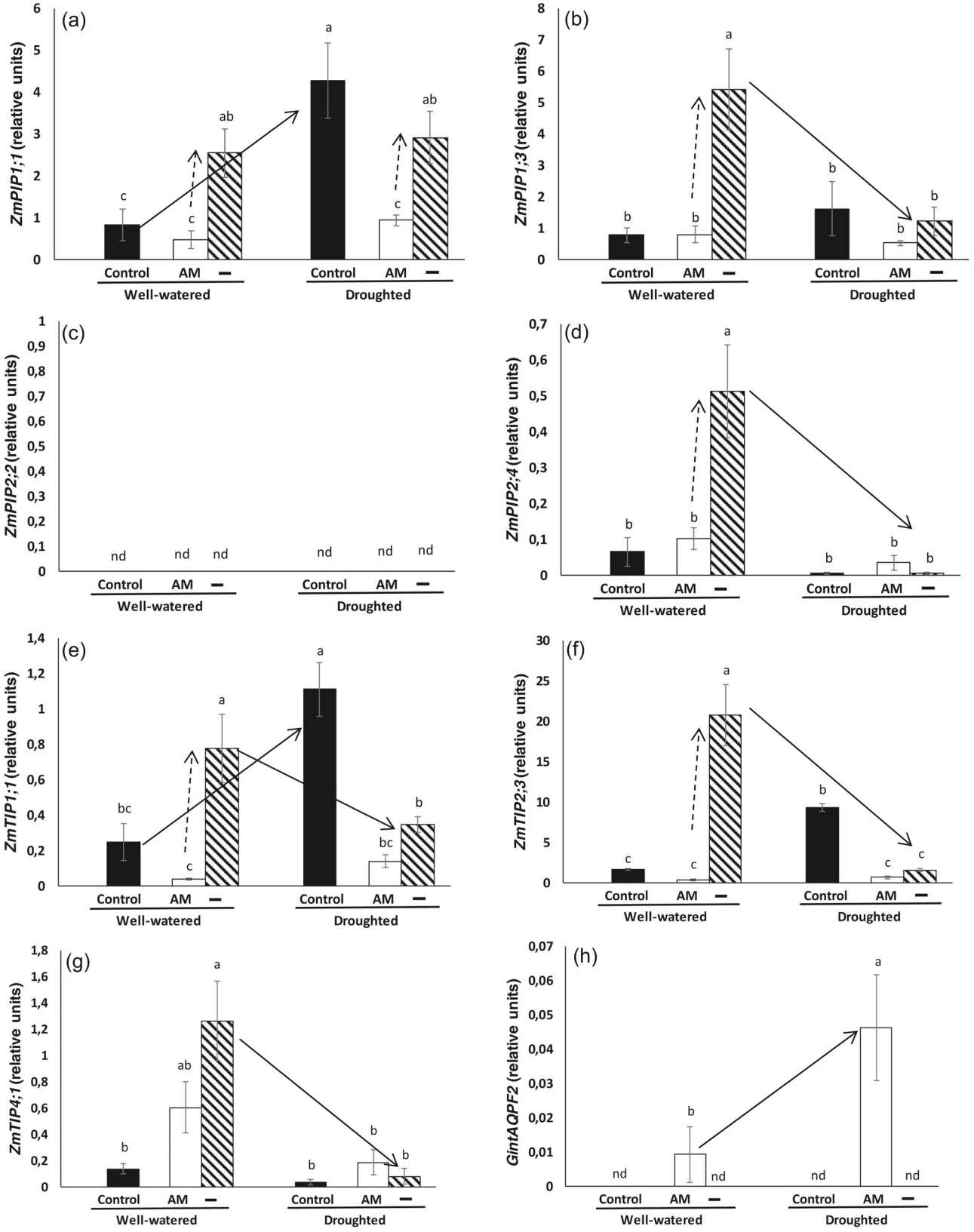

FIGURE 8 Aquaporin gene expression in root cells isolated from uninoculated maize plants (Control, black bars) or from the two root fractions of maize plants inoculated with the AM fungus *Rhizophagus intraradices* (AM). See the legend for Figure 7. AM, arbuscular mycorrhizal; Nd, non-detected.

radial water transport and also of cell osmoregulation being generally their expression is more abundant in roots than in leaves (Chaumont & Tyerman, 2014). In roots, an essential role of PIPs in transmembrane water diffusion has been proposed when its movement is hindered by the presence of apoplastic barriers (Prado et al., 2013; Shatil-Cohen et al., 2014). In the case of TIPs, it has been proposed that these proteins may control the exchange of water between vacuole and cytosol, providing a quick way for cellular osmotic balance (Forrest & Bhave, 2007). The AM regulation of the studied PIPs and TIPs in this study may have also contributed to the AM effects on L and Lo, as discussed below.

On the other hand, the hydrostatic root hydraulic conductivity (Lpr) estimates the water movement through the sum of both symplastic and apoplastic pathways and, by that reason is higher than L or Lo. In this study, Lpr was also enhanced by the AM symbiosis (Figure 5c). The positive effect of the AM symbiosis on L, Lo and Lpr may be also related to the enhanced JA-Ile content in AM roots, as evidenced in other studies (Sánchez-Romera et al., 2016). In addition, the positive effect of the AM symbiosis on L, Lo and Lpr has also been explained on the basis of the capacity of AM fungal hyphae to take water from soil pores which are inaccessible to the plant roots and with the effects of AM fungi on soil hydraulic properties and inside the root (Allen, 2009; Augé, Sylvia, et al., 2004; Hallett et al., 2009). In this sense, there is abundant literature demonstrating that the AM fungal mycelium can take and transport water from soil towards the roots (Allen, 2009; Kakouridis et al., 2022; Marulanda et al., 2003; Püschel et al., 2020; Ruth et al., 2011; Wu et al., 2024). Such water provided by the fungal mycelium can follow both a symplastic or an apoplastic pathway once it is transferred to the host root (Bárzana et al., 2012; Kakouridis et al., 2022; Wu et al., 2024), thus affecting L, Lo and Lpr.

In any case, the results obtained so far on aquaporin regulation by the AM symbiosis show that the effects of the symbiosis on these genes are complex and dependent on the own intrinsic properties of the applied stress. In this study, we analysed the expression of several maize PIPs and TIPs aquaporin genes that were previously selected as regulated by the AM symbiosis under drought stress conditions (Quiroga et al., 2017). Using a split root system, we found that the diverse analysed aquaporin genes were regulated differently in the root fraction containing the AM fungus (left root) and in the root fraction uninoculated with the fungus (right root). Moreover, such regulation was also different at the cell level when comparing the expression of arbusculated cells with that of uncolonized cells. At the root tissue level, the regulation varied depending on the watering conditions. Thus, under WW conditions, *ZmPIP1;1*, *ZmPIP1;3*, *ZmPIP2;4* and *ZmTIP2;3* decreased their expression in the uninoculated root fraction (right root) as compared to the inoculated AM fraction (left root). In contrast, under drought stress conditions, these genes increased their expression in the uninoculated root fraction (right root) as compared to the inoculated AM fraction (left root). This suggests that when water is sufficiently available, the presence of the fungus in the root allows a high water uptake, probably mediated by the fungal hyphae (Püschel et al., 2020; Ruth et al., 2011; Wu et al., 2024), and these aquaporins and the own fungal aquaporin *GintAQPF2* are involved in the mobilization of water. Indeed, among the three previously identified aquaporins in *R. intraradices*, only the isoform *GintAQPF2* has been described to feature high water transport capacity (Li et al., 2013), and this is the only gene detected under our growing conditions. This isoform may have accounted for the enhanced Lo and Lpr values in AM plants. In the case of the right root fraction that remains uninoculated with the fungus, there is a down-regulation of these plant aquaporins since there is no water uptake by the fungal hyphae and the amount of water mobilized is lower than in the AM left root fraction. Moreover, it can be suggested that the absence of AM fungal hyphae in the uninoculated right root fraction will also affect the own soil capacity for water retention (Augé et al., 2001; Augé, Sylvia, et al., 2004; Cheng et al., 2021). For instance, no glomalin is produced in such soil and less soil aggregates will be produced (Bedini et al., 2009; Rillig et al., 2002). Thus, the own roots will have less water availability in the soil where this uninoculated root fraction is growing. On the contrary, under drought stress the plant need to obtain as much water as possible from the growing substrate and, as in the uninoculated right root fraction there are no fungal hyphae to take up such water, the root expresses aquaporins to mobilize water in such root fraction. Indeed, mycorrhization enhanced Lpr in all cases as compared to control plants, and curiously, the Lpr values in the uninoculated right root fraction reached similar values than in the AM-colonized left root fraction, probably by means of the overexpression of several aquaporins. This hypothesis agrees with the model proposed recently by Wu et al. (2024) for the uptake of water-mediated by plant roots and AM fungal hyphae, depending on the soil moisture content.

To study the regulation of these aquaporins at the cellular level, a laser microdissection protocol has been applied to collect AM colonized cells (cells containing arbuscules) from the AM left root fraction and uncolonized cells from the uninoculated right root fraction (both cell types being from the same root system). Indeed, a mycorrhizal root represents a complex heterogeneous mixture of different cell and tissue types of both symbiotic partners, which complicates the interpretation of whole root expression profiling (Fiorilli et al., 2019). Moreover, the amount of aquaporin-facilitated water uptake differs between root developmental regions and root types (thin and thick roots) and also changes by AM colonization (Cruz et al., 2004; Liu et al., 2024). The expression pattern of some aquaporin genes was, in fact, found to be the opposite at the cell than at the root tissue level, with a higher expression in uncolonized cells than in arbusculated cells under WW conditions and no significant changes under drought stress conditions. However, we also observed that most aquaporin genes that resulted highly expressed in the uncolonized cells under WW conditions were down-regulated considerably under drought stress. This down-regulation of most aquaporins under drought must be considered as a cellular mechanism by these uncolonized cells to avoid cellular dehydration due to drought stress, as it has been previously proposed (Aharon et al., 2003; Bárzana et al., 2014; Porcel et al., 2006; Smart et al., 2001). In any case, the different aquaporin expression patterns

at root tissue and at root cells might be related to the two different levels of measurement. While at the whole root level, all cell types and root morphologies (thin and tick roots) were included, at the cell level, only RNA extracted from cortical cells of thin roots (those that are colonized by the AM fungus) were used to measure the aquaporin genes expression, and this may explain the different pattern of aquaporin regulation. It is known that aquaporin gene expression is regulated spatially and developmentally in a cell-specific manner by a wide range of environmental signals such as blue light, phytohormones, drought or salinity (Marjanović et al., 2005). Indeed, it has been reported that the regulation of up to seven PIP genes by ectomycorrhizal symbiosis in poplar plants was different in fine roots and in the main roots, being opposite in both kinds of roots for most of the aquaporin genes analysed (Marjanović et al., 2005). In agreement with the observed discrepancy between tissue and cell level, Recchia et al. (2018) reported the expression of a specific bean aquaporin gene (*PvPIP2;3*) exclusively in arbusculated cells, although a down-regulation was found in the whole root organ from AMF-inoculated plants, confirming the possibility that whole transcriptomics might mask the expression of relevant genes regulated by the symbiosis.

In addition to that, we also observed that drought stress enhanced the amount of root ABA accumulation, and in AM plants, such accumulation was higher in the uninoculated root fraction than in the inoculated root fraction, indicating that the level of drought stress was higher in the uninoculated root fraction and supporting the previous idea that such root fraction suffered more the drought stress imposed and need to try to mobilize as much water as possible by expressing aquaporin genes. The own ABA may have affected the aquaporin gene expression (Beaudette et al., 2007; Lian et al., 2006; Ruiz-Lozano et al., 2009, 2022) and may also explain why the expression of the aquaporin genes was different at the cell and root tissue levels. The other plant hormones measured in this study (IAA, JA and JA-Ile) were affected either by the drought stress imposed or by the mycorrhizal presence. In fact, it is known that plant development, interactions with other organisms or responses to stress are regulated by complex hormonal crosstalk (Foo et al., 2013; Munné-Bosch & Müller, 2013; Ullah et al., 2018) and this crosstalk has been shown to be altered by the AM symbiosis (Foo et al., 2013; Pozo et al., 2015; Ruiz-Lozano et al., 2022). Moreover, these hormones also affect root hydraulic parameters. Thus, jasmonates increased hydrostatic root hydraulic conductivity (Lpr) in three different plant species (*Solanum lycopersicum*, *Phaseolus vulgaris* and *Arabidopsis thaliana*) in a calcium- and ABA-dependent way (Sánchez-Romera et al., 2016), but also in an ABA-independent way (Sánchez-Romera et al., 2014). IAA decreased osmotic root hydraulic conductivity (Lo) during water stress conditions (Quiroga et al., 2020). Its exogenous application decreased Lo in both AM and non-AM plants during WD conditions. The effects of IAA on the internal cell component of root water conductivity (Lo) were mediated by altered levels of ABA or jasmonates, suggesting that aquaporins are involved in the hormone-dependent inhibition of this internal cell water transport pathway, in which aquaporins participate.

5 | CONCLUSION

To the best of our knowledge, the present study is the first work comparing regulation of the expression of several maize and AM fungal aquaporin genes at the cell and whole root system, focusing on AM-colonized and uncolonized cells. Results indicate the existence of both, local and systemic regulation of plants aquaporins by the AM symbiosis. This was observed both at root tissue and at cellular levels. However, depending on the watering conditions, the regulation was different (even opposite) at root tissue and at cell levels. Altogether, results suggest that the regulation of aquaporins by the AM symbiosis at root and cell levels is related to the higher or lower availability of water taken up by fungal hyphae in each root fraction. When water is sufficiently available, the presence of the fungus in the root allows a high water uptake and the studied plant aquaporins and the fungal aquaporin *GintAQPF2* are involved in the mobilization of water. The regulation of aquaporins by the AM symbiosis at root and cell levels is also related to the degree of stress that affects each root fraction, including the level of ABA accumulation, as well as the plant's need for water mobilization. Thus, under drought stress the plant need to obtain as much water as possible from the growing substrate and, as in the uninoculated right root fraction there are no fungal hyphae to take up such water, the root overexpresses aquaporins to mobilize water in such root fraction. The enhancement of root ABA level as a consequence of the stress suffered by each root fraction may be also a way to regulate the aquaporin gene expression and may explain why the expression of the aquaporin genes was different at the cell and root tissue levels. In any case, this aspect needs further investigation to be elucidated in deep. For that a split root system similar to the one used here may be used, but changing the set-up of irrigation to each root fraction and including treatments where only a root fraction is subjected to drought, while the other root fraction remains WW. This will allow obtaining plants with no physiological drought but where a root fraction is indeed affected by drought, for comparison with the plants suffering a physiological drought where both root fractions are subjected to drought, as was the case in the present study.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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