



Review Article

Plant Aquaporin Responses to Mycorrhizal Symbiosis under Abiotic Stress

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Abstract

Arbuscular mycorrhizal fungi (AMF) from soil microbial communities establish mycorrhizal symbionts with roots of terrestrial plants. Mycorrhizal extraradical hyphae directly participate in water absorption of plants. At present, six aquaporin (AQP) genes have been identified and cloned from two AMF namely *Glomus intraradices* and *Rhizophagus clarus*. These fungal AQP genes are involved in water transport. In addition to fungal AQP responses, host AQPs also give a quick response to mycorrhizal colonization under stress conditions. The mycorrhiza-modulated responses of host AQPs register a diverse pattern: up-regulation to accelerate water absorption, down-regulation to avoid water loss, or no changes. These host AQP-responded patterns are likely related to root hydraulic conductance and the complementary relation with mycorrhizal fungal AQPs. The physiological changes regarding water relations by mycorrhization may be the integrative result of different AQP expressions, depending on stress types, duration and intensity, AMF species, and host types. Understanding AQP-response patterns in both hosts and AMF is crucial to understand how mycorrhizal symbiosis enhances water relations of host plants. © 2020 Friends Science Publishers

Keywords: Aquaporin; Drought stress; *Glomus intraradices*; Mycorrhiza; Symbiosis

Introduction

Most terrestrial plants are capable of forming reciprocal symbiosis (arbuscular mycorrhizas, AMs) with soil arbuscular mycorrhizal fungi (AMF), belonging to Glomeromycotina (Plassard *et al.*, 2019). Under a suitable soil environment, spores of AMF germinate and form hyphopodia on the root surface of host plants. After passing through the outer-cell layers, mycorrhizal fungal hyphae in the inner cortical cells establish highly branched arbuscules (Keymer and Gutjahr, 2018). In addition, AMs develop external hyphae outside the root system, which is 10 to 40-fold more extensive than the roots and whose length ranges from 10 to 22 m per plant (Ferrol *et al.*, 2019). The external hyphae also colonize neighbor plants to establish common mycorrhizal networks between plants, which can deliver the signaling of disease resistance (Zhang *et al.*, 2019). In roots, plant sugars and lipids are transferred to AMF for its growth, and in return, AMs aid in nutrient acquisition of host plants. Such beneficial roles of AMs, positively stimulate plant growth performance and partly mitigate damage caused by abiotic and biotic stresses on host plants (Wu and Zou, 2017).

Many studies have shown a beneficial effect of AMF on increasing tolerance of abiotic stress in plants, associated with anatomical changes of the plants, physiological changes in the antioxidant protective system, osmotic

adjustment, polyamine, fatty acid, and nutrient and water acquisition, and molecular changes in aquaporin (AQP) genes, the 14-3-3 gene, and late embryogenesis (Wu and Zou, 2017; Wu *et al.*, 2019; Zhang *et al.*, 2020). Safir *et al.* (1971) found that inoculation with *Glomus mosseae* reduced resistance of water transportation in *Glycine max*, but did not change root morphology. Then, up-regulation of AQP expression in host plants by mycorrhization was found in parsley and *Medicago truncatula* (Roussel *et al.*, 1997; Krajinski *et al.*, 2000). Lately, many studies also analyzed the effects in maize, tomato, trifoliate orange and lettuce (Marulanda *et al.*, 2003; Li and Chen, 2012; Calvo-Polanco *et al.*, 2014; Ding *et al.*, 2019; He *et al.*, 2019). The induced AQP gene expression of *M. truncatula* by mycorrhiza facilitated the water transport through heterologous expression of *in-vitro*-transcribed RNA in *Xenopus* oocytes (Krajinski *et al.*, 2000), which further revealed the important role of mycorrhiza-regulated AQP expression in host plants. The regulation of AQP-encoding genes located in the root by AMF is a potential mechanism for mycorrhizas to enhance stress tolerance of plants (Navarro-Ródenas *et al.*, 2013). Nevertheless, the response of plant and fungal AQPs to water deficit is complex (Ruiz-Lozano and Aroca, 2017). This paper aims to review the classification and functions of AQPs, the advances of AQPs in AMF, and AQPs gene expression in mycorrhizal systems under stress conditions.

Overview of AQPs in Plants

AQPs belong to the major intrinsic proteins (MIPs) in many organisms for transporting certain small molecules across biological membranes (Maurel *et al.*, 2015). There are a large number of homologues of AQPs in dicotyledons and monocotyledons, as well as in C3 and C4 metabolic plants. These plant AQPs show abundant diversity and high abundance. According to amino acid sequence homology and subcellular localization, plant AQPs are classed into five categories: plasma intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), NOD26-like intrinsic proteins (NIPs), small intrinsic proteins (SIPs), and Glps-like intrinsic proteins (GIPs) (Johansson *et al.*, 2001). In *Sphaerotheciella sphaerocarpa*, two new AQP types, hybrid intrinsic proteins (HIP) and uncharacterized intrinsic (XIPs) proteins were identified (Bienert *et al.*, 2011).

The PIPs are highly conserved and narrow in pores, and they are typical high-moisture-selective channel proteins, including PIP1, PIP2, and PIP3 (Marty, 1999). TIPs are located on the tonoplast, which contain five subclasses viz., α , β , γ , δ , and ϵ (Johansson *et al.*, 2001). NIPs are located on the symbiotic membrane of soybean nodules and bacteria, and are divided into three categories: NIP I, NIP II, and NIP III (Mitani-Ueno *et al.*, 2011). SIPs are the smallest family of plant AQPs, located on the endoplasmic reticulum membrane, and divided into SIP1 and SIP2 (Ishikawa *et al.*, 2005). At present, AQP genes have been found and cloned in various plants such as *Arabidopsis thaliana* (Quigley *et al.*, 2001), *Oryza sativa* (Sakurai, 2005), *Zea mays* (Chaumont, 2001), *Citrus sinensis* (Martins *et al.*, 2015) etc. The discovery of these AQPs provides important guidance in systematic analysis of AQP diversity. These identified AQPs are the most abundant transporters of H₂O, as well as glycerol, urea, NH₃, CO₂, silicon, antimony, arsenite, boron, hydrogen peroxide, etc. (Afzal *et al.*, 2016). Meanwhile, PIPs can transport glycerol, hydrogen peroxide, water, and urea. TIPs function in the permeability of water. NIPs possess less activity of water transport, but are responsible for permeability of organic molecules. For example, NIP1 in *Medicago truncatula* is functioned in the inner membrane of symbiotic cells (Uehlein *et al.*, 2007), and NIP1;1 transports both glycerol and silicon (Bárzana *et al.*, 2014).

Overview of AQPs in AMF and Their Potential Roles

In addition to plants, mycorrhizal fungi also have AQPs. In an ectomycorrhizal fungus *Laccaria bicolor*, six AQPs including one orthodox aquaporin and five aquaglyceroporins were identified, and showed water transport capacity (Dietz *et al.*, 2011). In 2009, Aroca *et al.* (2009) first reported an AQP gene isolated from an AM fungus *G. intraradices*, named as *GintAQP1*. *GintAQP1* expression and host AQP expressions are a compensatory way. For example, under salt stress, the *GintAQP1* gene

expression was not changed, while *PIPs* expression of *P. vulgaris* up-regulated. Both drought and cold did not regulate expression of *GintAQP1*, whereas two of four *PIP* genes in *P. vulgaris* down-regulated their expression. After being heterologously expressed in *Xenopus laevis* oocytes, *GintAQP1* did not transport water. Therefore, the location of *GintAQP1* and its hypothetical transporter substrates need to be further studied.

Li *et al.* (2013) also isolated two full-length putative AQP genes in *G. intraradices*, *GintAQP1* and *GintAQP2*. *GintAQP1* is 1093 bp and located in the plasma membrane of yeast. *GintAQP2* is located in both plasma and intracellular membranes without any introns, supporting the roles in regulating water flux across plasma membranes for water transfer (Xu *et al.*, 2013). Drought stress strongly induced the expression of *GintAQP1* and *GintAQP2* in maize. Moreover, the fungal AQPs were enriched in cortical cells having arbuscules. This suggested potential water transport by fungal AQPs to host plants, further illuminating the AMF role in drought tolerance of plants.

Kikuchi *et al.* (2016) also identified three putative AQP genes from *Rhizophagus clarus*, viz., RcAQP1, RcAQP2, and RcAQP3, which are most similar to *GintAQP1*, *GintAQP2*, and *GintAQP3* of *R. irregularis*, respectively. Additionally, the fungal aquaglyceroporin *RcAQP3* is most highly expressed in intraradical hyphae to transport water across the plasma membrane, as well as to accelerate transpiration and polyp translocation towards the roots. Despite all this, the structure, function, and regulation of AQP in AMF are elusive.

When analyzing expression of fungal AQP genes, host AQP gene expressions are also considered (Ruiz-Lozano and Aroca, 2017). For example, Li and Chen (2012) analyzed expression of host *PIPs* and *GintAQP1* in maize roots inoculated with *G. intraradices* exposed to soil water deficit. They revealed the enhanced expression of eight *ZmPIP* genes, accompanied with the up-regulated expression of *GintAQP1*. Other studies also showed the enhanced expression of mycorrhizal fungal AQPs under the conditions of soil water deficit in this article, providing further evidence to support water movement in mycorrhizal plants by fungal AQPs. However, more AQPs from AMF need to be identified, and the functions of fungal AQPs remain to be examined.

Interestingly, based on transcriptomic data, Giovannetti *et al.* (2012) found two up-regulated AQP genes, *LjNIP1* and *LjXIP1*, in *Gigaspora margarita*-colonized roots of *Lotus japonicus*. Among them, *LjNIP1* was expressed exclusively in inner membrane systems of arbuscule-enriched cells. This indicated that *LjNIP1* could be used as an indicator of mycorrhizal status at arbuscule-developed process.

In short, fungal AQPs and their roles were identified to support the involvement in water transport and nutrient acquisition of mycorrhizal symbiosis, which is possibly important for mycorrhizal responses to abiotic stress.

Collective Physiological Roles of both Aquaporins and Arbuscular Mycorrhizas

Water transport in roots: There are three types of water absorption in mycorrhizal plants: (i) the apoplast pathway in which the water moves through the cell wall and the intercellular space without involving the cytoplasm, (ii) the symplastic or transmembrane pathway in which the water moves from one cell to another through the plasma membrane twice and through the vacuolar membrane, and (iii) the symbiotic pathway in which mycorrhizas provide a special way to absorb water by mycorrhizal hyphae from soils to root cortical cells (Zhu *et al.*, 2015). Early studies indicated a significantly higher water transport speed of mycorrhizal pine seedlings than that of the non-mycorrhizal control, providing evidence for mycorrhiza-reduced water transport resistance (Tataranni *et al.*, 2012). Ruth *et al.* (2011) used a high-resolution online water content sensor to quantitatively analyze mycorrhizal water contribution, accounting for 20% in whole water absorption rate of plants. AMF possesses multi-nucleated, diaphragm-free mycelium, which quickly transferred water with little resistance in the mycelium. After reaching the top of the mycelium, water seeped into cells of the host root, and shortened the water transport path in the root. As a result, mycorrhizal hyphae provided a special water absorption channel. In addition, mycorrhizal symbiosis affects root branching, root diameter, and root density without change in the total root biomass, which provide greater water absorptive capacity of mycorrhizal hosts subjected to adversity (Kabouw *et al.*, 2012).

Many AQPs as efficient transport membrane proteins are highly expressed in roots to transport water, while 70 to 90% of the water transport through roots is derived from AQPs and water transport via AQPs is mainly transmembrane transport (Kaldenhoff and Fischer, 2006). In roots of *Hordeum vulgare*, cortical cells of lateral roots have the highest water conductivity, and the smallest water conductivity is in the mature zone and transition zone of main roots, while the cortical cells of the adventitious root transition zone have relatively higher water conductivity (Knipfer *et al.*, 2011), which is consistent with expression of *HvPIP2;2*, *HvPIP2;5*, and *HvTIP1;1*. After being treated by water channel protein inhibitors, the water flow decreased by 83 to 95%, indicating that high expression of these AQPs in cortical cells is the main reason for maintaining high water conductivity. A recent study also showed that AQPs contributed 79 and 85% root water conductivity in rice roots when water was sufficient or deficient, respectively (Grondin *et al.*, 2016). This suggested that AQPs are important in root water transport under a stress environment.

Plant growth responses: AMF can promote plant growth behavior in various abiotic stress conditions (Lü *et al.*, 2018). Inoculation with AMF greatly improves root architecture (root length, surface area, and volume) of host plants, and the mycorrhiza-improved root morphological changes are associated with both the AMF species used and

mycorrhiza-induced changes in carbohydrates in host plants (Wu *et al.*, 2011). The increase of shoot and root biomass in cucumber plants was 24 and 13% respectively after AMF treatment (Wang *et al.*, 2003), which was related with AMF-increased nutrient acquisition. Higher gas exchange in both transpiration rate and stomatal conductance was found in mycorrhizal citrus versus non-mycorrhizal citrus after inoculation with *G. fasciculatus* during water stress and stress recovery (Levy and Krikun, 1980). As a result, mycorrhizal fungi facilitated water transport more smoothly and rapidly, thus maintaining normal plant growth under water deficit conditions.

AQP expressions are closely related with cell proliferation. For example, the expression pattern of *TIP1;1* in *A. thaliana* is associated with the cell elongation of roots, hypocotyls, leaves, and flower stems, and *TIP1;1* also participates in the exchange of water and solutes (Ludevid *et al.*, 1992). In addition, overexpression of *PIP1;2* from *Arabidopsis* significantly promoted plant growth in tobacco (Peng *et al.*, 2007). Expression of *PIPs* in *Vitis berlanderi* × *V. rupestris* was the highest in the tip of roots and decreased in the root-hair zone of roots (Gambetta *et al.*, 2013). This suggested that AQP expression might promote the transport of mineral elements and water in roots, thereby further stimulating cell proliferation and subsequent plant growth.

Phytohormone regulation: As an important chemical signal substance, endogenous hormones regulate plant growth and root development in order to alleviate environmental stress (Fahad *et al.*, 2015). AM symbiosis alters the levels of phytohormones such as cytokinin, auxin, auxin-related substances, abscisic acid (ABA), and jasmonic acid (Ludwig-Müller, 2010). Cruz *et al.* (2000) observed that mycorrhizal symbiosis increased the content of IAA, gibberellins, and CTK in host plants, while it decreased ABA and ethylene concentrations under drought stress. On the other hand, hyphae of AMF also produce ABA (Esch *et al.*, 1994). Hence, AM symbiosis regulates phytohormone levels of host plants to respond to environmental stress.

Drought-induced ABA not only stimulates stomatal closure, but also regulates the water channel function in plants (Peret *et al.*, 2012). Exogenous ABA treatment had a positive effect on root water conductivity and reduced the phosphorylation of several PIP2 in *Arabidopsis* (Kline *et al.*, 2010). IAA inhibits expressions of most PIP genes at transcription and translation levels through the auxin response factor 7-dependent pathway (Yamada *et al.*, 1995), and also reduces the water conductivity of root cortical cells (Hose *et al.*, 2000). Salicylic acid regulates PIP expression and root water conductivity through a mechanism mediated by reactive oxygen species (Boursiac *et al.*, 2008). Expression of *RhPIP1;1* in leaves of Chinese rose was increased after being treated by exogenous GA₃ application, while it was reduced by exogenous ABA (Yin *et al.*, 2014). Hence, environment stress-induced hormonal changes are associated with plant hydraulics, and thus mycorrhizas alter hydraulic characteristics of plants through regulation of plant hormones.

Osmotic regulation: Osmotic regulation refers to the accumulation of solutes in plant cells to decrease osmotic potential and maintain cell turgor pressure, and thus reduces stress damage and promotes plant growth. Solutes involved in osmotic regulation are divided into two categories: organic solutes, such as polyols, betaines, polyamines, proline, free amino acids, sugars, and alcohols, and inorganic ions, including Na^+ , K^+ , Mg^{2+} , Ca^{2+} , and H^+ (Zeng *et al.*, 2015). Previous studies showed that mycorrhizal inoculation improved the ability of osmotic regulation in host plants in response to a stress environment (Wu *et al.*, 2013a; Yang *et al.*, 2018; Zhang *et al.*, 2018). In a study conducted by Zhao *et al.* (2017), AMF inoculation increased concentrations of soluble sugar, soluble protein, and free proline in alfalfa under salt stress, which resulted in the promotion of water and nutrients and the stabilization of proteins and enzyme activities. Similarly, AQPs are not only involved in water absorption in plants, but also in the regulation of osmosis between vacuoles and the cytoplasm or between the cytoplasm and the apoplast (Yang *et al.*, 2005). AQPs can prevent water loss under stress environments. PIP1 is rich in the plasma membrane of mesophyll cells in *Arabidopsis* in order to regulate water exchange (Beebo *et al.*, 2009). In crux, AMF and AQPs are collectively involved in the osmotic regulation of plants. AMF enhances stress resistance by increasing solute contents, while AQPs strive for time to synthesize osmotic solutes by increasing water permeability and preventing water loss.

Responses of AQPs to Mycorrhization under Abiotic Stress

Both AMF and AQPs can respond to stress environments, but their mechanisms are not identical. The mechanisms of AMF-associated stress tolerance are mostly at the physiological level: water absorption of extraradical hyphae, enhancement of nutrient acquisition, superior root architecture, greater osmotic regulation and antioxidant protective systems, and improvement of the soil structure by mycorrhiza-released glomalin (Wu *et al.*, 2013b; Zhang *et al.*, 2018 a,b). Since most mycorrhiza-induced changes are in the cytoplasmic or vacuolar membrane of cells in roots, it is expected to find genes encoding membrane related proteins such as AQPs (Sade *et al.*, 2009). The accumulation pattern of AQPs in roots by mycorrhization versus non-mycorrhization has different roles in physiological regulation (Fig. 1). Therefore, many studies focused on whether AQP genes respond differently to abiotic stress under mycorrhization (Table 1).

Drought stress: Porcel *et al.* (2006) investigate the expression patterns of AQP genes in AMF-colonized and non-AMF-colonized soybean and lettuce roots exposed to drought stress. They found down-regulating expression of PIP genes in AM plants in response to drought stress. However, Alguacil *et al.* (2009) carried out a research, where *Lactuca sativa* seedlings were inoculated with *G.*

intraradices or *Pseudomonas mendocina* and also subjected to two water regimes and two atmospheric CO_2 levels. They showed that mycorrhizal treatment increased expression of the *LsPIP2* gene under two water regimes and two CO_2 levels, while *G. intraradices* was more effective than *P. mendocina*. This indicated that AMF may up- or down-regulate AQP gene expression in response to drought stress. Marulanda *et al.* (2003) reported that *G. intraradices* inoculation elevated water absorption via maintaining high levels of PIP gene expression, while *G. mosseae* seemed to protect plants against drought stress through down-regulating expression of PIP genes.

In addition to water transport, researchers also tried to a whole set of AQP gene expression patterns and transporting other molecules. Bárzana *et al.* (2014) revealed that AMF symbiosis regulated expression of a number of AQP genes in host plants, including members of different AQP subfamilies. AMF-modulated AQP expression patterns depended on the soil water status and the applied drought severity. In a short-term soil water deficit, AM symbiosis up-regulated the expression of 10 AQP genes, while 6 AQP gene expressions were not affected. In contrast, when the soil water deficit lasted, 6 AQP genes were down-regulated, 7 AQP were unaffected, and only 3 AQP genes were up-regulated. However, the AQP response was down-regulated in non-AMF plants by drought stress, regardless of the intensity of drought stress. Some AQP gene expressions can be modulated by the soil water deficit degree under mycorrhization suggests that AMF-regulated AQP expression might take part in water physiology and other potential physiological activities. In addition, functional characterization showed that different subtypes of AQPs can transport water, glycerol, urea, NH_3 , B, and H_2O_2 . Quiroga *et al.* (2017) showed that more host AQP genes were inhibited by AM symbiosis in the drought-sensitive cultivar of maize under drought stress, as compared with that in the drought-tolerant cultivar of maize. The down-regulation of AQPs by mycorrhization is a way to minimize water loss, thus, producing drought tolerance of plants (Min *et al.*, 2016). Recently, Quiroga *et al.* (2019) analyzed the accumulation of phosphorylated PIP2s in maize inoculated with *R. irregularis* subjected or not to water stress. They found that during water deficit stress, phosphorylation levels of PIP2 were increased in mycorrhizal plants, indicating that mycorrhizal symbiosis induces a relatively higher activity of PIP2 in AM plants. AQP expression regulated by arbuscular mycorrhizas under drought conditions has also been elucidated in other plants with differently results. He *et al.* (2016) reported that AM symbiosis down-regulated the *RpPIP1;3* gene expression in roots, stems, and leaves of *Robinia pseudoacacia*, which is a method to reduce water loss in host tissues. While, AM symbiosis also up-regulated *RpTIP2;1* and *RpPIP2;1* gene expression under drought stress, that could be a way to increase water flow. Liu *et al.* (2016) found that *R. irregularis* up-regulated the expressions of six PIP genes in leaves of *Populus* ×

Table 1: Aquaporin responsive patterns of host plants under drought stress, salt stress, and cold stress after inoculation with AMF

Abiotic stress	Host plant	Plant tissue	Species of AMF	Gene expression	Reference	
Drought stress	<i>Glycine max</i>	Root	<i>Glomus mosseae</i>	<i>GmPIP2</i> ↓	Porcel <i>et al.</i> 2006	
	<i>Lactuca sativa</i>	Root	<i>G. mosseae</i>	<i>LsPIP2</i> ↓	Porcel <i>et al.</i> 2006	
		Root	<i>G. intraradices</i>	<i>LpPIP2</i> ↑	Alguacil <i>et al.</i> 2009	
	<i>Populus × canadensis</i>	Leaf	<i>Rhizophagus irregularis</i>	<i>PIP1;1</i> ↑; <i>PIP1;2</i> ↑; <i>PIP1;3</i> ↑; <i>PIP1;4</i> ↑; <i>PIP1;5</i> ↑; <i>PIP2;2</i> ↑; <i>PIP2;3</i> ↓	Liu <i>et al.</i> 2016	
	<i>Poncirus trifoliata</i>	Root	<i>Funneliformis mosseae</i>	<i>PtTIP1;2</i> ↑; <i>PtTIP1;3</i> ↑; <i>PtTIP4;1</i> ↑; <i>PtTIP2;1</i> ↓; <i>PtTIP5;1</i> ↓; <i>PtTIP1;1^{ns}</i> ; <i>PtTIP2;2^{ns}</i>	He <i>et al.</i> 2019	
	<i>Zea mays</i>	Root	<i>R. irregularis</i>	Short-term drought <i>ZmTIP1;2</i> ↑; <i>ZmPIP1;4</i> ↑; <i>ZmTIP2;4</i> ↑; <i>ZmNIP2;1</i> ↑; <i>ZmTIP2;3</i> ↑; <i>ZmPIP2;2</i> ↑; <i>ZmTIP1;1</i> ↑; <i>ZmPIP1;2</i> ↑; <i>ZmTIP1;6</i> ↑; <i>ZmSIP2;1</i> ↑; <i>ZmNIP1;1^{ns}</i> ; <i>ZmTIP4;1^{ns}</i> ; <i>ZmTIP4;2^{ns}</i> ; <i>ZmPIP1;1^{ns}</i> ; <i>ZmPIP1;3^{ns}</i> ; <i>ZmNIP2;2^{ns}</i> . Sustained drought <i>ZmPIP1;4</i> ↓; <i>ZmPIP1;3</i> ↓; <i>ZmNIP2;2</i> ↓; <i>ZmTIP1;2</i> ↓; <i>ZmPIP2;2</i> ↓; <i>ZmTIP1;1</i> ↓; <i>ZmNIP1;1</i> ↑; <i>ZmTIP4;1</i> ↑; <i>ZmTIP4;2</i> ↑; <i>ZmPIP1;4^{ns}</i> ; <i>ZmPIP2;4^{ns}</i> ; <i>ZmNIP2;1^{ns}</i> ; <i>ZmTIP2;3^{ns}</i> ; <i>ZmPIP1;2^{ns}</i> ; <i>ZmPIP1;6^{ns}</i> ; <i>ZmSIP2;1^{ns}</i> . Drought-sensitive cultivar <i>ZmPIP1;1</i> ↓; <i>ZmPIP1;3</i> ↓; <i>ZmPIP1;6</i> ↓; <i>ZmPIP2;2</i> ↓; <i>ZmPIP2;4</i> ↓; <i>ZmTIP1;1</i> ↓; <i>ZmTIP2;3</i> ↓; <i>ZmTIP4;1^{ns}</i> ; <i>ZmNIP2;1^{ns}</i> . Drought-tolerant cultivar <i>ZmPIP1;1</i> ↑; <i>ZmTIP4;1</i> ↑; <i>ZmPIP2;4</i> ↑; <i>ZmPIP1;3</i> ↓; <i>ZmTIP1;1^{ns}</i> ; <i>ZmTIP2;3^{ns}</i> ; <i>ZmNIP2;1^{ns}</i> ; <i>ZmPIP1;6^{ns}</i> ; <i>ZmPIP2;2^{ns}</i> .	Bárcana <i>et al.</i> 2014	
Salinity stress	<i>Lycopersicon esculentum</i>	Leaf and Root	mixture of <i>G. geosporum</i> and <i>G. intraradices</i>	<i>LePIP1</i> ↓; <i>LeTIP</i> ↓; <i>LePIP2^{ns}</i>	Ouziad <i>et al.</i> 2006	
		Root	<i>G. mosseae</i>	<i>LePIP1</i> ↓; <i>LePIP2</i> ↓; <i>LeTRAMP</i> ↓; <i>LeTIP</i> ↓; <i>LeAQP2</i> ↑	He <i>et al.</i> 2011	
		Root	<i>G. intraradices</i>	<i>PvPIP1;2</i> ↓; <i>PvPIP1;1</i> ↑; <i>PvPIP1;3</i> ↑; <i>PvPIP2;1</i> ↑	Aroca <i>et al.</i> 2009	
	<i>Poncirus trifoliata</i>	Root	<i>Paraglomus occultum</i>	<i>PtTIP1;1</i> ↓; <i>PtTIP1;3</i> ↓; <i>PtTIP2;2</i> ↓; <i>PtTIP1;2</i> ↓; <i>PtTIP2;1</i> ↓; <i>PtTIP4;1</i> ↑; <i>PtTIP5;1^{ns}</i>	Ding <i>et al.</i> 2019	
	Cold stress	<i>Oryza sativa</i>	Root	<i>G. intraradices</i>	<i>OsPIP1;1</i> ↑; <i>OsPIP1;3</i> ↑; <i>OsPIP2;1</i> ↑; <i>OsPIP2;2</i> ↑; <i>OsPIP1;2^{ns}</i> ; <i>OsPIP2;3^{ns}</i>	Liu <i>et al.</i> 2014
		<i>Phaseolus vulgaris</i>	Root	<i>G. intraradices</i>	<i>PvPIP1;1</i> ↓; <i>PvPIP1;2</i> ↓; <i>PvPIP1;3^{ns}</i> ; <i>PvPIP2;1^{ns}</i>	Aroca <i>et al.</i> 2007

Note: the symbol “↑”, “↓” and “ns” means the up and down-regulation, and no changes in this aquaporin gene expression after mycorrhizal colonization

canadensis and reduced expression of two *PIP* genes under drought stress. In trifoliolate orange, He *et al.* (2019) observed the induced expression of *PtTIP2;1* and *PtTIP5;1* in trifoliolate orange after inoculation with *Funneliformis mosseae* under well-watered conditions and the inhibitive expression under drought stress. These results suggest relatively complex and diverse changes in expression patterns of AMF-modulated host *AQPs* exposed to water stress. In any case, AM symbiosis could regulate the water relation and physiological activity of host plants through modulating host *AQP* expression.

Salinity stress: Expression or activity of *AQPs* is also correlated with salt sensitivity of plants. Ouziad *et al.* (2006) compared tomato *AQP* expression under the condition of AMF inoculation and NaCl treatment. Transcript levels of both a *TIP* and a *PIP* gene were reduced by salt stress, while this effect was distinctly enhanced by AMF colonization. In another study by He *et al.* (2011), AMF symbiosis under salt stress promoted plant growth and water uptake of tomato under NaCl stress, followed with the decreased expression of *AQP* genes. Ding *et al.* (2019) reported that except for *PtTIP4;1* and *PtTIP5;1*, transcription levels of the other five *TIP* genes were down-regulated by the colonization of *Paraglomus occultum* under NaCl stress. However, the

biomass and water potential of AMF-colonized plants were higher than those of non-AMF plants subjected to NaCl, indicating that the water absorption of mycelium might be more important than *AQPs*. In addition, in a two-chambered rootbox, expression of *GintAQP1* in the root compartment of carrot roots was higher than in the hyphal compartment, when the hyphal compartment was treated by additional NaCl (Aroca *et al.*, 2009). When the root compartment was applied by additional NaCl, the hyphal compartment had higher expression of *GintAQP1* than the root compartment in monoxenic culture. Such results implied that fungal *GintAQP1* and host *AQPs* might be regulated by certain signal substances between NaCl-treated and untreated hyphae. Hence, mycorrhiza-regulated *AQP* expression patterns under salinity are a complex network depending on AMF species, *AQP* types, and host plant species.

Cold stress: Low temperatures generally reduce root water uptake by decreasing hydraulic conductivity of roots. At the same time, expression levels of several *AQP* genes are considered to modulate plant water response to cold stress. For example, two *PIP* genes in rice roots were up-regulated after subjected to low temperature for several days (Kuwaagata *et al.*, 2012). In rice, low temperature stress and mycorrhizal treatment collectively increased four *PIP*

homologous gene expressions (Liu *et al.*, 2014). At the same time, *GintAQP1* and *GintAQP2* were over expressed by low temperature treatment. This confirmed that both fungal AQP activities and host AQP gene expression could be collectively induced to transport water under cold stress. The *PIP* gene expression was also studied by Aroca *et al.* (2007) in roots of *Phaseolus vulgaris* under three stresses environments and mycorrhization. They observed that only under cold treatment conditions, mycorrhizal inoculation down-regulated the expressions of *PIP1;1* and *PIP1;2* genes but did not alter the expressions of *PIP1;3* and *PIP2;1* genes. It suggests that mycorrhizas induced diverse expression patterns of *PIP* homologous gene in response to cold stress. More studies still need to analyze the AMF species, stress conditions and relationship between host AQPs and mycorrhizal AQPs.

Contribution of Mycorrhiza-regulated AQP Expression to Host Plants Exposed to Abiotic Stress

Under abiotic stress, host AQP gene expressions are modulated by mycorrhization, suggesting the change of water physiological activities in plants. The induction or inhibition of host AQPs by AMF could reflect plant water strategy in any case. There are two opposite mechanisms by which AMF regulates the expression of AQPs in dehydration stress responses. Host AQP expression is induced by mycorrhization under dehydration stress, indicating the improvement of water permeability of the membrane and the promotion of water transport; the down-regulation of host AQP expression by mycorrhization under dehydration stress means a decrease of membrane permeability and consequently water retention by cells, thereby reducing water loss (Fig. 1) (Ruiz-Lozano *et al.*, 2008).

Additionally, mycorrhiza-regulated *PIP* expression patterns were clearly correlated with enhanced root hydraulic conductivity of maize plants after soil water deficit and water recovery (Fig. 2) (Ruiz-Lozano *et al.*, 2009). Lee *et al.* (2010) found that mycorrhizal effects on root *PIP* transcriptional levels could stimulate the increase in cell-to-cell water transport in roots, which was closely associated with root hydraulic conductivity. As a result, mycorrhiza-affected host AQP expression takes part in root hydraulic conductivity of drought-stressed plants, which is an important mechanism (Fig. 2).

Fungal AQP expressional patterns may be a compensatory way for host AQP expression under stressed conditions (Aroca *et al.*, 2009): fungal *GintAQP1* expression was unchanged; host *PIPs* were induced. And, host AQP expressional patterns may be a compensatory way for water absorption of extraradical hyphae of arbuscular mycorrhiza under stressed conditions (Zou *et al.*, 2019): water absorptive rate of extraradical mycorrhizal hyphae was enhanced by drought stress; host AQP expressions were inhibited or unchanged.

In the association of soil–fungus–plant pathway of

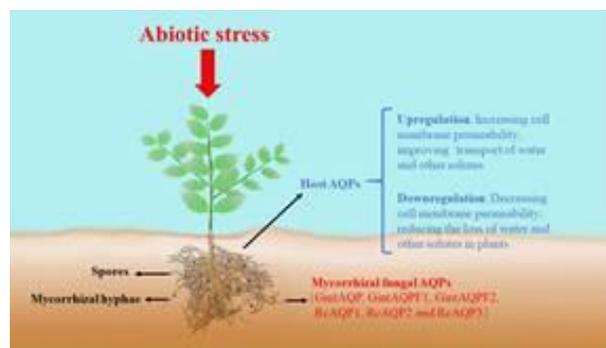


Fig. 1: The response pattern of AQPs by mycorrhization under abiotic stress. When plants are subjected to abiotic stress, mycorrhization up-regulates host AQPs expression to increase cell membrane permeability and improve transport of water and other solutes. Mycorrhizal symbiosis also down-regulates host AQP expression to reduce both cell membrane permeability and the loss of water and other solutes in plants. On the other hand, mycorrhizal fungal AQPs (*GintAQP*, *GintAQP1*, and *GintAQP2* from *Glomus intraradices*, and *RcAQP1*, *RcAQP2*, and *RcAQP3* from *Rhizophagus clarus*) respond to abiotic stress with a complementary mechanism, relative to host AQP expressions

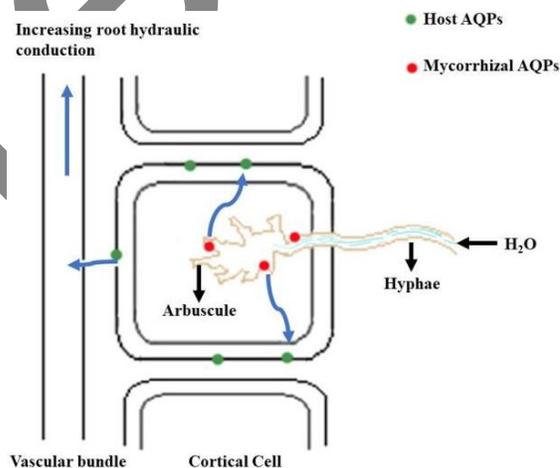


Fig. 2: A proposed synergistic mechanisms of both AMF and plant aquaporins to describe water movement. Here, mycorrhizal extraradical hyphae absorb water from growth substrates, and the water is further transferred in arbuscules of cortical cells. Fungal aquaporins located in arbuscules and intra-radical hyphae are involved in water movement across the membrane into cortical cells containing arbuscules. On the other hand, host aquaporin genes are induced to improve transport of water, resulting in the increasing of root hydraulic conduction; host aquaporin genes are down-regulated to reduce the loss of water or cell membrane permeability. The responses of both fungal and plant aquaporins collectively finish water absorption of hosts under abiotic stress

water transport, mycorrhizal fungal AQPs also contribute to efficient water absorption in mycorrhizal plants subjected to abiotic stress (Fig. 2) (Xu *et al.*, 2013), which is also an important mechanism of stress tolerance. In the identified AQPs of AMF, *GintAQP1* gene does not transport water,

whereas GintAQP1 and GintAQP2 gene is involved in water transport (Aroca *et al.*, 2009; Li *et al.*, 2013). In addition, drought stress induced the expressions of *GintAQP1* and *GintAQP2* in cortical cells containing arbuscules to regulate water flux across plasma membranes (Li *et al.*, 2013). *RcAQP3* and *LjNIP1* also expressed in mycorrhizal hyphae or inner membrane systems of arbuscule-enriched cells to take part in water transport across plasma membranes (Kikuchi *et al.*, 2016).

In brief, fungal and host AQPs could be regulated by abiotic stress for water absorption in mycorrhizal plants through root hydraulic conductivity, as well as for the regulation of osmosis between cytoplasm or between the cytoplasm and the apoplast (Yang *et al.*, 2005). Fungal AQP genes take part in the water movement across the membrane into cortical cells containing arbuscules (Fig. 2). Host AQP genes are induced to improve transport of water, resulting in the increasing of root hydraulic conduction; host AQP genes are down-regulated to reduce the loss of water or cell membrane permeability (Fig. 2). The responses of both fungal and plant AQPs synergistically finish water absorption of hosts under abiotic stress.

Conclusion and Outlook

AMF up- or down-regulates the plant AQP gene expression levels to increase root hydraulic conductivity or reduce water loss under stress conditions (Fig. 1). Fungal and host AQP genes collectively take part in water movement across the membrane (Fig. 2). AQP expression in some host plants are unchanged by mycorrhization under one kind of stress conditions, while they are induced under other stress conditions, indicating the complex behavior of AQP expression in response to mycorrhization under various stresses. More plant AQP isoforms should be studied to determine the regulation networks. AQPs are a multifunctional protein family from MIPs, some of which transport glycerol, urea, mineral nutrients, lactic acid, and hydrogen peroxide in addition to water. Future work should pay more attention to the role of AQP genes on solute transport under stress conditions. On the other hand, subcellular locations of AQPs in host should be analyzed to clarify their mycorrhizal roles in plant hydraulics, nutrient acquisition, and stressed responses. Currently, a small number of AQPs from *G. intraradices* and *R. clarus* only have been identified, but they are not enough to understand the AMF-enhanced tolerance in response to abiotic stress by mycorrhization with regard to AQPs. Future work should be done on other AMF species, and the location of these fungal AQPs in the mycorrhizal hyphae and the symbiotic cell organisms is also a hot research direction in the future.

Acknowledgements

This study was supported by the Hubei Provincial Department of Education (T201604), the National Key Research and Development Program of China

(2018YFD1000300), and the University of Hradec Kralove (Faculty of Science, VT2019-2021).

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(Received 23 October 2019; Accepted 13 December 2019)