Role of Aquaporins in the Maintenance of Xylem Hydraulic Capacity

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Abstract Terrestrial plants' well-being depends upon an uninterrupted supply of water from roots to leaves. Water stress or high transpirational demand results in an increase of water tension in the xylem, followed by an increased likelihood of embolism formation and reduction of xylem capacity to conduct water. The prolonged presence of xylem hydraulic dysfunction caused by embolism can have dramatic short- and long-term effects on plant function including the decrease of photosynthetic capacity, reduced vitality, or plant death. As the presence of embolisms is a negative trait, plants have evolved several strategies to prevent and/or mitigate the effects of hydraulic failure and restore xylem transport capacity. Recovery process requires a set of physiological activities that promote water flow into embolized conduits to restore its transport function. As hydraulic repair necessitates movement of water across xylem parenchyma cell membranes, an understanding of xylem-specific aquaporin expression patterns, their localization and activity are essential for the development of biological models describing embolism recovery process in woody plants. In this chapter, we provide an overview of aquaporin distributions and activity during development of drought stress, formation of embolism, and subsequent recovery from stress that result in restoration of xylem hydraulic capacity.

1 Water Transport and Embolism Formation

Terrestrial plants depend upon an uninterrupted supply of water from roots to photosynthetic tissue (Sperry 2003). This supply is guaranteed, in part, via the apoplastic axial transport of water through the lumens of interconnected dead cells characterized by thick, lignified walls. In the case of non-angiosperms, these cells

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are typically uniform in shape and length and connected one to the next via specialized bordered pits that include structures called the torus and margo (Fig. 1). Angiosperms, on the other hand, possess water transport conduits called vessels that are formed from continuous linear files of cells (vessel elements) with large diameters and are separated within a vessel by partially or completely digested walls referred to as perforation plates. One vessel is connected to the next by bordered pits, but these bordered pits do not include the torus and margo as they do in non-angiosperms (Fig. 1). The flow of a plant's water supply is driven by a decrease in water potential from soil to air and described using electrical analogs. Coupling this pressure-driven flow with the fact that the cellular conduits for flow are dead, we are led to consider the transport capacity of xylem in a purely physical context. Such a non-biological perspective on water transport has predominantly focused xylem research on the anatomy and morphology that protect transport from failure, however, while largely ignoring the role of living cells.

What is transport failure? Under drought stress or high transpirational demands, water tension (often described in plant literature as negative pressure) in the xylem increases, increasing the likelihood of embolism formation. When xylem tension
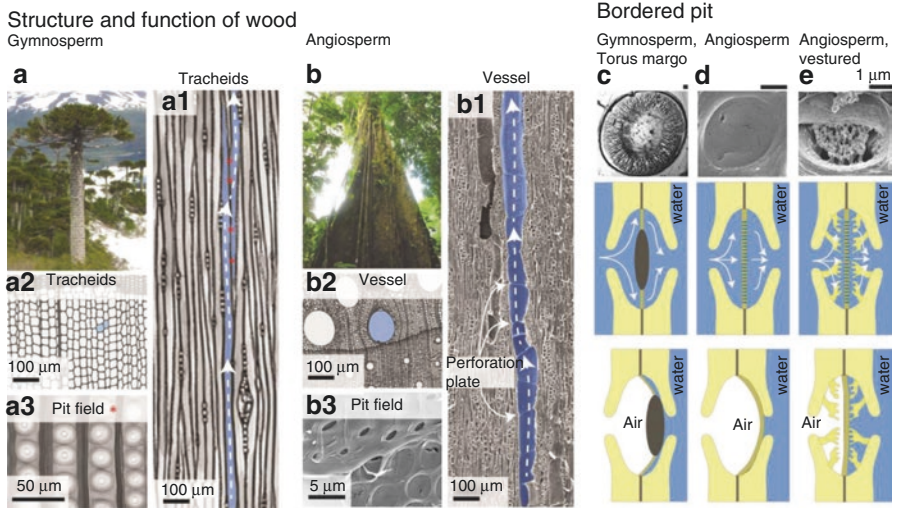
Fig. 1 Principles of wood structure in gymnosperms (**a**) and angiosperms (**b**) (**a1**) and (**b1**) depict typical features of conductive wood (xylem). In gymnosperms, the longitudinal conducting elements (tracheids (**a1**), cross-sectional view (**a2**)) are connected via linearly aligned bordered pits (**a3**). In angiosperm, wood vessels are composed from large cells that are vertically aligned and joined via fully or partially digested end walls (**b1**); vessels are also seen as large ovals in cross section (**b2**). Vessels are connected via fields composed from tens to hundreds of bordered pits (**b3**). The structure and function of xylem bordered pit pores vary between species (**c**–**e**). (**c**) Bordered pits of gymnosperm with torus and margo, (**d**) typical angiosperm pit, and (**e**) angiosperm with vestured pits. Drawings below (**c**–**e**) represent cross sections of bordered pits under normal nonembolized conditions and their potential function as protection from gas spread (Jensen et.al. 2016)

forces the radii of the air-water interface beyond a critical threshold, the tensile strength of water is overcome. This happens either as (1) air aspirates through the bordered pit membranes separating adjacent conduits or (2) preexisting gas bubbles spontaneously expand (Tyree and Zimmermann 2002). Embolism formation is considered to be a spatially and temporally unpredictable phenomenon related to the degree of tension in the xylem, the thermal environment, the physical properties of the xylem, the chemical properties of water, and a plant's previous embolism activity (Holbrook and Zwieniecki 1999; Hacke et al. 2001; Stiller and Sperry 2002; Tyree and Zimmermann 2002). As a consequence of embolism, a plant's water continuum is broken and transport is blocked via the vacuum or air-filled tracheid or vessel. Thus, the presence of embolism reduces a stem's capacity to transport water and can magnify leaf water stress, forcing stomatal closure and reducing leaf photosynthetic activity (Brodribb and Jordan 2008). In the event of a severe overload of the water transport system (when water loss exceeds transport capacity of xylem, runaway cavitation may occur resulting in plant death (Sperry et al. 1998). Therefore, the capacity of a plant to reduce the detrimental effects of embolism is an important trait for growth and survival (Tyree and Ewers 1991; Pockman et al. 1995; Choat et al. 2012; Barigah et al. 2013).

2   Why Do Plants Need to Remove Embolism?

The prolonged presence of xylem hydraulic dysfunction caused by embolism can have dramatic short- and long-term effects on plant function including the reduction of photosynthetic capacity, reduced vitality, or death. As the prolonged presence of embolisms is a negative trait, plants have evolved several strategies to prevent and/or mitigate the effects of hydraulic failure and restore xylem transport capacity post embolism. Although embolism formation is a purely physical process (Brenner 1995; Tyree and Zimmermann 2002), embolism removal requires that empty vessels fill with water against existing energy gradients as the bulk of water in the xylem remains under tension. Thus, recovery from embolism cannot happen spontaneously and necessitates some physiological activities that promote water flow into embolized conduits. The restoration of xylem capacity can be divided in two sets of strategies:

1. Strategies requiring both relief from water stress/transpiration and a prolonged period of time. This group includes shedding leaves or small branches (shrubs) to lower evaporative demand followed by the growth of new shoots, generating root pressure (small herbaceous plants) to refill embolized conduits, or growing new vessels or tracheids (radial xylem growth) to replace lost capacity with a new transport system (Sperry et al. 1987; Stiller and Sperry 2002). However, as these strategies depend on plant growth, they are slow and may result in the temporary loss of species competitiveness in a highly variable environment.
2. Strategies requiring cellular activities to dynamically repair embolized conduits and relieve tension. These strategies may be fast (minutes to hours) and thus

allow for greater flexibility in response to water stress (Zwieniecki and Holbrook 2009). They also provide protection from temporary reductions in photosynthetic capacity that might reduce competitiveness. Whether or not this type of refilling can occur in the presence of xylem tension has been difficult to prove although proposed conceptual theories attempt to reconcile experimental data with our physical understanding of xylem function (Tyree et al. 1999; Holbrook and Zwieniecki 1999).

Because the second group of strategies requires physiological activity in the xylem to maintain or restore transport function, it also requires that the xylem tissue is not dead. Indeed, even in woody plants, living cells constitute at least a few percent of the xylem and up to more than 80 % in baobabs where water storage is exceptional (Chapotin et al. 2006). The majority of living cells in the xylem are located in parenchyma rays – radially extending files of cells produced by the cambium alongside water conduits and often remaining in direct contact with vessels or tracheids. In many angiosperms, vessels are in contact with multiple parenchymal rays that link the vertical water transport system into an intricate network of interconnected pathways. At the extreme, vessels are fully surrounded by living cells – as in the case of the black locust (Robinia) (Fromard et al. 1995), multiple palm trees (Tomlinson et al. 2001; Tomlinson and Spangler 2002), and even maize roots (Barrieu et al. 1998). These parenchymal cells are often connected with vessels via simple pits with narrow straight walls. The role of living axial and radial parenchyma cells in the xylem remains ambiguous. They have been shown to store carbohydrates in the form of starch that may be used to support spring bloom or bud growth (Lebon et al. 2005, 2008; Sperling et al. 2015). In some cases, these cells are responsible for the formation of tyloses – vascular occlusions formed by the ingrowth of cells into the vessel through the pits. These ingrowths usually occur in winter (Cochard and Tyree 1990), in response to infection by pathogens (Beckman and Talboys 1981; Davison and Tay 1985) and/or in response to wounding (Sun et al. 2006, 2008) and completely cease the transport function of occluded vessels. Yet another potential function might be related to radial redistribution of water among functional vessels, cambium, and phloem, which may provide both water and energy to redistribute solutes and actively refill the embolized conduits.

Interestingly, both angiosperm and gymnosperm species transport water only in conduits adjoined to living parenchymal cells. The death of parenchyma cells inevitably is linked to the loss of water transport capacity and formation of heartwood. Cell death is most likely caused by a decrease in oxygen concentration rather than a loss of xylem water transport capacity (Spicer and Holbrook 2007). Thus the dependence of water transport on living parenchymal cells further suggests that cellular activity is the key aspect of xylem function maintenance over long time periods.

The major interruption of xylem water transport is embolism formation. The close association of viable xylem conduits and xylem parenchymal cells suggests that these living support cells are involved in xylem recovery from embolism, possibly enabling the mobilization of water against existing energy gradients. Visual evidence from cryo-scanning electron microscopy studies, magnetic resonance

imaging observations, and computed tomography scans shows that vessels indeed fill up with water during recovery (Holbrook et al. 2001; Clearwater and Goldstein 2005; Scheenen et al. 2007) and water droplets preferentially form and grow until the lumen completely refills on the vessel walls in contact with living parenchymal cells (Brodersen et al. 2010; Holbrook et al. 2001). However, these observational studies do not provide any indication of sources and pathways involved in moving the water required for recovery. As processes related to water transport across the cellular membrane involve the activity of specific water channels named aquaporins (AQP), the role of those and in particular the involvement of the plasma intrinsic proteins (PIPs) must be considered when contemplating how plants recover from embolism formation.

3 Aquaporins in the Vascular Tissue

The tissue-specific localization of AQP expression, with consideration of specific isoforms and rates of expression, can provide clues about the physiological roles of aquaporins and their temporal activity. The localization of AQPs is well described for leaves and roots of angiosperms, where they are expressed in the leaf sheath cells, in/around vascular bundles (*Arabidopsis*) and apoplastic barriers of roots (exodermis and endodermis), suggesting a crucial role in transmembrane water diffusion/control in the barriers separating the plant from its environment (Gambetta et al. 2013; Kirch et al. 2000; Perrone et al. 2012a; Chaumont and Tyerman 2014; Schaffner 1998; Suga et al. 2003; Hachez et al. 2006, 2008, 2012; Shatil-Cohen et al. 2011; Vandeleur et al. 2009; Prado et al. 2013). If xylem paren-chyma cells have to supply a significant fraction of the water required for refilling embolized vessels, water must pass through a cellular membrane, and therefore, the flow must be facilitated by aquaporins and can be controlled by the number, activity, and localization of these proteins. Despite reported observations of AQP abundance in stem tissues, only a few studies have focused on the xylem. Molecular and microscopic studies have revealed that AQPs are highly expressed in the xylem parenchymal cells. For instance, the ZmTIP1;1 is expressed in tono-plast of cells surrounding the mature xylem vessels of roots and stems and in the phloem companion cells of maize plants (Barrieu et al. 1998, Fig. 2). In spinach, the SoPIP1;2 is highly expressed in the phloem sieve elements of leaves, roots, and petioles while SoPIP1;1 is present in stomatal guard cells (Fraysse et al. 2005). A detailed description of tobacco NtAQP1 localization reports that younger stems express the protein in developing xylem vessels and internal phloem cells, while older stems accumulate the protein in the outer xylem border and internal phloem (Otto and Kaldenhoff 2000). This specific localization of AQP isoforms in or around conduits implicates their role in permitting a transcellular water transfer between xylem conduits and, potentially, phloem via xylem parenchyma cells. It also reflects geometry of water transport, as flux density is highest near the narrow conduits.
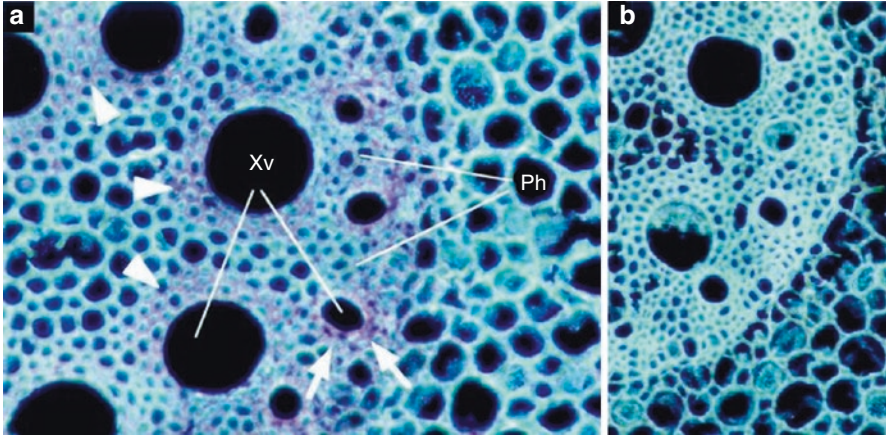
Fig. 2 *In situ* localization of ZmTIP1 mRNA in mature maize root. Transverse sections of the root (10–12 cm from the tip) were hybridized with ZmTIP1 antisense (**a**) or sense (**b**) digoxigenin-labeled RNA probes and photographed under dark-field conditions. The transcript signal is *red*. (**a**) Expression of ZmTIP1 in the parenchyma cells of early (*arrows*) and late (*arrowheads*) xylem vessels. *Xv* Xylem vessels, *Ph* phloem strand. (**b**) Control section hybridized with a ZmTIP1 sense probe (Barrieu et al. 1998)

Most studies on AQPs have applied bulk tissue analysis to herbaceous plants (i.e., total xylem and/or bark), while the stems of trees have received much less attention. However, detailed information for trees showing the localization of AQP expression in particular cells and tissue types is reported for the stems of a hybrid poplar (Almeida-Rodriguez and Hacke 2012). There, the greatest accumulation of expression occurred in the cambial region and adjacent xylem-phloem cells. Aquaporin accumulation was also detected in ray cells. Interestingly, the cells connected with vessels through pits, or contact cells, exhibited particularly high AQP protein expression, suggesting an increased potential for water exchange between apoplast and symplast. The ray cells not in contact with vessels, or isolation cells, accumulated water channels to varying degrees. Additional studies on walnut (*Juglans regia*) showed a higher expression of two aquaporin proteins (JrPIP2.1 and JrPIP2.2) in specific vessel-associated parenchyma cells (VACs), which are living cells in direct contact with vessels (Sakr et al. 2003, Fig. 3). In a study with another woody perennial, the aquaporin transcript profile was examined on VACs isolated from petioles (by laser microdissection) and on whole petioles of grapevine, confirming their specificity. While some of the *VvPIP1-* and *VvPIP2-* tested genes were activated by stress and subsequent recovery in whole petioles, some aquaporin genes *VvPIP1;1* and *VvPIP2;4N* were exclusively expressed in VACs (Chitarra et al. 2014).

Another omission in the AQP localization literature applies to non-angiosperm plant groups like ferns and gymnosperms. Available data on the expression of aquaporins in the needles of *Picea glauca* show that for drought-stressed trees, expression is abundant in the endodermis-like bundle sheath, in phloem cells, and in
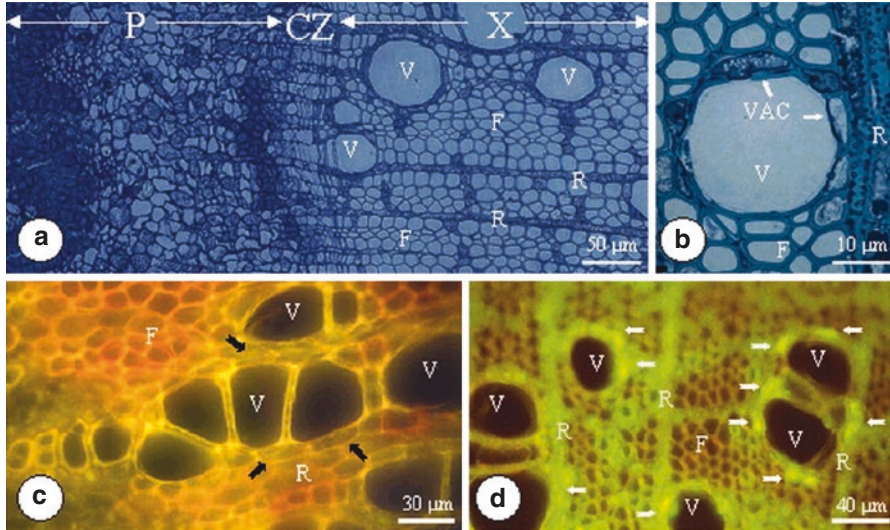
Fig. 3 Transversal section showing the general organization of the stem in walnut tree. (**a**) Cambium zone appears at the phloem-xylem interface. Xylem contains lignified xylem vessels and fibers (died cells) associated with parenchyma cells (living cells). The support tissue is constituted of fibers emptied of their content. (**b**) Cell types in walnut xylem. VACs (*white arrows*) are specialized cells that surround the xylem vessels. From their localization in the tissue, VACs have the ability to control nutrient exchanges between the parenchyma cells and the xylem vessels. Localization of aquaporins in xylem tissue of walnut tree sampled in February (winter period). (**c**) No recognition in control (AtPIP2 antiserum saturated by the purified JrPIP2,1 protein). *Black arrows* indicated the localization of VACs. (**d**) Section showing extensive green immunofluorescence (*white arrows*) in VACs. *X* xylem, *P* phloem, *CZ* cambial zone, *V* xylem vessel, *F* fiber, *R* ray cell (Sakr et al. 2003)

transfusion parenchyma tissue, further suggesting that water channels are localized in vascular tissue (Laur and Hacke 2014a). Similar results were reported by Mayr et al. 2014, showing higher amounts of PIP1 and PIP2 proteins in the endodermis and phloem cells of the needles of Norway spruce (*Picea abies*). In *Cheilanthes lanosa*, a xerophytic fern, it was shown that a PIP1 might have a key role in water balance mainly in the gametophyte stages (Diamond et al. 2012).

4 Aquaporins in Stems Under Water Stress and Embolism Formation

Water stress has a strong influence on AQP gene expression (see also chapter "Plant Aquaporins and Abiotic Stress"). However, studies attempting to relate physiological water stress responses to the expression patterns of different aquaporins have led to contrasting results. Upregulation, downregulation, and no change have all been reported (Baiges et al. 2002). Variation in the range of transcriptional responses

might be species or tissue specific, associated with stress level and duration, or dependent upon the specific physiological role of each AQP gene isoform (Alexandersson et al. 2005; Kaldenhoff et al. 2008; Galmes et al. 2007). Furthermore, differences between drought-adapted and nonadapted varieties can affect aquaporin expression (Lian et al. 2004). Thus, it is currently very difficult to provide a general pattern of AQP gene expression in response to water stress; aquaporin upregulation is thought to increase membrane permeability to water transport when water is less available (Yamada et al. 1997), but the downregulation of AQP gene expression may encourage cellular water conservation during periods of water stress (Smart et al. 2001; Li et al. 2004). It is probable that in order to maintain a suitable water status under abiotic stress, both increased water transport via AQP in some tissues and reduced water transport in other tissues are required (Jang et al. 2004).

The effects of drought treatment on the expression of *AQP* genes have been studied in numerous species, and the downregulation has been frequently observed. *PIP* and *TIP* genes are downregulated in the leaves, shoots, and roots of *Nicotiana glauca* (Smart et al. 2001). In the leaves of *Arabidopsis*, the gradual imposition of drought stress downregulated 10 out of 13 PIP aquaporins at both transcript and protein levels. Of the three remaining, one of the isoforms (*AtPIP2;6*) was maintained at the same expression level and two (*AtPIP1;4* and *AtPIP2;5*) were upregulated (Alexandersson et al. 2005). The strong downregulation of *PIP* gene transcription under drought stress was also observed in the roots and twigs of olives (Secchi et al. 2007a, b) as well as in tobacco roots (Mahdieh et al. 2008) and in the leaves of *Populus trichocarpa* (Laur and Hacke 2014b).

Evidence for the downregulation of AQPs in response to drought may be contrasted with data suggesting that some tissue-specific AQP isoforms show increased expression in response to drought. For example, the *VvPIP1;1* gene in the roots of grapevines was upregulated by drought stress in an anisohydric but not in an isohydric cultivar (Vandeleur et al. 2009). In the stems of *P. trichocarpa*, expression levels of the PIP2 subfamily did not change in response to water stress or embolism presence, while some genes from the PIP1 subfamily were highly upregulated (Secchi and Zwieniecki 2010). Similar results were found by Chitarra et al. 2014 showing that two aquaporin genes (*VvPIP2;1* and *VvPIP2;4N*) were activated upon stress in petioles. Two other studies performed on different rootstocks of *Vitis* sp. showed similar results where drought treatments resulted in significant variations (both up- and downregulation) in leaf aquaporin gene expression over time (Galmes et al. 2007; Pou et al. 2013).

It is difficult to isolate response to embolism specifically from response to water stress in general and, consequently, analyses of embolism-induced AQP expression may often be confounded by water-stress-induced AQP expression. As mentioned earlier, the expression of aquaporins in *J. regia* was induced in VACs in response to water stress, while xylem parenchyma cells not in contact with vessels varied in their AQP expression due to water stress (Sakr et al. 2003). Considering this pattern and after imposing stress levels large enough to cause widespread embolism in the xylem of *J. regia,* it was inferred that the presence of embolism is associated with the expression of at least two aquaporins (JrPIP2.1 and JrPIP2.2). The progression of recovery in both stem water potential and stem water conductance did not immediately reduce

the expression suggesting that embolism presence or rather a lack of vessel functionality in terms of water transport under tension was required to maintain this elevated expression level of studied AQPs in VACs. It is worth noting that this over expression of AQPs in VACs was ubiquitously occurring along the xylem vessels and continued to be present during recovery suggesting that the two studied proteins may play a role in the regulation of water flux between VACs and adjacent vessels (Sakr et al. 2003) by redistributing water between functional and embolized vessels.

The induction of water stress is usually a slow process that in natural conditions can extend for days or even weeks. The prolonged implementation of the stress might be the basis for the high variability in AQP expression observed in response to drought treatments. Embolism formation is, on the other hand, a very fast event that results in both the evacuation of vessels and the cessation of water movement due to the blocking of conduits by embolisms in distal locations. Can plants exclusively respond to embolism formation without stress-related changes? To answer this question, a study involving the induction of embolism in the stem of *P. trichocarpa* plants and the determination of concurrent expressions of AQPs was performed (Secchi et al. 2011; Secchi and Zwieniecki 2010). In these studies, embolism was induced by forcing air into the stem of non-stressed plants. Both a genome-wide analysis and the specific analysis of selected genes showed that the expression of some AQPs from the PIP1 subfamily (*PtPIP1.1* and *PtPIP1.3*) in poplar stems increased due to embolism presence alone without changes in stem water potential. This expression change occurred in less than half an hour following embolism formation (Secchi and Zwieniecki 2010; Secchi et al. 2011). These studies suggest that plants can possibly sense the formation of embolism (or its presence) separately from water stress.

There is no direct evidence that embolism formation independent of water stress can induce AQP expression in conifers. However, conifers growing at high elevations are often subjected to winter embolism (Sparks and Black 2000; Mayr et al. 2002, 2006), and some of these species were found to recover from hydraulic failure in late winter and spring when the snow on branch surfaces started to melt (Sparks et al. 2001; Limm et al. 2009). In such situations, water stress is minimal as humidity and water availability are very high despite the fact that conduits are mostly embolized. In these conditions, increased amounts of PIP1 and PIP2 proteins in the needle endodermis and phloem cells were detected (Mayr et al. 2014) (providing support that conifers also may detect embolism and respond to its presence with the upregulation of a few specific AQPs).

5   The Role of Aquaporins in the Maintenance of Xylem Water Transport

Observed changes in AQP expression during the onset of water stress may be interpreted and tested at the sites of water exchange between plant and environment. For example, an increase in AQP expression or activity might aim to reduce the

resistance to water flow at distally located water uptake sites such as roots in order to beneficially reduce stress. Conversely, decreasing AQP expression and activity and so increasing resistance to water movement across living cell membranes might be beneficial at sites of water loss like leaves. In fact, the roles of root and leaf aquaporins in relation to changes in hydraulic resistance and plant susceptibility to stress have been tested using genetic manipulation approaches. For example, tobacco plants (*Nicotiana tabacum)* with downregulated aquaporin 1 (NtAQP1) show reduced root hydraulic conductivity and lower water stress resistance (Siefritz et al. 2002). *Arabidopsis thaliana* plants expressing *PIP* antisense genes exhibited an impaired ability to recover from water stress (Martre et al. 2002), and *Arabidopsis* knockout mutants were characterized by reduced leaf hydraulic conductivity (Da Ines et al. 2010) and root hydrostatic hydraulic conductivity (Postaire et al. 2010). Plants with silenced *PIP1* genes demonstrated decreased transcript and protein levels and decreased mesophyll and bundle sheath osmotic water permeability among many other physiological parameters (Sade et al. 2014). On the other hand, *Arabidopsis* expressing an AQP (PIP1) from *Vicia faba* exhibit a faster growth rate, a lower transpiration rate, and a greater drought tolerance compared to control plants (Cui et al. 2008). A better tolerance to several abiotic stresses was also displayed in transgenic banana plants overexpressing an isoform of the PIP1 subfamily (Sreedharan et al. 2013). All of these studies have focused on distal locations (roots, leaves), or ubiquitous changes in expression throughout the entire plant, however, and so do not provide insight into the physiological role of AQPs expressed in vascular tissue.

Interestingly, applications of genetic manipulation technologies to elucidate the roles of particular aquaporins in woody plants have not been as successful as equivalent studies in herbaceous plants. For example, the overexpression of PIP2;4 root-specific aquaporin enhanced water transport in transformed *Vitis* spp. under well-watered conditions, but not under water stress (Perrone et al. 2012a), and *Eucalyptus* spp. hybrid clones overexpressing two *Raphanus sativus* genes (*RsPIP1;1* and *RsPIP2;1*) did not display any increase in drought tolerance (Tsuchihira et al. 2010). The downregulation of the entire PIP1 family in *Populus tremula x alba* by 80 % also had a very minimal effect on the majority of physiological functions prior to the onset of water stress (Secchi and Zwieniecki 2013). These ambiguous results might be related to the fact that in woody plants the xylem contributes significantly to a plant's stress response only. Considering that xylem functionality likely dominates hydraulic resistance under stress due to dynamic changes in embolism level, a tree's overall response might not depend on the distal locations of water exchange with the environment so much as AQPs that maintain xylem hydraulic capacity.

The indication that AQPs are indeed involved in the maintenance of xylem hydraulic function involves the earlier described localization of AQPs in xylem parenchymal cells, including VACs, observed increases in expression with the onset of embolism formation, and the fact that water droplets grow, form, and expand on walls in contact with living cells. This deductive interpretation of evidence is supported by observations of transgenic poplar trees characterized by the dramatic

downregulation of multiple isoforms belonging to the PIP1 subfamily (Secchi and Zwieniecki 2014). As different poplar PIP1 isoforms in the stem were upregulated in response to the induction of embolism in the presence of water stress and were downregulated soon after full recovery occurred (Secchi and Zwieniecki 2010), the downregulation of AQPs was expected to delay the hydraulic restoration process. Indeed, transgenic plants with significantly reduced amounts of various AQP isoform transcripts in *P. trichocarpa* leaves (Laur and Hacke 2014b) and stems (Secchi and Zwieniecki 2014) significantly delayed the restoration of leaf hydraulic conductance and xylem functionality upon recovery from water stress. In addition to the delay in recovery, an unexpected finding was that the downregulation of PIP1 expression resulted in a significant shift in the susceptibility of *P. tremula x alba* xylem to embolism formation; the transgenic poplars were found indeed to be more sensitive to imposed water stress resulting in increased vulnerability to embolism formation (Secchi and Zwieniecki 2014). This shift in susceptibility to embolism formation holds important clues to both the role of aquaporins in xylem responses to embolism and the recovery process itself. As embolism formation is considered a function of cellular wall chemistry and xylem anatomical features, aquaporins should not affect susceptibility to embolism. Thus, the observed shift must be the result of physiological processes happening in the xylem during its normal function and should be referred to as an "apparent susceptibility." As we currently understand it, apparent susceptibility to embolism is a balance between the rates of embolism formation (functionally linked to water stress) and the capacity of living parenchyma cells to refill (inversely linked to water stress) (Secchi and Zwieniecki 2012, 2014). Because the latter is related to the parenchymal capacity to redistribute water between living cells and the xylem apoplast, it is thus a function of aquaporin activity. In such a context, it is easy to see that the downregulation of AQPs may affect this balance by both reducing the capacity to refill and shifting apparent susceptibility.

The radial redistribution of stem water during the process of hydraulic recovery may only be a secondary role while the primary role of stem AQPs is related to the absorption of water from the environment. Some recent studies suggest that many plant species have the capacity to absorb rain, melting snow, or fog water directly into their leaves and even through bark. This strategy of water uptake provides a means to relieve localized disruptions to hydraulic conductivity and to reduce tracheid embolization (Limm et al. 2009; Mayr et al. 2014; Laur and Hacke 2014a; Earles et al. 2015). In such a case, stem AQPs may behave similarly to roots and leaves, following the typical function/expression patterns observed in distal locations, i.e., activation during times of water availability (wet bark) and deactivation during drought. For example, increased aquaporin expression in the endodermis-like bundle sheath, phloem cells, and transfusion parenchyma of drought-stressed *P. glauca* needles was observed when the same needles were exposed to high relative humidity, supporting the idea that there is a role for AQPs in transferring absorbed water to vascular tissue (Laur and Hacke 2014a). The reconciliation of observed increases in AQP expression in the stems of drought-stressed trees and the above-mentioned role of AQPs in the transfer of water from wet bark requires an analysis of the temporal and spatial aspects of expression distribution.

In general, drought results in the induction of AQP expression in the xylem but not necessarily in the outer part of the stem. Large concurrent changes in the expression of other gene groups, however, may complete the pathway. In poplar xylem the upregulation of ion transport, additional aquaporins, and carbon metabolism has been detected (Secchi et al. 2011). Carbon metabolism and aquaporin expression were also strongly upregulated in drought-stressed grapevine petioles (Perrone et al. 2012b). This type of drought upregulation may be an indication of the stem priming for recovery. Under natural conditions, prolonged drought should eventually end with a significant rain event and the recovery of stem function. Even if drought leads to the loss of leaves, a rain event would wet the entire tree, including bark, providing easily accessible water in a relatively short period of time. Uptake would be facilitated if the hydraulic resistance of the path is low, and the energy gradient favors the flow of water into the stem. The cambium with its limited apoplastic path and multiple layers of membrane serves as a significant barrier to water flow, and the reduction of resistance requires the presence of aquaporin (Barrowclough et al. 2000). In addition, if the plant can manage to direct flow into embolized vessels with the specific localized expression of AQPs in VACs (see Chitarra et al. 2014), then such global crown wetting may indeed provide a mechanism for the recovery of functional xylem transport.

6   Aquaporin Upregulation in the Xylem: Signaling

Very little is known about embolism-related regulation of aquaporins in the xylem. There are currently two, not mutually exclusive, experimentally supported views on the topic. The first idea about signaling relies on the sudden transition between high tension when water is in its liquid state to zero tension/pressure when water under tension converts to vapor, releasing mechanical stresses in the cellular wall as well as energy in the form of sound (Tyree and Sperry 1989; Nardini et al. 2011; Tyree and Dixon 1983; Johnson et al. 2009; Zweifel and Zeugin 2008). Such spatially specific events are thought to interact with mechanosensors in VAC membranes and trigger embolism-specific expression patterns that are upregulated until the restoration of tension is achieved (Salleo et al. 2000). This idea has some experimental support, as there have been experiments aimed to test generation of mechanosensor triggering without the implementation of water stress (see more details in chapter "Root Hydraulic and Aquaporin Responses to N Availability"). We do, however, know that induction of embolism in non-stressed plants (when mechanical stresses are at their minimum) also results in the triggering of AQP expression (Secchi and Zwieniecki 2010, 2011).

The second signaling path relates to the fact that embolism formation stops water transport around VAC cells, dramatically changing surrounding mass flow/diffusional paths including the rate of diffusion of respirational CO_2, thus facilitating its efflux to the void and changes in apoplastic pH, concentration of ions and sugars, and the cell vicinity by cessation of the washout effect of the transpirational stream (Nardini et al.

2011). All of these changes are known to be involved in multiple signaling paths including expression of AQPs. Recent experiments aimed at testing if the presence of sucrose in the stem would trigger a similar expression response as in the case of embolism only showed that indeed multiple gene ontology (GO) groups including ion transporters, AQPs, and reactive oxygen species responded similarly, suggesting that there might be some shared signaling pathways between sugar concentration and embolism presence (Secchi and Zwieniecki 2011). Despite these efforts, the question on what signaling paths lead to embolism-specific expression changes is wide open.

7 Conclusions

The role of aquaporins in the maintenance of xylem hydraulic function remains an active research field. The spatial distribution of AQPs in xylem parenchyma cells, the dynamics of expression in response to the development of water stress, and during the recovery from water stress only indirectly point to their role in facilitating recovery from embolism. With exception of few studies using chemical inhibitors of aquaporins (Lovisolo and Schubert 2006; Voicu and Zwiazek 2010), most of the observations aimed at testing radial water transport in stems and leaves in relation to AQPs were correlative and not manipulative and still need direct experimental proof of concept. The genetic manipulation of expression level designed specifically to test stem hydraulic recovery was only performed on one species (*P. tremula x alba*) and only for one subfamily of AQPs genes (PIP1) (Secchi and Zwieniecki 2014). These results proved that while the susceptibility of xylem hydraulic capacity to water stress was affected by the downregulation of AQPs, recovery was only marginally affected. This suggests that our understanding of the embolism formation-recovery cycle is the main obstacle to progress in our knowledge of the physiological role of xylem aquaporins.

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