AperTO - Archivio Istituzionale Open Access dell'Università di Torino

The functional role of xylem parenchyma cells and aquaporins during recovery from severe water stress

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1609328> since 2021-11-08T14:16:46Z

Published version:

DOI:10.1111/pce.12831

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

The functional role of xylem parenchyma cells and aquaporins during recovery from severe water stress.

Francesca Secchi¹, Chiara Pagliarani¹, and Maciej A. Zwieniecki²

¹Department of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, Italy

²Department of Plant Sciences, UC Davis, Davis, CA, USA

Corresponding author: Maciej Zwieniecki

Department of Plant Sciences, UC Davis, One Shields Avenue, Davis 95616, USA

Email: mzwienie@ucdavis.edu

Abstract

Xylem parenchyma cells (VACs) constitute a significant fraction of the xylem in woody plants. These cells are often closely connected with xylem vessels or tracheids via simple pores (remnants of plasmodesmata fields). The close contact and biological activity of VACs during times of severe water stress and recovery from stress suggest that they are involved in the maintenance of xylem transport capacity and responsible for the restoration of vessel/tracheid functionality following embolism events. As recovery from embolism requires the transport of water across xylem parenchyma cell membranes, an understanding of stem-specific aquaporin expression patterns, localization and activity is a crucial part of any biological model dealing with embolism recovery processes in woody plants. In this review we provide a short overview of xylem parenchyma cell biology with a special focus on aquaporins. In particular we address their distributions and activity during the development of drought stress, during the formation of embolism and the subsequent recovery from stress that may result in refilling. Complemented by the current biological model of parenchyma cell function during recovery from stress, this overview highlights recent breakthroughs on the unique ability of long-lived perennial plants to undergo cycles of embolism-recovery related to drought/rewetting or freeze/thaw events.

Keywords: living parenchyma cells, aquaporin, embolism, xylem refilling

Introduction

Trees regularly cope with environmental stressors, many of which have been exacerbated recently by climatic alterations across the planet. Over the years the plant physiology community has focused increasing attention on drought stress, which is known to induce a complex network of physiological effects including the xylem embolism formation/recovery cycle. The way in which plants sense and recover from embolism is a matter of particular research interest because of its relevance to their intrinsic ability to handle the transport of water under tension. However, while embolism formation is a purely physical phenomenon related to xylem chemistry and morphology, xylem refilling requires the generation of water flow against a pressure gradient. Thus, recovery from embolism can only be understood through consideration of biological activities capable of providing the energy and water needed to restore hydraulic conductivity. In recent years, significant efforts have been made to gain knowledge on this debated process, but a comprehensive understanding of the biological processes involved in xylem recovery from embolism in tree stems remains elusive.

This review explores recent research advances in woody plant embolism repair theories, which take into account the biological processes occurring at stem and cellular levels.

Xylem morphology and parenchyma cells in woody plants

Long-distance water transport in vascular plants occurs through a network of conduits built from nonliving cells (xylem) along the stem, branches, twigs, petioles, and leaf veins which connect roots to leaf mesophyll cells (Sperry *et al.*, 2003, Tyree & Zimmermann, 2002). The xylem represents about 99% of the entire length of the water transport pathway from roots to photosynthetic tissues and the remaining 1% consists of a few millimeters of extra-vascular

pathways that water follows when moving from the root surface to the stele and from leaf minor veins to evaporation sites (Cruiziat *et al.*, 2002, Nardini *et al.*, 2011b).

Xylem conduits are long hollow tubes with finite dimensions, dead at maturity (Carlquist, 2015, Comstock & Sperry, 2000). They are characterized by thick and lignified walls capable of sustaining large negative pressures (Hacke *et al.*, 2001a, Pittermann *et al.*, 2006). Gymnosperms possess xylem conduits (tracheids) that are uniform in shape and length and connected to each other through small openings in the secondary cell walls (bordered pits). Most conifers have a pit membrane structure with a porous margo and central torus assembly (Zimmermann, 1983, Choat *et al.*, 2008, Pittermann *et al.*, 2005). In Angiosperms, the water transport conduits are more specialized vessels consisting of drum-shaped cells (vessel elements). The end walls between vessels are open (perforation plates) with elements stacked end on end to form long tubes extending over several cm or even meters. Water transfer from vessel to vessel occurs through bordered pit-fields, which consist of multiple small openings separating adjacent vessels with thin cellulose/pectin membranes (Holbrook & Zwieniecki, 2005, Tyree & Zimmermann, 2002).

Tracheid and vessel elements are the key structural components of long-distance water transport, but the xylem as a whole is not made of solely dead conduits. The bulk of secondary xylem (functional xylem) contains, besides fibers, an interconnected network of living cells that links heartwood (non-functional xylem compartmentalized within the stem) and phloem (stem parenchyma cells). These parenchyma cells usually have thin walls and are rectangular or square in shape (Morris *et al.*, 2016). They are formed by fusiform and ray initials of the vascular cambium and are oriented both axially and radially. The living parenchyma cells can represent a large component of the tissue volume and the abundance of those varies across environments,

plants organs and species (Holbrook & Zwieniecki, 2005, Spicer, 2014). Stems have fewer, smaller and tighter ray parenchyma cells than the roots (Denne & Gasson, 2008, Morris *et al.*, 2016, Pratt *et al.*, 2007). Moreover, recent meta-analysis has found significant differences between volumes of ray and axial parenchyma across climatic zones with higher volumes observed in tropical trees and lower volumes in trees and shrubs growing in temperate and subtropical areas (Morris *et al.*, 2016). Conifers were found to have far less radial and axial parenchyma in xylem than angiosperms. Lianas and stem succulents represent some of the most parenchyma-rich stems in the plant world (Spicer, 2014), a pattern which reflects an increased demand for mechanical elasticity in climbing plants and the need for water storage in succulents (Brandes & Barros, 2008, Carlquist, 2015, Chapotin *et al.*, 2006, DeSmidt, 1922).

It is assumed that living parenchyma cells play many important functional roles. However, specific functions are often derived indirectly as the location of these cells makes them difficult to study. Among these functions, the loading/unloading of solutes into/from the transpiration stream (De Boer & Volkov, 2003) and the storage and transport of carbohydrates as soluble sugars, starch and/or lipids are most often considered (Bucci *et al.*, 2003, De Boer & Volkov, 2003, Salleo *et al.*, 2004, Secchi *et al.*, 2011b, Spicer, 2014, Zwieniecki & Holbrook, 2009). In addition, they may play a significant role in defense against pathogens by preventing their lateral and axial spread (Deflorio *et al.*, 2008, Morris & Jansen, 2016) and accumulating anti-microbial compounds. Some evidence of their involvement in mechanical support is also reported (attributed mostly to ray parenchyma) (Arbellay *et al.*, 2012, Reiterer *et al.*, 2002).

Recent studies point to the role of living stem parenchyma cells pathways between mature xylem and phloem, as xylem conduits are both physically and functionally associated with living phloem. Physical association is derived from a single cambium initial that produces both xylem

and phloem derivatives (Larson, 1994), thus the radially oriented parenchyma cells grouped together in rays extend from xylem to phloem. This functional association is demonstrated by several examples of the interchange of water and solutes between xylem and phloem (Metzner *et al.*, 2010, Nardini *et al.*, 2011b, Schneider *et al.*, 1994, Vanbel, 1990, Wang *et al.*, 1997).

Additionally, parenchyma cells are shown to be involved in water storage (as confirmed by the high amount of ray and axial parenchyma in stem succulents) and xylem hydraulic capacitance (Barnard *et al.*, 2013, Holbrook *et al.*, 2002, Pfautsch *et al.*, 2015, Salleo *et al.*, 2004). Therefore, a greater amount of axial and radial parenchyma cells in wood may confer higher stem hydraulic capacitance. These properties: (1) facilitation of transport between phloem and xylem, (2) energy and osmotic storage capacity, and (3) water storage capacity were the basis of the recently proposed role of stem parenchyma cells as a crucial component in the maintenance of xylem transport capacity and embolism removal (refilling) even under small negative tensions (Nardini *et al.*, 2011a, Nardini *et al.*, 2011b, Salleo *et al.*, 2004, Secchi & Zwieniecki, 2011, Zwieniecki & Holbrook, 2009). Specifics of the biology behind and the role of parenchyma cells in embolism-recovery are detailed in this review.

Although there is no single confirmed theory explaining the dynamics of embolism repair in all vascular plants, it is worthwhile noting that compared to angiosperms, gymnosperms tend to have little parenchyma in their wood. If we assume that the ability to rapidly repair embolisms relies on the presence of nearby parenchyma cells, this may explain the long length of conifer embolism recovery time (days or months) and their need for a larger safety margin when compared to angiosperms experiencing comparable levels of embolism (Johnson *et al.*, 2012; Johanson 2012). It is also proposed that conifers may utilize an entirely different approach to embolism recovery that depends on the tree's ability to restore functional water potentials when

exposed to fog or snow (Earles *et al.*, 2016, Mayr *et al.*, 2014). In this review we will focus on the biology of parenchyma cells in angiosperm species and discuss their biological role in xylem recovery from severe water stress.

Parenchyma function in xylem embolism repair

Water transport in the xylem is a purely physical process driven by a difference in water pressure. Water transport through a network of dead cellular conduits occurs under negative pressures (tension). Since the apoplastic water column in the xylem is under tension, it is considered to be in a metastable state (Stroock *et al.*, 2014) and at risk of cavitation. Although the tensions experienced by trees are far less than the tensions required to cause homogeneous cavitation, they may be large enough to trigger cavitation from seeding sites – like the micron or sub-micron sized air pockets present in the vessel crevices (Tyree & Sperry, 1989). Expansion of these gas bubbles results in the formation of embolisms that can quickly spread through an entire vessel. Further spread of embolism from vessel to vessel may occur via the penetration of air bubbles through bordered pit membranes (Brodersen *et al.*, 2013). Embolism formation is a purely physical process (Brenner, 1995, Tyree & Zimmermann, 2002) related to the degree of tension in the xylem, the chemical properties of water, the thermal environment and the physical properties of the xylem (Hacke *et al.*, 2001b, Holbrook & Zwieniecki, 1999, Stiller & Sperry, 2002, Tyree & Zimmermann, 2002). The presence of an embolism disrupts the plant's water continuum by reducing xylem transport capacity and causing short and long-term effects on plant functions. Effects include stomatal closure, reductions in the rate of photosynthesis, reductions of growth, loss of production, and even plant death (Brodribb & Cochard, 2009, Sperry *et al.*, 1998).

148 As the prolonged presence of embolisms is a threat for plant survival, species have evolved
149 several strategies to prevent and/or alleviate the effects of hydraulic failure and restore xylem
150 transport functionality. These include anatomical xylem adaptation (specialized pit membrane
151 structures, stem sectoriality, wood density), the shedding of leaves or small branches (shrubs) to
152 lower evaporative demand and growing new vessels to replace nonfunctional conduits
153 (Zwieniecki & Secchi, 2015). These processes are very slow and necessitate prolonged relief
154 from water stress/transpirational demand. We might consider these long-term strategies non-
155 competitive. However, some species growing in competitive environments demonstrate the
156 evolution of active physiological strategies that lead to the quick recovery of xylem hydraulic
157 functionality. These strategies include a) the generation of positive root pressure when the soil is
158 fully saturated (often found only in small herbaceous and smaller woody plants) (Cochard *et al.*,
159 1994, Ewers *et al.*, 1997, Yang *et al.*, 2012); b) access to external water sources (rain, fog or
160 snow) in order to facilitate water uptake and water flow into the xylem through leaves, buds
161 and/or bark, a strategy adopted principally by coniferous species (Earles *et al.*, 2016, Laur &
162 Hacke, 2014, Mayr *et al.*, 2014); c) cellular activities of living xylem parenchyma cells, resulting
163 in fast (minutes to hours) localized embolism removal in woody plants (observed primarily in
164 angiosperm species) (Brodersen & McElrone, 2013, Brodersen *et al.*, 2010, Nardini *et al.*,
165 2011b, Salleo *et al.*, 2004, Secchi & Zwieniecki, 2012), which may even occur during the
166 presence of active transpiration (Holbrook & Zwieniecki, 1999, Trifilò *et al.*, 2003). The
167 presence of great tension most likely precludes the occurrence of xylem refilling but recovery
168 has been reported under low-tension levels. Reconciling the presence of tension with embolism
169 recovery has been proved difficult to understand and only recently *in vivo* imaging has suggested
170 the ability of plants to refill embolized vessels in situations with very low tensions (below 0.5

171 MPa) (Brodersen *et al.*, 2010, Clearwater & Goldstein, 2005, Knipfer *et al.*, 2015, Zwieniecki *et*
172 *al.*, 2013). A recent work using X-ray micro CT analysis has shown that in grapevine refilling
173 can occur without presence of root pressure and it is osmotically driven against low negative
174 water potential (Knipfer *et al.*, 2016). However, despite scientific efforts (Nardini *et al.*, 2011a,
175 Salleo *et al.*, 1996, Secchi & Zwieniecki, 2010, Zwieniecki & Holbrook, 2009), the biological
176 mechanisms responsible for embolism recovery under low negative pressure are not resolved
177 beyond the general statement that living cells are involved in the recovery process.

178 Although embolism formation is a physical process, its removal requires that empty vessels fill
179 with water against existing energy gradients as the bulk of water in the xylem remains under
180 tension. In this scenario, recovery from embolism cannot happen spontaneously and necessitates:
181 1) some physiological activities in the xylem to maintain or restore transport function (promoting
182 water flow into empty conduits) and 2) the involvement of living parenchyma cells able to
183 perform physiological activities during the recovery process. Existing models of xylem repair
184 suggest that living parenchyma cells, adjacent to the xylem vessels, are at the forefront of the
185 refilling process (Salleo *et al.*, 2004). This assumption is supported by the observations of
186 inhibition or reduction of refilling in the case of either physical damage to phloem transport or
187 the metabolic inhibition of living cells in stems (Bucci *et al.*, 2003, Salleo *et al.*, 2004,
188 Zwieniecki *et al.*, 2004). It was concluded that phloem might be involved in supplying the
189 energy to sustain xylem recovery (Bucci *et al.*, 2003, Salleo *et al.*, 2004) and since there is a
190 physical separation of phloem from xylem conduits, living parenchyma cells can function as
191 bridges that allow solutes (and water) to flow from phloem to embolized conduits (Nardini *et al.*,
192 2011a). This pathway may involve multiple crossings of cellular membranes, thus being
193 mediated by the activity of water channels (aquaporins), sugar transporters, and plasmodesmata.

Xylem parenchyma cells in contact with xylem conduits are assumed to simultaneously generate the energy gradient (deposition of solutes in the form of sugars, ions, or a combination of both) that allows water to flow into empty vessels/tracheids, and supply water for refilling (Zwieniecki & Holbrook, 2009). Regarding the first task, the initial research focus has been on finding the source of osmoticum through analysis of carbohydrates in the parenchyma cells and the role of phloem in the delivery of sugars to sustain refilling. Both enzymatic analysis and visualization techniques have demonstrated that starch levels in living parenchyma cells adjacent to xylem vessels decrease on a timescale coincident with embolism refilling (Bucci *et al.*, 2003, Nardini *et al.*, 2011a, Sakr *et al.*, 2003, Salleo *et al.*, 2009, Secchi & Zwieniecki, 2011). Furthermore, a drop in starch content has been associated with an increase in parenchyma cell sucrose content (Nardini *et al.*, 2011a, Regier *et al.*, 2009, Salleo *et al.*, 2009, Secchi & Zwieniecki, 2010). These results are strongly supported by a transcriptome analysis that found, in response to embolism, both the down-regulation of genes transcribing the monosaccharide metabolic pathways and the strong up-regulation of those involved in the disaccharide metabolic pathways that include starch metabolism (Secchi *et al.*, 2011a). Similar changes in transcript expression were found in the petioles of grapevine during cycles of water stress and recovery (Perrone *et al.*, 2012b). Increased rates of starch depolymerization lead to an upsurge of simple, low-molecular weight sugar concentration that may be exported across membranes into the conduit wall establishing the gradient required to drive water movement into the embolized vessel (Brodersen & McElrone, 2013, Nardini *et al.*, 2011b, Secchi & Zwieniecki, 2012).

However, it has yet to be shown how the products of starch hydrolysis move from starch storing living cells in the stem to vessel-associated parenchyma and finally to the walls of the conduit network. It has been recently proposed that this process of sugar movement might be controlled

by tissue-level changes in stem chemistry (Figure 1):

- An increase in cellular sucrose concentration can be generated by either the long-distance transport of sugars (mostly sucrose) through the phloem or from the hydrolysis of starch stored in the stem; hypothesis confirmed by Bucci *et al.*, (2003), Nardini *et al.*, (2011a), Salleo *et al.*, (2004), Salleo *et al.*, (2009), Secchi & Zwieniecki, (2011), Zwieniecki & Holbrook, (2009).
- Starch hydrolysis results in an increased symplastic cellular soluble sugar concentration providing not only osmotic protection from stress but also shifting the membrane sucrose gradient. This shift may trigger proton-coupled sucrose efflux into apoplastic compartments through the plasma membrane sugar/proton co-transporters, as energized by membrane H⁺-ATPase (Geiger, 2011, Wipfel *et al.*, 2010); this hypothesis has to be proved directly in plants, although an active proton-sucrose efflux has been demonstrated in heterologous systems (Carpaneto *et al.*, 2005, Carpaneto *et al.*, 2010).
- Proton pumps have been localized in xylem-associated cells (De Boer & Volkov, 2003) and the chemical inhibition of H⁺-ATPase pumps in parenchyma cells prevent recovery, while the stimulation of their activities induces recovery (Salleo *et al.*, 2004, Secchi & Zwieniecki, 2016), confirming proton-coupled membrane transport.
- Sucrose-proton efflux may have two effects: an increase in apoplastic sucrose concentration and a drop in apoplastic pH values. This hypothesis is supported by a previous report showing that xylem sap collected from embolized vessels in poplar had a significantly lower pH than functional conduits (Secchi & Zwieniecki, 2012). Alteration in pH is one of the first chemical changes measurable in xylem sap from plants exposed to drought (Bahrin *et al.*, 2002, Sobeih *et al.*, 2004). Although the alkalization of sap is

often measured in transpiring plants (Wilkinson & Davies, 1997) and leads to the general conclusion that pH in xylem sap increases with water stress, a recent study showed that alkalization is not a universal phenomenon. It has been demonstrated that in woody plants, xylem sap alkalization is much less common than in annual species, and of the 22 species studied and exposed to water stress only four showed an increase in sap pH (Sharp & Davies, 2009). Indeed, a new study which uses a novel in vivo technique, has just confirmed that during embolism formation (induced water stress) xylem pH decreases (Secchi & Zwieniecki, 2016)

- A drop in pH and thus the acidification of the apoplast may stimulate the apoplastic activity of acidic invertases, promoting the reduction of sucrose concentrations and at the same time increasing the accumulation of monosaccharides (glucose and fructose; (Secchi & Zwieniecki, 2016) providing additional osmoticum for refilling.
- Lower apoplastic pH may trigger not only the activation of proton pumps but also the activation of metal ion antiporters. Xylem sap collected from embolized vessels in poplar contained up to five times more the osmotic potential of functional vessels. Inorganic ions accounted for half of the osmoticum and the rest was derived from soluble sugars (Secchi & Zwieniecki, 2012). Evidence of metal ion contributions to osmoticum is also provided by a previous study of transcriptome analysis showing an increase in the expression level of metal ion transporters in response to embolism formation (Secchi *et al.*, 2011b). Thus, sugars and ions together may account for the driving force that draws water into empty conduits under low water stress [i.e. during recovery from stress; (Secchi & Zwieniecki, 2012)]. Previously CryoSEM showed concentration of solutes in the xylem sap of maize in functional vessels in the range of ~100 mM (McCully *et al.*,

2000; CryoSEM does not detect sugar) i.e. osmotic levels similar to those observed in trees and adequately high ~0.2 MPa to drive water into conduit under low tension even if no sugar was present. It is worth to mention that majority of herbaceous species undergo an alkalization of apoplastic pH during stress conditions (Sharp & Davies, 2009), therefore sugar accumulation in the apoplast would be limited and other mechanisms of recovery from embolism might be in play. Thus trees and crops might adopt different strategies in order to recovery from embolism formation where trees depend on local recovery while herbaceous plants on root pressure.

- Mechanisms involved in embolism repair require that water enters empty conduits and fills the entire lumen. Indeed, visual evidence from cryo-SEM studies, MRI observations and CT-scans show that water reappears in previously empty conduits, confirming that plants do have the ability to remove embolisms in the xylem (Clearwater & Goldstein, 2005, Holbrook *et al.*, 2001, Scheenen *et al.*, 2007, Zwieniecki *et al.*, 2013). Recently, *in vivo* imaging of grapevine with high resolution x-ray computed tomography has shown that water droplets form on vessel walls in the proximity of parenchyma cells and that these droplets expand until the lumen completely refills (Knipfer *et al.*, 2015, Knipfer *et al.*, 2016, Brodersen *et al.*, 2010). This evidence supports the prediction that parenchyma cells draw water into embolized conduits.

In the proposed scenario, xylem parenchyma cells are the primary means for restoration of hydraulic continuity in the xylem. Although not previously tested, the amount of parenchyma cells, their relative contact with vessels and their physiological activity may be crucial in providing the energy for refilling. Preliminary support for this hypothesis has been provided by Choat *et al.*, (2015), the authors suggested that the lack of refilling in a conifer species (*Sequoia*

sempervirens) could be attributed to the lower amount of parenchyma cells. However, new evidence suggests presence of refilling activity in the same species exposed to fog (Earls *et al.*, 2016). In this case, radial parenchyma cells can provide path for water transport from the bark surface to tracheids. In addition, observations that water droplets form on vessel walls in contact with axial/radial parenchyma cells suggest that these cells may be highly active in water transport. If so, we can assume that water channel proteins (aquaporins) are critical to the refilling process and a closer look at aquaporin physiology is required to understand the potential for refilling in trees.

Aquaporins and their localization in the stem of woody species

In all developmental phases and responses to environmental cues, the maintenance of water flow across membranes is regulated by the activity and abundance of aquaporins (Hachez *et al.*, 2006). Five families of AQPs are known in higher plants on the basis of sequence similarities and common association with peculiar cell membrane localization (Maurel *et al.*, 2015). Among these, the PIP family, which is in turn divided into two subfamilies, PIP1 and PIP2, is the most prolific; examples can be found in woody plants, such as grapevine and poplar, where 28 and 56 MIP-encoding genes have been identified, respectively (Fouquet *et al.*, 2008, Gupta & Sankararamakrishnan, 2009, Sheldon *et al.*, 2009). In the last few years MIP aquaporins were found to act as multifunctional pores; highlighting that AQPs are able to perceive a wide array of signals crucial to cell metabolism, nutrition and signaling cascades [for details see recent reviews by Bienert & Chaumont (2014), Kaldenhoff *et al.*, (2014), Maurel *et al.*, (2015)]. Beyond single aquaporin type functions, further efforts have also recently improved our understanding of specific interactions among AQP isoforms, such as PIP1 and PIP2 members,

309 especially in woody species. In this context, the quantification of expression changes in response
310 to the imposed stress of a single and/or multiple groups of AQP isoforms coupled with studies on
311 their tissue-specific localization is certainly a relevant strategy for obtaining experimental
312 evidence about the physiological roles of aquaporins. Indeed, according to Heinen *et al.*, (2009)
313 there are three main ways by which AQPs regulate water movement across cell membranes:
314 expression level, trafficking, and gating. While all three are important, expression analysis in
315 particular is being used to guide our understanding of the specific localization and activity of
316 diverse AQP isoforms. Despite their importance in all plant species (annual, perennial) and all
317 tissues, the majority of investigations into AQP gene functions have been carried out on
318 herbaceous angiosperm species with special focus on leaves and roots [for details see the recent
319 review by Maurel *et al.*, (2015)], considering the rest of a plant bulk tissue (i.e. total xylem and
320 bark). Studies of aquaporins in woody angiosperms and the localization of AQPs in ferns and
321 gymnosperms are much less explored. A current and comprehensive list of PIP1 and PIP2
322 isoforms expressed in woody plant tissues is reported in Table 1. Several isoforms are tissue-
323 specific and some are almost exclusively expressed in the xylem. However, phylogenetic
324 analysis conducted on aquaporin sequences described in Table 1 show no obvious clustering
325 (Figure 2). The lack of a phylogenetic signal most likely precludes a simple computational
326 approach to detect the AQPs responsible for the maintenance of xylem hydraulic capacity.

327 The first indications of AQP presence in the stem of woody perennials was derived from gene
328 expression studies conducted on poplar species. Among trees, poplar is certainly the best
329 candidate for a woody model system for molecular biology experiments addressing the
330 functional characterization of genes such as aquaporins. Indeed, the genome of the species
331 *Populus trichocarpa* has already been fully sequenced and released (<http://genome.jgi->

332 psf.org/Poptr1_1/Poptr1_1.home.htm). In addition, these plants are relatively easy to genetically
333 transform. Despite the overall limited information on AQP activity in the stem, the majority of
334 the available data have been obtained on poplar. Although limited in scope, analyses have shown
335 highly variable expression in different stem sections, at different developmental stages, and in
336 response to stress treatments, thus suggesting that stem AQPs are an important part of stem
337 biology in woody plants.

338 For instance, significant insights on the expression profiles of AQP isoforms were provided in
339 poplar by Secchi *et al.*, (2009). Along with transcripts of PIP1 and PIP2 members found in roots
340 and leaves, they have also been found in bark and xylem samples of poplar stems, although with
341 highly diverse mixes of isoform characteristics for each tissue. Detailed investigations into
342 expression patterns of the previously characterized *PIP1* and *PIP2* genes in *P. trichocarpa* plants
343 responding to water stress and embolization events supports the idea that specific xylem
344 parenchyma AQPs are induced by stress and suggests some functional role of these proteins in
345 dealing with drought, embolism formation and recovery (Secchi *et al.*, 2011b, Secchi &
346 Zwieniecki, 2010, Secchi & Zwieniecki, 2011). In particular, an analysis of the temporal
347 dynamics of expression of all *PIP1* and *PIP2* transcriptional profiles, found a general strong
348 over-expression of the PIP1 subfamily when water stress occurred. However, response to
349 embolism formation has resulted in the selective activation of only *PIP1;1* and *PIP1;3* genes in
350 stem parenchyma (Secchi & Zwieniecki, 2010). Genome-wide analysis of *P. trichocarpa*
351 responding to embolism formation confirm the specificity of AQP expression patterns but also
352 show a correlation with changes in the expression of genes tied to carbohydrate metabolism and
353 sugar transport (Secchi *et al.*, 2011b).

354 Similarly, in a work performed on grapevine plants (*Vitis vinifera* cv Grenache) subjected to
355 either drought stress or artificially-induced embolization, changes in the expression of diverse
356 PIP1 and PIP2 aquaporin genes were profiled in both petioles (whole tissue level) and also in
357 vessel associated cells (VACs) isolated from the same tissue samples using a laser micro-
358 dissection technique (Chitarra *et al.*, 2014). Here, the comparison of AQPs expression trends in a
359 target cell type coupled with the transcriptional profiles of the same genes at the whole tissue
360 level provided the first consistent information about the specificity of some of these transcripts.
361 Indeed, while some of the *VvPIP1* and *VvPIP2* analyzed genes were on the whole induced by
362 stress and subsequent recovery in entire petioles, two transcripts encoding *VvPIP1;1* and
363 *VvPIP2;4N*, were exclusively activated in VACs, suggesting that the activity of the two derived
364 proteins was restricted to these cells (Chitarra *et al.*, 2014).

365 Although transcriptomic methods are certainly essential to the unravelling of AQP transcript
366 alterations occurring in target organs or tissues during either plant phenological phases or the
367 application of stress, they can only provide indirect evidence on the functional role of these
368 genes. They do not supply information about transcript localization. To better address the
369 characterization of aquaporins, the application of tissue and cellular level localization studies is
370 pivotal. The first cellular detection of AQPs in the stem of trees was in walnut (*Juglans regia*)
371 plants experiencing winter embolism formation. The expression signal of two members of the
372 PIP2 subfamily (*JrPIP2.1* and *JrPIP2.2*) was analyzed by an immunolocalization technique on
373 several divisions of xylem tissue including vessels, fibers and parenchyma cells (Sakr *et al.*,
374 2003). The immunolabeling results showed that the strongest detection of the two proteins
375 occurred in the living parenchyma cells in direct contact with xylem vessels (VACs), clearly

attesting that during winter months these AQPs are specifically located in the VACs of walnut stems.

Subsequent tissue- and cell-specific localizations of three AQP genes (two PIP2 isoforms and one TIP) in the secondary xylem of hybrid poplar stems directly observed through *in situ* hybridization experiments (Almeida-Rodriguez & Hacke, 2012) showed an increase in the abundance of all three in parenchyma cells when drought or high N fertilization was applied. This study validated and visually confirmed a previous work based solely on gene expression data (Hacke *et al.*, 2010). Drought and N fertilization resulted in significant changes in the abundance of target AQP transcripts in living tissues of the stem as well as in the ray cells adjacent to vessels (pith parenchyma), with various degrees of changes in expression patterns depending on the applied treatment. Surprisingly, this research revealed a strong increase in the AQP signal of parenchyma cells (often isolated) but only upon drought, suggesting an increased potential for water exchange between apoplast and symplast in response to imposed external conditions.

Finally, AQP tissue-localization studies in non-angiosperms (gymnosperms and ferns) are almost exclusively focused on leaves and gametophytes. For instance, interesting data were reported Laur & Hacke (2014) in a study dealing with the analysis of aquaporin expression in the needles of *Picea glauca*, where the expression of PIP1 and PIP2 aquaporins was measured and compared with immunolocalization and *in situ* hybridization experiments. The authors indicated that upon water deficit all tested PIP genes were significantly down-regulated in needles while a high humidity treatment resulted in an increased expression level for all transcripts, but to different extents depending on the period of exposure. These results were confirmed by the diverse patterns of tissue localization obtained for each AQP. Indeed, while PIP1s were mainly localized

in the endodermis, PIP2s showed a diffused signal within the central cylinder of the needle in both phloem tissue and in transfusion parenchyma cells. It was suggested that PIP1 isoforms mainly regulate water transport across the bundle sheath, from the needle epidermis towards the vascular tissue, while PIP2s may facilitate water movement from the needle towards stems (Laur & Hacke, 2014). These findings are consistent with previous aquaporin immunolocalization experiments carried out in *P. abies*, where a strong signal was detected in needles at the vascular tissue level (Olviusson *et al.*, 2001) and by observations of a higher abundance of PIP1 and PIP2 aquaporins at the endodermis and phloem cell level in the needles of *Picea abies* recovering from winter embolism (Mayr *et al.*, 2014). Information on AQP expression in ferns comes from work conducted on the xerophytic fern *Cheilanthes lanosa* (Diamond *et al.*, 2012) where analysis indicated that the role of PIP1 proteins is highlighted by the maintenance of water balance during gametophyte stages.

Despite limited information, we can conclude that AQPs are abundant in living cells associated with long distance transport tissue, including xylem axial and radial parenchyma and phloem. It is also apparent that the expression of stem AQPs is related to plant hydraulic status with drought causing species/tissue specific up or down regulation and recovery from stress (rain, re-watering, fog) causing significant up regulation of stem specific AQPs. This pattern of expression underlines the potential role of AQPs in the recovery of the hydraulic capacity of the xylem, a trait that long-lived perennial plants may rely upon for their survival.

Parenchyma aquaporins and recovery from water stress

Existing models of recovery processes occurring in trees indicate that, among other functions, living parenchyma cells associated with xylem conduits are key players in both supplying the water and generating the energy needed to refill non-functional vessels (Brodersen

423 & McElrone, 2013, Nardini *et al.*, 2011b, Salleo *et al.*, 2004a, Zwieniecki & Holbrook, 2009).

424 As water flow between the symplast and apoplast is mediated by aquaporins, xylem parenchyma

425 cells possess a significant ability to temporally and spatially control water efflux, by regulating

426 the expression and activity of specific AQP isoforms. As mentioned previously, AQP *in vivo*

427 localization studies have revealed a fine-tuning of AQP expression in vessels associated cells

428 (VACs) especially during transition periods between drought stress and stress recovery. It is thus

429 believed that the physiological function of AQPs is specifically needed not during the imposition

430 of environmental stress (drought, frost), but during the recovery from stresses that often requires

431 the restoration of xylem hydraulic conductivity.

432 The contribution of aquaporins to the restoration of xylem hydraulic conductivity throughout

433 periods of water stress and/or subsequent recovery have mainly been addressed in order to better

434 understand the plant water relations of distal organs (roots and leaves) (Perrone *et al.*, 2012a,

435 Perrone *et al.*, 2012b, Pou *et al.*, 2013, Tsuchihira *et al.*, 2010), whereas a comprehensive

436 understanding of AQPs in controlling xylem refilling in the stem is just emerging. Revisiting the

437 insights gained from walnut stems in which expression was correlated with embolism recovery

438 processes (Sakr *et al.*, 2003), both over-expression and an increased abundance of two walnut

439 PIP2 proteins was exclusively induced in winter months in the VACs. This increase was

440 simultaneous with an increase in the sucrose concentrations of xylem sap and a decrease of

441 starch content in parenchyma cells (Sakr *et al.*, 2003). It was assumed that overexpression and

442 increases in AQP abundance facilitate water flow from VACs into embolized vessels following

443 this newly generated osmotic gradient. These results support earlier observations of increased

444 xylem water content, which in parallel decreases in stem parenchyma water content reflecting the

445 flow of water from cells to embolized vessels (Ameglio *et al.*, 2001). Recently, additional reports

446 have highlighted the involvement of PIP2 genes in particular in facilitating the recovery process
447 (see Table 1). For instance, the increased abundance of *PIP2;3* and *PIP2;5* detected in the VACs
448 of drought-exposed poplar stems (Almeida-Rodriguez & Hacke, 2012) and the overexpression of
449 *PIP2;4N* and *PIP2;1* genes observed in the VACs of either embolized or recovering grape
450 petioles (Chitarra *et al.*, 2014) may both support the need for PIP2 activity during vessel refilling
451 along the xylem-VAC-phloem transport path. These observations are in agreement with the
452 current models of embolism repair involving the interaction of xylem and phloem cells (Nardini
453 *et al.*, 2011a, Secchi *et al.*, 2011b, Secchi & Zwieniecki, 2016) presented here (Figure 1).

454 The contribution of PIP1s to water stress and recovery in trees was initially less considered as,
455 unlike PIP2s, PIP1s were thought to have little to no water transport activity when individually
456 expressed in *Xenopus* oocytes (Chrispeels *et al.*, 2001). This implied that PIP1 proteins did not
457 work as water channels and it was consequently assumed that they were not necessary for
458 promoting transmembrane water flow. Nevertheless, it was later shown that membrane water
459 permeability significantly increased when PIP1s were co-expressed with PIP2s, implying a
460 physical interaction between the two (Fetter *et al.*, 2004, Secchi *et al.*, 2009, Zelazny *et al.*,
461 2007). Interestingly, only PIP1 genes were shown to undergo strong transcriptional increase
462 upon water stress and embolism formation in poplar stems (Secchi *et al.*, 2011b, Secchi &
463 Zwieniecki, 2010, Secchi & Zwieniecki, 2011). In addition, the transcripts encoding *PIP1.1* and
464 *PIP1.3* were the most expressed among PIP aquaporin genes in poplar stems (Secchi *et al.*,
465 2009). These two genes were highly up-regulated in response to artificially-induced embolism
466 and their expression was repressed when plants recovered from embolism, showing VACs ability
467 to sense both embolism formation and end of the refilling process (Secchi & Zwieniecki, 2010).
468 Similarly, a *Vitis PIP1;1* gene was reported to be activated in VACs during both embolism

469 formation and recovery, whereas the same transcript was not detected at the whole tissue level
470 (Chitarra *et al.*, 2014, Perrone *et al.*, 2012b, see also Table 1). These findings demonstrate that
471 PIP1 expression is affected by drought stress, embolism and duration of recovery from stress.
472 However, hypotheses about PIP1s role in refilling remain open as all evidence is based on
473 transcription analyses and no direct proof exists on the physiological activity of these proteins.
474 Reverse genetic techniques have successfully been applied to the functional characterization of
475 AQP genes mainly in herbaceous species (Aharon *et al.*, 2003, Da Ines *et al.*, 2010, Kaldenhoff
476 *et al.*, 1998, Martre *et al.*, 2002, Postaire *et al.*, 2010) and more recently in woody plants (Bi *et*
477 *al.*, 2015, Perrone *et al.*, 2012a, Secchi & Zwieniecki, 2013, Sreedharan *et al.*, 2013, Tsuchihira
478 *et al.*, 2010). A similar reverse genetic approach was used to generate transgenic poplar lines by
479 down-regulating the whole PIP1 gene family, with the goal of attesting the function of this PIP
480 group by gaining *in vivo* evidence of their role during xylem embolism and recovery (Secchi &
481 Zwieniecki, 2014). The down-regulation of PIP1s did not affect plant behavior under well-
482 watered conditions (Secchi & Zwieniecki, 2013), but it changed the physiological response of
483 poplar during the progression of water stress. Specifically, the suppression of PIP1 expression
484 activity significantly lowered refilling activity, resulting in an apparent increase in the
485 vulnerability to embolism formation of transgenic plants (Secchi & Zwieniecki, 2014). These
486 findings have clearly elucidated that, under water stress, the function of stem PIP1s is pivotal to
487 both the maintenance of xylem transport capacity under stress and plant recovery from stress.
488 In conifers, the occurrence of freeze-thaw induced embolism formation and recovery processes is
489 also documented (Limm *et al.*, 2009, Mayr *et al.*, 2006, Mayr *et al.*, 2002, Sparks & Black, 2000,
490 Sparks *et al.*, 2001), but to date very few research reports have provided experimental evidence
491 that these phenomena can affect AQP gene regulation. In *Picea abies*, frost-induced embolism

has been shown to significantly correlate with the accumulation of PIP1 and PIP2-type proteins in needle endodermal and phloem cells (Mayr *et al.*, 2014). In *Picea glauca* AQPs were shown to promote xylem refilling by facilitating water movement between VACs and the embolized xylem (Laur & Hacke, 2014). Although more experimental efforts are needed to deepen existing knowledge of conifer AQP roles in recovery from embolism, the findings described above provide some indication that gymnosperms are capable of sensing and managing xylem embolism.

Future directions

Despite research efforts, our understanding of the biophysical and cellular mechanisms responsible for embolism refilling in woody plants remains incomplete. General agreement exists that the living parenchyma cells associated with xylem conduits are involved in the recovery process from stress. They are credited with supplying water to fill the void and releasing osmotically active compounds to generate energy gradients needed to pull water from living cells and restore hydraulic function (Nardini *et al.*, 2011a, Salleo *et al.*, 2004, Zwieniecki & Holbrook, 2009). Thus we can postulate that a correlation between a plant's capacity to recover from severe drought and VAC volume, surface area, or bridges provided between phloem and xylem should also exist. If so, more detailed anatomical studies of xylem parenchyma in combination with species drought tolerance may lead to the discovery of interesting patterns linking drought tolerance and parenchyma activity evolution. As xylem refilling process might require water transport from living cells to xylem lumens, reductions of membrane hydraulic resistance would be beneficial during recovery from stress and thus observing patterns of expression and activity of specific AQP isoforms in living parenchyma cells might provide further clues to

biology of stem under drought conditions. So far only a handful of species have been studied and we can neither address the question of how common described expression patterns are among species, nor provide a comprehensive overview of specific PIPs involved in the recovery process.

Another important aspect of plant recovery from severe water stress that includes the restoration of xylem hydraulic capacity is related to signaling (triggers) for the biological responses of VACs. Although we can observe changes in VAC physiological and expression activity, we do not know what physical or chemical changes trigger these responses. Several ideas have been suggested like mechano-sensing of high frequency sound waves associated with embolism (Salleo *et al.*, 2008), changes in sucrose concentration in the xylem apoplast (Nardini *et al.*, 2011a, Secchi & Zwieniecki, 2011b) or changes in pH (Secchi & Zwieniecki, 2016). In this review's model (Figure 1), sucrose concentration is the proposed trigger for embolism repair processes as previous results suggest that increased sucrose concentration in the xylem follows an expression pattern similar to that of VAC gene expression in response to the formation of embolism (Secchi & Zwieniecki, 2011). A more recent work also suggests that xylem apoplastic pH may be a significant part of the signaling path responsible for refilling apart from its role in invertase activity and sugar accumulation (Secchi & Zwieniecki, 2016). Low pH was shown to affect aquaporin molecular gating (Maurel *et al.*, 2015, Tornroth-Horsefield *et al.*, 2006, Tournaire-Roux *et al.*, 2003), which depends on protonation of the conserved amino acid residue of loop D (His193 in SoPIP2;1). At acidic pH, charged hydrogen interacts with two additional amino acids (Asp28 and Glu31) and loop B (Ser115) to stabilize loop D in a closed pore conformation (Tornroth-Horsefield *et al.*, 2006). This activity has been observed in heterologous systems (*Xenopus* oocytes) or in herbaceous species (spinach and *Arabidopsis*) (Tornroth-

Horsefield *et al.*, 2006, Tournaire-Roux *et al.*, 2003). Because xylem apoplastic pH triggers multiple parts of the recovery process (i.e. membrane sucrose transport, AQP gating, and the activity of apoplastic invertase), it is crucial to study the *in vivo* chemistry of xylem and VACs following a whole system approach.

Acknowledgements

We would like to thank Jessica Orozco, Anna Davidson, and Jessie Godfrey for their comments and editorial help. F.S. acknowledges funding from the ‘Programma Giovani Ricercatori Rita Levi Montalcini’s grant.

References

- Abascal F., Irisarri I. & Zardoya R. (2014) Diversity and evolution of membrane intrinsic proteins. *Biochimica Et Biophysica Acta-General Subjects*, **1840**, 1468-1481.
- Aharon R., Shahak Y., Wininger S., Bendov R., Kapulnik Y. & Galili G. (2003) Overexpression of a plasma membrane aquaporin in transgenic tobacco improves plant vigor under favorable growth conditions but not under drought or salt stress. *Plant Cell*, **15**, 439-447.
- Almeida-Rodriguez A.M. & Hacke U.G. (2012) Cellular localization of aquaporin mRNA in hybrid poplar stems. *American Journal of Botany*, **99**, 1249-1254.
- Ameglio T., Ewers F.W., Cochard H., Martignac M., Vandame M., Bodet C. & Cruiziat P. (2001) Winter stem xylem pressure in walnut trees: effects of carbohydrates, cooling and freezing. *Tree Physiology*, **21**, 387-394.
- Arbellay E., Fonti P. & Stoffel M. (2012) Duration and extension of anatomical changes in wood structure after cambial injury. *Journal of Experimental Botany*, **63**, 3271-3277.

561 Bahrn A., Jensen C.R., Asch F. & Mogensen V.O. (2002) Drought-induced changes in xylem
 562 pH, ionic composition, and ABA concentration act as early signals in field-grown maize
 563 (*Zea mays* L.). *Journal of Experimental Botany*, **53**, 251-263.

564 Baiges I., Schaffner A.R. & Mas A. (2001) Eight cDNA encoding putative aquaporins in *Vitis*
 565 hybrid Richter-110 and their differential expression. *Journal of Experimental Botany*, **52**,
 566 1949-1951.

567 Barnard D.M., Lachenbruch B., McCulloh K.A., Kitin P. & Meinzer F.C. (2013) Do ray cells
 568 provide a pathway for radial water movement in the stems of conifer trees? *American*
 569 *Journal of Botany*, **100**, 322-331.

570 Bi Z., Merl-Pham J., Uehlein N., Zimmer I., Muehlhans S., Aichler M., Walch A.K., Kaldenhoff
 571 R., Palme K., Schnitzler J.-P. & Block K. (2015) RNAi-mediated downregulation of
 572 poplar plasma membrane intrinsic proteins (PIPs) changes plasma membrane proteome
 573 composition and affects leaf physiology. *Journal of Proteomics*, **128**, 321-332.

574 Bienert G.P. & Chaumont F. (2014) Aquaporin-facilitated transmembrane diffusion of hydrogen
 575 peroxide. *Biochimica Et Biophysica Acta-General Subjects*, **1840**, 1596-1604.

576 Brandes A. & Barros C.F. (2008) Wood anatomy of eight liana species of Leguminosae family
 577 from Atlantic Rain Forest. *Acta Botanica Brasilica*, **22**, 465-480.

578 Brenner C.E. (1995) *Cavitation and Bubble Dynamics* (vol. 44). Oxford University Press, Inc.,
 579 Oxford.

580 Brodersen C.R. & McElrone A.J. (2013) Maintenance of xylem network transport capacity: a
 581 review of embolism repair in vascular plants. *Frontiers in Plant Science*, **4**.

582 Brodersen C.R., McElrone A.J., Choat B., Lee E.F., Shackel K.A. & Matthews M.A. (2013) In
 583 vivo visualizations of drought-induced embolism spread in *Vitis vinifera*. *Plant*
 584 *Physiology*, **161**, 1820-1829.

585 Brodersen C.R., McElrone A.J., Choat B., Matthews M.A. & Shackel K.A. (2010) The dynamics
 586 of embolism repair in xylem: *in vivo* visualizations using high-resolution computed
 587 tomography. *Plant Physiology*, **154**, 1088-1095.

588 Brodribb T.J. & Cochard H. (2009) Hydraulic failure defines the recovery and point of death in
 589 water-stressed conifers. *Plant Physiology*, **149**, 575-584.

590 Bucci S.J., Scholz F.G., Goldstein G., Meinzer F.C., Da L. & Sternberg S.L. (2003) Dynamic
 591 changes in hydraulic conductivity in petioles of two savanna tree species: factors and
 592 mechanisms contributing to the refilling of embolized vessels. *Plant, Cell and*
 593 *Environment*, **26**, 1633-1645.

594 Carlquist S. (2015) Living cells in wood. 1. Absence, scarcity and histology of axial parenchyma
 595 as keys to function. *Botanical Journal of the Linnean Society*, **177**, 291-321.

596 Carpaneto A., Geiger D., Bamberg E., Sauer N., Fromm J. & Hedrich R. (2005) Phloem-
 597 localized, proton-coupled sucrose carrier ZmSUT1 mediates sucrose efflux under the
 598 control of the sucrose gradient and the proton motive force. *Journal of Biological*
 599 *Chemistry*, **280**, 21437-21443.

600 Carpaneto A., Koepsell H., Bamberg E., Hedrich R. & Geiger D. (2010) Sucrose- and H⁺-
 601 dependent charge movements associated with the gating of sucrose transporter ZmSUT1.
 602 *Plos One*, **5**.

603 Chapotin S.M., Razanameharizaka J.H. & Holbrook N.M. (2006) Abiomechanical perspective
 604 on the role of large stem volume and high water content in baobab trees (*Adansonia* spp.;
 605 Bombacaceae). *American Journal of Botany*, **93**, 1251-1264.

606 Chitarra W., Balestrini R., Vitali M., Pagliarani C., Perrone I., Schubert A. & Lovisolo C. (2014)
 607 Gene expression in vessel-associated cells upon xylem embolism repair in *Vitis vinifera* L.
 608 petioles. *Planta*, **239**, 887-899.

609 Choat B., Cobb A.R. & Jansen S. (2008) Structure and function of bordered pits: new discoveries
 610 and impacts on whole-plant hydraulic function. *New Phytologist*, **177**, 608-625.

611 Choat B., Brodersen C.R., McElrone A.J., (2015) Synchrotron X-ray microtomography of xylem
 612 embolism in *Sequoia sempervirens* saplings during cycles of drought and recovery. *New*
 613 *Phytologist*, **205**, 1095-1105.

614 Chrispeels M.J., Morillon R., Maurel C., Gerbeau P., Kjellbom P. & Johansson I. (2001)
 615 Aquaporins of plants: Structure, function, regulation, and role in plant water relations.
 616 *Current topics in membranes*, **51**, 277-334.

617 Clearwater M. & Goldstein G. (2005) Embolism repair and long distance transport. In: *Vascular*
 618 *Transport in Plants* (eds N.M. Holbrook & M.A. Zwieniecki), pp. 201-220. Elsevier.

619 Cochard H., Ewers F.W. & Tyree M.T. (1994) Water relations of a tropical vine-like bamboo
 620 (*Rhipidocladum racemiflorum*) - root pressures, vulnerability to cavitation and seasonal-
 621 changes in embolism. *Journal of Experimental Botany*, **45**, 1085-1089.

622 Comstock J.P. & Sperry J.S. (2000) Theoretical considerations of optimal conduit length for
 623 water transport in vascular plants. *New Phytologist*, **148**, 195-218.

624 Cruiziat P., Cochard H. & Ameglio T. (2002) Hydraulic architecture of trees: main concepts and
 625 results. *Annals of Forest Science*, **59**, 723-752.

626 Da Ines O., Graf W., Franck K.I., Albert A., Winkler J.B., Scherb H., Stichler W. & Schaffner
627 A.R. (2010) Kinetic analyses of plant water relocation using deuterium as tracer - reduced
628 water flux of *Arabidopsis* pip2 aquaporin knockout mutants. *Plant Biology*, **12**, 129-139.

629 De Boer A.H. & Volkov V. (2003) Logistics of water and salt transport through the plant:
630 structure and functioning of the xylem. *Plant Cell and Environment*, **26**, 87-101.

631 de Paula Santos Martins C., Pedrosa A.M., Du D., Goncalves L.P., Yu Q., Gmitter F.G., Jr. &
632 Costa M.G.C. (2015) Genome-wide characterization and expression analysis of major
633 intrinsic proteins during abiotic and biotic stresses in sweet orange (*Citrus sinensis* L.
634 Osb.). *Plos One*, **10**, e0138786-e0138786.

635 Deflorio G., Johnson C., Fink S. & Schwarze F. (2008) Decay development in living sapwood of
636 coniferous and deciduous trees inoculated with six wood decay fungi. *Forest Ecology and*
637 *Management*, **255**, 2373-2383.

638 Denne P. & Gasson P. (2008) Ray structure in root- and stem-wood of *Larix decidua*:
639 Implications for root identification and function. *Iawa Journal*, **29**, 17-23.

640 DeSmidt W.J. (1922) Studies of the distribution and volume of the wood rays in slippery elm
641 (*Ulmus fulva* Michx.). *Journal of Forestry*, **20**, 352-362.

642 Diamond H.L., Jones H.R. & Swatzell L.J. (2012) The role of aquaporins in water balance in
643 *cheilanthes lanosa* (*Adiantaceae*) gametophytes. *American Fern Journal*, **102**, 11-31.

644 Earles J.M., Sperling O., Silva L.C.R., McElrone A.J., Brodersen C.R., North M.P. & Zwieniecki
645 M.A. (2016) Bark water uptake promotes localized hydraulic recovery in coastal redwood
646 crown. *Plant Cell and Environment*, **39**, 320-328.

647 Ewers F.W., Cochard H. & Tyree M.T. (1997) A survey of root pressures in vines of a tropical
648 lowland forest. *Oecologia*, **110**, 191-196.

649 Fetter K., van Wilder V., Moshelion M. & Chaumont F. (2004) Interaction between plasma
650 membrane aquaporins modulate their water channel activity. *Plant Cell* **16**, 215-228

651 Fouquet R., Leon C., Ollat N. & Barrieu F. (2008) Identification of grapevine aquaporins and
652 expression analysis in developing berries. *Plant Cell Reports*, **27**, 1541-1550.

653 Galmes J., Pou A., Alsina M.M., Tomas M., Medrano H. & Flexas J. (2007) Aquaporin
654 expression in response to different water stress intensities and recovery in Richter-110
655 (*Vitis* sp.): relationship with ecophysiological status. *Planta*, **226**, 671-681.

656 Geiger D. (2011) Plant Sucrose Transporters from a Biophysical Point of View. *Molecular Plant*,
657 **4**, 395-406.

658 Gupta A.B. & Sankararamakrishnan R. (2009) Genome-wide analysis of major intrinsic proteins
659 in the tree plant *Populus trichocarpa*: Characterization of XIP subfamily of aquaporins
660 from evolutionary perspective. *Bmc Plant Biology*, **9**.

661 Hachez C., Moshelion M., Zelazny E., Cavez D. & Chaumont F. (2006) Localization and
662 quantification of plasma membrane aquaporin expression in maize primary root: A clue to
663 understanding their role as cellular plumbers. *Plant Molecular Biology*, **62**, 305-323.

664 Hacke U.G., Plavcova L., Almeida-Rodriguez A., King-Jones S., Zhou W. & Cooke J.E.K.
665 (2010) Influence of nitrogen fertilization on xylem traits and aquaporin expression in
666 stems of hybrid poplar. *Tree Physiology*, **30**, 1016-1025.

667 Hacke U.G., Sperry J.S., Pockman W.T., Davis S.D. & McCulloch K.A. (2001a) Trends in wood
668 density and structure are linked to prevention of xylem implosion by negative pressure.
669 *Oecologia*, **126**, 457-461.

670 Hacke U.G., Stiller V., Sperry J.S., Pittermann J. & McCulloh K.A. (2001b) Cavitation fatigue.
671 Embolism and refilling cycles can weaken the cavitation resistance of xylem. *Plant*
672 *Physiology*, **125**, 779-786.

673 Heinen R.B., Ye Q. & Chaumont F. (2009) Role of aquaporins in leaf physiology. *Journal of*
674 *Experimental Botany*, **60**, 2971-2985.

675 Holbrook N.M., Ahrens E.T., Burns M.J. & Zwieniecki M.A. (2001) *In vivo* observation of
676 cavitation and embolism repair using magnetic resonance imaging. *Plant Physiology*, **126**,
677 27-31.

678 Holbrook N.M. & Zwieniecki M.A. (1999) Embolism repair and xylem tension: Do we need a
679 miracle? *Plant Physiology*, **120**, 7-10.

680 Holbrook N.M. & Zwieniecki M.A. (2005) *Vascular transport in plants*. Elsevier Academic
681 Press, Amsterdam ; Boston.

682 Holbrook N.M., Zwieniecki M.A. & Melcher P.J. (2002) The dynamics of "dead wood":
683 Maintenance of water transport through plant stems. *Integrative and Comparative*
684 *Biology*, **42**, 492-496.

685 Johnson D.M., McCulloh K.A., Woodruff D.R. & Meinzer F.C. (2012) Hydraulic safety margins
686 and embolism reversal in stems and leaves: Why are conifers and angiosperms so
687 different? *Plant Science*, **195**, 48-53.

688 Kaldenhoff R., Grote K., Zhu J.J. & Zimmermann U. (1998) Significance of plasmalemma
689 aquaporins for water-transport in *Arabidopsis thaliana*. *Plant Journal*, **14**, 121-128.

690 Kaldenhoff R., Kai L. & Uehlein N. (2014) Aquaporins and membrane diffusion of CO₂ in living
691 organisms. *Biochimica Et Biophysica Acta-General Subjects*, **1840**, 1592-1595.

692 Knipfer T., Eustis A., Brodersen C., Walker A.M. & McElrone A.J. (2015) Grapevine species
693 from varied native habitats exhibit differences in embolism formation/repair associated
694 with leaf gas exchange and root pressure. *Plant Cell and Environment*, **8**, 1503-1513.

695 Knipfer T., Cuneo I.F., Brodersen C.R., & McElrone A.J. (2016) In situ visualization of the
696 dynamics in xylem embolism formation and removal in the absence of root pressure: a
697 study on excised grapevine stems. *Plant Physiology*, **171**, 1024-1036.

698 Larson P.R. (1994) *The vascular cambium : development and structure*. Springer-Verlag, Berlin ;
699 New York.

700 Laur J. & Hacke U.G. (2014) Exploring *Picea glauca* aquaporins in the context of needle water
701 uptake and xylem refilling. *New Phytologist*, **203**, 388-400.

702 Limm E.B., Simonin K.A., Bothman A.G. & Dawson T.E. (2009) Foliar water uptake: a
703 common water acquisition strategy for plants of the redwood forest. *Oecologia*, **161**, 449-
704 459.

705 Marjanovic Z., Uehlein N., Kaldenhoff R., Zwiazek J.J., Weiss M., Hampp R. & Nehls U. (2005)
706 Aquaporins in poplar: What a difference a symbiont makes! *Planta*, **222**, 258-268.

707 Martre P., Morillon R., Barrieu F., North G.B., Nobel P.S. & Chrispeels M.J. (2002) Plasma
708 membrane aquaporins play a significant role during recovery from water deficit. *Plant*
709 *Physiology*, **130**, 2101-2110.

710 Maurel C., Boursiac Y., Luu D.T., Santoni V., Shahzad Z. & Verdoucq L. (2015) Aquaporins in
711 plants. *Physiological Reviews*, **95**, 1321-1358.

712 Mayr S., Hacke U., Schmid P., Schwienbacher F. & Gruber A. (2006) Frost drought in conifers
713 at the alpine timberline: Xylem dysfunction and adaptations. *Ecology*, **87**, 3175-3185.

714 Mayr S., Schmid P., Laur J., Rosner S., Charra-Vaskou K., Daemon B. & Hacke U.G. (2014)
 715 Uptake of water via branches helps Timberline conifers refill embolized xylem in late
 716 winter. *Plant Physiology*, **164**, 1731-1740.

717 Mayr S., Wolfschwenger M. & Bauer H. (2002) Winter-drought induced embolism in Norway
 718 spruce (*Picea abies*) at the Alpine timberline. *Physiologia Plantarum*, **115**, 74-80.

719 McCully M.E., Shane M.W., Baker A.N., Huang C.X., Ling L.E.C. & Canny M.J. (2000) The
 720 reliability of cryoSEM for the observation and quantification of xylem embolisms and
 721 quantitative analysis of xylem sap *in situ*. *Journal of Microscopy*, **198**, 24-33.

722 Metzner R., Thorpe M.R., Breuer U., Blumler P., Schurr U., Schneider H.U. & Schroeder W.H.
 723 (2010) Contrasting dynamics of water and mineral nutrients in stems shown by stable
 724 isotope tracers and cryo-SIMS. *Plant Cell and Environment*, **33**, 1393-1407.

725 Miniussi M., Del Terra L., Savi T., Pallavicini A. & Nardini A. (2015) Aquaporins in *Coffea*
 726 *arabica* L.: Identification, expression, and impacts on plant water relations and hydraulics.
 727 *Plant Physiology and Biochemistry*, **95**, 92-102.

728 Morris H. & Jansen S. (2016) Secondary xylem parenchyma - from classical terminology to
 729 functional traits *Iawa Journal*, **37**, 1-15.

730 Morris H., Plavcova L., Cvecko P., Fichtler E., Gillingham M.A.F., Martinez-Cabrera H.I.,
 731 McGlinn D.J., Wheeler E., Zheng J., Zieminska K. & Jansen S. (2016) A global analysis
 732 of parenchyma tissue fractions in secondary xylem of seed plants. *New Phytologist*, **209**,
 733 1553-1565.

734 Nardini A., Lo Gullo M.A. & Salleo S. (2011a) Refilling embolized xylem conduits: Is it a
 735 matter of phloem unloading? *Plant Science*, **180**, 604-611.

736 Nardini A., Salleo S. & Jansen S. (2011b) More than just a vulnerable pipeline: xylem
 737 physiology in the light of ion-mediated regulation of plant water transport. *Journal of*
 738 *Experimental Botany*, **62**, 4701-4718.

739 Oliviusson P., Salaj J. & Hakman I. (2001) Expression pattern of transcripts encoding water
 740 channel-like proteins in Norway spruce (*Picea abies*). *Plant Molecular Biology*, **46**, 289-
 741 299.

742 Perrone I., Gambino G., Chitarra W., Vitali M., Pagliarani C., Riccomagno N., Balestrini R.,
 743 Kaldenhoff R., Uehlein N., Gribaudo I., Schubert A. & Lovisolo C. (2012a) The grapevine
 744 root-specific aquaporin VvPIP2;4N controls root hydraulic conductance and leaf gas
 745 exchange under well-watered conditions but not under water stress. *Plant Physiology*, **160**,
 746 965-977.

747 Perrone I., Pagliarani C., Lovisolo C., Chitarra W., Roman F. & Schubert A. (2012b) Recovery
 748 from water stress affects grape leaf petiole transcriptome. *Planta*, **235**, 1383-1396.

749 Pfautsch S., Renard J., Tjoelker M.G. & Salih A. (2015) Phloem as capacitor: radial transfer of
 750 water into xylem of tree stems occurs via symplastic transport in ray parenchyma. *Plant*
 751 *Physiology*, **167**, 963-+.

752 Picaud S., Becq F., Dedaldechamp F., Ageorges A. & Delrot S. (2003) Cloning and expression
 753 of two plasma membrane aquaporins expressed during the ripening of grape berry.
 754 *Functional Plant Biology*, **30**, 621-630.

755 Pittermann J., Sperry J.S., Hacke U.G., Wheeler J.K. & Sikkema E.H. (2005) Torus-margo pits
 756 help conifers compete with angiosperms. *Science*, **310**, 1924-1924.

757 Pittermann J., Sperry J.S., Hacke U.G., Wheeler J.K. & Sikkema E.H. (2006) Inter-tracheid
 758 pitting and the hydraulic efficiency of conifer wood: The role of tracheid allometry and
 759 cavitation protection. *American Journal of Botany*, **93**, 1265-1273.

760 Postaire O., Tournaire-Roux C., Grondin A., Boursiac Y., Morillon R., Schaffner A.R. & Maurel
 761 C. (2010) A PIP1 aquaporin contributes to hydrostatic pressure-induced water transport in
 762 both the root and rosette of *Arabidopsis*. *Plant Physiology*, **152**, 1418-1430.

763 Pou A., Medrano H., Flexas J. & Tyerman S.D. (2013) A putative role for TIP and PIP
 764 aquaporins in dynamics of leaf hydraulic and stomatal conductances in grapevine under
 765 water stress and re-watering. *Plant Cell and Environment*, **36**, 828-843.

766 Pratt R.B., Jacobsen A.L., Ewers F.W. & Davis S.D. (2007) Relationships among xylem
 767 transport, biomechanics and storage in stems and roots of nine *Rhamnaceae* species of the
 768 California chaparral. *New Phytologist*, **174**, 787-798.

769 Rasheed-Depardieu C., Parent C., Crevecoeur M., Parelle J., Tatin-Froux F., Le Provost G. &
 770 Capelli N. (2012) Identification and expression of nine oak aquaporin genes in the primary
 771 root axis of two oak species, *Quercus petraea* and *Quercus robur*. *Plos One*, **7**.

772 Regier N., Streb S., Coccozza C., Schaub M., Cherubini P., Zeeman S.C. & Frey B. (2009)
 773 Drought tolerance of two black poplar (*Populus nigra* L.) clones: contribution of
 774 carbohydrates and oxidative stress defence. *Plant Cell and Environment*, **32**, 1724-1736.

775 Reiterer A., Burgert I., Sinn G. & Tschegg S. (2002) The radial reinforcement of the wood
 776 structure and its implication on mechanical and fracture mechanical properties - A
 777 comparison between two tree species. *Journal of Materials Science*, **37**, 935-940.

778 Sakr S., Alves G., Morillon R.L., Maurel K., Decourteix M., Guilliot A., Fleurat-Lessard P.,
779 Julien J.L. & Chrispeels M.J. (2003) Plasma membrane aquaporins are involved in winter
780 embolism recovery in walnut tree. *Plant Physiology*, **133**, 630-641.

781 Salleo S., Lo Gullo M.A., De Paoli D. & Zippo M. (1996) Xylem recovery from cavitation-
782 induced embolism in young plants of *Laurus nobilis*: a possible mechanism. *New*
783 *Phytologist*, **132**, 47-56.

784 Salleo S., Lo Gullo M.A., Trifilo' P. & Nardini A. (2004) New evidence for a role of vessel-
785 associated cells and phloem in the rapid xylem refilling of cavitated stems of *Laurus*
786 *nobilis* L. *Plant, Cell and Environment*, **27**, 1065-1076.

787 Salleo S., Trifilo' P. Lo Gullo M.A. (2008) Vessel wall vibrations: trigger for embolism repair?
788 *Functional Plant Biology*, **35**, 289-297.

789 Salleo S., Trifilo' P., Esposito S., Nardini A. & Lo Gullo M.A. (2009) Starch-to-sugar conversion
790 in wood parenchyma of field-growing *Laurus nobilis* plants: a component of the signal
791 pathway for embolism repair? *Functional Plant Biology*, **36**, 815-825.

792 Scheenen T.W.J., Vergeldt F.J., Heemskerk A.M. & Van As H. (2007) Intact plant magnetic
793 resonance imaging to study dynamics in long-distance sap flow and flow-conducting
794 surface area. *Plant Physiology*, **144**, 1157-1165.

795 Schneider A., Schatten T. & Rennenberg H. (1994) Exchange between phloem and xylem during
796 long-distance transport of glutathione in spruce trees (*Picea-abies* Karst l). *Journal of*
797 *Experimental Botany*, **45**, 457-462.

798 Secchi F., Lovisolo C. & Schubert A. (2007a) Expression of OePIP2.1 aquaporin gene and water
799 relations of *Olea europaea* twigs during drought stress and recovery. *Annals of Applied*
800 *Biology*, **150**, 163-167.

801 Secchi F., Lovisolo C., Uehlein N., Kaldenhoff R. & Schubert A. (2007b) Isolation and
 802 functional characterization of three aquaporins from olive (*Olea europaea* L.). *Planta*,
 803 **225**, 381-392.

804 Secchi F., MacIver B., Zeidel M.L. & Zwieniecki M.A. (2009) Functional analysis of putative
 805 genes encoding the PIP2 water channel subfamily in *Populus trichocarpa*. *Tree*
 806 *Physiology*, **29**, 1467-1477.

807 Secchi F. & Zwieniecki M.A. (2016) Accumulation of sugars in the xylem apoplast observed
 808 under water stress conditions is controlled by xylem pH. *Plant Cell and Environment* doi:
 809 10.1111/pce.12767

810 Secchi F. & Zwieniecki M.A. (2010) Patterns of PIP gene expression in *Populus trichocarpa*
 811 during recovery from xylem embolism suggest a major role for the PIP1 aquaporin
 812 subfamily as moderators of refilling process. *Plant Cell and Environment*, **33**, 1285-1297.

813 Secchi F., Gilbert M.E. & Zwieniecki M.A. (2011a) Transcriptome response to embolism
 814 formation in stems of *Populus trichocarpa* provides insight into signaling and the biology
 815 of refilling. *Plant Physiology*, **157**, 1419-1429.

816 Secchi F. & Zwieniecki M.A. (2011b) Sensing embolism in xylem vessels: the role of sucrose as
 817 a trigger for refilling. *Plant, Cell and Environment*, **34**, 514-524.

818 Secchi F. & Zwieniecki M.A. (2012) Analysis of xylem sap from functional (nonembolized) and
 819 nonfunctional (embolized) vessels of *Populus nigra*: chemistry of refilling. *Plant*
 820 *Physiology*, **160**, 955-964.

821 Secchi F. & Zwieniecki M.A. (2013) The physiological response of *Populus tremula x alba*
 822 leaves to the down-regulation of PIP1 aquaporin gene expression under no water stress.
 823 *Frontiers in Plant Science*, **4**.

824 Secchi F. & Zwieniecki M.A. (2014) Down-regulation of plasma intrinsic protein1 aquaporin in
 825 poplar trees is detrimental to recovery from embolism. *Plant Physiology*, **164**, 1789-1799.

826 Sharp R.G. & Davies W.J. (2009) Variability among species in the apoplastic pH signalling
 827 response to drying soils. *Journal of Experimental Botany*, **60**, 4361-4370.

828 Sheldon M.C., Howitt S.M., Kaiser B.N. & Tyerman S.D. (2009) Identification and functional
 829 characterisation of aquaporins in the grapevine, *Vitis vinifera*. *Functional Plant Biology*,
 830 **36**, 1065-1078.

831 Sobeih W.Y., Dodd I.C., Bacon M.A., Grierson D. & Davies W.J. (2004) Long-distance signals
 832 regulating stomatal conductance and leaf growth in tomato (*Lycopersicon esculentum*)
 833 plants subjected to partial root-zone drying. *Journal of Experimental Botany*, **55**, 2353-
 834 2363.

835 Sparks J.P. & Black R.A. (2000) Winter hydraulic conductivity and xylem cavitation in
 836 coniferous trees from upper and lower treeline. *Arctic Antarctic and Alpine Research*, **32**,
 837 397-403.

838 Sparks J.P., Campbell G.S. & Black R.A. (2001) Water content, hydraulic conductivity, and ice
 839 formation in winter stems of *Pinus contorta*: a TDR case study. *Oecologia*, **127**, 468-475.

840 Sperry J.S., Adler F.R., Campbell G.S. & Comstock J.P. (1998) Limitation of plant water use by
 841 rhizosphere and xylem conductance: results from a model. *Plant Cell and Environment*,
 842 **21**, 347-359.

843 Sperry J.S., Stiller V. & Hacke U.G. (2003) Xylem hydraulics and the soil-plant-atmosphere
 844 continuum: Opportunities and unresolved issues. *Agronomy Journal*, **95**, 1362-1370.

845 Spicer R. (2014) Symplasmic networks in secondary vascular tissues: parenchyma distribution
846 and activity supporting long-distance transport. *Journal of Experimental Botany*, **65**, 1829-
847 1848.

848 Sreedharan S., Shekhawat U.K.S. & Ganapathi T.R. (2013) Transgenic banana plants
849 overexpressing a native plasma membrane aquaporin MusaPIP1;2 display high tolerance
850 levels to different abiotic stresses. *Plant Biotechnology Journal*, **8**, 942-952.

851 Sreedharan S., Shekhawat U.K.S. & Ganapathi T.R. (2015) Constitutive and stress-inducible
852 overexpression of a native aquaporin gene (MusaPIP2;6) in transgenic banana plants
853 signals its pivotal role in salt tolerance. *Plant Molecular Biology*, **88**, 41-52.

854 Stiller V. & Sperry J.S. (2002) Cavitation fatigue and its reversal in sunflower (*Helianthus*
855 *annuus* L.). *Journal of Experimental Botany*, **53**, 1155-1161.

856 Stroock A.D., Pagay V.V., Zwieniecki M.A. & Holbrook N.M. (2014) *The Physicochemical*
857 *Hydrodynamics of Vascular Plants*. In: Annual Review of Fluid Mechanics, Vol 46 (eds
858 S.H. Davis & P. Moin), pp. 615-642.

859 Tornroth-Horsefield S., Wang Y., Hedfalk K., Johanson U., Karlsson M., Tajkhorshid E., Neutze
860 R. & Kjellbom P. (2006) Structural mechanism of plant aquaporin gating. *Nature*, **439**,
861 688-694.

862 Tournaire-Roux C., Sutka M., Javot H., Gout E., Gerbeau P., Luu D.T., Bligny R. & Maurel C.
863 (2003) Cytosolic pH regulates root water transport during anoxic stress through gating of
864 aquaporins. *Nature*, **425**, 393-397.

865 Trifilo' P., Gasco A., Raimondo F., Nardini A. & Salleo S. (2003) Kinetics of recovery of leaf
866 hydraulic conductance and vein functionality from cavitation-induced embolism in
867 sunflower. *Journal of Experimental Botany*, **54**, 2323-2330.

868 Tsuchihira A., Hanba Y.T., Kato N., Doi T., Kawazu T. & Maeshima M. (2010) Effect of
869 overexpression of radish plasma membrane aquaporins on water-use efficiency,
870 photosynthesis and growth of Eucalyptus trees. *Tree Physiology*, **30**, 417-430.

871 Tyree M.T. & Sperry J.S. (1989) Vulnerability of xylem to cavitation and embolism. *Annual*
872 *Reviews of Plant Physiology and Molecular Biology*, **40**, 19-38.

873 Tyree M.T. & Zimmermann M.H. (2002) *Xylem Structure and the Ascent of Sap*. (2nd ed.).
874 Springer-Verlag, New York.

875 Vanbel A.J.E. (1990) Xylem-phloem exchange via the rays - the undervalued route of transport.
876 *Journal of Experimental Botany*, **41**, 631-644.

877 Vandeleur R., Mayo G., Shelden M., Gilliam M., Kaiser B. & Tyerman S.D. (2009) The role of
878 plasma membrane intrinsic protein aquaporins in water transport through roots: diurnal
879 and drought stress responses reveal different strategies between isohydric and anisohydric
880 cultivars of grapevine. *Plant Physiology*, **149**, 445-460.

881 Wang N., Zhang H.H. & Nobel P.S. (1997) Phloem-xylem water flow in developing cladodes of
882 *Opuntia ficus-indica* during sink-to-source transition. *Journal of Experimental Botany*, **48**,
883 675-682.

884 Wilkinson S. & Davies W.J. (1997) Xylem sap pH increase: A drought signal received at the
885 apoplastic face of the guard cell that involves the suppression of saturable abscisic acid
886 uptake by the epidermal symplast. *Plant Physiology*, **113**, 559-573.

887 Wipfel K., Wittek A., Hedrich R. & Sauer N. (2010) Inverse pH regulation of plant and fungal
888 sucrose transporters: a mechanism to regulate competition for sucrose at the host/pathogen
889 interface? *Plos One*, **5**.

890 Yan X., Zhou M., Dong X., Zou S., Xiao H. & Ma X.-F. (2015) Molecular mechanisms of foliar
891 water uptake in a desert tree. *AoB PLANTS*, **7**.

892 Yang S.J., Zhang Y.J., Sun M., Goldstein G. & Cao K.F. (2012) Recovery of diurnal depression
893 of leaf hydraulic conductance in a subtropical woody bamboo species: embolism refilling
894 by nocturnal root pressure. *Tree Physiology*, **32**, 414-422.

895 Yooyongwech S., Sugaya S., Sekozawa Y. & Gemma H. (2009) Differential adaptation of high-
896 and low-chill dormant peaches in winter through aquaporin gene expression and soluble
897 sugar content. *Plant Cell Reports*, **28**, 1709-1715.

898 Zelazny E., Borst J.W., Muylaert M., Batoko H., Hemminga M.A. & Chaumont F. (2007) FRET
899 imaging in living maize cells reveals that plasma membrane aquaporins interact to
900 regulate their subcellular localization. *Proc Natl Acad Sci*, **104**, 12359-12364.

901 Zimmermann M.H. (1983) *Xylem structure and the ascent of sap* (vol. 1). Springer-Verlag,
902 Berlin, New York

903 Zwieniecki M.A. & Holbrook N.M. (2009) Confronting Maxwell's demon: biophysics of xylem
904 embolism repair. *Trends in Plant Science*, **14**, 530-534.

905 Zwieniecki M.A., Melcher P.J. & Ahrens E.T. (2013) Analysis of spatial and temporal dynamics
906 of xylem refilling in *Acer rubrum* L. using magnetic resonance imaging. *Frontiers Plant*
907 *Science*, **4**, 265.

908 Zwieniecki M.A., Melcher P.J., Feild T. & Holbrook N.M. (2004) A potential role for xylem-
909 phloem interactions in the hydraulic architecture of trees: effects of phloem girdling on
910 xylem hydraulic conductance. *Tree Physiology*, **24**, 911-917.

911 Zwieniecki M.A., Secchi F. (2015) Threats to xylem hydraulic function of trees under "new
912 climate normal' conditions. *Plant Cell and Environment*, **38**, 1713-1724.

913

Figure and Table legends

Figure 1

Schematic illustration of membrane transporter activity during onset of water stress and recovery (Secchi & Zwieniecki 2016). Please refer to the text for the details of the overall scenario describing stem parenchyma cell activity,.

Figure 2

Neighbor-joining circle tree of the woody plant PIP1- and PIP2-type aquaporin proteins detailed in Table1. Only for *Vitis* aquaporins, the letters 110R, F or S at the end of the protein name identify the aquaporins characterized by Baiges *et al.* (2001), Fouquet *et al.* (2008) and Shelden *et al.* (2009), respectively. The significance of each node was tested using 2000 bootstrap replicates. Accession numbers of each aquaporin are reported in Table1 together with expression details and references. The green rectangular indicate, respectively, the specific PIP expression (++ or +++) in xylem, purple in the phloem and red in entire stem tissue (bark and wood). The filled red rectangles denote the presence of PIP expression exclusively in stem tissue (it was not detectable in the other tissues tested).

Table 1. List of known PIP aquaporins expressed in woody plants with focus on stem aquaporins.

934 **Figure 1**

935

Figure 2

Table 1.

Gene name	Species	Gene ID NCBI GenBank ^(a) / Phytozome ^(b)	Expression patterns	Response to embolism/ recovery	References
PtPIP1.1	<i>Populus trichocarpa</i>	POPTR_0008s06580 (b)	L (+++), R (++), W (++), B (+++)	**	(Secchi <i>et al.</i> , 2009, Secchi & Zwieniecki, 2010, Secchi & Zwieniecki, 2011)
PtPIP1.2	<i>Populus trichocarpa</i>	POPTR_0003s12870 (b)	L (++), R (++), W (++), B (++)	no changes	(Secchi <i>et al.</i> , 2009, Secchi & Zwieniecki, 2010, Secchi & Zwieniecki, 2011)
PtPIP1.3	<i>Populus trichocarpa</i>	POPTR_0010s19930 (b)	L (+++), R (++), W (++), B (+++)	*	(Secchi <i>et al.</i> , 2009, Secchi & Zwieniecki, 2010, Secchi & Zwieniecki, 2011)
PtPIP1.4	<i>Populus trichocarpa</i>	POPTR_0006s09920 (b)	L (+), R (+), W (+), B (+)	*	(Secchi <i>et al.</i> , 2009, Secchi & Zwieniecki, 2010, Secchi & Zwieniecki, 2011)
PtPIP1.5	<i>Populus trichocarpa</i>	POPTR_0016s12070 (b)	L (++), R (++), W (++), B (++)	*	(Secchi <i>et al.</i> , 2009, Secchi & Zwieniecki, 2010, Secchi & Zwieniecki, 2011)
PtPIP2.1	<i>Populus trichocarpa</i>	POPTR_0009s13890 (b)	L (+), R (+), W (+), B (+)	*	(Secchi <i>et al.</i> , 2009, Secchi & Zwieniecki, 2010, Secchi & Zwieniecki, 2011)
PtPIP2.2	<i>Populus trichocarpa</i>	POPTR_0004s18240 (b)	L (+++), R (+++), W (+++), B (+++)	*	(Secchi <i>et al.</i> , 2009, Secchi & Zwieniecki, 2010, Secchi & Zwieniecki, 2011)
PtPIP2.3	<i>Populus trichocarpa</i>	POPTR_0016s09090 (b)	L (+), R (+), W (++), B (++)	**	(Secchi <i>et al.</i> , 2009, Secchi & Zwieniecki, 2010, Secchi & Zwieniecki, 2011)
PtPIP2.4	<i>Populus trichocarpa</i>	POPTR_0010s22950 (b)	L (++), R (++), W (++), B (++)	*	(Secchi <i>et al.</i> , 2009, Secchi & Zwieniecki, 2010, Secchi & Zwieniecki, 2011)

PtPIP2.5	<i>Populus trichocarpa</i>	POPTR_0006s12980 (b)	L (+++), R (+++), W (++), B (+)	*	(Secchi <i>et al.</i> , 2009, Secchi & Zwieniecki, 2010, Secchi & Zwieniecki, 2011)
PtPIP2.6	<i>Populus trichocarpa</i>	POPTR_0008s03950 (b)	L (+++), R (++), W (++), B (++)	**	(Secchi <i>et al.</i> , 2009, Secchi & Zwieniecki, 2010, Secchi & Zwieniecki, 2011)
PtPIP2.7	<i>Populus trichocarpa</i>	POPTR_0009s01940 (b)	L (++), R (+++), W (+), B (++)	*	(Secchi <i>et al.</i> , 2009, Secchi & Zwieniecki, 2010, Secchi & Zwieniecki, 2011)
PtPIP2.8	<i>Populus trichocarpa</i>	POPTR_0005s11110 (b)	L (+++), R (+), W (+), B (++)	*	(Secchi <i>et al.</i> , 2009, Secchi & Zwieniecki, 2010, Secchi & Zwieniecki, 2011)
OePIP2.1	<i>Olea europea</i> L	DQ202709 (a)	L (+), R (+++), T (+)	**	(Secchi <i>et al.</i> , 2007a, Secchi <i>et al.</i> , 2007b)
OePIP1.1	<i>Olea europea</i> L	DQ202708 (a)	L (+), R (++), T (++)	**	(Secchi <i>et al.</i> , 2007a, Secchi <i>et al.</i> , 2007b)
PttPIP2.1	<i>Populus tremula x tremuloides</i>	AJ849324 (a)	L (++), S (++), MR (++), FR (++)		(Marjanovic <i>et al.</i> , 2005)
PttPIP2.2	<i>Populus tremula x tremuloides</i>	AJ849325 (a)	L (+), S (+), MR (++), FR (+++)		(Marjanovic <i>et al.</i> , 2005)
PttPIP2.3	<i>P. tremula · tremuloides</i>	AJ849326 (a)	L (+), S (+), MR (++), FR (+)		(Marjanovic <i>et al.</i> , 2005)
PttPIP2.4	<i>P. tremula · tremuloides</i>	AJ849327 (a)	L (++), S (+), MR (+), FR (++)		(Marjanovic <i>et al.</i> , 2005)
PttPIP2.5	<i>P. tremula · tremuloides</i>	AJ849328 (a)	L (+), S (-), MR (+), FR (++)		(Marjanovic <i>et al.</i> , 2005)
PttPIP1.1	<i>P. tremula · tremuloides</i>	AJ849323 (a)	L (+), S (+), MR (++), FR (+++)		(Marjanovic <i>et al.</i> , 2005)
PttPIP1.2	<i>P. tremula · tremuloides</i>	AJ849322 (a)	L (+), S (++), MR (++), FR (++)		(Marjanovic <i>et al.</i> , 2005)

JrPIP2.1	<i>Juglans regia</i>	AY189974 (a)	L (+++), B (++), W (++), R (+)	**	(Sakr <i>et al.</i> , 2003)
JrPIP2.2	<i>Juglans regia</i>	AAO39008 (a)	L (+++), B (+), W (+), R (+++)	**	(Sakr <i>et al.</i> , 2003)
PtPIP2.3	<i>Populus</i> x'Okanese'	567607 (b)	P (++), C (+++), R(CC) (+++), R(IC) (+)	**	(Almeida-Rodriguez & Hacke, 2012)
PtPIP2.5	<i>Populus</i> x'Okanese'	826419 (b)	P (++), C (+++), R(CC) (+++), R(IC) (+)	**	(Almeida-Rodriguez & Hacke, 2012)
PgPIP1;1	<i>Picea glauca</i>	BT113218 (a)	R, L, S, W	*	(Laur & Hacke, 2014)
PgPIP1;2	<i>Picea glauca</i>	BT115139 (a)	R, L, S	**	(Laur & Hacke, 2014)
PgPIP1;3	<i>Picea glauca</i>	BT105794 (a)	S, L		(Laur & Hacke, 2014)
PgPIP2;1	<i>Picea glauca</i>	BT107672 (a)	R, L, S	*	(Laur & Hacke, 2014)
PgPIP2;2	<i>Picea glauca</i>	BT105999 (a)	R, L, S, W	**	(Laur & Hacke, 2014)
PgPIP2;3	<i>Picea glauca</i>	BT115639 (a)	R, S		(Laur & Hacke, 2014)
PgPIP2;5	<i>Picea glauca</i>	BT108646 (a)	reproductive parts		(Laur & Hacke, 2014)
PgPIP2;7	<i>Picea glauca</i>	BT106222 (a)	R, S		(Laur & Hacke, 2014)
PgPIP2;8	<i>Picea glauca</i>	BT106086 (a) (partial sequence)	R, L, S		(Laur & Hacke, 2014)
PgPIP2;9	<i>Picea glauca</i>	BT106471 (a)	S		(Laur & Hacke, 2014)
PgPIP2;10	<i>Picea glauca</i>	BT106822 (a)	R, S		(Laur & Hacke, 2014)
PgPIP2;11	<i>Picea glauca</i>	BT106775 (a)	S		(Laur & Hacke, 2014)
PgPIP2;12	<i>Picea glauca</i>	BT106446 (a)	R, S		(Laur & Hacke, 2014)
PgPIP2;13	<i>Picea glauca</i>	BT110135 (a)	S		(Laur & Hacke, 2014)
CaPIP2;1	<i>Coffea arabica</i>	LM654169 (a)	L, R	**	(Miniussi <i>et al.</i> , 2015)
CaPIP2;2	<i>Coffea arabica</i>	LM654170 (a)	L, R	**	(Miniussi <i>et al.</i> , 2015)
CaPIP1;1	<i>Coffea arabica</i>	LM654171 (a)	L, R	**	(Miniussi <i>et al.</i> , 2015)

CaPIP1;2	<i>Coffea arabica</i>	LM654172 (a)	L, R	**	(Miniussi <i>et al.</i> , 2015)
ThPIP1-2	<i>Tamarix ramosissima</i>	Unigene23675 (a)	L	*	(Yan <i>et al.</i> , 2015)
ThPIP2-1	<i>Tamarix ramosissima</i>	Unigene38680 (a)	L	*	(Yan <i>et al.</i> , 2015)
QpPIP2:1	<i>Quercus petraea</i> , <i>Quercus robur</i>	JQ768372 (a)	R (+++)		(Rasheed-Depardieu <i>et al.</i> , 2012)
QpPIP2:2	<i>Quercus petraea</i> , <i>Quercus robur</i>	JQ846268 (a)	R (++)		(Rasheed-Depardieu <i>et al.</i> , 2012)
QpPIP2:3	<i>Quercus petraea</i> , <i>Quercus robur</i>	JQ846269 (a)	R (+)		(Rasheed-Depardieu <i>et al.</i> , 2012)
QpPIP1:1	<i>Quercus petraea</i> , <i>Quercus robur</i>	JQ846270 (a)	R (++)		(Rasheed-Depardieu <i>et al.</i> , 2012)
QpPIP1:2	<i>Quercus petraea</i> , <i>Quercus robur</i>	JQ846271 (a)	R (++)		(Rasheed-Depardieu <i>et al.</i> , 2012)
QpPIP1:3	<i>Quercus petraea</i> , <i>Quercus robur</i>	JQ846272 (a)	R (-)		(Rasheed-Depardieu <i>et al.</i> , 2012)
MusaPIP1;2	<i>Musa acuminata</i>	FF561783 (a)	L (++)		(Sreedharan <i>et al.</i> , 2013)
MusaPIP2;6	<i>Musa acuminata</i>	FL667907 (a)	R, L		(Sreedharan <i>et al.</i> , 2015)
EcPIP1	<i>Eucalyptus grandis</i>	XM_010057362.1 (a)	L (+)		(Tsuchihira <i>et al.</i> , 2010)
EcPIP2	<i>Eucalyptus camaldulensis</i>	XM_010033696.1 (a)	L (+)		(Tsuchihira <i>et al.</i> , 2010)
CsPIP1;1	<i>Citrus sinensis</i>	orange1.1g018895 (b)	R (+), L (+++), FL (+++), CL (+++), F (+++)		(de Paula Santos Martins <i>et al.</i> , 2015)

CsPIP1;2	<i>Citrus sinensis</i>	orange1.1g023021 (b)	R (+), L (+), FL (++), CL (++), F (++)		(de Paula Santos Martins <i>et al.</i> , 2015)
CsPIP1;3	<i>Citrus sinensis</i>	orange1.1g023107 (b)	R (+), L (+++), FL (+++), CL (++), F (++)		(de Paula Santos Martins <i>et al.</i> , 2015)
CsPIP1;4	<i>Citrus sinensis</i>	orange1.1g023069 (b)	R (+), L (++), FL (+++), CL (-), F (++)		(de Paula Santos Martins <i>et al.</i> , 2015)
CsPIP2;1	<i>Citrus sinensis</i>	orange1.1g023108 (b)	R (+), L (+), FL(-), CL (-), F (+)		(de Paula Santos Martins <i>et al.</i> , 2015)
CsPIP2;2	<i>Citrus sinensis</i>	orange1.1g022966 (b)	R (+), L (++), FL (++), CL (++), F (++)		(de Paula Santos Martins <i>et al.</i> , 2015)
CsPIP2;3	<i>Citrus sinensis</i>	orange1.1g019681 (b)	R (+), L (+++), FL (+++), CL (++), F (+++)		(de Paula Santos Martins <i>et al.</i> , 2015)
CsPIP2;4	<i>Citrus sinensis</i>	orange1.1g023370 (b)	R (+), L (+++), FL (+++), CL (+), F (+)		(de Paula Santos Martins <i>et al.</i> , 2015)
PpPIP1	<i>Prunus persica</i>	AB303644 (a)	Bu	Cold stress	(Yooyongwech <i>et al.</i> , 2009)
PpPIP2	<i>Prunus persica</i>	AB329725 (a) (partial seq)	Bu	Cold stress	(Yooyongwech <i>et al.</i> , 2009)
VvPIP1;1	<i>Vitis hybrid Richter-110</i>	AF141643 (a)	R (+++), RT (+++), L (+), S (+)		(Baiges <i>et al.</i> , 2001, Galmes <i>et al.</i> , 2007)
VvPIP1;2	<i>Vitis hybrid Richter-110</i>	AF141898 (a)	R (+), RT (+), L (+), S (++)		(Baiges <i>et al.</i> , 2001, Galmes <i>et al.</i> , 2007)
VvPIP1;3	<i>Vitis hybrid Richter-110</i>	AF141899 (a)	R (++), RT (++), L (+), S (++)		(Baiges <i>et al.</i> , 2001, Galmes <i>et al.</i> , 2007)
VvPIP2;1	<i>Vitis hybrid Richter-110</i>	AF141642 (a)	R (++), RT (+++), L (++), S (++)		(Baiges <i>et al.</i> , 2001, Galmes <i>et al.</i> , 2007)
VvPIP2;2	<i>Vitis hybrid Richter-110</i>	AF141900 (a)	R (++), RT (+++), L (++), S (+++)		(Baiges <i>et al.</i> , 2001, Galmes <i>et al.</i> , 2007)
VvPIP1a	<i>V.vinifera</i> cv. Chardonnay, Ugny Blanc, Pinot Meunier	AF188844 (a)	F (++)		(Picaud <i>et al.</i> , 2003)

VvPIP1b	<i>V.vinifera</i> cv. Chardonnay, Ugny Blanc, Pinot Meunier	AF188843 (a)	F (++)		(Picaud <i>et al.</i> , 2003)
VvPIP1;1	<i>V.vinifera</i> cv. Cabernet Sauvignon	DQ834694 (a)	F (+)		(Fouquet <i>et al.</i> , 2008)
VvPIP1;2	<i>V.vinifera</i> cv. Cabernet Sauvignon	DQ834695 (a)	F (+)		(Fouquet <i>et al.</i> , 2008)
VvPIP1;3	<i>V.vinifera</i> cv. Cabernet Sauvignon	DQ834696 (a)	F (+++)		(Fouquet <i>et al.</i> , 2008)
VvPIP2;1	<i>V.vinifera</i> cv. Cabernet Sauvignon	DQ834698 (a)	F (+++)		(Fouquet <i>et al.</i> , 2008)
VvPIP2;2	<i>V.vinifera</i> cv. Cabernet Sauvignon	DQ834699 (a)	F (+)		(Fouquet <i>et al.</i> , 2008)
VvPIP2;3	<i>V.vinifera</i> cv. Cabernet Sauvignon	DQ834700 (a)	F (++)		(Fouquet <i>et al.</i> , 2008)
VvPIP1;1	<i>Vitis vinifera</i> diverse cvs	EF364432 (a)	R (-, cv Nebbiolo; ++ cv. Grenache and Chardonnay), L (+), S (-), P (++)	**	(Chitarra <i>et al.</i> , 2014, Perrone <i>et al.</i> , 2012a, Perrone <i>et al.</i> , 2012b, Pou <i>et al.</i> , 2013, Shelden <i>et al.</i> , 2009, Vandeleur <i>et al.</i> , 2009)
VvPIP1;2	<i>Vitis vinifera</i> diverse cvs	EF364433 (a)	R (+), S (++), P(-)	*	(Vandeleur <i>et al.</i> , 2009, Perrone <i>et al.</i> , 2012b, Chitarra <i>et al.</i> , 2014, Shelden <i>et al.</i> , 2009)
VvPIP1;4	<i>Vitis vinifera</i> diverse cvs	EF364435 (a)	R (++)		(Vandeleur <i>et al.</i> , 2009, Shelden <i>et al.</i> , 2009)

VvPIP2;1	<i>Vitis vinifera</i> diverse cvs	AY823263 (a)	R (+), L (++), S (++), P (++)	**	(Vandeleur <i>et al.</i> , 2009, Perrone <i>et al.</i> , 2012a, Perrone <i>et al.</i> , 2012b, Chitarra <i>et al.</i> , 2014, Pou <i>et al.</i> , 2013, Shelden <i>et al.</i> , 2009)
VvPIP2;2	<i>Vitis vinifera</i> diverse cvs	EF364436 (a)	R (++), L (++), S (++)	**	(Vandeleur <i>et al.</i> , 2009, Perrone <i>et al.</i> , 2012a, Perrone <i>et al.</i> , 2012b, Pou <i>et al.</i> , 2013, Shelden <i>et al.</i> , 2009)
VvPIP2;3	<i>Vitis vinifera</i> diverse cvs	EF364437 (a)	R (+), L (-, cv. Nebbiolo; ++, cv. Chardonnay)	**	(Vandeleur <i>et al.</i> , 2009, Perrone <i>et al.</i> , 2012a, Pou <i>et al.</i> , 2013, Shelden <i>et al.</i> , 2009)
VvPIP2;4	<i>Vitis vinifera</i> diverse cvs	EF364438 (a)	R (+)		(Vandeleur <i>et al.</i> , 2009, Shelden <i>et al.</i> , 2009)
VvPIP2;4N	<i>Vitis vinifera</i> diverse cvs	DQ358107 (a)	R (+++), L (+), S (-), P (++)	**	(Perrone <i>et al.</i> , 2012a, Perrone <i>et al.</i> , 2012b, Chitarra <i>et al.</i> , 2014)

L – leaf, R – root, MP - main root, FR - fine root, RT -root tip, W – wood/xylem, B – bark, S – stem (wood and bark), T – twig, P - phloem (mainly in companion cells), C - cambial zone and derivatives, R – ray parenchyma, R(CC) – ray contact cells, R(IC) - ray, isolation cells, Bu – buds, FL – flower, F – Fruit, CL - Callus.

+++ : strong expression; ++ : expression; + : detectable expression; - : no expression

* not significant changes in gene expression; ** significant changes in gene expression.