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Review

Aquaporins and plant transpiration

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ABSTRACT

Although transpiration and aquaporins have long been identified as two key components influencing plant water status, it is only recently that their relations have been investigated in detail. The present review first examines the various facets of aquaporin function in stomatal guard cells and shows that it involves transport of water but also of other molecules such as carbon dioxide and hydrogen peroxide. At the whole plant level, changes in tissue hydraulics mediated by root and shoot aquaporins can indirectly impact plant transpiration. Recent studies also point to a feedback effect of transpiration on aquaporin function. These mechanisms may contribute to the difference between isohydric and anisohydric stomatal regulation of leaf water status. The contribution of aquaporins to transpiration control goes far beyond the issue of water transport during stomatal movements and involves emerging cellular and long-distance signalling mechanisms which ultimately act on plant growth.

Key-words: guard cell signalling; isohydric and anisohydric plants; stomatal movement.

INTRODUCTION

Gas exchange between plant shoots and the atmosphere plays a key role in plant function and performance through the intake of carbon dioxide (CO_2) to supply photosynthetic carbon fixation and the diffusion of water vapour by transpiration. The latter is driven by the evaporative demand, that is the dramatic drop in vapour pressure between plant tissues and the atmosphere. Water loss by transpiration cannot simply be considered as the detrimental component of a trade-off for optimizing CO_2 intake and carbon fixation. Transpiration is important in leaf cooling and drives xylem-mediated mass flow of nutrients from the soil into the uppermost parts of the plant (Cowan & Farquhar, 1977; Taiz & Zeiger, 1991; Medina & Gilbert, 2016). Because of these multiple and sometimes conflicting functions, gas exchanges by plant aerial parts must be tightly controlled. Gas exchanges are mediated to a large extent through stomata, specialized pores differentiated from epidermal cells at the surface of leaves and stems (Taiz & Zeiger, 1991; Murata *et al.,* 2015). The remaining outer surface of the epidermis is covered with a largely gas-tight cuticle (Yeats & Rose, 2013).

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Aquaporins are intrinsic membrane proteins present in the plasma membrane and most inner membranes of plants cells. While most aquaporins function as channels to facilitate transmembrane water transport, they can also transport small neutral molecules such as gases (CO_2, ammonia), reactive oxygen species (hydrogen peroxide: H_2O_2) and metalloids (boric acid, silicic acid, antimonite, arsenite) (Kaldenhoff, 2012; Bienert & Chaumont, 2014; Maurel *et al.,* 2015). The water transport function of aquaporins plays a key role in as diverse processes as root water uptake, leaf hydraulics, seed and pollen grain germination, expansive growth and lateral root emergence (For recent reviews, see Chaumont & Tyerman, 2014; Maurel *et al.,* 2015). In relation to their multiple cellular localizations and functions, plant aquaporins fall into at least four subclasses that each shows a high isoform multiplicity. Of specific interest for this article, are the Plasma membrane Intrinsic Proteins (PIP; 13 isoforms in *Arabidopsis*) and the Tonoplast Intrinsic Proteins (TIP; 9 isoforms in *Arabidopsis*) which represent the most abundant aquaporins in the plasma membrane and vacuolar membrane (tonoplast), respectively.

This review examines the relations between aquaporins and plant transpiration, two important components of plant water relations. We first discuss the various facets of aquaporin function in stomatal guard cells and show that it involves transport of water but also of other molecules. We also show that function of aquaporins in roots and shoots is intimately linked to transpiration. Thus, the contribution of aquaporins to transpiration control goes far beyond the issue of water transport during stomatal movements and involves emerging cellular and long-distance signalling mechanisms.

THE ROLE OF AQUAPORINS IN GUARD CELLS

Principles of stomatal movements

Stomata are microscopic pores delineated by a pair of guard cells. In certain plant families such as cereals, guard cells are themselves surrounded by specialized subsidiary cells to form so-called stomatal complexes. Stomatal aperture is itself determined by the volume and mechanics of guard cells. An increase in guard cell osmotic pressure leads to water intake, guard cell swelling and stomatal opening. Conversely, osmotically driven water efflux from guard cells leads to stomatal closure. These movements are under tight control by the circadian clock and numerous environmental and hormonal signals that promote stomatal opening (light, high air humidity, some phytotoxins) or closure (abscisic acid, jasmonic acid, high ambient CO_2,

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ozone, Pathogen Associated Molecular Patterns (PAMPs)) (Maurel *et al.,* 2015). Thus, stomatal aperture can adapt to daily fluctuations in evaporative demand or light, respond on the longer-term to soil water or atmospheric CO_2 availability and mediate elaborate plant strategies to counteract pathogen attacks.

Aquaporin expression in guard cells

As aquaporins can be found in essentially any plant tissue, it was no surprise to detect expression of aquaporins in guard cells of all higher plants examined, in species as diverse as *Arabidopsis* (Leonhardt *et al.,* 2004; Prasch *et al.,* 2015), sunflower (*Helianthus annuus*) (Sarda *et al.,* 1997), broad bean (*Vicia faba*) (Sun *et al.,* 2001), spinach (*Spinacia oleracea*) (Fraysse *et al.,* 2005), maize (*Zea mays*) (Heinen *et al.,* 2014), tree tobacco (*Nicotiana glauca*) (Smart *et al.,* 2001) and Norway spruce (*Picea abies*) (Oliviusson *et al.,* 2001). In contrast to certain plant organs such as seeds and pollen grains which express specific aquaporin isoforms, guard cells harbour PIPs and TIPs which are also expressed in other tissues (Smart *et al.,* 2001; Leonhardt *et al.,* 2004; Fraysse *et al.,* 2005; Heinen *et al.,* 2014; Prasch *et al.,* 2015). Yet, many studies have shown that regulation of aquaporin expression in guard cells is linked to key components of stomatal regulation. In *Arabidopsis* guard cells, for instance, the transcript abundance of several *PIPs* was enhanced after 4 h in response to spray of ABA on the leaf surface (Leonhardt *et al.,* 2004). In tree tobacco, expression of two *TIP* genes was reduced by drought after withholding water for 3–4 days (Smart *et al.,* 2001). In sunflower, expression of a TIP showed diurnal variation, was transiently induced in response to water stress and in both cases was synchronized with stomatal closure (Sarda *et al.,* 1997). In maize stomatal complexes, six out of seven *PIP* genes investigated showed a diurnal expression pattern with >3-fold higher expression in the morning than at night (Heinen *et al.,* 2014). Finally, expression of two *PIP* and three *TIP* genes was reduced in an *Arabidopsis* mutant lacking a guard cell β-amylase and defective in starch degradation and stomatal opening (Prasch *et al.,* 2015).

Cellular roles

Water transport

Initial evidence for a role of aquaporins in stomatal movements was rather indirect. For instance, the dose-dependent effects of extracellular calcium on stomatal aperture in broad bean leaf epidermal peals were tentatively associated to an activation and an inhibition of aquaporins by low and high calcium concentrations, respectively (Yang *et al.,* 2006). Shope & Mott (2006) also investigated broad bean guard cells and used the kinetics of osmotically induced changes in cell volume to estimate their apparent hydraulic conductivity. Interestingly, this parameter was sensitive to membrane trafficking inhibitors (cytochalasin D, wortmannin) provided that guard cells had received a hyperosmotic pretreatment. This was interpreted to mean that protein (aquaporin)-mediated transport of water had been induced in these conditions. Consistent with this idea, the water permeability of isolated *Arabidopsis* guard cell protoplasts was enhanced twofold in response to a 10 μM ABA treatment (Grondin *et al.,* 2015). The role of *At*PIP2;1 aquaporin in this process was assessed using protoplasts from *pip2;1* knock-out plants which showed a similar basal water permeability as wild types but lacked any response to ABA. In fact, *At*PIP2;1 can be phosphorylated at a specific cytosolic site (Ser121) and activated by the Snf1-Related protein Kinase 2.6 (SnRK2.6, also named OST1) (Grondin *et al.,* 2015). OST1 is itself released from inhibition by clade A Protein Phosphatases 2C (PP2C) such as ABA Insensitive 1 (ABI1), upon binding of ABA to RCAR/Pyr/PYL receptors which in turn capture PP2Cs (Cutler *et al.,* 2010) (Fig. 1). The role of Ser121 phosphorylation in activation of *At*PIP2;1 by ABA was definitely established by water transport measurements in guard cell protoplasts expressing phosphodeficient or phosphomimetic mutant forms of *At*PIP2;1 at Ser121. Consistent with an ABA-dependent activation of *At*PIP2;1, stomata

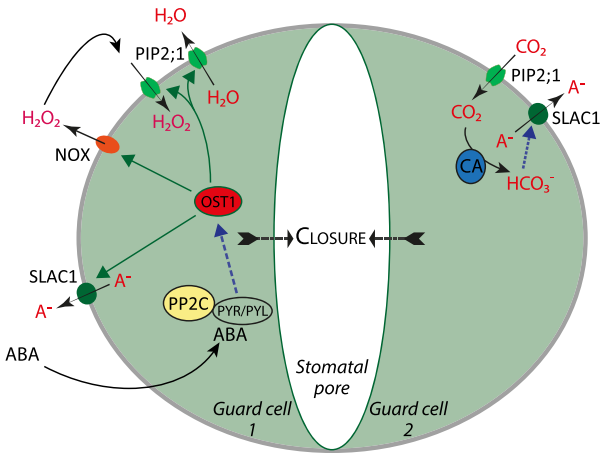

Figure 1. Simplified overview of implication of aquaporins in ABA- and CO_2-induced stomatal movements in *Arabidopsis*. The drawing represents two guard cells (light green) delineating a stomatal pore (white). **Left** (guard cell 1), under resting conditions, clade A Protein Phosphatase 2C (PP2C) family members, such as ABA Insensitive 1 (ABI1), act as negative regulators of ABA signalling, inhibiting the Snf1-Related protein Kinase 2.6 (SnRK2.6, also named OST1) via physical interaction, and leaving the S-type anion channel (SLAC1) with basal activity. Under drought, the ABA concentration in leaves increases and is perceived via Regulatory Component of ABA Receptor (RCAR) family members (Pyr/PYL). The ABA-induced formation of the RCAR/Pyr/PYL–PP2C complex breaks the PP2C–OST1 complex, thereby releasing active OST1 kinase. In turn, OST1 phosphorylates and activates several guard cell membrane proteins, such as the plasma membrane aquaporin *At*PIP2;1 (PIP2;1), NADPH oxidases (NOX) such as RbohD, and the SLAC1 anion channel. Apoplastic H_2O_2 resulting from NOX activity may diffuse into the guard cell through *At*PIP2;1 to trigger subsequent signalling events essential for stomatal closure. Release of anions through SLAC1 depolarizes the membrane and triggers cation efflux. The accompanying osmotic efflux of water mediated by *At*PIP2;1 leads to a drop in guard cell turgor and to stomatal closure. **Right** (guard cell 2), *At*PIP2;1 also physically interacts with plasma membrane β-Carbonic Anhydrase 4 (CA), facilitating the transmembrane diffusion of ambient CO_2 into the guard cell. Resulting intracellular bicarbonate binds to and activates SLAC1, thereby promoting stomatal closure.

of *pip2;1* plants opened and closed normally in response to changing light or ambient CO_2 whereas they failed to close in response to ABA. Further, this response could be restored after expression of the phosphomimetic but not the phosphodeficient form of *At*PIP2;1 at Ser121 (Grondin *et al.,* 2015). We note that a role for PIPs in stomatal responses to ABA had previously been suggested by the finding that overexpression of a *V. faba* PIP1 in *Arabidopsis* leaves accelerated ABA-induced stomatal closure in a peeled epidermal assay (Cui *et al.,* 2008). As indicated above, stomatal complexes of grasses have subsidiary cells which participate in stomatal movement via bulk water and ion flow into guard cells (Raschke & Fellows, 1971). It will be interesting to investigate whether aquaporins fulfil specific functions in these cells to assist stomatal movements. More generally, pharmacological and genetic experiments in algae (*Chara corallina*), moss (*Physcomitrella patens*) or higher plants (*Raphanus sativus*) have shown that aquaporins are not limiting for evaporation from outer cell wall surface (Tazawa & Okazaki, 1997; Lienard *et al.,* 2008), as would occur in stomatal chambers.

H_2O_2 transport

Grondin *et al.* (2015) have suggested that the failure of *pip2;1* guard cells to close in response to ABA may be because of combined defects in membrane water transport and hormonal signalling. In support for the latter hypothesis, *pip2;1* guard cells lacked the typical intracellular accumulation of reactive oxygen species that occurs over the 30 min following exposure to ABA. H_2O_2 is produced in the apoplasm through ABA-dependent activation of plasma membrane NADPH oxidases and plays a central role in ABA signalling (Murata *et al.,* 2015). However, the sites of action of H_2O_2 and its modes of diffusion into the cells have remained undetermined. Aquaporins, which were shown to transport H_2O_2 after functional expression in yeast (Bienert *et al.,* 2007; Dynowski *et al.,* 2008), represent interesting candidates for this function (Fig. 1). This hypothesis is currently being investigated in our laboratory using the HyPer genetic probe as an intracellular reporter of H_2O_2 (Rodrigues et al., unpublished data).

CO_2 transport

Functional expression in yeast cells or *Xenopus* oocytes have indicated that some of the PIP isoforms expressed in maize or *Arabidopsis* guard cells (*Zm*PIP1;5, *Zm*PIP1;6, *At*PIP2;1) can transport CO_2 in addition to water (Heinen *et al.,* 2014; Wang *et al.,* 2016). The preferential localization of *Zm*PIP1;5 and *Zm*PIP1;6 at the plasma membrane (and not the chloroplast envelope) suggested a role in CO_2 signalling rather than CO_2 fixation (Fig. 1). This idea was recently corroborated by the finding that *At*PIP2;1 physically interacts with plasma membrane β-Carbonic Anhydrase 4 (βCA4) (Wang *et al.,* 2016). Co-expression in *Xenopus* oocytes of the two partners with Slow Anion Associated-Channel 1 (SLAC1) and activating protein kinases (OST1, CPK6 or CPK23) was necessary to confer on SLAC1 an enhancement by extracellular CO_2. This study led to a model whereby *At*PIP2;1 and βCA4 cooperatively facilitate the transmembrane diffusion of ambient CO_2 to enhance the intracellular concentration of bicarbonate (Fig. 1). Bicarbonate in turns binds to and activates SLAC1, thereby promoting stomatal closure. However, *pip2;1* guard cells showed a normal response to external CO_2, likely because of a functional redundancy of *At*PIP2;1 with other PIP isoforms. Thus, this interesting model awaits confirmation in the plant.

Conclusion

Initial studies on the function of aquaporins in guard cells have focused on the key role of these proteins in membrane water transport. While this role definitely remains relevant, a few recent reports point to new aquaporin functions related to cell signalling, with potential significance beyond the context of stomatal regulation (Heinen *et al.,* 2014; Grondin *et al.,* 2015; Wang *et al.,* 2016). A future challenge will be to determine how these functions lead to integration of aquaporins in the numerous signalling pathways acting in guard cells and how aquaporins may themselves create cross-talks between these pathways. Phosphorylation of *At*PIP2;1 in guard cells is enhanced by ABA (Grondin *et al.,* 2015) while gene expression of this and other PIPs can be regulated by H_2O_2 in leaves and roots (Hooijmaijers *et al.,* 2012). Thus, it will be crucial to establish more precisely how the signalling pathways at work in guard cells can themselves act on the activity (or subcellular localization) of aquaporins. The functional redundancy of the numerous aquaporin isoforms expressed in guard cells may hinder the genetic validation of these analyses.

Whereas studies have so far focused on the role of PIPs, TIPs may also play key and original roles in stomatal responses. For instance, compartmental analyses of ion fluxes in osmotically challenged guard cells of *Commelina communis* have revealed that the tonoplast might be able to sense local osmotic gradients to promote vacuolar ion release. A tentative role in osmosensing has been assigned to aquaporins sitting in this membrane (MacRobbie, 2006).

WHOLE PLANT AQUAPORIN FUNCTIONS AND PLANT TRANSPIRATION

Principles of plant transpiration

Several steps possibly determine the transfer of water in the soil–plant–atmosphere continuum and, as a consequence, plant water use. For instance, reduced water equilibration in the rhizosphere can result in local dehydration and thereby a high soil hydraulic resistance (Draye *et al.,* 2010). The diffusion of water vapour from stomatal apertures is another strongly limiting process. It is directly determined by meteorological factors (e.g. vapour pressure deficit, leaf temperature) and stomatal aperture, which is itself governed by physiological (e.g. ABA) and environmental (e.g. light, ambient CO_2) variables. The previous section showed how aquaporins contribute to integrating some of these variables for optimized adjustment of stomatal aperture. By comparison to the rhizosphere and stomata, the hydraulic resistances of inner plant segments (roots, stems,

leaves) are assumed to be much lower (Taiz & Zeiger, 1991). Whereas their direct impact on the rate of transpiration (E) is reduced, these hydraulic resistances crucially determine water potential gradients throughout the plant and, thereby, its water status. In the present section, we examine the mutual links existing between aquaporin activity in these organs and plant transpiration and/or stomatal conductance (g_s). Although not exhaustive, this section emphasizes some of the mechanisms at work for optimizing stomatal functioning and plant transpiration efficiency (i.e. the ratio of mass accumulation to transpiration).

Aquaporin genetic manipulation

The literature abounds with reports showing that genetic manipulation of aquaporins can dramatically alter E or g_s. Table 1 lists a representative subset of these reports. It shows that a decrease or enhancement of g_s by up to 30–40% could be observed in several studies. While most of these addressed the function of PIPs, it is of note that genetic manipulation of TIPs can also lead to alterations in E and g_s (Lin *et al.,* 2007; Sade *et al.,* 2009). In some cases, the effects of aquaporin genetic manipulation on root or leaf hydraulics were also characterized. Overall, the spectacular plant phenotypes support a crucial role of aquaporins in plant water relations. Yet, their interpretation with regard to stomatal regulation remains uncertain.

First, transgenic overexpression of an aquaporin gene or targeting of several aquaporin genes using antisense, RNAi or miRNA strategies can lead to a dramatic deregulation of the whole plant hydraulics which in turn may impact stomatal conductance. These difficulties can be circumvented using different approaches. For instance, grafting experiments were used by Sade *et al.* (2010) to uncouple aquaporin effects in roots and shoots. With respect to transgenic tomato plants that ectopically expressed tobacco *Nt*AQP1, plants with wild-type root systems carrying transgenic shoots showed reduced E without significant difference in g_s. To address guard cell-specific aquaporin functions, Sade *et al.* (2014a) have expressed *Nt*AQP1 in transgenic *Arabidopsis* under the control of a KST1 stomata-specific promoter. Surprisingly, no alteration in g_s was observed whereas expression of *Nt*AQP1 using a photosynthetic tissue-promoter resulted in enhanced g_s. A limitation in these and other studies is because of heterologous aquaporin expression: the transgene-encoded aquaporin can escape tissue-specific molecular and cellular regulations targeting endogenous aquaporins and even lead to their deregulation (Jang *et al.,* 2007).

Another difficulty may arise from confounding effects of aquaporin CO_2 transport activity. Table 1 shows that a co-variation of g_s with mesophyll conductance to CO_2 (g_m) could be observed in all studies where the two parameters were investigated. The molecular and physiological mechanisms which possibly link these two parameters are as yet unknown (Flexas *et al.,* 2006; Flexas *et al.,* 2013). Nevertheless, it remains difficult to deduce the primary effects of the targeted aquaporin(s) based on such integrated phenotypes.

Overall, these genetic approaches provide significant and promising results to explore the integrated role of aquaporins and their impact on transpiration. They may be refined, by using for instance tissue-specific complementation in corresponding aquaporin knock-out mutants. Such strategy was used in leaf veins (Prado *et al.,* 2013) but remains to be developed for stomatal expression of aquaporins. For now, a safe approach may be to relate whole plant and isolated stomata phenotypes. In these respects, the reduced transpiration rate of transgenic *Arabidopsis* expressing a *V. faba* PIP1 (*Vf*PIP1) could be connected to an enhanced stomatal closing response to ABA or darkness in a peeled epidermis assay (Cui *et al.,* 2008).

Root and shoot hydraulic conductance and transpiration

Consistent with the genetic studies discussed above, a large body of physiological data can explain how changes in hydraulics and related aquaporin activities occurring throughout the plant body can dramatically impact stomatal function. For instance, xylem vessel embolism leads to a drop in leaf water potential which mechanically induces stomatal closure. By facilitating embolism refilling, aquaporins may thus indirectly promote stomatal opening (Secchi & Zwieniecki, 2014). The living cells wrapping leaf veins, including xylem parenchyma cells and bundle sheath cells, also represent another hydraulic constriction for leaf water supply (Ache *et al.,* 2010; Shatil-Cohen *et al.,* 2011; Prado *et al.,* 2013). As aquaporins critically determine the water permeability of bundle sheath cells, their down-regulation by ABA provides a powerful mechanism for promoting stomatal closure (Shatil-Cohen *et al.,* 2011; Pantin *et al.,* 2013). The dual effects of ABA on stomatal closure were elegantly demonstrated in *Arabidopsis* leaves by using an *ost2* mutant which guard cells have become insensitive to ABA (Pantin *et al.,* 2013). Yet, this mutant was able to close stomata in response to ABA through hydraulic regulation of leaf vein cells. Thus, in plants under water stress, ABA inhibits and enhances water transport (aquaporin activities), in bundle sheath and guard cells, respectively. These opposite effects both contribute to stomatal closure. Regulation of root hydraulic conductivity can also impact leaf water relations (Ehlert *et al.,* 2009). In maize plantlets, pharmacological inhibition of root aquaporins revealed that the expansive growth of leaves was more sensitive than transpiration to root water transport. Yet, an inhibition of root hydraulic conductivity by 50% was able to reduce E by >50%, provided that the plant was under high evaporative demand (2.8 kPa VPD and 400 μmol m^{-2} s^{-1} photosynthetic photon flux density) (Ehlert *et al.,* 2009). Models that integrate effects of ABA on root and shoot hydraulics and stomatal aperture are needed to apprehend the full impact of the hormone on plant transpiration.

The interplay between transpiration and aquaporin regulation

Regulation of aquaporins by transpiration

In agreement with transgenic approaches in tomato (Sade *et al.,* 2009), two recent studies on natural variation and water

Table 1. Effects of aquaporin genetic manipulation of plant water relations and photosynthesis. The function of the indicated aquaporin gene was manipulated in the original or in a heterologous plant species, using antisense, overexpression (overexpr.), RNAi, miRNA or T-DNA insertion strategies. These manipulations were performed at the whole plant level or with the indicated tissue specificity. The following parameters were examined: *E*: transpiration; Ψ: water potential; Lp_r: root hydraulic conductivity; K_{leaf}: leaf hydraulic conductance; K_{ros}: rosette hydraulic conductivity; P_f : osmotic water permeability coefficient; A_N: photosynthetic activity; g_s: stomatal conductance; g_m: mesophyll conductance. n.d.: not determined; n.s.: not significant. Note that this table is not exhaustive and shows a few representative reports.

Aquaporin name	Origin plant species	Host plant species	Genetic alteration	Tissue specificity	Hydraulics and water relations	Photosynthesis (A_N)	g_s	g_m	Reference
					Percentage change with respect to wild type				
*Nt*AQP1	*Nicotiana tabacum*	*N. tabacum*	Antisense	Whole plant	Lp_r: −42 Ψ_{stem}: −19 Ψ_{leaf}: −10 *E*: −7 (Control) *E*: −32 (Drought)	n.d.	−32	n.d.	(Siefritz *et al.*, 2002)
NtAQP1	*N. tabacum*	*N. tabacum*	Overexpr.	Whole plant	n.d.	+136	+43	+64	(Uehlein *et al.*, 2003)
NtAQP1	*N. tabacum*	*N. tabacum*	Antisense	Whole plant	n.d.	−57	−33	−32	(Uehlein *et al.*, 2003)
*Hv*PIP2;1	*Hordeum vulgare*	*Oryza sativa*	Overexpr.	Whole plant	n.d.	+18	+27	+40	(Hanba *et al.*, 2004)
*Nt*AQP1	*N. tabacum*	*N. tabacum*	Antisense	Whole plant	n.d.	−13	−30	−30	(Flexas *et al.*, 2006)
*Nt*AQP1	*N. tabacum*	*N. tabacum*	Overexpr.	Whole plant	n.d.	+20	+40	+20	(Flexas *et al.*, 2006)
*Nt*AQP1	*N. tabacum*	*N. tabacum*	RNAi	Whole Plant	n.d.	−5	−15	−20	(Uehlein *et al.*, 2008)
*Vf*PIP1	*Vicia faba*	*Arabidopsis thaliana*	Overexpr.	Whole plant	*E*: −21 (Drought)	n.d.	n.d.	n.d.	(Cui *et al.*, 2008)
*Nt*AQP1	*N. tabacum*	*N. tabacum*	Overexpr.	Whole plant	Lp_r : +113 (Stress) Mesophyll P_f : +178 *E*: +25	+41	+39	+8	(Sade *et al.*, 2010)
*At*PIP1;2	*A. thaliana*	*A. thaliana*	T-DNA insertion	Whole plant	n.d.	−23	Stomatal aperture : −25	−21	(Heckwolf *et al.*, 2011)
*Mc*MIPB	*Mesembryanthemum crystallinum*	*N. tabacum*	Overexpr.	Whole plant	n.d.	+31	+13	+35	(Kawase *et al.*, 2013)
*Nt*AQP1	*N. tabacum*	*A. thaliana*	Overexpr.	Mesophyll	n.d.	+21	+35	+24	(Sade *et al.*, 2014a)
*At*PIP1;1 −5	*A. thaliana*	*A. thaliana*	miRNA	Whole plant	K_{leaf} : −50 K_{ros} : −32 Bundle sheath P_f : −67 Mesophyll P_f : −68	−18	−21	−20	(Sade *et al.*, 2014b)
*At*PIP1;1 −5	*A. thaliana*	*A. thaliana*	miRNA	Bundle sheath	K_{leaf} : −62 Bundle sheath P_f : −55 Mesophyll P_f : −80	n.s.	n.s.	n.s.	(Sade *et al.*, 2014b)
*Pc*PIP1;1–1; 2–1;4	*Populus canescens*	*P. canescens*	RNAi	Whole plant	K_{leaf}: +60 *E*: +23	+37	+47	+133	(Bi *et al.*, 2015)
PgTIP1	*Panax ginseng*	*A. thaliana*	Overexpr.	Whole plant	*E* : +38	n.s.	+18	n.d.	(Lin *et al.*, 2007)
SlTIP2;1	*Solanum lycopersicum*	*S. lycopersicum*	Overexpr.	Whole plant	*E* : n.s. (Control) *E* : +56–100 (Stress)	n.d.	n.d.	n.d.	(Sade *et al.*, 2009)

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stress responses in grapevine (Vandeleur *et al.,* 2009; Pou *et al.,* 2013) have pointed to a positive relation between aquaporin expression and whole plant transpiration or g_s. Interestingly, this relation not only applies to PIPs which fulfil obvious roles in transcellular water transport and tissue hydraulics (Vandeleur *et al.,* 2009) but also to TIPs (Pou *et al.,* 2013). Because they rely on wild-type plants, these studies (Vandeleur *et al.,* 2009; Pou *et al.,* 2013) avoid the drawback of current aquaporin genetic manipulations. However, they could not elucidate the causal origin of the relationship between aquaporin expression and plant transpiration.

While the physiological studies discussed above establish a general frame for understanding the direct and indirect impacts of aquaporins on plant transpiration, a feedback effect of transpiration on aquaporin function is emerging. Establishing such effect is not trivial as transpiration has to be uncoupled from other types of parameters such as light or temperature that usually co-vary with transpiration, during diurnal cycles in particular. In relation to diurnal changes in root hydraulic conductivity of rice plants, *Os*PIP2;5 was shown to be specifically responsive to transpiration as its mRNA and protein accumulation in roots during the day could be reduced when shoots were exposed to high humidity (Sakurai-Ishikawa *et al.,* 2011). A similar approach for uncoupling transpiration from light was developed in hybrid poplar (Laur & Hacke, 2013). In this case, an increase in PIP1 and PIP2 expression in roots could be induced by a low relative humidity treatment whereas g_s remained unchanged. Aquaporin expression in roots, root hydraulic conductivity and evaporative demand (based on meteorological factors) were also determined in rice plants grown under field conditions, and were nicely correlated, in good agreement with observations made in growth chamber experiments (Murai-Hatano *et al.,* 2015). In these approaches, expression of five PIPs and a TIP showed a strong positive correlation to evaporation potential, whereas expression of a PIP and a TIP homolog, which seem to be associated with cell elongation, showed a negative correlation. The effects of a low relative humidity (i.e. high transpiration) are not restricted to root hydraulics. In *Arabidopsis*, a high evaporative demand resulted in an increase by >3-fold in leaf hydraulic conductance (K_{leaf}) (Levin *et al.,* 2007). In rice leaves, a coordinated up-regulation of several *PIP* and *TIP* genes could be observed as soon as 4 h after a dry air treatment (Kuwagata *et al.,* 2012).

The signalling mechanisms which link plant transpiration to aquaporin activity in shoots and roots are as yet unclear. The rapid down-regulation of root hydraulics observed after shoot topping or defoliation may pertain to the shoot-to-root signalling involved (Liu *et al.,* 2014; Vandeleur *et al.,* 2014). This process was more specifically investigated in soybean and grapevine. It was proposed that a xylem-mediated hydraulic signal could be responsible for the change in root aquaporin expression observed within the 0.5–1 h following shoot topping (Vandeleur *et al.,* 2014). Conversely, the negative pressure (tension) present in xylem vessels of intact, transpiring plants could be perceived as an activating signal for aquaporin expression in root and shoot tissues.

Isohydric versus anisohydric plants

These recent studies may provide new ways to mechanistically understand the distinct behaviours of isohydric and anisohydric plants. The former ones have a conservative behaviour to maintain variations of leaf water potential at a minimum whereas the latter favour gas exchange at the expense of leaf water potential. Anisohydric plants have a more risky behaviour but are of potential agronomic interest as they can perform better than isohydric plants under mild water stress (Moshelion *et al.,* 2015). Reduced transpiration, which allows optimizing daily transpiration efficiency and long-term soil water availability, can also be an interesting agronomic trait. In a set of field-grown maize genotypes, reduced transpiration was associated to increased plant productivity in drought-prone environments (Messina *et al.,* 2015).

The current idea to explain plant anisohydric behaviour is that enhanced aquaporin expression and activity in roots and shoots promote whole plant hydraulic conductance thereby buffering water potential and favouring open stomata. Stomatal opening, as a consequence, promotes carbon fixation and plant growth (Sade *et al.,* 2009; Vandeleur *et al.,* 2009; Moshelion *et al.,* 2015). For instance, an anisohydric grapevine cultivar (Chardonnay) was found, at variance with an isohydric cultivar (Grenache), to maintain the diurnal activation of root aquaporins under water stress to match the plant transpiration demand (Vandeleur *et al.,* 2009). Thus, all mechanisms which provide a mutual coupling of tissue hydraulics and transpiration could be of prime importance for plant productivity. However, it is as yet unclear whether isohydric and anisohydric cultivars differ on direct aquaporin sensitivity to transpiration (see above) or on integrated ABA regulations. In particular, it is not yet known whether the guard cell mechanisms discussed in the first part of this review may differ between the two types of plants. Interestingly, some plant species such as grapevine or olive tree can switch between isohydric and anisohydric behaviours, depending on their environmental or developmental context (Moshelion *et al.,* 2015). These materials will be useful to further explore the mechanistic bases of these two behaviours.

CONCLUSIONS

Although transpiration and aquaporins have long been identified as two key components influencing plant water status, it is only recently that their relations have been examined in detail. Multiple and unanticipated facets are currently emerging. In particular, recent studies indicate how guard cells can be used as a model to address new roles of aquaporins in cell signalling and movements. In addition, we now have a better understanding on how aquaporins throughout the plant exert multiple indirect effects on stomatal aperture. The latter impacts the leaf water status and carbon fixation, which in turn interfere with expansive growth and biomass accumulation. Future studies will have to further explore intimate links between aquaporins and plant growth (Maurel *et al.,* 2015). They may provide new directions for controlling or engineering the transpiration efficiency of crop plants, under replete or limiting water supply,

thereby improving crop performance and productivity (Moshelion *et al.,* 2015).

REFERENCES

Ache P., Bauer H., Kollist H., Al-Rasheid K.A., Lautner S., Hartung W. & Hedrich R. (2010) Stomatal action directly feeds back on leaf turgor: new insights into the regulation of the plant water status from non-invasive pressure probe measurements. *Plant Journal* **62**, 1072–1082.

Bi Z., Merl-Pham J., Uehlein N., Zimmer I., Muhlhans S., Aichler M., … Block K. (2015) RNAi-mediated downregulation of poplar plasma membrane intrinsic proteins (PIPs) changes plasma membrane proteome composition and affects leaf physiology. *Journal of Proteomics* **128**, 321–332.

Bienert G.P. & Chaumont F. (2014) Aquaporin-facilitated transmembrane diffusion of hydrogen peroxide. *Biochimica et Biophysica Acta* **1840**, 1596–1604.

Bienert G.P., Moller A.L., Kristiansen K.A., Schulz A., Moller I.M., Schjoerring J. K. & Jahn T.P. (2007) Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. *Journal of Biological Chemistry* **282**, 1183–1192.

Chaumont F. & Tyerman S.D. (2014) Aquaporins: highly regulated channels controlling plant water relations. *Plant Physiology* **164**, 1600–1618.

Cowan I.R. & Farquhar G.D. (1977) Stomatal function in relation to leaf metabolism and environment. *Symposia of the Society for Experimental Biology* **31**, 471–505.

Cui X.H., Hao F.S., Chen H., Chen J. & Wang X.C. (2008) Expression of the *Vicia faba VfPIP1* gene in *Arabidopsis thaliana* plants improves their drought resistance. *Journal of Plant Research* **121**, 207–214.

Cutler S.R., Rodriguez P.L., Finkelstein R.R. & Abrams S.R. (2010) Abscisic acid: emergence of a core signaling network. *Annual Review of Plant Biology* **61**, 651–679.

Draye X., Kim Y., Lobet G. & Javaux M. (2010) Model-assisted integration of physiological and environmental constraints affecting the dynamic and spatial patterns of root water uptake from soils. *Journal of Experimental Botany* **8**, 2145–2155.

Dynowski M., Schaaf G., Loque D., Moran O. & Ludewig U. (2008) Plant plasma membrane water channels conduct the signalling molecule H_2O_2. *Biochemical Journal* **414**, 53–61.

Ehlert C., Maurel C., Tardieu F. & Simonneau T. (2009) Aquaporin-mediated reduction in maize root hydraulic conductivity impacts cell turgor and leaf elongation even without changing transpiration. *Plant Physiology* **150**, 1093–1104.

Flexas J., Ribas-Carbo M., Hanson D.T., Bota J., Otto B., Cifre J., … Kaldenhoff R. (2006) Tobacco aquaporin *Nt*AQP1 is involved in mesophyll conductance to CO_2 *in vivo*. *Plant Journal* **48**, 427–439.

Flexas J., Scoffoni C., Gago J. & Sack L. (2013) Leaf mesophyll conductance and leaf hydraulic conductance: an introduction to their measurement and coordination. *Journal of Experimental Botany* **64**.

Fraysse L., Wells B., McCann M.C. & Kjellbom P. (2005) Specific plasma membrane aquaporins of the PIP1 subfamily are expressed in sieve elements and guard cells. *Biology of the Cell* **97**, 519–534.

Grondin A., Rodrigues O., Verdoucq L., Merlot S., Leonhardt N. & Maurel C. (2015) Aquaporins contribute to ABA-triggered stomatal closure through OST1-mediated phosphorylation. *Plant Cell* **27**, 1945–1954.

Hanba Y.T., Shibasaka M., Hayashi Y., Hayakawa T., Kasamo K., Terashima I. & Katsuhara M. (2004) Overexpression of the barley aquaporin HvPIP2;1 increases internal CO_2 conductance and CO_2 assimilation in the leaves of transgenic rice plants. *Plant and Cell Physiology* **45**, 521–529.

Heckwolf M., Pater D., Hanson D.T. & Kaldenhoff R. (2011) The *Arabidopsis thaliana* aquaporin *At*PIP1;2 is a physiologically relevant CO_2 transport facilitator. *Plant Journal* **67**, 795–804.

Heinen R.B., Bienert G.P., Cohen D., Chevalier A.S., Uehlein N., Hachez C., … Chaumont F. (2014) Expression and characterization of plasma membrane aquaporins in stomatal complexes of *Zea mays*. *Plant Molecular Biology* **86**, 335–350.

Hooijmaijers C., Rhee J.Y., Kwak K.J., Chung G.C., Horie T., Katsuhara M. & Kang H. (2012) Hydrogen peroxide permeability of plasma membrane aquaporins of *Arabidopsis thaliana*. *Journal of Plant Research* **125**, 147–153.

Jang J.Y., Rhee J.Y., Kim D.G., Chung G.C., Lee J.H. & Kang H. (2007) Ectopic expression of a foreign aquaporin disrupts the natural expression patterns of endogenous aquaporin genes and alters plant responses to different stress conditions. *Plant and Cell Physiology* **48**, 1331–1339.

Kaldenhoff R. (2012) Mechanisms underlying CO_2 diffusion in leaves. *Current Opinion in Plant Biology* **15**, 276–281.

Kawase M., Hanba Y.T. & Katsuhara M. (2013) The photosynthetic response of tobacco plants overexpressing ice plant aquaporin McMIPB to a soil water deficit and high vapor pressure deficit. *Journal of Plant Research* **126**, 517–527.

Kuwagata T., Ishikawa-Sakurai J., Hayashi H., Nagasuga K., Fukushi K., Ahamed A., … Murai-Hatano M. (2012) Influence of low air humidity and low root temperature on water uptake, growth and aquaporin expression in rice plants. *Plant and Cell Physiology* **53**, 1418–1431.

Laur J. & Hacke U.G. (2013) Transpirational demand affects aquaporin expression in poplar roots. *Journal of Experimental Botany* **64**, 2283–2293.

Leonhardt N., Kwak J.M., Robert N., Waner D., Leonhardt G. & Schroeder J.I. (2004) Microarray expression analyses of *Arabidopsis* guard cells and isolation of a recessive abscisic acid hypersensitive protein phosphatase 2C mutant. *Plant Cell* **16**, 596–615.

Levin M., Lemcoff J.H., Cohen S. & Kapulnik Y. (2007) Low air humidity increases leaf-specific hydraulic conductance of *Arabidopsis thaliana* (L.) Heynh (Brassicaceae). *Journal of Experimental Botany* **58**, 3711–3718.

Lienard D., Durambur G., Kiefer-Meyer M.C., Nogue F., Menu-Bouaouiche L., Charlot F., Gomord V. & Lassalles J.P. (2008) Water transport by aquaporins in the extant plant *Physcomitrella patens*. *Plant Physiology* **146**, 1207–1218.

Lin W., Peng Y., Li G., Arora R., Tang Z., Su W. & Cai W. (2007) Isolation and functional characterization of *Pg*TIP1, a hormone-autotrophic cells-specific tonoplast aquaporin in ginseng. *Journal of Experimental Botany* **58**, 947–956.

Liu J., Equiza M.A., Navarro-Rodenas A., Lee S.H. & Zwiazek J.J. (2014) Hydraulic adjustments in aspen (*Populus tremuloides*) seedlings following defoliation involve root and leaf aquaporins. *Planta* **240**, 553–564.

MacRobbie E.A. (2006) Osmotic effects on vacuolar ion release in guard cells. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 1135–1140.

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

Medina V. & Gilbert M.E. (2016) Physiological trade-offs of stomatal closure under high evaporative gradients in field grown soybean. *Functional Plant Biology* **43**, 40–51.

Messina C.D., Sinclair T.R., Hammer G.L., Curan D., Thompson J., Oler Z., Gho C. & Cooper M. (2015) Limited-transpiration trait may increase maize drought tolerance in the US corn belt. *Agronomy Journal* **107**, 1978–1986.

Moshelion M., Halperin O., Wallach R., Oren R. & Way D.A. (2015) Role of aquaporins in determining transpiration and photosynthesis in water-stressed plants: crop water-use efficiency, growth and yield. *Plant, Cell and Environment* **38**, 1785–1793.

Murai-Hatano M., Kuwagata T., Hayashi H., Ishikawa-Sakurai J., Moriyama M. & Okada M. (2015) Rice plants sense daily weather and regulate aquaporin gene expressions in the roots—close correlation with potential evaporation. *Journal of Agricultural Meteorolog* **71**, 124–135.

Murata Y., Mori I.C. & Munemasa S. (2015) Diverse stomatal signaling and the signal integration mechanism. *Annual Review of Plant Biology* **66**, 369–392.

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

Pantin F., Monnet F., Jannaud D., Costa J.M., Renaud J., Muller B., Simonneau T. & Genty B. (2013) The dual effect of abscisic acid on stomata. *New Phytologist* **197**, 65–72.

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

Prado K., Boursiac Y., Tournaire-Roux C., Monneuse J.-M., Postaire O., Da Ines O., … Maurel C. (2013) Regulation of *Arabidopsis* leaf hydraulics involves light-dependent phosphorylation of aquaporins in veins. *Plant Cell* **25**, 1029–1039.

Prasch C.M., Ott K.V., Bauer H., Ache P., Hedrich R. & Sonnewald U. (2015) β-amylase1 mutant *Arabidopsis* plants show improved drought tolerance due to reduced starch breakdown in guard cells. *Journal of Experimental Botany* **66**, 6059–6067.

Raschke K. & Fellows M.P. (1971) Stomatal movement in *Zea mays*: shuttle of potassium and chloride between guard cells and subsidiary cells. *Planta* **101**, 296–316.

Sade N., Galle A., Flexas J., Lerner S., Peleg G., Yaaran A. & Moshelion M. (2014a) Differential tissue-specific expression of *Nt*AQP1 in *Arabidopsis thaliana* reveals a role for this protein in stomatal and mesophyll conductance of CO_2 under standard and salt-stress conditions. *Planta* **239**, 357–366.

Sade N., Gebretsadik M., Seligmann R., Schwartz A., Wallach R. & Moshelion M. (2010) The role of tobacco Aquaporin1 in improving water use efficiency,

hydraulic conductivity, and yield production under salt stress. *Plant Physiology* **152**, 245–254.

Sade N., Shatil-Cohen A., Attia Z., Maurel C., Boursiac Y., Kelly G., … Moshelion M. (2014b) The role of plasma membrane aquaporins in regulating the bundle sheath-mesophyll continuum and leaf hydraulics. *Plant Physiology* **166**, 1609–1620.

Sade N., Vinocur B.J., Diber A., Shatil A., Ronen G., Nissan H., … Moshelion M. (2009) Improving plant stress tolerance and yield production: is the tonoplast aquaporin *Sl*TIP2;2 a key to isohydric to anisohydric conversion? *New Phytologist* **181**, 651–661.

Sakurai-Ishikawa J., Murai-Hatano M., Hayashi H., Ahamed A., Fukushi K., Matsumoto T. & Kitagawa Y. (2011) Transpiration from shoots triggers diurnal changes in root aquaporin expression. *Plant, Cell and Environment* **34**, 1150–1163.

Sarda X., Tousch D., Ferrare K., Legrand E., Dupuis J.M., Casse-Delbart F. & Lamaze T. (1997) Two TIP-like genes encoding aquaporins are expressed in sunflower guard cells. *Plant Journal* **12**, 1103–1111.

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

Shatil-Cohen A., Attia Z. & Moshelion M. (2011) Bundle-sheath cell regulation of xylem-mesophyll water transport via aquaporins under drought stress: a target of xylem-borne ABA? *Plant Journal* **67**, 72–80.

Shope J.C. & Mott K.A. (2006) Membrane trafficking and osmotically induced volume changes in guard cells. *Journal of Experimental Botany* **57**, 4123–4131.

Siefritz F., Tyree M.T., Lovisolo C., Schubert A. & Kaldenhoff R. (2002) PIP1 plasma membrane aquaporins in tobacco: from cellular effects to function in plants. *Plant Cell* **14**, 869–876.

Smart L.B., Moskal W.A., Cameron K.D. & Bennett A.B. (2001) MIP genes are down-regulated under drought stress in *Nicotiana glauca*. *Plant and Cell Physiology* **42**, 686–693.

Sun M.-H., Xu W., Zhu Y.-F., Su W.-A. & Tang Z.-C. (2001) A simple method for in situ hybridization to RNA in guard cells of *Vicia faba* L.: the expression of aquaporins in guard cells. *Plant Molecular Biology Reporter* **19**, 129–135.

Taiz L. & Zeiger E. (1991) *Water Balance of the Plant*, pp. 81–99. Plant Physiology. Benjamin/Cummings Publishing Company, Redwood City, California.

Tazawa M. & Okazaki Y. (1997) Water channel does not limit evaporation of water from plant cells. *Journal of Plant Research* **110**.

Uehlein N., Lovisolo C., Siefritz F. & Kaldenhoff R. (2003) The tobacco aquaporin NtAQP1 is a membrane CO_2 pore with physiological functions. *Nature* **425**, 734–737.

Uehlein N., Otto B., Hanson D.T., Fischer M., McDowell N. & Kaldenhoff R. (2008) Function of *Nicotiana tabacum* aquaporins as chloroplast gas pores challenges the concept of membrane CO_2 permeability. *Plant Cell* **20**, 648–657.

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

Vandeleur R.K., Sullivan W., Athman A., Jordans C., Gilliham M., Kaiser B.N. & Tyerman S.D. (2014) Rapid shoot-to-root signalling regulates root hydraulic conductance via aquaporins. *Plant, Cell and Environment* **37**, 520–538.

Wang C., Hu H., Qin X., Zeise B., Xu D., Rappel W.J., Boron W.F. & Schroeder J.I. (2016) Reconstitution of CO_2 regulation of SLAC1 anion channel and function of CO_2-permeable PIP2;1 aquaporin as CARBONIC ANHYDRASE4 interactor. *Plant Cell* **28**, 568–582.

Yang H.M., Zhang X.Y., Tang Q.L. & Wang G.X. (2006) Extracellular calcium is involved in stomatal movement through the regulation of water channels in broad bean. *Plant Growth Regulation* **50**, 79–83.

Yeats T.H. & Rose J.K. (2013) The formation and function of plant cuticles. *Plant Physiology* **163**, 5–20.

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