Regulation of leaf hydraulics: from molecular to whole plant levels.
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The water status of plant leaves is dependent on both stomatal regulation and water supply from the vasculature to inner tissues. The present review addresses the multiple physiological and mechanistic facets of the latter process. Inner leaf tissues contribute to at least a third of the whole resistance to water flow within the plant. Physiological studies indicated that leaf hydraulic conductance ($K_{\text{leaf}}$) is highly dependent on the anatomy, development and age of the leaf and can vary rapidly in response to physiological or environmental factors such as leaf hydration, light, temperature, or nutrient supply. Differences in venation pattern provide a basis for variations in $K_{\text{leaf}}$ during development and between species. On a short time (hour) scale, the hydraulic resistance of the vessels can be influenced by transpiration-induced cavitations, wall collapses, and changes in xylem sap composition. The extravascular compartment includes all living tissues (xylem parenchyma, bundle sheath, and mesophyll) that transport water from xylem vessels to substomatal chambers. Pharmacological inhibition and reverse genetics studies have shown that this compartment involves water channel proteins called aquaporins (AQPs) that facilitate water transport across cell membranes. In many plant species, AQPs are present in all leaf tissues with a preferential expression in the vascular bundles. The various mechanisms that allow adjustment of $K_{\text{leaf}}$ to specific environmental conditions include transcriptional regulation of AQPs and changes in their abundance, trafficking, and intrinsic activity. Finally, the hydraulics of inner leaf tissues can have a strong impact on the dynamic responses of leaf water potential and stomata, and as a consequence on plant carbon economy and leaf expansion growth. The manipulation of these functions could help optimize the entire plant performance and its adaptation to extreme conditions over short and long time scales.

Keywords: aquaporin, hydraulic conductance, leaf growth, veins, xylem
stomata to water vapor diffusion. $K_{leaf}$ integrates all water transport paths working in parallel or in series within the inner leaf tissues, each having its own physical characteristics.

**TECHNIQUES FOR MEASURING WATER TRANSPORT IN WHOLE LEAVES**

Experimentally, $K_{leaf}$ is determined as the ratio of water flow rate through the leaf to the driving force, that is, the water potential difference between the petiole and leaf lamina (ideally, the substomatal chambers). $K_{leaf}$ is usually normalized by leaf area (Sack and Holbrook, 2006). At the whole leaf level, three major techniques have been developed to measure $K_{leaf}$.

The evaporative flux method (EFM) is the most commonly used. It relies on the relationship that exists, under steady state conditions, between the flux of transpiration across the plant or an excised leaf and the corresponding drop in water potential (Martial et al., 2002; Sack et al., 2002). $K_{leaf}$ is deduced from the ratio of transpiration flow to the difference of water potential between the stem and the leaf. In practice, water potentials are measured in a fully transpiring leaf and in a leaf covered with a bag to locally prevent any transpiration. The latter leaf reports on the stem water potential.

The high pressure method (HPM) requires a flow of solution to be pushed using a pump, from the petiole throughout the leaf (Sack et al., 2002; Tyree et al., 2005). Alternatively, an excised leaf or rosette can be inserted into a pressure chamber whereby a flow of solution is pressed through the stomata and exits the leaf through the hypodermal layer (Postaire et al., 2010). $K_{leaf}$ can be deduced from the flow vs. pressure relationship. It has been argued that stomatal constrictions could dominate the measured $K_{leaf}$. However, Poiseulle’s law indicates that, by contrast to vapor phase transport, the pore apertures must represent a negligible resistance under conditions of liquid flow. These assumptions were supported experimentally in walnut (Juglans regia) leaves which showed a marked stomatal closure in response to abscisic acid (ABA), without any alteration in $K_{leaf}$ (Tyree et al., 2005) and in model species Arabidopsis thaliana which rosette hydraulic conductivity was increased under darkness, simultaneously to stomatal closure (Postaire et al., 2010).

The vacuum pump method (VPM) represents the third type of $K_{leaf}$ measuring method. In this case, water enters an excised leaf through its sectioned petiole. The leaf blade is carefully maintained to other methods, EFM most closely reports on the natural pathway for water movement, in addition to those utilized during transpiration. Yet, several comparative studies, including one with six woody angiosperm species, showed that similar $K_{leaf}$ values (with differences around 10%) could be determined by the three methods (Sack et al., 2002). From this, it was inferred that the mesophyll pathway that may be shunted when using the HPM may be of negligible resistance (Sack et al., 2002).

**LEAF HYDRAULIC CONDUCTANCE VALUES ACROSS PLANT SPECIES**

A comprehensive set of $K_{leaf}$ data has now been collected in the whole plant kingdom. These studies revealed that $K_{leaf}$ is highly variable, by up to 65-fold across plant species (Sack et al., 2005).

These studies also established that, with respect to roots and stems, leaf tissues can represent a substantial part of the inner resistance to whole plant water flow. Within a sample of 34 species, the leaf contributed on average a third of the whole plant resistance (Sack et al., 2003) but in some cases it could represent up to 86% of this resistance (Sack and Holbrook, 2006). Of outstanding interest for the physiologist is also the observation that $K_{leaf}$ can be highly variable and dynamic during plant life. Thus, $K_{leaf}$ depends on the anatomy and developmental stage of the leaf, it can also vary according to plant growing conditions, over a wide range of time scales, from minutes to months.

**VARIATION OF LEAF HYDRAULIC CONDUCTANCE IN RESPONSE TO DEVELOPMENTAL AND ENVIRONMENTAL FACTORS**

**DEVELOPMENT**

$K_{leaf}$ shows dynamic changes over the whole leaf lifetime, with patterns specific for each species (Anamaa et al., 2005; Nardini et al., 2005). Generally, $K_{leaf}$ increases in developing leaves as the vascular network matures. In the weeks or months following its maximum, $K_{leaf}$ begins to decline, by up to 80–90% at abscission (Anamaa et al., 2005; Brodribb et al., 2005). Some authors have hypothesized that senodal decline of $K_{leaf}$ is a trigger for leaf senescence (Sack and Holbrook, 2006).

**IRRADIANCE**

Variations in $K_{leaf}$ due to changes in irradiances have been reported in numerous plant species. In most cases, $K_{leaf}$ is the lowest at low irradiance (<10 μmol photons m−2 s−1) or under darkness (Sack et al., 2002; Nardini and Salleo, 2005; Tyree et al., 2005). In sunflower (Helianthus annuus) for instance, $K_{leaf}$ is reduced by 30–40% during the night compared to the day (Nardini and Salleo, 2005). Conversely, $K_{leaf}$ can rapidly increase by several-fold in response to a high irradiance (up to >1000 μmol photons m−2 s−1; Sack et al., 2002; Lo Gallo et al., 2005). For instance, $K_{leaf}$ was increased in the 30 min following a transition to high light in 6 out of 11 tropical plant species (Tyree et al., 2005). Light quality also has an important impact on leaf hydraulic properties. In silver birch (Betula pendula; Sellin et al., 2011) and cucumber (Cucumis sativus) leaves (Savvides et al., 2012), $K_{leaf}$ was the highest under blue light, intermediate under white light, and the lowest under red light. It is of note that the $K_{leaf}$ of Arabidopsis is also regulated during HPM measurements (and perhaps to some degree with the VPM), the leaf or rosette is flooded with a liquid solution and leaf airspaces rapidly become infiltrated. This may create novel pathways for water movement, in addition to those utilized during transpiration. Yet, several comparative studies, including one with six woody angiosperm species, showed that similar $K_{leaf}$ values (with differences around 10%) could be determined by the three methods (Sack et al., 2002). From this, it was inferred that the mesophyll pathway that may be shunted when using the HPM may be of negligible resistance (Sack et al., 2002).
ABA decreased Kleaf on inner leaf water transport (Postaire et al., 2010). More generally, Kleaf follows diurnal and seasonal rhythms. For sunflower and some tree species, Kleaf increased by up to two to threefold over a few hours from morning to midday and then declined by evening (Lo Gullo et al., 2005; Cochard et al., 2007). When sunflower plants were kept in the dark for several days, Kleaf continued to oscillate in phase with the subjective light period, indicating that these changes were driven by the circadian clock (Nardini and Salleo, 2005).

DROUGHT STRESS

Leaves are able to sense and respond to various types of water shortage (Sack and Holbrook, 2006). When Arabidopsis plants were exposed to low air humidity (implying higher transpiration) a concomitant increase in Kleaf and whole plant hydraulic conductance was observed (Levin et al., 2007). ABA is also a central mediator of plant response to drought stress. Inhibiting effects of ABA on inner leaf water transport (Kleaf) were recently revealed in Arabidopsis (Shahit-Cohen et al., 2011). In this study, ABA was fed to excised leaves through the xylem via transpiration. Pantin et al. (2013) confirmed these effects and showed that xylem-fed ABA decreased Kleaf and stomatal conductance (g_s) in mutants that are known to be insensitive to ABA-induced stomatal closure. This suggested that the stomatal regulation was mediated via a hydraulic feedback in a tissue upstream of the stomata (Pantin et al., 2013).

INTERACTION BETWEEN FACTORS ACTING ON LEAF HYDRAULIC CONDUCTANCE

Although most studies have addressed the effects of individual factors on Kleaf, an integrated view of the dynamics and combined impacts of light, leaf water status and development of Kleaf is now critically needed. This question was recently investigated in sunflower and three shrub species (Guyot et al., 2012). In each case, the amplitude of Kleaf response to light or leaf dehydration was positively correlated to the intensity of the other parameter. These properties may allow optimal adjustment of the leaf water status under contrasting conditions when light tends to enhance transpiration whereas soil water availability is declining. These few examples illustrate the diversity of physiological contexts leading to changes in Kleaf. The following sections address the variety of molecular and cellular mechanisms involved and the physiological significance of these regulations.

VASCULAR WATER TRANSPORT

CONTRIBUTION OF THE VASCULAR PATHWAY TO LEAF HYDRAULIC CONDUCTANCE

The vascular pathway is composed of a highly structured network of differentiated (non-living) vessels that deliver xylem sap through the entire leaf, close to the evaporation sites. The minimization of transport distances out of the vascular pathway is one key feature of the hydraulic performance of leaves (Sack and Holbrook, 2006). In most dicots, venation is constructed according to a hierarchical order: midvein, second- and third-order veins, and finally minor veins that confer the reticulate pattern (Figure 1A). It is generally assumed that, whatever the leaf vascular anatomy, the bulk of transpired water follows the path of lesser resistance down the vein network, from midrib to minor veins before exiting the vessels (Sack and Holbrook, 2006). This means that water subsequently follows the vascular and extravascular paths. The respective contributions of these two paths to Kleaf and to its variations have been the object of numerous studies. Models based on electrical analogies and using Poiseuille’s law have been developed to calculate the hydraulic conductance of leaf xylem networks (Lewis and Bosee, 1995). They demonstrated the importance of hierarchy in the vein network to optimize water transport (Cochard et al., 2004; McKown et al., 2010). One first method to determine the contribution of veins to the leaf hydraulic resistance (Rveinleaf, the inverse of Kleaf) is to cut an increasing number of minor veins. The measured Rvein leaf progressively decreases and converges toward a stable value, the supposed vascular resistance (Sack et al., 2004; Nardini and Salleo, 2005). A second method consists in disrupting the living structures of the leaf by freezing or boiling the entire organ. The measured Rveinleaf is then reduced to its vascular component (Cochard et al., 2004) provided that the treatments do not alter the xylem vessel diameter or the extensibility of the walls. All these studies have revealed that the hydraulic resistances of the vascular and extravascular compartments are of the same order of magnitude. Either one may prevail, depending on species or environmental factors (Zwieniecki et al., 2002; Sack et al., 2004; Nardini and Salleo, 2005).

VARIATIONS BETWEEN SPECIES

Leaf vascular anatomy is highly variable across species with respect to vein arrangement and density. The number, size, and geometry of the vascular bundles in the veins and of the xylem conduits within the bundles are also very diverse (Roth-Nebelsick et al., 2001). Yet, common principles of organization can be found, such as a global scaling between leaf size and vein characteristics. In particular, larger leaves have major veins of larger diameter, but lower length per leaf area, whereas minor vein traits are independent of leaf size (Sack et al., 2012; Sack and Scoffoni, 2013). This great anatomic variability could explain to a large extent the dramatic differences in Kleaf observed between species. Several main trends relative to measured Kleaf have been validated through modeling (Cochard et al., 2007; McKown et al., 2010). Firstly, the conductance of the main veins appeared as a major limiting factor of Kleaf. By contrast, the arrangement and density of these veins had a marginal impact on Kleaf (Sack and Frolo, 2006) and would rather contribute to a uniform distribution of water across the lamina (Roth-Nebelsick et al., 2001; Zwieniecki et al., 2002) and avoid cavitation (Sack and Holbrook, 2006). Secondly, the Kleaf of plants with a higher minor vein density tended to be greater. This is not due to an increase in conductance of the xylem system per se (Cochard et al., 2004), but rather to an increase in the surface area for exchange of xylem sap with surrounding mesophyll and reduced distances in extravascular pathway (Roth-Nebelsick et al., 2001; Sack and Frolo, 2006). A high vein density also favors water potential equilibration across the leaf and prevents the damage or blockade of higher-order veins (Sack and Scoffoni, 2013).
Regulation of leaf hydraulics

**FIGURE 1 | Leaf vascular pathway and regulation of water transport.**

(A) Based on the example of *Arabidopsis thaliana*, the figure shows that following uptake by roots and transfer to shoots, water is delivered throughout the whole leaf lamina by a highly organized network of veins. The non-living vessels form the leaf vascular pathway which is constructed according to a hierarchical order with midvein, second-order veins, third-order veins, and minor veins. The midvein runs from the petiole to the leaf apex, with second-order veins branching at regular intervals, and third-order veins branching on the latter. (B) The hydraulic resistance of the vascular pathway can be influenced by various developmental and environmental factors acting on the venation pattern or the indicated xylem properties.

**THE CONSTRUCTION COST OF VASCULAR PATHWAYS**

The development of a dense vein network represents a massive investment for the plant because lignified tissues are net carbon sinks that do not directly contribute to photosynthesis (Pantin et al., 2012). However, maximum net assimilation rate of photosynthesis depends on the capacity of the leaf vascular system to supply water to photosynthesizing mesophyll cells (Brodribb et al., 2007). Hydraulic modeling of leaves revealed that the conductivity and density profiles of veins of various orders contribute to optimizing the hydraulic efficiency of the xylem network. A high vein density only becomes economically viable compared to the photosynthetic costs when it is supported by a highly conductive low order venation. A high vein density limits the distance of photosynthate and water transport between veins, photosynthesizing mesophyll cells, and evaporative surfaces of the leaf (Amiard et al., 2005; Brodribb et al., 2007; McKown et al., 2010).

Hence, the hydraulic properties of the leaf tissue play a fundamental role in linking leaf construction with photosynthetic capacity.

**ENVIRONMENTAL EFFECTS**

It is of note that, beyond developmental factors, the functioning and hydraulic resistance of the vascular pathway depends on the plant growth conditions (Brodribb et al., 2010). The combined use of a xylem pressure probe and a Scholander–Hammel pressure bomb in intact maize (*Zea mays*) plants was used to demonstrate that leaf xylem pressure can change rapidly and reversibly with environmental modifications, such as light intensity or soil water potential (Wei et al., 1999). One striking consequence is water stress-induced xylem cavitations that result in marked reductions in $K_{leaf}$ (Bucci et al., 2003; Nardini et al., 2003; Johnson et al., 2009). However, decrease of $K_{leaf}$ in dehydrating pine needles...
WATER then crosses bundle sheath extensions or the mesophyll to a broad range of hydraulic configurations between species, due to reversible regulations of leaf structures that can provide complementary means for rapid and et al., 2010). Hence, different plant species may exhibit contrasting vulnerability to water stress- or winter-induced embolism, depending on the anatomy of their vessels.

The xylem sap composition, and in particular its potassium concentration, can interfere with the wall permeability of tracheids (Zwieniecki et al., 2001). These effects may be due to a shrinking and swelling of the pectin hydrogel forming the intervessel pit membranes. This mechanism which impacts $K_{up}$ has been invoked to explain the effects of light on stem hydraulics in laurel and silver birch (Betula pendula, Nardini et al., 2010; Sellin et al., 2010).

In conclusion, the vascular compartment of leaves allows a broad range of hydraulic configurations between species, during development or in response to environment fluctuations (Figure 1B). As explained in the next sections, the extravascular structures can provide complementary means for rapid and reversible regulations of $K_{up}$ (Sack and Holbrook, 2006).

THE EXTRAVASCULAR COMPARTMENT
WATER PATHWAYS INSIDE THE EXTRAVASCULAR COMPARTMENT

The extravascular compartment includes all living tissues that transport water from xylem vessels to substomatal chambers. Following its exit from xylem conduits, water flows through xylem parenchyma cells and the bundle sheath made up of parenchymatous cells wrapped around the veins (Leegood, 2008). Water then crosses bundle sheath extensions or the mesophyll to reach the epidermis and evaporations sites, respectively. The location and surface area of the latter sites may vary according to leaf anatomy; some species having huge leaf internal airspaces (Brodribb and Field, 2011; Figure 2A). Recently, a shift has been made from the simple idea that leaves can be reduced to a single pool of evaporating water to a more complex leaf representation with well-organized water pools separated by hydraulic resistances (Zwieniecki et al., 2007).

It is classically assumed that water can follow different paths to flow across living tissues, from cell-to-cell, through cell membranes (apoplastic path) and plasmodesmata (symplastic path), or through the continuity of walls (apoplastic path; Steudle and Peterson, 1998). The relative contribution of these different paths in leaves is currently unclear and could vary according to species, leaf developmental stage (Voicu and Zwieniecki, 2010b), or physiological conditions (Sack et al., 2004; Nardini and Salleo, 2005; Cochard et al., 2007; Ye et al., 2008). Tissue anatomy can provide preliminary hints at these questions. Mesophyll tissues often have a low cell packing and are largely composed of airspaces. This, and experiments whereby apoplastic transport was traced using dyes such as 8-hydroxyphrene-1,3,6-trisulfonic acid (HPTS), have suggested that apoplastic water movement predominates during transpiration (Sack and Holbrook, 2006; Voicu et al., 2008, 2009).

Water may cross cell membranes only for cell water homeostasis, during rehydration and expansion growth (Heinen et al., 2009). In contrast, the vascular bundles show physically tight cell layers (Figure 2A). In addition, recent work indicated that bundle sheath cells may have suberin lamellae and/or apoplastic barriers on radial walls, thereby decreasing the apoplastic flow of water (Leuten and Curtis, 1997). Thus, transcellular water flow may be critical at this site.

THE DYNAMICS OF LEAF CELL WATER PERMEABILITY IN RESPONSE TO DEVELOPMENTAL AND ENVIRONMENTAL FACTORS

Several techniques have been developed to measure the water permeability of leaf cells and therefore dissect the functional behavior of the extravascular pathway. The cell pressure probe technique which gives access to cell water relation parameters in intact plant tissues has been applied to several cell types including the stomata, epidermis, mesophyll (Franke, 2003), and midrib parenchyma (Kim and Steudle, 2007, 2009). Since this technique is not applicable to small sized or deeply embedded cells, cell water permeability can also be characterized by means of osmotic swelling assays in protoplasts. The protoplasts are isolated according to their morphology or to cell-specific expression of fluorescent reporter proteins. This approach has been developed firstly in mesophyll protoplasts of various plant species (Ramahaleo et al., 1999; Mörillon and Chrissipeels, 2001; Martre et al., 2002) and more recently in protoplasts from Arabidopsis bundle sheath (Shari'd-Cohen et al., 2011) and xylem parenchyma (Prado and Maurel, 2013). In general, the water permeability of protoplasts is lower than in intact cells (Moshelion et al., 2004; Chaumont et al., 2005; Hachez et al., 2006, 2008; Volkov et al., 2007).

These techniques have first revealed that cell water permeability can vary according to leaf developmental stage (Figure 2B). In barley (Hordeum vulgare) and maize leaves, the water permeability of protoplasts isolated from the zones of emergence, elongation, and maturation was the highest in the former zone (Volkov et al., 2007; Hachez et al., 2008). A high cell water permeability may be beneficial during tissue expansion.

Measurements in individual leaf cells have also indicated that changes in $K_{up}$ induced by environmental factors on the short-term may be mediated through changes in cell membrane water permeability (Figure 2B). For instance, the water permeability of individual parenchyma cells, as measured with a cell pressure probe in the midrib of maize leaves, was increased by up to three-fold at low light intensities (Kim and Steudle, 2007). Other studies using protoplast swelling assays showed that, in maize, the leaf cell water permeability was the highest during the early hours of the day (Hachez et al., 2008). A similar approach revealed that diurnal leaf movements in rain tree (Samaneus saman) and tobacco were linked to regulation of cell water transport in pulvini and peti-ole, respectively (Moshelion et al., 2002; Siefritz et al., 2004). The transpiration demand can also impact leaf cell water permeability. In Arabidopsis plants grown under various transpiring regimes or ABA treatments (Mörillon and Chrissipeels, 2001), an inverse relationship was found between mesophyll protoplast water permeability and the rate of plant transpiration which, however, could not be attributed to a direct action of ABA on the mesophyll. Bundle sheath cells seem to have, by contrast, a specific responsiveness...
FIGURE 2 | Leaf extravascular pathway and regulation of water transport. (A) The figure shows, using the Arabidopsis leaf as an example, the various components of the extravascular pathway, from whole organ to molecular levels. The pathway followed by water between xylem vessels and substomatal chambers is not entirely understood. Whereas the role of xylem parenchyma and bundle sheath is emerging (see text), the contribution of the mesophyll may depend on leaf anatomy. Water transport across living cells is mediated in part by water channel proteins called aquaporins (AQPs) formed by six α-helical transmembrane domains linked by five loops (A–E), and N- and C-terminal ends localized in the cytosol. Two specific, highly conserved structural motifs (NPA) are located in the pore and contribute to AQP selectivity. AQPs are expressed in all leaf living cells but preferentially in veins. (B) Various developmental and environmental factors act on the indicated components of the extravascular pathway to alter its hydraulic properties. AQP regulation occurs at various levels including gene expression, AQP trafficking and gating (see text).

The nature of the living cells that, within the leaf, oppose the major hydraulic resistance to the transpiration flow is still under debate (Cochard et al., 2004; Sack et al., 2004; Nardini and Salleo, 2005; Voicu et al., 2008). One recent approach made use of a non-invasive leaf pressure probe in Arabidopsis leaves (Ache et al., 2010). This new technique indicated that mesophyll cell turgor was markedly reduced at high transpiration rate, suggesting that an upstream structure, possibly the bundle sheath, was hydraulically limiting. In support for this, Shatil-Cohen et al. (2011) observed a correlation between the effects of ABA on $K_{\text{leaf}}$ and the water permeability of protoplasts from the bundle sheath but not from mesophyll. This correlative approach was recently extended by Prado et al. (2013) who considered a larger set of vein protoplasts in Arabidopsis leaves. The data indicated that xylem parenchyma, in addition to bundle sheath, may be limiting during $K_{\text{leaf}}$ regulation by light. A hydraulic limitation due to the xylem parenchyma was already suggested in maize leaf (Yang and Boyer, 2002). We note, however, that these conclusions may not apply to tobacco which showed no correlation between the hydraulic conductivities of whole leaves and bundle sheath cells (Lee et al., 2009). In addition, bundle sheath extensions which in some species link the bundle sheath to the epidermis and separate the leaf into chambers to ABA which could explain the down-regulating effects of this hormone on $K_{\text{leaf}}$ (Shatil-Cohen et al., 2011).

HYDRAULIC LIMITATIONS IN THE EXTRAVASCULAR COMPARTMENT

The nature of the living cells that, within the leaf, oppose the major hydraulic resistance to the transpiration flow is still under debate (Cochard et al., 2004; Sack et al., 2004; Nardini and Salleo, 2005; Voicu et al., 2008). One recent approach made use of a non-invasive leaf pressure probe in Arabidopsis leaves (Ache et al., 2010). This new technique indicated that mesophyll cell turgor was markedly reduced at high transpiration rate, suggesting that an upstream structure, possibly the bundle sheath, was hydraulically limiting. In support for this, Shatil-Cohen et al. (2011) observed a correlation between the effects of ABA on $K_{\text{leaf}}$ and the water permeability of protoplasts from the bundle sheath but not from mesophyll. This correlative approach was recently extended by Prado et al. (2013) who considered a larger set of vein protoplasts in Arabidopsis leaves. The data indicated that xylem parenchyma, in addition to bundle sheath, may be limiting during $K_{\text{leaf}}$ regulation by light. A hydraulic limitation due to the xylem parenchyma was already suggested in maize leaf (Yang and Boyer, 2002). We note, however, that these conclusions may not apply to tobacco which showed no correlation between the hydraulic conductivities of whole leaves and bundle sheath cells (Lee et al., 2009). In addition, bundle sheath extensions which in some species link the bundle sheath to the epidermis and separate the leaf into chambers to ABA which could explain the down-regulating effects of this hormone on $K_{\text{leaf}}$ (Shatil-Cohen et al., 2011).
AQPs have a characteristically conserved structure with monomers. AQP isoforms can transport non-polar solutes such as metalloids. AQPs may fulfill multiple roles in the mesophyll: transcellular (At signaling.ing multiple functions, in water and nutrient transport, and cell specific proteins (TIPs) represent the most abundant AQPs in the plasma membrane and in the tonoplast, respectively. The great diversity of plant AQPs also reflects a broad range of transport specificities (Tyerman et al., 2002). For instance, the plasma membrane intrinsic proteins (PIPs) and the tonoplast intran-10158555temporal variations of cell membrane water transport. Aquaporin (AQP) channels are membrane proteins that facilitate the exchange of water across cell membranes and can be responsible for up to 95% of the water permeability of plant plasma membranes (Maurel et al., 2008). This explains the intensive research recently developed on the function and regulation of AQPs in leaves.

**AQPFAMILY OFWATER CHANNEL PROTEINS**

AQPs have a characteristically conserved structure with monomers (25–31 kDa) comprising six α-helical transmembrane domains linked by five loops (A–E) and N- and C-terminal ends local-ized in the cytosol (Figure 2A). AQPs assemble as tetramers, each monomer forming an individual transmembrane pore (Wang and TeKkokshrid, 2007). Plant AQPs show a great diversity, with >30 isoforms in higher plant species. They fall into at least four major homology subgroups that somehow reflect specific subcellular localizations (Maured et al., 2008). For instance, the plasma membrane intrinsic proteins (PIPs) and the tonoplast intran-sic proteins (TIPs) represent the most abundant AQPs in the plasma membrane and in the tonoplast, respectively. The great diversity of plant AQPs also reflects a broad range of transport specificities (Tyerman et al., 2002). In addition to water, some AQPs can transport non-polar solutes such as metalloids (Bieneset et al., 2008), gases (Uehlein et al., 2003), or reactive oxygen species (ROS; Bienert et al., 2007; Dynowski et al., 2008), suggest-ing multiple functions, in water and nutrient transport, and cell signaling.

**TISSUESPECIFIC EXPRESSION OF AQPSON PUTATIVE ROLES**

Expression profiling of the AQP gene family in several plant species has indicated that leaves are equipped with multiple AQP isoforms. By contrast to what was observed in pollen or seeds, no AQP transcript was strictly specific for leaves. In the Arabidopsis leaf, two TIP (AtTIP2;2 and AtTIP2;1) and three PIP (AtPIP2;3, AtPIP2;1, and AtPIP2;6) genes are strongly expressed and AtPIP2;6 shows preferential expression in this organ (Jang et al., 2004; Figure 3). Quantitative proteomics of plasma membranes purified from Arabidopsis leaves confirmed the pattern and showed that AtPIP1;2, AtPIP2;3, and AtPIP2;7 were the most abundant among the nine PIPs isoforms detected (Monnense et al., 2011).

Beyond these global studies, the marked cell-specific expres-sion patterns of some isoforms can provide interesting hints at a variety of AQP functions in the leaf. In tobacco for instance, strong expression of a PIP1 homolog, NtAQP1, was observed in spongy parenchyma cells of mesophyll, with the highest concentra-tion around substomatal cavities (Otto and Kaldenhoff, 2000). AQPs may fulfill multiple roles in the mesophyll: transcellular water transport during transpiration, as suggested for NtAQP1;2, but also cell osmotic adjustment under varying water demand, or CO2 transport (Otto and Kaldenhoff, 2000). Yet, a preferential expression of AQPs in the vascular bun-dles was observed in many plant species, suggesting a special role for AQPs in delivering water from the vessels to the mesophyll (Kaldenhoff et al., 2008). In particular, bundle sheath cells were shown to have high PIP and TIP expression levels in rapeseed (Brassica napus; Fragne et al., 2001), Arabidopsis (Kaldenhoff et al., 1995), Prado et al., 2013), ice plant (Mesembryanthemum crystallinum; Kirch et al., 2000), Norway spruce (Picea abies; Oliviersson et al., 2001), maize (Hachez et al., 2008), and rice (Oryza sativa; Sakurai et al., 2008). This expression pattern is con-sistent with the observation that the bundle sheath is formed of highly compacted cells, with sometimes lignified or suberized cell walls (see Water Pathways Inside the Extravascular Compartment). Strong expression of AQPs in the xylem parenchyma has also been described in several species (Barrieu et al., 1998; Otto and Kalden-hoff, 2000; Sakai et al., 2003; Hachez et al., 2008; Prado et al., 2013). This site of expression may be crucial for radial cell to cell water movement during exit from the xylem vessels (Prado et al., 2013) and for osmotically driven water loading in xylem vessels during embolism refilling (Sakai et al., 2003; Sechi and Zwieniecki, 2010). AQPs were also found to be abundant in phloem companion cells (Kirch et al., 2000; Fraysse et al., 2003) suggesting a role in phloem sap loading and in maintaining vascular tissue functions under drought stress (Montaño-Hernández et al., 2008). Finally, AQPs are expressed in epidermis (Cui et al., 2008), trichomes, stomata (Heinem et al., 2009), and dividing cells (Barrieu et al., 1998) where their role still needs to be established.

This survey should not give a static view of AQP expression, which is constantly adjusted during leaf development. In maize and barley leaves for instance, some isoforms were highly expressed in young, elongating leaf tissues whereas others were preferentially expressed in fully developed, matured tissues (Wei et al., 2007; Hachez et al., 2008; Bosse et al., 2011; Yue et al., 2012).

**INVOLVEMENT OF AQPS IN LEAF HYDRAULICS: PHARMACOLOGICAL AND GENETIC EVIDENCES**

The contribution of AQPs to leaf water transport was first demon-strated using pharmacological inhibition. Treatment of mesophyll and bundle sheath protoplasts with mercury, which blocks AQPs through oxidation of Cys residues, resulted in a fivefold reduction in cell water permeability (Kaldenhoff et al., 1998; Shanti-Cohen et al., 2011). At the whole leaf level, mercury treatment decreased Ksat by 33% in sunflower (Nardini and Salleo, 2005) and by around 40% in six temperate deciduous trees (Asaamaa et al., 2005). Although it is also rather unspecific and toxic, azide, which induces cell acidosis and a pH-dependent closure of PIPs (Tournaire-Roux et al., 2003), was used in Arabidopsis as an inde-pendent type of AQP blocker. The similar inhibiting effects of mercury and azide supported the idea that, in this species, PIPs truly contribute to the enhancement of rosette hydraulic conductivity under darkness (Postaire et al., 2010).

Given the lack of specific inhibitors, genetic approaches provide a more reliable approach for studying the physiological function of plant AQPs. Arabidopsis plants expressing AtPIP1;2 or AtPIP2;3 antisense transgenes, individually or in combination, showed in parallel to a reduced expression of PIP1s and/or PIP2s, a 5- to 30-fold reduction in water permeability of isolated mesophyll
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FIGURE 3 | Expression pattern of three highly expressed PIP genes during leaf development in Arabidopsis. The figure shows expression in transgenic plants of chimeric genes expressing a β-glucuronidase gene (GUS) under the control of PIP promoter sequences (ProPIP). The three indicated AQP isoforms (AtPIP1;2, AtPIP2;1, AtPIP2;6) contribute to rosette hydraulic conductivity (Prado et al., 2013). Cross-sections show intense staining in the veins of each type of transgenic plants (black scale bar = 2.5 mm, red scale bar = 0.1 mm).

protoplasts (Kaldenhoff et al., 1998; Martre et al., 2002). The antisense lines also showed a leaf water potential and a $K_{\text{leaf}}$ significantly lower than in control plants, only under water limiting conditions. The differences were stronger during re-watering, suggesting that AQP-mediated water transport was directly involved in leaf tissue rehydration (Martre et al., 2002). In tobacco, the phenotype of an antisense NaAQP1 line suggested that this AQP is involved in the differential expansion growth of the upper and lower surfaces of the petiole during leaf unfolding (Siefritz et al., 2004). The contribution of individual AQPs to water leaf transport was thoroughly dissected in Arabidopsis. Plant lines carrying an individual T-DNA insertion in three out of four highly expressed PIP genes (AtPIP1;2, AtPIP2;1, AtPIP2;6) displayed, when grown in the dark, reduction in $K_{\text{leaf}}$ by approximately 30%, similar to the reduction displayed by a corresponding triple pip mutant (Prado et al., 2013). Another study using a deuterium tracer method to assess water relocation in Arabidopsis showed that $K_{\text{leaf}}$ was significantly reduced by about 20% in pip2;1 and pip2;2 knock-out plants (Da Ines et al., 2010).

AQUAPORINS IN LEAVES: MODES OF REGULATION

RESPONSE TO LIGHT AND CIRCADIAN RHYTHM

Understanding the molecular and cellular bases of AQP regulation in leaves, and therefore the modes of $K_{\text{leaf}}$ regulation in response to developmental or environmental cues, represents an important focus in current research. Because of the dominating role of light and circadian rhythms in regulating $K_{\text{leaf}}$, most of recent studies have been performed in this context. Combined HPM and quantitative RT-PCR analyses in detached walnut leaves revealed a positive correlation between the increase in $K_{\text{leaf}}$ under high irradiance and the transcript abundance of two PIPs, JrPIP2;1 and JrPIP2;2 (Cochard et al., 2007). The light-dependent stimulation of $K_{\text{leaf}}$ in European beech (Fagus sylvatica) and pedunculate oak (Quercus robur) was also associated to enhanced expression of PIP1 genes (Baaziz et al., 2012). Diurnal oscillations in expression of NaAQP1 in tobacco leaf petioles (Siefritz et al., 2004), SsAQP2 in motor cells of Samanea saman leaves (Moshelion et al., 2002), and most of ZmPIP genes in maize leaves (Hachez et al., 2008) were correlated to changes in water permeability of corresponding protoplasts. However, light-dependent $K_{\text{leaf}}$ was not associated to any AQP transcriptional control in certain species such as bur oak (Voicu et al., 2009). Quantitative proteomic analysis in the Arabidopsis rosette showed that the abundance of each of the nine detected PIP isoforms was perfectly stable regardless the light regime (Prado et al., 2013). In contrast, the diphosphorylation of AtPIP2;1 at two C-terminal sites (Ser280 and Ser283) was enhanced by twofold under the same conditions. Whereas the rosette hydraulic conductivity of a pip2;1 knock-out mutant had
lost any responsiveness to the light regime, expression in the same 
background of phosphomimetic and phosphorylation deficient 
forms of AtPIP2;1 demonstrated that phosphorylation at Ser280 
and Ser283 was necessary for \( K_{\text{sat}} \) enhancement under darkness 
(Prado et al., 2013).

**WATER STRESS**

Plants can undergo water stress in response to numerous environ-
mental constraints such as drought, low atmospheric humidity, 
salinity, or cold. Studies trying to relate physiological responses to 
water stress with expression profile of AQP still have led to contrast-
ing results depending on the time courses and intensity of water 
stress (Tyerian et al., 2002; Galíneti et al., 2007). Some studies have 
shown, however, that water stress can coordinate alters AQP 
expression and activity in the leaf. In grapevine (Vitis vinifera) 
under reduced irrigation for instance, the \( K_{\text{sat}} \) was decreased by 
about 30% together with the expression of VvTIP2;1 and VvPIP2;1 
(Piva et al., 2013). A low humidity treatment also induced a coor-
dinated up-regulation of many PIP and TIP genes in rice leaves 
(Kuwagata et al., 2012). Enhanced expression of some AQP may 
also support a role in embolism refilling. For instance, \( \delta \text{STOP} \) 
which was highly expressed in vessel-associated cells of walnut 
leaves during the winter period (Sakr et al., 2003).

Protomorphic approaches have provided complimentary insights 
to the mode of AQP regulation under drought. A label-free 
quantitative shotgun approach in rice leaves under moderate or 
extreme drought or re-watering conditions showed that most of 
the nine AQP identified were responsive to drought, with six 
decreasing rapidly during plant re-watering (Mirzaei et al., 2012). Phosphoprotromic analyses of Arabidopsis seedlings indi-
cated that the C-terminal phosphorylation of AtPIP2;1 decreased 
after 30 min of an ABA treatment (Klime et al., 2010). This obser-
vation is consistent with the down-regulating effects of ABA on 
Arabidopsis \( K_{\text{sat}} \) through a mechanism that involves bundle sheath 
cells (Sheb-Cohen et al., 2011; Furrin et al., 2013). Thus, simi-
lar to what was described in leaves under changing light (Prado 
et al., 2013), altered phosphorylation of AQP in veins may act 
on their trafficking and gating (Törnroth-Horsefield et al., 2006; 
Prak et al., 2008; Eso et al., 2010) to adjust leaf hydraulics dur-
ing plant response to drought. The decreased phosphorylation 
of spinach \( \delta \text{STOP} \) following a hypotonic stress in leaf 
fragments (Johansson et al., 1996) was initially interpreted in the 
context of leaf cell turgor regulation, whereby an enhanced activ-
ity (phosphorylation) of \( \delta \text{STOP} \) would favor water influx under 
fully hydrated conditions. It could also correspond to a water 
stress-dependent regulation of \( K_{\text{sat}} \).

**SIGNALING MECHANISMS ACTING UPSTREAM OF AQP REGULATION**

The signaling mechanisms that act upstream of leaf AQP regula-
tion now represent a critical challenge for future research. They 
likely involve ROS and calcium (Ca\(^{2+}\)), which both display spe-
cific signatures during leaf response to environmental or hormonal 
stimuli.

Hydrogen peroxide (H\(_2\)O\(_2\)) is now recognized as a potent reg-
ulator of plant AQPs. H\(_2\)O\(_2\) perfusion via the petiole decreased by 
up to 30-fold the water permeability of epidermal and parenchyma 
cells, in wandering jew (Tradescantia fluminensis; Ye et al., 2008) 
and maize (Kim and Steudle, 2009) leaves, respectively. A ROS-
dependent down-regulation of AQP has also been invoked to 
explain the inhibition at high light intensities of the hydraulic con-
ductivity of parenchyma cells, in the midrib tissues of maize leaves 
(Kim and Steudle, 2009). The mode of action of ROS on water 
transport is still debated. Hydroxyl radicals produced from exoge-
nously supplied H\(_2\)O\(_2\) may act on AQP gating by direct oxidation 
(Henzi et al., 2004). Such effects were not observed in Arabidopsis 
whereby H\(_2\)O\(_2\) triggers a cell signaling cascade ultimately leading 
to PIP down-regulation, through altered phosphorylation and/or 
cellular internalisation (Bouriac et al., 2008; Prak et al., 2008).

Ca\(^{2+}\) plays key structural and signaling roles in plants. It can 
directly inhibit PIP activity in vitro (Gerbeau et al., 2002; Alleva 
et al., 2006; Verdasco et al., 2008) by a molecular mechanism 
that involves Ca\(^{2+}\) binding to the cytosolic side of the AQP to 
stabilize its closed conformation (Hedfalk et al., 2006; Törnroth-
Horsefield et al., 2006). This effect has not yet been related to any 
physiological process in the plant. Plant AQPs can also undergo 
Ca\(^{2+}\)-dependent phosphorylation, which in turn increases their 
water channel activity. For instance, in vitro phosphorylation of 
spinach leaf PM2A (\( \delta \text{STOP} \)) was mediated by a plasma 
membrane-associated protein kinase that was strictly dependent on 
submicromolar concentrations of Ca\(^{2+}\) (Johansson et al., 1996; 
Sjöwall-Larsen et al., 2006). This and other protein kinases acting 
on leaf AQPs still await biochemical and molecular characteriza-
tion. An integrative model that links the water flow pathways and 
Ca\(^{2+}\) distribution in leaves was recently proposed (Gilliam et al., 
2011). According to this model, the delivery of apoplastic Ca\(^{2+}\) 
and its storage could determine most of hydraulic regulations involving 
leaf AQPs.

**INTEGRATION AND MANIPULATION OF LEAF HYDRAULICS**

LEAF HYDRAULIC CONDUCTANCE AND WATER STATUS

Because the leaf water status is at the cross-road of fundamen-
tal physiological functions including carbon fixation and growth, 
its manipulation or genetic improvement could help optimize 
the entire plant performance, including yield and adaptation to 
environmental constraints, over short and long time scales. How-
ever, several important principles first need to be emphasized to 
understand the integrative aspects of plant leaf hydraulics and the 
potential and possible pitfalls of its manipulation.

The present review addressed plant leaf hydraulics, essentially 
by looking at the multiple facets of \( K_{\text{sat}} \). It is of note that, in plants 
derunning transpiration, the dominating resistance for water 
transport across the plant does not operate in inner leaf tissues 
but on vapor diffusion, through stomata and at the leaf surface. 
Thus, the direct impact of \( K_{\text{sat}} \) on the intensity of the leaf trans-
piration may be marginal. The physiological importance of \( K_{\text{sat}} \) 
should not be underestimated, however, since under a fixed tran-
spiration regime, \( K_{\text{sat}} \) strongly impacts on the hydration status of 
the inner leaf tissues (Tsuda and Tyerian, 2008). As explained below, 
leaf hydraulics has a great significance for growth, due to crucial 
links between this process and leaf water potential. Water poten-
tial maintenance in inner leaf tissues is also linked to hydraulic 
conductance of vessels and stomata and, as a result, interferes with 
the transpiration flow. For instance, stimuli such as light 
that enhance \( K_{\text{sat}} \) actually promote water supply to the inner leaf
tissues to prevent an excessive drop in water potential through-out the transpiring leaf (Tsuda and Tyye, 2000). This may help reduce tensions and avoid cavitations in xylem vessels. Conversely, a hydraulic limitation in veins, which can typically be enhanced by ABA-dependent down-regulation of AQPs in these territories, can result in a hydraulic signal to promote stomatal closure in plants under water stress (Pantin et al., 2013). This example emphasizes the fundamental interplay that exists between leaf water potential, $K_{lw}$ and $g_s$.

**AQPs and Hydraulic Control of Leaf Growth**

While most of the water absorbed by the plant is lost by transpiration, a minor fraction is retained for supporting leaf growth (Pantin et al., 2012). Leaf expansion growth primarily results from a fine interplay between cell wall relaxation and cell water potential, which both determine the rate of water inflow (Cousarone, 1987). It is therefore highly sensitive to the leaf water status and has to be protected from environmental disturbances.

The finding of growth-induced water potential gradients (Fricke, 2002; Tang and Broyer, 2002) provided the first direct evidence that leaf growth can be hydraulically limited. This idea is also supported by enhanced function of AQPs in expanding tissues. In cereal leaves for instance, cell water permeability was higher in the elongation zone than in the emerged non-growing zone (Volkov et al., 2007; Hachez et al., 2008). Preferential expression of AQP isoforms in leaf expanding tissues was described in several plant species (We et al., 2007; Hachez et al., 2008). This pattern was not restricted to plasma membrane AQPs since expression of *AtTIP1;2* was associated with cell enlargement in Arabidopsis leaves (Luede-vid et al., 1992) and enhanced by the growth-promoting hormone gibberellic acid (GA3; Phillips and Hatch, 1994). Vascular AQPs may favor the differentiation of a large central vacuole that is characteristic of fully elongated cells (Luedevid et al., 1992). Whole plant measurements have also provided evidence for hydraulic limitation of leaf growth. In Arabidopsis, it occurs during leaf ontogeny, with leaf growth becoming slower during the day than at night (for a review, see Pantin et al., 2012). In maize, leaf growth was highly sensitive to alterations of inner plant hydraulic conductance, through pharmacological inhibition of AQPs (Ehler et al., 2009) or genetic alteration of ABA biosynthesis which in turn altered AQP expression (Parent et al., 2009).

In summary, a hydraulic resistance between vascular and peripheral expanding tissues may result in marked growth-induced water potential gradients, which would in turn collapse cell turgor and result in an immediate growth arrest. Thus, high AQP-mediated cell water permeability can be highly beneficial to enhance cell-to-cell water transport in expanding tissues. Under water stress conditions, however, solute deposition rate in the elongation zone may become the limiting factor to sustain water inflow, resulting in a hydraulic signal to promote stomatal closure in plants under water stress (Pantin et al., 2013). This indicates that this AQP can indeed play a role in drought resistance and ultimately promote plant growth.

**AQPs, Carbon Fixation and Growth**

Following the initial phase of turgor-driven cell expansion, a proper supply of carbon and therefore efficient photosynthesis are necessary for new cell wall deposition and an overall increase in dry matter (Pantin et al., 2012). Thus, the ability of some plant AQPs to transport CO$_2$, in addition to water, may also be highly relevant to their beneficial role in plant growth. In particular, functional expression in oocytes or yeast of a tobacco PIP AQP, NtAQP1, has shown that this AQP can enhance membrane permeability to gaseous CO$_2$ (Uehlein et al., 2003). Immunological and translational fusion approaches further showed that NtAQP1 was present in guard cells and mesophyll cells, where it localized to both the plasma membrane and in the inner chloroplast membranes. The latter localization is particularly suggestive of a role in CO$_2$ assimilation (Uehlein and Kalderhoff, 2008).

In transgenic tobacco plants with altered expression of NtAQP1, the rate of $^{13}$C incorporation in leaf disks fed with $^{14}$CO$_2$ (Uehlein et al., 2003), the intensity of gas exchange, chlorophyll fluorescence, and $^{13}$C discrimination (Flexas et al., 2008) were positively correlated to the level of NtAQP1 expression. These results were interpreted to mean that NtAQP1 functions as a CO$_2$ channel in the mesophyll. These initial observations have now been extended to rice (Hanba et al., 2004) and Arabidopsis (Heckwell et al., 2011). In the latter study, Arabidopsis *pip1;2* knock-out plants displayed a reduction by 40% of their mesophyll conductance ($g_{m}$), to CO$_2$. With respect to previous reports, this work defines a clear molecular and genetic context in which to address the function of PIPs in CO$_2$ transport. In view of other possible contributors of $g_{m}$, such as cell walls and carbonic anhydrases (Evans et al., 2009), it remains to be understood, however, how a single AQP isoform can contribute...
up to 40% of $g_w$. Also, it is intriguing that the ArPIP1;2 isoform was also identified as an important component of root and leaf hydraulics (Postaire et al., 2010). Thus, much remains to be learnt about the interplay and regulation of water and CO$_2$ transport by AQPs. The possible coupling of tissue hydraulics with growth and carbon assimilation provides unique research perspectives in plant integrative biology.

CONCLUSION

Recent research indicates that the veins, and the AQPs that are expressed in these territories, represent key determinants of leaf hydraulics. Understanding how the vascular architecture of leaves optimizes their hydraulic behavior or, in other words, understanding the adaptive value of leaf venation according to species and/or natural habitats represents an important challenge for future studies. Besides studies on xylem differentiation, a better knowledge of the function and regulation of the numerous AQP homologs expressed in plant leaves is also critically needed to understand how multiple environmental factors such as day/night cycles or water stress act alone or in combination to alter leaf hydraulics. While a role for AQPs in phloem loading, leaf movement, and CO$_2$ transport is emerging, we also anticipate that genetically altered plants will help decipher these and other new AQP functions. Finally, integrative studies have shown how the hydraulics of inner leaf tissues can have a strong impact on the dynamic responses of leaf water potential and stomata, and as a consequence on plant carbon economy and leaf expansion growth. These studies point to the power but also complexity of biotechnological strategies where plant AQP function is manipulated to potentially improve plant growth and tolerance to water stress.

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