



Review

Aquaporin structure–function relationships: Water flow through plant living cells

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Abstract

Plant aquaporins play an important role in water uptake and movement—an aquaporin that opens and closes a gate that regulates water movement in and out of cells. Some plant aquaporins also play an important role in response to water stress. Since their discovery, advancing knowledge of their structures and properties led to an understanding of the basic features of the water transport mechanism and increased illumination to water relations. Meanwhile, molecular and functional characterization of aquaporins has revealed the significance of their regulation in response to the adverse environments such as salinity and drought. This paper reviews the structure, species diversity, physiology function, regulation of plant aquaporins, and the relations between environmental factors and plant aquaporins. Complete understanding of aquaporin function and regulation is to integrate those mechanisms in time and space and to well regulate the permeation of water across biological membranes under changing environmental and developmental conditions.

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Keywords: Aquaporin; Water channel; Drought stress; Membrane interface; Water uptake

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Abbreviations: AQPs, aquaporins; MIPs, major Intrinsic Proteins; TIPs, tonoplast intrinsic proteins; NIPs, nodulin like plasma membrane intrinsic proteins; SIPs, small intrinsic proteins; PIPs, plasma membrane intrinsic proteins; NPA, asparagine–proline–alanine; ABA, Abscisic acid.

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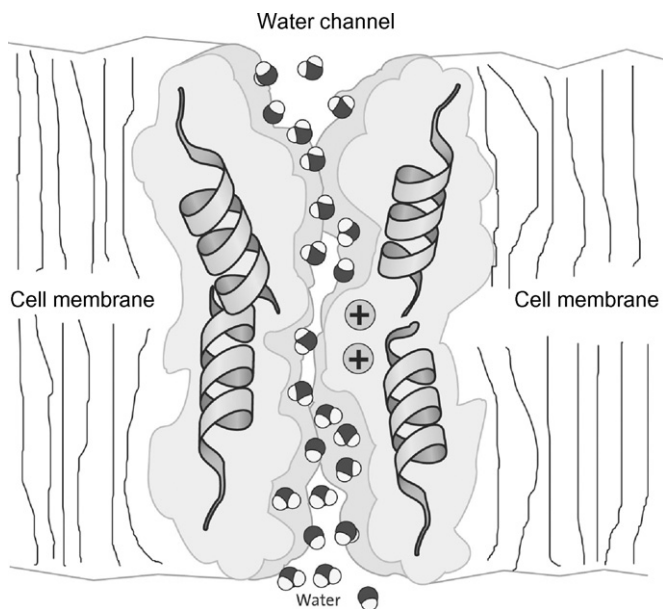


Fig. 1. Models for water molecules through the aquaporin in the membrane (adapted from Chrispeels and Agre [7]).

1. Introduction

The discovery of aquaporins showed a new insight into the mechanism of water-transmembrane transportation (the model in Fig. 1), which provided solid molecular basis for fast and reversible regulation of transmembrane water transport and gave strong support to the idea that such high water permeability might be required for certain physiological processes [1,2]. Aquaporins, or major intrinsic proteins (MIPs), are channel-forming membrane proteins with the extraordinary ability to combine a high flux with a high specificity for water across biological membranes. They belong to a well-conserved and ancient family of proteins called the major intrinsic proteins (MIPs) with molecular weights in the range of 26–34 kDa [3], with members found in nearly all living organisms. The aquaporin family in plants is large, indicating complex and regulated water transport within the plant in order to adapt to different environmental conditions, which includes more than 150 membrane channel proteins [4]. Regulation of aquaporin-mediated water flow, through indirect or direct means, appears to be a mechanism by which plants can control cellular and tissue water movement [5]. All aquaporin isoforms probably work together in an orchestrated manner, where each individual aquaporin isoform displays a specific localization pattern, substrate specificity, and regulatory mechanism [6]. The structure, function and gene regulation of aquaporins as well as research methodology are reviewed as following.

2. Diversity of plant aquaporins

The physiological role of water channel proteins is particularly important in plants because of their continuous water recruitment [7,8]. Many more MIP family genes have since been identified in plants, with additional members in *Arabidopsis*,

tobacco, spinach, tomato, the ice plant (*Mesembryanthemum crystallinum*), radish, and snapdragon [2,3,9]. The permeability values establish limits on aquaporin tissue densities required for physiological function and suggest significant structural and functional differences among the aquaporins [10].

There are two highly important aspects of plant aquaporins [2]. One aspect is their tremendous diversity in plants: in the *Arabidopsis* genome, 35 AQP genes have been identified [2,11,12]. The other aspect is the discovery that some aquaporins are multifunctional channel proteins, allowing some small neutral solutes across cellular membranes, such as glycerol, CO₂, ammonia (NH₃), urea, boron, and hydrogen peroxide [13–17]. The diversity of aquaporin isoforms in plants can also be explained in part by their presence in multiple subcellular compartments [7,9,18].

Aquaporins are differentially expressed in different organs and membranes. In the plant kingdom, a single plant expresses a considerably large number of MIP homologues. These homologues can be subdivided into four groups with highly conserved amino acid sequences and intron positions in each group: the tonoplast intrinsic proteins (TIPs) [19–21], the plasma membrane intrinsic proteins (PIPs) [22,23], the nodulin like plasma membrane intrinsic proteins (NIP) [24], and the small intrinsic proteins (SIP) [25]. There are various isoforms of plant TIP: alpha (seed), gamma, root (Rt), and water-stress induced (Wsi). These proteins may allow the diffusion of water, amino acids and/or peptides from the tonoplast interior to the cytoplasm [21–26]. The plasma membrane aquaporins (PIPs) can be divided into two subfamilies: PIP1 with inactive or low water-channel activity and PIP2 with high water-channel activity [27]. The high diversity of aquaporins reveals novel facets of plant membrane functions [6].

Discovery of the aquaporin family of water channel proteins has provided a molecular explanation for rapid water movements across the plasma membranes of cells while diffusion still works in parallel [9,26,28]. This may be one reason for the abundance and diversity of aquaporins, in particular in plants. Their activity may be required for fine regulation at the gene and/or protein levels, which is influenced directly or indirectly by cell metabolism, for example, via phosphorylation of the aquaporin proteins and water stress [6,29–31].

The numerous aquaporin isoforms of plants have specific expression patterns throughout plant development and in response to environmental stimuli. Once a protein is involved, the cell has the ability to regulate its abundance (transcriptional or post-transcriptional) or to modulate its activity [2,32]. Water permeability of the plasma membrane and tonoplast through the aquaporins depends on their quantity, activity, and substrate specificity [33]. In part because of this very high diversity, the knowledge about plant aquaporin expression and subcellular localization is far from complete [34]. Thus, future investigations on the aquaporin family of proteins will provide important information not only on the physiology of membrane transport processes in many cell types, but also on the targeting and trafficking signals that allow proteins to enter distinct intracellular vesicular pathways in cells [6,32,34].

3. Aquaporin gene expression and diurnal fluctuations

Because of aquaporin potentially important role in regulating water flow in plants, studies documenting aquaporin gene expression in specialized tissues involved in water and solute transport are important [35,36]. The high level of expression of ZmTIP1 in maize tissues (root epidermis, root endodermis, small parenchyma cells surrounding mature xylem vessels in the root, and so on) facilitates rapid flow of water through the tonoplast to permit osmotic equilibration between the cytosol and the vacuolar content, and to permit rapid transcellular water flow through living cells when required. Specific plasma membrane aquaporins of the PIP1 subfamily are expressed in sieve elements and guard cells [37]. Barley HvPIP2:1 is a plasma membrane aquaporin and its expression was down-regulated after salt stress in barley [38].

The abundance of water channel proteins is a critical parameter to understand their function at the tissue, cell, or subcellular levels. Studies regarding the aquaporin mRNA abundance have led to the following conclusions: (1) many aquaporins are highly expressed in the vascular tissues; (2) some tonoplast aquaporins are highly expressed in meristems, where vacuolar biogenesis takes place; (3) aquaporins are highly expressed in the tissues that can experience high water or metabolite flux [39–41]. It is therefore of interest to study the expression patterns of AQP isoforms in order to further elucidate their involvement in plant water transport [6,18,19].

Under water-deficit conditions, expression of the tonoplast aquaporin gene in cauliflower is subject to a precise regulation that can be correlated with important cytological changes in the cells [43]. Therefore, the expression of aquaporin genes may be independently regulated in an organ and stage-specific manner, while the amount of aquaporin protein can be regulated at the translational level and by the rate of protein turnover [13,33,43,44]. Meanwhile there are numerous reports providing clear evidence that the abundance of aquaporins can be regulated by developmental and environmental factors [6,13,32]. Monitoring aquaporin gene expression patterns in many plant species in specific tissues, cell types or in response to phytohormones or environmental factors has highlighted the putative roles of water channels [13,32,44,45]. Currently, modulation of aquaporin expression in plant is considered the strategy of choice for elucidating the role of aquaporins in plant physiology [13].

In *Lotus japonicus* roots, the PIP1-type water channel showed diurnal fluctuations that were correlated with the diurnal variation in root hydraulic conductivity [46]. Harmer et al. found that the mRNA level of an *Arabidopsis* tonoplast aquaporin, GTIP, cycled in a circadian manner [47]. The plasma membrane aquaporin in the motor cells of *Samanea saman* also showed a diurnal and a circadian regulation [48,49].

4. Aquaporin cellular and subcellular localization

Regardless of whether all or only the majority of the plant MIPs are aquaporins, it is clear that a large number of aquaporins are present in plants, some localized in the tonoplast,

some in the plasma membrane and some possibly localized in endomembranes [4,6,11,18,20,21,26,32,45]. MIP-B was found in fractions containing tonoplast proteins and possibly in a fraction of intermediate density, distinct from both plasma membrane and tonoplast, and also distinct from the fractions in which MIP-A was located [19,34,50]. Only a small amount of MIP-B was located in the plasma membrane fractions, as predicted by sequence alignments. Based on the distribution of PIP proteins within plant cells, Kirch et al. suggested that the control of plant water-channel activity might appear through an analogous vesicle shuttle mechanism [51]. It is possible that the subcellular localization (in the plasma membrane or intracellular vesicles) of aquaporin tetramers is dependent on the level of phosphorylation of the subunits [5,52].

Multiple aquaporin genes could allow for variable expression in different tissues, cells and intracellular membranes during development, under changing external conditions and in response to rapidly changing demands for water movement [4,6,11,18,20,21,26,32,45,50,52]. Höfte et al. found that a presumptive tonoplast aquaporin (Phaseollus α -TIP) is located in multivesicular bodies in transgenic tobacco that expresses Phaseollus α -TIP [53]. Aquaporins have been also localized in plasmalemmasomes, invaginating domains of the plasma membrane that protrude into the vacuole [54,55]. As suggested by Robinson et al., clustering of aquaporins in the plasmalemmasomes may provide the means for achieving a rapid osmotic balance, and therefore, turgor maintenance in mesophyll cells [54]. Repeat experiments produced the same distribution, and clearly indicated that putatively plasma membrane-located aquaporins show a more complex pattern, and might be localized to different subcellular membranes or compartments.

5. Aquaporin structure and selectivity

The structure of aquaporins is highly conserved in animals, plants, yeast, and bacteria [4]. All MIP family proteins share six putative transmembrane domains with the N- and C-termini facing the cytosol (Fig. 2). The six transmembrane domains were predicted to be α -helices, packed together with the pore-forming domains outside and towards the center of an aquaporin tetramer [4,26,28,56]. There are five loops (A–E) joining the transmembrane helices. The first cytosolic loop and the third extracytosolic loop are hydrophobic and contain the conserved asparagine–proline–alanine (NPA) sequence [8,3,57]. The two halves of the protein show obverse symmetry, with the hydrophobic loops containing the NPA motif overlapping in the middle of the lipid bilayer to form two hemipores that together create a narrow channel proposed to be similar in shape to an hour-glass [3,57,58].

Aquaporin polypeptides generally form homotetramers in the membrane [59–61], with each monomer forming a single water pore [58]. Water molecules passing the channel are forced, by the protein's electrostatic forces, to flip at the center of the channel. Water is thought to flow across the water channel pore in either direction down its potential gradient [1,62].

To account for the molecular selectivity of MIP family channels, the pore so formed was hypothesized to function via a

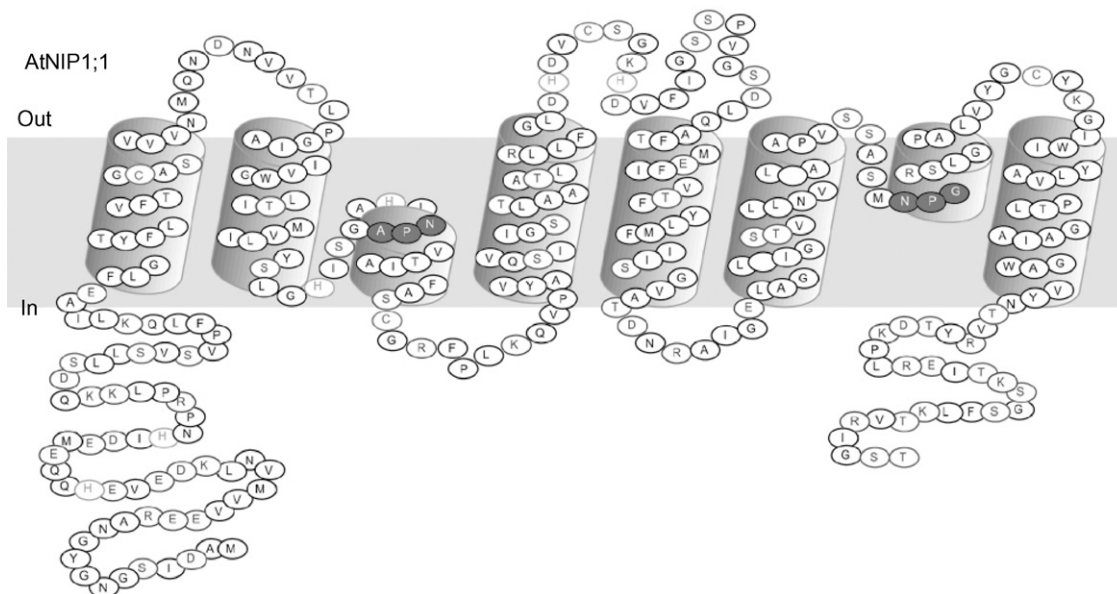


Fig. 2. Aquaporin structure: a schematic three-dimensional structure of AQP based on structural studies of human AQP1 and *E. coli* GLP-F (modified from Postaire et al. [120]).

size-exclusion mechanism [1]. Inhibition of the water channel by mercuric chloride is thought to be the result of the sulfhydryl reagent binding a cysteine residue located in close proximity to the pore, resulting in the physical blockage of the molecular flow through the pore [5,62,63]. The gating mechanism successfully unifies a significant body of biochemical and genetic evidence that has identified specific amino-acid residues governing plant aquaporin gating, and immediately suggests how the closed structure might be stabilized or destabilized [5,64,65].

Nevertheless, plant aquaporin structures have been reported only at low resolution. Further investigation is necessary to determine the atomic structure of the water pore and the mechanism of its selectivity [5,64,66]. Moreover, the specificity of more plant aquaporins also needs to be determined, and plausible plant metabolites need to be tested, in order to identify regions important to specificity [5,9,20,32].

6. Change and regulation of aquaporin water permeability

Land plants have evolved to cope with rapid changes in the availability of water by regulating all aquaporins that lie within the plasma membrane [1]. Regulation of aquaporin trafficking may also represent a way to modulate membrane water permeability, and the factors affecting and regulating aquaporin behaviors possibly involve phosphorylation, heteromerization, pH, Ca^{2+} , pressure, solute gradients and temperature drought, flooding and so on (Fig. 3), which suggests aquaporins are involved in a versatile and dynamic regulation of water movement [1–3,5,9,20,32,50]. The abundance and activity of aquaporins in the plasma membrane and tonoplast may be regulated, hence enabling the plant to tightly control water fluxes into and out of its cells, as well as within the cells [5,6,19,50].

6.1. Functional regulation of aquaporin activity by phosphorylation

Aquaporin activity may also be directly regulated. Phosphorylation is a major mechanism used by cells as a molecular ‘switch’ to regulate protein activity [5,19,20,30]. The kinases responsible for protein phosphorylation are induced in response to a number of signals, including drought or water stress [19,63], attack by pathogens [67], the plant hormones auxin and abscisic acid [19,68], and light [6,20].

Mutation of the putative phosphorylation sites in α -TIP (Ser7Ala, Ser23Ala and Ser99Ala) reduced the apparent water transport activity of α -TIP in oocytes, suggesting that phosphorylation of α -TIP occurs in the oocytes and participates in the control of water-channel activity [29,35,69]. Phosphorylation of the putative PM-AQP was thought to activate the water channel composed of PM-AQP. Dephosphorylation of the phosphorylated PM-AQP was also observed during petal closing at 5 °C, suggesting the inactivation of the water channel [70].

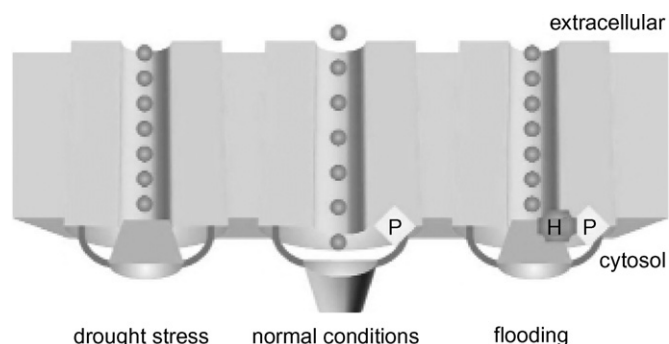


Fig. 3. Schematic illustration of how plant plasma membrane aquaporins are gated (adapted from Törnroth-Horsefield et al. [121]).

As demonstrated by Johansson et al., species- and stress-specific changes in cell hydraulic conductivity may be caused by phosphorylation of aquaporins [26,35]. The phosphorylation of Soybean nodulin 26 on Ser-262, which is catalyzed by a symbiosome membrane-associated calcium-dependent protein kinase, stimulates its intrinsic water transport rate. Soybean nodulin 26 phosphorylation is enhanced further by osmotic stresses (water deprivation and salinity) [71].

Phosphorylation of the plasma membrane aquaporin PM28A is carried out by a Ca^{2+} -dependent membrane-bound protein kinase and depends also on the apoplastic water potential [26,35]. Three PIP2 isoforms (ZmPIP2;1, ZmPIP2;3 and ZmPIP2;4) were detected in the phosphorylated band. The water-channel activity of these isoforms was partially inhibited by H7, a PKC inhibitor, suggesting an important effect of phosphorylation on channel function [72]. Both tonoplast and plasma membrane aquaporins have putative phosphorylation sites that are conserved among different members of this family [24].

6.2. Effects of turgor and osmotic pressure on aquaporin activity

There is no direct evidence for mechanosensitivity of aquaporin proteins, although some studies illustrate the potential for MIPS to be involved in volume or turgor homeostasis in plants [3,73,74], and a different mechanism of mechanical inhibition was recently reported in young maize roots [75]. The dependence of mercury-induced closure of water channels on tissue turgor indicates that changes in turgor can induce changes in the conformation of the plasma membrane, or even in the aquaporins themselves [76]. In more recent experiments, membrane permeability parameters (hydraulic conductivity, permeability and reflection coefficient) of *Chara* cells were measured using a cell pressure probe as the concentration of variously sized osmolytes was increased [77]. Water-channel activity, inferred from the membrane permeability parameters, was seen to decrease with increasing osmotic pressure and with increasing osmolyte size. A cohesion/tension mechanism was proposed to account for the gating of *Chara* water channels, which suggests that the osmotic tension generated within the channel by a high osmotic potential leads to the structural collapse and closure of the protein channel. Similar effects of high salinity on membrane water permeability as reported in several species could result, at least partly, from the same mechano-sensitive mechanism [78–80].

6.3. Effect of pH variation on aquaporin activity

There is not much known regarding the pH regulation of aquaporins in plants. However, several studies suggested the involvement of pH in regulating the aquaporin activity. Thus, structural pH sensors must reside in these proteins. Furthermore, the position of histidines in different members of the aquaporin family can “tune” the pH sensitivity toward alkaline or acid pH ranges. The water permeability of plasma membrane from *Arabidopsis* suspension cells or root cells was reduced in the presence of low pH [14,81]. In contrast, acidic pH activated the

water conductance of the mammalian aquaporin AQP0 [82]. The water permeability of outer cortex cells of an acid sensitive maize variety was decreased by acidic pH, while no effect was recorded in a variety that was acid tolerant [83]. On the other hand, in the killifish AQP0 homologue, MIPfun, with His at position 39 in loop A, alkaline rather than acid pH increased water permeability. A novel molecular mechanism for aquaporin gating by cytosolic pH was uncovered, which permits coordinate inhibition of plasma membrane aquaporins and, as a consequence, a general block of root water transport [81].

6.4. Sensitivity of aquaporin to cations

To date there is no doubt that the cellular biochemistry and physiology of a living organism is seriously affected by heavy metal ions. Mercury (Hg^{2+}) has been used extensively to provide evidence for the involvement of aquaporins in water transport process in animal and plant cells [66]. Due to mercury-induced conformational changes and identification of conserved surface loops in plasma membrane aquaporins from higher plants, mercury is thought to bind to sulphhydryl groups of the aquaporin proteins, physically blocking the channels and reducing their hydraulic conductivity [9]. Partial recovery of the water flow rate following the application of mercuric chloride was also observed in tomato and aspen root systems, implying the presence of aquaporins as the regulators of plant water status [84,85]. However, the inhibition of water flow with mercurial reagents is neither completely understood nor a general characteristic of aquaporins [28]. Some mercurial reagents, especially mercuric chloride, are highly membrane-permeant and are powerful metabolic inhibitors. That is why the effect of HgCl_2 on water permeation across the living cells should be interpreted with caution, since a possible outcome of HgCl_2 application could be the reduced phosphorylation of water channels [9]. As proven by Barone et al., mercury can also induce conformational changes in the plasma membrane aquaporins of higher plants [86].

Calcium signalling is a common path in the response of plants to stresses or hormones and cell-specific fluctuations in cytosolic Ca^{2+} occur in the epidermis, endodermis and pericycle of *Arabidopsis* roots in response to drought and salt [87]. Aquaporins in plant membranes can undergo Ca^{2+} -dependent phosphorylation, which can increase their water-channel activity [19,29,35]. On the other hand, calcium showed a clear effect on aquaporin activity, with two distinct ranges of sensitivity to free Ca^{2+} concentration (pCa 8 and pCa 4). Since the normal cytoplasmic free Ca^{2+} sits between these ranges it allows for the possibility of changes in Ca^{2+} to finely up- or down-regulate water-channel activity [88]. Ca^{2+} decreased the osmotic water permeability of PM vesicles from *Arabidopsis*, suggesting a potential relevance to intracellular Ca^{2+} signaling [14]. At the whole plant level, Ca^{2+} has been shown to ameliorate the reduction of root hydraulic conductivity produced by salinity [80]. The calcium effect is predominantly on the cytoplasmic face, and inhibition corresponds to an increase in the activation energy for water transport. However, a link between these observations and cell signalling and/or calcium-dependent water channel gating remains to be established.

Zinc (Zn^{2+}) inhibited the water transport across the membranes of *Chara* cells [89] and the water transport activity of *Actinidia deliciosa* protoplasts [90]. There is increasing evidence that zinc may be an integral part of the biomembranes, required for the stability and control of lateral mobility of membrane molecules [91]. As suggested by Tazawa et al., zinc might bind to the proteinaceous water-pore (aquaporins) [89].

The effect of other cations on water-channel activity has been tested. Sodium (Na^+), magnesium (Mg^{2+}), manganese (Mn^{2+}), potassium (K^+) had no effect on the water transport activity of *Actinidia deliciosa* protoplasts [90]. On the other hand, Ba^{2+} , Mg^{2+} , and Sr^{2+} reduced the osmotic water permeability of *Arabidopsis* PM, suggesting a direct blockage of aquaporins by direct cation binding [14]. The Mg^{2+} -dependent protein kinase phosphorylates the lentil seed aquaporins [69]. Magnesium (Mg^{2+}) can physically interact with the aquaporin proteins or with their neighboring lipids as suggested by Ren et al. [92], and Fu et al. [93]. AQPs in changes of hydraulic conductivity provoked by K^+ -deprivation was examined in a preliminary study. K^+ -deficiency reduced four ZmPIP and three ZmTIP members [94].

The effect of cations on osmotic water permeability of the plasma membrane is complex and not well understood. In plants exposed to environmental stimuli, there is a complex interaction of different cations with direct or indirect effects on the aquaporin water transport activity [95].

6.5. Relationships between plant aquaporins and hormones

Abscisic acid (ABA) is a well-recognized mediator of water stress responses. The responsiveness of each aquaporin to ABA were different, implying that the regulation of aquaporin expression involves both ABA-dependent and ABA-independent signaling pathways. Both energy-input and tension-gating mechanisms might be used by the plant to sense changes in turgor pressure and surrounding water availability, and to adapt the membrane water permeability in an ABA-dependent manner [75]. In many species including sunflower, barley, sorghum and maize [96–98], exogenous ABA enhanced root L_p . Short-term effects (i.e. within 1–3 h) of ABA on root aquaporin gene expression have also been described in different species including rice, *C. plantagineum* and *Arabidopsis* [11,99–101]. In tobacco flowers, ABA induced expression of the aquaporin gene NtAQP1 [102]. The effect of ABA in rapid positive regulation of aquaporin gating might suggest a direct binding to the channel. However, for both water stress and ABA, comprehensive studies using macro- or micro-arrays are needed to determine whether the complement of aquaporin genes varies in a coordinated fashion, and parallels changes in root water transport [68,97].

Expression of certain plant aquaporins is regulated by gibberellic acid, or water deprivation. Gibberellic acid is also known to activate the promoter of the aquaporin PIP1b [99,103]. The significance of promoter regions for an abscisic acid- and gibberellic acid-induced gene expression could be restricted to a region between –1450 and –1112 upstream of the transcrip-

tion start point by transient transformation of a bicistronic vector into tobacco protoplasts [102]. Gibberellic acid and abscisic acid suppressed the levels of mRNAs of RsPIP2-1, RsPIP2-2 and RsPIP2-3 and the protein level of RsPIP2-1 in roots. On the other hand, the protein levels of RsPIP1-group members and RsTIPs were scarcely changed by these phytohormones [104].

Brassinolide may control aquaporin activities in *Arabidopsis thaliana*, which has been shown to be involved in the modification of the water-transport properties of cell membranes [105], however, mRNA and protein levels of aquaporin isoforms in root and shoot of radish were not affected by brassinolide treatment [104]. Ethylene significantly increased stomatal conductance, root hydraulic conductivity (L_p), and root oxygen uptake in hypoxic seedlings. Ethylene can increase plasma membrane permeability permitting more water to cross the cells [106]. At the whole plant level, increased water transport in hypoxic aspen seedlings exposed to ethylene was explained in terms of enhanced aquaporin activity, probably due to a direct effect of ethylene on the phosphorylation of aquaporins [107]. The L_p of root cortex cells was maximally stimulated ≥ 30 -fold after a 1 h exposure to 1 μ M ABA, but these effects disappeared over the next hour. In the same studies it was also noted that in contrast to ABA, auxin (IAA) and cytokinin (kinetin) reduced the cell L_p by three- to four-fold [98]. Studies at the cell level provide more direct evidence for cell membranes and aquaporins being involved in stimulus-induced regulation of root L_p [1–3,5,7,108].

6.6. Effects of water and nutrient stress on aquaporins

The role of aquaporins in plant water status under water stress is a complex issue, because the expression of different aquaporin genes may be stimulated, reduced, or unchanged under abiotic stress [1,51,109–110]. The expression of some genes that encode plasma membrane aquaporins, such as *Arabidopsis* RD28 and the NeMip2 and NeMip3 genes of *Nicotiana excelsior*, is stimulated under drought stress [111,112]. Conversely, expression of the *M. crystallinum* MIPA plasma membrane aquaporin gene is down-regulated under salt stress [112], whereas expression of the *Arabidopsis* PIP1a gene is not altered significantly by stress conditions [113].

Drought stress induced in the rice seedlings appeared to increase the physiological functioning of water channels by increasing the root water-channel activity or by increasing the aquaporin number compared with unstressed control seedlings [114]. Under water-deficit conditions, expression of the tonoplast aquaporin gene in cauliflower is subject to a precise regulation that can be correlated with important cytological changes in the cells [42]. PIP1b overexpression had no beneficial effect on transgenic tobacco under salt stress, whereas during drought stress it had a negative effect, causing faster wilting [115]. Since drought stress decreased the osmotic permeability of root protoplasts but did not have any influence on the amount of PIP1 and PIP2 aquaporins, it is plausible that drought affected the protein functionality [52]. The accumulation of transcripts arising from *NgMIP2*, *NgMIP3* and *NgMIP4* diminished dramatically in drought-stressed plants. This down-regulation of

MIP gene expression may result in reduced membrane water permeability and may encourage cellular water conservation during periods of dehydration stress [116,122,123].

Aquaporin functionality fits well with the overall water relations response is very relevant, since the two-phase adaptation to salinity may imply two types of aquaporin regulation. Salinity can negatively affect root water uptake. The work shows that exposure of roots to salt induces changes in aquaporin expression at multiple levels. These changes include a coordinated transcriptional down-regulation and subcellular relocalization of both PIPs and TIPs [117]. On the other hand, salt and water stresses induced the accumulation of *ZmTIP2-3* transcripts [118]. Similar findings on the dominance of symplastic water transport were obtained using transgenic tobacco plants expressing an antisense construct of the tobacco NtAQP1 gene that encodes another PIP1b isoform [25], while Barley HvPIP2;1 is a plasma membrane aquaporin and its expression was down-regulated after salt stress in barley [38]. Two aquaporin-encoding transcripts were found to be down-regulated during the first 15 and 60 min of a salt treatment, respectively. The expression level of the two transcripts then recovered and, after 7 d, expression had become higher than in plants grown in standard conditions [118].

It is the incoming nutrient supply that is registered as deficient, not the plant's nutrient status. At some point, close to the initiation of these responses, changes in water-channel activity may be involved. The reduction of root hydraulic conductivity in wheat plants affected by nutrient (N-, P- and S-) deficiencies, suggested that either the activity or the density of water channels in the root cell plasma membrane is diminished during nutrient deficiency, but the manner in which monitoring of nutrient stress is transduced into an hydraulic response is also unknown [119].

7. Conclusions and future perspectives

The discovery of aquaporins in plants has resulted in a paradigm shift in the understanding of plant water relations. Water flux across cell membranes has been shown to occur not only through the lipid bilayer, but also through aquaporins, which are members of the major intrinsic protein super-family of channel proteins [2,3,5,9,20,32]. As has been found in other organisms, plant MIPs function as membrane channels permeable to water (aquaporins) and in some cases to small nonelectrolytes. Aquaporins greatly increase the membrane permeability for water, but may also be regulated, allowing cellular control over the rate of water influx/efflux [1–7]. As a result, aquaporins provide a unique molecular entry point into the water relations of plants and establish fascinating connections between water transport, plant development and the adaptive responses of plants to their ever-changing environment [2].

Plants counteract fluctuations in water supply by regulating all aquaporins in the cell plasma membrane. Aquaporins can provide spatial markers to explore the intricate flows of water and solutes that play a critical role throughout all stages of plant development [2]. The rate of transmembrane water flux may be controlled by changing the abundance or the activity of the aqua-

porins. Actually, there are observations showing the alteration of water permeabilities in responses of plants to biotic or abiotic stresses such as high salinity, nutrient deprivation, or extreme temperatures [1–3,5,9,20]. In plants, aquaporins are likely to be important both at the whole plant level, for transport of water to and from the vascular tissues, and at the cellular level, for buffering osmotic fluctuations in the cytosol [19].

By combining molecular biology with plant physiology, it should be possible to determine the role that aquaporins play in water transport in the plant [1–7]. There is growing evidence that suggests that aquaporins play different roles throughout plant development [120–125]. Therefore, aquaporin genomic information is important because, assigning physiological function via transgenic reduction or removal of gene expression requires sequence information for precise targeting. Direct determination of the location of each aquaporin within tissues is still required to understand its function in the plant. A powerful tool in elucidating the aquaporin function is given by the reverse genetics that can also reveal unexpected function of water channel proteins, which benefit to our understanding of sequence-structure and structure–function relationships in plants [2,4,5,9,121,122]. This should be done both at the transcript and at the protein level because aquaporin turnover appears to be variable, such as when comparing constitutively expressed and inducible aquaporins. The transcriptional and/or post-translational regulation of aquaporins would determine changes in membrane water permeability. Both phosphorylation and translocation to/from vesicles have been reported as post-translational mechanisms [123–125]. However, translocation in plants has not yet been shown. Here, the aquaporin family is a set of genes whose functions are intuitively perceived as important, much isolated information has been accumulated, yet their function is far from being understood in living plant, and we still have a long way to go to fully understand the significance of these proteins.

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