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AQUAPORINS IN PLANTS

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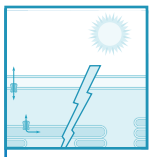

Maurel C, Boursiac Y, Luu D-T, Santoni V, Shahzad Z, Verdoucq L. Aquaporins in Plants. *Physiol Rev* 95: 1321–1358, 2015. Published September 2, 2015; doi:10.1152/physrev.00008.2015.—Aquaporins are membrane channels that facilitate the transport of water and small neutral molecules across biological membranes of most living organisms. In plants, aquaporins occur as multiple isoforms reflecting a high diversity of cellular localizations, transport selectivity, and regulation properties. Plant aquaporins are localized in the plasma membrane, endoplasmic reticulum, vacuoles, plastids and, in some species, in membrane compartments interacting with symbiotic organisms. Plant aquaporins can transport various physiological substrates in addition to water. Of particular relevance for plants is the transport of dissolved gases such as carbon dioxide and ammonia or metalloids such as boron and silicon. Structure-function studies are developed to address the molecular and cellular mechanisms of plant aquaporin gating and subcellular trafficking. Phosphorylation plays a central role in these two processes. These mechanisms allow aquaporin regulation in response to signaling intermediates such as cytosolic pH and calcium, and reactive oxygen species. Combined genetic and physiological approaches are now integrating this knowledge, showing that aquaporins play key roles in hydraulic regulation in roots and leaves, during drought but also in response to stimuli as diverse as flooding, nutrient availability, temperature, or light. A general hydraulic control of plant tissue expansion by aquaporins is emerging, and their role in key developmental processes (seed germination, emergence of lateral roots) has been established. Plants with genetically altered aquaporin functions are now tested for their ability to improve plant tolerance to stresses. In conclusion, research on aquaporins delineates ever expanding fields in plant integrative biology thereby establishing their crucial role in plants.

I. INTRODUCTION

In their large majority, plants are autotrophic organisms that just require light and a carbon source (usually carbon dioxide, CO_2), on the one hand, and water and mineral nutrients, on the other hand, to achieve their life cycle. Because they are sessile, plants have, however, to efficiently absorb water and mineral nutrients from their close surroundings. This function is mostly fulfilled by the root system, which shows a remarkable ability to grow continuously and explore the soil for available resources. With no short-term escape strategies, plants also have to continuously face severe constraints from both the soil and aerial environments. These include abiotic stresses such as a lack or excess of water (drought, flooding) or beneficial or toxic mineral ions at varying concentrations in the soil. High or low extremes in temperature or light intensity also impose severe stresses, on the shoot especially. In addition, plants are under constant attack by a myriad of herbivores and pathogens including viruses, bacteria, insects, or fungi. Many plant species are also able to develop symbioses with specific soil micro-organisms which, in particular, optimize plant mineral nutrition. Plants therefore continuously adjust their metabolic functions, growth and development, to adapt to an ever-changing abiotic and biotic environment.

With respect to their water status, plants exhibit remarkable features. Their aerial parts mediate a tricky trade-off with the atmosphere, by absorbing CO_2, a fundamental brick for photosynthesis, and releasing water by transpiration. This exchange is realized and tightly controlled by stomata, microscopic pores located in the epidermis of the plant's aerial parts **(FIGURE 1)**. Transpiration is made possible by an intense flow of water (sap) traveling throughout the plant body, from the roots to the substomatal chambers where it evaporates. This stream is particularly useful to drive water and nutrient ascent to the uppermost parts of

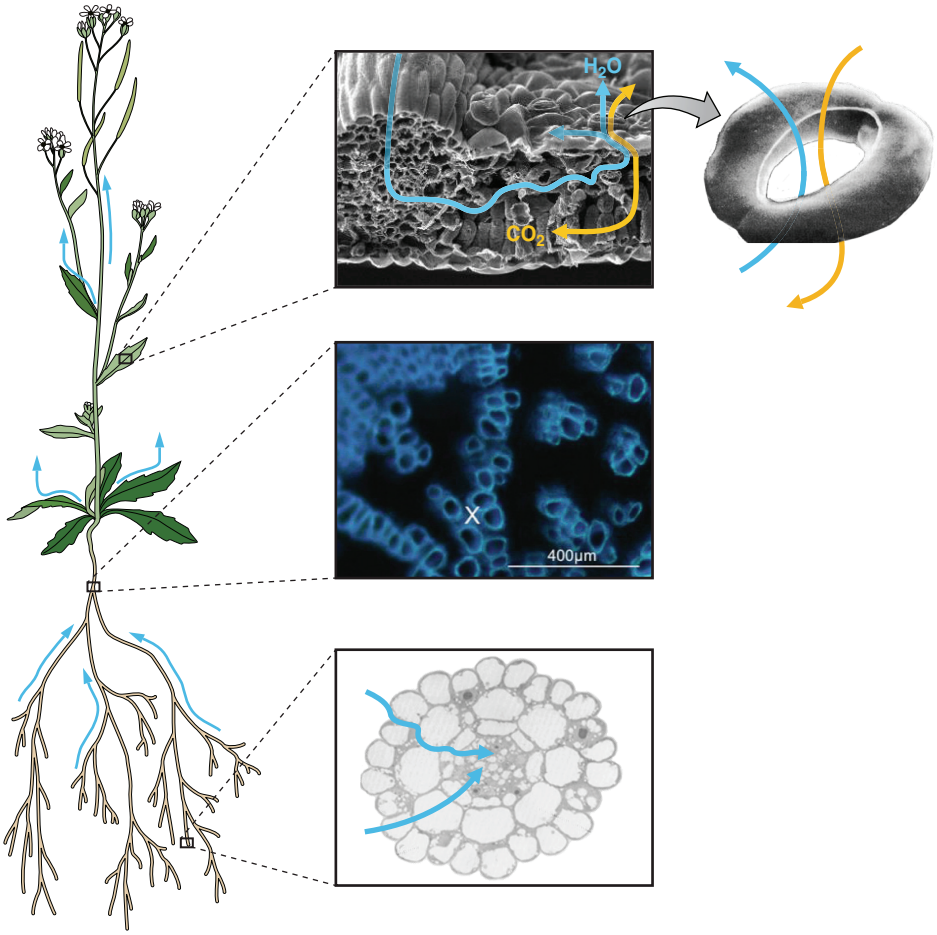

FIGURE 1. Short- and long-distance transport of water in a vascular plant. During transpiration, an intense stream of water occurs from the roots to the shoots. Soil water flows radially across concentric cell layers in the root before reaching the xylem vessels (*middle panel*, X) and being exported to the shoots. In leaves, water flows out of vascular tissues and reaches substomatal chambers where it diffuses as a gas (vapor) through the stomata.

the shoots. Whereas long-distance water transport mostly occurs through the xylem, which is formed of specialized dead vessels, water uptake by roots and delivery to shoots requires transport through living tissues **(FIGURE 1)**. Controlling the intensity and direction of these flows is particularly important for maintaining the whole plant water status. At the cellular level, the presence of a cell wall allows buildup of intracellular hydrostatic pressure (turgor) of several atmospheres which largely supports the erect shape of the plant. In addition, plant growth and development are determined by cell divisions in well-defined territories called meristems and subsequent expansion growth of the living cells. Cell turgor provides the motive force for the latter process. The metabolic activity of the plant and its growth and development are therefore highly dependent on its water status.

Despite a few pioneering works (reviewed in Ref. 185), the identification of water channel proteins in plants in the early 1990s (187), shortly after their discovery in animals, was fairly unexpected and provided a strong momentum for studies on plant water transport. Early studies of these so-called aquaporins contributed to a general boost in the molecular characterization of membrane transport systems in plants and brought new paradigms to address the molecular bases of plant water relations. After 20 years, plant aquaporins are recognized as multifunctional proteins transporting water but also gases such as CO_2, nutriments [e.g., boron (B), silicon (Si)] or reactive oxygen species (ROS). Thus aquaporin studies have spread to many fields of plant biology, from molecular membrane biophysics to cell biology and signaling. Their function also appears central for the physiology of plant growth and responses to abiotic stresses. This review covers the many facets of aquaporin functions in plants and stresses their importance and functional originality in these organisms.

II. PLANT AQUAPORIN DIVERSITY

A. Phylogeny of Plant Aquaporins

Aquaporins are now assimilated in a broad sense to the ancient superfamily of major intrinsic proteins (MIPs). MIPs are present throughout the living kingdom, with an exception for thermophilic Archaea and intracellular bacteria (1). MIP homologs have a reduced (~25%) overall sequence conservation but show a typical sequence signature, with six putative membrane spanning domains and two highly conserved Asn-Pro-Ala (NPA) motifs. In addition, MIP coding sequences are formed from a direct sequence repeat, which creates an internal symmetry of the

channel protein, with its two halves (each with 3 transmembrane domains) showing an inverted insertion in the membrane. With an increasing number of plant genome sequences available, aquaporin genes have now been fully described in several herbaceous (*Arabidopsis thaliana*, maize, rice, soybean, tomato, and cotton) and ligneous (poplar) higher plant species (45, 93, 121, 224, 235, 239, 252, 334). Thorough sequence analyses have also been performed in other plant species of agronomical interest such as wheat (76) or grapevine (265).

These studies have revealed a great diversity of aquaporins in higher plants, with more than 30 isoforms in all examined species **(TABLE 1)**. Due to a higher degree of ploidy, the genomes of soybean and upland cotton even encode 66 and 71 homologs, respectively. Higher plant aquaporins fall into five subfamilies. Three of these, the plasma membrane intrinsic proteins (PIPs), the tonoplast intrinsic proteins (TIPs), and the nodulin26-like intrinsic proteins (NIPs) are now well-described, with respect to protein localization and function. Two additional subfamilies, the small basic intrinsic proteins (SIPs) (120) and the uncategorized (X) intrinsic proteins (XIPs) (59) were discovered more recently. The latter are absent in some higher plant species such as the monocots or the *Brassicaceae* **(FIGURE 2)**.

Genomic studies have also provided insights into the aquaporin family in algae or early branched land plants (1, 7, 8, 59) **(TABLE 1)**. With respect to seed plants, mosses (e.g., *Physcomitrella patens*) have two additional subfamilies, the hybrid intrinsic proteins (HIPs) and GlpF-like intrinsic proteins (GIPs), whereas spike mosses (e.g., *Selaginella moellendorffii*) only have HIPs, in addition to the PIPs, TIPs, NIPs, SIPs, and XIPs (8, 59). Algae (e.g., *Chlamydomonas reinhardtii*, *Chlorella*, *Ostreococcus tauri*) reside upstream in the plant lineage. They also have PIP and GIP homologs in addition to five subclasses (MIP A-E) that, in contrast, seem largely unrelated to other plant aquaporin subclasses (7). Whereas animal and bacterial MIPs fall into both the water channel (aquaporin) and aquaglyceroporin clades, all plant subfamilies except GIPs belong to the first clade (1).

B. Plant Aquaporin Evolution

1. Interspecific variations

Phylogenic analyses have drawn a general scheme of plant aquaporin evolution. The GIPs may originate by horizontal gene transfer from an ancestral bacterial gene (94) and together with the PIPs have been transmitted from algae to land plants. Whereas XIPs and SIPs likely trace back to early plant (algae) ancestors, other subclasses (HIPs, TIPs) seem to have emerged during land plant evolution, possibly from a PIP ancestor. Interestingly, plant TIPs and mammalian aquaporin-8 share sequence homology, and it is as yet unclear whether this reflects a distant evolutionary relationship (1). The origin of NIPs is also uncertain. They may derive by horizontal gene transfer from an ancestral bacterial gene encoding an aquaglyceroporin with solute transport functions. Alternatively, NIPs may reflect an evolutionary convergence toward this subclass of aquaporins (1, 330). Finally, some subclasses (such as XIPs, HIPs, or GIPs) were lost during evolution of certain plant lineages pointing to functional redundancies.

The genetic diversity of aquaporins in plants also reflects the great dynamics of their genomes. While some subfamilies were lost in certain plant lineages (e.g., the XIPs in monocots), key genomic rearrangements have shaped the general expansion of the plant aquaporin family. These events have occurred at different stages during plant evolution. The subdivision of PIPs in PIP1s and PIP2s, or of TIPs in five subfamilies may have occurred early since they are conserved throughout all higher plants **(FIGURE 2)**. In contrast, the XIP subfamily may have evolved later as it shows taxon-specific clade divergences (162). In poplar (52), 48 of 54 aquaporin genes belong to pairs of genes. At variance to what is found in vertebrates (1), most of these pairs (17) are due to

Table 1. *Diversity of aquaporin gene family in plants*

Species	Common Name	PIPs	TIPs	NIPs	SIPs	XIPs	HIPs	GIPs	Total	Reference Nos.
Selaginella moellendorffii	Spike moss	3	2	8	1	3	2		19	8
Physcomitrella patens	Moss	8	4	5	2	2	1	1	23	59
Oryza sativa	Rice	11	10	10	2				33	252
Arabidopsis thaliana	Mouse ear-cress	13	10	9	3				35	121, 235
Solanum lycopersicum	Garden tomato	14	11	12	4	6			47	239
Populus trichocarpa	Black cottonwood	15	17	11	6	6			55	93
Glycine max	Soybean	22	23	13	6	2			66	334
Gossypium hirsutum	Upland cotton	28	23	12	7	1			71	224

Shown for all plant species, the genome of which was fully sequenced, are the number of homologs present in each of the indicated aquaporin subclasses.

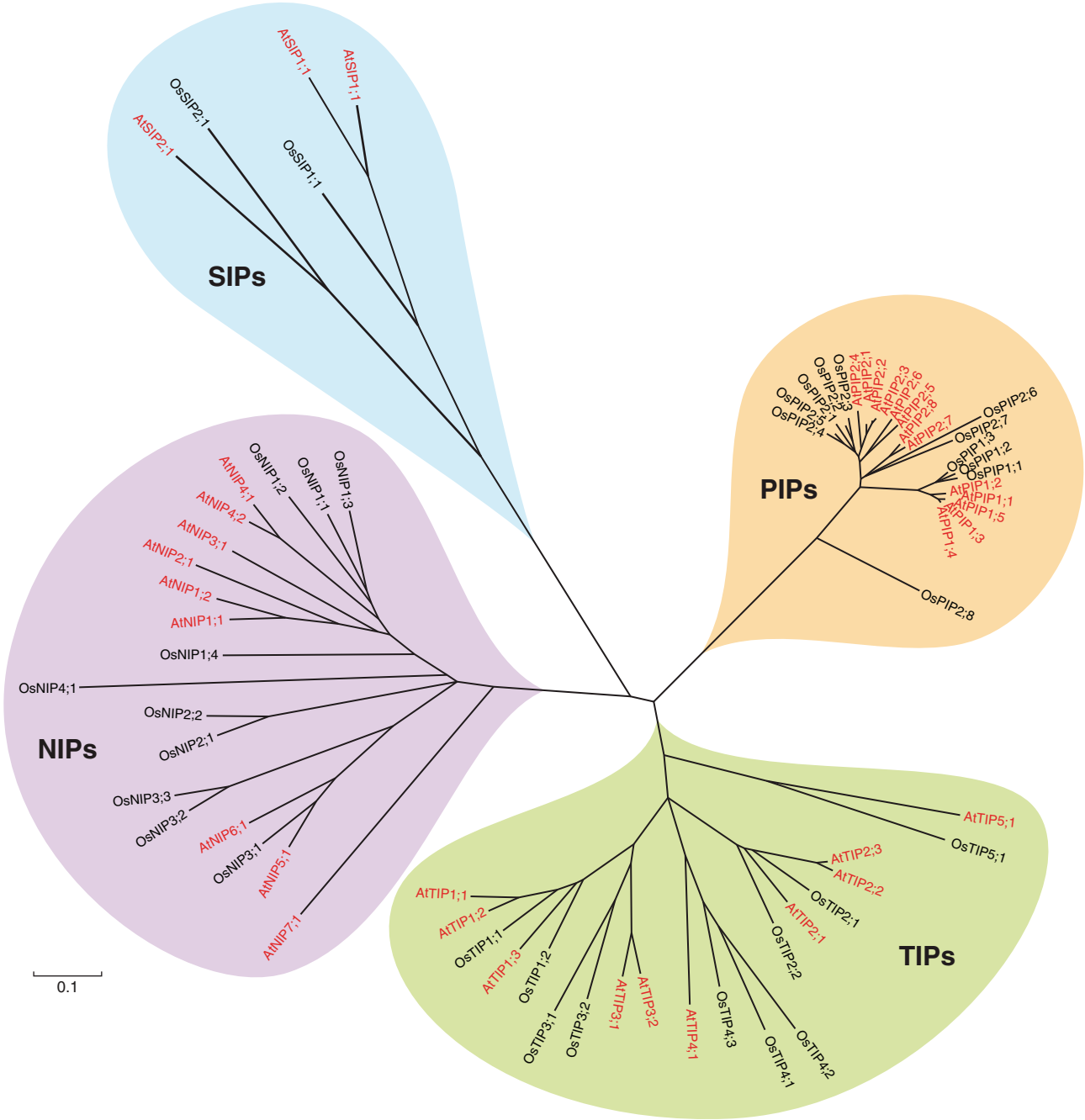

FIGURE 2. Phylogenetic tree of the *Arabidopsis* (red) and rice (black) aquaporin families. The evolutionary history of aquaporins of the two plant species was analyzed using a MEGA4 software (280). It was first inferred using the Neighbor-Joining method. The bootstrap consensus tree inferred from 1,000 replicates was taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in <50% bootstrap replicates were collapsed. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). There were a total of 189 positions in the final dataset.

genomic duplication, whereas a few of them (3) appeared through tandem gene duplications.

Regarding regulation of MIP expression, some of duplicated genes (principally TIPs) present in poplar showed a significant coregulation whereas others (PIPs) mostly showed divergent gene regulation (52). A precise analysis of tissue-specific expression of the 35 aquaporins in *Arabidopsis* showed that, in this species, there is no strict overlap in expression between isoforms (1), pointing to quick genetic divergence. Evidence of balancing selection has even been provided in PIPs of five tropical trees species, providing a putative response to variable environmental conditions (11).

2. Intraspecific variations

Plant aquaporin evolution is still at work as indicated by intraspecific variations. In particular, marked differences in aquaporin gene expression between rice cultivars or *Arabidopsis* natural accessions exist under normal or water stress conditions (4, 152, 275). In rice cultivars, differences in aquaporin gene regulation seem to underlie distinct adaptive mechanisms to water deficit (152). The natural variation of plant aquaporin expression and function provides a promising avenue for exploring their integrated function. For instance, genome-wide association studies have pointed to the putative contribution of *Os*TIP3;1 to grain width in rice (109) or to the role of *At*TIP2;1 in adaptation of wild *Arabidopsis* to local habitats (77).

III. MOLECULAR FUNCTIONS OF PLANT AQUAPORINS

A. Functional Expression and Selectivity

1. Expression systems

Xenopus oocytes have been the first expression system used to investigate plant aquaporin function (128, 187). These huge cells are prone to intracellular microinjection of complementary RNAs and allow simple transport assays based on measurements of cell volume, intracellular pH, or content in radiolabeled molecules. The oocyte system allows addressing transport by aquaporins of water and many other substrates such as organic compounds (glycerol, urea), metalloids (B, Si), or even dissolved gases [CO_2, ammonia (NH_3)]. The oocyte system is still frequently used but displays some disadvantages. Although some intracellular plant aquaporins such as TIPs can be efficiently expressed at the cell surface, some other plant aquaporin subclasses (e.g., PIP1s, SIPs) seem to be recalcitrant to functional expression. In addition, an accurate quantification of proteins present at the plasma membrane remains difficult, restricting the quantitative resolution of transport measurements.

Yeast cells provide an alternative and have been successfully used for characterization of all five plant aquaporin subclasses. Growth assays can easily be performed, revealing how expression of an aquaporin can enhance the assimilation of some compounds (urea, NH_3) or the toxicity of others. Toxic compounds include hydrogen peroxide (H_2O_2) (29), germanium dioxide ($Ge0_2$) used as a toxic analog of Si (17), boric acid [$B(OH)_3$], arsenous acid [$As(OH)_3$], and antimonite [$Sb(OH)_3$] (31). However, growth assays provide a rather indirect evidence for transport. Genuine transport measurements on whole cells or spheroblasts are more difficult to develop, but were applied to CO_2 (221), ROS (29), NH_3 (22), or selenous acid [H_2SeO_3] (337). Even more challenging is functional reconstitution of recombinant aquaporins in proteoliposomes. This approach was used to dissect the transport activities of PIPs, NIPs, and SIPs for water (60, 214, 304), glycerol (60), and NH_3 (112). Reconstitution of aquaporins in gas-tight triblock-copolymer membranes is particularly efficient to detect their CO_2 transport activity (294).

2. Selectivity in five subclasses

All expression systems have revealed that plant aquaporins are multifunctional channels, with a wide range of selectivity profiles **(TABLE 2)**. Most of the PIPs and TIPs function as efficient water channels with additional substrates such as H_2O_2 (28, 105) and CO_2 for PIPs, or NH_3 (115, 164) and urea (86) for TIPs. Glycerol has also been investigated as a test solute, by reference to studies in animal and microbial aquaporins (25, 86). Yet, the physiological significance of this transport in plants, for osmotic tolerance in particular, remains uncertain.

In contrast to PIPs and TIPs, all NIPs investigated showed at most a reduced water transport activity. NIPs are mostly permeable to small organic solutes and mineral nutrients (169, 278). In particular, they mediate the transport of beneficial [B, Si, selenium (Se)] or toxic [arsenic (As), antimony (Sb)] metalloids (30, 337).

Although SIPs potentially display an original pore conformation (see sect. III*A3*) (120, 214), their functional characterization has only revealed a moderate water transport activity. In contrast, XIPs appear as multifunctional channels (26, 162) permeable to water, metalloids, and ROS.

Whereas mammalian aquaporin-6 is truly permeable to ions (113), no such transport function could be established for plant aquaporins. The electrical conductance induced upon reconstitution of a soybean NIP (nodulin-26) in lipid bilayers was likely due to protein aggregation artefacts (315) and could not be reproduced in other expression systems.

3. Overall molecular organization and pore conformations

Whereas aquaporin structures have been mostly investigated in animal or microbial homologs, a handful of studies have established crucial structural features of plant aquaporins. Cryoelectron microscopy of PIPs (139) and TIPs (57) confirmed their overall organization as tetramers. More importantly, X-ray crystallography of spinach *So*PIP2;1 revealed with an unprecedented resolution the structure of an aquaporin in its closed or open states (217, 286), shedding light onto the original gating properties of PIPs.

These data and information deduced by homology modeling have established that plant aquaporins are formed by an

Table 2. *Functional expression and substrate specificity of representative plant aquaporins*

Subclass	Isoform	Substrate	Expression System	Transport Assay	Reference Nos.
PIP	*At*PIP2;1	Water	Proteoliposome	Shrinkage	304
	*At*PIP2;1	H_2O_2	Yeast	Toxicity growth assay	66
	*At*PIP2;2	Water	*Xenopus* oocyte	Swelling	287
	*Nt*AQP1	Glycerol	*Xenopus* oocyte	Radiolabeling	25
	*Nt*AQP1	CO_2	*Xenopus* oocyte	Intracellular pH	293
	*Nt*AQP1	CO_2	Yeast	Intracellular pH	221
	*Nt*AQP1	CO_2	Planar lipid bilayer	Local pH	294
TIP	*At*TIP1;1	Water	*Xenopus* oocyte	Swelling	187
	*Nt*TIPa	Urea	*Xenopus* oocyte	Radiolabeling	86
	*Nt*TIPa	Glycerol	*Xenopus* oocyte	Radiolabeling	86
	*At*TIP1;2	H_2O_2	Yeast	Intracellular fluorescence	29
	*Ta*TIP2	NH_3	Yeast	Extracellular pH	115
	*Zm*TIP1;1	H_2O_2	Yeast	Toxicity growth assay	17
	*At*TIP2.3	NH_3	*Xenopus* oocyte	Radiolabeling	164
NIP	*At*NIP5;1	$B(OH)_3$	*Xenopus* oocyte	Intracellular dosage	278
	*Os*NIP2;1	$Si(OH)_4$	*Xenopus* oocyte	^{68}Ge-radiolabeling	169
	*At*NIP5;1	$As(OH)_3$	*Xenopus* oocyte	Intracellular dosage	193
	*Zm*NIP2;1	GeO_2	Yeast	Toxicity growth assay	117
	*Bj*NOD26	Water	Proteoliposome	Shrinkage	112
	*Bj*NOD26	NH_3	Proteoliposome	Internal pH	112
SIP	*Vv*SIP1	Water	Yeast	Shrinkage	214
	*Vv*SIP1	Water	Proteoliposome	Shrinkage	214
XIP	*Nt*XIP1;1	H_2O_2	Yeast	Toxicity growth assay	26
	*Pt*XIP2;1	Water	*Xenopus* oocyte	Swelling	162

This table is not intended to be exhaustive. It illustrates, using selected examples, the diversity of expression systems and transport assays used to determine the wide of range of substrates found for members of each plant aquaporin subclass.

assembly of four monomers, each with six transmembrane spanning domains (1-6) and five connecting loops (*A*–*E*) localized on the intra- (*B*, *D*) or extracytosolic (*A*, *C*, *E*) sides of the membrane **(FIGURES 3 AND 4)**. Each monomer is able to form an individual transmembrane pore, and remarkably, the *A* and *D* loops, which both carry a NPA motif, fold as half-membrane-spanning α-helices and dip into the membrane to position each of their NPA motif at the center of the pore. These two motifs contribute to a central pore constriction and, in conjunction with the dipole moment of the two half-membrane-spanning α-helices, prevent proton (H^+) permeation. Furthermore, four conserved residues form, close to the extracytosolic mouth of the pore, a typical aromatic/arginine (Ar/R) constriction functioning as the main selectivity filter.

Whereas functional selectivity assays have been restricted to a few representative plant isoforms, homology modeling was used to explore in a broader sense the transport selectivity of plant aquaporins, with a main emphasis on the Ar/R selectivity filter (16, 309). Each aquaporin subfamily was found to show specific pore profile(s) and could be further refined in subclasses with distinct predicted selectivities. For instance, three putative selectivity subclasses can be identified in NIPs (194, 242, 308). A critical residue of transmembrane spanning domain 2 differs between subclasses I and II. Reverse substitution of this residue in a class I (nodulin-26) and class II (*At*NIP6;1) homolog showed how, in class I, a Ala residue enhances permeation of large solutes at the expense of water transport, whereas in class II, a Trp residue has opposite effects (308). Class III NIPs have small size residues in the Ar/R selectivity filter allowing the passage of large diameter solutes such as silicic acid (194, 242). TIPs also display a great diversity of substrates and Ar/R selectivity filter configurations. A Val-to-Ile substitution at a critical position in *loop E* was shown to determine the range of transported solutes (14). In barley PIPs, a Ile residue at the end of *loop E* is also crucial for transport and would confer CO_2 permeability through interaction with a Leu residue in *loop C* (203). There are also concerns that, in addition to the four individual monomer pores, a fifth pore may be formed at the center of the aquaporin tetramer. The differential inhibition by mercury (see sect. III*B2*) of water and NH_3 transport in a wheat TIP was interpreted to mean that the preferential permeation pathways for these two substrates would be the four monomer pores and the central pore, respectively (22). In X-ray structures, the central region of spinach *So*PIP2;1 tetramers can

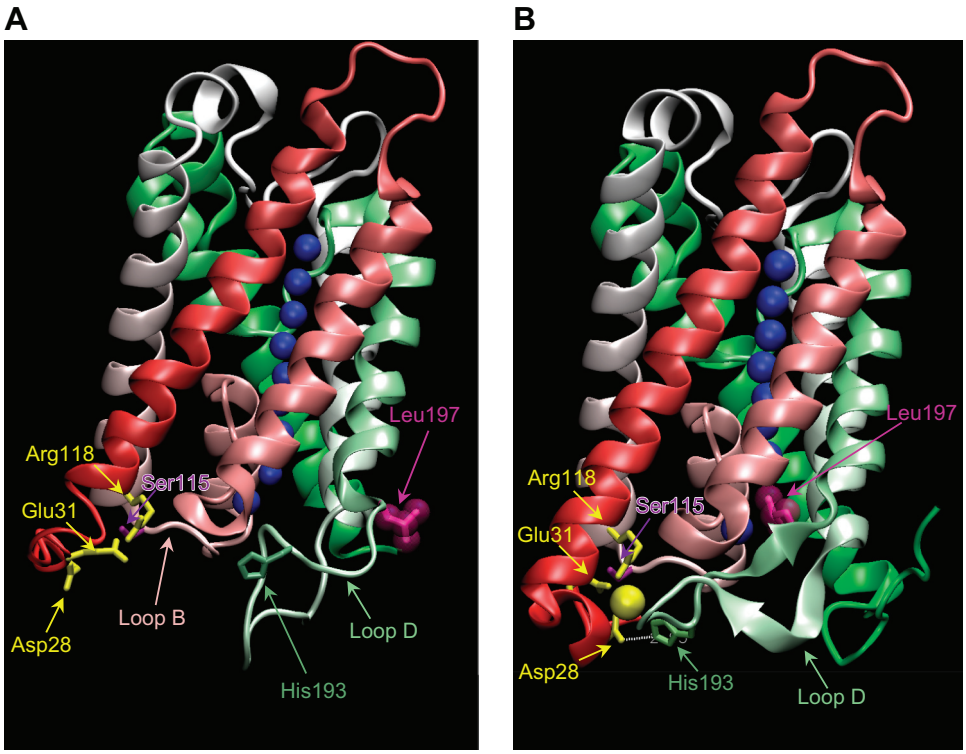

FIGURE 3. Molecular mechanisms of PIP gating. The structure of spinach *So*PIP2;1 was solved in an open (*A*: PDB code 2B5F) and a closed (*B*: PDB code 1Z98) conformation (286). In the open conformation (*A*), the His193 (green) is not protonated and *loop D* is distal from the other cytoplasmic loop (*loop B*). In the closed conformation (*B*), the protonation of His193 allows interaction with an acidic residue (Asp28, in yellow) of the NH_2 terminus. This in turns drives a conformational change of *loop D* and occlusion of the pore by displacement, into the cytosolic pore mouth, of the hydrophobic side chain of Leu197 (in pink). Binding of divalent cations (Cd^{2+} in the atomic structure, in yellow) also involves Asp28 and an adjacent acidic residue (Glu31, in yellow). *Loop D* is thereby stabilized in the closed pore conformation through a network of H-bond and hydrostatic interactions, involving Arg118 (yellow). In this model, phosphorylation of *loop B*, at Ser115 (in purple), would disrupt this network of interactions and unlock *loop D* to allow the open conformation.

accommodate a detergent molecule instead of a lipid, indicating that CO_2 permeation through this pathway may be possible, at least from a steric point of view (78).

B. Gating

1. Molecular mechanisms

In vitro studies on purified plant membranes or proteoliposomes have demonstrated the role of cations, H^+, and phosphorylation in controlling plant aquaporin activity (85, 90, 304). In the case of PIPs, a unifying model of pore opening and closing (gating) was proposed, based on *So*PIP2;1 molecular structure (286) **(FIGURE 3)**. This model indicates how the reversible motion of a hydrophobic residue (Leu197 in *So*PIP2;1) into and out of the cytoplasmic opening of the pore controls the aquaporin water permeability. This hydrophobic gating residue is carried by the second cytosolic loop (*D*), the conformational changes of which are mediated by ionic and H-bond interactions with the NH_2-terminal tail (Asp28 and Glu31) and first cytosolic loop (*B*; Ser 115 and Arg118).

The pH-dependent gating of PIPs primary relies on protonation of a perfectly conserved His residue of *loop D* (His193 in *So*PIP2;1) (287) **(FIGURE 3)**. At acidic pH, charged His193 interacts with Asp28, Glu31, and *loop B* (Ser115) to stabilize *loop D* in a closed pore conformation (79, 286). There also are reports of H^+-dependent inhibition of TIPs (146, 272), which however involves a His residue located in the second extracytoplasmic (intravacuolar) *loop C*. The structural basis of this gating mechanism is as yet unknown.

Similar to H^+-dependent gating, the inhibition of PIP water transport by divalent cations can be explained by a direct binding of the cation, which in turn mediates H-bonding between the NH_2 terminus (Gly30 and Asp31) and *loop D* through *loop B*, thereby stabilizing the closed pore conformation **(FIGURE 3)**. Although this mechanism was revealed by Cd^{2+} binding in the crystal structure of *So*PIP2;1, the binding site may rather be occupied by Ca^{2+} in vivo. In agreement with the biphasic dose-dependent effects of Ca^{2+} on the water permeability of certain plant plasma membranes (6), a second binding site was recently identified between *loop D* and the COOH terminus of *So*PIP2;1 (78). This second binding site may reflect a stabilizing role of the COOH terminus on *loop D* folding.

Structure-function analyses of TIPs, PIPs, and NIPs in *Xenopus* oocytes (90, 122, 186) have pointed to the role of several cytosol-exposed phosphorylation sites in controlling water transport. Structural models of PIPs now support this view indicating how phosphorylation of a conserved Ser residue in *loop B* (Ser115 in *So*PIP2;1) would destabilize the *loop D*-*loop B* anchor, thereby favoring the open-pore conformation. Interestingly, phosphorylation of Ser274 on *So*PIP2;1 COOH-terminal tail would act similarly but through a transactivation process, whereby interaction between the COOH-terminal tail and the *loop D* of an adja-

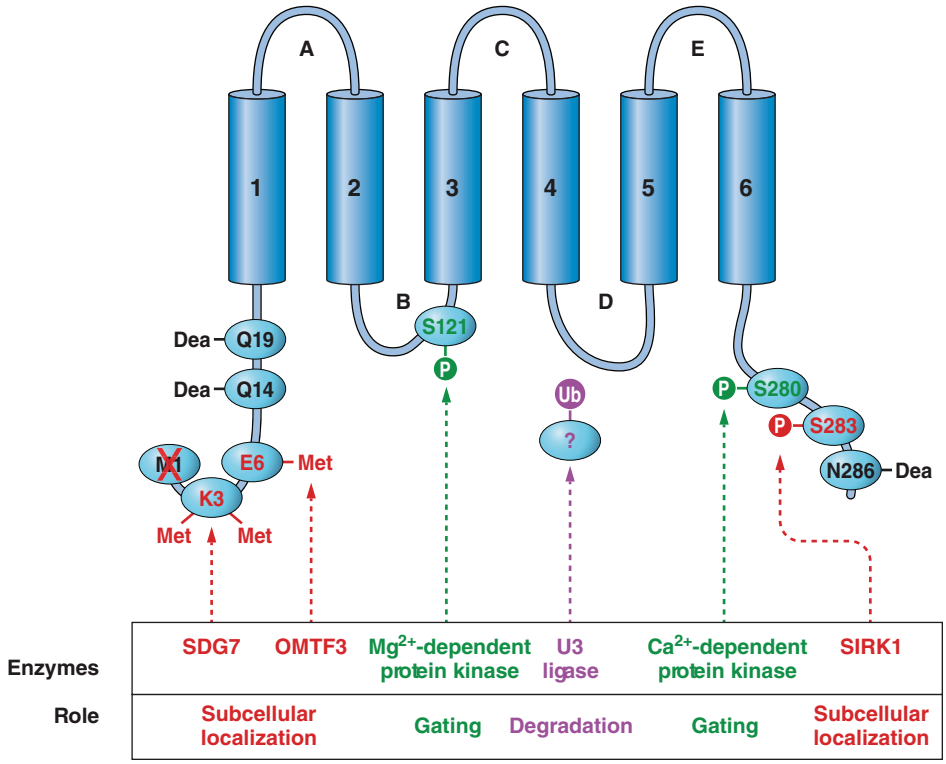

FIGURE 4. Multiple covalent posttranslational mechanisms acting on plant plasma membrane aquaporins. The figure shows a schematic representation of *Arabidopsis At*PIP2;1 with its six transmembrane domains (1–6), five connecting loops (*A*–*E*), and NH_2- and COOH-terminal tails bathing in the cytosol. The indicated modifications were mostly determined by mass spectrometry. They include cleavage of the initiating methionine (cross), dimethylation (Met Met) of a Lys residue (K3), monomethylation (Met) of a Glu residue (E6) (254), deamidation (Dea) of Gln and Asn residues (Q14, Q19, N286) (62), and ubiquitination (Ub), the ubiquitinated residue(s) being unknown (143). Phosphorylation of some Ser residues (S280 and S283) was established experimentally (62, 234). In contrast, the phosphorylation of *loop B* at S121 was inferred from studies on spinach *So*PIP2;1 (122, 286). Some enzymes involved in covalent modification of PIPs have been identified. They include methyl-transferases SDG7 (At2g44150) and OMTF3 (At3g61990) (247), a U3 ligase (143), and several protein kinases including Mg^{2+}- and Ca^{2+}-dependent protein kinases (268) and SIRK1 (At5g10020) (318). The figure is color-coded to show the enzymatic targets and the main molecular and cellular function associated with the posttranslational modification. Thus posttranslational modifications interfere with the subcellular localization (red), gating (green), and degradation of the aquaporin (purple). The functional role of PIP deamidation is unknown.

cent monomer would be destabilized after Ser274 phosphorylation.

Due to these great advances, PIP gating is emerging as a paradigm in the aquaporin field. Although distinct protein domains may be involved, similar mechanisms seem to be at work in other plant, animal, or microbial aquaporins. For instance, the gating of *Arabidopsis* NIP7;1 is largely controlled by a unique Tyr residue (Tyr81), located in transmembrane spanning domain 2 and interacting with neighboring Arg220 of the Ar/R selectivity filter to stabilize closed channel conformation (148). In mammalian aquaporin-0 and bacterial aquaporin-z, the corresponding Arg189 residue was shown to adopt distinct conformational states leading to channel gating (102).

Despite recent progress, a refined structural model of plant aquaporin gating is still needed. For instance, *So*PIP2;1 mutants carrying phosphomimetic mutations at Ser115 and Ser274 did not show enhanced water transport activity after reconstitution in proteoliposomes and, accordingly, their X-ray structures displayed a closed conformation (217). Thus these mutations may not be sufficient to stabilize the open pore conformation in vitro, whereas they have an activating role in vivo (232). Also, a control of aquaporin activity by osmotic or hydrostatic pressures has been reported in some plant cells or purified membranes (206, 212, 310, 327). It is not yet clear whether these effects are mediated through solute- or pressure-induced changes in aquaporin conformation or through membrane-associated cell signaling events (327). Finally, the role for membrane lipids in aquaporin gating is emerging in animals but has not yet been explored in plants (284, 285). In particular, its significance with respect to protein partitioning in membrane microdomains and stress-induced changes in lipid composition will have to be carefully examined (see sect. IV*B2*).

2. Mercury and other aquaporin blockers

Mercury is the most common aquaporin blocker used in plants and animals. It is thought to inhibit the water permeability of purified membranes, cells, or tissues through binding to the thiol groups of crucial aquaporin Cys residues. Mercury may also target His residues as recently revealed in rice *Os*NIP3;3 (130). The use of mercury must be considered with care, because of its strong cellular toxicity (see sect. VI*B1*) (175, 336). The generality of its inhibiting effects has also been questioned. First, some plant aquaporins are insensitive to mercury because they do not harbor the critical Cys residues identified in other isoforms (58). In addition, mercury did not block but rather activated *So*PIP2;1 in proteoliposomes (78). This activation, which was Cys independent, may reflect a mechanical gating through effects on the lipid environment. In plants as in other organisms (328), there is therefore a strong need for new aquaporin blockers with reduced cellular toxicity. Weak acids or respiration inhibitors (azide, cyanide) decrease cytosolic pH, thereby inducing a H^+-dependent PIP closure. Although they can potentially confirm mercury inhibition (275), these treatments are also toxic. Silver and gold compounds offer an interesting alternative (213), but proper application to living cells and tissues seems to be challenging.

C. Posttranslational Modifications

Although their high hydrophobicity hinders classical biochemical analyses, plant aquaporins are, together with H^+-ATPases, the most abundant intrinsic proteins of plant plasma and vacuolar membranes. Aquaporins have therefore been prone to extensive proteomic characterizations. In-depth mass spectrometry analyses have revealed that plant aquaporins carry numerous co- and posttranslational modifications, including phosphorylation, methylation, deamidation, NH_2-terminal acetylation, and ubiquitination (44, 62, 132, 216, 254, 255, 297) **(FIGURE 4)**. The latter modification and *N*-glycosylation were also revealed using immunodetection techniques (143, 303). Thus the molecular diversity of plant aquaporins goes far beyond a high number of isoforms. The multiple posttranslational modifications of aquaporins point to a variety of regulatory mechanisms targeting aquaporin expression and function. Quantitative proteomics is now revealing how these modifications can vary in abundance, depending on plant tissues or physiological contexts (62, 232). Whereas a general role of phosphorylation in aquaporin gating and trafficking (see sect. IV*B2*) is now well established, the significance of other aquaporin posttranslational modifications is still elusive. Ubiquitination of *Arabidopsis At*PIP2;1 and *N*-acetylation of ice plant (*Mesembryenthemum crystallinum*) *Mc*TIP1;2 seem to act on endoplasmic reticulum (ER) degradation and redistribution to endosomal compartments, respectively (143, 303). *At*PIP2;1 was the first plant membrane protein and first aquaporin shown to be methylated. Two adjacent residues, Lys3 and Asp6, serve as methylation sites on its cytosolic NH_2-terminal tail (254). These residues overlap with a di-acidic motif involved in ER export of the protein (see sect. IV*B1*), suggesting a role for *At*PIP2;1 methylation in protein subcellular trafficking. Yet, any mutation at these sites had dominating effect on aquaporin trafficking, thereby preventing proper structure-function analyses (254). Although deamidation of PIPs shows multiple stimulus-induced changes in *Arabidopsis* roots, the mechanisms leading to this modification and its role are as yet unknown (62).

D. Heteromerization

1. Oligomeric structures

Due to their high structural similarity, members of a same plant aquaporin subclass may physically assemble as heterotetramers, thereby enabling multiple molecular and functional combinations. Although their existence remains to be formally demonstrated, heteromers formed of either PIPs or TIPs have been suggested from functional coexpression in *Xenopus* oocytes or yeast, or protein-protein interaction assays such as bimolecular fluorescence complementation (72, 209, 282, 331). Furthermore, molecular modeling of PIPs pointed to a critical role of first extracytosolic loop (*loop A*) in oligomer interactions. This loop contributes to a disulfide bond that stabilizes PIP dimers, which in turn may associate as tetramers (27). Accordingly, site-directed mutations within this loop were able to modify the interaction properties of *Beta vulgaris* (common beet) PIP1s and PIP2s (124).

2. Functional effects

In plants, heteromerization may be critical for combining PIP1s and PIP2s with distinct functional properties (221). Coexpression studies in *Xenopus* oocytes and maize protoplasts have revealed a clear incidence on trafficking. Whereas singly expressed PIP1s were retained intracellularly, interactions with PIP2s allowed them to reach the plasma membrane (72, 331). Converse effects, whereby PIP1s may alter the overall tetramer pH sensitivity (20) and transactivate their interacting PIP2 partner (324), have also been revealed using oocyte expression. Functional expression in yeast showed that heteromeric assembly of PIP isoforms with a preferential role in water or gas (CO_2) transport may also provide means for adjusting the overall PIP tetramer selectivity (221). Whereas these studies point to very complex combinatorial regulations, their significance in the whole plant remains difficult to assess. For instance, overexpression in transgenic *Arabidopsis* of a PIP2 mutant form deficient in trafficking to the plasma membrane induced an intracellular retention of native PIPs (and a decrease in root water transport) (270). Although this was not strictly demonstrated, these effects

may be due to molecular interactions between PIPs in the plant.

E. Perspectives

The present section shows that we have got a fair understanding of plant aquaporin molecular functions. This knowledge is central for addressing aquaporin function and regulations at the plant cell and whole organism level. A powerful, but challenging integrative approach is expression in transgenic plants of site-directed mutant forms of aquaporins. The sections below exemplify how aquaporin phosphorylation and trafficking are now analyzed along these lines. By comparison, in planta analysis of aquaporin properties such as transport specificity or H^+- or Ca^{2+}-dependent gating is lagging behind. Addressing the functions and regulations emerging from aquaporin heteromeric assembly also represents an important but highly challenging objective.

IV. CELL BIOLOGY OF PLANT AQUAPORINS

A. Subcellular Localization Patterns

In relation to their unique metabolic capacities (e.g., photosynthesis, N_2-fixation through endosymbiosis), plant cells are characterized by a great diversity of intracellular compartments and subcellular membranes. Accordingly, plant cells display a wide palette of aquaporin subcellular localization patterns.

1. Plasma membrane

As in animals, the plant plasma membrane represents a crucial barrier and exchange platform. Accordingly, this membrane harbors three subclasses of aquaporins, namely, the PIPs **(FIGURE 5*A*)**, NIPs, and XIPs (26). Fusions with fluorescent protein reporters have indicated that most of these aquaporins are expressed on the entire cell surface. Yet, some isoforms can be confined to membrane subdomains. For instance, two NIP homologs, *Arabidopsis At*NIP5;1 (277) and rice Lsi1 (*Os*NIP2;1) (277), are specifically expressed on the endofacial side of root endodermal cells, to mediate a passive cellular efflux of B and Si, respectively. Transporters specialized in the cellular influx of the same solutes (BOR1 and Lsi2, respectively) show an opposite polar localization. Thus these complementary arrangements create an efficient path for transcellular transport of B and Si, and centripetal transfer into vascular tissues (xylem vessels).

A few PIP isoforms also show a somewhat polar expression, with preferential accumulation on the exofacial and endofacial sides of root cells in rice and maize, respectively (95, 250). The PIP isoform counterparts that must be expressed

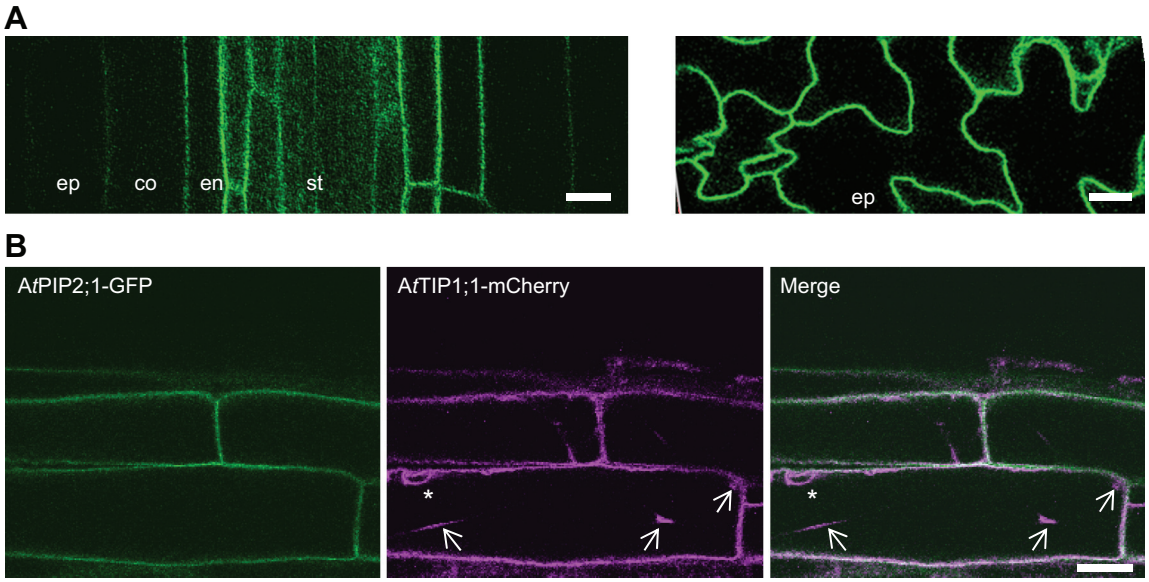

FIGURE 5. Localization of aquaporins in the plant plasma and vacuolar membranes. Transgenic *Arabidopsis* plants expressing *At*PIP2;1 fused to the green fluorescent protein (PIP2;1-GFP) (*A*), or coexpressing PIP2;1-GFP and *At*TIP1;1 fused to the fluorescent protein mCherry (TIP1;1-mCherry) (*B*), were observed by laser scanning confocal microscopy. *A*: plasma membrane localization of PIP2;1-GFP in a cross section of a root (*left panel*) with indicated cell layers (ep, epidermis; co, cortex; en, endodermis; st, stele) and in epidermal cells of cotyledons (*right panel*). *B*: localization in root epidermal cells of PIP2;1-GFP and TIP1;1-mCherry, in the plasma membrane and vacuolar membrane (tonoplast), respectively. The arrows indicate cytoplasmic strands formed within grooves at the surface of the invaginated vacuole, and the asterisk indicates a nucleus skirted by the tonoplast. Bar size = 20 μm.

on the opposite cell side to mediate transcellular water transport remain to be identified.

Recent imaging of PIPs using fluorescence recovery after photobleaching (FRAP) or evanescent wave microscopy techniques also revealed their extremely low lateral mobility at the cell surface (107, 150, 167, 181). This feature may partly result from hindering interactions with the cell wall (181). Single particle tracking further showed a confinement of PIPs in membrane microdomains associated with specific sterol compositions (150). Accordingly, PIPs were found to be enriched in detergent-resistant membrane fractions tentatively associated with membrane lipid rafts (131, 199, 202). This membrane partitioning likely underlies the dynamics of PIP cycling between the cell surface and endosomal compartment (see sect. IV*B2*).

2. Vacuoles

Vacuoles represent the most voluminous intracellular organelles of plant cells. Vacuoles are not uniform, with distinct subtypes present in a same cell and showing specialized lytic or protein storage functions. Although vacuoles harbor a single subclass of aquaporins, the so-called TIPs (319) **(FIGURE 5*B*)**, each vacuolar subtype is characterized by a specific set of isoforms (118). It has been argued that these patterns must be determined by targeting pathways specific of each vacuolar subtype. However, they are more likely generated through one or several common trafficking machinery(ies) and simply result from distinct developmental expression of TIP isoforms during the course of cell differentiation (111, 118). The vacuolar membrane (tonoplast) is more fluid than the plasma membrane and, by comparison to PIPs, TIPs show a much higher (>10-fold) lateral membrane mobility (107, 167). Yet, their expression is not uniform and preferential expression of TIP fused to GFP has been observed in intravacuolar invaginations (bulbs) and in apposing tonoplast regions of two adjacent vacuoles (19, 248). This pattern may allow privileged exchanges between neighboring vacuoles. It may also partly result from antiparallel dimerization of GFP expressed in two apposing membranes (262).

3. Other intracellular compartments

Whereas most plant aquaporins can be observed in the ER, during biogenesis and transfer to their destination membrane, some isoforms such as SIPs (114, 214) and some NIPs (197) seem to be resident of this compartment. Their mode of targeting and cellular function in this compartment are as yet unknown.

The chloroplast is a plant specific organelle, delineated by an envelope formed by a double membrane. The chloroplast also contains numerous stacks of thylakoid membranes, which harbor the light-collecting antenna and electron transfer chains crucial for photosynthesis. Thorough proteomic analyses in *Arabidopsis* (70, 71) have suggested the presence of PIPs and TIPs, in the inner envelope and thylakoids, respectively. Localization of a PIP1 (*Nt*AQP1) in the chloroplast envelope of tobacco leaves has also been revealed by immunocytochemistry (295). Whereas these localizations point to highly relevant plant specific function (see sect. V*D*), they now call for complementary data and confirmation in other plant materials.

Mammalian aquaporin-8, which shows a somewhat atypical sequence among other animal aquaporins and significant homology to TIPs, has been localized to mitochondria (38). A putative mitochondrial targeting signal was also detected in pollen-specific *Arabidopsis At*TIP5;1 (272). Ectopic expression in the vegetative cell of transgenic pollen of *At*TIP5;1 fused to a fluorescent reporter led to the proposal that *At*TIP5;1 localizes to mitochondria. Yet, more adequate reporter fusion and colocalization studies revealed that this conclusion is erroneous and that *At*TIP5;1 actually localizes to the tiny vacuoles of pollen sperm cells (320). Thus, as far as we know, plant mitochondria seem to be deprived of aquaporins.

4. Dual localization patterns

It is assumed that the high degree of cell compartmentalization in plants has represented a major driving force during the molecular and functional diversification of aquaporins in these organisms. In summary, plant aquaporins have been localized throughout the cell secretory system including ER, Golgi, endosomes, autophagosomes, and vacuoles (98, 168). Specific isoforms are also expressed in chloroplasts and in the symbiosome membrane that surrounds, in legumes, the intracellular N_2-fixing bacteroids of *Rhizobiaceae*-infected roots (see sect. VIII*A*). In contrast, and for some unknown reasons, aquaporins seem to be excluded from some compartments such as plant mitochondria and peroxisomes.

Interestingly, examples of dual subcellular localizations of aquaporins have recently emerged in plants. For instance, the PIP1 homolog *Nt*AQP1 is expressed in the plasma membrane and chloroplast of tobacco leaves (295), while TIP3s have been localized in both the plasma membrane and tonoplast of maturating *Arabidopsis* seeds (82). A vacuolar TIP1 homolog can also be transiently expressed on the symbiosome membrane of *Medicago truncatula* (barrel clover) root nodules (84). As discussed below, these patterns must be determined by exquisite trafficking signals, which most often remain to be identified.

B. Aquaporin Trafficking

1. Multiple pathways in the secretory system

The modes of plant aquaporin targeting to their destination membranes have been addressed using various ap-

proaches. First, site-directed mutations of maize and *Arabidopsis* PIP2s revealed that inner sequence motifs direct their trafficking to the plasma membrane. In particular, diacidic motifs located on their NH_2-terminal tail likely interact with the COPII sorting machinery and critically determine their export from the ER (270, 332) **(FIGURE 6*A*)**. Anterograde transport of PIP2s in the secretory pathway also involves a LxxxA motif (48) in the third membrane spanning helix and a phosphorylation site located on the COOH-terminal tail (234). Most PIP1s do not have these motifs but are likely dragged to the plasma membrane through heteromer assembly with PIP2s.

The motifs that determine TIP routing to the vacuole remain largely unknown. A pharmacological screening in *Arabidopsis* (241) revealed that TIP1 targeting is sensitive to Brefeldin A and likely involves a Golgi-dependent route **(FIGURE 6*A*)**. In contrast, trafficking of TIP2s and TIP3s does not pass through the same route and is sensitive to a newly identified compound named C834 (241). In the future, a chemical genetic approach may be used to identify the molecular components of these different pathways.

Q-SNAREs of the syntaxin family are well identified molecular players of vesicular trafficking in eukaryotes. In plants, they mediate the trafficking of PIPs and TIPs at specific stages along the secretory pathway (24, 291). In particular, post-Golgi trafficking of *Arabidopsis At*PIP2;7 was shown to depend on a direct physical interaction with two specific syntaxins, SYP61 and SYP121 (96).

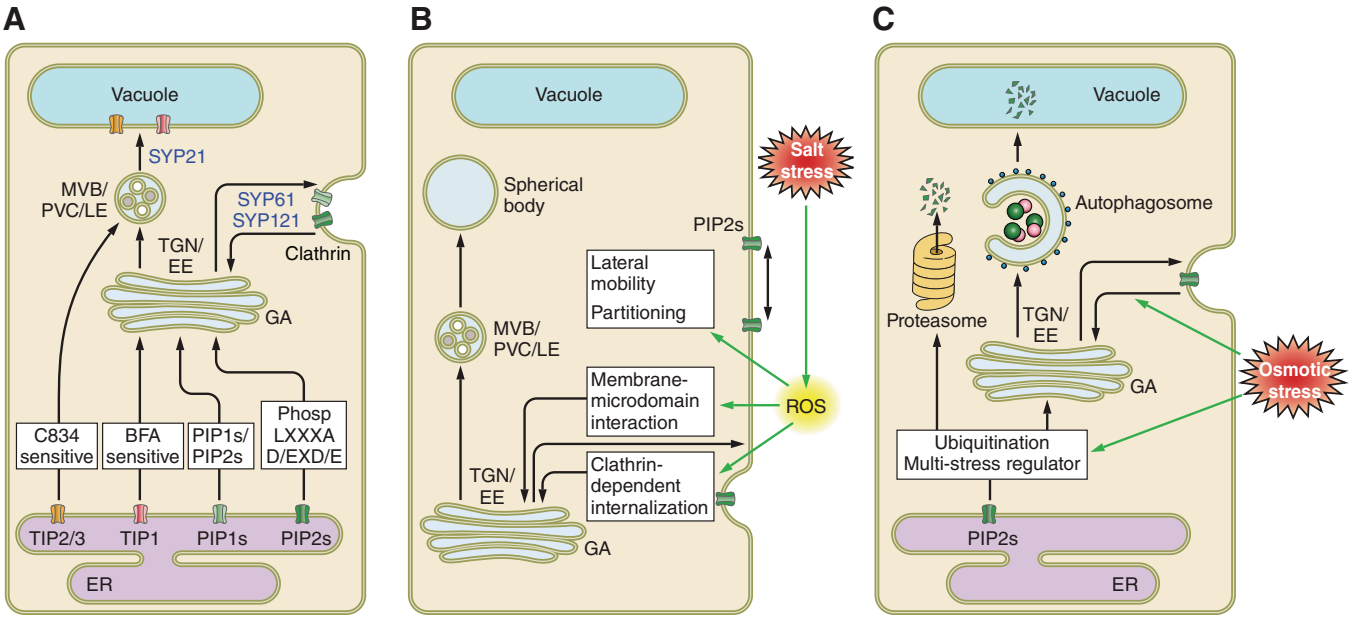

FIGURE 6. Subcellular dynamics of PIPs and TIPs. *A*: targeting of aquaporins to the plasma membrane and tonoplast. Both PIPs and TIPs follow the secretory system to be targeted to their final destinations. All isoforms are synthesized in the endoplasmic reticulum (ER). Specific signals or mechanisms are requested for the exit of PIPs out of this compartment. These include diacidic (D/EXD/E) or LXXXA motifs, or phosphorylation (Phosp) for PIP2s, or physical interactions with PIP2s (PIP1s/PIP2s) for PIP1s. The existence of similar mechanisms or motifs is not documented for TIPs. The targeting pathways followed by TIP1s and TIP2/3s are sensitive to Brefeldin A (BFA) or C834, respectively. The Golgi apparatus (GA) and *trans*-Golgi network (TGN) continuum provide intermediate compartments for trafficking of PIPs, prior to their exocytosis towards the PM. In contrast, TIP1s traffic through the GA/TGN and multivesicular body (MVB) compartments before reaching the tonoplast. Note that, in plants, MVB is also termed prevacuolar compartment (PVC) or late endosomal compartment (LE). The precise targeting path followed by TIP2/3s is not yet known. Once at the plasma membrane, PIPs undergo constitutive cycling, through endocytosis from the cell surface towards early endosomes (EE) using a clathrin-dependent pathway and subsequent exocytosis back to the PM. The TGN and the EE are considered to form a single compartment. The syntaxins (SYP) that are known to play a role in some steps of PIP or TIP trafficking are indicated. *B*: subcellular dynamics of PIPs, with mobilization from the plasma membrane to intracellular compartments, in salt-stressed root cells. The effects of salt are mediated through ROS which enhance the lateral mobility of PIPs at the cell surface and their partitioning in membrane microdomains. PIP cycling, by endocytosis through clathrin-dependent and clathrin-independent pathways and exocytosis, is also enhanced by ROS. Some of the internalized PIPs are directed toward the MVB and spherical bodies. Abbreviations are as in *A*. *C*: osmotic stress-induced degradation of PIPs. In addition to enhanced cycling, PIPs undergo ubiquitination or interaction with multi stress regulator proteins. As a consequence, PIPs are directed toward the proteasome and vacuolar degradation pathways. The latter pathway involves autophagosomes (see main text for details). Abbreviations are as in *A*.

2. Constitutive cycling of PIPs and response to environmental stresses

Although they have a reduced lateral mobility, PIPs are far from static at the plasma membrane. Pharmacological and genetic studies have revealed that they continuously cycle between the plasma membrane and early endosomes (*trans*-Golgi network) (167) **(FIGURE 6*A*)**. Under resting conditions, this process is mediated through clathrin-mediated endocytosis (61) and is blocked in part by the auxin analog NAA using an as yet unknown mechanism. In root epidermal cells under osmotic or salt stress, the cycling rate of PIPs is enhanced due in part to the activation of a complementary endocytic pathway, independent of clathrin, and associated with membrane micro-domains (150, 167) **(FIGURE 6*B*)**. These processes are probably linked to the enhanced mobility of PIP molecules at the cell surface observed under osmotic and salt stress conditions (107, 150).

In connection to these processes, the same stresses induce a partial internalization of PIPs (34, 35) **(FIGURE 6*B*)**. This leads to a reduced abundance of PIPs at the root cell surface, which may contribute to the observed decrease of root water permeability. In addition, osmotic stresses induce functional and morphological rearrangements of the vacuolar apparatus. For instance, they trigger a relocalization of TIPs, from the vacuole to endosomal compartments or intravacuolar bulbs, in ice plant and *Arabidopsis*, respectively (34, 303).

These profound effects of stresses on aquaporin subcellular localization and dynamics are central in plant responses to their environment. Beyond their impact on plant water relations, they represent one of the earliest cellular responses to stresses and can possibly be extended to other membrane proteins. In addition, stimulus-induced PIP trafficking can be antagonized by ROS scavengers, in agreement with the central role played by ROS in stress and hormonal signaling in plants (35) **(FIGURE 6*B*)**. Deciphering the molecular and cellular mechanisms that govern PIP and TIP dynamics may therefore be central to understand the perception and transduction of stress signals in plants. In *Arabidopsis* roots, both salt and oxidative stresses induce quantitative changes in the double COOH-terminal phosphorylation of *At*PIP2;1 (62, 234). Phosphorylation of a specific residue (Ser283) interferes with trafficking of internalized PIPs to fuzzy structures or spherical bodies. These structures have tentatively been identified as sorting endosomes and prevacuolar compartment (multivesicular bodies), respectively (234) **(FIGURE 6*B*)**. Whether this sorting permits a reversible sequestration of PIPs, prior to remobilization to the plasma membrane, or whether it directs them towards vacuolar degradation is unknown. Nevertheless, these mechanisms are reminiscent of the vasopressin- and phosphorylation-dependent trafficking of aquaporin-2 in renal epithelial cells (198).

3. Degradation pathways

Although aquaporin abundance can markedly vary in response to abiotic stresses or oscillate along circadian rhythms, information on the modes of plant aquaporin degradation has remained scarce. A RING membrane-anchor E3 ubiquitin ligase from pepper, and its *Arabidopsis* homologs, were shown to localize in the ER and ubiquitinate *At*PIP2;1 leading to its retention in this compartment (143). This process was enhanced under osmotic stress, leading to aquaporin degradation through the proteasome pathway **(FIGURE 6*C*)**. A more recent study established a very novel link between aquaporin degradation and intracellular trafficking in *Arabidopsis* (98). *At*PIP2;7 was shown to interact in the ER and Golgi, with a membrane protein, named TSPO for tryptophan-rich sensory protein/translocator and serving as multistress regulator **(FIGURE 6*C*)**. The complex was then directed towards vacuolar degradation, using the autophagosome pathway, this process being stimulated by drought-induced hormone, abscisic acid (ABA). Thus plant cells under water stress can use various pathways, for aquaporin degradation and long-term downregulation of plasma membrane water permeability.

C. Perspectives

The present section showed that, in addition to multiple substrates and selectivity profiles, plant aquaporins show very diverse subcellular localization patterns, and cell responses to hormonal and environmental stimuli. These properties can provide a first hint at their high genetic diversity. We also note that aquaporins have initially been used as mere reference markers for the plasma membrane or the vacuole. An accumulating body of data now reveals their remarkable trafficking properties. Aquaporins are therefore emerging as one of major membrane protein models in plant cell biology (168).

While evanescent wave fluorescent microscopy and associated single particle tracking techniques provide novel insights into plant aquaporin dynamics, much remains to be learned about the molecular and cellular mechanisms that direct their trafficking, both under resting and stress conditions. The recent characterization of individual aquaporin partners has provided insights into specific cell sorting or degradation steps (96, 98, 143) **(TABLE 3)**. Genomic studies based on classical (53) or split-ubiquitin (123, 140) yeast two-hybrid systems are now revealing a myriad of additional aquaporin partners **(FIGURE 7)**. These genome-wide aquaporin interactomes and their functional genomic characterization will undoubtedly reveal novel mechanisms related to aquaporin trafficking. It should also reveal hormonal and environmental signaling chains acting on aquaporins or large protein complexes coupling water transport to other membrane processes **(TABLE 3)**.

Table 3. *Molecular and functional characterization of aquaporin-interacting proteins*

Aquaporin	Interacting Protein Name	Interacting Protein Function	Method	Reference Nos.
*Zm*PIP1;1, *Zm*PIP1;2, *Zm*PIP1;6	*Zm*PIP2;1 and/or *Zm*PIP2;5	Aquaporin	FRET/FLIM	331
*Rh*PIP1;1	*Rh*PIP2;1	Aquaporin	Split-Ub, BiFC	47
*Gh*PIP2;6	*Gh*PIP1	Aquaporin	Y2H, BiFC	147
*At*PIP2;1	Rma1H1	E3 Ub ligase	Y2H Pull-down	143
*Zm*PIP2;5	ZmSYP121	Syntaxin	Affinity purification, BiFC	24
*At*PIP2;7	*At*SYP61 (At1g28490)	Syntaxin	Pull-down, BiFC, Split-Ub	96
*At*PIP2;7	*At*SYP121 (At3g11820)	Syntaxin	Pull-down, BiFC, Split-Ub	96
*At*PIP2;7	TSPO (At2g47770)	Tryptophan-rich sensory protein/translocator	Pull-down, BiFC	98
*At*PIP1;3, 2;2, 2;6, 2;1, 1;1, 1;4, 1;5, 2;7, 2;4	SIRK1 (At5g10020)	LRR-receptor-like kinase family	Pull-down	318
*Gm*NOD26	Soybean GS1 β1, β2, γ1, γ2	Glutamine synthase	Split-Ub, BiFC	184
*At*TIP1;1, 1;2, 1;3, 2;1, 2;2, 2;3	CMV 1a	Cucumber mosaic virus 1a	Y2H, SRS, co-IP	133

The table lists all plant aquaporin-interacting proteins that have been individually validated. The molecular function of each partner is indicated. The methods used to demonstrate a protein-protein interaction are also shown. FRET, fluorescence resonance energy transfer; FLIM, fluorescence-lifetime imaging microscopy; BiFC, bimolecular fluorescence complementation; Split-Ub, split-ubiquitin; Y2H, yeast two-hybrid; SRS, Sos recruitment; co-IP, co-immunoprecipitation.

V. CELL-AUTONOMOUS AND INTRACELLULAR FUNCTIONS

A. Osmoregulation

Addressing the cell-autonomous functions of aquaporins provides a useful step towards integration of their role at the organ and whole plant levels. One major cellular function of aquaporins is osmoregulation.

In agreement with the targeting of specific aquaporin isoforms to distinct subcellular compartments, water transport assays in purified membrane vesicles, isolated vacuoles, and protoplasts have revealed that membranes from a same cell can exhibit strikingly different permeability profiles. In tobacco cells for instance, purified tonoplast vesicles were 100-fold more permeable to water than their plasma membrane counterparts (188). An extremely high water permeability in the tonoplast seems to be common to most of plant cells (188, 204, 212). According to mathematical models, this property would provide the cell with the capacity of reducing peak fluctuations of cytosolic volume in case of a sudden change in extracellular water potential (208, 290). Thus plant cells use their large vacuoles as buffering compartments during cell osmoregulation.

The delayed volume response of isolated protoplasts subjected to an osmotic challenge indicated that dynamic changes in water permeability may also contribute to plant cell responses to osmotic stresses (206). A direct dependence of water permeability on transmembrane osmotic gradients has been observed in tonoplast vesicles purified from wheat roots, but not in tobacco suspension cells (188, 212). Although the mechanisms leading to rapid and possibly direct changes in aquaporin activity have not yet been addressed in these contexts, we assume that they pertain to the general gating and trafficking properties described in the two previous sections (see sects. III and IV).

The dynamic responses of aquaporins to osmotic stress have led to the idea that aquaporins, localized on the vacuolar or plasma membrane may serve as osmosensors, in guard cells during stomatal movements or in growing pollen tubes for instance (173, 263). These ideas remain highly speculative and rely at most on experiments showing that osmoregulation is altered by mercury, a nonselective aquaporin inhibitor. More solid genetic evidence is now needed to support such models. Despite the presence of aquaporins in most intracellular compartments, we also ignore the volume dynamics and osmotic or mechanical constraints at work on their membranes during volume or turgor regulation. Finally, most studies have focused on plasma membrane and vacuolar aquaporins, and a closer characterization of SIPs, aquaporins that are confined in the ER, may reveal novel aspects of plant cell osmoregulation (114, 214).

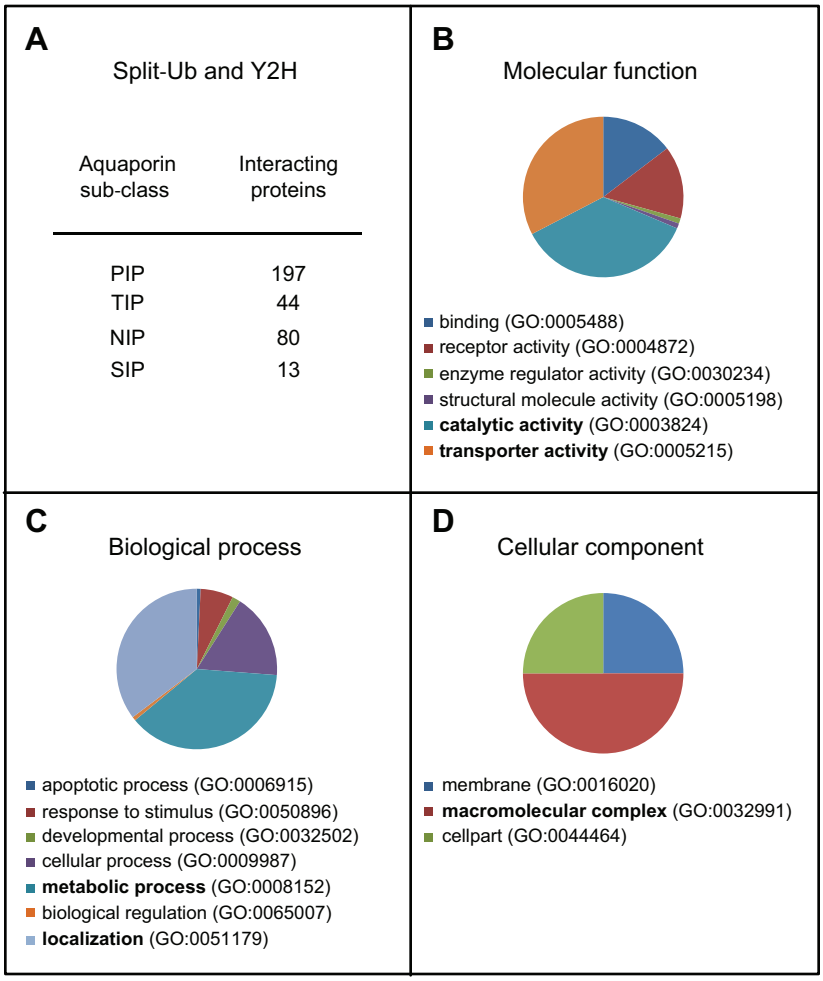

FIGURE 7. Large-scale determination of plant aquaporin interactomes. The figure refers to two independent studies using split-Ubiquitin (Split-Ub) (123) and yeast two-hybrid (Y2H) (53), respectively, that were used for genome-wide identification of plant aquaporin-interacting proteins in *Arabidopsis*. *A*: number of aquaporin-interacting partners. In the Split-Ub screen, an interaction between two partners was validated provided that the interaction was reproducible in both a primary and a secondary interaction screen. Thus 22 aquaporin isoforms showed interactions with at least one protein. In contrast, 10 isoforms (*At*PIP1;1, *At*PIP1;5, *At*PIP2;6, *At*TIP1;2, *At*TIP2;1, *At*TIP3;1, *At*NIP1;2, *At*NIP3;1, *At*NIP5;1, *At*SIP1;1) did not exhibit any interacting proteins. No information is available for *At*PIP2;4, *At*TIP3;2, and *At*NIP4;2. For more details, see https://associomics.org. The Y2H screen identified interacting proteins for 18 aquaporins. For more details, see Reference 53. *B–D*: Gene Ontology (GO) classification of proteins interacting with aquaporins using Panther (http://www.pantherdb.org/). Bold characters indicate major GO terms.

B. ROS Detoxification and Signaling

Consistent with the ability of TIPs to transport H_2O_2 in yeast (29), transgenic overexpression (312) or inactivation of these aquaporins (258) can confer plant tolerance or sensitivity to oxidative stress, respectively. Among ROS, H_2O_2 has a relatively long lifetime and its import by TIPs into the vacuole may efficiently contribute to ROS detoxication. This implies, however, that a detoxication machinery is expressed in vacuoles. Up to now, most of these systems have been characterized in the cytoplasm.

PIP2s can also have a significant H_2O_2 transport capacity (66), and the expression of some of these aquaporins is reduced by H_2O_2 itself (105). Considering that H_2O_2 produced in the apoplasm by combined NADPH-oxidase and superoxide dismutase activities can serve as a signaling intermediate, a role of PIPs in plant cell signaling is very likely. Such signaling role, which has been established for mammalian aquaporin-1, aquaporin-3, and aquaporin-8 (28), still awaits experimental demonstration in plants. Guard cells, which respond to ABA through H_2O_2 production, may represent an interesting model to address this issue.

C. Vacuolar Storage

The permeability of tonoplast aquaporins to compounds such as NH_3 (164) or urea (86, 160) has suggested a role for TIPs in vacuolar storage and remobilization of nitrogen metabolites. However, this role could not be validated in planta using reverse genetic approaches (164). In addition, plant membranes are equipped with other transport systems that mediate a more specific and vectorial transport of NH_3/NH_4^+ and urea. Thus the role of TIPs in vacuolar partitioning of nitrogen metabolites seems at most minor.

D. Chloroplast Transport

Because of its roles in cell bioenergetics and photosynthesis, the plant chloroplast is a unique organelle in eukaryotic cells **(FIGURE 8)**. One key reaction is water oxidation to molecular oxygen that occurs in the thylakoid lumen. It was calculated that this consumption of water molecules represents 60- to 170-fold the thylakoid lumen volume per day (18). Although NMR measurements indicate a moderate diffusional water permeability of the thyla-

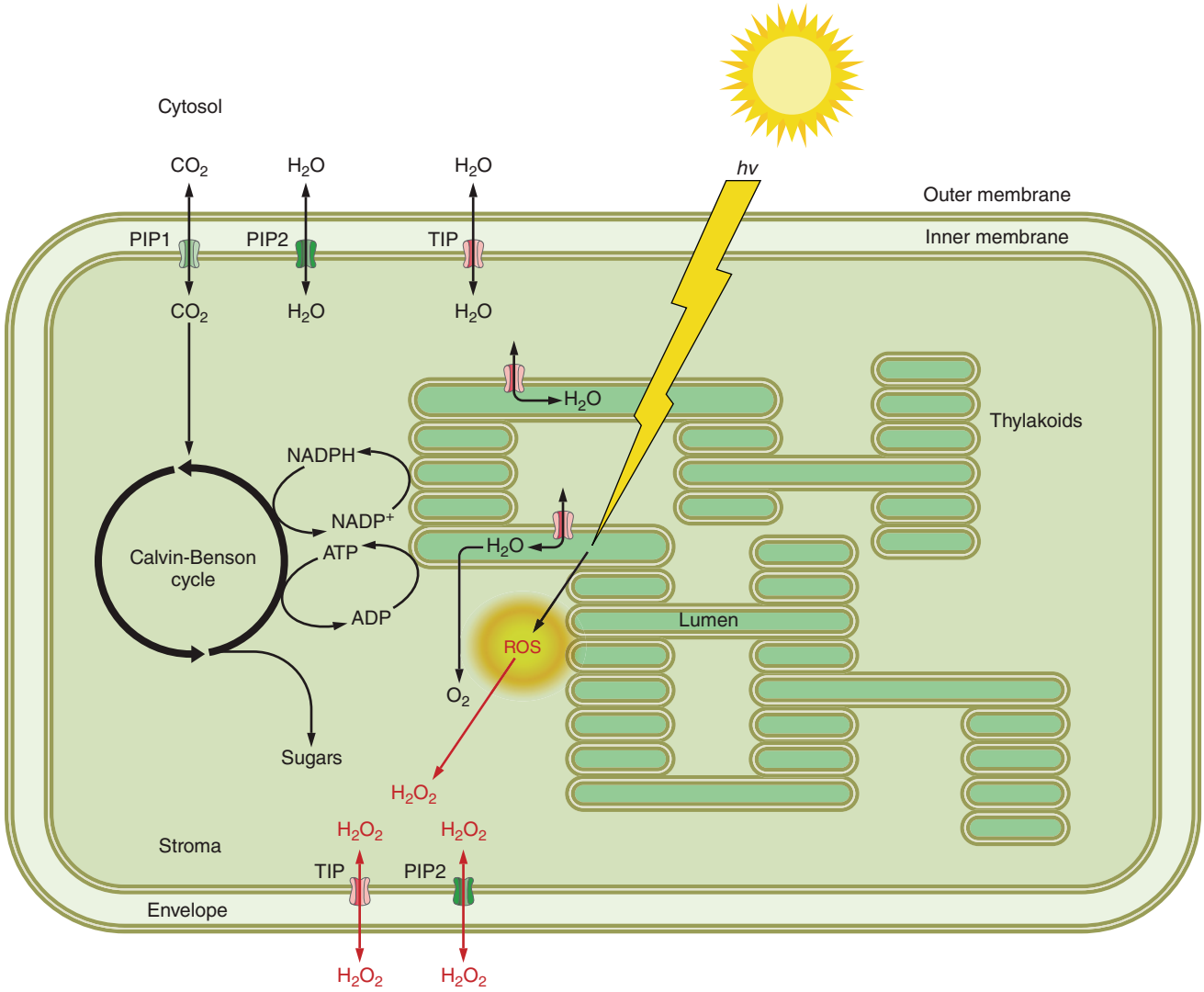

FIGURE 8. Putative functions of aquaporins in the chloroplast. Several PIPs and TIPs have been tentatively localized in the inner envelope and thylakoid membranes. They may contribute to water transport into the stroma and thylakoid lumen. In the latter compartment, photosynthesis leads to oxidation of water to molecular oxygen. Changes in incident light can result in adjustments of thylakoid and overall chloroplast volumes. A role of PIP1s in CO_2 transport across the inner envelope membrane is also indicated. Carbon fixation occurs in the stroma through carboxylation reactions within the Calvin-Benson cycle. Finally, ROS can be formed as by-products of photosynthetic activities. A putative role of PIP and TIP in facilitating H_2O_2 export from the chloroplast is shown.

koid membrane, a minute osmotic gradient (=3 mosM) might be sufficient to support the steady uptake of water required for thylakoid lumen refilling (18). Thus aquaporins may be dispensable during this process. In contrast, the thylakoid lumen shows fast and marked volume increase in response to light, which may be facilitated by aquaporin activity.

CO_2 is another potentially relevant substrate of aquaporins in the chloroplast. Several physical and biochemical components of plant cells, such as cell wall composition and tortuosity, unstirred layers and carbonic anhydrase activities in the vicinity of membranes, or CO_2 permeability of the membranes themselves possibly restrict CO_2 diffusion throughout plant cells (69). The chloroplast envelope may represent a critical barrier, limiting carboxylation reactions performed in the stroma through the Calvin-Benson cycle. Interestingly, the CO_2 permeability of chloroplast envelopes purified from tobacco leaves was fivefold lower than that of PM vesicles and was reduced by ~90% after antisense inhibition of *Nt*AQP1 in transgenic tobacco (295). It is now critically needed that these data are extended to other plant materials.

Finally, aquaporins located on the chloroplast envelope may help dissipate ROS produced in the chloroplast under high-light conditions through H_2O_2 transport into the cytosol (207).

VI. WHOLE PLANT WATER TRANSPORT AND TRANSPIRATION

A. Context

Terrestrial plants establish a continuum of water between the soil and the atmosphere (273) **(FIGURE 1)**. One illustration is plant transpiration which drives an ascending flow of water, its intensity being primarily determined by the hydraulic resistances crossed along the soil-plant-atmosphere continuum. The hydraulic processes operating at the soil-root (rhizosphere) and leaf-air (stomata) interfaces are crucial during transpiration but will not be discussed here, as they do not involve cell membranes and aquaporins. This section rather focuses on water transport within the plant. The functional characterization of aquaporins has brought a strong momentum to earlier studies on this topic (273), building up on their physical concepts and providing molecular and cellular insights into previously described physiological controls of cell and tissue hydraulics (46, 189).

Water transport in inner plant tissues is indeed targeted by multiple regulations, which tend to stabilize the plant water status and provide means for adaptation of the plant to its environment. For instance, the water permeability of roots (root hydraulic conductivity; Lp_r) is highly dependent on the soil content in water, nutrients, and oxygen, whereas leaf hydraulics is sensitive to air humidity and light regime. Temperatures and diurnal rhythms also exert a general impact on plant hydraulics. The coordinated transcriptional regulation of aquaporins by hormonal and environmental factors (5, 34, 116) and abiotic stresses in particular (92, 172, 340) has provided a first hint at the general role of aquaporins during these processes. As detailed below, multiple posttranscriptional mechanisms provide additional means for fine tuning aquaporins and water transport in roots and leaves.

B. Root Water Transport

1. Structure-function analyses

In all higher plants, root water uptake is mediated by radial (centripetal) transport from the soil into xylem vessels, through the epidermis, cortex, endodermis, and stele tissues **(FIGURE 9*A*)**. Once in vessels, water (xylem sap) flows axially towards the plant shoots. During radial transport, water can flow along cell wall structures (apoplastic path) or from cell to cell, along cytoplasmic continuities formed by plasmodesmata (symplastic path) or across cell membranes (transcellular path). A so-called composite model of the root was developed to formalize water transport along this

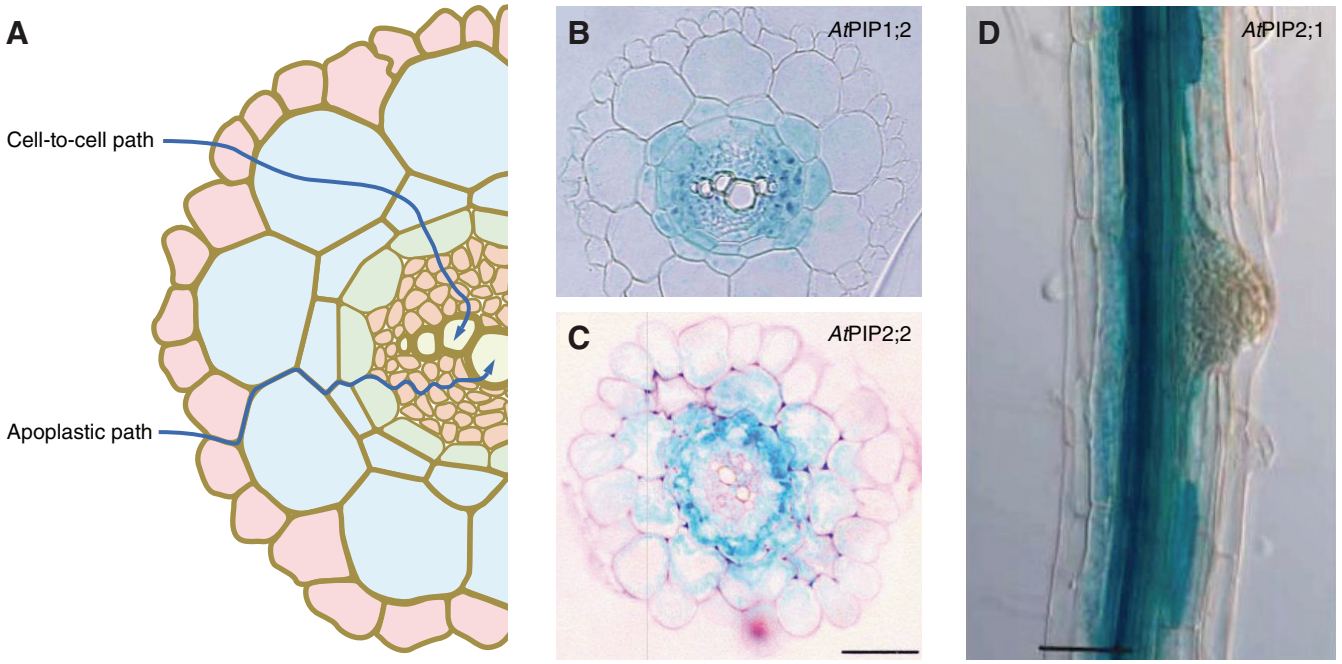

FIGURE 9. Water transport and aquaporin expression in roots. *A*: schematic representation of an *Arabidopsis* root cross section (pink, epidermis; blue, cortex and endodermis; green, pericycle; brown, stele and xylem vessels). Water flows through the cell-to-cell and apoplastic paths are represented by blue arrows. *B–D*: radial and longitudinal cuts of *Arabidopsis* roots expressing *AtPIP1;2-GUS* (*B*; Ref. 230), *AtPIP2;2-GUS* (*C*; Ref. 119) or *AtPIP2;1-GUS* (*D*; Ref. 226) transgenes, which allow expression of β-glucuronidase (*GUS*) reporter gene under the control of the indicated PIP promoters. These pictures show how various aquaporin isoforms exhibit different expression patterns although all are expressed in the stele. Bars = 50 μm (*B*), 50 μm (*C*), or 25 μm (*D*).

network (274). The model can account for water transport under transpiring conditions, when hydrostatic driving forces (pressures and tensions) predominate, or under root pressure conditions when water transport is mostly driven by solute transport and osmotic forces. The model also explains how water transport paths and therefore Lp_r can vary depending on the plant water transport regime (274). While most studies have considered that water flows in root tissues along water potential gradients, by diffusion through cell walls, lipids, or water channels, a role of solute transporters for localized uphill water pumping has recently been hypothesized (316).

Aquaporin blockers, and mercury in particular, have been used as convenient tools for testing the contribution of aquaporins to the composite model of root water transport. Mercury inhibition of Lp_r indicated that the relative importance of the transcellular (aquaporin-dependent) path can vary between plant species and is predominant in tomato or *Arabidopsis*, where it represents 57% and up to 64% of Lp_r, respectively (175, 275). At present, plant aquaporin pharmacology remains highly limiting, all inhibitors described being potentially toxic and exerting numerous secondary effects. Nevertheless, a higher reliability can be obtained when inhibitors with different modes of action (mercury versus weak acids or azide) yield similar estimates of the aquaporin-dependent path (275).

Aquaporin expression patterns can also provide useful hints at the contribution of water channels to root water transport. These patterns have been investigated in roots of many plant species with most accurate descriptions in *Arabidopsis* (83, 119, 230) **(FIGURE 9, *B–D*)** and cereals such as rice (251), maize (97), and barley (136). In addition to their agronomical importance, cereals offer an interesting context for studying root function, with seminal and shoot-borne roots showing distinct morphologies and functions. Aquaporin expression studies in roots have focused on PIPs, which supposedly play a crucial role in plasma membrane and transcellular water transport, and TIPs, which show high expression levels in roots. For instance, the *Arabidopsis* root expresses at least seven PIP and six TIP isoforms with specific expression patterns, the latter being expressed in all tissues except the vasculature and the root tip meristem (83). In all plant species examined, very precise and distinct cell-specific expression patterns could be established for the numerous aquaporin isoforms present in roots (97, 251). Overall, the data stress the importance of controlling local hydraulic properties all along the radial pathway, with preferential expression of some isoforms in the exodermis or endodermis (97). Apoplastic barriers are formed in these two cell layers, through lignin deposition at Casparian strips and subsequent suberization of the whole apoplasm, thereby creating specific limitation for water transport. In addition, high expression of some other isoforms in the stele is consistent with centripetal transport of water towards the root vasculature, whereby water flows have to be mediated by reduced exchange surfaces (80, 230) **(FIGURE 9, *B–D*)**. Aquaporin expression profiles were also shown to vary along the root axis, indicating that the radial pathway itself varies during root growth and differentiation. In some cases, these studies were coupled to water transport measurements in well-defined root zones (80, 136). Recent comparisons of water transport and aquaporin expression patterns in woody perennial and herbaceous plants have indicated distinct root hydraulic differentiation profiles between these types of plants (80). In grapevine for instance, the radial pathway seems to be extremely tight in differentiated root segments, due to the formation of a periderm, suggesting that soil water is taken up through root tips, mostly.

In complement to expression analyses, reverse genetics have brought unequivocal evidence for the contribution of PIPs to Lp_r (119, 226, 230, 267). Although of modest amplitude (10–20% change in Lp_r), significant water transport phenotypes could be observed in single knockout mutants. Yet, these studies have remained restricted to a few isoforms and a genetic redundancy between close homologs has to be considered (226). Thus we still lack a comprehensive molecular view of root hydraulics, whereby each isoform may be associated with water transport at specific cell sites and under a given type of force.

Modeling of root water transport, along concentric cell layers and the root axis, or in distinct root subtypes (37, 137), can provide a significant support to these functional and genetic analyses. These modeling studies indicate that the site of water absorption (root tips versus whole root) may vary between species. A future challenge will be to integrate root hydraulic functioning in a soil to apprehend all kinetic and spatial refinement of water uptake in a natural environment (64). These studies should not be restricted to herbaceous species. For instance, the use of natural caves revealed that aquaporins of live oak and gum bumelia (*Sideroxylon lanuginosum*) contribute to root water uptake deep into the soil (18–20 m) where they can respond to seasonal and diurnal rhythms (190).

2. Water stress

Direct exposure of roots to water stress usually results in inhibition of aquaporin activity and water transport at the cell and whole organ levels (34, 43, 99, 227, 275). Yet, some plant varieties or natural accessions show opposite responses with an enhancement of root cell hydraulic conductivity (99, 275). The effects of external (106, 176) or endogenous ABA (223) are also contrasting, during time and between species. For instance, ABA transiently enhances cell hydraulic conductivity in maize root cells (106), whereas it inhibits Lp_r in aspen (311). These observations highlight the variety of hydraulic strategies in response to soil drying. Whereas increased Lp_r during the early phase of

drought could help optimize the capture of soil water resources, a long-term inhibition provides a more conservative mechanism for the plant, to prevent a reverse flow of water, from the plant root into a dry soil. Regulation of whole root water transport can also have a dramatic hydraulic impact on leaves, to favor or reduce growth or act on stomatal aperture (transpiration) (see sect. VII*A3*).

It is now required that we go beyond classical and abrupt water stress settings used in laboratories. In particular, the heterogeneity of soil water content in natural conditions will have to be considered more carefully in future experimental studies, to understand how plant roots sense soil water deficit and manage soil water on the long term. Partial root-zone drying in a riparian tree (*Melaleuca argentea*) resulted in threefold enhancement of root hydraulic conductance in the wet soil portion, with a slight increase in PIP1 expression, by a signaling mechanism that remains to be identified (191). Also, heterogeneities in local hydraulic conductivities could interfere in water redistribution (hydraulic lift) between soil layers varying in water content (40).

3. Nutrient availability

Root water transport and aquaporin activities are extremely sensitive to nutrient availability in the soil. Plant deprivation in most macronutrients (phosphorus, nitrogen, or sulfur) leads over a couple of days to a decrease in Lp_r (42, 62) through aquaporin downregulation **(FIGURE 10)**. It is assumed that these regulations may favor whole water uptake in conditions favorable to plant growth. Considering possible soil heterogeneities, these regulations may also enhance nutrient drag to the root in soil zones that are the richest in nutrients. At variance with these effects, potassium (K^+) starvation enhances Lp_r (157, 236). The modes of aquaporin regulation by nutrients seem to be complex since they involve mixed effects on aquaporin expression and phosphorylation (62, 88, 157, 313).

The signaling mechanisms that mediate these effects are as yet unknown but clearly involve hormone responses. Thus ethylene was shown to mediate the inhibiting effects of phosphorus starvation on Lp_r in *Medicago falcata* (151), whereas ABA enhanced Lp_r stimulation by K^+ starvation in sunflower (236). It will also be important to explore the respective contributions of local and systemic signals in these regulations (87).

4. Other soil signals

Both low temperatures and oxygen deprivation in the soil exert a general inhibiting effect on root water uptake through decrease of cell and whole root hydraulic conductivities. Cold exerts complex up- and downregulating effects on *PIP* expression in roots (144, 329). ROS and calcium, which accumulate in these conditions, and aquaporin phosphorylation may provide complementary mechanisms for cold-induced Lp_r regulation (144, 145). In rice, a long-term (2–5 days) exposure of roots to low temperature induced a compensatory increase in Lp_r, due in large part to enhanced expression of *Os*PIP2;5 (2). Interestingly, this induction required that the shoot was maintained both at a control temperature and in its integrity, suggesting that a shoot-to-root signal was involved (see sect. VI*B6*).

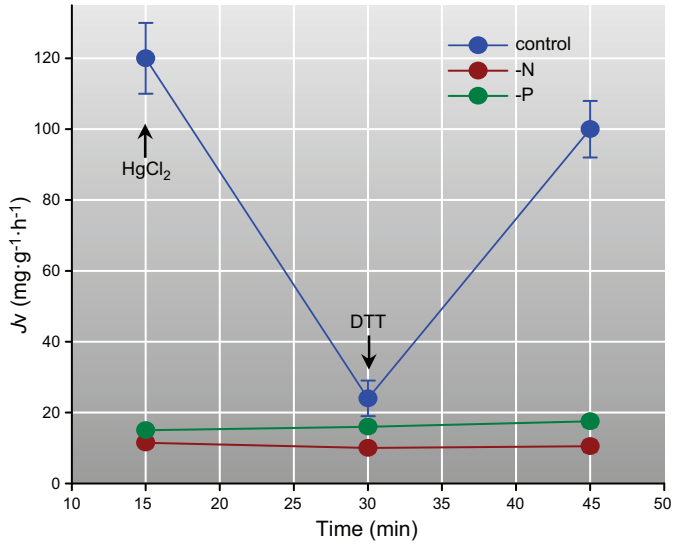

FIGURE 10. Aquaporin-dependent downregulation of root water transport during abiotic stresses. The figure shows sap flow (J_v) exuded by detached root systems of wheat. Roots of control plants treated first with 50 μM $HgCl_2$ and then with 5 mM DTT highlight the role of the aquaporin-dependent path in root water transport (blue filled circles). J_v in roots of nitrogen (N)- and phosphorus (P)-deprived plants (red and green filled circles, respectively) is much lower and shows no sensitivity to $HgCl_2$ inhibition. The conclusion of this landmark experiment is that inhibition of root water transport in plants under N- and P- deficiency is mediated through aquaporin regulation. [Redrawn from Carvajal et al. (42).]

Soil flooding and compaction prevent oxygen diffusion, thereby creating severe anoxic stress, especially onto actively growing root tips. This stress results in a strong metabolic imbalance, with a marked cytosolic acidosis, which in turn mediates H^+-dependent gating of PIPs (287). The long-term effects of anoxia are mediated through a general inhibition of aquaporin gene expression (156). Interestingly, the *AtNIP2;1* gene shows a strong induction in *Arabidopsis* roots under anoxic stress. This aquaporin, which transports lactic acid, may facilitate the leak into the soil of this fermentation product, thereby preventing excessive cell acidification (50).

5. Diurnal and circadian rhythms

In relation to diurnal changes in transpiration, many plant species show a peak in Lp_r during the day (103, 250). This regulation can be accounted for by an increase in root aqua-

porin transcripts during the early phase of the day (163, 279, 298) and a slightly delayed peak in protein abundance (99, 250). Experiments under constant light and/or in circadian clock mutants have shown that at least in maize and *Arabidopsis*, these oscillations are under circadian control (39, 279). In maize, the amplitude of these oscillations is dramatically amplified by previous exposure of plants to water-limiting conditions, in the air or in the soil. This climatic control would optimize water uptake in moderate dry soils, by preserving rhizosphere hydration (39). Roots are also able to directly sense light. For instance, the photoreceptor phytochrome A controls the enhancement of *At*-TIP2;2 expression during adaptation of the *Arabidopsis* root to darkness (296).

6. Transpirational demand and other shoot-to-root signals

Recent studies in rice and poplar (142, 250) have established a direct role of the transpirational demand in Lp_r regulation. This was made possible by independent manipulation of several factors affecting transpiration (i.e., light or relative air humidity) to discard primary effects of these factors on aquaporin functions. In both plant species, transpiration triggered a dramatic increase in *PIP* gene expression in roots.

Identifying systemic signals conveyed from the shoots to regulate root hydraulics represents one of most exciting questions in this field. Several studies have identified context to address this topic. For instance, shoot topping in grapevine, soybean, and maize was shown to reduce Lp_r and root aquaporin activity and expression in a few tens of minutes (299). Whereas auxin or phloem-borne signals seem to be excluded, hydraulic signals transmitted through the xylem are more likely. In aspen (*Populus tremuloides*) seedlings, defoliation results in adjustments of both leaf and root hydraulics. The decrease in Lp_r observed after 1 day was associated with downregulation of a major PIP1 isoform (159). In wheat, partial root excision did not alter stomatal conductance nor transpiration but increased after 1.5 h the hydraulic conductivity of the remaining root by severalfold, in relation to enhanced accumulation of ABA in this organ (307).

C. Leaf Water Transport

1. Hydraulic resistances in the leaf

Following root uptake, the soil water enriched in nutriments is transported as sap to the plant shoot through xylem vessels formed of dead cell structures. Except under severe drought (49), xylem transport in the stem is nonlimiting. In contrast, the leaf represents, in addition to the root, a major checkpoint for plant water transport. In fact, the hydraulics of inner leaf tissues is designed for efficient sap delivery through a network of fine xylem vessels down to substomatal chambers, where water evaporates and is transpired through the stomatal pores.

Leaf hydraulics is therefore determined by vascular (xylem) and extravascular resistances, the respective contributions of which vary according to plant species and leaf morphology (233). The extravascular pathway is formed of parenchyma cells packed along the vessels, surrounded by a tight bundle sheath and by the mesophyll, which shows a looser compaction with numerous lacunas **(FIGURE 11*A*)**. Similar to roots, water can flow in leaves along the apoplastic path or from cell to cell.

The contribution of aquaporins to leaf water transport has been dissected using approaches similar to those used in roots. Expression studies revealed a high aquaporin abundance in the elongating zone of cereal leaves, in the vascular bundles of most plant species and to a lesser extent in the mesophyll (23, 95, 232) **(FIGURE 11*B*)**. Reverse genetic analysis in *Arabidopsis* identified three PIP isoforms that contribute to leaf hydraulics (230, 232). Several lines of evidence indicate that, in *Arabidopsis* at least, the veins (xylem parenchyma and bundle sheath cells) rather than the mesophyll are hydraulically limiting. First, all PIPs contributing to rosette hydraulic conductivity shared a common expression in the vascular bundles (232). Second, the water permeability of protoplasts purified from the veins but not from the mesophyll showed a regulation by light and ABA that paralleled that of the whole leaf (232, 264). Third, complementation, using vein-specific expression of *At-PIP2;1*, of a *Atpip2;1* knockout mutant lacking light-dependent regulation of leaf hydraulics was sufficient to restore a wild-type response (232).

2. Signals

Leaf hydraulics is controlled by numerous environmental or hormonal signals, which most often establish a functional coupling between regulation of inner leaf water transport and stomatal movements. For instance, ABA inhibits inner leaf water transport by downregulating aquaporin activity in bundle sheath cells (264). These effects are physiologically consistent with an additional and direct induction of stomatal closure by the hormone. More generally, a strong correlation between leaf hydraulic and stomatal conductances and expression of a TIP2 homolog was observed in grapevine leaves under both water sufficient and water stress conditions (231).

Besides water stress, the light regime is the other signal that dominates leaf hydraulic regulation. In most plant species, leaf hydraulic conductance is maximal during day time, and a circadian control was established in sunflower (210). In several tree species including walnut (*Juglans regia*), pedunculate oak (*Quercus robur*), and common beech (*Fagus sylvatica*), light-dependent leaf hydraulic conductance showed

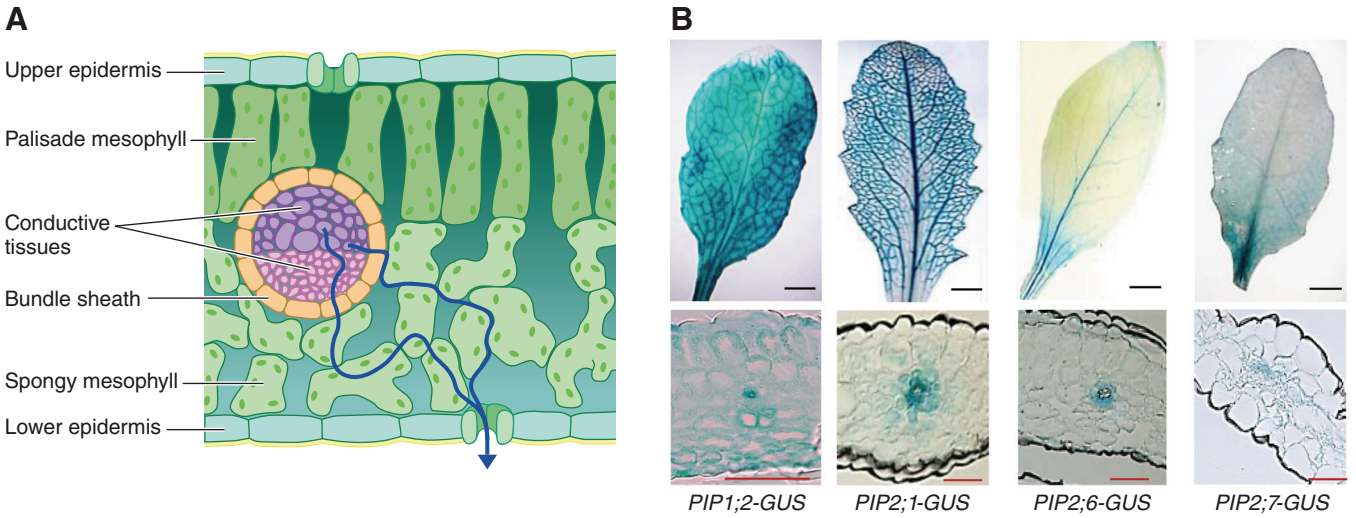

FIGURE 11. Water transport and aquaporin expression in leaves. *A*: schematic representation of an *Arabidopsis* leaf section (blue, epidermis; green, mesophyll; brown, bundle sheath; purple, conductive tissues). Water flows through the cell-to-cell and apoplastic paths are represented by purple arrows. *B*: staining of whole organ or sections in *Arabidopsis* leaves expressing the *PIP1;2-GUS*, *PIP2;1-GUS*, *PIP2;6-GUS*, or *PIP2;7-GUS* transgenes (230, 232). The pictures show a unique leaf expression pattern for each isoform. Black bars = 2.5 mm; red bars = 0.1 mm.

a good correlation to *PIP* gene expression (15, 51). In *Arabidopsis*, in contrast, quantitative proteomics revealed that the diphosphorylated form of *At*PIP2;1 was closely linked to changes in rosette hydraulic conductivity under varying light or dark regimes (232). The ability of a phosphomimetic but not of a phosphodeficient form of *At*PIP2;1 to restore the light response of a *Atpip2;1* knockout mutant established that phosphorylation of this single isoform is necessary for light-dependent regulation of leaf hydraulics.

Crassulacean acid metabolism (CAM) allows some plants to adapt to low-water environment by day/night variation in CO_2 uptake and fixation. In leaves of ice plant, the osmotic water permeability of purified protoplasts and expression of three PIPs and a TIP isoforms peaked at the end of the light period, together with accumulation of CAM cycle metabolites. Thus diurnal regulation of aquaporins may favor cell osmoregulation and leaf water balance throughout the CAM cycle (302).

3. Guard cells and leaf movements

Stomatal aperture is constantly adjusted through reversible changes in guard cell volume and plays a major role in controlling plant transpiration. Whereas a role for aquaporins in stomatal movement has long been hypothesized, supporting evidence has remained scarce, rather suggesting a role in stomatal closure. For instance, overexpression of a *Vicia faba* PIP1 in *Arabidopsis* accelerated the stomatal response to dark and ABA (55). In sunflower guard cells, a TIP isoform showed a diurnal peak in expression that coincides with stomatal closure (256). With regard to the plethora of information on hormone signaling and membrane transport regulation in guard cells, getting a better understanding of aquaporin function in these cells is now becoming critical.

Other types of movements in leaves seem to be associated with circadian regulation of aquaporins. In rain tree (*Samanea saman*), such regulation contributes to circadian variation of osmotic water permeability in motor cells and as a consequence to diurnal leaf movements (205). Similarly, epinastic movement of tobacco leaves is dependent on circadian expression of a PIP1 homolog (*Nt*AQP1) in the petiole (266).

4. Embolism repair

Long-distance transport of water in vascular plants can be dramatically impeded by the formation of embolisms in xylem vessels, following tissue freezing during winter (249) or under extreme drought, when intense transpiration and limiting water supply result in high tension within the vessels (165). Changes in aquaporin expression in relation to embolism recovery and inhibition of this process by mercury have suggested that aquaporin-facilitated water transport can contribute to embolism refilling (165, 249, 260). Accordingly, transgenic poplar and *Arabidopsis* with reduced expression of PIPs were more susceptible to drought-induced embolisms and showed reduced recovery response of whole plant water conductance upon rewatering, respectively (182, 259). Yet, the modes of water and solute cotransport to allow embolism refilling in tissues under strong water tensions are still debated (104, 316).

5. Water uptake

In some very specific physiological contexts or species, leaves can substitute for roots in taking up external water. In atmospheric epiphytes for instance, water absorption of air moisture through trichomes can provide a strong adaptation to drought (218). In conifers, leaf absorption of melting snow may support embolism refilling after winter (141). In both cases, a role of aquaporins in water equilibration within leaf tissues was suggested by aquaporin expression patterns or pharmacological inhibition.

D. Perspectives

1. Molecular dissection of plant tissue hydraulics

Despite an expected genetic redundancy within the aquaporin family, a few studies have identified single aquaporin isoforms that, under laboratory conditions, significantly contribute to root or leaf hydraulics (56, 119, 226, 230, 232). These advances will hopefully encourage a more thorough genetic dissection of plant tissue water transport. Also, a special focus should be put onto aquaporin function in cell layers that are thought to be hydraulically limiting, such as the endodermis and exodermis in roots or the bundle sheath cells in leaves. For instance, a crucial role of the aquaporin pathway in suberized root tissues has been hypothesized in most text books but was only investigated in a handful of studies (80, 238). One critical approach in the future may be to use tissue-specific promoters for cell sorting or directed expression of aquaporins (232, 244, 264).

Most importantly, the identification of master regulators of aquaporins in these cell contexts will be critically needed. In the future, quantitative genetics of plant hydraulics in plants under control or stress conditions (275) may provide a privileged access to these regulators. For now, and as outlined below, the dissection of aquaporin regulation networks using the power of transcriptomic and proteomic approaches provides more operational strategies.

2. Transcription factors and transcriptional networks

A myriad of literature reports describe how environmental, developmental, or hormonal signals interfere with water relations in many plant species, and consistently act on regulation of individual aquaporins. It is now required to go beyond these case-by-case studies and access a unified understanding of the mechanisms involved and of their integration in whole plant responses.

With respect to aquaporin gene regulation, significant progress can be expected from genomic studies. Several studies have addressed regulation of the whole aquaporin family (5, 34, 92, 116, 252, 340), and gene coexpression networks may reveal how regulation of individual aquaporins connects with that of other isoforms or great physiological functions (4). Another important direction will be to identify transcription factors that act as master regulators of aquaporin genes. A role for water stress responsive factors belonging to the DREB (237) and ASR1 (240) families was recently established. Regulation of aquaporin expression by additional ethylene (225) or abiotic stress responsive (325, 341) transcription factors is also emerging. In addition, plant aquaporins are predicted targets of miRNAs in cotton (333) and potato (335). The impact of these and other epigenetic regulations on plant water relations has not yet been evaluated.

Similar advances are foreseen at the protein level. Protein interaction network comprising aquaporins (53, 123) should reveal as yet unknown functional interactions and help identify the interplay of aquaporins with novel cellular functions.

3. General role of phosphorylation

Maintaining the plant water status under ever changing light, temperature, or water availability requires constant cell and tissue hydraulic adjustments. Aquaporin phosphorylation provides a crucial means for such rapid and reversible regulation, without the costs associated with protein synthesis and degradation. Quantitative proteomics in *Arabidopsis* roots has revealed a general correlation across abiotic and nutritional stimuli between Lp_r and aquaporin phosphorylation (62). This type of approach (200, 232, 234) will have to be refined for identifying the most relevant signals and aquaporin isoforms involved in roots and other organs. Most importantly, the putative regulations will have to be functionally validated by expression in transgenic plants of phosphomimetic and phosphodeficient forms of the aquaporin isoform involved (232).

Exploring upstream signaling events also represents an important challenge for future studies. A pioneering work recently identified SIRK1, a protein kinase involved in plant responses to sugar and targeting PIPs (318). Hypothetical phosphorylation cascades triggered by ethylene and negatively regulating aquaporin phosphorylation have recently been modeled (326). Yet, tremendous work remains for identifying the numerous protein kinases and protein phosphatases that likely regulate aquaporins. ABA-dependent phosphorylation may be of central importance (135), but we still ignore the signaling components involved.

VII. PLANT GROWTH AND DEVELOPMENT

A. Hydraulics of Plant Growth

1. Fundamental principles and role of aquaporins

Plants show continuous apical growth of their roots and shoots, through active cell multiplication in meristems and

subsequent cell expansion. These growth processes are remarkably plastic, providing efficient means for long-term adaptation of plants to their environment. The plant water and nutrient status in particular exert multiple controls on these processes.

Plant cell expansion has been formalized in the so-called Lockhart model. It is primarily driven by the intracellular positive pressure (turgor) and mostly restricted by the cell wall that yields beyond a turgor threshold (54). Since cell expansion requires a steady water intake, the model also predicts that the rate of tissue growth can be restricted due to a drop in turgor, when water delivery, through transcellular transport from the vasculature, encounters significant hydraulic resistances. These ideas have been validated by measuring growth-associated water potential gradients (36). They provide a frame for understanding the role of aquaporins in plant tissue growth. Accordingly, aquaporins were shown to be strongly expressed in tissues that can be hydraulically limiting during growth, in barley or maize leaves or hypocotyls of *Arabidopsis* or castor oil plants (*Ricinus communis*) (68, 95, 166, 317). Aquaporin expression in expanding tissues of barley leaves was also associated with a high cell hydraulic conductivity (306).

A direct assessment of the role of aquaporins in tissue growth is experimentally challenging. It was attempted using mercury, which reversibly inhibited root tip growth in maize and embryo expansion in germinating broad bean seeds (110, 215). A hydraulic control of growth can also be revealed at the whole plant level, when water supply from roots becomes limiting for leaf growth. A role of aquaporins in this control was established by pharmacological inhibition of root water transport using a panel of treatments (mercury, acid loads, H_2O_2) with distinct modes of action on aquaporins. A good correlation between inhibition of Lp_r, drop of leaf cell turgor, and leaf growth retardation (67) was observed across these treatments. Interestingly, leaf growth was even more sensitive than stomatal conductance to root hydraulics. Recently, this type of control was nicely integrated in the context of diurnal variations of leaf growth (39). Growth was shown to be tightly linked to circadian oscillations of plant hydraulic conductance and *PIP* transcript abundance in root. In addition, these oscillations were amplified under drought, to adjust growth to water availability in the soil and improve plant performance (39). These findings indicate that Lp_r regulation by numerous environmental and hormonal stimuli provides a general means for quickly and reversibly adjusting shoot growth to environmental constraints.

Whereas PIPs can play an obvious role in transcellular water transport towards expanding tissues, vacuolar aquaporins (TIPs) also seem critical for plant growth. In *Arabidopsis* for instance, expression of a specific TIP isoform (*At*TIP1;1) was associated with cell expansion (166) and regulated by the growth-promoting hormone gibberellic acid (GA_3) (228). Overexpression in the same plant of a ginseng TIP ortholog enhanced overall plant growth through increased leaf cell size (154). It has been suggested that TIPs critically contribute to osmoregulation and vacuolar differentiation in expanding cells. Yet, the enhancement of tobacco protoplast division after TIP overexpression has remained unexplained (219).

2. Particular developmental processes in shoots and roots

Auxin was recently found to potently downregulate the tissue-specific expression and function of most root aquaporins. This regulation shed light onto a new role of aquaporins in root growth, to favor the emergence of secondary roots (226). In brief, physiological analyses in plants with various genetic alterations in PIP function were coupled to mathematical modeling to show that a fine spatial and temporal control of PIP expression favors water entry in the lateral root primordium. This flow is made at the expense of overlaying cells, thereby reducing their mechanical resistance and facilitating lateral root emergence. These results are crucial since they reveal for the first time a link between the capacity of the root to transport water and its ability to grow and potentially adjust its architecture to water availability.

A role for aquaporins in local stimulation of growth has also been proposed in gravistimulated rice leaves. Accumulation of GA_3 on the abaxial side was shown to promote expression of a *PIP* gene (*OsRWC3*) thereby favoring local growth and leaf bending (108).

3. Growth of plants under water deficit

Water deficit usually results in leaf growth arrest, in part because downregulation of root or leaf hydraulic conductance induces hydraulic limitations for growth (67, 223). Yet, there is a large array of responses to water deficit across all plant species. For instance, some cultivated plants such as maize or tomato exhibit an ABA-induced aquaporin upregulation, to enhance soil water uptake and whole plant conductance and maintain plant growth under mild water deficit (39, 223). Under water-limiting conditions, the so-called isohydric plants protect the leaf water status at midday through a tight stomatal regulation. In contrast, anisohydric plants have a less conservative strategy and favor gas exchange and photosynthesis at the expenses of water consumption. As long as water resources are not sharply limiting, the latter plants can exhibit better growth performance than the former. Transgenic tomato plants ectopically expressing a TIP gene or grapevine cultivars differing in drought tolerance (246, 298) have revealed that enhanced aquaporin activity typically confers an anisohydric behavior by favoring plant water transport and preventing sto-

matal closure. These phenomena certainly involve the general link found in many species, between root water transport (Lp_r) and transpiration (142) (see sect. VI*B6*).

B. CO_2 Fixation

1. Mesophyll conductance to CO_2

Strikingly enough, the plant photosynthetic capacity, and therefore plant growth, are primarily limited by CO_2 delivery from the atmosphere to the sites of carboxylation in the chloroplasts (314). Whereas stomatal conductance (g_s) accounts for gas diffusion through the stomatal pore, the mesophyll conductance to CO_2 (g_m) corresponds to the subsequent transport of CO_2, from the substomatal chamber to the chloroplast stroma (74, 314). g_m can differ by more than 10-fold between species. It also varies according to physiological conditions such as water availability or atmospheric CO_2 (69). Several molecular and cellular entities such as cell walls, carbonic anhydrases, or aquaporins have been proposed to contribute to g_m (69). Each of these components may therefore underlie the genetic and physiological variations of g_m in plants.

2. Role of aquaporins

Several lines of converging evidence indicate that aquaporins significantly contribute to g_m. For instance, a 60–70% inhibition of g_m by millimolar concentrations of mercuric chloride was reported in broad bean (*Vicia faba*) and French bean (*Phaseolus vulgaris*) leaves (283). Genetic studies in transgenic tobacco (75, 293, 295), rice (100), or *Arabidopsis* (101) with enhanced or reduced PIP functions have revealed parallel variations in g_m. Finally, it was proposed that the dependency of g_m to atmospheric CO_2 reflects a contribution of PIPs to g_m. High CO_2 would result in cell acidification, which in turn would reduce g_m through pH-dependent gating of PIPs (73).

3. Open questions

Although a physiological role of PIPs in leaf CO_2 transport is emerging, this idea is not fully settled yet and requires a few notes of caution. First, the methodology for measuring g_m and its CO_2 dependency, in particular, is still disputed (73). Second, the g_m variations observed after pharmacological or genetic manipulation of PIP functions are larger than those anticipated for the membrane path (69). Finally, some genetic studies are difficult to interpret because of confounding effects on stomatal gas exchanges (g_s) that would indirectly alter g_m. In these respects, ABA has recently been identified as a dual regulator of g_s and g_m (196), but its mode of action on g_m is as yet unknown. Thus studies where PIP genetic manipulation allows to uncouple effects on g_m and g_s are more reliable (261).

In any case, the role of aquaporins in CO_2 transport certainly represents a very exciting and significant field of research. Some biological membranes seem to have lower than expected permeability to CO_2, which would call for a significant contribution of membrane channels (125). More specifically, aerial plants have to achieve a critical tradeoff between water conservation and carbon fixation, and aquaporins might be at the center of these mechanisms. Future studies on this topic will surely shed light onto the molecular bases of g_m and of its physiological regulations.

C. Nutrient Allocation and Toxicity

1. Boron

Boron (B) is necessary for plant growth. It is taken up from the soil and deposited in tissues to reinforce cell walls. *Arabidopsis* plants strongly react to B limiting conditions by decreased root length, burst in root hair development, and strong induction of the *AtNIP5;1* gene (278) **(FIGURE 12)**. Reverse genetic studies have shown that this aquaporin is central for root B uptake and plant growth under B limiting conditions. Maize *tsl1* plants mutated in a probable *At*NIP5;1 ortholog also show growth defects in these conditions, with altered root growth and reduced size of the shoot apical meristem (65). In *Arabidopsis*, the *At*NIP6;1 and *At*NIP7;1 homologs also serve in B transport and mediate its allocation to shoots and pollen, respectively (148, 281). Additional aquaporins may be involved in B transport and, for instance, a role of XIPs in deposition of B within growing tissues has been proposed in tobacco (26).

Whereas B is an essential micronutrient, an excess in the soil can be toxic for the plant. Thus the activity of some NIPs can become detrimental for plant growth. For instance, a quantitative trait locus for B toxicity in barley was associated with elevated expression of a NIP homolog in roots of the parental line showing the highest sensitivity to high B (257).

2. Silicon

Silicon (Si), an abundant element of the soil, can accumulate in some plants (mostly cereals), to promote their growth and resistance to abiotic and biotic stresses. In particular, Si was shown to alleviate drought stress in sorghum, by enhancing root water uptake and aquaporin activity (161). Elegant genetic studies in rice have revealed a role for several NIP homologs (*Os*NIP2;1/Lsi1 and *Os*NIP2;2/Lsi6) in Si uptake and allocation to shoots (169, 323). As for B, Si transporting NIPs function as efflux channel. They mediate Si transcellular transport by coexpression with secondary active transporters involved in Si import. Similar Si transport equipment exists in maize (195).

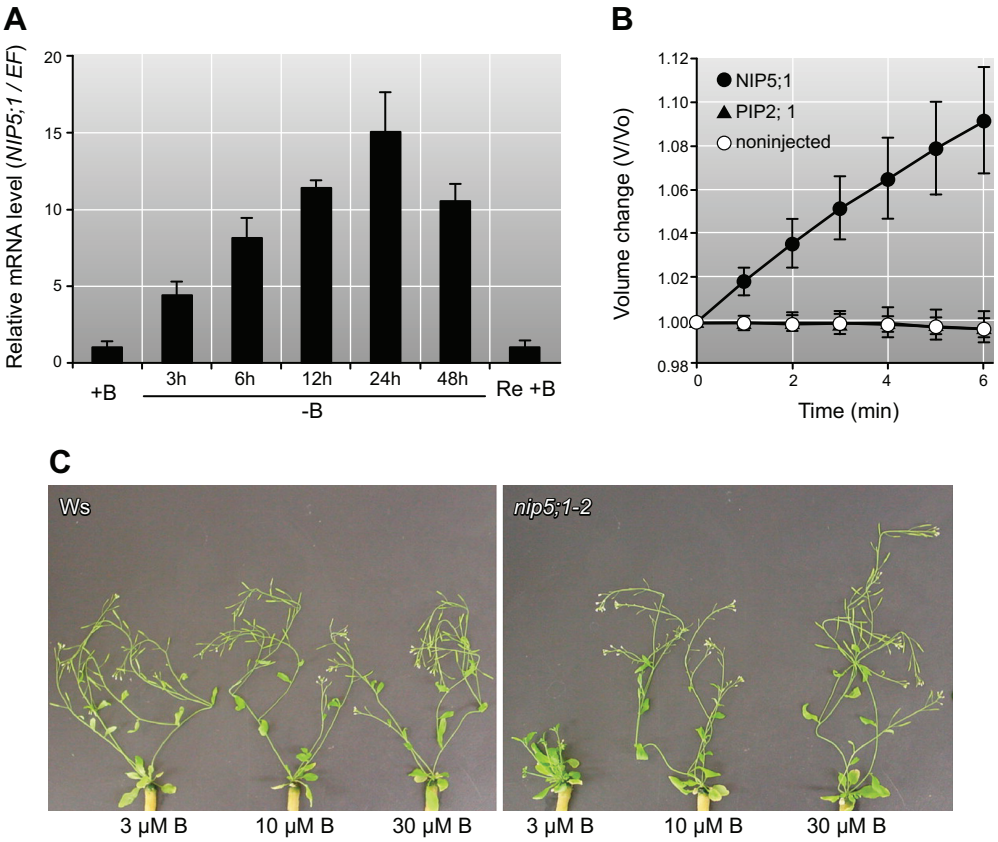

FIGURE 12. Key experiments showing the role of *At*NIP5;1 in B transport (278). *A*: time-dependent accumulation of *AtNIP5;1* mRNA in roots of B-deprived plants, as revealed by quantitative RT-PCR. *B*: swelling assay in *Xenopus* oocytes incubated in an isotonic B solution, showing that, in contrast to *At*PIP2;1, *At*NIP5;1 is permeable to B. *C*: the reproductive growth of a *nip5;1* knockout mutant (*nip5;1–2*) is specifically altered under B-limiting conditions. For more experimental details, see the original publication (278). [Modified from Takano et al. (278). Copyright American Society of Plant Biologists.]

3. Other metalloids

Other metalloids than B and Si, such as arsenic (As), antimony (Sb), or selenium (Se), naturally occur in soils and are absorbed by plants. When entering the food chain, through contamination of crops or drinking water, As and Sb are highly toxic for humans. In contrast, Se is beneficial for human health. At high levels, As and Sb compounds are also toxic for the plants. Consistent with the ability of NIPs to transport arsenous acid in heterologous expression assays, direct genetic studies have revealed a central role for *At*NIP1;1 in arsenite sensitivity of *Arabidopsis* (127). Accumulation of As in rice seeds was shown to be mediated by Si transporters including *Os*NIP2;1 (Lsi1) (170). Interestingly, this toxic accumulation could be counteracted by an excess of Si. *Os*NIP2;1 also mediates selenous acid uptake in rice (337). Due to these multiple roles, members of the NIP subfamily seem to play a crucial role in plant health and food quality. Analyzing their selectivity profile and tissue specific expression is therefore crucial to understand how accumulation of toxic compounds can be avoided, especially in edible parts of crops.

D. Plant Reproduction

Two desiccated forms of plant life, pollen and seeds, play a central role in the life cycle of higher plants and in their dissemination. In contrast to other plant organs, pollen and seeds express specific aquaporin isoforms of the TIP5 and TIP3 subclasses, respectively (271, 301, 320) **(FIGURE 13)**. This specificity may be due to the highly specialized growth and germination processes observed in these organs. More generally, plant reproduction offers striking examples of specialized cell water transport.

1. Flowers

The sophisticated beauty and function of flowers owe much to aquaporins. For instance, the blue sepal color of hydrangea (*Hydrangea macrophylla*) grown in acidic soil is due to a vacuolar accumulation of aluminum (Al) complexed with anthocyanins. To reach the vacuole, Al is transported as $Al(OH)_3$ through the plasma and vacuolar membrane, by a NIP and a TIP aquaporin, respectively (211). It is as yet unclear whether and how TIPs and NIPs work in concert with other Al transporters of the NRAMP family to mediate Al homeostasis in other plant organs (321).

Flowers are also remarkable by their blooming and diurnal movements. These processes require highly controlled cell expansion processes. Accordingly, PIP1s and PIP2s were found to play an interacting role during ethylene-dependent expansion of rose petals (47, 171). In addition, the opening and closing of tulip sepals induced at normal (20°C) and low (5°C) temperature, respectively, were associated with reversible phosphorylation of PIP homologs (13).

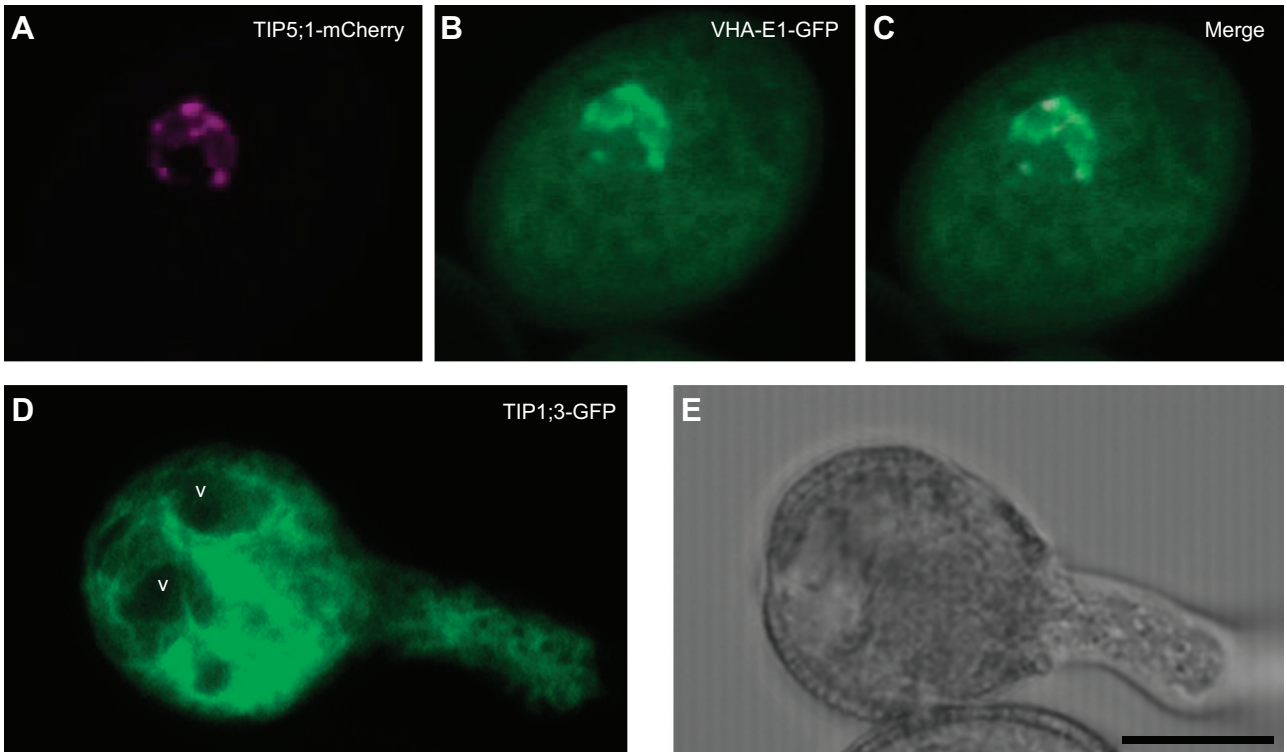

FIGURE 13. Cell-specific and subcellular localization of pollen TIPs. Expression in transgenic *Arabidopsis* of fluorescent protein fusion constructs show that *At*TIP5;1 (*A*) and *At*TIP1;3 (*D*) are specifically expressed in the sperm and the vegetative cell of pollen, respectively. Colocalization (*C*) of TIP5;1-mCherry (*A*) with an *Arabidopsis* vacuolar H^+-ATPase subunit E construct (VHA-E1-GFP) (*B*) indicates the vacuolar localization of *At*TIP5;1. In a germinating pollen grain (see transmission image in *E*), TIP1:3-GFP shows an intricate labeling of tonoplast and endoplasmic reticulum membranes. The position of two vacuoles is indicated (v). Bar size = 10 μm.

In flowers, the reproductive function itself requires a tight control of tissue desiccation, involving aquaporins at various stages. For instance, anther dehydration is necessary for dehiscence and release of mature pollen and was hampered in tobacco plants with reduced expression of PIP2s (32). The formation of pollen grains is accompanied by a progressive dehydration, whereas its imbibition triggers germination through dramatic growth of the pollen tube. *Arabidopsis* plants invalidated for two pollen TIPs specific of the vegetative and sperms cells, respectively, showed reduced fertility under limited water or nutrient supply (320). However, the modes of water transport from the receptive papilla into dry pollen and the possible role of plasma membrane aquaporins during pollen tube growth remain as yet unknown (178, 269). The numerous reproductive development defects of the maize B transport mutant *tsl1* (65) indicate that, in addition to water, micronutrients such as B also are critical for proper growth and function of flowers.

2. Seeds

Seed maturation is accompanied by a profound reorganization of the vacuolar apparatus, with formation of numerous protein storage vacuoles. Although genetic evidence is lacking, the expression of seed-specific TIPs is thought to be crucial during this process (82, 276, 301). Studies in developing bean seeds also revealed a specific aquaporin equipment, for phloem-mediated import of water and nutrients into developing coat, and for water recycling in the xylem and its delivery to cotyledons (339).

Seed germination is triggered by a rapid imbibition of desiccated tissues and shows a second phase of water uptake associated with embryo growth. Mercury inhibition experiments have indicated that aquaporins may be limiting during the first phase of water uptake in species such as pea (305), whereas in *Arabidopsis* or broad bean, they would contribute to embryo growth (215, 301). The transition from protein storage vacuoles to a large central vacuole, with a shift from TIP3 to TIP1 expression, also seems crucial during this process.

Despite the presumed importance of aquaporins in seeds, we note that, so far, genetic evidence is only available in rice. In this species, the knockout and/or overexpression of *OsPIP1;1* and *OsPIP1;3* was shown to alter both the rate and speed of germination (155, 158). Interestingly, the two genes are under the control of nitric oxide. The control of seed aquaporin function by osmotic or hormonal signals will deserve more efforts in future research.

VIII. BIOTIC INTERACTIONS

A. Rhizobium-Legume Symbiosis

Besides plant responses to abiotic stimuli, aquaporins also serve in plant biotic interactions. The best described examples are root symbiosis.

Nitrogen is, together with potassium and phosphorus, a major macronutrient of plants. It is most often limiting for plant growth, hence the intensive use of nitrogen fertilizers in agriculture. Legumes have acquired the remarkable capacity to develop symbiotic root nodules able to fix atmospheric nitrogen (N_2). For this, specific soil bacteria (*Rhizobiaceae*) infect plant root cells and differentiate into intracellular bacteroids, each surrounded by a plant symbiosome membrane (292). During root nodule differentiation in barrel clover (*Medicago truncatula*), a TIP1 homolog was shown to be transiently retargeted from the tonoplast to the symbiosome membrane, this process being necessary for functional nodule formation (84). The fully differentiated symbiosome membrane then expresses NIPs which transport both water and NH_3 (91, 112) and likely play a dual role in cell osmoregulation and nitrogen assimilation. One of these NIP homologs, named nodulin-26 and present in soybean root nodule, was actually the first aquaporin identified in plants. Nodulin-26 molecularly interacts with cytosolic glutamine synthase (184). Upon import of NH_4^+/NH_3 into the plant cytosol by paths that still need to be unambiguously identified, this molecular interaction may optimize fixation of NH_4^+ onto glutamate, to prevent a feedback leak of NH_4^+/NH_3 to the peribacteroid space through nodulin-26.

B. Mycorrizhae

Mycorrizhae represent a very common symbiotic interaction between soil fungi and plant roots. Depending on the two partners, the plant interface with the fungus is either intracellular (arbuscular mycorrhizae) or extracellular (ecto-mycorrhizae). In all cases, the mycelia optimize soil exploration and nutrient capture, whereas the plant root provides carbon metabolites for nutrient assimilation. Besides plant mineral nutrition, mycorrhizae seem to ameliorate plant water relations and stress responses. For instance, numerous reports indicate that, both in crops (maize, bean, soybean, lettuce) and trees (poplar), mycorrhizae can enhance plant tolerance to drought, flooding, cold, or salinity stress (10, 17, 41, 179, 229). However, the molecular and physiological bases of these effects are not fully elucidated yet. For instance, root water transport and overall aquaporin expression were enhanced in poplar, whereas an opposite response was seen in soybean and lettuce (179, 229). In the latter two species, arbuscular mycorrhizae may help the plant anticipate root responses to stress. In maize, interactive effects of mycorrhizae with plant ABA enhance root aquaporin inhibition and water conservation under drought (243).

The role of plant aquaporin-mediated solute transport in arbuscular mycorrhizae was recently examined (17). Gene profiling indicated that, in symbiotic roots, glycerol export from the plant to the microbe, and NH_4^+/NH_3 import into the plant, for storage and detoxification, may be enhanced through activation of proper plant aquaporin genes. In contrast, the transport of B and Si would be downregulated. Indeed, the B released by the fungus can be toxic for the plant, and Si is known to antagonize mycorrhizal infection.

A current challenge is now to characterize the aquaporin equipment of fungi, a task that was accelerated through fungal genome sequencing (63, 149, 322). Similar to their plant counterparts, aquaporins of arbuscular or ecto-mycorrhizal fungi such as *Glomus intraradices* or *Laccaria bicolor*, respectively, appear to play a crucial role in both water and nutrient transport. An ultimate challenge will be to understand the molecular dialog between plant and fungal cells, and how this coordinates expression of aquaporins and complementary transport systems between the two partners (9). Even though the underlying mechanism are not known, it was recently shown that genetic manipulation of aquaporin expression in *Laccaria bicolor* alters expression of the plant host aquaporins (322).

C. New Directions: Investigating Beneficial or Pathogenic Interactions

Recent studies on plant root symbiosis have revealed how aquaporins can contribute to a functional dialog between transport proteins of distinct organisms. Yet, many other biotic interactions remain to be explored to ultimately apprehend the full ecological integration of aquaporin functions.

For instance, the plant-growth-promoting rhizobacterium *Bacillus megatorium* exerts ameliorative effects on plant salt tolerance. Accordingly, it enhances L_{p_r} in maize and modifies aquaporin expression in both control and salt stress conditions (183). Yet, the precise modes of aquaporin regulation and the role of bacterial signals, possibly auxin, remain unknown. The plant hormone methyl jasmonate also represents an important signal in plant defense. It was shown to enhance aquaporin activity in roots of French bean, tomato, and *Arabidopsis* through interaction with calcium- and/or ABA-dependent signaling pathways (253).

The effects of pathogenic infection on aquaporin expression and their significance in plant disease also deserve specific attention. One of most striking example is the induction of a tobacco TIP1 expression in giant root cells induced upon nematode infection (220). This induction may favor the

osmoregulation of these cells that serve as feeding sites for the pathogen. Infection of soybean leaves by *Pseudomonas syringae* resulted after 8 h downregulation in 24 of 32 aquaporin genes (342), but the significance of this regulation was not assessed.

IX. AQUAPORINS AND PLANT GENETIC IMPROVEMENT

A. Success Stories and Failures

Water relations and mineral nutrition represent major traits in crop improvement. Aquaporins, which provide genetic and molecular tools to act on these traits, are therefore attractive targets for plant molecular breeders (180). Genetic manipulation of PIPs, and NIPs has now been explored in many plant species, with varying rates of success.

Transgenic strategies aimed at altering aquaporin function have first been developed in herbaceous species, leading to contrasting effects on plant growth and stress response. Ectopic expression of an aquaporin in a heterologous plant species seems hazardous, since the fine regulations that govern each individual isoform in the native plant may not work in a distinct transgenic species. Yet, overexpression of a *Vicia faba* PIP1 (*Vf*PIP1) or a wheat PIP2 (*Ta*AQP7) in *Arabidopsis* and tobacco, respectively, enhanced plant drought resistance (55, 338). Identification of stress-regulated aquaporin isoforms and subsequent genetic manipulation in the same species seems to be a more solid approach. For instance, constitutive expression of a stress-responsive TIP2 of tomato (*Sl*TIP2;2) enhanced the growth performance of transgenic tomato plants under both normal and water stress conditions by favoring their anisohydric behavior (246). *Os*PIP1;3 (RWC3) was initially identified as stress-induced in a upland rice cultivar. Its expression in a lowland rice cultivar, using an engineered stress-induced promoter, conferred on transgenic plants drought avoidance properties (such as maintenance of leaf water potential) reminiscent of those observed in the upland cultivar (153). In rice again, tolerance of root and leaf growth to salt stress and ameliorated germinative properties of seeds were obtained upon constitutive expression of *Os*PIP1;1, provided that the transgene was expressed at a moderate level (155).

In addition to these few success stories, the literature abounds with more mitigated reports. These works are informative with respect to aquaporin physiology but represent clear failures in terms of biotechnology. For instance, tobacco plants overexpressing *Arabidopsis At*PIP1;2 grew better than control plants in normal conditions but became dramatically more sensitive to water deprivation (3), probably because of a stomatal deregulation. Expression of cucumber and figleaf gourd aquaporins in *Arabidopsis* had either beneficial or detrimental effects on plant survival and seed germination, whether plant where subjected to a dehydration (mimicked by PEG application) or a salt (NaCl) stress (117).

By comparison to herbaceous crops, the genetic improvement of woody plants poses special difficulties due to slower growth and longer reproduction cycles. Yet, with the emergence of genomic tools and reverse genetics, aquaporin functions and the potentialities of their genetic alteration are now being explored in these species. For instance, overexpression of a root PIP (*Vv*PIP2;4N) in transgenic grapevine (227) enhanced root hydraulics, transpiration, and shoot growth, and therefore water consumption, under control conditions, whereas the plant exhibited a normal water conservation response under water stress. In poplar, RNAi was recently used as an efficient means for downregulating expression of several PIP1 homologs. Whereas no major phenotype was observed in transgenic trees with fast growth under no stress conditions, trees under water stress unfortunately showed severe defects in recovery from embolism (259, 261). *Eucalyptus* is another forest tree of great use in the paper industry. Aquaporin function was explored in the species using transgenesis (288). Finally, targeting ethylene-regulated aquaporins in *Hevea* may help improve latex production (289).

B. Mechanisms

The phenotype of plants with genetically altered aquaporins is usually complex to decipher, as it integrates far more than direct effects of the manipulated aquaporin on tissue hydraulics or carbon fixation. For instance, several studies have revealed that ectopic expression of a foreign aquaporin gene can perturb the expression pattern of endogenous aquaporin genes, thereby resulting in unpredictable beneficial or detrimental effects on plant growth and stress responses (117, 288). Heteromerization of an artificially expressed aquaporin with endogenous ones may also result in dominant negative effects on plant water transport (270). In addition, altered water relations can influence other physiological responses to stress, which in turn contribute to most of plant phenotype. For instance, PIP overexpression in grapevine may have disturbed water stress sensing in root, thereby enhancing ABA synthesis, specifically under water stress (227). These hormonal effects likely explain why water conservation was improved in these conditions (227). At high concentrations, sodium (Na^+) is toxic for plants. Controlling its accumulation and compartmentalization is therefore critical in plants under salt stress. In *Arabidopsis*, overexpression of a wheat NIP homolog (81) or a TIP from the halophyte *Thelungiella salsuginea* (312) reduced salt loading or favored its vacuolar accumulation, respectively. In both cases, plant stress tolerance was improved. ROS metabolism, which is central to plant stress responses, can also be altered after aquaporin genetic manipulation. Overexpression of a wheat PIP2 homolog (*Ta*AQP7) in tobacco resulted in enhanced superoxide dismu-

tase and catalase activities through an unknown mechanism (338), thereby contributing to drought tolerance. Conversely, an *Arabidopsis* line inactivated for *TIP1;1* and *TIP1;2* showed a reduced catalase activity and higher anthocyanin content that somewhat mimicked a response to high light. This phenotype was tentatively associated with the capacity of the two TIP homologs to channel H_2O_2 (258). In most studies, information on root and shoot hydraulics, but also leaf morphology, stomatal density, and shoot/root ratio is lacking to fully interpret how aquaporin manipulation can interfere with plant growth properties. Yet, elegant grafting experiments using wild-type and transgenic tobacco showed that overexpression of *Nt*AQP1 in roots was sufficient to sustain a high transpiration in wild-type shoots. In contrast, the beneficial effects of NtAQP1 on stomatal conductance and photosynthesis required its expression in shoots (245).

C. Targeting Micronutrients

Besides water relations and growth under drought or salt stress, the plant micronutrient status represents another, easier target for aquaporin genetic manipulations, which, however, has barely been explored. Thus enhanced expression of *At*NIP5;1 and *At*TIP5;1 in *Arabidopsis* improved plant tolerance to low B and high B, respectively (129, 222). In the latter case, the effects were likely due to facilitated storage of B in the vacuole. Reduced expression of NIPs that transport B at the plasma membrane should also enhance plant tolerance to high B toxicity (257). We note however that, as for water transport, ectopic expression of aquaporins transporting B and Si can lead to detrimental effects on growth. Thus organ- or tissue-specific expression seems to be crucial, to prevent mislocalization of these micronutrients (129, 201).

X. CONCLUSIONS AND PERSPECTIVES

A. Conclusions

Two decades after their discovery, it is now established that aquaporins play a broad role in plant physiology. Genetic and physiological studies, in support of gene expression data and functional characterization in heterologous systems, have revealed that, despite their multiplicity, plant aquaporins can individually fulfill multiple functions. For instance, some PIP1s seem to play a dual role in water and CO_2 transport in *Arabidopsis* and tobacco (75, 230, 264, 267). In the former species, a single PIP2 isoform (*At*PIP2;1) is involved in root water uptake, lateral root formation, and water transport in leaf veins (226, 232). Therefore, a next challenge will be to dissect, at least for a few representative isoforms, the cell specificity of aquaporin functions and regulations. One direction would be targeted proteomics, to access cell-specific aquaporin phosphorylation profiles and interacting partners. Transgenic plants expressing cell-type specific reporters (232, 264) and aquaporin knockout plants with cell-specific complementation (232, 244) will be crucial in these studies. Beyond these generic approaches, we believe that a few, more specific topics will deserve a specific attention.

B. Emerging Research Directions

1. Aquaporins and plant cell signaling: interplay with ROS and hormones

Through their multiple transport functions and regulations, aquaporins emerge as critical nodes that integrate cell metabolism and signaling into whole plant responses to environmental and hormonal signals. The interplay of aquaporins with ROS appears as a particularly central topic. On the one hand, ROS mediate the effects of salt, salicylic acid, and cold (33, 145) on water transport and aquaporin regulation in roots. Yet, we do not understand why ROS have contrasting effects in bean (21) and *Arabidopsis* roots (35). On the other hand, the capacity of plant aquaporins to transport H_2O_2 points to emerging roles in cell signaling and ROS detoxification. The former has been established in animals (192), and the latter is supported by recent studies in transgenic plants. The regulation of aquaporins by hormones in plants under environmental stress will also deserve more attention. For instance, ethylene may play a crucial role for water transport regulation during anoxia (126) or potassium starvation. Finally, the circadian regulation of aquaporins and the significance of hydraulics as an input or output signal of the clock will represent an exciting challenge in coming years (39, 279). Mutants such as *early flowering 3* will allow investigating how aquaporin function is coupled to the circadian clock (279). In addition, understanding the synchronizing role of light but also ABA on oscillating aquaporin functions may provide clues on how plant growth is optimized according to soil water ability and transpiration demand (39, 279).

2. Role of intracellular aquaporins

Whereas it definitely pertains to original plant specific functions, the role of intracellular aquaporins has remained a kind of black box. Since reverse genetics of TIPs and SIPs has failed to reveal macroscopic phenotypes (19, 174, 258), cellular phenotypes will have to be inspected in closer detail. In particular, an accurate monitoring of compartmental volumes in osmotically challenged cells will be required to establish the putative role of these aquaporins in cell osmoregulation. Also, the actual substrates of these aquaporins are as yet undetermined. It was suggested that SIPs may transport ethylene across the ER membrane (174) but, to our knowledge, this hypothesis has not been further explored. Finally, the role of PIPs in CO_2 transport across the chloroplast envelope is indicated by a single study in tobacco (295) and definitely deserves more attention.

3. Exploring more diverse plant systems

While initially performed in a few plant model species (*Arabidopsis*, tobacco) or crops (maize, rice), studies on aquaporins are now expanding to more and more plant species. Plant biodiversity is definitely worth being further explored. In particular, extremophile plants such as resurrection plants or halophytes (177, 300, 312) may reveal novel physiological regulations. In ice plant, aquaporin function is dramatically changed during the salt-induced switch from C3 to CAM metabolism (134, 302). Original information is also expected from unicellular photosynthetic organisms such as *Synecocystis* (12) or *Chlamydomonas reinhardtii* (138), from mosses (154) or representative species of early evolved plants (8). In particular, recent studies in horsetail (*Equisetum arvense*), a primitive vascular plant that was identified for its remarkably high Si content, allowed identification of a new subfamily of aquaporin Si channels (89).

By delineating ever-expanding fields in plant integrative biology, aquaporins have been fascinating research objects over the last two decades. This is quite not finished: we have outlined here a few research directions which, we believe, are particularly relevant and have been somewhat neglected. We are confident that these and other directions will provide exciting discoveries and establish further the crucial role of aquaporins in plants.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES

1. Abascal F, Irisarri I, Zardoya R. Diversity and evolution of membrane intrinsic proteins. *Biochim Biophys Acta* 1840: 1468–1481, 2014.

2. Ahamed A, Murai-Hatano M, Ishikawa-Sakurai J, Hayashi H, Kawamura Y, Uemura M. Cold stress-induced acclimation in rice is mediated by root-specific aquaporins. *Plant Cell Physiol* 53: 1445–1456, 2012.

3. Aharon R, Shahak Y, Wininger S, Bendov R, Kapulnik Y, Galili G. Overexpression of a plasma membrane aquaporin in transgenic tobacco improves plant vigor under favorable growth conditions but not under drought or salt stress. *Plant Cell* 15: 439–447, 2003.

4. Alexandersson E, Danielson JA, Råde J, Moparthi VK, Fontes M, Kjellbom P, Johanson U. Transcriptional regulation of aquaporins in accessions of *Arabidopsis* in response to drought stress. *Plant J* 61: 650–660, 2010.

5. Alexandersson E, Fraysse L, Sjovall-Larsen S, Gustavsson S, Fellert M, Karlsson M, Johanson U, Kjellbom P. Whole gene family expression and drought stress regulation of aquaporins. *Plant Mol Biol* 59: 469–484, 2005.

6. Alleva K, Niemietz CM, Sutka M, Maurel C, Parisi M, Tyerman SD, Amodeo G. Plasma membrane of *Beta vulgaris* storage root shows high water channel activity regulated by cytoplasmic pH and a dual range of calcium concentrations. *J Exp Bot* 57: 609–621, 2006.

7. Anderberg HI, Danielson JA, Johanson U. Algal MIPs, high diversity and conserved motifs. *BMC Evol Biol* 11: 110, 2011.

8. Anderberg HI, Kjellbom P, Johanson U. Annotation of *Selaginella moellendorffii* Major Intrinsic Proteins and the evolution of the protein family in terrestrial plants. *Front Plant Sci* 3: 33, 2012.

9. Aroca R, Bago A, Sutka M, Paz JA, Cano C, Amodeo G, Ruiz-Lozano JM. Expression analysis of the first arbuscular mycorrhizal fungi aquaporin described reveals concerted gene expression between salt-stressed and nonstressed mycelium. *Mol Plant Microbe Interact* 22: 1169–1178, 2009.

10. Aroca R, Porcel R, Ruiz-Lozano JM. How does arbuscular mycorrhizal symbiosis regulate root hydraulic properties and plasma membrane aquaporins in *Phaseolus vulgaris* under drought, cold or salinity stresses? *New Phytol* 173: 808–816, 2007.

11. Audigeos D, Buonamici A, Belkadi L, Rymer P, Boshier D, Scotti-Saintagne C, Vendramin GG, Scotti I. Aquaporins in the wild: natural genetic diversity and selective pressure in the PIP gene family in five Neotropical tree species. *BMC Evol Biol* 10: 202, 2010.

12. Azad AK, Sato R, Ohtani K, Sawa Y, Ishikawa T, Shibata H. Functional characterization and hyperosmotic regulation of aquaporin in *Synechocystis* sp. PCC 6803. *Plant Sci* 180: 375–382, 2011.

13. Azad AK, Sawa Y, Ishikawa T, Shibata H. Phosphorylation of plasma membrane aquaporin regulates temperature-dependent opening of tulip petals. *Plant Cell Physiol* 45: 608–617, 2004.

14. Azad AK, Yoshikawa N, Ishikawa T, Sawa Y, Shibata H. Substitution of a single amino acid residue in the aromatic/arginine selectivity filter alters the transport profiles of tonoplast aquaporin homologs. *Biochim Biophys Acta* 1818: 1–11, 2012.

15. Baaziz KB, Lopez D, Rabot A, Combes D, Gousset A, Bouzid S, Cochard H, Sakr S, Venisse JS. Light-mediated K_{leaf} induction and contribution of both the PIP1s and PIP2s aquaporins in five tree species: walnut (*Juglans regia*) case study. *Tree Physiol* 32: 423–434, 2012.

16. Bansal A, Sankararamakrishnan R. Homology modeling of major intrinsic proteins in rice, maize and *Arabidopsis*: comparative analysis of transmembrane helix association and aromatic/arginine selectivity filters. *BMC Struct Biol* 7: 27, 2007.

17. Barzana G, Aroca R, Bienert GP, Chaumont F, Ruiz-Lozano JM. New insights into the regulation of aquaporins by the arbuscular mycorrhizal symbiosis in maize plants under drought stress and possible implications for plant performance. *Mol Plant Microbe Interact* 27: 349–363, 2014.

18. Beebo A, Mathai JC, Schoefs B, Spetea C. Assessment of the requirement for aquaporins in the thylakoid membrane of plant chloroplasts to sustain photosynthetic water oxidation. *FEBS Lett* 587: 2083–2089, 2013.

19. Beebo A, Thomas D, Der C, Sanchez L, Leborgne-Castel N, Marty F, Schoefs B, Bouhidel K. Life with and without *AtTIP1;1*, an *Arabidopsis* aquaporin preferentially localized in the apposing tonoplasts of adjacent vacuoles. *Plant Mol Biol* 70: 193–209, 2009.

20. Bellati J, Alleva K, Soto G, Vitali V, Jozefkowicz C, Amodeo G. Intracellular pH sensing is altered by plasma membrane PIP aquaporin co-expression. *Plant Mol Biol* 74: 105–118, 2010.

21. Benabdellah K, Ruiz-Lozano JM, Aroca R. Hydrogen peroxide effects on root hydraulic properties and plasma membrane aquaporin regulation in *Phaseolus vulgaris*. *Plant Mol Biol* 70: 647–661, 2009.

22. Bertl A, Kaldenhoff R. Function of a separate NH_3-pore in aquaporin TIP2;2 from wheat. *FEBS Lett* 581: 5413–5417, 2007.

23. Besse M, Knipfer T, Miller AJ, Verdeil JL, Jahn TP, Fricke W. Developmental pattern of aquaporin expression in barley (*Hordeum vulgare L.*) leaves. *J Exp Bot* 62: 4127–4142, 2011.

24. Besserer A, Burnotte E, Bienert GP, Chevalier AS, Errachid A, Grefen C, Blatt MR, Chaumont F. Selective regulation of maize plasma membrane aquaporin trafficking and activity by the SNARE SYP121. *Plant Cell* 24: 3463–3481, 2012.

25. Biela A, Grote K, Otto B, Hoth S, Hedrich R, Kaldenhoff R. The *Nicotiana tabacum* plasma membrane aquaporin NtAQP1 is mercury-insensitive and permeable for glycerol. *Plant J* 18: 565–570, 1999.

26. Bienert GP, Bienert MD, Jahn TP, Boutry M, Chaumont F. Solanaceae XIPs are plasma membrane aquaporins that facilitate the transport of many uncharged substrates. *Plant J* 66: 306–317, 2011.

27. Bienert GP, Cavez D, Besserer A, Berny MC, Gilis D, Rooman M, Chaumont F. A conserved cysteine residue is involved in disulfide bond formation between plant plasma membrane aquaporin monomers. *Biochem J* 445: 101–111, 2012.

28. Bienert GP, Chaumont F. Aquaporin-facilitated transmembrane diffusion of hydrogen peroxide. *Biochim Biophys Acta* 1840: 1596–1604, 2014.

29. Bienert GP, Moller AL, Kristiansen KA, Schulz A, Moller IM, Schjoerring JK, Jahn TP. Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. *J Biol Chem* 282: 1183–1192, 2007.

30. Bienert GP, Schüssler MD, Jahn TP. Metalloids: essential, beneficial or toxic? Major intrinsic proteins sort it out. *Trends Biochem Sci* 33: 20–26, 2007.

31. Bienert GP, Thorsen M, Schüssler MD, Nilsson HR, Wagner A, Tamás MJ, Jahn TP. A subgroup of plant aquaporins facilitate the bi-directional diffusion of $As(OH)_3$ and $Sb(OH)_3$ across membranes. *BMC Plant Biol* 6: 26, 2008.

32. Bots M, Vergeldt F, Wolters-Arts M, Weterings K, van As H, Mariani C. Aquaporins of the PIP2 class are required for efficient anther dehiscence in tobacco. *Plant Physiol* 137: 1049–1056, 2005.

33. Boursiac Y, Boudet J, Postaire O, Luu DT, Tournaire-Roux C, Maurel C. Stimulus-induced downregulation of root water transport involves reactive oxygen species-activated cell signalling and plasma membrane intrinsic protein internalization. *Plant J* 56: 207–218, 2008.

34. Boursiac Y, Chen S, Luu DT, Sorieul M, van den Dries N, Maurel C. Early effects of salinity on water transport in *Arabidopsis* roots. Molecular and cellular features of aquaporin expression. *Plant Physiol* 139: 790–805, 2005.

35. Boursiac Y, Prak S, Boudet J, Postaire O, Luu DT, Tournaire-Roux C, Santoni V, Maurel C. The response of *Arabidopsis* root water transport to a challenging environment implicates reactive oxygen species- and phosphorylation-dependent internalization of aquaporins. *Plant Signal Behav* 3: 1096–1098, 2008.

36. Boyer JS, Silk WK. Hydraulics of plant growth. *Funct Plant Biol* 31: 761–773, 2004.

37. Bramley H, Turner NC, Turner DW, Tyerman SD. Roles of morphology, anatomy, and aquaporins in determining contrasting hydraulic behavior of roots. *Plant Physiol* 150: 348–364, 2009.

38. Calamita G, Ferri D, Gena P, Liquori G, Cavalier A, Thomas D, Svelto M. The inner mitochondrial membrane has aquaporin-8 water channels and is highly permeable to water. *J Biol Chem* 280: 17149–17153, 2005.

39. Caldeira CF, Jeanguenin L, Chaumont F, Tardieu F. Circadian rhythms of hydraulic conductance and growth are enhanced by drought and improve plant performance. *Nat Commun* 5: 5365, 2014.

40. Caldwell MM, Dawson TE, Richards JH. Hydraulic lift: consequences of water efflux from the roots of plants. *Oecologia* 113: 151–161, 1998.

41. Calvo-Polanco M, Molina S, Zamarreno AM, Garcia-Mina JM, Aroca R. The symbiosis with the arbuscular mycorrhizal fungus *Rhizophagus irregularis* drives root water transport in flooded tomato plants. *Plant Cell Physiol* 55: 1017–1029, 2014.

42. Carvajal M, Cooke DT, Clarkson DT. Responses of wheat plants to nutrient deprivation may involve the regulation of water-channel function. *Planta* 199: 372–381, 1996.

43. Carvajal M, Martinez V, Alcaraz CF. Physiological function of water channels as affected by salinity in roots of paprika pepper. *Physiol Plant* 105: 95–101, 1999.

44. Casado-Vela J, Muries B, Carvajal M, Iloro I, Elortza F, Martinez-Ballesta MC. Analysis of root plasma membrane aquaporins from Brassica oleracea: post-translational modifications, de novo sequencing and detection of isoforms by high resolution mass spectrometry. *J Proteome Res* 9: 3479–3494, 2010.

45. Chaumont F, Barrieu F, Wojcik E, Chrispeels MJ, Jung R. Aquaporins constitute a large and highly divergent protein family in maize. *Plant Physiol* 125: 1206–1215, 2001.

46. Chaumont F, Tyerman SD. Aquaporins: highly regulated channels controlling plant water relations. *Plant Physiol* 164: 1600–1618, 2014.

47. Chen W, Yin X, Wang L, Tian J, Yang R, Liu D, Yu Z, Ma N, Gao J. Involvement of rose aquaporin RhPIP1;1 in ethylene-regulated petal expansion through interaction with RhPIP2;1. *Plant Mol Biol* 83: 219–233, 2013.

48. Chevalier AS, Bienert GP, Chaumont F. A new LxxxA motif in the transmembrane Helix3 of maize aquaporins belonging to the plasma membrane intrinsic protein PIP2 group is required for their trafficking to the plasma membrane. *Plant Physiol* 166: 125–138, 2014.

49. Choat B, Jansen S, Brodribb TJ, Cochard H, Delzon S, Bhaskar R, Bucci SJ, Feild TS, Gleason SM, Hacke UG, Jacobsen AL, Lens F, Maherali H, Martinez-Vilalta J, Mayr S, Mencuccini M, Mitchell PJ, Nardini A, Pittermann J, Pratt RB, Sperry JS, Westoby M, Wright IJ, Zanne AE. Global convergence in the vulnerability of forests to drought. *Nature* 491: 752–755, 2012.

50. Choi WG, Roberts DM. Arabidopsis NIP2;1, a Major Intrinsic Protein transporter of lactic acid induced by anoxic stress. *J Biol Chem* 282: 24209–24218, 2007.

51. Cochard H, Venisse JS, Barigah TS, Brunel N, Herbette S, Guilliot A, Tyree MT, Sakr S. New insights into the understanding of variable hydraulic conductances in leaves. Evidence for a possible implication of plasma membrane aquaporins. *Plant Physiol* 143: 122–133, 2007.

52. Cohen D, Bogeat-Triboulot MB, Vialet-Chabrand S, Merret R, Courty PE, Moretti S, Bizet F, Guilliot A, Hummel I. Developmental and environmental regulation of *Aquaporin* gene expression across *Populus* species: divergence or redundancy? *PLoS One* 8: e55506, 2013.

53. Consortium AIM. Evidence for network evolution in an *Arabidopsis* interactome map. *Science* 333: 601–607, 2011.

54. Cosgrove DJ. Water uptake by growing cells: an assessment of the controlling roles of wall relaxation, solute uptake and hydraulic conductance. *Int J Plant Sci* 154: 10–21, 1993.

55. Cui XH, Hao FS, Chen H, Chen J, Wang XC. Expression of the *Vicia faba VfPIP1* gene in *Arabidopsis thaliana* plants improves their drought resistance. *J Plant Res* 121: 207–214, 2008.

56. Da Ines O, Graf W, Franck KI, Albert A, Winkler JB, Scherb H, Stichler W, Schäffner AR. Kinetic analyses of plant water relocation using deuterium as tracer—reduced water flux of *Arabidopsis* pip2 aquaporin knockout mutants. Plant Biol Suppl 1: 129–139, 2010.

57. Daniels MJ, Chrispeels MJ, Yeager M. Projection structure of a plant vacuole membrane aquaporin by electron cryo-crystallography. *J Mol Biol* 294: 1337–1349, 1999.

58. Daniels MJ, Mirkov TE, Chrispeels MJ. The plasma membrane of *Arabidopsis thaliana* contains a mercury-insensitive aquaporin that is a homolog of the tonoplast water channel protein TIP. *Plant Physiol* 106: 1325–1333, 1994.

59. Danielson JA, Johanson U. Unexpected complexity of the aquaporin gene family in the moss *Physcomitrella patens*. *BMC Plant Biol* 8: 45, 2008.

60. Dean RM, Rivers RL, Zeidel ML, Roberts DM. Purification and functional reconstitution of soybean Nodulin 26. An aquaporin with water and glycerol transport properties. *Biochemistry* 38: 347–353, 1999.

61. Dhonukshe P, Aniento F, Hwang I, Robinson DG, Mravec J, Stierhof YD, Frim LJ. Clathrin-mediated constitutive endocytosis of PIN auxin efflux carriers in *Arabidopsis*. *Curr Biol* 17: 520–527, 2007.

62. Di Pietro M, Vialaret J, Li G, Hem S, Rossignol M, Maurel C, Santoni V. Coordinated post-translational responses of aquaporins to abiotic and nutritional stimuli in *Arabidopsis* roots. *Mol Cell Proteomics* 12: 3886–3897, 2013.

63. Dietz S, von Bulow J, Beitz E, Nehls U. The aquaporin gene family of the ectomycorrhizal fungus *Laccaria bicolor*: lessons for symbiotic functions. *New Phytol* 190: 927–940, 2011.

64. Draye X, Kim Y, Lobet G, Javaux M. Model-assisted integration of physiological and environmental constraints affecting the dynamic and spatial patterns of root water uptake from soils. *J Exp Bot* 8: 2145–2155, 2010.

65. Durbak AR, Phillips KA, Pike S, O'Neill MA, Mares J, Gallavotti A, Malcomber ST, Gassmann W, McSteen P. Transport of boron by the *tassel-less1* aquaporin is critical

for vegetative and reproductive development in maize. *Plant Cell* 26: 2978–2995, 2014.

66. Dynowski M, Schaaf G, Loque D, Moran O, Ludewig U. Plant plasma membrane water channels conduct the signalling molecule H_2O_2. *Biochem J* 414: 53–61, 2008.

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

68. Eisenbarth DA, Weig AR. Dynamics of aquaporins and water relations during hypocotyl elongation in *Ricinus communis L*. seedlings. *J Exp Bot* 56: 1831–1842, 2005.

69. Evans JR, Kaldenhoff R, Genty B, Terashima I. Resistances along the CO_2 diffusion pathway inside leaves. *J Exp Bot* 60: 2235–2248, 2009.

70. Ferro M, Brugiere S, Salvi D, Seigneurin-Berny D, Court M, Moyet L, Ramus C, Miras S, Mellal M, Le Gall S, Kieffer-Jaquinod S, Bruley C, Garin J, Joyard J, Masselon C, Rolland N. AT_CHLORO, a comprehensive chloroplast proteome database with subplastidial localization and curated information on envelope proteins. *Mol Cell Proteomics* 9: 1063–1084, 2010.

71. Ferro M, Salvi D, Brugiere S, Miras S, Kowalski S, Louwagie M, Garin J, Joyard J, Rolland N. Proteomics of the chloroplast envelope membranes from *Arabidopsis thaliana*. *Mol Cell Proteomics* 2: 325–345, 2003.

72. Fetter K, Van Wilder V, Moshelion M, Chaumont F. Interactions between plasma membrane aquaporins modulate their water channel activity. *Plant Cell* 16: 215–228, 2004.

73. Flexas J, Diaz-Espejo A, Galmes J, Kaldenhoff R, Medrano H, Ribas-Carbo M. Rapid variations of mesophyll conductance in response to changes in CO_2 concentration around leaves. *Plant Cell Environ* 30: 1284–1298, 2007.

74. Flexas J, Ribas-Carbó M, Diaz-Espejo A, Galmés J, Medrano H. Mesophyll conductance to CO_2: current knowledge and future prospects. *Plant Cell Environ* 31: 602–621, 2008.

75. Flexas J, Ribas-Carbo M, Hanson DT, Bota J, Otto B, Cifre J, McDowell N, Medrano H, Kaldenhoff R. Tobacco aquaporin *NtAQP1* is involved in mesophyll conductance to CO_2 in vivo. *Plant J* 48: 427–439, 2006.

76. Forrest KL, Bhave M. The PIP and TIP aquaporins in wheat form a large and diverse family with unique gene structures and functionally important features. *Funct Integr Genomics* 8: 115–133, 2008.

77. Fournier-Level A, Korte A, Cooper MD, Nordborg M, Schmitt J, Wilczek AM. A map of local adaptation in *Arabidopsis thaliana*. *Science* 334: 86–89, 2011.

78. Frick A, Jarva M, Ekvall M, Uzdavinys P, Nyblom M, Tornroth-Horsefield S. Mercury increases water permeability of a plant aquaporin through a non-cysteine-related mechanism. *Biochem J* 454: 491–499, 2013.

79. Frick A, Jarva M, Tornroth-Horsefield S. Structural basis for pH gating of plant aquaporins. *FEBS Lett* 587: 989–993, 2013.

80. Gambetta GA, Fei J, Rost TL, Knipfer T, Matthews MA, Shackel KA, Walker MA, McElrone AJ. Water uptake along the length of grapevine fine roots: developmental anatomy, tissue-specific aquaporin expression, and pathways of water transport. *Plant Physiol* 163: 1254–1265, 2013.

81. Gao Z, He X, Zhao B, Zhou C, Liang Y, Ge R, Shen Y, Huang Z. Overexpressing a putative aquaporin gene from wheat, *TaNIP*, enhances salt tolerance in transgenic *Arabidopsis*. *Plant Cell Physiol* 51: 767–775, 2010.

82. Gattolin S, Sorieul M, Frigerio L. Mapping of tonoplast intrinsic proteins in maturing and germinating *Arabidopsis* seeds reveals dual localization of embryonic TIPs to the tonoplast and plasma membrane. *Mol Plant* 4: 180–189, 2011.

83. Gattolin S, Sorieul M, Hunter PR, Khonsari RH, Frigerio L. In vivo imaging of the tonoplast intrinsic protein family in *Arabidopsis* roots. *BMC Plant Biol* 9: 133, 2009.

84. Gavrin A, Kaiser BN, Geiger D, Tyerman SD, Wen Z, Bisseling T, Fedorova EE. Adjustment of host cells for accommodation of symbiotic bacteria: vacuole defunctionalization, HOPS suppression, and TIP1g retargeting in *Medicago*. *Plant Cell* 26: 3809–3822, 2014.

85. Gerbeau P, Amodeo G, Henzler T, Santoni V, Ripoche P, Maurel C. The water permeability of *Arabidopsis* plasma membrane is regulated by divalent cations and pH. *Plant J* 30: 71–81, 2002.

86. Gerbeau P, Guclu J, Ripoche P, Maurel C. Aquaporin Nt-TIPa can account for the high permeability of tobacco cell vacuolar membrane to small neutral solutes. *Plant J* 18: 577–587, 1999.

87. Gorska A, Ye Q, Holbrook NM, Zwieniecki MA. Nitrate control of root hydraulic properties in plants: translating local information to whole plant response. *Plant Physiol* 148: 1159–1167, 2008.

88. Gorska A, Zwieniecka A, Holbrook NM, Zwieniecki MA. Nitrate induction of root hydraulic conductivity in maize is not correlated with aquaporin expression. *Planta* 228: 989–998, 2008.

89. Gregoire C, Remus-Borel W, Vivancos J, Labbe C, Belzile F, Belanger RR. Discovery of a multigene family of aquaporin silicon transporters in the primitive plant *Equisetum arvense*. *Plant J* 72: 320–330, 2012.

90. Guenther JF, Chanmanivone N, Galetovic MP, Wallace IS, Cobb JA, Roberts DM. Phosphorylation of soybean Nodulin 26 on serine 262 enhances water permeability and is regulated developmentally and by osmotic signals. *Plant Cell* 15: 981–991, 2003.

91. Guenther JF, Roberts DM. Water-selective and multifunctional aquaporins from *Lotus japonicus* nodules. *Planta* 210: 741–748, 2000.

92. Guo L, Wang ZY, Lin H, Cui WE, Chen J, Liu M, Chen ZL, Qu LJ, Gu H. Expression and functional analysis of the rice plasma-membrane intrinsic protein gene family. *Cell Res* 16: 277–286, 2006.

93. Gupta AB, Sankararamakrishnan R. Genome-wide analysis of major intrinsic proteins in the tree plant *Populus trichocarpa*: characterization of XIP subfamily of aquaporins from evolutionary perspective. *BMC Plant Biol* 9: 134, 2009.

94. Gustavsson S, Lebrun A, Norden K, Chaumont F, Johanson U. A novel plant major intrinsic protein in *Physcomitrella patens* most similar to bacterial glycerol channels. *Plant Physiol* 139: 287–295, 2005.

95. Hachez C, Heinen RB, Draye X, Chaumont F. The expression pattern of plasma membrane aquaporins in maize leaf highlights their role in hydraulic regulation. *Plant Mol Biol* 68: 337–353, 2008.

96. Hachez C, Laloux T, Reinhardt H, Cavez D, Degand H, Grefen C, De Rycke R, Inze D, Blatt MR, Russinova E, Chaumont F. Arabidopsis SNAREs SYP61 and SYP121 coordinate the trafficking of plasma membrane aquaporin PIP2;7 to modulate the cell membrane water permeability. *Plant Cell* 26: 3132–3147, 2014.

97. Hachez C, Moshelion M, Zelazny E, Cavez D, Chaumont F. Localization and quantification of plasma membrane aquaporin expression in maize primary root: a clue to understanding their role as cellular plumbers. *Plant Mol Biol* 62: 305–323, 2006.

98. Hachez C, Veljanovski V, Reinhardt H, Guillaumot D, Vanhee C, Chaumont F, Batoko H. The *Arabidopsis* abiotic stress-induced TSPO-related protein reduces cell-surface expression of the aquaporin PIP2;7 through protein-protein interactions and autophagic degradation. *Plant Cell* 26: 4974–4990, 2014.

99. Hachez C, Veselov D, Ye Q, Reinhardt H, Knipfer T, Fricke W, Chaumont F. Short-term control of maize cell and root water permeability through plasma membrane aquaporin isoforms. *Plant Cell Environ* 35: 185–198, 2012.

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

101. Heckwolf M, Pater D, Hanson DT, Kaldenhoff R. The *Arabidopsis thaliana* aquaporin *AtPIP1;2* is a physiologically relevant CO_2 transport facilitator. *Plant J* 67: 795–804, 2011.

102. Hedfalk K, Tornroth-Horsefield S, Nyblom M, Johanson U, Kjellbom P, Neutze R. Aquaporin gating. *Curr Opin Struct Biol* 16: 447–456, 2006.

103. Henzler T, Waterhouse RN, Smyth AJ, Carvajal M, Cooke DT, Schaffner AR, Steudle E, Clarkson DT. Diurnal variations in hydraulic conductivity and root pressure can be correlated with the expression of putative aquaporins in the roots of *Lotus japonicus*. *Planta* 210: 50–60, 1999.

104. Holbrook NM, Zwieniecki MA. Embolism repair and xylem tension: do we need a miracle? *Plant Physiol* 120: 7–10, 1999.

105. Hooijmaijers C, Rhee JY, Kwak KJ, Chung GC, Horie T, Katsuhara M, Kang H. Hydrogen peroxide permeability of plasma membrane aquaporins of *Arabidopsis thaliana*. *J Plant Res* 125: 147–153, 2012.

106. Hose E, Steudle E, Hartung W. Abscisic acid and hydraulic conductivity of maize roots: a study using cell- and root-pressure probes. *Planta* 211: 874–882, 2000.

107. Hosy E, Martiniere A, Choquet D, Maurel C, Luu DT. Super-resolved and dynamic imaging of membrane proteins in plant cells reveal contrasting kinetic profiles and multiple confinement mechanisms. *Mol Plant* 8: 339–342, 2015.

108. Hu L, Cui D, Neill S, Cai W. *OsEXPA4* and *OsRWC3* are involved in asymmetric growth during gravitropic bending of rice leaf sheath bases. *Physiol Plant* 130: 560–571, 2007.

109. Huang X, Zhao Y, Wei X, Li C, Wang A, Zhao Q, Li W, Guo Y, Deng L, Zhu C, Fan D, Lu Y, Weng Q, Liu K, Zhou T, Jing Y, Si L, Dong G, Huang T, Lu T, Feng Q, Qian Q, Li J, Han B. Genome-wide association study of flowering time and grain yield traits in a worldwide collection of rice germplasm. *Nat Genet* 44: 32–39, 2012.

110. Hukin D, Doering-Saad C, Thomas CR, Pritchard J. Sensitivity of cell hydraulic conductivity to mercury is coincident with symplasmic isolation and expression of plasmalemma aquaporin genes in growing maize roots. *Planta* 215: 1047–1056, 2002.

111. Hunter PR, Craddock CP, Di Benedetto S, Roberts LM, Frigerio L. Fluorescent reporter proteins for the tonoplast and the vacuolar lumen identify a single vacuolar compartment in *Arabidopsis* cells. *Plant Physiol* 145: 1371–1382, 2007.

112. Hwang JH, Ellingson SR, Roberts DM. Ammonia permeability of the soybean nodulin 26 channel. *FEBS Lett* 584: 4339–4343, 2010.

113. Ikeda M, Beitz E, Kozono D, Guggino WB, Agre P, Yasui M. Characterization of aquaporin-6 as a nitrate channel in mammalian cells. Requirement of pore-lining residue threonine 63. *J Biol Chem* 277: 39873–39879, 2002.

114. Ishikawa F, Suga S, Uemura T, Sato MH, Maeshima M. Novel type aquaporin SIPs are mainly localized to the ER membrane and show cell-specific expression in *Arabidopsis thaliana*. *FEBS Lett* 579: 5814–5820, 2005.

115. Jahn TP, Moller AL, Zeuthen T, Holm LM, Klaerke DA, Mohsin B, Kuhlbrandt W, Schjoerring JK. Aquaporin homologues in plants and mammals transport ammonia. *FEBS Lett* 574: 31–36, 2004.

116. Jang JY, Kim DG, Kim YO, Kim JS, Kang H. An expression analysis of a gene family encoding plasma membrane aquaporins in response to abiotic stresses in *Arabidopsis thaliana*. *Plant Mol Biol* 54: 713–725, 2004.

117. Jang JY, Rhee JY, Kim DG, Chung GC, Lee JH, Kang H. Ectopic expression of a foreign aquaporin disrupts the natural expression patterns of endogenous aquaporin genes and alters plant responses to different stress conditions. *Plant Cell Physiol* 48: 1331–1339, 2007.

118. Jauh GY, Phillips TE, Rogers JC. Tonoplast intrinsic protein isoforms as markers for vacuolar functions. *Plant Cell* 11: 1867–1882, 1999.

119. Javot H, Lauvergeat V, Santoni V, Martin-Laurent F, Guclu J, Vinh J, Heyes J, Franck KI, Schäffner AR, Bouchez D, Maurel C. Role of a single aquaporin isoform in root water uptake. *Plant Cell* 15: 509–522, 2003.

120. Johanson U, Gustavsson S. A new subfamily of Major Intrinsic Proteins in plants. *Mol Biol Evol* 19: 456–461, 2002.

121. Johanson U, Karlsson M, Gustavsson S, Sjovall S, Fraysse L, Weig AR, Kjellbom P. The complete set of genes encoding Major Intrinsic Proteins in *Arabidopsis* provides a framework for a new nomenclature for Major Intrinsic Proteins in plants. *Plant Physiol* 126: 1358–1369, 2001.

122. Johansson I, Karlsson M, Shukla VK, Chrispeels MJ, Larsson C, Kjellbom P. Water transport activity of the plasma membrane aquaporin PM28A is regulated by phosphorylation. *Plant Cell* 10: 451–460, 1998.

123. Jones AM, Xuan Y, Xu M, Wang RS, Ho CH, Lalonde S, You CH, Sardi MI, Parsa SA, Smith-Valle E, Su T, Frazer KA, Pilot G, Pratelli R, Grossmann G, Acharya BR, Hu HC, Engineer C, Villiers F, Ju C, Takeda K, Su Z, Dong Q, Assmann SM, Chen J, Kwak JM, Schroeder JI, Albert R, Rhee SY, Frommer WB. Border control–a membrane-linked interactome of *Arabidopsis*. *Science* 344: 711–716, 2014.

124. Jozefkowicz C, Rosi P, Sigaut L, Soto G, Pietrasanta LI, Amodeo G, Alleva K. Loop A is critical for the functional interaction of two *Beta vulgaris* PIP aquaporins. *PLoS One* 8: e57993, 2013.

125. Kaldenhoff R. Mechanisms underlying CO_2 diffusion in leaves. *Curr Opin Plant Biol* 15: 276–281, 2012.

126. Kamaluddin M, Zwiazek JJ. Ethylene enhances water transport in hypoxic aspen. *Plant Physiol* 128: 962–969, 2002.

127. Kamiya T, Tanaka M, Mitani N, Ma JF, Maeshima M, Fujiwara T. NIP1;1, an aquaporin homolog, determines the arsenite sensitivity of *Arabidopsis thaliana*. *J Biol Chem* 284: 2114–2120, 2009.

128. Kammerloher W, Fischer U, Piechottka GP, Schäffner AR. Water channels in the plant plasma membrane cloned by immunoselection from a mammalian expression system. *Plant J* 6: 187–199, 1994.

129. Kato Y, Miwa K, Takano J, Wada M, Fujiwara T. Highly boron deficiency-tolerant plants generated by enhanced expression of NIP5;1, a boric acid channel. *Plant Cell Physiol* 50: 58–66, 2009.

130. Katsuhara M, Sasano S, Horie T, Matsumoto T, Rhee J, Shibasaka M. Functional and molecular characteristics of rice and barley NIP aquaporins transporting water, hydrogen peroxide and arsenite. *Plant Biotech* 31: 213–219, 2014.

131. Kierszniowska S, Seiwert B, Schulze WX. Definition of *Arabidopsis* sterol-rich membrane microdomains by differential treatment with methyl-β-cyclodextrin and quantitative proteomics. *Mol Cell Proteomics* 8: 612–623, 2009.

132. Kim DY, Scalf M, Smith LM, Vierstra RD. Advanced proteomic analyses yield a deep catalog of ubiquitylation targets in *Arabidopsis*. *Plant Cell* 25: 1523–1540, 2013.

133. Kim MJ, Kim HR, Paek KH. *Arabidopsis* tonoplast proteins TIP1 and TIP2 interact with the cucumber mosaic virus 1a replication protein. *J Gen Virol* 87: 3425–3431, 2006.

134. Kirch HH, Vera-Estrella R, Golldack D, Quigley F, Michalowski CB, Barkla BJ, Bohnert HJ. Expression of water channel proteins in *Mesembryanthemum crystallinum*. *Plant Physiol* 123: 111–124, 2000.

135. Kline KG, Barrett-Wilt GA, Sussman MR. In planta changes in protein phosphorylation induced by the plant hormone abscisic acid. *Proc Natl Acad Sci USA* 107: 15986–15991, 2010.

136. Knipfer T, Besse M, Verdeil JL, Fricke W. Aquaporin-facilitated water uptake in barley (*Hordeum vulgare L.*) roots. *J Exp Bot* 62: 4115–4126, 2011.

137. Knipfer T, Fricke W. Water uptake by seminal and adventitious roots in relation to whole-plant water flow in barley (*Hordeum vulgare L.*). *J Exp Bot* 62: 717–733, 2011.

138. Komsic-Buchmann K, Wostehoff L, Becker B. The contractile vacuole as a key regulator of cellular water flow in *Chlamydomonas reinhardtii*. *Eukaryot Cell* 13: 1421–1430, 2014.

139. Kukulski W, Schenk AD, Johanson U, Braun T, de Groot BL, Fotiadis D, Kjellbom P, Engel A. The 5A structure of heterologously expressed plant aquaporin SoPIP2;1. *J Mol Biol* 350: 611–616, 2005.

140. Lalonde S, Sero A, Pratelli R, Pilot G, Chen J, Sardi MI, Parsa SA, Kim DY, Acharya BR, Stein EV, Hu HC, Villiers F, Takeda K, Yang Y, Han YS, Schwacke R, Chiang W, Kato N, Loque D, Assmann SM, Kwak JM, Schroeder JI, Rhee SY, Frommer WB. A membrane protein/signaling protein interaction network for *Arabidopsis* version AMPv2. *Front Physiol* 1: 24, 2010.

141. Laur J, Hacke UG. Exploring *Picea glauca* aquaporins in the context of needle water uptake and xylem refilling. *New Phytol* 203: 388–400, 2014.

142. Laur J, Hacke UG. Transpirational demand affects aquaporin expression in poplar roots. *J Exp Bot* 64: 2283–2293, 2013.

143. Lee HK, Cho SK, Son O, Xu Z, Hwang I, Kim WT. Drought stress-induced Rma1H1, a RING membrane-anchor E3 ubiquitin ligase homolog, regulates aquaporin levels via ubiquitination in transgenic *Arabidopsis* plants. *Plant Cell* 21: 622–641, 2009.

144. Lee SH, Chung GC, Jang JY, Ahn SJ, Zwiazek JJ. Overexpression of PIP2;5 aquaporin alleviates effects of low root temperature on cell hydraulic conductivity and growth in *Arabidopsis*. *Plant Physiol* 159: 479–488, 2012.

145. Lee SH, Singh AP, Chung GC. Rapid accumulation of hydrogen peroxide in cucumber roots due to exposure to low temperature appears to mediate decreases in water transport. *J Exp Bot* 55: 1733–1741, 2004.

146. Leitao L, Prista C, Moura TF, Loureiro-Dias MC, Soveral G. Grapevine aquaporins: gating of a tonoplast intrinsic protein (TIP2;1) by cytosolic pH. *PLoS One* 7: e33219, 2012.

147. Li DD, Ruan XM, Zhang J, Wu YJ, Wang XL, Li XB. Cotton plasma membrane intrinsic protein 2s (PIP2s) selectively interact to regulate their water channel activities and are required for fibre development. *New Phytol* 199: 695–707, 2013.

148. Li T, Choi WG, Wallace IS, Baudry J, Roberts DM. *Arabidopsis thaliana* NIP7;1: an anther-specific boric acid transporter of the aquaporin superfamily regulated by an unusual tyrosine in helix 2 of the transport pore. *Biochemistry* 50: 6633–6641, 2011.

149. Li T, Hu YJ, Hao ZP, Li H, Wang YS, Chen BD. First cloning and characterization of two functional aquaporin genes from an arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytol* 197: 617–630, 2013.

150. Li X, Wang X, Yang Y, Li R, He Q, Fang X, Luu DT, Maurel C, Lin J. Single-molecule analysis of PIP2;1 dynamics and partitioning reveals multiple modes of *Arabidopsis* plasma membrane aquaporin regulation. *Plant Cell* 23: 3780–3797, 2011.

151. Li Y, Mao X, Tian Q, Li L, Zhang W. Phosphorus deficiency-induced reduction in root hydraulic conductivity in *Medicago falcata* is associated with ethylene production. *Environ Exp Bot* 67: 172–177, 2009.

152. Lian HL, Yu X, Lane D, Sun WN, Tang ZC, Su W. Upland rice and lowland rice exhibited different PIP expression under water deficit and ABA treatment. *Cell Res* 16: 651–660, 2006.

153. Lian HL, Yu X, Ye Q, Ding XS, Kitagawa Y, Kwak SS, Su WA, Tang ZC. The role of aquaporin RWC3 in drought avoidance in rice. *Plant Cell Physiol* 45: 481–489, 2004.

154. Lin W, Peng Y, Li G, Arora R, Tang Z, Su W, Cai W. Isolation and functional characterization of *PgTIP1*, a hormone-autotrophic cells-specific tonoplast aquaporin in ginseng. *J Exp Bot* 58: 947–956, 2007.

155. Liu C, Fukumoto T, Matsumoto T, Gena P, Frascaria D, Kaneko T, Katsuhara M, Zhong S, Sun X, Zhu Y, Iwasaki I, Ding X, Calamita G, Kitagawa Y. Aquaporin OsPIP1;1 promotes rice salt resistance and seed germination. *Plant Physiol Biochem* 63: 151–158, 2013.

156. Liu F, Vantoai T, Moy LP, Bock G, Linford LD, Quackenbush J. Global transcription profiling reveals comprehensive insights into hypoxic response in *Arabidopsis*. *Plant Physiol* 137: 1115–1129, 2005.

157. Liu HY, Sun WN, Su WA, Tang ZC. Co-regulation of water channels and potassium channels in rice. *Physiol Plant* 128: 58–69, 2006.

158. Liu HY, Yu X, Cui DY, Sun MH, Sun WN, Tang ZC, Kwak SS, Su WA. The role of water channel proteins and nitric oxide signaling in rice seed germination. *Cell Res* 17: 638–649, 2007.

159. Liu J, Equiza MA, Navarro-Rodenas A, Lee SH, Zwiazek JJ. Hydraulic adjustments in aspen (*Populus tremuloides*) seedlings following defoliation involve root and leaf aquaporins. *Planta* 240: 553–564, 2014.

160. Liu LH, Ludewig U, Gassert B, Frommer WB, von Wiren N. Urea transport by nitrogen-regulated Tonoplast Intrinsic Proteins in *Arabidopsis*. *Plant Physiol* 133: 1220–1228, 2003.

161. Liu P, Yin L, Deng X, Wang S, Tanaka K, Zhang S. Aquaporin-mediated increase in root hydraulic conductance is involved in silicon-induced improved root water uptake under osmotic stress in *Sorghum bicolor* L. *J Exp Bot* 65: 4747–4756, 2014.

162. Lopez D, Bronner G, Brunel N, Auguin D, Bourgerie S, Brignolas F, Carpin S, Tournaire-Roux C, Maurel C, Fumanal B, Martin F, Sakr S, Label P, Julien JL, Gousset-Dupont A, Venisse JS. Insights into *Populus* XIP aquaporins: evolutionary expansion, protein functionality, and environmental regulation. *J Exp Bot* 63: 2217–2230, 2012.

163. Lopez F, Bousser A, Sissoeff I, Gaspar M, Lachaise B, Hoarau J, Mahe A. Diurnal regulation of water transport and aquaporin gene expression in maize roots: contribution of PIP_2 proteins. *Plant Cell Physiol* 44: 1384–1395, 2003.

164. Loque D, Ludewig U, Yuan L, von Wiren N. Tonoplast Intrinsic Proteins AtTIP2;1 and AtTIP2;3 facilitate NH_3 transport into the vacuole. *Plant Physiol* 137: 671–680, 2005.

165. Lovisolo C, Schubert A. Mercury hinders recovery of shoot hydraulic conductivity during grapevine rehydration: evidence from a whole-plant approach. *New Phytol* 172: 469–478, 2006.

166. Ludevid D, Höfte H, Himelblau E, Chrispeels MJ. The expression pattern of the Tonoplast Intrinsic Protein γ-TIP in *Arabidopsis thaliana* is correlated with cell enlargement. *Plant Physiol* 100: 1633–1639, 1992.

167. Luu DT, Martinière A, Sorieul M, Runions J, Maurel C. Fluorescence recovery after photobleaching reveals high cycling dynamics of plasma membrane aquaporins in *Arabidopsis* roots under salt stress. *Plant J* 69: 894–905, 2012.

168. Luu DT, Maurel C. Aquaporin trafficking in plant cells: an emerging membrane-protein model. *Traffic* 14: 629–635, 2013.

169. Ma JF, Tamai K, Yamaji N, Mitani N, Konishi S, Katsuhara M, Ishiguro M, Murata Y, Yano M. A silicon transporter in rice. *Nature* 440: 688–691, 2006.

170. Ma JF, Yamaji N, Mitani N, Xu XY, Su YH, McGrath SP, Zhao FJ. Transporters of arsenite in rice and their role in arsenic accumulation in rice grain. *Proc Natl Acad Sci USA* 105: 9931–9935, 2008.

171. Ma N, Xue J, Li Y, Liu X, Dai F, Jia W, Luo Y, Gao J. *Rh-PIP2;1*, a rose aquaporin gene, is involved in ethylene-regulated petal expansion. *Plant Physiol* 148: 894–907, 2008.

172. Maathuis FJ, Filatov V, Herzyk P, Krijger GC, Axelsen KB, Chen S, Green BJ, Li Y, Madagan KL, Sanchez-Fernandez R, Forde BG, Palmgren MG, Rea PA, Williams LE, Sanders D, Amtmann A. Transcriptome analysis of root transporters reveals participation of multiple gene families in the response to cation stress. *Plant J* 35: 675–692, 2003.

173. MacRobbie EA. Osmotic effects on vacuolar ion release in guard cells. *Proc Natl Acad Sci USA* 103: 1135–1140, 2006.

174. Maeshima M, Ishikawa F. ER membrane aquaporins in plants. *Pflügers Arch* 456: 709–716, 2008.

175. Maggio A, Joly RJ. Effects of mercuric chloride on the hydraulic conductivity of tomato root systems. Evidence for a channel-mediated water pathway. *Plant Physiol* 109: 331–335, 1995.

176. Mahdieh M, Mostajeran A, Horie T, Katsuhara M. Drought stress alters water relations and expression of *PIP*-type aquaporin genes in *Nicotiana tabacum* plants. *Plant Cell Physiol* 49: 801–813, 2008.

177. Mariaux JB, Bockel C, Salamini F, Bartels D. Desiccation- and abscisic acid-responsive genes encoding major intrinsic proteins (MIPs) from the resurrection plant *Craterostigma plantagineum*. *Plant Mol Biol* 38: 1089–1099, 1998.

178. Marin-Olivier M, Chevalier T, Fobis-Loisy I, Dumas C, Gaude T. Aquaporin PIP genes are not expressed in the stigma papillae in *Brassica oleracea*. *Plant J* 24: 231–240, 2000.

179. Marjanovic Z, Uehlein N, Kaldenhoff R, Zwiazek JJ, Weiss M, Hampp R, Nehls U. Aquaporins in poplar: what a difference a symbiont makes! *Planta* 222: 258–268, 2005.

180. Martinez-Ballesta Mdel C, Carvajal M. New challenges in plant aquaporin biotechnology. *Plant Sci* 217–218: 71–77, 2014.

181. Martiniere A, Lavagi I, Nageswaran G, Rolfe DJ, Maneta-Peyret L, Luu DT, Botchway SW, Webb SE, Mongrand S, Maurel C, Martin-Fernandez ML, Kleine-Vehn J, Friml J, Moreau P, Runions J. Cell wall constrains lateral diffusion of plant plasma-membrane proteins. *Proc Natl Acad Sci USA* 109: 12805–12810, 2012.

182. Martre P, Morillon R, Barrieu F, North GB, Nobel PS, Chrispeels MJ. Plasma membrane aquaporins play a significant role during recovery from water deficit. *Plant Physiol* 130: 2101–2110, 2002.

183. Marulanda A, Azcon R, Chaumont F, Ruiz-Lozano JM, Aroca R. Regulation of plasma membrane aquaporins by inoculation with a *Bacillus megaterium* strain in maize (*Zea mays L.*) plants under unstressed and salt-stressed conditions. *Planta* 232: 533–543, 2010.

184. Masalkar P, Wallace IS, Hwang JH, Roberts DM. Interaction of cytosolic glutamine synthetase of soybean root nodules with the C-terminal domain of the symbiosome membrane nodulin 26 aquaglyceroporin. *J Biol Chem* 285: 23880–23888, 2010.

185. Maurel C. Aquaporins and water permeability of plant membranes. *Annu Rev Plant Physiol Plant Mol Biol* 48: 399–429, 1997.

186. Maurel C, Kado RT, Guern J, Chrispeels MJ. Phosphorylation regulates the water channel activity of the seed-specific aquaporin α-TIP. *EMBO J* 14: 3028–3035, 1995.

187. Maurel C, Reizer J, Schroeder JI, Chrispeels MJ. The vacuolar membrane protein γ-TIP creates water specific channels in *Xenopus* oocytes. *EMBO J* 12: 2241–2247, 1993.

188. Maurel C, Tacnet F, Guclu J, Guern J, Ripoche P. Purified vesicles of tobacco cell vacuolar and plasma membranes exhibit dramatically different water permeability and water channel activity. *Proc Natl Acad Sci USA* 94: 7103–7108, 1997.

189. Maurel C, Verdoucq L, Luu DT, Santoni V. Plant aquaporins: membrane channels with multiple integrated functions. *Annu Rev Plant Biol* 59: 595–624, 2008.

190. McElrone AJ, Bichler J, Pockman WT, Addington RN, Linder CR, Jackson RB. Aquaporin-mediated changes in hydraulic conductivity of deep tree roots accessed via caves. *Plant Cell Environ* 30: 1411–1421, 2007.

191. McLean EH, Ludwig M, Grierson PF. Root hydraulic conductance and aquaporin abundance respond rapidly to partial root-zone drying events in a riparian *Melaleuca* species. *New Phytol* 192: 664–675, 2011.

192. Miller EW, Dickinson BC, Chang CJ. Aquaporin-3 mediates hydrogen peroxide uptake to regulate downstream intracellular signaling. *Proc Natl Acad Sci USA* 107: 15681–15686, 2010.

193. Mitani-Ueno N, Yamaji N, Zhao FJ, Ma JF. The aromatic/arginine selectivity filter of NIP aquaporins plays a critical role in substrate selectivity for silicon, boron, and arsenic. *J Exp Bot* 62: 4391–4398, 2011.

194. Mitani N, Yamaji N, Ma JF. Characterization of substrate specificity of a rice silicon transporter, Lsi1. *Pflügers Arch* 456: 679–686, 2008.

195. Mitani N, Yamaji N, Ma JF. Identification of maize silicon influx transporters. *Plant Cell Physiol* 50: 5–12, 2009.

196. Mizokami Y, Noguchi K, Kojima M, Sakakibara H, Terashima I. Mesophyll conductance decreases in the wild type but not in an ABA-deficient mutant (*aba1*) of *Nicotiana plumbaginifolia* under drought conditions. *Plant Cell Environ* 38: 388–398, 2015.

197. Mizutani M, Watanabe S, Nakagawa T, Maeshima M. Aquaporin NIP2;1 is mainly localized to the ER membrane and shows root-specific accumulation in *Arabidopsis thaliana*. *Plant Cell Physiol* 47: 1420–1426, 2006.

198. Moeller HB, Fenton RA. Cell biology of vasopressin-regulated aquaporin-2 trafficking. *Pflügers Arch* 464: 133–144, 2012.

199. Mongrand S, Morel J, Laroche J, Claverol S, Carde JP, Hartmann MA, Bonneu M, Simon-Plas F, Lessire R, Bessoule JJ. Lipid rafts in higher plant cells: purification and characterization of Triton X-100-insoluble microdomains from tobacco plasma membrane. *J Biol Chem* 279: 36277–36286, 2004.

200. Monneuse JM, Sugano M, Becue T, Santoni V, Hem S, Rossignol M. Towards the profiling of the *Arabidopsis thaliana* plasma membrane transportome by targeted proteomics. *Proteomics* 11: 1789–1797, 2011.

201. Montpetit J, Vivancos J, Mitani-Ueno N, Yamaji N, Remus-Borel W, Belzile F, Ma JF, Belanger RR. Cloning, functional characterization and heterologous expression of *TaLsi1*, a wheat silicon transporter gene. *Plant Mol Biol* 79: 35–46, 2012.

202. Morel J, Claverol S, Mongrand S, Furt F, Fromentin J, Bessoule JJ, Blein JP, Simon-Plas F. Proteomics of plant detergent-resistant membranes. *Mol Cell Proteomics* 5: 1396–1411, 2006.

203. Mori IC, Rhee J, Shibasaka M, Sasano S, Kaneko T, Horie T, Katsuhara M. CO_2 transport by PIP2 aquaporins of barley. *Plant Cell Physiol* 55: 251–257, 2014.

204. Morillon R, Lassalles JP. Osmotic water permeability of isolated vacuoles. *Planta* 210: 80–84, 1999.

205. Moshelion M, Becker D, Biela A, Uehlein N, Hedrich R, Otto B, Levi H, Moran N, Kaldenhoff R. Plasma membrane aquaporins in the motor cells of *Samanea saman*: diurnal and circadian regulation. *Plant Cell* 14: 727–739, 2002.

206. Moshelion M, Moran N, Chaumont F. Dynamic changes in the osmotic water permeability of protoplast plasma membrane. *Plant Physiol* 135: 2301–2317, 2004.

207. Mubarakshina-Borisova MM, Kozuleva MA, Rudenko NN, Naydov IA, Klenina IB, Ivanov BN. Photosynthetic electron flow to oxygen and diffusion of hydrogen peroxide through the chloroplast envelope via aquaporins. *Biochim Biophys Acta* 1817: 1314–1321, 2012.

208. Murai-Hatano M, Kuwagata T. Osmotic water permeability of plasma and vacuolar membranes in protoplasts I: high osmotic water permeability in radish (*Raphanus sativus*) root cells as measured by a new method. *J Plant Res* 120: 175–189, 2007.

209. Murozuka E, Hanisch S, Pomorski TG, Jahn TP, Schjoerring JK. Bimolecular fluorescence complementation and interaction of various *Arabidopsis* major intrinsic proteins expressed in yeast. *Physiol Plant* 148: 422–431, 2013.

210. Nardini A, Salleo S, Andri S. Circadian regulation of leaf hydraulic conductance in sunflower (*Helianthus annuus L. cv Margot*). *Plant Cell Environ* 28: 750–759, 2005.

211. Negishi T, Oshima K, Hattori M, Kanai M, Mano S, Nishimura M, Yoshida K. Tonoplast- and plasma membrane-localized aquaporin-family transporters in blue hydrangea sepals of aluminum hyperaccumulating plant. *PLoS One* 7: e43189, 2012.

212. Niemietz CM, Tyerman SD. Characterization of water channels in wheat root membrane vesicles. *Plant Physiol* 115: 561–567, 1997.

213. Niemietz CM, Tyerman SD. New potent inhibitors of aquaporins: silver and gold compounds inhibit aquaporins of plant and human origin. *FEBS Lett* 531: 443–447, 2002.

214. Noronha H, Agasse A, Martins AP, Berny MC, Gomes D, Zarrouk O, Thiebaud P, Delrot S, Soveral G, Chaumont F, Geros H. The grape aquaporin VvSIP1 transports water across the ER membrane. *J Exp Bot* 65: 981–993, 2014.

215. Novikova GV, Tournaire-Roux C, Sinkevich IA, Lityagina SV, Maurel C, Obroucheva N. Vacuolar biogenesis and aquaporin expression at early germination of broad bean seeds. *Plant Physiol Biochem* 82: 123–132, 2014.

216. Nuhse TS, Stensballe A, Jensen ON, Peck SC. Phosphoproteomics of the *Arabidopsis* plasma membrane and a new phosphorylation site database. *Plant Cell* 16: 2394–2405, 2004.

217. Nyblom M, Frick A, Wang Y, Ekvall M, Hallgren K, Hedfalk K, Neutze R, Tajkhorshid E, Törnroth-Horsefield S. Structural and functional analysis of SoPIP2;1 mutants add insight into plant aquaporin gating. *J Mol Biol* 387: 653–668, 2009.

218. Ohrui T, Nobira H, Sakata Y, Taji T, Yamamoto C, Nishida K, Yamakawa T, Sasuga Y, Yaguchi Y, Takenaga H, Tanaka S. Foliar trichome- and aquaporin-aided water uptake in a drought-resistant epiphyte *Tillandsia ionantha* Planchon. *Planta* 227: 47–56, 2007.

219. Okubo-Kurihara E, Sano T, Higaki T, Kutsuna N, Hasezawa S. Acceleration of vacuolar regeneration and cell growth by overexpression of an aquaporin *Nt*TIP1;1 in tobacco BY-2 cells. *Plant Cell Physiol* 50: 151–160, 2009.

220. Opperman CH, Taylor CG, Conkling MA. Root-knot nematode-directed expression of a plant root-specific gene. *Science* 263: 221–223, 1994.

221. Otto B, Uehlein N, Sdorra S, Fischer M, Ayaz M, Belastegui-Macadam X, Heckwolf M, Lachnit M, Pede N, Priem N, Reinhard A, Siegfart S, Urban M, Kaldenhoff R. Aquaporin tetramer composition modifies the function of tobacco aquaporins. *J Biol Chem* 285: 31253–31260, 2010.

222. Pang Y, Li L, Ren F, Lu P, Wei P, Cai J, Xin L, Zhang J, Chen J, Wang X. Overexpression of the tonoplast aquaporin AtTIP5;1 conferred tolerance to boron toxicity in *Arabidopsis*. *J Genet Genomics* 37: 389–397, 2010.

223. Parent B, Hachez C, Redondo E, Simonneau T, Chaumont F, Tardieu F. Drought and abscisic acid effects on aquaporin content translate into changes in hydraulic conductivity and leaf growth rate: a trans-scale approach. *Plant Physiol* 149: 2000–2012, 2009.

224. Park W, Scheffler BE, Bauer PJ, Campbell BT. Identification of the family of aquaporin genes and their expression in upland cotton (*Gossypium hirsutum L.*). *BMC Plant Biol* 10: 142, 2010.

225. Pei H, Ma N, Tian J, Luo J, Chen J, Li J, Zheng Y, Chen X, Fei Z, Gao J. An NAC transcription factor controls ethylene-regulated cell expansion in flower petals. *Plant Physiol* 163: 775–791, 2013.

226. Péret B, Li GW, Zhao J, Band LR, Voß U, Postaire O, Luu DT, Da Ines O, Casimiro I, Lucas M, Wells DM, Lazzerini L, Nacry P, King JR, Jensen OE, Schäffner AR, Maurel C, Bennett MJ. Auxin regulates aquaporin function to facilitate lateral root emergence. *Nature Cell Biol* 14: 991–998, 2012.

227. Perrone I, Gambino G, Chitarra W, Vitali M, Pagliarani C, Riccomagno N, Balestrini R, Kaldenhoff R, Uehlein N, Gribaudo I, Schubert A, Lovisolo C. The grapevine root-specific aquaporin VvPIP2;4N controls root hydraulic conductance and leaf gas exchange under well-watered conditions but not under water stress. *Plant Physiol* 160: 965–977, 2012.

228. Phillips AL, Huttly AK. Cloning of two gibberellin-regulated cDNAs from *Arabidopsis thaliana* by subtractive hybridization: expression of the tonoplast water channel, γ-TIP, is increased by GA_3. *Plant Mol Biol* 24: 603–615, 1994.

229. Porcel R, Aroca R, Azcon R, Ruiz-Lozano JM. PIP aquaporin gene expression in arbuscular mycorrhizal Glycine max and *Lactuca sativa* plants in relation to drought stress tolerance. *Plant Mol Biol* 60: 389–404, 2006.

230. Postaire O, Tournaire-Roux C, Grondin A, Boursiac Y, Morillon R, Schäffner T, Maurel C. A PIP1 aquaporin contributes to hydrostatic pressure-induced water transport in both the root and rosette of *Arabidopsis*. *Plant Physiol* 152: 1418–1430, 2010.

231. Pou A, Medrano H, Flexas J, Tyerman SD. 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 Environ* 36: 828–843, 2013.

232. Prado K, Boursiac Y, Tournaire-Roux C, Monneuse JM, Postaire O, Da Ines O, Schäffner AR, Hem S, Santoni V, Maurel C. Regulation of *Arabidopsis* leaf hydraulics involves light-dependent phosphorylation of aquaporins in veins. *Plant Cell* 25: 1029–1039, 2013.

233. Prado K, Maurel C. Regulation of leaf hydraulics: from molecular to whole plant levels. *Front Plant Sci* 4: 255, 2013.

234. Prak S, Hem S, Boudet J, Viennois G, Sommerer N, Rossignol M, Maurel C, Santoni V. Multiple phosphorylations in the C-terminal tail of plant plasma membrane aquaporins. Role in sub-cellular trafficking of AtPIP2;1 in response to salt stress. *Mol Cell Proteomics* 7: 1019–1030, 2008.

235. Quigley F, Rosenberg JM, Shachar-Hill Y, Bohnert HJ. From genome to function: the *Arabidopsis* aquaporins. *Genome Biol* 3: 1–17, 2001.

236. Quintero J, Fournier J, Ramos J, Benlloch M. K^+ status and ABA affect both exudation rate and hydraulic conductivity in sunflower roots. *Physiol Plant* 102: 279–284, 1998.

237. Rae L, Lao NT, Kavanagh TA. Regulation of multiple aquaporin genes in *Arabidopsis* by a pair of recently duplicated DREB transcription factors. *Planta* 234: 429–444, 2011.

238. Ranathunge K, Schreiber L. Water and solute permeabilities of *Arabidopsis* roots in relation to the amount and composition of aliphatic suberin. *J Exp Bot* 62: 1961–1974, 2011.

239. Reuscher S, Akiyama M, Mori C, Aoki K, Shibata D, Shiratake K. Genome-wide identification and expression analysis of aquaporins in tomato. *PLoS One* 8: e79052, 2013.

240. Ricardi MM, Gonzalez RM, Zhong S, Dominguez PG, Duffy T, Turjanski PG, Salgado Salter JD, Alleva K, Carrari F, Giovannoni JJ, Estevez JM, Iusem ND. Genome-wide data (ChIP-seq) enabled identification of cell wall-related and aquaporin genes as targets of tomato ASR1, a drought stress-responsive transcription factor. *BMC Plant Biol* 14: 29, 2014.

241. Rivera-Serrano EE, Rodriguez-Welsh MF, Hicks GR, Rojas-Pierce M. A small molecule inhibitor partitions two distinct pathways for trafficking of tonoplast intrinsic proteins in *Arabidopsis*. *PLoS One* 7: e44735, 2012.

242. Rougé P, Barre A. A molecular modeling approach defines a new group of Nodulin 26-like aquaporins in plants. *Biochem Biophys Res Commun* 367: 60–66, 2008.

243. Ruiz-Lozano JM, del Mar Alguacil M, Barzana G, Vernieri P, Aroca R. Exogenous ABA accentuates the differences in root hydraulic properties between mycorrhizal and non mycorrhizal maize plants through regulation of PIP aquaporins. *Plant Mol Biol* 70: 565–579, 2009.

244. Sade N, Galle A, Flexas J, Lerner S, Peleg G, Yaaran A, Moshelion M. Differential tissue-specific expression of *NtAQP1* 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, 2014.

245. Sade N, Gebretsadik M, Seligmann R, Schwartz A, Wallach R, Moshelion M. The role of tobacco Aquaporin1 in improving water use efficiency, hydraulic conductivity, and yield production under salt stress. *Plant Physiol* 152: 245–254, 2010.

246. Sade N, Vinocur BJ, Diber A, Shatil A, Ronen G, Nissan H, Wallach R, Karchi H, Moshelion M. Improving plant stress tolerance and yield production: is the tonoplast aquaporin *SlTIP2;2* a key to isohydric to anisohydric conversion? *New Phytol* 181: 651–661, 2008.

247. Sahr T, Adam T, Fizames C, Maurel C, Santoni V. *O*-carboxyl- and *N*-methyltransferases active on plant aquaporins. *Plant Cell Physiol* 51: 2092–2104, 2010.

248. Saito C, Ueda T, Abe H, Wada Y, Kuroiwa T, Hisada A, Furuya M, Nakano A. A complex and mobile structure forms a distinct subregion within the continuous vacuolar membrane in young cotyledons of *Arabidopsis*. *Plant J* 29: 245–255, 2002.

249. Sakr S, Alves G, Morillon R, Maurel K, Decourteix M, Guilliot A, Fleurat-Lessard P, Julien JL, Chrispeels MJ. Plasma membrane aquaporins are involved in winter embolism recovery in walnut tree. *Plant Physiol* 133: 630–641, 2003.

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

251. Sakurai J, Ahamed A, Murai M, Maeshima M, Uemura M. Tissue and cell-specific localization of rice aquaporins and their water transport activities. *Plant Cell Physiol* 49: 30–39, 2008.

252. Sakurai J, Ishikawa F, Yamaguchi T, Uemura M, Maeshima M. Identification of 33 rice aquaporin genes and analysis of their expression and function. *Plant Cell Physiol* 46: 1568–1577, 2005.

253. Sanchez-Romera B, Ruiz-Lozano JM, Li G, Luu DT, Martinez-Ballesta Mdel C, Carvajal M, Zamarreno AM, Garcia-Mina JM, Maurel C, Aroca R. Enhancement of root hydraulic conductivity by methyl jasmonate and the role of calcium and abscisic acid in this process. *Plant Cell Environ* 37: 995–1008, 2014.

254. Santoni V, Verdoucq L, Sommerer N, Vinh J, Pflieger D, Maurel C. Methylation of aquaporins in plant plasma membrane. *Biochem J* 400: 189–197, 2006.

255. Santoni V, Vinh J, Pflieger D, Sommerer N, Maurel C. A proteomic study reveals novel insights into the diversity of aquaporin forms expressed in the plasma membrane of plant roots. *Biochem J* 372: 289–296, 2003.

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

257. Schnurbusch T, Hayes J, Hrmova M, Baumann U, Ramesh SA, Tyerman SD, Langridge P, Sutton T. Boron toxicity tolerance in barley through reduced expression of the multifunctional aquaporin HvNIP2;1. *Plant Physiol* 153: 1706–1715, 2010.

258. Schüssler MD, Alexandersson E, Bienert GP, Kichey T, Laursen KH, Johanson U, Kjellbom P, Schjoerring JK, Jahn TP. The effects of the loss of TIP1;1 and TIP1;2 aquaporins in *Arabidopsis thaliana*. *Plant J* 56: 756–767, 2008.

259. Secchi F, Zwieniecki MA. Down-regulation of plasma intrinsic protein1 aquaporin in poplar trees is detrimental to recovery from embolism. *Plant Physiol* 164: 1789–1799, 2014.

260. Secchi F, Zwieniecki MA. Patterns of PIP gene expression in *Populus trichocarpa* during recovery from xylem embolism suggest a major role for the PIP1 aquaporin subfamily as moderators of refilling process. *Plant Cell Environ* 33: 1285–1297, 2010.

261. Secchi F, Zwieniecki MA. The physiological response of *Populus tremula* × *alba* leaves to the down-regulation of PIP1 aquaporin gene expression under no water stress. *Front Plant Sci* 4: 507, 2013.

262. Segami S, Makino S, Miyake A, Asaoka M, Maeshima M. Dynamics of vacuoles and H^+-pyrophosphatase visualized by monomeric green fluorescent protein in *Arabidopsis*: artifactual bulbs and native intravacuolar spherical structures. *Plant Cell* 26: 3416–3434, 2014.

263. Shachar-Hill B, Hill AE, Powell J, Skepper JN, Shachar-Hill Y. Mercury-sensitive water channels as possible sensors of water potentials in pollen. *J Exp Bot* 64: 5195–5205, 2013.

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

265. Shelden MC, Howitt SM, Brent NK, Tyerman SD. Identification and functional characterisation of aquaporins in the grapevine, *Vitis vinifera*. *Funct Plant Biol* 36: 1065–1078, 2009.

266. Siefritz F, Otto B, Bienert GP, Van Der Krol A, Kaldenhoff R. The plasma membrane aquaporin NtAQP1 is a key component of the leaf unfolding mechanism in tobacco. *Plant J* 37: 147–155, 2004.

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

268. Sjövall-Larsen S, Alexandersson E, Johansson I, Karlsson M, Johanson U, Kjellbom P. Purification and characterization of two protein kinases acting on the aquaporin SoPIP2;1. *Biochim Biophys Acta* 1758: 1157–1164, 2006.

269. Sommer A, Geist B, Da Ines O, Gehwolf R, Schäffner AR, Obermeyer G. Ectopic expression of *Arabidopsis thaliana* plasma membrane intrinsic protein 2 aquaporins in lily pollen increases the plasma membrane water permeability of grain but not of tube protoplasts. *New Phytol* 180: 787–797, 2008.

270. Sorieul M, Santoni V, Maurel C, Luu DT. Mechanisms and effects of retention of over-expressed aquaporin *AtPIP2;1* in the endoplasmic reticulum. *Traffic* 12: 473–482, 2011.

271. Soto G, Alleva K, Mazzella MA, Amodeo G, Muschietti JP. AtTIP1;3 and AtTIP5;1, the only highly expressed *Arabidopsis* pollen-specific aquaporins, transport water and urea. *FEBS Lett* 582: 4077–4082, 2008.

272. Soto G, Fox R, Ayub N, Alleva K, Guaimas F, Erijman E, Mazzella A, Amodeo G, Muschietti J. TIP5;1 is an aquaporin specifically targeted to pollen mitochondria and is probably involved in nitrogen remobilization in *Arabidopsis thaliana*. *Plant J* 64: 1038–1047, 2010.

273. Steudle E. The cohesion-tension mechanism and the acquisition of water by plant roots. *Annu Rev Plant Physiol Plant Mol Biol* 52: 847–875, 2001.

274. Steudle E, Peterson CA. How does water get through roots? *J Exp Bot* 49: 775–788, 1998.

275. Sutka M, Li G, Boudet J, Boursiac Y, Doumas P, Maurel C. Natural variation of root hydraulics in *Arabidopsis* grown in normal and salt stress conditions. *Plant Physiol* 155: 1264–1276, 2011.

276. Takahashi H, Rai M, Kitagawa T, Morita S, Masumura T, Tanaka K. Differential localization of tonoplast intrinsic proteins on the membrane of protein body Type II and aleurone grain in rice seeds. *Biosci Biotechnol Biochem* 68: 1728–1736, 2004.

277. Takano J, Tanaka M, Toyoda A, Miwa K, Kasai K, Fuji K, Onouchi H, Naito S, Fujiwara T. Polar localization and degradation of *Arabidopsis* boron transporters through distinct trafficking pathways. *Proc Natl Acad Sci USA* 107: 5220–5225, 2010.

278. Takano J, Wada M, Ludewig U, Schaaf G, von Wiren N, Fujiwara T. The *Arabidopsis* major intrinsic protein NIP5;1 is essential for efficient boron uptake and plant development under boron limitation. *Plant Cell* 18: 1498–1509, 2006.

279. Takase T, Ishikawa H, Murakami H, Kikuchi J, Sato-Nara K, Suzuki H. The circadian clock modulates water dynamics and aquaporin expression in *Arabidopsis* roots. *Plant Cell Physiol* 52: 373–383, 2011.

280. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 24: 1596–1599, 2007.

281. Tanaka M, Wallace IS, Takano J, Roberts DM, Fujiwara T. NIP6;1 is a boric acid channel for preferential transport of boron to growing shoot tissues in *Arabidopsis*. *Plant Cell* 20: 2860–2875, 2008.

282. Temmei Y, Uchida S, Hoshino D, Kanzawa N, Kuwahara M, Sasaki S, Tsuchiya T. Water channel activities of *Mimosa pudica* plasma membrane intrinsic proteins are regulated by direct interaction and phosphorylation. *FEBS Lett* 579: 4417–4422, 2005.

283. Terashima I, Ono K. Effects of $HgCl_2$ on CO_2 dependence of leaf photosynthesis: evidence indicating involvement of aquaporins in CO_2 diffusion across the plasma membrane. *Plant Cell Physiol* 43: 70–78, 2002.

284. Tong J, Briggs MM, McIntosh TJ. Water permeability of aquaporin-4 channel depends on bilayer composition, thickness, and elasticity. *Biophys J* 103: 1899–1908, 2012.

285. Tong J, Canty JT, Briggs MM, McIntosh TJ. The water permeability of lens aquaporin-0 depends on its lipid bilayer environment. *Exp Eye Res* 113: 32–40, 2013.

286. Törnroth-Horsefield S, Wang Y, Hedfalk K, Johanson U, Karlsson M, Tajkhorshid E, Neutze R, Kjellbom P. Structural mechanism of plant aquaporin gating. *Nature* 439: 688–694, 2006.

287. Tournaire-Roux C, Sutka M, Javot H, Gout E, Gerbeau P, Luu DT, Bligny R, Maurel C. Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. *Nature* 425: 393–397, 2003.

288. Tsuchihira A, Hanba YT, Kato N, Doi T, Kawazu T, Maeshima M. Effect of overexpression of radish plasma membrane aquaporins on water-use efficiency, photosynthesis and growth of Eucalyptus trees. *Tree Physiol* 30: 417–430, 2010.

289. Tungngoen K, Kongsawadworakul P, Viboonjun U, Katsuhara M, Brunel N, Sakr S, Narangajavana J, Chrestin H. Involvement of HbPIP2;1 and HbTIP1;1 aquaporins in ethylene stimulation of latex yield through regulation of water exchanges between inner liber and latex cells in *Hevea brasiliensis*. *Plant Physiol* 151: 843–856, 2009.

290. Tyerman SD, Bohnert HJ, Maurel C, Steudle E, Smith JA. Plant aquaporins: their molecular biology, biophysics and significance for plant water relations. *J Exp Bot* 50: 1055–1071, 1999.

291. Tyrrell M, Campanoni P, Sutter JU, Pratelli R, Paneque M, Sokolovski S, Blatt MR. Selective targeting of plasma membrane and tonoplast traffic by inhibitory (dominant-negative) SNARE fragments. *Plant J* 51: 1099–1115, 2007.

292. Udvardi M, Poole PS. Transport and metabolism in legume-rhizobia symbioses. *Annu Rev Plant Biol* 64: 781–805, 2013.

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

294. Uehlein N, Otto B, Eilingsfeld A, Itel F, Meier W, Kaldenhoff R. Gas-tight triblock-copolymer membranes are converted to CO_2 permeable by insertion of plant aquaporins. *Sci Rep* 2: 538, 2012.

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

296. Uenishi Y, Nakabayashi Y, Tsuchihira A, Takusagawa M, Hashimoto K, Maeshima M, Kumi Sato-Nara K. Accumulation of TIP2;2 aquaporin during dark adaptation is partially PhyA dependent in roots of *Arabidopsis* seedlings. *Plants* 3: 177–195, 2014.

297. Van Wilder V, Miecielica U, Degand H, Derua R, Waelkens E, Chaumont F. Maize plasma membrane aquaporins belonging to the PIP_1 and PIP_2 subgroups are in vivo phosphorylated. *Plant Cell Physiol* 49: 1364–1377, 2008.

298. Vandeleur RK, Mayo G, Shelden MC, Gilliham M, Kaiser BN, Tyerman SD. 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 Physiol* 149: 445–460, 2009.

299. Vandeleur RK, Sullivan W, Athman A, Jordans C, Gilliham M, Kaiser BN, Tyerman SD. Rapid shoot-to-root signalling regulates root hydraulic conductance via aquaporins. *Plant Cell Environ* 37: 520–538, 2014.

300. Vander Willigen C, Pammenter NW, Mundree SG, Farrant JM. Mechanical stabilization of desiccated vegetative tissues of the resurrection grass *Eragrostis nindensis*: does a TIP3;1 and/or compartmentalization of subcellular components and metabolites play a role? *J Exp Bot* 55: 651–661, 2004.

301. Vander Willigen C, Postaire O, Tournaire-Roux C, Boursiac Y, Maurel C. Expression and inhibition of aquaporins in germinating *Arabidopsis* seeds. *Plant Cell Physiol* 47: 1241–1250, 2006.

302. Vera-Estrella R, Barkla BJ, Amezcua-Romero JC, Pantoja O. Day/night regulation of aquaporins during the CAM cycle in *Mesembryanthemum crystallinum*. *Plant Cell Environ* 35: 485–501, 2012.

303. Vera-Estrella R, Barkla BJ, Bohnert HJ, Pantoja O. Novel regulation of aquaporins during osmotic stress. *Plant Physiol* 135: 2318–2329, 2004.

304. Verdoucq L, Grondin A, Maurel C. Structure-function analysis of plant aquaporin *AtPIP2;1* gating by divalent cations and protons. *Biochem J* 415: 409–416, 2008.

305. Veselova TV, Veselovskii VA, Usmanov PD, Usmanova OV, Kozar VI. Hypoxia and imbibition injuries to aging seeds. *Russ J Plant Physiol* 50: 835–842, 2003.

306. Volkov V, Hachez C, Moshelion M, Draye X, Chaumont F, Fricke W. Water permeability differs between growing and non-growing barley leaf tissues. *J Exp Bot* 58: 377–390, 2007.

307. Vysotskaya LB, Arkhipova TN, Timergalina LN, Dedov AV, Veselov SY, Kudoyarova GR. Effect of partial root excision on transpiration, root hydraulic conductance and leaf growth in wheat seedlings. *Plant Physiol Biochem* 42: 251–255, 2004.

308. Wallace IS, Roberts DM. Distinct transport selectivity of two structural subclasses of the nodulin-like intrinsic protein family of plant aquaglyceroporin channels. *Biochemistry* 44: 16826–16834, 2005.

309. Wallace IS, Roberts DM. Homology modeling of representative subfamilies of *Arabidopsis* Major Intrinsic Proteins. Classification based on the Aromatic/Arginine selectivity filter. *Plant Physiol* 135: 1059–1068, 2004.

310. Wan X, Steudle E, Hartung W. Gating of water channels (aquaporins) in cortical cells of young corn roots by mechanical stimuli (pressure pulses): effects of ABA and of $HgCl_2$. *J Exp Bot* 55: 411–422, 2004.

311. Wan X, Zwiazek JJ. Root water flow and leaf stomatal conductance in aspen (*Populus tremuloides*) seedlings treated with abscisic acid. *Planta* 213: 741–747, 2001.

312. Wang LL, Chen AP, Zhong NQ, Liu N, Wu XM, Wang F, Yang CL, Romero MF, Xia GX. The *Thellungiella salsuginea* tonoplast aquaporin TsTIP1;2 functions in protection against multiple abiotic stresses. *Plant Cell Physiol* 55: 148–161, 2014.

313. Wang YH, Garvin DF, Kochian LV. Nitrate-induced genes in tomato roots. Array analysis reveals novel genes that may play a role in nitrogen nutrition. *Plant Physiol* 127: 345–359, 2001.

314. Warren CR. Stand aside stomata, another actor deserves centre stage: the forgotten role of the internal conductance to CO_2 transfer. *J Exp Bot* 59: 1475–1487, 2008.

315. Weaver CD, Shomer NH, Louis CF, Roberts DM. Nodulin 26, a nodule-specific symbiosome membrane protein from soybean, is an ion channel. *J Biol Chem* 269: 17858–17862, 1994.

316. Wegner LH. Root pressure and beyond: energetically uphill water transport into xylem vessels? *J Exp Bot* 65: 381–393, 2014.

317. Wei W, Alexandersson E, Golldack D, Miller AJ, Kjellbom PO, Fricke W. HvPIP1;6, a barley (*Hordeum vulgare L.*) plasma membrane water channel particularly expressed in growing compared with non-growing leaf tissues. *Plant Cell Physiol* 48: 1132–1147, 2007.

318. Wu XN, Sanchez Rodriguez C, Pertl-Obermeyer H, Obermeyer G, Schulze WX. Sucrose-induced receptor kinase SIRK1 regulates a plasma membrane aquaporin in *Arabidopsis*. *Mol Cell Proteomics* 12: 2856–2873, 2013.

319. Wudick MM, Luu DT, Maurel C. A look inside: localisation patterns and functions of intracellular plant aquaporins. *New Phytol* 184: 289–302, 2009.

320. Wudick MM, Luu DT, Tournaire-Roux C, Sakamoto W, Maurel C. Vegetative and sperm cell-specific aquaporins of *Arabidopsis* highlight the vacuolar equipment of pollen and contribute to plant reproduction. *Plant Physiol* 164: 1697–1706, 2014.

321. Xia J, Yamaji N, Kasai T, Ma JF. Plasma membrane-localized transporter for aluminum in rice. *Proc Natl Acad Sci USA* 107: 18381–18385, 2010.

322. Xu H, Kemppainen M, El Kayal W, Lee SH, Pardo AG, Cooke JE, Zwiazek JJ. Overexpression of *Laccaria bicolor* aquaporin JQ585595 alters root water transport properties in ectomycorrhizal white spruce (*Picea glauca*) seedlings. *New Phytol* 205: 757–770, 2015.

323. Yamaji N, Ma JF. A transporter at the node responsible for intervascular transfer of silicon in rice. *Plant Cell* 21: 2878–2883, 2009.

324. Yaneff A, Sigaut L, Marquez M, Alleva K, Pietrasanta LI, Amodeo G. Heteromerization of PIP aquaporins affects their intrinsic permeability. *Proc Natl Acad Sci USA* 111: 231–236, 2014.

325. Yang O, Popova OV, Suthoff U, Luking I, Dietz KJ, Golldack D. The *Arabidopsis* basic leucine zipper transcription factor AtbZIP24 regulates complex transcriptional networks involved in abiotic stress resistance. *Gene* 436: 45–55, 2009.

326. Yang Z, Guo G, Zhang M, Liu CY, Hu Q, Lam H, Cheng H, Xue Y, Li J, Li N. Stable isotope metabolic labeling-based quantitative phosphoproteomic analysis of *Arabidopsis* mutants reveals ethylene-regulated time-dependent phosphoproteins and putative substrates of constitutive triple response 1 kinase. *Mol Cell Proteomics* 12: 3559–3582, 2013.

327. Ye Q, Wiera B, Steudle E. A cohesion/tension mechanism explains the gating of water channels (aquaporins) in *Chara* internodes by high concentration. *J Exp Bot* 55: 449–461, 2004.

328. Yool AJ, Brown EA, Flynn GA. Roles for novel pharmacological blockers of aquaporins in the treatment of brain oedema and cancer. *Clin Exp Pharmacol Physiol* 37: 403–409, 2010.

329. Yu X, Peng YH, Zhang MH, Shao YJ, Su WA, Tang ZC. Water relations and expression analysis of plasma membrane intrinsic proteins in sensitive and tolerant rice during chiling and recovery. *Cell Res* 16: 599–608, 2006.

330. Zardoya R, Ding X, Kitagawa Y, Chrispeels MJ. Origin of plant glycerol transporters by horizontal gene transfer and functional recruitment. *Proc Natl Acad Sci USA* 99: 14893–14896, 2002.

331. Zelazny E, Borst JW, Muylaert M, Batoko H, Hemminga MA, Chaumont F. FRET imaging in living maize cells reveals that plasma membrane aquaporins interact to regulate their subcellular localization. *Proc Natl Acad Sci USA* 104: 12359–12364, 2007.

332. Zelazny E, Miecielica U, Borst JW, Hemminga MA, Chaumont F. An N-terminal diacidic motif is required for the trafficking of maize aquaporins *ZmPIP2;4* and *Zm*PIP2;5 to the plasma membrane. *Plant J* 57: 346–355, 2009.

333. Zhang B, Wang Q, Wang K, Pan X, Liu F, Guo T, Cobb GP, Anderson TA. Identification of cotton microRNAs and their targets. *Gene* 397: 26–37, 2007.

334. Zhang DY, Ali Z, Wang CB, Xu L, Yi JX, Xu ZL, Liu XQ, He XL, Huang YH, Khan IA, Trethowan RM, Ma HX. Genome-wide sequence characterization and expression analysis of major intrinsic proteins in soybean (*Glycine max L.*). *PLoS One* 8: e56312, 2013.

335. Zhang N, Yang J, Wang Z, Wen Y, Wang J, He W, Liu B, Si H, Wang D. Identification of novel and conserved microRNAs related to drought stress in potato by deep sequencing. *PLoS One* 9: e95489, 2014.

336. Zhang WH, Tyerman SD. Inhibition of water channels by $HgCl_2$ in intact wheat root cells. *Plant Physiol* 120: 849–858, 1999.

337. Zhao XQ, Mitani N, Yamaji N, Shen RF, Ma JF. Involvement of silicon influx transporter OsNIP2;1 in selenite uptake in rice. *Plant Physiol* 153: 1871–1877, 2010.

338. Zhou S, Hu W, Deng X, Ma Z, Chen L, Huang C, Wang C, Wang J, He Y, Yang G, He G. Overexpression of the wheat aquaporin gene, TaAQP7, enhances drought tolerance in transgenic tobacco. *PLoS One* 7: e52439, 2012.

339. Zhou Y, Setz N, Niemietz C, Qu H, Offler CE, Tyerman SD, Patrick JW. Aquaporins and unloading of phloem-imported water in coats of developing bean seeds. *Plant Cell Environ* 30: 1566–1577, 2007.

340. Zhu C, Schraut D, Hartung W, Schaffner AR. Differential responses of maize *MIP* genes to salt stress and ABA. *J Exp Bot* 56: 2971–2981, 2005.

341. Zhu D, Wu Z, Cao G, Li J, Wei J, Tsuge T, Gu H, Aoyama T, Qu LJ. TRANSLUCENT GREEN, an ERF family transcription factor, controls water balance in *Arabidopsis* by activating the expression of aquaporin genes. *Mol Plant* 7: 601–615, 2014.

342. Zou J, Rodriguez-Zas S, Aldea M, Li M, Zhu J, Gonzalez DO, Vodkin LO, DeLucia E, Clough SJ. Expression profiling soybean response to *Pseudomonas syringae* reveals new defense-related genes and rapid HR-specific downregulation of photosynthesis. *Mol Plant Microbe Interact* 18: 1161–1174, 2005.