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Review

Modulating the expression of aquaporin genes in planta: A key to understand their physiological functions?

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Abstract

Aquaporins (AQPs) are believed to act as "cellular plumbers", allowing plants to rapidly alter their membrane water permeability in response to environmental cues. This study of AQP regulation at both the RNA and protein levels has revealed a large number of possible mechanisms. Currently, modulation of AQP expression in planta is considered the strategy of choice for elucidating the role of AQPs in plant physiology. This review highlights the fact that this strategy is complicated by many factors, such as the incomplete characterization of transport selectivity of the targeted AQP, the fact that AQPs might act as multifunctional channels with multiple physiological roles, and the number of post-translational regulation mechanisms. The classification of AQPs as constitutive or stress-responsive isoforms is also proposed.

Keywords: Aquaporin; Water relation; Membrane permeability; Hydraulic conductivity

Contents

1. Introduction

Water absorption from the soil into the root tissue is how plants maintain their water status in suitable range. The water is then distributed in plant tissues, and approximately 95% evaporates from the leaf through stomata, a process known as evapotranspiration. Large amounts of water can be lost this way: a rapidly transpiring sunflower leaf loses the equivalent of the entire leaf water content every 20 min [1] and, consequently, a plant can transport 200–1000 times its dry body weight of water in its lifetime [2].

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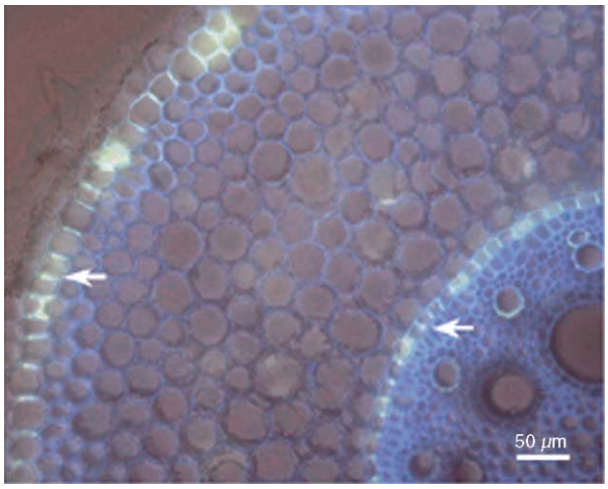

Fig. 1. Detection of apoplastic barriers in maize roots. In aeroponically-grown maize roots, monolayers of suberized endodermis and exodermis hindering apoplastic water movement are detected using a fluorescent dye, berberine hemisulphate, under UV excitation (350–380 nm). Suberin and lignin deposits are seen in the radial cell walls as yellowish fluorescent signal (white arrows; for staining protocol, see [110]). Strong aquaporin protein expression has been recorded in the adjacent cell layers (Hachez and Chaumont., unpublished data).

Long distance transport of water within the plant occurs through xylem vessels and phloem sieve tubes that have low hydraulic resistance. In contrast, water molecules that enter or exit these conduits pass through living tissues and may encounter membrane barriers. Three different pathways of water transport through plant tissues have been described [3]: these are the apoplastic pathway around the protoplast (across the cell wall and intercellular space when filled with liquid), the symplastic pathway from cell to cell through the plasmodesmata, and the transcellular path across the cell membranes. The last two cannot currently be experimentally separated and are referred to as the cell-to-cell path. Depending on the species, growth conditions, and developmental stages, these pathways contribute differently to overall water flow in all parts of the plants. Based on measurements of the hydraulic conductivity of the overall root or individual cells, a composite transport model has been proposed to explain the variability in the ability of roots to take up water in response to different developmental and environmental factors [4]. Water movement via the apoplastic pathway is driven by physical forces and is mainly regulated by differences in water potential between the soil, the plant, and the atmosphere. During the day under high transpiration conditions (i.e., the tension in the xylem is increased), it is accepted that the driving force for the radial movement of water across the root is mainly hydrostatic, the apoplastic pathway being predominant. As a consequence, the root hydraulic conductivity (Lp_r, m s^{-1}. MPa^{-1}) is high. At night and during periods of water stress, when transpiration is low, water flow occurs by an active osmoregulation mechanism, with an osmotic gradient being build up by solute accumulation in the xylem. Under these conditions, the cell-to-cell pathways is the preferred one and the Lp_r is low [4].

Thanks to certain adaptations, the plant is able to control both the apoplastic and cell-to-cell pathways to a certain extent. Movement via the apoplast can be limited by the presence of apoplastic barriers (Fig. 1). The Casparian bands, mainly composed of lignin in and suberin, are located in the primary walls of the endodermal and exodermal cell layers and are linked structurally to the plasma membranes. These barriers are very common in plant species. Virtually all vascular plants have an endodermis, and 91% of all investigated angiosperms show a clearly suberized exodermis with Casparian bands [5]. The Casparian bands constitute a hydraulic barrier that forces water to enter the symplast and cross the cell membranes, a process which might necessitate a high water membrane permeability [3,6]. It seems that the Casparian bands do not constitute an absolute barrier to water flow, as apoplastic bypasses still exist

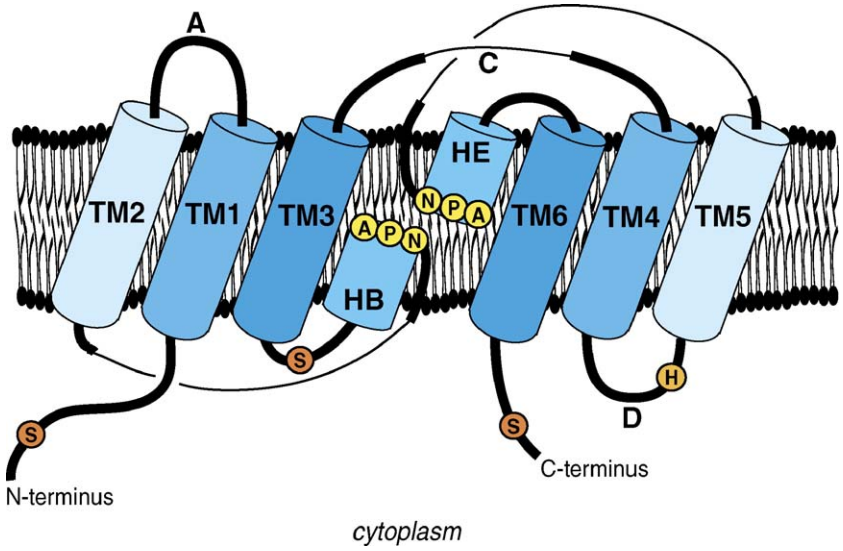

Fig. 2. Model of an AQP showing its principal features. Six transmembrane alpha helices (TM1–TM6) are connected by five loops (A–E). Two helical domains (HB and HE) containing the highly conserved NPA motifs dip halfway into the membrane from opposite sides and participate in the formation of the aqueous pore (adapted from [15]). This model shows the serine amino acid residues (S) potentially phosphorylated in plants at the N- and C-termini and in loop B (phosphorylation at this last position has not been demonstrated in planta). The pH-sensitive histidine residue (H) in loop D involved in AQP gating is also indicated [34].

to a certain extent [7]; however, they limit water flow and significantly reduce the Lp_r [8,9].

The movement of water via the cell-to-cell pathway is under fine regulation. At the physiological level, it can be controlled by the regulation of water channels, known as aquaporins (AQPs). These are ubiquitous in plants and are detected in high amounts at regions of high symplastic water transport, such as the endodermis ([10,11] Hachez, Moshelion and Chaumont, unpublished data). AQPs, are major integral proteins that facilitate the movement of water or other small solutes through the membranes, and are widespread in biological membranes of many organisms from vertebrates, insects, and plants to fungi and bacteria. AQP monomers, with molecular masses ranging from 21 to 34 kDa, contain six membrane-spanning alpha helices which, together with two short hydrophobic helices dipping halfway into the membranes from opposite sides (loops B and E), create a pore with a high solute specificity (Fig. 2) [12,13]. Monomers cluster as homo- and possibly heterotetramers in the membrane, but each monomer acts as separate water channel; however its gating might be regulated by interaction with other members of the tetramer (see below; [14]).

Angiosperm species possess approximately 35 different AQPs grouped on the basis of sequence similarity into four subfamilies: these are the plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), nodulin 26-like intrinsic proteins (NIPs), and small basic intrinsic proteins (SIPs) [15–17]. The large number of AQPs found in plants is puzzling. One explanation could be that it reflects their importance in maintaining both the extensive water transport from the roots to the leaves and cell homeostasis at all developmental stages and under all environmental conditions. Indeed, the function of AQPs is generally believed to be to facilitate the movement of water across membranes by increasing their osmotic water permeability coefficient (P_f) by up to 20-fold. However, water is not the only molecule capable of transiting through AQPs. Indeed, whereas some AQPs, such as AtTIP;1 [18], are highly selective for water, others are less specific and facilitate the passage of other small solutes, such as glycerol, urea, ammonia, ammonium, and carbon dioxide (reviewed in [19,20]). Clearly, the physiological relevance of small non-electrolyte, gas, and perhaps ion transport by AQPs is of major interest. Indeed, the expression of so many AQP isoforms in plants raises the question whether their ability to conduct water is their sole role in the membrane. Evidence that plant AQPs exhibit diverse transport properties and can behave as multifunctional channels suggests an increasingly large number of putative functions for these proteins and could partly explain the isoform diversity.

The relationship between AQP expression and regulation of plant water status is still unclear, as it is complicated by the large number of AQP isoforms and the number of possible regulatory events. However, despite extensive research in this field, little is known about the exact physiological role of AQPs in planta under normal growth conditions or during salt or water stresses. What is the real extent to which AQP contribute to maintaining the global water status? How do they act in planta? How do they physiologically alter key processes, such as elongation or carbon bioavailability? Before considering the role of AQPs in plant physiology by analysis of their expression and the study of water-related parameters in plants with modified AQP expression patterns, it is important to bear in mind that the water channel activity of AQPs in the membrane is dependent on the transport properties of the specific protein, its trafficking to the membrane, and the aperture or closure state of the channel (reviewed in [20,21]). These regulatory aspects are summarized in the following section.

2. Post-translational regulation of aquaporin activity

In vivo phosphorylation of serine residues has been seen in bean seed PvTIP3;1, spinach SoPIP2;1, soybean NOD26, and *Arabidopsis* and maize PIP1 and PIP2 isoforms (PIP isoforms are classified into two subgroups by their sequences) ([22–26] Van Wilder, Degand, Derua, Waelkens and Chaumont, unpublished data). The effect of phosphorylation on plant AQP regulation has been demonstrated by expression of the mutated proteins in *Xenopus* oocytes and the use of kinase and phosphatase inhibitors or agonists. For instance, replacement of Ser7 or Ser23 (N-terminus) or Ser99 (loop B) of PvTIP3;1 by Ala reduces its water transport activity, and cAMP agonist activation of oocyte protein kinase A increases the water transport activity of the channel [27]. Similarly, phosphorylation of Ser115 (loop B) or Ser274 (C-terminus) of spinach SoPIP2;1 activates water channel activity in *Xenopus* oocytes (Fig. 2) [28]. An influence of C-terminal serine phosphorylation on PIP2 activity has also been suggested for radish (*Raphanus sativus*) RsPIP2;2 heterologously expressed in yeast [29]. Plant AQP phosphorylation status is dependent on environmental parameters. SoPIP2;1 phosphorylation decreases when the apoplastic water potential is reduced, suggesting closure of the channel during osmotic stress [23]. On the other hand, NOD26 phosphorylation on Ser262, which increases water permeability, is increased in vivo under osmotic stress, and is dependent on the developmental stage of the symbiotic nodule, reaching a peak when the nodules are mature [30]. Temperature is also an important factor regulating phosphorylation. In the tulip, the opening and closing of the flowers is regulated by the phosphorylation and dephosphorylation of an undefined plasma membrane AQP [31]. At 20 °C, this AQP is phosphorylated in a Ca^{2+}-dependent manner and this probably facilitates water transport from the stem to the petals, allowing the flower to open. At 5 °C, the plasma membrane AQP is dephosphorylated decreasing water influx and, as a result, the flower closes. Together, these data establish a direct link between AQP phosphorylation and physiological processes.

Calcium ions and the pH modulate plant AQP activity. The P_f of *Arabidopsis* cells is reduced by up to 4-fold in the presence of Ca^{2+} [32]. In addition, plasma membrane vesicles in standard medium have a low P_f, whereas, in the presence of chelators of divalent cations, they show a higher P_f, indicating down-regulation of plasma membrane AQPs by Ca^{2+}. A decrease in the pH of the medium from 8.3 to 7.2–7.5 also induces a 50% reduction in plasma membrane water permeability [32]. A similar reduction is seen in tonoplast vesicles isolated from

storage roots of *Beta vulgaris* at low pH [33]. In *Arabidopsis*, oxygen deprivation (anoxia) induces a decrease in the cystolic pH and a reduction in the Lp_r [34]. Expression studies in *Xenopus* oocytes showed that acidification caused an inhibition of PIP activity. Mutagenesis analysis of AtPIP2;2 revealed the importance of His197 (located in cytosolic loop D) in the pH regulation mechanisms (Fig. 2); H197A mutation leads to a much less pronounced inhibition of the P_f in acid conditions and H197D mutation, which introduces a negatively charged amino acid residue, makes PIP2;2 pH-insensitive, with a constitutively high activity. Other charged amino acids in loop D also seem to be involved in this pH-dependent gating [34].

Recent high-resolution structures of SoPIP2;1 in closed and open conformations together with molecular dynamics simulations allowed to examine the gating of the channel [35]. The conformation of the loop D defines the open or closed state of the pore which depends on the phosphorylation status of Ser115 and Ser274 and the protonation of H193 (corresponding to H197 of AtPIP2;2). These important results clearly unify the functional and biochemical data that have highlighted the role of specific amino acid residues in AQP activity regulation [35].

Structural studies have demonstrated that mammalian and bacterial AQPs form tetramers in the membranes [12,13, 36,37]. In plants, this oligomerization state seems to be conserved, as shown by scanning transmission electron microscopy mass analysis of SoPIP2;1 and SoPIP1;2 particles, cryo-electron microscopy of SoPIP2;1 and PvTIP3;1 2D crystals and X-ray diffraction from SoPIP2;1 [35,38–40]. Although all AQPs examined seem to form homotetramers, several plant isoforms have also been found to associate, probably as heterotetramers. Heteromers of two tonoplast AQPs from lentil seeds were detected in cross-linking experiments [41], and recent data [14] show that maize ZmPIP1s and ZmPIP2s physically interact to modify their trafficking and modulate their water channel activity. When expressed in *Xenopus* oocytes, ZmPIP1s are inactive, whereas ZmPIP2s cause a large increase in the P_f. Interestingly, when ZmPIP2s are co-expressed with increasing amounts of ZmPIP1;2, a synergistic effect is observed, with an increase in the P_f compared to oocytes expressing ZmPIP2s alone. Co-expression of ZmPIP1;2-GFP chimera protein and ZmPIP2;1 leads to better trafficking and/or stability of ZmPIP1;2 in the plasma membrane, and the two isoforms co-purify as heterotetramers. A positive interaction between *Mimosa pudica* MpPIP1;1 and MpPIP2;1 was also demonstrated in *Xenopus* oocytes and, interestingly, although Ser131 phosphorylation of MpPIP1;1 has no effect on the MpPIP1;1/MpPIP2;1 interaction in COS7 cells, this post-translational modification is necessary for enhancing osmotic water permeability during co-expression with MpPIP2;1 [42].

High concentrations of osmotic solutes can induce a decrease in water transport in the alga *Chara corallina* [43]. To explain this phenomenon, the authors proposed a cohesion/tension model of AQP gating. Solute exclusion from AQPs would create tension in the pore (negative pressure) which would cause deformation of the protein and reversible channel closure, and the larger the molecular mass of the solute, the stronger the gating of the pore. Recently, this cohesion/tension theory was used to evaluate pore volumes of *Chara* AQPs [44]. Mechanical stimuli could also be involved in pore gating. Wan et al. [45] observed that small or medium pulses of turgor pressure (0.1 to 0.2 MPa) cause reversible inhibition of the Lp of maize root cortical cells, whereas larger pulses (greater than 0.2 Mpa) induce irreversible Lp inhibition.

In addition to mechanisms regulating the activity of AQPs in the membrane, control of the subcellular distribution of AQPs seems to play an important role for some isoforms. *Mesembryanthemum crystallinum* McTIP1;2, originally present in the tonoplast, is relocated in other vesicular membranes during mannitol-induced osmotic stress [46]. This redistribution correlates with the appearance of glycosylated McTIP1;2 and is abolished by tunicamycin, which blocks the formation of N-glycosidic protein carbohydrate linkages. McTIP1;2 redistribution is also perturbed by brefeldin A, which triggers disassembly of the Golgi and disruption of vesicular trafficking, and by wortmannin or cytochalasin D, which, respectively, inhibit endocytosis or disassemble the actin cytoskeleton [46]. Salt stress can also trigger relocalization of tonoplastic AtTIP1;1-GFP to intravacuolar invaginations, possibly corresponding to special compartments involved in TIP1;1 degradation or to modification of the vacuolar apparatus with the formation of a new subtype of vacuole [47]. AtPIP1 proteins have also been shown to be present in plasma membrane invaginations called plasmalemmasomes, independently of any stress [48].

3. AQP expression in wild-type plants

The number of AQPs expressed also regulates water movement across membranes. The study of *AQP* gene expression patterns in many plant species in specific tissues and cell types or in response to environmental cues has highlighted the putative roles of water channels [19,21,49]. As water channels, AQPs seem to fulfill two main functions in plants, namely individual cell osmoregulation and control of transcellular and tissue water transport [49,50]. The presence of these proteins in both the tonoplast and plasma membranes allows them to play a role at both the cellular (osmoregulation of the cytosol via movement of water from the vocuole compartment to the cytosol, or vice-versa) and tissue (water transport through the cell-to-cell pathway) levels. For instance, AQPs are abundantly expressed in roots, where they mediate water uptake from the soil ([6,15,47,51], Hachez, Moshelion and Chaumont, unpublished data). Expression of AQPs in aerial parts of the plant, such as cotyledons, leaves, stems, and petioles, has been reported [11,23,52]. AQPs seem to be preferentially expressed in the vascular tissue or in cells undergoing rapid elongation and/or differentiation [53,54]. The timing and localization of their expression suggest that transmembrane water transport may be relevant to many other processes unrelated to transpiration. At the organ level, AQP expression has been described in flowers and during seed maturation and germination [55–58]. Flower expansion and blooming require accurate control of water relationships in the sepals and petals, as well as in the anther, stigma, and pollen grains.

Light-dependent expression of *AQP* genes and, more specifically, circadian fluctuations in transcript levels have been reported in several plant species. mRNA levels of several AQPs in *Oryza sativa*, *Zea mays*, and *Lotus japonicus* show a clear diurnal fluctuation in roots, peaking 3 h after light onset and reaching a minimum 3 h after onset of darkness [17,59,60]. AQPs are also involved in diurnal leaf unfolding [61]. The movement of pulvini (motor organs responsible for the movement of leaves and leaflets) in the leguminous Mimosacea tree, *Samanea saman*, results from coordinated and simultaneous changes in the volume of cortex cells on opposing sides of the pulvinus and that correlate with changes in *AQP* mRNA levels.

AQP also play an important role in plant development and their adaptation to an ever changing environment, as they allow the plant to respond quickly to anoxic stress, water deficit, and other harmful conditions. At the transcriptional level, many reports have shown responsiveness of plant AQPs to drought, low temperature, salinity, light, pathogens, and hormones. AQP isoforms have been shown to be either up- or down-regulated in various plant species in response to these environmental stimuli [51,62,63]. Down-regulation might be interpreted as a means by which the plant can reduce both water loss and uptake and/or decrease water fluxes through tissues [47,64,65]. Up-regulation of specific drought resistant *AQP* genes has also been reported [62]. At the transcriptional level, there is a body of data showing that the plant response in terms of transcriptional regulation of *AQP* genes is complex and requires differential and specific regulation of membrane water permeability that might require the activation of specific isoforms. Cold also influences *AQP* expression [17,66]. In *O. sativa*, mRNA levels of 10 AQP genes markedly decrease in roots during chilling and recover after warming, and these changes correlate with the change in bleeding sap volume. These results suggest a relationship between root water uptake and mRNA levels of several *AQPs* with higher water channel activity [17]. Similarly, upon cold stress, most of *Arabidopsis PIP* genes (except *AtPIP2;5*) are down-regulated [51].

Salt exposure triggers considerable changes in gene expression, as shown by microarray analysis [62]. Expression of *AQP* genes is modulated by salt and water stresses [67–72]. For example, several AQP genes from salt-tolerant rice lines have been shown to be up-regulated following 150 mM NaCl stress [62]. On the other hand, AQPs are down-regulated both transiently and permanently in several plants upon salt exposure [65,70,72,73]. Maathuis et al. [63] reported that, during the first 2–5 h of NaCl treatment, as the tissue water content is declining, there is usually extensive down-regulation of *AQP* gene expression, probably to limit the initial water loss, then, accompanying the recovery of tissue water content, up-regulation of stress-responsive isoforms is seen, permitting water influx, as uptake of ions, such as Na^+ and Cl^-, and the synthesis of compatible osmolytes lower the cellular water potential. Jang et al. [51] found that salinity has less marked effects on gene expression modulation than drought stress.

However, although information on transcriptional effects is abundant, information on protein accumulation under stress conditions is still limited. A study on the regulation of radish AQP proteins following drought and salt stresses came to the conclusion that RsPIP2 group members are responsive to water stress (caused by NaCl, PEG, or mannitol treatment), while RsPIP1s are more constitutively expressed, suggesting distinct physiological functions of these proteins (see below) [74]. Boursiac et al. [47] showed that salt exposure of roots triggers changes in AQP expression at multiple levels, including transcriptional down-regulation, dynamic control at the protein level (altered translation and/or degradation rates), and subcellular relocation of AQP proteins (see above). A significant decrease in PIP1 proteins was seen in cell extracts 30 min after onset of salt exposure, suggesting that a rapid response to salt stress could be mediated by the dynamic control of AQP translation or degradation [47]. Surprisingly, the amount of PIP2 proteins was found to remain fairly constant even after 6 h of salt exposure; however, PIP1, PIP2, and TIP1 were all present in reduced amounts 24 h after onset of salt exposure. Alexandersson et al. [65], showed that *AtPIP* transcripts in leaves were down-regulated following drought stress, except for two genes (*AtPIP1;4* and *AtPIP2;5*) that were up-regulated and another (*AtPIP2;6*) that was shown to be constitutively expressed and not significantly affected by the stress. This distinction between constitutive and stress-responsive isoforms will be discussed later in terms of their different contributions to water transport upon stress application. The expression profile of the different genes might explain their different behavior. At the protein level, this down-regulation in leaves is also observed for PIP1 and PIP2 isoforms, showing a clear link between RNA and protein levels [65]. However, after 26 h of rehydration, AQP transcript levels return to normal, but no increase in AQP protein is seen.

4. Physiological roles of AQPs deduced from plants with altered AQP expression

A traditional method for determining the involvement of AQPs in physiological processes is the use of AQP inhibitors, such as mercury chloride, Ag (as $AgNO_3$ or silver sulfadiazine), or gold (as $HAuCL_4$). The last two have only been tested on root plasma membranes or peribacteroid membrane vesicles [75]. Blocking of AQPs is seen with compounds that can oxidize the cysteine residues associated with the pore region of the protein or bind to protein sulfhydryl groups [50]. The blocking effect of silver and gold is mainly due to their ability to interact with protein sulfhydryl groups and, more precisely, with the sulfhydryl group of a cysteine in the vicinity of the pore region. However, the non-reversibility of the blocking by mercaptoethanol suggests a different mode of inhibition than that of mercury. According to Niemitz and Tyerman [75], only a few types of proteins are inhibited by silver, so silver is a more selective agent for testing for the presence of active AQPs than mercurials, which show many side effects.

Table 1
Deregulation of plant AQP expression

Author	Species	Protein/modification	Effect	Interpretation
Kaldenhoff et al., 1998 [88]	*Arabidopsis*	PIP1;2 Anti-sense plants	3-fold decrease in cell P_f. Root system 5× more developed than in wild type.	Anti-sense plants compensate the reduced cell Lp by increasing root mass to ensure sufficient water supply to the plant.
Martre et al., 2002 [86]	*Arabidopsis*	PIP1 and PIP2 Double anti-sense mutants	5- to 30-fold reduction in P_f from isolated root and leaf protoplasts. Normal conditions or water stress: identical to WT Mutant recover significantly more slowly from stress than the WT. Root/leaf dry mass ratio 2.5-fold higher than WT.	PIPs play an important role in the recovery process of *Arabidopsis* under water deficient conditios. Under water-sufficient conditions, the anti-sense plants compensate for the lower hydraulic conductivity by investing more carbon in root production.
Javot et al., 2003 [89]	*Arabidopsis*	PIP2;2 Silencing (T-DNA insertion)	Abundantly expressed in roots (cortex, stele, endodermis). Mutants: reduced P_f of root cortex protoplasts (25 to 30%), reduced Lp_r (14%). No difference in phenotype between wild-type and mutants.	PIP2;2 is involved in the movement of fluid through the root.
Ma et al., 2004 [93]	*Arabidopsis*	TIP1;1 Silencing	Miniature plants: slow growth rate, late bolting, slow seed formation or inability to form siliques. Silencing is lethal in its severe form. Small plants, early senescence, lesion formation. Disturbance in carbon metabolism. Accumulation of starch in chloroplasts.	TIP1;1 would be a marker for vesicles targeted to the central vacuole. Absence of TIP1;1 leads to the loss of an intermediate involved in cellular routing of vesicles to the central vacuole resulting in a carbon distribution that alter source/sink relationships. Effect on vacuolar loading of sucrose (which could explain the absence of glucose and fructose in metabolite profiles).
Katsuhara et al., 2003 [102]	*Oryza sativa*	HvPIP2;1 (barley gene) Overexpression	Down-regulated under water stress in barley. Overexpression increases radial Lp_r by up to 140%. Increase of up to 150% in the shoot/root mass ratio. In normal conditions: identical phenotype of WT and transgenic lines. One line showed a higher shoot/root ratio. A smaller amount of root can sustain the growth of AQP overexpressing plants. Transgenic lines: increased salt sensitivity (reduced growth).	Involvement of AQP in salt tolerance. Overexpression of HvPIP2;1 in rice makes transgenic rice sensitive to 100 mM NaCl. An increase or decrease in AQP levels affects the water permeability of the root surface: plant compensate with root mass to obtain a constant water uptake by roots.
Hanba et al., 2004 [107]	*Oryza sativa*	HvPIP2;1 (barley gene) Overexpression	Altered photosynthesis: 40% increase in internal CO_2 conductance. 14% increase in CO_2 assimilation rate, 27% increase in stomatal conductance. Increased water loss by the leaf (triggering anatomical adaptations). Smaller mesophyll cells. Thickening of epidermis and mesophyll cell walls (leaves become xeromorphic).	AQP increases internal CO_2 conductance and CO_2 as simulation. Higher growth rate than WT, but plants are more sensitive to water stress, as AQP overexpression induces an increase in transpiration Suggests that, if AQP levels exceed a certain threshold, anatomical alterations are induced by water stress.
Lian et al., 2004 [95]	*Oryza sativa*	OsPIP1;3 Normal conditions	Under drought stress (PEG): OsPIP1;3 increases in upland rice (drought resistance mechanism), decreases in low land rice (sensitive)	OsPIP1;3 plays a role in drought resistance in rice.

(continued on next page)

Table 1 (*continued*)

Author	Species	Protein/modification	Effect	Interpretation
			after 10h of water deficit.	
		Overexpression under control of a stress-inducible promoter	Overexpressing lines (WT is drought sensitive): higher Lp_r, leaf water potential, and transpiration than WT, but no morphological differences.	
Otto and Kaldenhoff, 2000 [11]	*Nicotiana tabacum*	NtAQP1	Localization at places of high symplastic water movement. NtAQP1 is a CO_2 facilitator.	Increased growth velocity could be the result of a higher photosynthetic activity, but could also be a synergistic effect of the increased CO_2 and plant water permeability.
		Overexpression	Overexpression leads to improved leaf growth. No effect on plant height or root mass compared to WT.	
Siefritz et al., 2002 [87]	*Nicotiana tabacum*	NtAQP1 Silencing (anti-sense)	Decreased water permeability at the cell level. Reduced Lp and water stress resistance: transpiration rate, Ψ_{stem} and Ψ_{leaf} dissimilar in the mutant and wild-type under standard conditions. No variation in the stem/root ratio compared to WT.	Importance of symplastic water transport. Absence of NtAQP1 causes stomatal closure (water stress signal).
Reisen et al., 2003 [100]	*Nicotiana tabacum* suspension cells (cauliflower gene)	BobTIP26;1 Overexpression of a fusion protein tagged with GFP	Marker for acidic and lytic vacuoles. Overexpression has no effect on growth rate, but has an effect on cell volume (increased) due to a larger vacuole size.	The fusion protein, by allowing a vacuolar volume increase, triggers a cell volume increase. A concomitant transport of solute would build up a *trans*-tonoplastic osmotic gradient that would trigger the swelling of the vacuole, allowing cells to enlarge under lower differences of osmotic potential.
Aharon et al., 2003 [101]	*Nicotiana tabacum*	PIP1;2 (*Arabidopsis* gene) Overexpression	Overexpression significantly increases plant growth rate, transpiration rate, stomatal density, photosynthetic efficiency. Plants are taller than WT and stem diameter is larger.	Symplastic transport via AQP limits growth and vigor even under favorable conditions.
			Size of leaves is comparable, but transgenic plants are taller with more internodes. Transgenic lines: lower root/shoot mass ratio due to a 50% increase in shoot fresh weight. Leaves contain 30% more dry mass than WT. No beneficial effect upon salt stress. Under drought stress: overexpression is detrimental, causing faster wilting.	Enhanced transport via AQP is not beneficial under salt stress and has a deleterious effect in water shortage conditions. Plants limit their symplastic transport by AQP (and hence their transpiration) under favorable growth conditions. Defensive mechanism to prevent fast wilting under water stress.
Uehlein et al., 2003 [106]	*Nicotiana tabacum*	NtAQP1	NtAQP1 overexpression increases membrane permeability for CO_2 and water and increases leaf growth.	An increase in CO_2 membrane permeability increases net photosynthesis.
		Overexpression	Photosynthesis increased by 136%. Plants with low level of NtAQP1 incorporate less carbon.	NtAQP1-mediated CO_2 permeability is of physiological importance in plants.
		Silencing	Net photosynthesis is reduced by 57%.	The reduced CO_2 availability in anti-sense plants is rate limiting for photosynthesis.
Siefritz et al., 2004 [108]	*Nicotiana tabacum*	NtAQP1 silencing (anti-sense)	Diurnal epinastic leaf movement is reduced in the transgenic lines.	Cyclic expression of PIP1 aquaporins is important for leaf (un)folding.
Bots et al., 2005 [109]	*Nicotiana tabacum*	PIP2 anti-sense lines	Primary roots of the mutants are significantly longer.	Importance of PIP2 in root water uptake and root development.

Table 1 (*continued*)

Author	Species	Protein/modification	Effect	Interpretation
			PIP2 proteins are modulated during anther development. Mutants display slower anther dehydration and delayed dehiscence.	PIP2 proteins are required for efficient anther dehydration prior to dehiscence.
Yu et al., 2005 [97]	*Nicotiana tabacum*	BnPIP1 (*Brassica napus* gene)	Overexpression increases tolerance to water stress at the whole plant level, leaf protoplasts swell faster than WT, seeds germinate faster than WT in osmotic stress conditions (soil containing 20% PEG).	Increased aquaporin levels might provide plants with additional ability to withstand drought stress.
		Silencing	Antisense plants show morphological deformation, developmental delay and decreased tolerance to water stress. Antisense leaf protoplasts remain intact and stable for a longer period of time than WT in hypotonic solution, germination of antisense seeds is seriously inhibited in soil containing 20% PEG.	Aquaporins are involved in plant adaptation to dehydration conditions.
Ding et al., 2004 [98]	*Nicotiana tabacum*	AqpL1 (*Lilium philadelphicum*)	Transgenic plants consume more water than WT plants. Leaf cells overexpressing AqpL1 have 3 to 4 times the hydraulic conductivity of WT leaf cells.	Overexpression of AqpL1 in tobacco improved water permeability fold.
		Overexpression	Stomatal aperture of transgenic plants is bigger than for control; stomatal density is higher in young leaf tissue. Overexpression of AqpL1 in tobacco greatly increases osmotic water permeability of leaf protoplasts (6-fold increase in P_f compared to WT).	Overexpression might either enhance the cell-to-cell pathway or might be an internal factor to induce high stomatal density and increased leaf transpiration.

Mercurial compounds have been used to assess the contribution of AQPs to plant water relationships. Inhibition by mercurial compounds has been extensively studied at the root level. For instance, mercurials reduce the Lp_r by 32–90% in Buckhorn cholla cactus, tomato, wheat, paprika pepper, melon, sugar beet, and barley [76–82]. Using 20 μM mercury, Hukin et al. [83] also showed a rapid 4-fold decrease in root elongation rate, suggesting a central role of AQPs in this process. Although this general AQP inhibition is a good indication for water channel activity at the cellular level, the lack of specificity of these agents makes any analysis of AQP function at the tissue level difficult. Inhibition of water transport in plant cells and tissues can be caused by direct blockage of AQPs and by indirect effects through altered cell metabolism and solute homeostasis. These indirect effects could result in down-regulation of AQP activity or in the collapse of local water-potential gradients [84]. In addition, the contribution of AQPs to root water transport could still be underestimated using mercury, as several AQP isoforms have been shown to be mercury-insensitive [85].

Suppression of gene expression, or reverse genetics, provides a more specific approach than blockers to probing the function of AQPs in planta and has become the strategy of choice for pinpointing the physiological function of a (set of) gene(s). Different approaches can be performed, each yielding different kinds of answer. If the role of a given isoform is to be investigated, disruption of its gene by T-DNA or transposon insertion should provide precise information. The targeted isoform should be chosen on the basis of its subcellular localization or tissue-specific expression pattern. This approach can, however, be complicated by possible phenotypic compensation from close homologs of the disrupted gene. As a large number of AQP genes are found in plant genomes, gene silencing by antisense or RNA interference represents another efficient approach, as the concomitant down-regulation of several AQP homologs, and thus more pronounced phenotypes, is expected. Overexpression of a given AQP is another possible approach to determining their function in planta. Finally, heterologous expression is a commonly used method to elucidate the function and specificity of AQPs. However, although heterologous expression of AQPs in *Xenopus* oocytes is a powerful tool for probing water channel activity, results from other heterologous expression systems (culture cells, other plant species, etc.) should be treated with caution, as the subcellular targeting of an AQP can differ in different organisms, so that the observed phenotype might not be related to the physiological function in the source organism. In the following sections, we attempt to identify common trends by comparing the reverse genetics results obtained on AQPs over the last decade. After careful analysis, we identified some general trends when AQP genes are overexpressed or silenced that are shared by different isoforms. On the other hand, some effects seem to be quite difficult to analyze in a global way. Comparison of the phenotypes caused by up- or down-regulation of AQP genes must take into account when and where the gene is expressed in the wild-type plant. For example, it is meaningless to try to

correlate the down-regulation of leaf-specific AQP in tobacco with the down-regulation of root-specific AQP in *Arabidopsis*, as the effect will not primarily affect the same organ or even the same physiological process. Table 1 summarizes the data obtained by deregulating AQP expression in different plant species.

5. AQP silenced plants have lower single cell water permeability

The silencing of plasma membrane AQPs usually results in a decrease in cell water permeability. When examining *Arabidopsis* plants with reduced expression of PIP1s and PIP2s, produced by crossing two different antisense lines (double antisense plants, dAS), Martre et al. [86] found that the osmotic hydraulic conductivity of isolated root and leaf protoplasts was reduced by 5- to 30-fold. Down-regulation of *PIP1* gene expression (using *NtAQP1* antisense constructs) in *Nicotiana tabacum* plants resulted in a shift from high P_f cells (P_f values of 16–64 μm/s) in control plants to low P_f cells (P_f, 8–16 μm/s) [87]. Similarly, *Arabidopsis* lines with down-regulated PIP1 expression were found to have a reduced P_f in leaf protoplasts (shift in P_f from 11 to 3 μm/s) [88]. Finally, characterization of an *Arabidopsis PIP2;2* knock-out line obtained by T-DNA insertion revealed a 27–28% reduction in the cell Lp [89], indicating that this single isoform was responsible for roughly one third of water transport in root cortical cells. Despite this huge change in the single cell Lp, the growth and development of the *PIP2;2* knock-out plants were not different from those in the wild-type plant.

From these data, we can infer that reduced cell osmotic water permeability is a common event in PIP down-regulated plants. This confirms the role of PIPs as active water channels in planta. PIP2s are indeed considered good water channels, but the role of PIPs in facilitating water transport is still unclear. While *Arabidopsis* PIP1;1, PIP1;2 and PIP1;3 exhibit water channel activity when expressed in oocytes, PIP1 isoforms from maize or other species have very low, or no, activity [90,91]. Maize proteins from these two subgroups do, however, interact in *Xenopus* oocytes, resulting in better PIP1 trafficking to the plasma membrane and higher activity than in oocytes expressing PIP2 isoforms alone, suggesting that heterotetramerization of PIP2 and PIP1 isoforms might activate the water channel activity of the latter (see above; [14,42]). Interestingly, Martre et al. [86] pointed out that, when comparing the water parameter data obtained from PIP1 and/or PIP2 antisense plants, the PIP subgroup that was not down-regulated appeared inactive, providing further support for the theory of a positive interaction between PIP subgroups in regulating the cell P_f, possibly through heteromerization.

6. Different compensation mechanisms exist for lower cell water conductivity

Although the phenotypes of silenced PIP plants are quite similar at the single cell level, it is harder to generalize for results at the tissue level. This is probably a consequence of the different mechanisms used by plants to compensate for lower water permeability. Plants can try to avoid reduction of water flow either by increasing their absorption capacity or by reducing water loss without increasing water absorption by the roots. These compensation mechanisms involve either an increase in the root/shoot ratio or a decrease in evapotranspiration. Importantly, when studying antisense lines, one should pay attention to the number of genes showing altered expression (either reduced, or increased by a gene compensation mechanism). Indeed, RNA interference might not be totally gene-specific (depending on the specificity of the sequence used to silence the gene) and may trigger the down-regulation of close homologs. Moreover, compensation by close homologs is always possible and should not be neglected.

In *Arabidopsis*, PIP dAS plants compensated for a 3-fold decrease in root hydraulic conductivity (expressed on a root dry mass basis) by a 2.5-fold increase in the root/leaf dry mass ratio, with the result that the hydraulic conductance of the whole plant was almost unchanged [86]. The phenotype of PIP1 antisense *Arabidopsis* plants was quite similar to that of dAS mutants, with a 5-fold increase in the root/leaf mass ratio [88]. These observations suggest that, under water-sufficient conditions, *Arabidopsis* antisense plants compensate for the lower hydraulic conductivity by investing more carbon in root production, so that the overall Lp_r tends to remain the same even though the single cell P_f is decreased [86]. Due to the higher root surface area, the drought resistance is not significantly different from that of control plants, but these plants recover more slowly from water stress, as recovery involves water crossing the cell membrane, and reduced cell water permeability would therefore hinder fast recovery.

Down-regulation of PIP1 aquaporins (NtAQP1) from *N. tabacum* was also shown to reduce the osmotic water permeability of leaf and root protoplasts compared to wild-type plants, resulting in a lower Lp_r [87]. However, these plants did not compensate this lower Lp_r by increasing the root/shoot ratio. Instead, the physiological adaptation generated by reduced PIP expression was a reduction in the driving force for evapotranspiration, i.e., limiting evapotranspiration by stomatal closure and decreasing the plant water potential. However, these silenced plants were more sensitive to water stress than controls. The nature of the osmotic sensor triggering these physiological adaptations is still unknown. A recent review [92] suggests that one major function of AQPs might be to sense differences in osmotic and/or turgor pressure and transduce signals to regulate the cellular water homeostasis. This concept of AQPs as osmosensors is quite innovative. However, even if this theory might seem quite radical, the true situation might lie somewhere in between where some AQPs could act as osmosensors, but experimental evidences are currently missing.

The data on TIP isoforms indicate that they play a central role in cell homeostasis, cell elongation processes, and possibly vesicle trafficking. The physiological role of *Arabidopsis* tonoplast TIP1;1 was investigated in planta using RNA interference [93]. Strong down-regulation of *AtTIP1;1* led to plant death. Transcript and metabolite profiling and GFP-

TIP1;1 cellular localization suggested a role of TIP1;1 in the control of carbon distribution, which alters source/sink relationships, possibly by regulation of vesicle trafficking towards the central vacuole. The lethality in plants with down-regulated *AtTIP1;1* indicated an essential physiological role of this highly expressed isoform. Five subclasses of TIPs are currently known. The TIP1, 2, and 5 subclasses have been studied extensively and proposed as markers for vacuoles with different functions [94]. However, instead of being a passenger, TIP proteins could be the "driver" of these vesicles to the vacuolar compartment [93] and a mutation would cause perturbation of the shuttling that could account for the observed phenotypes. A similar crucial function was deduced from the characterization of maize *ZmTIP1;1* knockout mutants. Screening of a Pioneer Hi-Bred maize mutant collection identified 9 independent F1 lines with a transposon insertion in the *ZmTIP1;1* gene, However, molecular characterization (PCR amplification, Southern and Northern blot hybridizations) performed on F2 plants did not confirm any of these insertions (except one insertion downstream of the open reading frame), suggesting that the presence of the transposon in *ZmTIP1;1* in F1 plants was due to somatic events that were not inherited and that true knockout mutants were not viable [Chaumont, Meeley, Chrispeels, unpublished data].

7. AQP overexpression highlights their roles in numerous physiological processes

Overexpression consists of expressing an endogenous or heterologous gene under the control of a strong and/or inducible promoter. Overexpression of plant AQPs could provide the plant with an increased water absorption capacity in conditions under which the osmotic gradient between the soil and plant is very weak, but, nevertheless, in favor of the plant. However, this improved absorption capacity could be detrimental in conditions of water stress, as excess AQP activity could favor more rapid water loss to the soil or atmosphere (evapotranspiration) and, therefore, faster wilting of the plant. *OsPIP1;3* is a rice *AQP* gene from the *PIP1* subfamily putatively implicated in drought resistance [95]. Its expression was increased in drought-tolerant rice lines and was decreased in drought-sensitive lines. Overexpression of this gene under the control of a drought-inducible promoter resulted in a higher Lp_r, leaf water potential, and cumulative transpiration than in the wild-type, but no morphological differences were seen between wild-type and transgenic plants [95]. In transgenic rice expressing a barley gene (*HvPIP2;1*), the radial root hydraulic conductivity was up to 140% higher than in controls [96]. The phenotype of these plants was identical to that of the controls, except for one line, which had an increased shoot/root mass ratio due to a reduction in root mass. The explanation might be that a lower root surface area is sufficient to supply water to the plant. However these transgenic rice plants were more affected by salt stress than controls (see below). These data correlate well with the expected phenotype if AQPs are considered as pure water channels.

Experiments analyzing the water permeability of plant cells overexpressing AQPs are still scarce. Leaf protoplasts extracted from transgenic tobacco overexpressing rape BnPIP1 swelled and burst faster than WT cells when transferred into hypotonic medium [97]. Similarly, overexpression of a lily *PIP1* gene in tobacco increased the osmotic water permeability of leaf cells more than 5-fold [98].

Overexpression of TIP proteins has not been documented in whole plant systems. Barrieu et al. [99] reported up-regulation of two cauliflower tonoplast AQPs, BobTIP26;1 and BobTIP26;2 (orthologous to AtTIP1;1), following desiccation stress. Heterologous expression of *BobTIP26;1* in tobacco suspension cells had no effect on the growth rate, but the cells were larger than in the wild-type [100]. This agrees with the hypothesis that TIP proteins are involved in cell enlargement. This volume increase might be triggered by enlargement of the vacuolar compartment facilitated by the higher tonoplast permeability, allowing increased water movement from the cytosol to the vacuole. This greater swelling of the cells is dependent on the inwardly directed movement of water across the plasmalemma and the tonoplast which requires the built-up of a *trans*-tonoplastic osmotic potential.

AQP overexpression might highlight other physiological processes in which AQPs act as bottlenecks. As already mentioned, AQPs might have physiological functions other than just facilitating water movement, thus complicating the situation. This could explain the observed phenotypes which would not be expected if facilitated water transport was the only issue to be taken into account. However, whether the effects seen at the physiological level are linked directly to AQP activity or are a side-effect due by an effect on the expression of another protein is still a matter of debate, and the data should be interpreted with care. Transgenic expression of *AtPIP1;2* in tobacco significantly increased plant growth and transpiration rate, stomatal density, and photosynthetic activity. The global phenotype was quite different from that of control plants; the transgenic lines were taller, the number of stem internodes greater, and the stem diameter larger, but the size of the leaves was comparable to wild-type. This resulted in a 50% decrease in the root/shoot ratio caused by a 50% increase in stem fresh weight. The leaves contained 30% more dry mass than in the wild-type. Overexpression may have been an internal factor to induce high stomatal densities and aperture and subsequently increased leaf transpiration rate causing a stimulation of cellular and physiological processes regulating plant vigor [98]. Similarly, overexpression of *HvPIP2;1* in transgenic rice increased the internal CO_2 conductance (40%), stomatal aperture (27%), and CO_2 assimilation (14%), showing a clear role of this protein in these processes [96]. This transgenic rice had a faster growth rate than the wild-type, but, as with transgenic tobacco, these plants were more affected by water stress due to their higher water loss by transpiration. This overexpression also led to a change in leaf anatomical structure, with smaller mesophyll cells and thicker epidermis and mesophyll cell walls, indicating that the leaves became xeromorphic. These features are normally observed during water stress. These observations suggest that, if AQP expression exceeds a certain threshold, the resulting plant water status can induce anatomical changes in the organs [101]. Facilitated

passage of CO_2 has been demonstrated by heterologous expression of human AQP1 in *Xenopus* oocytes [102]. However, two studies have cast doubt on this interpretation. The first showed that erythrocytes from AQP1 null mice had the same CO_2 permeability as the wild-type [103], while the second showed that carbonic anhydrase, used to accelerate the pH changes associated with CO_2 fluxes in some previous studies, was itself inhibited by $HgCl_2$, so the observed inhibitory effects, previously thought to be due to an effect on AQP activity, might in fact be nonspecific [104]. Recently, tobacco NtAQP1 was shown to facilitate the passage of CO_2 through membranes [105]. Moreover, NtAQP1 seems to have a significant role in photosynthesis and stomatal opening. *NtAQP1* overexpression increased membrane permeability for CO_2 and water and increased leaf growth, but there was no effect on plant height or root mass development [105]. NtAQP-related CO_2 permeability seems to be of physiological importance under conditions in which the CO_2 gradient across a membrane is small, as is the case between the atmosphere and the plant cell cytosol [105]. Since ribulose 1,5 biphosphate carboxylase, a key enzyme in photosynthesis, has a relatively low affinity for CO_2, it would be preferable to have an overall low resistance to internal diffusion of CO_2. A decrease in membrane resistance to CO_2 transport would increase the apparent CO_2 bio-availability for the plant and thus would improve photosynthesis, which would ultimately affect the degree of photorespiration and the efficiency of nitrogen and water use of the leaf. In support of this hypothesis, some phenotypes seen following overexpression of AQP genes might be connected to improved carbon bio-availability [11,105,106]. Is this enhanced carbon bio-availability a side-effect of AQPs acting as water channels or is it a direct effect of AQPs acting as gas movement facilitators? The observed phenotype might also be due to a synergistic effect of increased water permeability and CO_2 diffusion, but it is currently not possible to distinguish the contributions of these two factors. Altogether, these data suggest that AQPs are key regulators of plant vigor.

8. Resistance to salt and water stresses: 2 classes of aquaporins?

The overall behavior of AQP-deregulated (both silenced or overexpressing) plants following water and salt stresses is highly variable and puzzling. Effects can range from none (compared to control plants) to increased or decreased water or salt stress sensitivity, and predictions of increased or decreased resistance following up- or down-regulation of a given isoform are not possible. Some examples found in the literature highlight this point. Transgenic rice overexpressing *HvPIP2;1* was found to be more salt-sensitive (100 mM NaCl), showing reduced growth of the AQP-overexpressing plants, pointing out the importance of AQPs in salt tolerance [101]. Similarly, overexpression of *AtPIP1;2* in tobacco had no beneficial effect under salt stress, but was detrimental upon drought stress, causing faster wilting [107]. On the other hand, OsPIP1;3, a rice AQP, seems to improve resistance to drought stress (drought avoidance mechanism) when expressed in drought-sensitive lines under the control of a stress-induced promoter [95]. Overexpression of this isoform increased the Lp_r, leaf water potential, and cumulative transpiration in drough-sensitive lines, improving their overall resistance, but the transformed plants had a normal phenotype. Although effects are quite divergent, they emphasize the central role of AQPs in stress resistance. However caution must be taken when interpreting these results obtained either by heterologous expression under a constitutive promoter or by homologous expression under a stress-responsive promoter. The latter strategy seems more relevant to study the role of an aquaporin in water resistance.

Two opposite views exist today concerning the functions of AQPs in plant under water stress. The first one is that increased AQP levels might provide the plant with additional ability to handle drought stress, which relies on the observation that some AQPs are induced or activated upon drought stress. The second opinion is that plants try to avoid excessive loss of water by down-regulating AQPs during dehydration. We would like to suggest the grouping of plant AQPs into two "classes". The first would consist of AQPs involved in the maintenance of cellular water (osmotic) status, which are not involved in controlling (huge) stress-related variations in water potential, but which buffer local variations at the cell level or allow the movement of some gasses or small non-electrolytes through membranes. These isoforms would be expressed in a constitutive way to allow a basal mode of action of the cellular machinery and their regulation would not be primarily involved in stress response mechanisms. The second class of AQPs would consists of isoforms that are specifically expressed and/or regulated following stresses in appropriate organs in order to compensate for the altered water potential (stress-responsive isoforms). Mutation of these isoforms would markedly alter the overall resistance of the plants to these stresses. The existence of these two classes of AQPs integrates the two views on the role of AQPs in stress conditions: down-regulation of "constitutive isoforms" and activation "stress-responsive" ones.

The identifications of characteristics making these isoforms stress-responsive should be a prime interest in the field. A non-exhaustive list of such characteristics is:

- Subcellular localization of the protein: plasma membrane or internal membrane?
- Transport specificity: is water the only substrate of this isoforms?
- Tissue expression: is it specifically expressed in roots, shoots, or reproductive organs?
- Method of regulation: is expression modulated by transcriptional or post-translational modifications? What are the effects of these modifications? Is the promoter of the gene activated by stress-induced signals?

In a given plant species, quantitative expression analysis (at both the RNA and protein level) following stress should help to identify these stress-responsive isoforms. This type of experiment has been carried out in rice [74] and *Arabidopsis* [47,65].

For instance, Alexandersson et al. [65] showed different expression patterns of *AQP* genes upon drought stress. Whereas the general trend was a down-regulation of the *PIP* genes, the expression of three of them, *AtPIP1;4 AtPIP2;5 and AtPIP2;6*, was stable or enhanced. Interestingly, these three genes are lowly expressed in roots and highly expressed in leaves and/or flower, and all *PIP* genes shown to be down-regulated are more highly expressed in roots. These observations further support the theory of different physiological functions for AQP genes upon stress. However, interpretation of the expression data is complicated by the large number of regulatory events (both post-transcriptional and post-translational) occurring in planta (see above). Finally, we believe that the substrate specificity of each AQP should be determined to know whether it facilitates only water transport or also facilitates the passage of other molecules and thus plays a role in physiological processes not directly concerned with water transport.

9. Conclusions

It is now well established that AQPs are widely expressed in both the vegetative and reproductive organs of the plant, allowing fine physiological regulation of water transport. Over the last decade, their physiological contributions have been investigated in planta by reverse genetics. Assuming that overexpression or down-regulation of a gene can help identify its function, this kind of approach is of key importance in the field. Such studies have already provided results demonstrating the central role of AQPs in plant physiology. Their role in physiological water flow control is now clearly established and their involvement in CO_2 conductance has also been recently identified. This emphasizes the central role of AQPs in plants, which, as photosynthetic autotrophs, mainly require two compounds, water and CO_2 the availability of which might be directly modulated in plant tissues by AQP activity.

However, in the case of plant AQPs, the procedure of identifying their physiological functions is complicated by the high isoform diversity (with possible compensation mechanisms between close homologs) and the high number of regulatory processes at different levels (transcriptional and post-translational). The situation is often complicated by a lack of characterization of transport selectivity, especially in the case of CO_2 conductance. AQPs are often characterized solely in terms of their water transport ability. After considering the large number of phenotypes seen following deregulation (overexpression) of these genes, we believe that, when carrying out reverse genetic experiments, careful transport characterization, in terms of selectivity, should be performed (e.g., in heterologous systems, such as *Xenopus* oocytes) which might help to analyze the data obtained by modulating the expression of these genes. A distinction between constitutive and stress-responsive isoforms should also be made by examining RNA and protein expression profiles in different growth conditions. Finally, the diversity of mechanisms regulating AQP activity under these conditions requires careful characterization and should be taken in account to generate a complete picture of AQP function in planta.

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