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Review

Functional aquaporin diversity in plants

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Abstract

Due to the fact that most plants are immobile, a rapid response of physiological processes to changing environmental conditions is essential for their survival. Thus, in comparison to many other organisms, plants might need a more sophisticated tuning of water balance. Among others, this is reflected by the comparable large amount of aquaporin genes in plant genomes. So far, aquaporins were shown to be involved in many physiological processes like root water uptake, reproduction or photosynthesis. Their classification as simple water pores has changed according to their molecular function into channels permeable for water, small solutes and/or gases. An adjustment of the corresponding physiological process could be achieved by regulation mechanisms. Concerning aquaporins these range from posttranslational modification, molecular trafficking to heteromerization of aquaporin isoforms. The aim of this review is to underline the function of the four plant aquaporin family subclasses with regard to the substrate specificity, regulation and physiological relevance.

Keywords: Plant; Aquaporin; Water channel; Gas transport

Contents

1. Introduction

Aquaporins in plants are more abundant and show greater diversity than in animals or bacteria. In *Arabidopsis* for example 35 [1] and in maize 33 [2] MIP (major intrinsic protein) like isoforms were identified by genome and transcriptome analysis. Whereas in vertebrates 11 to 13 different types of aquaporin genes exist [3]. Higher plant aquaporins form four subfamilies: tonoplast intrinsic proteins (TIPs), plasma membrane intrinsic proteins (PIPs), Nodulin26-like intrinsic proteins (NIPs) and small basic intrinsic proteins (SIPs) [4] (Table 1).

2. TIPs

The plant vacuole is a cellular storage compartment with functions in turgor regulation, cell signaling and degradation. The flux of water and small solutes across the vacuolar membrane (tonoplast) suggests that aquaporins participate in

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Table 1
Properties of aquaporin representatives

Subfamily	Name	Substrate specificity	Regulation	Physiological relevance	References
TIPs	AtTIP1;1	H_2O, urea	gibberellic acid, salinity, membrane relocalization, drought stress	osmoregulation	[5,10,12,13,29,30]
	AtTIP1;2	urea	N-availability in roots, salinity, drought stress	vacuolar urea loading/unloading	[10,12,13,30]
	AtTIP2;1	urea, NH_4^+/NH_3, methylammonium	$N/NH_4^+/NH_3$ availability in roots, drought stress	vacuolar urea loading/unloading, NH_4^+/NH_3 detoxification	[12,13,16,30]
	AtTIP2;3	NH_4^+/NH_3, methylammonium	$N/NH_4^+/NH_3$ availability in roots, salinity	NH_4^+/NH_3 detoxification	[10,16]
	AtTIP4;1	urea	N-availability in roots	vacuolar urea loading/unloading	[13]
	TaTIP2;1	NH_4^+/NH_3, methylammonium, formamide	not determined	not determined	[14,15]
	NtTIPa	H_2O, urea, glycerol	not determined	osmoregulation	[11]
	PvTIP3;1	H_2O	phosphorylation	osmoregulation	[24]
	McTIP1;2	H_2O	Osmotic stress, abscisic acid, membrane distribution, glycosylation	osmoregulation	[26]
NIPs	Nodulin26	H_2O, glycerol, formamide, malat, NH_3	osmotic/drought stress, phosphorylation	osmoregulation/metabolite exchange in rhizobia-legume symbiosis	[22,31,32]
	LIMP2	H_2O, glycerol	phosphorylation	not determined	[33,34]
	AtNIP1;1	glycerol	not determined	not determined	[35]
	AtNIP1;2	glycerol	not determined	not determined	[35]
	CpNIP1	H_2O, urea	not determined	transcellular urea/ H_2O transport	[12]
SIPs	AtSIP1;1	H_2O	not determined	not determined	[36]
	AtSIP1;2	H_2O	not determined	not determined	[36]
PIP1s	NtAQP1	H_2O, glycerol, CO_2, urea	gibberellic acid, abscisic acid	root water transport, epinastic leaf movement, photosynthesis, stomatal opening	[37–39]
	PsPIP1;1	glycerol, glycine	not determined	rehydration of dry seed	[40]
	AtPIP1;2	H_2O	blue light, gibberellic acid, abscisic acid, pH-dependent gating	cellular water transport	[41–44]
	ZmPIP1;1	not determined	diurnal expression, heteromerization of PIP isoforms	not determined	[45–47]
	ZmPIP1;2	not determined	heteromerization of PIP isoforms	not determined	[45,47]
PIP2s	AtPIP2;1	H_2O	pH-dependent gating	not determined	[44]
	AtPIP2;2	H_2O	pH-dependent gating, drought stress	osmotic water transport in roots	[30,44,48]
	ZmPIP2;1	H_2O	diurnal expression, heteromerization of PIP isoforms	root water transport	[45,47]
	SsAQP2	H_2O	diurnal/circadian expression	leaflet movement	[49]
	HvPIP2;1	H_2O, CO_2	not determined	CO_2 assimilation	[50]
	SoPIP2;1	H_2O	Phosphorylation, appoplastic water potential, pH-dependent gating	regulation cell turgor	[51–54]

these processes. Accordingly, one of the first proteins with aquaporin function in plants was identified in vacuolar membranes from *Arabidopsis thaliana* [5,6]. This tonoplast intrinsic protein (TIP1;1, initially named γ-TIP) was found being highly water selective when expressed in *Xenopus* oocytes. It did not promote permeability for glycerol. Further osmotic water permeability measurements on isolated vacuoles or tonoplast vesicles and purified plasma membranes exhibited a comparable 100-fold higher permeance of the tonoplast [7–9]. This implicates an involvement of the tonoplast in regulating water flow in response to osmotic challenges like drought or salinity for a plant cell. Hence, TIP expression correlated with water stress after salinity in *Arabidopsis* [10]. Abundance of TIP transcripts decreased to 75% after exposure to salt. Furthermore, TIP fusions with the green fluorescent protein showed a delocalization into vacuolar membrane invaginations [10]. In 1999, Gerbeau et al. [11] analyzed the water flux in purified tobacco tonoplast vesicles and showed an increased permeability of solutes like urea and glycerol compared to plasma membrane vesicles. The authors isolated a cDNA encoding a tonoplast intrinsic protein (NtTIPa) homologue localized in the tonoplast membrane. Functional expression of NtTIPa in *Xenopus* oocytes indicated that this protein was permeable to water, urea and glycerol. Also, in *Arabidopsis* different TIP isoforms (TIP1;1, TIP1;2, TIP2;1, TIP4;1) could be isolated and were characterized in the oocyte system and yeast complementation assays as urea permeases [12,13]. All four genes conferred growth of a urea uptake defective yeast mutant in a phloretin-sensitive and a pH-independent manner. Thus, in addition to their role as water channels, some TIPs may play a role in equilibrating urea concentrations between the vacuole and the cytoplasm.

Ammonium (NH_4^+) and its gaseous conjugated base (NH_3, ammonia) as additional N sources and primary substrates for the synthesis of amino acids were as well suggested to be transported by tonoplast intrinsic proteins [14–16]. Cellular uptake of NH_4^+/NH_3 in plant cells is mediated by ammonium transporters of the AMT protein family [17–20]. Recent studies encouraged participation of TIPs to transport NH_4^+/NH_3 from the cytoplasm into the vacuole. TIPs of *Arabidopsis* [16] and wheat [14, 15] could be identified in yeast complementation assays that conferred permeability to ammonium and its analogs methylammonium or formamide. Expression of TIPs in *Xenopus* oocytes showed an increased permeability for NH_4^+/NH_3 at rising pH. A comparable observation came from yeast complementation approaches. At applied pH values of 6.5 and 7.5, NH_4^+/NH_3 equilibration is shifted towards the formation of NH_3 and provides a preferred substrate for these aquaporins. Consequently, TIPs could be involved in the detoxification of the cytoplasm by an acid-trap mechanism as suggested by Loque et al. [16]. NH_3 was transported into the acidic vacuolar lumen, subsequently binds a proton to form NH_4^+. Results from voltage clamp experiments indicated for a different model of NH_3 conductance [14]. In oocytes expressing several mammalian aquaporins or wheat TIP2;1, inward clamp currents at high NH_3 concentration in the bathing solution were carried by NH_4^+. The authors suggested that NH_3 enters the aqueous pore, reacts with an H^+ and NH_4^+ is released into the cell. However, until now no physiological consequences of TIP mediated NH_3 transport could be identified, since transgenic *Arabidopsis* plants overexpressing AtTIP2;1 showed no differences in capacity and rate of ammonium accumulation in root cells compared to wild type plants [16]. With regard to the high diversity in the putative pore regions within the TIP subfamily [21] different physiological functions are possible.

A recent study investigating the permeation of ammonia gas through the peribacteroid membrane of soybean nodules correlated the phosphorylation of an aquaporin to decreased NH_3 conductance [22]. Phosphorylation of a TIP was initially shown for seed specific bean PvTIP3;1 [23,24]. Expressed in *Xenopus* oocytes PvTIP3;1 conferred an increase in osmotic water permeability of the membrane. Incubation of oocyte plasma membrane vesicles with cAMP agonists led to a further increase in water channel activity. The results imply a membrane associated cAMP dependent protein kinase phosphorylating PvTIP3;1 in vivo [24]. Homology modeling of PvTIP3;1 suggested that a serin residue at position 7 is the only residue accessible to kinases [25]. Expression in yeast and mass spectrometry of the purified protein confirmed in vitro phosphorylation of Ser7. Treatments with phosphatases and kinase suggested Ser7 as the unique phosphorylation site in PvTIP3;1.

Tonoplast intrinsic protein regulation and redistribution in response to osmotic stress was further investigated using ice plant (*Mesembryanthemum crystallinum*) [26]. Exposure to salt slightly decreased McTIP1;2 abundance while treatment with mannitol, sorbitol or abscisic acid caused an up-regulation [27,28]. Osmotic stress induced by mannitol increased McTIP1;2 amount, which was accompanied by fast and persistent changes in aquaporin membrane allocation [26]. Using indirect immunofluorescence microscopy McTIP1;2 associated fluorescence was observed in the cytosol in conjunction with vesicle like structures upon mannitol treatment. Membrane distribution was accompanied by glycosylation of McTIP1;2. Treatment with tunicamycin, which blocks the formation of protein carbohydrate bonds, prevented both membrane distribution and formation of the glycoprotein. Effects of salinity on aquaporin expression was also investigated in *Arabidopsis* roots [10]. TIP and other aquaporin transcripts showed an extensive decrease in abundance after 4 h following exposure to salt. AtTIP1;1 green fluorescent protein fusion showed a relocalization into intracellular spherical structures identified as intravacuolar invaginations after 45 min. Consequently, aquaporin relocalization seem to be a short-term regulation mechanism after osmotic stress while transcriptional regulation modifies root water transport properties in the long term.

Taken together, on the basis of the high water permeability found for several TIPs, an involvement in osmoregulation and non-limiting water flow through the tonoplast is apparent. The additionally observed conductance of small solutes and gas may link tonoplast intrinsic proteins to important metabolic pathways like the urea cycle or amino acid synthesis. The further investigation of potential regulation mechanisms could define aquaporins of the TIP subfamily as multifunctional channels.

3. NIPs

Under nitrogen-limiting soil conditions, plants of the *Leguminosae* family can be infected by nitrogen fixing bacteria. The Infection results in the formation of a nitrogen fixing root organ, the nodule [55–57]. The core of the nodule filled with bacteroids is surrounded by an external symbiosome space and a highly specialized membrane referred as symbiosome membrane (SM). The symbiosome membrane takes part in the efflux of fixed nitrogen from the bacteria to the plant and carbon supply in the opposite direction. During the nodule formation several proteins (nodulins) were expressed by the plant and targeted to the SM [58]. Soybean Nodulin 26 (Nod26) was described as a major integral protein of SM, constituting approximately 10% of total membrane protein [58,59]. Nod26 was classified into the major intrinsic protein (MIP) cluster and describes the archetype of the subfamily. Consequently, all proteins related to nodulin26-like intrinsic proteins (NIPs) were named accordingly. Nod26 heterologously expressed in *Xenopus* oocytes showed a mercury-sensitive osmotic water permeability and conductance to glycerol [31]. Stopped flow spectrophotometry analysis of native symbiosome vesicles approved a high mercury sensitive water permeability of the Nod26 containing membrane. In the same approach, SM vesicles were shown to be additionally permeable for glycerol and formamide. Due to the mercury sensitivity, the transport was referred to nodulin 26 function [31]. These results were confirmed by purification and functional reconstitution of Nod26 into proteoliposomes [60]. Besides water and small solutes Nod26 may also permeate gaseous NH_3 [22]. Ammonia uptake into isolated SM vesicles was inhibited by mercury to about 40%. NH_3 permeability was

mercaptoethanol-reversible but decreased subsequently to ATP incubation. In preceding studies, Nod26 was shown to be phosphorylated in vitro and in vivo at serin residue 262, catalyzed by an SM associated Ca^{2+}-dependent protein kinase (CDPK) [59,61,62]. Phosphorylation enhanced water and solute transport while it apparently decreased gas permeability (see above). The results suggest that either NH_3 and H_2O may permeate Nod26 via different pathways or different components of the SM are required for gas conductivity. A recent study analyzing the developmentally regulated phosphorylation state of Nod26 supported participation of the protein in osmoregulation [63]. Phosphorylation of Nod26 was enhanced by osmotic stress (both drought and salt stress) and could be detected at steady state levels until the onset of senescence. Nod26 is phosphorylated by calcium-signaling pathways in response to osmotic stress. The physiological functions of the demonstrated malat and glycerol conductivities seem unclear and have to be revealed in further studies.

Besides Nod26 a few NIP of nonlegumes were characterized and were shown to be expressed in vegetative and reproductive tissues, suggesting a larger role of NIPs in plant water relations than the symbiotic function of Nod26 [1,64]. Sequence analysis identified specific amino acid residues to distinguish between aquaporins and aquaglyceroporins within the MIP cluster [65]. While most of plant TIPs and PIPs belong to the aquaporin group of MIP, NIPs exhibit amino acid signatures of both subgroups.

In different experimental approaches NIPs of *Arabidopsis* (AtNIP1;1, AtNIP1;2), and the legume *Lotus japonicus* (LIMP2) were analyzed by heterologous expression, formed functional glycerol permeases and exhibited partially low water conductivity [33,35,64].

A NIP from zucchini (CpNIP1) complemented growth of a urea-transporter defective yeast mutant and features water permeability, but lacks glycerol conductivity [12].

In conclusion, Nod26 and other NIP proteins share the general multifunctional transport properties of water and uncharged solutes like glycerol. Compared to most water-selective aquaporins NIPs have a lower rate of water transport and the physiological function of NIPs found in nonlegumes will most likely be different from Nod26. NIP gene transcripts were found in seed coat, shoot and root while Nod26 is only expressed in nodules [35,40,64,66].

4. SIPs

The small basic intrinsic protein subfamily is the smallest in the MIP cluster in plants. It was identified by database mining and phylogenic analysis [4]. Proteins of the new subfamily are also small, like TIPs, but still different being highly basic. The main reason for their small size is a very short cytosolic N-terminal region compared to the other plant MIPs. The N-terminal region in SIPs is even shorter than in TIPs but similar to AqpZ from *Escherichia coli*.

In recent studies, fusions of *Arabidopsis* SIPs with green fluorescent protein (GFP) were expressed in suspension cultured cells [36]. The GFP-fusion could be detected in the ER and was not abundant in plasma or vacuolar membranes. By heterologous expression in yeast and vesicle permeability studies, SIP1;1 and SIP1;2 were characterized as aquaporins, while SIP2;1 showed only a slow water influx into membrane vesicles. This was the first approach to analyze the SIP physiological function and substrate specificity.

5. PIPs

The plasma membrane intrinsic proteins constitute the largest plant aquaporin subfamily with 13 members in *Arabidopsis* and 14 in maize [1,45]. As the nomenclature implies, the majority of PIPs is localized in the plasma membrane [67]. The PIPs can be divided into two phylogenetic subgroups, named PIP1 and PIP2 [3]. They differ in the length of the N- and C-termini and in water permeability characteristics as analyzed in different heterologous expression systems.

6. PIP1s

PIP1 isoforms of *Arabidopsis* show 90% amino acid sequence identity [1]. Although, the amino acid residues at the selectivity filter are similar in PIP1 and PIP2 [21] their permeability and cellular function diverge. Tobacco plants with a decreased expression of NtAQP1, a member of the PIP1 family, showed reduced root hydraulic conductivity and lower water stress resistance [37]. These results were confirmed by a study of double PIP1/PIP2 antisense *Arabidopsis* plants [68]. In pea (*Pisum sativum*), a PIP1 is suggested to play a role in water absorption during seed imbibition [40]. PIP1 isoforms expressed heterologously in *Xenopus* oocytes or other expression systems show no or very low aquaporin activity [45,47,49,69,70]. In contrast, the permeability for small solutes like glycerol or gases such as CO_2 seems to be increased in some instances [39,69]. Experimental evidences from studies with *Xenopus* oocytes expressing tobacco NtAQP1 showed a CO_2 transport function [39]. The oocytes were injected with NtAQP1 cRNA and carbonic anhydrase, which accelerates the conversion of CO_2 to HCO_3^-. In this experimental setup, CO_2 membrane transport is rate limiting for HCO_3^- accumulation rather than the conversion reaction to HCO_3^- [71]. Eventually, CO_2 transport into the cells generates a decrease in intracellular pH that is monitored [72]. In oocytes expressing NtAQP1, it was found that CO_2 uptake was 45% higher compared to control oocytes injected with water. The CO_2 transport was initially shown for the human AQP1 [71] and could be associated to gas channel functions of aquaporins. Effects of the membrane lipid composition or expression pattern of intrinsic genes that could modify oocyte CO_2 permeability were excluded [73].

The physiological relevance of AQP mediated CO_2 transport in animals is discussed rather than a controversy. Physiological studies in plants provided evidences for relevance of an aquaporin mediated CO_2 conductance. Tobacco plants with an increased intrinsic aquaporin expression or an aquaporin anti-sense construct show changed attributes towards water transport as well as CO_2 dependent processes like photosynthesis [37,39]. When *Vicia faba* or *Phaseolous vulgaris* leaf discs were treated with minimum concentrations of $HgCl_2$ the hydraulic permeability of the plasma membrane was decreased by 70–80% as well as

photosynthetic CO_2 fixation and conductance for CO_2 from the intercellular spaces to the chloroplast stroma. Although, the $HgCl_2$ treatment should be considered with the same carefulness as in experiments investigating water conductivity, it was assumed that the photosynthetic CO_2 uptake across the plasma membrane of the mesophyll cells was facilitated by mercury sensitive aquaporins [74]. Under favorable growth conditions *Arabidopsis* AtPIP1;2 overexpressing tobacco plants showed a significantly increased transpiration rate, higher stomatal density and a greater photosynthetic efficiency [43]. Nevertheless, the authors did not relate the effects to an increase in CO_2 transport rate, but to facilitated water transport. Consequently PIP1 aquaporins could be transporters for small solutes and/or gases, or they need to be activated in the plant in order to function as water channels. Regulation mechanism like phosphorylation mentioned for TIPs and NIPs were absent for PIP1 aquaporins so far, since oocytes expressing maize PIP1;2 showed no increase in osmotic water permeability after addition of protein kinase A activators or phosphatases inhibitors [45]. Posttranslational modification is suggested for tobacco NtAQP1 [B. Otto, unpublished]. Antibody detection in root and leaf extracts led to signals of different size depending on the origin of proteins. In roots a second signal larger than the aquaporin-specific 28 kDa could be detected which was not present in protein preparations from leaf cells and could be interpreted as a result of a posttranslational modification like phosphorylation or glycosylation. A recent study, investigating the influence of heteromerization of aquaporins on their water permeability showed that coexpression of two PIP1 isoforms from maize increased the plasma membrane water permeability compared to individual assays [47]. These results implicate that heteromerization is required for PIP1 isoforms to act as functional water channel.

7. PIP2s

Aquaporins of the PIP2 subfamily seem to be more efficient water channels than members of the PIP1 cluster. In various studies using *Xenopus* oocytes or yeast membrane vesicles as heterologous expression system PIP2 aquaporins exhibit 5- to 20-fold increased water permeability compared to control values [52,64,75]. In general PIP2 aquaporins possess a shorter amino-terminal extension and a longer carboxy-terminal end. In addition PIP2s have an additional stretch of 4–10 amino acids located in the first extracytosolic loop.

To date members of the PIP2 subfamily are functional characterized in different species and a role in different physiological processes has been assumed. The proteins could be involved in cellular water transport in roots, leaves [46,68], reproduction organs [76,77] and seed germination [40].

Sequence comparison of maize EST clones led to the identification of a member of the PIP2 family named ZmPIP2;1 [45]. Transient expression in *Xenopus* oocytes increased the osmotic water permeability of the membranes 8-fold above water-injected control cells. $HgCl_2$ reversibly inhibited its water channel activity. Another example for the water channel activity of PIP2 aquaporins from plants is given by SsAQP2, which was found in membranes of the pulvini of the leguminous Mimosa tree *Samanea saman* [49]. Pulvini are motor organs responsible for movement of leaves and leaflets. Functional characterization of SsAQP2 induced up to 20-fold increased P_f values compared to oocytes expressing SsAQP1 affiliated to the PIP1 family. Like ZmPIP2;1 the water permeability could be inhibited by $HgCl_2$ and additionally by millimolar concentrations of phloretin, another transport blocker [78].

Besides water also CO_2 transport mediated by PIP2 aquaporins is suggested. The barley aquaporin HvPIP2;1 was overexpressed in rice in order to confirm the hypothesis that a PIP2 contributes to facilitated CO_2 transport [50]. Mesophyll conductance (g_i) was determined for intact leaves by concurrent measurements of gas exchange and carbon isotope ratio. The level of HvPIP2;1 was found to be strongly related to g_i and the results were interpreted in a way that HvPIP2;1 has a role in CO_2 diffusion in rice leaves. A molecular characterization of HvPIP2;1 in the oocyte system was however not provided in this study. It remained to be determined whether the correlation between aquaporin expression and CO_2 permeability increase was just a side effect or causal to HvPIP2;1 expression.

A structural and functional well characterized member of the PIP2 class is the SoPIP2;1 (formerly known as PM28A) from *Spinacia oleracea* [53]. It is localized in leaf plasma membranes. In the C-terminal region a Serin residue was found being phosphorylated in vivo by a plasma membrane-associated Ca^{2+}-dependent protein kinase in response to increasing appoplastic water potential [52]. In recent studies the crystal structure of the tetrameric protein could be solved [79]. The 3D structure of the tetramer reveals novel insides into the regulation of aquaporins. Besides the suggested phosphorylation at serin 274, a highly conserved cystein residue at the C terminus of loop A is proposed to be involved in regulation of the channel. Together with ascertained amino acid residues at the N-terminal part of helix 2 the conserved cystein may stabilize the SoPIP2;1 monomer by hydrogen bonds or complexing a metal ion initiating an opening or closure of the protein.

The plasma membrane permeability appears also to be influenced by divalent cations and the pH [80]. Cell hydraulic conductivity (L_p) was measured on *Arabidopsis* suspension cells using a cell pressure probe and varying bathing solutions. Ca^{2+} added to the pipette and bathing solution reduced L_p 4-fold. The results were confirmed on purified plasma membrane vesicles and stopped flow spectrophotometer measurements. Intracellular acidification through anoxic stress led to a decrease in water permeability of root cell membranes in Arabidopsis [44]. In experiments expressing *Arabidopsis* aquaporins in *Xenopus* oocytes a drop of intracellular pH resulted in a diminishment of water conductance implicating a closure of aquaporins by protons. A histidin (his) residue at position 197 in Loop D of AtPIP2;2 was identified to be the major pH-sensing site under physiological conditions [44,81]. In a structural model of AtPIP2;2 with protonated His^{197}, Loop D is folded over the pore and caused the closure of the protein.

Besides the regulation through mechanisms mentioned above, a multimerization of membrane proteins can regulate their activity and function [82]. To investigate influences of heteromerization on aquaporin function of PIPs, different PIP1s and PIP2s

were coexpressed in *Xenopus* oocytes (as mentioned before in the PIP1 chapter) [47,70]. Injecting of 3 ng PIP2 cRNA and increasing amounts (3–12 ng) of PIP1 cRNA from maize aquaporins led to increased osmotic water permeability (P_f) according to augmented PIP1 cRNA. Using PIP1-GFP constructs and confocal analysis techniques a 4-fold increased amount of PIP1 protein was incorporated into the membrane compared to PIP2 protein. The results attributed an increase in P_f to the formation of heteromers containing PIP1 and PIP2 isoforms. Direct interaction was confirmed by copurification on a nickel chromatography column. The increased P_f may explained by stabilization, better folding or more efficient trafficking of the proteins.

Taken together, PIP1 and PIP2 isoforms were localized in nearly all parts of the plant like roots or leaves. These plant organs are completely different in morphology and physiological function, e.g. with regard to water or CO_2 conductance. Thus, in planta, cells more permeable for water or gas could be distributed differently and it can be speculated that the PIP1 aquaporin function is modified according to the requirements of the respective tissue or cells. In membranes of leaf cells some PIP1 might act as transporters for small solutes or gases and in root these cells could display water channel activity mediated by modifications or interaction with other aquaporins. Due to the high water permeability displayed in several assays (see above) PIP2 isoforms may the major pathways for symplastic and appoplastic water transport. Through different regulation mechanisms aquaporins can provide a fine control of the hydraulic conductivity of the cell to cell pathway in response to biotic and abiotic challenges.

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