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TIP Aquaporins in Plants: Role in Abiotic Stress Tolerance

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Abstract

Tonoplast Intrinsic Proteins (TIP) are one of five subfamilies of aquaporins in higher plants. Plants typically contain a large number of TIP genes, ranging from 6 to 35 compared to humans. The molecular weight of the TIP subfamily members ranges from 25 to 28 kDa. Despite their sequence diversity, all TIP monomers have the same structure, which consists of six transmembrane helices and five inter-helical loops that form an hourglass shape with a central pore. Four monomers form tetramers, which are functional units in the membrane. TIPs form channels in the tonoplast that basically function as regulators of the intracellular water flow, which implies that they have a role in regulating cell turgor. TIPs are responsible for precisely regulating the movement of not only water, but also some small neutral molecules such as glycerol, urea, ammonia, hydrogen peroxide and formamide. The expression of TIPs may be affected by different environmental stresses, including drought, salinity and cold. TIPs expression is also altered by phytohormones and the appropriate *cis*-regulatory motifs are identified in the promoter region of the genes encoding TIPs in different plant species. It was shown that manipulating TIP-encoding genes expression in plants could have the potential to improve abiotic stress tolerance.

Keywords: aquaporins, tonoplast intrinsic proteins, abiotic stress, drought, salinity, cold, phytohormones, gene expression analysis, *cis*-regulatory motifs, plants

1. Introduction

Aquaporins (aqua-pore, AQP), which are members of the major intrinsic proteins (MIPs) superfamily, facilitate the bidirectional flux of water and non-aqua substrates across the cell membranes [1–4]. These transmembrane proteins form pores in the lipid bilayers of archaea, bacteria, fungi, plants, non-mammalian metazoans and mammals including humans. In plants, the first AQP Nodulin-26 (GmNOD26) was identified in soybean (*Glycine max* L.) [5], whereas their ability to conduct water in plants was demonstrated for the first time for an Arabidopsis tonoplast intrinsic protein homologue (AtTIP1; 1, gamma-TIP) *via* its heterologous expression in *Xenopus* oocytes [6]. In higher plants, MIPs are divided into five evolutionarily distinct subfamilies in terms of the similarities of their amino acid sequence and intercellular localisation: plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), nodulin-26 like intrinsic proteins (NIPs), small basic intrinsic proteins (SIPs) and X intrinsic proteins / uncharacterised-intrinsic proteins (XIPs) [7]. In turn, the TIP subfamily can be further divided into different subgroups. In Arabidopsis (*Arabidopsis thaliana*), the TIP subfamily has been

divided into five subgroups from TIP1 to TIP5. Each subgroup is then again divided into different isoforms. In Arabidopsis, the TIP subfamily consists of 11 isoforms. Some of these have synonymous name such as TIP1;1 (gamma-TIP, γ -TIP, RB7), TIP1;2 (gamma-TIP2, γ -TIP2), TIP2;1 (delta-TIP, δ -TIP), TIP3;1 (alpha-TIP, α -TIP) and TIP3;2 (beta-TIP, β -TIP) [8].

Water, whose transport is facilitated by aquaporins and, among them TIPs, is their most important substrate. TIPs are responsible for precisely regulating the movement of not only water, but also some small neutral molecules that have great physiological significance such as glycerol, urea, ammonia, hydrogen peroxide (H_2O_2) and formamide [7]. The transport of these substrates was predicted using a bioinformatic analysis and some of them have also been proven experimentally by using their *in vitro* expression in yeast or in a *Xenopus* oocyte system in which the expression of gene of interest led to, for example, an increase water permeability (Table 1).

Species	TIP name	Substrate					References
		Water (H ₂ O)	Hydrogen peroxide (H ₂ O ₂)	Urea	Ammonia (NH ₃)	Glycerol (GLY)	
Arabidopsis (<i>Arabidopsis thaliana</i>)	AtTIP1;1	+	+	+	+		[6, 9–13]
	AtTIP1;2	+	+	+	+		
	AtTIP2;1	+		+			
	AtTIP2;3			+			
	AtTIP4;1						
	AtTIP5;1						
Barley (<i>Hordeum vulgare</i> L.)	HvTIP1;1	+					[14]
	HvTIP1;2	+					
	HvTIP2;3	+					
Bread wheat (<i>Triticum aestivum</i> L.)	TaTIP2;1				+		[15–17]
	TaTIP2;2				+		
Maize (<i>Zea mays</i> L.)	ZmTIP1;1	+	+	+	+	-	[18–20]
	ZmTIP1;2	+	+	+	+	-	
	ZmTIP2;3	+		+			
	ZmTIP4;4						
Rice (<i>Oryza sativa</i> L.)	OsTIP1;2	+				+	[21]
	OsTIP2;2	+				+	
	OsTIP3;2	+				+	
	OsTIP4;1	+					
	OsTIP5;1						
Radish (<i>Raphanus sativus</i>)	RsTIP1;1	+					[22]
	RsTIP2;1	+					
Sunflower (<i>Helianthus annuus</i> L.)	TIP7	+					[23]
	TIP20	+					
Tobacco (<i>Nicotiana tabacum</i> L.)	NtTIPa	+		+		+	[24]

+ transport of the substrate has been confirmed, – transport of the substrate has been excluded.

Table 1. The transport of different substrates by TIPs, which was demonstrated via their *in vitro* expression in yeast or oocytes.

The central vacuole occupies most of the intracellular space of most mature plant cells [25]. The plant vacuole plays important roles not only in space filling, but also in osmotic adjustment, storage and digestion [26, 27]. TIPs form channels in the vacuolar membrane (tonoplast) that basically function as regulators of the intracellular water flow, which implies that they have a role in regulating cell turgor. It has been proposed that the presence of a specific TIP isoform may define the function of the vacuole and that specific TIP proteins could be used as markers for the different types of vacuoles that occur in different tissue types or in response to developmental and environmental stimuli [28, 29]. Vacuoles whose tonoplast contains TIP2;1 (delta-TIP, δ -TIP) are used by plant cells to store pigments and vegetative storage proteins (VSPs) [28]. Moreover, in vegetative tissues, the lytic or degradative vacuoles (LVs) are marked by the presence of TIP1;2 (gamma-TIP, γ -TIP) [26, 28, 30]. TIP1;2 may be expressed primarily at the time when the large central vacuoles are being formed during cell enlargement [30], while the protein storage vacuoles (PSVs) in mature seeds are marked by the presence of TIP3;1 (alpha-TIP, α -TIP) [26, 31, 32].

TIP proteins are most abundant in the tonoplast, but some isoforms have also been detected in the chloroplast, e.g. on the luminal site of the thylakoid, the chloroplast membranes or the envelope fraction using mass-spectrometry-based proteomics. Such a location was detected for TIP1;1, TIP1;2 and TIP2;1 in Arabidopsis [33–35].

2. Structure and diversity of TIPs

Although it has been suggested that AQPs and, among them, TIPs are multi-functional proteins, their structure is unique across all kingdoms of life. Despite their sequence diversity, all TIPs have a similar structure that consists of six trans-membrane helices (TM1 to TM6) with five connecting inter-helical loops (LA to LE) and two half helices that contain one highly conserved Asn-Pro-Ala (NPA) motif each of which forms its functional pore (**Figure 1**). The hydrophobic NPA motif is located at the first intracellular (loop B) and the third extracellular loop (loop E) and the overlap in the middle of the lipid bilayer ('hourglass' model) and forms two hemipores that generate a narrow part of the channel [7, 36]. A second filter region is the aromatic/Arginine (ar/R) selectivity filter, which includes four amino acids (aa), which are located at the non-cytosolic end of the pore [37]. These two constriction regions are very important for the transport selectivity of the channel, which have been predicted based on structural knowledge combined with

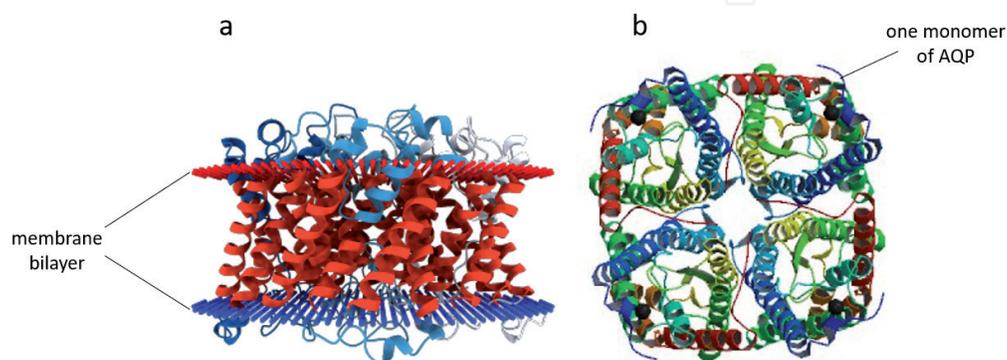


Figure 1.

The structure of aquaporin as represented by the crystal structure of spinach SoPIP2;1 (PDB 1Z98). The view from different sides of the holoprotein, which consists of four monomers. (a) the side view, (b) the tetrameric assembly from the cytoplasmic side. Each monomer functions as a single channel pore.

Species	Genome size/ ploidy x/no. of chromosomes	AQP	PIP	TIP	NIP	SIP	XIP	References
Arabidopsis (<i>Arabidopsis thaliana</i>)	~135 Mbp 2n = 2x = 10	38	13	11	11	3	0	[8]
Barley (<i>Hordeum vulgare</i> L.)	~5.3 Gbp; 2n = 2x = 14	39	18	11	8	2	0	[51]
Bread wheat (<i>Triticum aestivum</i> L.)	~17 Gb 2n = 6x = 42, AABBDD, hexaploid	113 (65-A, 42-B, 36-D)	51	29	29	4	0	[52]
Cucumber (<i>Cucumis sativus</i> L.)	~350 Mbp 2n = 2x = 14	39	19	8	9	2	1	[53]
Foxtail millet (<i>Setaria italica</i> L.)	~490 Mb 2n = 2x = 18	39	12	11	13	3	0	[54]
Maize (<i>Zea mays</i> L.)	2.4 Gb 2n = 2x = 20	41	12	18	8	3	0	[55]
Potato (<i>Solanum tuberosum</i> L.)	~840 Mb 2n = 4x = 48	41	15	11	10	3	8	[56]
Rice Japonica (<i>Oryza sativa</i> L.)	500 Mb 2n = 2x = 24	33	11	10	10	2	0	[57]
Sorghum (<i>Sorghum bicolor</i>)	~730 Mb 2n = 2x = 20	37	13	11	11	2	0	[58]
Tomato (<i>Solanum lycopersicum</i> L.)	~828 Mb 2n = 2x = 24	47	14	11	12	4	6	[59]

*Based on the Ensemble Plants Database.

The number of Tonoplast Intrinsic Proteins (TIP) has been marked in bold.

Table 2.

The number of AQP isoforms in the selected plant species.

simulation studies [38, 39]. Both filters at the pore “mouth” constitute barriers to the passage of inorganic cations (such as Na⁺ and K⁺) and protons [39]. The NPA motif not only plays a role in regulating membrane transport but also in protein localisation [40]. The substrate selectivity of AQPs is controlled by the amino acid residues of the NPA and ar/R filters as well as another conserved region, which is known as the Froger’s residues, which consist of five conserved residues [41]. This region enables the aquaglyceroporins that transport glycerol from the AQPs that facilitate the transport of water to be distinguished [39].

All MIP sequences take on a typical hour-glass MIP helical fold with six transmembrane helices and two half-helices [42]. This fold has been maintained during evolution via its conservation around 40 positions within the transmembrane region [42]. The stability of the functionally important half-helix is modulated by a stabilising intra-helical salt-bridge interaction and/or two helix destabilising residues, glycine and proline, which was demonstrated by an analysis of the loop E region of 1468 MIP sequences and their structural models from six different groups of organisms [43]. In the cell membrane, MIPs are grouped as homotetramers that are located in the lipid bilayer (**Figure 1**). Each monomer functions independently as a single channel pore [44]. In plants, the only the crystal structure of the MIPs for two species is currently available in the Protein Data Bank (PDB: <http://www.pdb.org>), the spinach (*Spinacia oleracea*) aquaporin SoPIP2;1 was in its closed confirmation at a 2.1 Å resolution and its open confirmation at a 3.9 Å resolution [45] and the Arabidopsis aquaporins

AtPIP2;4 at a 3.7 Å resolution [46] all of which were determined using X-ray diffraction, which showed their structural identity. However, the functions and regulation of the majority of the MIP channels have not yet been fully characterised [42].

A large number of MIP genes and, among them TIP genes, have been identified in the genomes of different plant species. In plants, the MIP family contains around 2008 members while the TIP subfamily contains 585 members from different plant species, which have been deposited in the MIPDB [47, 48]. The length of the TIP sequences ranges from 55 to 780 aa. Plants typically contain a large number of AQPs genes and among them the TIP genes, when are compared with for example humans, ranges from 6 to 35 in moso bamboo (*Phyllostachys edulis*) and oilseed rape (*Brassica napus* L.), respectively (Table 2) [49, 50]. The molecular weights of the TIP subfamily members ranges from 25 to 28 kDa [47].

3. Study of the tissue- and developmental-dependent expression of TIPs

Aquaporins are postulated to be the main water transporter protein in plants. However their presence and expression may vary among plant species, tissues and time during development. In plants, AQPs are present in almost all organs, including the roots, leaves, stems, flowers, fruits, seeds, dry seeds [60], pollen [13, 61–63], anther and specific cells such as the guard cells [64, 65]. Moreover, some TIPs are expressed in specific tissues, e.g. *AtTIP5;1* was localised in the pollen mitochondria, which are probably involved in the remobilisation of N via the transport of mitochondrial urea to the cytoplasm [61] as well as in a time-specific manner, e.g. the *RsTip1;1* (*gamma-VM23*) gene expression was high not only in the hypocotyls but also in the developing tap roots and young growing leaves and the pattern of its expression was connected with cell elongation [22] (Table 3).

Species	TIP name	Organ/tissue/process	References
Arabidopsis (<i>Arabidopsis thaliana</i>)	<i>AtTIP1;3</i> , <i>AtTIP5;1</i>	Pollen mitochondria	[61, 66, 67]
	<i>AtTIP3;2</i>	Senescence	
	<i>AtTIP3;1</i>	Seed-specific	
	<i>AtTIP1;1</i>	Entire vegetative body	
	<i>AtTIP2;1</i>	Developing vascular tissue, primary shoot	
Barley (<i>Hordeum vulgare</i> L.)	<i>HvTIP1;1</i> , <i>HvTIP2;3</i> , <i>HvTIP1;2</i> , <i>HvTIP3;1</i>	Leaf	[14]
	<i>HvTIP1;1</i> , <i>HvTIP2;3</i> , <i>HvTIP1;2</i>	Root	
Bread wheat (<i>Triticum aestivum</i> L.)	<i>TaTIP1;12</i>	Root-specific	[52]
	<i>TaTIP2;22</i>	Root-specific	
Eucalyptus (<i>Eucalyptus grandis</i> L.)	<i>EgTIP1;1</i> , <i>EgTIP2;1</i>	Flower bud	[68]
	<i>EgTIP1;1</i> , <i>EgTIP1;4</i> , <i>EgTIP2;1</i> , <i>EgTIP2;2</i>	Flower	
	<i>EgTIP1;4</i>	Root	
	<i>EgTIP1;1</i> , <i>EgTIP1;2</i> , <i>EgTIP4;1</i>	Stem	
	<i>EgTIP1;3</i>	Cambium	
	<i>EgTIP1;2</i> , <i>EgTIP3;2</i>	Leaf	
	<i>EgTIP3;1</i> , <i>EgTIP3;2</i>	Fruit	

Species	TIP name	Organ/tissue/process	References
Maize (<i>Zea mays</i> L.)	<i>ZmTIP2;3</i>	Root-specific	[20]
Radish (<i>Raphanus sativus</i>)	<i>RsTip1;1</i> (<i>gamma-VM23</i>)	Hypocotyl of seedlings, developing tap roots and young growing leaves	[22]
	<i>RsTIP2;1</i> (<i>delta-VM23</i>)	Not expressed in young roots or leaf mesophyll	
Rice (<i>Oryza sativa</i> L.)	<i>OsTIP3;2</i>	Mature seed, spikelet, callus	[21, 69]
	<i>OsTIP1;1</i>	Shoots, roots	
Sunflower (<i>Helianthus annuus</i> L.)	<i>TIP7</i> (<i>delta-TIP</i>)	Guard cells	[23]
	<i>TIP27</i> (<i>delta-TIP</i>)	Guard cells	
Tobacco (<i>Nicotiana tabacum</i> L.)	<i>NtTIP1;1</i> (<i>TobRB7</i>)	Root-specific	[70]

Table 3.
The expression of the selected TIP genes in different plant tissues.

4. Study of the expression of TIPs under abiotic stress or phytohormone treatment, and their role in maintaining cell homeostasis under stress conditions

The gene expression is the process in which gene information is converted into a protein [7]. Analysing the gene expression profiles is a prerequisite for determining the physiological function of a gene [71]. The function of AQPs can generally be modified in two ways: by regulating the gene expression, which leads to changes in protein abundance or by changing its permeability – gating. However, it should be kept in mind that changes in the mRNA expression level often reflect the protein abundance, but that this is not necessarily strictly connected in any cell or tissue [37]. In term, AQP trafficking and activity could be affected by the post-translational modifications (PTMs), like deamination, phosphorylation, methylation, ubiquitination and acetylation [37]. Water homeostasis is fundamental for cell survival [72]. AQP facilitate the transport of water and play roles in response to abiotic stress conditions during which the cell homeostasis is disturbed. Different studies have suggested that a low abundance of the AQP proteins reduces the water permeability of membranes and that a high abundance of them increases it. Although it can be assumed that all AQPs are able to transport water, an experimental study was performed for only a few members of the TIP subfamily in different plant species (**Table 1**). The expression of TIPs may be affected by different environmental conditions, including drought, salinity and cold and this has been investigated in many plant species. Comparative transcriptome studies using RNA sequencing (RNA-seq), which is a next generation sequencing technology, could lead to the discovery of a differential expression of multiple aquaporin homologues in different tissues and/or in different conditions of growth, e.g. under abiotic stress. The transcriptome defines as a set of coding and non-coding RNA molecules that are present in a single cell or a population of cells [73]. Nowadays, RNA-seq technology is used more and more frequently [74, 75]. On the other hand, the reverse-transcription quantitative PCR (RT-qPCR) technique still serves as the method of choice to perform the expression analysis of specific genes in many researches.

Abiotic stresses, which have a significant impact on the plants, are involved in changes at the transcriptome, cellular and physiological levels. When the stress is

prolonged, plant growth and productivity are severely diminished [76, 77]. The response of plants is very complicated and many mechanisms are initiated simultaneously to restore cellular homeostasis and promote survival [78]. Aquaporins are thought to be responsive to the stress signalling pathways and are also thought to be involved in the stress-coping mechanisms such as altering the tissue hydraulic conductivity [79, 80]. Drought and salinity are two major factors that significantly limit plant growth and productivity [81, 82]. The huge yield losses caused by drought stress ranging 45–92 (%) have been reported in major field crops like maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), chickpea (*Cicer arietinum* L.), soybean (*Glycine max* L.), sunflower (*Helianthus annuus* L.) [83]. Cold is also often associated with climate change [84]. Drought, salinity and freezing stresses cause a changing water status in plant cells and all of these result in cellular dehydration [85]. In order to improve the tolerance of crop plants to unfavourable environmental conditions, a detailed understanding of their stress responses is required.

4.1 Drought, salinity and cold stress

Aquaporins are responsible for precisely regulating the movement of water and therefore may play a crucial role in the drought-stress response as well as in drought-stress tolerance [78]. A transcriptome analysis of the leaf and root tissues in plant species revealed significant changes in the expression of the *TIP* genes in response to drought stress. Under drought stress, when water is limited, the down-regulation of the *TIP* genes can lead to a decrease in the water permeability of the tonoplast in order to avoid water loss and to minimise the water flow through the cell membranes in order to prevent the further loss of leaf turgor. This expression pattern has been shown in many studies. In *Nicotiana glauca*, the *NgMIP2* and *NgMIP3* genes, which are homologous to *TIP*, were down-regulated in leaves under drought stress when the leaves were wilting, usually three to four days after water was withheld [86] and in *Arabidopsis*, the levels of *AtTIP1;1*, *AtTIP1;2*, *AtTIP2;1* and *AtTIP2;2* were more than four-fold down-regulated after 12 days of drought [87]. In contrast to these results, the expression level of *AtTIP1;1* and *AtTIP2;1* under drought treatment was up-regulated, but the time that the stress was applied was only 24 hours [88]. Moreover, in barley (*Hordeum vulgare* L.), the expression of five *HvTIP* aquaporins in leaves: *HvTIP1;1*, *HvTIP1;2*, *HvTIP2;1*, *HvTIP2;2* and *HvTIP2;3* was down-regulated after ten days of severe drought treatment [89]. In turn, in *Festuca* species, the transcript level of the *TIP1;1* aquaporin decreased in leaves after 11 days of a water deficit [90]. The possible functional role of coffee (*Coffea arabica* L.) *TIPs* in regulating the water balance has been explored by measuring the *TIP* gene expression in leaf and root tissues that had been subjected to drought [91]. In root tissue, the expression of three *CsTIP* genes (*CaTIP1;1*, *CsTIP2;1*, *CsTIP4;1*) decreased as the severity of the drought stress increased. In the leaf, a similar expression profile of their expression was obtained only for of *CaTIP1;1* [91]. By contrast, the level of *TIP* expression increased in response to drought in some plant species. In soybean (*Glycine max*), transcriptome analysis data revealed that under dehydration stress, the expression of *GmTIP2;1*, *GmTIP1;7* and *GmTIP1;8* was up-regulated. The authors suggested that these three genes are probably the major AQPs that are involved in the stress response [75].

Interestingly, the expression profile of *TIP* genes can be compared for cultivars that have contrasting responses to drought stress under control and stress conditions. In two cultivars of strawberry (*Fragaria x ananassa*) that have contrasting drought stress phenotypes, the gene expression of *FaTIPs* in the roots was analysed [79]. During severe drought stress, the *FaTIPs* were only up-regulated in the

drought-tolerant cultivar. This was connected with a gradual stomatal closure in this cultivar, which was further associated with conservative water use [79]. In turn, in soybean (*Glycine max*), the difference in gene expression was investigated using RNA-seq and it was found that the expression level of seven *TIP* genes (*GmTIP2;1*, *2;2*, *2;3*, *2;4*, *2;5*, *3;2* and *5;1*) in the leaves was lower in the slow-wilting soybean lines, but that it increased in the fast-wilting soybean lines after drought stress [74]. Slow wilting is an important physiological parameter that is used in stress tolerance studies of plants. A cultivar-specific *TIP* expression under drought has also been observed in the common bean (*Phaseolus vulgaris* L.) [92]. In this study, the expression of *PvTIP1;1* and *PvTIP4;1* decreased under drought compared to the control conditions in two cultivars, which has different phenotypes in terms of their tolerance to drought and yield at harvest, which were determined by time that was required for the plants to wilt after withholding water. Interestingly, the gene expression of *PvTIP4;1* during drought was cultivar specific with greater down-regulation of these gene in the drought-tolerant cultivar [92]. The differences in the relative water content (RWC) and water potential of leaves between these cultivars indicate that there is greater prevention of water loss in the tolerant cultivar during drought, which may be associated with the rapid and adequate down-regulation of the *AQPs* [92]. However, it should be kept in mind that the adaptive mechanism for reducing the effects of water deficit stress in which the *TIP* or other *AQP* expression is changed should be further analysed by a functional characterisation of these candidate genes or proteins [52].

The regulation of water transport has been postulated as being the most important and the best recognised function of all of the *AQPs* including *TIPs* under drought stress. A second important function could be their involvement in the movement of hydrogen peroxide (H_2O_2), which is one of the reactive oxygen species (ROS), which include the unstable, highly reactive molecules that are produced during abiotic stress, e.g. drought [83, 93]. This activity makes the *AQPs* important players in both the redox signalling network and in H_2O_2 detoxification [94]. *TIPs* can facilitate the movement of H_2O_2 across the membranes that have already been shown by their *in vitro* expression in the yeast or oocyte (**Table 1**). In barley under drought stress, the expression of the *HvTIP3;1* gene increase 5000-fold compared to their expression under optimal water conditions [89].

Soil salination and a high salt accumulation affects the growth, development and metabolism and yield of plants [95]. A wide range of physiological and biochemical alterations in plants are induced by salinity, which causes a lower water potential in the soil solution, ionic disequilibrium and also a higher accumulation of reactive oxygen species (ROS) [96]. Salinity stress leads to changes in the expression profiles of the *TIP* genes, which was shown in many plant species. In *Arabidopsis thaliana*, the plants were treated with 100 mM NaCl, which led to osmotic stress. Macroarray experiments with gene-specific tags were performed to investigate the expression of all of the *AQPs* including the *TIP* subfamily in roots that had been under salt treatment for 24 hours [97]. The *AtTIP1;1*, *AtTIP1;2*, *AtTIP2;2* and *AtTIP2;3* genes were highly expressed in the roots and their expression was maximally reduced at 6 h of treatment and remained low until 24 h of treatment, except for *AtTIP1;1*. In turn, in *Festuca arundinacea*, the plants were exposed to salt stress by irrigating them with 250 mM NaCl solution over 21 days, which resulted in an increase in the abundance of the *FaTIP1;1* transcript in two contrasting salt tolerance genotypes compared to the control conditions. This increase was noticed earlier on day six of the NaCl treatment in the high-salt tolerant (HST) genotypes and later on day 11 in the low-salt tolerant (LST) genotype [90].

Similar to water and salt stresses, cold stress is an important abiotic stress factor that significantly limits plant growth and development [98]. In *Festuca pratensis*,

cold acclimation was associated with a lower transcript level of the *FpTIP1;1* gene in the leaves in both the high and low frost-tolerant genotypes. The authors concluded that the down-regulation of these isoforms during the exposure to cold may be one of the cold-acclimation components that prevents frost-induced cellular dehydration [90]. A similar pattern of expression was observed in roots in rice (*Oryza sativa* L.) under cold treatment. A 4°C treatment caused a decrease in the transcript level of the *OsTIP1;1* and *OsTIP2;2* genes. The mRNA levels of these genes started to decrease after 48 hours and continued to decrease after 72 hours of a chilling treatment [57]. One more example of a change in the expression of the TIP member genes comes from the cold treatment of cotton (*Gossypium hirsutum*). The *GhTIP1;1* transcripts mainly accumulated in the roots and hypocotyls under normal conditions, but were dramatically up-regulated in the cotyledons and down-regulated in the roots within a few hours after the cotton seedlings had been cold treated [99].

4.2 Phytohormone treatment

Phytohormones play vital roles in the ability of plants to acclimatise to varying environments, by mediating growth, development, and nutrient allocation [100]. Interestingly, it has been shown that the TIPs expression is also altered by phytohormones, including abscisic acid (ABA), cytokinin (CK), gibberellins (GA), and jasmonates (JA). Abscisic acid (ABA) is a plant growth regulator that plays an essential role in the abiotic stress response mainly during water stress [101]. It regulates the movement of the stomatal guard cells [102]. Jasmonates act as stress hormones that play an important role in the plant response to biotic and abiotic stresses [103]. In general, JA inhibits plant growth and modulate many growth and developmental processes of plants including root, shoot and leaf growth, trichome and tuber formation, fruit ripening and leaf senescence [104]. The gibberellins stimulate the growth of most organs via cell elongation and also respond to abiotic stresses [105]. Cytokinins play a role in regulating plant growth, development and their acclimation to environmental stresses [106, 107].

Cytokinin has been reported to influence the expression of the aquaporin genes. The expression of *PgTIP1* from ginseng (*Panax ginseng*) was positively regulated by applying cytokinin in suspension-cultured hormone-autotrophic ginseng cells [108]. Methyl jasmonate treatment caused a decrease in the nitrogen content in barley leaves, which was associated with an increased expression of the four tonoplast aquaporin genes (*HvTIP1;2*, *HvTIP2;2*, *HvTIP4;1* and *HvTIP4;2*), which are predicted to transport the nitrogen compounds from the vacuole to the cytosol. The up-regulation of the nitrogen-transporting *HvTIPs* may lead to a vacuolar unloading of ammonia and urea, which could both be remobilised when the nitrogen content in barley leaves decreases [109]. In barley, the changes in the expression of all of the investigated *HvTIPs* in response to MeJA treatment were associated with the presence of the *cis*-regulatory elements in their promotor regions, which are recognised by the jasmonate-related transcription factors [109]. Other types of elements that possibly participate in the response to phytohormones in the *HvTIP* promotor regions such as abscisic acid (ABA), gibberellins (GA) and auxin were also identified [89]. In barley, the effect of ABA and GA on the expression of the *HvTIP1;2*, *HvTIP2;1*, *HvTIP2;2*, *HvTIP2;3* and *HvTIP4;1* genes after 24 hours of treatment was studied in both the shoots and roots using quantitative real-time RT-PCR [110]. Interestingly, the exogenous application of ABA increased the expression of *HvTIP2;1* and *HvTIP4;1*, while the GA-treatment led to an increase of *HvTIP1;2* and *HvTIP4;1* in the roots compared to the control conditions. However, in the shoots, the ABA

treatment decreased the expression of *HvTIP1;2*, while the GA treatment led to the up-regulation of *HvTIP4;1* [110].

5. Genetic manipulation of the TIP-encoding genes

The tonoplast intrinsic proteins genes have been reported to be involved in the increasing the abiotic stress tolerance in several plant species using genetic modification technology (Table 4). It was shown that manipulations of the expression of

TIP name/species	Method/expression in species	Phenotype	References
AQUA1 (TIP) <i>Populus alba</i> L.	OX <i>Populus alba</i>	Increased plant growth rate and water use efficiency under excess Zn conditions	[114]
SITIP2;2 <i>Solanum lycopersicum</i> L.	OX <i>Solanum lycopersicum</i>	Increased drought tolerance due to the ability of plant to regulate its transpiration under drought stress conditions, improved CO ₂ uptake and a balanced nutrient supply	[111]
SITIP2;2 <i>Solanum lycopersicum</i> L.	OX <i>Arabidopsis thaliana</i>	Enhances the tolerance to salt stress	[115]
PgTIP1 <i>Panax ginseng</i>	OX <i>Glycine max</i>	Higher tolerance to salinity	[116]
PgTIP1 <i>Panax ginseng</i>	OX <i>Arabidopsis thaliana</i>	Acceleration of plant development, with faster growth, precocious flowering and a higher accumulation of biomass, increased seed size and seed mass, greatly increased growth rate	[85]
PgTIP1 <i>Panax ginseng</i>	OX <i>Arabidopsis thaliana</i>	Accumulated more Na ⁺ under salt stress, and exhibited superior performance under drought stress	[108]
CsTIP2;1 <i>Citrus spp.</i>	OX <i>Nicotiana tabacum</i>	Increased plant growth and tolerance to drought and salinity	[117]
JcTIP1;3 <i>Jatropha curcas</i>	OX <i>Arabidopsis thaliana</i>	Increased tolerance to drought and salinity, improved germination under high salt and mannitol stress	[118]
AtTIP5;1 <i>Arabidopsis thaliana</i>	OX <i>Arabidopsis thaliana</i>	Increased tolerance to boron toxicity	[119]
AtTIP1;1 <i>Arabidopsis thaliana</i>	Down-regulation /RNAi technology	Early senescence and plant death	[112]
AtTIP1;1 -mutant AtTIP1;2 and AtTIP1;2 -double mutant <i>Arabidopsis thaliana</i>	KO Transposon insertion	Single mutant -no significant effect on the metabolism or elemental composition of the plants Double mutant -minor increase in the anthocyanin content, and a decrease in catalase activity, no changes in the water status, mutant alive and thriving	[113]
AtTIP2;2 <i>Arabidopsis thaliana</i>	KO T-DNA insertion	Less sensitive to abiotic stresses (mannitol, NaCl and PEG)	[120]

OX-overexpression, KO- knockout, RNAi-RNA interference, PEG- polyethylene glycol.

Table 4. Genetic manipulation of the TIP genes in different plant species and its effect on the phenotype.

the *TIP*-encoding genes in plants could have the potential to improve their abiotic stress tolerance (**Table 4**). In tomato (*Solanum lycopersicum* L.), overexpression of *SlTIP2;2* has been shown to substantially improve the water homeostasis under drought stress. Conversely, this overexpression also promoted more prodigal water use [111]. Another gene, *TIP1;1*, was artificially manipulated in a few studies *via* genetic engineering (**Table 4**). It is not only believed to play an important role during plant growth and development, but also to be beneficial for growth under stress when it is overexpressed. The overexpression of the *PgTIP1;1* gene from *Panax ginseng* in Arabidopsis led to a general acceleration of plant development, which included a faster growth, precocious flowering and a higher accumulation of biomass compared to the wild type (wt), or the accumulation of more Na⁺ under salt stress and also caused a superior performance under drought stress compared to wt [85, 108]. The authors concluded that *PgTIP1;1* plays an important role in the growth and development of plant cells and suggested that regulating it in the water movement across the tonoplast has a great impact on plant vigour under favourable growth conditions and also in the responses of plants to drought, salt and cold stresses [85]. Therefore, *TIP1;1* might be a potential target in biotechnology and agriculture. It was interesting to determine whether silencing this gene would have the opposite effect on growth in control and stress condition. First, it was shown that the down-regulation of *AtTIP1;1* using RNA interference (RNAi) resulted in early senescence and plant death [112]. However, later research using transposon insertion did not confirm these results [113]. A single mutant (*tip1;1-1*) that had been generated using transposon insertion showed any significant effect on the metabolism or elemental composition of the plants. Additionally, a double mutant in the *AtTIP1;1* and *AtTIP1;2* genes (*tip1;1-1 tip1;2-1*) had a small increase in its anthocyanin content and a decrease in its catalase activity, but showed no changes in its water status. Moreover, the mutant was alive and thriving [113].

6. Conclusions

It is believed that aquaporins and, among them, the tonoplast intrinsic proteins may be important players in the plant water relations at the cell, tissue, organ and whole plant levels. TIP proteins could have a vast impact on the acquisition of knowledge about plant tolerance against abiotic stress and could serve as a target sequence for genetic modifications. A drawback in this approach is that in the genome of each plant species, many genes encode the same subgroup of isoforms. It is possible that some of these have a redundant function. Taken together, TIPs probably play complex and diversified roles in the response of plants to abiotic stresses depending on TIP isoforms and the type and degree of stress treatment.

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Conflict of interest

The author declares no conflict of interest.

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