

A tonoplast intrinsic protein in *Gardenia jasminoides*

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Abstract: Physiological and molecular studies proved that plasma membrane intrinsic proteins (PIPs) and tonoplast intrinsic proteins (TIPs) subfamily of aquaporins play key functions in plant water homeostasis. Five specialized subgroups (TIP1-5) of TIPs have been found in higher plants, in which the TIP1 and TIP2 isoforms are the largest arbitrary groups. TIPs have high water-transport activity than PIPs, some TIPs can transport other small molecule such as urea, ammonia, hydrogen peroxide, and carbon dioxide. In this work, the structure of the putative tonoplast aquaporin from *Gardenia jasminoides* (GjTIP) was analyzed. Its transcript level has increased during fruit maturation. A phylogenetic analysis indicates that the protein belongs to TIP1 subfamily. A three-dimensional model structure of GjTIP was built based on crystal structure of an ammonia-permeable AtTIP2-1 from *Arabidopsis thaliana*. The model structure displayed as a homo-tetramer, each monomer has six trans-membrane and two half-membrane-spanning α helices. The data suggests that the GjTIP has tendency to be a mixed function aquaporin, might involve in water, urea and hydrogen peroxide transport, and the gating mechanism founded in some AQPs involving pH and phosphorylation response have not been proved in GjTIP.

1 Introduction

Maintenance of water balance in cells is critically important for plants to sustain cellular and functional homeostasis, and this process is regulated by aquaporins in plants. Aquaporins (AQPs) are water channels/major intrinsic proteins (MIPs), which facilitate the passive movement of water and down the water potential gradient. In order to maintain proper water homeostasis, plants express a larger number of aquaporin isoforms than for mammals and yeast, including 30 to more than 70 homologs, compared with 13 AQP isoforms have been identified in mammals [1]. Plant AQPs were divided into five subfamilies: the plasma membrane intrinsic proteins (PIPs), the tonoplast intrinsic proteins (TIPs), the nodulin26-like intrinsic proteins (NIPs), the small basic intrinsic proteins (SIPs), and the X intrinsic proteins (XIPs) [7]. PIPs and TIPs play key functions in water homeostasis [3]. There are 35 aquaporins have been found in *Arabidopsis* (including 11TIPs) [7], 34 in cotton (11TIPs) [11], 36 in maize (12TIPs) [2] and 59 in *Brassica rapa* (16 TIPs) [8].

The localization of TIP aquaporins is in the vacuolar membrane which has 100-fold higher osmotic permeabilities to water than those of the plasma membrane [12]. The 40% of the protein in the vacuolar membrane are TIPs. TIPs are present in all land plants, five specialized subgroups (TIP1-5) have being found in higher plants, but whereas primitive plants like mosses only have one type of TIP (TIP6). Regon et al. has analyzed 100 TIPs from ten different plants including both monocots and di-



cots, two types TIP2 and TIP1 isoforms of aquaporins are found to be present in greater number in the genome as compared to others [14].

Expression of TIPs genes can be altered under various environmental conditions as well as according to cell/tissue type, plant developmental stages. TIP1 and TIP2 isoforms are largely expressed in vegetative tissues and are thought to be preferentially associated with the large lytic vacuoles and vacuoles accumulating vegetative storage proteins, respectively [6], TIP3 are expressed in seeds, and TIP5;1 is exclusively expressed in dry seeds and pollen grains [17]. Studies have shown that TIPs regulate water flow in response to drought and salinity stress in plant. The expression of AQPs are regulated in transcript level, translational level and can be further regulated by various post-translational modifications such as methylation, membrane trafficking, ubiquitination, glycosylation, heteromerization, phosphorylation and gating [10]. The phosphorylation is prevalent mode of regulation for PIPs.

The MIPs have a common molecular weight of 23-31 kDa, six transmembrane helices, asparagine-proline-alanine (NPA) motifs, and cytosolic C- and N-terminal. Crystal structures of aquaporins from bacterial, archaeal, yeast, plasmodium, plant, mammalian, and human have established [16]. Plant aquaporins are not simple water channels but can exhibit varied transport-selectivity properties. TIP subfamily are also as transporters of small solutes such as $\text{NH}_3/\text{NH}_4^+$, glycerol, urea and H_2O_2 . Putative substrate specificities of aquaporins is thought to be achieved by the aromatic/arginine (ar/R) selectivity filter, which has been defined as four residues located at TM helix2 and 5 and Loop B and E respectively, and five key amino residues have been proposed to discriminate whether AQPs transports water or glycerol by Froger et al. [4], P1 in loop C, P2-3 within loop E and P4-5 within TM6. Kirscht et al. suggested an extended filters in Loop C (LC) may decide the permeability of ammonia [9].

Gardenia jasminoides originates in Asia and has been in cultivation for at least a thousand years. The fruit of *G. jasminoides* is used in Asian countries as a natural colorant, and as a traditional herbal medicine. Crocin, crocetin and geniposide are the main secondary metabolites in the fruit, and they all exhibit a wide range of pharmacological activities. We have isolated a tonoplast intrinsic protein (GjTIP) gene from *G. jasminoides* [5], and found the transcript levels of GjTIP have increased during fruit maturation. In this paper, we analyzed the structure of GjTIP.

2 Materials and Methods

2.1 Alignment and Phylogenetic Analysis of AQPs

The Aquaporin (GjTIP) from *Gardenia jasminoides* was aligned with aquaporins from other species using Clustal Omega program (<http://www.ebi.ac.uk>) and Blast (<http://www.ncbi.nlm.nih.gov>). A consensus tree was computed in MEGA4.

2.2 Building Three-dimensional model

Three-dimensional model building of deduced amino acid sequences was performed using the Swiss-model Workspace (<http://swissmodel.expasy.org>). We constructed homology model of GjTIP based on X-ray crystallography of *Arabidopsis thaliana* tonoplast aquaporin AtTIP2-1 (PDB 5I32). And the crystal structure of spinach plasma membrane aquaporin SoPIP2-1 (PDB 2B5F and 1Z98) was used for gating analysis.

3 Results And Discussion

The predicted amino acid sequence of GjTIP is a polypeptide with 257 amino acids. A phylogenetic analysis conducted with 31 previously characterized aquaporins from other plant species indicates that the protein belongs to TIP1 subfamily (Fig.1). In table 1, the amino acid composition of ar/R selectivity filters and extended filters (LC) of AQPs from plant, human and *E.coli* were compared.

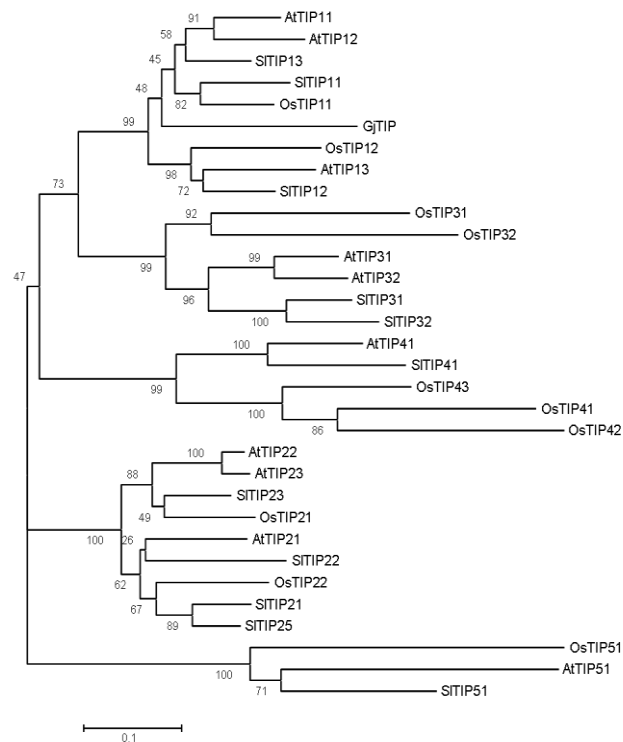


Fig.1 Phylogeny of 32 TIP proteins. TIP1s were shown in bold. The Genbank accession numbers are : AtTIP1-1, AAD31569; AtTIP1-2, BAB01832; SITIP1-3, Solyc10g083880.1.1; SITIP1-1, BAO18632; OsTIP1-1, P50156; GJTIP, AEF59492; OsTIP1-2, Q94CS9; AtTIP1-3, O82598; SITIP1-2, BAO18633; OsTIP3-1, Q9FWV6; OsTIP3-2, Q7XKI5; AtTIP3-1, P26587; AtTIP3-2, AAF97261; SITIP3-1, FC17BG08; SITIP3-2, BAO18639; AtTIP4-1, AAC4224; SITIP4-1, BAO18640; OsTIP4-3, Q9LWR2; OsTIP4-1, Q75GA5; OsTIP4-2, Q9LWR0; AtTIP2-2, Q41975; AtTIP2-3, BAB09071; SITIP2-3, BAO18636; OsTIP2-1, Q7XA61; AtTIP2-1, BAB01264; SITIP2-2, BAO18635; OsTIP2-2, Q5Z6F0; SITIP2-1, BAO18634; SITIP2-5, Solyc06g066560.1.1; OsTIP5-1, Q7XU31; AtTIP5-1, Q9STX9; SITIP5-1, Solyc03 g093230 .2.1.

31 TIPs from three plant species have H (Q, T, N)-I (V, T, M)-A (G)-R (V, C, Y) residues at ar/R filter, GJTIP has Y-I-A-V, the Y at H2 position is unique. It is predicated by Kirscht et al [9] that a histidine at position H2 in helix2 and an aromatic residues at position LC in loop C seem to be a common feature among ammonia-permeable AQPs both in plants and animals, 31 TIPs from three plant species have H (F, T, Y) at LC while GJTIP has L, it's unlikely that the GJTIP may transport ammonia. 31 TIPs from three plant species have T (S, V)-S (A, V)-A (S)-Y-W residues at Froger's position (P1-P5) positions. GJTIP has T-A-A-Y-W, it's relatively conserved.

AtTIP1;1 in Arabidopsis, the first intracellular plant AQP to be expressed and characterized in oocytes, induced a high membrane water permeability. Urea transport has been identified to members of almost all TIP subclasses in Arabidopsis, including AtTIP1;1, 1;2, 1;3, 2;1, 4;1 and 5;1. GJTIP may transport urea. It is predicated that TIPs containing the H-I-A-V or H-I-G-R residues in the ar/R filter and T-A-A-Y-W or T-S-A-Y-W residues at P1-P5 positions, have been shown to transport urea and H_2O_2 and it was suggested that TIPs triggered the ROS translocation into the vacuoles for their detoxification.

Table 1 Ar/R filter and LC in AQPs

Protein	Ar/R filter and LC					Substrate specificity
	H2	H5	LB	LE	LC	
GjTIP	Y	I	A	V	L	Not defined
AtTIP1-1	H	I	A	V	F	Water, urea, ammonia, H ₂ O ₂
AtTIP2-1	H	I	G	R	H	Water, urea, ammonia, H ₂ O ₂
TIPs(Arabispis, tomato and rice)	H(Q,T, N)	I(V,T, M)	A(G)	R(V,C, Y)	H(F,T, Y)	
SoPIP2-1	F	H	T	R	G	Water
NtPIP1-2	P	H	T	R	G	Water, CO ₂
AtNIP5-1	A	I	G	R	G	Water, urea, boron, silicon
AQP4	F	H	A	R	V	Water, NO
AQPZ	F	H	T	R	L	Water
GlpF	W	G	F	R	Y	Water, glycerol

It suggests that the GjTIP may transport water, urea and H₂O₂ and the amino acid at H2 position is unique compared to AQPs from bacterial, plants and animals.

In plant cells, the vacuoles have various functions, including metabolite storage, pH regulation and

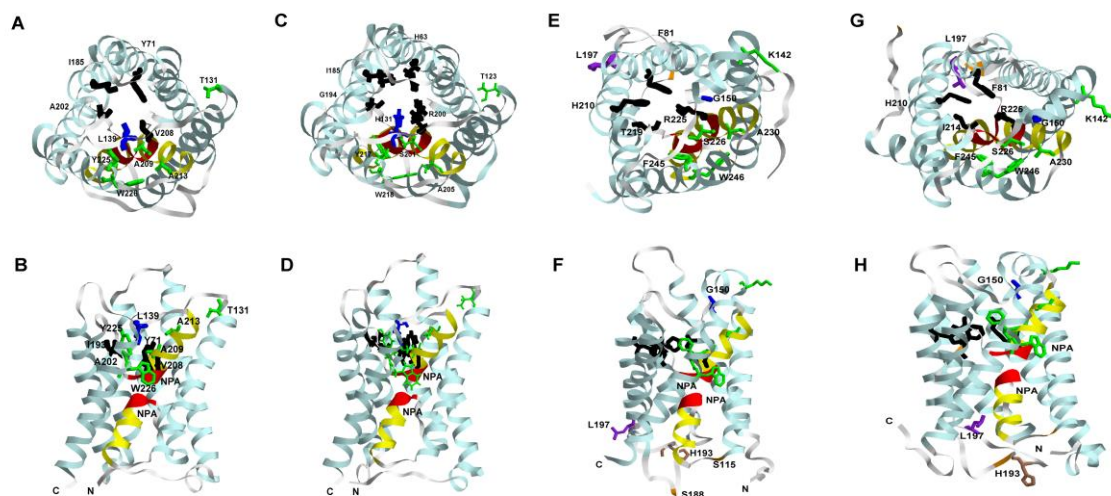


Fig. 2 Three-dimensional structure of AQPs. (A, C, E, G) Top view into the pore for monomeric GjTIP, AtTIP2-1, open structure of SoPIP2-1 and closed structure of SoPIP2-1 prespectivliy. (B, D, F, H) Viewed from the periplasmic side of monomeric GjTIP, AtTIP2-1, open structure of SoPIP2-1 and closed structure of SoPIP2-1 prespectivliy. The N and C termini that face the cytosol, HB, HE are represented by yellow ribbon, NPA domains are shown in red ribbon. ar/R selectivity filter amino acids are shown as black sticks, the LC residues are shown as blue sticks. Frøger's positions (P1-P5) are shown in green sticks.

cell signalling. And it is implicated in the exchange of water between the vacuole and the cell exterior, the cytosol may also be subjected to volume fluctuations. There are mainly two types of vacuole, the vacuoles in elongating cells express TIP1 families, while the vacuoles in protein storage in seed cells express TIP3 family's protein. TIPs with high water-transport activity are abundant in expanding cells. During rapid cell elongation, the up-regulated expression of aquaporin tonoplast genes increased the water channel number, while the water inflow through opened water channels made by TIP1 and TIP2

aquaporins are accelerated. It was shown that over-expression of a NtTIP1;1-GFP fusion in protoplasts derived from tobacco BY-2 cells leads to a 24% increase in expansion rate [13].

During fruit and flower development, there are rapid enlargement took place and increase of water content in fruits and floral primordia of buds. The TIP1;1 homolog in pear is highly expressed in the young fruit, whereas TIP proteins levels in grape gradually increase along with ripening. Several SIAQPs were found to be expressed in fruits pecific pattern, indicating a role in fruit development, they most likely transport of water or solutes. Expression of SITIP3;2 started at 14 DAP (days after pollination) and increased with proceeding fruit development. SITIP3;1 was expressed exclusively in fruits during mid-development (around 21 DAP) [15]. GjTIP may expressed in the vacuoles in expanding cells in fruits.

The 3D (three dimension) model structure of GjTIP was constructed using SWISS-PDB software (Fig. 2A, 2B), the X-ray crystallography at resolutions down to 1.18 Å of AtTIP2-1 was used as template (Fig.2C, 2D). The amino acid sequence of GjTIP (from 10 to 246 residue range) has 48.6% amino acid identity with AtTIP2-1 (from 26 to 250 residue range).

In the model, each of the monomers in tetramer has the typical six membrane spanning helices (shown in light turquoise), N- and C-terminal regions at the cytoplasmic vestibule. The two NPA motifs meet in the middle of the membrane, shown in red, HB and HE shown in yellow; the ar/R selectivity filter shown in black and the fifth extended filter residue L139 was shown in blue, they formed the narrowest part of the pore, near the vacuolar vestibule. The patterns of ar/R residues surrounding the pore regions are similar in AtTIP2-1 and GjTIP 3D structure, and the sites of the fifth residue in the pore regions are equitable. Spinach aquaporin SoPIP2-1 channel has a gating mechanism conducted by the intracellular loopD residue Leu197, response to pH through protonation of His 193 and phosphorylation of two serines, Ser115 and Ser274 (which is not in the 3D structure) [16]. The open and closed conformations of SoPIP2-1 are shown in Fig. 2E-2H. From alignment, these four conserved residues have no counterpart in GjTIP sequence. VvTnTIP2;1 in grapevine enhanced the water permeability of water in yeast, and acidification of yeast cytosol resulted in loss of VvTnTIP2;1 activity, it's involed the His131 [10], which is unusual in TIPs and not in GjTIP.

4 Conclusions

During plant cell elongation, the up-regulated expression of aquaporin tonoplast genes lead to the increase in water channel. Some tonoplast aquaporins was detected in samples from fruits in the cell expansion stage. We predicted that the GjTIP might have water, urea and H₂O₂ permeability and also facilitate water movement within the fruit and on the intercellular level between the cytosol and the vacuole.

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