Gene Expression and Phylogenetic Analysis of Plant Aquaporins

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ABSTRACT

Plant aquaporins play indispensible roles in plant growth and development through their function in regulating membrane permeability to water which leads to plant adaptation to environmental stresses including drought and salt stress. Plant aquaporin full length cDNA sequences obtained from the gene bank database were used to study the expression and phylogenetic relation among plant aquaporins using semiquantitative PCR and sequence alignment. Amino acid sequence alignment revealed some amino acid substitution in the NPA motifs and various conserved amino acid, such as the two glycine residues before and after the second NPA motif by three residues. Comparing the phylogenetic trees obtained using nucleotide sequence and amino acid sequence showed that plant aquaporins were distributed into the four main groups, PIP, TIP, NIP, SIP. Estimation of plant aquaporin gene expression using semiquantitative PCR analysis revealed natural inter and intra sub-family variations among plant aquaporins under the same conditions. The gene expression variations give indications that different plant species modulate plant aquaporin genes differently which open the door for targeting plant aquaporins to enhance resistance of plants to abiotic stresses.

Key words: Plant aquaporins, gene expression, NPA motifs, phylogenetic, amino acid substitution.

INTRODUCTION

The major plant biological processes including plant growth and development are dependent on water movement through various molecular and physiological processes. Water movement through membranes is facilitated by a group of membrane channel proteins (aquaporins, AQPs) that represent a subgroup of the major intrinsic protein (MIP) family. Aquaporrins are found in all living organisms (Agre *et al*., 1998). The aquaporin pore has six membrane-spanning domains and cytoplasmic N- and C- termini. It also, has two loops; the cytosolic loop between the second and third transmembrane domains and the extracellular loop positioned between the fifth and sixth transmembrane domain. These two loops form short hydrophobic helices that are buried in the membrane from opposite sides. They contain the conserved NPA motifs which lie in the middle of the pore (Murata *et al*., 2000). Aquaporins are arranged in tetramers in the membrane each polypeptide forms single channel that transport water bidirectionally (Agre *et al*., 2002; Hub *et al*., 2008; Wang and Tajkhorshid, 2007). Aquaporins include two main types; although some of them work as water channels, others transfer wide range of solutes, such as urea or glycerol, gases, such as carbon dioxide and ammonia (Bienert *et al*., 2008; Maurel *et al*., 2008). Both kinds of aquaporin channels control the passive transport of water and other molecules across biological membranes in all forms of life (Heller *et al*., 1980).

Aquaporins in higher plants are classified into five groups; plasma membrane intrinsic proteins (PIP), tonoplast intrinsic proteins (TIPs) which are localized in the plasma and tonoplast membranes respectively, nodulin26-like intrinsic proteins (NIPs) which is a homologue of GmNod26, abundant in prebacteroid membrane of nitrogen fixing nodules in soybean, small basic intrinsic proteins (SIPs) that are mostly present in the endoplasmic reticulium, and X intrinsic proteins (XIPs) (Chaumont *et al*., 2001; Johanson *et al*., 2001; Sakurai *et al*., 2005; Danielson and Johanson, 2008). Plant aquaporins determine the plant hydraulic relations from cell to whole plant level and they are responsible for up to 95% of the water transfer through the plasma membranes (Henzler and Steudle, 2004). Generally, aquaporins can regulate water exchange across cell membrane in three strategies; their expression level, their trafficking after synthesis in the ER, and channel gating; the opening or closing of the aquaporin pore.

Many studies at the whole genome level of aquaporins have been showed the abundance of their genes. Genome wide study of major intrinsic proteins (MIPs) and their expression was designed to analyze the soybean genome (Zhang *et al* 2013). In this study, 66 GmMIPs were identified in the soybean genome that represented the five subfamilies. Expression analyses were performed for a selected set of GmMIPs using semiquantitative reverse transcription and qPCR. Results suggested that GmMIPs contains aquaporins, glyceroporins, aquaglyceroporins to regulate its water relations

(Zhang *et al* 2013). Aquaporin family of cotton (*G. hirsutum*) was identified and found to include 71 aquaporin genes that consist of 28 PIPs, 23 TIPs, 12 NIPs, 7 SIPs, and 1 XIPs. Gene expression, sequencing and phylogenetic analysis showed high similarity and distributed the cotton aquaporins into the five known subfamilies (Park *et al*, 2010). Tomato (*Solanum lycopersicum*) genome was screened and found to have 47 aquaporin genes. Phylogenetic analysis of the deduced amino acid sequences revealed that aquaporin genes were distributed among the five subfamilies; PIPs, TIPs, NIPs, SIPs and XIPs. Tissue and development-specific expression of tomato aquaporin genes also were studied (Reuscher *et al* 2013).

etExpression of specific aquaporin genes was associated with some physiological and environmental conditions in various plant species. PIP aquaporin were found to be down regulated in reresponses to drought to prevent water loss. Ten *Fragaria* PIP genes were identified from strawberry (*Fragaria vesca L.*) genome. Four PIP genes were down regulated with different intensities of drought stress in roots and leaves. It was suggested that transcription of PIP aquaporins is altered in response to low water availability (Surbanovski *et al*, 2013). Various studies have shown that water stresses, including drought and salinity, can modulate aquaporin expression. Drought stress induced by 250 mM mannitol in the aerial part of *Arabidopsis* changes the expression of most PIP genes (Jang *et al*., 2004). In rice, different responses of aquaporin expression to water stress were observed in upland (drought-resistant) and lowland (drought-sensitive) rice (Lian *et al*., 2006). Based on this conclusion, it was suggested that different cultivars of the same species may respond in different fashion to water stress by changes in the expression of aquaporin genes. Overexpression of aquaporin genes improved drought resistance in *Arabidopsis* (Cui *et al*., 2008; Zhou *et al* 2012). It has been documented that aquaporins also have different roles in cell expansion, fiber development (Aslam *et al*, 2013), and salt tolerance (Zhou *et al* 2014, Lian *et al*., 2014). The genetic natural variations among plant aquaporin as well as their expression have not been studied. Therefore, in this study, full length aquaporin sequences were studied and compared at the protein sequence and the phylogenetic relationship among them was established. Also, the expression of selected plant aquaporins was estimated from different plants. This is to investigate the molecular features among plant aquaporins which may enhance our understanding of their structure and expression and exploit that to enhance plant resistance to environmental stresses including drought and salinity.

MATERIALS AND METHODS

2.1. Plant aquaporin sequences

Various full length plant aquaporin cDNA sequences were obtained from the nucleotide database at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). The amino acid sequences of the same cDNA accessions were obtained. When more than one accession were found for specific plant aquaporin various accessions were aligned and only one sequence was used. Table 1 summarizes general information about plant aquaporin accessions used in this study.

2.2. Sequence alignment

Clastual Omega for multiple alignments at the European Bioinformatic Institute web site (http://www.ebi.ac.uk/Tools/msa/clustalo) was used to align nucleotide and amino acid sequences. Only the coding region sequence of the nucleotide sequence was used after removing the 5' and 3' untranslated regions. The aligned sequences (nucleotide and protein) were used to construct the phylogenetic tree at the same web site.

2.3. Primer design

Forward and reverse specific primers were designed on cDNAs used in this study using primer3 plus (http://biotools.umassmed.edu/cgi-bin/primer3plus/primer3plus.cgi). Table 2 has a summary of primer sequence and the accession number used.

2.4. Aquaporin expression

2.4.1. RNA isolation

RNA was isolated from 10 day old etiolated shoots grown of 10 different genotypes (Table 2) in the dark. Plant tissues were collected and lyophilized in Alpha2 lyophilizer, ground to fine powder, and kept at -80°C or used for RNA isolation. One ml of QIAzol was added to 10 mg of lyophilized powder and mixed (QIAGEN Inc., Valencia, CA). A volume of 0.3 ml chloroform was added to the homogenate. The mixture was then shaken for 30 s followed by centrifugation at 4°C and 13000 rpm for 20 min. The supernatant was transferred to a new tube. One fourth volume of 12 M lithium chloride was added and kept at -20°C overnight. Samples were centrifuged for 15 min at 4°C and 13000 rpm. RNA pellet was washed with 70% ethanol, briefly dried, and dissolved in DEPC water. The integrity of RNA was checked by agarose gel electrophoresis and its concentration and purity were determined at 260 nm and the OD260/280 ratio.

2.4.2. Synthesis of cDNA

A mixture of 2 µg of total RNA and 0.5 ng oligodT primer in a total volume of 11 µl sterilized DEPC- water was incubated in the Multigene thermal Cycler (Labnet, USA) at 65°C for 10 min for denaturation. Then, 4 µl of 5X RT-buffer, 2 µl of 10 mM dNTPs and 100 U M-MuLV Reverse Transcriptase

Table 1: Molecular information of plant aquaporins used in the study including plant species, accession number, length in bp, length of coded proteins, tissue of activity, and function.

Name	Accession No	Bp	No aa	Organ/Tissue	Function
Arabidopsis thaliana SIP1 (At-S1P1)	NM_111280	1100	240	ER	SIP
Arabidopsis thaliana SIP2 (At-S1P1)	NM_001203184	1109	260	ER	SIP
Lotus japonicas NIP5 (Lj- NIP5)	EU294214	909	302	Nodulin	NIP
Lotus japonicas NIP6 (Lj-NIP6)	EU294215	942	313	Nodulin	NIP
Malus hupehensis PIP1 (Mh- PIP1)	JN632528	870	289	PM	PIP
Pisum sativum PIP1 (Ps- PIP1)	KF770828	1204	289	PM	PIP
Nicotiana tabacum PIP1 (Nt-PIP1)	AF440271	1083	286	PM	PIP
Zea mays PIP1 (Zm-PIP1)	NM_001111661	1364	288	PM	PIP
Hordeum vulgare PIP2 (Hv-PIP2)	AB377270	1082	290	PM	PIP
Triticum aestivum PIP1(Ta- PIP1)	AF139814	1283	290	PM	PIP
Cucumis sativus PIP2 (Cs- PIP2)	KF641178	1108	278	PM	PIP
Brassica napus PIP1 (Bn- PIP1)	AF118382	1093	278	PM	PIP
*Helianthemum almeriense PIP2 (*Ha- PIP2)	JF491352	1059	281	PM	PIP
*Vitis vinifera PIP2 (*Vv- PIP2)	AY823263	1216	284	PM	PIP
Zea mays TIP3 (Zm-TIP3)	NM_001111562	989	262	Tonoplast	TIP
Vitis vinifera TIP1 (Vv-TIP1)	AY839872	993	251	Tonoplast	TIP
Coffea racemosa TIP1 (Cr-T1P1)	GAJU01000003	852	251	Tonoplast	TIP
Brassica napus TIP2 (Bn-T1P2)	AF118381	1020	253	Tonoplast	TIP
Helianthemum almeriense TIP1 (Ha-T1P1)	HQ234609	998	252	Tonoplast	TIP

PIP: plasma membrane intrinsic proteins; TIP: tonoplast intrinsic proteins; NIP: nodulin26-like intrinsic proteins; SIP: small basic intrinsic proteins.

Table 2 : Primer sequence used in this study and the expected size of PCR products.

Name	Accession No	5' primer	3' primer	Product size
At-SIP1	NM_111280	TGCATTGGCGATCATGGAGT	CGCAATGGCAGGATTCATGG	256
Ps-PIP1	KF770828	GGAAAAGTTCACGGTGGTGC	GCACCAAGACTCCTAGCAGG	251
Zm-PIP1	NM_001111661	CGTCTACACCGTCTTCTCCG	AAGAGCAGCACCGATGAAGG	241
Hv-PIP2	AB377270	GAGATCATCGGCACCTTCGT	CCGACCCAGAAGATCCACTG	240
Ta-PIP1	AF139814	CTCTACATCACCGTGGCCAC	GGCACTGCGCAATCATGTAG	242
Bn-PIP1	AF118382	TGCATTGGCGATCATGGAGT	CGCAATGGCAGGATTCATGG	256
Vv-PIP2	AY823263	GGTCTCGTCAAAGCCTTCCA	TACCGGTGCCAGTGATAGGA	260
Zm-TIP3	NM_001111562	CCTACTACGCCACGGTGATC	CCAGGTACTCGTACACCAGC	253
Vv-TIP1	AY839872	TTTCGGTGGGTGCGAACATA	CACTGTGTAAACCAGGCCGA	260
Bn-TIP2	AF118381	CGTTAACCCTGCCGTTACCT	TACCGTTCTTGGGGTCAACG	259

(SibEnzyme Ltd. AK, Novosibirsk, Russia) were added and the total volume was brought to 20 µl by DEPC water. The mixture was then re-incubated in the thermal Cycler at 37°C for 1h, then at 90°C for 10 min to inactivate the enzyme then, hold at 4°C. cDNA was kept at -20°C until used.

2.4.3. Semi-quantitative PCR

Primers were designed using Primer 3 software based on the nucleotide sequence published in Genebank (Table 2) and synthesized by Macrogen campany (http://dna.macrogen.com , Korea). PCR was conducted in a final volume of 25 µl consisting of 1 µl cDNA, 1 µl of 10 picomol of each primer (forward and reverse), and 12.5 µl PCR master mix (Promega Corporation, Madison, WI, USA)PCR

was carried out using cycle sequence of denaturing at 94 °C for 5 minute for one cycle, followed by 35 cycles which consisted of denaturation at 94 °C for one minute, annealing at 55 °C for 40 s, and extension at 72 °C for one minute with additional cycle as a final extension at 72 °C for 5 minutes. As a reference, expression of β-actin mRNA was tested using specific primers. PCR products were electrophoresed on 2% agarose gel in TBE buffer at 100 volt for 50 minutes with ethidium bromide staining. PCR products were visualized under UV light and photographed. Densitometeric analysis of band intensities was determined using NIH imageJ program (http://rsb.info.nih.gov/nih-imageJ).

RESULTS

3.1. Sequence alignment

Sequence alignment of amino acids of plant aquaporins revealed many molecular features. First, the first NPA motif is not conserved in the studied aquaporins because it was NPT in *Arabidopsis thaliana* SIP1, NPL in *Arabidopsis thaliana* SIP2, and NPS in *Lotus japonicus* NIP5. The second NPA motif also was NPV in *Lotus-japonicus* NIP5 and NIP6 (Figure 1). Second, the spacing between the two NPA motifs differed among plant aquaporin. It ranged from 96 to 120 residues (Table 3). The spacer length was 120 in *Pisum sativum* PIP1, whereas it was 96 in *Arabidopsis thaliana* SIP2. Third, the N-terminus, sequence before the first NPA motif, and C-terminus, sequence after the second NPA motif, of plant aquaporins also differed. The N-terminus ranged from 67 to 141 residues. It was 67 in *Arabidopsis thaliana* SIP2 and 141 in *Lotus japonicas* NIP6. The C-terminus ranged from 47 to 78 amino acids (Table 3, Figure 2).

Protein sequence alignment revealed some more molecular characteristics. There are some conserved amino acids around the two NPA motifs including highly conserved residues. Two residues, EF, near to the N-terminus are conserved in the studied aquaporins except *Arabidopsis thaliana* SIP1 and SIP2. An alanine residue is found in all aquaporins and is located at 27 residues before the first NPA motif except in *Arabidopsis thaliana* SIP1 and SIP2. Two residues, SG, are located before the first NPA by 3 amino acids except *Arabidopsis thaliana* SIP1 and SIP2. A histidine residue is located one residue before the first NPA motif except *Arabidopsis thaliana* SIP1 and SIP2. A Q residue is conserved in all studied aquaporins except *Arabidopsis thaliana* SIP2 where it is replaced by E residue. It is located 24 residues after the first NPA motif. F residue is present 50 amino acid residues before the second NPA motif that is conserved in all studied aquaporins. Also, an A residue is located in all aquaporins at 23 residues before the second NPA motif. Two G residues are conserved in all aquaporins at 3 residues before and after the second NPA motif. Therefore, the second NPA motif is surrounded by two glycines 3 residues apart. An alanine residue is present 5 residues after the second NPA motif. A proline residue is located 23 residues after the second NPA motif is also conserved in all aquaporins except *Arabidopsis thaliana* SIP1 (Figure 2).

3.2. Phylogenetic tree

The phylogenetic tree of plant aquaporin sequences was constructed using the nucleotide sequence (Figure 3) and the amino acid sequence (Figure 4). Imbalanced trees were obtained using the nucleotide sequence and the amino acid sequence. Also, both trees were characterized with accelerating diversification. One interesting feature of both trees is that the four different types of aquaporins, PIP, TIP, NIP, and SIP are distributed in four distinct clusters. The two trees have similar topology with different phylogenetic distribution of plant aquaporins. The tree obtained from the nucleotide sequence started with three clades A, B, and C. Clade C is the oldest aquaporins which included Ha-PIP2. Clade B has both of VvPIP2 and Cs-PIP2. Clade A was diverged into two sub-clades A1 and A2. Sub-clade A1 has Ta-PIP1 and Hv-PIP2, whereas sub-clade A2 was diverged into two sub-sub-clades A3 and A4. Sub-sub-clade A3 has two branches A5 which has Zm-PIP1 and branch A6 which carry Mh-PIP1, PsPIP1, and Nt-PIP1. The sub-sub-clade A4 was diverged into two main branches A7 and A8. Branch A7 is diverged into to sub-branches A9 and A10 which has Zm-TIP3, whereas A9 has Ha-TIP1, Bn-TIP2, Cr-TIP1, and Vv-TIP1. Branch A8 is diverged into two sub-branches A11 and A12. A11 has Lj-NIP5 and Lj-NIP6, while A12 has At-SIP1 and SIP2 and Bn-PIP1 (Figure 3).

Using the amino acid sequence of plant aquaporins resulted in a more distinct phylogenetic tree with minute different topology. The subgroups of plant aquaporins are separated as PIP, TIP, NIP, and SIP from bottom to the top of the tree indicating their consecutive origin. The tree has three main clades A, B and C. Clade C, the oldest, has Bn-PIP1, whereas clade B has two branches one of them has Vv-PIP2 and Ha-PIP2 while the other branch has Ta-PIP1 and Hv-PIP2. Clade A was diverged into two sub-clades A1 which has Cc-PIP2 and A2 which was diverged into two sub-sub clades A3 and A4. Sub-sub clade A3 has two branches one of them, A5, has Nt-PIP1 and A6 which is diverged into two sub-branches one of them carry Zm-PIP1 and the other carry Ps-PIP1 and Mh-PIP1. Sub-sub clade A4 was diverged into two branches A7 and A8. Branch A7 is diverged into two sub-branches A9 which has Ha-TIP1, Bn-TIP2, Cr-TIP1, and Vv-TIP1, while sub-branch A10 has Zm_TIP3. Branch A8 has Lj-NIP5, LJ-NIP6, At-SIP1, and At-SIP2 (Figure 4).

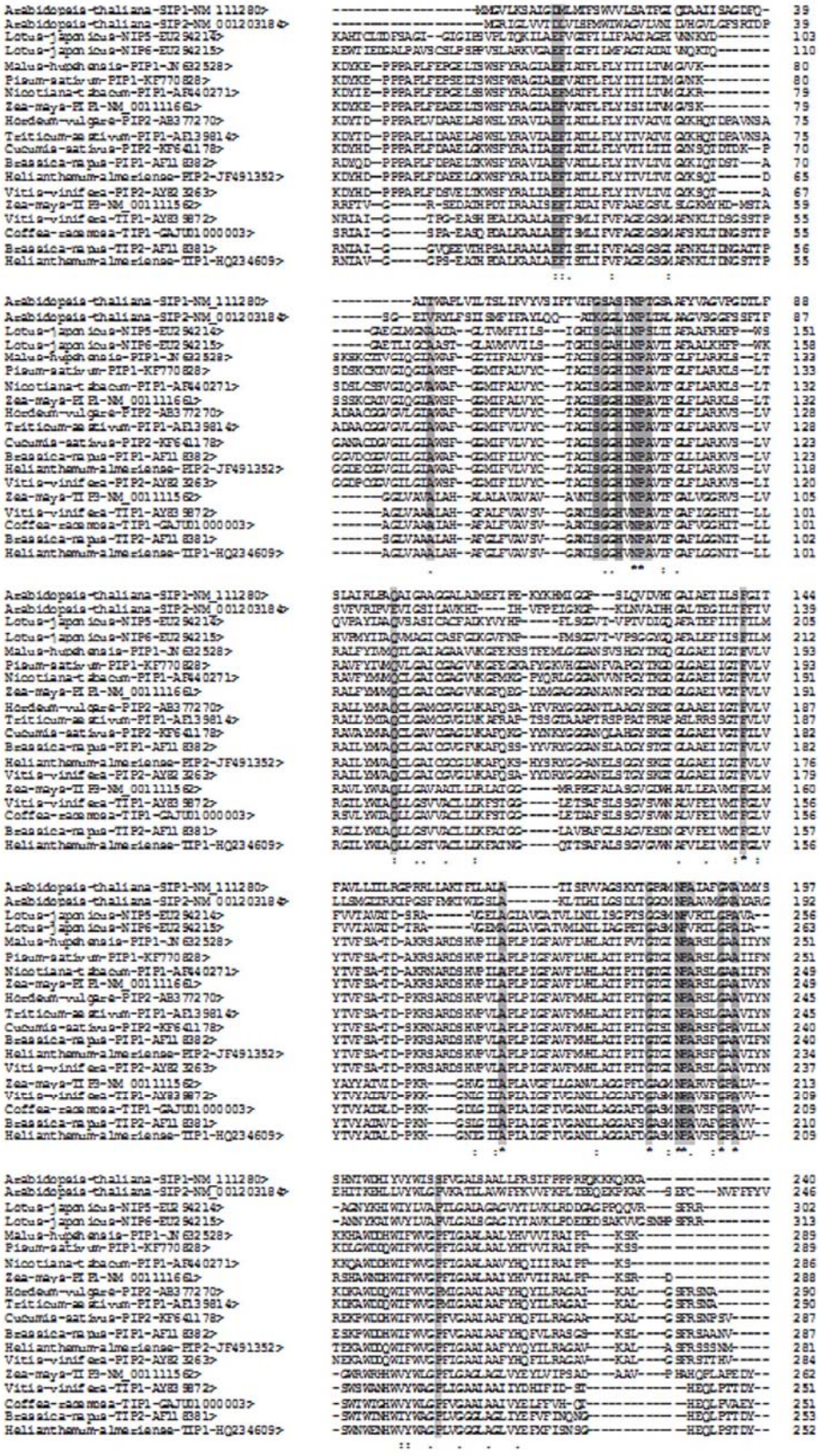

Figure 1: Amino acid sequence alignment of 19 plant aquaporins showing the two NPA motifs and the most conserved residues. Definition of aquaporins contains the species name followed by the aquaporin subfamily and the accession number. Conserved amino acids residues are highlighted.

Table 3: Summary of amino acid substitution in NPA motifs, length of C-terminus and N-terminus, and length of spacer between the NPA motifs of plant aquaporins.

Name	Accession No	First NPA motif			Spacer	second NPA motif			AA No
		N-term	Sequence	Location		Sequence	Location	C-term	
At-SIP1	NM_111280	69	NPT	70-73	112	NPA	185-187	53	240
At-SIP2	NM_001203184	67	NPL	68-70	96	NPA	166-168	78	246
Lj-NIP5	EU294214	134	NPS	145-147	99	NPV	246-248	54	302
Lj-NIP6	EU294215	141	NPA	142-144	109	NPV	253-255	58	313
Mh-PIP1	JN632528	116	NPA	117-119	120	NPA	239-241	48	289
Ps-PIP1	KF770828	116	NPA	117-119	120	NPA	239-241	48	289
Nt-PIP1	AF440271	115	NPA	116-118	119	NPA	237-239	47	286
Zm-PIP1	NM_001111661	115	NPA	116-118	119	NPA	239-241	47	288
Hv-PIP2	AB377270	111	NPA	112-114	119	NPA	233-235	55	290
Ta-PIP1	AF139814	111	NPA	112-114	119	NPA	233-235	55	290
Cs-PIP2	KF641178	106	NPA	107-109	119	NPA	228-230	57	287
Bn-PIP1	AF118382	106	NPA	107-109	119	NPA	228-230	57	287
Ha-PIP2	JF491352	101	NPA	102-104	118	NPA	222-224	57	281
Vv-PIP2	AY823263	103	NPA	104-106	119	NPA	225-227	57	284
Zm-TIP3	NM_001111562	88	NPA	89-91	112	NPA	203-205	57	262
Vv-TIP1	AY839872	84	NPA	85-87	112	NPA	199-201	50	251
Cr-TIP1	GAJU01000003	84	NPA	85-87	112	NPA	199-201	50	251
Bn-TIP2	AF118381	85	NPA	86-88	112	NPA	200-202	51	253
Ha-TIP1	HQ234609	84	NPA	85-87	112	NPA	199-201	51	252

At: *Arabidopsis thaliana*; Lj: *Lotus japonicas*; Mh: *Malus hupehensis*; Ps: *Pisum sativum*; Nt: *Nicotiana tabacum*; Zm: *Zea mays*; Hv: *Hordeum vulgare*; Ta: *Triticum aestivum*; Cs: *Cucumis sativus*; Bn: *Brassica napus*; Ha: *Helianthemum almeriense*; Vv: *Vitis vinifera*; Cr: *Coffea racemosa*

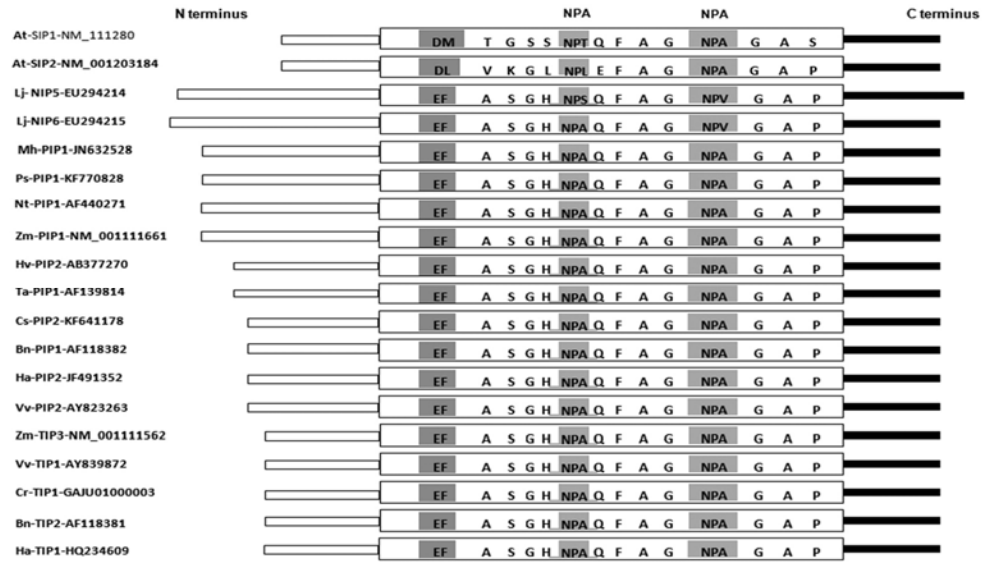

Figure 2: Schematic drawing of plant aquaporin protein sequence showing the two NPA motifs, the conserved amino acids, and the relative length of the N and C-terminus. The numerical data are summarized in Table 3. Definitions are as shown in Table 3 legend.

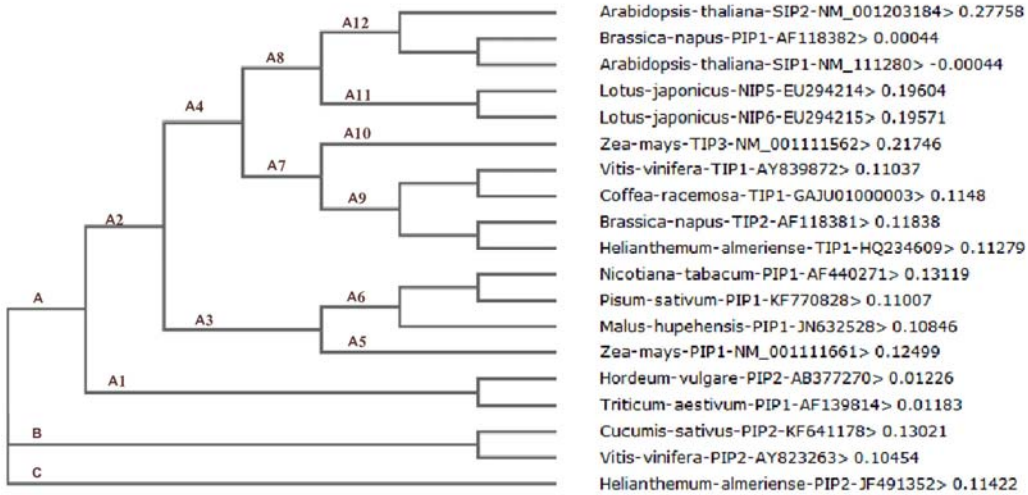

Figure 3: Phylogenetic tree of plant aquaporins using the nucleotide sequence of the coding regions. Definitions are as shown in Figure 1 legend. Distance between neighboring aquaporins is shown next to each accession on the tree.

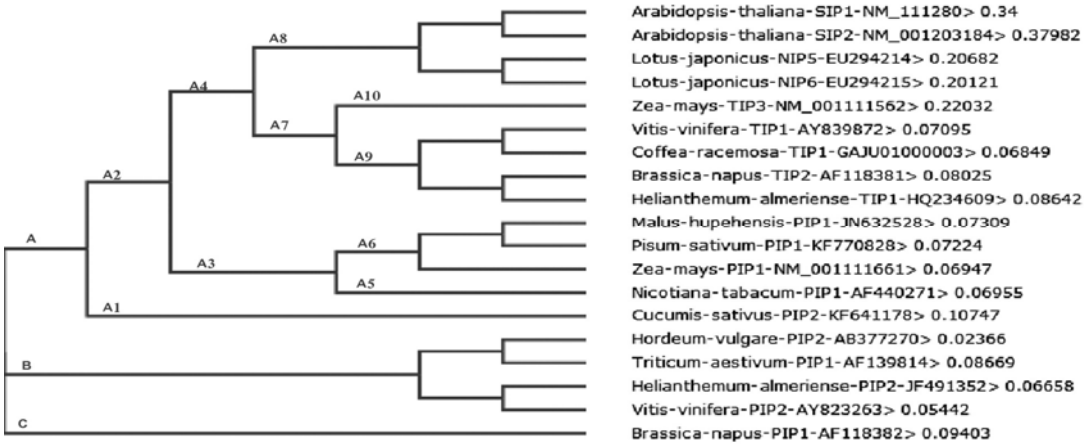

Figure 4: Phylogenetic tree of plant aquaporins using the amino acid sequence. Definitions are as shown in Figure 3 legend.

3.3. Expression of plant aquaporin genes

Specific primers (Table 2) designed on the cDNA sequences obtained from the GeneBank database were used to check the gene expression of 10 plant aquaporins under study (Figure 5a). The expression of nine of the 10 tested aquaporins was detected in their respective species, whereas the expression of only one species, Bn-TIP2, was not detected using the designed primers. Expression level varied among different aquaporins (Figure 5a). Image J software was used to estimate the gene expression in a semiquantitative way which revealed differences in gene expression level. The Hv-PIP2 and Zm-PIP1 showed the highest level of gene expression followed byTa-PIP1 and Bn-PIP1, whereas At-SIP1 showed the lowest expression level (Figure 5c).

DISCUSSION

Various full length cDNA of plant aquaporin genes were used to study their expression and the phylogenetic relationship among these vital molecules for all life forms. Sequence alignment showed differences in the amino acid sequence, especially the NPA motifs and the highly conserved amino acid residues. One interesting molecular feature of the studied plant aquaporins is that the length of the N-terminus and the C-terminus which varied drastically among plant aquaporins under study. Both termini are localized in the cytoplasm. Their variation must play a molecular role in the functions of different aquaporins. They may respond to different signals, therefore more studies are needed to investigate the detailed functions of the N and C domains of different plant aquaporins.

Phylogenetic relationship among plant aquaporins using nucleotide or amino acid sequences revealed some characteristics among the two trees. In the nucleotide tree (Figure 3), Bn-PIP1 was located on the same branch with At-SIP1 and At-SIP2, whereas on the protein tree Bn-PIP1 was located at the bottom of the tree representing the oldest plant aquaporin in this study from which other PIPs were diverged (Figure 4). Ta-PIP1 and Hv-PIP2 lie on the same branch and the same location on both trees; they come third and fourth from the bottom of the trees (Figure 3, 4). Phylogenetic trees showed that plant aquaporins were distributed in the four categories PIP, TIP, NIP, and SIP. This was very clear in the protein phylogenetic tree. Similar distribution was obtained by Maurel (2007) when he studied a number of 35 aquaporin of *Arabidopsis thaliana* which were distributed in the known four subfamilies; PIP, TIP, NIP, and SIP.

Various SNP mutations have been mapped in some human aquaporin genes which were proved to be associated with genetic variants of certain health problems. For example, different SNPs have been reported in human aquaporin7 (Murata *et al*., 2000; Heymann and Engel, 2000; Kondo *et al*., 2002). In one of these variants G264 in Aqua7 is substituted with Val that causes obesity (Maeda *et al*., 2008). This glycine is identical in almost all human aquaporins. Similar studies are absent in plant aquaporins, no SNP studies have been reported on plant aquaporins. Therefore, more studies of plant aquaporins are needed to test the amino acid substitution effects on the function of aquaporins

Plant aquaporins under study showed various gene expression levels among different species (Figure 5c). PIP2 from barely showed the highest level of expression. PIP1 of Zea maze and PIP1 of *Arabidopsis thaliana* came next in expression level. SIP1 of *Arabidopsis thaliana* showed the lowest expression level. TIP2 of *Brassica napus* expression was not detected using our primers. It is worth noted that this is endogenous variation in gene expression among species under study which reflects the natural variations in plant aquaporins expression under the same conditions. This is very clear among PIP genes in this study. This could be related to the various responses of different species toward biotic or abiotic stresses, especially drought and salinity. Similar results were obtained by Lian *et al*. (2006) in upland and lowland rice cultivars. In this study, they found different responses of aquaporin expression to water stress in upland (drought-resistant) and lowland (drought-sensitive) rice cultivars. They concluded that different cultivars of the same species may respond differently to water stress by modulating the expression of aquaporin genes.

Although there are few studies that have been carried out to design blockers or inhibitors for human aquaporins, no similar research have been conducted on plant aquaporins. It is well known that mercury decreases water flow of aquaporins (Savage and Stroud, 2007; Yukutake *et al*., 2008). Mercury binds to the cysteine residue Cys183 in AquaZ of bacteria. In another study, human aquaporin4 permeability of water was decreased by 5 μM concentration of $HgCl_2$ and this effect was reversed by 2-mercaptoethanol (Yukutake *et al*., 2008). Gold-based inhibitors of aquaporins were developed (Martins *et al*., 2012). There are no similar studies on plant aquaporins. Therefore, great research efforts need to be targeted to develop inhibitors or modulators of plant aquaporins. This would lead to controlling water flow through plant cell membrane which could be exploited in enhancing plant resistance to abiotic stresses such as drought.

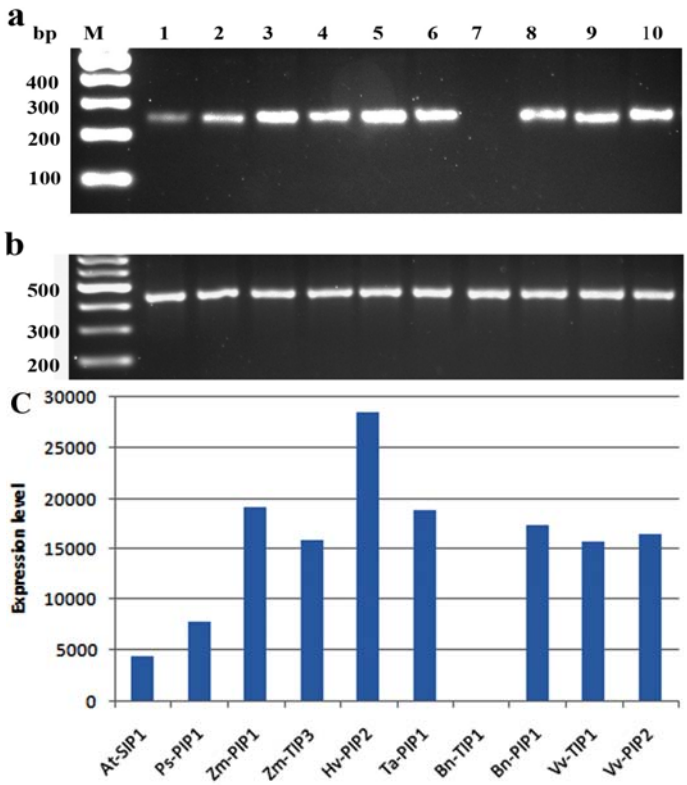

Figure 5: Gene expression of ten aquaporin genes. a. aquaporin gene expression; b. expression of actin gene as reference. M: 100 bp ladder; 1: At-SIP1; 2: Ps-PIP1; 3: Zm-PIP1, 4: Zm-TIP3; 5: Hv-PIP2; 6: Ta-PIP1; 7: Bn-TIP2; 8: Bn-PIP1; 9: Vv-TIP1; 10: Vv-PIP2. c. Semiquantitative analysis of gene expression of panel a. Abbreviations as described in Table (2).

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الملخص العربى

التعبير الجينى وتحليل درجة القرابة لجينات الأكوابورين النباتية

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تلعب بروتينات الأكوابورين دور مهم في نمو وتطور النباتات من خلال وظائفها في تنظيم نفاذية الأغشية الخلوية للماء والتي بدورها تمثل عامل مهم في أقلمة النباتات للظروف البيئية مثل الجفاف والملوحة. في هذه الدراسة، تم إستخدام تتابعات cDNA الكاملة من قاعدة بيانات بنك الجينات لدراسة التعبير الجيني والعلاقة الوراثية الفيلوجينية بين جينات الأكوابورين النباتية بواسطة تقنية تفاعل البلمرة المتسلسل PCR شبه الكمي، ومقارنة التتابعات. بمقارنة تتابعات الأحماض الأمينية لجينات الأكوابورين النباتية وجد عديد من إستبدالات الاحماض الأمينية في NPA motifs وكذلك بعض الأحماض الأمينية المحافظة في هذه البروتينات مثل حامض الجليسين الذي يقع علي بعد ثلاثة أحماض أمينية قبل وبعد تتابع NPA الثاني. بمقارنة الشجرة الفيلوجينية الناتجة من إستخدام تتابعات القواعد النيتروجينية مع نظيرتها الناتجة من إستخدام تتابعات الأحماض الأمينية وجد أن بروتينات الأكوابورين النباتية تتوزع في الأربعة مجاميع الرئيسية لهذه البروتينات وهي PIP، TIP، NIP، SIP. بقياس التعبير الجيني لجينات الأكوابورين النباتية تحت الدراسة بواسطة تقنية PCR الشبة كمية وجد إختلاف طبيعي في مستوي التعبير الجيني بين وداخل مجاميع بروتينات الأكوابورين النباتية تحت نفس الظروف. هذا الإختلاف في التعبير الجيني يدلل علي أن النباتات المختلفة تنظم عمل جينات الأكوابورين بطرق مختلفة والذي يفتح المجال لإستهداف هذه الجزيئات لتحسين مقاومة النبات للظروف البيئية وخاصة الجفاف.