Aquaporins as targets for stress tolerance in plants: genomic complexity and perspectives

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Abstract: As a multigene family, plant aquaporins function not only in water transport but also in transport of small elements that are important for vegetative and reproductive growth of plants. Increasing evidence exhibits the relevance of aquaporins to tolerance against abiotic and biotic stresses such as drought, nutrient deficiency, and herbivore attack. With the accumulation of crop genome sequencing, it is suggested that several aquaporin genes are conserved in subchromosomal locations as tandem duplicated members. In this review, we will discuss the compelling nature of aquaporins as multifunctional transport channels that are often encoded in clustered regions of genomes and relevant to stress resistance in plants.

Key words: Aquaporin, boron, drought, silicon, stress, tandem duplication, water

1. Introduction

Since the discovery of aquaporins as water channel proteins in human erythrocytes and renal cells, a dramatic diversity of aquaporin isoforms has been subsequently described across living organisms from prokaryotes to eukaryotes including unicellular algae, fungi, and higher plants (Preston et al., 1992; Maurel et al., 1993; Agre et al., 1998; Hohmann et al., 2000). Current knowledge of aquaporin multichannel proteins in plants indicates that they are generally classified in five distinct subfamilies by sequence homology and subcellular localization in association with versatile cellular transport functions (Kruse et al., 2006; Maurel et al., 2008; Abascal et al., 2014). These evolutionarily and phylogenically distinct subfamilies are the plasma membrane intrinsic proteins (PIPs), the tonoplast intrinsic proteins (TIPs), the small basic intrinsic proteins (SIPs), the nodulin26-like intrinsic proteins (NIPs), and the uncharacterized X intrinsic proteins (XIPs) (Johanson et al., 2001; Danielson and Johanson, 2008) (Figure). Unique from animals, the number of aquaporin genes is substantially expanded in higher plants and reaches more than 50 genes in the case of crop species that possess highly complex genomes (Table 1).

In actively growing plant cells, water and nutrients need to be constantly supplied for proper cell expansion, division, and reproduction (Chrispeels and Maurel,

1994). Although initially triggered by transpiration through stomata in leaves, water uptake and transport are multifaceted processes in which water can move apoplastically, simplastically, and transcellularly (Chaumont and Tyerman, 2014). Consequently, orchestrated management in highly compartmentalized plant cellular organization necessitates the presence of the abundant aquaporin multigene family in plants in comparison to metazoans in which small numbers of aquaporins are present (Maurel et al., 2015).

Multiple copies of aquaporin isoforms are localized in cellular and subcellular membranes across plant bodies and play diverse roles in water transport (Wudick et al., 2009). In Arabidopsis (*Arabidopsis thaliana* L.), although aquaporin genes are highly redundant, single gene knockout mutant studies demonstrate that a single aquaporin gene has its own phenotypic consequence (Peret et al., 2012). Peret et al. (2012) have shown that lateral root emergence is temporally and spatially regulated by auxin, which negatively regulates accumulation of aquaporin *AtPIP2;1* thereby altering water flow to the lateral root primordia. Moreover, phylogenetic analysis and functional study of various aquaporin members reveal that neofunctionalization of duplicated aquaporin genes allows more diverse roles of aquaporins in uptake and transport of other substrates in addition to water molecules (Kruse et al., 2006) (Figure).

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Table 1. Number of aquaporins in genomes of different species.

| Species	Genome size	Ploidy of genome	Number of aquaporin	Reference	Number of tandem array	Tandem arrayed genes
Homo sapiens	2.5 Gb	2x	12	(Kruse et al., 2006)	0	
Arabidopsis thaliana	120 Mb	2x	35	(Quigley et al., 2002)	2	2 PIP2s, 2 NIP4s
Physcomitrella patens	473 Mb	2x	23	(Danielson and Johanson, 2008)	0	0
Gossypium raimondi	885 Mb	2x	59	(Paterson et al., 2012)	2	3 PIP1s, 2 XIPs
Gossypium arboreum	1.75 Gb	2x	52	(Li et al., 2014)	2	3 PIP1s, 2 XIPs
Gossypium hirsutum	2.2 – 2.4 Gb	4x	>71	(Park et al., 2010)	NA	NA
Populus trichocarpa	423 Mb	2x	55	(Lopez et al., 2012)	1	6 XIPs
Oryza sativa	389 Mb	2x	34	(Nguyen et al., 2013)	3	2 TIP4s, 4 PIP2s, 2 NIP3s
Zea mays	2.3 Gb	2x	44	(Schnable et al., 2009)	NA	NA
Phaseolus vulgaris	521 Mb	2x	41	(Ariana, 2015)	4	2 NIP1, 2PIP2, 2 NIP1,2 XIPs

NA: Not available
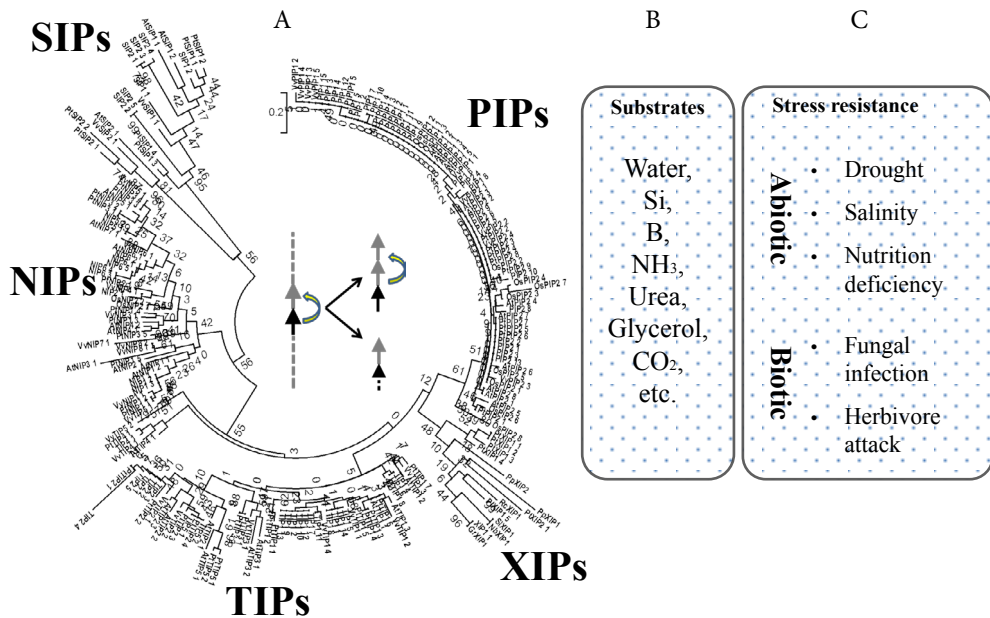
Figure. Aquaporins, a multigene family consisting of 5 main subfamilies (PIPs, TIPs, NIPs, SIPs, and XIPS) are diversified by duplication or tandem repeats as shown by arrows (A), known to facilitate transport of water and other essential/beneficial molecules such as Si, B, and glycerol (B), and related to resistance to abiotic and biotic stresses (C).

Land plants have adapted by altering their genomic structure to facilitate better strategies for survival in changing environments. In higher eukaryotes including many crop species, gene duplication has contributed largely to genomic novelty by a combination of functional divergence of duplicated gene pairs followed by selective retention or silencing (Flagel and Wendel, 2009; Fedoroff, 2012). In a number of plant species, it has been shown

that genes in orthologous groups expanded via tandem duplication are more associated with biotic and abiotic stress responses (Hanada et al., 2008).

In this review, we describe the multigene aquaporin family focusing on two main subjects: 1) aquaporin gene family members localized in genomes by tandem duplication, and 2) aquaporin channel proteins playing diverse roles in plants in conjunction with stress resistance. It is within this context that we will discuss aquaporins as targets for stress tolerance.

2. Genomic distribution of aquaporin genes in plants

2.1. Clustered aquaporins

Plant genomes have diversified structurally and functionally over time, leading to genetic and trait diversity largely due to the alteration of gene expression contributed by modification of regulatory mechanisms. Gene regulatory mechanisms include factors acting as cis- or trans-, elements working along with epigenetic regulation, and insertion/deletion of inter/intragenic regions (Fedoroff, 2012). Comparative crop genomics studies dramatically increased our knowledge of the genomic structure of the plant lineage with the help of recent advances in massively parallel nucleic acid sequencing approaches. A primary outcome is that many plant genes or gene families are highly conserved across the plant kingdom and experienced gene duplication over time (Flagel and Wendel, 2009).

Interestingly, from the available chromosomal maps of aquaporin genes in plant genomes, two to five regions in the genome harbor clustered aquaporin genes (Table 1). Aquaporins from these clustered sequences are highly similar and may function redundantly. In Arabidopsis, 35 aquaporin genes are distributed across the genome and two sets of duplicated aquaporin genes are clustered in a tandem arrayed manner (Johanson et al., 2001; Quigley et al., 2002). Considering the origination of tandem duplicated genes, expression of the tandem arrayed orthologues tends to be orchestrated in a collinear fashion because of the shared cis-acting elements (Schmid et al., 2005). Although there is frequent overlap in expression patterns, there are still considerable occasions in which tandem duplicated genes do not act alike. One of the tandem duplicated genes, *AtPIP2;2*, is shown to function nonredundantly as determined by transfer DNA (T-DNA) mutants in which a 30% reduction in hydraulic conductivity occurs in root cortex cells despite the presence of the highly homologous *AtPIP2;3*. These two genes are physically arrayed by head to head gene arrangement and therefore share the cis-regulatory regions (Javot et al., 2003). In rice (*Oryza sativa* L.), three independent chromosomal locations show tandem repeats in which 2 *TIP4*s in chromosome 1, 4 *PIP2*s in chromosome 7, and 2 *NIP3*s in chromosome 8 are highly similar in sequence and grouped into a single

subfamily of aquaporins in each clustered locus (Sakurai et al., 2005; Nguyen et al., 2013). The fact that 25% of aquaporin genes have been generated in the rice genome by tandem repeats coincides with the finding that 30% of all rice genes are localized with tandem arrays (Jiang et al., 2013).

2.2. Gene expression

In Arabidopsis and rice, gene ontology analysis indicates that tandem arrayed genes are enriched in the coding sequences categorized in proteins such as membrane localized proteins and stress-related proteins. These features are strikingly conserved between two plant species (Rizzon et al., 2006), although receptor-like protein coding genes constitute the majority of this group (Meyers et al., 2005). In the case of tandem arrayed aquaporin members in rice, expression analysis of four *OsPIP2* copies in chromosome 7 reveals partial overlap of expression patterns in tissues examined, but these genes are expressed with considerably different levels when examined under different stress conditions (Nguyen et al., 2013). In another example of tandem arrayed genes, *OsTIP4;2* and *OsTIP4;3*, show sequence similarity in general, but also exhibit unique patterns of tissue specific expression different from each other (Nguyen et al., 2013). In common bean (*Phaseolus vulgaris* L.), four pairs of aquaporin genes are clustered among 41 aquaporin members. *PvPIP2;1* and *PvPIP2;2* show 98% sequence homology and similar expression patterns among tissues tested, while expression patterns and gene structure of the other three pairs are not shared between counterparts (Ariana, 2015). A possible modification of gene expression at the genomic level has been suggested because of two silencing events detected in two out of four clustered aquaporins (Ariana, 2015).

2.3. XIP subfamily

The subfamily XIP was initially identified in the moss *Physcomitrella patens*, a model organism of the basal lineage of land plants in which 23 aquaporins were found including two members of the XIP subfamily (Danielson and Johanson, 2008). This novel aquaporin subfamily has less similarity with previously identified aquaporin members, resulting in delay of the identification of the XIP subfamily. While the XIP subfamily is present from unicellular algae to higher plants, it has been noted that XIPs are not found in the family *Brassica* including Arabidopsis and in all monocotyledon genomes sequenced so far (Danielson and Johanson, 2008). Functional analysis of XIP members from various plants among the family *Solanaceae* have shown their roles as membrane channels for different small, nonpolar molecules such as glycerol, urea, and boric acid, but not for water molecules (Bienert et al., 2011). Interestingly, *XIP* genes often locate in plants genomes in a tandem arrayed manner, implicating possible duplicated origins followed by functional diversification (Table 1).

Among 55 poplar (*Populus trichocarpa* L.) aquaporins, six *XIP*s (including one pseudogene) are clustered on chromosome 9 and are expressed nonredundantly (Gupta and Sankararamakrishnan, 2009; Lopez et al., 2012; Cohen et al., 2013). Interestingly, these *XIP* members harbor alternative solute specificities, demonstrating unique but overlapping features of this clustered subfamily members under stressed growth environment (Lopez et al., 2012).

2.4. Polyploidization

Clustered in an aquaporin-rich region, it would be interesting to explore allelic expression variation of these highly homologous genes not only in a single genotype but also across different genotypes of the same species. In maize (*Zea mays* L.), all of eight functionally unrelated genes in the *Bz1-Sh1* gene rich region show 1.3- to 36-fold of strikingly biased allelic expression variation in at least one of the tissues examined (Dooner and Martinez-Ferez, 1997; Hawkins et al., 2014). Distribution of aquaporin genes in polyploid plant genomes such as upland cotton (*Gossypium hirsutum* L.) has proven to be extremely complicated (Park et al., 2010). Upland cotton is known to have evolved as a result of the hybridization between two ancestral species tracing back to diploid A- and D- genome progenitors (Wendel and Cronn, 2003). Evolutionary genomic events present within *Gossypium* species reveal a dynamic fluctuation in gene copy number ascribed to gene duplication phenomena followed by pseudogenization and deletion (Small and Wendel, 2000). *Gossypium raimondii* (D_5 genome) has the smallest genome size among *Gossypium* species and thus was the first *Gossypium* species sequenced at the whole genome level (Paterson et al., 2012). In this genome, two clustered chromosomal loci of aquaporin genes are localized in a tandemly duplicated manner among 59 aquaporins (Table 1). Recently, another ancestral A_2 cultivated diploid, *Gossypium arboreum*, was fully sequenced (Li et al., 2014), and preliminary physical localization of A-genome aquaporin genes revealed similar clustered patterns in both diploid genomes (http://cgp.genomics.org.cn/page/species/index.jsp). It will be interesting to determine the genomic organization of clustered aquaporin genes in tetraploid cotton and how tandem duplicated genes are functionally diverged.

3. Stress

3.1. Complexity in aquaporin study

Plants are often exposed to challenging environments such as drought, salinity, and cold shock in which circumstances require balanced water uptake and transport against adverse osmotic potential for survival (Luu and Maurel, 2005). Water deficit stress is one of the major challenging abiotic stresses that prompts complicated physiological, cellular, and genetic changes such as stomatal closure, transcriptomic, epigenetic, and metabolic modifications. Consequently, these changes result in decreased photosynthetic activity, leading to decreased crop production (Morison et al., 2008; Ahuja et al., 2010). Therefore, it is fundamental to understand underlying mechanisms by which plants control the water balance, water transport, and responses to water-deficit stress and recovery after drought stress. Despite the idea that aquaporins can play roles in plant response to water-deficit stress based upon their basal water-transporting activity, related studies have often been unclear. This lack of clarity results from the complex expression patterns of aquaporins, high sequence similarity, and limitation of tools to measure accurate movement across transmembrane aquaporin channels (Kaldenhoff et al., 2008).

3.2. Gene suppression study

In addition to aquaporin gene expression analyses that have shown the relevance of aquaporins to the water deficit stress, there are other sophisticated approaches that have used forward and reverse genetics and gene knockout mutant systems to support the idea linking aquaporin functions and water stress responsiveness in plants (Table 2). In the moss *P. patens*, two *PIP2* knockout mutants exhibited a severe stress phenotype when grown under drought conditions and protoplast plasma membranes of these mutants have reduced water conductivity. This demonstrates that *PpPIP2;1* and *PpPIP2;2* take part in transporting water molecules and in managing plant water balance under stress conditions (Lienard et al., 2008). When a tobacco (*Nicotiana tabacum* L.) aquaporin, *NtAQP1*, was silenced to downregulate *NtAQP1* transcripts, this antisense construct-expressing plant exhibited reduced root hydraulic conductivity and lowered resistance to water deficit stress in comparison to the control plants. This illustrated the functional importance of *NtAQP1* in the water balance under a water stressed growth environment (Siefritz et al., 2002). T-DNA knockout mutations of the Arabidopsis *PIP2;2* gene showed reduced hydraulic conductivity in root cortex cells in spite of the presence of the neighboring, highly homologous *PIP2;3* gene (Javot et al., 2003). It is noteworthy to point out the suggested relevance of aquaporins to CO_2 transport, because the presence of a functional CO_2 transporter in drought conditions may offer alternate routes of photosynthetic activity (Kaldenhoff et al., 1998; Boudichevskaia et al., 2015). In accordance with this idea, a differential CO_2 concentration was measured using a micro pH electrode to support the possible role of aquaporins in CO_2 transport across biological membranes with and without AtPIP1;2 (Uehlein et al., 2012).

3.3. Drought stress

From a long-term germplasm screening program under field drought conditions, a slow-wilting soybean genotype,

PI416937, was identified. This phenotype was attributed to the low hydraulic conductance in leaves and might be caused by a lack of specific aquaporins that displayed sensitivity to silver (Ag), an inhibitor of aquaporin protein in leaves of PI416937 (Sinclair et al., 2008; Sadok and Sinclair, 2010). Two poplar clones differing in response to drought stress were chosen to analyze aquaporin gene expression under drought conditions. In this study six *PIP* genes including *PIP1;3* showed increased expression in response to water deficit conditions only in leaves of *P. simonii × balsamifera* in which rapid reduction of stomatal conductance occurred, resulting in growth retardation under drought conditions (Almeida-Rodriguez et al., 2010). In upland cotton, two highly homologous aquaporin-coding sequences belonging to a PIP1 subfamily were identified to have spatial and contrasting expression patterns in root and leaf tissues when field-grown cotton plants were compared in two different water conditions (Park et al., 2012). This implicated the involvement of duplicated aquaporin genes with functional diversification in response to drought.

3.4. Boron (B)
In addition to their function as essential water transporters, the major intrinsic protein aquaporin family members are also considered ancient and multifaceted channel proteins for metalloids (Ma et al., 2006; Bienert et al., 2008). As an essential element in plant growth and reproduction, boron (B) has to be supplied to plants from soil for proper cell wall biosynthesis (Goldbach and Wimmer, 2007). Deficiency of B in arable areas has been problematic across crop species worldwide (Takano et al., 2008). In Arabidopsis, an aquaporin gene responsible for B uptake was identified. T-DNA insertion mutations in the *NIP5;1* gene displayed decreased ability of mutant plants to take up B in root tissue and a striking growth retardation occurred in the case of low B supply (Table 2) (Takano et al., 2006). Additionally, for the purpose of acclimation of plants exposed to high B supply, it was shown that there was a feedback mechanism by which *AtNIP5;1* was negatively regulated through the regulation of the 5'-untranslated region (5'-UTR) of the gene (Tanaka et al., 2011). Recently, it was shown by positional cloning of the *tasselless-1* (*tls1*) mutation in maize that a B transporter ZmNIP3;1 (an orthologue of Arabidopsis boron transporter AtNIP5;1) was responsible for the phenotype caused by B deficiency in the mutant maize plant. This boron transporter was essential for normal vegetative and reproductive growth in maize (Durbak et al., 2014).

3.5. Silicon (Si)
Similar to B, Si is ubiquitous in soil and is absorbed into plant root as a form of monosilicic acid, $Si(OH)_4$. After absorption, Si is transported to aerial parts through the transpiration stream, and deposited in plant cell walls as a form of amorphous silica, $SiO_2 \cdot nH_2O$. Alleviation of abiotic and biotic stress mediated by Si is a well-known phenomenon in many plants (Ma and Yamaji, 2008; Van Bockhaven et al., 2013). Specifically, the prophylactic effects of Si on disease were noted in rice, which is known as a strong Si accumulator (Rodrigues et al., 2003; Ma and Yamaji, 2008). The influx Si transporter Lsi1 (for Low silicon1) is the first plant Si transporter identified by quantitative trait loci (QTL) mapping using a rice mutant defective in Si uptake (Ma et al., 2006). Belonging to the aquaporin subfamily NIP2, Lsi1 is widely conserved in the plant kingdom. In addition, a second aquaporin type Si transporter has been identified in rice where NIP2;2 (Lsi6) facilitates Si transport by unloading silicic acid from the xylem to leaf tissues (Yamaji et al., 2008). In fescue grasses (*Festuca* spp.) the microstructure and shapes of phytoliths have shown dramatic effects in preventing damage from herbivore attack, indicating that not only the Si accumulation itself but also the morphological structure of accumulated Si on leaf surface provide cues of more effective defense against herbivore attacks (Hartley et al., 2015).

3.6. Urea
Lastly, in Arabidopsis, *AtTIP1;3* and *AtTIP5;1* showed strong and limited expression in mature pollens and capacity to transport water and urea in a heterologous system (Soto et al., 2008). Each single mutation in these two genes generated a phenotype with a 30% reduction in pollen tube elongation and an 80% decrease in mitochondrial volume in pollen tubes when germinated in vitro in the absence of exogenous nitrogen sources. Because AtTIP5;1 protein was subcellularly localized in mitochondria, it was suggested that AtTIP5;1 might have roles in nitrogen remobilization across cellular organelles (Table 2) (Soto et al., 2010).

4. Prospects
Redundancy and novelty are equally important in the history of crop genome diversification. There are increasing circumstances that support a role of aquaporins as selective transporters of different substrates. For example, aquaporins can facilitate membrane transport of gaseous molecules such as carbon dioxide, ammonia, and nitroxide (Hwang et al., 2010; Herrera and Garvin, 2011; Kaldenhoff, 2012). As cross membrane transporting components for water and different nutrients, aquaporins also function across kingdoms, in symbiosis between plants and microbes (Aroca et al., 2007; Bonfante and Genre, 2008; Aroca et al., 2009). In this relationship, colonized microorganisms such as ectomycorrhizas and arbuscular mycorrhizas present in the plant root rhizosphere are beneficial to plant nutrition especially in stressed conditions such as water deficit and nutrient

Table 2. A list of genetic studies of aquaporins in stress response.

Gene	Plant	Function	Stress	Reference	Mutation
PpPIP2;1/PpPIP2;2	*P. patens*	Water transport	Water-deficit	(Lienard et al., 2008)	Antisense silencing
AtPIP1a/b	Arabidopsis	Water transport	Hypotonic	(Kaldenhoff et al., 1998)	Antisense silencing
AtPIP2;2	Arabidopsis	Water transport	Hypotonic	(Javot et al., 2003)	T-DNA
AtNIP3-1	Arabidopsis	As root-shoot translocation	As tolerance	(Xu et al., 2015)	T-DNA
AtTIP1;3/AtTIP5;1	Arabidopsis	N remobilization	N deficiency	(Soto et al., 2010)	T-DNA
NtAQP1	Tobacco	Water transport	Drought	(Siefritz et al., 2002)	Antisense silencing
AtPIP1;2	Arabidopsis	CO_2 transport	Low CO_2	(Uehlein et al., 2012; Boudichevskaia et al., 2015)	T-DNA
PIP1	Poplar	Water/CO_2	Drought	(Secchi and Zwieniecki, 2013)	Antisense silencing
Lsi1	Rice	Silicon uptake	Leaf blight	(Ma et al., 2006)	Point mutation
NIP3;1 (tls1)	Maize	Boron uptake	Boron deficiency	(Durbak et al., 2014)	Point mutation
NIP5;1	Arabidopsis	Boron uptake	Boron deficiency	(Takano et al., 2006)	T-DNA

deficiency. Aquaporins present on both sides of symbiotic relationships are considered functionally important. Further exploration of this relationship is a very interesting topic and may assist with strategies important in coping with crop production in adverse conditions.

In conclusion, this review described that aquaporin genes appeared as tandem duplication in genomes of many plants and crop species and those tandem localized aquaporins were often diverged in gene expression patterns and possible functions. Multifaceted functions of aquaporins in transporting water and other small molecules were shown to be essential mechanisms that play important roles in proper growth, reproduction, and resistance to abiotic and biotic stresses. The aquaporin studies mentioned in this review and on-going research on this compelling topic will lead to a new generation in biotic and abiotic stress resistance in crop production.

Acknowledgments

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