AQUAPORINS IN PLANTS - EXPRESSION AND REGULATION UNDER STRESS

ARCHANA KUMARI, ADITI TAILOR AND SATISH C BHATLA

Laboratory of Plant Physiology and Biochemistry,
Department of Botany, University of Delhi, Delhi-110007, India
Email: bhatlasc@gmail.com
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Plants continuously adjust their water status in response to external cues, which requires rapid and efficient transmembrane water transport, a feat which is made possible by highly specialized channels, collectively referred to as 'aquaporins'. Aquaporins (AQPs), belonging to the Major Intrinsic Protein superfamily of membrane-associated proteins, are located on cellular as well as various subcellular membranes, where they conduct not only water but also other substrates, such as urea, glycerol, boric acid, arsenite, CO₂, NO and H₂O. AQPs are also involved in defense against biotic stress factors, including pathogen attack and herbivory, through deployment of H₂O₂ as defense response, which may also activate further immunity responses. TIPs and NIPs play significant roles in establishing symbiotic relationships via facilitation of bidirectional exchange of nutrients and water between host and symbiont. An interplay between ABA levels and changes in expression and activity of AQPs isoforms has been established to be crucial for stress tolerance, which may involve H₂O₂, an important player in ABA-mediated responses, most important being regulation of stomatal movements. Therefore, a complex mechanism of AQP regulation exists in plants which may be elicited in response to various stress conditions. This review attempts to summarize the function, regulation and modulation of different aquaporin isoforms under abiotic stress.

Keywords: Abiotic stress, aquaporin, MIPs, water channel AQP - Aquaporins

Plant cells are capable of adjusting their cellular water status in response to various stimuli by altering osmotic and water potential which may be achieved by regulation of transport of ions and accumulation of osmolytes inside the cell, and consequential influx or efflux of water. Transmembrane transport of water is facilitated by highly specialized water channels, categorized as 'aquaporins' (AQP) which allow rapid fluxes of water across the membranes to facilitate events, such as cell elongation and stomatal movement, and also maintain overall plant water relations. The discovery of AQPs dates back to early 1990s when first ever water permeable protein, CHIP28, was characterized as an integral protein spanning the membranes of human erythrocytes (Preston et al. 1993). CHIP28 was later renamed as AQP1 and was placed in the family 'Major Intrinsic Proteins' as it formed a major constituent of total membrane proteins, along with other aquaporins that share substantial homology with each other. The first MIP reported in plants was a 26.5 kDa transmembrane protein, Nodulin-26 (NOD26), isolated from the...
peribacteriod membrane of soybean nodules (Fortin et al. 1987). However, its identification as a water channel was not established until a few years later, when it was shown to form channel in artificial membranes that conducts cations, water and glycerol (Dean et al. 1999). The first plants AQP observed to exhibit water transporting activity was the vacuolar membrane protein γ-TIP (Maurel et al. 1993). Since then, a diverse array of MIPs have been identified and functionally characterized with respect to their substrate specificity and selectivity.

The Major Intrinsic Protein superfamily

Major Intrinsic Proteins (MIPs) constitute a large, conserved family of widely distributed integral membrane proteins that form channels facilitating water transport across the hydrophobic membranes (Maurel et al. 2008). The plant MIP superfamily is highly diverse, comprising more than 150 members. Plant AQP belong to the superfamily of MIP. The term 'aquaporin' has been used to refer only to the MIPs with water transporting activities, as they are almost exclusively involved in water transport. Despite the water-selective nature of some AQP isoforms, ample evidence highlight the permeability of other members of the AQP family for substrates other than, or in addition, to water, such as neutral solutes (urea, glycerol, lactic acid), metalloids (boric acid, silicic acid, zinc, arsenite, antimony), gases (ammonia, CO and NO) and ROS (H$_2$O$_2$) (Maurel et al. 2008, Kapilan et al. 2018). All AQP isoforms exhibit high degree of conservation, right from the level of primary up to quaternary structure, with minor deviations, and thus, share unique structural characteristics (Verma et al. 2015). They have molecular weights in the range of 23–31 kDa, and adopt a conserved 'hourglass' structure. This hourglass architecture achieved by AQP isoforms has been retained through evolution in all the members despite the diversification of functions. The conserved AQP structure is composed of six transmembrane α-helical domains (1–6), two 'half-helices', five interconnecting loops (A–E), and the amino (N) and carboxy (C) termini, which are together arranged in a right-hand fold (Fig. 1). The α-helices are tilted at a slight angle around the central axis of the channel (Ludewig and Dynowski 2009). The half-transmembrane helices are formed by the organization of two conserved NPA motifs present in the loops B and E, along with some adjacent residues, which fold back into the membrane from either side. The two 'half-membrane-spanning' helices together form a seventh 'broken or pseudo' helix upon folding. Out of the five loops, B and D are located on the cytoplasmic side of the membrane while the other three loops (A, C and E) are extracytoplasmic. The loops B and E are hydrophobic and remain partially embedded in the membrane. The N- and C-termini are both cytosolic. The N- and the C-terminal halves of AQP are homologous to each other, located in an inverse manner with respect to each other, with each half consisting of 2 transmembrane helices, a half-transmembrane helix followed by another transmembrane helix. This two-fold symmetry is also reflected in the AQP structure which consists of two hemipores, each arising from the N- and C-terminal halves, respectively, and assume the shape of a conical vestibule. A functional pore is constituted by the interaction of the two hemipores, giving an appearance to the structure similar to that of an hourglass with the tip of the two hemipores facing each other. A narrow region is created at the interface of two hemipores as result of juxtaposition of the two NPA motifs situated in the middle of the pore. A typical AQP channel pore has a radius between 4 to 6 Å and runs 20 to 28 Å in length (Luang and Hrmova 2017). Among the conserved structural features shared by AQP isoforms, two regions are of extreme importance with regard to their permeability and transport properties. These highly specialized regions, an electrostatic repulsion filter (ar/A selectivity filter) and two NPA motifs, form two regions of constriction in the pore, and function to provide a combination of high transport rate as well as selectivity.
Figure 1: Structure and assembly of aquaporins (AQP) in cell membrane. A, B The structure of AQPs is characteristically composed of 6 transmembrane helices (1–6), 5 interconnecting loops (A–B) and N- and C-terminals with conserved NPA motifs in loops B and E. C, D The assembly of an AQP monomer which, in association with 3 other monomeric subunits, gets organized as a functional tetramer in the membrane.
characteristic of AQPs. In addition to these two highly conserved motifs, a region composed of 5 relatively conserved amino acid residues, known as Froger's positions (FPs), has also been deemed important in determining the substrate selectivity (Verma et al. 2015).

Types of Aquaporins
Plant AQP superfamily is categorized into five main subfamilies largely depending on the sequence homology and subcellular localization—TIP, NIP, PIP, SIP and XIP (Table 1). In addition to these, 2 more subfamilies have been identified in moss—the GIP (GLP-like Intrinsic Protein) and the HIP (Hybrid Intrinsic Protein)—which have not yet been reported in higher plants (Danielson and Johanson 2008). The GIP subfamily comprises members closely related to the glyceroporin (GLP) group of AQPs involved in glycerol transport in bacteria. The members belonging to HIP subfamily display hybrid features, sharing certain similarities with both the PIP and TIP subfamilies, hence given the name. A unique subfamily of AQPs, the LIP (Large Intrinsic Protein), a subfamily closely related with the SIP subfamily, has been identified in diatoms (Khabudaev et al. 2014). The names of the subfamilies PIP and TIP have been designated to indicate their common subcellular location, i.e. the plasma membrane and tonoplast, respectively (Maurel et al. 2008). Each subfamily is further divided into groups based on sequence homology. PIPs, together with TIPS, are the major AQPs expressed in most plant tissues.

PIPs (Plasma membrane Intrinsic Proteins) represent the most highly conserved group of water-selective plant AQPs and constitute the largest AQP subfamily, comprising members with an average molecular weight of 30 kDa and an isoelectric point (pI) of 9. They are generally localized in plant organs exhibiting characteristic large water fluxes, for example, guard cells, vascular tissue of leaves and roots, and flower petals (Azad et al. 2012, Heinen et al. 2014, Chaumont and Tyerman 2014). PIPs are the main modulators of cellular water transport since they manage the bidirectional transport of water across cellular membrane. They are characterized by the presence of an ar/R filter region and a narrow pore characteristic of the 'orthodox' water-specific AQPs, and are specifically involved in water transport and remain impermeable to other substances (Wallace and Roberts 2004, Bansal and Sankararamakrishnan 2007, Maurel et al. 2008).

Most PIP isoforms are located on the plasma membrane. However, some PIPs are also associated with internal membranes (Zelazny et al. 2009). The PIP subfamily is further divided into two subgroups, i.e. PIP1 and PIP2, with each group having a set of unique features. The PIP1 and PIP2 subgroups have so far been found to comprise 5 (PIP1;1 to PIP1;5) and 8 (PIP2;1 to PIP2;8) isoforms (Kapilan et al. 2018). These subgroups mainly differ in the length of the N- and C-terminal domains and the loop A as well as their water transporting activity and cellular functions. PIP1s are characterized by a longer N-terminal and shorter C-terminal as compared to PIP2s. They exhibit a low permeability to water, with some isoforms being unable to act independently, and thus, requiring co-expression and assembly into heterotetramers with PIP2 to function as facilitators of water transport (Horie et al. 2011). However, reports have also demonstrated their significant role in regulating hydraulic conductivity during water uptake by different tissues and in transport of glycerol and CO₂ (Schuurmans et al. 2003, Uehlein et al. 2003, Hanba et al. 2004, Uehlein et al. 2008). PIP2s, on the other hand, are highly efficient in transporting water, and are main facilitators of transmembrane water transport. CO₂ has been suggested as alternative solute for PIPs as well (Hanba et al. 2004). Dynowski et al. (2008) also proposed involvement of PIPs in H₂O₂ transport.

TIPs (Tonoplast Intrinsic Proteins) form the most abundant group of AQPs predominantly located in the tonoplast of vacuoles, with isoforms ranging in molecular weights between 25 to 28 kDa (Johanson and
They are more acidic and smaller as compared to PIPs and have an isoelectric point of 6. Five subgroups of TIPs (TIP1 to TIP5) have been identified in higher plants, which belong to either group I, II or III TIPs, and a sixth subgroup is specific to moss Physcomitrella patens (Danielson and Johanson 2008). Different TIP isoforms are expressed characteristically in the membranes of functionally diverse vacuoles, and thus, have been used as markers to identify vacuolar types, supporting the diversification of the TIP subfamily as a function of vacuolar differentiation (Jauh et al. 1999). In addition to their predominance occurrence in tonoplas, TIPs have also been located in plasma membranes during seed maturation and germination in Arabidopsis, which might be helpful in facilitating rapid water intake to accelerate the process of imbibition (Gattolin et al. 2011). Among plant AQPs, TIP isoforms contain the highest degree of diversity in the ar/R region (Wallace and Roberts 2004). Various isoforms of TIPs have been reported to transport a variety of solutes, such as NH$_3$/NH$_4^+$, glycerol, urea and H$_2$O$_2$ in addition to water (Wudick et al. 2009). They are also important for regulating water fluxes in response to drought and salinity stress (Wudicket al 2009). Recently, the transport ability of arsenic for a TIP has also been demonstrated in the fern Pteris vittata (He et al. 2015).

Members of the NIP (Nodulin-26 like Intrinsic Protein) subfamily were thought to be specifically expressed in the peribacteriod membrane in the nodules of nitrogen-fixing plants. However, they are now known to be present in non-leguminous plants as well. NOD26 was the first plant AQP to be discovered (Fortin et al. 1987). Most of the NIPs are similar in sequence to NOD26 protein. NIPs play significant role in transfer of water between the bacterial symbiont and the host plant. Other roles for NIPs have been identified in maintenance of water balance and responses to various stresses (Roberts and Routray 2017). Different NIP isoforms transport small uncharged solutes, like formamide and glycerol, large but uncharged solutes, like urea and metalloids, like silicon, boron, arsenic and antimony (Wallace et al. 2006, Pommerrenig et al. 2015, Roberts and Routray 2017). The NIP subfamily is functionally classified into three subgroups based on the ar/R selectivity region: the NIP I, NIP II and NIP III (Wallace et al. 2006). The NIP subgroup I includes members resembling the archetype NOD26, and exhibit permeability to water, glycerol and formamide. The other two NIP subgroups, i.e. NIP II and NIP III, include members capable of transporting metalloids.

SIP (Small and basic Intrinsic Protein) subfamily, as the name suggests, includes all the small basic intrinsic proteins. They were named after two of their characteristics: 1) smaller size than PIPs and NIPs, and 2) more basic nature in comparison with TIPs (Johanson and Gustavsson 2002). SIPs possess a shorter N-terminal. Hence, they are smaller in size; and possess unusual NPA boxes and highly divergent ar/R selectivity filter regions (Ishikawa et al 2005). They are localized in the ER membrane. They have mainly been shown to have water transporting activity (Ishikawa et al. 2005). However, the great degree of diversity displayed by SIP isoforms in the ar/R filter and other residues may be a reflection of their different solutes permeabilities, an assumption which needs to be further explored.

The XIP (X Intrinsic Protein) subfamily represents the most recently discovered and the least characterized subfamily of AQPs in terms of both structure and function. It

<table>
<thead>
<tr>
<th>AQP subfamilies</th>
<th>Molecular weight (kDa)</th>
<th>Isoelectric point (pI)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean (range)</td>
<td>Mean (range)</td>
</tr>
<tr>
<td>PIP</td>
<td>30 (29–32)</td>
<td>9</td>
</tr>
<tr>
<td>TIP</td>
<td>25 (25–29)</td>
<td>6 (4.5–7)</td>
</tr>
<tr>
<td>NIP</td>
<td>30 (28–35)</td>
<td>7 (4.5–9)</td>
</tr>
<tr>
<td>SIP</td>
<td>25 (25–29)</td>
<td>(9.5–10)</td>
</tr>
<tr>
<td>XIP</td>
<td>28 (25–35)</td>
<td>(8.5–9)</td>
</tr>
</tbody>
</table>

Table 1: Major aquaporin (AQP) subfamilies in plants and their characteristic features.
comprises the uncharacterized isoforms of plant AQPs discovered so far, homologs of which are absent in many plant species. They were first reported in the moss *Physcomitrella patens* and later reported in poplar tree and cotton (Danielson and Johanson 2008, Gupta and Sankaramakrishnan 2009, Park *et al.* 2010). Since then, many other members of XIPs have been described in different species of plants. Functional characterization of the XIP superfamily is yet to be done. XIPs are impermeable to water due to the presence of more hydrophobic residues in the ar/R selectivity filters (Bienert *et al.* 2011). However, some plant XIPs transport glycerol, $\text{H}_2\text{O}$, urea and boric acid (Noronha *et al.* 2016).

**Tetrameric assembly**: PIPs and TIPs exist as multi-subunit complexes in native environments, i.e. as tetramers, which is now believed to be a common feature shared by all the aquaporins. The tetrameric assembly of AQPs may be constituted by the association of homo- or heteromeric subunits (as in the case of PIP1 and PIP2) around a four-fold axis, and are stabilized by H-bonds and hydrophobic interactions (Luang and Hrmova 2017). Loops A and E are crucial for interaction between monomers, and thus, tetrameric assembly (Jozefkowicz *et al.* 2013). The AQP monomers can dimerize through the formation of disulphide linkage between the Cys residues in loop A of two monomers and then finally assemble into tetramers (Bienert *et al.* 2012). Although each monomer in itself is a functional unit capable of carrying out transport of water independently of the rest of the subunits, organization into a tetrameric unit is suggested to be important for the correct folding of individual units and imparting stability to the conformation as well as for targeting of the protein to the membranes (Fetter *et al.* 2004). The tight association of AQP monomers into tetrameric assembly creates a central pore which may be as wide as 10 Å. The central pore has been speculated to serve as a potential path for conductance of ions and gases (Fu *et al.* 2000, Uehlein *et al.* 2008, Herrera and Garvin 2011).

**Aquaporin expression and regulation in stress conditions**: Functional aspects of AQPs go beyond water and solute transport in normal growth conditions as they are also involved in plant responses against biotic and abiotic stress conditions. They are considered to be the zenith contender for decoding the mechanism of stress tolerance in plants. Many investigations have revealed that accumulation and abundance of AQPs is under the regulation of numerous developmental processes and environmental stress conditions, comprising both biotic (pathogen attack) and abiotic (flooding, drought, change in temperature etc.) factors. Drought and salinity cause rapid and constant loss of water from plants. Abundance and expression of AQPs alter, according to variations in environmental conditions (Kapilan *et al.* 2018). Overexpression of *HvPIP2;5*, an AQP from *Hordeum vulgare*, improves the tolerance in Arabidopsis and yeast against drought and salinity (Javot and Maurel 2002). This improvement in tolerance level is a consequence of improved expression and activity of ROS quenching enzymes (catalase, superoxide dismutase etc.). Zhu *et al.* (2005) has also suggested a correlation between ABA (stress hormone) biosynthesis and AQP expression (Deshmukh *et al.* 2017, Kapilan *et al.* 2018). Discovery of several isoforms of aquaporins highlights their versatile nature in regulating water transport. Several factors, such as stress (biotic or abiotic), pH and hormones, regulate the abundance and expression of these isoforms in plants (Kapilan *et al.* 2018). Ability of few AQP isoforms to transport small molecules ($\text{Na}^+$, $\text{K}^-$), compounds ($\text{H}_2\text{O}_2$), and gases ($\text{CO}_2$) in addition to water, further complicates the still existing challenge in understanding the nature and function of aquaporins in plants (Kapilan *et al.* 2018).

Genetic transformation of plants with aquaporin genes sometimes causes phenotypic
alterations in plants relative to their wild type. For example, transgenic of Arabidopsis, overexpressing GoPIP1 gene of Galega orientalis, develops high rosette/root ratio as compared to its wild type in controlled environmental conditions (Li et al. 2015). Similar shift in morphology is observed in transgenics of rice and tobacco, overexpressing barley HvPIP2;1 and Arabidopsis AtPIP1b genes, respectively (Aharon et al. 2003, Katsuhara et al. 2003, Li et al. 2015). Thus, it is plausible that the change in plant phenotype is a consequence of the mechanisms employed by aquaporins to maintain water status under normal and stress conditions. It can be an outcome of cellular level feedback reaction or a compensation mechanism which reduces root/shoot ratio in plants (Li et al. 2015) (Table 2). Transgenic Arabidopsis overexpressing GoPIP1 gene grown under water deficit conditions develops a rosette of low weight and faded colour (Kapilan et al. 2018). Significant difference in plant phenotype is also reported in transgenics during salt stress (Katsuhara et al. 2003, Ayadi et al. Li et al (2015) claimed that no clear difference was observed in transgenic Arabidopsis and tobacco plants grown under salt stress with respect to its wild type. It is interesting to note that genetic modification of

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Stress</th>
<th>AQP gene</th>
<th>Host plant of AQP gene</th>
<th>Transgenic plants</th>
<th>Response</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Drought</td>
<td>VfPIP1</td>
<td>Vicia faba</td>
<td>Arabidopsis thaliana</td>
<td>Resistance</td>
<td>Cui et al. 2008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VvPIP2;4 N</td>
<td>Vitis vinifera</td>
<td>Vitis vinifera</td>
<td>Susceptible</td>
<td>Perrone et al. 2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BnPIP1</td>
<td>Brassica Napus</td>
<td>Nicotiana tabacum</td>
<td>Resistance</td>
<td>Yu et al. 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RWC3</td>
<td>Oryza sativa</td>
<td>Oryza sativa</td>
<td>Resistance</td>
<td>Lian et al. 2004</td>
</tr>
<tr>
<td>2.</td>
<td>Salt</td>
<td>AtPIP1;b</td>
<td>Arabidopsis Thaliana</td>
<td>Nicotiana tabacum</td>
<td>Susceptible</td>
<td>Aharon et al. 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TdPIP1;1</td>
<td>Triticum Durum</td>
<td>Nicotiana tabacum</td>
<td>Resistance</td>
<td>Ayadi et al. 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TaAQP7</td>
<td>Triticum aestivum</td>
<td>Nicotiana tabacum</td>
<td>Resistance</td>
<td>Zhou et al. 2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AtPIP1;4, AtPIP2;5</td>
<td>Arabidopsis thaliana; Nicotiana tabacum</td>
<td>No effect</td>
<td>Jang et al. 2007</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HvPIP2;1</td>
<td>Hordeum Vulgare</td>
<td>Oryza sativa</td>
<td>Susceptible</td>
<td>Kapilan et al. 2018</td>
</tr>
<tr>
<td>3.</td>
<td>Cold</td>
<td>AtPIP1;4, AtPIP2;5</td>
<td>Arabidopsis thaliana; Nicotiana tabacum</td>
<td>No effect</td>
<td>Jang et al. 2007</td>
<td></td>
</tr>
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</table>

Table 2: Aquaporin (AQP) expression in plants in relation with abiotic stress tolerance
some plants with aquaporin genes also shows reverse and negative impact on the growth and development, and yet in others, it shows no change or difference under stress conditions. As reported by Aharon et al. (2003) overexpression of AtPIP1b in tobacco causes rapid wilting in transgenic plants relative to its wild plants under drought stress. Also, overexpression of PIP1 gene of Galega orientalis negatively affects the growth of transgenic Arabidopsis (Li et al. 2015). Reduced growth is a consequence of enhanced water transport in transgenic plants, which is harmful for plant parts under stress conditions. These results support the preposition of transgenic lines being susceptible to stress conditions and be able to concentrate less photosynthates in leaves.

Drought: Relative transcriptome analysis of different AQP isoforms (PIP and TIP) has revealed their distinctive patterns of expression when subjected to drought stress, and thus it has defined their explicit roles in regulating water status under water deficit condition.

Yamaguchi-Shinozaki et al. (1992) presented first report of AQPs having a role under drought stress. They observed that drought stress up regulates AtPIP2;3 expression in Arabidopsis. An investigation of all 35 isoforms of AQPs from Arabidopsis also revealed that expression of each PIP and TIP isoform is induced under water stress. NIPs exhibit minor modulation in their accumulation and function under drought stress (Alexandersson et al. 2005). Drought stress up regulates expression level of AtPIP1;4 and AtPIP2;5, while all other PIPs undergo down regulation. Furthermore, AtPIP2;6 and AtSIP1;1 display no striking change in their expression, and are constitutively expressed under drought stress. When both drought and heat stress are applied in combination, only AtPIP2;5 gene shows significant up regulation in leaves (Rizhsky et al. 2004). Several previous reports suggest that PIP1;4 and PIP2;5 genes are responsible for rapid water loss and stunted growth of seedlings under drought (Jang et al. 2007, Li et al. 2015). Moreover, AtPIP1b overexpression in tobacco plants results in an early and swift wilting in comparison to its wild type during drought stress (Aharon et al. 2003, Li et al. 2015). Enhanced expression of Galega orientalis derived PIP1 in Arabidopsis also induces negative effect on plant growth during drought stress (Jang et al 2007, Li et al. 2015).

In the past few years, PIPs have emerged as the most responsive water transporting channels under drought conditions and, in general, most of them undergo down regulation (Jang et al 2004, Alexandersson et al. 2010, Afzal et al 2016). All these down regulated AQP genes are significantly expressed in roots of Arabidopsis, tobacco, olive's twigs (Secchi et al. 2007, Afzal et al. 2016) and in peach fruit (Sugaya et al. 2002, Afzal et al. 2016). Response pattern of AQPs changes with tissue (leaves and roots), cultivars and plant species. For example, expression of PIP1;1 enhanced in roots of anisohydric cultivars but the same was not observed in isohydric cultivars of Vitis vinifera under water deficit condition (Vandeleur et al. 2009). Additionally, structural similarity of AQPs does not guarantee similar expression pattern of two similar isoforms. A comparative transcriptome research on cotton (Gossypium hirsutum) raised in control and drought conditions has revealed that PIP1;1 and PIP1;3 have distinct patterns of expression in leaves and roots even though they share high level of structural similarity (Park et al. 2012, Li et al. 2013 and Afzal et al. 2016). Furthermore, water deficit imposed through artificial means, such as polyethylene glycol (10%), mannitol etc., induces contrasting expression of AQPs as compared to natural water deficient conditions. For example, no change of expression is obtained in OsPIP1;3, when water stress is induced by the application of PEG (10%). However, expression of OsPIP1;2 and OsPIP1;1 genes is enhanced under induced drought conditions (Guo et al. 2006). Alternatively, OsPIP1;1 expression is down regulated under drought (Malz and Sauter 1999, Afzal et al. 2016) and mannitol...
stimulated osmotic stress (Li et al. 2000, Afzal et al 2016). GoPIP1 of forage legume Galega orientalis is also involved as a stress response against water loss (Li et al. 2015, Kapilan et al 2018). Accumulation of GoPIP1 transcripts increases strikingly in roots during osmotic stress (by NaCl or PEG). Interestingly, enhanced expression of GoPIP1 made transgenic Arabidopsis susceptible to water loss but invincible to salinity (Li et al. 2015). In rice (Oryza sativa), induction of drought stress results in up regulation of OsTIP1;1 expression both in root cells and leaves (Li et al. 2000, Katsuhasha et al 2003). Functions of NIPs, SIPs and XIPs during water stress are still not clear. Transcriptome analysis of these AQPs in citrus plants subjected to drought stress showed that all CsTIPS and CsXIPS exhibit differential expression in roots and leaves. Expression of CsTIPS and CsXIPS undergoes up regulation in leaves and down regulation in roots during water stress. In citrus leaves, expression of two CsPIP-2 CsPIP2;4, CsPIP1;1, one CsSIP - CsSIP1;2 and one CsNIP - CsNIP1;1 up regulates during drought stress (Martins et al. 2015, Kapilan et al. 2018).

It is presumed that PIPs have the most crucial role during drought stress. However, the distinctive mechanism of AQPs to regulate the water balance during salt stress is still unknown. Response of AQPs against drought stress varies greatly depending upon the extent of stress level, AQP isoforms, host organ/tissue, plant cultivar/variety, nature of stimuli and symbionts available that causes water loss. However, universal down regulation of most PIPs restricts water loss and prevents backflow of water. TIPs also modulates water homeostasis of cells. They maintains an efficient water transport across the vacuolar membranes. However, experimental evidences of their roles are limited in comparison to PIPs.

**Salt**: Constant accumulation of excess salts of chloride (NaCl) or sulphates (CuSO₄) in soil is the prime reason for soil salinity. High salt accumulation in soil alters plant growth by two modes: water stress and osmotic stress. To combat, plants decrease water conductance by shutting down water uptake from roots. Water loss is a primary effect of salt stress in plants and evidence of AQPs response under salt stress has shown a direct relation to drought stress. For example, salinity causes a striking decrease in water conductance, and transcript abundance of PIPs and TIPs in Arabidopsis (Afzal et al. 2016). Enhanced expression of MusaPIP1;2 and MusaPIP1;2 in transgenic banana has been reported to confer high tolerance both under salt and water stress (Shekawat and Ganapathi 2013). Transcriptome and proteome analysis of AQPs in barley showed that HvPIP2;1 gene is specifically expresses under salt stress and is regulated distinctly in leaves and roots of barley. Salt stress also mediates differential modulation of HvPIP2;1 expression in barley. Salt stress down regulates the expression of HvPIP2;1 in roots and up regulates it in leaves (Katsuhasha et al. 2002, 2003). Mesembryanthemum crystallinum (ice plant), a salt-tolerant plant, also displays alteration in AQP's expression and abundance under salt stress, with many PIPs and TIPs getting down regulated in roots and leaves, respectively (Yamada et al. 1995, Kirch et al. 2002). Regulation of the expression of ZmPIP1 and ZmPIP2 under salt stress. However, ZmPIP1;1, ZmPIP1;5, and ZmPIP2;4 expression is enhanced transiently, especially in the apical of roots. ZmTIPs shows no difference in its expression (Zhu et al. 2005). In rice, expression level of OsTIP1;1 is down regulated under cold stress (Sakurai et al. 2005) but water and salinity stress up regulates its expression (Liu et al. 1994). Overexpression of tonoplast AQP- PgTIP1 (Panax ginseng), in transgenics of Arabidopsis promotes optimal growth in plants during favourable environment and also improves the ability of tolerance of plants against salinity and drought (Peng et al. 2007). Various investigations done so far to analyze AQPs distribution under saline environment have reported that severity of salinity determines the abundance, redistribution and re-localization of AQPs in...
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Low temperature: Low temperature stress is yet another abiotic factor which strikingly stunts the growth and development of plants. Transgenic of banana, overexpressing a gene of PIP homologue, i.e. MusaPIP1:2 and transgenic tobacco overexpressing AQP gene of wheat, [TaAQP7 (PIP2)] exhibit a better tolerance against drought and low temperature stress, relative to their non-transgenic counterparts (Zhou et al 2012, Shekhawat and Ganapathi 2013, Huang et al. 2014). Contrastingly, AtPIP1:4 or AtPIP2:5 overexpression in transgenics of Arabidopsis display enhanced adaptability during cold stress, but excessive loss of water makes them vulnerable to drought stress. Prolonged exposure of low temperature to rice plants (cold sensitive) results in up regulation of OsPIP2:5 expression (Ahamed et al. 2012). Most of the TIPs, such as OsTIP2:2 and OsTIP1:1 down-regulates at transcript level in response to cold stress in the roots of rice plants, whereas expression of some PIPs, such as PIP1:3 is enhanced by about 60% (Sakurai et al. 2005). All PIP isoforms in rice display decreased expression under cold/chilling stress, except the two low expressing PIPs, i.e. PIP2:5 and PIP2:6, which get upregulated both in roots and shoots under cold stress (Suga et al. 2002, Jang et al. 2004). Aroca et al. (2005) investigated impact of short but continued induction of chilling stress on AQPs expression and reported that both treatments (short or continued exposure of chilling stress) provoke similar response pattern in both plant genotypes, i.e. cold-tolerant and cold-susceptible maize plants. PIP's expression is down regulated in both the genotypes. Similar results were also obtained in rice and sugarcane (Li et al. 2000, Nogueira et al. 2003, Aroca et al. 2005). Increase in AQPs expression under low temperature and high salinity stress results in improved hydraulic conductivity in roots. Moreover, revival from the impact of cold stress is especially linked to the expression pattern of PIPs in plants (Afzal et al. 2016).

Micronutrient homeostasis and heavy metals: It is a well-established fact that AQPs are involved in the mobilization of metal ions, gaseous compounds and small neutral solutes (Afzal et al. 2016). Several PIPs and TIPs are involved in such transport, along with their role in supporting water movement. However, central role in transportation of these substances is performed by NIPs (Maurel et al. 2015). Even though the efficiency of NIPs in transporting water is highly restricted, but they are efficient transporters of nutrients involved in various metabolic pathways. For example, knockout mutant of NIP5;1 in Arabidopsis shows significant role of AQP (NIP5;1) in transfer of essential element Boron. Boron is a necessary element in plant growth, extension and development. Boron also required in reproductive phase of plants (Pollent tube germination). Suppression of growth in NIP5;1 knockout mutants of Arabidopsis supports this theory (Takano et al. 2006) .
Another evidence confirming the role of NIP5;1 in boron transport and homeostasis has been obtained from transgenic Arabidopsis where high supply of boron inhibits AtNIP5;1 gene expression as a feedback mechanism (Takano et al. 2006). Parallel results are obtained in maize, where loss of function mutation of maize NIP, i.e. ZmNIP3;1 (an ortholog of AtNIP5;1) results in aberrant phenotype in transgenic plants, owing to low supply of boron. NIP7;1, NIP6;1 and NIP5;1 isoforms of NIP family of AQP are reported to be involved in the distribution of boron in shoot and anthers of Arabidopsis plants, respectively (Li et al. 2011). In Arabidopsis, TIP5;1 mediates allocation of boron as borate into vacuole (Pang et al. 2010). Under high boron availability, reduced level of NIP2;1 enhances tolerance level in barley (Schnurbusch et al. 2010). Furthermore, some reports suggest that NIPs can also transport silicon. In plants, silicon, also have a major role to play in mediating defense reactions (biotic and abiotic). Si-induced mitigation pathway for abiotic stress is also well-established in plants (Yamaji et al. 2008). NIP2;1 and NIP2;2 are the two transporters of silicon discovered in rice. NIPs have a significant role in translocation of elements and small solutes in plants during deficiency or excess of the respective elements. NIPs are also involved in transport and defense response against heavy metals. Evidence for mobilization of heavy metals, such as arsenic and antimony through NIPs has recently been found in plants. For example, NIPs (NIP1;1 and NIP3;1) present in Arabidopsis are involved in transporting arsenic (Kamiya et al. 2009). Knockout mutant of t-DNA of these genes in Arabidopsis shows better tolerance against arsenic stress. A double knockout mutant of NIP genes, i.e. NIP1;1 and NIP3;1 provides better tolerance under arsenic stress than the single knockout mutant (Bienert et al. 2008, Kamiya et al. 2009). Analysis of level of expression of other NIPs in yeast and oocytes has revealed that some other isoforms of NIPs, such as NIP1;2, NIP5;1, NIP6;1 and NIP7;1 also have the ability to transport arsenic (Bienert et al. 2008). NIPs have also been identified for antimony transport in Arabidopsis. Loss of function mutant of NIP1;1 in Arabidopsis displays better tolerance against antimony stress, indicating the role of NIP1;1 gene in antimony transport (Kamiya and Fujiwara 2009). In Arabidopsis, AtNIP2;1 is anticipated to be a transporter of lactic acid because of its association with anoxic condition (oxygen deprivation) and water logging. It is postulated that Arabidopsis AtNIP2;1 is a transporter of lactic acid and are functionally important for plant’s adaptation from lactic acid fermentation under anoxic environment (Choi and Roberts 2007).

**Hormone:** Plants initiate the production of stress hormones, such as ABA and ethylene, when subjected to any abiotic stress, such as chilling stress. ABA maintains water status of plants by regulating aquaporin activity, and thus provides a long lasting effect on plant's hydraulic conductivity (Parent et al. 2009, Li et al. 2015). Variations in ABA levels induce a positive effect in Arabidopsis overexpressing GoPIP1 gene from Galega orientalis during drought stress (Afzal et al. 2016). But each aquaporin isoform is different and, therefore, responds differently (characteristic to itself) to ABA treatment. Plants exhibit both ABA-dependent and ABA-independent regulatory channels of aquaporin expression (activity and abundance) (Suga et al. 2002, Jang et al. 2004). Treatment of radish roots with phytohormones, such as GA₃ (0.1mM) and ABA (1mM) results in reduced mRNA levels of RsPIP2-3, RsPIP2-2 and RsPIP2-1) as well as RsPIP2-1 protein levels. However, protein content of RsTIPs and RsPIP1 members is not altered by ABA (Suga et al 2002). ABA also enhances expression levels of PIP1b (PIP1-2) in Arabidopsis and γ-TIP (TIP1-1) of rice (Suga et al 2002). Exogenous ABA application enhances transcript levels of Cp-PIPa (Craterostigma plantagineum) but has no effect on mRNA levels of Cp-TIPs. ABA treatment of Craterostigma plantagineum reduces mRNA level of Cp-TIPs. GA₃
treatment also provides similar results. GA treatment increases mRNA levels of TIP1-1 and PIP1-2 in Arabidopsis. Another hormone, 'BL,' a brassinosteroid, shows no effect on the abundance of any AQP in radish seedlings (Suga et al. 2002). BL treatment, however, increases the permeability of water in protoplasts extensively in mutants of Arabidopsis, which lack in their capability to synthesize BL. However, no prominent effect of BL is observed on aquaporin expression. It has also been observed that the effect of different phytohormones differs with different AQPs isoforms of different plants (Suga et al. 2002).

**Biotic interactions:** Symbiotic association of plants with bacteria/fungi causes a significant effect on plant growth and development. In addition, it regulates AQP's expression and reaction under various biotic and abiotic stress conditions (Aroca et al. 2005, 2007, Barzana et al. 2014). In a symbiotic relationship, redistribution of nutrients and water takes place between the host and the symbionts and, therefore, there is a great possibility that these bilateral transfers are being mediated by AQP genes. Symbiotic association of plant with arbuscular mycorrhiza is reported to benefit its host plants by improving their tolerance against drought stress. Barzana et al. (2014) reported that AM fungi (Rhizophagus sp.) enhance the expression level of AQP genes in their host plants. Similarly, Rhizophagus claru, another AM fungus, mediates long distance translocation of polyphosphate from fungal vacuoles to root and leaf cells of plants through its aquaglycoprotein RcAQP3 (glycerol/water transporting AQP) (Kikuchi et al. 2016). Nodulin-26, a NIP, is involved in symbiotic collaboration between the plant, soybean and the symbiont Rhizobium (a nitrogen fixing bacteria) (Maurel et al. 2015, Kapilan et al. 2018). NIPs are expressed on symbiosome membrane after the development of associative interaction between the plant and the bacteria. They are involved in transport of nitrogen available in various forms, such as urea and ammonia. Analysis of symbiotic relationship between plant and arbuscular mycorrhizae through gene profiling has revealed that AQP genes, including NIPs, are involved in transfer of glycerol and ammonia (NH$_4$/NH$_3$) from plant to mycorrhizae and vice-versa, respectively (Barzana et al. 2014, Maurel et al. 2015).

Many pathogens of plants depend upon plant nutrients for their survival and growth in a way similar to the symbionts. Dehydration occurs because pathogen attack serves as a regulatory mechanism in maintaining the water and nutrient homeostasis in plant. Additionally, plants also generate several ROS, such as H$_2$O$_2$ as a defense response (signaling). Transportation of ROS molecules is mediated by AQP isoforms, but a complete understanding of this pathway is still not known (Dynowski et al. 2008, Hooijmaijers et al. 2012). PIPs consist of an extracellular region exposed to the external environment, which makes them susceptible to pathogen attack. However, during pathogen infection, PIPs shift their function from water and substrate transfer to regulation of plant's stress responses against the pathogen in real time. PIPs are involved in regulating the function of effectors (pathogen determinant). Interaction with the pathogen determinant i.e. effectors, decides the impact (positive or negative) of PIP's regulation on its growth and development (Zhang et al. 2018, Li et al. 2019, Zhang et al. 2019). AtPIP1;4 of Arabidopsis facilitates the movement of H$_2$O$_2$ from apoplast to cell cytoplasm and induces immunity signaling cascades. These signals ultimately activate the immunity response in Arabidopsis either through PTI (Pattern-Triggered Immunity) or SAR (Systemic Acquired Resistance) (Zhang et al. 2019). Zou et al. (2005) reported that expression of 24 out of 32 AQP genes is down regulated in soybean in response to Pseudomonas syringae infection. Also, CsMIPs i.e. CsSIP1;1, CsNIP6;1, CsNIP5;2, CsNIP2;2, CsPIP2;2 and CsPIP1;2 display differential expression in citrus plants, solely because of stress forced by
Candidatus liberibacter (proteobacterium) infection. Comparative analysis of CsMIPs (CsNIP5;1, CsPIP2;2, CsTIP2;1, CsTIP2;2, and CsTIP1;2) in tolerant rough lemon cultivars and sensitive sweet orange has confirmed that many of the CsMIPs genes undergo negative regulation in response to any pathogen attack and thereby they might have some relation to the process of disease development. (Aritua et al. 2013, Martins et al. 2015). NIP's and TIP's (TIP1) role in maize and tobacco, respectively, has also been reported under biotic stress, such as nematode infection (Opperman et al. 1994, Lawrence et al. 2013). Accumulation of silicon in grasses, such as Festuca sp., improves its tolerance against herbivore infections. Therefore, it is postulated that transport of silicone through NIPs may have a relation with plant's defense response against herbivory (Hartley et al. 2015). TIPs (TIP1 and TIP2) of host plant interact with replication protein of cucumber mosaic virus (CMV1a), recruit SOS system and thus potentially affect CMV replication (Kim et al. 2006). The results highlights the significant role of AQP's in defense mechanism of plants against pathogen attack.

**Integrated roles of aquaporins in plants facing stress**: Interaction between proteins and other biomolecules defines the precise role of proteins in cell functioning. These interactions often lead to the formation of complexes, ranging from the stable complexes produced in cells to the transitory complexes formed during cell signaling pathways. Membrane proteins are an important unit of cell and are responsible for mobilization of water and small solutes across the membrane (Martinez-Ballesta and Carvajal 2016). Functions of AQP's are regulated by various physiological signals, interactions between various AQP monomers and their association with other biomolecules. Integrated contribution of AQP's under several physiological situations is more significant in transgenic plants transformed with alien AQP genes. Hetero-oligomerization of different AQP isoforms also occurs in plants. Heteromolecular interactions between PIP1 and PIP2 enhance water permeability of PIP1 isoform and stimulate water transport from ER to PM. Covalent bonding between the conserved Cys residues (located at loop A of both the isoforms) facilitates interaction between PIP1 and PIP2. This interaction (PIP1-PIP2) is regulated by the abundance of PIP transcripts, and different combinations are formed by plant as per requirement, under different environmental conditions (Yaneff et al. 2015, Martinez-Ballesta and Carvajal 2016). Strong interaction and tetrameric structure formation between TIPs (TIP1;2, TIP2;1, and TIP3;1) has also been described (Murozuka et al. 2013, Martinez-Ballesta and Carvajal 2016).

The degree of tolerance or susceptibility of transgensics is decided by the extent of association of foreign genes with the host plant. Overexpression of PIP1 gene of Vicia faba (VfPIP1) in transgensics of Arabidopsis provides better tolerance of water deficit by initiating stomatal closure and, therefore, averting water loss under drought. Interaction between VfPIP1 and regulatory genes of stomatal movement in Arabidopsis increases drought tolerance of Arabidopsis (Zhou et al. 2012). In contrast, endogenous PIP genes (EgPIP1 and EgPIP2) of transgenic Eucalyptus gets silenced completely after it is genetically transformed with Raphanus sativus aquaporin (RsPIP1;1) gene (Tsuchihira et al. 2010, Afzal et al. 2016). Transgenic tobacco, overexpressing AtPIP1b, displays an increase in growth and stomatal intensity under favorable conditions. However, during water stress it results in extensive water loss (Aharon et al. 2003). Overexpression of wheat AQP [TaAQP7 (PIP2)] increases the expression of ROS scavenging enzymes, which as a result reduces ROS level in tobacco (Afzal et al. 2016). Thus, defense response of tobacco against antioxidative and osmotic stress improves through genetic recombination of TaAQP7 gene in tobacco plants (Zhou et al. 2012). Similarly, over-expression of another wheat AQP
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TdPIP1;1 gene in tobacco also improves plant health and tolerance level under abiotic stress conditions. Their overexpression results in increased surface area of leaves and longer roots (Ayadi et al. 2011). Co-transformation in Arabidopsis with two foreign AQP genes, each from Cucurbita ficifolia (CfPIP2;1) and C. sativus (CsPIP2;1), disturbs the natural expression of AQPs of Arabidopsis, and results in altered defense response (Jang et al. 2007). Overexpression of PIP1 gene from Brassica napus in transgenic tobacco, increases plant growth, seed germination rate and enhances tolerance against drought stress. However, plants with its antisense (antisense BnPIP1) gene show several deformities in development and increase their susceptibility toward drought stress (Yu et al. 2005). Moreover, physical interactions among different isoforms of AQPs have also been observed along with their interaction with other plant proteins involved in different biological functions. This highlights the integrated and complex nature of the roles of AQP in a number of physiological and stress induced reactions (Besserer et al. 2012, Wu et al. 2013, Hachez et al. 2014).

To sum up, each member of AQP family is functionally different from the other, but they share some common traits, such as structural redundancy, overlapping functions and roles other than solute transport (Zhang et al. 2019). Recent investigations suggest that along with their basic roles in regulating the water status in cells, AQPs are also involved actively in the multifaceted defense mechanism employed by plants under various stress conditions (abiotic and biotic). PIPs and TIPs hold major responsibility in this context. Water deficit and salt stress bring about major changes in expression pattern of AQP genes. PIP family of AQPs is the most significant and active transmembrane channels involved in the water and solute transport under normal and stress conditions. Investigations using knockout mutants and overexpressing transgenics with PIPs suggest that regulatory mechanisms of AQPs are complex and are integrated with other stress-related pathways. They also function in regulating the growth and developmental processes in plants by regulating water status and maintaining the homeostasis of different solutes and ROS molecules. AQPs interact with other AQP isoforms and proteins involved in stress responses to provide better adaptability to plants under stress. Unlike PIPs, information available about the regulatory roles of other AQPs, such as TIPs and NIPs, is very less and require further investigations to unveil their appropriate functional properties under stress conditions. Some reports indicate that TIPs are also involved in modulating osmotic stress responses in plants (Kim et al. 2006, Pang et al. 2010). TIPs, also facilitates the uptake and transport of few micronutrients, such as boron and silicone, thereby regulating the homeostasis of micronutrients in plants (Yamaji et al. 2008, Schnurbusch et al. 2010). NIPs mainly mediate transport of nutrients or heavy metals (arsenic and antimony), and their role in maintaining the nutrient homeostasis and responses under heavy metal stress is much more pronounced than any other AQP isoforms. TIPs and NIPs support nutrient uptake between host plant and symbiont (bacteria/fungi) during their symbiotic relationship, and it highlights their significance under biotic stress conditions. AQPs undergo rapid modulations as a response to different stress conditions, but the cellular and molecular mechanisms triggering these modulations are still far from full understanding. According to a hypothesis, specific localization of AQPs at their respective membranes and sub-cellular compartments, along with their redistribution in response to various stress plays a crucial role in defining their expression and efficiency level in plants (Mueller et al. 2012, Chevalier and Chaumont 2015). AQP also interacts with pathways of some stress hormones, such as ABA and ethylene. But to reach a conclusive
decision, further investigations are needed. Interactions of AQP isoforms with other plant proteins also indicates a complex mechanism of AQP regulation and various stress responses. Due to such integrated and complex roles of AQPs under various abiotic or biotic stress, a defined and individual roles of AQP has still not been fully decoded yet and to map every role performed by AQP in different physiological activities occurring in plants under normal and stress conditions, more detailed investigations are required in the future.

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