12 Aquaporins
A Promising Gene Family for Tackling Stresses for Crop Improvement

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CONTENTS

12.1 INTRODUCTION

Water is the most abundant molecule found in all living cells, and the molecular processes that define life take place largely in an aqueous environment. One of the remarkable features of life in an aqueous environment was that it facilitated the production of membrane structures that allow the compartmentation of biological processes. During the course of evolution, specific protein molecules were created that allow for the selective transport of the necessary compounds into and out of the cell to maintain cellular processes and homeostasis, thus enhancing the capabilities of the membrane. As a result of this flux of metabolites, the transport of water across membranes became more complex, and the need for osmoregulation to maintain osmotic balance and cell volume became critical. Water could penetrate the cell membranes by diffusion and by transport through water channels (pores). AQPs were first demonstrated in erythrocytes and kidney collecting ducts. Later on, AQP-like proteins homologous to animal AQPs were found in some bacteria, fungi, and plants. On the basis of sequence conservation and localization, plant AQPs are classified into several subfamilies including plasma membrane intrinsic protein (PIPs), tonoplast intrinsic protein (TIPs), nodulin intrinsic proteins (NIPs), and small intrinsic proteins (SIPs) (Postaire et al., 2008). In its turn, the subfamily PIP is subdivided into two phylogenetic groups, PIP1 and PIP2, differing mainly in the structure of their N terminal domains: it is longer in PIP1 AQPs (Chaumont et al., 2001). Moreover, the recently discovered X intrinsic proteins (XIPs) are also part of the MIPs family now

and also considered as the fifth subfamily of MIPs. The XIPs have been characterized in protozoa, fungi, mosses, and dicots. Interestingly, XIP homologs were absent from monocots (Danielson and Johanson 2008). PIP and NIPs are generally localized in the plasma membrane and expressed on the entire cell surface, while TIPs are localized to the tonoplast, the membrane of the vacuole. For most plant AQPs, localization on the endoplasmic reticulum (ER) can be observed during the processes of post-transcription, translation, and modification. However, SIPs and some NIPs have been found to localize in the ER, although the mechanism of targeting and their cellular functions are still not clear. Another difference between various MIP subfamilies is their substrate selectivity. Two factors that contribute to their substrate selectivity are the conserved NPA motifs and amino acid residues including the ar/R (aromatic/arginine) region (Forrest and Bhave 2007), which are highly conserved in plant PIPs and TIPs, while alternative motifs have been found only in the NIP or SIP groups (Ishibashi, 2006; Ishibashi et al., 2000) (Figure 12.1).

The persistence of AQPs during evolution indicates their key role in water transport and common occurrence in the plant kingdom. AQP molecules form tetramers in the membrane, and each of four subunits is an individual water-transporting pore (Postaire et al., 2008, Maurel et al., 2008). AQP monomers are hydrophobic transmembrane proteins comprising six α-helical domains connected through two cytosolic and three extracytosolic loops. The N and C terminals of the AQP molecule are located in the cytoplasm. In the second and third loops connecting transmembrane domains, there are two short α-helical domains directed toward each other. Just these domains are involved in the formation of the water channel, being closely positioned within the molecule. Such a structure of the AQP molecule was called the "hour glass model". Water transport through the AQPs occurs passively along the gradient of the water potential and without energy consumption (Maurel and Chrispeels 2001). It can occur in both directions (into and from the cell), being driven by the direction of the osmotic gradient. AQPs are involved in three pathways for water inflow to plant organs, namely in the transcellular water transport, in apoplastic, and in symplastic transport. The transcellular pathway represents sequential membrane crossing during water transport.

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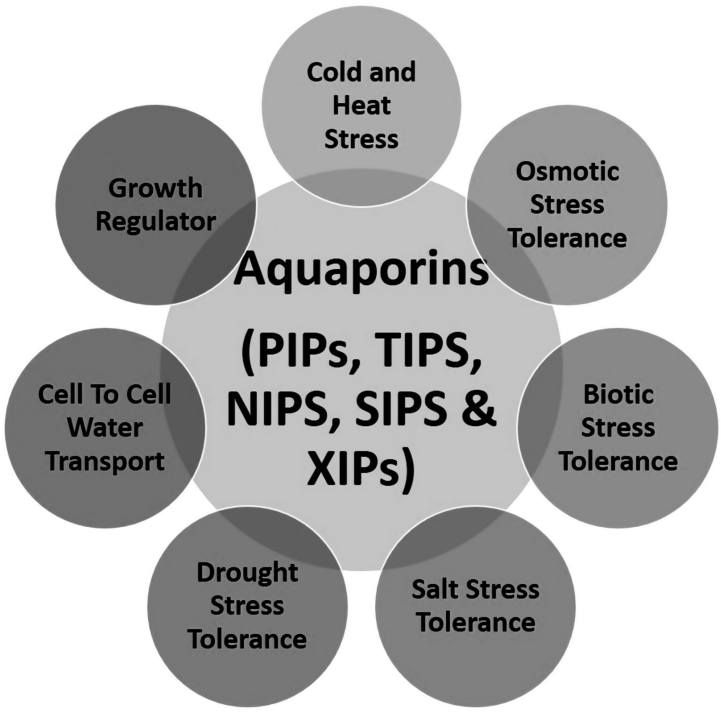

FIGURE 12.1 Summary of various functions of AQPs.

Water influx into the cell is limited only by plasmalemma AQPs, whereas the delivery of excessive water to the vacuole is limited by the tonoplast AQPs, the osmotic permeability of which for water is much higher (Javot and Maurel 2002; Trofimova et al., 2001). The rate of transmembrane transport of water depends on AQP content in the membranes and a specific permeability of each individual isoform for water.

Functional genomics and reverse genetics approaches allow a more rigorous approach and may reveal unexpected functions of AQPs. Transgenic *Arabidopsis* plants expressing an antisense copy of the *pip1b* gene showed reduced expression of several *PIP1* homologs and provided definitive evidence for the contribution of AQPs to plasma membrane water transport. Surprisingly, these antisense plants showed an increased root mass, whereas the development of the shoot was unchanged. Even though this phenotype might be related to the old observation that the root/shoot ratio of plants adjusts in response to their water status, it directly emphasizes how membrane transport can influence the developmental plasticity of plants. In the near future, analysis of single knockout aquaporin mutants will hopefully provide evidence for the multiple functions of AQPs in the growth and development of plants and in their adaptive response to stresses. It has been fascinating to observe during the last few years, how the discovery of AQPs has challenged general concepts about the role of membranes in plant–water relations. At one time it was assumed by most plant biologists that the residual water permeability of plant membrane lipids was sufficient for water flow in plants. Enthusiasm about the discovery of AQPs led to the unrealistic proposition that transmembrane water flow must be necessarily mediated by these proteins. The truth must lie somewhere in between, and we still have a long way to go to fully understand the significance of these proteins. Nevertheless, AQPs provide a unique molecular entry point into the water relations of plants and establish fascinating connections between water transport, plant development, and the adaptive responses of plants to their ever-changing environment. Abiotic stress tolerances are governed by multiple gene families involved in multiple mechanisms that may be expressed at different plant growth stages (Foolad, 1999). Functional genomics employs multiple parallel approaches including global transcriptional profiling coupled with the use of mutants and transgenic, to study gene function in a high-throughput mode (Vij and Tyagi, 2007). Since the area of functional genomics is very extensive, this chapter will focus on recent updates on the role of *AQP* genes in crop improvement in terms of achieving stress tolerance (Table 12.1).

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12.2 DISCOVERY AND DIVERSITY OF THE AQPS

For many years it was believed that the transport of water across biological membranes occurred by simple passive diffusion through the lipid bilayer. However, some membranes exhibited such high water permeabilities that they could not be explained by simple diffusion, leading to the hypothesis of proteinaceous membrane components which could facilitate the rapid low-energy transport of water across the bilayer (Finkelstein, 1987). The conductance of water and control of transpiration steam is mainly done by vascular tissues and guard cells. Water flows through living cells while getting in or out of vascular tissues. When water flows across living tissues, it can follow apoplastic, symplastic, or traversing the cell membranes. It is believed that the discovery of a class of water channel proteins named AQPs (Agre et al., 1998) has become the milestone in understanding the conductance of living cells. Because of their abundance, plant MIP homologs were identified in the late 1980s (Fortin et al., 1987), but it was recognized much later that some of them can function as highly efficient water channels and facilitate the diffusion of enormous amounts of water along transmembrane water potential gradients (Maurel et al., 1993). The discovery of AQPs establishes a conceptual advance in plants. It had been hypothesized that in certain specialized membranes, the high rate of water conductance was caused by proteinaceous membrane channels, but the molecular characterization of these channels remained elusive until the latter part of the 20th century. Aquaporin discovery allowed the understanding of a new notion about the dynamics of rapid and controlled water transport across membranes at the rate exceeding that of water diffusion.

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TABLE 12.1
Summarizing a Few Illustrations of Aquaporins Transgenics Developed to Tackle Abiotic Stress Conditions like Drought (Water), Salt (Osmotic and Salinity) and Cold

S. No.	AQP Gene	Transgenic Plant	Stress	Type of Response	Reference
1	*ScPIP1*	*Arabidopsis thaliana*	Drought	Resistance	Wang et al. (2019)
2	*ThPIP2;5*	*Tamarix hispida Arabidopsis thaliana*	Osmotic and salt	Enhanced seed germination, ROS-scavenging capability, antioxidant enzymes activities	Wang et al. (2018)
3	*PeTIP4;1*	*Arabidopsis thaliana*	Drought and salinity	Resistance	Sun et al. (2017)
4	*MdPIP1;3*	*Solanum lycopersicum* L.	Drought	Resistance	Wang et al. (2017)
5	*MaPIP1;1*	*Arabidopsis thaliana*	Drought	Resistance	Xu et al. (2014)
6	*OsPIP1;1*	*Oryza sativa*	Salinity	Resistance	Liu et al. (2013)
7	*MusaPIP1;2*	*Musa acuminata*	Cold and drought	Resistance	Shekhawat et al. (2013)
8	*TaAQP7*	*Nicotiana tabacum*	Drought and osmotic stress	Resistance	Zhou et al. (2012)
9	*VfPIP1*	*Arabidopsis thaliana*	Drought	Resistance	Uehlein et al. (2012)
10	*TdPIP1;1*	*Nicotiana tabacum*	Salinity, water stress	Resistance	Ayadi et al. (2011)
11	*SlTIP2;2*	*Solanum lycopersicum*	Salinity	Resistance	Sade et al. (2009)
12	*VvPIP2;4*	*Vitis vinifera*	Water stress	Sensitive	Vandeleur et al. (2009)
13	*StPIP1*	*Nicotiana tabacum*	Water and osmotic stress	Sensitive	Wu et al. (2009)
14	*AtPIP1;4, AtPIP2;5*	*Arabidopsis thaliana*, *Nicotiana tabacum*	Water, salinity, and cold stress	No effect	Jang et al. (2007)
15	*PgTIP1*	*Arabidopsis thaliana*	Salt and drought	Resistance	Peng et al. (2007)
16	*BnPIP1*	*Nicotiana tabacum*	Drought	Resistance	Yu et al. (2005)
17	*RWC3*	*Oryza sativa*	Water stress	Resistance	Lian et al. (2004)
18	*AtPIP1;b*	*Nicotiana tabacum*	Salinity	Sensitive	Aharon et al. (2003)
19	*HvPIP2;1*	*Oryza sativa*	Salinity	Sensitive	Katsuhara et al. (2002)

The genome sequencing projects have enabled many researchers to study aquaporins from different models and crops plants too, viz. in *Arabidopsis* (Johanson et al., 2001 Quigley et al., 2001), sorghum (Reddy et al., 2015), maize (Chaumont et al., 2001), populus (Gupta and Sankararamakrishnan, 2009), upland cotton (Park et al., 2010), soybean (Zhang et al., 2013), potato (Venkatesh et al., 2013) and tomato (Reuscher et al., 2013), and rice (Sakurai et al., 2005). In addition, expressed sequence tags corresponding to 36 aquaporin isoforms have been identified in maize (Chaumont et al., 2001). Plant AQPs have been classified into their subfamilies based on substrate specificity and protein sequence homology. They can be subdivided into four subgroups which to

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some extent correspond to distinct sub-cellular localizations (Johanson et al., 2001 Quigley et al., 2001l Chaumont et al., 2001). The TIPs and PIPs correspond to aquaporins that are abundantly expressed in the vacuolar and plasma membranes, respectively. PIPs are further subdivided into two phylogenetic subgroups, PIP1 and PIP2. Because of their abundance, PIPs and TIPs represent central pathways for transcellular and intracellular water transport (Maurel et al., 2002 Tyerman et al., 1999; Wallace et al., 2006). A third subgroup comprises nodulin26-like intrinsic membrane proteins (NIP), i.e. aquaporins that are close homologs of GmNod26, an abundant aquaporin in the peribacteroid membrane of symbiotic nitrogen-fixing nodules of soybean roots (Wallace et al., 2006). NIPs are present in non-leguminous plants where they have been localized in plasma and intracellular membranes (Ma et al., 2006; Mizutani et al., 2006; Takano et al., 2006). The small basic intrinsic proteins (SIP) define the fourth plant aquaporin subgroup that was first uncovered from genome sequence analysis (Johanson and Gustavsson 2002). SIPs form a small class of two to three divergent aquaporin homologs and are mostly localized in the endoplasmic reticulum (ER) (Ishikawa et al., 2005). Moreover, the recently discovered X intrinsic proteins (XIPs) are also part of MIPs family now and also considered as the fifth subfamily of MIPs. They were first discovered in upland cotton (Park et al., 2010). The XIPs have been characterized in protozoa, fungi, mosses, and dicots. In grapevine, VvXIP1 was found to play a role in osmotic regulation in addition to H_2O_2 transport and metal homeostasis (Noronha et al., 2016). Interestingly, XIP homologs were absent from monocots (Danielson and Johanson 2008).

To date, the expression in *Xenopus* oocytes is still widely used because in these cells individual gene products can be tested in a well-characterized membrane environment (Zhang and Verkman, 1991). However, other systems like expression in slime mold, yeast secretory vesicles, or baculovirus-infected insect cells have been successfully used to demonstrate aquaporin activity and to determine transport rates (LaizeÂ et al., 1995; Verkman, 1995; Chaumont et al., 1997; Yang and Verkman, 1997). It is really tough to explain the exact role of AQPs in maintaining the plant water status under different stress conditions, because different *AQP* genes may be differentially expressed or may remain unchanged under abiotic stresses. Considering that AQPs may function in transport processes of other molecules in addition to water, understanding the nature of these complex changes may prove challenging. These complex expression patterns also suggest that the water budget levels are maintained by increased or reduced cell-to-cell water transport via AQPs under abiotic stress conditions (Javot and Maurel, 2002). Therefore, the functional validation of each *AQP* gene in the plant system is of the utmost necessity and is a bottleneck for understanding the water transport mechanism.

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12.3 AQUAPORIN AS POWERFUL ARSENAL TO COMBAT VARIOUS STRESSES

12.3.1 Drought

It is a well-established fact that the water uptake and transcellular water flow in roots are largely mediated by PIPs and TIPs in most plant species. Furthermore, these two subfamilies are the most abundantly present in plant cells (Boursiac et al., 2008). Comparative transcriptome studies have shown differential expression of multiple AQP homologs in response to drought stress suggesting definite roles in stress responses. The key molecular mechanisms underlying the regulation of AQP function in plants overexpressing AQP genes were believed to be a milestone in aquaporin operational in stress tolerance (Rizhsky et al., 2004). Elevated levels of *AtPIP2;3* under drought stress conditions are one of the earliest pieces of evidence that AQP plays central role in drought tolerance (Yamaguchi-Shinozaki, et al., 1992). Plant water retention and improving water use efficiency in the cell are considered the important goals for achieving abiotic stress tolerance. Correspondingly, the expression of *PIP1* of *Vicia faba* (*VfPIP1*) in transgenic *Arabidopsis* improved drought resistance by the reduction of transpiration rates through stomatal closure (Uehlein et al., 2012). Ranganathan et al. (2016) demonstrated that the gas exchange rates, water use efficiency, and hydraulic conductivity

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of the transgenic aspen lines were significantly higher than the wild-types; however, plant biomass and dry weight were unchanged. The plants exposed to a combination of heat and drought stress presented significantly higher expression of *AtPIP2;5* (Rizhsky et al., 2004). Contradictorily, substantial down-regulation of *PIP* genes under drought stress was detected in peach fruits (Sugaya et al., 2002), in the roots and twigs of olive plants (Secchi et al., 2007), and in the roots of tobacco (Mahdieh et al., 2008). Noticeably, in many other plant species, differential responses by the same aquaporin homologs have been seen between different cultivars of the same plant species. For instance, in grapevines the expression of *VvPIP1;1* in the root was up-regulated by drought stress in an isohydric cultivar but not in an isohydric cultivar (Vandeleur et al., 2009). Furthermore, expression of the *VvPIP2;1* gene was down-regulated under drought conditions (Cramer et al., 2007). Recently, the characterization of *GoPIP1* from the legume forage *Galega orientalis* displayed its association with drought tolerance. The transcripts levels of *GoPIP1* increased significantly in roots upon exposure to the osmotic stress imposed by both high NaCl concentration and PEG. Overexpression of this gene in transgenic *Arabidopsis* made the plants more vulnerable to drought stress but not to salinity stress (Li et al., 2015). In a drought-tolerant Vitis hybrid, Richter-110 (*Vitis berlandieri* X *Vitis rupestris*) expression of five PIPs and two TIPs was checked at different levels of water stress, and it was found that the AQP genes in the leaves showed differential regulation in response to moderate and drastic water stress. A moderate decrease in water availability results in down-regulation of the AQPs. However, in roots, aquaporin expression revealed complex patterns, with no generality among different AQPs (Galmes et al., 2007). Kaldenhoff et al. (1998) showed a higher expression of *AtPIP2;2* in several root cell types including the endodermis of *Arabidopsis*. Additionally knockout mutant plants showed reduced Lpr by 14% in comparison to corresponding wild-type plants. Lately, the transcript abundance of several *PIPs* (*AtPIP1;1*, *AtPIP1;2*, *AtPIP1;4*, *AtPIP2;1*, *AtPIP2;3*, *AtPIP2;4*, and *AtPIP2;5*) in *Arabidopsis* roots was positively correlated with Lpr; this was in decent agreement with published genetic data (Lopez et al., 2013). Wang et al. (2017) screened out an ectopically expressing *MdPIP1;3* that enhanced the drought tolerance of transgenic tomatoes.

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Water transport by AQPs was also found to be coupled with diurnal rhythms. The diurnal expression of PIPs in response to different intensities of drought was investigated in *Fragaria vesca*, where the PIPs were down-regulated in roots, and the expression of *FvPIP1;1* and *FvPIP2;1* was strongly correlated to the decrease in substrate moisture contents. In leaves, the amplitude of the diurnal aquaporin expression was lower in response to drought (Surbanovski et al., 2013). Many studies revealed the roles of PIPs and TIPs in drought response or water-deficient conditions. In rice, the expression of *OsTIP1;1* was up-regulated in roots and shoots in response to water stress (Liu et al., 1994). *Arabidopsis* knockout mutants of *AtPIP1;2* and *AtPIP2;2* genes were studied by Kaldenhoff et al. (1998); the study showed that there was a noteworthy reduction in the water permeability of protoplasts and a 14% decrease in Lpr, respectively, making these mutants more vulnerable to drought stress (Javot et al., 2003). On other hand, double antisense lines with lowered expression of *AtPIP1* and *AtPIP2* in *Arabidopsis* exhibited a 30-fold decrease in Lpr. Similar results were obtained in tobacco plants with antisense *NtPIP1* gene targeting, resulting in a 55% reduction in Lpr and increased sensitivity to drought. In addition to decreased Lpr, the reduced expression of *NtPIP1* also showed a significant decrease in the transpiration rate (Tr) (Siefritz et al., 2002). In moss, *Physcomitrella patens*, knockout mutants of *PpPIP2;1* and *PpPIP2;2* exhibited severe stress phenotypes when grown under water-limited conditions. These observations led researchers to believe that AQPs play a collective role as water transporters, and their reduced expressions make plants susceptible to water stress due to a lowered Lpr. Additionally, it was also postulated that decreased Tr caused reduced photosynthesis, ultimately affecting the overall survivability of the plant (Lienard et al., 2008). Consistently, transgenic plants overexpressing AQPs exhibited improved drought tolerance. Likewise, the overexpression of *BnPIP1* from *Brassica napus* in transgenic tobacco resulted in increased tolerance to drought (Yu et al., 2005). In the same way, transgenic tobacco plants overexpressing the wheat aquaporin gene *TaAQP7* (*PIP2*) were more tolerant to drought stress when

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compared with wild-type tobacco plants due to enhanced water retention capabilities (Zhou et al., 2012). Transgenic *Arabidopsis* plants expressing a *Vicia faba PIP1* (*VfPIP1*) showed improved drought resistance by preventing water loss through transpiration because of the induction of stomatal closure (Cui et al., 2008). In *Arabidopsis*, the overexpression of a banana PIP gene *MaPIP1;1* showed increased root growth and enhanced survival rates of transgenic plants under drought stress, when compared to wild-type plants (Xu et al., 2014). The overexpression of *PeTIP4;1–1*, a bamboo aquaporin gene, confers drought and salinity tolerance in transgenic *Arabidopsis* (Sun et al., 2017). Also, transgenic banana plants expressing banana *PIP1;2* driven by two diverse promoters deliberated higher drought tolerance. The overexpression of a tomato *SlTIP2;2* gene in transgenic tomato plants resulted in increased drought tolerance due to the ability of the plant to regulate its Tr under drought stress conditions (Sade et al., 2009). Similarly, the overexpression of Jojoba *ScPIP1* led to improved tolerance to drought stress in transgenic *Arabidopsis* by reducing membrane damage and improving osmotic adjustment (Wang et al., 2019). The above experimental evidence suggests that the overexpression of AQPs makes plants more resistant to drought stress. However, some contrasting results have also been observed because rapid water loss due to increased leaf and root hydraulic conductivity makes some plants even more vulnerable to drought stress conditions. It can be concluded that the drought stress response of AQPs is highly variable depending on stress levels, aquaporin isoform, tissue, species, presence of symbionts, and the nature of stimuli causing dehydration similar to drought stress. However, a general down-regulation of most of the *PIP* genes is thought to reduce water loss and to help prevent backflow of water to drying soil. Although TIPs are found to play a key role in controlling cell water homeostasis by rapid water transport between the vacuole and cytoplasm of plant cells, experimental evidence on their roles in response to drought stress is limited in comparison to PIPs.

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12.3.2 Salt Stress

The osmatic balance of a cell is altered by salt stress which affects plant growth and development. Plant responds primarily to the salt stress by the inhibition of root-water uptake and subsequently decrease in root hydraulic conductivity (Lpr) (Boursiac et al., 2005), which is also displayed in response to drought stress. The overexpression of *PeTIP4;1–1*, a bamboo AQP gene, confers drought and salinity tolerance in transgenic *Arabidopsis* (Sun et al., 2017). The overexpression of Jojoba *ScPIP1* led to improved tolerance to salt stress in transgenic *Arabidopsis* by reducing membrane damage and improving osmotic adjustment (Wang et al., 2019). In the roots of barley, the downregulation of the *HvPIP2;1* transcript and protein product levels under osmotic stress was seen whereas up-regulation of the same transcript was recorded under salt stress (Katsuhara et al., 2002). The same results were obtained when the expression of *OsPIP1;1* was studied in leaves and roots (Liu et al., 2013). Under salt stress, *Mesembryanthemum crystallinum*, an ice plant, displayed down-regulation of *PIP* genes in roots and a *TIP* gene in leaves (Yamada et al., 1995 and Kirch et al., 2000). In maize plants, down regulation of most of the members of *ZmPIP1* and *ZmPIP2* was detected, but a transiently boosted expression of *ZmPIP1;1*, *ZmPIP1;5*, and *ZmPIP2;4* was also seen preferentially in the outer parts of the roots when maize plants were exposed to ABA-mediated salt stress. However, ZmTIPs' expression was unchanged in similar conditions (Zhu et al., 2005).

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The transgenic *Arabidopsis* plant, harboring the *AtTIP5;1* gene, showed tolerance to high borate concentrations, possibly suggestive of AQPs' involvement in vacuolar compartmentation of salt particles, in this case borate (Pang et al., 2010). Many genes differentially express themselves under diverse abiotic stress. The same results were obtained when Liu et al. (1994) studied the expression of *OsTIP1;1.* It showed down-regulation in response to cold stress (Sakurai et al., 2005) but up-regulation during responses to water and salinity stress. The overexpression of *PgTIP1*, *Panax ginseng* aquaporin, in *Arabidopsis* revealed improved plant growth under optimal conditions and also better tolerance to salt and drought stress (Peng et al., 2007). Similarly, in drought-tolerant, salinity-sensitive grapevine the expression of the *PIP2;1* gene was up-regulated under salt stress

but down-regulated under drought (Cramer et al., 2007). Gao et al. (2010), suggested the salt stress tolerance in wheat plant is in association with ABA and other regulated pathways, by validating up-regulated expression of *TaNIP*. Overexpressed *ThPIP2;5 Tamarix hispida* aquaporin genes conferred salt and osmotic stress tolerance to transgenic *Tamarix* and *Arabidopsis* and also enhanced seed germination, ROS-scavenging capability, antioxidant enzymes activities, and seedling growth under salt and osmotic stresses (Wang et al., 2018).

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12.3.3 Cold Stress

Like other stresses, cold stress is a vital abiotic stress factor that significantly confines plant growth and development. There are several research groups who have reported the early response of AQPs to cold stress. The transgenic banana plants overexpressing *MusaPIP1;2* and *MusaPIP1;2* exhibited improved tolerance to both cold and drought stress (Shekhawat et al., 2013). Cold stress tolerance was observed in transgenic tobacco plants overexpressing a wheat aquaporin *TaAQP7 (PIP2)* gene. The same plants also showed increased drought tolerance (Huang et al., 2014). The transgenic *Arabidopsis* plants overexpressing *AtPIP1;4* or *AtPIP2;5* showed enhanced tolerance to cold stress but are more susceptible to drought due to rapid water loss. On other hand, only *PIP2;5* and *PIP2;6*, which are normally among the low-expressed PIPs, are significantly up-regulated by cold stress in both the roots and aerial parts of the plant. All other *PIP* genes were found to be down regulated by cold stress, and their patterns of expression vary with the application of salt or drought stress (Jang et al., 2007). In cold-sensitive plants like rice, prolonged exposure to cold stress cause an increase in Lpr which should be regulated through root AQPs, as most, particularly *OsPIP2;5*, are found to be up-regulated (Ahamed et al., 2012). The mRNA levels of ten genes including *OsTIP1;1* and *OsTIP2;2* were significantly down-regulated, but the expression of *OsPIP1;3* increased up to 60% in roots on exposure to chilling treatment in rice (Sakurai et al., 2005). Significant up-regulation in *OsPIP1;3* was recorded in response to drought stress in a drought-tolerant rice cultivar (Lian et al., 2004). Contrastingly, *OsTIP1;1* showed up-regulation in response to water and salinity stress (Liu et al., 1994). Most abiotic stresses, including chilling, induce the production of ABA (Jang et al., 2004 and Suga et al., 2002).

12.3.4 Biotic Stress

Plant AQPs expressions are often triggered or suppressed by the symbiotic association of microorganisms. It also affects the response of aquaporin genes under various biotic and abiotic stress. This is reported in *Phaseolus vulgaris* and maize (Aroca et al., 2007 and Barzana et al., 2014). In order to elucidate the definite role of AQPs in plant disease combat, many putative members within the AQPs family need to be analyzed by using a reverse genetic-based approach. RNAseq (transcriptomic Approach) projects have provided further insight into the involvement of AQPs in host–pathogen interactions. It was assumed that in plant symbiotic relations, the re-distribution of water and nutrients takes place between the host and symbionts. The roles of NIPs in various forms of nitrogen transport (urea, ammonia) are well-known but experimental evidence is limited. Nodulin-26 was first identified in soybean root nodules and was assumed to have formed as a result of a symbiotic interaction between the plant and nitrogen-fixing bacteria, i.e. rhizobium. Many researchers are involved in the mechanistic involvement of NIPs in driving these nutrient exchanges between plants and symbionts or plants and pathogens needs. The gene profiling studies done by Barzana et al. (2014) showed the roles of plant aquaporin-mediated solute transport during plant symbiosis with arbuscular mycorrhizae. These results demonstrated the aquaporin-mediated transport of glycerol from the plant to the microbe, in addition to NH_4/NH_3 from microbe to plant. Expression profiling of *Pseudomonas syringae*-infected soybean leaves showed down-regulation in a majority of the *AQP* genes (Zou et al., 2005). In citrus plants, six CsMIPs (*CsPIP1;2*, *CsPIP2;2*, *CsNIP2;2*, *CsNIP5;2*, *CsNIP6;1*, and *CsSIP1;1*) were found to be differentially expressed

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under the biotic stress imposed by the citrus-infecting proteobacterium, *Candidatus* Liberibacter. Comparisons of *CsPIP2;2*, *CsTIP1;2*, *CsTIP2;1*, *CsTIP2;2*, and *CsNIP5;1* expression patterns of susceptible sweet orange and tolerant rough lemon cultivars revealed that most CsMIPs are down regulated. Therefore, they could be correlated with the disease development (Martins et al., 2015 and Aritua et al., 2013). The foreseen role of AQPs in response to pathogen infection led to many studies which gave the lacking evidence of involvement of AQPs in disease combat. Later, NIPs' involvement in biotic stress responses in maize was reported by Lawrence et al. (2013). The regulation of Si uptake through NIPs was found to be associated with plant defense against herbivory in *Festuca* spp. grasses (Hartley et al., 2015). Protein–protein interaction studies carried out by yeast two-hybrid systems revealed interactions of AQPs with bacterial and oomycete effectors (Mukhtar et al., 2011). Interactions of a cucumber mosaic virus (CMV) replication protein with TIP1 and TIP2 in the CMV1a SOS recruitment system suggested that the TIPs–CMV1a interaction potentially affects CMV replication in the host plant's tonoplasts (Kim et al., 2006).

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12.4 AQUAPORIN AS TRANSPORT OF ESSENTIAL MICRONUTRIENTS

Apart from conductance of water, AQPs exhibit other functions like the transport of other substrates in various cellular processes. NIPs are known for playing a fundamental role in transporting other substrates involved in numerous cellular processes. Most NIP homologs also execute the transport of nutrients in plants. Boron is one of those minor elements, a crucial one for plant growth; however, it can be toxic when present at high concentrations. A role of AQPs in boric acid transport was first proposed by Dordas et al. (2000). B is an essential element for plant growth, development, and reproduction, and its deficiency in arable areas has drastically affected crop production worldwide. The report showing aquaporin contributing to boron transport came from *Arabidopsis*. *AtNIP5;1* signifies boron uptake in plants, and its gene is strikingly induced in response to boron deficiency, and boric acid transport activity of the protein was demonstrated after oocyte expression or using nip5;1 knockout plants (Takano et al., 2006). In the event of high B supply, a feedback inhibition of the *AtNIP5;1* gene is observed, thus providing strong evidence of the involvement of *NIP5;1* in B homeostasis and the adaptation of plants to B toxicity in soil. These results were further confirmed in maize, where a loss of function mutation in *ZmNIP3;1*, a maize ortholog of *AtNIP5;1*, was shown to be responsible for an abnormal phenotype caused by B deficiency. In addition, *AtNIP6;1* and *AtNIP7;1* homologs serve in B transport in *Arabidopsis*, facilitating its distribution in the shoots and anthers, respectively (Li et al., 2011). Tolerance to high B was observed in barley by a reduced expression of the *HvNIP2;1* gene (Schnurbusch et al., 2010). In addition to NIPs, an overexpression of *TIP5;1* in *Arabidopsis* suggested its involvement in the vacuolar compartmentation of borate (Pang et al., 2010).

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One more key mineral is silicon (Si), which is crucial for cereal plants. It accounts for 10% of shoot dry weight in rice. Silicon plays an important role in plant defense against biotic and abiotic stresses. Si-uptake in rice plants is a well-known phenomenon. The quantitative trait loci (QTL) mapping for an Si uptake experiment in rice led to the identification of an Si transporter (OsNIP2;1) (Ma et al., 2006). Furthermore, the Si transporter NIP2;2 (Lsi6) was also identified from rice (Yamaji et al., 2008). Therefore, OsNIPs seem to play a significant role in Si transport in rice plants. Furthermore, ammonium/ammonia (NH_4+/NH_3) is an important nitrogen fertilizer for crops. Whereas NH_4+ transporters have long been identified in plants, NH_3 was initially proposed to cross the membrane by free diffusion. Yet, several TIP2 homologs of *Arabidopsis* and wheat were found to have a significant permeability to NH_3, and may therefore participate in NH_3 compartmentalization in vacuoles. This idea remains to be assessed at the whole plant level and, for instance, the overexpression of *AtTIP2;1* and *AtTIP2;3* in *Arabidopsis* failed to enhance whole-plant NH_4+/NH_3 accumulation (Loque et al., 2005). Moreover, A T-DNA knockout mutant of *Arabidopsis NIP1;1* exhibited arsenate (As) tolerance, signifying its role as an As transporter (Kamiya et al., 2009). Recently, *AtNIP3;1* has also shown to be involved in As transport. Double knockout mutants for

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both *AtNIP1;1* and *AtNIP3;1* show more pronounced tolerance against As stress. An additional four other isoforms, *NIP5;1*, *NIP6;1*, *NIP7;1*, and *NIP1;2*, are reported to be capable of As(III) transport on the basis of their expressions in yeast and oocytes (Bienert et al., 2008). TIP aquaporin from *P. vittata; PvTIP4;1* was shown to be involved in As(III) uptake (He et al., 2016). The transport of antimonite (Sb) by *AtNIP1;1* has also been established, and its loss of function mutant displayed an improved tolerance to high Sb stress (Kamiya et al., 2009). Plant aquaporin-mediated transport of H_2O_2 also contributed to plant defense; however a full understanding of these pathways has not been elucidated (Dynowski et al., 2008 and Hooijmaijers et al., 2012). Rodrigues et al.'s (2017) study filled the gap in our understanding of stomatal regulation and suggests a general signaling role of aquaporin in contexts involving H_2O_2.

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12.5 MULTIFACETED PHYSIOLOGICAL ROLE OF AQPS

Water is a key element of all physiological processes including the growth and metabolism of the plant. The bulk flow of water through plants can take three different routes: the apoplastic route along the cell wall structure, the symplastic route from cell to cell through the plasmodesmata, and the transcellular path across the cellular membranes (Steudle and Henzler, 1995). AQPs are transmembrane water and solute transporter channels and are also considered potential regulators of plant cell–water relations, that also regulate the osmolality of cell (Wallace at al., 2006), root hydraulic conductivity (Lpr), leaf hydraulic conductivity (Siefritz et al., 2002), transpiration (Sade et al., 2010), and cell elongation (Hukin et al., 2002). Abiotic stress conditions directly influence plant–water relations and trigger an array of complicated cellular and physiological responses that lead to plant water-saving mechanisms from stomatal closure to cut off water loss during transpiration. To maintain balance, water-saving photosynthetic activity is reduced due to the unavailability of CO_2, ultimately leading to the production of less plant biomass. Park et al. (2010) studied the underlying mechanisms that control plant–water relations in relation to photosynthesis and their response to biotic and abiotic stresses. AQPs are explored as potential targets in developing stress-resistant crop plants as they are vital regulators of plant–water relations. A similar approach for uncoupling transpiration from light was developed in hybrid poplar (Laur and Hacke, 2013). In this particular experiment, an increased expression of *PIP1* and *PIP2* in roots was observed, and this expression was studied under low relative humidity conditions. Aquaporin expression in roots, root hydraulic conductivity, and evaporative demand (based on meteorological factors) were also determined in rice plants grown under field conditions, and were significantly correlated, in good agreement with observations made in growth chamber experiments (Hayashi et al., 2015). In these approaches, the expression of five *PIPs* and a *TIP* showed a strong positive correlation to evaporation potential, whereas the expression of a *PIP* and a *TIP* homolog, which seem to be associated with cell elongation, showed a negative correlation. The effects of low relative humidity (i.e. high transpiration) were restricted to root hydraulics. In *Arabidopsis*, a high evaporative demand resulted in an increase by > three-fold in leaf hydraulic conductance (*K* leaf) (Levin et al., 2007). In rice leaves, a coordinated up-regulation of several *PIP* and *TIP* genes could be observed as soon as 4 h after a dry air treatment (Kuwagata et al., 2012). The signaling mechanisms which link plant transpiration to aquaporin activity in shoots and roots are as yet unclear. The rapid down-regulation of root hydraulics observed after shoot topping or defoliation may pertain to the shoot-to-root signaling involved (Liu et al., 2013; Vandeleur et al., 2009). This process was more specifically investigated in soybean and grapevine. It was proposed that a xylem-mediated hydraulic signal could be responsible for the change in root aquaporin expression observed within 0.5–1 h following shoot topping (Vandeleur et al., 2009). Conversely, the negative pressure present in xylem vessels of intact, transpiring plants perceived as an activating signal for aquaporin expression in root and shoot tissues. Their significance in all facets of plant growth and development is well-established, but the mechanistic pathways behind their roles in plant defense responses are under elucidation (Forrest and Bhave, 2007). The contribution of AQPs to transpiration control goes far beyond the issue of water transport

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during stomatal movements and involves emerging cellular and long-distance signaling mechanisms which ultimately act on plant growth (Maurel et al., 2016). In addition to water deficiency dehydration caused by other environmental stimuli exhibited differential responses in some species. For example, osmotic stress induced by 10% polyethylene glycol (PEG) in rice revealed no effect on *OsPIP1;3*, but the expression of *OsPIP1;1* and *OsPIP1;2* was up-regulated (Guo et al., 2006). Contrastingly, the expression of *OsPIP1;1* was down-regulated in osmotic stress administrated by mannitol and also by drought stress (Malz and Sauter 1999). Similar alterations were seen in reddish AQPs in response to salt-, PEG-, and mannitol-induced osmotic stress (Suga et al., 2002).

12.6 CONCLUSIONS

The discovery of AQPs in plants has resulted in a paradigm shift in the understanding of plant–water relations. Water flux across cell membranes has been shown to occur not only through the lipid bilayer, but also through AQPs, which are members of the major intrinsic protein super-family of channel proteins. As has been found in other organisms, plant MIPs function as membrane channels permeable to water (AQPs) and in some cases to small nonelectrolytes. AQPs greatly increase the membrane permeability for water, but may also be regulated, allowing cellular control over the rate of water influx/efflux. As a result, AQPs provide a unique molecular entry point into the water relations of plants and establish fascinating connections between water transport, plant development, and the adaptive responses of plants to their ever-changing environment. Plants counteract fluctuations in water supply by regulating all AQPs in the cell plasma membrane. AQPs can serve as markers to explore the intricate flows of water and solutes that play a critical role throughout all stages of plant development. The rate of transmembrane water flux may be controlled by changing the abundance or the activity of the AQPs; actually, there are observations showing the alteration of water permeabilities in the responses of plants to biotic or abiotic stresses such as high salinity, nutrient deprivation, and extreme temperatures. In plants, AQPs are likely to be important both at the whole plant level, for transport of water to and from the vascular tissues, and at the cellular level, for buffering osmotic fluctuations in the cytosol. By combining molecular biology with plant physiology, it should be possible to determine the role that AQPs play in water transport in the plant. There is growing evidence that suggests that AQPs play different roles throughout plant development. Therefore, aquaporin genomic information is important because assigning physiological function via transgenic reduction or removal of gene expression requires sequence information for precise targeting. Direct determination of the location of each aquaporin within tissues is still required to understand its function in the plant. A powerful tool in elucidating the aquaporin function is given by reverse genetics that can also reveal unexpected functions of water channel proteins, which benefit our understanding of sequence–structure and structure–function relationships in plants. This should be done both at the transcript and at the protein level because aquaporin turnover appears to be variable, such as when comparing constitutively expressed and inducible AQPs. The transcriptional and/or post-translational regulation of AQPs would determine changes in membrane water permeability. Both phosphorylation and translocation to/from vesicles have been reported as post-translational mechanisms. However, translocation in plants has not yet been shown. Here, the aquaporin family is a set of genes whose functions are intuitively perceived as important; much isolated information has been accumulated, yet their function is far from being understood in living plants, and we still have a long way to go to fully understand the significance of these proteins.

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