Physiological Roles of Aquaporins in the Improvement of Plant Water and Nutrient Usage

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Abstract

Crops with an enormous food and feed security role and trade potential are sensitive to any kind of water deficit. Transportation of solutes across membrane has vital role in plant normal physiology and resistance toward stresses. H2O2 involves in cell signaling, boron results in leaf expansion, root elongation, flower, fruit and seed development. Silicon improves resistance against pathogens. CO2 transport is important for green plants. Ammonia and urea play significant roles in cell metabolism. Members of major intrinsic proteins (MIPs) consist of aquaporin channel proteins (AQPs) which facilitate the passive transport of water and small solutes across membranes. Four important subfamilies of MIPs are plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), nodolin-26 like intrinsic proteins (NIPs) and small basic intrinsic proteins (SIPs). In this study, functional properties of MIP channels which have been demonstrated experimentally are reviewed. While PIPs are specific as CO2 transporters, ammonia and silicon are only transported by TIPs and NIPs respectively. H2O2, urea and boron are common substrates for PIP, TIP and NIP subfamilies. Identification of MIP channels which are involved in resistance against biotic and abiotic stresses results in the improvement of cultivar traits.

Keywords: Major intrinsic proteins; Aquaporin; Physiology; Transporter; Plant

Abbreviations: PIP: Plasma Membrane Intrinsic Protein; TIP: Tonoplast Intrinsic Protein; NIP: Nodolin-26 Like Intrinsic Protein; SIP: Small Basic Intrinsic Protein, Si: Silicon; CO_2: Carbon Dioxide; NH_3: Ammonia

Introduction

The hydrophobic nature of membrane acts as a rate limiting step in the rapid movement of small polar molecules such as water and natural solutes [1]. Major intrinsic proteins (MIPs) as pore forming proteins in membrane are distributed in

organisms from all kingdoms of life. Most members of this superfamily increase the water permeability coefficient by affecting hydraulic conductivity of the membrane, which in turn results in the transportation of billions of water molecules in a second [2]. Other substrates like glycerol, urea, ammonia (NH_3), carbon dioxide (CO_2), silicon (Si) and hydrogen peroxide (H_2O_2) are also transported by MIPs in plants [3]. Usually, the former subfamilies of MIPs which are selective/specific for the transportation of water are called aquaporins (AQPs). The later subfamily named aquaglyceroporins (GlpFs), are permeable channels to water and other small molecules [4]. However, AQP is the common term for both subfamilies. Several gene duplications and sub-functionalization result in a large diversification in structure and function of AQPs in plants [5]. In contrast to the thirteen identified MIPs in human [6], this number ratchets up to 47 in tomato [7], 66 in soybean [8], 53 in *Brassica rapa* [9], 41 in potato [10], 55 in *Populus trichocarpa* [11], 35 in *Arabidopsis thaliana* [12], 71 in cotton [13] 31 in maize [14] and 34 in rice [15,16]. Based on sequence homology and subcellular localization, plant AQPs are divided into five subfamilies including plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), nodolin-26 like intrinsic proteins (NIPs), small basic intrinsic proteins (SIPs), and uncategorized X intrinsic proteins (XIPs) [17]. In the early land plants like *Selaginella moellendorffii* and *Physcomitrella patens* additional MIPs called hybrid intrinsic proteins (HIPs) are also included [18,19].

Optimum availability of water and solutes is important for health and growth yield of plants. Identification and characterization of MIPs, which are involved in the transport of small molecules like H_2O_2, boron, Si, NH_3 and urea would have a vital role in defense against biotic and abiotic stresses. Although, it is possible to predict the physiological properties of MIPs by *in silico* analysis [20]; we herein emphasize on the functionality of those MIPs that have been demonstrated experimentally.

Experimental

MIPs as transporters of H_2O_2

H_2O_2, which is known as a reactive oxygen species (ROS), initiates signaling to provide adaptation to biotic and abiotic stresses at low concentrations. At high concentrations, H_2O_2 involves in programmed cell death [21]. H_2O_2 plays a crucial role in plant cell wall lignification, phytoalexin production and induction of defense genes under pathogen attack. Other functions like involvement in plant growth and development, regulation of cell cycle, plant senescence and ABA induced opening/closure of stomata have been attributed to H_2O_2 [22]. On the other hand, a mechanism to protect cells at toxic concentrations of the molecule is inevitable. Decisive roles of this inter/intra-cellular messenger require tightly regulation and a fast transport mechanism across plant membranes. Several MIPs channel H_2O_2 in plants [23]. The members of PIP2, TIP1 and TIP2 subfamilies of *Arabidopsis* and NIP3 subfamily of rice are transporters of H_2O_2 [24,25]. Yeast expression assays showed that AtPIP2-1, AtPIP2-2, AtPIP2-4, AtPIP2-5 and AtPIP2-7 from *Arabidopsis*, and ZmPIP2-5 of maize are permeable to H_2O_2 [26,27,24]. Increased H_2O_2 permeability in yeasts was observed when several *Arabidopsis* TIPs like AtTIP1-1, AtTIP1-2, AtTIP2-3 were expressed [28]. Members of the NIP subfamily have also been shown to conduct H_2O_2. AtNIP1-2, OsNIP3-2 and OsNIP3-3 are transporters of H_2O_2 [27,25]. Moderating H_2O_2 effect after pathogen attack might be one of the origins of vast distribution of H_2O_2 transporters in different MIP subfamilies.

MIPs as transporters of CO_2

Considering its crucial role as the substrate in sugar biosynthesis during photosynthesis, CO_2 transport is of extraordinary importance for green plants [29]. After crossing the stomata numerous resistances exist in the path of CO_2 to chloroplast stroma, one of which is mesophyll CO_2 conductance and/or resistance (g_m) [30]. Environmental factors such as temperature, water stress and biotic stresses limit CO_2 conductance and photosynthesis [31]. Involvement of MIPs (sometimes called COO porins) for an efficient CO_2 transport has been well established [32]. CO_2 transporters are distributed within PIP1 and PIP2 subfamilies in plants (FIG. 1a) [33,34]. NtAQP1-a member of PIP1-was the first candidate as a CO_2 conductor [35]. AtPIP1-2, HvPIP2-1, HvPIP2-2, HvPIP2-3, HvPIP2-5 and NtPIP2-1 are AQPs which have shown CO_2 permeability [36,33,34,37,38]. A 40% increase in the g_m of leaves has been observed when barly's HvPIP2-1 was over-expressed in rice [36]. McMIPB an AQP channel from ice plant, which was ectopically expressed in tobacco, transports CO_2 and regulates stomata under soil water deficit stress to compensate CO_2 deficiency [39]. Photosynthetic efficiency is a sensitive factor of overall plant health. Over-expression of MaPIP1-2 and MaPIP2-6 in banana plants had a pivotal role in drought and salinity tolerance [40,41]. These transgenic plants also exhibited higher photosynthetic efficiency. Suppression of photosynthesis related genes has been widely observed in different stresses. MIP related CO_2 transporters might be involved in this response [42]. Identification of CO_2 transporters which affect photosynthetic efficiency will increase tolerance to abiotic stresses such as drought or salinity conditions.

MIPs as transporters of Ammonia

Ammonia (NH_3) and its conjugated acid ammonium ion (NH_4^+) play a major role in cell metabolism; for example they are the source of nitrogen for amino acid biosynthesis in the cell [43]. Ammonium is found as the byproduct of metabolic pathways and as a nitrogen source in fertilizers. Ammonium transporter (AMT) family is responsible for NH_4^+ uptake [44]. MIPs called aqua-ammoniaporins have been introduced as passive transporters of NH_3 [45]. Involvement of *Arabidopsis* TIP2 members have been shown in this process [46]. Accumulation of NH_3 inside the vacuole and photorespiratory released NH_3 in the leaf mitochondria; all require NH_3 passage across subcellular compartments. Extra-cytosolic transport of NH_3 and cytosolic detoxification can be moderated mainly by MIP channels (FIG. 1b) [44]. AtTIP2-1 and AtTIP2-3 have been identified as NH_3 transporter at high medium pH. AtTIP2-3 (which under prolonged nitrogen deficiency was down-regulated) involves in vacuolar loading of NH_3 [46]. Involvement of TaTIP2-1 and TaTIP2-2 from wheat in NH_3 transport is elucidated [47,45]. Manipulation of these transporters would have beneficial effects on nitrogen use efficiency (NUE). Since nitrogen fertilizers might have negative impact on the environment, attentions are toward biological nitrogen fixation (BNF) by bacteria [48]. Presence of MIP transporters of NH_3 in plant symbiosome has long been demonstrated [49]. Identification of MIPs involved in such an interaction would have tremendous effect on the growth and quality of plants.
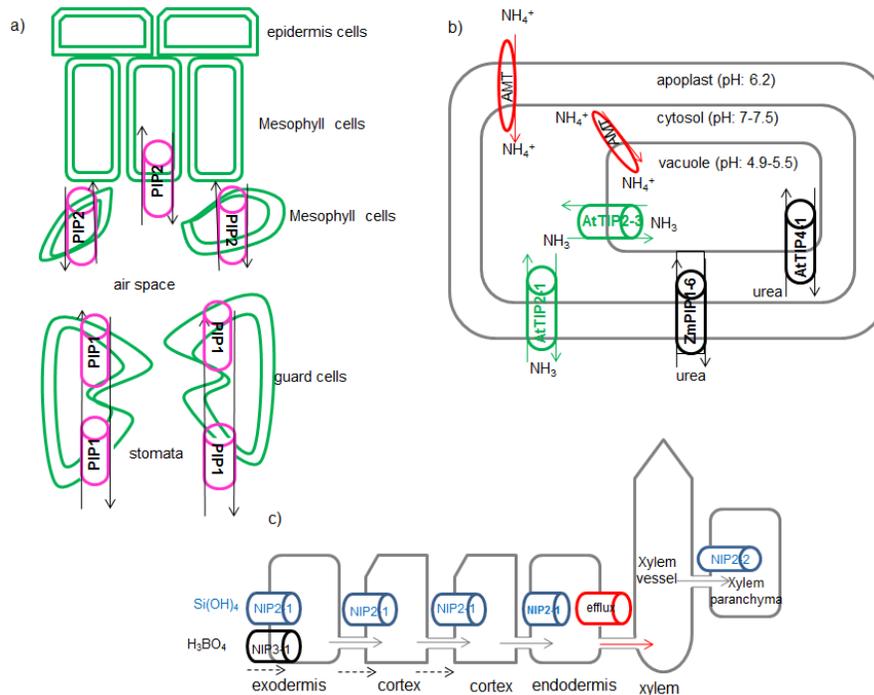
FIG. 1. **A visual guide schematically represents the potential role of various MIP subfamilies. a) PIP1 and PIP2 subfamilies are involved in CO$_2$ conductance *via* stomata guard cells and mesophyll cells in the leaves. b) ZmPIP1-6 transports urea from soil to the cytosol across plasma membrane. AtTIP4-1 loads urea into vacuole for storage under N deficiency or detoxification under high fertilization rate. AtTIP2-1 and AtTIP2-3 diffuse NH$_3$ by passive transport to the cytosol and vacuole respectively. NH$_4^+$ is transported actively by ammonium transferase (AMT). c) Si is transported from soil solutions to the root exodermis by NIP2-1. At the proximal side of the endodermis an efflux transporter actively transports Si to the xylem, which is then unloaded by NIP2-2. Filled and dotted arrows represent symplastic and apoplastic pathway respectively. A NIP3-1 is responsible for boron transport.**

MIPs as transporters of Urea

Urea, ammonium and nitrate are three major sources of native soil or applied fertilizers for optimal growth and yield improvement [50]. Increasing chemical input of nitrogen fertilizers destructively affect global climate. Less than 50% NUE is considered as serious economic loss [51]. Strategies to improve fertilizer use efficiency will lead to NUE. Due to high nitrogen content and cost effectiveness, more than 50% of nitrogen fertilizers are urea (http://faostat.fao.org). Urea is transported into plant cells either in the intact form or in the form of NH$_3$ after degradation by soil bacteria [51]. Urea acquisition at low concentrations in the soil is mediated by high affinity secondary active transport systems, called DUR3 transporters [52]. In agricultural soils fertilized by urea, AQP mediated passive transport system facilitates urea transport [53]. Urea is also the catabolic product of arginine in mitochondria, which is exported to cytosol probably by an AQP [54]. To detoxify excess urea from cytosol, it transiently stores in vacuole, where AQPs act as a transporter. This is usually the case when leaves are sprayed with urea fertilizers [55]. Members of PIP, TIP and NIP subfamilies have been involved in urea transport in plants, which is a comparable situation to that in mammals. While PIPs and NIPs move urea between apoplast and symplast in plants, TIPs equilibrate urea concentration over different cellular compartments [56-58]. NtAQP1 a tobacco

PIP channel and ZmPIP1-5b, which is mainly expressed in maize roots, have shown urea transportation activity [59,60]. NtTIPa (a homologue of AtTIP2-1) from tobacco is highly urea permeable [61]. Tobacco tonoplast vesicles have 75 times higher permeability to urea than plasma membrane preparations. AtTIP1-2, AtTIP2-1 and AtTIP4-1 from *Arabidopsis* have shown urea permeability when expressed in yeast [57]. Acquisition of nitrogen from urea is either from roots or from leaves after foliar spray. Compartmentalization to vacuole mediated by TIPs is inevitable for storage or detoxification of urea under high fertilization rate [55]. AtTIP4-1 which mainly expresses in *Arabidopsis* roots loads urea from cytosol to vacuole and is inducible by nitrogen starvation [62]. AtTIP1-3 and AtTIP5-1 with high expression level in *Arabidopsis* pollen have intermediate and high permeability to water and urea respectively [63]. AtTIP5-1 is responsible for transportation of urea produced from mitochondrial arginase [54]. ZmTIP4-4 (up-regulated in roots and leaves under nitrogen starvation) is also involved in urea passive transport [56]. In a yeast complementation assay, CpNIP1 from zucchini (*Cucurbita pepo*) has shown urea transport activity [64]. ZmNIP2-1 (expressed constitutively in different organs) and ZmNIP2-4 (abundant in roots and reproductive organs) are involved in urea passive transport [56]. Boosting urea uptake or partitioning by over-expression of genes encoding AQPs promotes urea utilization as nitrogen fertilizer and reduces nitrogen loss from agricultural systems.

MIPs as transporters of Boron

The structural role of boron as the cross linker of pectin in cell wall has been confirmed [65]. A 1:2 borate diol diester is responsible for the cross linkage of dimeric rhamnogalacturonan II, one of the main components of the pectin polymer [66]. This linkage is essential for normal leaf expansion. Proteomic analysis of *Arabidopsis* leaves has revealed the major role of boron in nucleic acid metabolism, photosynthesis and protein synthesis [67]. Growing organs are affected by boron deficiency so that leaf expansion, root elongation, flower, fruit and seed development are inhibited. Chlorotic and necrotic symptoms are observed in the marginal regions of leaves in toxic conditions. [65]. In high rainfall regions of the world, boron, which in soil solution usually exists as boric acid is washed out of the soil and leads in boron deficiency. In contrast, in dried and semidried regions with high boron in the soil water, concentration of boron reaches to toxic levels for plants [65]. Although both conditions decrease crop yield, boron deficiency is more common than boron toxicity. Active transport by BOR transporters and facilitated transport of boron by MIP channels control boron uptake by plants [65]. Members of PIP, TIP and NIP subfamily have been shown to be involved in boron transport in *Arabidopsis*, rice and barley [68-70]. Experimental evidence exists on tolerance of rice to toxic levels of boron provided by OsPIP2-4 and OsPIP2-7 [68]. Over-expression of AtTIP5-1 in *Arabidopsis* has increased tolerance to boron toxicity [69]. Role of NIP5-1 in boron uptake from root surface, NIP6-1 in boron distribution in shoots and NIP7-1 in floral anthers of *Arabidopsis* is confirmed [71-73]. Under boron limitation, AtNIP5-1 transcript was increased more than ten folds. OsNIP3-1 has been identified as a boron transporter under boron limitation in rice [74]. Boron toxicity tolerance in barley has been possible by reduced expression of NIP2-1 and NIP2-2, respectively [70,75]. Identification and manipulation of MIPs involved in boron transport would alleviate symptoms of boron deficiency and toxicity.

MIPs as transporters of Silicon

Metalloids have various roles ranging from essential (boron) to highly toxic (arsenic) [76]. Si has not been recognized as an essential element for plant growth but its beneficial roles classify it as a quasi-essential element for healthy growth, when plants are exposed to stress [77]. Si is known as an alleviator of different plant stresses, including salt, drought, metal toxicity and nutrient imbalances. Since Si improves resistance against biotic stresses such as plant pathogens and herbivores, it has

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been used extensively for disease resistance [78]. Si as the second richest element in the soil is absorbed from roots in the form of uncharged monomeric orthosilicic acid (H_4SiO_4) and then transports from roots to transpiration site wherein silicic acid precipitates in the form of biogenic silica ($SiO_2.nH_2O$), called phytolithis [79]. Si beneficial is due to the silica deposition, which acts as physical barrier to prevent penetration of pathogens and activation of defense mechanisms such as production of phenolics, phytoalexins and lignin [79]. In contrast to dicots, monocots are generally considered as either intermediate or high accumulator of Si [80]. Active, passive, and rejective are three possible mechanisms of Si uptake in higher plants [81]. The influx channel type transporters and efflux transporters are responsible for Si uptake and translocation [78]. The efflux transporter is a putative anion transporter, which by proton gradient promotes active transport of Si. NIP subgroup of MIPs is responsible for AQP mediated transportation of Si in plants. The specific ability for Si transportation has made the NIP2 subfamily as a distinctive feature to predict Si absorption potential by plant species [80]. The first identified MIP, which facilitates the passive Si uptake was OsLsi1 (OsNIP2-1) in rice roots [82]. The other passive transporter of Si, OsLsi6 (OsNIP2-2) is responsible for distribution of Si in the shoot and leaves, which results in an increase excretion of Si in the guttation fluid by unloading Si from the xylem [83,84]. Homologous of Si influx transporters has been reported from other plant species as well. Barley (HvLsi1, HvLsi6), maize (ZmLsi1), wheat (TaLsi1), soybean (GmLsi1), cucumber (CsiT1, CsiT2) and pumpkin (CmLsi1) are the NIP AQPs, which facilitate Si uptake and distribution [78]. EaNIP3-1, EaNIP3-3 and EaNIP3-4 are horsetail MIP transporters of Si in the roots and shoots [85]. It has been shown that in the two diploid species of *Musa acuminata* Banksi (AA genome) and *Musa balbisiana* Tani (BB genome) at the high concentration levels of Si supply, plant Si concentration was less than proportional [86]. This suggests not only the involvement of passive transport by MIPs but also the operation of exclusion mechanisms. When the concentration of Si is low to intermediate in soil, Si has been totally depleted, which is associated to the active transport. Henriet proposed that Si moves across four compartments in banana (root, pseudostem, petiol and lamina) [86]. X-ray and SEM analysis have shown that Si concentration increases in banana root epidermis. The inner cortex of banana has the highest deposition of Si, and then a decrease in Si concentration is observed in endodermis and stele [87]. Absorption and distribution of Si has been schematically elaborated in FIG. 1c. The involvement of at least two NIP passive transport sites has been suggested. The model assumes that Si is taken by root exodermis, cortex and endodermis, *via* NIP2-1. Xylem unloading then proceeds through NIP2-2.

Results, Discussion and Conclusion

By affecting water permeability and ion transport, different isoforms of MIPs provide a tight cell osmoregulation under stress. This manuscript deciphers the role of different AQPs during development and stress and provides the opportunity to manipulate carbon gain and water use efficiency, which in turn allow crops to cope with environmental stresses. MIPs in higher plants are at least divided into five subfamilies. XIPs with unknown function have been found in dicots but not monocots. PIP channels results in higher tolerance to salt and drought stress. Experimental evidences have demonstrated that PIPs are transporters of H_2O_2, urea, CO_2 and boron. In comparison to the PIP2 subgroup, many PIP1s have little or no water permeability. TIPs are transporters of H_2O_2, urea, NH_3 and boron. NIPs are transporters of H_2O_2, boron and Si. Although AtSIPs have shown water transport activity, the non-aqua substrate specificity of SIPs have not been yet demonstrated.

Acknowledgments

Conflict of Interest

The author declares that there is no conflict of interest.

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