Review article

Molecular mechanisms of water uptake and transport in plant roots: research progress with water channel aquaporins

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Abstract: Aquaporins (water channels) are membrane proteins which facilitate the transport of water and low molecular weight compounds across biological membranes. The author and collaborators identified barley aquaporins and investigated the level of transcripts in roots, water transport activity, tissue localization, expression reduction by salt stress, and diurnal changes in the expression of a particular aquaporin. Over-expression of a barley aquaporin, HvPIP2;1, increased the shoot/root ratio and raised salt sensitivity in transgenic rice plants. Aquaporin research is providing significant insights into the water relations of plant roots.

Keywords: aquaporin, barley, salt stress, root water permeability, transgenic rice, water transport.

Abbreviations: G, conductance; J, water flow; V, motive force; Lp, water permeability; Lp_r, root water permeability; PIP, plasma-membrane intrinsic protein (aquaporin); TIP, tonoplast intrinsic protein (aquaporin); NIP, Nod26-like intrinsic protein (aquaporin); SIP, small basic intrinsic protein (aquaporin).

Introduction

Water uptake is one of the most important functions of plant roots for terrestrial plants except "air plants" which utilize aerial water. If water uptake through roots is seriously inhibited by drought or salt stress, plant growth stops, and such plants will die. Physiological and agronomical attention has been focused on the mechanism of root water uptake and water transport to the shoot, as this determines cell growth and ultimately plant yield.

Water flow (J) is the product of the motive force (V) and conductance (G), that is:

$$J = V \cdot G \quad (1)$$

where V is described as the water potential difference between two compartments, for example, the inside and outside of a root cell. Plants can regulate the internal water potential in several ways: via accumulations of ions and compatible solutes, or the opening/closing of stomata. Such biochemical and molecular biological mechanisms have been investigated for years. At the molecular level, however, almost nothing concerning water permeability was considered until Dr P. Agre discovered a water transporter, an aquaporin, in 1992. Soon after the first aquaporin (AQP1) from erythrocytes was reported, plant aquaporins were reported. Then, substantial investigations of water uptake and its regulation were launched at the molecular level. The discovery and establishment of aquaporins marked such an important turning point in the field of water relations in biology that Dr. Agre was awarded the Nobel Prize in 2003.

Structure and function of aquaporins

Many isomers of plant aquaporins exist. More than 30 aquaporin genes have been identified in *Arabidopsis*, rice, or maize. Plant aquaporins are classified to 4 subgroups: plasma-membrane intrinsic protein (PIP), tonoplast intrinsic protein (TIP), Nod26-like intrinsic protein (NIP), and small basic intrinsic protein (SIP). Most aquaporin proteins (except some SIPs) have 6 membrane-spanning regions and 2 Asn-Pro-Ala motifs. Tow asparagines in the motifs were supposed to function to recognize and select water molecules. Recently, the fine structure of spinach PIP type aquaporin was established, and the opening/closing mechanisms were consequently revealed in detail (Törnroth-Horsefield et al. 2006).

In addition to water molecules, some aquaporins can transport other low molecular weight molecules (Tyerman et al. 2002). Ma et al. (2006) found that one

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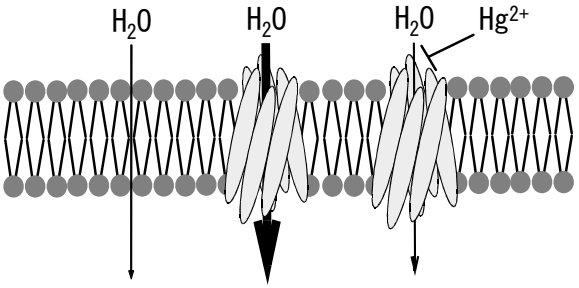

Fig. 1. Water permeation across biological membranes. Water molecules permeate across the membrane at less than 1/10 of the speed in the case of diffusion (left) compared to aquaporin-driven transport (center). Water permeability can be regulated with inhibitors like Hg ions (right), aquaporin levels, or channel opening/closing.

Table 1. Amounts the three PIP transcripts in young barley roots with or without salt stress (200 mM NaCl, 24h)

	HvPIP2;1	***HvPIP1;3***	***HvPIP1;5***
Control	22.0 ± 2.0	2.2 ± 0.3	1.3
Salt stress	12.5 ± 2.10	3.5 ± 1.1	0.8

(million molecular copies in 1 μg total RNA)
Data are means±SE. (Modified from Katsuhara et al. 2002)

rice aquaporin (OsNIP2;1) functioned to transport Si. Takano et al. (2006) reported that NIP5;1 was a boron transporter in *A. thaliana*.

In most plant cells, the water permeability (Lp) of living cells shows a ten-fold higher value than the water diffusion constant (Ld) across the cell membrane or reconstituted lipid-bilayer (Fig. 1). If aquaporins are inhibited by Hg ions or if their expression is reduced (Clarkson et al. 2000), Lp decreases to the level of Ld. These results suggest that aquaporins function in most living cells and most Lp is under the control of aquaporins. A high root water permeability (radial direction) is important to support plant evaporation. High water permeability is also important for plant cells to realize rapid elongating growth. Aquaporins play an indispensable role in this process. Aquaporins provide another option for plant cells, that is, regulating the cellular water permeability by the amount (expression) of aquaporins and/or molecular modification of proteins such as phosphorylation/dephosphorylation to activate/inactivate aquaporins. This regulatory ability can be advantageous for the survival and adaptation of plants under various environments from moist to dry conditions. In the case of cell elongation, increase in cellular hydraulic conductivity by activation of and/or increase in aquaporins can be advantageous if enough extracellular water is available. However, during very dry periods (that is, in the case of extracellular water potential is lower than the intracellar water potential), decrease in and/or inactivation of aquaporins can be advantageous to prevent dehydration, and to survive.

In general, cell water permeability is supposed to be determined by the characteristics of the plasma-membrane. Usually, tonoplasts do not provide a barrier for cellular water transport, because tonoplasts show a higher water permeability than the plasma-membrane. This is consistent with the usual greater abundance of TIPs than PIPs. A higher tonoplast permeability is also theoretically reasonable to maintain the cytoplasmic volume against changes of extracellular osmolarity (Tyerman et al. 1999). Because TIPs are less probable to be a limiting factor of cell water permeability as mentioned, PIPs are mostly being focused, especially in relation to water-related stresses, in the investigation of the molecular regulation of water permeability of root cells.

Barley aquaporins

Barley is one of the most important cereals and shows a high salt and drought tolerance in the grass (*Gramineae*) family. Analyzing and characterizing the barley aquaporins may provide significant information to reveal the molecular mechanisms of the water-use strategy in *Gramineae* crops. Barley aquaporins, *HvPIP2;1*. *HvPIP1;3*, and *HvPIP1;5*, were identified by the author and collaborators (Katsuhara et al. 2002). Transcript abundance was investigated in the roots of barley seedlings (Table 1). Among the 3 genes, transcripts of *HvPIP2;1* were ten-fold more abundant than those of *HvPIP1;3* and *HvPIP1;5*. Although a probable 10 PIP genes exist in the barley genome, no other PIP transcripts more abundant than *HvPIP2;1* have been detected in the roots of seedlings (unpublished data).

Salt stress, on the one hand, reduced the amount of *HvPIP2;1* transcripts in roots to half. On the other hand, amounts of *HvPIP1;3* and *HvPIP1;5* transcripts were relatively low and constant during salt stress (Table 1). Western blot analysis showed that HvPIP2;1 protein also decreased during salt stress. Due to these results, HvPIP2;1 was further investigated.

Enhanced water permeability was confirmed in *Xenopus leavis* oocytes injected with *HvPIP2;1* cRNA (Fig. 2), indicating that *HvPIP2;1* codes for a functional aquaporin with water transport activity (Katsuhara et al. 2002). In some PIPs (spinach

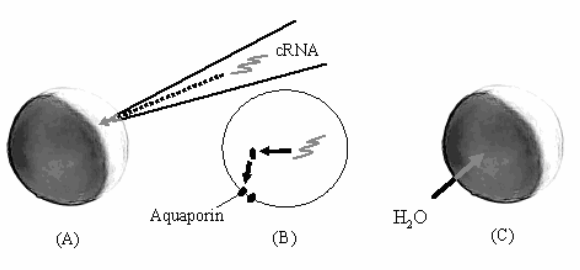

Fig. 2. Heterogeneous gene expression in oocytes. Synthetic cRNA according to the target cDNA was micro-injected into oocytes about 1 mm in diameter (A). An aquaporin protein was synthesized in the cytosol and then was targeted into the plasma-membrane (B). The culture medium was substituted with hypotonic solution to induce swelling due to water influx (C). Water permeability can be calculated from the volume change and surface area.

PM23A and others), phosphorylation in certain Ser residues activates water transport (Kjellbom et al. 1999), but no effect of phosphorylation was observed in HvPIP2;1, at least in the oocyte system. Chaumont et al. (2000) proposed that PIPs can be classified into two subgroups: A PIP1 subfamily with less or no water transport activities, and a PIP2 subfamily with high water transport activities. This was consistent with barley aquaporins, that is, HvPIP1;3 showed no water transport activities in the oocyte system (unpublished data).

As for tissue localization, HvPIP2;1 protein was detected in cells surrounding the vascular cylinder and in the epidermis or outer layer of the cortex, but was less prominent inside the vascular cylinder. These results suggest the possibility that HvPIP2;1 is more involved in water uptake into the symplast than in water loading into the xylem (Katsuhara et al. 2003a).

In several plants, diurnal changes in root water permeability (Lp_r) or root pressure have been reported. Henzler at al. (1999) reported well synchronized diurnal changes of Lp_r and amounts of aquaporin

Table 2. Plant fresh weight （FW）, relative growth rate for 2 weeks (RGR), and fresh weight ratio of shoot/root (shoot/root) of 4-week old rice plants （non-transgenic: WT, and transgenic: 6322） grown via hydroponic culture

	FW (g)	RGR (%)	Shoot/root
WT	26±7 (n = 4)	148	2.4 (100%)*
6322	30±14 (n= 6)	137	3.5 (146%)**

Data are means±SE. Means of the mass ratio of shoot/root followed by different number of asterisks are significantly different (P <0.05) according to the t-test. (Modified from Katsuhara et al. 2003b)

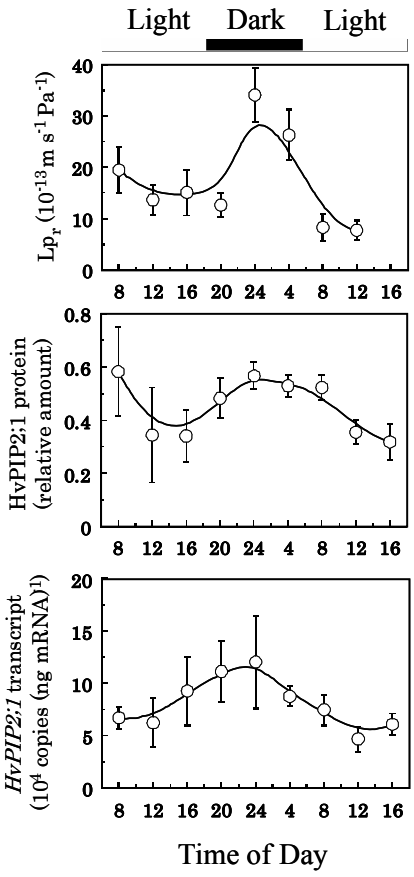

Fig. 3. Diurnal changes in the root water permeability (upper), amount of HvPIP2;1 protein (middle), and amount of *HvPIP2:1* mRNA (bottom). Barley seedlings were grown in hydroponic culture. After two cycles of light (AM5 to PM7, 14 h) and dark (10 h), monitoring was started from the third morning. HvPIP2;1 protein was normalized using the amount of cyt *c*. Data are means±SE (Modified from Katsuhara et al. 2003a).

transcripts. In most cases, Lp_r or aquaporin expressions were high in the morning, daytime, or evening. Diurnal changes of Lp_r, HvPIP2;1 protein, and *HvPIP2;1* transcript were also investigated in barley seedlings, and they showed high peaks at night (Fig. 3, Katsuhara et al. 2003a). These findings and previous work suggest that each tissue of individual plant species shows unique diurnal changes in water permeability and aquaporin expression, probably depending on the developmental stage and surroundings. Barley seedlings show rapid growth at night when almost no transpiration occurs. It is assumed that J in formula (1) is much reduced according to the marked decrease in motive force (transpiration) at night. In such a case, an increase in Lp_r (that is, increase in G) may, at lease partially, compensate for the reduction of J (Katsuhara et al. 2003a).

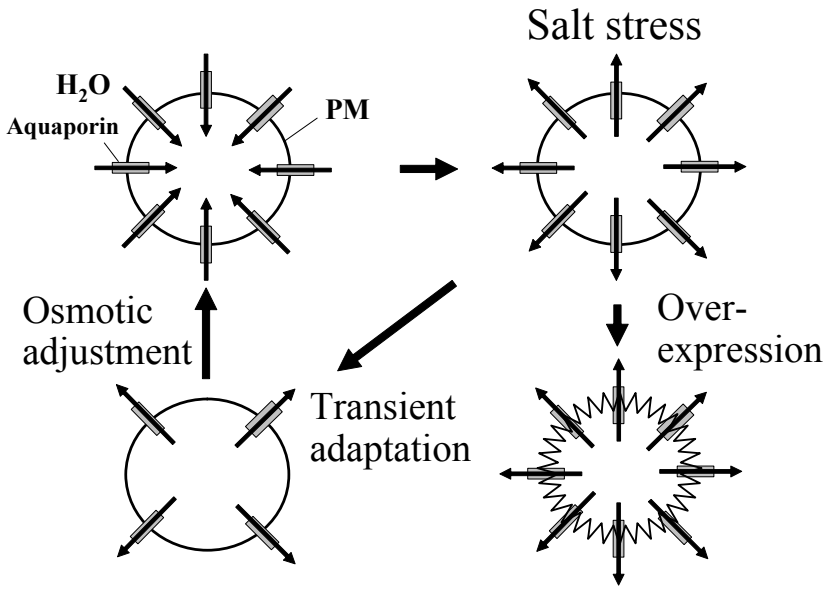

Fig. 4. A hypothetic model of aquaporins in salt tolerance. During the low outer cellular water potential under salt stress, plant cells reduce levels of aquaporins, cellular water permeability, and then water loss. After osmotic adjustments via accumulations of ions and/or compatible solutes, water uptake resumes. Plants over-expressing aquaporins cannot survive because of continuous water loss. Below right: water loss in a cell over-expressing aquaporins. Below left: a cell with reduced levels of aquaporins. Middle arrow (from upper right to below left): adaptive transition in wild type cells.

Transgenic plants over-expressing aquaporins

Transgenic techniques provide powerful tools to investigate gene functions in tissue and individuals. Because transgenic barley is difficult to produce, *HvPIP2;1* was introduced into rice plants (Katsuhara et al. 2003b). In line 6322 of T_2 generations, an over-expression of HvPIP2;1 and increase in Lp_r were observed. This increase was accompanied by a higher shoot/root ratio of line 6522 plants than of non-transgenic rice plants. In transgenic plants, relatively small roots could uptake sufficient water to sustain the shoot. An increase in the shoot/root ratio was also observed in *A. thaliana* over-expressing a tobacco aquaporin (Aharon et al. 2003).

In hydroponic culture, line 6322 rice plants were subjected to salt stress of 100 mM NaCl. Non-transgenic plants showed some growth reduction with salt stress of 100 mM NaCl, but maintained green leaves and survived. Transgenic plants, however, stopped growing and died within 2 weeks. In plants over-expressing aquaporins driven by 35S-promoter, it was supposed that down-regulation of aquaporin expression hardly occurred and water loss through aquaporins continued in root and/or leaf cells during salt stress (Fig. 4).

In the transgenic 6322 line, leaf CO_2 conductance (permeability) was also investigated (Hanba et al. 2004) and a 20% higher value than in non-transgenic leaves was confirmed. This result suggested that HvPIP2;1 aquaporin could promote CO_2 permeation. Over-expressing HvPIP2;1 enhanced not only CO_2 conductance, but also the assimilation and transpiration rates. Cellular conductance of CO_2 is one of the most influential factors on photosynthesis. However, the molecular mechanisms of CO_2 permeation were not investigated until our work mentioned above and other studies (Uehlein et al. 2003). In the non-photosynthetic root, CO_2 permeation through aquaporins may function in CO_2 extrusion outside of cells.

Conclusion

Much progress in aquaporin research has been achieved since the first aquaporin was reported in 1992. It has been indicated that aquaporins are involved in many important physiological phenomena such as, in addition to the above-mentioned, the chilling-sensitivity of rice (Li et al. 2000) and acidosis (Tournaire-Roux et al. 2003). The classical topic of water uptake by roots has been re-investigated and its molecular mechanism is under intensive investigation today. Further findings are expected in aquaporin research which will give us further insights into the water relations of plant roots.

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Associate Prof. Maki Katsuhara has an interest in ionic and water relations in plant cells under salt and water stress, and now focuses on aquaporins recent years.