WATER TRANSPORT IN PLANTS: FROM MOLECULES TO WHOLE PLANT

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Abstract

Aquaporins are membrane proteins which provide a very efficient membrane transport system for water and low molecular weight compounds in living cells. We identified 23 barley aquaporin genes. Among tonoplast-type aqauporins (TIPs), TIP3;1 seems to be essential for seed maturation. Expressions of several plasma-membrane type aquaporins (PIPs) were down-regulated after strong salt (200 mM NaCl) or osmotic (360 mM mannitol) stresses, probably to prevent dehydration during salt/osmotic stresses. Inactivation of PIPs by dephosphorylation and internalization is also functional mechanism to prevent dehydration. Another regulation mechanism of PIP activity, heteromerization between PIP1 and PIP2 subgroups, was also investigated. Our research indicates that aquaporins are also involved in low-temperature tolerance. Molecular engineering/enhancement of aquaporins' functions may improve water relations, tolerance to some stresses, nutrition uptake, and finally increase of crop yield in future.

Key words: Aquaporin, barley, salt stress, water

1.0 Introduction

Water absorption/transport is a classical topic in biology and agricultural science; because all living cells require water. Drought (water-shortage) is one of the most serious limiting factors for crop yield. Although many scientists have worked in the field of plant water relations, almost no molecular mechanisms of water transport at cellular level were until aquaporins, membrane proteins which provide a very efficient membrane transport system for water, were identified in human erythrocyte (1992) and in plants (1993). Before the discovery of aquaporins, that water simply diffused "somehow'' across plants membrane and proteins were not involved in these processes (Schaffner 1998).

Plant aquaporins are classified as: PIP, TIP, NIP, SIP, and XIP (Table 1). In rice, 33 aquaporin genes are identified. In Arabidopsis, 35 aquaporins are known. Aquaporins were determined as a water channel at first, but now some aquaporins species are evidenced to transport low molecular weight compounds like H_2O_2, $Si(OH)_4$, $B(OH)_3$ NH_3, and CO_2 (Katsuhara *et al.,* 2008). However, profiling of substrate specificity in each aquaporin species is still on the way.

2.0 Materials and Methods

The seedlings of barley (*Hordeum vulgare*) were hydroponically grown as described (Katsuhara *et al.* 2002). Root samples were collected after NaCl/mannitol treatments and immediately frozen in liquid nitrogen, and then total RNA was extracted. Real-time RT-PCR was used for absolute quantification of RNAs (Mahdie *et al*. 2008). Water transport activity of each HvPIP was determined in *Xenopus* oocytes (Katsuhara *et al.* 2002). *In situ* localization of PIPs were investigated with the indirect immunofluorescence microscopy (Figure 1). Root hydraulic conductivity (Lp_r, that is, water permeability) was calculated from a sap flow rate and driving pressure using the pressure chamber (Figure 2).

3.0 Results and Discussion

To date we identified 23 barley aquaporins. Among TIPs, TIP3;1 is more interesting because this aquaporin highly expressed in immature seed, especially in aleurone layers. TIP3;1 is thought to have essential role in seed maturation. Our collaborators have suggested that TIP3;1 highly expresses in maturing seeds of rice, too (Hayashi, personal communication).

From the aspect of water-related stress tolerance, we mainly focused on PIPs because, at the plasma-membrane, PIPs face stressful environment more directly than other aquaporins in endomembranes. PIPs were divided in to two subgroups, PIP1s and PIP2s. In roots, barley PIP1s (HvPIP1s) were detected in the vicinity of the xylem and the cortex. HvPIP2;2 was found in the epidermis, especially in cells developing root hair, and also detected in the stele. Among 10 HvPIPs, transcripts of some major HvPIPs (HvPIP1;2, HvPIP2;1, HvPIP2;2 and HvPIP2;3) were down-regulated after strong salt (200 mM NaCl) or osmotic (360 mM mannitol) stress (Figure 3). This may be an example of tolerance mechanism to prevent dehydration during strong salt/osmotic stresses (Figure 4). During mild stress (100 mM NaCl or isotonic 180 mM mannitol), no reduction

of RNAs was observed, but other inactivation mechanisms are assumed to function to decrease PIP activity and Lp_r. Aquaporins are known to be activated by phosphorylation. A protein kinase inhibitor (Staurosporin) reduces Lp_r. Salt stress induced decrease of Lp_r but this reduction was partially inhibited by a protein-phosphates inhibitor (Okadaic acid), indicating that dephosphorylation of aquaporins is involved in the reduction of Lp_r during salt stress. Supplemental endocytosis inhibitor (Wartmannin) strongly inhibited the reduction of Lp_r by salt stress, suggesting that internalization of PIPs via endocytosis may occur during salt stress.

Another regulatory mechanism of PIP activity, heteromerization, was also investigated. Plant aquaporins are thought to form a tetramer to act as an active water channel protein (Törnroth-Horsefield *et al.* 2006). Heteromerization of PIP 1 and PIP2 aquaporins was proposed (Fetter *et al.* 2004). Co-expression of HvPIP1;2 with each HvPIP2 isoform significantly increased the water permeability in oocytes. Physiological function of heteromerization in the regulation of water transport activity awaits elucidation by further studies.

Aquaporins are also involved in low-temperature tolerance (Katsuhara *et al.* 2007). On the one hand, low-temperature tolerant figleaf-gourd plant can maintain root water permeability during chilling stress by increasing amounts of PIPs. On the other hand, sensitive cucumber plants cannot keep expression of aquaporins during chilling stress resulting in loss of water uptake. In addition to water transport and stress tolerance, recent studies have shown some aquaporins are involved in the transport of CO_2, ammonia, silica or boron.

4.0 Conclusions

Recent studies have revealed that aquaporins have essential roles in several physiological functions of plant cells. Molecular engineering to enhance of aquaporins' functions may play a useful role in improving water relations, tolerance to some stresses, nutrition uptake, and finally increase of crop yield in future.

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Table 1: Description/localization of plant aquaporins and number of detected genes in barley

		Localization	No. of genes (Barley)
PIP	Plasma membrane Intrinsic Protein	Plasma membrane (PM)	10
TIP	Tonoplast Intrinsic Protein	Tonoplast (Vacuolar membrane)	8
NIP	Nodulin 26-like Intrinsic Protein	Bacteroid membrane, ER, PM	3
SIP	Small basic Intrinsic Protein	ER membrane	2
XIP	X Intrinsic Protein	Unknown (recently discovered)	-

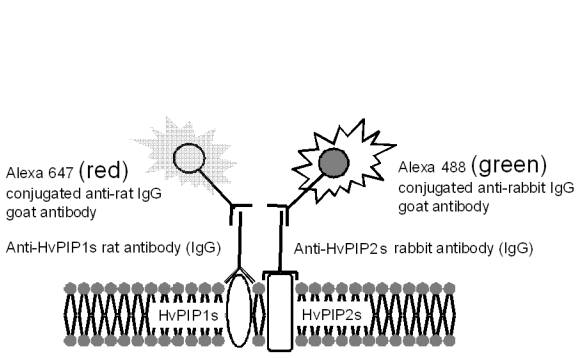

Figure 1: Indirect immunofluorescence

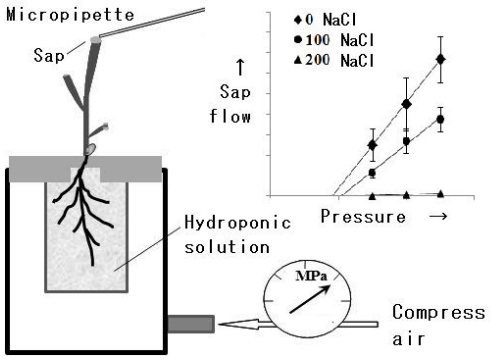

Figure 2: Pressure chamber

Sap flow = Lp_r x pressure – constant

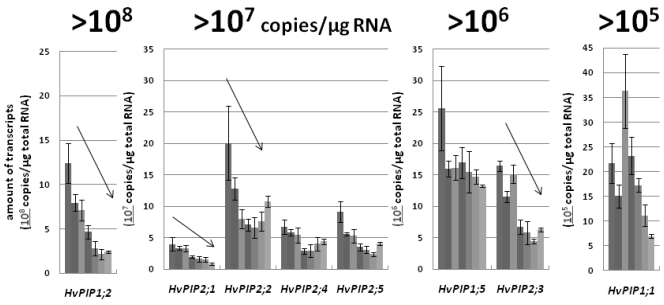

Figure 3: 200 mM NaCl reduced HvPIPs expression (left to right: 0, 2,4,6,8,12 and 24h NaCl)

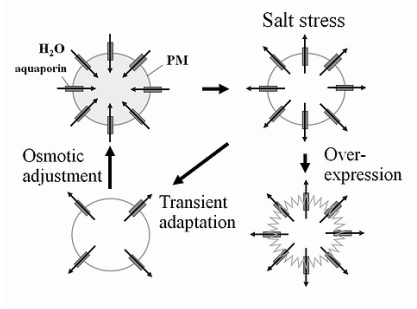

Figure 4: Dehydration model