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Barley plasma membrane intrinsic proteins (PIP aquaporins) as water and CO2 transporters

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Barley Plasma membrane Intrinsic Proteins (PIP Aquaporins) as water and CO_2 transporters.

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Abstract

We identified barley aquaporins, and demonstrated that one, HvPIP2;1, transports water and CO_2. Regarding water homeostasis in plants, regulations of aquaporin expression were observed in many plants under several environmental stresses. Under salt stress, a number of plasma-membrane type aquaporins were down-regulated, which can prevent continuous dehydration resulting in cell death. The leaves of transgenic rice plants that expressed the largest amount of HvPIP2;1 showed a 40% increase in internal CO_2 conductance compared with leaves of wild-type rice plants. The rate of CO_2 assimilation also increased in the transgenic plants. The goal of our plant aquaporin research is to determine the key aquaporin species responsible for water and CO_2 transport, and to improve plant water relations, stress tolerance, CO_2 uptake/assimilation, and plant productivity via molecular breeding of aquaporins.

Keywords
Barley, CO_2, Plant aquaporins, Salt stress, Water transport

Introduction

Water uptake is an indispensible function of plant roots for the survival of terrestrial plants. If water uptake through the roots is reduced or blocked by water-related stress such as drought, salt stress (salt accumulation in soil inducing osmotic stress), or low temperatures, plant growth is seriously or lethally inhibited. 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 plant yield. Since water flow is determined as the product of the motive force (V) and water permeability (Lp), V and/or Lp should be adjectively regulated to maintain the growth of plants under water-related stress. Water motive force is described as the water potential difference between the inside and outside of a root cell. For decades, researchers have investigated the biochemical and molecular biological regulatory mechanisms of the internal water potential in several ways: via accumulations of ions and compatible solutes, or the opening/closing of plant stomata. At the molecular level, however, almost nothing concerning water permeability was considered until Dr. P. Agre discovered the first aquaporin in 1992. Soon after this first aquaporin (AQP1) from erythrocytes was reported, plant aquaporins were identified. Not long following this, it was established that Lp of the plasma membrane and tonoplasts (vacuolar membrane) in plant cells mainly depends on two types of plant aquaporins: plasma membrane intrinsic proteins (PIPs) and tonoplast intrinsic proteins (TIPs), respectively, although the membrane lipid compositions and other membrane proteins can also affect water permeability. In the plant genome, more than 30 aquaporin genes have been detected [11,21]. Some of them specifically transport water molecules, but others can mediate other low molecular weight compounds such as CO_2 [7, 8, 28], silicon [19], boron [24], ammonia [9], and H_2O_2 [2].

To date, it has been established that aquaporins play a critical role in the transport of many essential molecules in plants. Except for light reception, aquaporins partially or mainly contribute to the transport of water, minerals, and CO_2 that are essential for plant life. In this article, two functions of plant aquaporins are described: i) the regulation of water transport in various environments, and ii) CO_2 transport. The authors have investigated barley aquaporins, because barley is a crop showing fairly good tolerance to drought, salt stress, and low temperatures compared to many other crops, including rice. Analyses of barley aquaporins are providing a good insight into the molecular mechanisms involved in the transport of water and other essential molecules in *Gramineous* crops.

Water transport of barley PIPs

The peripheral plasma membrane is composed of two major membranes of plant cells together with the membrane of the vacuole (tonoplast). These two major serial conducting parts are involved in cellular hydraulic conductivity. Because Lp of the tonoplast is basically higher than that of the plasma membrane according to the abundance of TIPs [27], and because Lp of the whole system principally obeys the Lp of the lower conducting part (that is, the plasma membrane), PIPs are the most important factors regarding the characteristics of cellular water uptake/water loss. This is the reason why PIPs have been the most intensively investigated in terms of the water-relations of plant cells under various environmental conditions.

Three PIPs: *HvPIP2;1*, *HvPIP1;3*, and *HvPIP1;5*, were first identified and barley PIPs were analyzed by the authors and collaborators [12]. Absolute amounts of these 3 transcripts were determined in the roots of barley seedlings. It was revealed that transcripts of *HvPIP2;1* (22 million copies/μg total RNA) were ten-fold more abundant than those of *HvPIP1;3* (2 million copies/μg total RNA) and *HvPIP1;5* (1 million copies/μg total RNA). Water transport activity of HvPIP2;1 and HvPIP1;3 was assayed in a *Xenopus laevi* oocyte heterologous expression system. The former (HvPIP2;1) markedly increased the water permeability (P_f) of oocytes, but HvPIP1;3 did not [15]. This result is consistent with the general feature of plant PIP aquaporins [3], whereby aquaporins of the PIP2-subfamily show marked water transport activity in the *Xenopus* oocyte system but PIP1 aquaporins do not.

Under water-related stress, aquaporins play an important role in plant osmotic and ion homeostasis [16,18,27]. Phosphorylation of certain serine residues activates some PIPs, and dephosphorylation rapidly closes them [26]. This aquaporin inactivation can prevent water loss from cells under drought or strong salt stress [16]. This activation/inactivation can be effective for short-term (within a few hours) adjustment of the cellular water balance.

During several hours to days of salt or osmotic stress, the down-regulation of several aquaporins including HvPIP2;1 was observed in many plant species [10,12,29,32]. A decrease in the level of aquaporins and reduction of Lp can prevent cell death due to continuous dehydration and gain time for intracellular osmotic adjustment. Consistently, continuous over-expression of HvPIP2;1 increased the water permeability of roots and raised the salt sensitivity of transgenic rice plants [14]. In such transgenic plants, a lower down-regulation of cellular water permeability might induce a

relatively higher rate of water loss from roots/shoots, and result in death under salt stress with induced osmotic stress and dehydration.

Recently, the authors and collaborators detected novel barley aquaporin genes (Table 1) in addition to those previously reported, and a total of 10 barley PIP genes (*HvPIPs*) were preliminarily analyzed (Table 2). Among them, 5 genes were classified into the sub-class *PIP1* and the others into the sub-class *PIP2* according to the sequence homology. Transcript amounts of *HvPIP1s*, except for *HvPIP1;2*, were lower than *HvPIP2s*. However, the *HvPIP1;2* transcript was markedly more abundant (> 10-fold) than *HvPIP2;1*. Down-regulation of *HvPIP1;2* and many *HvPIP2s* due to salt stress was observed. Water transport activities of some HvPIP2s were detected in the *Xenopus laevis* oocyte heterologous expression system. Co-injection of *HvPIP1;2* and *HvPIP2;1* increased the Pf of oocytes, but such an enhancement of Pf was not observed when *HvPIP1;2* cRNA was injected alone. This activation mechanism of "heteromerization" was first proposed in maize PIPs [6]. Interactions between ZmPIP1s and ZmPIP2s were clearly demonstrated in living maize cells using a FRET imaging system [31]. At present, however, there is no information suggesting whether heteromerization regulation is or is not involved in the regulation of aquaporin activity and cellular water transport in plant cells under salt or osmotic stress.

In addition to salt and osmotic stress, many environmental factors markedly change the expression of plant aquaporins. Nutritional deficiency in general decreases the expression of Lp and aquaporin genes in roots [4], however, specific genes were induced under special conditions, such as *Arabidopsis* NIP5;1 by boron-deficiency [24] and *Arabidopsis* TIP2;1 by nitrogen-deficiency [17]. Low temperature [13], phytohormones [23], and far-red light [22] also regulated the expression of plant aquaporins. These observations indicate that adequate regulation of water homeostasis via aquaporins is common in plant cells adjusting to various environments.

Aquaporins as CO_2 transporters

In mammalian cells, there were some indications that CO_2 could permeate AQP1 [5,20], but Yang et al. raised a serious question over this interpretation[30]. Mostly negative data regarding animal aquaporins mediating CO_2 transport were discussed in a recent conference (The 5th International Aquaporin Conference, Nara), suggesting that sufficient CO_2 transport can be achieved by simple diffusion across the lipid bilayer, because a high CO_2 pressure gradient between the inside and outside of

cells is generated by respiration in animal cells. However, the simple diffusion of CO_2 without an efficient transport system seems to be inadequate to maintain photosynthesis (CO_2 assimilation) in chloroplasts within mesohyll cells because the atmospheric CO_2 concentration is low (0.03 %) and CO_2 is rapidly consumed internally if CO_2-fixing enzymes (RubisCO) function properly. Aquaporin is one candidate facilitating CO_2 transport.

Photosynthesis is the most basic and important function of plants. Except for chemoautotrophic bacteria, all plants and animals, including humans, depend on photosynthetic products. On the one hand, stomata are one of the limiting factors for CO_2 uptake, and the regulation of stomatal conductance has been investigated for many years. On the other, internal conductance (g_i), conductance regarding CO_2 diffusion from stomata to chloroplasts, is known to be another limiting step. The limitation of photosynthesis by g_i is often greater than that by stomata. Although many factors are involved in g_i, recent research has indicated that aquaporins are probably the most important factor determining g_i. In the pioneering work of Terashima and Ono [25], a significant decline of g_i in the presence of $HgCl_2$, an inhibitor of most of the aquaporins, was demonstrated.

The authors demonstrated that one of the barley aquaporins, HvPIP2;1, transports CO_2 in addition to water. Because the generation of transgenic barley was not established, *HvPIP2;1* was introduced into rice plants to be analyzed ***in planta.*** The leaves of transgenic rice plants that expressed the greatest amount of HvPIP2;1 showed a 40% increase in g_i compared with the leaves of wild-type rice plants [8]. This was the first evidence of a direct relation between aquaporins and g_i (Fig.1). Although Uehlein et al. showed that membrane permeability to CO_2 increased in *Xenopus* oocytes expressing the tobacco aquaporin NtAQP1 [28], they did not perform the measurement of g_i. Recently, Flexas et al. confirmed an increase in g_i of leaves of transgenic plants over-expressing NtAQP1[7].

In transgenic rice leaves showing a high g_i, CO_2 assimilation also increased [8]. A rise in g_i is supposed to effectively promote the CO_2 supply to the photosynthetic center in chloroplasts and result in a high CO_2 assimilation rate. The same phenomenon was observed in tobacco plants, that is, the CO_2 assimilation rate increased in leaves over-expressing AtPIP1;2 (PIP1b) [1], or NtAQP1[28]. Recently, we also observed increases in CO_2 assimilation in tobacco leaves over-expressing ice plant (*Mesembryanthemum crystallinum*) aquaporins (unpublished data). These results suggest that CO_2 assimilation is commonly limited by g_i, which depends on aquaporins, and the enhancement of aquaporin activity is a potentially promising way to promote

plant CO_2 assimilation via improving g_i.

Future direction

All aquaporin genes in *Arabidopsis thaliana* and rice have been identified [11,21]. However, profiles (substrate specificity, spatial and developmental expression pattern, and stress responses) of most plant aquaporins are waiting to be clarified. Although some aquaporins (AtPIP1;2, NtAQP1, or HvPIP2;1) showed CO_2 transport activities, as described previously, many aquaporin species have not yet been analyzed and characterized from the view point of CO_2 transport at present. It is possible that one of such aquaporins can show a higher CO_2 permeability and be the most important for CO_2 assimilation. It is also necessary to establish which aquaporin species is the most crucial for water uptake *in planta*. Therefore, first, all aquaporin species should be investigated, analyzed, and profiled. Second, special aquaporin species should be selected as targets for molecular engineering to improve plant water relations, stress tolerance, CO_2 uptake/assimilation, and plant productivity.

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Table 1

Number of barley aquaporin genes estimated and analyzed

	Estimated gene number	Identified with RT-PCR	Full length cDNA isolated and sequenced	Quantitative analysis of transcript	Swelling assay performed
PIPs	10	10	10	10	5
TIPs	8	5	2	5	2
NIPs	3	1	1	1	1
SIPs	2	0	0	0	0

Putative aquaporin genes were estimated as contigs identified from the HarvEST barley database (http://harvest.ucr.edu/). Data from November 2007.

Table 2

Expression profile of barley PIP transcripts in roots

	PIP1 subfamily					*PIP2* subfamily				
	1;1	*1;2*	*1;3*	*1;4*	*1;5*	*2;1*	*2;2*	*2;3*	*2;4*	*2;5*
Amount of transcript in control roots	+	+++++	++++	++	++	+++	++++	++	+++	+++
Amount of transcript under salt stress	→	↓	↓	↓	→	↓	↓	↓	→	→

Transcripts were absolutely quantified using real-time PCR, and 200 mM NaCl was added to the hydroponic solution as salt stress up to 24 h. +, $>10^5$ copies/μg total RNA: ++, $>10^6$ copies/μg total RNA: +++, $>10^7$ copies/μg total RNA: ++++, $>10^8$ copies/μg total RNA: +++++, $>10^9$ copies/μg total RNA: →, no change (comparison with no-stress control): ↓, down-regulated.

Figure legend

Fig.1

Transport of ambient CO_2 to the chloroplast through several barriers. 1, leaf surface with stomata; 2, intercellular space; 3, cell wall; 4, plasma membrane of mesophyll cell; 5, cytoplasm; 6, chloroplast. An aquaporin can mediate CO2 transport in the plasma membrane (right path) via simple diffusion (left path). Internal conductance of CO_2 involves steps 2 to 6 mentioned above. Tissue and cellular structures are not to scale.

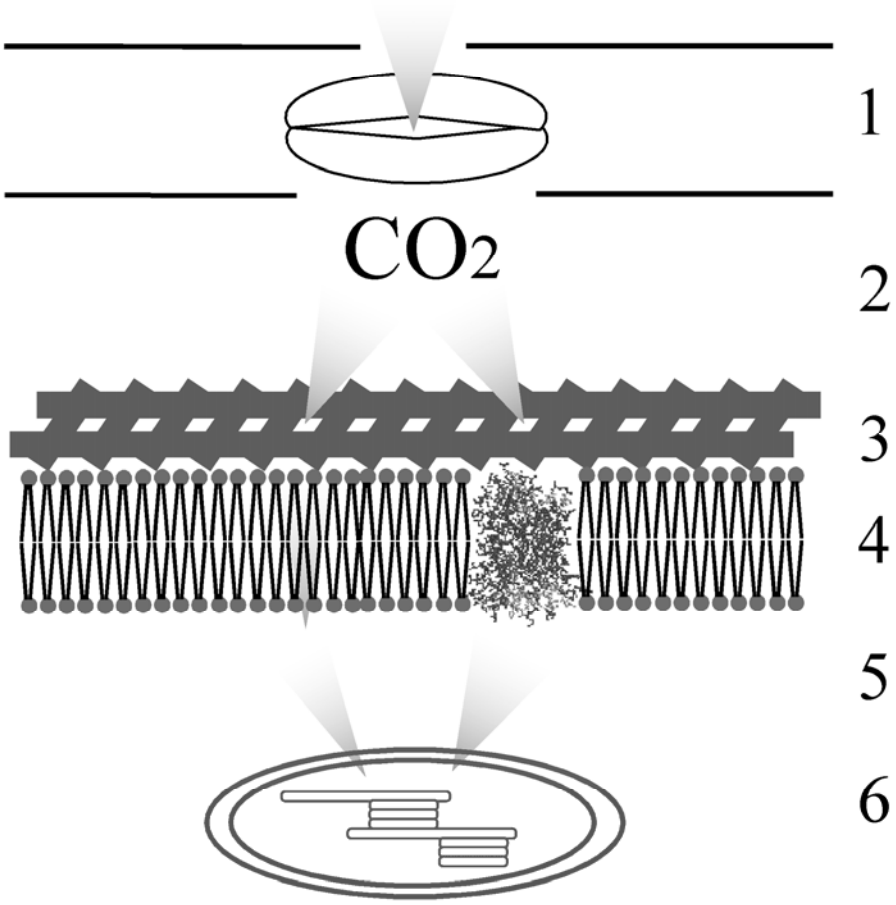

Fig.1