Rice Plants Sense Daily Weather and Regulate Aquaporin Gene Expressions in the Roots—Close correlation with potential evaporation—

Mari Murai-HATANO a, Tsuneo KUWAGATA b, Hidehiro HAYASHI a,c, Junko Ishikawa-SAKURAI a,† Masahisa MORIYAMA a, and Masumi OKADA d

a NARO Tohoku Agricultural Research Center (NARO/TARC), 4 Akahira Shimo-kuriyagawa, Morioka 020-0198, Japan
b Agro-Meteorology Division, National Institute for Agro-environmental Sciences, 3–1–3 Kannondai, Tsukuba 305–8604, Japan
c United Graduate School of Agricultural Sciences, Iwate University, Ueda 3–18–8, Morioka 020–8550, Japan
d Faculty of Agriculture, Iwate University, Ueda 3–18–8, Morioka 020–8550, Japan
† present address, NARO Institute of Crop Science (NICS), 2–1–18 Kannondai, Tsukuba 305–8518, Japan

Abstract

Transpiration, the evaporation from aerial parts of plants, is major driving force for the roots to uptake water from the soil. Aquaporins, the water channel proteins, are thought to play crucial roles in regulation of root hydraulic conductivity. Here we demonstrate that the evaporative demand plays a dominant role in the induction of specific aquaporins in rice (Oryza sativa) roots. Aquaporins in the plasma and intracellular membranes, facilitates the transport of water across the membrane, in the plasma membrane intrinsic proteins (PIPs, with 11 rice isoforms), tonoplast intrinsic proteins (TIPs, with 10 rice isoforms), nodulin 26-like intrinsic proteins (NIPs, with 10 rice isoforms), small basic intrinsic proteins (SIPs, with 2 rice isoforms), and the uncharacterized X intrinsic proteins (XIPs, not detected in rice). Several aquaporins with higher correlation to potential evaporation showed higher diurnal amplitude of their expression. Our study suggests that rice plants sense daily weather and responded to it by adjusting the expression of specific root aquaporin genes.

Key words: Aquaporin, Evaporative demand, Rice, Roots, Weather.

1. Introduction

The ability of roots to supply more water to the shoot under intense transpiration is critical for the growth and productivity of terrestrial plants, because it can be a limiting factor for higher stomatal conductance and hence for higher CO2 supply for photosynthesis (Hirasawa et al. 1992). Transpiration is induced by the evaporative demand from the atmosphere. Depending on the day-night cycles (of light intensity, temperature and humidity) and on daily weather conditions, the evaporative demand changes dynamically from nearly zero to up to 1,000 W m−2. How do the plants adapt successfully to such rapid environmental changes?

A large family of water channel proteins (aquaporins), located in the plasma and intracellular membranes, facilitates the transport of water and small neutral solutes (Maurel et al., 2008). Based on sequence similarities, plant aquaporins are divided into 5 major subfamilies: plasma membrane intrinsic proteins (PIPs, with 11 rice isoforms), tonoplast intrinsic proteins (TIPs, with 10 rice isoforms), nodulin 26-like intrinsic proteins (NIPs, with 10 rice isoforms), small basic intrinsic proteins (SIPs, with 2 rice isoforms), and the uncharacterized X intrinsic proteins (XIPs, not detected in rice) (Chaumont et al., 2001; Johanson et al., 2001; Sakurai et al., 2005; Bienert et al., 2011; Reuscher et al., 2013). Studies using mercury as an aquaporin inhibitor or transgenic plants for specific aquaporins suggested that aquaporins significantly contribute to water uptake by roots (reviewed by Maurel et al., 2008). Extensive studies of the effects of the water conditions in the root zone (such as soil water deficit, high salinity, high osmolality, and flooding) on the function and regulation of aquaporins suggested marked effects of these environmental stresses on aquaporin abundance in the roots (Tournaire-Roux et al., 2003; Jang et al., 2004; Alexandersson et al., 2005; Boursiac et al., 2005; Bramley et al., 2007; Li et al., 2008; Mahdieh et al., 2008). However, relatively little information is available on how root aquaporins respond to fluctuating evaporative demand (Levin et al., 2009; Sakurai-Ishikawa et al., 2011; Almeida-Rodriguez et al., 2011; Kuwagata et al., 2012; Vandeleur et al., 2014).

We have previously found that shoot transpiration triggers diurnal changes in root aquaporin expressions (Sakurai-Ishikawa et al., 2011). The abundance of many isoforms of PIPs and TIPs in the roots of rice plants grown in controlled growth chambers showed clear diurnal changes at both mRNA and protein levels: it is low at night and gradually increases toward the onset of the light period, although the amplitudes differ significantly among the aquaporin isoforms. For example, for diurnally regulated OsPIP2;5 the mRNA level was 100 times and the protein level was 4 times the respective minimum levels during dark period (Sakurai-Ishikawa et al., 2011). However, up-regulation of root aquaporins during the light period was strongly inhibited when the shoots are exposed to over-humid condition (Sakurai-Ishikawa et al., 2011). These results suggest that daily re-synthesis of root aquaporins is regulated by the evaporative demand, which varies with weather conditions.

The aim of the present study is to evaluate the importance of the evaporative demand for root aquaporin expressions under
natural weather conditions. We investigated the day-to-day morning variation of aquaporin expression levels and its relation to the evaporative demand and other meteorological factors, and also the effect of daily weather on aquaporin diurnal expression patterns. We also investigated whether the evaporative demand affects root hydraulic conductivity under controlled laboratory conditions. We used potential evaporation (Ep) as an appropriate indicator of evaporative demand. Ep is defined as evaporation from a standardized wet-surface, and can be calculated from energy budget, using routine hourly data for air temperature, relative humidity, wind speed, solar radiation, and downward longwave radiation (Kondo and Xu, 1997; Xu and Haginoya, 2001; Xu et al., 2005). To the best of our knowledge, this is the first report of a close connection between aquaporin expression and a meteorological parameter under natural weather conditions.

2. Materials and Methods

2.1 Plant material

We used hydroponically grown rice seedlings (cv. Akitakomachi) for all experiments. Akitakomachi is a major cultivar grown in our study area, the Tohoku region of Japan.

2.2 Day-to-day variation of aquaporin expression levels at 8:00 a.m. and its relation to meteorological factors (Experiment 1)

To obtain homogeneous plant material which experienced the same growth condition, the seedlings were pre-grown in a growth chamber for 14 days including germination. The plants were then exposed into an open field 1 day before the roots are harvested for analysis of aquaporin expression as follows. Seeds were germinated in the dark for 4 days under 12 h 25°C/12 h 20°C temperature cycles. The 4-d-old seedlings were then transferred to a hydroponic culture solution as in experiment 1, and grown in a growth chamber (PGW36; Conviron, Winnipeg, Canada) with a 12-h of light period, air temperatures of 25/20°C (light/dark), and RH of 75% until 8-d-old. The 8-d-old plants were then transferred to the same experimental field as in experiment 1. The plants were grown in the field condition for 8 days, and the roots of 16-d-old plants (fourth leaf stage) were harvested. We monitored diurnal changes in the mRNA levels of the root aquaporin genes on 3 days with different weather conditions from 2 July to 4 July 2011.

2.3 Diurnal changes in the mRNA levels of the root aquaporin genes for 3 different weather days (Experiment 2)

Seeds were germinated in the dark for 4 days under 12 h 25°C/12 h 20°C temperature cycles, and the 4-d-old seedlings were transferred to the same hydroponic culture solution as in experiment 1, and grown in a growth chamber (PGW36; Conviron, Winnipeg, Canada) with a 12-h of light period, air temperatures of 25/20°C (light/dark), and RH of 75% until 8-d-old. The 8-d-old plants were then transferred to the same experimental field as in experiment 1. The plants were grown in the field condition for 8 days, and the roots of 16-d-old plants (fourth leaf stage) were harvested. We monitored diurnal changes in the mRNA levels of the root aquaporin genes on 3 days with different weather conditions from 2 July to 4 July 2011.

2.4 Analysis of meteorological data and calculation of potential evaporation

Routine hourly data, including Ta, RH, W speed, Sd, and Ld, were obtained from the meteorological station at the headquarter of NARO/TARC. Ep is defined as evaporation from a standardized wet surface (as mentioned in Introduction) and can be calculated from the energy budget equations (Kondo and Xu 1997; Xu et al., 2005). The standardized wet surface means a virtual surface where evaporation efficiency equals 1, the ground heat flux equals 0, and aerodynamic roughness is assumed to be 0.005 m. Ep has usually been evaluated as daily mean values, using daily mean meteorological data (Xu et al., 2005, Kuwagata et al., 2011). Because changes in gene expression in response to environmental cues occur within several hours, daily resolution is not adequate for studies of gene expression. Thus, in the present study, Ep was calculated as hourly mean value, because it can also be used as an hourly indicator of the evaporative demand.

Focusing the morning Ep after sunrise, we averaged the meteorological data for 4 h in the morning (4:00–8:00 a.m.) as shown in Fig. 1. Throughout the root sampling period (12 June to 11 August), air temperature gradually increased, ranging between 16.5 to 25.1°C, while other meteorological factors, such as Sd, Ld, W, RH, VPD and Ep, fluctuated day-to-day depending on the weather conditions. Throughout the period, RH was constantly high (79–99%), reflecting the fact that this was the rainy season in the Tohoku region of Japan. Ep had a variation pattern similar to that of Sd, and it fluctuated between 10 and 117 W m⁻².

2.5 RNA extraction and quantitative real-time RT-PCR analysis

We collected almost all roots deeper than 1 cm below the stem base. Roots were frozen immediately in liquid nitrogen and then ground with a mortar and pestle, and total RNA was extracted by using an RNeasy Plant Mini kit (Qiagen) according to the manufacturer’s instructions. First-strand cDNA was synthesized from 1.5 μg of total RNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The primers and probes were described by Sakurai-Ishikawa et al. (2011). Transcript levels of 9 OspPis and 4 OspTis and the amount of 18S rRNA (internal control) were determined by quantitative real-time RT-PCR performed with cDNA (diluted 1:50) using a StepOne Real-Time PCR System (Applied Biosystems). The absolute copy numbers of aquaporin mRNAs were evaluated using standard plasmids carrying individual aquaporin genes, as described previously (Sa-
2.6 Measurement of osmotic hydraulic conductivity of the root system $L_{pr}(os)$ under different conditions of evaporative demand (Experiment 3)

Rice seeds were grown hydroponically in a growth chamber under a 12 h light/12 h dark cycle (photosynthetic photon flux density of 400 μmol s$^{-1}$ m$^{-2}$) at 25/20°C and RH of 75%. The 15-d-old plants (fourth leaf stage) were separated into 2 groups, and were exposed to either low (RH > 95%, wind speed 0.01 m s$^{-1}$) or high (RH 40–50%, wind speed 1.5–2.5 m s$^{-1}$) evaporative demand immediately before the onset of the light period (450–500 μmol s$^{-1}$ m$^{-2}$). Here, wind speed was measured by hot-wire anemometer (Kanomax Anemomaster Model 6071, Nihon Kagaku Kogyo, Osaka, Japan). Four h after exposure of the shoot to low or high evaporative demand, $L_{pr}(os)$ was measured according to the method of Sakurai-Ishikawa et al., (2011). Shoots were removed by a razor blade at the root base, and xylem sap was collected into pre-weighed cotton, and its volume was calculated from the change in the cotton weight. Xylem sap was discarded during the first 5 min, and then the sap was collected for the following 20 min for calculating the $L_{pr}(os)$.

Xylem sap from other seedlings (15-d-old plants) was collected simultaneously by a micropipette. Xylem sap from 10–15 plants was combined on each measurement to obtain sufficient volume (> 20 μL) for osmotic potential measurements. Osmotic potential of the hydroponic medium and xylem sap exudate was determined by using a VAPRO 5520 osmometer (Wescor Inc., Logan, UT, USA).

We calculated $L_{pr}(os)$ (m MPa$^{-1}$ s$^{-1}$) using the following equation:

$$L_{pr}(os) = J_V / \sigma \Delta \Psi_s$$  \hspace{1cm} (1)

where $J_V$ is the volumetric xylem sap flow rate per unit root surface area (m$^2$ m$^{-2}$ s$^{-1}$), $\sigma$ is the reflection coefficient for nutrient salts in the xylem, and $\Delta \Psi_s$ is the difference in osmotic potential (MPa) between the xylem sap exudate and the hydroponic solution (~0.0077MPa). The $\sigma$ value of 0.4 was used in the present study, because Miyamoto et al. (2001) estimated it as 0.4 for rice roots. Total root surface area was measured using the WinRHIZO software (Regent Instruments, Inc., Quebec City, QC, Canada) after measuring the volumetric xylem sap flow rate.

3. Results

3.1 Day-to-day variation of aquaporin expression levels and its relation to meteorological factors (Experiment 1)

We focused on the relationship between mRNA levels of each
aquaporin and weather conditions in the morning. We took 23-day data set from June to August (Figs 1, 2 and 3). Among 33 rice aquaporin genes, we investigated 9 PIPs (OsPIP1;1, OsPIP1;2, OsPIP1;3, OsPIP2;1, OsPIP2;2, OsPIP2;4, OsPIP2;5 and OsPIP2;6) and 4 TIPs (OsTIP1;1, OsTIP1;2, OsTIP2;1 and OsTIP2;2), because these were abundantly expressed in the roots (> 0.1 copies per pg of total RNA; Sakurai et al. 2008; Sakurai-Ishikawa et al. 2011; Fig. 2).

Three days with the highest and the lowest Ep (from 4:00 to 8:00 a.m.) were chosen from the 23-day data, and the abundance of aquaporin transcripts was compared (Fig. 2). The cumulative transcript number of all 13 aquaporin genes of high-Ep days was 1.52 times higher than that on low-Ep days. The mRNA levels of 5 PIPs (OsPIP1;2, OsPIP1;3, OsPIP2;1, OsPIP2;4 and OsPIP2;5) and 1 TIP (OsTIP2;1) were significantly (p < 0.05) higher on the high-Ep days than on the low-Ep days (Fig. 2). The mRNA level of OsPIP2;1 was the highest among all aquaporin transcripts regardless of Ep. OsPIP2;5 had the highest ratio (5.21) of the mRNA levels on high-Ep to those on low-Ep days (Fig. 2, Table 1).

The day-to-day variation of the OsPIP2;2 transcript level at 8:00 a.m. and its relation to meteorological factors (averaged over 4:00–8:00 a.m.) are shown in Fig. 3. The OsPIP2;2 transcript level was higher on sunny weather days and lower on rainy and cloudy days, and its day-to-day fluctuation paralleled Ep (Fig. 3a). The R² value (0.74, p < 0.01) indicated that 74% of the day-to-day variance for OsPIP2;2 could be explained by Ep (Fig. 3b). A significant correlation was also found between the OsPIP2;2 level and relative humidity (RH), although the R² value (0.33, p < 0.01) was lower than that for Ep (Fig. 3c). No significant correlation was observed between the OsPIP2;5 level and air temperature (R = 0.064, p > 0.05; Fig. 3d). There was high correlation between Ep and solar radiation (R = 0.98, p < 0.01; Fig. 3e), but not between Ep and air temperature (R = −0.0063, p > 0.05, data not shown). Correlation of the OsPIP2;5 level to Sd was high (R = 0.88, p < 0.01, data not shown), as much as that to Ep (R = 0.86, p < 0.01, Fig. 3b).

Correlation coefficients between aquaporin expression levels at 8:00 a.m. and Ep are summarized in Table 1. Among 13 aquaporins examined, positive and significant (p < 0.05) correlation was observed in 5 PIPs (OsPIP1;2, OsPIP1;3, OsPIP2;1, OsPIP2;4, OsPIP2;5) and 1 TIP (OsTIP2;1). All of them, except OsPIP1;2 and OsPIP2;1, are highly root specific (Sakurai et al., 2005; Sakurai et al., 2008; Sakurai-Ishikawa et al., 2011; Kuwagata et al., 2012). The highest correlation was observed in OsPIP2;5 (R = 0.86, p < 0.01). In contrast, no significant correlation was observed for OsPIP1;1, OsPIP2;2 and OsTIP1;1 and OsTIP1;2. A significant negative correlation with Ep was found for OsPIP2;6 and OsTIP2;2.

3.2 Diurnal changes in the aquaporin expression levels under natural weather conditions (Experiment 2)

The relationship between the diurnal amplitude and responsiveness to Ep for each aquaporin isoform are shown in Fig. 4. We plotted the amplitude data from Sakurai-Ishikawa et al. (2011) against the correlation coefficients between the expression levels and Ep (Table 1). As shown in Fig. 4, aquaporins with higher correlation to Ep, such as OsPIP1;3, OsPIP2;4 and OsPIP2;5, had higher diurnal amplitude of their expression levels. This relationship suggests that Ep is the dominant factor determining the diurnal amplitude of these aquaporins in rice roots.
To examine this idea under field conditions, we monitored the diurnal changes in mRNA levels of the root aquaporin genes for 3 days with different weather. In this experiment, rice seedlings were grown hydroponically under field conditions for 8 days until root harvest. (Plant age: 16-d-old on the harvest day.) Typical results for root-specific (OsPIP2;4, OsPIP2;5 and OsTIP2;1) and other (OsTIP1;1 and OsTIP2;2) aquaporins are shown in Fig. 5. Diurnal amplitude of the transcript levels of root-specific aquaporins (OsPIP2;4 and OsPIP2;5) was affected by weather of the day (Fig. 5a, b). The mRNA level was the highest at 10:00 a.m. when Ep was high (2 July, sunny day), but was generally low (3 July, cloudy; 4 July, rainy day) when Ep was low. A similar tendency was observed for other aquaporins including OsTIP2;1 (Fig. 5c). OsPIP1;2, OsPIP1;3 and OsTIP1;2 (data not shown), although the diurnal amplitude was smaller than that of OsPIP2;4 and OsPIP2;5 (see also Fig. 6). In contrast, there was no clear relationship between Ep and the mRNA levels of OsTIP1;1 (Fig. 5d), OsTIP2;2 (Fig. 5e) and other aquaporins, including OsPIP1;1, OsPIP2;2 and OsTIP2;6 (data not shown). Relationship between the amplitude of diurnal changes in the mRNA level of each aquaporin on a sunny weather day (2 July; Y-axis) and that observed in a growth chamber (Sakurai-Ishikawa et al., 2011; X-axis) are shown in Fig. 6. Strong correlation between these 2 parameters ($R^2 = 0.97$) confirmed that the order of the diurnal amplitude among root aquaporins in growth chamber experiments is consistent with that observed on a sunny weather day in the field experiment.

3.3 Effect of evaporative demand on root hydraulic conductivity (Experiment 3)
Plants were exposed to low or high (control plants) evaporative demand at the onset of the light period, and the treatment was continued for 4 h. The volumetric xylem sap flow rate per unit root surface area ($J_P$) of plants exposed to high evaporative demand was 1.3 times that of the control plants exposed to low evaporative demand (Fig. 7). A $J_P$ of the control plants was 3.2 times that of the plants exposed to high evaporative demand. $L_P(\cos)$ of the plants exposed to the high evaporative demand was significantly 4.2 times higher than that of control plants. (Fig. 7).

4. Discussion

4.1 Ep is a dominant factor determining the diurnal variation in aquaporin expression levels in the roots
We hypothesized that aquaporin expression in rice roots is strongly regulated by evaporative demand, which fluctuates with daily weather conditions. Our observations of young rice seedlings support this hypothesis. Among various meteorological parameters, Ep explained well day-to-day morning fluctuation of the mRNA levels of 7 of 13 aquaporins expressed in roots (in particular OsPIP2;5) (Figs. 2 and 3, Table 1). We are surprised that the correlation between Ep and aquaporin expression was significant ($p < 0.01$) in a narrow and high range (79 to 99%) of relative humidity (Table 1, Figs. 1, 3b, 3c). Aquaporin isoforms with higher responsiveness to Ep also exhibited higher diurnal amplitude of their expression levels (Figs. 3–5). Thus, diurnal induc-

<table>
<thead>
<tr>
<th>Aquaporin name</th>
<th>$R$ to Ep</th>
<th>$R$ to RH</th>
<th>Expression ratio for High Ep days / Low Ep days</th>
<th>Organ specific localization</th>
<th>Water activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>OsPIP1;1</td>
<td>0.12 ns</td>
<td>−0.30 ns</td>
<td>1.26</td>
<td>Constitutively expressed in various organs including root, leaf, node, internode and panicle</td>
<td>Low</td>
</tr>
<tr>
<td>OsPIP1;2</td>
<td>0.59 **</td>
<td>−0.50 *</td>
<td>1.86</td>
<td>Constitutively expressed in various organs including root, leaf, node, internode and panicle</td>
<td>Low</td>
</tr>
<tr>
<td>OsPIP1;3</td>
<td>0.75 **</td>
<td>−0.57 **</td>
<td>1.97</td>
<td>Highly root specific</td>
<td>Not examined</td>
</tr>
<tr>
<td>OsPIP2;1</td>
<td>0.46 *</td>
<td>−0.46 *</td>
<td>1.40</td>
<td>Constitutively expressed in various organs including root, leaf, node, internode and panicle</td>
<td>Low</td>
</tr>
<tr>
<td>OsPIP2;2</td>
<td>0.17 ns</td>
<td>−0.52 *</td>
<td>1.32</td>
<td>Abundant in root, leaf and panicle</td>
<td>High</td>
</tr>
<tr>
<td>OsPIP2;3</td>
<td>0.39 +</td>
<td>−0.56 **</td>
<td>1.49</td>
<td>Highly root specific</td>
<td>High</td>
</tr>
<tr>
<td>OsPIP2;4</td>
<td>0.65 **</td>
<td>−0.49 *</td>
<td>2.79</td>
<td>Highly root specific</td>
<td>High</td>
</tr>
<tr>
<td>OsPIP2;5</td>
<td>0.86 **</td>
<td>−0.57 **</td>
<td>5.21</td>
<td>Highly root specific (particulary in endodermis)</td>
<td>High</td>
</tr>
<tr>
<td>OsPIP2;6</td>
<td>−0.53 **</td>
<td>0.11 ns</td>
<td>0.49</td>
<td>Highly accumulated in elongating internode and leaf sheath</td>
<td>Not examined</td>
</tr>
<tr>
<td>OsTIP1;1</td>
<td>−0.07 ns</td>
<td>−0.14 ns</td>
<td>1.14</td>
<td>Abundant in various organs including root (particularly in outer part of roots), leaf, node, internode and panicle</td>
<td>Low</td>
</tr>
<tr>
<td>OsTIP1;2</td>
<td>0.28 ns</td>
<td>−0.25 ns</td>
<td>3.01</td>
<td>Highly leaf specific</td>
<td>Low</td>
</tr>
<tr>
<td>OsTIP2;1</td>
<td>0.66 **</td>
<td>−0.61 **</td>
<td>2.23</td>
<td>Highly root specific</td>
<td>Not examined</td>
</tr>
<tr>
<td>OsTIP2;2</td>
<td>−0.55 **</td>
<td>0.22 ns</td>
<td>0.50</td>
<td>Highly accumulated in elongating internode and leaf sheath</td>
<td>High</td>
</tr>
</tbody>
</table>
tion of these aquaporin genes can mainly be explained by the evaporative demand.

4.2 Ep as a good indicator for evaporative demand

The evaporative demand fluctuates dynamically, depending on the variation in several meteorological factors, such as solar radiation, air humidity, temperature, wind speed and longwave radiation. The evaporative demand increases with increasing solar radiation, with decreasing relative humidity, and with increasing wind speed. For instance, the evaporative demand is increased by a “foehn wind” (strong, dry and warm wind), which sometimes causes white head (severe damage of rice plants in the field at the heading stage) due to water stress (Hirasawa 2000). Ep is a direct indicator of the evaporative demand, which can be evaluated from these meteorological factors (Kondo and Xu, 1997; Xu et al., 2005).

It should be noted that Ep was strongly correlated to solar radiation (Sd) under our experimental conditions (Fig. 3e), although correlation between Ep and air temperature is low as described above. The close correlation between Ep and Sd was probably due to the weather conditions in the morning, when the contribution of day-to-day variation of other meteorological factors (which also strongly affect Ep) was relatively small at least

Fig. 3. Relationship between OsPIP2;5 expression level in rice roots at 8:00 a.m. and meteorological factors averaged for 4:00–8:00 a.m.

a, Day-to-day variations in the OsPIP2;5 mRNA levels and Ep. Weather at 8:00 a.m. is indicated by symbols. b–d, Correlation between OsPIP2;5 expression level and Ep (b), relative humidity (c) and air temperature (d). (e), Relationship between solar radiation and Ep. **, Correlation coefficient is significant at 1% level.
in this study (Fig. 1). This arises the question of whether the evaporative demand as such, or increased light intensity (solar radiation) induces aquaporin expression. Our previous study showed that shoot exposure to high humidity at a constant light level abolished the dynamic induction of root aquaporins during the light period (Sakurai-Ishikawa et al., 2011). Therefore, we consider that difference in the light intensity not be the most important factor to explain the day-to-day variation of root aquaporin expression levels in rice. This is also supported by the data of study by Laur and Hacke (2013), who showed that altering relative humidity without changing irradiance triggered up-regulation of PIP expression (and root water flow) in hybrid popular sapling roots. Future investigation is required to clarify whether there are any contributions of increase in the light intensity to root aquaporin expressions.

According to the reason as wrote above, we hypothesize that change in the plant water status, rather than the light intensity, functions as a signal to induce gene expression of root specific aquaporins. The shoot to root signaling mechanism is an important issue for future studies. By using a method to apply artificial pressure to the root, we are now testing a possibility whether do the changes in xylem water potential affect aquaporin expressions under intense transpiration from the shoot. We expect that these experiments will help to elucidate the long-distance shoot to root signaling mechanism involved in plant water relations.

Up-regulation of root aquaporins depending on an increased evaporative demand has directly been reported for a limited number of plant species, including rice (Sakurai-Ishikawa et al., 2011; Kuwagata et al., 2012), Arabidopsis (Levin et al., 2009) and hybrid popular (Almedia-Rodriguez et al., 2011; Laur and Hacke, 2013). Close relation between aquaporin abundance in the roots and transpiration (or stomatal conductance) has recently been suggested in grapevine (Gambetta et al., 2012, Perrone et al., 2012). Thus, Ep may be one of the major environmental stimuli to induce root aquaporin expression, not only in rice but in a wide variety of herbaceous and woody plant species.

4.3 Physiological significance of aquaporin response to Ep

In the present study, hydraulic conductivity (Lp,os) of rice seedlings exposed to high-evaporative demand for 4 h was 4 times that of seedlings exposed to low-evaporative demand. This result is line with previous reports for other plants, including hybrid popular (Almeida-Rodriguez et al., 2011), Quercus fusiformis (McElrone et al., 2007), Bunelia lannuginosa (McElrone et al., 2007) and wheat (Kudoyarova et al., 2011). McElrone et al., 2007 found that hydraulic conductivity of fine roots in common tree species in Edwards Pleateau of central Texas fluctuates diurnally with peaks corresponding to the period of highest transpiration demand (evaporative demand) at midday, and this cycling ceased during whole-tree shading.

Since increased Lp,os in rice roots was associated with the increase in the abundance of aquaporin transcripts in the roots, this suggests that changes in aquaporin levels caused by increased Ep lead to changes in hydraulic conductivity. Even in rice plants growing in submerged soil, leaf stomatal conductance and the photosynthetic rate increase with high evaporative demand because of the development of a water deficit on sunny mid-days (Ishihara and Saito, 1987; Hirawasa et al., 1992). Stomatal conductance under intense transpiration rate depends on the water uptake ability of the roots (Jiang et al., 1988; Hirawasa et al., 1992; Taylaran et al., 2011), and the increase in the root hydraulic conductivity via up-regulation of aquaporins could be an important factor for increasing photosynthesis and dry matter pro-

![Fig. 4.](image) Relationship between the correlation coefficient R (aquaporin mRNA level vs. Ep in rice roots under field conditions) and the diurnal amplitude of aquaporin expression levels at mRNA (a) and protein (b) levels evaluated in growth chamber experiments.

Correlation coefficients (X-axis) were obtained in the present study (Table 1). The diurnal amplitude data (Y-axis) are from Sakurai-Ishikawa et al. (2011), where rice plants were hydropponically grown in a growth chamber (12h light/12 h dark cycle, 25/20°C, 75% relative humidity, PPFD of 370μmol s−1 m−2). a, mRNA was quantified by real-time PCR (for the values, see Table 1 in Sakurai-Ishikawa et al. 2011); the amplitude of diurnal changes of OsPIP2;6, OsTIP1;1 and OsTIP2;2 was small, and here we considered it as 1 (triangles). b, Protein levels were evaluated by immunoblotting with antibodies specific to each isoform (Fig. 7 in Sakurai-Ishikawa et al. 2011). The amplitude was calculated as the ratio of the band intensity 6 h after the onset of the light period to that 3 h before the onset of the light period.
M. Murai-Hatano et al.: Evaporative demand and aquaporins in rice roots

...duction, thereby resulting in higher crop yield.

While the present study focused on the roots, there is a possibility that shoot hydraulics is also affected by aquaporins in leaves, as suggested by Laur and Hacke (2013). In our previous study (Kuwagata et al., 2012) on rice grown hydroponically in controlled growth chambers, exposure of the shoots to low air humidity induced coordinated up-regulation of aquaporin genes not only in the roots, but also in the leaf blades. We are currently investigating how rice plants growing in paddy fields perceive daily weather signals and then regulate gene expression in shoots and...
roots for daily optimization of whole-plant hydraulics.

4.4 Individual roles of root aquaporins

All 33 aquaporin isoforms encoded in the rice genome (Sakurai et al., 2005) have been characterized in terms of their abundance, location, gene expression in response to environmental factors, and water transport activity (Sakurai et al., 2005; Sakurai et al., 2008; Sakurai-Ishikawa et al., 2011; Muto et al., 2011; Ahamed et al., 2012; Kuwagata et al., 2012). These results suggest that each aquaporin plays an individual role in plant water relations or the transport of various solutes. Among 13 PIP and TIPs isoforms in rice roots (Fig. 2), 3 PIPs (OsPIP1;3, OsPIP2;4, OsPIP2;5) and OsTIP2;2 are highly root-specific (Sakurai et al., 2005, 2008; Sakurai-Ishikawa et al., 2011; summarized in Table 1). These aquaporins probably share a common role with respect to regulating root hydraulic conductivity in response to evaporative demand, as evidenced by their high responsiveness to Ep (Table 1).
OsPIP2;5 showed the highest correlation to Ep. OsPIP2;5 also showed the highest correlation to the diurnal pattern in root hydraulic conductivity (Sakurai-Ishikawa et al., 2011), to acclimation of root hydraulic conductivity under continuous low root temperature (Ahamed et al., 2012), and to down- and up-regulation of root hydraulic conductivity during and after nitrogen deprivation (Ishikawa-Sakurai et al., 2014). Overall, these results suggest that OsPIP2;5 plays a crucial role in regulating water uptake across roots in a fluctuating environment. This idea is also supported by high water transport activity of OsPIP2;5 (Sakurai et al., 2005, 2008), and by its predominant accumulation in the root endodermis (Sakurai-Ishikawa et al., 2011; see Table 1). Root endodermis is important for cell-to-cell water movement because apoplastic water movement is blocked at the endodermis by Casparian bands (Steudle 2000), and the endodermis is the rate-limiting site for radial water movement in the rice roots (Ranathunge et al., 2003). These results suggest that OsPIP2;5 is responsible for the regulation of cell-to-cell water movement from the endodermis to the central cylinder.

OsPIP2;1 has the most abundant aquaporin transcripts in the roots, although the responsiveness to Ep is moderate (Fig. 2, Table 1). The water transport activity of OsPIP2;1 is high and similar to that of OsPIP2;5 (Sakurai et al., 2008). The OsPIP2;1 was detected not only in the roots, but also in various organs such as leaf blades (Sakurai et al., 2005, 2008; Sakurai-Ishikawa et al., 2011), internodes, nodes, flowers and maturing seeds (H. Hayashi et al., unpublished data). Thus, OsPIP2;1 possibly plays a constitutive role in whole-plant water relations.

Several aquaporins, including, OsPIP2;6 and OsTIP2;2, showed no correlation or even a negative correlation with Ep (Table 1). The day-to-day fluctuations in the expression levels of OsPIP2;6 and OsTIP2;2 were very similar (R = 0.92 between OsPIP2;6 and OsTIP2;2), suggesting that expression of these aquaporins is regulated by the same environmental stimuli. The transcript abundance of both aquaporins was positively correlated with the downward longwave radiation, Ld (R = 0.68 for OsPIP2;6 and R = 0.79 for OsTIP2;2). The Ld value is high under cloudy, humid, or warm conditions. Since plant cell elongation is promoted under such environmental conditions, we hypothesize that OsPIP2;6 and OsTIP2;2 play a role in plant growth. The transcripts of these aquaporins are particularly abundant in the elongation zones of internodes at the heading stage (H. Hayashi et al., unpublished data). Upon submersion of deepwater rice, the OsTIP2;2 expression in internodes increases markedly at both mRNA and protein levels, and is associated with rapid inter-node elongation (Muto et al., 2011).

Aquaporins are regulated by some additional mechanisms, such as co- and post-translational modifications, channel gating, tetramer assembly, and cellular trafficking (Maurel et al., 2008). In the present study, we focused on aquaporin abundance. Whether the above mechanisms are also involved in the overall hydraulic adjustment of plants to Ep is an exciting and important topic for future studies.

5. Conclusion

Among the various meteorological factors, we found that the transcript levels of the root-specific aquaporins in the morning are highly correlated with the evaporative demand as evaluated by potential evaporation (Ep). The aquaporins with a higher correlation to Ep showed higher diurnal amplitude of their expression levels. Thus, diurnal variation in the aquaporin expression levels appears to be induced by the atmospheric evaporative demand; in other words, rice plants sense daily weather and respond to it by adjusting gene expression in the roots.

Acknowledgements

The authors are grateful to Prof. Keiichi Nakayama (Chiba University), Prof. Shizuo Yoshida (Hokkaido University), Prof. Masayoshi Maeshima (Nagoya University) and Prof. Junsei Kondo (Tohoku University) for stimulating this study. We are also grateful to Dr. Kaori Sasaki (NARO/ARC) and Dr. Yoshikazu Kawakata (NARCT) for providing meteorological data for this study. We also thank Dr. Mitsoshi Masaki (Akitsu Keioskku, Japan) for technical supports for the experiments using growth chambers. We also thank Dr. Yoshiaki Nagamura (NIAS) for suggestions on this study, which is valuable for our future work. We also thank reviewers on this manuscript for helpful comments and suggestions.

This study was partially supported by the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN, to M. M.-H.)., Grants-in-Aid from the Ministry of Education, Sports, Culture, Science and Technology of Japan (24570034 and 25292152) to M. M.-H. and K. T., and NARO Gender Equality Program to M. M.-H.

References


Hirasawa, T., 2000: Panicle dehydration causing a white head of paddy rice under the conditions of high temperature, low humidity and high wind velocity. *The Hokuriku Crop Science*, **35**, 81–82.


**Abbreviations:**

- $E_p$, potential evaporation
- $J_v$, volumetric xylem sap flow rate per unit root surface area
- $L_d$, downward longwave radiation
- $L_{p,(os)}$, osmotic hydraulic conductivity of root system
- NIP, Nod26-like intrinsic protein
- PIP, plasma membrane intrinsic protein
- RH, relative humidity
- $S_d$, solar radiation
- SIP, small basic intrinsic proteins
- $T_a$, air temperature
- TIP, tonoplasf intrinsic protein
- $V_PD$, vapor pressure deficit
- $W$, wind speed
- XIP, uncharacterized X intrinsic proteins