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ORIGINAL PAPER

Effects of abiotic stress, light, phytochromes and phytohormones on the expression of *OsAQP*, a rice aquaporin gene

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Abstract Water uptake across cell membranes is a principal requirement for plant growth at both the cellular and whole-plant levels. Water movement through plant membranes is regulated by aquaporins. We examined the expression of the *OsAQP* gene, encodes a tonoplast intrinsic protein (TIP), which was isolated from a rice panicle cDNA library. Semi-quantitative RT-PCR revealed that the gene was ubiquitously expressed in rice roots and leaves. Expression of the gene was up-regulated by drought, salinity and cold in leaves, down-regulated by these abiotic factors in roots, and the gene was also induced by the phytohormones gibberellic acid and abscisic acid in both leaves and roots. Expression of the gene was inhibited by salicylic acid, especially in roots. White light decreased levels of *OsAQP* transcript, whereas blue light increased expression of the gene. Given that the *OsAQP* gene is strongly expressed in response to drought, salinity, cold, abscisic acid and gibberellic acid, we propose that *OsAQP* is a stress-induced gene and that it plays an essential role in the defense of rice against several stresses.

Keywords Rice · OsAQP gene · Abiotic stress · Phytohormones · Light · Phytochromes · Expression profile

Introduction

Aquaporins (AQPs) are integral membrane proteins that occur in both prokaryotic and eukaryotic cells (Agre et al. 1995). They serve as channels that permit the rapid bidirectional movement of water through cellular membranes. In higher plants, AQPs consist of seven subfamilies: the plasma membrane intrinsic proteins (PIP), tonoplast intrinsic proteins (TIP), NOD26-like intrinsic proteins (NIP), small basic intrinsic proteins (SIP), GlpF-like intrinsic protein (GIP) from moss *Physcomitrella patens*, hybrid intrinsic protein (HIP) and X intrinsic proteins (XIP) (Bienert et al. 2011). Whereas some AQPs behave as 'strict' water channels, several have also been reported to transport physiologically important molecules, such as boron, silicon, NH_3, H_2O_2, and CO_2. Plant AQPs are involved in various physiological processes by regulating rapid transportation of water, including stomatal movement, seed germination, cell division, and cell elongation (Chaumont et al. 2005; Bienert et al. 2008; Boursiac et al. 2008; Eisenbarth and Weig 2005).

The rice genome encodes 33 AQPs, including 10 tonoplast intrinsic proteins (TIPs) (Sakurai et al. 2005). Expression and transport functions of several TIP isoforms have been reported (Liu et al. 1994; Takahashi et al. 2004; Li et al. 2008; Forrest and Bhave 2008). However, the function of each individual TIP isoform and the integrated function of TIPs under various physiological conditions remain elusive. Previously, a rice AQP gene *OsAQP* (GenBank accession number EF495246), which encodes a tonoplast intrinsic protein, was isolated by screening a cDNA library prepared from young rice panicles (Liu and Liang 2008). Sequence alignment showed that it was identical to *OsTIP1;1* in the cDNA coding region, but lacked a 44-bp sequence present within the 3′ untranslated region (UTR) of *OsTIP1;1*. It is known that TIP activity is regulated by developmental cues as well as environmental signals, both at the transcriptional and the post-transcriptional levels (Li et al. 2008). Analysis of TIP expression in

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response to abiotic stresses from *Arabidopsis* and maize indicated that most TIPs were repressed by drought and salinity (Zhu et al. 2005; Alexandersson et al. 2005). Several reports have suggested that TIPs mediate water exchange between the cytoplasm and vacuolar compartments and might be involved in nearly all vacuolar functions. Nonetheless, reports on the regulation of *OsTIP1;1* (also named as γ-Tip1) gave inconsistent, and sometimes even opposite, results (Liu et al. 1994; Forrest and Bhave 2008). The response of TIPs to environmental factors, such as abiotic stress, light and exogenous phytohormones, has yet to be systematically analyzed.

We have studied the expression of *OsAQP*, which was not previously described. The gene is ubiquitously expressed in rice leaves and roots, and the abundance of *OsAQP* transcripts in the guard cells of rice leaves, which was verified by RNA in situ hybridization (Liu et al. 2008), raised the necessity of characterizing the new rice TIP in order to understand the water transport mechanism mediated by OsAQP in rice stomatal regulation. In the present work, we have focused on the expression profile of the TIP isoform that was confirmed to be expressed throughout rice development, and the molecular basis of the physiological response to various environmental factors. We used semi-quantitative RT-PCR to investigate *OsAQP* expression in leaves and roots under different stresses, as well as in phytochrome mutants under different light conditions. We discuss the significance of these expression patterns in the response of rice to environmental stresses.

Materials and methods

Plant materials

The *japonica* Rice (*Oryza sativa*) cultivar Nipponbare was used in this study. The rice phytochrome mutants phyA, phyB and phyAphyB are all Nipponbare background. The mutants seeds were kindly provided by XIE Xian-zhi from Shandong Academy of Agricultural Sciences.

Enzymes and reagents

Taq DNA polymerase, RNAprep Total RNA Extraction kit and DNA Marker were purchased from TIANGEN, MMLV First Strand cDNA Synthesis kit was purchased from Promega.

Plant treatments

Rice was cultured hydroponically in a phytotron with photon flux density of 350–400 μmol/m^2/s, 16/8 h day–night, 28 °C and 60–80 % relative humidity. Shoots and roots at 5, 10, 15 days by blue light (400–550 nm) and darkness treatment, 7 days by red light (620–770 nm) and darkness treatment were collected. Plants at the three-leaf stages were treated with salt (addition of 1 % NaCl in the hydroponic culture medium), cold (exposure of plants to 4 °C) and drought (addition of 30 % polyethylene glycol 6000 in the hydroponic culture medium), followed by sampling at the designated time. The phytohormones treatment were 100 μmol/L abscisic acid (ABA), 5 mg/L gibberellic acid (GA), and 500 μmol/L salicylic acid (SA) also at the three-leaf stages. Rice shoots and roots were sampled at every 0.5–2 h during treatments.

Total RNA extraction and the first chain cDNA synthesis

Total RNA were isolated from rice leaves and roots using RNAprep Total RNA Extraction kit (TIANGEN). cDNA templates were synthesized using MMLV First Strand cDNA Synthesis kit (Promega) according to the manufacturer's instructions. 1 μg total RNA were used to synthesized the cDNA first strand which initiated with the Oligo$(dT)_{18}$ primer.

Effects of abiotic stresses

After abiotic stress treatment, the cDNA of these samples treated with NaCl, PEG6000 and 4 °C, respectively at 0, 1, 3, 6 and 12 h were obtained using the method described above. The constitutively expressed rice actin gene *OsAct1* was used to normalize samples. The primers of *OsAct1* were P1 (5′-CATGCTATCCCTCGTCTCGACCT-3′) and P2 (5′-CGCACTTCATGATGGAGTTGTAT-3′). The PCR was carried out as follows: predenaturation at 94 °C for 3 min; then 23 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min; and the final extension at 72 °C for 7 min. The primers of *OsAQP* were P3 (5′-AGCCTTCTGCTCAACCTATC-3′) and P4 (5′-CACCGAACCAACTGCTTTAC-3′). The thermocycler program had an initial 94 °C denaturation step followed by 24 cycles consisting of denaturation at 94 °C for 45 s, annealing at 58 °C for 30 s, and extension at 72 °C for 30 s, and then with a final extension at 72 °C for 5 min. 10 μl PCR products were electrophoresed on 1.5 % agarose gel. All treatments were repeated three times with similar results.

Effects of phytohormones

The rice leaves and roots after treated with plant hormones were collected as followed, 0, 0.5, 1, 1.5 and 2 h after treated with ABA; 0, 2, 5, 10 and 24 h after treated with SA; 0, 1, 6, 12 and 18 h after treated with GA3. The RT-PCR was conducted as described above, and the PCR

amplification cycles were 26 and 28 for *OsAct1* and *OsAQP,* respectively.

Effects of light and photoreceptors

The blue light treated rice leaves and roots were collected at 5, 10 and 15 days, the RT-PCR were conducted as described above. The rice leaves of phytochrome mutant phyA, phyB and phyAphyB in 7 days were collected after treated, the internal control was *ubiquitin (UBI)*,the primer were P5 (5′-ATCACGCTGGAGGTGGAGT-3′) and P6 (5′-AGGCCTTCTGGTTGTAGACG-3′). The PCR was carried out as follows: predenaturation at 94 °C for 3 min; then 23 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min; and the final extension at 72 °C for 3 min. The RT-PCR was conducted as described above.

Results

Sequence analysis of *OsAQP*

The 945-bp *OsAQP* cDNA comprises a 753-bp open reading frame (ORF), an 18-bp 5′-UTR, and a 174-bp 3′-UTR. Blast search and multiple alignment results showed that *OsAQP* and *OsTIP1;1* contain the same cDNA coding region, the sequence difference between them being a 44-bp sequence (5′-AACTGTGCATGCATTTGCCTGAG TTCCTTCGTTTTTTCCTAGTC-3′) that is present only in the 3′-UTR of *OsTIP1;1*, and may result from tissue-specific or developmentally regulated differences in splicing. A BLAST search of the rice genome database revealed that the 1,507-bp coding region of the gene maps to chromosome 3, and that the gene comprises two exons and one intron. The OsAQP protein is predicted to encode 250 amino acids, with a molecular mass of 25.7 kDa and a pI of 6.02, as predicted using the ExPASy—Compute pI/Mw tool (http://www.expasy.org/tools/pi_tool.html). Two conserved NPA motifs, which are characteristic of plant AQPs, are found at amino acid positions 85–87 and 198–200.

Abiotic stresses on the expression of *OsAQP*

To analyze *OsAQP* expression under conditions of abiotic stress, rice seedlings were exposed to 1 % NaCl (high salt), 30 % PEG (simulated drought) and 4 °C (low temperature), respectively. Levels of *OsAQP* mRNAs in seedling subjected to these treatments were detected using semi-quantitative RT-PCR (Fig. 1). Time-course assays showed that the expression of the gene was up-regulated by drought, salt and cold in leaves, with a noticeable strong accumulation in 3 h after salt treatment, but the levels of *OsAQP* transcript in roots was all decreased following exposure to these stresses.

Phytohormones and the expression of *OsAQP*

Given the well-documented roles of phytohormones in responses to various stimuli, we next investigated the effects of ABA, GA and SA on levels of *OsAQP* transcripts. As shown in Fig. 2, levels of *OsAQP* mRNA were significantly induced by ABA, with transcript accumulation evident within 0.5 h of treatment. A similar quickly change occurred in leaves and roots treated with GA, but *OsAQP* expression in leaves and roots declined 1 h after the treatment, and then recovered slowly, reaching a maximum around 18 h after treatment. But the response to SA seems much slower, levels of *OsAQP* mRNAs in roots declined significantly up to 24-h after the treatment, but no obviously change observed in leaves.

Our observations that *OsAQP* was up-regulated by ABA and GA, but down-regulated by SA, suggest that *OsAQP* may be involved in stress responses controlled by the ABA, GA and SA signaling pathways.

Effects of light quality on the expression of *OsAQP* and the roles of phytochrome photoreceptors

Light is a critical environmental factor for plant growth and development, and the phytochrome and cryptochrome photoreceptors are critical for perceiving the quantity and

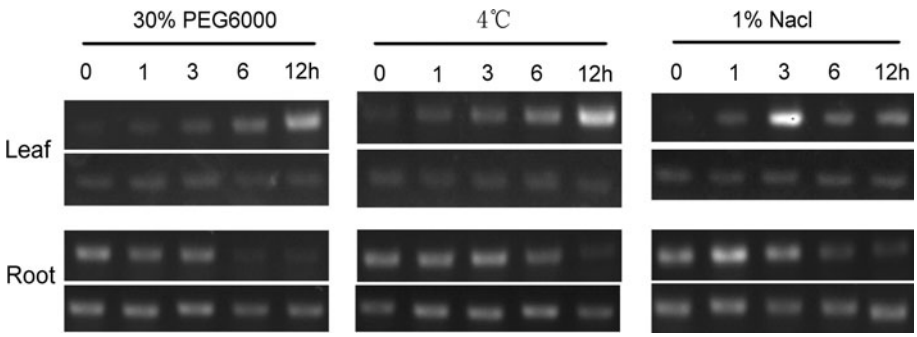

Fig. 1 Expression profile of *OsAQP* gene (*upper panel*) under abiotic stress, rice actin (*lower panel*) was used as a loading control. In salt, PEG and cold treatments, the leaves and roots were separately analyzed

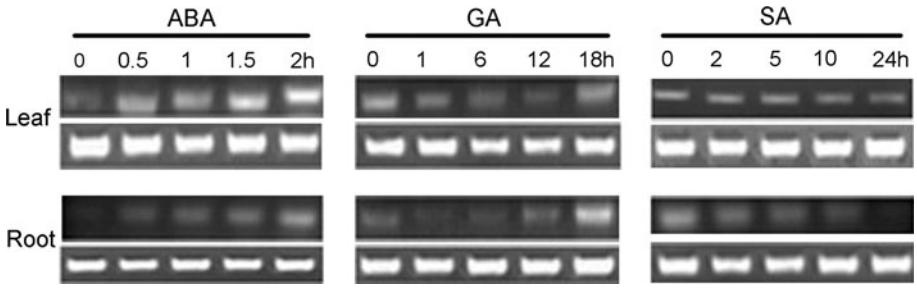

Fig. 2 Expression of the *OsAQP* gene (*upper panel*) in leaves and roots following ABA, SA and GA treatments. Rice actin (*lower panel*) was as a loading control

spectral quality of light. To investigate the regulation of *OsAQP* expression by light, we analyzed the distribution of *OsAQP* mRNAs under various light conditions and compared the expression profiles in wild-type rice with those of three phytochrome mutants. We analyzed the accumulation of *OsAQP* mRNAs in 7-day-old rice seedlings of wild type rice and phyA, phyB and phyAphyB phytochrome mutants. As shown in Fig. 3, levels of *OsAQP* mRNAs were always higher in darkness than after exposure of wild-type seedlings or any of the mutants to light. This suggests that light inhibits expression of the gene even in the absence of both phytochromes A and B. A failure to detect significant changes between materials treated with white light and red light implies that light with a longer wavelength had little effect on the accumulation of *OsAQP* mRNA. Levels of *OsAQP* expression in the phytochrome double mutant phyAphyB were higher than that in the two single mutants. This suggests that the regulation of *OsAQP* by light was not limited to phytochromes A and B, and that these two rice phytochromes may be functionally redundant.

In order to identify the effects of blue light, we used RT-PCR to analyze *OsAQP* expression in 5-, 10- and 15-day-old rice seedlings exposed to blue light or darkness. The results showed that blue light decreased *OsAQP* expression in roots, but increased accumulation of *OsAQP* mRNA in shoots relative to the same plant parts from seedlings exposed to darkness (Fig. 4).

Discussion

Aquaporins belong to the family of major intrinsic proteins and are best known for their ability to facilitate water flow. Over the past several years, several reports have described the participation of AQPs in responses to a large variety of environmental stresses, although some of the conclusions differ. The relationships between TIPs, water status and plant tolerance of abiotic stress remain unclear (Wang et al. 2011). In this report, we used semi-quantitative RT-PCR to study the effects of plant hormones and environmental factors, such as drought, salinity, chilling/freezing, and light, on the expression of *OsAQP* in wild-type rice and three rice phytochrome mutants.

Drought, salinity and low temperature are common stress conditions that adversely affect plant growth and crop production, because these stress conditions can affect water status in plants and inflict osmotic stress. Plant tolerance of these stresses depends largely on the regulation of water status. Nonetheless, reports demonstrating the effect of TIPs on plant tolerance to abiotic stresses remain limited (Peng et al. 2007; Sade et al. 2009). Some reports showed that osmotic stress and ion toxicity appear to be common consequences of exposure to abiotic stresses. Osmotic adjustment during the stress response appears to play a major role in the maintenance of osmotic homeostasis (Hauser and Horie 2010; Fricke and Peters 2002; Huang et al. 2012). Until now, studies concerning the regulation of stomatal movements focused more on extracellular stimulation, signal transduction, osmoregulatory compounds, ion channels and the cytoskeleton (Netting 2000; Yang and Wang 2001; Lu et al. 1995; Hwang et al. 1997; Ritte et al. 1999; Talbott and Zeiger 1996) than on role of the availability of water (Takase et al. 2011). The present study showed that drought, cold and salt stress increase levels of *OsAQP* mRNAs in leaves, whereas inhibit the accumulation of *OsAQP* transcripts in roots. This suggests that OsAQP may contribute to osmotic adjustment during rice responses to abiotic extremes by

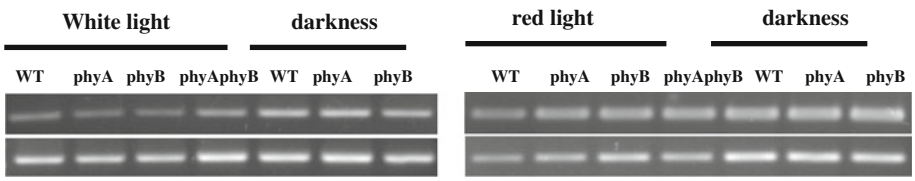

Fig. 3 Expression of *OsAQP* (*upper panel*) in wild-type rice and phytochrome mutants exposed to white light, red light or darkness. Rice ubiquitin (*lower panel*) was used as a loading control

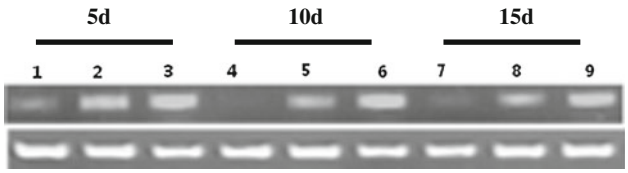

Fig. 4 Expression of *OsAQP* in response to blue light. *1,4,7* roots in 5, 10 and 15 days treated by darkness; *2,5,8* shoots in 5, 10 and 15 days treated by darkness; *3,6,9* shoots in 5, 10 and 15 days treated by blue light. Rice actin (*lower panel*) was used as a loading control

accelerating the flow of water in leaves. But for roots, the plant water absorption organs, the underlying mechanism is more complex. Because unlike animals, plants are sessile and may be subjected to diverse environmental stresses throughout their life cycle. By decreasing the expression level of *OsAQP* in roots, plants may protect themselves from the stress damages by reducing water flow and decreasing rates of metabolism in the abiotic stress responses.

Light regulates various developmental and movement responses, including de-etiolation, phototropic bending, cotyledon opening, photoperiodic flowering, chloroplast movement, stomatal opening, leaf flattening, and leaf positioning (Cashmore et al. 1999; Voicu et al. 2009; Inoue et al. 2008). Plants perceive diverse light signals from their environment by using a family of plant photoreceptors that includes the phytochromes, cryptochromes, and phototropins. The phytochromes regulate the expression of a large number of light-responsive genes, and thus influence many photomorphogenic events (Neff et al. 2000; Quail 2002a, b; Wang and Deng 2003). Rice has only three phytochrome genes—*PHYA, PHYB* and *PHYC* (Kay et al. 1989; Dehesh et al. 1991; Basu et al. 2000)—and rice mutants deficient in these photoreceptors have been isolated by *Tos17*-tagged knockout and γ-ray irradiation(Takano et al. 2001, 2005). In the present study, we compared expression patterns of *OsAQP* in three phytochrome mutants and wild type rice. Our results showed that white light inhibited expression of the *OsAQP* gene compared with that in shaded leaves, the levels of *OsAQP* mRNAs were comparable in leaves exposed to red light compared with leaves from plants left in darkness, and that levels of *OsAQP* transcript were higher in leaves exposed to blue light than in shaded leaves. These findings suggest that *OsAQP* is mainly regulated by blue light, and that the perception of red light by phytochrome probably does not play a role in the light-mediated regulation of hydraulic conductance at the level of *OsAQP* transcript accumulation. This is consistent with the conclusion that blue light has a greater effect than red light on the induction of stomatal opening (Sharkey and Raschke 1981).

Plant hormones are crucial signaling molecules that coordinate all aspects of plant growth, development and defense. The role of ABA in regulating several aspects of plant development, including seed development, desiccation tolerance of seeds and seed dormancy, is well documented. Accordingly, ABA plays a crucial role in plant responses to both abiotic stresses, such as drought, salinity, cold, and hypoxia, as well as biotic stresses (Wan and Li 2006; Chinnusamy et al. 2008; Wang et al. 2011). Crosstalk between molecular responses to salt stress and ABA signaling has been demonstrated (Uno et al. 2000; Chinnusamy et al. 2004). Our study also showed that salt and ABA both induced the transcriptional activation of *OsAQP*. Salicylic acid plays an important regulatory role in multiple physiological processes, including plant defense responses. In recent years, SA has been the focus of intensive research owing to its role as an endogenous signal that mediates local and systemic plant defense responses against pathogens. It has also been found that SA regulates plant responses to abiotic stresses, such as drought, chilling, heavy metal toxicity, heat, and osmotic stress (Rivas-San and Plasencia 2011). Our observation that levels of *OsAQP* transcripts are down-regulated by SA suggests that the abiotic stresses regulation network in the context of phytohormones is complex. There may be condition-specific positive and/or negative interactions among the phytohormones. Although each plant hormone has its specific and indispensable role in the regulation of plant physiological processes, every plant response is usually modulated by the action of more than one hormone, and the mechanisms of crosstalk among the hormone signaling pathways are still poorly understood (Shan et al. 2012). Although GAs commonly oppose ABA action, for instance during seed germination, there is remarkably little evidence for this antagonism in the regulation of guard cell behavior. Application of GA to the deseeded pericarps of pea fruits increased levels of γ-TIP mRNA (Ozga et al. 2002), and identified its relative contributions to cell division. It can be deduced that the expression of *OsAQP* may act as a qualitative marker for expanding tissue during rice early growth, which is regulated by GA. The GA-induced growth may, however, change the water status of cells, which in turn affects TIP abundance.

Our results reveal that expression of the rice TIP gene *OsAQP* is controlled by multiple pathways involved in the responses to abiotic stresses, and likely plays a critical role in the stress-tolerance response that maintains homeostasis under adverse environmental conditions. Identifying the function of OsAQP and better understanding the mechanisms underlying its regulation are of considerable potential value for stabilizing crop performance.

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