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REGULAR PAPER

# Hydrogen peroxide permeability of plasma membrane aquaporins of *Arabidopsis thaliana*

**Cortwa Hooijmaijers · Ji Ye Rhee · Kyung Jin Kwak · Gap Chae Chung · Tomoaki Horie · Maki Katsuhara · Hunseung Kang**

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**Abstract** Although aquaporins have been known to transport hydrogen peroxide ($H_2O_2$) across cell membranes, the $H_2O_2$-regulated expression patterns and the permeability of every family member of the plasma membrane intrinsic protein (PIP) toward $H_2O_2$ have not been determined. This study investigates the $H_2O_2$-regulated expression levels of all plasma membrane aquaporins of *Arabidopsis thaliana* (*AtPIP*s), and determines the permeability of every AtPIP for $H_2O_2$ in yeast. Hydrogen peroxide treatment of Arabidopsis down-regulated the expression of *AtPIP2* subfamily in roots but not in leaves, whereas the expression of *AtPIP1* subfamily was not affected by $H_2O_2$ treatment. The growth and survival of yeast cells that expressed AtPIP2;2, AtPIP2;4, AtPIP2;5, or AtPIP2;7 was reduced in the presence of $H_2O_2$, while the growth of yeast cells expressing any other AtPIP family member was not affected by $H_2O_2$. These results show that only certain isoforms of AtPIPs whose expression is regulated by $H_2O_2$ treatment are permeable for $H_2O_2$ in yeast cells, and suggest that the integrated regulation of aquaporin expression by $H_2O_2$ and the capacity of individual aquaporin to transport $H_2O_2$ are important for plant response to $H_2O_2$.

**Keywords** Aquaporin · Arabidopsis · Hydrogen peroxide · Transporter · Water channel

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C. Hooijmaijers · J. Y. Rhee · K. J. Kwak · G. C. Chung · H. Kang (✉)
Department of Plant Biotechnology and Agricultural Plant Stress Research Center, College of Agriculture and Life Sciences, Chonnam National University, 300 Yongbong-dong, Buk-gu, Gwangju 500-757, South Korea
e-mail: hskang@jnu.ac.kr

J. Y. Rhee · M. Katsuhara
Institute of Plant Science and Resources, Okayama University, Chuo 2-20-1, Kurashiki 710-0046, Japan

T. Horie
Division of Applied Biology, Faculty of Textile Science and Technology, Shinsu University, Ueda, Nagano 386-8567, Japan

## Introduction

Hydrogen peroxide ($H_2O_2$) is a reactive oxygen species (ROS) that is generated during diverse metabolic processes, and has an important role in biological systems. As an oxidant it directly affects intracellular redox systems, can initiate lipid peroxidation, degrade nucleic acids and inactivate enzymes, and, therefore, has the potential to affect physiological functions and cause cell damage or even death (Halliwell and Gutteridge 1999). At relatively low concentrations, $H_2O_2$ appears to perform a function in signal transduction processes within biological systems. It can influence gene expression and activate or modify redox-sensitive transcription factors, and thereby trigger tolerance to environmental stress (Orozco-Cárdenas et al. 2001; Laloi et al. 2004; Miller and Mittler 2006). Hydrogen peroxide has been implied to act as an intercellular messenger through activation and/or modulation of cellular antioxidants and disease resistance genes in plants (Levine et al. 1994; Chappell et al. 1997; Hu et al. 2005), and during xylem differentiation and lignification processes (Bienert et al. 2006). Signal transduction processes are only effective if rapid transport of the intercellular messenger molecule across the plasma membrane can be achieved. Movement of $H_2O_2$ across the plasma membrane of the

signal-producing as well as the signal-perceiving cell by diffusion is slow, and many membranes are poorly permeable to $H_2O_2$ (Bienert et al. 2006). Efficient transport of $H_2O_2$ across plasma membranes could be greatly increased by specific channel proteins. Indeed, experimental evidence suggests that certain aquaporins (AQPs) act as peroxoporins and thus facilitate the diffusion of $H_2O_2$ across biological membranes (Henzler and Steudle 2000; Bienert et al. 2007; Dynowski et al. 2008).

Aquaporins are membrane channels that belong to the major intrinsic protein (MIP) super family. These integral membrane proteins have highly conserved regions, and facilitate the transport not only of water but also other substrates, such as glycerol, ammonia, boric acid, carbon dioxide, nitric oxide, and $H_2O_2$ (Tyerman et al. 2002; Wu and Beitz 2007). *Arabidopsis thaliana* has 35 AQP isoforms which can be divided into four distinct groups based on sequence homology and subcellular location (Chaumont et al. 2005; Maurel 2007). A recent study investigated the involvement of several AQPs of Arabidopsis in $H_2O_2$ transport, and confirmed that several tonoplast intrinsic proteins (TIP1;1, TIP1;2, and TIP2;3) are permeable for $H_2O_2$ in yeast cells (Bienert et al. 2007; Dynowski et al. 2008). Despite the fact that the importance of an intercellular messenger like $H_2O_2$ to be quickly transported across plasma membranes is unquestionable, reports demonstrating the permeability of aquaporins of the plasma membrane intrinsic protein (PIP) family toward $H_2O_2$ are severely limited.

This study aims to analyze the $H_2O_2$-regulated expression patterns of plasma membrane AQPs of *A. thaliana* (AtPIPs), and to determine their possible involvement in $H_2O_2$ transport. The AtPIPs are divided into two subgroups, AtPIP1 and AtPIP2 with 5 and 8 isoforms, respectively (Johanson et al. 2001). Heterologous expression studies of plant aquaporins revealed that PIP2 is mainly involved in water transport, whereas PIP1 facilitates the diffusion of small electrolytes and gases as well as water (Kaldenhoff and Fischer 2006). Here, we examine $H_2O_2$-responsive expression patterns of thirteen *AtPIP* genes in Arabidopsis, and determine the permeability of AtPIPs to $H_2O_2$ in yeast cells.

## Materials and methods

### Plant materials and growth conditions

Seeds of *A. thaliana* (Columbia ecotype) were placed on water saturated rock wool and held at 4°C in darkness for 3 days for stratification. The seeds were germinated in a growth chamber at 23°C with 12 h-day/12 h-night cycle. Light was provided at an irradiance of 100 μmol $m^{-2}$ $s^{-1}$ by fluorescence tubes (FL40EX-D 40W, Eaglite). Three days after germination, the seedlings were transferred to an aerated hydroponic solution (Cooper 1975). To determine the effect of $H_2O_2$ on *AtPIPs* expression, three-week-old Arabidopsis plants were placed into distilled water (control) or exposed to exogenous $H_2O_2$ concentrations of 1 mM for 0.5, 1, 2, or 4 h.

### Quantitative real-time RT-PCR

The expression patterns of endogenous *AtPIP*s in response to $H_2O_2$ were examined by quantitative real-time RT-PCR. Total RNA was extracted from roots and leaves using an RNeasy extraction kit (Qiagen). Real-time RT-PCR was performed in a Rotor-Gene 2000 (Corbett Research) using a QuantiTect SYBR Green RT-PCR kit (Qiagen), essentially as described by Jang et al. (2004). Three independent experiments with subsequent RNA extractions were used as replicates. The expression levels of *AtPIPs* at each time point after $H_2O_2$ treatment were compared with that of the untreated-control sample. The expression level of actin was used as a reference.

### Gene cloning and yeast transformation

The coding regions of *AtPIP*s were first cloned into a pET-22b(+) vector (Novagen) to tag the protein with 6 histidine residues at the C-terminal end for later protein expression analysis. The insert was then cut out of the vector by enzymatic digestion, and finally ligated into pYES2 (Invitrogen), which was used as an expression vector for *Saccharomyces cerevisiae*. Two yeast strains were used in this study; the JC0176 mutant strain in which two AQPs (aqy1 and aqy2) have been deleted (Carbrey et al. 2001; Meyrial et al. 2001) and the Δ*skn7* mutant strain which has an impaired oxidative stress defense response and is sensitive to $H_2O_2$ (Bienert et al. 2007). The yeast strains were transformed with *AtPIPs* using the lithium acetate/single stranded carrier DNA/PEG method (Gietz and Schiestl 2007). Transformants were selected by colony PCR after 4 days incubation at 30°C on synthetic drop-out (SD) medium (Sigma) containing uracil and histidine as selective markers.

### Analysis of RNA and protein expression in yeast

RNA expression of *AtPIPs* in yeast was analyzed by RT-PCR and protein expression by western blotting. The expression of *AtPIPs* was induced by growing the yeast cells at 30°C on SD medium containing 2% galactose (SG) as a carbon source. For RNA extraction, the yeast cells were disrupted mechanically with 0.5 mm glass beads using a mini bead-beater (BioSpec Products Inc.) at 4°C

with five 1 min cycles and subsequent 1 min cooling intervals on ice. Total RNA was extracted using an RNeasy extraction kit (Qiagen), and the expression of *AtPIPs* in yeast was determined via RT-PCR using an Omniscript RT kit (Qiagen) with gene specific primers. RNA transcripts were loaded onto a 1% agarose gel and visualized after staining with ethidium bromide. In addition, the expression of His-tagged AtPIPs in yeast was analyzed by western blot analysis. For protein extraction, the yeast cells were suspended in protein sample buffer (45 mM Tris, pH 6.8, 10% glycerol, 1% sodium dodecyl sulfate, 50 mM dithiothreitol, 0.01% bromophenol blue). To denature highly hydrophobic proteins, the suspension was incubated at 55°C for 1 h, which prevents precipitation and multimerization of proteins with multiple transmembrane domains (Sambrook and Russell 2001). The proteins were then separated by 12% SDS-PAGE and the gel was transferred to an Immobilon-P membrane (Millipore) using a tank-blotting procedure (Qiagen). Immunodetection with penta-His HRP conjugates was used for the detection of AQP according to the manufacturer's protocol (Qiagen). After the final washing step, the membrane was incubated in WEST-ZOL plus (iNtRON Biotechnology), and the proteins on the membrane were visualized by chemiluminescence (ECL, Amersham Pharmacia Biotech).

### Growth and survival assay of yeast cells in response to $H_2O_2$

The sensitivity of AtPIP transformed yeast cells to $H_2O_2$ was examined by a growth and survival assay on the two mutant yeast strains. For the JC0176 mutant yeast strain in which the two endogenous AQPs (aqy1 and aqy2) have been deleted (Meyrial et al. 2001), transgenic yeast cells harbouring pYES2:AtPIP or an empty pYES2 vector (control) were grown at 30°C in liquid SG, and the $OD_{600}$ was adjusted to 1.0. The cells were then serially diluted, and 10 µL of cells were spotted on solid SG medium without or with $H_2O_2$. For the Δ*skn7* mutant yeast strain, previously used by Bienert et al. (2007), transformants harbouring pYES2:AtPIP or an empty pYES2 vector (control) were pre-cultured on solid SG medium for 2 days, and fresh yeast cells were diluted in 0.5 ml sterile water and then streaked on solid SG medium without or with $H_2O_2$. The plates were incubated at 30°C for 3–5 days, and the growth and survival of the cells was recorded.

## Results and discussion

### The effect of $H_2O_2$ on the endogenous expression levels of *AtPIPs* in leaves and roots of *A. thaliana*

To determine whether exogenously applied $H_2O_2$ affects the expression of *AtPIPs* in Arabidopsis, the transcript level of each *AtPIP* was measured via real-time RT-PCR analysis. To compensate for any circadian effects on *AtPIP* gene expression, the expression levels of *AtPIPs* at each time point after $H_2O_2$ treatment were compared with that of untreated-control samples. A hydrogen peroxide treatment of 1 mM over the course of 4 h did not greatly affect the endogenous expression levels of *AtPIP1* in the leaves and roots of Arabidopsis (Fig. 1), although the expression of *AtPIP1;3* in the leaves was slightly upregulated, especially during the first hour of $H_2O_2$ treatment. Hydrogen peroxide treatment did not affect the expression of *AtPIP2* genes in

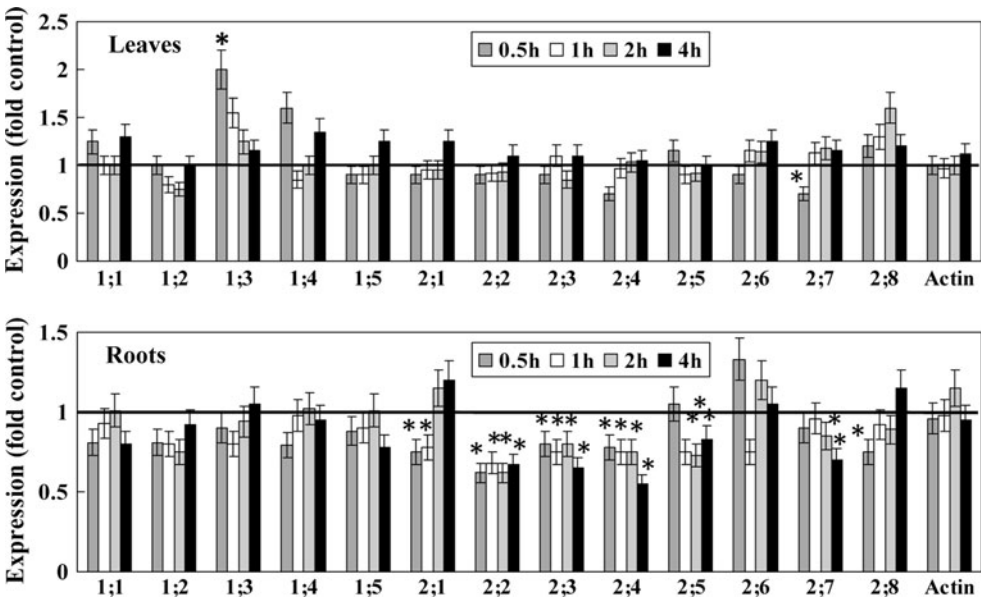


**Fig. 1** The effect of $H_2O_2$ on the endogenous expression levels of *AtPIPs* in the leaves and roots of *A. thaliana*. Hydrogen peroxide (1 mM) was applied for 0.5, 1, 2, and 4 h, and the expression levels of each AQPs were plotted relative to non-treated control plants. Actin was used as a reference to show that equal amounts of RNA were present in the samples. Values are means ± SE ($n = 6$). *Asterisks* above the columns indicate values that are statistically different from control values ($P < 0.05$). The numbers *1;1*, *1;2*, *1;3*, etc., in the figure represent AtPIP1;1, AtPIP1;2, AtPIP1;3, etc., respectively

the leaves. By contrast, *AtPIP2* genes in the roots were generally downregulated during the course of $H_2O_2$ treatment. The expression of *AtPIP2;1* and *AtPIP2;8* decreased during the first hour of $H_2O_2$ treatment and then returned to the pretreated values. The expression of *AtPIP2;2*, *AtPIP2;3*, *AtPIP2;4*, *AtPIP2;5* and *AtPIP2;7* decreased during the entire period of $H_2O_2$ treatment. GENEVESTIGATOR (http://www.genevestigator.com) analysis of microarray data on the transcript levels of *AtPIPs* in Arabidopsis also revealed that the expression of *AtPIP2;1*, *AtPIP2;2*, *AtPIP2;3*, *AtPIP2;4*, *AtPIP2;5* and *AtPIP2;7* decreased slightly at 1 h of $H_2O_2$ treatment (data not shown), which supports the results of our current quantitative real-time RT-PCR analysis. Although we do not presently know the physiological significance of different regulation of *AtPIPs* expression by $H_2O_2$, it is noteworthy that permeability of AtPIPs for $H_2O_2$ appears to be exclusive to certain members of the AtPIP2 subfamily only (see below). The transcript levels of each *AtPIP* in Arabidopsis have been determined in previous report, and the 13 *AtPIP* genes are classified into three groups based on their expression levels; the highest, intermediate, and low expression groups have the copy numbers in the range of 5,000–7,000 copies, 2,000–5,000 copies, and 500–2,000 copies per nanogram of total RNA, respectively (Jang et al. 2004). The high expression group includes *PIP1;1, PIP1;2,* and *PIP2;7,* the intermediate expression group includes *PIP1;3, PIP1;5, PIP2;2,* and *PIP2;3,* and the low expression group consists of *PIP1;4, PIP2;1, PIP2;4, PIP2;5, PIP2;6,* and *PIP2;8* (Jang et al., 2004). The *AtPIP2;2*, *AtPIP2;3*, *AtPIP2;4*, *AtPIP2;5* and *AtPIP2;7*, the expression of which is modulated by $H_2O_2$ treatment, belong to all three groups, indicating that the absolute transcript levels of *AtPIPs* are not directly related to $H_2O_2$ responsiveness. It seems that Arabidopsis responds mainly to the signalling molecule $H_2O_2$ by regulating the expression of *AtPIP2* family members. It has been reported that exogenously applied $H_2O_2$ to the roots of cucumber, wheat, and maize decreased the hydraulic conductivity (Ktitorova et al. 2002; Lee et al. 2004; Aroca et al. 2005), and this reduction of water transport into the root cells could well be an effect of genetic down-regulation of root AQPs. The expression of AQPs in Arabidopsis is tissue specific, and not only depends on the developmental stage of the plants but can also be affected by environmental signals such as cold, drought, salt, osmotic stress, and ABA (Jang et al. 2004; Kaldenhoff and Fischer 2006). Our current results directly demonstrate that $H_2O_2$ is another environmental factor that regulates the expression of AQPs in plants.

## AtPIP expression in yeast cells

To examine whether AtPIPs possess the ability to transport $H_2O_2$ across the plasma membrane, each AtPIP gene was expressed in the JC0176 mutant yeast strain in which the two endogenous AQPs (aqy1 and aqy2) have been deleted (Meyrial et al. 2001). We first determined the expression of each AtPIP gene in the yeast cells at both mRNA and protein levels. Yeast cells expressing each AtPIP were investigated for the expression levels of *AtPIPs*, and Supplementary Fig. S1 shows the results of representative experiments including AtPIP1;1, 1;5, 2;1, 2;7, and 2;8. When the yeast cells transformed with each AtPIP gene from Arabidopsis were grown in synthetic drop-out medium containing the inducer galactose (gal), the expression levels of *AtPIPs* transcripts were similar to each other (Supplementary Fig. S1a). In contrast, when the yeast cells transformed with AtPIP2;1 were grown in a medium containing glucose (glu) instead of galactose, no *AtPIP2;1* transcript was detected in the cells. The absence of *PIP2;1* expression in the medium with glucose as a carbon source clearly indicates that the Arabidopsis AtPIP genes are induced in the yeast cells by the addition of galactose. With the observation that the transcript levels of *AtPIP* genes are similar to each other, we then investigated the expression of AtPIP at protein level in the yeast cells. The total proteins in the cellular extract were separated by 12% SDS-PAGE, and the expression levels of AtPIP proteins were determined by western analysis with an anti-His antibody. Yeast cells expressing either AtPIP1;4, 2;1, or 2;6 were selected for $H_2O_2$-insensitive cells and yeast cells expressing either AtPIP2;2, 2;4, or 2;5 were selected for $H_2O_2$-sensitive cells, and the expression levels of AtPIP proteins were analyzed by western analysis. The protein levels of AtPIP in the yeast cells are comparable with each other (Supplementary Fig. S1b), further indicating that the Arabidopsis AtPIPs are expressed in a similar amount in the yeast cells. Since the His-tag was fused in-frame at the C-terminal end of the protein, these results also indicate that the full-length AtPIPs are successfully translated in the yeast cells.

## Sensitivity of AtPIP-expressing yeast cells to $H_2O_2$

To examine whether AtPIPs possess the ability to transport $H_2O_2$ across the plasma membrane, the growth and survival of two mutant yeast strains expressing each *AtPIP* gene was analyzed on solid SG medium containing 0.1–1 mM $H_2O_2$. Similar growth patterns were observed at different $H_2O_2$ concentrations, and the results showing most striking differences (1 mM $H_2O_2$ for JC0176 strain and 0.5 mM $H_2O_2$ for Δskn7 strain) are presented in Figs. 2 and 3. We first employed the JC0176 strain in which two native AQPs (aqy1 and aqy2) have been deleted (Carbrey et al. 2001; Meyrial et al. 2001). The yeast cells harbouring *AtPIPs* or an empty pYES2 expression vector grew well on SG medium without $H_2O_2$ with no noticeable difference (Fig. 2a). Similarly, the yeast cells harbouring any member

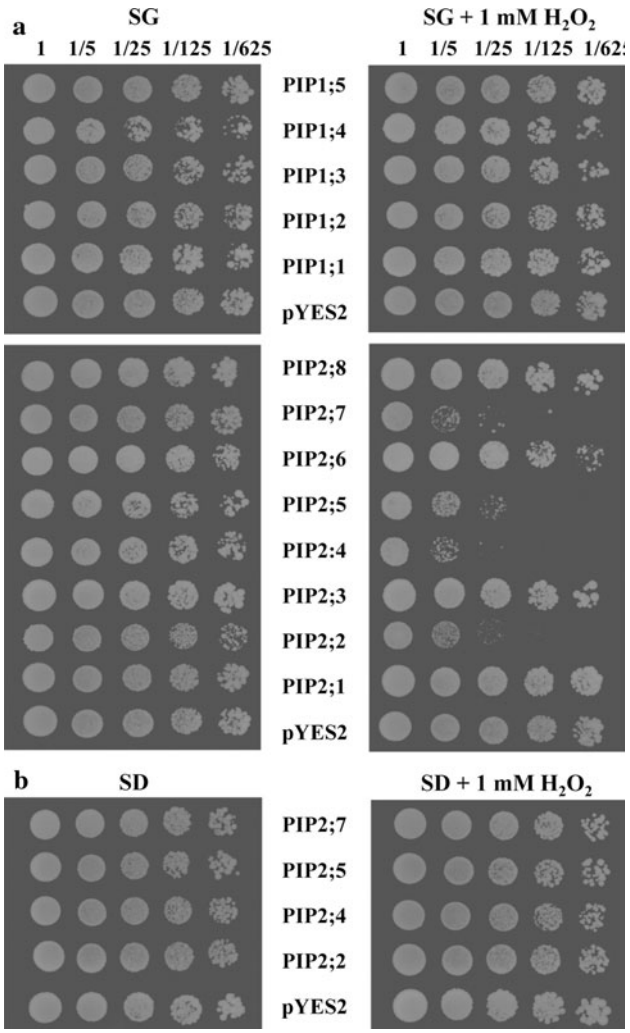


**Fig. 2** The effect of $H_2O_2$ on the growth and survival of JC0176 mutant yeast cells expressing AtPIPs. **a** Yeast was transformed with either an empty pYES2 vector (control) or an AtPIP gene, and 10 μL of serially diluted yeast cells were spotted on galactose medium (SG) without or with 1 mM $H_2O_2$. **b** As control experiment, transgenic yeast cells were spotted on glucose medium (SD) without or with 1 mM $H_2O_2$. The plates were photographed after 5 days incubation at 30°C. The experiments were repeated at least three times with consistent results

of the *AtPIP1* subfamily grew well on SG medium containing 1 mM $H_2O_2$ and thus did not show increased sensitivity towards $H_2O_2$. However, the growth and survival of yeast cells expressing *AtPIP2;2*, *AtPIP2;4*, *AtPIP2;5,* or *AtPIP2;7* was markedly reduced on $H_2O_2$ containing medium when compared to the empty vector control (Fig. 2a), whereas the yeast cells harbouring *AtPIP2;1, AtPIP2;3, AtPIP2;6,* or *AtPIP2;8* grew well in the presence of 1 mM $H_2O_2$. This increase in sensitivity to $H_2O_2$ disappeared completely when the cells were grown on SD medium containing glucose as a carbon source (Fig. 2b), indicating that the decreased growth of the yeast cells is due to the expression of AtPIPs in the yeast cells. These

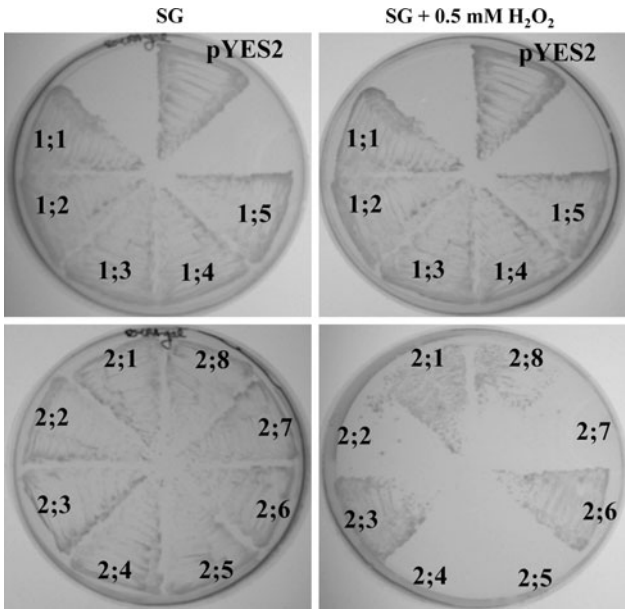


**Fig. 3** The effect of $H_2O_2$ on the growth and survival of Δ*skn7* mutant yeast cells expressing AtPIPs. Yeast cells transformed with either an empty pYES2 vector (control) or an AtPIP gene were pre-cultured on galactose medium (SG), and fresh yeast cells were streaked on SG medium without or with 0.5 mM $H_2O_2$. The plates were photographed after 3 days incubation at 30°C. The experiments were repeated at least three times with consistent results

results indicate that AtPIP2;2, AtPIP2;4, AtPIP2;5, and AtPIP2;7 are permeable for $H_2O_2$. Similar results were observed in the analysis of the second mutant yeast strain (Δ*skn7*) that has a defective oxidative stress defense response and is sensitive to $H_2O_2$ (Bienert et al. 2007).The yeast cells harbouring any member of the *AtPIP1* subfamily did not show any increased sensitivity towards $H_2O_2$, whereas the growth and survival of yeast cells expressing *AtPIP2;2*, *AtPIP2;4*, *AtPIP2;5,* or *AtPIP2;7* was markedly reduced on $H_2O_2$ containing medium when compared to the empty vector control (Fig. 3). Increased sensitivity of these AtPIP2-expressing yeast cells towards $H_2O_2$ in the medium could be the result of a decreased capacity to scavenge $H_2O_2$ inside the mutant yeast cells. This increase in sensitivity to $H_2O_2$ disappeared completely when the cells were grown on SD medium containing glucose as a carbon source (data not shown), indicating that the decreased growth of the yeast cells is due to the expression of AtPIPs in the yeast cells. These results further support the above observation that AtPIP2;2, AtPIP2;4, AtPIP2;5, and AtPIP2;7 are permeable for $H_2O_2$.

This study supports previous reports which suggest that only certain AQPs are selectively permeable for $H_2O_2$ (Henzler and Steudle 2000; Bienert et al. 2007; Dynowski et al. 2008). However, contrary to the report by Dynowski et al. (2008) who showed permeability of AtPIP2;1 for $H_2O_2$, our analysis demonstrates that AtPIP2;1 is not

permeable for $H_2O_2$. This discrepancy may arise from the fact that different yeast strains were used in each study. Interestingly, permeability of AtPIPs for $H_2O_2$ appeared to be exclusive to certain members of the PIP2 subfamily. It is not understood presently why only certain members of the AtPIP2 subfamily but not the AtPIP1 subfamily are permeable for $H_2O_2$ in yeast cells. It has been proposed that hydrogen peroxide can increase the phosphorylation of AQPs and thereby improve functionality (Aroca et al. 2005). Considering that AtPIP2s have a longer C-terminal end than AtPIP1s (Johanson et al. 2001), and that the serine residues in the C-terminus are the primary target for phosphorylation by $H_2O_2$-activated signalling cascade (Prak et al. 2008), it is likely that permeability toward $H_2O_2$ of certain members of AtPIP2s but not any members of AtPIP1s is the result of an increased AtPIP2 activity through phosphorylation by $H_2O_2$.

## The effect of aquaporin inhibitors on the sensitivity of yeast cells to $H_2O_2$

To test the effect of aquaporin inhibitors on the response of the yeast to $H_2O_2$, the growth and survival of the JC0176 yeast strain on medium containing $AgNO_3$, one of the commonly used aquaporin blockers, was investigated. In the absence of $H_2O_2$, increasing concentrations of $AgNO_3$ above 10 μM reduced the growth and survival of yeast cells (data not shown), a concentration that is similar to the toxic concentration (6 μM) determined by Bienert et al. (2007). The yeast cells expressing either *AtPIP2;2*, *AtPIP2;4*, *AtPIP2;5,* or *AtPIP2;7* as well as the control yeast cells harbouring the empty pYES2 vector showed a very similar growth pattern at 10 μM $AgNO_3$ in both the absence and presence of $H_2O_2$ (Fig. 4). These results suggest that $AgNO_3$ does not block the diffusion of $H_2O_2$ through these AtPIPs under the current experimental conditions.

The channeling activity of AQPs can be inhibited by mercury or silver ions, which can bind to cysteine residues and thereby block the channel path (Niemietz and Tyerman 2002; Maurel et al. 2008). In the present study, however, the toxicity of $H_2O_2$ for JC0176 yeast strain was not noticeably reduced when $AgNO_3$ (Fig. 4) or $HgCl_2$ (data not shown) was added to the medium. An insensitivity of AQPs to channel inhibitors has also been demonstrated previously. An AQP from *Nicotiana tabacum*, which shows great homology with the AtPIP1 subfamily, appeared to be mercury-insensitive (Biela et al. 1999). Moreover, a membrane AQP from Arabidopsis was also found to be insensitive to mercury (Daniels et al. 1994). Hydrogen peroxide can readily oxidize the sulfhydryl group of cysteine residues in proteins (Rhee et al. 2000), and might affect the activity of AQPs. Therefore, the addition of an AQP blocker to $H_2O_2$ containing medium would not have reduced the sensitivity of *AtPIP*-expressing yeast cells for $H_2O_2$ because of analogous effects of both molecules on AQP activity. Since the AtPIPs tested in this study seemed to be marginally sensitive to AQP blockers in yeast cells, it would be of interest to determine whether these AQPs are not inhibited equivalently by channel blockers in Arabidopsis.

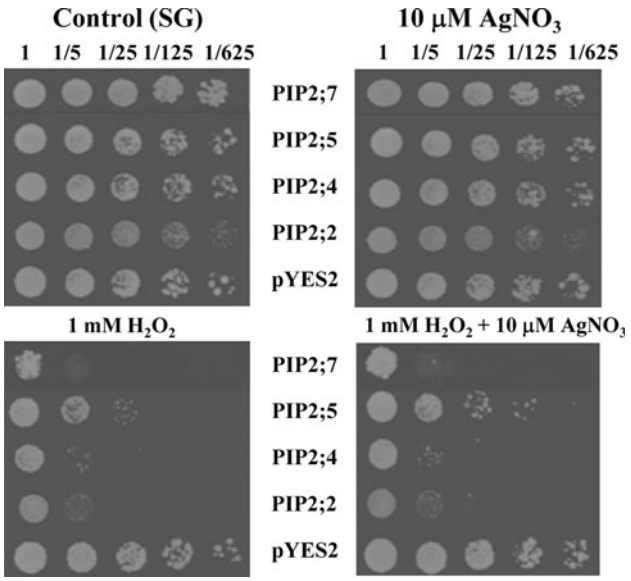


**Fig. 4** The effect of the aquaporin blocker $AgNO_3$ on the growth and survival of JC0176 yeast cells expressing AtPIPs. Yeast was transformed with an empty pYES2 vector (control) or an AtPIP gene, and 10 μL of serially diluted yeast cells were spotted on SG medium with 1 mM $H_2O_2$, 10 μM $AgNO_3$, or both 1 mM $H_2O_2$ and 10 μM $AgNO_3$. The growth and survival of the yeast cells was recorded after 5 days incubation at 30°C. The experiments were repeated at least two times with consistent results

## Conclusions

The current heterologous analysis of PIP-type aquaporins in two different mutant yeast strains clearly demonstrated that specific isoforms of AtPIPs function as $H_2O_2$ transporters in yeast. The heterologous expression of *AtPIP2;2*, *AtPIP2;4*, *AtPIP2;5* and *AtPIP2;7* increased the sensitivity of yeast cells for $H_2O_2$, which indicates that these plasma membrane AQPs are permeable for $H_2O_2$. Concurrently, the endogenous expression of *AtPIP2*s that appeared to be permeable for $H_2O_2$ in yeast was downregulated in the Arabidopsis roots in response to $H_2O_2$. Further investigation is needed to establish the effect of $H_2O_2$ on AQP functionality and activity in plants.

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