Co-regulation of water channels and potassium channels in rice
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Aquaporins and potassium channel proteins are probably critical for a plant to maintain proper cytosolic osmolarity in response to drought or other stresses. However, evidence linking water channel and potassium channel functions in plants remains to be demonstrated. The present study examined K⁺ channel/transporters and water channels in rice (Oryza sativa L. spp. indica cv. Guanglui 4) to reveal a potential functional correlation. The mRNA expression levels of plasma membrane intrinsic proteins (PIPs) and K⁺ channel/transporters responded similarly to K⁺ starvation or water deprivation. Transcription of the PIP- and K⁺ channel-encoding genes was induced by K⁺ starvation and could be downregulated by polyethylene glycol (PEG)-mediated water deficit. Consistent with the induced PIP expression, root hydraulic conductivity (Lp) also increased during K⁺ starvation. Furthermore, the K⁺ uptake capacity, but not the K⁺ content, was probably influenced by K⁺ starvation. Caesium chloride treatment decreased K⁺ content in the rice seedlings and reduced root Lp as did mercuric chloride. These results are compatible with the conclusion that PIP and K⁺ channel/transporters are functionally co-regulated in rice osmoregulation.

Introduction
Plants occasionally experience various environmental stresses including drought, salinity and temperature extremes. Exposure to these stressors may cause general and specific destructive effects on plant growth and development. These different environmental conditions often induce osmotic stress. Therefore, maintenance of osmotic homeostasis is critical for plant acclimatization to these stresses. Osmoregulation is achieved by absorption, transport and compartmentation of water and solutes, with potassium as an important component. Consequently, transmembrane channels for water and K⁺ are probably co-regulated and may function in a coordinated manner to maintain the appropriate cytosolic osmolarity. However, reports on the co-regulation of water and K⁺ channels in plants are scarce.

Many fundamental plant processes, such as cell enlargement and transpiration, depend greatly on water absorption from the surrounding soil, and water transport across tissues. In plants, long-distance water transport is accomplished by the xylem. Short-distance water transport in non-vascular tissues involves diffusion across membranes and is facilitated by water channel proteins known as aquaporins (Martre et al. 2002). Aquaporins exhibit the extraordinary ability to combine an elevated water flux with a high specificity for water (Johansson et al. 2000). Aquaporins have influenced thinking on plant water relations (Maurel and Chrispeels 2001), because they are

Abbreviations – CsCl, caesium chloride; HgCl₂, mercuric chloride; Lp, hydraulic conductivity; PEG, polyethylene glycol; PIPs, plasma membrane intrinsic proteins; RT, reverse transcription; TEA, tetraethylammonium chloride; UBQ, ubiquitin.
ubiquitous among plant tissues (Maurel 1997), and play a role in many important physiological processes. Some of these proteins may also transport other small non-ionic molecules such as glycerol and CO₂ (Wallace et al. 2002, Uehlein et al. 2003), in addition to water. Water channels are regulated by many factors, including day/night cycles, water stress and nutrient deprivation (Carvajal et al. 1996, North and Nobel 2000, Moshelion et al. 2002). Water channel quantity and activity may be regulated to control the transmembrane water flux that endows plants with a remarkable capacity to modulate water absorption, transport and compartmentation within tissues.

Potassium is also crucial to plants, as it is required for maintaining membrane potential and turgor, activation of enzymes, regulation of osmotic pressure, stoma movement and tropisms (Dennison et al. 2001, Cherel 2004). The K⁺ uptake mechanism in higher plant roots has been investigated for many years. According to Epstein et al. (1963), who studied K⁺ influx kinetics in barley roots, at least two distinct import mechanisms for K⁺ activity exist: high- and low-affinity uptake systems. In addition, the existence of several types of K⁺-transporting membrane proteins has been reported, including the AKT/KAT-type channels, HKT-type transporters and HAK/AT/KUP-like transporters (Maser et al. 2001). The first nutrient ion transport systems identified in plants were the Arabidopsis K⁺ channels AKT1 and KAT1, obtained by examining the functional complementation of yeast mutants defective for K⁺ uptake (Anderson et al. 1992, Sentenac et al. 1992). Arabidopsis AKT1 encodes an inward rectifying K⁺ channel (Sentenac et al. 1992, Gaymard et al. 1996) located in the plasma membrane. This channel is expressed primarily in roots and is localized to the epidermis, cortex and endodermal tissues (Lagarde et al. 1996). The AKT1 loss-of-function plants exhibit severe defects in K⁺ uptake when exposed to micromolar K⁺ and millimolar NH₄⁺ concentrations, indicating its pivotal role in K⁺ nutrition (Lagarde et al. 1996, Hirsch et al. 1998).

In the present paper, we explored channel co-regulation between the K⁺ channel/transporters, OsAKT1 and OsHAK1, and water channels, the plasma membrane intrinsic proteins (PIPs) in rice (Oryza sativa). The OsAKT1 K⁺-channel gene is homologous to the inward-rectifying K⁺ channels of the AKT/KAT sub-family, located predominantly in roots, and at a low abundance in leaves (Golldack et al. 2003). Another plasma membrane K⁺-transporter, OsHAK1, is expressed almost exclusively in roots (Banuelos et al. 2002). OsHKT1, a Na⁺ transporter gene in rice roots, accumulates under low external K⁺ concentrations, which may help to compensate for K⁺ deficiency by transporting Na⁺ into the cell (Horie et al. 2001). For this reason, OsHKT1 was investigated herein. Sakurai et al. (2005) identified 11 PIPs in rice and divided the PIP subfamily into two groups, PIP1 and PIP2 as same as those of Arabidopsis thaliana (Johnson et al. 2001). So far, the analyses of plants with impaired PIP protein expression capacities have shown that PIP functions in root water uptake and stress water recovery (Kaldenhoff et al. 1998, Siefritz et al. 2002, Javot et al. 2003). In onion roots, water transport is sensitive to water channel and K⁺-channel inhibitors, and the reduction in hydraulic conductivity (Lp) by treatment with a K⁺-channel inhibitor suggests that fluxes of K⁺ are linked to water channel activity in the plasma membrane (Tazawa et al. 2001). Furthermore, mRNA encoding water channel proteins respond strongly to cationic stresses, such as K⁺ starvation, even in the absence of water stress (Maathuis et al. 2003). The transcripts encoding water channels PIP1;2 (PIP1b), PIP2;2 (PIP2b) and TIP1;2 (TIP), and the K⁺ transporter HAK5, were reduced in Arabidopsis roots with growth on the K⁺-channel blocker, caesium chloride (CsCl) (Sahr et al. 2005). On the basis of these observations, we hypothesized that water and K⁺ channels are co-regulated during plant osmoregulation.

Materials and methods

Plant material and culture conditions

Seeds of O. sativa L. spp. indica cv. Guanglui 4 were germinated in the light on wet filter paper for 4–5 days. The seedlings were grown in nutrient solution (Ni 1985) at a photon flux density of 350–400 µmol m⁻² s⁻¹, 60–80% relative humidity, 12 h/12 h day-night cycle at 28°C (day/night) in a phytotron. The nutrient solution (pH 5.0) consisted of macronutrients (mg l⁻¹): 18.5 KNO₃, 59.9 Ca(NO₃)₂, 65.9 MgSO₄, 24.8 K₂HPO₄, 48.2 (NH₄)₂SO₄, 15.9 K₂SO₄, 200 Na₂SiO₃; and micronutrients (mg l⁻¹): 38.3 Fe-EDTA, 2.86 H₃BO₃, 0.08 CuSO₄.5H₂O, 0.22 ZnSO₄.7H₂O, 1.81 MnCl₂.4H₂O, 0.09 H₂MoO₄. Sterile nutrient solution was renewed twice a week. All experiments were performed with 3-week-old rice seedlings. For water deficit treatment, the nutrient solution contained 15% polyethylene glycol (PEG) 6000 (SanYo, Tokyo, Japan) providing −0.62 MPa of water stress determined using a Vapor Pressure Osmometer (Wescor, Logan, UT). For K⁺ starvation experiments, NaNO₃ replaced KNO₃, Na₂SO₄ replaced K₂SO₄ and NaH₂PO₄ replaced KH₂PO₄ in the nutrient solution. Seedlings were cultured with three different hydropic conditions, 0, 0.25 (K⁺-starvation treatment) and 0.55 mM K⁺ (control). Inhibitor treatments, 30 mM tetraethylammonium
chlordetrin (TEA), 30 mM CsCl or 30 μM mercuric chloride (HgCl$_2$) were performed by adding salts solutions to the nutrient solution.

**K$^+$ analysis**

Leaves and roots of the plants were rinsed three times with deionized water, dried on filter paper and then dried at 80°C to constant weight. 0.1 g dry samples were extracted with 5 ml 0.1 N acetic acid at 90°C for 2 h to release the free cations. Supernatants of the extracts were diluted after centrifugation at 10 000 g for 10 min. The concentration of K$^+$ was determined with a Shimadzu AA-680 atomic absorption/flame spectrophotometer (Shimadzu, Japan).

**Osmotic root hydraulic conductivity**

Osmotic root hydraulic conductivity was determined as described previously (Miyamoto et al. 2001). Surface areas of root systems were measured as described elsewhere (Lian et al. 2004).

**Real-time polymerase chain reaction**

For the detection of the RNA transcripts in potassium starvation and drought-treated samples, the real-time quantification of RNA targets was performed in the Rotor-Gene 3000 real-time thermal-cycling system (Corbett, Australia). A rice polyubiquitin gene (Nishi et al. 1993) was used as a standard control in the real-time polymerase chain reactions (PCRs). A two-step Reverse transcription-PCR (RT-PCR) procedure was performed in all experiments. First, total RNA samples (2 μg per reaction) from roots were reversely transcribed into cDNAs by AMV reverse transcriptase according to the manufacturer’s instructions (TaKaRa, Japan). Then, the cDNAs were used as templates in real-time PCR reactions with gene-specific primers and probes. The oligonucleotide primers and TaqMan-MGB probes (designed and synthesized by GeneCore, ShangHai, China; Table 1) were designed using the Primer Express 2.0 software (Applied Biosystems-Perkin-Elmer, Foster City, CA). The amplification reactions were performed in a total volume of 25 μl. The reaction mixture contained 2 μl of cDNA, 0.5 μl of the forward and reverse primers (10 μM) along with 0.5 μl of the TaqMan-MGB probe (5 μM) corresponding to each primer and probe, 0.25 μl of enzymes (TaKaRa, Japan), 0.75 μl of dNTP (10 mM); 0.5 μl of MgCl$_2$ (250 mM); 5 μl of ∝5 real-time PCR buffer and 15 μl of PCR-grade sterile water. The Rotor-Gene 3000 cycler was programmed as follows: 4 min at 95°C; 60 cycles of 30 s at 95°C and 30 s at 60°C. A control without the corresponding template DNA was included in every quantitative PCR assay for each primer and probe set. The fluorescence signal was carried out during the anneal step. All experiments were repeated three times. The real-time amplification data were analysed using Rotor-Gene 3000 software ver. 5.0.

**RT-PCR**

Semi-quantitative RT-PCR was performed to analyse the expression of OsAKT1. The cDNA was synthesized using AMV reverse transcriptase (TaKaRa, Japan), and 2 μl of cDNA was used for PCR amplification. The OsAKT1 fragment (356 bp) was amplified with the specific primers, 5’-acgaccgcctgaacgguu-3’ and 5’-ggagacgccacagcagaa-3’ at 94°C for 3 min, followed by 26–28 cycles at 94°C for 40 s, 53°C for 50 s, 72°C for 45 s and finished by an extension at 72°C for 10 min. The ubiquitin fragment (396 bp) amplified with the primers, 5’-gacggagacgccacgcttggtgaactc-3’ and 5’-tgctgctacatattatatacatc-3’, was used as internal control. The expression of OsAKT1 was quantified by comparing the optical density of their RT-PCR products using the UVP Labwork software (UVP Inc, Cambridge, UK).

**Results**

**Physiological response to K$^+$ starvation stress**

We investigated the effects of K$^+$ starvation on K$^+$ content and Lp in rice seedlings. Starvation of 0.3 mM K$^+$ induced a substantial decrease of the K$^+$ content in roots, and a significant increase of K$^+$ in the leaves (Fig. 1A, B). K$^+$-starvation treatment and control seedlings have similar K$^+$ contents (Fig. 1C); therefore, short-term K$^+$ deficiency treatment affects the K$^+$ uptake capacity, but not the K$^+$ content, and probably affects K$^+$ transport from the root to the shoot.

A doubling of Lp was observed in the roots after treatment with 0.25 mM K$^+$ for 4–6 h compared with that in the control plants (Fig. 2). These results suggest that K$^+$ starvation may induce water channel gene expression and may increase water channel activity, leading to an enhanced water transport in the roots.

**The response of plants to K$^+$ channel and water-channel inhibitors**

The water-channel inhibitor HgCl$_2$ and the potassium-channel inhibitors TEA and CsCl were used herein to investigate the possibility of co-regulation between
water and potassium channels. After treating the rice roots for 1 or 3 h with CsCl, the K⁺ content decreased from $5.50 \times 10^{-4}$ mol g⁻¹ DW to $5.10 \times 10^{-4}$ mol g⁻¹ DW and from $5.41 \times 10^{-4}$ mol g⁻¹ DW to $4.99 \times 10^{-4}$ mol g⁻¹ DW, respectively (Fig. 3A). A decline was also observed in the leaves of the treated plants, relative to those in the control plants (Fig. 3B). No significant difference in the K⁺ contents was detected between the TEA-treated and control plants (data not shown). The HgCl₂ treatment did not cause

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any significant K⁺ content differences in the roots or the leaves (Fig. 3).

According to Tazawa et al. (1997), HgCl₂ greatly reduces symplastic water transport by inhibiting water channel activity. We measured the $L_p$ in rice roots, in the presence and absence of low HgCl₂ concentrations. As shown in Fig. 4, HgCl₂ decreased the $L_p$ by more than 70%, relative to those in the controls, within 10 min. Moreover, the potassium-channel inhibitor, CsCl, decreased the $L_p$ in the roots by about 40% compared with that in the controls, within 3 h. Water transport in onion roots has been shown to be sensitive to water channel and K⁺-channel inhibitors (Tazawa et al. 2001).

Effects of K⁺ starvation on K⁺ channel/transporter and PIP expression

We used real-time PCR to determine whether the OsAKT1, OsHAK1 and OsHKT1 genes were altered by K⁺ starvation in rice. The two K⁺ starvation conditions resulted in a significant increase in the expression levels of OsAKT1, OsHAK1 and OsHKT1 (Fig. 5). Depleting
the K⁺ levels rapidly increased the OsAKT1 expression levels, while starving the roots of K⁺ for 1 h enhanced the OsHAK1 expression levels from 2.2- to 2.9-fold in the rice roots. A 1.3- to 2.9-fold increase in OsHKT1 expression was also detected in the rice roots during K⁺ starvation stress. In conclusion, these data show that K⁺ starvation induces the expression of specific K⁺-channel genes in the rice root system.

The increase of Lp due to K⁺ starvation presumably results from enhanced water channel gene expression and subsequent water channel activation. An increase in the PIP mRNA levels, by 1- to 3-fold compared with the controls, was detectable after K⁺ starvation for 6 and 24 h. Deprivation of nearly half or all K⁺ from the nutrient solution for 24 caused an observed PIP upregulation (Fig. 5). The water channel gene most strongly affected in all experiments after 1- and 24-h K⁺ starvation was the OsPIP2;7, which showed over a 2.5-fold change in its expression level, relative to the control values. Note that for the 1-h starvation period, a repression of the OsPIP1;2, OsPIP2;2, OsPIP2;6 and OsPIP2;8 transcript levels was observed under 0.25 mM K⁺ conditions.

Effects of water deficit stress on the expression of PIPs and K⁺ channel/transporters

To broaden our understanding of the co-regulation between PIP and K⁺ channel/transporters, we examined the variable expression responses of the PIPs and K⁺ channel/transporters influenced by the various water deficit treatments in the rice roots. Analysis of PIP and K⁺ channel/transporter expression levels after treatment with 15% PEG 6000 solutions showed a significant correlation among these genes. As shown in Fig. 6, the drought treatment significantly altered the expression of the rice PIPs. The transcript levels of OsPIP1;3, OsPIP2;1, OsPIP2;2, OsPIP2;4, OsPIP2;6 and OsPIP2;8 in the plant roots rapidly decreased to one-tenth, one-twentieth and even one-thirtieth of their original mRNA levels. The expression levels of OsPIP2;3 and OsPIP2;5 in the roots of the control plants are hundreds of folds above those in the drought-stressed plants. The expression levels of OsPIP1;1 and OsPIP1;2 decreased initially and then increased gradually to a level higher than the original, while OsPIP2;7 decreased initially, and then increased to its primary level and then decreased again (Fig. 6). At the same time, the levels of AKT1, HKT1 and TaAKT1 were strongly reduced after the rice seedlings were treated with PEG 6000. The OsAKT1 and OsHKT1 transcript levels decreased to 5% of their original levels while that of OsHAK1 decreased to 1% after the water limitation (Fig. 6). In conclusion, in response to water deficit, rice roots resulted in a similar trend in the expression of OsAKT1, OsHAK1, OsHKT1 channel/transporters and PIPs. However, the kinetics of gene regulation in response to these different stresses seems to be more or less unique for each gene.

Discussion

K⁺ starvation affects K⁺ uptake and water transport

Starving a plant of its essential mineral nutrients may result in an improved nutrient uptake capacity, which may be reflected in an enhanced transcript abundance for specific uptake systems (Maathuis et al. 2003). Herein, we report that K⁺ starvation led to decreased K⁺ levels in the rice roots and increased levels in the leaves (Fig. 1A, B), but the K⁺ content in the whole rice seedlings was not reduced (Fig. 1C). This finding suggests that K⁺ starvation may influence K⁺ channel/transporter activity or transcript abundance; thus, the K⁺ content in the whole rice seedlings was not affected. Our following results demonstrated that K⁺ starvation indeed induced OsAKT1 and OsHAK1 expression (Fig. 5), an observation that agrees with other recently reported results (Buschmann et al. 2000, Wang et al. 2002, Gierth et al. 2005). The upregulation of AtHAK5 and the homologue of HAK5 in tomato upon K⁺ starvation was confirmed (Wang et al. 2002, Gierth et al. 2005). The wheat root potassium channel homologue TaAKT1 was also enhanced in response to K⁺ deprivations (Buschmann et al. 2000).

The rice root Lp was dramatically increased in response to K⁺ starvation in our experiments (Fig. 2). It is possible that the enhanced root Lp results from an increase in either the density or the activity of water channels. Indeed, some of the timed K⁺-starvation...
treatments resulted in higher PIP expression levels compared with those in the controls (Fig. 5). What is the signal that activates the osmo-regulatory mechanism during short-term K\(^+\) starvation in rice roots? Clarkson et al. (2000) proposed that roots can monitor the nutrient content of the solution in which they are exposed and that this function occurs in the root apoplasm, where the root initiates specific responses, such as the change of water channel activity, to nutrient deficiencies.

It seems unlikely that separate biological processes caused the observed similar responses of the PIPs and K\(^+\) channel/transporters to K\(^+\) starvation. Moreover, it is probable that these similarities may reflect the
co-regulation of PIPs and K⁺ channel/transporters in rice roots. However, it is unclear how plants sense the range of external K⁺ concentrations and how a similar gene response may occur. There may be a common regulatory factor for ion and water channels. Pilot et al. (2003) demonstrated that treatment of plants with abscissic acid (ABA), cytokinins or auxin strongly affects K⁺-channel gene expression, thus providing support for the hypothesis that K⁺ transport in plants is under hormonal control and/or that changes in K⁺ fluxes underlie hormonal effects. Previous studies have demonstrated that the Arabidopsis water channel PIP1;2 was regulated by ABA (Jang et al. 2004) and that the rice water channels OsPIP1;1 (OsPIP1a) and OsPIP2;1 (OsPIP2a) were

Fig. 6. Real-time polymerase chain reaction analysis of PIP and K⁺-channel/transporter transcripts in rice roots. The transcript levels of each PIP and three K⁺-channel/transporter genes in the rice roots of the stress-treated plants that were exposed to drought conditions (PEG 6000) for 0.5, 1, 2, 4, 8 and 24 h were plotted as the relative expression of the non-stressed control plants. The expression level of ubiquitin was used as an internal control. Each data point represents the mean ± se of three independent experiments.
regulated by GA and ABA (Malz and Sauter 1999). Therefore, the common regulatory factor for ion and water channels may be hormone.

**Cs** and TEA block K⁺ uptake in roots

K⁺-channel inhibitors are convenient tools to investigate K⁺ uptake and transport. In this paper, we detected that exposing rice roots to a 3 h Cs⁺ treatment inhibited K⁺ uptake by 7.8% (Fig. 3), while TEA had no significant effect. Previous work by Smith and Epstein (1964) showed that NH₄⁺ inhibited K⁺ uptake by competing for a binding site on the transporter in maize leaves. Spalding et al. (1999) assumed that NH₄⁺ inhibited the non-AKT1 K⁺ channels in *Arabidopsis*. However, we observed decreased expression levels of *OsAKT1* in response to both CsCl and TEA treatments (Fig. 7). It was reported that the *OsHKT1* transcript level was also reduced by treatment with 150 mM CsCl for 24 h in rice roots (Gollack et al. 2002). Alternatively, it cannot be excluded that CsCl, especially at these high concentrations (30 and 150 mM), may have other effects on the metabolism besides blocking the K⁺ channels. A previous study showed that Cs⁺ affected chlorophyll biosynthesis by inhibiting uroporphyrinogen decarboxylation in barley leaves (Shalygo et al. 1997). In addition, Cs⁺ induced many genes, even at lower concentrations in *Arabidopsis* (Sahr et al. 2005). Therefore, the effect of K⁺-channel inhibitors on K⁺ uptake is a complex process in plants.

**HgCl₂ and Cs⁺ reduces Lp in roots**

In this present study, we show that HgCl₂ led to a decrease in Lp by more than 70% (Fig. 4), which indicates the possibility that HgCl₂ decreases cell-to-cell water transport by inhibiting water channel activity. We did not measure any significant effect of HgCl₂ on the K⁺ content in the plants that were preincubated in low concentrations of HgCl₂ for 10 min compared with the control plants. However, the relatively long-term treatment (1 and 3 h) decreased the *OsAKT1* transcript levels (Fig. 7), which may decrease K⁺ uptake. This suggests a sequence of events, whereby the water channel activity was reduced by HgCl₂ and then the signal was conducted to the K⁺ channels. Additionally, CsCl decreased the root Lp by about 40% compared with that of the controls within 3 h (Fig. 4). It was reported previously that the expression levels of the transcripts encoding aquaporins, PIP1;2, PIP2;2 and TIP1;2, as well as the K⁺ transporter HAK5 were reduced in *Arabidopsis* roots with growth on Cs⁺ (Sahr et al. 2005). In addition, TEA was shown previously to block the water permeability of human AQP1 channels expressed in kidney and kidney-derived cells, as well as in *Xenopus* oocytes (Yool et al. 2002). These results raise the possibility that K⁺-channel inhibitors decrease the hydraulic conductivity of the plasma membrane by downregulating or blocking water channels. There is another possibility that the decreased Lp with K⁺-channel inhibitor treatment is caused by the decreasing driving force of the reduced K⁺ uptake. These data further indicate that the transporters, water channels and potassium channels are co-regulated and act not independently to each other. When we starved the plants of K⁺, they appeared to compensate by upregulating the K⁺-channel/transporters and water channels. However, when they were treated with K⁺-channel inhibitors, they could not enhance K⁺ uptake and therefore reduced their water uptake to maintain the proper cytosolic osmolarity.

**Water deficiency strongly affects PIPs and K⁺ channel/transporters**

For these studies, we planted the rice seedlings in liquid media, supplemented with the non-ionic agent PEG 6000 to impose water stress on the plants. For the rice seedlings, 15% PEG 6000 can result in a modest stress as indicated by a moderate rolling of the leaves. We determined that the *PIP* and the *OsAKT1*, *OsHAK1* and *OsHKT1* expression levels in the roots generally showed a decrease in response to the 15% PEG 6000 treatment (Fig. 6). These results suggest that the roots decrease their water and ion uptake capacity by regulating channel activity at the transcription level to cope with the water deficiency. This downregulation of *PIP* and K⁺ channel/transporter expression levels may result in reduced membrane water permeability and may promote cellular water conservation during periods of

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**Fig. 7.** Effects of caesium chloride (CsCl), tetraethylammonium chloride (TEA) and mercuric chloride (HgCl₂) on the expression of *OsAKT1* in rice roots. The transcript levels were quantified using reverse transcription polymerase chain reaction. The rice plants were adapted to nutrient solution in the presence or absence of CsCl (30 mM, 1 and 3 h), TEA (30 mM, 1 and 3 h) or HgCl₂ (30 μM, 1 and 3 h). Expression levels of *OsAKT1* were normalized to ubiquitin (UBQ) levels (numbers are given below the gel photographs).
dehydration stress. In previous work, Smart et al. (2001) observed that genes encoding other aquaporins or aquaporin homologues were downregulated under drought conditions. These genes included *Nicotiana glauca* NgMIP1, NgMIP2, NgMIP3, NgMIP4 and NgMIP5. It should be pointed out that there are some water channels, such as OsPIP1;1 and OsPIP1;2, for which expression levels are inducible by a relative long-term water-deficit treatment (Fig. 6), which should result in greater osmotic water permeability and facilitated water flux. Previous studies indicated that the tobacco *NeMip2* and *NeMip3* were induced by drought treatment (Yamada et al. 1997). Recently, Alexandersson et al. (2005) observed that *AtPIP1;4* and *AtPIP2;5* were also upregulated in leaves upon gradual drought stress.

How can the *OsAKT1*, *OsHAK1* and *OsHKT1* response to drought be explained? Following drought treatment, rice seedlings may reduce water loss by decreasing their water channel activities. To maintain a proper ion equilibrium, plants reduced the functional K⁺- and Na⁺-channel/transporter stores to decrease potassium and sodium transport into cells. The expression levels of the PIP- and K⁺ channel/transporter-encoding genes responded similarly to water stress and co-regulated.

References


