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Salinity stress-tolerant and -sensitive rice (*Oryza sativa* L.) regulate AKT1-type potassium channel transcripts differently

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Abstract

In the indica rice (*Oryza sativa* L.) a cDNA was characterized that encoded *OsAKT1* homologous to inward-rectifying potassium channels of the AKT/KAT subfamily. Transcript analysis located *OsAKT1* predominantly in roots with low abundance in leaves. Cell-specificity of *OsAKT* expression was analyzed by *in situ* hybridizations. In roots, strongest signals were localized to the epidermis and the endodermis, whereas lower transcript levels were detected in cells of the vasculature and the cortex. In leaves, expression was detected in xylem parenchyma, phloem, and mesophyll cells. Transcriptional regulation and cell specificity of *OsAKT1* during salt stress was compared in rice lines showing different salinity tolerance. In the salt-tolerant, sodium-excluding varieties Pokkali and BK, *OsAKT1* transcripts disappeared from the exodermis in plants treated with 150 mM NaCl for 48 h but *OsAKT1* transcription was not repressed in these cells in the salt-sensitive, sodium-accumulating variety IR29. Significantly, all lines were able to maintain potassium levels under sodium stress conditions, while sodium concentrations in the leaves of IR29 increased 5–10-fold relative to the sodium concentration in BK or Pokkali. The divergent, line-dependent and salt-dependent, regulation of this channel does not significantly affect potassium homeostasis under salinity stress. Rather, repression in Pokkali/BK and lack of repression in IR29 correlate with the overall tolerance character of these lines.

Introduction

Potassium is a macronutrient for plants that is required for physiological processes such as the maintenance of membrane potential and turgor, activation of enzymes, regulation of osmotic pressure, stoma movement, and tropisms. Early kinetic analyses of K^+ uptake with barley roots suggested the existence of at least two distinct import mechanisms for the ion: a high-affinity uptake system with a K_m in the micromolar range and a low-affinity uptake system with a millimolar K_m (Epstein *et al.*, 1963). During the last decade genes encoding K^+ channels and transporters have been identified from higher plants where at least three families of proteins exist that maintain potassium homeostasis. They are high-affinity transporters, dual-affinity transporters and channels (Amtmann and Sanders, 1999). HKT1, a wheat K^+ transporter was shown to mediate K^+/Na^+ symport when expressed in *Xenopu*s oocytes (Schachtman and Schroeder, 1994; Rubio *et al.*, 1995; Gassmann *et al.*, 1996), and the *Arabidopsis* and rice homologues are now known to sustain substantial sodium currents (Uozumi *et al.*, 2000; Horie *et al.*, 2001). Mutant analysis in *Arabidopsis* recently indicated that AtHKT1 contributes significantly to sodium influx *in planta* (Rus *et al.*, 2001). K^+transporters

with affinity characteristics that are variable, both high and low depending on potassium concentrations, have been identified in the HAK/KT/KUP family (Santa-Maria *et al.*, 1997; Quintero and Blatt, 1997; Fu and Luan, 1998; Kim *et al.*, 1998; Rubio *et al.*, 2000; Rigas *et al.*, 2001). Multiple isoforms for these transporters exist in the *Arabidopsis* genome.

Inward-rectifying K^+ channels, such as AKT1 and KAT1, from *Arabidopsis*, KST1 from potato, maize ZMK1/ZMK2 and wheat TaAKT1, have been functionally characterized (Sentenac *et al.*, 1992; Anderson *et al.*, 1992; Müller-Röber *et al.*, 1995; Philippar *et al.*, 1999; Bauer *et al.*, 2000; Buschmann *et al.*, 2000). Plant K^+ channels of the AKT/KAT family show topological homology to animal Shaker-type K^+ channels, which contain 6 transmembrane domains and a pore domain, but in contrast to the outward-rectifying animal Shaker channels the plant channels are predominantly inward-rectifying (Zimmermann and Sentenac, 1999). Plant inward-rectifying K^+ channels show high selectivity for K^+ over other monovalent cations, and the channels are reputed to specifically mediate K^+ uptake and transport in plant cells (Schachtman *et al.*, 1992). The K^+ channels of the AKT/KAT subfamily are differently expressed in root and leaf tissues. For example, *Arabidopsis* KAT1 and its potato homologue KST1 are expressed in guard cells (Nakamura *et al.*, 1995; Müller-Röber *et al.*, 1995). *Arabidopsis* AKT1 is primarily expressed in root tissue and has been localized to epidermis, cortex, and endodermis by promoter activity analysis (Cao *et al.*, 1995; Lagarde *et al.*, 1996) whereas tomato LKT1 was localized to root hairs (Hartje *et al.*, 2000). In contrast, the *Arabidopsis* K^+ channel AKT2/AKT3 and the *Vicia faba* K^+ channel VFK1 transcripts are most abundant in leaves in cells associated with phloem indicating possible involvement in potassium transport (Cao *et al.*, 1995; Marten *et al.*, 1999; Deeken *et al.*, 2000; Lacombe *et al.*, 2000; Ache *et al.*, 2001). In *Brassica napus* the expression of AKT1 was not affected by different external K^+ concentrations ranging from 5 μM to 5 mM suggesting that AKT-type K^+ channels may be constitutively expressed mediating K^+ uptake both at micromolar and the millimolar concentrations (Lagarde *et al.*, 1996). Reduced seed germination in the presence of low external K^+ and reduced growth of an *Arabidopsis akt1* mutant (Spalding *et al.*, 1999) support this finding.

We have isolated and characterized, through transcript analysis and *in situ* hybridization, the rice K^+-channel transcript *OsAKT1*. Our emphasis was on the comparative analysis of *OsAKT1* expression in rice lines with different tolerance to salinity stress. The *OsAKT1* K^+-channel transcripts are regulated in a cell-specific manner; and this regulation distinguishes sodium-excluding and sodium-accumulating rice lines indicating correlation of K^+ homeostasis and OsAKT1 transcription in salt-stressed rice.

Materials and methods

Plant material

Seeds of *Oryza sativa* L. *indica* cvs. IR29, Pokkali, and BK were provided by the International Rice Research Institute (IRRI, Los Baños, Phillipines). Seedlings were transferred to aerated hydroponic tanks 7 d after germination in silica sand. Rice plants were grown in half-strength Hoagland's solution (Ostrem *et al.*, 1987) with double iron and 4 mM K^+. For studies with K^+ concentrations of 0.1 mM the plants were adapted to these concentrations for 7 d prior to the experiments. In experiments with reduced K^+, K_3NO_4 was substituted by NH_4NO_3. Plants were grown in a controlled environment chamber (ConViron, Asheville, NC) with a photoperiod of 12 h light at 28 °C/12 h dark at 22 °C. Experiments were performed with plants at the age of 3 weeks. For salt stress, plants were subjected to 150 mM NaCl. Control plants were grown in parallel and harvested at the same time. For analyses of leaf tissue the second youngest leaf of the plants was used.

Isolation and characterization of transcripts

Degenerate primers corresponding to conserved regions of AKT1 (Anderson *et al.*, 1992) and KAT1 (Sentenac *et al.*, 1992) were used in PCR reactions with first-strand cDNA synthesized from total RNA of cv. IR29. A 300 bp cDNA fragment was obtained and used to screen a cDNA library synthesized from total RNA of IR29. The 5′ end of the sequence was obtained by 5′-RACE PCR (Gibco-BRL, Rockville, MD) with sequence-specific 3′-oligonucleotide primers. *OsAKT1* cDNA was sequenced and sequence data analyses were performed with programs of the Wisconsin Genetics Computer Group package (GCG package version 9.0; University of Wisconsin, Madison, WI).

Southern hybridization and RT-PCR

Genomic DNA was prepared from leaves of rice plants cv. IR29 and cv. Pokkali by dodecyltrimethyl ammonium bromide extraction and subsequent LiCl precipitation to remove RNA (Gustinich *et al.*, 1991). The genomic DNA was digested with restriction endonucleases, and DNA fragments were separated in 0.8% agarose gels, blotted and hybridized by standard procedures (Sambrook *et al.*, 1989). Detection probes corresponding to the coding region of the gene and to the untranslated region of the gene were labeled with ^{32}P-dCTP with random primers. The final wash of the filters was in 2× SSC at 55 °C.

For transcript analysis by RT-PCR, total RNA of rice cv. IR29 was isolated by guanidinium thioisocyanate extraction (Chomczynski and Sacci, 1987). cDNA for RT-PCR amplification was synthesized from 3 μg of total RNA with SuperScript RT II (Gibco-BRL) in 20 μl reactions. After synthesis, the cDNA was diluted 1:10 and 10 μl aliquots of cDNA were used as template for PCR amplifications in 50 μl standard reactions. The following cycle parameters were used for PCR: 90 s 94 °C in the first cycle, followed by 1 min 94 °C, 1 min 55 °C, 2 min 72 °C with cycle numbers as indicated, and a final extension at 72 °C for 10 min. The sequences of the primers used were 5′-GGCTGCAAGATCAGATGA-3′ and 5′-ACGCAACAACTGGCACAA-3′. The PCR products were separated on 1.7% w/v agarose gels and blotted and hybridized according to standard procedures (Sambrook *et al.*, 1989). The detection probe corresponding to the coding region of *OsAKT1* was prepared by PCR with digoxigenin-dUTP (Roche Diagnostics, Mannheim, Germany) as a label. Signal detection was performed with anti-digoxigenin alkaline phosphatase-conjugated Fab fragments and CSPD as substrate. Filters were washed with 0.5× SSC at 42 °C for 30 min. Rice actin was amplified as a loading control from cDNA with the same PCR parameters described above and the following primers: 5′-GTGATCTCCTTGCTCATACG-3′ and 5′-GGNACTGGAATGGTNAAGG-3′. The PCR products were separated in 1.7% w/v agarose gels, stained with ethidium bromide, and photographic images were obtained.

HPLC analysis of cations

Roots and the second-youngest leaf of each 7 plants of the rice lines IR29, Pokkali, and BK were collected and ground in liquid nitrogen. The tissue was homogenized in ethanol/chloroform/water (15:5:3) and re-extracted with water. The aqueous phase was used for cation HPLC analysis (IonPac cation exchange, Dionex, Sunnyvale, CA).

In situ *hybridization*

Root tissue about 5 cm from the root tip, tissue about 200 μm from the root tip, and tissue from the second youngest leaf of rice plants were used for *in situ* hybridizations. *In situ* hybridization was performed with the same probe from the coding region of the gene as described above for the Southern hybridizations according to Golldack and Dietz (2001).

Results

Isolation of rice AKT1

The cDNA of rice *OsAKT1*, 2899 bp in length, homologous to *Arabidopsis* AKT1/KAT1 sequences, was obtained by a combination of RT-PCR and 5′-RACE extension. BLAST searches of the rice genomic sequence revealed a BAC clone (AP003340), which contained a sequence identical to the cDNA sequence. The start codon was deduced by comparison with the AKT1-like potassium channel sequences from wheat (AAF36832) and maize (CAA68912). *OsAKT1* consists of an open reading frame of 935 amino acid residues that shares sequence homology with inward-rectifying K^+ channels from other grasses (Figure 1). The amino acid sequence identity is 73% with maize ZMK1 (CAA68912) and 74% with wheat TaAKT1 (AAF36832). *Arabidopsis* AKT1 (AAA96810) and the rice homologue share 61% amino acid identity. Hydrophobicity of the deduced amino acid sequence of *OsAKT1* predicts at least six membrane-spanning domains and an overall structure very similar to that predicted for the *Arabidopsis* protein (not shown).

Southern-type hybridizations were performed with probes from the 3′-untranslated region of the *OsAKT1* transcript and with a probe from the conserved coding region of the gene (Figure 2). Whereas hybridization of a single gene was detected in the rice genome when using the probe from the non-coding region of the gene, results using the probe from the coding part of *OsAKT1* indicated the existence of a small gene family. A search of the available rice genome sequences indicated the existence of three additional sequences homologous to AKT1-type inward-

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OsAKT1     MARWGAARMAACGPWGRNRRVGAGDAFEASEVRRDGRSRMMPA-CGPWGAGHG--GGD
ZMK1       ---------------------------------MAGCAPSRVASCGPWG-------G-
TaAKT1     -------------------------------MSSRSGAARMRA-CGPWGEGGSGVVGD
                                *
OsAKT1    -PALERELSRDGSHYSISSAILPSLGARSNRRIKLRRFIISPYDRRYRIWETFLIVLVVY
ZMK1      RAALESELSWDGSHYSISSGILPSLGARSNRRVKLRPFIVSPYADRYRCWETFLIILVVY
TaAKT1    AHALEREMSRDGSHYSLSSGILPSLGARSNRRVKLRRFIISPYDRRYRLWETFLIVLVVY

OsAKT1    SAWVSPFEFGFIPKPTGALATADNVVNAFFAVDIILTFFVAYLDKMSYMLEDDPKKIAWR
ZMK1      SAWVSPFEFGFIQKPTGALAAVDNVVNAFFAVDIILTFFVAYLDRMTYLLEDDPKRIAWR
TaAKT1    SAWVSPFEFGFIRIPTGGLAATDNAVNAIFAVDIILTFFVAYLDRLTYLLEDDPKRIAWR

OsAKT1    YSTTWLVLDVASTIPSEFARRILPSKLRSYGFFNMLRLWRLRRVSSLFSRLEKDRHFNYF
ZMK1      YTTSWFVLDVASTIPSEFARKILPPDLRSYGFFNMLRLWRLRRVSSLFARLEKDRHFNYF
TaAKT1    YATSWLVLDVASTIPSEIARRMLPSKLRSYGFFNMLRLWRLRRVSSLFARLEKDRHFNYF

OsAKT1    WVRCAKLICVTLFAVHCAACFYYLLADRYPVPTSTWIGNYMADFHERSLWIRYVTSVYWS
ZMK1      WVRCAKLICVTLFAVHCSACFYYLLADMYPTPTDTWIGNSMPDFHQRGLWIRYVVSVYWS
TaAKT1    WVRCAKLICVTLFAVHCAACFYYLLADRYPDPKETWIGNTMPDFHSKGLWIRYVTSVYWS

OsAKT1    ITTLTTVGYGDLHAENTREMIFNIFYMLFNLGLTAYLIGNMTNLVVHGTSRTRNYRDTIQ
ZMK1      ITTLTTVGYGDLHAENTREMIFNILYMLFNLGLTAYLIGNMTNLVVHGTSRTRKYRDTIQ
TaAKT1    ITTLTTVGYGDYHAENIREMIFNIFYMFFNLGLTAYLIGNMTNLVVHGTSRTRKYRDTIQ

OsAKT1    AATSFGVRNQLPPRLQDQMISHISLKYRTDSEGLQQQEILDSLPKAIKSSISQYLFFHLV
ZMK1      AATSFALRNQLPSRLQDQMMSHLCLKFRTDSEGLQQQETLDVLPKAIRSSISQYLFFNLV
TaAKT1    AATSFALRNQLPPRLQDQMISHLSLKFRTDSEGLQQQETLDALPKAIRSSISQYLFLNLV

OsAKT1    QNVYLFQGVSNDLIFQLVSEMKAEYFPPREDVILQNEAPTDFYILVSGSVELVEQQN---
ZMK1      QKVYLFEGVSNDLIFQLVSEMKAEYFPPREDVILQNEAPTDFYILVIGSAELIELQN---
TaAKT1    QNIYLFQGVSNDLIFQLVSEMKAEYFPPREDVILQNEAPTDFYILVSGSVELVEVPNGAE

OsAKT1    -GADQVIQVATSGEVVGEIGVLCYRPQLFTVRTRSLCQLLRLNRTAFLSIVQSNVGDGTI
ZMK1      -GGEQMVGVAKAGDVVGEIGVLCYRPQLFTVRTKSLCQLLRMNRTAFLSLVQSNVADGTI
TaAKT1    HGAEQVVGVAKSGEVIGEIGVLCYRPQLFTVRTRSLCQLLRMNRTAFLSIVQSNVGDGTI

OsAKT1    IMNNLIQFLKEQKENSVMAGVVKEIESMLARGNLDLPITLCFAVTRGDDFLLHQLLKRGM
ZMK1      IMNNLIRLLKQQNDNSVMMGVLKEYENMLARGRLDLPVTLCFAVNKGDDFMLHQLLKRGL
TaAKT1    IMNNLIQLLKEQTDG-VMVGVLKEIESMLARGRLDLPITLCFAVTRGDDHLLHQLLKRNL

OsAKT1    DPNESDNDGHTALHIAASKGNEQCVRLLLEYGADPNARDSEGKVPLWEALCEKHAAVVQL
ZMK1      DPNESDNNGHTALHIAASKGDEQCVKLLLEHGADPNARDSEGKVPLWEALCEKQNPVVEL
TaAKT1    DPNESDQDGRTALHIAASKGNEQCVKLLLEYGADPNARDSEGKVPLWEAVYAKHDTVVQL

OsAKT1    LVEGGADLSSGDTGLYACIAVEESDTELLNDIIHYGGDVNRARRDGTTALHRAVCDGNVQ
ZMK1      LVQSGAGLSSGDVALYSCVAVEENDPELLENIIRYGGNVNSSMKDGTTPLHRAVCDGNVQ
TaAKT1    LVKGGAELSSGDTSLYACTAVEQNNIELLKQILKHVIDVNRPSKDGNIPLHRAVCDGNVE

OsAKT1    MAELLLEHGADIDKQDGNGWTPRALAEQQGHDDIQLLFRSRKAATASGHHHVPSSTTTRV
ZMK1      MVELLLEHGADIDKQDNNGWSARALADQQGHDDIHSLSRSRKAHRQQHASKG--------
TaAKT1    MVELLLRHGADIDKQDSNGWTPRALAEQQGHEEIQNLFRSVIAPRKYTSNGR--------

OsAKT1    APAAAAASLIGRFNSEPMMKNMIHED-ADLPSRVLPEKLRRKRVTFQNSLFGVISSSQAQ
ZMK1      ---TVAPVPIWRFNSEPTMPNIKHEEDAELRGKVVPQKLLRKRVSFQNSLFGVISSSHAR
TaAKT1    ----VTPMLLGRFSSDPSMQKVIHEDVEQQPSKVLPQRR---KVSFHNSLFGVISSAHPR

OsAKT1    RETDHPLSRGGLAATGSPNPSSGSRNAVIRVTISCPEKGNTAGKLVLLPQTLDMLLELGA
ZMK1      QDTGRLLSKG----LAGPGSPGCSHGSLVRVTIGCPEKGNAAGKLVLLPRSMTEVLELGA
TaAKT1    RETDHLLSRG-LAATGGPTYPQAHHNPLIRVTISCPEMGNTAGKLVILPGSIKELLQLGA

OsAKT1    KKFDFAPTKVLTVEGAEVDEVELIRDGDHLVLVSDEWDAEKMKCKS---
ZMK1      RKFGFKPTKVLTTGGAEIDEVELIRDGDHVVLVSDDWAPDVAQLRPNDK
TaAKT1    KKFDMMPTKVLTIEGAEVDEVELIRDGDHLVLASDDWVPDDTQIRGKN-
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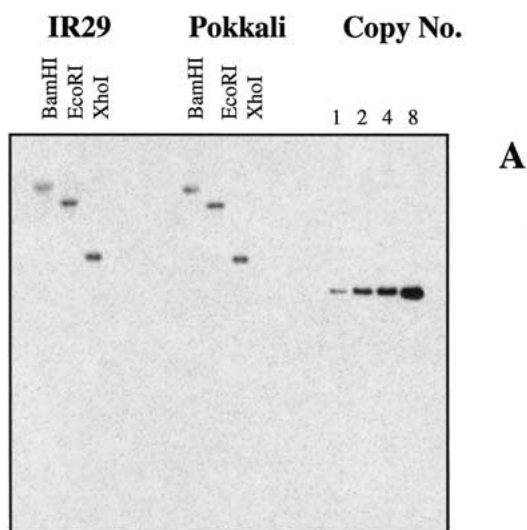
Figure 1. Characterization of the *OsAKT1* transcript from rice IR29. Alignment of the predicted amino acid sequence of OsAKT1 with maize ZMK1 (CAA68912) and wheat TaAKT1 (AAF36832). The asterisk marks the start of the OsAKT1 cloned cDNA.



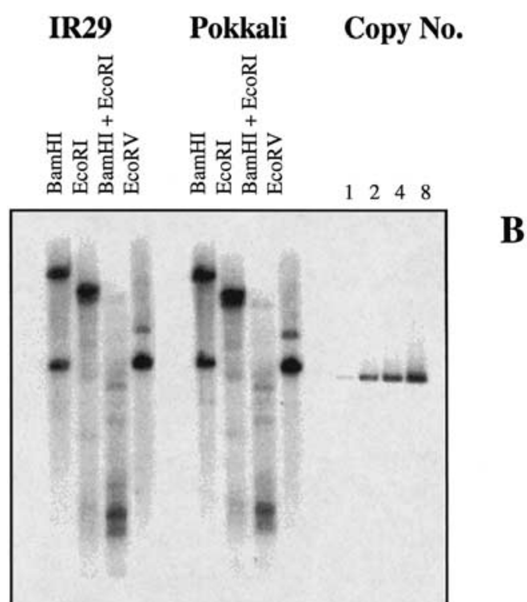


Figure 2. Southern-type hybridization of OsAKT1 to rice genomic DNA. Rice genomic DNA from lines IR29 and Pokkali was digested by restriction enzymes as indicated and hybridized with a probe of the 3′-non-coding region (A) and a probe of the coding region (B).

rectifying K^+ channels. The hybridization patterns with both probes were the same for the rice lines IR29 and Pokkali indicating sequence conservation and the conservation of gene number for *OsAKT1*-type K^+ channels in both rice lines (Figure 2).

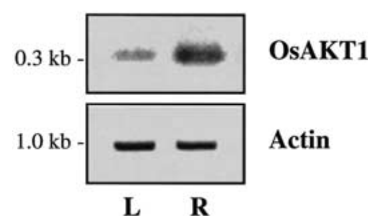


Figure 3. Tissue specificity of *OsAKT1* expression in rice. Transcript amounts of *OsAKT1* were quantitated in leaves (L) and roots (R) of the rice line IR29 by RT-PCR amplification and subsequent Southern-type hybridization. Fragments of the coding region of *OsAKT1* were amplified by RT-PCR and a probe of the coding region was used for hybridization. L, transcript abundance of *OsAKT1* in leaves, 22 cycles of PCR amplification; R, transcript abundance of *OsAKT1* in roots, 22 cycles of PCR amplification.

Tissue and cell specificity of OsAKT1 *transcript abundance*

The expression of *OsAKT1* was studied in the rice lines IR29, Pokkali, and BK. Abundance of the transcripts was quantified by RT-PCR amplification with gene-specific oligonucleotide primers designed from the coding region of the gene and subsequent Southern-type hybridization. Rice actin was amplified as a loading control. Expression of the *OsAKT1* potassium channel transcript, using a gene-specific primer pair, could be detected in root and leaf tissue with higher transcript amounts in roots than in leaves (shown for IR29 in Figure 3).

To study the cell specificity of *OsAKT1* expression, *in situ* hybridizations were performed in root and leaf tissue sections of 3-week old rice plants (Figure 4). In root tips the highest signal strength was found in the exodermis and the epidermal cell layer as well as in the endodermis and in pericycle cells. *OsAKT1* showed weaker signals in cortex cells and in protoxylem and phloem cells within the vasculature. In mature roots transcripts were highest in the epidermis and exodermis, and in the vasculature surrounding metaxylem vessels. In leaf cross-sections, *OsAKT1* signals were detected in xylem parenchyma cells, the phloem, and in mesophyll cells. The three rice lines, IR29 (shown in Figure 4), BK and Pokkali (not shown), showed identical cell specificity of *OsAKT1* expression.

To study the effects of the external K^+ concentration on tissue-specificity of *OsAKT1* expression, rice plants were adapted to 100 μM K^+. Cell specificity of the expression pattern of the rice potassium channel homologue was identical in all tissues for the three rice lines irrespective of whether the plants were grown in 100 μM or 4 mM K^+ (not shown).

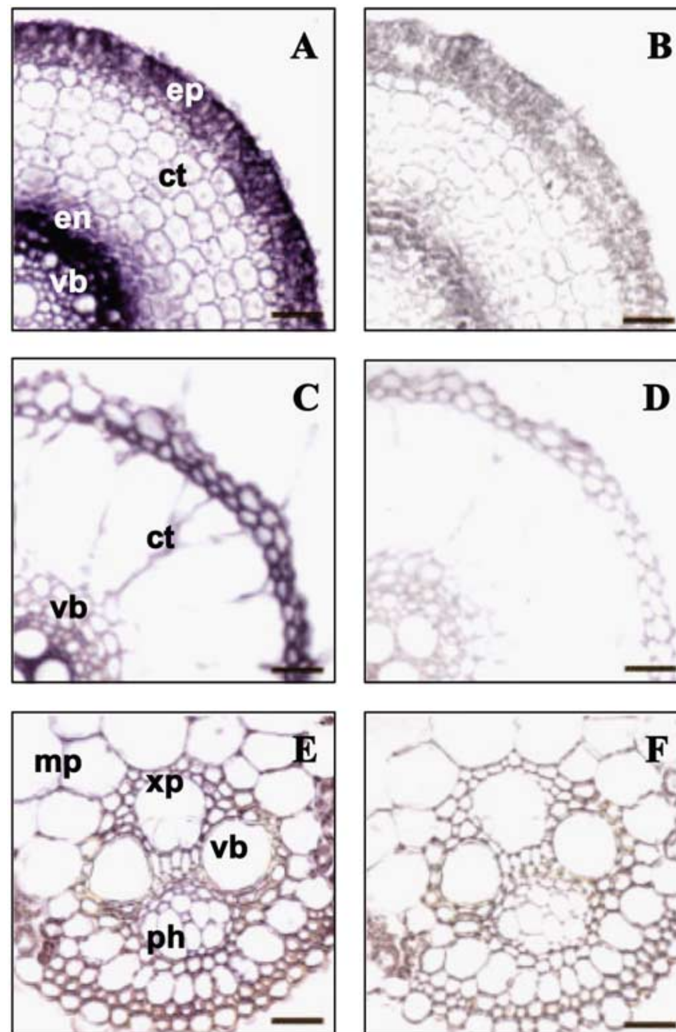


Figure 4. *In situ* hybridization of IR29 OsAKT1. Hybridization of an antisense probe (A) and a sense probe (B) to cross-sections of roots about 200 μm from the meristem. Hybridization of an antisense probe (C) and sense probe (D) to cross sections of roots about 5 cm from tip. E. Hybridization to a leaf cross-section focusing on a vascular bundle using antisense probes and sense probe hybridization (F) to a leaf cross section. The bars equal 50 μm. vb, vascular bundle; ct, cortex; ep, epidermis; en, endodermis; mp, mesophyll cells; xp, xylem parenchyma; ph, phloem.

Salt stress-dependent expression of OsAKT1

We were interested in analyzing salinity stress induced responses of *OsAKT1* expression in rice lines with different sensitivity to salinity. IR29, Pokkali, and BK plants were either grown under control conditions or treated for 24 and 48 h with 150 mM NaCl. Under these stress conditions the plants of line IR29 accumulated Na^+ in leaf tissue to concentrations of 600 μmol per gram fresh weight after 24 h of exposure to 150 mM NaCl, and about 1400 μmol/g Na^+ after 48 h (Figure 5). At this time the K^+concentration had increased by about 50% with respect to the value in IR29 control plants. In contrast, the rice lines Pokkali and BK were acting as Na^+ excluders under identical conditions. After a 48 h exposure to 150 mM NaCl, Pokkali had accumulated 200 μmol/g Na^+ in the leaves, and BK about 100 μmol/g Na^+. Changes in leaf potassium content, compared to the control values, amounted to about 50% in Pokkali and 10% in BK.

To obtain information on *OsAKT1* expression in plants exposed to 150 mM NaCl, transcript abundance was studied by RT-PCR amplification and by *in situ* hybridization. No differences in transcript levels be-

tween control plants and plants treated for 24 and 48 h with 150 mM NaCl could be detected in leaves and roots of IR29, Pokkali, and BK (not shown). Also, cell specificity of expression of the K^+-channel homologue was not changed in mature roots and in leaves of the 3 rice lines (not shown). Root tips, however, showed a difference in the signal that distinguished IR29 from Pokkali and BK. The signal intensity declined in the exodermis and endodermis of the sodium-excluding lines Pokkali and BK (Figure 6) but increased slightly in the vascular tissue. In contrast, in the sodium-accumulating line IR29 the cell-specificity of *OsAKT1* expression was not changed in response to salinity stress (Figure 6).

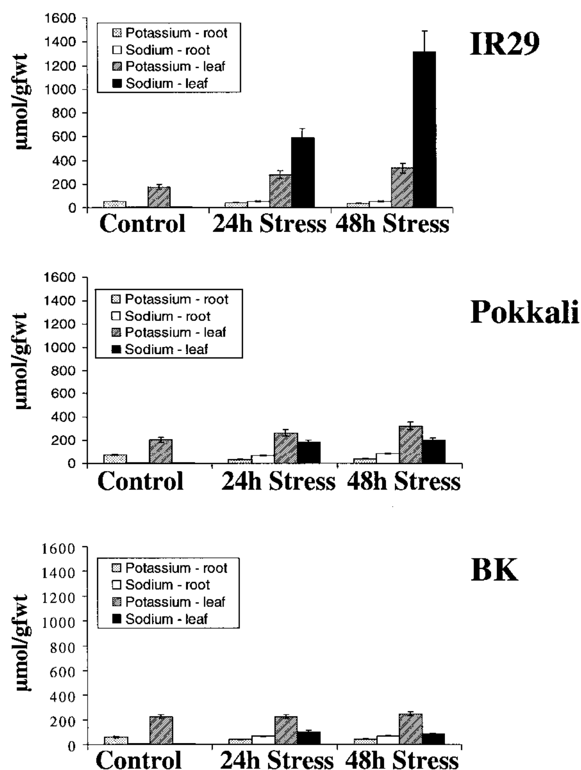


Figure 5. Sodium and potassium accumulation in the rice lines IR29, Pokkali, and BK. The plants were adapted either to control conditions or treated with 150 mM NaCl for 24 and 48 h. Ion concentrations were measured by cation HPLC ($n = 7$).

Discussion

OsAKT K^+-channel homologues are expressed cell-specifically in roots and leaves

Here we report the isolation of a rice cDNA, *OsAKT1*, homologous to inward-rectifying K^+channels, and focus on the analysis of cell specificity of its expression by *in situ* hybridization. By using a probe from a region of *OsAKT1* that is conserved when compared to other plant K^+ channels we studied the transcripts of the rice *OsAKT* genes. As demonstrated by Southern hybridization, *OsAKT* constitutes a small gene family (Figure 2). Accordingly, transcripts were observed in different tissues and cells. In roots, we found *OsAKT* expression in cell types known as primary sites for K^+ uptake in plants. In differentiated elongating root tips close to the meristematic tip, *OsAKT* showed the highest transcript abundance in the epidermis and exodermis as well as in the endodermis and in pericycle cells whereas the signals in mature roots were most concentrated in the exodermis and in stelar cells.

Root hairs and root epidermal cells are primary sites for the uptake of inorganic ions. Ions may pass cortex cell layers via diffusion both in the apoplastic and the symplastic space. The main diffusion barrier for root ion uptake is the endodermis with its Casparian strips in the cell wall that prevent further diffusion into the vasculature thus requiring uptake of the ions into the symplast (e.g., Peterson and Enstone, 1996; White, 2001). The cell specificity of expression of *OsAKT* in rice root cells (Figure 4) corresponded with data presented for *Arabidopsis. AKT1* promoter-GUS constructs reported expression in the epidermis, cortex, and endodermis of mature roots (Lagarde *et al.*, 1996). Our results demonstrate that the root expression pattern of the AKT-type subgroup of inward-rectifying K^+channels shows similarities in monocot and dicot plants.

Interestingly, cell specificity of *OsAKT* abundance was not changed by K^+ depletion to 100 μM in the nutrition medium. Using RNA-blot experiments, Lagarde *et al.* (1996) reported *AKT1* expression to be independent of the external K^+ concentration ranging from micro- to millimolar concentrations of the ion in *Brassica napus.* Buschmann *et al.* (2000) found transcripts of the wheat K^+ channel TaAKT1 to be enhanced in root cells by K^+ starvation. The *Arabidopsis* T-DNA insertion mutant *akt1* showed reduced growth rates at low micromolar external K^+ concentrations (Spalding *et al.*, 1999) indicating that K^+ uptake via inward-rectifying K^+ channels is not restricted to millimolar external potassium concentrations but that the channel has a physiological role even at micromolar concentrations.

Apart from the expression in roots, we detected *OsAKT* transcripts in leaves in mesophyll cells and

cells neighboring the metaxylem vessels indicating involvement of these channels in K^+ transport and distribution. An additional component mediating potassium long-distance transport, supporting OsAKT action, will be homologues of outward-rectifying channels of the plant Shaker family, such as SKOR, which is expressed in the root vascular tissue in *Arabidopsis* (Gaymard *et al.*, 1998). Also, *OsAKT* transcripts were localized to phloem cells suggesting involvement in phloem loading by this potassium channel type in rice. This result is comparable to that obtained with the *Arabidopsis* K^+ channel AKT2/AKT3 that has been detected in phloem elements (Marten *et al.*, 1999; Lacombe *et al.*, 2000). A function of K^+ recycling from shoots to roots in higher plants has previously been hypothesized by White (1997) based on kinetic studies with rye. There, the K^+ influx rate into roots is regulated by the K^+ concentration in the shoot and the root phloem acts by negative feedback.

Salt-dependent expression of OsAKT *is different in rice lines differing in tolerance to salinity*

Whereas no specific uptake system for sodium has been identified with certainty at the molecular level in plants, non-specific uptake of Na^+ via potassium transport systems is likely (Schachtman and Liu, 1999; Blumwald *et al.*, 2000). In general, successful adaptation of plants to high salinity requires maintenance of Na^+:K^+ homeostasis with high discrimination of K^+ over Na^+. Currently, several families of ion transport systems are known that might have a role in plant Na^+ uptake. The recent characterization of *Arabidopsis* mutants with extreme NaCl sensitivity and the suppression of this phenotype by a defect in AtHKT1, which then leads to lowered sodium influx, suggests that *AtHKT1*, at least in some species, could be a major sodium uptake protein (Rus *et al.*, 2001). Candidate proteins for non-specific Na^+ uptake, in addition to or apart from the HKT-type transporters, are HAK/KT/KUP-type transporters, inward-rectifying potassium channels, low-affinity cation transporters of the LCT1-type (Schachtman *et al.*, 1997), and voltage-independent channels.

In this study we compared the transcription of OsAKT in rice lines differing in tolerance to salinity stress: the Na^+-accumulating line IR29 and the Na^+-excluding lines BK and Pokkali (Yeo *et al.*, 1990; Garcia *et al.*, 1995). Interestingly, the ability of BK and Pokkali to exclude Na^+ depends on the external K^+ concentration. In the range from 100 μM to low-mM K^+ concentrations, Pokkali and BK selectively exclude Na^+ while maintaining the internal K^+ concentration. At low-μM K^+ these lines accumulate, however, Na^+ similarly to rice cv. IR29 (Golldack *et al.*, 1997; Golldack, Su, Quigley, Kamasani, Muñoz-Garay, Balders, Popova, Bennett, Bohnert, and Pantoja, unpublished results) supporting the hypothesis that K^+ transport systems are of physiological relevance for salt tolerance in plants. The fact that the tolerant rice lines loose tolerance at low external K^+ is most likely the consequence of a failure to maintain high Na^+:K^+ ratios under K^+ depletion.

We found a clear correlation of OsAKT expression in differentiated roots close to the meristematic root tip with whole-plant Na^+ selectivity. Whereas in the salt-sensitive IR29 the cell specificity of OsAKT was not changed in the endodermis and transcript amounts increased in the exodermis, down-regulation occurred in these cells in the Na^+-excluding lines BK and Pokkali in response to salinity. These results indicated that major nutrient uptake seems to be mediated from the root tip region rather than through the mature segments of the roots.

Regulation of AKT1 homologues in response to salt stress have also been reported for the halophytic *M. crystallinum.* The expression of *MKT1* decreased in response to salt stress and in roots whereas cell specificity did not change (Su *et al.*, 2001). As known from heterologous expression studies, inward-rectifying K^+channels are highly selective for potassium but other cations, such as Na^+, are not completely excluded (Schachtman *et al.*, 1992; Maathuis *et al.*, 1997). Amtmann and Sanders (1999) demonstrated that under conditions of low Na^+ concentrations no significant import of Na^+ through inward-rectifying K^+-channels occurs. However, these channels could mediate comparable uptake rates for Na^+ and K^+ at high salinity (Amtmann and Sanders, 1999). Taken together, the available data, including our study showing salt-dependent regulation of *OsAKT* transcripts, indicate involvement of inward-rectifying channels in the regulation of the K^+ to Na^+ ratio under salt stress.

Additional potassium uptake systems exist and additional mechanisms have been suggested to be of physiological relevance for Na^+ uptake *in planta.* HAK/KT/KUP-type transporters that mediate K^+ transport both in the low-affinity and the high-affinity range of K^+ concentrations have been shown to be affected by Na^+ when expressed in yeast (Santa-Maria *et al.*, 1997; Quintero and Blatt, 1997; Fu and

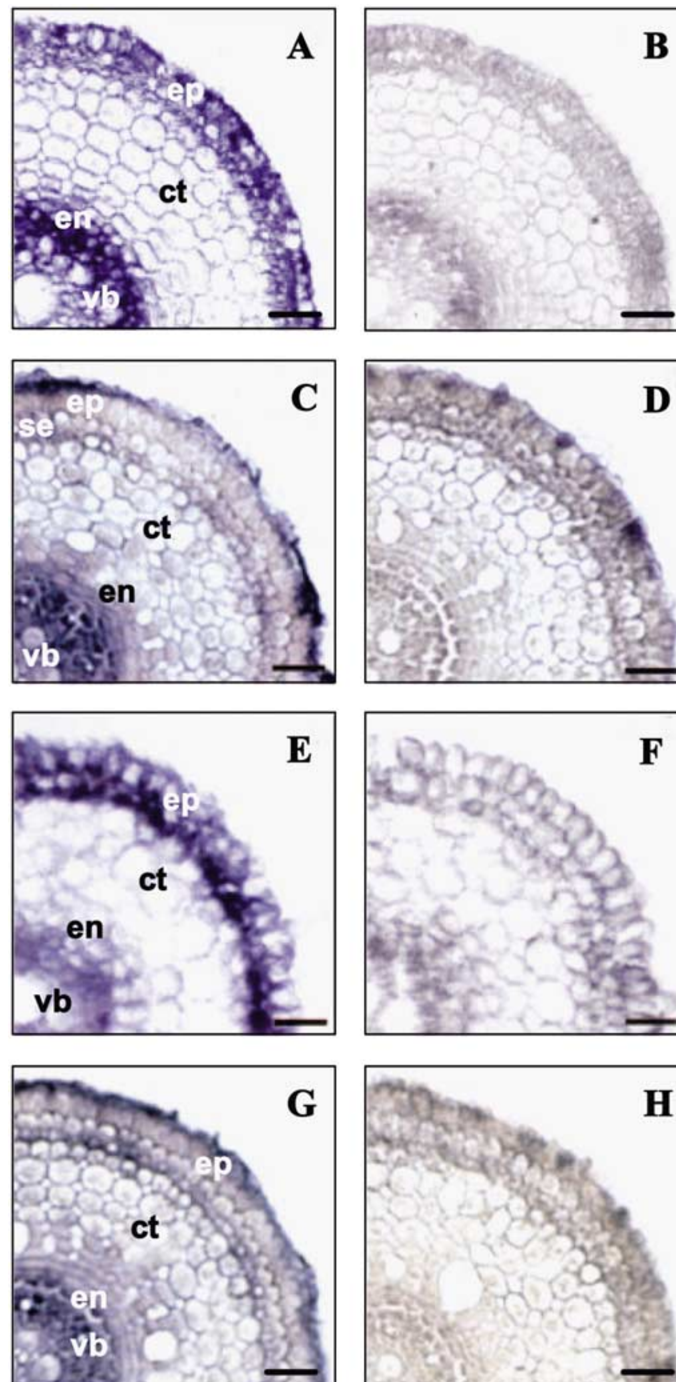


Figure 6. In situ hybridization of *OsAKT1* to cross-sections of roots about 200 μm from the meristematic tip. Hybridization of an antisense probe (A) and a sense probe (B) to plants of rice line BK grown under control conditions. *In situ* hybridization in antisense (C) or sense (D) orientation in rice line BK plants treated with 150 mM NaCl for 48 h. *In situ* hybridization in rice line IR29 treated with 150 mM NaCl for 48 h in antisense (G) and sense (H) orientation. *In situ* hybridization in rice line Pokkali treated with 150 mM NaCl for 48 h in antisense (G) and sense (H) orientation. The bars equal 50 μm. vb, vascular bundle; ct, cortex; ep, epidermis; en, endodermis; se, subepidermis.

Luan, 1998). Another possible pathway for Na^+ import might be via HKT-type transporters. Transcripts of one of these, OsHKT1, have been shown to act as a general alkali cation transporter in rice (Golldack *et al.*, 1997; Horie *et al.*, 2001; Golldack, Su, Quigley, Kamasani, Muñoz-Garay, Balders, Popova, Bennett, Bohnert, and Pantoja, unpublished results). We have, however, observed down-regulation of transcripts for this transporter in root tissue of the rice lines IR29 and Pokkali in response to salt stress. Thus, judged by their ion specificity, HKT-type transporters are not a major pathway for the uptake of K^+ and, possibly, also contribute little to Na^+ influx in rice roots at high salinity, at least not in species that down-regulated HKT transcripts and proteins during salt stress episodes.

Other possible pathways for Na^+ entry in plants might be LCT1-homologous transporters and voltage-independent channels that have a low selectivity of K^+ over Na^+. However, the function of both transport systems is inhibited by Ca^{2+} concentrations of 0.5 mM and higher (Amtmann and Sanders, 1999; Schachtman and Liu, 1999). All experiments in this study were performed with plants grown in nutrition medium with 2 mM Ca^{2+} and, accordingly, the involvement of LCT homologues and voltage-independent channels in Na^+ uptake in our experiments is unlikely or should be minimal, although these transports systems are not completely blocked by low-mM Ca^{2+} and may nevertheless provide a pathway for Na^+ uptake. However, for the rice lines investigated a direct correlation of Na^+ uptake and the regulation of expression of AKT-type K^+ channels seems to exist. Our findings suggest that whole-plant Na^+ accumulation or exclusion depends on the regulation of cell specificity of expression in response to salt stress and on the regulation of transcript abundance.

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