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Original Paper

Characterization of rice KT/HAK/KUP potassium transporters and K^+ uptake by HAK1 from *Oryza sativa*

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Abstract Plant high-affinity K^+ (HAK) transporters are divided into four major clusters. Cluster I transporters, in particular, are thought to have high-affinity for K^+. Of the 27 *HAK* genes in rice, eight HAK transporters belong to cluster I. In this study, we investigated the temporal expression patterns during K^+ deficiency and K^+ transport activity of these eight HAK transporters. The expression of seven *HAK* genes except *OsHAK20* was detected. Expression of *OsHAK1*, *OsHAK5* and *OsHAK21* was induced in response to K^+ deficiency; however, that of other genes was not. Six of the eight HAK transporters—OsHAK1, OsHAK5, OsHAK19, OsHAK20, OsHAK21, and OsHAK27—complemented the K^+-transporter-deficient yeast or bacterial strain. Further, the yeast cells expressing *OsHAK1* were more sensitive to Na^+ than those expressing *OsHAK5*. Mutant analysis showed that the high-affinity K^+ uptake activity was almost undetectable in *oshak1* mutants in a low-K^+ medium (0.02 mM). In addition, the high-affinity K^+ uptake activity of wild-type plants was inhibited by mild salt stress (20 mM NaCl); however, Na^+ permeability of OsHAK1 was not detected in *Escherichia coli* cells. The high-affinity K^+ uptake activity by leaf blades was detected in wild-type plants, while it was not detected in *oshak1* mutants. Our results suggest that OsHAK1 and OsHAK5 are the two important components of cluster I corresponding to low-K^+ conditions, and that the transport activity of OsHAK1, unlike that of OsHAK5, is sensitive to Na^+. Further, *OsHAK1* is suggested to involve in foliar K^+ uptake.

Key words: HAK, potassium, rice, salt stress, transporter.

Introduction

Crop plants might always be slightly starved for nutrients because, in most cases, fertilizers cause an increase in their size, and they then require greater intake of nutrients. Potassium (K^+) is a major component of fertilizers and the most abundant cation in glycophytes. It plays important roles not only in biological events such as stomatal opening in response to osmotic conditions, but also in the regulation of enzymatic activities, including translation (Amrutha et al. 2007). In addition, the intracellular K^+/Na^+ ratio is a key feature of plant salt tolerance (Maathuis and Amtmann 1999) and the ability to retain K^+ in root cells under salt stress correlates with salt tolerance in many species (Cuin et al. 2008; Shabala et al. 2016). Therefore, the regulation of K^+ acquisition and transport is extremely important for plant growth and agriculture. Minerals from soil are distributed to the entire plant via the xylem vessels, and many K^+ transporters and channels are involved in this process. Identification and detailed analysis of K^+ transporters and channels are useful for understanding nutrient transport and appropriate fertilizer application.

Plants have three K^+ transporter families: the K^+ transporters (HKT), the cation proton antiporters (CPA), and the K^+ uptake permease transporters (KT/HAK/KUP) (Gierth and Mäser 2007; Sharma et al. 2013). The HAK transporters form a large family in plants. In all, 13 and 27 paralogs of HAK transporters have been annotated in *Arabidopsis thaliana* and *Oryza sativa*, respectively. They are classified into four clusters based on their amino acid sequence (Bañuelos et al. 2002). Cluster I HAK transporters have high-affinity for K^+ (Santa-María et al. 1997). AtHAK5, the only cluster I

Abbreviations: HAK, high-affinity K^+; T-DNA, transfer DNA; RT-PCR, Reverse Transcription-Polymerase Chain Reaction; *E. coli*, *Escherichia coli*.
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HAK transporter in *A. thaliana*, mediates high-affinity K^+ uptake from the soil. The expression of *AtHAK5* is induced in response to K^+ deficiency, and AtHAK5 is the only system involved in K^+ uptake at concentrations below 0.01 mM (Rubio et al. 2010). The transport activity of AtHAK5 is severely impaired under saline conditions, although it is an important pathway to sustain plant growth at low-K^+ in the presence of salinity (30 mM NaCl) (Nieves-Cordones et al. 2010). *HvHAK1*, a cluster I HAK transporter in *Hordeum vulgare*, is also thought to mediate K^+ uptake (Santa-María et al. 2000; Fulgenzi et al. 2008). The expression of *HvHAK1* and Rb^+ uptake activity are transiently enhanced under saline conditions (Fulgenzi et al. 2008).

The HAK type transporters in *O. sativa* also have been studied well. The analysis of mutants and overexpression lines analysis revealed that OsHAK5 not only mediates high-affinity K^+ acquisition, but also participates in root-to-shoot K^+ transport as well as in K^+-regulated salt tolerance (Yang et al. 2014). Overexpression of *OsHAK5* in tobacco BY-2 cells increases salt tolerance; further it functions as a Na^+-insensitive K^+ transporter, whereas a cluster II HAK, OsHAK2, is sensitive to extracellular Na^+ and exhibits higher Na^+ over K^+ transport activities (Horie et al. 2011). OsHAK21 localizes to the plasma membrane and is expressed in xylem parenchyma and individual endodermal cells (putative passage cells). Knockout of *OsHAK21* led to an increased Na^+/K^+ ratio and salt sensitivity (Shen et al. 2015). Expression levels of rice HAK transporters in 27 tissues were investigated using previously reported transcriptome data (Gupta et al. 2008). The data revealed that the amino acid homology among HAK transporters and the tissue specificity of their expression was rarely related, suggesting that, even if multiple HAK transporters belong to the same cluster, their physiological functions are different.

Bañuelos et al. (2002) showed that *OsHAK1-1* (a partial chimera gene fused with *HvHAK1*), belonging to cluster I, has the ability to transport K^+ in *Saccharomyces cerevisiae* cells. They suggested that the HAK transporters in cluster I mediate K^+ uptake from the soil on the basis of the similarity of K^+ uptake kinetics between yeasts expressing *OsHAK1-1* and K^+-starved rice roots. Chen et al. (2015) showed that the expression of *OsHAK1* is up-regulated by K^+ deficiency, particularly in the root and shoot apical meristem, the epidermis and steles of roots, and vascular bundles of shoots. Knockout of OsHAK1 reduced the total K^+ uptake by approximately 80% and 65% at K^+ levels of 0.05–0.1 mM and 1 mM, respectively.

Thus, although rice has eight HAK transporters in cluster I, only a part of these eight cluster I HAK transporters has been characterized well. In this study, we targeted the eight rice cluster I HAK transporters with higher affinity for K^+ among the four HAK clusters. We determined the expression levels of all eight cluster I *HAK* genes by using real-time polymerase chain reaction (PCR), as well as the K^+ transport activity in yeast and *E. coli* cells.

Materials and methods

Plant culture and real-time PCR

Sterilized wild-type rice seeds (*Oryza sativa* L. cultivar Nipponbare) were germinated and grown on a nutrient medium for 11 days. The composition of the buffer was 1.0 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 0.5 mM H_3PO_4, 50 μM Fe-EDTA, 45 μM H_3BO_3, 7.1 μM $MnSO_4$, 7.6 μM $ZnSO_4$, 0.8 μM $CuSO_4$, 0.5 μM Na_2MoO_4, 2.5 mM MES (pH 5.5) with $Ca(OH)_2$ containing 0.8% [w v^{-1}] Agarose S (Nippon gene, Tokyo, Japan) and KCl at 10 or 0.1 mM. Total RNA was isolated from the shoots and roots of K^+-supplied or K^+-starved rice plants by using the RNeasy kit (Qiagen Hilden, Germany) and was treated with RQ1 DNase (Promega, Wisconsin, USA) for 30 min. First-strand cDNA was synthesized using the Rever Tra Ace kit (TOYOBO, Osaka, Japan). The resultant cDNA sample was diluted 1 : 40, and 2-μl aliquots of the diluted solution were used as the template for PCR in a 10-μl standard reaction mixture. Expression levels were analyzed using Thunderbird SYBR qPCR mix (TOYOBO, Osaka, Japan) in a Prism 7300 Real-time PCR system (Applied Biosystems, California, USA) with the following cycle parameters: once at 95°C for 1 min and 40 cycles at 95°C for 15 s, 58°C for 15 s, and 72°C for 30 s. Primer sequences for *OsHAK1, OsHAK5*, and *OsHAK16* and *actin* genes are as per Okada et al. (2008). Other specific primers used in this study were as follows: for *OsHAK19*, 5′-GTC AAC TAC GTC TAC ACA TTA C-3′ (forward) and 5′-GCC CTA AAA ATA AGA CAA GTG AG-3′ (reverse); for *OsHAK20*, 5′-CAT AGA GCT AGA CAT GTA CC-3′ (forward) and 5′-CTG AGT AGA AGT AGA ATT GAT GC-3′ (reverse); for *OsHAK21*, 5′-CTG TAA CAT GTA GTC AGA TGG-3′ (forward) and 5′-CTC AAA AGC TAG CCA CAA TGG-3′ (reverse); for *OsHAK22*, 5′-CTG ATC GAT CTC TCA TGC ATC-3′ (forward) and 5′-CAG TGG AGT ATA CTG GAG TAG C-3′ (reverse); for *OsHAK27*, 5′-GGA GTA CTC GCT TGT GAA CC-3′ (forward) and 5′-CTC TGC CCA GCA CAT CGT CAC C-3′ (reverse).

Complementation test of a yeast mutant

Rice *HAK* cDNAs were subcloned into the expression vector pYES2, which includes a 2-μm replicon, the GAL1 promoter, and a selective marker *URA3* (Invitrogen, California, USA). The plasmid constructs were used to transform a K^+ uptake-deficient budding yeast strain (*MATa*, *trk1*, *trk2::pCK64*, *his3*, *ura3*) (Becker et al. 1996). For complementation studies, the transformed yeast cells were pre-cultured overnight in liquid SD medium (2% [w v^{-1}] glucose, 0.67% [w v^{-1}] bacto-yeast nitrogen w/o amino acids, 0.5% [w v^{-1}] casamino acids, 14 mM adenine, 15.4 mM histidine, 23.5 mM tryptophan, pH 5.8). Pre-cultured yeast cells were washed three times with 20 mM

$MgCl_2$. After cell densities were adjusted with $MgCl_2$, the cells were spotted on a modified AP medium. The composition of the medium was 8 mM phosphoric acid, 10 mM L-arginine, 2 mM $MgSO_4$, 0.2 mM $CaCl_2$, 2% [wv^{-1}] galactose, 0.6% [wv^{-1}] sucrose, 10 μM NaCl, 6 mM adenine sulfate dehydrate, 11.4 mM L-histidine monohydrochloride monohydrate, 23.5 mM L-tryptophan, and 2% [wv^{-1}] agar, supplemented with 10, 0.3, or 0.1 mM KCl. In the growth inhibition tests, additional NaCl at 25 or 50 mM was added to the AP medium. The yeasts were incubated at 30°C for 3 or 7 days.

Growth tests of E. coli

The *HAK* cDNAs were cloned into the pPAB404 vector (Buurman et al. 1995). The plasmids were introduced into *E. coli* strain LB2003 (*trk*, *kup*, *kdp*) (Uozumi 2001) for the complementation tests and Na^+ sensitivity assays, and *E. coli* strain KNabc (*nhaA*, *nhaB*, *chaA*) (Ito et al. 2001) for growth inhibition assays. For complementation tests, each LB2003 cell line was pre-cultured at 30°C in a synthetic liquid medium (5 mM H_3PO_4, 0.4 mM $MgSO_4$, 6 μM $FeSO_4$, 1 mM citric acid, 1 mg l^{-1} thiamine-HCl, 0.2% [wv^{-1}] glycerol, 8 mM L-asparagine, 10 mM MES, 20 μM $CaCl_2$, 50 μg l^{-1} ampicillin, supplemented with 30 mM KCl), the pH of which was adjusted to 5.5 by using L-arginine. Cultures saturated overnight were washed and spotted equally onto the synthetic solid medium supplemented with 5 or 30 mM KCl, and with or without isopropylthio-β-galactoside (IPTG). The media were incubated at 30°C for 2 or 3 days. For Na^+ resistance assays of LB2003 cells expressing *OsHAK21* and *OsHAK27*, each cell line was pre-cultured at 30°C for 1 day on solid LBK medium (10 g l^{-1} tryptone, 5 g l^{-1} yeast extract, 10 g l^{-1} KCl, 50 μg l^{-1} ampicillin, and 1.5% agar). Cultures were scraped together and washed twice, and then spotted equally onto modified LB medium supplemented with 0.5 mM IPTG and the indicated concentration of KCl or NaCl. These dishes were incubated at 30°C for 12, 20, 48, and 96 h. For growth inhibition assays of KNabc cells expressing *OsHAK1* and *OsHAK5*, each cell line was pre-cultured at 30°C in an LBK medium. Cultures saturated overnight were washed and spotted equally onto solid LBK medium supplemented with 0.5 mM IPTG and indicated amounts of NaCl.

Identification of T-DNA mutant

Two putative T-DNA insertion lines were identified from the Rice Functional Genomic Express Database (http://signal.salk.edu/cgi-bin/RiceGE), and two homozygous lines, *oshak1-D* (3A60207, cultivar Dongjin) and *oshak1-M2* (1B22215, cultivar Manan) were purchased from POSTECH RISD. Each T-DNA insert of *oshak1-D* and *oshak1-M2* was located in the eighth and fourth exons, respectively. The defect of *OsHAK1* transcripts was confirmed by RT-PCR by using mRNA from the roots of K^+-starved plants. Primers used for RT-PCR were as follows: for *OsHAK1*, 5′-CAG CAA GCT AAG CTA GTA G-3′ (forward) and 5′-CCA ACA TTT CAG CTG AAA C-3′ (reverse), for *ubiquitin* (accession no.: D12629), 5′-CCA GGA CAA GAT GAT CTG CC-3′ (forward), 5′-AAG AAG CTG AAG CAT CCA GC-3′ (reverse).

K^+ uptake experiments

For K^+ uptake from roots, two-week-old *oshak1* mutant and wild-type plants, which were grown on K^+-rich medium (nutrient solution supplemented with KCl and gellan gum at 10 mM and 0.4% [wv^{-1}], respectively), were subjected to K^+ deprivation for 8 days in a K^+-free nutrient solution. The solution was replaced every 3 days. Subsequently, the roots of these K^+-starved plants were soaked in 10 ml of low-K^+ medium at 0.02 mM KCl. They were incubated for 30, 60, 90 and 120 min at 30°C. Subsequently, the K^+ concentration of the low-K^+ medium was determined using atomic absorption spectrometry (AA-6800; Shimadzu) as previously described (Chen et al. 2013). The weight of each tube containing the low-K^+ medium was measured before and after K^+ absorption, and the change in the volume of the medium was estimated as 1 g ml^{-1}. Then, the change in the amount of K^+ in the low-K^+ medium was used to calculate the amount of absorbed K^+. Soaked roots were dried completely, and their dry weight was measured. The inhibition effect of external Na^+ was determined by adding additional NaCl to the medium to adjust the final concentration to 2 and 20 mM, respectively, before the roots were soaked. They were incubated for 1 h at 30°C. For foliar K^+ uptake, two-week-old *oshak1* mutant and wild-type plants, which were grown on K^+-rich medium, were transferred to a nutrient solution supplemented with KCl at 1 mM. They were grown for 6 days and then subjected to K^+ deprivation for 3 days in a K^+-free nutrient solution. Subsequently, the shoots were well rinsed with ion-exchanged water and two leaf blades of those K^+-starved plants, about 6 cm from the leaf tip, were soaked in 10 ml of low-K^+ medium at 0.1 mM KCl. They were incubated for 24 h in a growth chamber at 27.5°C during the daytime and 24°C during the nighttime. Subsequently, the amounts of absorbed K^+ were calculated from the K^+ concentration and the volume of the low-K^+ medium as described above. Additional ammonium chloride was also supplemented to the low-K^+ medium as an inhibitor of HAK-type transporters at 1 mM.

Results

Expression profile of the eight cluster I HAK transporter genes under K^+ deficiency

Previously, we analyzed the expression of the known 17 *HAK* genes (Okada et al. 2008). Subsequently, 27 *HAK* genes were reported to be encoded in the rice genome, of which 8 belonged to cluster I (Gupta et al. 2008). HAK transporters belonging to cluster I have high-affinity K^+ transport activity to adapt to K^+ deficiency (Bañuelos et al. 2002; Gierth et al. 2005; Horie et al. 2011; Senn et al. 2001; Shen et al. 2015). The expression patterns of the 8 *HAK* genes under low-K^+ condition, were comapared by using real-time PCR analysis. The total RNA extracted from 11-day-old plants revealed that seven *HAK* genes,

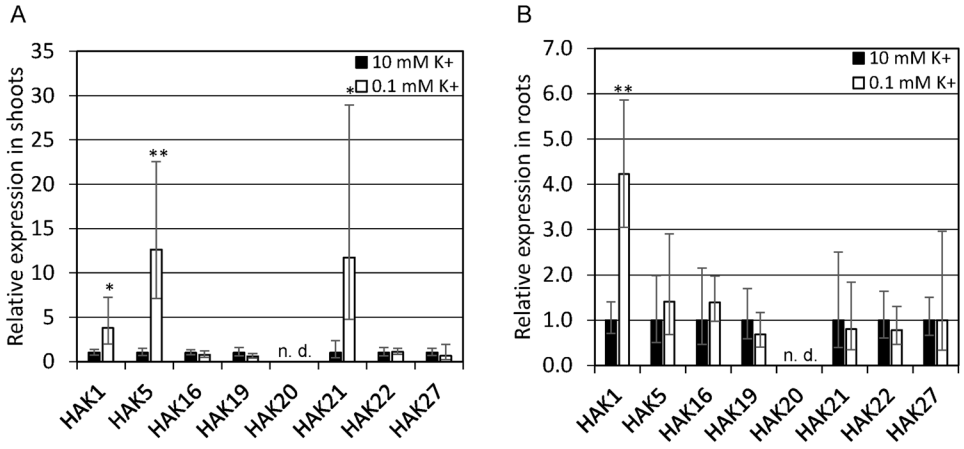

Figure 1. Expression levels of *OsHAK* transcripts belonging to cluster I under low-K^+ condition in rice (A) shoots and (B) roots. Rice plants were germinated and grown for 11 days on nutrition medium containing 10 mM or 0.1 mM K^+. The relative expression level of *OsHAK* genes was normalized to that of *actin* gene. Black and white bars indicate the non-stress (10 mM K^+) and low-K^+ (0.1 mM K^+) conditions, respectively. The relative expression levels under the non-stress condition were standardized as 1. Error bars indicate standard deviation ($n=5$). n.d. means not detected. Significant differences between the expression levels of 10 mM and 0.1 mM K^+ were calculated using Student's *t*-test and are indicated by *($p<0.05$) and **($p<0.01$).

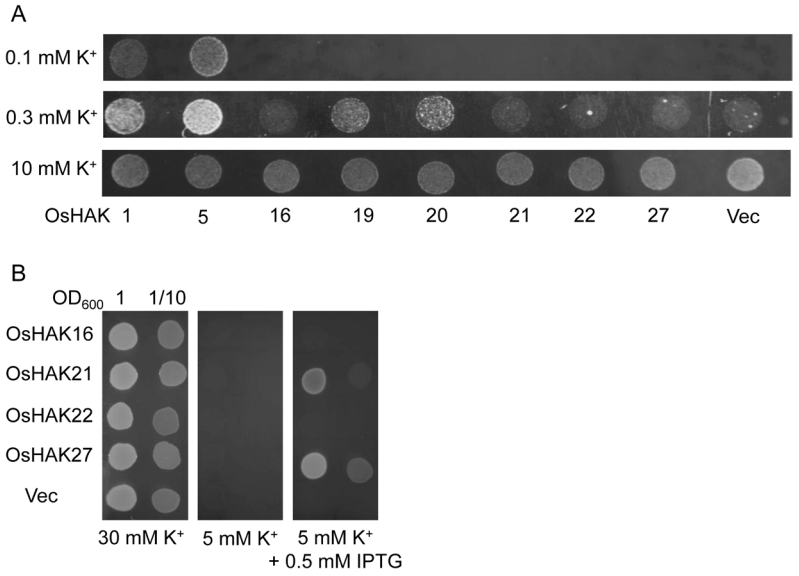

Figure 2. K^+ transport activity of rice cluster I HAK transporters. (A) Complementation of a yeast strain defective in high-affinity K^+ uptake with HAK transporters belonging to cluster I. The *trk1 trk2* yeast cells carrying empty vector pYES2 or the vector containing rice *HAK* cDNA were grown on arginine phosphate medium supplemented with 10, 0.3, or 0.1 mM KCl. Images of the cultures were obtained after incubation for 3 (10 mM KCl) or 7 (0.3, 0.1 mM KCl) days at 30°C. (B) Complementation of an *E. coli* strain defective in K^+ uptake with the four HAK transporters belonging to cluster I. *E. coli* strain (LB 2003), deficient in the K^+ transport system, was transformed with an empty vector or with the vector containing *OsHAK* cDNAs under the control of an IPTG-responsive promoter. Ten-fold dilutions of cells were inoculated, as shown on the upper side. Images of the cultures were obtained after incubation for 2 (30 mM KCl) or 3 (5 mM KCl) days at 30°C.

OsHAK1, OsHAK5, OsHAK16, OsHAK19, OsHAK21, OsHAK22, and *OsHAK27*, were expressed in both the shoots and roots, while the expression of *OsHAK20* was not detected (Figure 1). The expression of *OsHAK1* increased significantly under low-K^+ conditions in both the shoots and roots, whereas that of *OsHAK5* and *OsHAK21* was induced only in the shoots. The expression of other genes, *OsHAK16, OsHAK19*, *OsHAK22*, and *OsHAK27*, did not change in response to K^+ deficiency.

Cluster I HAK transporters showed K^+ transport activity in heterogeneous expression systems

The transport activity of HAK transporters in cluster I was compared by expressing them in a *trk1 trk2* yeast strain. The two main K^+ transporters, Trk1 and Trk2 (Becker et al. 1996) were disrupted in this strain; this strain was used to test the high-affinity K^+ transport activity (Rubio et al. 2000; Santa-María et al. 1997). When the eight rice cluster I *HAK* transporters were

introduced in the *trk1 trk2* yeast strain, yeast cells expressing all the eight *HAK* transporters and the empty vector grew on a K^+-rich medium (10 mM K^+). However, on a low-K^+ medium containing 0.3 mM K^+, only cells expressing *OsHAK1, OsHAK5, OsHAK19*, and *OsHAK20* could grow (Figure 2A). In particular, the growth of cells expressing *OsHAK1* and *OsHAK5* was faster than that of cells expressing *OsHAK19* and *OsHAK20*. Furthermore, *OsHAK1* and *OsHAK5* transformants could grow on a low-K^+ medium containing 0.1 mM K^+. In contrast, yeast cells expressing the remaining four *HAK* genes, *OsHAK16*, *OsHAK21*, *OsHAK22*, and *OsHAK27*, did not grow on these low-K^+ media. To further investigate whether the four HAK transporters that could not complement the yeast defect have the K^+ transport activity, we adopted the K^+ uptake-deficient *E. coli* strain (LB2003) (Uozumi 2001) for the K^+ transport activity analysis. In the absence of IPTG, all transformants grew on a K^+-rich medium containing 30 mM K^+, but not on a low-K^+ medium containing 5 mM K^+. In the presence of IPTG, cells expressing *OsHAK21* and *OsHAK27* could grow on the low-K^+ medium (Figure 2B). These results suggest that six out of eight cluster I HAK transporters, OsHAK1, OsHAK5, OsHAK19, OsHAK20, OsHAK21, and OsHAK27, potentially have K^+ transport activity. Further, only OsHAK1 and OsHAK5 could complement the yeast defect on the low-K^+ medium at 0.1 mM.

Na^+ sensitivity of the cluster I HAK transporters

Expressions of *OsHAK1* and *OsHAK5* were induced by K^+ deficiency and the yeast strains expressing them showed similar phenotypes. The K^+ transport activity of only HvHAK1, the closest paralog to *OsHAK1*in *H. vulgare*, was inhibited by external Na^+, while that of OsHAK5 was not (Horie et al. 2011), suggesting that OsHAK1 and OsHAK5 have different sensitivities to Na^+. Therefore,

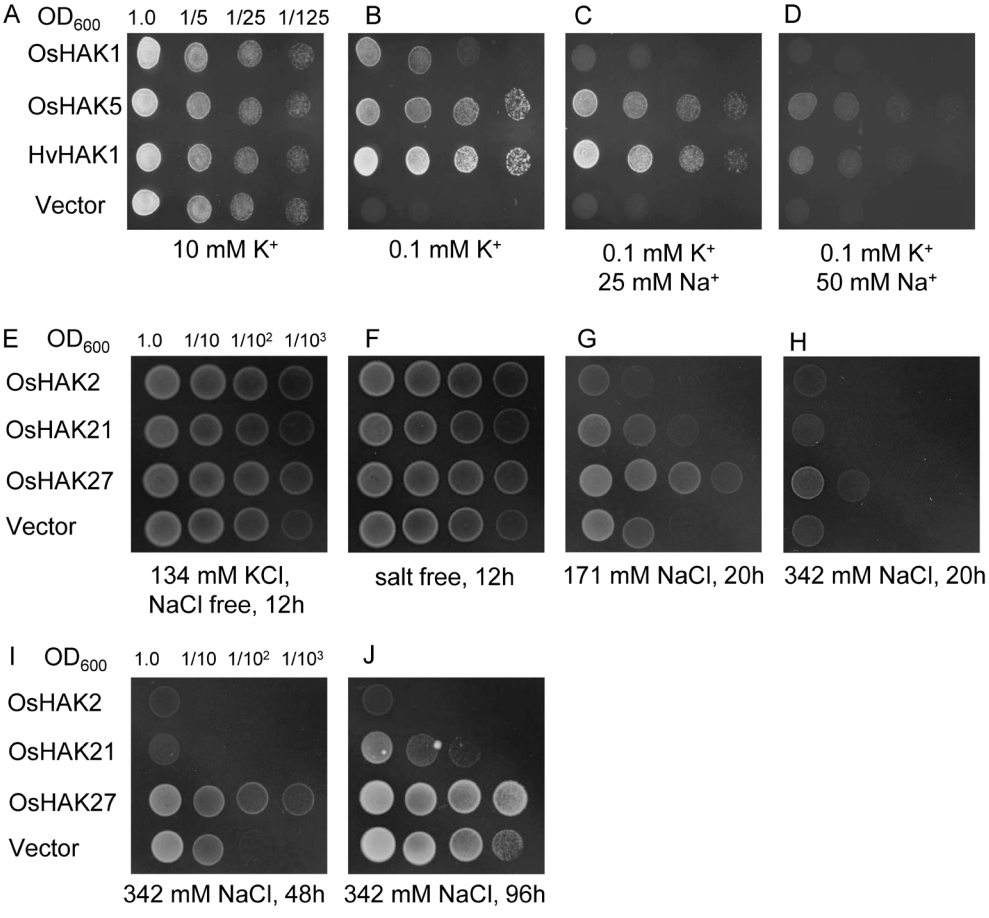

Figure 3. Na^+-dependent inhibition of K^+ uptake activity (A–D). Growth of the *trk1 trk2* cells transformed with negative control plasmid (vector), Na^+-insensitive or Na^+-sensitive positive control plasmids (OsHAK5 or HvHAK1), or the plasmid containing yeast expression cassette of *OsHAK1* on AP medium supplemented with (A) 10 mM KCl. Growth of the same cell lines on AP medium supplemented with (B) 0.1 mM KCl, or in combination with (C) 25 mM NaCl or (D) 50 mM NaCl. Five-fold dilutions of the cells were inoculated, as shown on the upper side. Images of the cultures were obtained after incubation for 3 (10 mM KCl) or 7 (0.1 mM KCl) days at 30°C. Na^+ resistance of LB2003 strain expressing HAK transporters (E–J). Growth of the LB2003 cells transformed with negative control plasmid (vector), an Na^+-permeable control plasmid (OsHAK2), or a plasmid containing an *E. coli* expression cassette encoding either *OsHAK21* or *OsHAK27* on the modified LB medium. Growth of the same cell lines on LB-based medium supplemented with (E) 134 mM KCl, (F) no salts, (G) 171 mM NaCl (a standard concentration), or (H, I, J) 342 mM NaCl. Ten-fold dilutions of the cells were inoculated, as shown on the upper side. The concentration of KCl or NaCl and the incubation times are shown on the lower side.

their Na^+ sensitivity was compared by incubating *trk1 trk2* yeast strains expressing three *HAK* transporters, *OsHAK1*, *OsHAK5* and *HvHAK1*, on the Na^+ supplemented low-K^+ medium. Although all the strains grew well on low-K^+ medium, the growth inhibition of strains expressing *OsHAK1* and *HvHAK1* was greater than those expressing *OsHAK5* (Figure 3). The growth of *OsHAK1*-expressing strain was very poor, similar to the vector control when the medium was supplemented with NaCl. This result clearly suggests that the K^+ transport activity of OsHAK1 is more sensitive to Na^+ than that of OsHAK5. OsHAK21 and OsHAK27 did not complement the *trk1 trk2* yeast strain, but they did complement the LB2003 strain. A previous study showed that OsHAK21 functions in mediating Na^+/K^+ homeostasis and OsHAK21-mediated K^+ transport likely enhances the salt tolerance of yeast cells (Shen et al. 2015). To study the Na^+ resistance of the LB2003 strains expressing *OsHAK21* and *OsHAK27*, they were grown on modified LB media supplemented with different concentrations of salt. The empty vector-transformed cells grew quickly on the modified LB medium containing no NaCl, but not on the standard LB medium containing 171 mM NaCl. The cells expressing *OsHAK27* grew faster than the empty vector-transformed control cells on the LB media containing 171 and 342 mM NaCl, while the cells expressing *OsHAK2*—a control that was confirmed to have Na^+ permeability (Horie et al. 2011)—and *OsHAK21* grew more slowly than the empty vector-transformed cells. These results suggested that OsHAK27 was superior to OsHAK21 for conferring Na^+ resistance in the *E. coli* cells.

The oshak1 mutants scarcely uptake K^+ in low-K^+ medium

The expression patterns and K^+ transport activity of cluster I HAK transporters suggested that OsHAK1 and OsHAK5 have more important roles than other transporters under low-K^+ condition. The two transporters have been shown to function in the adaptation to K^+ deficiency. K^+ absorption decreased by about 80% in *oshak1* mutants at 0.05–0.1 mM K^+,

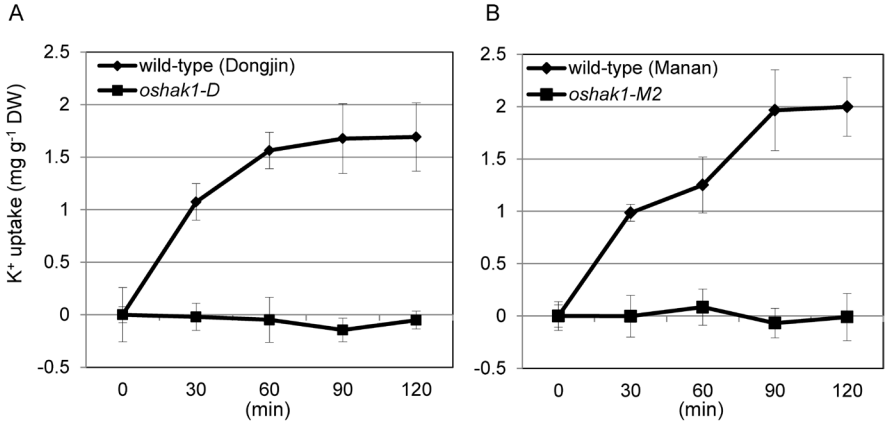

Figure 4. K^+ uptake activity of wild-type and *oshak1* plants. After 8 days of K^+ starvation in the K^+ free medium, roots of *oshak1* mutants and each wild-type, (A) Dongjin and (B) Manan, were soaked in the nutrient solution containing KCl at 0.02 mM for 0, 30, 60, 90, and 120 min. For 0 min incubation, plant samples were removed immediately after soaking. K^+ concentration of the solution was measured after absorption; the amount of absorbed K^+ was calculated from the dry weight of roots. Error bars indicate ± standard deviation (n=4).

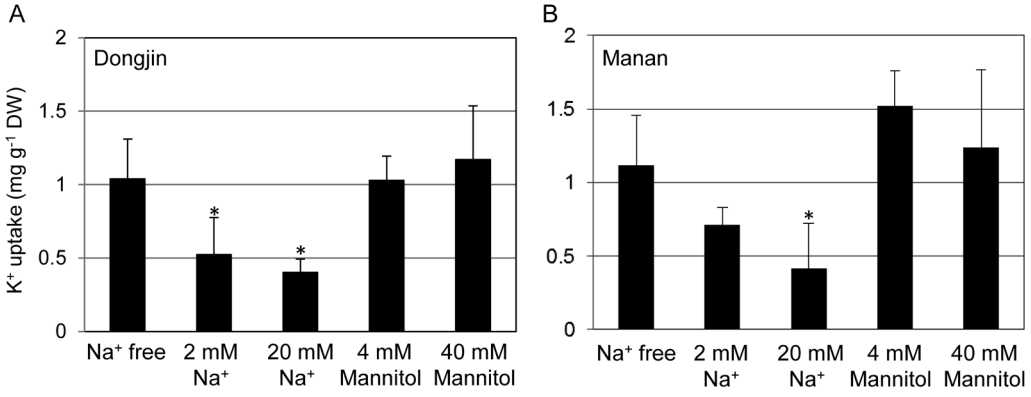

Figure 5. High-affinity K^+ uptake of wild-type plants (A) cultivars Dongjin and (B) Manan was inhibited by Na^+. After 8 days of K^+ starvation, roots were soaked in the nutrient solution containing 0.02 mM KCl and 2 or 20 mM NaCl for 1 h. Mannitol was used as an osmotic control. K^+ concentration of the solution was measured after incubation, and the amount of absorbed K^+ was calculated from the dry weight of roots. Error bars indicate standard deviation (n=4). Significant differences were calculated using Student's t-test and are indicated by *(p<0.05).

whereas it decreased about 20% in *oshak5* mutants at 0.3 mM K^+ (Chen et al. 2015; Yang et al. 2014). Because AtHAK5 mediate K^+ uptake at concentration below 0.01 mM (Rubio et al. 2010), the K^+ uptake activity of OsHAK1 in *planta* was analyzed by using lower K^+ concentration than that of Chen et al. (2015)—0.02 mM. We used *OsHAK1* T-DNA insertion homozygous mutants (*oshak1-D* cultivar Dongjin and *oshak1-M2* cultivar Manan, Supplemental Figure 1A), which have no detectable transcripts of *OsHAK1* (Supplemental Figure 1B). Two-week-old *oshak1* mutants and wild-type plants were transferred to the K^+ free media and grown for 8 days, and then they were subjected to K^+ uptake experiments. The *in planta* analysis shows that the K^+ uptake of both *oshak1* alleles was almost undetectable (Figure 4A, B), suggesting that the K^+ uptake activity of wild-type plants was mediated mainly by OsHAK1 at the K^+ concentration used (0.02 mM). In addition, when external Na^+ was added at 20 mM in the wild-type plants, the high-affinity K^+ uptake activity of OsHAK1 was inhibited (Figure 5). This result suggests that K^+ uptake was mainly mediated by OsHAK1 at 0.02 mM K^+, and that the activity was easily inhibited by mild salt stress even *in planta*.

OsHAK1 has lower Na^+ permeability than OsHAK2 in E. coli

To investigate Na^+ permeability of OsHAK1, OsHAK1 and OsHAK2 were transformed into *E. coli* mutants. Initially, the K^+ uptake-deficient LB2003 strain expressing both *OsHAK1* and *OsHAK2* grew on the low-K^+ medium in the presence of IPTG (Figure 6A), confirming that OsHAK1 and OsHAK2 mediate K^+ influx in *E. coli*. Next, Na^+ permeability was assayed by expressing *OsHAK1* and *OsHAK2* genes in the salt-sensitive *E. coli* strain KNabc (Ito et al. 2001). All transformants grew only on the medium without additional Na^+; however, the growth of the strain expressing *OsHAK2* was inhibited in the medium containing additional Na^+ (Figure 6B). In contrast, the growth of the strain expressing *OsHAK1* was comparable to that of the control strain. These results suggest that OsHAK1 does not mediate Na^+ transport.

OsHAK1 mediates K^+ uptake from leaf blades

The expression of *OsHAK1* was induced not only in the roots but also in the shoots (Figure 1). Recent reports showed that cesium uptake by rice roots largely depends upon OsHAK1 (Rai et al. 2017) and flooded rice can take up radiocesium by foliar uptake via the exposed stem base (Uematsu et al. 2017). Taking those reports into consideration, we tested the K^+ uptake activity in shoots using leaf blades of *oshak1* mutants and wild-type plants. Plants were starved of K^+ for 3 days in K^+-free medium before the K^+ uptake assay. Two leaf blades per plant were soaked in low-K^+ medium at 0.1 mM K^+ for 24 h with or without NH_4^+, which is an inhibitor of HAK-type transporters. Then, the amounts of K^+ uptake were calculated from the change in the K^+ concentration of the low-K^+ medium. The leaf blades of both wild-type plants showed K^+ uptake activity, and the activity was inhibited by NH_4^+ (Figure 7). However, K^+ uptake activity was not detected in the *oshak1* mutants. These results suggested that OsHAK1 mediates foliar K^+ uptake by leaf blades.

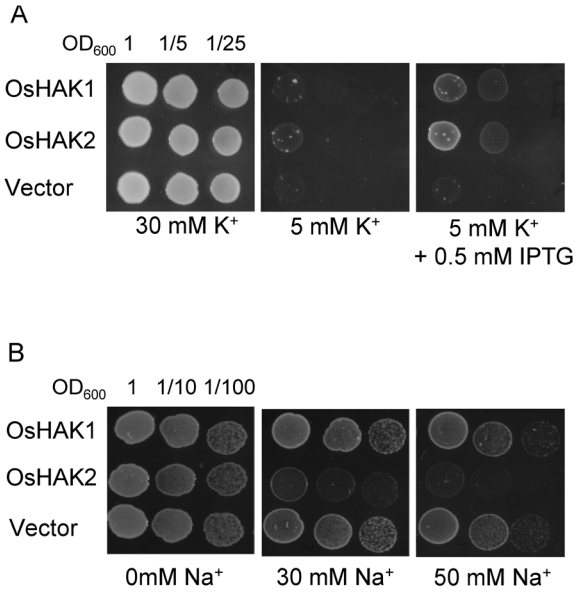

Figure 6. OsHAK1 does not have Na^+ permeability in *E. coli*. (A) Complementation of the *E. coli* strain defective in K^+ uptake with OsHAK1. The *E. coli* LB 2003 strain was transformed with an empty vector or with the vector containing *OsHAK1* or *OsHAK2* under the control of the IPTG-responsive promoter. Five-fold dilutions of cells were inoculated, as shown on the upper side. (B) Growth of the salt-sensitive *E. coli* strain (KNabc). KNabc strain carrying an empty vector or the vector containing *OsHAK1*and *OsHAK2*, a control mediates Na^+ transport, was grown on medium supplemented with 0.5 mM IPTG under non-stress (0 mM NaCl) or salt stress conditions (30 or 50 mM NaCl). Ten-fold dilutions of cells were inoculated, as shown on upper side.

Discussion

At least some of the eight HAK transporters in *O. sativa* have been speculated to play important roles in K^+ deficiency; hence, we investigated the expression pattern and K^+ transport activity of the transporters in this study. Seven *HAK* genes were expressed in the shoots and roots (Figure 1). Although the expression of *HvHAK1* and *AtHAK5* belonging to cluster I in *H. vulgare* and *A. thaliana* was induced under K^+ deficiency (Fulgenzi et al. 2008; Gierth et al. 2005), expression of most *HAK* genes, *OsHAK16, OsHAK19, OsHAK22*, and *OsHAK27*, belonging to cluster I in *O. sativa* was not induced. The expression of *OsHAK22* is up-regulated by Fe deficiency in the roots and is suggested to be involved in K^+ transport associated with the secretion of mugineic acids

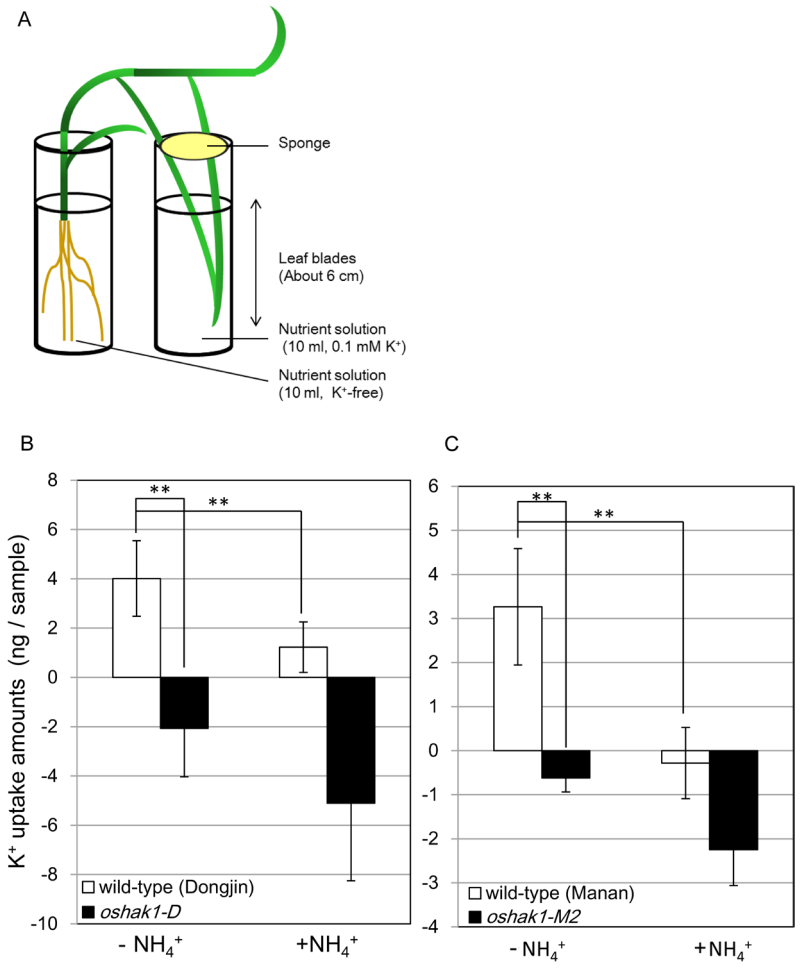

Figure 7. K^+ uptake via the leaf blades of wild-type and *oshak1* mutant plants. (A) A scheme of the K^+ uptake experiment using leaf blades. After 3 days of K^+ starvation in the K^+ free nutrient solution, two leaf blades of *oshak1* mutants and each wild-type variety, (B) Dongjin and (C) Manan, were soaked in the nutrient solution containing 0.1 mM KCl. The base of the leaf sheath was bent to soak an appropriate length of the leaf blades in the nutrient solution and the leaf blades were fixed with a sponge to keep them soaking during the incubation period. A portion of each leaf blade about 6 cm from the leaf tip was soaked in 10 ml of nutrient solution for 24 h. Afterwards, the amount of K^+ uptake was calculated from the K^+ concentration of the nutrient solution. The K^+ uptake activity was tested with or without 1 mM NH_4Cl, which is an inhibitor of HAK-type transporters. Error bars indicate standard deviation ($n \geq 4$). Statistical significance was calculated using Student's *t*-test and significant differences are indicated by **($p<0.01$).

(Ogo et al. 2014). Although the physiological function of OsHAK27 was not revealed, its expression was down-regulated by NAA treatment in seedlings (Gupta et al. 2008). Although the expression of *OsHAK20* was not detected in the shoots or roots (Figure 1), the expression was relatively higher in the stamen and endosperm than in the other tissues (Gupta et al. 2008). *OsHAK20* might not be a concern in external K^+ starvation. Four of eight HAK genes were not upregulated by the K^+ deficiency condition, whereas the expression of *OsHAK1*, *OsHAK5*, and *OsHAK21* was induced (Figure 1). According to previous microarray data, *OsHAK21* was strongly expressed in the panicle and hull (Gupta et al. 2008), *OsHAK21* was expressed in whole rice seedling as revealed by promoter-GUS analysis, and GUS staining was also observed in the xylem parenchyma and in some endodermis cells (Shen et al. 2015). OsHAK21 is suggested to play an important role in the maintenance of Na^+/K^+ homeostasis under salt stress (Shen et al. 2015). The transcripts of *OsHAK5* were increased in shoots but not in roots (Figure 1B). Yang et al. (2014) reported that short-term K^+ deprivation (1–24 h) induced the expression of *OsHAK5* in the roots, but long-term K^+ deprivation (72 h) did not. Because low-K^+ treatment was maintained over a long-term in our experiments, the expression of *OsHAK5* would not be induced in the roots. Gupta et al. (2008) divided cluster I into two sub-groups, cluster IA and cluster IB, based on their amino acid sequences. Interestingly, all four HAK transporters, OsHAK1, OsHAK5, OsHAK19, and OsHAK20, which showed K^+ transport activity in yeast, belong to cluster IB, and the remaining four HAK transporters belonged to cluster IA (Figure 2A, Supplemental Figure 2). The expressions of *OsHAK1* and *OsHAK5* were up-regulated under K^+ deficiency, and yeast strains expressing these genes grew on low-K^+ medium (0.1 mM). Thus,

transporters induced under K^+ deficiency can be assumed to have high-affinity for K^+. These high-affinity activities would be necessary to utilize apoplastic K^+ under K^+ deficiency condition. Our experiment using *E. coli* showed that OsHAK21 has a K^+ transport activity (Figure 2). This finding is consistent with a previous report, which showed that the expression of *OsHAK21* rescued the low-K^+-sensitive phenotype of *athak5* mutant cells (Shen et al. 2015). OsHAK27 showed K^+ transporter activity in *E. coli* but not in yeast (Figure 2). Because *E. coli* also has a HAK type transporter (Schleyer and Bakker 1993), and its membrane structure is simpler than that in yeasts; the exogenous HAK transporters might more easily function in *E. coli*. Previous studies have shown that a tonoplast-localized transporter or a low-affinity K^+ transporter also rescued the K^+-transporter-deficient *E. coli* strain (Bañuelos et al. 2002; Senn et al. 2001). Whether OsHAK27 has high-affinity K^+ transport activity is not yet known; nonetheless, OsHAK27 was suggested to have K^+ transport activity. Interestingly, the growth of cells expressing *OsHAK27* was faster than that of cells expressing *OsHAK21*, and the Na^+ resistance of cells expressing *OsHAK27* was higher than that of cells expressing *OsHAK21* (Figures 2 and 3). These results suggested that OsHAK27 has a high selectivity for K^+. Our results indicate that *HAK* genes, which were up-regulated under K^+ deficiency, were exclusive in cluster I, and the K^+ transport activity of 6 out of 8 *HAK* genes was confirmed in heterogeneous expression systems.

A. thaliana has two high-affinity K^+ transport proteins, AtHAK5 and AtAKT1. Mutant analysis of these genes revealed that AtHAK5 has the highest affinity for K^+. AtHAK5 could uptake K^+ at concentrations of less than 0.01 mM (Rubio et al. 2008). Previous studies revealed that OsHAK1, OsHAK5, and OsAKT1 mediate high-affinity K^+ uptake in rice (Chen et al. 2015; Li et al. 2014; Yang et al. 2014). Mutant analysis showed that, even if *OsHAK5* or *OsAKT1* was disrupted, K^+ uptake activity could be detected at low-K^+ concentrations (0.3 or 0.25 mM) (Li et al. 2014; Yang et al. 2014). In *oshak1* mutants, the K^+ uptake activity was reduced to 20% of that in the wild-type at 0.05–0.1 mM K^+ (Chen et al. 2015). In the present study, the K^+ uptake activity of *oshak1* mutants was analyzed under low-K^+ conditions, the absorption of K^+ at 0.02 mM K^+ was negligible in both the mutants (Figure 4). These results suggested that high-affinity K^+ uptake activity was mediated mainly by OsHAK1 at 0.02 mM K^+. The K^+ transport activity of OsHAK1 was inhibited by mild salt stress (Figure 5), however, Na^+ permeability was not recognized in *E. coli* cells (Figure 6). Chen et al. (2015) reported that K^+/Na^+ ratio is elevated in *OsHAK1* overexpression plants, whereas it is decreased in *oshak1* mutants. This observation is consistent with our findings on *E. coli* that OsHAK1 does not mediate Na^+ transport. The availability of Na^+ and K^+ is generally similar; OsHKT2; 1, a Na^+ uptake transporter, was shown to contribute to rice growth under low-K^+ condition (Horie et al. 2007). The K^+ transport activity of OsAKT1 was also sensitive to Na^+. Patch clamp analysis showed that protoplasts isolated from salt-stressed rice roots showed significantly reduced K^+ inward currents (Fuchs et al. 2005). Interestingly, the activity of OsHAK5 was not considerably impaired by Na^+ (Horie et al. 2011) (Figure 3), *oshak5* mutants were more sensitive to Na^+ stress, and the transcriptional level of *OsHAK5* was elevated under salt stress (Yang et al. 2014). In our study, the expression of *OsHAK1* was induced in roots under long-term low-K^+ conditions (Figure 1B), and the major part of K^+ uptake in low-K^+ medium at 0.02 mM was attributed to OsHAK1 (Figure 4). Rice has at least two HAK type transporters, *OsHAK1* and *OsHAK5*, for K^+ uptake. We speculate that rice might differentially use these two transporters, depending on the availability of external Na^+. The foliar K^+ uptake was detected in wild-type plants but not in *oshak1* mutants (Figure 7). Although in situ hybridization showed that transcripts of *OsHAK1* were mainly detected in the cells at the inner side of the vascular bundles of the leaf sheath, it seemed that GUS staining was observed throughout the leaf blade (Chen et al. 2015). Cations are considered to flow into the apoplast through the stomata (Eichert and Goldbach 2008). Apoplastic cations are then absorbed by transporters or channels that are localized to the plasma membrane. Because OsHAK1 is localized to the plasma membrane (Chen et al. 2015), it is possible for it to mediate foliar K^+ uptake directly. The foliar uptake activity in wild-type plants was detected at 0.1 mM K^+ and was inhibited by ammonium ions, suggesting that this uptake activity was mediated by HAK-type transporters. However, it is likely that the K^+ uptake activity by the leaf blades is lower than that by the roots, since the activity by leaf blades was difficult to detect during incubation periods of 1 to 4 h (data not shown). Recently, OsHAK1 has been indicated to be the main pathway to absorb cesium (Rai et al. 2017). Radiocesium uptake activity by the stem base was detected in flooded rice and reducing the K^+ supply to the roots increased the uptake activity by the stem base (Uematsu et al. 2017). Since *OsHAK1* expression is induced in shoots under the K^+ starved condition (Figure 1A), the foliar K^+ uptake activity of OsHAK1 might be important for the plants to adapt to changing environmental conditions.

In this study, we characterized eight rice HAK transporters that belong to cluster I and are thought to have high-affinity for K^+. The expression levels of three transporters—*OsHAK1*, *OsHAK5*, and *OsHAK21*—were elevated under K^+-deficient conditions, and only *OsHAK1* and *OsHAK5* could complement the yeast defects at 0.1 mM K^+, suggesting that not all of the

cluster I HAK transporters function in K^+ deficiency. Interestingly, OsHAK1 function was more sensitive to Na^+ compared to that of OsHAK5, despite their high sequence homology. Under low-K^+ conditions, K^+ uptake activity of the wild-type is inhibited by mild salt stress. In addition, K^+ uptake activity is significantly decreased in *oshak1* mutants under low-K^+ conditions. It has been reported that OsHAK5 also mediates K^+ uptake and that *OsHAK5* expression is induced by NaCl treatment (Yang et al. 2014). Taken together, our finding suggest that two cluster I HAK transporters have important functions in K^+ transport under conditions of K^+ deficit. OsHAK1 is the main transporter under low-K^+ conditions and is more sensitive to Na^+ than OsHAK5; however, it has high selectivity to K^+. Further, it is suggested that OsHAK1 is involved in foliar K^+ uptake.

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References

Amrutha RN, Sekhar PN, Varshney RK, Kishor PBK (2007) Genome-wide analysis and identification of genes related to potassium transporter families in rice (*Oryza sativa* L.). *Plant Sci* 172: 708–721

Bañuelos MA, Garciadeblas B, Cubero B, Rodríguez-Navarro A (2002) Inventory and functional characterization of the HAK potassium transporters of rice. *Plant Physiol* 130: 784–795

Becker D, Dreyer I, Hoth S, Reid JD, Busch H, Lehnen M, Palme K, Hedrich R (1996) Changes in voltage activation, Cs^+ sensitivity, and ion permeability in H5 mutants of the plant K^+ channel KAT1. *Proc Natl Acad Sci USA* 93: 8123–8128

Buurman ET, Kim KT, Epstein W (1995) Genetic evidence for two sequentially occupied K^+ binding sites in the Kdp transport ATPase. *J Biol Chem* 270: 6678–6685

Chen G, Hu Q, Luo LE, Yang T, Zhang S, Hu Y, Yu L, Xu G (2015) Rice potassium transporter OsHAK1 is essential for maintaining potassium-mediated growth and functions in salt tolerance over low and high potassium concentration ranges. *Plant Cell Environ* 38: 2747–2765

Chen Z, Fujii Y, Yamaji N, Masuda S, Takemoto Y, Kamiya T, Yusuyin Y, Iwasaki K, Kato S, Maeshima M, et al. (2013) Mn tolerance in rice is mediated by MTP8.1, a member of the cation diffusion facilitator family. *J Exp Bot* 64: 4375–4387

Cuin TA, Betts SA, Chalmandrier R, Shabala S (2008) A root's ability to retain K^+ correlates with salt tolerance in wheat. *J Exp Bot* 59: 2697–2706

Eichert T, Goldbach HE (2008) Equivalent pore radii of hydrophilic foliar uptake routes in stomatous and astomatous leaf surfaces: Further evidence for a stomatal pathway. *Physiol Plant* 132: 491–502

Fuchs I, Stölzle S, Ivashikina N, Hedrich R (2005) Rice K^+ uptake channel OsAKT1 is sensitive to salt stress. *Planta* 221: 212–221

Fulgenzi FR, Peralta ML, Mangano S, Danna CH, Vallejo AJ, Puigdomenech P, Santa-María GE (2008) The ionic environment controls the contribution of the barley HvHAK1 transporter to potassium acquisition. *Plant Physiol* 147: 252–262

Gierth M, Mäser P (2007) Potassium transporters in plants: Involvement in K^+ acquisition, redistribution and homeostasis. *FEBS Lett* 581: 2348–2356

Gierth M, Mäser P, Schroeder JI (2005) The potassium transporter AtHAK5 functions in K^+ deprivation-induced high-affinity K^+ uptake and AKT1 K^+ channel contribution to K^+ uptake kinetics in Arabidopsis roots. *Plant Physiol* 137: 1105–1114

Gupta M, Qiu X, Wang L, Xie W, Zhang C, Xiong L, Lian X, Zhang Q (2008) KT/HAK/KUP potassium transporters gene family and their whole-life cycle expression profile in rice (*Oryza sativa*). *Mol Genet Genomics* 280: 437–452

Horie T, Costa A, Kim TH, Han MJ, Horie R, Leung HY, Miyao A, Hirochika H, An G, Schroeder JI (2007) Rice OsHKT2; 1 transporter mediates large Na^+ influx component into K^+-starved roots for growth. *EMBO J* 26: 3003–3014

Horie T, Sugawara M, Okada T, Taira K, Kaothien-Nakayama P, Katsuhara M, Shinmyo A, Nakayama H (2011) Rice sodium-insensitive potassium transporter, OsHAK5, confers increased salt tolerance in tobacco BY2 cells. *J Biosci Bioeng* 111: 346–356

Ito M, Guffanti A, Krulwich T (2001) Mrp-dependent Na^+/H^+ antiporters of *Bacillus* exhibit characteristics that are unanticipated for completely secondary active transporters. *FEBS Lett* 496: 117–120

Li J, Long Y, Qi G, Li J, Xu Z, Wu W, Wang Y (2014) The Os-AKT1 channel is critical for K^+ uptake in rice roots and is modulated by the rice CBL1-CIPK23 complex. *Plant Cell* 26: 3387–3402

Maathuis FJM, Amtmann A (1999) K^+ nutrition and Na^+ toxicity: The basis of cellular K^+/Na^+ ratios. *Ann Bot* 84: 123–133

Nieves-Cordones M, Alemán F, Martínez V, Rubio F (2010) The *Arabidopsis thaliana* HAK5 K^+ transporter is required for plant growth and K^+ acquisition from low K^+ solutions under saline conditions. *Mol Plant* 3: 326–333

Ogo Y, Kakei Y, Itai R, Kobayashi T, Nakanishi H, Nishizawa N (2014) Tissue-specific transcriptional profiling of iron-deficient and cadmium-stressed rice using laser capture microdissection. *Plant Signal Behav* 9: e29427

Okada T, Nakayama H, Shinmyo A, Yoshida K (2008) Expression of *OsHAK* genes encoding potassium ion transporters in rice. *Plant Biotechnol* 25: 241–245

Rai H, Yokoyama S, Satoh-Nagasawa N, Furukawa J, Nomi T, Ito Y, Fujimura S, Takahashi H, Suzuki R, Yousra E, et al. (2017) Cesium uptake by rice roots largely depends upon a single gene, *HAK1*, which encodes a potassium transporter. *Plant Cell Physiol* 58: 1486–1493

Rubio F, Alemán F, Nieves-Cordones M, Martínez V (2010) Studies on Arabidopsis athak5, atakt1 double mutants disclose the range of concentrations at which AtHAK5, AtAKT1 and unknown systems mediate K uptake. *Physiol Plant* 139: 220–228

Rubio F, Nieves-Cordones M, Alemán F, Martínez V (2008) Relative contribution of AtHAK5 and AtAKT1 to K^+ uptake in the high-affinity range of concentrations. *Physiol Plant* 134: 598–608

Rubio F, Santa-María GE, Rodríguez-Navarro A (2000) Cloning of *Arabidopsis* and barley cDNAs encoding HAK potassium transporters in root and shoot cells. *Physiol Plant* 109: 34–43

Santa-María GE, Danna CH, Czibener C (2000) High-affinity

potassium transport in barley roots. Ammonium-sensitive and -insensitive pathways. *Plant Physiol* 123: 297–306

Santa-María GE, Rubio F, Dubcovsky J, Rodríguez-Navarro A (1997) The HAK1 gene of barley is a member of a large gene family and encodes a high-affinity potassium transporter. *Plant Cell* 9: 2281–2289

Schleyer M, Bakker EP (1993) Nucleotide sequence and 3′-end deletion studies indicate that the K^+-uptake protein kup from *Escherichia coli* is composed of a hydrophobic core linked to a large and partially essential hydrophilic C terminus. *J Bacteriol* 175: 6925–6931

Senn ME, Rubio F, Bañuelos MA, Rodríguez-Navarro A (2001) Comparative functional features of plant potassium HvHAK1 and HvHAK2 transporters. *J Biol Chem* 276: 44563–44569

Shabala S, Bose J, Fuglsang AT, Pottosin I (2016) On a quest for stress tolerance genes: Membrane transporters in sensing and adapting to hostile soils. *J Exp Bot* 67: 1015–1031

Sharma T, Dreyer I, Riedelsberger J (2013) The role of K^+ channels in uptake and redistribution of potassium in the model plant *Arabidopsis thaliana*. *Front Plant Sci* 4: 1–16

Shen Y, Shen L, Shen Z, Jing W, Ge H, Zhao J, Zhang W (2015) The potassium transporter OsHAK21 functions in the maintenance of ion homeostasis and tolerance to salt stress in rice. *Plant Cell Environ* 38: 2766–2779

Uematsu S, Vandenhove H, Sweeck L, Hees MV, Wannijn J, Smolders E (2017) Foliar uptake of radiocaesium from irrigation water by paddy rice (*Oryza sativa*): An overlooked pathway in contaminated environments. *New Phytol* 214: 820–829

Uozumi N (2001) *Escherichia coli* as an expression system for K^+ transport systems from plants. *Am J Physiol Cell Physiol* 281: C733–C739

Yang T, Zhang S, Hu Y, Wu F, Hu Q, Chen G, Cai J, Wu T, Moran N, Yu L, et al. (2014) The role of a potassium transporter OsHAK5 in potassium acquisition and transport from roots to shoots in rice at low potassium supply levels. *Plant Physiol* 166: 945–959