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Ion Transporter Note

Expression of *OsHAK* genes encoding potassium ion transporters in rice

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Abstract Regulation of K^+/Na^+ homeostasis is an important mechanism in plant growth. The high-affinity K^+ uptake system plays a central role in this mechanism especially when plants experience environmental stresses of K^+ starvation and high salinity. K^+ channels and the K^+/Na^+ co-transporter like HKT have been closely studied as principal members of the K^+ transport system in plants. The KT/HAK/KUP family is a major K^+ transporter family present in bacteria, fungi and plants. In plants, the KT/HAK/KUP transporters form a large family. *Arabidopsis thaliana* has 13 genes and rice has at least 25. KT/HAK/KUP transporters serve various functions in various K^+ traffic, but their physiological roles are still unclear. Plant KT/HAK/KUP transporters could be divided into 4 related clusters according to their amino acid sequences, the character of transporters belongs to each cluster was summarized. We analyzed the expression of *OsHAK* genes in rice roots under both K^+ starvation and salt stress conditions.

Key words: KT/HAK/KUP family, K^+ transporter, *Oryza sativa*, phylogenetic tree, rice root.

The potassium ion (K^+) is the most abundant cation in plant cells and is involved in enzyme activity, stomatal opening, and regulation of osmolarity. K^+ is present at ~100 mM in cytosol and ~200 mM in the vacuole. Since K^+ concentration is present at the micro-molar level in ordinary soil, high-affinity K^+ transporters are required for K^+ uptake in roots. In addition to K^+ channels, three other types of K^+ transporter families involved in potassium transport have been investigated in plants: the K^+ transporters (KT/HAK/KUP), high-affinity K^+ transporters (Trk/HKT), and cation proton antiporters (CPA) (Gierth and Mäser 2007). Since both K^+ and Na^+ are monovalent cations, Na^+ can be passed through some K^+ transporters. In high salt conditions, K^+ uptake mediated by TaHKT2;1 is blocked and low-affinity Na^+ uptake occurs (Gassmann et al. 1996). K^+ transporters may be influx routes for Na^+ under high-salinity conditions (Kader and Lindberg 2005, Takahashi et al. 2007). Thus, high-affinity K^+ transport systems are also essential for preventing Na^+ toxicity. We wish to identify transporters that mediate high-affinity K^+ uptake from soil in rice (*Oryza sativa*), a globally important crop.

K^+ uptake system in plants

AtAKT1 was initially identified as the K^+ uptake transporter in plants by reverse genetics. Root cells of *atakt1* mutants have no inward current, as determined by patch-clamp and their growth is inhibited in low K^+ media (100 μM or less) (Hirsch et al. 1998). In an analysis of *atakt1* mutant, Spalding et al. characterized other K^+ uptake transporters (Spalding et al. 1999). K^+ uptake of *atakt1* mutants is inhibited by NH_4^+, and was activated by Na^+ and H^+. KT/HAK/KUP transporters are inhibited by NH_4^+, and Trk/HKT transporters are K^+/Na^+ symporters that require an H^+ gradient for activation; consequently, these two transporters are considered K^+ uptake transporters in *A. thaliana*. Further studies have also suggested that *AtHAK5* function in K^+ uptake (Gierth et al. 2005). However, *AtHKT1*, which is the only one Trk/HKT transporter in *A. thaliana*, is a Na^+ transporter (Berthomieu et al. 2003).

The *OsAKT1* gene was isolated as the homologue of *AtAKT1* in rice. Golldack et al. comparatively analyzed the expression of *OsAKT1* gene in the salt-sensitive cultivar IR29 and the salt-tolerant cultivar BK. The difference in salt-tolerance of these cultivars is caused by the difference in *OsAKT1* expression: the mRNA of *OsAKT1* in root epidermis is reduced by salt stress in BK but not IR29. Although the expression of *OsAKT1* is down-regulated under salt stress in BK, the level of K^+ accumulation does not change under these conditions. These results suggest the presence of high-affinity K^+ transporters other than *OsAKT1* in rice (Golldack et al. 2003) (Figure 1). Whole-cell clamp studies on rice root

protoplasts indicated that OsAKT1-like K^+ transport is inhibited in response to salt stress (Fuchs et al. 2005); treatments with tetraethylammonium (TEA), an inhibitor of K^+ channels including *OsAKT1*, reduced the accumulation of Na^+ in the protoplasts of the salt-sensitive cultivar, BRRI Dhan 29 (Kader and Lindberg 2005). It is considered that the rice K^+ channels including *OsAKT1* participate in the Na^+ influx from soil. Two types of HKT transporters — *OsHKT2;1*, a Na^+ transporter, and *OsHKT2*; 2 a K^+/Na^+ co-transporter–have been characterized in rice (Horie et al. 2001, 2008). *OsHKT2;1* is the central transporter for nutritional Na^+ uptake into K^+-starved rice roots (Horie et al. 2007).

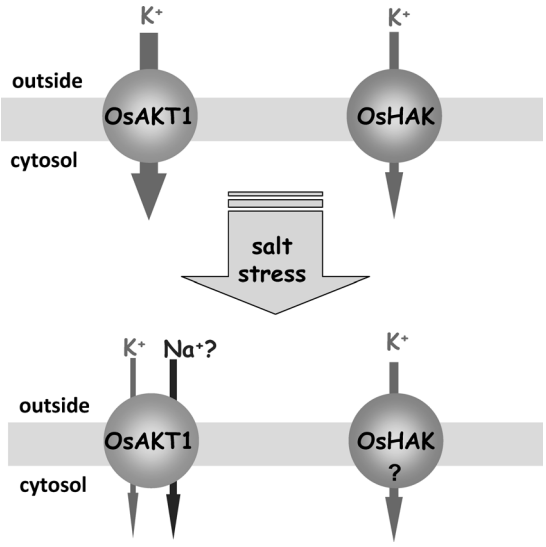

Figure 1. A schematic diagram of K^+ transport system in rice root.

Phylogenetic tree of KT/HAK/KUP transporters in plants

KT/HAK/KUP transporters compose a large family: 13 proteins are encoded in the genome of *A. thaliana*, 25 to 27 genes in the rice genome, and 24 genes in the poplar genome (Gierth and Mäser 2007, Grabov et al. 2007). These proteins can be classified into four clusters based on their amino acid sequence (Figure 2). Mutant strains of budding yeast (*Saccharomyces cerevisiae*) and *E. coli* that are defective in the high-affinity K^+ transport system are often used as host cells for complementation tests to assess whether a given transporter has K^+ transport activity. The *TRK1* and *TRK2* genes encode the high-affinity K^+ transporters in *S. cerevisiae*. Complementation tests with *trk1* and *trk2* mutant of yeast and analysis of T-DNA mutant *Arabidopsis* have revealed that KT/HAK/KUP transporters in cluster I, *AtHAK5*, *HvHAK1*, and *OsHAK1-1*, had high-affinity K^+ transport activity (Banuelos et al. 2002, Gierth et al. 2005, Santa-Maria et al. 1997). In contrast, *OsHAK7*, *OsHAK10* and *HvHAK2* transporters in cluster II could not complement the *trk1* and *trk2* mutations of yeast (Banuelos et al. 2002; Santa-Maria et al. 1997). The chimeric protein *OsHAK10*-GFP is localized to the tonoplast in onion epidermal cells (Banuelos et al. 2002). It is thought that *OsHAK10* protein cannot be localized to the plasma membrane in yeast cells (Banuelos et al. 2002). In some reports, the K^+ transport activity of KT/HAK/KUP transporters in cluster II was assessed using *E. coli* mutants (Ahn et al. 2004; Banuelos et al. 2002; Senn et al. 2001). Results of complementation tests with

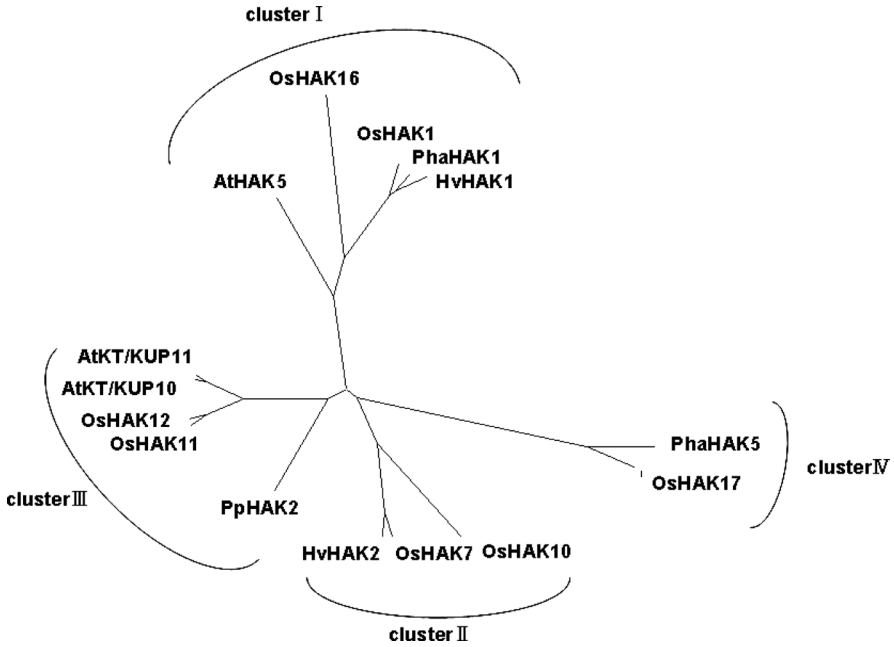

Figure 2. Phylogenetic tree of KT/HAK/KUP transporters. Alignments of the amino acid sequences were performed with the Clustal W program (http://clustalw.ddbj.nig.ac.jp/top-j.html). The phylogenetic tree was reconstructed by neighbor-joining algorithm.

Table 1. Primer sequences used for RT-PCR

genes	forward primer	reverse primer
OsHAK1	gttgatgatgctgatgttggaag	ccaacactttcagctgaaac
OsHAK2	ctacctgtggccgccttttttg	ctgaaactgaaaacgcatgg
OsHAK3	cagtactaggtcgtttattagg	gcatatgtggtttattaagctcg
OsHAK4	gtaaagtagatttaggaaaccg	cgggtgtattatagatctgacgatc
OsHAK5	cttggaaatctgagtaagtactc	cgaatctccatgcatgttctg
OsHAK6	cttgcagaagaactttaggtc	gattaatatccatcatcagctgc
OsHAK7	tgaatcttctgttggtcatcctca	ctcggcaactacattacatg
OsHAK8	gatatggtcacccaaaacaacg	gcaaaaggatgaccaaacatg
OsHAK9	cgtcaccacgagatctcatcgatc	cgcccaattttcttccaatctg
OsHAK10	gaagtttcgcttgtatatcctcg	gagcccatgatccagctgccc
OsHAK11	gtgtaggagtagggctccatg	gatccattcatttgtcatatgc
OsHAK12	gtttctgattcagagagtgagcag	ctacagcatcatttcatactgacag
OsHAK13	ctccgtatatacaactatacg	ccttgccagttttggttatc
OsHAK14	ctagagagtgaccaatacgac	caatggttgggtgctcggtag
OsHAK15	gttcgttgctatcaagtagagataac	gacgtggattcctaaaacagatg
OsHAK16	catgccaacaatcagtaag	catttgcaagtaagcaaacc
OsHAK17	ctaggaatcagacggttagaag	gtatacagtttacataacgc
OsRAC1	gctaagccaagaggagct	ctttgtccacgctaatgaag

E. coli revealed that the K^+ affinities of KT/HAK/KUP transporters in cluster II is lower than those of cluster I (Senn et al. 2001). It is thought that some of the cluster II KT/HAK/KUP transporters mediate K^+ efflux from the vacuole, in order to maintain intracellular ion homeostasis (Rodríguez-Navarro and Rubio 2006). *AtKT/KUP10* and *AtKT/KUP11* transporters in cluster III were confirmed to have K^+ transport activity (Ahn et al. 2004), but *PpHAK2* transporter could not complement the yeast *trk* mutations and the *PpHAK2*-GFP chimeric protein localized to tonoplast in yeast cells (Garciadeblas et al. 2007). In cluster IV, *PhaHAK5* could complement the *trk1* and *trk2* mutations of yeast. Since *PhaHAK5* can mediate Rb^+ uptake in the micromolar range in the yeast cells with the *trk1* and *trk2* mutations, it was classified as a high affinity K^+ transporter. However, Na^+ and K^+ transport activity of yeast cells expressing *PhaHAK5* was significantly inhibited when the concentration of Na^+ is higher than that of K^+ in media. In contrast, the K^+ transport activity of *PhaHAK1* (cluster I) is not inhibited even when the concentration of Na^+ is one hundred times higher than that of K^+ (Takahashi et al. 2007).

For the transporters involved in K^+ uptake, it is necessary that they 1) have inward transport activity of potassium ion, 2) localize plasma membrane, and 3) are expressed in root epidermis cells. There is no report that any transporters belonging to the KT/HAK/KUP family meet all of these three requirements. However, some transporters in the cluster I may be the first candidates to meet these criteria, as judged from the results of the following experiment. Transcripts of *AtHAK5* accumulated under K^+ starvation, and a GFP reporter driven by the *AtHAK5* promoter was observed in the epidermis of main and lateral roots and in the stele of main roots in *A. thaliana*. In low K^+ media, the accumulation level of K^+ in the *athak5* mutant of *A. thaliana* was lower than that of wild type (Gierth et al. 2005). Although these results suggested that *AtHAK5* functions in K^+ uptake, its intracellular localization is still unknown. *HvHAK1* of barley belongs to cluster I. Rb^+ uptake is inhibited by NH_4^+, and the transcripts of *HvHAK1* are induced by K^+ starvation (Senn et al. 2001, Augusto et al. 2005). However, *HvHAK1*-mediated intracellular K^+ uptake and subcellular localization have not been closely examined. *OsHAK1* and *OsHAK16* are KT/HAK/KUP transporters of cluster I in rice; expression of the *OsHAK1* gene is induced by K^+ starvation (Banuelos et al. 2002). The subcellular and tissue localization and properties of K^+ transport of *OsHAK1* and *OsHAK16* have not been characterized.

Expression of seventeen OsHAK genes in root of rice

At least seventeen genes encoding KT/HAK/KUP transporters are present in the genome of rice (Banuelos et al. 2002). To identify the function of each OsHAK transporter on the K^+ uptake in rice, we investigated the mode of expression of each gene in rice root under the stress conditions of K^+ starvation and 50 mM NaCl, using the real time (RT) PCR. The expression of *OsHAK1*, *OsHAK7*, and *OsHAK16* is much higher than that of other *OsHAK* genes; mRNA of *OsHAK3* and *OsHAK6* could not be detected by RT-PCR (Fig. 3A and B). Not only relative expression levels but also relative fold change is an important factor for a transporter functioning as a high-affinity K^+ uptake in the epidermis. The transcripts of *OsHAK1*, *OsHAK7*, and *OsHAK12* under K^+ starvation, and those of *OsHAK11* and *OsHAK16* under salt stress, were increased ~3-fold relative to the non-stress condition (Fig. 3C and D). Thus, *OsHAK1* (cluster I) and *OsHAK12* (cluster III) are candidates for high-affinity K^+ uptake transporters in rice root. Although level of expression of *OsHAK* genes belong to cluster III and IV is not high in rice root, transcription of both *OsHAK11* and *OsHAK12* (cluster III) is significantly induced by salt stress and K^+ starvation, respectively. In *A. thaliana*, transcripts of *AtKUP/HAK11* (cluster III) is increased ~3-fold by salt stress of 80 mM NaCl (Maathuis 2006). Therefore, KT/HAK/KUP transporters in cluster III may function in maintenance of K^+/Na^+ homeostasis under the stress conditions of salinity and/or low K^+. To clarify the physiological function of the whole KT/HAK/KUP family, K^+ ion selectivity and intracellular and tissue specific localization of each protein should be studied.

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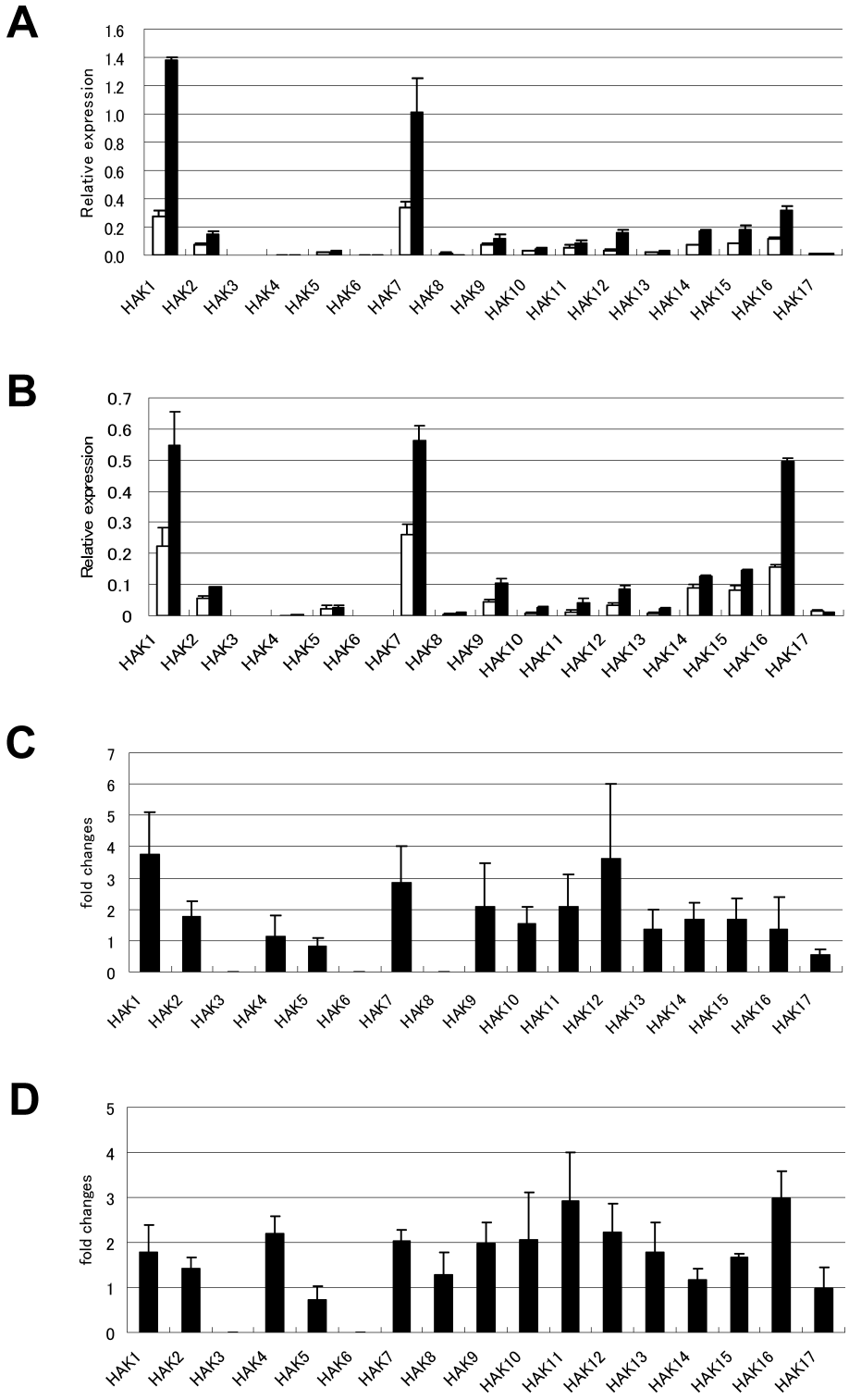

Figure 3. Relative expression levels of *OsHAK* transcripts in rice root under stress condition of potassium starvation (A) and salt stress (B) were analyzed with the RT-PCR assays (Real-Time PCR System Light-cycler 480, Roche). The relative expression level of *OsHAK* genes was normalized to the expression level of *OsRAC1*. Open bar indicates the non-stress condition (control), and closed bar indicates the stress condition. The relative fold changes of transcripts under stress condition are shown in each lower panel (C, D). Stress samples were germinated and grown in K^+-free medium for 8–11 days (A) or treated with 1/2 MS medium contained 50 mM NaCl 24 hours after germination on 1/2 MS medium (B). Control samples were germinated and grown in 1/2 MS medium during 8–11 days. The low-identity regions of the C terminus and 3′-UTR were used for primer design.

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