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Genome-wide analysis and identification of genes related to potassium transporter families in rice (*Oryza sativa* L.)

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Abstract

Potassium (K^+) is an important macronutrient and the most abundant cation in higher plants which plays a key role in various cellular processes. Its accumulation from soil and its distribution throughout diverse plant tissues is mediated by transporter proteins. In plants, different transport systems are known to be involved in the uptake and release of K^+ from the cells. Though most of the information about the putative K^+ transporters and their phylogenetic relationships is available in *Arabidopsis*, it is not the best model for plants with agronomic applications. Recent completion of rice genome sequencing project offered the opportunity to make an inventory of all putative K^+ transporter proteins. More than 5% of the rice genome appears to encode membrane transport proteins. Unfortunately, several hundreds of putative transporter proteins have not yet been assigned to any families or subfamilies or functions. Therefore, phylogenetic relationships of many K^+ transporters in rice are analyzed since rice is considered as a model plant because of its high degree of co-linearity with other cereals. Phylogenetic analysis of all K^+ transporters in rice revealed that they fall into five major branches. Phylogenetic trees of each family define the evolutionary relationships of the members to each other. In each family, closely related isoforms and separate subfamilies existed, indicating possible redundancies and specialized functions. The HAK family is represented by 26 genes and formed the tightest and most distinct branch in the phylogenetic tree. Around 14 genes with conserved P-loop were found in K^+ channel family out of which 11 genes belong to 1P/6TM (Shaker-type), and three genes to the 2P/4TM (ORK-type). On searching rice genome, it was found that nine genes belonged to Trk family. In rice, K^+/H^+ antiporter family is represented by a single gene. Comparative analysis of rice K^+ channels with that of *Arabidopsis*, wheat and maize revealed that while cereals are closely related, *Arabidopsis* appeared quite distant from rice.

Keywords: Potassium; Ion transporters; Salt stress; *Oryza sativa*; Genome analysis

1. Introduction

K^+ is an important macronutrient and the most abundant cation in higher plants. This cation plays a key role in various cellular processes such as: (1) charge balancing in the cytoplasm, (2) activation of crucial enzymatic reactions and (3) a substantial contribution to the osmotic pressure of the vacuole and hence to cell turgor [1,2]. Furthermore, K^+ is necessary for phloem solute transport and maintenance of cation:anion balance in the cytosol as well as in the vacuole. Apart from these, K^+ is also involved in processes such as cell elongation, stomatal movements and regulation of gaseous exchange and the transduction of various signals [3–5]. Accumulation of K^+ from soil and its distribution throughout diverse plant tissues is mediated by transporter proteins. The ratio of K^+/Na^+ in plant cells will depend on the concerted action of transport systems located at plasma and vacuolar membranes and probably involves K^+ selective, Na^+ selective and non-selective pathways. In plants, different transport systems are known to be involved in the uptake and release of K^+ from the cells. Currently, plant genomics allow the fast identification of molecular components related to mineral homeostasis. The recent completion of Arabidopsis genome sequencing project offered the opportunity to make an inventory of all of plants putative cation transporter proteins [6]. This was possible through database searches for sequences homologous to proteins already characterized. The IRGSP, formally established in 1998, pooled the resources of sequencing groups in 10 nations to obtain a complete finished quality sequence of the rice genome (*Oryza sativa* L. ssp.

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Japonica cv. Nipponbare). The IRGSP released a high-quality map-based draft sequence in December 2002, and the sequencing project was completed in December 2004, and the results were published in August 2005. At present, IRGSP submitted sequences to GenBank that comprise over 95% of the genome (http://www.tigr.org/tdb/e2k1/osa1/BACmapping/description.shtml). Most of the information about putative K^+ transporters that might play a role in K^+ transport came from sequence analyses and phylogenetic relationships [6]. Though functional identification and characterization of many K^+ transporters is possible in Arabidopsis, it is not the best model for plants with agronomic applications. Rice perhaps is an ideal crop having the smallest genome of the major cereals, dense genetic maps and relative ease of genetic transformation [7]. Discovery of extensive genome colinearity among the Poaceae [8] established rice as the model organism for the cereal grasses. These properties, along with the finished sequence and other tools under development, set the platform for a complete functional characterization of rice genome. A total of 37,544 protein-coding genes were identified in rice of which 71% have a putative homologue in Arabidopsis [9]. The first insight in the molecular analysis of membrane K^+ transport in plants came in 1992 with the identification of two *Arabidopsis* K^+ channels, AKT1 [10] and KAT1 [11]. Most plant K^+ channels were found to be members of Shaker family and were successfully expressed and characterized in heterologous systems. Another breakthrough in functional identification of plant potassium transporters came in the year 1994, with the cloning of HKT1 from wheat [12] that corresponds to HKT family. Members of HKT gene family act as Na^+ and Na^+/K^+ transporters in controlling Na^+ accumulation [13–17]. Based on PCR [18], *in silico* analyses [19] and functional complementation of yeast [20], one more family of plant K^+ transporters was identified. This family is named as HAK [21] or KUP/HAK/KT [6]. Further *in silico* approaches for new plant counter parts of animal K^+ channels lead to the identification of the KCO channels [22,23]. Also using *in silico* approaches, several authors [6,24–27] identified putative K^+ transporters (CNGC) and also cation transporters (LCT) that might play a role in K^+ transport in plants. The recently identified CNGC family was assumed to conduct K^+ transport in an unspecific manner [28]. Unfortunately, several of putative transporter proteins were not yet assigned to any families or subfamilies in rice. Therefore, an analysis of the genomic sequences related to K^+ transporter families in rice was carried out in the present study by searching the Japonica variety genome in public databanks. The purpose of this study is to contribute to the understanding of molecular mechanisms of K^+ transport and functional characterization of identified new K^+ transporter genes that play a major role in salt tolerance.

2. Materials and methods

Reference proteins of well established molecular function, representing each of the protein families investigated, were chosen as query sequences for searches in the rice (*O. sativa*) genome databases. These reference proteins were AtKUP1 (GenBank accession no. AAB87687), AtAKT1 (GenBank accession no. AAP21250), AtKCO1 (GenBank accession no. AAM64705), AtHKT (GenBank accession no. AAF68393) and AtKEA1 (GenBank accession no. NP_171684). Searches were made using the TBLASTN tool [29] against GenBank database non-redundant (NR), with search specifications for *O. sativa*. The other databases used were Rice Genome Research Program (RGP) (http://rgp.dna.affrc.go.jp/), The Institute of Genome Research (TIGR), rice genome annotation database (http://www.tigr.org/tdb/e2k1/osa1/index.shtml) and Universal Protein resource Uniprot (http://www.ebi.uniprot.org/uniprot-srv/protein/uniProtView). The BLAST server used was that of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/). As selection criteria of BLAST hits for genomic sequences, a cut off *e*-value of *e*-10 was previously set. The genomic sequences found were used to predict putative genes contained within them. Whenever possible, genes were predicted on the basis of sequences generated by the IRGSP, since these sequences present a higher degree of accuracy. To that end, a mixed procedure was adopted combining *ab initio* gene prediction algorithms of genomic sequence alignments with similar sequences from expressed genes (ESTs and cDNAs). The prediction algorithms were GenScan (Burge and Karlin, 1997; http:// genes.mit.edu/GENSCAN.html), GenomeScan [30]; http://genes.mit.edu/genomescan.html), FGENESH [31]; http://www.softberry.-com/berry.phtml?topic=gfind), GeneMark.hmm (Borodovsky and Lukashin, unpublished; http://opal.biology.gatech.edu/GeneMark/eukhmm.cgi) and GrailEXP [32]; http://compbio.ornl.gov/grailexp/). Such expressed sequences were found by BLAST searches against EST and NR databases of GenBank, using the genomic sequence as query. The algorithm of choice for the multiple alignments of protein sequences was ClustalW 1.8 [33], available through the BCM Search Launcher server (http://searchlauncher.bcm.tmc.edu/multi-align/multi-align.html). The multiple alignments were edited with the help of GENEDOC (Free Software Foundation Inc.). All the proteins with greater than 30% identity, with at least one of the reference proteins used in the searches, were regarded as functionally similar (homologous) to the reference proteins, receiving the same name [34–37]. Those sequences that did not conform to this criterion were discarded. Only in case of OsAKT the identity degree of 26% was accepted due to the conserved functional domains between this protein and the reference proteins. Prediction of homology and signature sequences for the putative K^+ transporter proteins were carried out with PROSITE (http://www.ebi.ac.uk/InterProScan/) [38] and Pfam databases [39]. Sequences were included into families based on homology and presence of signature sequences. For topology prediction, HMMTOP [40] was used. RGI gene codes for families were obtained from http://www.gramene.org/Multi/blastview and http://tigrblast.tigr.org/euk-blast/index. Protein alignments obtained with ClustalW 1.8 [33] were used as starting points for phylogenetic analysis. Unrooted trees were prepared by the neighbor-joining method using either Clustal, PHYLIP [41], or and 1000 bootstrap replicates were performed. Bold lines on trees indicate protein

sequences that were confirmed by cDNA sequencing. Chromosome locations of the putative K^+ transporter genes were estimated in accordance to genetic markers assigned to the BAC/PAC clones. The site used for genetic distance identification was http://rgp.dna.affrc.go.jp/cgibin/statusdb/stattable.pl?chr=X&lab=RGP. Chromosomal mapping for all the genes was carried out using MAPINSPECT software.

3. Results

3.1. Genomic overview

With the availability of rice genome data in NCBI, RGP and TIGR it is possible to construct an overview of K^+ transporter genes in rice. As a starting point, the protein families HAK, AKT, KCO, HKT/Trk and K^+/H^+ that have positive molecular implications on K^+ uptake and transport in Arabidopsis were chosen for analysis. Taking specific members of these families as query sequences, searches were carried out for orthologous sequences in GenBank, RGP and Uniprot current databases using TBLASTN. After searching the databanks with TBLASTN sequences, clones having genomic sequences to the related family were taken and converted to amino acid sequences. In each family, similar sequences were removed and the sequences were subjected to PROSITE and Pfam databases to see the presence of signature sequences for the corresponding families. After subjecting the sequences to PROSITE, 35

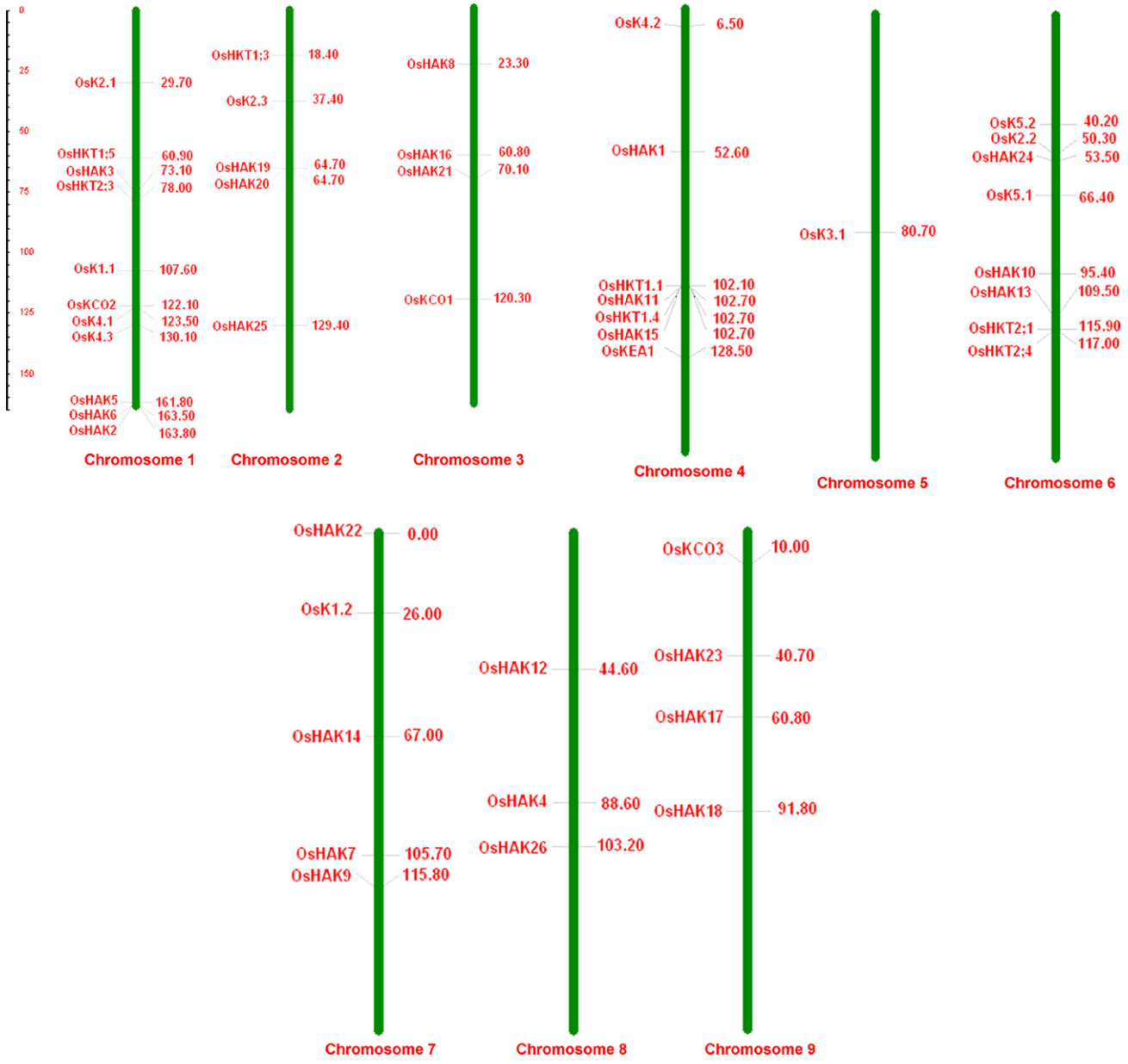

Fig. 1. Chromosome locations of the putative K^+ transporter genes. All positions in centMorgans (cM) were estimated in accordance to genetic markers assigned to the BAC/PAC clones http://rgp.dna.affrc.go.jp/cgibin/statusdb/stattable.pl?chr=X&lab=RGP) related to the predicted sequences. The proposed gene nomenclature is as follows: OsHAK, *Oryza sativa* K^+ transporter, OsK, *O. sativa* Shaker like K^+ channels, OsKCO, *O. sativa* ORK like K^+ channel, OsHKT, *O. sativa* high affinity K^+ transporters and OsKEA, *O. sativa* K^+/H^+ antiporter.

sequences related to HAK, 16 to AKT, 4 to KCO, 9 to HKT and 4 to K^+/H^+ antiporter families were obtained. The percent identity for all the sequences was calculated in each family with the corresponding query sequence using GENEDOC. Proteins that are showing more than 30% identity were taken for the construction of phylogenetic tree. In case of AKT family, proteins having more than 25% identity with the query sequence were taken. Together with the previously reported sequences, in this study, a total of 50 genes possibly involved with K^+ transport in rice (26 genes related to HAK family, 11 genes to AKT family, 3 sequences to KCO family, 9 genes to Trk family and one putative K^+/H^+ antiporter gene) were analyzed. Since the PAC and BAC clones of the IRGSP are linked to genetic markers, it was possible to estimate the relative position of the sequences in the genetic map (Fig. 1). The location of OsHKT2;2 gene still remains unknown, which was predicted from the Indica data, whose scaffolds were not anchored to genetic maps. OsHKT1;2 was not mapped since it is a pseudogene. The HAK family forms the tightest and most distinct branch in the phylogenetic tree of rice K^+ transporters. Out of 26 genes in HAK family, 17 genes were earlier identified by Bañuelos et al. [42]. In this study, we named the previously identified genes in this family as OsHAK1-17 and newly identified genes are represented from OsHAK18-26 (Fig. 2). Out of 26 sequences, 11 sequences have full-length cDNAs. All putative OsHAK transporters exhibited more than 30% identity with *Arabidopsis* AtHAK1. The presence of transmembrane domains was also predicted for all 26 transporter proteins. The result was heterogeneous with majority of proteins presenting 12–15 transmembrane domains (Table 1), which is close to the 12 observed in *Arabidopsis*. Phylogenetic analysis of the sequences of HAK transporters revealed that the rice transporters were divergent, except for the pairs formed by OsHAK8 and OsHAK9, with 80% identity, OsHAK13 and OsHAK24 with 77% identity and OsHAK11 and OsHAK12 with 76% identity. All these OsHAK transporter proteins exhibited more than 30% identity with the *Arabidopsis* AtHAK1 (Fig. 3). Through systematic searching in public databanks with *Arabidopsis* sequences AKT1 and KCO1 as query sequences, we found a total of 14 orthologous genes containing conserved K^+ channel P-loops, three of which are available as full-length

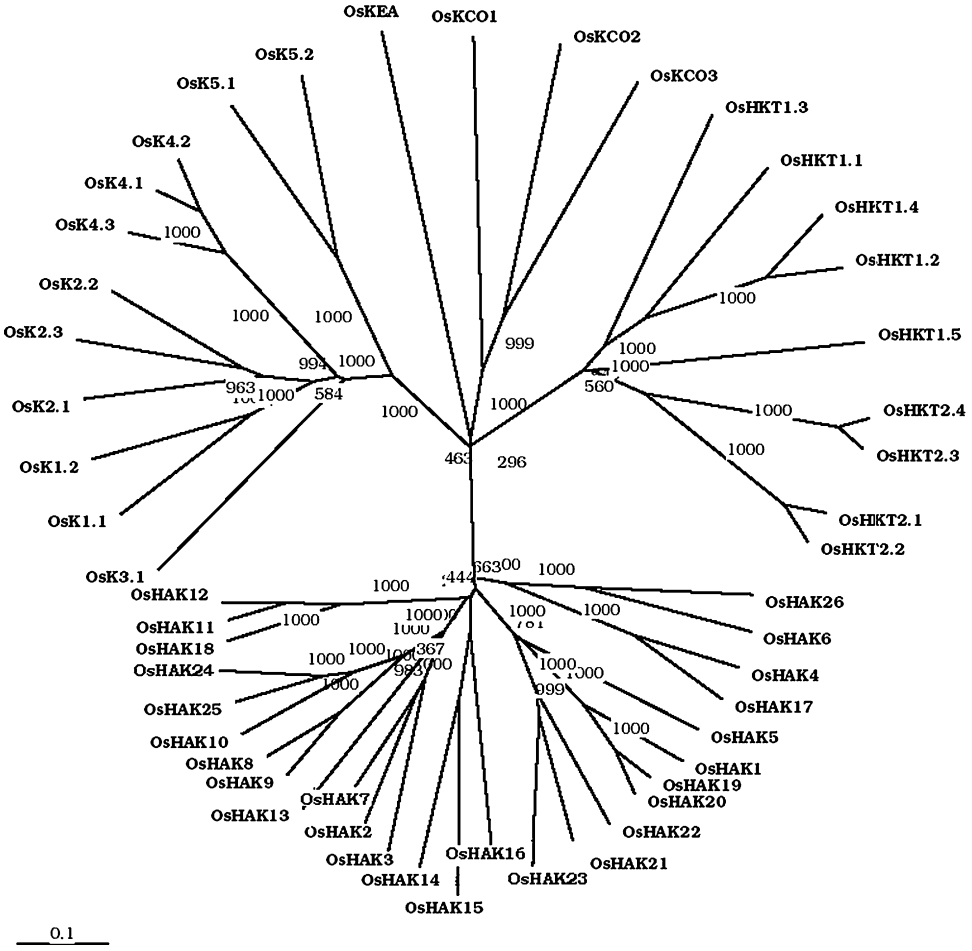

Fig. 2. Overview of rice K^+ transporters. A tree of all K^+ transporters from rice has five major branches: a, HAK transporters (26 genes); b, Trk/HKT transporters (Na^+ transporter; 9 genes); c, KCO (2P/4TM) K^+ channels (3 genes); d, Shaker-type (1P/6TM) K^+ channels (11 genes); e, K^+/H^+ antiporter homologs (one gene). Programs used were ClustalX [116] for alignments, and graphical output produced by Treeview [117]. Values indicate the number of times of 1000 bootstraps that each branch topology was found during bootstrap analysis. Scale bar corresponds a distance of 10 changes per amino acid positions.

Table 1
Potassium transporter gene families found in the rice genome orthologous to Arabidopsis K^+ transporter genes and their GenBank accession numbers and BAC/PAC clone accession numbers

Sequence name	BAC/PAC clone accession number	GenBank accession number	TIGR locus	Number of TM segments	% identity with query	Gene name	Full length cDNA	cM distance
KUP/HAK/KT family								
OsHAK1	AL606610	AAQ74384	Os04g32920	12	33	OSJNBb0012E08.7		52.6–56.1
OsHAK2	AP003266	BAB64197	Os01g70940	13	50	P0492G09	*	163.8
OsHAK3	AP003236	BAD61453	Os01g27170	15	45	OSJNBa0010P20.20		73.1
OsHAK4	AP006461	BAD10774	Os08g36340	12	35	P0104B02.21		88.6
OsHAK5	AP004330	BAD87321	Os01g70490	13	33	OSJNBa0052O12.31		161.8
OsHAK6	AP003246	BAD87162	Os01g70660	11	29	P0423A12.11		163.5
OsHAK7	AP004570	BAC83599	Os07g47350	15	46	P0625E02.129	*	105.7–114.5
OsHAK8	AC134885	CAD20998	Os03g21890	14	46			23.3
OsHAK9	AP003757	BAC79545	Os07g48130	14	49	OJ1409_C08.19	*	115.8
OsHAK10	AP004737	BAD37744	Os06g42030	13	40	OSJNBa0072A21.17		95.4
OsHAK11	AL731610	CAE05216	Os04g52390	15	36	OSJNBa0070C17.23		102.7–107.4
OsHAK12	AP003875	CAD21002	Os08g10550	13	42			44.6–45.4
OsHAK13	AP005610	BAD45996	Os06g45940	13	50	OSJNBa0032M14.1		109.5
OsHAK14	AP005185	BAD31109	Os07g32530	15	36	P0409B11.4		67–69.2
OsHAK15	AL606684	CAE03568	Os04g52120	13	30	OSJNBa0085I10.13	*	102.7–107.4
OsHAK16	AC146468	AAR10864	Os03g37840	11	32	OSJNBa0029P07.17		60.8–62.4
OsHAK17	AP005308	BAD37951	Os09g27580	13	35	OJ1596_C06.35–1	*	60.8–62.4
OsHAK18	AP005396	BAD46101	Os09g38960	15	40	P0635G10.34		91.8
OsHAK19	AP005845	BAD26327	Os02g31910	12	37	P0461D06	*	64.7
OsHAK20	AP005845	BAD26330	Os02g31940	12	37	P0461D06	*	64.7
OsHAK21	AC146468	AAR10865	Os03g37930	13	33	OSJNBa0008D12		70.1–70.6
OsHAK22	AP005869	BAC84608	Os07g01214	13	35	B1026C12	*	0
OsHAK23	AP005637	BAD26044	Os09g21000	14	52	P0711A01	*	40.7
OsHAK24	AP005387	BAD54410	Os06g15910	15	49	OSJNBa0084K06		53.5
OsHAK25	AP005751	BAD16364	Os02g49760	14	50	OSJNBa0072H09	*	129.4
OsHAK26	AP006049	BAC57399	Os08g39950	13	34	OSJNBa0016N23	*	103.2
AKT family								
OsK1.1	AP003234	BAC05546	Os01g45990	6	55 (AKT1)[a]	P0038D11.22		107.6–110.2
OsK1.2	AP003843	BAC24865	Os07g07910	6	51 (AKT1)[a]	OJ1656_E11.135		26–31
OsK2.1	AP002093	BAA96192	Os01g11250	6	47 (KAT1)[a]	Ozsa8120	*	29.7
OsK2.2	AP003375	BAB90143	Os01g55200	6	47 (KAT1)[a]	OJ1414_E05.8	*	130.1
OsK2.3	AP005614	BAD19939	Os02g14840	4	50 (KAT1)[a]	OSJNBa0090H18.7	*	37.4
OsK3.1	AC135429	AAS90668	Os05g35410	6	55 (AKT2/3)[a]	P0636F09.19		80.7–85.7
OsK4.1	AP003453	BAB68056	Os01g52070	5	43 (KAT3)[a]	P0480C01.16		123.5
OsK4.2	AL606631	CAE01724	Os04g02720	4	26 (KAT3)[a]	OSJNBb0050O03.14		6.5
OsK4.3	AP005518	BAD46168	Os06g14310	6	42 (KAT3)[a]	P0046H10.36		50.3
OsK5.1	AL731593	CAD40970	Os04g36740	6	47 (GORK)[a]	OSJNBa0027P08.8		66.4
OsK5.2	AP005107	BAD45977	Os06g14030	6	63 (SKOR)[a]	P0431E05		40.2–50.3
OsKCO1	AC092556	AAR87255	Os03g54100	4	49 (KCO1)[a]	OSJNBa0047E24.7		120.3–122.9
OsKCO2	AP003248	BAC10733	Os01g50120	5	25 (KCO1)[a]	OJ1116_H09.4		122.1
OsKCO3	AP004736	BAD33183	Os09g12790	6	26 (KCO1)[a]	OSJNBa0062A09.6		10–20.7
Trk family								
OsHKT2;1	AB061312	BAB61790	Os06g48810	12	36	P0596H10.9		115.9
OsHKT2;2	AB061313	BAB61791	Os06g48810	8	36			
OsHKT2;3	AP003447	BAB90322	Os01g34850	12	32	OJ1619_F12.10		78
OsHKT2;4	AP003726	BAD53774	Os06g0701600	12	32	P0596H10		117
OsHKT1;1	AJ491815	CAD37182	Os04g51820	12	34	OSJNBa0060N03.3		102.1
OsHKT1;2	AJ506745	–	–	–	–	–	–	–
OsHKT1;3	AP005772	BAD26198	Os02g07830	10	38	OSJNBa0073A21.31		18.4
OsHKT1;4	AL606691	CAE03639	Os04g51830	15	12	OSJNBa0060N03.4		102.7
OsHKT1;5	AP003567	BAB93392	Os01g20160	12	41	OSJNBb0022N24.25	*	60.9–62.5
K^+/H^+ antiporter family								
OsKEA1	AL606641	CAE03437	Os04g5862	14	45	OSJNBa0032F06.20	*	128.5

[a] For K^+ channel family of rice percentage identities with most similar sequences in *Arabidopsis* is given in parenthesis. Since OsHKT1;2 is a pseudo-gene we could not provide any information related to the gene.
* Presence of full length cDNA.

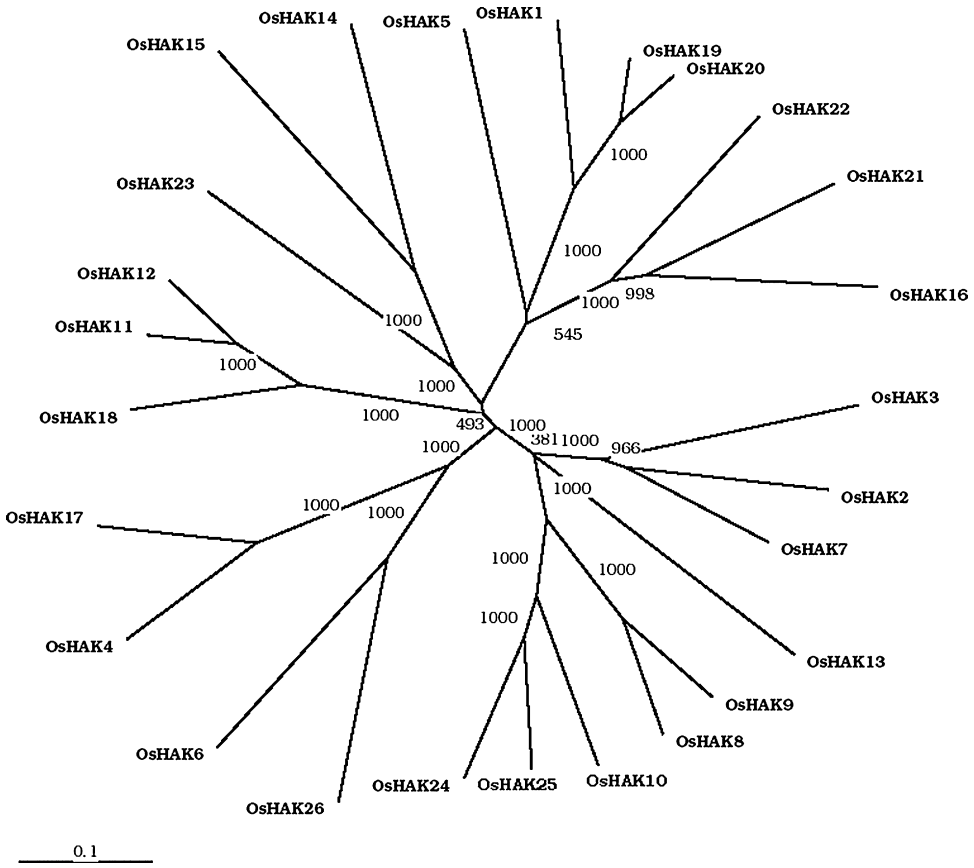

Fig. 3. Phylogenetic tree of rice HAK transporters. Programs used were ClustalX [116] for alignments, and Treeview [117] for graphical output. Values indicate the number of times (in %) that each branch topology was found during bootstrap analysis. Scale bar corresponds a distance of 10 changes per amino acid positions.

cDNA clones. All predicted proteins belonged to either 1P/6TM (11 proteins to Shaker type) or to the 2P/4TM (three proteins to ORK-type) family. A non-rooted phylogenetic tree of all 14 genes revealed two major branches: 1P/6TM K^+ channels and 2P/4TM K^+ channels (Fig. 4). Thus, the channels segregated according to the number of their P-loops. For K^+ channel family the TIGR genome codes and GenBank accession numbers are shown in Table 1. For the better visualization of the relationships between the AKT protein sequences, phylogenetic tree was constructed with AKT members of rice, maize, wheat, potato, carrot and tomato (Fig. 5 and Fig. 6). In the present analysis of rice genome, we found nine sequences related to HKT transporters. In public databanks, protein sequence was not found for OsHKT5 which was reported by Garciadeblas et al. [43], so we converted the nucleotide sequence of OsHKT5 into protein sequence using Genome scan [30] and included in the analysis. In UniProt, wrong GenBank accession no. AL606691 was given to OsHKT4 sequence. A phylogenetic analysis of the sequences of HKT transporters showed that rice transporters were very divergent, except for the pairs formed by OsHKT3 and OsHKT9, and by OsHKT1 and Po-OsHKT2, which showed 93 and 91% identities, respectively. HKT4 and HKT5 kept an identity of roughly 60%, while all the other sequences kept identities between 40 and 50% [43].

4. Discussion

4.1. HAK family

The largest gene family of K^+ transporters (KTs) was originally described by Schleyer and Bakker [44] in Bacteria, which is a constitutive low affinity K^+ uptake system [45]. In the soil-borne fungus *Schwanniomyces occidentalis*, HAKs is a major K^+ transport system [46] and K^+ uptake permease (KUP)acts as H^+-K^+ symport [21]. In plants, the homologues of KUP/HAK/KT transporters exist as multigene families in both dicots and monocots. By systematic BLAST searches in public databases, 13 members of this family were detected earlier in *Arabidopsis* [6], while at least 17 members are identified in rice [42]. Although HAK transporters do not show extensive conserved regions, up to 40 amino acid residues are conserved exactly in the same position [21]. The characteristic feature of HAK transporters is presence of consensus motif **G**VVY**GD**LGT**SPLY** (the amino acids conserved in all sequences are in bold) [21] where all the HAK transporters vary very little from it. In the present study, a total of 26 genes were identified under this family. Most of the KUP/HAK transporters identified so far in Arabidopsis [6] and in rice [42], belong to the cluster of high-affinity transporters [42]. They mediate high-affinity K^+ transport in root cells [42,47,48] but

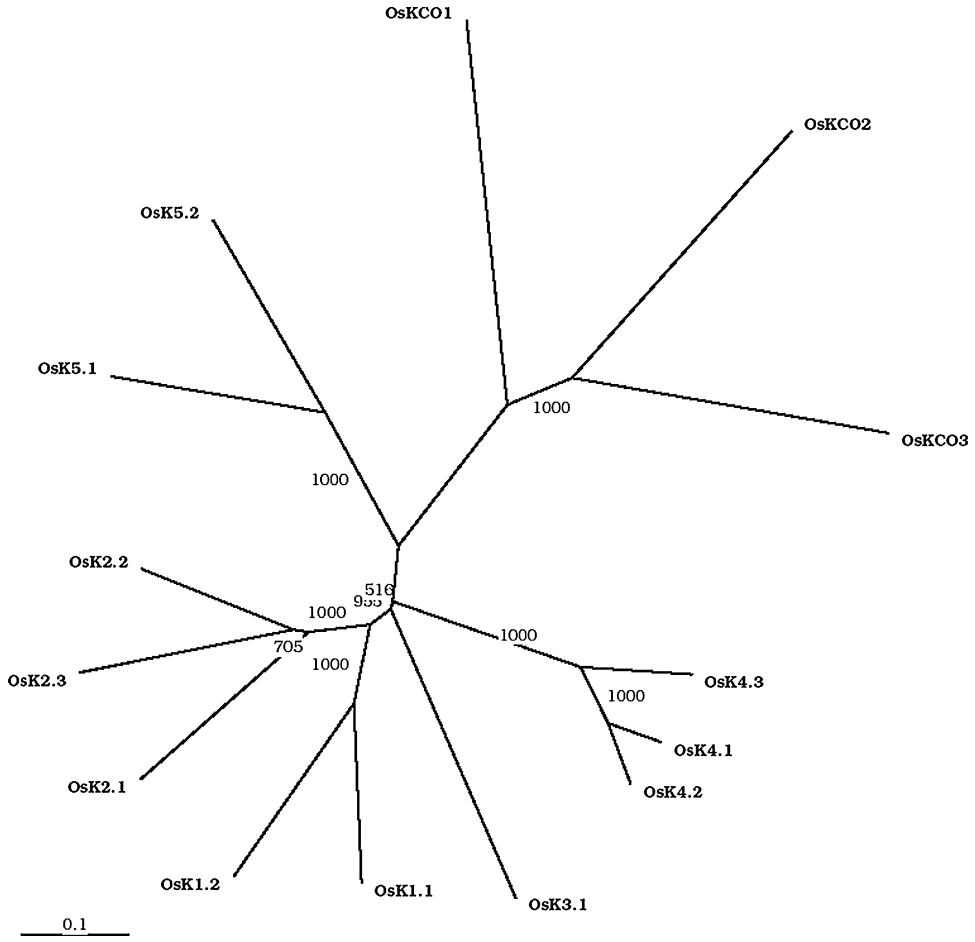

Fig. 4. Phylogenetic tree of rice K^+ channels. A non-rooted tree reflects distribution of K^+ channels into groups based on the structural and functional properties. The two major branches are the 2P/4TM-type and the 1P/6TM (Shaker)-type channels. Programs used were ClustalX [116] for alignments, and Treeview [117] for graphical output. Values indicate the number of times (in %) that each branch topology was found during bootstrap analysis. Scale bar corresponds a distance of 10 changes per amino acid positions.

some of them were demonstrated as low-affinity K^+ transporters [49,50]. The low affinity transporters are highly permeable to K^+ and Rb^+, but are less permeable to Na^+ and Cs^+ [19,49,51]. Phylogenetic analyses of the encoded transporters showed a great diversity and were grouped into four branches [42,47]. Transporters from groups I and II, have been characterized at the functional level. Three distinct HAK transporters of rice, OsHAK1, OsHAK7, and OsHAK10 were expressed in yeast (*Saccharomyces cerevisiae*) and bacterial mutants to determine their functions [42]. The three transporters mediate K^+ influxes or effluxes, depending on the conditions. OsHAK1 was expressed in the whole plant, but maximum expression was observed in roots and up-regulated in conditions of K^+ deficiency [42]. This resembles the expression pattern of its closest homologue in barley, HvHAK1 [18]. OsHAK7 and OsHAK10 were similarly expressed in seedlings of roots and shoots, where the expression of OsHAK10 was noticed in the panicle at flowering stage [42]. Most of the HAK transporters are expected to be located at plasma membrane having a role in high-affinity K^+ transport by root cells. But GFP-tagged transient expression of OsHAK10, which is a low-affinity transporter in onion epidermal cells showed that some of these transporters are located at the tonoplast with a probable function of mediating the release of K^+ from the vacuole [42]. A comparative kinetic analysis of HAK-mediated K^+ influx in yeast and in roots of K^+-starved rice seedlings demonstrated the involvement of HAK transporters in root K^+ uptake. In K^+-starved cells of the *E. coli* mutant, expression of the OsHAK7 and OsHAK10 transporters at high arabinose concentrations elicited K^+ or Rb^+ uptake at higher rates than the endogenous uptake of the bacterial mutant [42].

4.2. K^+ channels

K^+ channels are a structurally diverse group of proteins that facilitate the flow of K^+ ions across cell membranes. They are ubiquitous, being present virtually in all cell types. K^+ channels are the best understood transporter family in plants in terms of gating, second messenger regulation, transport properties, and other predicted functions in different plant cells and membranes. Plant K^+ channels also play a key role in K^+ uptake, translocation and osmotic regulation too [52,53]. K^+ channel families can be categorized by the number of P-loops and presence of transmembrane (TM) domains per monomer.

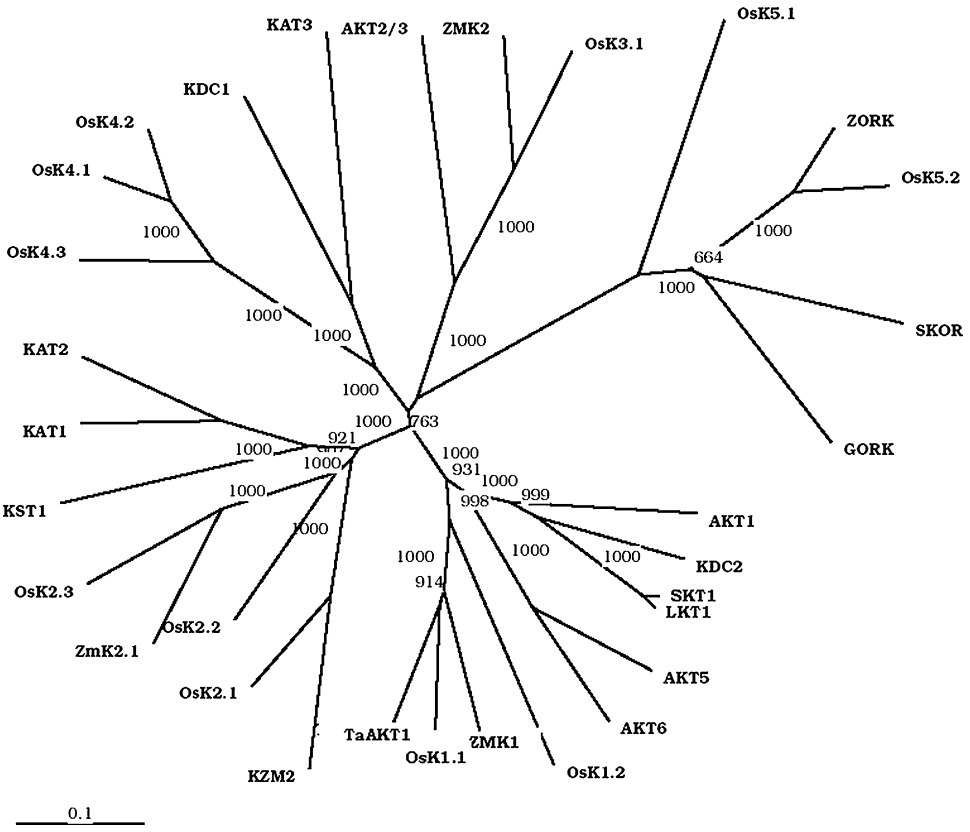

Fig. 5. Phylogenetic relationsips of *O. sativa* K^+ channels to other plant K^+ channels. The proteins are from *O. sativa*, *Arabidopsis thaliana*, *Zea mays*, *Triticum aestivum*, *Solanum tuberosum*, *Lycopersicon esculentum* and *Daucus carota*. Programs used were ClustalX [116] for alignments, and Treeview [117] for graphical output. Values indicate the number of times (in %) that each branch topology was found during bootstrap analysis. Scale bar corresponds a distance of 10 changes per amino acid positions.

Typical examples of these channels are *Shaker*-type 1P/6TM channels [54], the 1P/2TM channels [55], the ORK like 2P/4TM KCO channels [56] and the TOK like 2P/8TM channels [57]. Most of the plant K^+ channels are voltage dependent and display high sequence homology with animal Shaker channels and particularly with channels belonging to the *ether a gogo* (EAG) subfamily [58]. The functioning of the plasma membrane K^+ channels depends on the regulation of their number in the plasma membrane. Trafficking to the plasma membrane was found to be controlled by intrinsic sequence motifs in many mammalian ion channels. In such sequences, diacidic motifs are present that function as endoplasmic reticulum export signals. It is not clear till date if such motifs also exist in plant ion channels or not. Mikosch et al. [59], recently showed that such a diacidic motif is required for efficient transport of the plant K^+ channel KAT1 to the plasma membrane. Plant K^+ channels belonging to *Shaker*-family are characterized by the presence of hydrophobic core with six transmembrane segments (S1–S6), and a pore-forming domain P, located between S5 and S6 [60]. The K^+ channel signature sequence comprises **TXXTXGYGD** motif [61,62], a hallmark for majority of potassium-selective channels in plant and animal cells [63]. This is well conserved and forms the selectivity filter for K^+ ion transport. The glycine residue present in the inner helix hinge point in most K^+ channels allow the inner helices to switch between a closed KcsA-like conformation and an opened MthK like conformation [64]. Plant *Shaker*-type K^+ channels possess a cyclic nucleotide binding domain (cNBD) downstream of the S6 segment [58,65], that is responsible for K^+ subunit interactions. Another motif, the ankyrin domain, present downstream of the cNBD in some types of plant K^+ channels facilitate the binding of the channels to cytoskeleton, or protein-protein interactions or modulation by cytosolic factors [10,65–74]. According to the voltage-dependence within which they are active, plant *Shaker*-type K^+ channels are classified as hyper polarization activated inward-rectifying channels that mediate K^+ uptake, depolarization activated outward-rectifying channels that mediate K^+ release and weakly rectifying K^+ channels that mediate both uptake and release, depending on the electrochemical K^+ gradients. Plant *Shaker*-type K^+ channel genes were classified into five groups based on sequence similarity and functional properties [58]. Properties of K^+ channels such as structure, function, localization, membrane topogenesis, their expression, modulation and physiological roles was extensively reviewed by Gamblae and Uozumi [75]. KCOs are classified as 2P/4TMS or 1P/2TMS channels and possess K^+ channel signature sequence which is a hallmark for all K^+ channel proteins. These channels do not possess any TMS for voltage sensing. Most of them possess putative Ca^{2+}-binding sites (one or two EF hands in their cytosolic C-terminal region [23,76]. Most of the 2P/4TM channels were described as leak-like channels, with some

of them gated by membrane stretch [77]. Based on systematic sequencing programs and DNA based strategies, nine genes encoding *Shaker*-type K^+ channels and six genes encoding 2P/4TM channels were identified in *Arabidopsis* [6]. In the present analysis, we identified a total of 11 genes corresponding to *Shaker*-type K^+ channel proteins and our data is consistent with K^+ channel genes identified by Pilot et al. [58] in rice and three genes to 2P/4TM KCO family. Out of 3 putative genes of KCO family, OsKCO1 and OsKCO3 possess EF hand motifs, whereas it is absent in OsKCO2. Among the 11 identified genes in Shaker-family, 2 genes belonged to group I (inward-rectifying channels), 3 to group II (inward-rectifying channels), only 1 to group III (weakly inward-rectifying channels), 3 to group IV (helper family) and 2 genes to group V (outward-rectifying channels). All the putative K^+ channel genes were designated according to the nomenclature given by Pilot et al. [58]. In the present analysis, the nomenclature of the proteins OsK2.2 and OsK4.3 given by Pilot et al. [58] is changed as OsK4.3 and OsK2.2, respectively, as they were showing homology with the corresponding groups of proteins. OsK3.1 protein given by Pilot et al. [59] is a truncated protein of AAS90668. So, this protein sequence was taken for analysis in place of OsK3.1. The record for the protein sequence OsK2.1 was discontinued from the public data bank, so we gave the protein accession number **BAD73049** for OsK2.1 as both the sequences showed 100% homology. The first *Shaker*-type K^+ channels identified and cloned from plants were AKT1 [10] and KAT1 [11] from *Arabidopsis*. Genes encoding root K^+ uptake channels were identified in many other plant species; for example SKT1 in potato, LKT1 in tomato, KDC1 in carrot and ZMK1 in maize and OsAKT1 (OsK1;1) in rice [68–71,78–80]. Recently, OsAKT1 (OsK1;1), which is homologous to *Arabidopsis* AKT1 was cloned and functionally characterized as an inward-rectifying K^+ channel [81]. In contrast to ZMK1, OsAKT1 (OsK1;1) transcripts were predominantly expressed in coleoptile and roots of young rice seedlings and share expression pattern with that of *Arabidopsis* AKT1. It is a dominant salt sensitive K^+ uptake channel in rice roots. In general, channels are widely distributed in plant tissues. Channels of group I and IV are mostly present in the root and

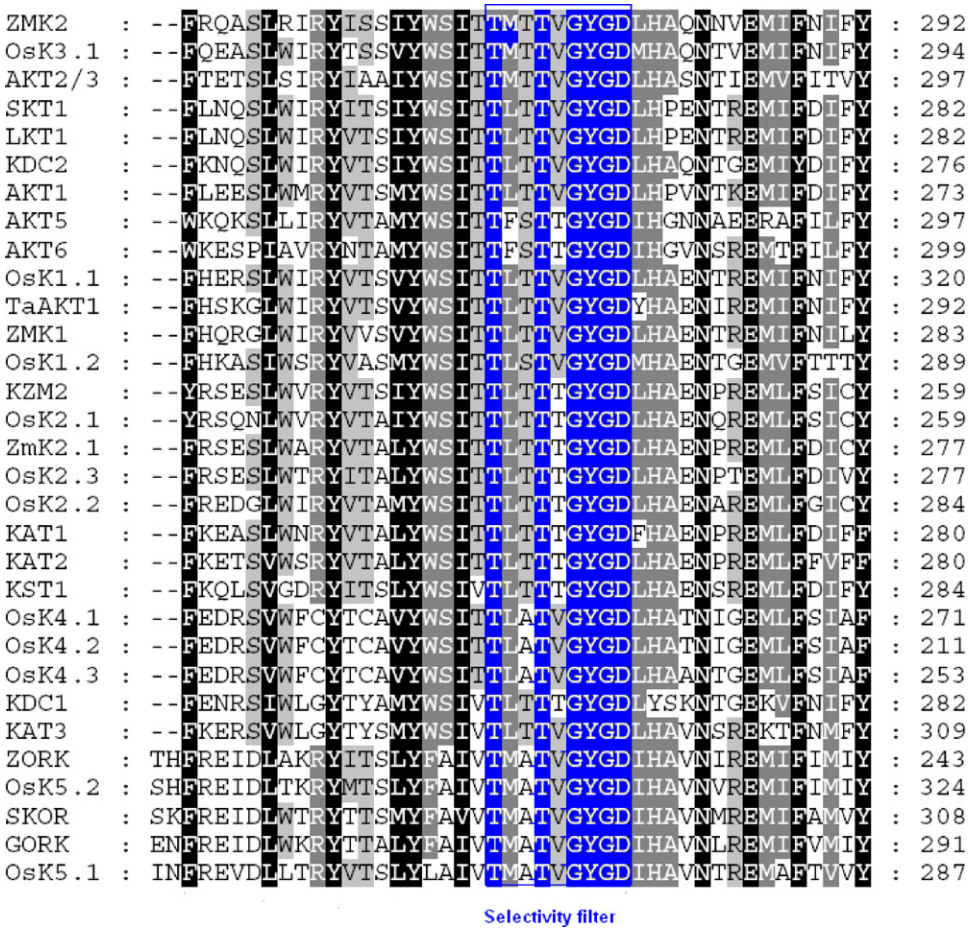

Fig. 6. Alignment of K^+ channel proteins showing conserved regions in rice, arabidopsis, wheat, maize, potato, carrot and tomato. Amino acids in putative transmembrane fragments are shadowed, and amino acids, which are conserved in most sequences, are highlighted (the selectivity filter sequence is highlighted with blue and the glycine gating hinge with red). Alignments were made using the ClustalX [116] program. Sequence accession numbers: SKT1 (CAA60016); KST1 (CAA56175); LKT1 (CAA65254); ZMK1 (CAA68912); ZMK2 (CAB54856); ZmK2.1 (AAR21352); KZM2 (AAX15943); TaAKT1 (AAF36832); KDC1 (CAB62555); KDC2 (CAG27094); KAT1 (AAA32824); AKT2/3 (AAA97865); KAT3 (CAB05669); AKT1 (AAB95299); AKT5 (CAB79967); AKT6 (AAD31377); KAT2 (CAA16801). SKOR (CAA11280), GORK (CAC17380), ZORK (AAW82753). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

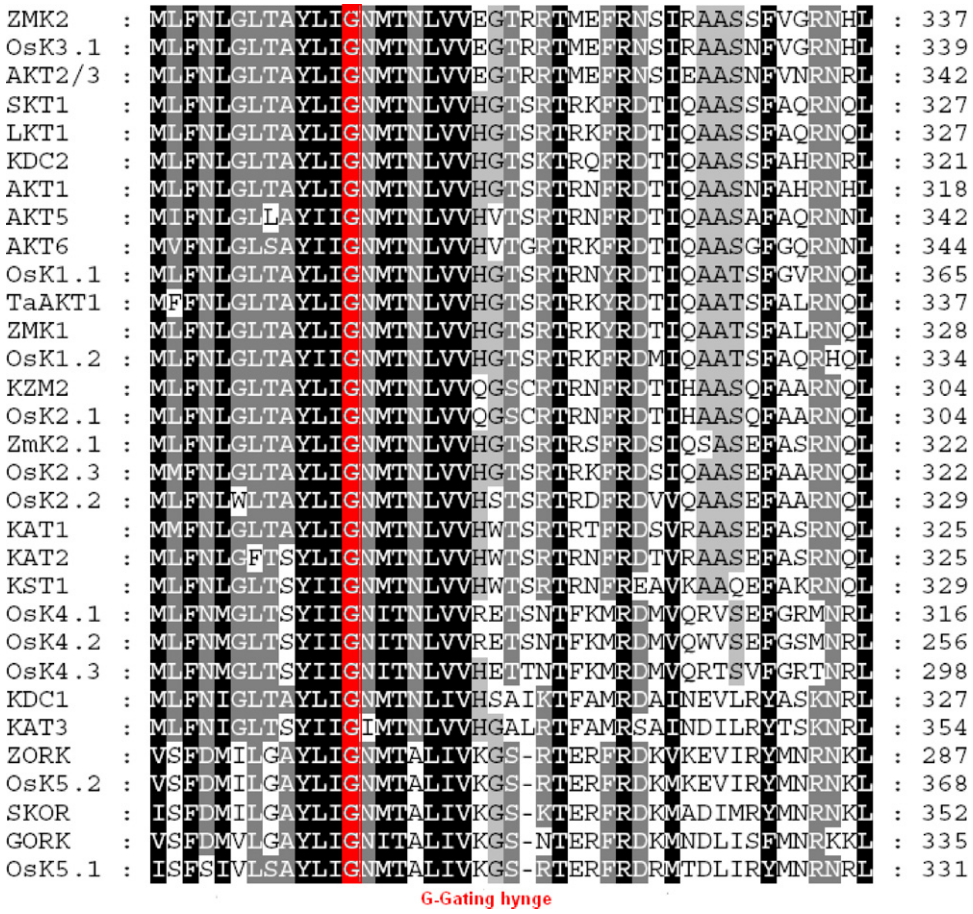

Fig. 6. (*Continued*).

that of group II in leaves. Whereas channels of group III are expressed in the phloem, group V have been identified in roots, as well as in guard cells and phloem [75]. From the phylogenetic analysis of rice K^+ channels and a comparison of it with other plant K^+ channels, it is evident that rice AKT members were closely related to maize and wheat but divergent from *Arabidopsis*. Consistent with our results, studies on *Shaker* type K^+ channels by Pilot et al. [58] showed that monocots (maize, rice and wheat) do not form specific groups but are included in the five groups previously defined by the analysis of *Arabidopsis* sequences. OsAKT1 (OsK1;1), which was a characterized protein, showed more identity with TaAKT1 (73%) and Zmk1 (71%) than *Arabidopsis* AKT1. On the other hand, OsAKT3 (OsK3;1) showed 77% identity with ZMK2 of maize. All other OsAKTs are closely clustered with maize K^+ channels. These results indicate that OsAKTs found in rice are homologous to wheat and maize K^+ channels and probably have similar transport functions (Fig. 6).

4.3. HKT/TRK family

Yeast Trk (transport of K^+), HKT and bacterial KtrB transporters are all members of this super family. Trk1 from *S. cerviciae* was the first gene cloned from this family encoding K^+ transport [82]. However, HKT1 from wheat was the first plant K^+ transporter cloned and identified by functional complementation studies using yeast. Most of these transporters are believed to work as K^+:H^+/Na^+ co-transporters [83–85] or K^+:K^+ co-transporters [86,87] depending on the transporter and ionic conditions. It was hypothesized that they were evolved from bacterial 2TM K^+ channels [21]. They display a core structure with 8TMs and 4P forming domains. This was evident from AtHKT1 having 8TMs and N- and C-terminal cytosolic regions. In eukaryotic transporters, there is a conserved **GNTXFP** motif in M2b, and an F (**V** or **I**) (**V** or **I**) **SXYG** motif in Pd. It is remarkable that the P loops of both prokaryotic and eukaryotic transporters keep only a glycine residue of the selective filter residues **GYG**, which are absolutely required for K^+ selectivity in K^+ channels [63]. HKT1 homologues were cloned from *Arabidopsis* [88], eucalyptus [89] and rice [90]. Molecular analyses of Na^+/K^+ permeability of these transporters also identified a glycine residue highly conserved in all P domains of HKT transporters but replaced by serine in the first P domain of AtHKT1 and OsHKT1, which are weakly permeable to K^+ [90,91]. Site directed mutagenesis of S-G in AtHKT and OsHKT1, G-S in HKT of wheat gave evidence about the prominent role of glycine in K^+ permeability [92]. From these studies it is evident that glycine residue is probably necessary for K^+ transport in some species [13,90], but serine residue allows K^+ transport in others [89,93,94]. Recently, phylogenetic tree of all the publicly available HKT sequences showed that it was divided

into two subfamilies [95], based on the substitution of glycine/serine in the first pore loop of the protein [13,43]. All HKT genes from dicots fall within the first major subfamily. In contrast, the nine HKT genes from rice [95] showed more divergence and were divided between the two major branches. In the phylogenetic tree, the clade containing HKT sequences from dicots plus the rice OsHKT4-OsHKT8 were designated as subfamily 1. Thus, the nomenclature of these genes, for example OsHKT4 was changed to OsHKT1;1 and OsHKT5 to OsHKT1;2. The second clade includes sequences from graminaceous species and was designated as subfamily 2 and the numbering was given according to the order in which they have been identified. For example OsHKT1 was named as OsHKT2;1. The second number in the designation of family is to differentiate genes within a species. The members of subfamily 1 have serine at the pore loop whereas members of subfamily 2 have glycine at this position [95] and these residues play a key role in determining the Na^+ selectivity of the HKT transporters [13,43,90]. Therefore, the division into two major subfamilies might reflect an important division of function. All HKT transporters so far identified are expressed in roots. Functional analysis of HKT1 in wheat, rice and barley [96,97] revealed that it functions as a low-affinity Na^+ transporter under salt stress and a determinant of salt sensitivity in plants [98]. Initially, two transporters of HKT family were identified in rice, OsHKT1 and Po-OsHKT2, but neither of them were reported as K^+ sensitive [90,94]. The predicted amino acid sequence of Ni-OsHKT1 shared 100% identity with Po-OsHKT1 and 91% identity with Po-OsHKT2. By searching the rice genome in public databases, we identified nine genes representing Trk/HKT family. In this study, we followed the nomenclature given by Platten et al. [95] for designating the genes in HKT family. All rice OsHKT transporters could be classified as KcsA-related transporters, which are made up of 4MPM motifs and have characteristics in common with fungal and bacterial Trk transporters [21,99,100].

HKT1 from wheat was originally characterized as the K^+–H^+ symporter that mediate the high-affinity K^+ uptake in wheat roots [12], but it was later found to co-transport Na^+–K^+ when expressed in yeast or *Xenopus* oocytes [98]. Although there is no strong evidence on functioning of HKT1 as K^+–H^+ symporter in the roots of any cereal [101–103], the notion that HKT1 is a high-affinity root K^+ transporter still persists [104]. From the available evidences it was proposed that high-affinity K^+ uptake is not only mediated by HAK transporters [18,42], but also by HKT transporters [43]. This was supported by partial expression of wheat HKT1, which shows Na^+ uptake, but not K^+ uptake [105]. The controversy about the function of HKT transporters was well reviewed by Rodriguez-Navarro and Rubio [106]. Plant and yeast HKT/TRK transporters posses two cation binding sites [21,43,86,107] and depends on the availability of Na^+/K^+ in the external environment for their function. When K^+ or Na^+ are present at micromolar concentrations and occupy the two sites, the result is a uniport of either K^+ or Na^+, as in the case of K^+ transport in *Saccharomyces cerevisiae* and Na^+ transport by OsHKT1, TaHKT1, HvHKT1 and AtHKT1. If both Na^+/K^+ bind and cross the membrane, the result is a Na^+–K^+ symport, as observed in case of TaHKT1 and HvHKT1 or Na^+ uniport and its function is strongly inhibited in the presence of K^+ as described for OsHKT1. This means to say that this transporter mediates Na^+ uptake when K^+ is not available. Some of these transporters may uniport Na^+, K^+ or Rb^+ regardless of the function that they exhibit when K^+ is present at millimolar concentrations [108,43]. From the literature it is observed that high-affinity Na^+ uptake in barely, wheat [96,109], and rice [43] was greatly enhanced when plants are K^+-starved and Na^+ uptake was inhibited by K^+ in K^+ starved plants [43]. In the light of the above findings, the proposal of Epstein may be modified where mechanism I is operated by HAK1 K^+ tranasporter and HKT1 Na^+ transporter in parallel, when the barley plants are grown in the absence of NH_4^+. By cloning and functional expression of rice (*O. sativa* cv. Nipponbare) *HKT* cDNAs in yeast, it was concluded that HKT transporters mediate high-affinity Na^+ uptake in rice roots but other processes of Na^+ transport in shoots. However, the function of HKT1 in roots is controversial. Cloning and expression studies of HvHKT1 cDNA in yeast cells demonstrated that high affinity Na^+ uptake by plant roots is a uniport. But it is inhibited by external K^+ and the heterologous systems fail to reproduce this mechanism as it depends on the construct from which the transporter was expressed [109]. OsHKT1 is a Na^+ transporter and does not mediate K^+ flux. It corresponds to the transporting properties of AtHKT1. On the other hand, OsHKT2 is a Na^+ and K^+ coupled transporter, the character of which is similar to those of TaHKT1 [91,94]. Yeast expressions of OsHKT1 and OsHKT4 proved that they are Na^+ transporters of high and low-affinity respectively, which are sensitive to K^+ and Ba^{2+}. Parallel experiments of K^+ and Na^+ uptake in yeast expressing the wheat or rice HKT1 transporters proved that they were very different; TaHKT1 transported K^+ and Na^+, while that of OsHKT1 only Na^+. Transcript expressions of the *OsHKT* genes in shoots were fairly constant and insensitive to changes in the K^+ and Na^+ concentrations of the nutrient solution [43]. In roots, the expressions were much lower than in shoots, except for OsHKT4 and OsHKT1 in K^+-starved plants [43]. From the literature it becomes clear that most of the HKT transporters are involved in Na^+ transport and mediate Na^+ distribution in *Arabidopsis* [92,110,111] and help in removal of Na^+ from the xylem sap. This may be a key process to limit the ascent of Na^+ to leaves. Some of the HKT transporters like AtHKT1 [88], McHKT1 [112], and OsHKT4 [43] expressed in plants exhibited low affinity for Na^+. Besides, another striking characteristic of these transporters was, they function as Cl^- channels [113,114]. In rice, OsHKT1 specifically mediates Na^+ uptake in roots when the plants are K^+ deficient. The incidence of *HKT* ESTs in several plant species suggests that the rice model with many *HKT* genes applies to other plants too. However, high affinity HKT transporters may transport Na^+ in different modes. Therefore, a comprehensive understanding of the functions of these transporters needs more extensive research.

4.4. K^+/H^+ antiporters

K^+/H^+ antiporters were first described from gram-negative bacteria, where they are gated by glutathione-S conjugates and

inactivated by glutathione. These antiporters provide a means for acidification of the cytosol as a defense to toxic electrophiles such as methylglyoxal [115]. A search of the rice genome, revealed only one K^+/H^+ antiporter gene (Fig. 2) which is named as OsKEA, and complete full length cDNA sequence is available for this one. The KEAs belong to the monovalent cation: proton antiporter family 2 (CPA2 family). However, none of the plant KEAs was experimentally characterized so far. From the phylogenetic tree it was observed that KEA family clade is falling in between Shaker and KCO families. Since the function of this protein is not characterized, we assume that this may share functional properties of plant K^+ channels. Functional characterization of this protein may help us in understanding its role in K^+ transport. The analysis of rice genome for K^+ transporter genes certainly helps in understanding the mechanism of accumulation and transport of K^+ *in planta* that can acquire K^+ under NaCl stress.

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