Letters

Nomenclature for *HKT* transporters, key determinants of plant salinity tolerance

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Salinity tolerance in many plants is inversely related to the extent of Na^+ accumulation in the shoot, notably in the major cereals such as wheat and rice [1]. In *Arabidopsis* and rice, there is evidence indicating a central role for members of the *HKT* gene family of Na^+ and Na^+/K^+ transporters in controlling Na^+ accumulation [2–6] and, thus, in determining salinity tolerance. However, in heterologous systems, whereas the wheat TaHKT1 protein transports both Na^+ and K^+, AtHKT1 is more Na^+-specific [7,8] and their sequences are not particularly closely related. Recent studies suggest that members of the HKT gene family in rice and *Arabidopsis* are expressed in xylem parenchyma cells and protect leaves from salinity stress by removing sodium from the xylem sap [5,6]. Given the wealth of sequences becoming available and the potential for confusion inherent in the current nomenclature, it is timely to propose an internationally agreed nomenclature for the family.

Phylogenetic trees of publicly available full-length *HKT* coding sequences or HKT amino acid sequences show that the gene family splits into two major branches (Figure 1). The major division is stable, as are the clusters of closely related genes, although the precise relationships between gene clusters in the larger subfamily 1 vary slightly with the analysis method used [e.g. minimum-evolution, neighbor-joining, UPGMA (Unweighted Pair Group Method with Arithmetic Mean) and maximum parsimony]. The two HKT subfamilies can also be distinguished on the basis of gene organization (Figure 2). Although all *HKT* genes contain two introns, these are significantly larger in group 1 than in group 2 genes ($p = 0.0085$, Mann–Whitney test).

HKT genes from dicot species fall within the first major subfamily. By contrast, the nine *HKT* genes found in rice [9] are more diverse and divide between the two major branches. A search of publicly available EST sequences, supplemented by targeted cloning of further members from wheat, barley and sorghum (J.D. Platten and O. Cotsaftis, unpublished) indicates that this family structure is general for many graminaceous species.

It is proposed that the clade containing sequences from dicot species plus the rice *OsHKT4*– *OsHKT8* genes [9] be designated subfamily 1 (Table 1). Thus, for example, *AtHKT1* becomes *AtHKT1;1* and *OsHKT4* becomes *OsHKT1;1*. The second clade, which so far only contains genes from graminaceous species, is designated subfamily 2. Within these two clades, we propose that genes within a species would be numbered according to the order in which they were identified (or, for those already named, in the current numbering order). For example, *OsHKT9* will now be named *OsHKT2;4*. The revised nomenclature is outlined in Table 1.

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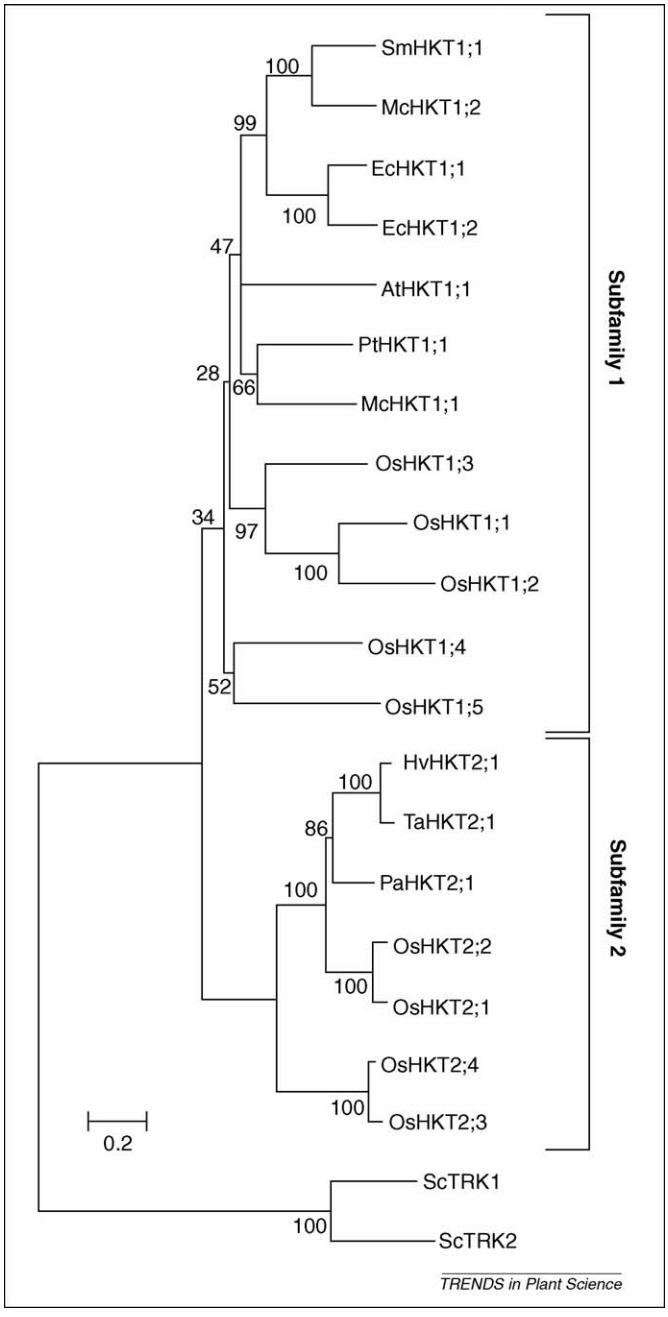

Figure 1. Unrooted minimum-evolution tree of known full-length protein sequences encoded by *HKT* genes from higher plants showing the division into two major clades. *Saccharomyces cerevisiae* (Sc) *TRK1* and *TRK2* genes (Accession numbers AAA34728 and AAA35172, respectively) are included as an out-group. Abbreviations: *At, Arabidopsis thaliana; Ec, Eucalyptus camaldulensis; Hv, Hordeum vulgare; Mc, Mesembryanthemum crystallinum; Os, Oryza sativa; Pa, Phragmites australis; Pt, Populus trichocarpa; Sm, Suaeda maritima; Ta, Triticum aestivum*. The tree was constructed using MEGA3 [15] with a random number seed of 92 702 and 10 000 bootstrap replicates. The numbers indicate percentage bootstrap support. The scale bar indicates 0.2 substitutions per site.

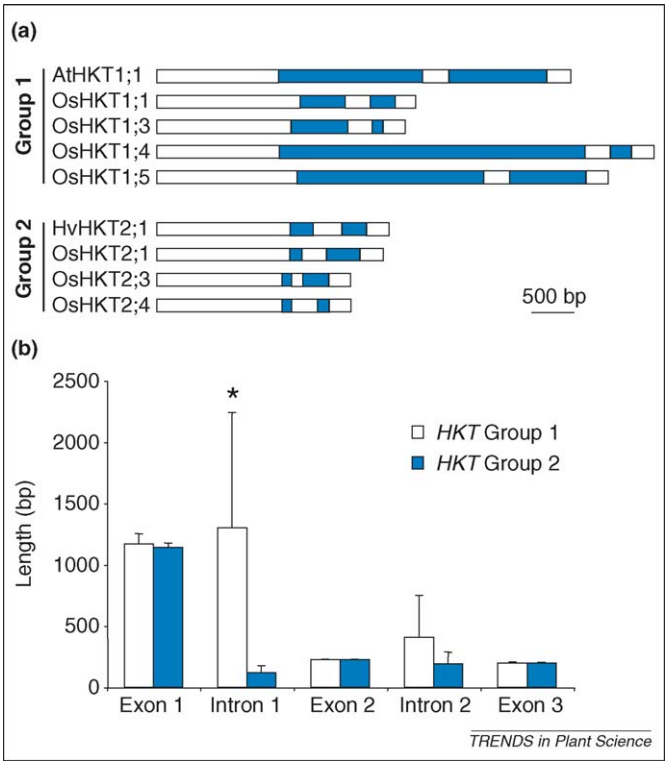

Figure 2. The two HKT subfamilies have different intron sizes. **(a)** Structures from the start-to-stop codon of some plant *HKT* genes for which genomic DNA sequences are available. Coding sequences (exons) are depicted in white, introns in blue. All *HKT* genes contain two introns, but they are significantly longer in group 1 than in group 2 genes ($p = 0.0085$, two-tailed Mann–Whitney test). **(b)** Building blocks of plant *HKT* genes for which genomic DNA sequences are available. Although there are no differences in exon sizes, group 1 genes (white; n = 5) contain longer introns than group 2 genes (blue; n = 4; *, $p = 0.016$, two-tailed Mann–Whitney test).

This naming system has the advantages of clearly assigning membership within the gene family to the two functionally and evolutionarily distinct clades – for different members of the family within a species and for similar members of the family from different species. Thus, sequence differences between *TaHKT1* (*TaHKT2;1*) and *AtHKT1* (*AtHKT1;1*) are supported by their separation between the two clades, whereas the similarities between *TaHKT1* (*TaHKT2;1*) and *OsHKT2* (*OsHKT2;2*) are more clearly indicated. Note that the second number is solely to differentiate genes within a species, and does not reflect relationships – thus, the name *TaHKT2;1* does not indicate a relationship closer to *OsHKT2;1* than to *OsHKT2;3*.

The division of the family into two major branches is associated with a glycine/serine substitution of a residue predicted to be in the first pore loop of the protein [7,9]. All members of subfamily 1 have a serine at this position, whereas members of subfamily 2 (except for the likely revertant *OsHKT2;1*) have a glycine. Functional analyses of the *TaHKT2;1*, *AtHKT1;1* and rice genes suggest that this particular residue could play a central role in determining the Na^+ selectivity of the transporter [7,9,10], results consistent with those from the related bacterial Na^+-dependent K^+ transporter KtrAB [11]. Therefore, the division into two major subfamilies might reflect an important division of function. There could be other structural determinants of selectivity, which might explain possible effects on selectivity in heterologous systems of the N-terminus [12] and the K^+ transport activity of HKTs from *Eucalyptus* [13,14].

Furthermore, the tree suggests that dicot plants lack members of subfamily 2. The function of these subfamilies and how these genes contribute to salinity tolerance and other aspects of whole-plant function requires further investigation.

Table 1. Revised nomenclature for *HKT* family members

Previous name	Nucleotide Accession no.	Locus identification no.	Protein Accession no.	Revised name
AtHKT1	AF237672	At4g10310	AAF68393	***AtHKT1;1***
McHKT1	AF367366	–	AAK52962	***McHKT1;1***
McHKT2	AY231175	–	AAO73474	***McHKT1;2***
PtHKT1	grail3 LG_XVIII[a]	–	[a]	***PtHKT1;1***
EcHKT1	AF176035	–	AAF97728	***EcHKT1;1***
EcHKT2	AF176036	–	AAD53890	***EcHKT1;2***
SmHKT1	AY530754	–	AAS20529	***SmHKT1;1***
OsHKT4	AJ491816	Os04g51820	CAD37183	***OsHKT1;1***
OsHKT5	AJ506745	–	[b,c]	***OsHKT1;2***
OsHKT6	AJ491818	Os02g07830	CAD37185	***OsHKT1;3***
OsHKT7	AJ491853	Os04g51830	CAD37197	***OsHKT1;4***
OsHKT8, SKC1	AK108663	Os01g20160	BAB93392	***OsHKT1;5***
TaHKT1	U16709	–	AAA52749	***TaHKT2;1***
HvHKT1	AM000056	–	CAJ01326	***HvHKT2;1***
PaHKT1	AB234304	–	BAE44385	***PaHKT2;1***
OsHKT1	AB061311	Os06g48810	BAB61789	***OsHKT2;1***
OsHKT2	AB061313	–	BAB61791	***OsHKT2;2***
OsHKT3	AJ491820	Os01g34850	CAD37187	***OsHKT2;3***
OsHKT9	AJ491855	Os06g48800	CAD37199	***OsHKT2;4***

[a]From the poplar genome project, at http://genome.jgi-psf.org.
[b]Translated from nucleotide sequence.
[c]Because *OsHKT1;2* is a pseudogene in rice cv. Nipponbare, internal stop codons were overridden to create a full-length amino acid sequence

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