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RESEARCH PAPER

Comparative mapping of *HKT* genes in wheat, barley, and rice, key determinants of Na^+ transport, and salt tolerance

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Abstract

Salt tolerance of plants depends on HKT transporters (High-affinity K^+ Transporter), which mediate Na^+-specific transport or Na^+-K^+ co-transport. Gene sequences closely related to rice *HKT* genes were isolated from hexaploid bread wheat (*Triticum aestivum*) or barley (*Hordeum vulgare*) for genomic DNA southern hybridization analysis. *HKT* gene sequences were mapped on chromosomal arms of wheat and barley using wheat chromosome substitution lines and barley–wheat chromosome addition lines. In addition, *HKT* gene members in the wild diploid wheat ancestors, *T. monococcum* (A^m genome), *T. urartu* (A^u genome), and *Ae. tauschii* (D^t genome) were investigated. Variation in copy number for individual *HKT* gene members was observed between the barley, wheat, and rice genomes, and between the different wheat genomes. *HKT2;1/2*-like, *HKT2;3/4*-like, *HKT1;1/2*-like, *HKT1;3*-like, *HKT1;4*-like, and *HKT1;5*-like genes were mapped to the wheat–barley chromosome groups 7, 7, 2, 6, 2, and 4, respectively. Chromosomal regions containing *HKT* genes were syntenic between wheat and rice except for the chromosome regions containing the *HKT1;5*-like gene. Potential roles of *HKT* genes in Na^+ transport in rice, wheat, and barley are discussed. Determination of the chromosome locations of *HKT* genes provides a framework for future physiological and genetic studies investigating the relationships between *HKT* genes and salt tolerance in wheat and barley.

Key words: Barley, comparative mapping, HKT, rice, salt tolerance, sodium transport, wheat.

Introduction

Soil salinity is one of the major abiotic environmental problems affecting agricultural production. The problem of salinization is increasing due to land clearing or irrigation (Rengasamy, 2006). To meet this challenge, it is important to understand the mechanisms of salt tolerance to improve further salt tolerance of crops such as wheat and barley (Colmer *et al*., 2005). In wheat, sodium exclusion is one of the major mechanisms conferring salt tolerance (Gorham *et al*., 1990*b*; Munns *et al*., 2006). Bread wheat (*Triticum aestivum*, AABBDD) has a low rate of Na^+ transport to the shoot and maintains a high ratio of K^+/Na^+ in the leaves (Gorham *et al*., 1990*b*). This trait is conferred, at least partially, by the *Kna1* gene on chromosome 4DL (Dubcovsky *et al*., 1996). Durum wheat (*Triticum turgidum* ssp. *durum*, AABB) is more salt-sensitive than bread wheat (Rawson *et al*., 1988) due to its poorer ability to exclude Na^+ from the shoot (Gorham *et al*., 1990*b*). However, an unusual durum wheat, Line 149, has the low Na^+ concentrations and high K^+/Na^+ ratios in the leaf blade typical of bread wheat. Line 149 is derived from a cross between *Triticum monococcum* (A^mA^m) accession C68-101 and the durum wheat cultivar Marrocos (The, 1973). The Na^+ exclusion trait in Line 149 is controlled by two major genes (Munns *et al*., 2003), namely *Nax1* and *Nax2*, interacting via net Na^+ xylem loading and net leaf sheath sequestration (Davenport *et al*., 2005). In barley (*Hordeum vulgare*), Na^+ exclusion and K^+/Na^+ selectivity is lower than in bread wheat (Gorham *et al*., 1990*a*); however, in barley leaves, Na^+ is compartmentalized into the vacuole and therefore its toxicity is reduced (Greenway and Munns, 1980). Furthermore, K^+ in barley leaves is partitioned more into mesophyll cells than into epidermal cells, resulting in a higher K^+:Na^+ ratio in the cytoplasm of

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mesophyll cells, which is beneficial for maintaining photosynthesis (James *et al*., 2006*b*).

Salt tolerance of plants could depend on HKT transporters (High-affinity K^+ Transporter), which mediate Na^+-specific transport or Na^+-K^+ transport and play a key role in regulation of Na^+ homeostasis (Rodríguez-Navarro and Rubio, 2006; Munns and Tester, 2008). In *Arabidopsis thaliana* there is only one *HKT* gene (Uozumi *et al*., 2000). In rice (*Oryza sativa*) there are eight *HKT* genes (Horie *et al*., 2001; Garciadeblás *et al*., 2003). Based on amino acid sequence similarity, *HKT* genes have been grouped into two main subfamilies (Platten *et al*., 2006). The division into the two subfamilies is associated with differences in a key amino acid in the first pore loop of the protein (Mäser *et al*., 2002*b*; Garciadeblás *et al*., 2003); all gene members of subfamily 1 have a serine residue which is replaced by glycine in most members of subfamily 2. The division is also associated with differences in Na^+ and K^+ selectivity (Horie *et al*., 2001; Mäser *et al*., 2002*a*; Garciadeblás *et al*., 2003).

Gene members of subfamily 1 are all Na^+-specific transporters. Some of them are expressed in cells in the stele rather than the root cortex, and regulate root-to-shoot transport of Na^+ by removing Na^+ from the xylem sap as it flows to the shoot. The AtHKT1;1 (AtHKT1) transporter plays an important role in regulation of Na^+ homeostasis (Rus *et al*., 2001; Mäser *et al*., 2002*a*), but its mechanism of action is not fully resolved (Munns and Tester, 2008). *AtHKT1;1* is expressed in xylem parenchyma cells in roots and leaves (Sunarpi *et al*., 2005), and *athkt1;1* mutants have a higher concentration of Na^+ in xylem sap than their wild type (Berthomieu *et al*., 2003; Sunarpi *et al*., 2005). A quantitative flux analysis study using $^{22}Na^+$ showed that AtHKT1;1 controls the rate of Na^+ transport from root to shoot by the retrieval of Na^+ from the xylem in the roots (Davenport *et al*., 2007). *AtHKT1;1* expression has also been detected in phloem tissue (Berthomieu *et al*., 2003; Sunarpi *et al*., 2005), but there is no evidence that AtHKT1;1 contributes significantly to recirculation in the phloem and thereby the control of shoot Na^+ concentration in *Arabidopsis* (Davenport *et al*., 2007).

The rice gene *OsHKT1;5* (*OsHKT8*), first identified as the quantitative trait locus *SKC1* (Lin *et al*., 2004), codes for a transporter that unloads Na^+ from the root xylem (Ren *et al*., 2005). In wheat, *HKT1;5*-like (*HKT8*-like) genes are likely candidates for two major genes controlling Na^+ exclusion from leaves: *Nax2* in durum wheat, and *Kna1* in bread wheat (Byrt *et al*., 2007). *Nax2* is associated with Na^+ exclusion from leaves via the retrieval of Na^+ from the xylem in roots (James *et al*., 2006*a*), and *Kna1* with Na^+ exclusion from leaves via the control of net xylem loading in roots (Gorham *et al*., 1990*b*). In wheat also, an *HKT1;4*-like (*HKT7*-like) gene is a candidate for the major gene *Nax1* (Huang *et al*., 2006). *Nax1* was first identified as a quantitative trait locus for Na^+ exclusion in durum wheat by Lindsay *et al*. (2004), and is associated with the retrieval of Na^+ from the xylem in roots and leaf bases (James *et al*., 2006*a*).

Gene members of subfamily 2 are Na^+-K^+ co-transporters or Na^+ and K^+ uni-porters, except *OsHKT2;2* (*OsHKT2*). Some of them are specifically expressed in the root cortex, and may serve to scavenge Na^+ under conditions of K^+ deficiency and so provide ionic homeostasis. Under saline conditions the expression of those genes may be down-regulated. This was recently shown to be the case for *OsHKT2;1* (*OsHKT1*) (Horie *et al*., 2007). OsHKT2;1 mediated the transport of Na^+ into roots of K^+-starved plants and enhanced their growth, but was down-regulated when plants were exposed to 30 mM NaCl (Horie *et al*., 2007). In wheat and barley roots, TaHKT2;1 (TaHKT1) and HvHKT2;1 (HvHKT1) also mediated Na^+ uptake into roots of K^+-starved plants (Laurie *et al*., 2002; Haro *et al*., 2005).

In determining the copy number of individual *HKT* genes and their chromosome locations in wheat and barley, the rice genome sequence provides a useful reference for comparative mapping (Yu *et al*., 2002). Using the available *HKT* gene sequences in rice (Horie *et al*., 2001; Garciadeblás *et al*., 2003), closely related EST sequences in wheat and barley were identified, and mapped by Southern hybridization. The complex hexaploid wheat genome (Fig. 1) originates from the

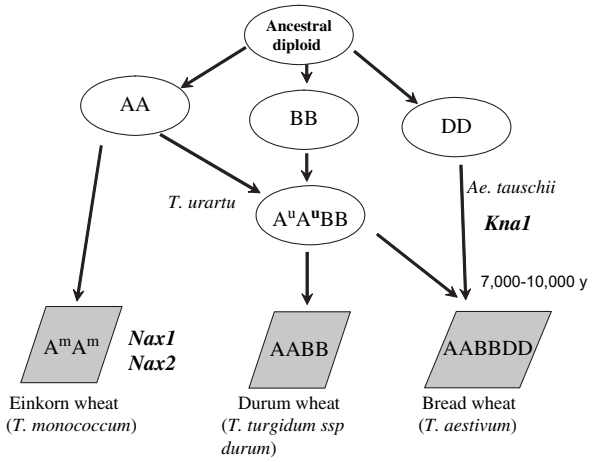

Fig. 1. Evolution of the wheat genome and known salt-tolerant loci in wheat. Bread wheat (*Triticum aestivum* L., AABBDD) originated from hybridization of three diploid species, *T. urartu* (A genome), an extinct or undiscovered population closely related to *Ae. speltoides* (B genome), and *Ae. tauschii* (D genome). The A genome of tetraploid durum wheat (AABB) and hexaploid bread wheat (AABBDD) may share a common ancestor, and *T. uaratu* is considered to be the closest diploid ancestor surviving today (Dvořák *et al*., 1988). Salt-tolerant durum wheat Line 149, containing *Nax1* and *Nax2*, was derived from a cross between *T. monococcum* L. (AA) accession C68-101 and the durum cultivar Marrocos (The, 1973). *Nax1*, *Nax2*, and *Kna1* are *HKT* genes conferring Na^+ exclusion in wheat (Gorham *et al*., 1990*a*; Huang *et al*., 2006; James *et al*., 2006*a*; Byrt *et al*., 2007).

hybridization of three wild diploid species: *T. urartu* (A genome), an unknown species closely related to *Aegilops speltoides* (B genome), and *Aegilops tauschii* (D genome). Wheat ancestors and wild relatives are a likely source of new genes for salt tolerance in wheat (Colmer *et al*., 2006). *HKT* genes were therefore also examined in *T. monococcum*, *T. urartu*, and *Ae. tauschii*.

This study presents information on the copy number of individual *HKT* gene members in the barley genome, the different genomes of bread wheat, and related wild diploid wheats, and their chromosome locations of individual *HKT* members in the barley and bread wheat genomes. The potential role of *HKT* genes in Na^+ transport and salt tolerance in rice, wheat, and barley is discussed.

Materials and methods

Genetic materials

Genetic materials used for *HKT* gene mapping included the hexaploid bread wheat cv. Chinese Spring, the tetraploid durum wheat cvs Langdon and Tamaroi, the diploid wheats *Triticum urartu* AUS1789 and AUS1790, *Triticum monococcum* C68-101 and DV92, *Aegilops tauschii* AUS18913, and the barley cv. Betzes. For wheat chromosome mapping, nulli-tetrasomic and ditelosomic aneuploid stocks developed in Chinese Spring were used (Sears *et al*., 1954). In the nulli-tetrasomic lines, a deleted pair of chromosomes is compensated for by two copies of a pair of homoeologous chromosomes. Ditelosomic lines carry a centromeric deletion of one chromosome arm (Sears *et al*., 1954). For barley chromosome mapping, wheat–barley addition lines were used. These were developed by adding one barley chromosome from Betzes barley (chromosome addition line) or chromosome arm (ditelosomic addition line) into Chinese Spring (Islam *et al*., 1981).

Database searches

In rice, there are eight *HKT*-like genes (*OsHKT2;1–4*, *OsHKT1;1*, *OsHKT1;3–5*; Horie *et al*., 2001; Garciadeblás *et al*., 2003). *OsHKT2;1* has been annotated in the Nipponbare genome sequence as *Os06g48810* on chromosome 6 and shares 93% identity at the nucleotide level with *OsHKT2;2* (AB061313), a gene isolated from salt-tolerant cultivar Pokkali but which could not be identified in the *japonica* or *indica* rice genome sequences. *OsHKT2;3* (AJ491820; Os01g34850) was positioned on chromosome 1 and was 95% identical to *OsHKT2;4* (AJ491855; Os06g48800) on chromosome 6. *OsHKT2;4* is therefore tightly linked to *OsHKT2;1* but has only 73–76% sequence identity in three regions of *OsHKT2;1* at positions 669–855, 1016–1170, and 1366–1502 (Fig. 2). *OsHKT1;1* on chromosome 4 (Os4g51820) was separated by ~3 kb from a pseudogene *OsHKT1;2*. *OsHKT1;1* and *OsHKT1;2* gene sequences are ~80% identical at the nucleotide level. *OsHKT1;3* was located on chromosome 2 (Os02g07830), *OsHKT1;4* on chromosome 4 (Os4g51830), and *OsHKT1;5* on chromosome 1 (Os01g20160). Garciadeblás *et al*. (2003) described the sequence similarity between *OsHKT* genes using phylogenetic trees. NCBI (www.ncbi.nlm.nih.gov) and the Gramene (www.gramene.org) database were used to search closely related wheat or barley sequences for probe design (Table 1).

Cloning and sequencing of wheat or barley ESTs related to HKT *genes in rice*

Primers were designed on the basis of wheat or barley EST sequences that were closely related to rice *HKT* genes (Table 1). The amplified products from wheat or barley were cloned using pGEM-T Easy vector system (Promega) and confirmed by sequencing. The probe developed from the wheat orthologue *TaHKT2;1* was not expected to hybridize to *HKT2;3* or *HKT2;4*-like genes in wheat, although *OsHKT2;1* has some similarity (73–76% identity) at the positions 669–855, 1016–1170, and 1366–1502 with *OsHKT2;3/4* in rice (Fig. 2).

DNA extraction and Southern hybridization

Plants were grown in soil for 4 weeks. The leaves were harvested for DNA extraction as described by Lagudah *et al*. (1991). DNAs were digested with different restriction enzymes (*Eco*RI, *Eco*RV, *Hin*dIII, *Nco*I) and electrophoretically fractionated in 1% agarose gel and transferred to Hybond N^+ nylon membranes (Amersham) by capillary transfer. Prehybridization and hybridization were

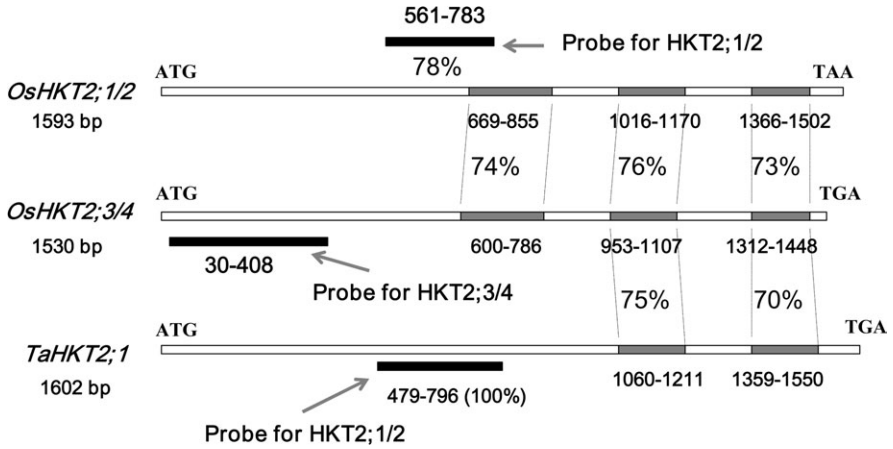

Fig. 2. Diagram of matches between sequences of specific probes for *HKT2;1/2*-like and *HKT2;3/4*-like genes and sequences of *OsHKT2;1/2* and *OsHKT2;3/4*. *OsHKT2;1/2* and *OsHKT2;3/4* have some similarity (73–76% identity) in three regions of *OsHKT2;1* at positions 669–855, 1016–1170, and 1366–1502. The sequence of specific probe for *HKT2;1/2*-like genes matched *OsHKT2;1/2* (Table 1) and was 100% identical to *TaHKT2;1* but had no similarity to *OsHKT2;3/4*. The sequence of specific probe for *HKT2;3/4*-like genes matched *OsHKT2;3/4* (Table 1) but had no similarity to *OsHKT2;1/2* and *TaHKT2;1*.

performed in a rotary hybridization chamber at 65 °C in a solution containing 1% sodium dodecyl sulphate (SDS), 50 mM 1 TRIS–HCl (pH 8.0), 10 mM EDTA, 3.3× SSC buffer, 10% dextran sulphate, 0.1% BSA, 0.1% PVP, 0.1% Ficoll-400, and 0.03% salmon DNA. The immobilized DNAs were hybridized overnight in buffer at 65 °C with probes ^{32}P-labelled by the random primer method using Megaprime DNA Labelling Kit (Amersham). The membranes were washed at 65 °C twice, 20 min each time, in 2× SSC/0.1% SDS, once for 20 min in 1× SSC/0.1% SDS and once for 15 min in 0.5× SSC/0.1% SDS. Autoradiograms were exposed for 1–3 d at –80 °C with intensifying screens.

Results

Chromosome mapping

DNA probes were used to localize each *HKT*-like gene to a specific chromosome or chromosome arm in wheat and barley, utilizing the nulli-tetrasomic and ditelosomic aneuploid stocks developed in Chinese Spring hexaploid wheat. The mapping results for each *HKT*-like gene are given in detail below and are summarized in Table 2 and Fig. 3. A representative set of blots is shown for the *HKT2;1/2*-like gene and described in detail in the legend of Fig. 4A–C. *HKT* gene map locations in hexaploid wheat and barley are shown in Fig. 5A, B, respectively.

HKT2;1/2*-like genes in wheat and barley*

The *HKT2;1/2* probe which was developed from *TaHKT2;1* (*TaHKT1*, Genbank accession: U16709) hybridized to five bands in genomic DNA of hexaploid wheat (see Fig. 4) suggesting that up to five members of the *HKT2;1/2*-like family could be present in the bread wheat genome, which is consistent with results of Laurie *et al*. (2002). Up to two bands were mapped on the long arm of chromosomes 7A and 7B, while only one band mapped to chromosome 7D (Figs 3, 4). In barley, only one band was detected (Table 2; Fig. 5), which was mapped on chromosome 7H (Fig. 3). Two bands were detected in the A genome of the diploid *T. urartu*, but only one band was present in the A genome of the diploid *T. monococcum* (Table 2; Fig. 4). In the D genome of the diploid *Ae. tauschii*, two bands were detected (Table 2; Fig. 4). The location of *HKT1/2*-like genes within the syntenic wheat

Table 1. *Probes developed for detection of* HKT *genes in wheat and barley genomes*

HKT genes	Old name	Matched wheat/barley sequence	Probe size (bp)	*E*-value	Identity	Primers
HKT2;1	*HKT1*	U16709	318	7E-34	78%	5′-TATGTGATGAGTCGCAGCTTGAA
HKT2;2	*HKT2*			1E-35	78%	3′-GCAACAAGAGGCCTGAATTCTTT
HKT2;3	*HKT3*	DR733562	387	2E-89	82%	5′-TCTTAGTTCGGCAAGGCATATCA
HKT2;4	*HKT9*			6E-101	83%	3′-TGCACGGTAACCGATGTAACTCT
HKT1;1	*HKT4*	BJ472463	431	4E-11	76%	5′-TTAAAAATATTCGGGCCAACACC
HKT1;2	*HKT5*			1E-54	79%	3′-TGGGGTAAGCAGAAGAAGGAAAG
HKT1;3	*HKT6*	BJ473256	368	1E-91	85%	5′-CTATTTTGCCAAATCTGCACAGC 3′-TCTGGTCCCTTCTGTTGAATGAA
HKT1;4	*HKT7*	BE604162	453	3E-30	83%	5′-ATTCAGGCAACACCTAATCATGC 3′-GCATCACAAGAATGAGGATGAGC
HKT1;5	*HKT8*	DQ646342	315	3E-55	87%	5′-CGTGCTAGCGCAGCTGTCGCTCT 3′-ATCATACCATTAGATGCGTCATG

Table 2. *Summary of* HKT*-like gene copy numbers detected in barley and wheat genomes by probes using genomic DNA Southern hybridization*

HKT genes	Old name	Rice genome	Barley genome	Wheat genome[a]					
				A^mA^m	A^uA^u	AA	BB	DD	D^tD^t
HKT2;1	*HKT1*	1–2	1	1	2	2	2	1	2
HKT2;2	*HKT2*						*TaHKT2;1*		
HKT2;3	*HKT3*	2	2	1	1	1	1	1	1
HKT2;4	*HKT9*								
HKT1;1	*HKT4*	1	1	0	0	0	1	1	1
HKT1;2	*HKT5*								
HKT1;3	*HKT6*	1	1	1	1	0	1	1	1
HKT1;4	*HKT7*	1	2	2 *Nax1*	2	2	3	3	3
HKT1;5	*HKT8*	1 *SKC1*	1	1 *Nax2*	0	0	3	1 *Kna1*	1

[a] A^mA^m represents the A genome from *Triticum monococcum*; A^uA^u represents the A genome from *Triticum urartu*; D^tD^t represents the D genome of *Ae. tauschii*.

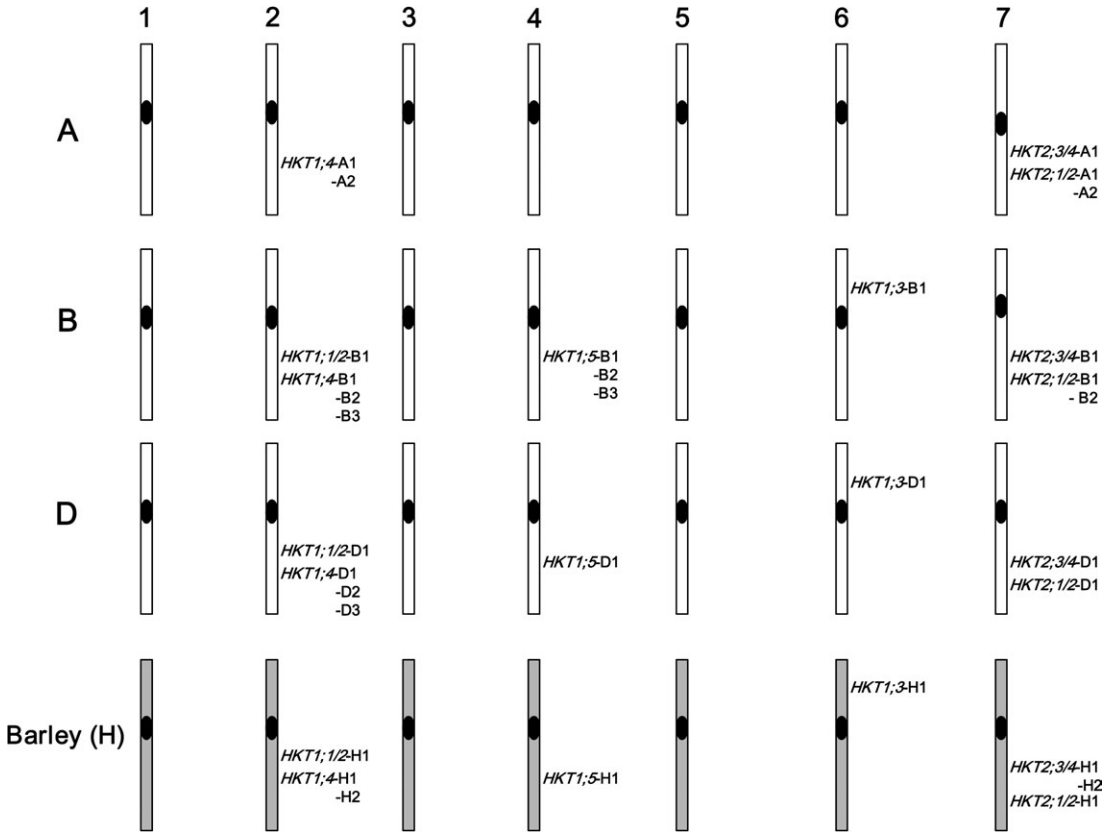

Fig. 3. Diagram of detected chromosome arm locations of *HKT* genes using Southern blot analyses in hexaploid bread wheat Chinese Spring (AABBDD) and barley cultivar Betzes. A, B, and D represent three different genomes from bread wheat Chinese Spring. The black circles on the chromosome represent the centromere. For wheat chromosome location mapping, nulli-tetrasomic and ditelosomic aneuploid stocks developed in Chinese Spring (Sears *et al*., 1954) were used. For barley chromosome location mapping, wheat–barley addition lines by addition of particular barley chromosome (addition line) or chromosome arm (ditelosomic line) into Chinese Spring (Islam *et al*., 1981) were used.

region to rice chromosome 6 suggests that these genes are orthologous to *OsHKT2;1* (Sorrells *et al*., 2003).

HKT2;3/4-*like genes in wheat and barley*

In rice, *OsHKT2;3* (AJ491820) and *OsHKT2;4* (AJ491855) are 95% identical at the nucleotide sequence level and are located on chromosomes 1 and 6, respectively. Using a probe developed from a closely related barley EST (DR733562), three bands were detected in hexaploid wheat (Table 2; Fig. 5) with one band mapping to chromosomes 7A, 7B, and 7D (Fig. 3). In barley, two bands were detected and mapped to chromosome 7H (Table 2; Fig. 5). There was one band present in *T. monococcum*, *T. urartu*, and *Ae. tauschii*, indicating that only one member of *HKT2;3/4*-like genes were present in those diploid wheats (Table 2). The location of *HKT2;3/4*-like genes on the long arm of wheat chromosome 7 suggests that these genes are orthologues of *OsHKT2;4* (Os06g48800) located within the syntenic region on rice chromosome 6 (Sorrells *et al*., 2003) but not of *OsHKT2;3* (Os01g34850) on chromosome 1 (Table 3). An *HKT2;3*-like orthologue could be absent from the wheat genome.

HKT1;1/2-*like genes in wheat and barley*

The barley probe which was derived from EST BJ472463 detected two members of the *HKT1;1/2*-like gene family present in hexaploid wheat (Table 2; Fig. 5). Those two members were mapped on the long arm of chromosome 2B and 2D, respectively (Fig. 3), but no *HKT1;1/2*-like gene was detected in the A genome of hexaploid wheat (Table 2). In the A genome of *T. monococcum* and *T. urartu*, an *HKT1;1/2*-like gene was also absent (Table 2). In the D genome of *Ae. tauschii*, one copy of *HKT1;1/2*-like gene was detected (Table 2). In barley, one band was present and mapped on the long arm of chromosome 2H (Table 2; Figs 3, 5). The map location of *HKT1;1/2*-like genes in wheat is syntenic to rice chromosome 4 (Sorrells *et al*., 2003) containing *OsHKT1;1* (Os04g51820) and *OsHKT1;2* (Table 3).

HKT1;3-*like genes in wheat and barley*

Two members of the *HKT1;3*-like genes were detected in hexaploid wheat (Table 2; Fig. 5) and mapped to the short arm of chromosomes 6B and 6D, respectively (Fig. 3). No *HKT1;3*-like gene was detected in the A genome of bread

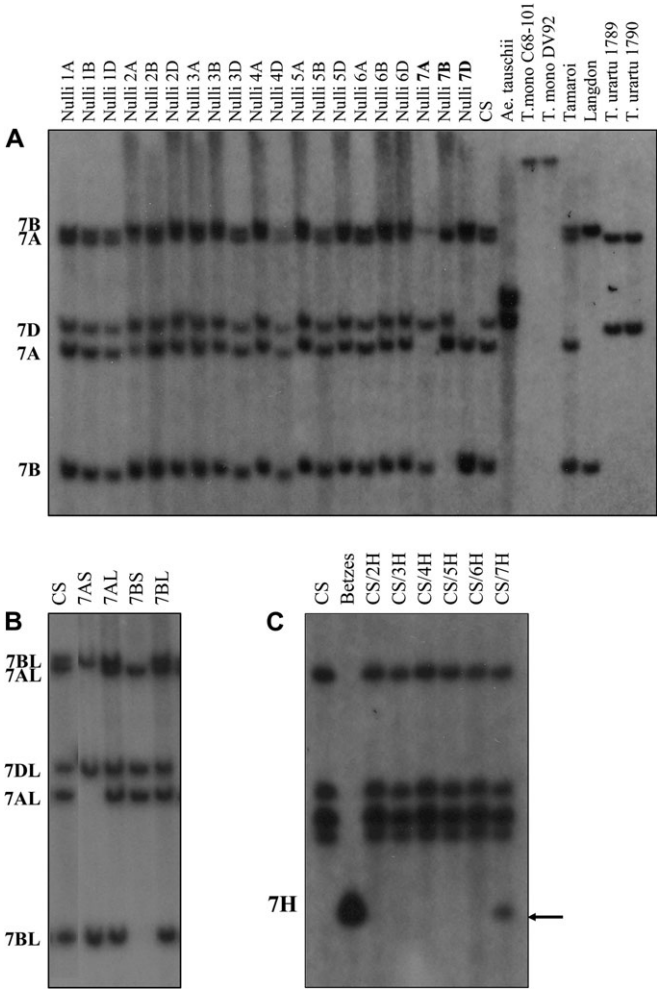

Fig. 4. DNA blot analysis of the *HKT2;1/2*-like gene. (A) Autoradiogram of a DNA blot containing digested genomic DNA (*Hin*dIII) of nulli-tetrasomic Chinese Spring aneuploid stocks. Polymorphism detected between *HKT2;1/2*-like gene members enables individual gene members to be assigned to chromosomes 7A, 7B, and 7D as indicated on the left side. (B) Autoradiogram of a DNA blot containing digested genomic DNA (*Hin*dIII) of ditelosomic Chinese Spring aneuploid stocks for group 7. No ditelosomic 7DS and ditelosomic 7DL are used because those lines are not reliable. *HKT2;1/2*-like genes were mapped on the long arm of 7A and 7B; therefore the mapped *HKT2;1/2*-like gene on chromosome 7D was likely located on the long arm. (C) Autoradiogram of a DNA blot containing digested genomic DNA (*Nco*I) of Chinese Spring wheat/Betzes barley chromosome addition lines. The arrow indicates a single band in barley which was only found in the wheat addition line containing barley chromosome 7. Therefore it is concluded that the band is present on chromosome 7H. No ditelosomic 7HS and ditelosomic 7HL lines were used because those lines are not reliable, but the mapped *HKT2;1/2*-like gene on chromosome 7H was likely to be located on the long arm based on the fact that the *HKT2;1/2*-like gene was located on the long arm of 7A and 7B in wheat.

wheat (Table 2); however, in the A genomes of *T. monococcum* and *T. urartu*, at least one copy of the *HKT1;3*-like gene was present in each genome (Table 2). This result differs from that of the *HKT1;1/2*-like genes, which are absent from all homoeologous A genomes of bread wheat, *T. monococcum* and *T. urartu* (Table 2). In *Ae. tauschii*, one *HKT1;3*-like gene was found (Table 2). In barley, one copy of *HKT1;3*-like gene was present and mapped on the short arm of chromosome 6H (Table 2; Figs 3, 5). The location of *HKT1;3*-like genes on wheat chromosome 6 is syntenic to rice chromosome 2 (Sorrells *et al.*, 2003) that contains *OsHKT1;3* (Os02g07830) (Table 3).

HKT1;4-*like genes in wheat and barley*

The *HKT1;4* probe showed that up to eight bands were present in hexaploid wheat (Table 2; Fig. 5). Three hybridization bands were mapped to the long arm of chromosomes 2B and 2D, respectively, and two bands were mapped on the long arm of chromosome 2A (Figs 4, 5) consistent with the previous report (Huang *et al.*, 2006). Two bands were present in the A genomes of *T. monococcum* and *T. urartu* (Table 2), the same number as in the A genome of hexaploid wheat. One *HKT1;4*-like gene in *T. monococcum* (EF062819) is a strong candidate for *Nax1*, a gene conferring sodium exclusion in durum wheat (Huang *et al.*, 2006; James *et al.*, 2006*a*). In *Ae. tauschii*, three bands were detected, the same number as for the D genome of hexaploid wheat (Table 2). In barley, two bands were detected and mapped to the long arm of chromosome 2H (Table 2; Figs 3, 5). The location of *HKT1;4*-like genes on the long arm of chromosome 2 is syntenic to rice chromosome 4 (Sorrells *et al.*, 2003) containing *OsHKT1;4* (Os04g51830) (Table 3).

HKT1;5-*like genes in wheat and barley*

Up to four bands of *HKT1;5*-like genes were detected in hexaploid wheat using *HKT1;5* probe (Table 2; Fig. 5). Three hybridization bands were mapped on the long arm of chromosome 4B and one band was mapped on the long arm of chromosome 4D (Fig. 3). Notably, the *HKT1;5*-like gene is absent in the A genome of hexaploid wheat (Table 2). A single copy *HKT1;5*-like gene was present in *T. monococcum* while no member was found in the accession of *T. urartu* used in the study. Byrt *et al.* (2007) previously showed that the single copy *HKT1;5*-like gene found in *T. monococcum* was located in the distal region of the long arm of chromosome 5A, reflecting the ancient reciprocal translocation which occurred between the distal segment of the long arm of chromosomes 4A and 5A in an ancestral wheat genome. The *HKT1;5*-like gene in *T. monococcum* (DQ646339) was considered a strong candidate for *Nax2*, a gene conferring sodium exclusion in durum wheat (James *et al.*, 2006*a*; Byrt *et al.*, 2007). The *HKT1;5*-like gene in the D genome of hexaploid wheat is a candidate for *Kna1* (DQ646342), a gene conferring sodium exclusion in hexaploid wheat (Gorham *et al.*, 1990*b*; Dubcovsky *et al.*, 1996; Byrt *et al.*, 2007). One band was also detected in *Ae. tauschii* (Table 2). In barley, one band was

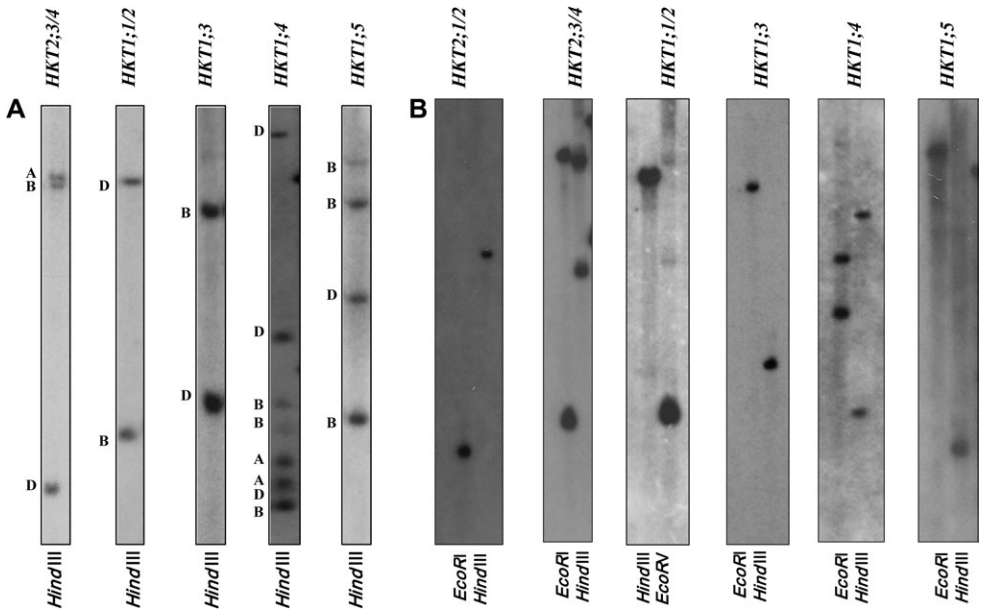

Fig. 5. DNA blot analysis of *HKT* genes in Chinese Spring wheat and Betzes barley. (A) Autoradiogram of a DNA blot of *HKT* genes detected in wheat with the letters on the left side indicating to which genome they belong, using the same method as described in Fig. 4. (B) Autoradiogram of a DNA blot of *HKT* genes detected in barley.

Table 3. *Syntenic regions of rice and wheat chromosomes containing* HKT *genes*

*HKT*s		Rice chromosome	EST	Wheat chromosome	Synteny[a]
HKT2;1	*HKT1*	Loc_Os06g48810	U16709	7L	Yes
HKT2;2[b]	*HKT2*	–			
HKT2;3	*HKT3*	Loc_Os01g34850	DR733562	7L	No
HKT2;4	*HKT9*	Loc_Os06g48800			Yes
HKT1;1	*HKT4*	Loc_Os04g51820	BJ472463	2L	Yes
HKT1;2[c]	*HKT5*	–			Yes
HKT1;3	*HKT6*	Loc_Os02g07830	BE604162	6S	Yes
HKT1;4	*HKT7*	Loc_Os04g51830	BJ472463	2L	Yes
HKT1;5	*HKT8*	Loc_Os01g20160	DQ646339	4L	No

[a] Syntenic chromosome regions between wheat and rice are described by Sorrells *et al.* (2003).
[b] *OsHKT2;2* is present in salt-tolerant cultivar Pokkali but not in Nipponbare (Horie *et al.*, 2001). The chromosome location of *OsHKT2;2* remains unknown.
[c] *OsHKT1;2* is a pseudogene without any gene chromosome annotation. Based on the result of sequence blast, *OsHKT1;2* locates about 3 kb beside *OsHKT1;1*.

detected and mapped to the long arm of chromosome 4H (Table 2; Figs 3, 5).

No syntenic relationship between *HKT1;5*-like genes in wheat and rice was found (Table 3) since *HKT1;5* (Os01g20160) is located on rice chromosome 1, which is syntenic to wheat chromosome 3 (Sorrells *et al.*, 2003).

Discussion

In the three different genomes of bread wheat (Fig. 1), variations in copy numbers of *HKT* genes were observed (Table 2). Differences in copy number were also found between the A genome of the wild diploid relatives (*T. monococcum* and *T. urartu*) and the A genome of bread wheat. There were variations in copy number of *HKT2;1/2*-like, *HKT2;3/4*-like, *HKT1;3*-like, and *HKT1;5*-like genes (Table 2). It is not known whether these variations in numbers are widely present in the A genome of bread wheat and ancestor diploid relatives, or confined to the individual genotypes that were used in this study. It is possible that the variation in copy number may affect salt tolerance of wheat, and that allelic variations in gene sequence may also affect salt tolerance.

The syntenic chromosome regions between wheat and rice have been described comprehensively by Sorrells *et al.* (2003). In the present study, *HKT2;1/2*-like, *HKT2;3/4*-like, *HKT1;1/2*-like, *HKT1;3*-like, and *HKT1;4*-like genes

were mapped on wheat and barley chromosome groups 7L, 7L, 2L, 6S, and 2L, respectively, which are synentic to chromosome regions containing putative orthologues of *HKT* genes in rice (Table 3). No synteny between wheat and rice is known for the chromosomal regions containing *HKT1;5*-like genes (Table 3).

The Na^+-K^+ co-transporters—subfamily 2

These are high-affinity transporters of K^+ and/or Na^+, and are important in K^+-deficient conditions where they may take up Na^+ and thereby promote growth. Some of them are specifically expressed in plasma membrane of cells in the epidermis and cortex of roots and their expression could be down-regulated in conditions of salinity.

TaHKT2;1 (*TaHKT1*) was the first *HKT* gene isolated from higher plants (Schachtman and Schroeder, 1994). Bread wheat could have five copies of *HKT2;1*-like genes on the basis of DNA hybridization from the present study (Fig. 3) and the previous report (Laurie *et al*., 2002). *TaHKT2;1* is probably one of the two copies located on the B genome as (i) the wheat EST (BE428877) isolated from roots of tetraploid durum wheat was 100% identical to *TaHKT2;1* (U16709), and (ii) primers designed on the basis of *TaHKT2;1* amplified a product only from the long arm of chromosome 7B (Mullan *et al*., 2007). *TaHKT2;1* was found to be expressed in cortical cells of bread wheat roots by *in situ* hybridization (Schachtman and Schroeder, 1994), but this finding may be confounded by potential cross hybridization with other *HKT2;1*-like members in bread wheat.

In barley, there is only a single copy of the *HKT2;1*-like gene (*HvHKT1*, AM000056) (Haro *et al*., 2005), which is consistent with the present study (Table 2). *HvHKT2;1* and *TaHKT2;1* have 92% identity at nucleotide sequence level and both encoded as a Na^+-K^+ co-transporter in a yeast transformation system (Rubio *et al*., 1995; Haro *et al*., 2005). In a root uptake system, however, HvHKT2;1 and TaHKT2;1 functioned as a putative Na^+ uniport (Haro *et al*., 2005; Fig. 6). The reason for the different behaviour in yeast is considered to be an alternative initiation of translation, which produced a different protein with different kinetic properties from that in roots (Haro *et al*., 2005). This hypothesis was supported by another study using a *TaHKT2;1* anti-sense transgenic line (Laurie *et al*., 2002). The down-regulation of *TaHKT2;1* in wheat was associated with an increase in shoot fresh weight in 200 mM NaCl under conditions of K^+ deficiency (Laurie *et al*., 2002). Following the down-regulation of *TaHKT2;1*, the transgenic wheat had smaller Na^+-induced depolarization in root cortical cells than the control, and lower $^{22}Na^+$ influx, indicating that TaHKT2;1 mediates Na^+ influx (Laurie *et al*., 2002; Fig. 6).

OsHKT2;1 functions as a relatively Na^+-specific transporter that mediates Na^+ influx in K^+-starved roots and so promotes their growth (Horie *et al*., 2001, 2007; Fig. 6). It is expressed in the cortex of rice roots, and is down-regulated under saline conditions, and so does not cause Na^+ toxicity (Horie *et al*., 2007). It is also expressed in leaves (Kader *et al*., 2006). OsHKT2;2 could function as a Na^+-K^+ co-transporter (Mäser *et al*., 2002*a*). Using a yeast transformation system, OsHKT2;2 showed Na^+-K^+ co-transport activity (Horie *et al*., 2001). Treatment with 150 mM NaCl greatly enhanced the expression of *OsHKT2;2* in the leaves of salt-tolerant Pokkali, particularly in the mesophyll cells (Kader *et al*., 2006), although these results have to be treated with caution as the 150 mM NaCl was administered as an osmotic shock. The expression of *OsHKT2;1* was down-regulated in the phloem and xylem tissues, as well as the mesophyll cells in the leaves (Kader *et al*., 2006). It would be interesting to investigate any tissue differential expression of *HKT2;1*/*2*-like genes in wheat, as observed for *OsHKT2;1* and *OsHKT2;2* in rice (Kader *et al*., 2006) and wheat (Mullan *et al*., 2007).

In rice, *OsHKT2;3*/*4* is expressed mainly in the shoot with little expression in the roots (Garciadeblás *et al*., 2003). No EST matching *OsHKT2;3*/*4* has been isolated so far from roots of wheat and barley. In wheat, *OsHKT2;3*/*4*-like ESTs (DR733562 and CA663666) were isolated from leaf and crown, suggesting that the *OsHKT2;3*/*4*-like genes were expressed in those tissues. In barley, *OsHKT2;3*/*4*-like ESTs (DN180983 and DN187639) were isolated from the leaf epidermis (Zierold

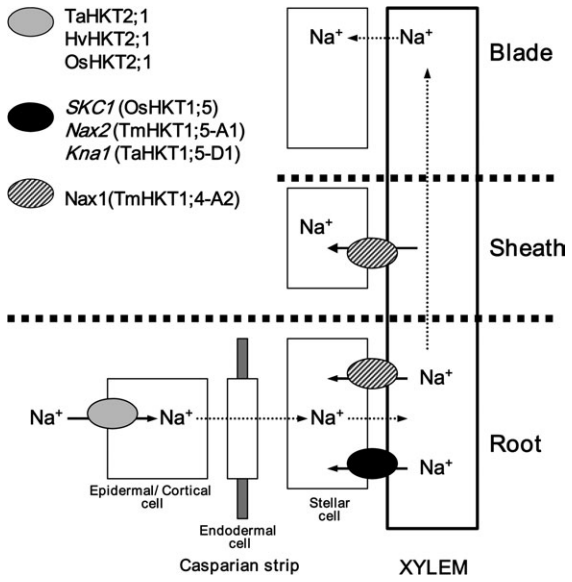

Fig. 6. Possible roles of known HKT transporters in controlling Na^+ flux in wheat, barley, and rice. TaHKT2;1, HvHKT2;1, and OsHKT2;1 could function as an Na^+ uptake system in the epidermal/cortical cells (Horie *et al*., 2001, 2007; Laurie *et al*., 2002; Garciadeblás *et al*., 2003; Haro *et al*., 2005). *SKC1* (OsHKT1;5), *Nax2* (TmHKT1;5-A1), and *Kna1* (TaHKT1;5-D1) could unload Na^+ from the xylem of the root (Gorham *et al*., 1990*a*; Ren *et al*., 2005; James *et al*., 2006*a*; Byrt *et al*., 2007). *Nax1* (TmHKT1;4-A2) could unload Na^+ from the xylem of the root and leaf sheath (Huang *et al*., 2006; James *et al*., 2006*a*).

et al., 2005). Future research is required to determine whether the expression of *OsHKT2;3/4*-like genes is specific to the epidermis. This investigation is of particular interest because differential partitioning of K^+ between epidermal and mesophyll cells was observed in salt-treated barley and wheat, leading to a desirably high K^+/Na^+ ratio in the cytoplasm of mesophyll cells (James *et al*., 2006*b*). Furthermore, Garciadeblás *et al*. (2003) found that OsHKT2;3 and OsHKT2;4 did not mediate any type of transport in transformed yeast, and suggested that their toxicity effects might be consistent with their expression in internal membrane and not in plasma membrane. It would be particularly interesting to investigate any *HKT2;3/4*-like genes targeted to the tonoplast which may be related to tissue tolerance in barley.

The Na^+-specific transporters—subfamily 1

These are low-affinity transporters specific to Na^+. Some of them are located in the plasma membrane of cells in the stele of roots, particularly the xylem parenchyma cells where they retrieve Na^+ from the xylem sap, and so prevent it reaching the shoots.

OsHKT1;1 can be expressed in rice shoots and roots (Garciadeblás *et al*., 2003). In a transformed yeast system, OsHKT1;1 mediated low-affinity Na^+ uptake (Garciadeblás *et al*., 2003). Although *OsHKT1;2* is a pseudogene, a transcript was isolated from rice roots (Garciadeblás *et al*., 2003). In the barley genome and wheat B and D genomes, there is only one copy of the *HKT1;1/2*-like gene (Table 2; Fig. 3). It is not clear which gene member is absent from the wheat and barley genome. In wheat, *HKT1;1/2*-like ESTs (CJ594572, CJ700470, CJ594562, and CJ700475) were isolated from the shoots, showing that the *HKT1;1/2*-like gene can be expressed in wheat shoots. In barley, *HKT1;1/2*-like ESTs (BJ472463, BM816866, CD058368, BF262602, and DN17794) were isolated from leaves or leaf epidermis. Those barley ESTs could come from different regions of the same gene because they have 100% or 99% (sequence variation) identity in the overlapped region. This may provide additional information that there is only one *HKT1;1/2*-like gene in barley. Future research is required to test any tissue-specific expression of the *HKT1;1/2*-like gene in barley.

OsHKT1;3 is mainly expressed in shoots of rice, with little expression in roots (Garciadeblás *et al*., 2003). The present study found no wheat EST matching *OsHKT1;3*, although a single copy of an *HKT1;3*-like gene was present in the B and D genomes of bread wheat (Table 2). In barley, 18 *HKT1;3*-like ESTs (e.g. BJ476674) were isolated from one cDNA library from the leaves of adult plants, indicating that an *HKT1;3*-like gene may be highly expressed in barley leaves. From experiments with transformed yeast, Garciadeblás *et al*. (2003) suggested that OsHKT1;3 might not be located on the plasma membrane but on an internal membrane. In barley, a species known for its tolerance of high internal Na^+ concentrations, presumably because of compartmentalization in the vacuole, the presence of an *HKT1;3*-like transporter on the tonoplast warrants investigation.

OsHKT1;4 is mainly expressed in shoots (Garciadeblás *et al*., 2003). A barley *OsHKT1;4*-like gene (BQ739876) was also expressed in the leaves of drought-stressed plants (Ozturk *et al*., 2002). The matched wheat EST (BE604162) was isolated from a drought-stressed wheat leaf cDNA library, indicating it was expressed in leaf tissues. Using a comparative mapping approach, an *OsHKT1;4*-like gene, *TmHKT1;4*-A2 (*TmHKT7*-A2) was cloned from *Triticum monococcum* as the candidate for *Nax1* conferring sodium exclusion and salt tolerance to durum wheat (Huang *et al*., 2006; James *et al*., 2006*a*). *TmHKT1;4*-A2 co-segregated with *Nax1* and its expression pattern in roots and leaf sheath was consistent with its proposed physiological role in removing Na^+ from the xylem of the roots and leaf sheaths (Huang *et al*., 2006; James *et al*., 2006*a*; Fig. 6). The distinctive phenotype of *TmHKT1;4*-A2 in wheat is a high Na^+ sheath:blade ratio (Davenport *et al*., 2005; James *et al*., 2006*a*).

OsHKT1;5 (SKC1) regulates K^+/Na^+ selectivity in rice, and maintains high shoot K^+ and low Na^+ under salt stress by controlling the unloading of Na^+ from the root xylem (Ren *et al*., 2005; Fig. 6). *OsHKT1;5* was preferentially expressed in the parenchyma cells surrounding xylem vessels (Ren *et al*., 2005). Voltage-clamp analysis of *Xenopus laevis* oocytes showed that OsHKT1;5 functions as a Na^+-selective transporter (Ren *et al*., 2005). *HKT1;5*-like genes are considered as candidates for *Nax2* in durum wheat and *Kna1* in bread wheat (Byrt *et al*., 2007). *Nax2* and *Kna1* have the same phenotype as *SKC1*, namely low leaf Na^+ concentration and high K^+:Na^+ ratio, enhanced discrimination of K^+ over Na^+ in transport from roots to shoots, and no effect on root Na^+ concentration (Gorham *et al*., 1990*b*; Davenport *et al*., 2005; James *et al*., 2006*a*). With *Nax2* and *Kna1* there is no effect on the sheath:blade Na^+ ratio, and no evidence of removal of Na^+ from the xylem in the leaf sheath (data for *SKC1* is lacking). The lack of a high sheath:blade Na^+ ratio distinguishes *Nax2* and *Kna1* from *Nax1* (James *et al*., 2006*a*). In durum wheat, *TmHKT1;5-A* on chromosome 5A segregated perfectly with Na^+ exclusion and K^+/Na^+ selectivity, and is the candidate gene for *Nax2* (Byrt *et al*., 2007). In bread wheat, *TaHKT1;5-D* on chromosome 4D is the candidate gene for *Kna1* (Byrt *et al*., 2007).

In summary, the copy numbers of individual *HKT* gene members vary between barley, wheat, and rice genomes and among different wheat genomes. *HKT2;1/2*-like, *HKT2;3/4*-like, *HKT1;1/2*-like, *HKT1;3*-like, *HKT1;4*-like, and *HKT1;5*-like genes were mapped on wheat and barley chromosome groups 7L, 7L, 2L, 6S, 2L, and 4L, respectively. Chromosomal regions containing *HKT*-like

genes between wheat and rice were syntenic except for the chromosome regions containing the *HKT1;5*-like gene. Some *HKT1;4* and *HKT1;5*-like genes are candidates for *Nax1*, *Nax2*, and *Kna1* in wheat, conferring salt tolerance by controlling sodium exclusion from xylem unloading (Huang *et al*., 2006; James *et al*., 2006*a*; Byrt *et al*., 2007). Further research will elucidate the full range of important functions of *HKT* genes in wheat and barley, and their role in salt tolerance.

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