The Plant Cell, Vol. 9, 2281–2289, December 1997 © 1997 American Society of Plant Physiologists

The *HAK1* Gene of Barley Is a Member of a Large Gene Family and Encodes a High-Affinity Potassium Transporter

Guillermo E. Santa-María,[a] Francisco Rubio,[a] Jorge Dubcovsky,[b] and Alonso Rodríguez-Navarro[a,1]

[a]Departamento de Biotecnología, Escuela Técnica Superior de Ingenieros Agrónomos, 28040 Madrid, Spain
[b]Department of Agronomy and Range Science, University of California, Davis, California 95616

The high-affinity K^+ uptake system of plants plays a crucial role in nutrition and has been the subject of extensive kinetic studies. However, major components of this system remain to be identified. We isolated a cDNA from barley roots, *HvHAK1*, whose translated sequence shows homology to the *Escherichia coli* Kup and *Schwanniomyces occidentalis* HAK1 K^+ transporters. *HvHAK1* conferred high-affinity K^+ uptake to a K^+-uptake–deficient yeast mutant exhibiting the hallmark characteristics of the high-affinity K^+ uptake described for barley roots. HvHAK1 also mediated low-affinity Na^+ uptake. Another cDNA (*HvHAK2*) encoding a polypeptide 42% identical to HvHAK1 was also isolated. Analysis of several genomes of Triticeae indicates that *HvHAK1* belongs to a multigene family. Translated sequences from bacterial DNAs and Arabidopsis, rice, and possibly human cDNAs show homology to the Kup–HAK1–HvHAK1 family of K^+ transporters.

INTRODUCTION

Potassium is a major essential nutrient in higher plants. It plays important physiological roles in enzyme activity, osmoregulation, and movement; large amounts of K^+ are necessary to sustain normal growth and development (Flowers and Läuchli, 1983). To satisfy the K^+ requirements of the plant, roots take up K^+ from solution in the soil, where its concentration can be very low, especially in the depletion zone around the roots. Therefore, plant roots are furnished with K^+ transport systems of high affinity and high capacity to concentrate this cation (Epstein, 1973). An additional problem affecting K^+ acquisition occurs in many saline soils, in which K^+ uptake is impaired by the presence of high sodium concentrations (Greenway and Munns, 1980). In this case, Na^+ substitutes for K^+, and the plant suffers both K^+ nutritional imbalance and Na^+ toxicity (Flowers and Läuchli, 1983).

An accurate description of K^+ uptake by plant roots was made in the early 1960s by Epstein and co-workers, who observed that the rate of K^+ uptake in barley roots exhibited a biphasic response to the increase in the K^+ concentration in the external medium (Epstein et al., 1963). As K^+ concentration increases, the kinetics of K^+ uptake show a first saturable component in the micromolar range and a second saturable component in the millimolar range. These complex kinetics can be described with precision as the sum of two Michaelis–Menten equations, which are ascribed to the activity of mechanism 1 or a high-affinity uptake system and mechanism 2 or a low-affinity uptake system. Identical or similar responses have been found in other plant species (Kochian and Lucas, 1982; Benlloch et al., 1989), suggesting that this pattern could be general in higher plants.

Although the existence of high- and low-affinity K^+ uptake systems in plants is an accepted paradigm, the number and characteristics of the transporters have yet to be determined. In the search for plant genes encoding plant K^+ transporters, researchers have cloned several cDNAs encoding inward- (Anderson et al., 1992; Sentenac et al., 1992; Cao et al., 1995; Müller-Röber et al., 1995; Ketchum and Slayman, 1996) and outward-rectifying (Czempinski et al., 1997) K^+ channels and a cDNA encoding a high-affinity K^+–Na^+ symporter (Schachtman and Schroeder, 1994; Rubio et al., 1995). Some of these cDNAs were isolated by complementing yeast mutants with defective K^+ uptake. This procedure has been critical to the advancement of the understanding of K^+ transport in plants. Two of the isolated cDNAs correspond to the root-expressed genes *AKT1* (Sentenac et al., 1992; Lagarde et al., 1996) and *HKT1* (Schachtman and Schroeder, 1994). *AKT1* encodes an inward-rectifying K^+ channel (Sentenac et al., 1992), and *HKT1* encodes a high-affinity, Na^+-coupled K^+ transporter (Rubio et al., 1995). Although the role of these transporters remains unclear, it seems unlikely that they play major roles in the high-affinity K^+ uptake system. This system mediates the uptake of K^+ from very dilute solutions, and the involvement of a channel can be ruled out because, under those conditions, the K^+ diffusion potential is negative in relation to the membrane potential (Maathuis and Sanders, 1993,1996; Basset et al., 1995).

[1]To whom correspondence should be addressed. E-mail arodrignavar @bit.etsia.upm.es; fax 34-1-336-5757.

A K^+–Na^+ symporter does not have thermodynamic restriction, but it should give rise to Na^+-enhanced K^+ uptake. This enhancement has not been detected in K^+ uptake experiments with plant roots (Epstein, 1973; Maathuis et al., 1996), indicating that HKT1 is not a major contributor to K^+ uptake from micromolar concentrations (Rubio et al., 1996; Walker et al., 1996). Thus, major components of the high-affinity K^+ uptake system that display characteristics consistent with those described for roots remain to be identified.

As an alternative to the procedure of complementing yeast mutants defective in K^+ uptake, we tried to identify plant transporters homologous to fungal K^+ transporters, because fungal K^+ transport exhibits kinetic (Ramos and Rodríguez-Navarro, 1985) and mechanistic (Rodríguez-Navarro et al., 1986; Maathuis and Sanders, 1994) characteristics similar to those found for K^+ transport in plant roots. Two types of high-affinity K^+ transporters have been described in fungal species, TRK and HAK1. The TRK transporters were identified in *Saccharomyces cerevisiae* (Gaber et al., 1988; Ko and Gaber, 1991), *S. uvarum* (Anderson et al., 1991), and *Schizosaccharomyces pombe* (Soldatenkov et al., 1995), and the HAK1 transporter (Bañuelos et al., 1995), homologous to the *Escherichia coli* Kup K^+ transporter (Schleyer and Bakker, 1993), was identified in *Schwanniomyces occidentalis.* Using a method based on polymerase chain reaction coupled with reverse transcription (RT-PCR), we identified in barley and closely related species a multigene family that encodes proteins homologous to SoHAK1 and Kup. One of the isolated cDNAs (*HvHAK1*) conferred high-affinity K^+ uptake to a K^+ uptake–deficient yeast mutant. This uptake exhibited the hallmark characteristics of the high-affinity K^+ uptake described for barley roots.

RESULTS

Cloning of *HvHAK1* and *HvHAK2*

By performing RT-PCR with RNA obtained from roots of barley seedlings grown in the absence of K^+, we isolated two 0.75-kb cDNA fragments whose translated sequences showed a high degree of homology to SoHAK1 (Bañuelos et al., 1995) and Kup (Schleyer and Bakker, 1993). One of the fragments was further extended, yielding a full-length 2.7-kb cDNA (*HvHAK1*); for the other fragment, we obtained a partial-length 2.4-kb cDNA (*HvHAK2*), which could not be completed at the 5′ end. The *HvHAK1* cDNA contains an open reading frame (ORF) of 2319 bp that encodes an 86.2-kD polypeptide of 773 amino acid residues. The partial-length *HvHAK2* cDNA could encode a polypeptide of 721 amino acid residues. A hydrophobicity profile of the deduced polypeptides predicted 12 membrane-spanning regions, as already described for the SoHAK1 and Kup transporters. Figure 1 shows the amino acid sequence alignment of the translated *HvHAK1* and *HvHAK2* cDNAs with those of SoHAK1

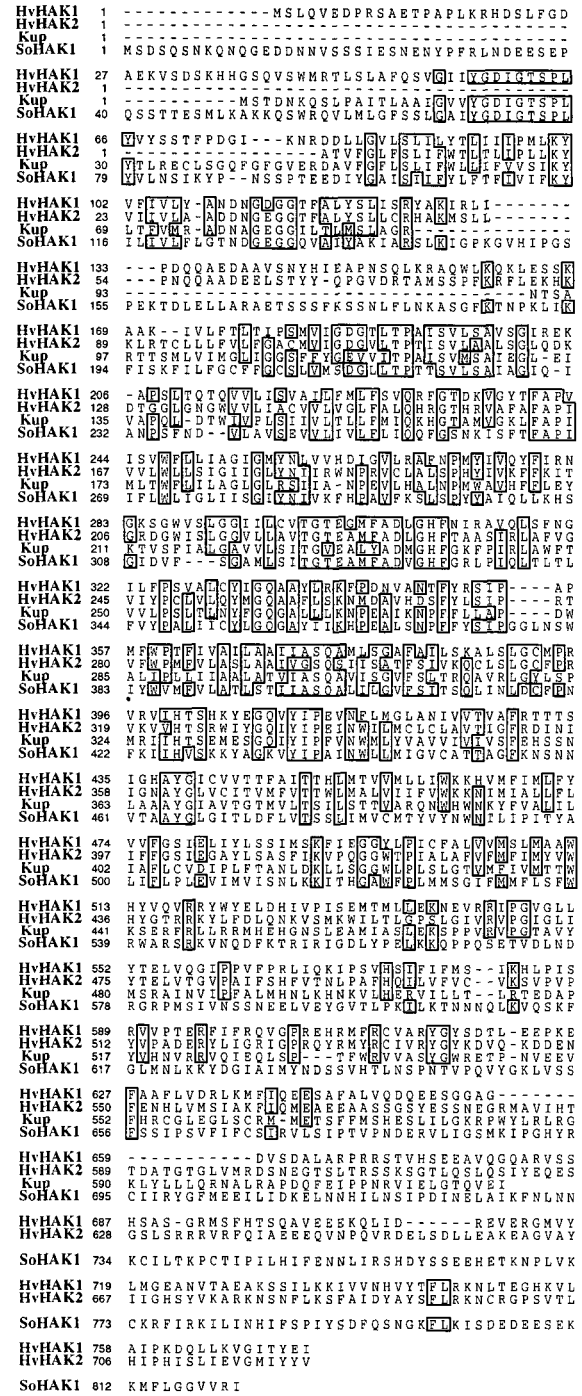

Figure 1. Amino Acid Sequence Alignment of the Translated *HvHAK1* and *HvHAK2* cDNAs and *E. coli kup* and *S. occidentalis HAK1* Genes.

Amino acids common to three or four sequences are boxed. Gaps were introduced to optimize alignment.

and Kup, revealing that they display considerable identity. In addition, the alignment required the insertion of a limited number of gaps. The binary comparisons of the four proteins, summarized in Table 1, suggest that they belong to the same family of proteins.

HvHAK1 Encodes a K^+ Transporter

To determine whether HvHAK1 was a K^+ transporter, we inserted the *HvHAK1* cDNA into a yeast expression vector, and the resulting plasmid was transformed into a yeast mutant defective in K^+ uptake. Growth of the transformant was assayed in low K^+ media, in which the mutant would not grow. Figure 2A shows the growth of the transformant in an ammonium-free medium, with Figure 2B showing growth in a standard ammonium mineral medium. These experiments revealed that HvHAK1 conferred on the yeast mutant the capacity to grow as fast as the wild-type strain in the medium without ammonium, whereas it poorly enhanced growth in the ammonium medium. Tests of K^+ uptake in the presence or absence of ammonium showed that K^+ uptake was strongly inhibited by ammonium (Figure 3) at a much lower concentration than that present in standard yeast mineral medium (76 mM). The inhibition of the HvHAK1-mediated K^+ uptake by ammonium is consistent with the inhibition of K^+ uptake by ammonium observed in plant roots (Rufty et al., 1982).

Figure 4A shows that yeast-expressed HvHAK1 mediated high-affinity Rb^+ uptake and that it was competitively inhibited by K^+. The K_m values of HvHAK1 for Rb^+ and K^+ were 18 and 27 μM, respectively. At micromolar concentrations, Na^+ did not inhibit or enhance Rb^+ uptake (data not shown), whereas at millimolar concentrations, it exerted a competitive inhibition, with a K_i of 15 mM (Figure 4B). HvHAK1-mediated Rb^+ uptake in the yeast mutant was not affected by the K^+ status of the cells, suggesting that in yeast, HvHAK1 activity was not regulated by the internal K^+ concentration.

According to the classical model of Epstein et al. (1963), K^+ and Na^+ uptake in barley roots occurs with high and low affinities, mediated by so-called mechanism 1 and mechanism 2, respectively. Although mechanisms 1 and 2 are thought to be different entities, it cannot be ruled out that a single transporter could participate in both mechanisms (Epstein et al., 1963). To test the possibility that HvHAK1 also mediated low-affinity Rb^+ and Na^+ uptake, we studied HvHAK1-mediated uptake of Rb^+ and Na^+ in the millimolar range of concentrations. However, drawing conclusions from these experiments is difficult because *trk1 trk2* yeast mutants exhibit high rates of Rb^+ and Na^+ uptake when the cations are present at high concentrations (Ramos et al., 1994). Because of this high background of uptake, HvHAK1-mediated uptake of Na^+ and Rb^+ in the millimolar range cannot be detected unless it reaches high rates. We found that above 1 mM Rb^+, the difference in the rates of Rb^+ uptake between the transformed strain and the control strain remained fairly constant, suggesting that HvHAK1 did not exhibit a second phase of Rb^+ uptake in the millimolar range. In the case of Na^+ uptake, we observed that HvHAK1 increased the uptake with reference to the control strain but that the increase was at the limits of detection.

A different construct lacking the 5′ untranslated region of *HvHAK1* and with three changes in the first 15 bp of the ORF (see Methods) displayed higher Rb^+ uptake activity than did the original construct when expressed in yeast (K_m of 17 μM Rb^+ and V_{max} of 33 nmol of Rb^+ mg^{-1} min^{-1}). Using this construct, we observed neither high-affinity Na^+ uptake nor low-affinity Rb^+ uptake mediated by HvHAK1. However, as shown in Figure 5, we found that HvHAK1 transported Na^+ when this cation was present at millimolar concentrations.

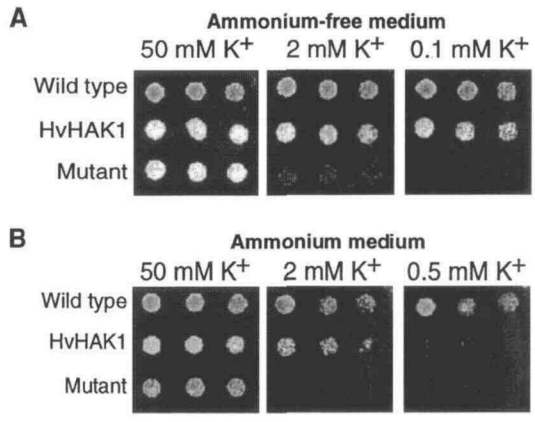

Figure 2. A Yeast Potassium Transport Mutant Transformed with the *HvHAK1* cDNA under the Control of the Yeast *PGK1* Gene Promoter Acquired the Capacity to Grow in a Low K^+ Medium.

(A) A tenfold dilution series of cell suspensions of the wild-type strain, a mutant strain transformed with *HvHAK1*, and a mutant strain inoculated in solid arginine medium (ammonium free) containing different concentrations of K^+.

(B) The same as shown in **(A)**, but in ammonium medium (76 mM ammonium).

Table 1. Binary Comparison Scores for Members of the Kup–HAK Family of Proteins[a]

Protein	HvHAK2	SoHAK1	Kup
HvHAK1	41.7 (54)	26.8 (39)	31.3 (45)
HvHAK2		27.5 (41)	30.4 (42)
SoHAK1			25.1 (37)

[a]Percentage of identity is given first; percentage of similarity is given in parentheses.

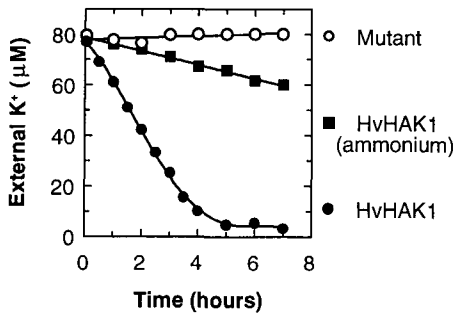

Figure 3. HvHAK1-Mediated K^+ Uptake from Micromolar K^+ Concentrations.

The mutant and the mutant transformed with *HvHAK1* were grown overnight in arginine medium, K^+ starved for 1 hr, and transferred to K^+-free arginine mineral medium, either standard or supplemented with 5 mM ammonium. K^+ was then added, and K^+ depletion was measured for 7 hr.

Expression of HvHAK1

To define the conditions and organs in which HvHAK1 is expressed, we performed gel blot hybridizations with RNA from shoots and roots of plants grown in the presence or absence of K^+. Figure 6 shows that the *HvHAK1* transcript was detected exclusively in roots and that its expression was enhanced at least fivefold in K^+-starved plants relative to plants grown in medium with a 1 mM K^+ concentration. Unlike *HvHAK1*, the *HvHAK2* transcript was not detected in either roots or shoots, regardless of the K^+ concentration in which the plants had been grown.

Mapping of *HvHAK1* and *HvHAK2* Homologs in Triticeae Genomes

Gel blot hybridization of genomic barley DNA revealed that four to seven restriction fragments, depending on the enzyme used, hybridized with *HvHAK1* (data not shown). This observation suggested that *HvHAK1* belongs to a large gene family, and we proceeded to map its members in several genomes of the Triticeae tribe (i.e., genome A^m, *Triticum monococcum*; A, B, and D, *T. aestivum*; E, *Lophopyrum elongatum*; and H, *Hordeum vulgare*). We found genes that hybridized with *HvHAK1* on chromosomes or chromosome arms (S, short; L, long) of homeologous groups 2 (2AL, 2DL, 2EL, and 2H; genome B was not analyzed), 3 (3AL, 3DL, 3EL, and 3H; genome B was not analyzed), 4 (4A, 4BL, 4DL, 4EL, and 4HL), and 6 ($6A^mS$, 6B, 6DS, 6ES; no polymorphism was detected on 6A and 6H). Figure 7 shows the positions of these loci in the four homeologous groups.

In *T. aestivum*, a K^+/Na^+ discrimination locus (*Kna1*) conferring resistance to salt stress maps on the long arm of chromosome 4D (Dvorák et al., 1994; Dubcovsky et al., 1996b). Considering that this gene affects the concentration of K^+ in leaves and that both *Kna1* and one member of the *HvHAK1* family are located on chromosome 4DL, we investigated the possibility that they are the same gene. *XHvHAK1* hybridized with restriction fragment length polymorphism fragments that were 12.5 centimorgans apart from *Kna1*; consequently, we concluded that *XHvHAK1* and *Kna1* were different loci. Unlike *HvHAK1*, DNA gel blot hybridization showed that only one restriction fragment hybridizes with *HvHAK2* in barley DNA, and we found a single *XHvHAK2* locus on the short arm of chromosome 2 of the A^m, B, D, E, and H genomes (Figure 7).

The Kup–HAK Family of Transporters

A database search for protein sequences related to HvHAK1 and HvHAK2 revealed that in addition to SoHAK1 and Kup, putative proteins from *Lactococcus lactis*, Arabidopsis, rice, and possibly from humans very likely belong to the family of transporters defined by the Kup transporter of *E. coli* and the HAK1 transporter of *S. occidentalis*. Furthermore, in our laboratory, two genes encoding the same type of transporters have been isolated from *Rhizobium leguminosarum* and *Sinorhizobium meliloti*. Table 2 summarizes the species in

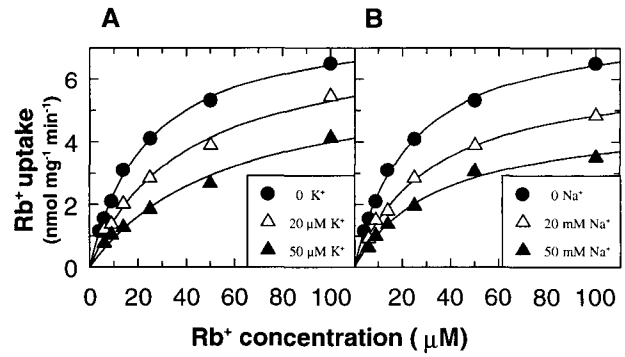

Figure 4. Competitive Inhibition of HvHAK1-Mediated High-Affinity Rb^+ Uptake by Micromolar Concentrations of K^+ and Millimolar Concentrations of Na^+.

(A) Concentration dependence of the initial rates of Rb^+ uptake exhibited by the yeast potassium transport mutant transformed with the *HvHAK1* cDNA under the control of the yeast *PGK1* gene promoter in the absence or presence of two concentrations of K^+.

(B) The same as shown in **(A)**, but in the absence or presence of two concentrations of Na^+.

Data were fitted without constraints to Michaelis–Menten functions. The Michaelis–Menten parameter values (means ±SE of three independent experiments) are V_{max} = 7.1 ± 2.1 nmol mg^{-1} min^{-1}, K_m = 18 ± 3 μM Rb^+, K_i = 27 ± 2 μM K^+, and K_i = 15 ± 2 mM Na^+. In the mutant strain transformed with the plasmid lacking an insert, Rb^+ uptake was undetectable in the recorded concentration range.

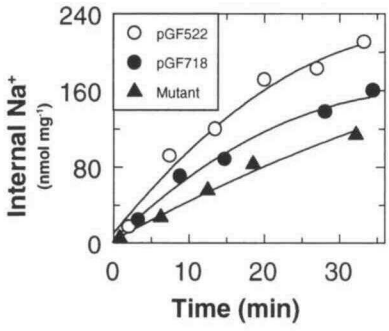

Figure 5. HvHAK1-Mediated Na^+ Uptake in the Millimolar Range.

Shown is the time course of Na^+ uptake by the yeast K^+ transport mutant, the mutant transformed with the *HvHAK1* cDNA under the control of the yeast *PGK1* gene promoter (pGF718), and the mutant transformed with a similar construct but modified at the 5′ end of the cDNA to increase HvHAK1 activity (pGF522).

which putative transporters of the Kup-HAK family have been found.

DISCUSSION

Here, we report the isolation of a full-length *HvHAK1* cDNA clone from barley roots encoding a polypeptide with marked amino acid sequence similarity to the K^+ transporters Kup of *E. coli* (Schleyer and Bakker, 1993) and HAK1 of *S. occidentalis* (Bañuelos et al., 1995). To characterize this transporter functionally, we expressed the *HvHAK1* clone in a *trk1 trk2* mutant strain of *S. cerevisiae*.

The kinetic characteristics of K^+ and Rb^+ uptake by roots have been studied extensively in barley and other members of the Triticeae tribe. In these (Epstein et al., 1963; Elzam and Epstein, 1969) as well as in other plant species (Kochian and Lucas, 1982; Benlloch et al., 1989), the kinetics of Rb^+ and K^+ uptake are biphasic, exhibiting a high-affinity component that may dominate K^+ acquisition under most field conditions (Epstein, 1973). Considering only this component, barley roots exhibit K_m values of 17 and 21 μM for Rb^+ and K^+ uptake, respectively, which are remarkably coincident with the Rb^+ K_m of 18 μM and the K^+ K_i of 27 μM reported here for the yeast-expressed HvHAK1 transporter. These K_m values illustrate the lack of discrimination between K^+ and Rb^+ in barley and HvHAK1. This characteristic is also found in *L. elongatum* and bread wheat (Huang et al., 1992). In *L. elongatum* and *Tenophyrum intermedia*, the K_m values for K^+ ($^{86}Rb^+$) uptake are 8 and 9 μM, respectively (Elzam and Epstein, 1969).

The inhibitory effect of Na^+ on the uptake of K^+ or Rb^+ and the transport of Na^+ have also been studied in barley (Rains and Epstein, 1965; Rains and Epstein, 1967; Welch and Epstein, 1968), *L. elongatum*, and *T. intermedia* (Elzam and Epstein, 1969). The results of these studies are complex, showing higher variability between species than in the case of high-affinity K^+ uptake. However, a common observation for all of these species is that micromolar Na^+ concentrations do not affect high-affinity K^+ uptake, as we found in yeast expressing HvHAK1. At millimolar Na^+ concentrations, high-affinity K^+ uptake was inhibited in the three species and also in HvHAK1, showing K_i values of 1.5 mM in barley (Epstein et al., 1963), 3.9 mM in *L. elongatum*, and 19 mM in *T. intermedia* (Elzam and Epstein, 1969). The 15 mM Na^+ K_i found in HvHAK1 was closer to the K_i in *T. intermedia* than to the K_i in barley. Similarly, the lack of Na^+ uptake by HvHAK1 in the low range of concentrations (<0.2 mM) was also closer to the results obtained with *T. intermedia* (Elzam and Epstein, 1969) than to those reported for barley (Rains and Epstein, 1967) and *L. elongatum* (Elzam and Epstein, 1969). To date, it is not possible to explain why species that are so similar in K^+ uptake are different in both Na^+ uptake from low Na^+ concentrations and in the sensitivity of K^+ uptake to Na^+. An interesting hypothesis is that different members of the HvHAK1 family, with different K^+/Na^+ selectivities, are involved in this variability.

A likely explanation for the observation that Na^+ in the millimolar range of concentrations inhibits the Rb^+ uptake mediated by HvHAK1 is that both cations compete for transport. Although other explanations cannot be ruled out, the fact that HvHAK1 transports Na^+ provides support for the first view. The finding that a probable major contributor to the high-affinity K^+ transport system can mediate low-affinity Na^+ uptake poses interesting questions regarding Na^+ tolerance in plants. In the case of the HvHAK1 transporter, its contribution to Na^+ uptake would be low under field conditions because the ratio between the affinities of both cations is very high ($\sim 10^3$). However, if other members of the HAK1 family of transporters present lower K^+/Na^+ selectivities, their contribution to Na^+ uptake might be important. The notion that a high-affinity K^+ transporter can be a low-affinity Na^+ transporter is a common characteristic for HKT1 (Gassmann et al., 1996) and for HvHAK1.

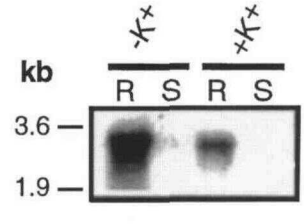

Figure 6. HvHAK1 Is Expressed Exclusively in Barley Roots, and Its Expression Is Enhanced When Deprived of K^+.

The results of RNA gel blot analyses of *HvHAK1* transcripts in barley roots (R) and shoots (S) of seedlings grown in the absence or presence of K^+ (1 mM) are shown. Molecular length markers are indicated at left.

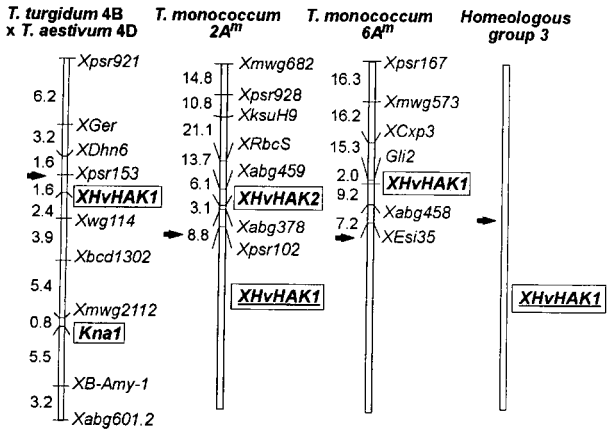

Figure 7. Chromosomal Location of the *XHvHAK2* and Four Loci Complementary to *HvHAK1*.

The genetic material used for linkage mapping is indicated at top. *XHvHAK1* and *Kna1* loci are boxed. Underlined loci were assigned to chromosome arms but not mapped on linkage maps. Distances are given in centimorgans and were calculated using the Kosambi function. Positions of the centromeres are indicated by arrows. Short arms are drawn on top.

Measurements of K^+ influx into barley roots of plants grown in the absence and in the presence of K^+ have shown the existence of basal high-affinity transport of this cation, which is greatly enhanced by K^+ starvation (Glass and Dunlop, 1978). This finding is consistent with the pattern of expression of the *HvHAK1* transcript, which was detected only in roots, with a low level of expression in K^+-grown plants and a much higher level of expression in K^+-starved plants. These results and those discussed above lend support to the idea that one or several members of the HvHAK1 family of transporters are major components of the high-affinity K^+ uptake system in species of the Triticeae tribe.

The second cDNA isolated, *HvHAK2*, also encoding a polypeptide with marked amino acid sequence similarity to the K^+ transporters Kup of *E. coli* and HAK1 of *S. occidentalis*, could not be completed, and its activity could not be tested. In addition, the *HvHAK2* transcript was not detected. Therefore, it is not yet possible to predict a physiological function for the putative HvHAK2 transporter.

The conservation of genes with high DNA homology to *HvHAK1* in several homeologous chromosomes in *Lophopyrum* spp, *Triticum* spp, and *Hordeum* spp suggests that duplications of an *XHvHAK1* gene preceded the divergence of the Triticeae tribe. The low percentage of amino acid identity between sequences of the HvHAK1 and HvHAK2 polypeptides suggests that their divergence occurred even earlier than duplication of *XHvHAK1*. Conservation of these genes in the Triticeae may reflect that they have various and essential functions. In addition, translated amino acid sequences of cDNAs from seedling hypocotyls of Arabidopsis (GenBank accession numbers W43757 and W43758) and from etiolated shoots of rice (GenBank accession number D40091) show homology to HvHAK1, indicating that the HAK transporters are not restricted to the Triticeae tribe and that they operate in shoots as well as in roots. Because of their potential diversity of functions, characterization of HAK transporters can advance our understanding of K^+ movements in plants and offer a way to identify alleles of practical interest, especially for K^+ nutrition and Na^+ tolerance.

The K^+ and Na^+ transporters studied to date seem to be specific to a group or a few groups of living organisms. In contrast, the Kup–HAK type of transporters described here is widely distributed in bacteria, fungi, higher plants, and possibly in mammals (Table 2).

METHODS

Cloning of the *HvHAK1* and *HvHAK2* cDNAs

Barley (*Hordeum vulgare* cv Albacete) seedlings were grown in the dark for 7 days in 10 mM Mes buffer brought to pH 5.5 with $Ca(OH)_2$.

Table 2. The Kup–HAK Type of Transporters Is Widely Distributed among Living Organisms

Organism	Species	Evidence
Bacteria	*E. coli*	Sequence and function[a]
	R. leguminosarum	Sequence[b]
	Sinorhizobium meliloti	Sequence[b]
	Lactoccocus lactis	Sequence[c]
Fungi	*Schwanniomyces occidentalis*	Sequence and function[d]
Plants	*H. vulgare*	Sequence and function[e]
	Lophopyrum elongatum	DNA gel blot identification[e]
	T. aestivum	DNA gel blot identification[e]
	T. monococcum	DNA gel blot identification[e]
	Oryza sativa	Sequence[f]
	A. thaliana	Sequence[g]
Animals	*Homo sapiens*	Sequence[h]

[a]Schleyer and Bakker (1993).
[b]DNA fragments were obtained by PCR with primers deduced from homologous regions of Kup and HAK1 transporters. Their presence in the genome was confirmed by DNA gel blot analysis (R. Madrid and A. Rodríguez-Navarro, unpublished results).
[c]GenBank accession number U74322.
[d]Bañuelos et al. (1995).
[e]This report.
[f]GenBank accession number D40091.
[g]GenBank accession numbers W43757, W43758, N96203, T20469, T13770, and T04361.
[h]GenBank accession numbers Z15484 and Z13060.

Total root RNA (6 μg), extracted from these seedlings, was reverse transcribed by use of a degenerate primer deduced from conserved regions of Kup and HAK1 (degenerate nucleotide sequence 5′-CCRAARTGICCIAYRTCIGCRWA-3′ deduced from the sense amino acid sequence [M/L][Y/F]AD[M/V]GHF) and avian myeloblastosis virus reverse transcriptase (Amersham). The reverse transcription (RT) products were amplified by polymerase chain reaction (PCR) with the Expand high-fidelity PCR system (Boehringer Mannheim), using the antisense primer described above and a sense degenerate primer deduced also from conserved regions of Kup and HAK1 (degenerate nucleotide sequence 5′-TAYGGIGAYATHGGIACIWSICC-3′, which corresponds to the sense amino acid sequence YDGIGTSP). This procedure yielded two cDNA fragments of ~750 bp, whose translations showed sequence homology to Kup and SoHAK1. The fragments were extended to the 3′ end by RT-PCR, using an anchored NotI$(dT)_{18}$ primer (Pharmacia) for RT and an antisense anchor-specific primer and sense fragment-specific primers for PCR. Extension of the cDNAs up to the 5′ ends of the mRNAs was also performed by RT-PCR, using the 5′/3′ rapid amplification of cDNA ends kit (Boehringer Mannheim), following the manufacturer's instructions. A full-length cDNA was obtained from one of the fragments (*HvHAK1*; GenBank accession number AF025292) starting 84 bp upstream of the first ATG of the open reading frame (ORF). However, use of the same procedure did not yield a full-length cDNA containing the second fragment (*HvHAK2*).

PCR products were inserted into the pCR2.1 vector by using the TA cloning kit (Invitrogen, Carlsbad, CA). *HvHAK1* was inserted in the correct orientation at the EcoRI site of the yeast expression vector pYPGE15 (Brunelli and Pall, 1993), under the control of the *PGK1* promoter, producing plasmid pGF718. An additional pYPGE15-based construct lacking the 5′ untranslated region of *HvHAK1* and with three changes in the first 15 bp of the ORF (ATATCGATGCAAGGC instead of ATGTCGCTGCAAGTC) gave rise to plasmid pGF522.

RNA Gel Blot Hybridization

Barley seedlings were grown, as described above, in the presence or in the absence of 1 mM KCl. Total RNA (30 μg per lane) prepared from shoots and roots was separated by electrophoresis on a formaldehyde–1.1% agarose gel and transferred to a nylon membrane (Hybond-N; Amersham). Membranes were hybridized overnight at 42°C in the presence of 50% formamide with probes prepared from the 0.75-kb cDNA fragments of *HvHAK1* and *HvHAK2* that were labeled with α-^{32}P-dATP by the random priming method. Membranes were washed twice in 2 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate) plus 0.1% SDS at 42°C and then twice in 0.2 × SSC plus 0.1% SDS at 65°C. Membranes were exposed at −70°C for 3 days to Curix RP-2 (Agfa, Mortsel, Belgium) films.

Yeast Strain and Growth Tests

A *Saccharomyces cerevisiae trk1Δ trk2Δ* (Ko and Gaber, 1991) double mutant strain (WΔ3), obtained from laboratory strain W303.1A by gene disruption, was the recipient strain for plasmids pYPGE15 and pGF718. Yeast strains were grown in a mineral medium with ammonium or with arginine as the nitrogen source (Rodríguez-Navarro and Ramos, 1984). The basic medium contained neither K^+ nor Na^+, which we added as indicated.

Cation Uptake Experiments

Yeast cells were grown in arginine medium, supplemented with either 30 mM KCl (for WΔ3 and WΔ3 transformed with pYPGE15) or 3 mM K^+ (for WΔ3 transformed with pGF718 and pGF522), and then were K^+ starved for 1 hr in K^+-free arginine medium. Cells were suspended in a 2% glucose and 10 mM Mes buffer brought to pH 6.0 with $Ca(OH)_2$. At intervals, samples were taken, filtered through a 0.8-μm pore nitrocellulose membrane filter (Millipore, Bedford, MA), and washed with 20 mM $MgCl_2$. Filters were incubated overnight in 0.1 M HCl. Rb^+ and Na^+ uptakes were determined by atomic emission spectrophotometry of acid-extracted cells (Rodríguez-Navarro and Ramos, 1984). Results are expressed on a cell dry weight basis, and the parameters of the concentration dependence of the initial rates of Rb^+ uptake were obtained by fitting the rates to a Michaelis–Menten function. Control experiments were performed with the WΔ3 strain and with the WΔ3 strain transformed with plasmid pYPGE15 without an insert.

Mapping of *HvHAK1* and *HvHAK2* Homologs in Triticeae Genomes

The same DNA probes described for RNA gel blot hybridizations were used for DNA gel blot hybridizations of genomic DNA from *H. vulgare*, *Lophopyrum elongatum*, *Triticum aestivum*, and *T. monococcum*. Hybridizations were performed as previously described (Dubcovsky et al., 1996a). Restriction fragments were assigned to chromosomes and chromosome arms by using nullisomic–tetrasomic and ditelosomic cytogenetic stocks of *T. aestivum* (cv Chinese Spring) (Sears, 1954), substitution lines and ditelosomic addition lines of *L. elongatum* (Dvorák, 1979; Hart and Tuleen, 1983), and addition lines of *H. vulgare* (Islam et al., 1981). The *XHvHAK1* locus on chromosome 4DL was mapped in the same mapping population as the *Kna1* locus (Dubcovsky et al., 1996b). *XHvHAK1* and *XHvHAK2* loci on chromosomes 2 and 6 were mapped in *T. monococcum* (Dubcovsky et al., 1996a).

ACKNOWLEDGMENTS

We thank Rosario Haro for providing the yeast mutant strain. This work was supported by grants from the European Community (Nos. BI02-CT-0400 and BI04-CT96-0775) and from the Promoción General del Conocimiento Program of the Ministerio de Educación y Cultura of Spain (No. PB92-0907). G.E.S.-M. is recipient of a fellowship from Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina.

Received July 2, 1997; accepted September 30, 1997.

REFERENCES

Anderson, J.A., Best, L.A., and Gaber, R.F. (1991). Structural and functional conservation between the high-affinity transporters of *Saccharomyces uvarum* and *Saccharomyces cerevisiae*. Gene **99,** 39–46.

Anderson, J.A., Huprikar, S.S., Kochian, L.V., Lucas, W.J., and Gaber, R.F. (1992). Functional expression of a probable *Arabidopsis thaliana* potassium channel in *Saccharomyces cerevisiae.* Proc. Natl. Acad. Sci. USA **89,** 3736–3740.

Bañuelos, M.A., Klein, R.D., Alexander, S.J., and Rodriguez-Navarro, A. (1995). A potassium transporter of the yeast *Schwanniomyces occidentalis* homologous to the Kup system of *Escherichia coli* has a high concentrative capacity. EMBO J. **14,** 3021–3027.

Basset, M., Conejero, G., Lepetit, M., Fourcroy, P., and Sentenac, H. (1995). Organization and expression of the gene coding for the potassium transport system AKT1 of *Arabidopsis thaliana.* Plant Mol. Biol. **24,** 947–958.

Benlloch, M., Moreno, I., and Rodriguez-Navarro, A. (1989). Two modes of rubidium uptake in sunflower plants. Plant Physiol. **90,** 939–942.

Brunelli, J.P., and Pall, M.L. (1993). A series of yeast/*Escherichia coli* λ expression vectors designed for directional cloning of cDNAs and cre/lox–mediated plasmid excision. Yeast **9,** 1309–1318.

Cao, Y., Ward, J.M., Kelly, W.B., Ichida, A.M., Gaber, R.F., Anderson, J.A., Uozumi, N., Schroeder, J.I., and Crawford, N.M. (1995). Multiple genes, tissue specificity, and expression-dependent modulation contribute to the functional diversity of potassium channels in *Arabidopsis thaliana.* Plant Physiol. **109,** 1093–1106.

Czempinski, K., Zimmermann, S., Ehrhardt, T., and Müller-Röber, B. (1997). New structure and function in plant K^+ channels: KCO1, an outward rectifier with a steep Ca^{2+} dependency. EMBO J. **16,** 2565–2575.

Dubcovsky, J., Luo, M.-C., Zhong, G.-Y., Bransteiner, R., Desai, A., Kleinhofs, A., and Dvorák, J. (1996a). Genetic map of diploid wheat, *Triticum monococcum* L., and its comparison with maps of *Hordeum vulgare* L. Genetics **143,** 983–999.

Dubcovsky, J., Santa-Maria, G., Epstein, E., Luo, M.-C., and Dvorák, J. (1996b). Mapping of the K^+/Na^+ discrimination locus *Kna1* in wheat. Theor. Appl. Genet. **92,** 448–454.

Dvorák, J. (1979). Metaphase I pairing frequencies of individual *Agropyrum elongatum* chromosome arms with *Triticum* chromosomes. Can. J. Genet. Cytol. **21,** 243–254.

Dvorák, J., Noaman, M.M., Goyal, S., and Gorham, J. (1994). Enhancement of the salt tolerance of *Triticum turgidum* L. by the *Kna1* locus transferred from the *Triticum aestivum* L. chromosome 4D by homoeologous recombination. Theor. Appl. Genet. **87,** 872–877.

Elzam, O.E., and Epstein, E. (1969). Salt relations of two grass species differing in salt tolerance. II. Kinetics of the absorption of K, Na, and Cl by their excised roots. Agrochimica **13,** 196–205.

Epstein, E. (1973). Mechanisms of ion transport through plant cell membranes. Int. Rev. Cytol. **34,** 123–168.

Epstein, E., Rains, D.W., and Elzam, O.E. (1963). Resolution of dual mechanisms of potassium absorption by barley roots. Proc. Natl. Acad. Sci. USA **49,** 684–692.

Flowers, T.J., and Läuchli, A. (1983). Sodium versus potassium: Substitution and compartmentation. In Inorganic Plant Nutrition, A. Läuchli and R.L. Bieleski, eds (Berlin: Springer-Verlag), pp. 651–681.

Gaber, R.F., Styles, C.A., and Fink, G.R. (1988). *TRK1* encodes a plasma membrane protein required for high-affinity potassium transport in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **8,** 2848–2859.

Gassmann, W., Rubio, F., and Schroeder, J.I. (1996). Alkali cation selectivity of the wheat root high-affinity potassium transporter HKT1. Plant J. **10,** 869–882.

Glass, A.D.M., and Dunlop, J. (1978). The influence of potassium content on the kinetics of potassium influx into excised ryegrass and barley roots. Planta **141,** 117–119.

Greenway, H., and Munns, R. (1980). Mechanism of salt tolerance in non-halophytes. Annu. Rev. Plant Physiol. **31,** 149–190.

Hart, G.E., and Tuleen, N.A. (1983). Introduction and characterization of alien genetic material. In Isozymes in Plant Genetics and Breeding, S.D. Tanskley and T.J. Orton, eds (Amsterdam: Elsevier), pp. 339–361.

Huang, Z.Z., Yan, X., Jalil, A., Norlyn, J.D., and Epstein, E. (1992). Short-term experiments on ion transport by seedlings and excised roots. Plant Physiol. **100,** 1914–1920.

Islam, A.K.M.R., Shepherd, K.M., and Sparrow, D.H.B. (1981). Isolation and characterization of euplasmic wheat-barley chromosome addition lines. Heredity **46,** 161–174.

Ketchum, K.A., and Slayman, C.W. (1996). Isolation of an ion channel gene from *Arabidopsis thaliana* using the H5 signature sequence from voltage-dependent K^+ channels. FEBS Lett. **378,** 19–26.

Ko, C.H., and Gaber, R.F. (1991). *TRK1* and *TRK2* encode structurally related K^+ transporters in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **11,** 4266–4273.

Kochian, L.V., and Lucas, W.J. (1982). Potassium transport in corn roots. I. Resolution of kinetics into a saturable and linear component. Plant Physiol. **70,** 1723–1731.

Lagarde, D., Basset, M., Lepetit, M., Conejero, G., Gaymard, F., Astruc, S., and Grignon, C. (1996). Tissue-specific expression of the *Arabidopsis AKT1* gene is consistent with a role in K^+ nutrition. Plant J. **9,** 195–203.

Maathuis, F.J.M., and Sanders, D. (1993). Energization of potassium uptake in *Arabidopsis thaliana.* Planta **191,** 302–307.

Maathuis, F.J.M., and Sanders, D. (1994). Mechanism of high-affinity potassium uptake in roots of *Arabidopsis thaliana.* Proc. Natl. Acad. Sci. USA **91,** 9272–9276.

Maathuis, F.J.M., and Sanders, D. (1996). Mechanisms of potassium absorption by higher plant roots. Physiol. Plant. **96,** 158–168.

Maathuis, F.J.M., Verlin, D., Smith, F.A., Sanders, D., Fernández, J.A., and Walker, N.A. (1996). The physiological relevance of Na^+-coupled K^+-transport. Plant Physiol. **112,** 1609–1616.

Müller-Röber, D., Ellemberg, J., Provart, N., Willmitzer, L., Busch, H., Becker, D., Dietrich, P., Hoth, S., and Hedrich, R. (1995). Cloning and electrophysiological analysis of KST1, an inward rectifying K^+ channel expressed in potato guard cells. EMBO J. **14,** 2409–2416.

Rains, D.W., and Epstein, E. (1965). Transport of sodium in plant tissue. Science **148,** 1611.

Rains, D.W., and Epstein, E. (1967). Sodium absorption by barley roots: The role of the dual mechanisms of alkali cation transport. Plant Physiol. **42,** 314–318.

Ramos, J., and Rodríguez-Navarro, A. (1985). Rubidium transport in *Neurospora crassa.* Biochim. Biophys. Acta **815,** 97–101.

Ramos, J., Alijo, R., Haro, R., and Rodríguez-Navarro, A. (1994). TRK2 is not a low-affinity potassium transporter in *Saccharomyces cerevisiae.* J. Bacteriol. **1176,** 249–252.

Rodríguez-Navarro, A., and Ramos, J. (1984). Dual system for potassium transport in *Saccharomyces cerevisiae.* J. Bacteriol. **159,** 940–945.

Rodríguez-Navarro, A., Blatt, M.R., and Slayman, C.L. (1986). A potassium-proton symport in *Neurospora crassa.* J. Gen. Physiol. **87,** 649–674.

Rubio, F., Gassmann, W., and Schroeder, J.I. (1995). Na^+-driven K^+ uptake by the plant K^+ transporter HKT1 and mutations conferring salt tolerance. Science **270,** 1660–1663.

Rubio, F., Gassmann, W., and Schroeder, J.I. (1996). High-affinity potassium uptake in plants. Science **273,** 977–979.

Rufty, T.W., Jackson, W.A., and Raper, C.D. (1982). Inhibition of nitrate assimilation in roots in the presence of ammonium: The moderating influence of potassium. J. Exp. Bot. **33,** 1122–1137.

Schachtman, D.P., and Schroeder, J.I. (1994). Structure and transport mechanism of a high-affinity potassium uptake transporter from higher plants. Nature **370,** 655–658.

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

Sears, E.R. (1954). The aneuploids of common wheat. Res. Bull. Mo. Argric. Exp. Stn. **572,** 1–59.

Sentenac, H., Bonneaud, N., Minet, M., Lacroute, F., Salmon, J.-M., Gaymard, F., and Grignon, C. (1992). Cloning and expression in yeast of a plant potassium ion transport system. Science **256,** 663–665.

Soldatenkov, V.A., Velasco, J.A., Avila, M.A., Dritschilo, A., and Notario, V. (1995). Isolation and characterization of *SpTRK*, a gene from *Schizosaccharomyces pombe* predicted to encode a K^+ transporter protein. Gene **161,** 97–101.

Walker, N.A., Sanders, D., and Maathuis, F.J.M. (1996). High-affinity potassium uptake in plants. Science **273,** 977–978.

Welch, R.M., and Epstein, E. (1968). The dual mechanism of alkali cation absorption by plant cells: Their parallel operation across the plasmalemma. Proc. Natl. Acad. Sci. USA **61,** 447–453.