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Functional characterization of a potassium transporter gene *NrHAK1* in *Nicotiana rustica*[*]

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Abstract: The purpose of this study is to investigate the function of a novel potassium transporter gene (*NrHAK1*) isolated from *Nicotiana rustica* roots using yeast complement and real-time PCR technique. The complementary DNA (cDNA) of *NrHAK1*, 2 488 bp long, contains an open reading frame (ORF) of 2334 bp encoding a protein of 777 amino acids (87.6 kDa) with 12 predicted transmembrane domains. The NrHAK1 protein shows a high sequence similarity to those of high-affinity potassium transporters in *Mesembryanthemum*, *Phytolacca acinosa*, *Arabidopsis thaliana*, and so on. We found that the *NrHAK1* gene could complement the yeast-mutant defect in K^+ uptake. Among several tissues surveyed, the expression level of *NrHAK1* was most abundant in the root tip and was up-regulated when exposed to potassium starvation. Moreover, the transcript accumulation was significantly reduced by adding 5 mmol/L NH_4^+ to the solution. These results suggest that *NrHAK1* plays an important role in potassium absorption in *N. rustica*.

Key words: Functional characterization, K^+ transporter, *Nicotiana rustica*
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INTRODUCTION

Potassium (K^+) is an essential macronutrient in plants and functions as an important osmoticum and a cofactor for enzymes that affect protein synthesis, photosynthesis, and regulation of pH and the cation-anion balance. Root absorption of K^+ has been known as biphasic: a high-affinity system sustains K^+ accumulation in plants when external K^+ is in the micro-molar range; and a low-affinity transporter is responsible for K^+ uptake at the milli-molar K^+ concentrations (Epstein *et al*., 1963). The high-affinity uptake mechanism has been shown to be inducible, whereas the low-affinity system may be constitutive (Glass and Dunlop, 1978; Fernando *et al*., 1990). There is a functional overlap between the high- and low-affinity uptake mechanisms (Hirsch *et al*., 1998; Santa-María *et al*., 2000). Absorption of K^+ may involve multiple transporter proteins, including AKT (arabidopsis K^+ transporter)-type channels, HKT (high-affinity K^+ transporter)-type transporters and HAK/KUP (high-affinity K^+ transporter/K^+ uptake permeases)-type transporters.

HAK/KUP transporters were originally identified in *Escherichia coli* as KUPs (K^+ uptake permeases) (Schleyer and Bakker, 1993). Later, their homologous genes were identified in soil-borne fungus *Schwanniomyces occidentalis* as HAKs (high-affinity K^+). Plant HAK/KUP transporters are homologous to the *S. occidentalis* HAK and the bacterium *E. coli* KUP (Santa-María *et al*., 1997; Fu and Luan, 1998; Kim *et al*., 1998). HAK/KUP transporters are located in roots and shoots, and they may also be in the plasma membrane and the tonoplast (Rodríguez-Navarro, 2000). Phylogenetic analyses indicate that plant HAK/KUP transporters can be

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classified into four clusters (Rubio *et al*., 2000). Hydrophobicity plots show that the HAK/KUP protein contains 12 putative transmembrane domains (Kim *et al*., 1998; Rubio *et al*., 2000).

The purpose of this study was to investigate the function of a novel potassium transporter gene (*NrHAK1*) isolated from the root of *Nicotiana rustica*, which has higher potassium content than any other species of tobacco. We isolated and analyzed the *NrHAK1* cDNA, performed functional complementation tests in yeast, and determined the *NrHAK1* transcription levels under different ionic environments.

MATERIALS AND METHODS

Strains and media

The *E. coli* strain *DH5α* was used for plasmidic DNA propagation. *E. coli* cells were cultured in Luria-Burtani (LB) medium with an appropriate antibiotic for selecting transformants. A *Saccharomyces cerevisiae* mutant strain *trk1Δtrk2Δ*, which was obtained from *EGY48* (*MATα*, *his3 trp1 ura3-52 leu::pLeu2-LexAop6*) (Invitrogen, CA, USA) by gene disruption, was the recipient strain for the yeast shuttle vector pPC87 plasmid. Yeast cells were grown in rich medium YPD [1% (w/w) yeast extract, 2% (w/w) peptone and 2% (w/w) glucose] or mineral medium APG (10 mmol/L arginine, 8 mmol/L phosphoric acid, 2% (w/w) glucose, 2 mmol/L $MgSO_4$, 1 mmol/L KCl, 0.2 mmol/L $CaCl_2$, trace minerals and vitamins, pH 4.0). The APG basic medium containing neither K^+ nor Na^+ was added as indicated, and was adjusted to pH 4.0 with diluted acetic acid. The effect of NH_4^+ on the mutant strain was examined with ammonium as the nitrogen source in place of arginine. Solid media contained 1.5% (w/w) agar.

Isolation of *NrHAK1*

Manipulation of nucleic acids was performed according to the standard protocols or the manufacturer's instructions. The total RNA was isolated from the K^+-starvated roots of *Nicotiana rustica* with the RNeasy Plant Mini Kit (Qiagen, Germany) and then treated with Dnase I (Promega, USA) to remove any remaining genomic DNA. The concentration of RNA was determined by the spectro-photometric analysis at the wavelength of 260 nm. As the template for polymerase chain reaction (PCR) amplification, the first-strand complementary DNA (cDNA) was synthesized from 2 μg total RNA with the SMART RACE cDNA Amplification Kit (Clontech, CA, USA). Degenerate primers for PCR were designed based on two conserved regions of HAK/KUP potassium transporters among several species. The primer for Kup1F was 5′-GCTGATGATAA(Y)GG(W)GA (R)GG(W)GG(W)AC(W)TTTGC-3′, deduced from the sense amino acid sequence GVVYGDL; and the primer for KuP3R was 5′-ATAAT(R)CTAAA(W) GT(W)GC(W)GAAAT-3′, corresponding to the sense amino acid sequence MFADLGHF. The gene-specific primers (GSPs) were designed based on degenerate PCR products. The 5′- and 3′-RACE PCRs were performed with the GSPs and the universal primers (UPs) supplied in the SMART RACE cDNA Amplification Kit (Clontech, CA, USA). A full-length cDNA of 2488 bp was obtained and named *NrHAK1*. The nucleotide sequence was deposited in the National Center for Biotechnology Information (NCBI) database (accession No. DQ535884).

Amino acid sequence analysis

Multiple sequence alignments of amino acid sequences were generated by the CLUSTALW program (http://www.ebi.ac.uk/clustalw/), and the phylogenetic tree was produced by Tree View based on complete amino acid sequences. The BOXSHADE program (http://www.ch.embnet.org/software/BOX_form.html) was used to analyze conserved amino acid regions after the initial alignment was obtained.

Vector construction

The full-length *NrHAK1* was amplified with the open reading frame (ORF) primers 5′-AAGAATTCA TGTTACCCATGTTTGAAAA-3′ and 5′-AAAGA GCTCTTATACATGGTAAATCATTCC-3′ with the first-strand cDNA as a template. After the amplified gene was purified from agarose gel, the products were digested with *Eco*RI and *Sac*I and inserted into the yeast shuttle vector pPC87 plasmid that was digested in the same way. Fidelity of the constructs was checked by DNA sequencing, and the obtained plasmid was designated as pPCHAK.

Expression of *NrHAK1* in yeast

For the functional complementation analysis, the plasmid pPCHAK was introduced into the mutant yeast strain *EGY4 trk1Δtrk2Δ* with the lithium acetate method (Gietz *et al*., 1995). The transformed yeast cells were selected in APG medium and then harvested and adjusted to OD_{600} (optical density at 600 nm)=0.8 with double-distilled water. A serial of 10-fold dilutions of the strains starting at OD_{600}=0.8 were prepared, and 5 μl of each dilution was spotted onto the solid APG basic medium supplemented with selected amounts of K^+, Na^+ and NH_4^+ provided as chloride salts. Cultures were grown at 30 °C for 7 d.

Salt stress experiments

Tobacco seeds were surface-sterilized [5% (w/w) sodium hypochloride] and germinated in vermiculite soaked with modified one-third strength Hoagland solution containing 2.5 mmol/L KNO_3, 1.0 mmol/L $Ca(NO_3)_2$, 2 mmol/L $MgSO_4$, 100 μmol/L EDTA-FeNa (ethylene diamine tetraacetic acid ferric sodium salt), 200 μmol/L KH_2PO_4, 50 μmol/L H_3BO_3, 0.5 μmol/L $CuSO_4$, 2 μmol/L $MnSO_4$, 0.5 μmol/L Na_2MoO_4, 0.1 μmol/L $CaCl_2$, 2 μmol/L $ZnSO_4$ and 0.1 μmol/L KI. Experiments were carried out in a photoperiod with light:dark=16:8 (25 °C). Three-week-old seedlings were transferred to the hydroponic culture for ionic environmental experiments. Solutions of KNO_3 (2.5 mmol/L) and KH_2PO_4 (200 μmol/L) were replaced by the ones with the same concentrations of $Ca(NO_3)_2$ and $Ca(H_2PO_4)_2$ for K^+ deprivation treatment. In the salt stress experiment, 100 mmol/L Na^+ or 5 mmol/L NH_4^+ was prepared by adding NaCl or NH_4Cl to the modified one-third Hoagland solution up to the concentration indicated. Treatments were performed by transferring seedlings to each solution. Seedling roots were harvested after two days of treatments. Total RNA was isolated and subjected to the *NrHAK1* expression analysis with real-time PCR.

Rb^+ uptake experiments

The strains contained the empty vector and the vectors containing *NrHAK1* cDNAs were grown in YPD medium. After 30-min K^+ starvation, the cells were suspended in a 2% (w/w) glucose, 10 mmol/L MES [2-(*N*-morpholine)-ethane sulphonic acid] buffer brought to pH 6.0 with $Ca(OH)_2$. After the addition of RbCl to a final concentration of 0, 7.5, 15, 30, 60, 90, 120, or 180 μmol/L for 5 min, 1 ml sample cells in the absence or presence of two concentrations of K^+ (25 and 50 μmol/L) were taken, filtered through the 0.8-μm pore nitrocellulose membrane filters (Millipore, MA, USA), and washed with 20 mmol/L $MgCl_2$. The filters were incubated overnight in 0.1 mol/L HCl, and the concentration of Rb^+ or K^+ was determined based on atomic emission of acid-extracted cells by a Perkin-Elmer 5500 spectrophotometer (Boston, MA, USA). The average value of three independent experiments was reported.

Real-time PCR

To generate the standard curves, the PCR products of *NrHAK1* and tobacco β-actin (accession No. AB158612, used as a house-keeping gene for normalization) were purified with the QIA Quick Spin Purifying Kit (Qiagen, Germany). The concentration of purified DNA fragments was determined and further adjusted to 10^2, 10^3, 10^4, 10^5, and 10^6 copies per microliter according to the OD_{260} of PCR products and the amounts of four nuclei acids in the DNA fragment. The primers used in the qRT-PCR (real-time quantity PCR) were as follows: NrHAK1-F, 5′-CCTTATTGTGCCGTCATGCC-3′; NrHAK1-R, 5′-CTGAGATTGCAGGAGTAAGG-3′; ACT-F, 5′-GATCTTGCTGGTCGTGATCT-3′; and ACT-R, 5′-ACTTCCGGACATCTGAACCT-3′. The Ct (cycle threshold) values were estimated at a threshold value of 0.1, which was at least twice as high as the detection limit (corresponding to 10 times the standard deviation of the fluorescence in cycles 1~10). The *NrHAK1* expression level in each sample was calculated as a ratio of the copy number of *NrHAK1* to that of β-actin. Statistical analyses were performed in SPSS (version 10.0 for Microsoft Windows). Data were presented as the mean±*SE* (standard error) and $P<0.05$ was considered statistically significant. After PCR, all reaction products were analyzed by agarose gel electrophoresis to confirm that only the expected PCR products were produced.

RESULTS

Analysis of the *NrHAK1* amino acid sequence

We first isolated a 0.73-kb cDNA fragment by RT-PCR, whose translated sequence showed a high

sequence similarity to HAK/KUP potassium transporters of *Mesembryanthemum*, *Phytolacca acinosa* and *Arabidopsis thaliana*, and so on. The fragment was further extended by 5′- and 3′-RACE amplification, leading to a full-length cDNA of 2488 bp containing a 2334-bp ORF (777 amino acids). A BLASTx search for protein sequences related to *NrHAK1* revealed a moderate sequence similarity to the HAK/KUP transporters. *NrHAK1* appears to share a homology with HAK-type proteins in other plants: the similarity to *Arabidopsis* HAK/KUP transporters varies from 60% to 87% and the identity from 41% to 76%, respectively. *NrHAK1* shows a particularly high homology to *AtHAK8* (76% identity).

The existence of closely related orthologs among different species suggests that HAK/KUP transporters are highly conserved not only in structure but also maybe in function. According to Rubio *et al*.(2000), all plant HAK/KUP transporters fall into four clusters. Fig.1 shows the phylogenetic relationships among *NrHAK1* and other HAK/KUP proteins in *Arabidopsis thaliana*, *Phytolacca*, *Mesembryanthemum*, and *Hordeum vulgare*, indicating that the NrHAK1 protein belongs to Group II. NrHAK1 appears to be in the same cluster of *PaHAK1*, *McHAK1*, *AtHAK8* and *AtHAK6*.

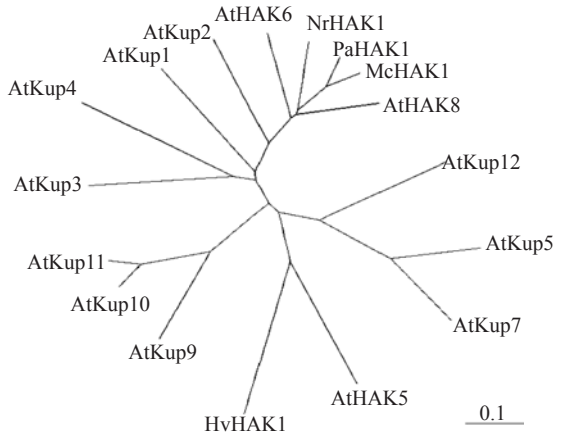

Fig.1 Phylogenetic analysis of the potassium transporter
The bootstrapped tree was produced by CLUSTALW with 1000 repetitions. A consensus tree for plant HAKs was generated with TREEVIEW software. The origins of the proteins are as follows: AtKup1 (At2g30070), AtKup2 (At2g40540), AtKup4 (At4g23640), AtKup3 (AAF19432), AtHAK5 (AAF19432), AtKup5 (At4g33530), AtHAK6 (At1g70300), AtKup7 (At5g09400), AtHAK8 (At5g14880), AtKup9 (At4g19960), AtKup10 (At1g31120), AtKup11 (At2g35060), AtKup12 (At1g60160), HvHAK1 (AF025 292), PaHAK1 (AAX13997), McHAK1 (AF367864), and NrHAK1 (DQ535884)

Functional complementation of *NrHAK1* in yeast mutants

The *NrHAK1* cDNA was inserted into the yeast expression vector pPC87 and transformed into the mutant strain *EGY4 trk1Δtrk2Δ*. Our results show that all strains grew at similar rates in the medium containing 50 mmol/L KCl (Fig.2a). The growth of mutant strains lacking the K^+ transporters *trk1* and *trk2* was rescued by the *NrHAK1* expression in 0.1 mmol/L K^+ medium (Fig.2b), whereas the mutants containing the empty vector pPC87 did not grow at the concentration of 0.1 mmol/L K^+. Thus, we confirmed that *NrHAK1* was able to support yeast growth at a concentration as low as 0.1 mmol/L K^+ in the culture medium, suggesting that the *NrHAK1* protein functioned as a potassium transporter in *N. rustica*.

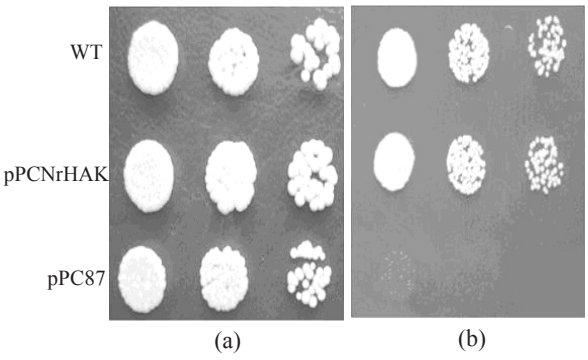

Fig.2 Complementation of the yeast mutant strain by *NrHAK1*
Ten-fold dilution series of cell suspensions were prepared from yeast *EGY48* [wild type (WT)], mutant yeast *EGY4 trk1Δtrk2Δ* transformed with *NrHAK1* (pPCNrHAK) and mutant strain with empty pPC87 plasmid (pPC87). Suspensions were spotted on solid APG medium (pH 4.0) supplemented with (a) 50 mmol/L or (b) 0.1 mmol/L KCl. The plates were incubated at 30 °C for 7 d

As shown in Fig.3, during potassium starvation treatments, the rate of Rb^+ influx in the K^+-uptake-deficient yeast mutant with overexpressing *NrHAK1* was fitted to a Michaelis-Menten function. We also found that the rate of Rb^+ influx in *NrHAK1* transformant was four times larger than that in the control cells and had an apparent K_m of 27.6 μmol/L for Rb^+.

Fig.4 shows that the yeast-expressed *NrHAK1* mediated the high-affinity Rb^+ uptake and it was competitively inhibited by K^+ at micromolar concentrations.

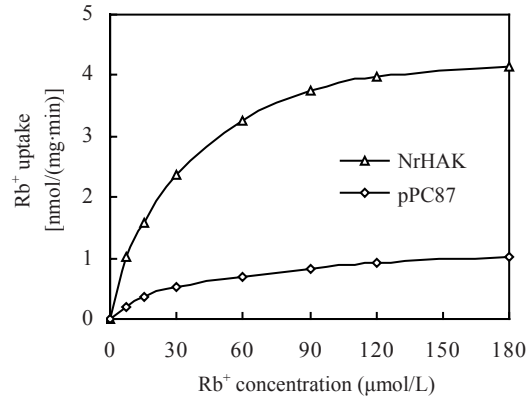

Fig.3 *NrHAK1* mediated Rb^+ influx in yeast
Rb^+ uptake in the mutant yeast strain *EGY48 trk1Δtrk2Δ* transformed with *NrHAK1* (NrHAK) or with the empty pPC87 plasmid (pPC87)

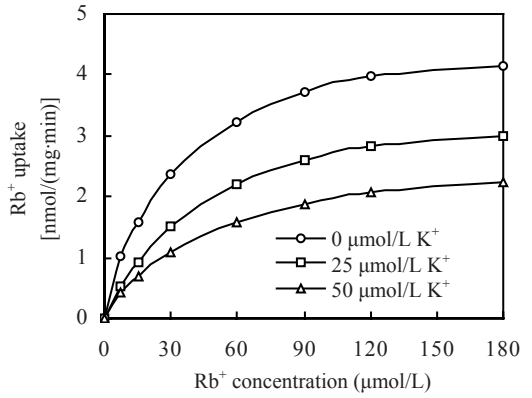

Fig.4 Competitive inhibition of *NrHAK1* mediated Rb^+ uptake by micromolar concentrations of K^+
Concentration dependence of the initial rates of Rb^+ uptake exhibited by the yeast potassium transport mutant transformed with the *NrHAK1* cDNA in the absence or presence of two concentrations of K^+ (25 and 50 μmol/L)

Growth of *NrHAK1* transformant in the presence of Na^+ or NH_4^+

It has been reported that the non-specific uptake of potassium into cells is impaired when high extracellular concentrations of competing monovalent cations are available (Wang *et al*., 2000). We investtigated the growth patterns of *NrHAK1* transformant strains in the medium containing 100 mmol/L NaCl or 5 mmol/L NH_4Cl. We found that *NrHAK1* conferred on the yeast mutant a capacity to grow as fast as the wild-type strain in the NaCl medium (Fig.5a), but not in the NH_4Cl medium (Fig.5b). This inhibition of the *NrHAK1*-mediated K^+ uptake by ammonium is consistent with the ammonium inhibition in other plant roots (Rufty *et al*., 1982).

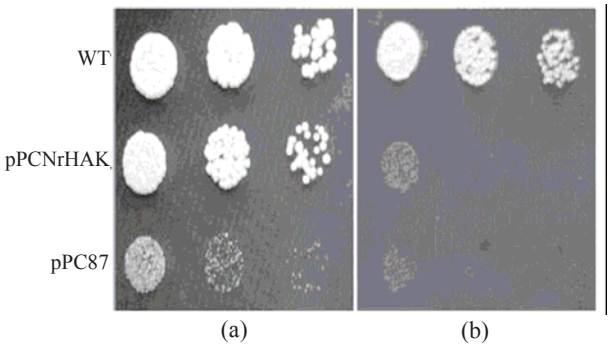

Fig.5 Growth of *NrHAK1* transformant in the presence of Na^+ or NH_4^+
Ten-fold dilution series of cell suspensions were prepared from yeast *EGY48* [wild type (WT)], mutant yeast *EGY48 trk1Δtrk2Δ* transformed with *NrHAK1* (pPCNrHAK) and mutant strain with empty pPC87 plasmid (pPC87). Suspensions were spotted on solid APG medium (pH 4.0, containing 50 mmo/L KCl) supplemented with (a) 100 mmol/L NaCl or (b) 5 mmol/L NH_4Cl. The plates were incubated at 30 °C for 7 d

Specific expression of *NrHAK1* in *N. rustica*

We performed the real-time PCR analysis on reverse-transcribed RNA isolated from roots (R), root tips (RT), young leaves (YL), old leaves (OL) and flowers (F) with the gene-specific primers. Through analyzing the expression data, we found that the *NrHAK1* transcripts were most abundant in the root tip of seedlings (Fig.6).

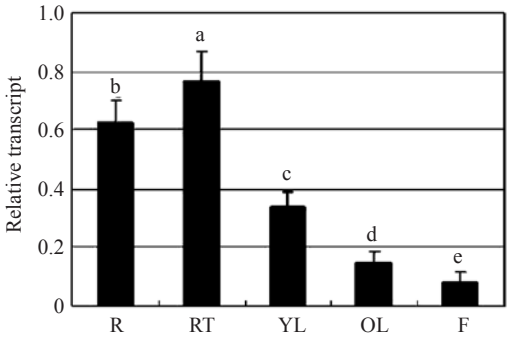

Fig.6 mRNA analysis of *NrHAK1* in organs of *N. rustica*
Ten-μg aliquots of total RNA from roots (R), root tips (RT), young leaves (YL), old leaves (OL) and flowers (F) were subjected to real-time PCR quantification of *NrHAK1*. The relative amounts were calculated and normalized with a ratio of copy number of *NrHAK1* to that of β-actin. Data were presented as mean±*SE* of triplicate measurements (n=15). The presence of the same letter on two columns indicates the absence of significant differences at P=0.05

Expression patterns of *NrHAK1* under different ionic environments

Involvement of *NrHAK1* in response to

environmental conditions was tested by examining the effects of potassium, sodium, and ammonium on *NrHAK1* expression in the root of *N. rustica*. Comparing the relative transcript abundance of treatments [($+K^+ + NH_4^+$) vs ($+K^+ - NH_4^+$) and ($-K^+ + NH_4^+$) vs ($-K^+ - NH_4^+$), as shown in Fig.7a], we found that the *NrHAK1* transcription was sharply decreased by the addition of NH_4^+. This is consistent with previous studies suggesting that NH_4^+ blocks the high-affinity K^+ uptake systems and has an inhibitory effect on plant growth, especially in low K^+ environments (Hirsch *et al*., 1998; Santa-María *et al*., 2000). Additionally, in plants grown in solution without NH_4^+ ($-K^+ - NH_4^+$), the *NrHAK1* transcription level was reduced to 25% by adding 2.5 mmol/L K^+ ($+K^+ - NH_4^+$). This indicates that transcription of *NrHAK1* was more abundant in tobacco roots subjected to potassium starvation, which was supported by the comparison of ($+K^+ + NH_4^+$) vs ($-K^+ + NH_4^+$) and ($+K^+ + Na^+$) vs ($-K^+ + Na^+$) (as shown in Fig.7b).

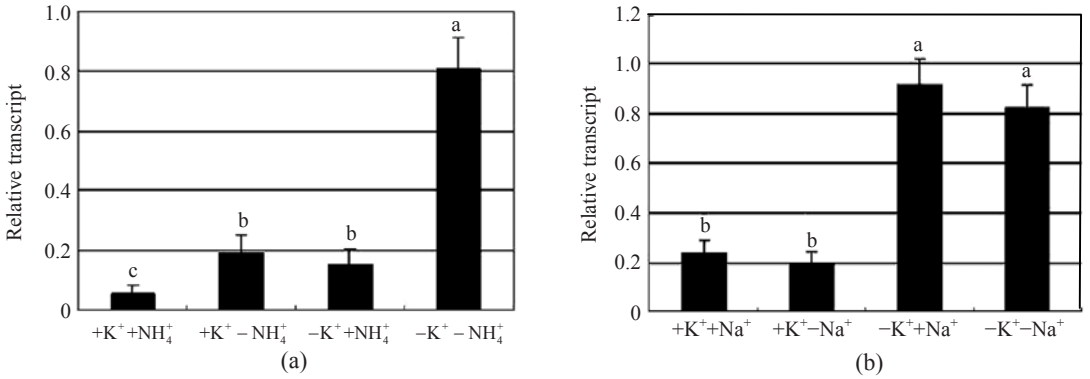

Fig.7 Expression of *NrHAK1* in tobacco roots treated with K^+, Na^+ and NH_4^+

Plants were treated with solutions lacking potassium in combination with added Na^+ or NH_4^+ stresses, including absence ($-K^+$) or 2.5 mmol/L KCl ($+K^+$), absence ($-Na^+$) or 100 mmol/L NaCl ($+Na^+$) and absence ($-NH_4^+$) or 5 mmol/L NH_4Cl ($+NH_4^+$), for 2 d. Total RNA extracted from roots was subjected to real-time PCR to analyze the expression of *NrHAK1*. The relative transcripts were calculated and normalized with a ratio of the copy number of *NrHAK1* to that of β-actin. Data are presented as mean±*SE* of triplicate measurements (n=12). The presence of the same letter on two columns indicates the absence of significant differences at P=0.05

DISCUSSION

Plants have developed flexible strategies to deal with changes in their environment and to minimize adverse effects of nutrient deficiency and excess toxic ions. Adaptive response includes a significant alteration in gene expression, particularly for membrane transporters responsible for the uptake, efflux, and translocation of beneficial and toxic minerals (Maathuis *et al*., 2003). Many inorganic nutrient transporters in plants are encoded by multi-gene families whose members often exhibit strongly overlapping expression patterns. Studies in plants and microorganisms have shown that these transporters have a wide variety of functional properties, including both high- and low-affinity K^+ transports (Fu and Luan, 1998; Senn *et al*., 2001). The difference in functional properties and spatial distributions of the transport systems is thought to allow the fine tuning of K^+ under varying environmental conditions in different plant tissues. Plant HAK/KUP transporter genes have been cloned and characterized from *Arabidopsis thaliana* (*AtHAK1-13*), *Hordeum vulgare* (*HvHAK1-2*), *Oryza sativa* (*OsHAK1-17*), *Lycopericon esculentum* (*LeHAK5*), *Capsicum annuum* (*CaHAK1-2*) and *Mesembryanthemum crystallinum* (*McHAK1-4*) (Su *et al*., 2002). Most of the HAK transporters characterized so far are high-affinity K^+ ones, and only a few of them are low-affinity K^+ transporters (Santa-María *et al*., 1997; Senn *et al*., 2001). As recently proposed (Rubio *et al*., 2000; Banuelos *et al*., 2002), all HAK/KUP transporters can

be grouped into four clusters. The cluster I genes such as *OsHAK1*, *HvHAK1*, *CaHAK1* and *AtHAK5* are involved in high-affinity K^+ uptake. For these transporters, the K_m values for K^+ are 6, 27, 6.2 and 24 μmol/L, respectively (Banuelos *et al*., 2002; Martínez-Cordero *et al*., 2004; Gierth *et al*., 2005), which are remarkably close to the K_m range (4 to 35 μmol/L) for the high-affinity K^+ uptake systems (Epstein *et al*., 1963). The affinity of cluster II HAK/KUP transporters for K^+ is less clearly defined. For this cluster, the barley transporter HvHAK2 has been reported to facilitate low-affinity transport with a K_m of 5 mmol/L for K^+ (Rubio *et al*., 2000; Senn *et al*., 2001). Growth of the *E. coli* and yeast mutants is restored at micromolar K^+ concentrations by expressing cluster II genes such as *AtKT/HAK/KUP1*, *AtKT/HAK/KUP2*, *AtKT/HAK/KUP4* and *McHAK1*, *McHAK2*, *McHAK4* (Ahn *et al*., 2004; Su *et al*., 2002). Transgenic *Arabidopsis* suspension cells with over-expressing *AtKUP1* showed an increased Rb^+ uptake at micromolar concentrations with an apparent K_m of 22 μmol/L, indicating that *AtKUP1* encodes a high-affinity K^+ uptake (Kim *et al*., 1998). But when expressed in a yeast mutant, *AtKUP1* was shown to mediate a dual-affinity K^+ transporter (Fu and Luan, 1998). The expression of *AtKUP3*, which belongs to cluster II, was up-regulated by K^+ starvation treatment (Kim *et al*., 1998). Several studies have confirmed that different members of HAK/KUP transporters have tissue-specific expression patterns (Kim *et al*., 1998; Rigas *et al*., 2001; Langer *et al*., 2002; Su *et al*., 2002). *Arabidopsis AtKT2* was found in roots and leaves (Quintero and Blatt, 1997), whereas *Arabidopsis AtKUP1* was predominantly expressed in roots. The barley *HvHAK1* was only detected in roots. At the transcript level, many genes encoding K^+ channels and transporters are known to be regulated by external K^+ (Pilot *et al*., 2003). The transcription of some members of the HAK/KUP family in *Arabidopsis*, *Oryza sativa* and *Lycopersicon esculentum* are up-regulated in response to K^+ deprivation (Kim *et al*., 1998; Maathuis *et al*., 2003; Qi *et al*., 2008; Banuelos *et al*., 2002; Wang *et al*., 2002). High-affinity K^+ uptake activity of both *Arabidopsis AtKUP1* and barley *HvHAK1* is inhibited by millimolar concentrations of Na^+ when these transporters are expressed in yeast cells (Santa-María *et al*., 1997; Fu and Luan, 1998; Fulgenzi *et al*., 2008). In *Mesembryanthemum crystalline*, *HAK* gene expression increases transiently in response to high salt concentrations (Su *et al*., 2002; Rodríuez-Navarro and Rubio, 2006). HAK K^+ transporters are NH_4^+ sensitive for K^+ uptake in contrast to the K^+ channel that is insensitive to NH_4^+ (Santa-María *et al*., 2000). The inhibition of the CaHAK1 expression by external NH_4^+ has been reported (Martínez-Cordero *et al*., 2005). LeHAK5, a high-affinity K^+ uptake system in tomato plants, is induced in K^+-starvated plants, and the presence of NH_4^+ in the growth solution further increases LeHAK5 expression (Nieves-Cordones *et al*., 2007).

In this study, we found that the transcription of *NrHAK1* in *N. rustica* was primarily accumulated in root tips and the expression of *NrHAK1* was induced by K^+ starvation or high salt stress. The parsimony analysis of *NrHAK1* with the *Arabidopsis* HAK/KUP family confirmed that *NrHAK1* maintained a high amino acid sequence identity to *AtHAK6*, a K^+ transporter belonging to cluster II. The rate of Rb^+ influx of *NrHAK1* transformant was competitively inhibited by K^+ at micromolar concentrations and had an apparent K_m of 27.6 μmol/L for Rb^+. Therefore, our results suggest that *NrHAK1* isolated in this study functions as a high-affinity K^+ transporter.

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