

Altered shoot/root Na⁺ distribution and bifurcating salt sensitivity in *Arabidopsis* by genetic disruption of the Na⁺ transporter *AtHKT1*

Pascal Mäser^{a,1}, Brendan Eckelman^{a,1}, Rama Vaidyanathan^a, Tomoaki Horie^a,
David J. Fairbairn^b, Masahiro Kubo^c, Mutsumi Yamagami^d, Katsushi Yamaguchi^e,
Mikio Nishimura^e, Nobuyuki Uozumi^c, Whitney Robertson^f, Michael R. Sussman^f,
Julian I. Schroeder^{a,*}

^aDivision of Biological Sciences, Cell and Developmental Biology Section, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0116, USA

^bDepartment of Botany, University of Queensland, St. Lucia, Qld 4072, Australia

^cBioscience Center, Nagoya University, Nagoya 464-8601, Japan

^dInstitute of Environmental Sciences, Aomori 039-3212, Japan

^eDepartment of Cell Biology, National Institute for Basic Biology, Okazaki 444-8585, Japan

^fBiotechnology Center, University of Wisconsin, Madison, WI 53706, USA

Received 30 April 2002; revised 29 August 2002; accepted 29 August 2002

First published online 8 October 2002

Edited by Marc Van Montagu

Abstract Sodium (Na⁺) is toxic to most plants, but the molecular mechanisms of plant Na⁺ uptake and distribution remain largely unknown. Here we analyze *Arabidopsis* lines disrupted in the Na⁺ transporter *AtHKT1*. *AtHKT1* is expressed in the root stele and leaf vasculature. *athkt1* null plants exhibit lower root Na⁺ levels and are more salt resistant than wild-type in short-term root growth assays. In shoot tissues, however, *athkt1* disruption produces higher Na⁺ levels, and *athkt1* and *athkt1/sos3* shoots are Na⁺-hypersensitive in long-term growth assays. Thus wild-type *AtHKT1* controls root/shoot Na⁺ distribution and counteracts salt stress in leaves by reducing leaf Na⁺ accumulation.

© 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Sodium transport; Salt stress; T-DNA insertion; *Arabidopsis thaliana*

1. Introduction

Salinization of soils due to irrigation is increasingly detrimental to agricultural productivity, because sodium (Na⁺) is toxic to most plants at high millimolar concentrations [1]. Potassium (K⁺), in contrast, is a plant macronutrient that fulfills a multitude of physiological functions: it is an essential factor for protein synthesis, photosynthesis and for glycolytic enzymes, and as an osmoticum K⁺ mediates cell expansion, turgor and turgor-driven movements [2,3]. Salt toxicity includes competition of Na⁺ with K⁺ at different levels: in the extracellular space for uptake into the cytosol, in the cytosol and in the chloroplast for potassium-specific functions. In the vacuole, however, Na⁺ may replace K⁺ in its osmotic function and thereby make available more potassium to the cytosol [4]. Thus under K⁺ starvation moderate amounts of Na⁺ can promote plant growth [5–7]. [K⁺]:[Na⁺] ratios in diverse

cellular compartments and plant tissues are determined by the activity of transporter proteins. Identification and characterization of Na⁺-permeable transporters is therefore pivotal to an understanding of the effects of Na⁺ on plants, adverse or beneficial. Elucidation of the molecular mechanisms underlying Na⁺ uptake into roots and Na⁺ distribution throughout the plant can allow engineering of salt tolerance [8] and improved potassium nutrition.

HKT1 from wheat was isolated by complementation of a K⁺ uptake-deficient yeast mutant and was shown to function as a K⁺/Na⁺ symporter [9–12]. In wheat *HKT1* is expressed in the root cortex and in the leaf vasculature [9]. In rice *HKT1* homologs were expressed in the root epidermis and endodermis [13], suggesting differential localization in different plant species. HKT1 has been proposed to be a determinant of Na⁺ sensitivity and point mutations in HKT1 have been genetically isolated that reduce Na⁺ transport while enhancing K⁺ selectivity [10,12]. HKT1 homologs have been cloned from *Arabidopsis* [14], eucalyptus [15], and rice [16]. Surprisingly, *Arabidopsis* *AtHKT1* transported only Na⁺ when expressed in yeast or in *Xenopus* oocytes [14]. In *Arabidopsis*, *AtHKT1* has recently been shown to be involved in salt stress: a screen for suppressor mutations of the salt overly-sensitive *sos3* mutant [17] revealed that disruption of *AtHKT1* suppresses the Na⁺-hypersensitive phenotype of *sos3* plants [18]. However, because *AtHKT1* mediates Na⁺ stress, the reason for expression of this gene and its physiological beneficial function in a wild-type background have remained unknown. Here we analyze the expression pattern of *AtHKT1* and the effects of genetic disruption of *AtHKT1* in an *Arabidopsis* wild-type background, and reveal that *AtHKT1* plays a central and specific role in Na⁺ sensitivity and distribution between shoots and roots in *Arabidopsis*.

2. Materials and methods

2.1. Identification of an *Arabidopsis* line disrupted in *AtHKT1*

The DNA collection of the *Arabidopsis* Knock-out Facility, University of Wisconsin (Madison, WI, USA), was screened for a T-DNA insertion into *AtHKT1* by polymerase chain reaction (PCR) [19,20].

*Corresponding author. Fax: (1)-858-534 7108.

E-mail address: julian@biomail.ucsd.edu (J.I. Schroeder).

¹ These authors contributed equally to this work.

AtHKT1-sense (GCACGAATCCTCTCCACCTTTTCAGGC) and *AtHKT1*-antisense (CAAACTACATTACGTGAGATAATA) primers were used in combination with a T-DNA (left border) -specific primer (CATTTTATAATAACGCTGCGGACATCTAC). One positive pool was identified with the sense primer. The corresponding *Arabidopsis* line was isolated by PCR on individual plants. *SOS3* and *ATHKT1* mutations in *athkt1-1*, *sos3/athkt1-1* and *sos3/athkt1-2* were confirmed by PCR amplification and sequencing.

2.2. Extraction and analysis of nucleic acids from *Arabidopsis*

Genomic DNA was extracted from young leaves using the DNeasy plant maxi kit (Qiagen, Valencia, CA, USA). For Southern blotting, 20 µg DNA were digested with *EcoRI*. A fragment of the neomycin phosphotransferase gene *NPT2* was used as a T-DNA-specific probe. RNA was extracted from seedlings with Trizol reagent (Invitrogen, Carlsbad, CA, USA) and DNaseI treated (Ambion, Austin, TX, USA). cDNA was made with the first strand cDNA synthesis kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA) using (dT)₁₆ primer. Reverse transcriptase (RT)-PCR was performed on cDNA corresponding to 100 ng total RNA with the primers s2 (GTCC-CCACGAATGAGAACATGAT), as3 (CCAAGATAGCTGGGGA-AAGTGTA), as5 (GTTCCGTCAACGGCATAAAT), EF1α-s (GG-CCACGTCGATTCTGGAAA) and EF1α-as (GGCTTGGTTGGA-GTCATCTT). Aliquots were sampled after each cycle from 20 to 30 and run on agarose gels.

2.3. Cultivation of *Arabidopsis*

Root growth assays were performed on plates in a basic ('minimal') medium of 0.5 mM KH₂PO₄, 1.25 mM KNO₃, 0.5 mM MgSO₄, 20 µM FeNa-EDTA, and the additional micronutrients 7 µM H₃BO₃, 1.4 µM MnSO₄, 1 µM ZnSO₄, 4.5 µM KI, 0.1 µM CuSO₄, 0.2 µM Na₂MoO₄, 10 nM CoCl₂, 1% agar, supplemented with the indicated amounts of NaCl and Ca(NO₃)₂. Hydroponic cultures of *Arabidopsis* were maintained in 0.5 mM KH₂PO₄, 1.25 mM KNO₃, 0.5 mM MgSO₄, 40 µM FeNa-EDTA, micronutrients as above, and the indicated amounts of NaCl and Ca(NO₃)₂. The medium was exchanged weekly.

2.4. Inductively coupled plasma-optic emission spectroscopy (ICP-OES) analysis

Shoots and roots from plants grown hydroponically for 14 to 17 days were separated, washed twice in 5 mM CaCl₂ for 3 min, dried in 15 ml polypropylene tubes (Fisher Scientific, Pittsburgh, PA, USA), and digested in concentrated ultrapure nitric acid (Sigma, St. Louis, MO, USA). In each experiment three sets of wild-type and mutant plants were grown in parallel pools and ICP samples analyzed. Samples were analyzed with an inductively coupled plasma optic emission spectrometer (ICP-OES; Perkin Elmer Optima 3000XL, Applied Biosystems, Foster City, CA, USA). Na⁺ values were normalized to those of Mg²⁺ or to plant dry weight.

2.5. *AtHKT1* promoter-GUS expression analysis

A 837 bp fragment of genomic DNA lying immediately upstream the *AtHKT1* start codon was amplified by PCR with primers TTAAGCTTACTCCATGTGTCAATACCAAAA (sense) and TTC-CCGGGTCCATTTTAGTTCTCGAGTCGG (antisense), cloned into vector pBI101-Hm [21], and transformed into *Arabidopsis thaliana*

Col-1 via *Agrobacterium tumefaciens*. GUS activity in hygromycin-resistant plants was monitored following standard protocols [21] on 3 weeks old plants. GUS staining was performed for 16 h with 0.5 mM X-Gluc (5-bromo-4-chloro-3-indolyl-β-D-glucuronide), 0.5 mM K₃Fe(CN)₆, 0.5 mM K₄Fe(CN)₆, 0.3% Triton X-100, 20% methanol, 0.1 M phosphate buffer (pH 7.0). The reaction was stopped by addition of 70% ethanol.

3. Results

3.1. *AtHKT1* is expressed in the root stele and leaf vasculature

RT-PCR analysis showed that *AtHKT1* is highly expressed in roots [14]. In order to further determine the tissue specificity of *AtHKT1* expression, a 837 bp fragment of genomic DNA lying immediately upstream of the *AtHKT1* start codon was inserted into a plant expression vector containing the β-glucuronidase (GUS) reporter gene. *Arabidopsis* seedlings transformed with this construct were incubated with the chromogenic substrate X-Gluc. GUS activity was detected mainly in the root stele (Fig. 1A, B) and in the leaf vasculature (Fig. 1C), indicating that these are important physiological sites of *AtHKT1* function in *Arabidopsis*. No GUS activity was detected in root tips (Fig. 1B).

3.2. Identification of an *Arabidopsis* line disrupted in *AtHKT1*

AtHKT1 is a single gene in *Arabidopsis* [14]. Genetic disruption of *AtHKT1* was pursued to analyze its physiological function. We screened the T-DNA insertion collection of the *Arabidopsis* Knock-out Facility (University of Wisconsin; <http://www.biotech.wisc.edu/Arabidopsis/>) by PCR, combining a primer specific to *AtHKT1* with one complementary to the T-DNA. A single insertion was detected that lies in the first intron of *AtHKT1* (Fig. 2A), and an *Arabidopsis* line harboring that insertion was isolated. After several rounds of self-fertilization, that line – called *athkt1-3* – was confirmed to be homozygously disrupted in *AtHKT1* and contained no other T-DNA insertions (Fig. 2B). *athkt1-3* plants have no full-length *AtHKT1* mRNA, but they produce small amounts of a truncated *AtHKT1* transcript as determined by RT-PCR (Fig. 2C). Whether this RNA is actually translated remains to be investigated.

3.3. Root growth of *athkt1-3* seedlings is more salt tolerant

athkt1-3 plants have no visible phenotype during their life-cycle when grown on soil under standard greenhouse conditions. Root growth assays have been successfully used to identify and characterize salt sensitivity mutants of *Arabidopsis* [22,23]. Wild-type Ws-0 and *athkt1-3* seeds were germinated

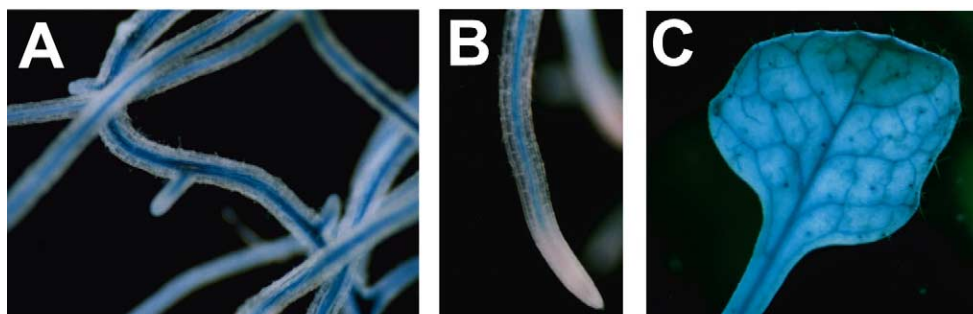


Fig. 1. The *AtHKT1* promoter is active in the root stele (A) and leaf vasculature (C), but not in root tips (B) of *Arabidopsis* seedlings. *Arabidopsis* plants expressing the GUS gene under control of the *AtHKT1* promoter (837 bp 5' of start codon) were grown for 3 weeks. GUS expression was monitored with the chromogenic substrate X-Gluc (see Section 2 for details).

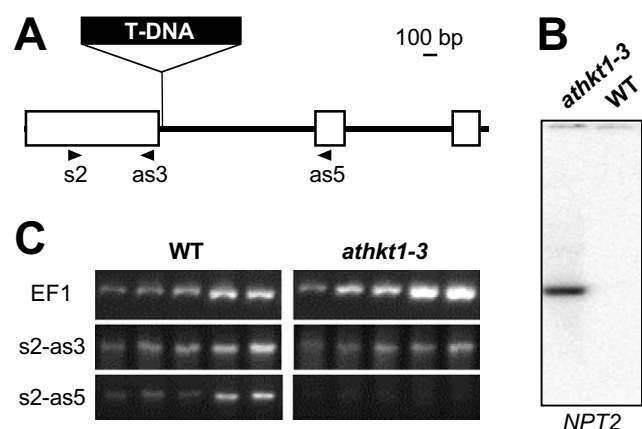


Fig. 2. *athkt1-3* is disrupted in *AtHKT1*. A: Map of the *AtHKT1* locus in *athkt1-3*. The T-DNA is inserted into the first intron of the *AtHKT1* gene, in the sense direction from left to right border (T-DNA is not drawn to scale). Sequence sites of primers used for RT-PCR are indicated with triangles. B: T-DNA insertion in *athkt1-3* is at a single locus. Genomic DNA from *athkt1-3* and wild-type plants was hybridized with a radiolabeled *NPT2* probe, the selectable marker on the T-DNA. The single band in *athkt1-3* indicates insertion of the T-DNA at a single locus (left lane). No signal was observed from wild-type plants (right lane). C: *athkt1-3* does not express a full-length *AtHKT1* mRNA. RT-PCR was performed with the *AtHKT1*-specific primer pairs s2-as3 and s2-as5 (cycles 26–30 shown for both), and EF1 α primers as a positive control (cycles 23–27 shown). No full-length *AtHKT1* transcript was detected in *athkt1-3*. However, using the primer pair that binds upstream of the T-DNA insertion (s2-as3) a signal was obtained from *athkt1-3*, albeit weaker than in wild-type.

and grown vertically on minimal media agar plates which were supplemented with varying concentrations of NaCl and $\text{Ca}(\text{NO}_3)_2$. When 75 mM Na^+ and 1 mM Ca^{2+} were added to the growth medium, *athkt1-3* plants were more salt resistant than wild-type (Fig. 3A). At 10 mM Ca^{2+} in 75 mM NaCl medium, however, wild-type and *athkt1-3* roots grew equally well (Fig. 3B). Ca^{2+} counteracted Na^+ toxicity in both the *athkt1-3* and the wild-type Ws-0 ecotype. Ca^{2+} -induced salt resistance is in accordance with previous findings [24–27] (Fig. 3).

3.4. *athkt1-3* plants show an altered root/shoot distribution of Na^+

Cation contents of hydroponically grown *Arabidopsis* were measured by ICP-OES. The Na^+ contents of both roots and shoots responded sharply to the external Na^+ concentration, while the concentrations of Mg^{2+} were stable. Under all external Na^+ concentrations tested, root Na^+ contents were lower in *athkt1-3* than in wild-type plants (Fig. 4A; $n=3$ experiments, two-tailed paired Wilcoxon test, $P<0.01$), consistent with the enhanced Na^+ resistance of root growth in *athkt1-3* (Fig. 3A). Surprisingly, however, Na^+ contents of the shoots were significantly higher in *athkt1-3* plants than in wild-type (Fig. 4B; $n=3$ experiments, two-tailed paired Wilcoxon test, $P<0.01$). *athkt1-3* and wild-type plants showed opposite Na^+ distribution patterns in roots and shoots in each individual experiment, although total Na^+ contents varied somewhat from experiment to experiment. Thus *athkt1-3* and wild-type plants showed diametrically opposed Na^+ distribution patterns in roots (Fig. 4A) and shoots (Fig. 4B). The higher root Na^+ content at low $[\text{Ca}^{2+}]$ (Fig. 4A) is in accordance with the model that Ca^{2+} blocks Na^+ -permeable cyclic nucleotide-dependent ‘VIC’ or ‘NSCC’ channels in roots [28].

3.5. Leaves in *athkt1* disruption alleles are hypersensitive to salt stress

The finding that *AtHKT1* disruption enhances Na^+ accumulation in the aerial parts of *Arabidopsis* (Fig. 4B) led us to analyze long-term effects of salt stress on leaves. Plants were grown to adult size on minimal media agar plates supplemented with 1 mM $\text{Ca}(\text{NO}_3)_2$ and either 1 mM NaCl (Fig. 5A) or 75 mM NaCl (Fig. 5B, C). After 40 days of growth, root growth was slightly reduced in *athkt1-3* compared to wild-type plants (Fig. 5B). The aerial parts of *athkt1-3* showed severe chlorosis and small leaves and the plants eventually died, whereas wild-type plants showed no such symptoms (Fig. 5B). The Na^+ -hypersensitive shoot phenotype was also observed for the independent null allele, *athkt1-1* (Fig. 5C) (kindly provided by P.M. Hasegawa and A. Rus, Purdue University). Leaves of *sos3/athkt1-1* and *sos3/athkt1-2* double mutant plants [18] were more salt-sensitive than *athkt1-1* and showed strong chlorosis after ≤ 10 days of growth in the same media (data not shown).

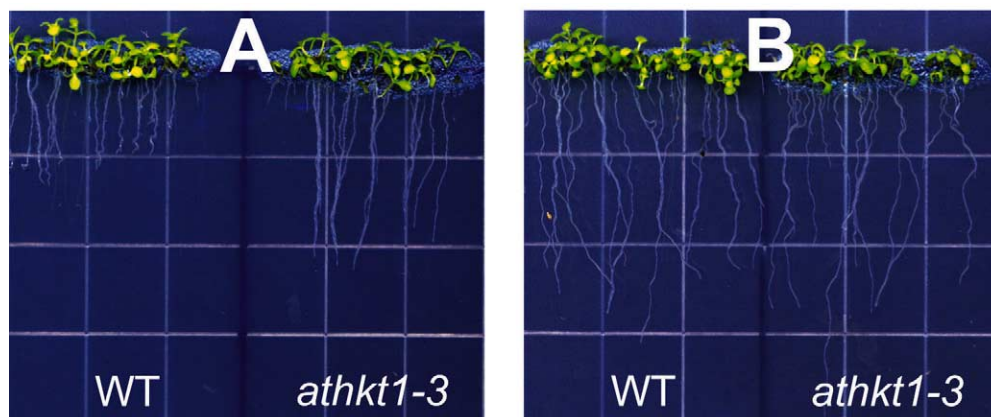


Fig. 3. Increased salt resistance of short-term *athkt1-3* root growth. *athkt1-3* and wild-type (Ws-0) seedlings were grown vertically on agar plates with minimal medium supplemented with 75 mM NaCl and 1 mM $\text{Ca}(\text{NO}_3)_2$ (A), or 75 mM NaCl and 10 mM $\text{Ca}(\text{NO}_3)_2$ (B) for 8 days. At 1 mM Ca^{2+} *athkt1-3* is more salt resistant than wild-type in seedling root growth. The distance between gridlines is 1 cm.

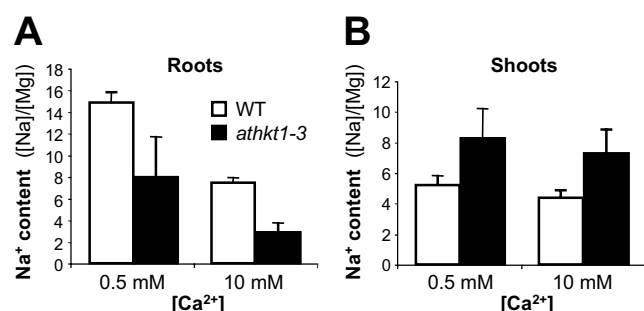


Fig. 4. Na⁺ content and distribution in *athkt1-3* plants. Hydroponic cultures of *athkt1-3* (black bars) and wild-type plants (white bars) were subjected to salt stress (100 mM NaCl) for 48 h in media supplied with 0.5 mM or 10 mM Ca(NO₃)₂. Roots (A) and aerial parts (B) were harvested separately and cation contents were measured by ICP-OES. *athkt1-3* roots contained significantly less Na⁺ than those of wild-type. For the aerial parts, however, the situation was reversed with *athkt1-3* having higher Na⁺ contents than wild-type. Na⁺ levels were normalized to those of Mg²⁺, which showed no significant changes; the same distributions were observed when normalizing to plant dry weight (data not shown). Error bars represent standard error of *n* = 3 experiments, with each experiment consisting of three pooled samples.

Thus, under permanent and prolonged exposure, *Arabidopsis athkt1* null mutants are hypersensitive to salt stress in leaves (Fig. 5B, C). Under shorter or transient exposure to salt, however, *athkt1* null mutants show reduced Na⁺ accumulation in roots and increased Na⁺ tolerance in roots (Figs. 3A and 4A; [18]).

4. Discussion

HKT transporters have been proposed to be involved in Na⁺ uptake and salt toxicity in plants [9,10]. These proteins transport Na⁺ when expressed in *Saccharomyces cerevisiae* and *Xenopus* oocytes, and in plants they are expressed in roots and in the leaf vasculature [9,10,13–16]. Recently, AtHKT1 has directly been shown to mediate salt stress in *Arabidopsis* since null mutations in *AtHKT1* suppressed the Na⁺-hypersensitive phenotype of the *sos3* mutant [18]. *athkt1/sos3* double mutant seedlings had a lower total Na⁺ content per dry

weight than *sos3* and wild-type (Col-0) plants [18]. However, whether *athkt1/sos3* affects Na⁺ levels in shoots and/or roots has not been investigated. The beneficial physiological function of AtHKT1 has remained a mystery, given its Na⁺ selectivity [14]. To gain insight into the physiological function of AtHKT1 in *Arabidopsis*, phenotypes of mutations of *AtHKT1* in a wild-type background were analyzed here. Genetic disruption of *AtHKT1* lowered the Na⁺ content of *Arabidopsis* roots and rendered root growth less Na⁺-sensitive (Fig. 3A), which correlates to *athkt1/sos3* double mutant analyses. Our findings also correlate with studies in wheat root cells, in which K⁺ starvation induced *HKT1* expression in root cortex cells [9,29] and enhanced inward Na⁺ currents that included HKT1-like properties in root cortex cells [30].

In *Arabidopsis*, the *AtHKT1* promoter is highly active in the root stele and in the leaf vasculature (Fig. 1). We therefore analyzed the effects of *athkt1-3* on leaf Na⁺ accumulation and on Na⁺ sensitivity of aerial tissues. Interestingly, *athkt1-3* exhibited higher Na⁺ contents than wild-type plants in aerial tissues, and consistent with these findings, *athkt1* null plants were Na⁺-hypersensitive when exposed to intermediate salt concentrations for a prolonged time (Figs. 4B and 5B, C). The fact that *athkt1* null plants have a higher shoot Na⁺ content than wild-type suggests that (i) there are pathways in *Arabidopsis* for Na⁺ translocation from root to shoot that function in the absence of AtHKT1; and that (ii) under salt stress AtHKT1 counteracts these Na⁺ transport pathways. Thus disruption of *AtHKT1* facilitates net transfer of Na⁺ to shoots. AtHKT1 could counteract shoot-ward Na⁺ transport either by sequestering Na⁺ in the roots and/or preventing it from being loaded into the xylem, by re-extracting Na⁺ that is being loaded into the xylem, or by removing Na⁺ from leaves and transporting Na⁺ to roots via the phloem. To further elucidate the physiological function of AtHKT1, determination of its cell type-specific expression and subcellular location is pivotal. The identification of possible protein–protein interactions will also be of interest. Given the different expression patterns of the K⁺ transporting Na⁺/K⁺ symporter isoforms of HKT1 in wheat [9] and rice [13] compared to the Na⁺ selective AtHKT1, it is plausible that the Na⁺/K⁺ symporter HKT1 isoforms [10,16,31] have fundamentally different physiological and cellular functions from the Na⁺-se-

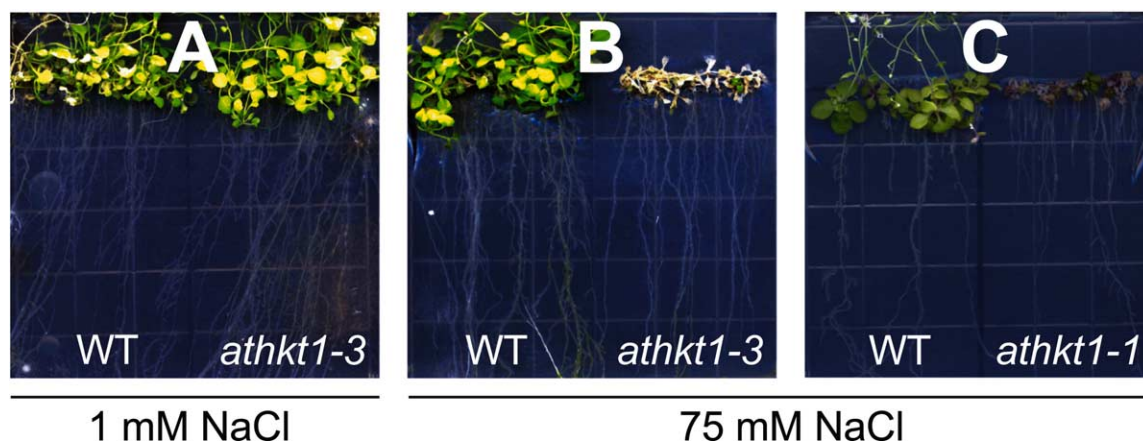


Fig. 5. Increased salt sensitivity of *athkt1-3* and *athkt1-1* shoots. Wild-type *Ws-0*, *athkt1-3*, and *athkt1-1* plants were grown vertically on agar plates with minimal medium and 1 mM Ca(NO₃)₂ (see Section 2), supplemented with 1 mM (A) or 75 mM (B, C) NaCl, for 40 days (A, B) or 30 days (C). *athkt1* null shoots showed severe chlorosis in the presence of salt and the plants eventually died (B, C). No such harmful effects of 75 mM NaCl were observed in wild-type plants under the imposed conditions. The distance between gridlines is 1 cm.

lective HKT1 isoforms [14,16], even though a single amino acid change determines K^+ permeability [32].

A role in counteracting root to shoot Na^+ transport was also proposed for the *Arabidopsis* Na^+/H^+ antiporter SOS1 [33]. *sos1* null plants are Na^+ -hypersensitive and they accumulate less Na^+ than wild-type under standard growth conditions [34] but more Na^+ than wild-type under salt stress [33]. SOS1 is expressed in the root and leaf vasculature and has been proposed to re-extract Na^+ from the root xylem under salt stress via a proposed reversal of the SOS1 transporter [33]. AtHKT1 may counteract Na^+ root to shoot transport in a different or complementary way to SOS1 because, in contrast to *sos1*, *athkt1-3* plants contain less Na^+ in roots than wild-type upon salt stress (Fig. 4A). Furthermore, SOS1 is expressed predominately in the root tips [33] whereas the AtHKT1 promoter is not active in root tips (Fig. 1B).

Here we demonstrate an important role for an HKT transporter in improving salt tolerance (Figs. 4B and 5). Previous studies predicted detrimental effects of HKT transporters under salt stress [9,10,14,32], as also confirmed here (Fig. 3) and in *athkt1/sos3* [18]. Expression patterns of HKT1 in wheat root cortex cells [9] and in rice epidermal cells [13] implicate roles in Na^+ uptake and salinity stress in those plants. However, the reason for expression of highly Na^+ -permeable AtHKT1-like transporters [14,32] in plants remained unknown. The presented results reveal the importance of AtHKT1 to physiological Na^+ resistance and Na^+ transport within *Arabidopsis*. Our data suggest a new model in which a major function of AtHKT1 in *Arabidopsis* lies in controlling the root/shoot distribution of Na^+ within the plant and reducing Na^+ accumulation in leaves. The expression of wheat HKT1 in vascular tissues [9] indicates that this newly recognized Na^+ distribution function of HKT transporters reported here may apply to other plants as well.

Acknowledgements: We wish to thank Kevin Walda (Scripps Institute of Oceanography, University of California San Diego) for help with ICP-OES, Dr. June Kwak (University of California San Diego) for providing the *NPT2* probe, and Paul M. Hasegawa and A. Rus (Purdue University) for providing *athkt1-1* and *sos3/athkt1* seeds. This work was supported by United States Department of Agriculture grant 2001-35304-09988 and NIH grant 1P42ES10337 to J.I.S. and Human Frontiers Science Program and Swiss National Science Foundation fellowships to P.M. Promoter-GUS analyses were supported by a grant-in-aid for scientific research and Center of Excellence (COE) research from the Ministry of Education, Science, Sports and Culture of Japan to N.U., M.Y. and M.N.

References

- [1] Flowers, T.J. (1999) *Sci. Hort.* 78, 1–4.
- [2] Marschner, H. (1995) *Mineral Nutrition of Higher Plants*, Academic Press, London.
- [3] Schroeder, J.I., Ward, J.M. and Gassmann, W. (1994) *Annu. Rev. Biophys. Biomol. Struct.* 23, 441–471.
- [4] Serrano, R. and Rodriguez-Navarro, A. (2001) *Curr. Opin. Cell Biol.* 13, 399–404.
- [5] Flowers, T. and Läuchli, A. (1983) *Inorganic Plant Nutrition*, Springer Verlag, Berlin.
- [6] Maathuis, F.J.M. and Sanders, D. (1993) *Planta* 191, 302–307.
- [7] Subbarao, G.V., Wheeler, R.M., Stutte, G.W. and Levine, L.H. (1999) *J. Plant Nutr.* 22, 1745–1761.
- [8] Apse, M.P., Aharon, G.S., Snedden, W.A. and Blumwald, E. (1999) *Science* 285, 1256–1258.
- [9] Schachtman, D.P. and Schroeder, J.I. (1994) *Nature* 370, 655–658.
- [10] Rubio, F., Gassmann, W. and Schroeder, J.I. (1995) *Science* 270, 1660–1663.
- [11] Gassmann, W., Rubio, F. and Schroeder, J.I. (1996) *Plant J.* 10, 869–882.
- [12] Rubio, F., Schwarz, M., Gassmann, W. and Schroeder, J.I. (1999) *J. Biol. Chem.* 274, 6839–6847.
- [13] Goldack, D., Kamasani, U.R., Quigley, F., Bennett, J. and Bohnert, H.J. (1997) *Plant Physiol.* 114, S529.
- [14] Uozumi, N. et al. (2000) *Plant Physiol.* 122, 1249–1259.
- [15] Fairbairn, D.J., Liu, W., Schachtman, D.P., Gomez-Gallego, S., Day, S.R. and Teasdale, R.D. (2000) *Plant Mol. Biol.* 43, 515–525.
- [16] Horie, T., Yoshida, K., Nakayama, H., Yamada, K., Oiki, S. and Shinmyo, A. (2001) *Plant J.* 27, 129–138.
- [17] Liu, J.P. and Zhu, J.K. (1998) *Science* 280, 1943–1945.
- [18] Rus, A. et al. (2001) *Proc. Natl. Acad. Sci. USA* 98, 14150–14155.
- [19] McKinney, E.C., Ali, N., Traut, A., Feldmann, K.A., Belostotsky, D.A., McDowell, J.M. and Meagher, R.B. (1995) *Plant J.* 8, 613–622.
- [20] Krysan, P.H., Young, J.C., Tax, F. and Sussman, M.R. (1996) *Proc. Natl. Acad. Sci. USA* 93, 8145–8150.
- [21] Ohta, S., Mita, S., Hattori, T. and Nakamura, K. (1990) *Plant Cell Physiol.* 31, 805–813.
- [22] Wu, S.J., Ding, L. and Zhu, J.K. (1996) *Plant Cell* 8, 617–627.
- [23] Zhu, J.K. (2000) *Plant Physiol.* 124, 941–948.
- [24] Epstein, E. (1998) *Science* 280, 1906–1907.
- [25] Hasegawa, P.M., Bressan, R. and Pardo, J.M. (2000) *Trends Plant Sci.* 5, 317–319.
- [26] Sanders, D. (2000) *Curr. Biol.* 10, R486–R488.
- [27] Zhu, J.K. (2001) *Trends Plant Sci.* 6, 66–71.
- [28] Maathuis, F.J.M. and Sanders, D. (2001) *Plant Physiol.* 127, 1617–1625.
- [29] Wang, T.B., Gassmann, W., Rubio, F., Schroeder, J.I. and Glass, A.D. (1998) *Plant Physiol.* 118, 651–659.
- [30] Buschmann, P.H., Vaidyanathan, R., Gassmann, W. and Schroeder, J.I. (2000) *Plant Physiol.* 122, 1387–1397.
- [31] Liu, W., Fairbairn, D.J., Reid, R.J. and Schachtman, D.P. (2001) *Plant Physiol.* 127, 283–294.
- [32] Mäser, P. et al. (2002) *Proc. Natl. Acad. Sci. USA* 99, 6428–6433.
- [33] Shi, H., Quintero, F.J., Pardo, J.M. and Zhu, J.K. (2002) *Plant Cell* 14, 465–477.
- [34] Ding, L. and Zhu, J.K. (1997) *Plant Physiol.* 113, 795–799.