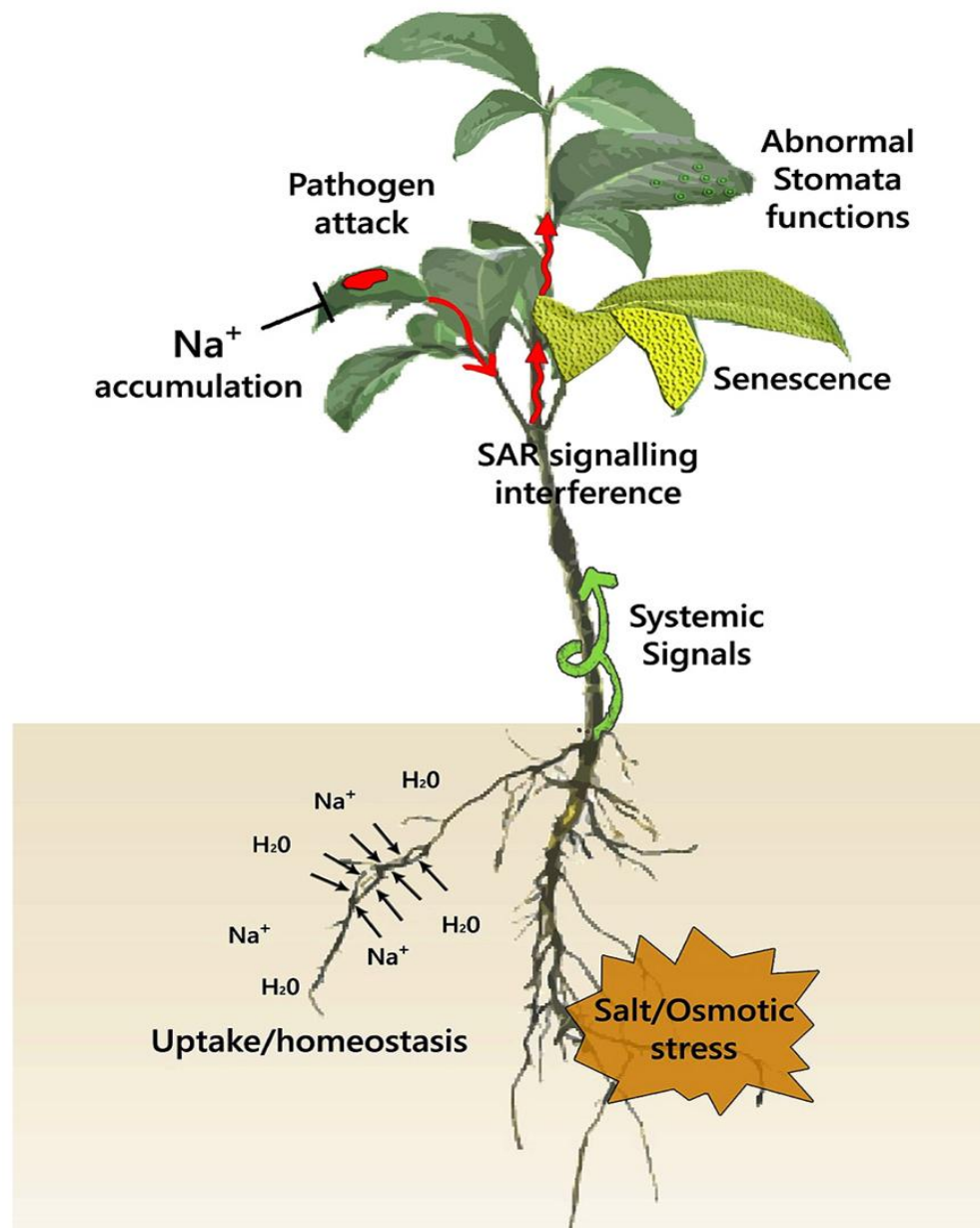


Soil salinity; What do we learn from Halophytes?

Akhtar Ali, CPSBB

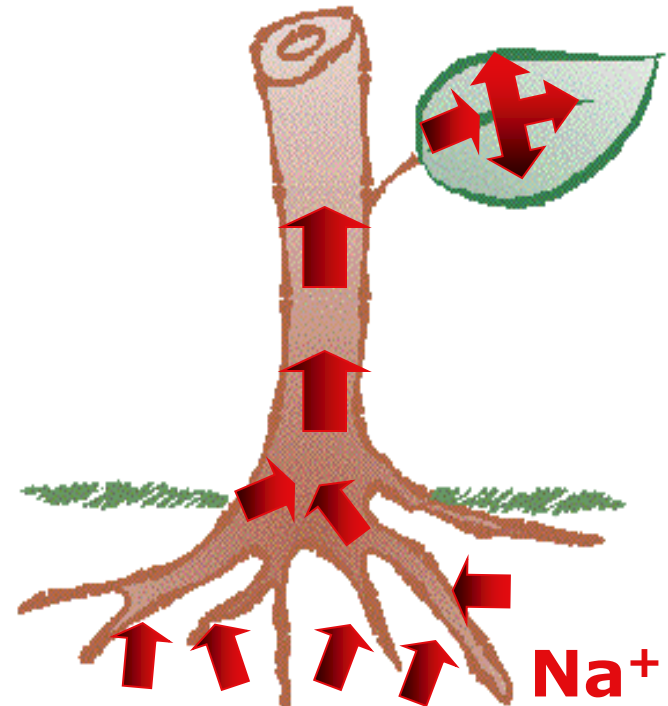
Environmental challenges



How do plants deal with salt stress?

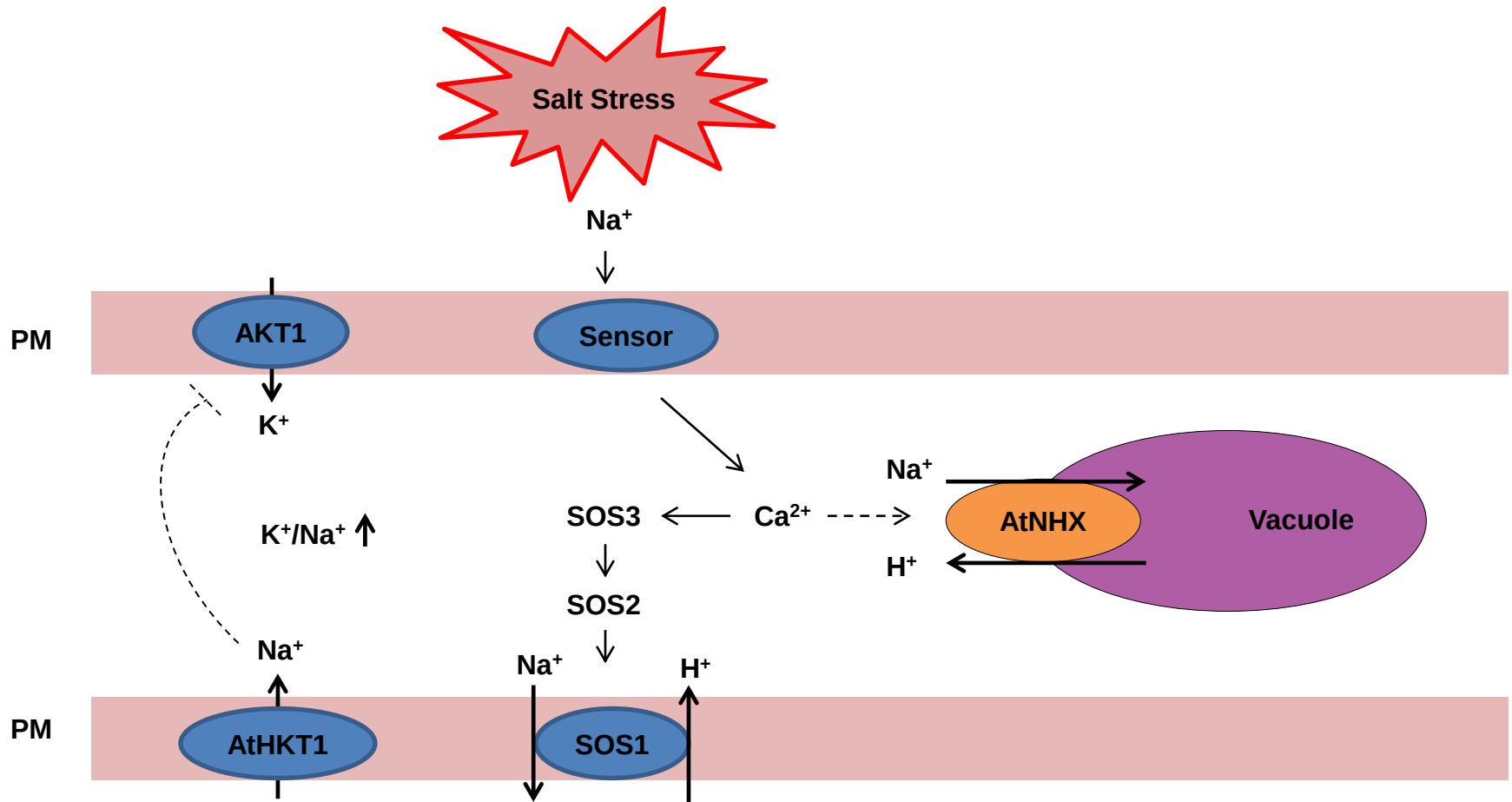


Exclusion by rejection
or efflux



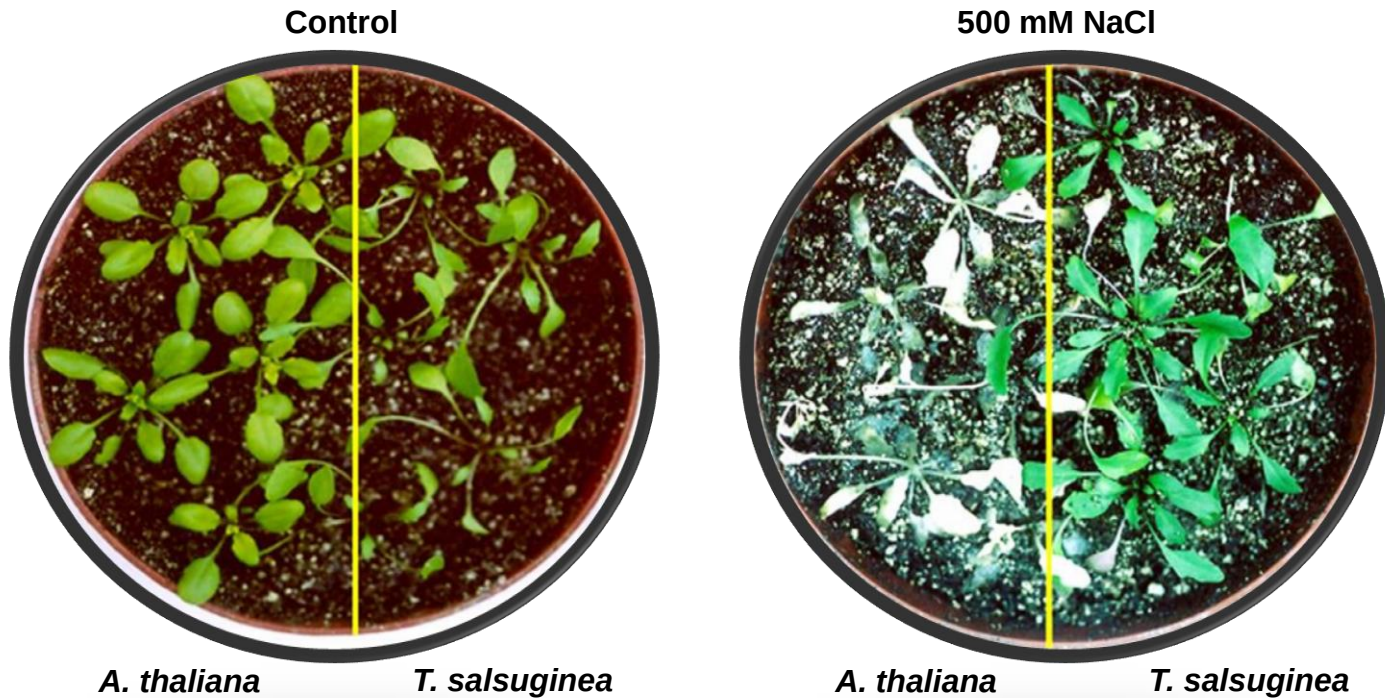
Long distance transport
and compartmentation

Salt stress regulation in plants

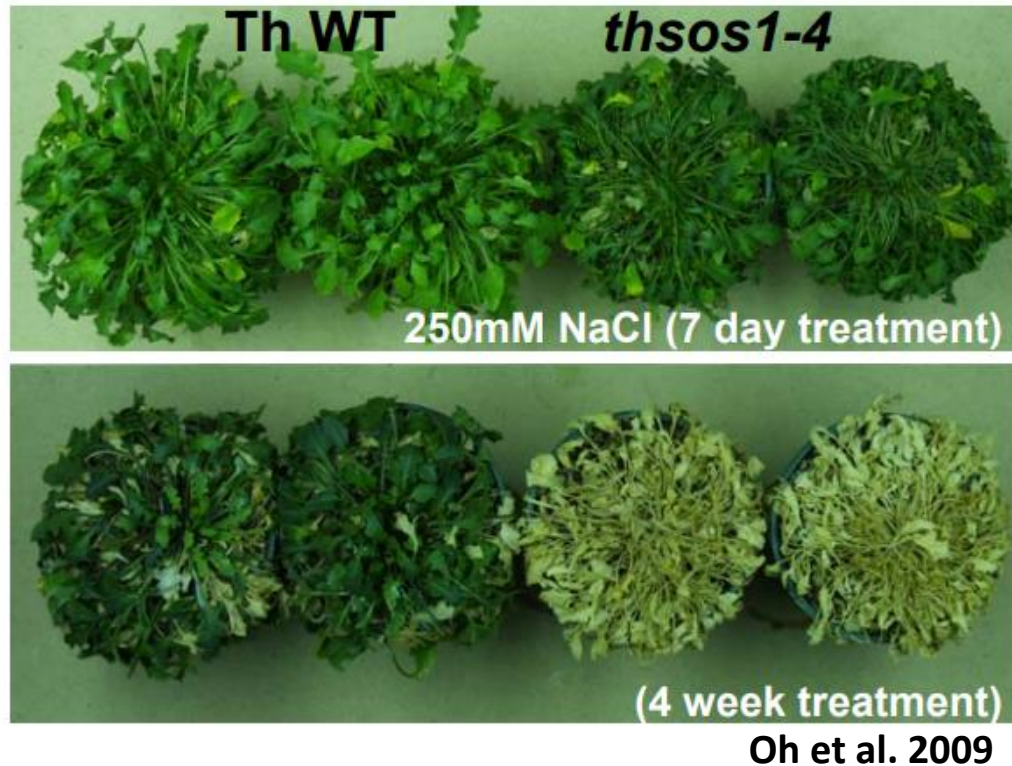


***T. salsuginea* – a close relative of *Arabidopsis* and a halophyte**

T. salsuginea shows ~93% amino acid & ~90% Nucleic acid similarities with *Arabidopsis*



TsSOS1 RNAi plants loss halophytism



Role of HKT1 in Halophytism???

PART I

**Role of Na⁺ transporter, HKT1, in *Thellungiella salsuginea*,
a model halophyte plant**

PART II

**Functional mutation in *Arabidopsis* Na⁺ transporter
AtHKT1, leads to salt stress tolerance**

PART I

**Role of Na⁺ transporter, HKT1, in *Thellungiella salsuginea*,
a model halophyte**

PART II

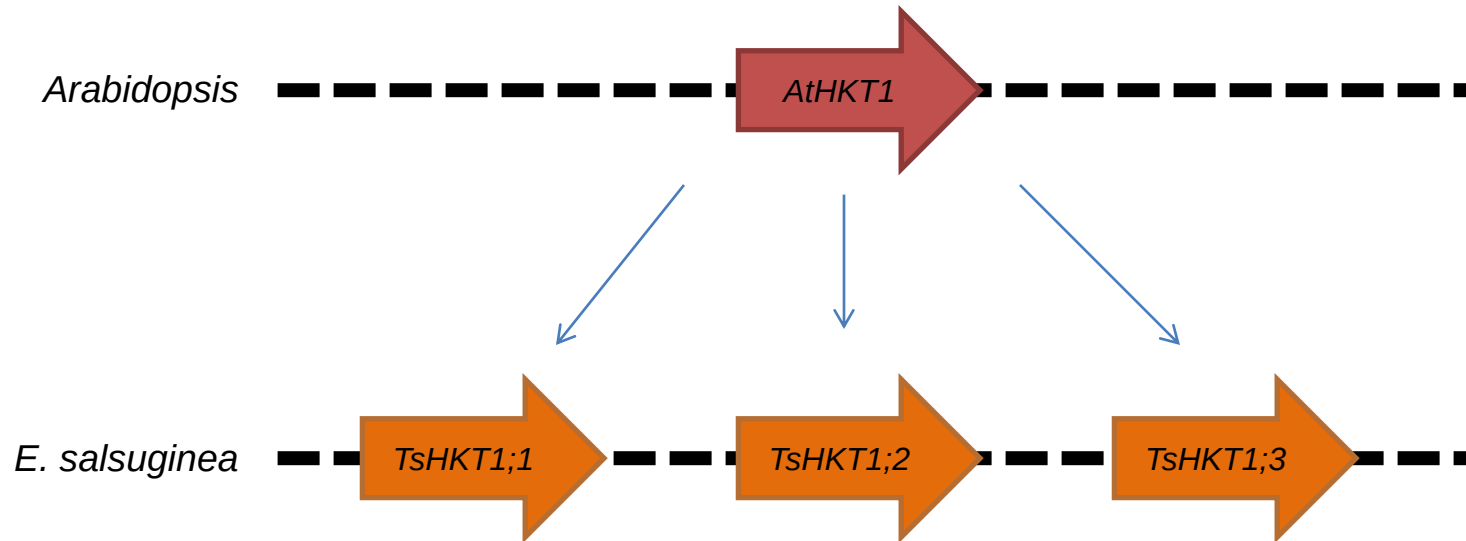
Functional mutation in *Arabidopsis* Na⁺ transporter
AtHKT1, leads to salt stress tolerance

Background

What is HKT1 and how does it regulate salt stress?

- I. HKT1 - first isolated and described in wheat as a High affinity K⁺ uptake Transporter1 with a K⁺/Na⁺ co-transport activity (Rubio et al., 1995).
- II. In *Arabidopsis*, however, HKT1 functions as a selective Na⁺ transporter (Uozumi et al., 2000).
- III. *Thellungiella salsuginea*, an *Arabidopsis* closely relative halophyte contains three homologs of HKT1, among which TsHKT1;2 shows K⁺/Na⁺ co-transporter activity.
- IV. The conc. of K⁺ in the cytoplasm has consistently been found to be between 100 and 200 mM (Shabala and Pottosin, 2010).
- V. Maintaining a high cytosolic K⁺/Na⁺ ratio (either by retaining K⁺ or preventing Na⁺ from accumulating in the leaves) is critical for plant growth and salt tolerance (Shabala and Pottosin, 2010).

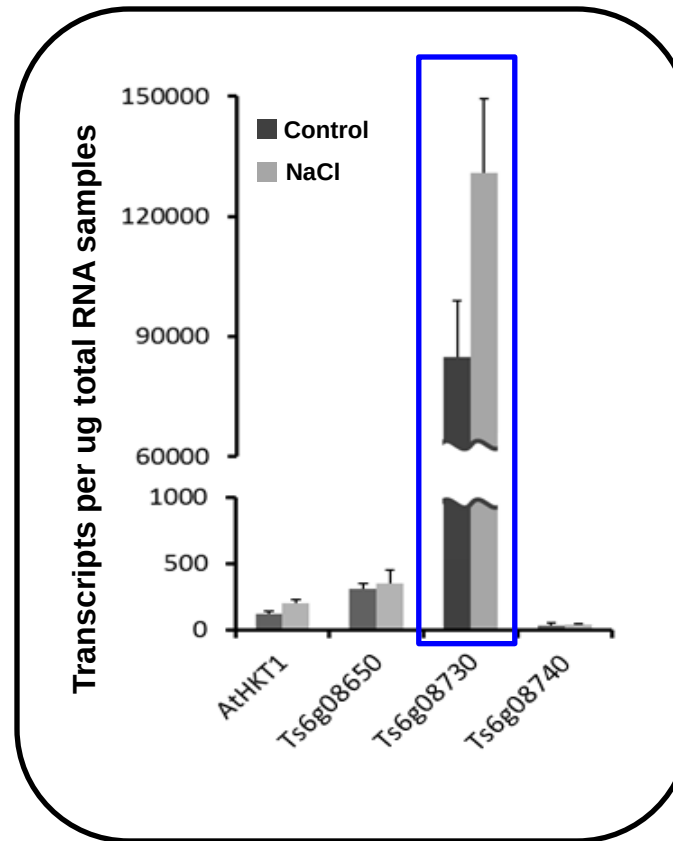
HKT1 Duplication in *Thellungiella* compare to *Arabidopsis*



- 1 *AtHKT1* (At4g10310)
- 2 *TsHKT1;1* (PAC:20197319)
- 3 *TsHKT1;2* (PAC:20197527)
- 4 *TsHKT1;3* (PAC:20196940)

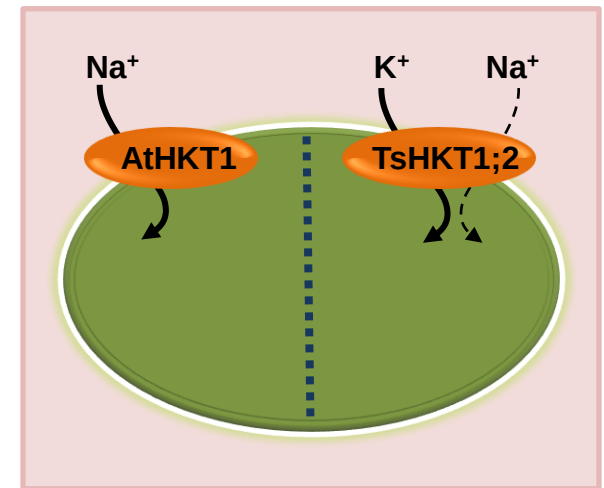
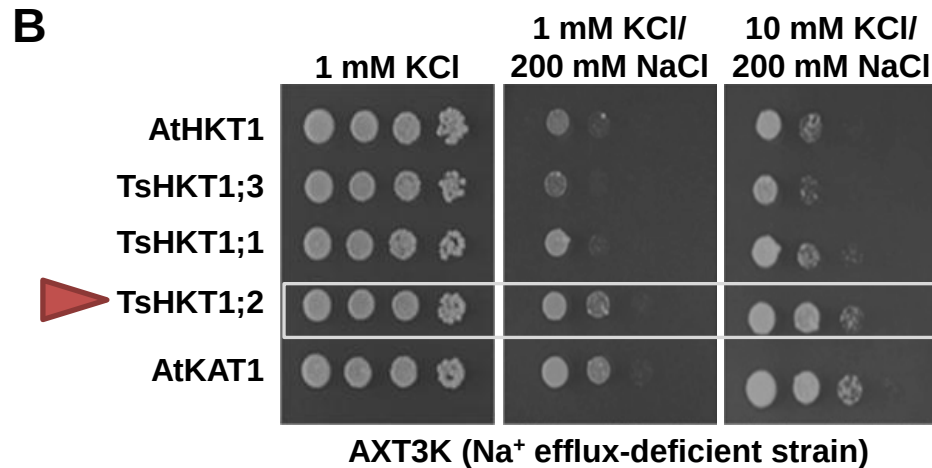
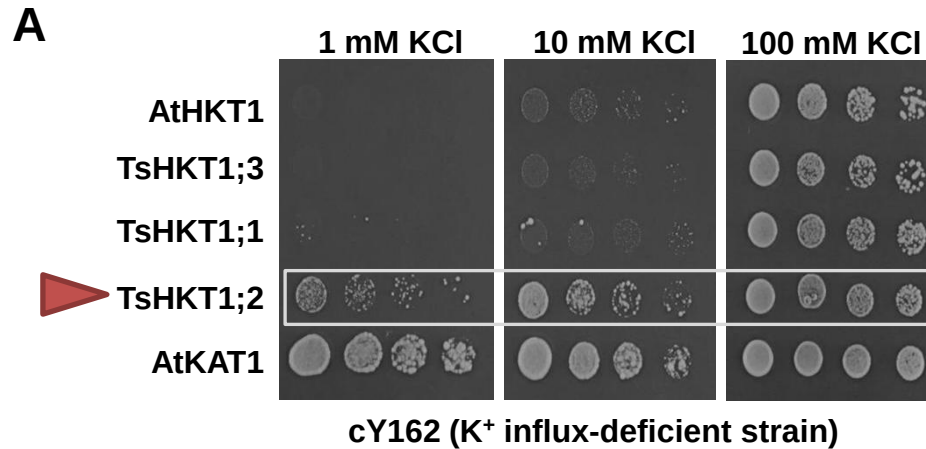
The abundant TsHKT1;2 is transcriptionally up-regulated by salt stress

1. TsHKT1;1 (Ts6g08750)
2. TsHKT1;2 (Ts6g08730)
3. TsHKT1;3 (Ts6g08740)

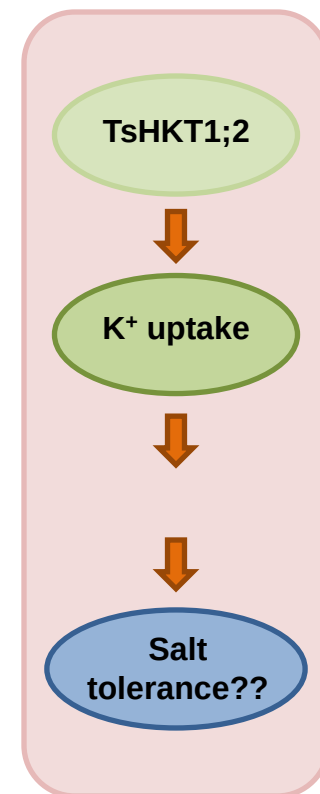
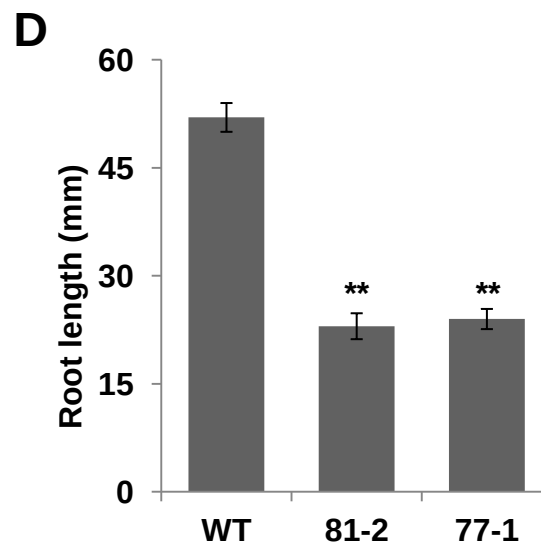
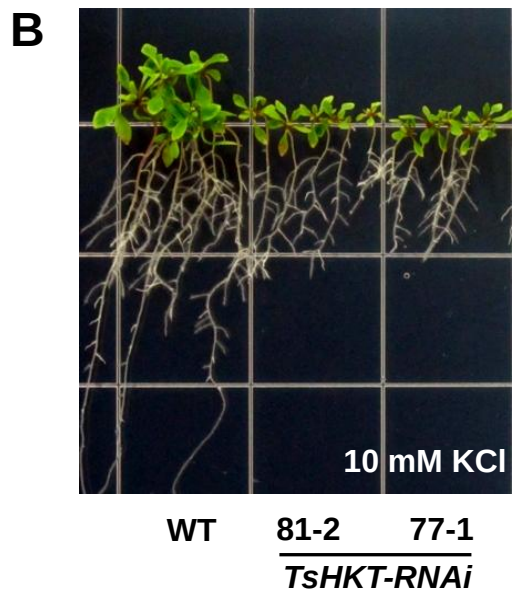
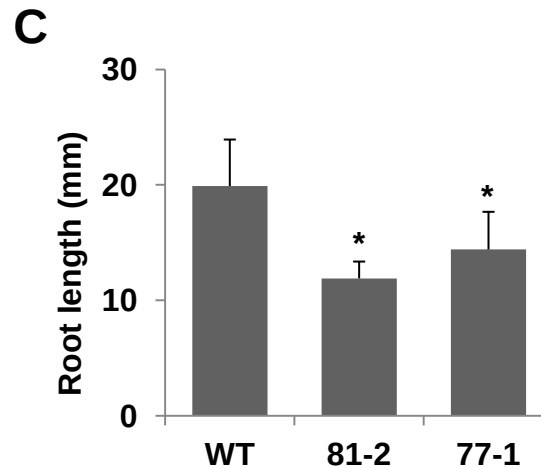
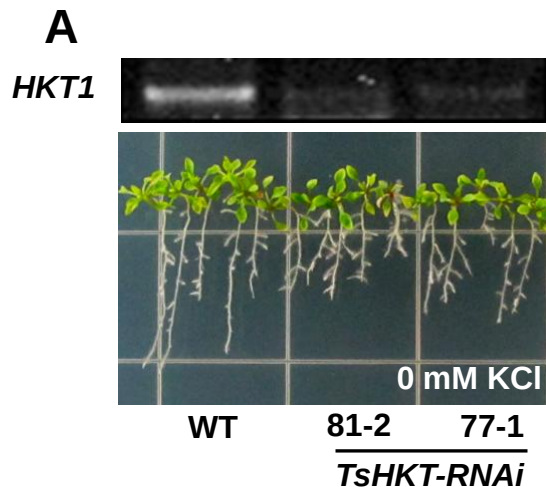


2 week-old *A. thaliana* and 3 week-old *T. salsuginea* plants treated with 200 mM NaCl for 12 hours

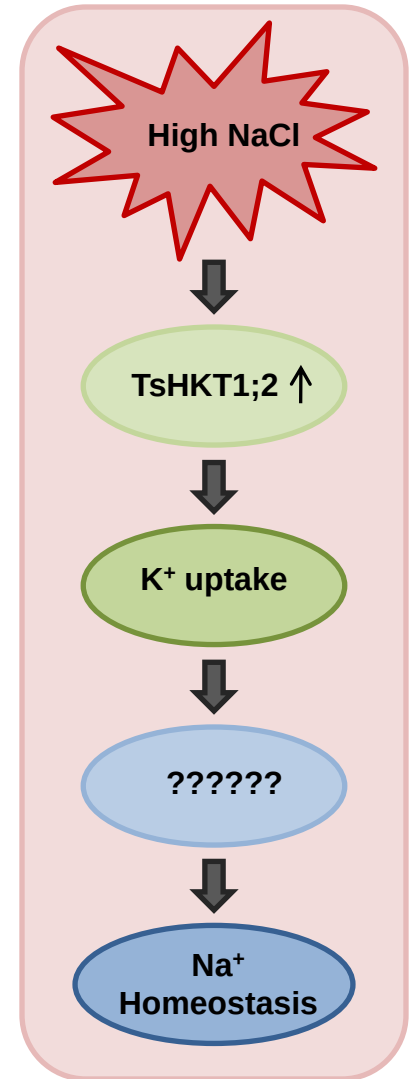
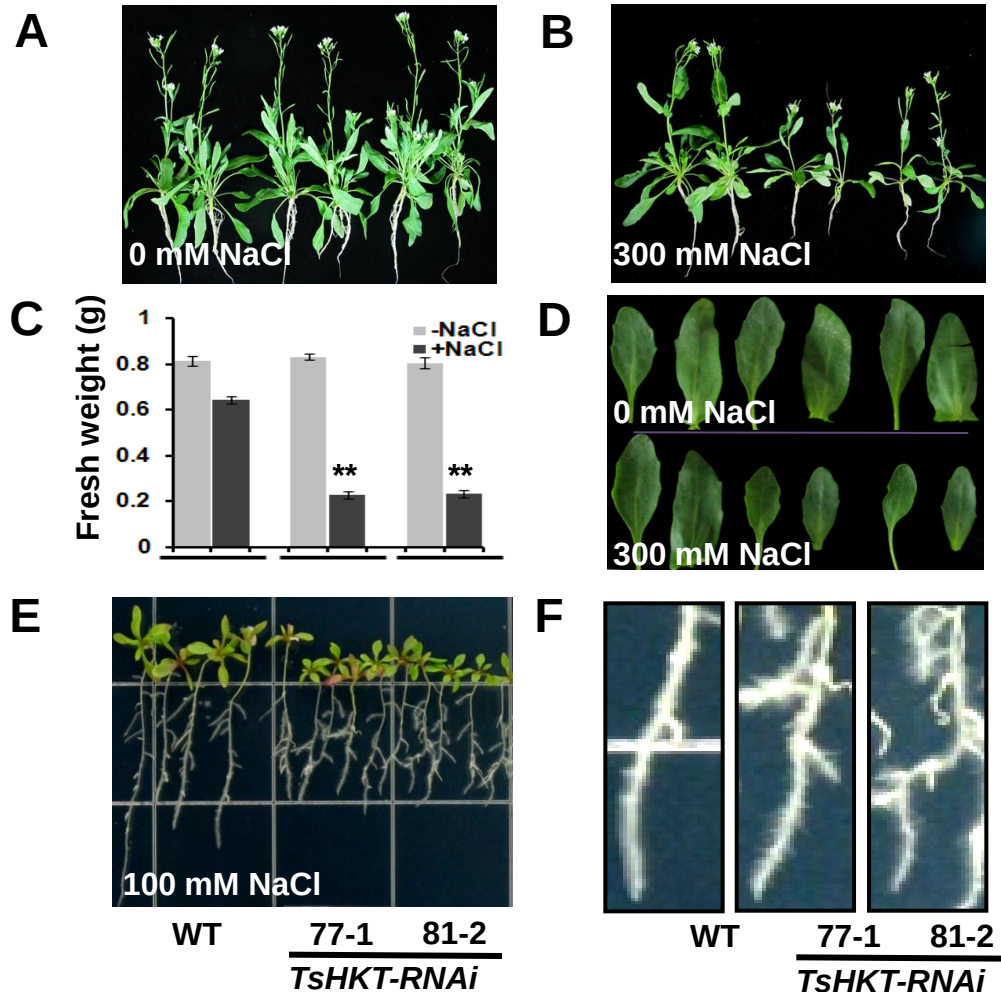
TsHKT1;2 complements K⁺-deficiency of yeast cells and has very low affinity for Na⁺



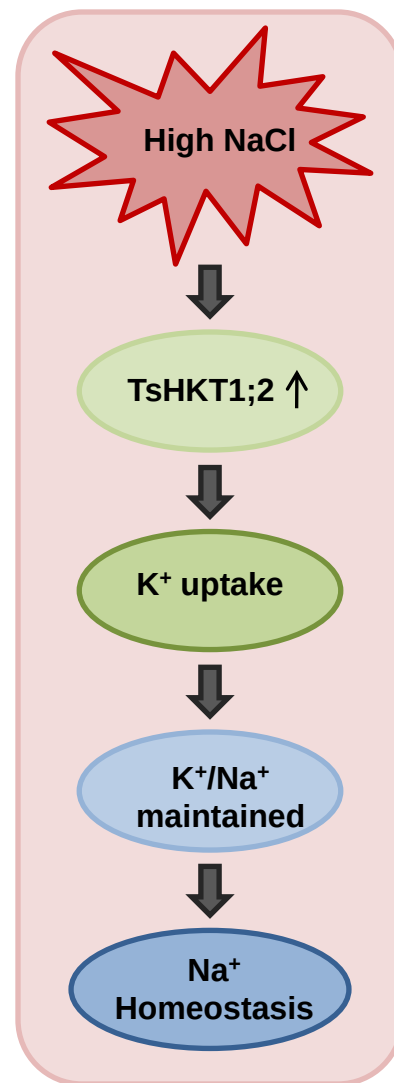
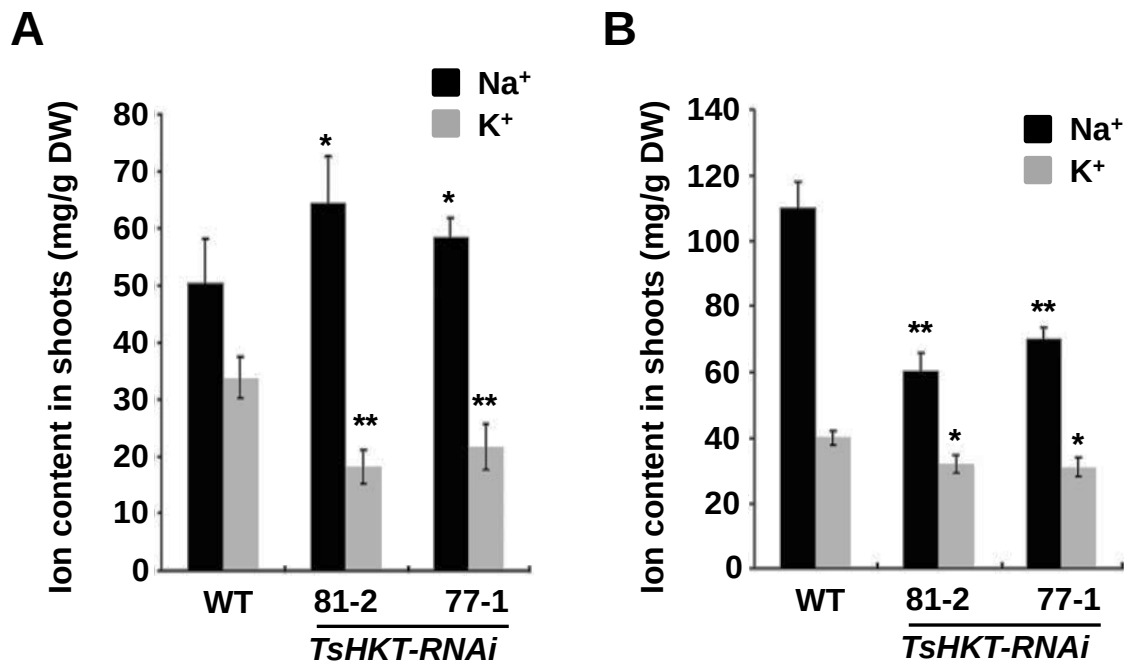
TsHKT1;2-RNAi leads to root growth retardation under K^+ -limiting condition



TsHKT-RNAi plants show sodium hypersensitive phenotypes

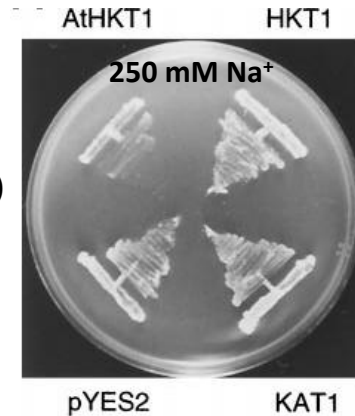


Down-regulation of *TsHKT1;2* disturbs the balance between Na^+ and K^+ under salt stress



The Arabidopsis *HKT1* Gene encodes a sodium specific transporter

G19
(Na⁺ efflux-deficient strain)



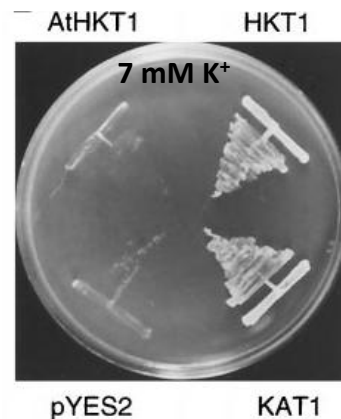
Col-0

35S::AtHKT1 #1

35S::AtHKT1 #2

Møller et al. 2009

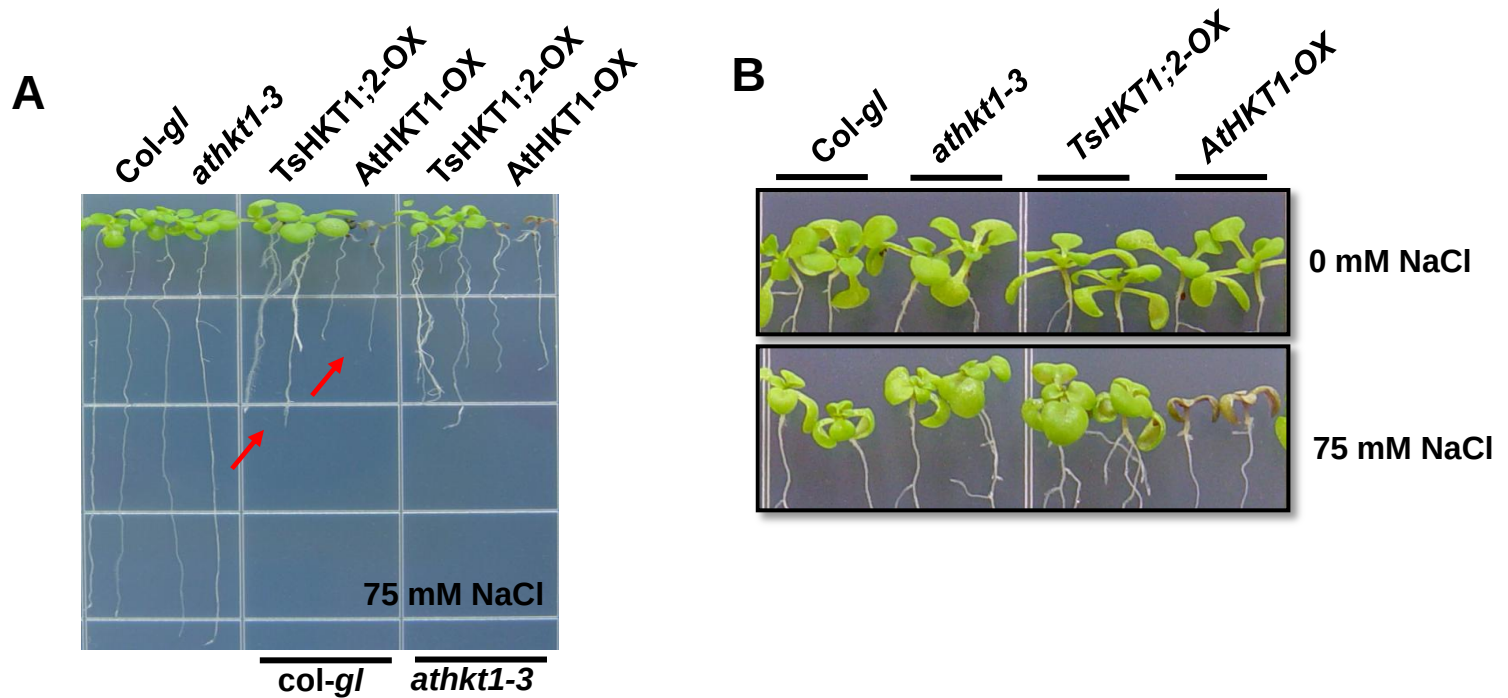
cY162
(K⁺ influx-deficient strain)



Uozumi et al. 2000

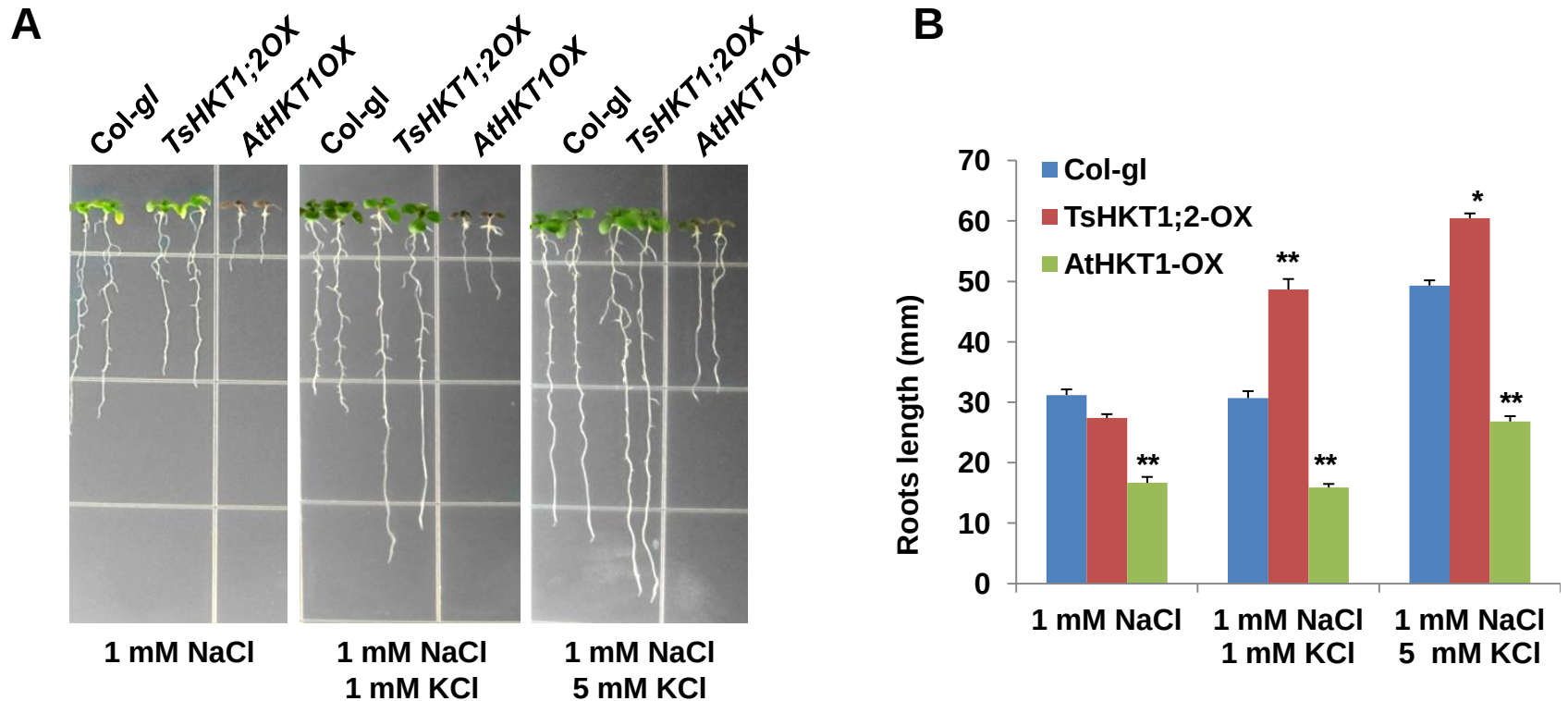
**How about TsHKT1;2 OX
in Arabidopsis ????**

TsHKT1;2 is different from *AtHKT1* in the Na⁺ Hypersensitive response

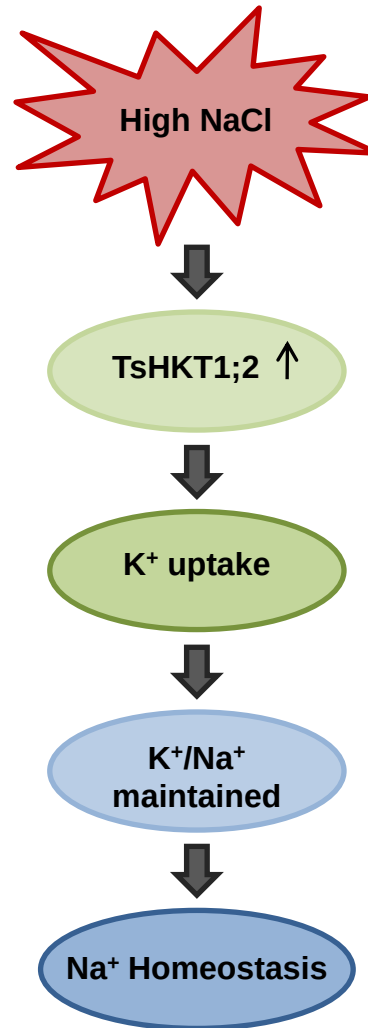


HKT1 proteins permit Na⁺ influx during salt stress

TsHKT1;2-OX leads to salt tolerance in the Presence of K^+



Hypothetical model of salt stress regulation by TsHKT1;2 in *Thellungiella salsuginea*



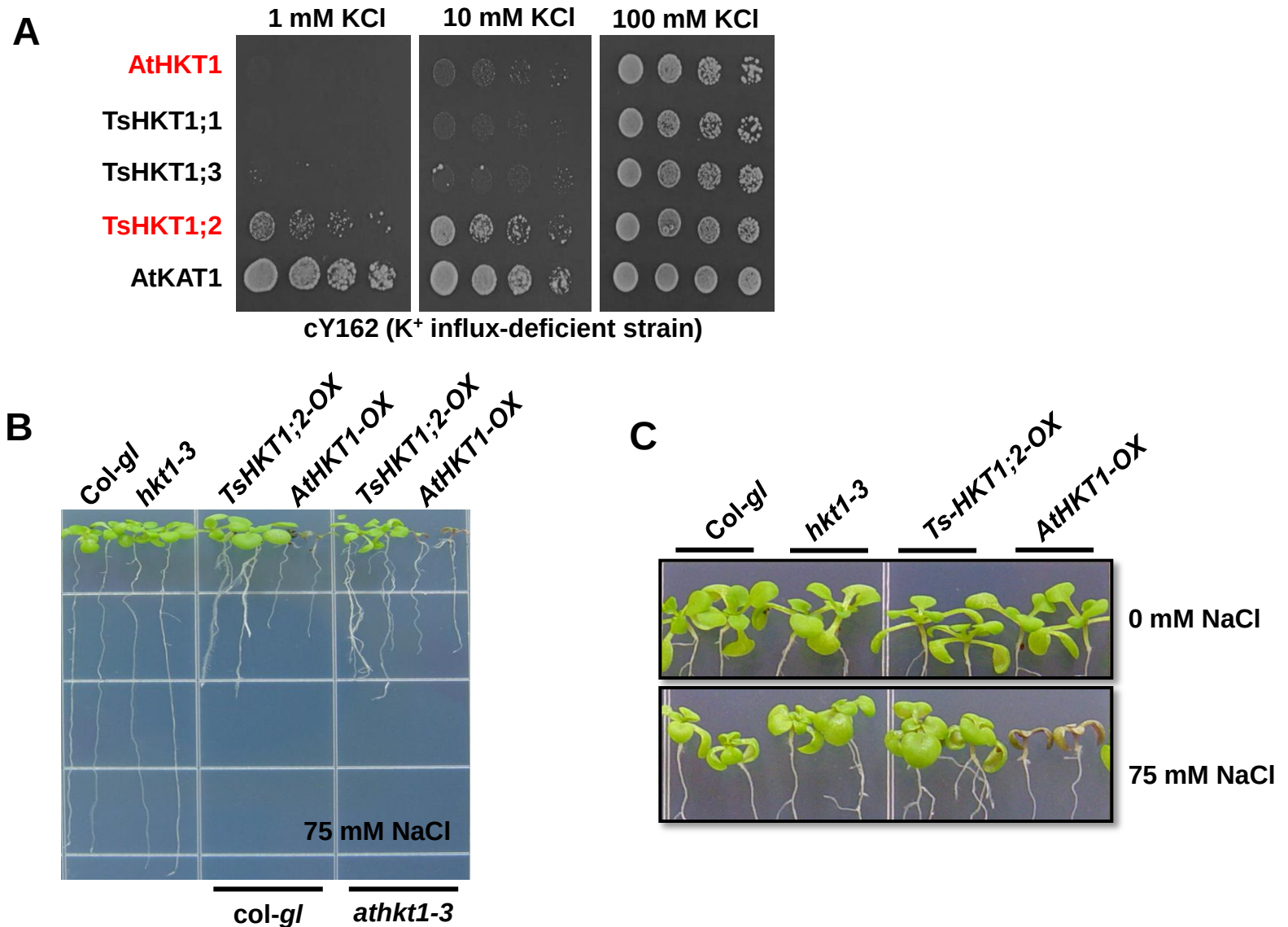
PART I

Role of Na⁺ transporter, HKT1, in *Thellungiella salsuginea*,
a model halophyte

PART II

Functional mutation in *Arabidopsis* Na⁺ transporter
AtHKT1, leads to salt stress tolerance

TsHKT1;2 is different from AtHKT1 in cation selectivity



QUESTION?

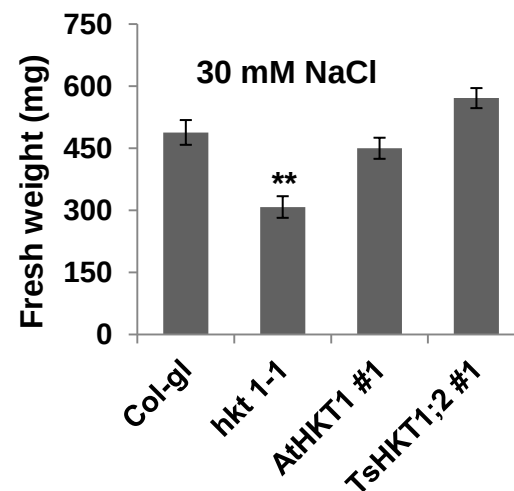
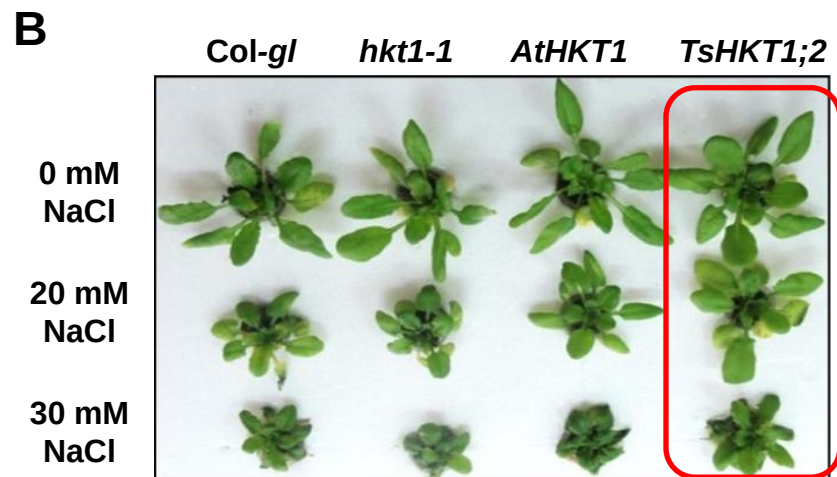
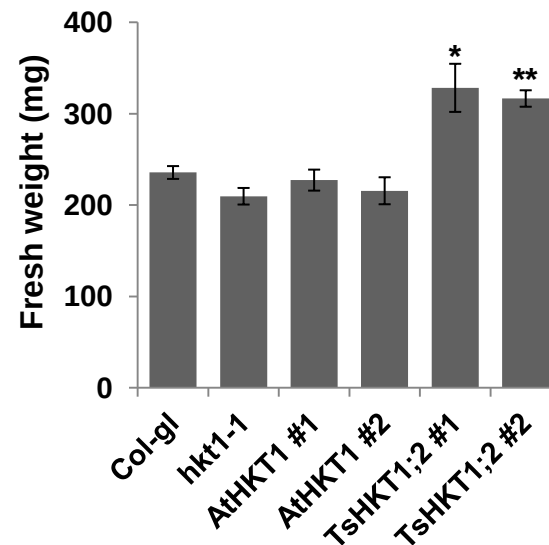
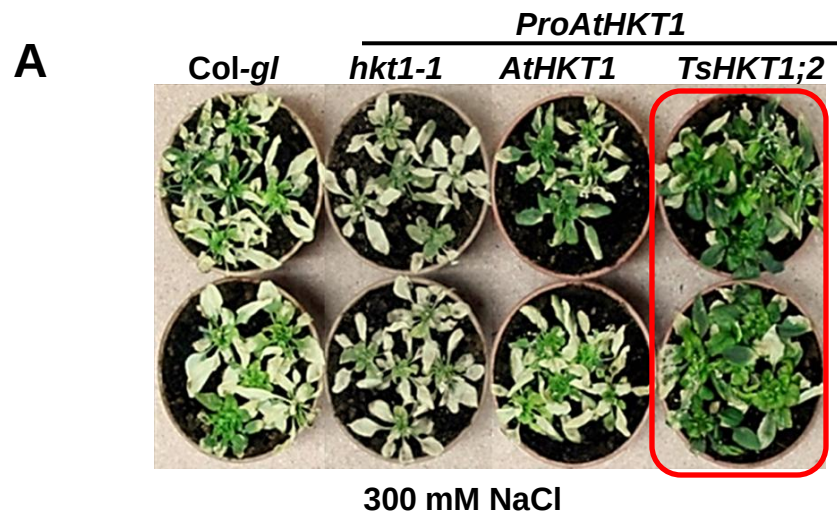
What are the basic differences between AtHKT1 and TsHKT1;2 that approaches differently towards ions selection??

Transgenic plants expressing *AtHKT1* and *TsHKT1;2* under *AtHKT1* promoter

1. ProAtHKT1::AtHKT1

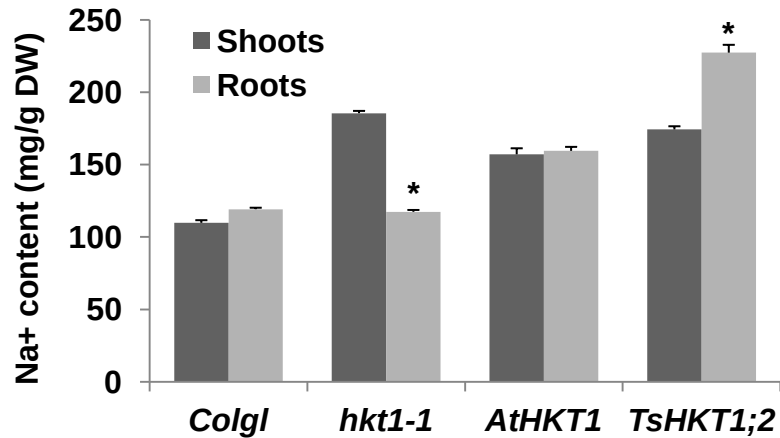
2. ProAtHKT1::TsHKT1;2

TsHKT1;2 expressing plants are tolerant to salt stress compared to *AtHKT1*

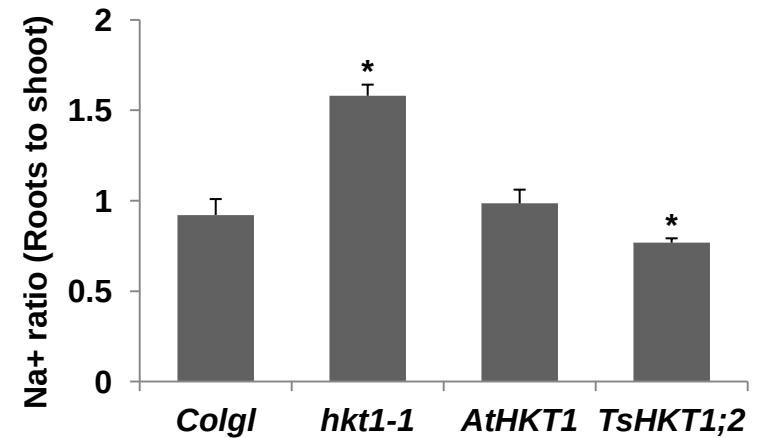


TsHKT1;2 expressing plants accumulate less Na⁺ in the shoots than roots

A



B



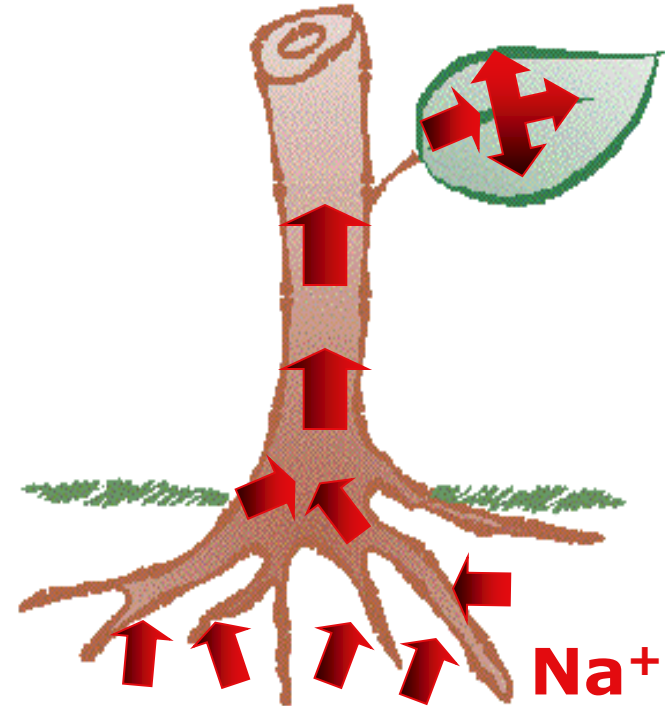
How does *TsHKT1;2*-expressing plants tolerate salt stress?

TsHKT1;2



Salt tolerant

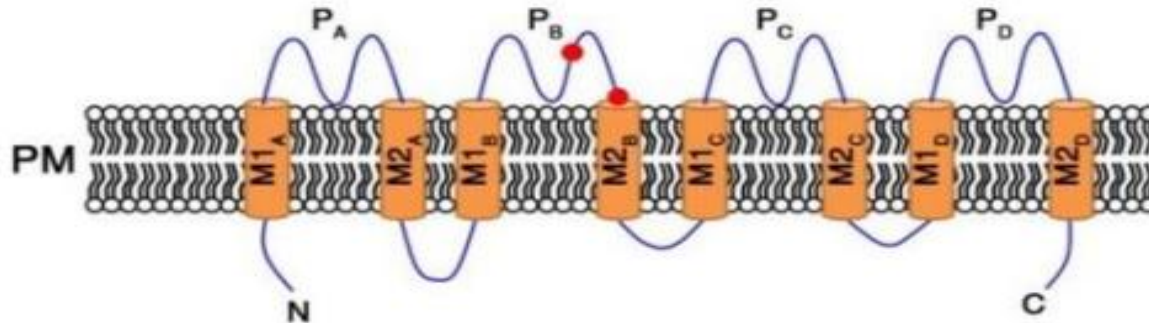
AtHKT1



Salt sensitive

1. *ProAtHKT1::AtHKT1*
2. *ProAtHKT1::TsHKT1;2*

Structure and Sequence Alignment of HKTs

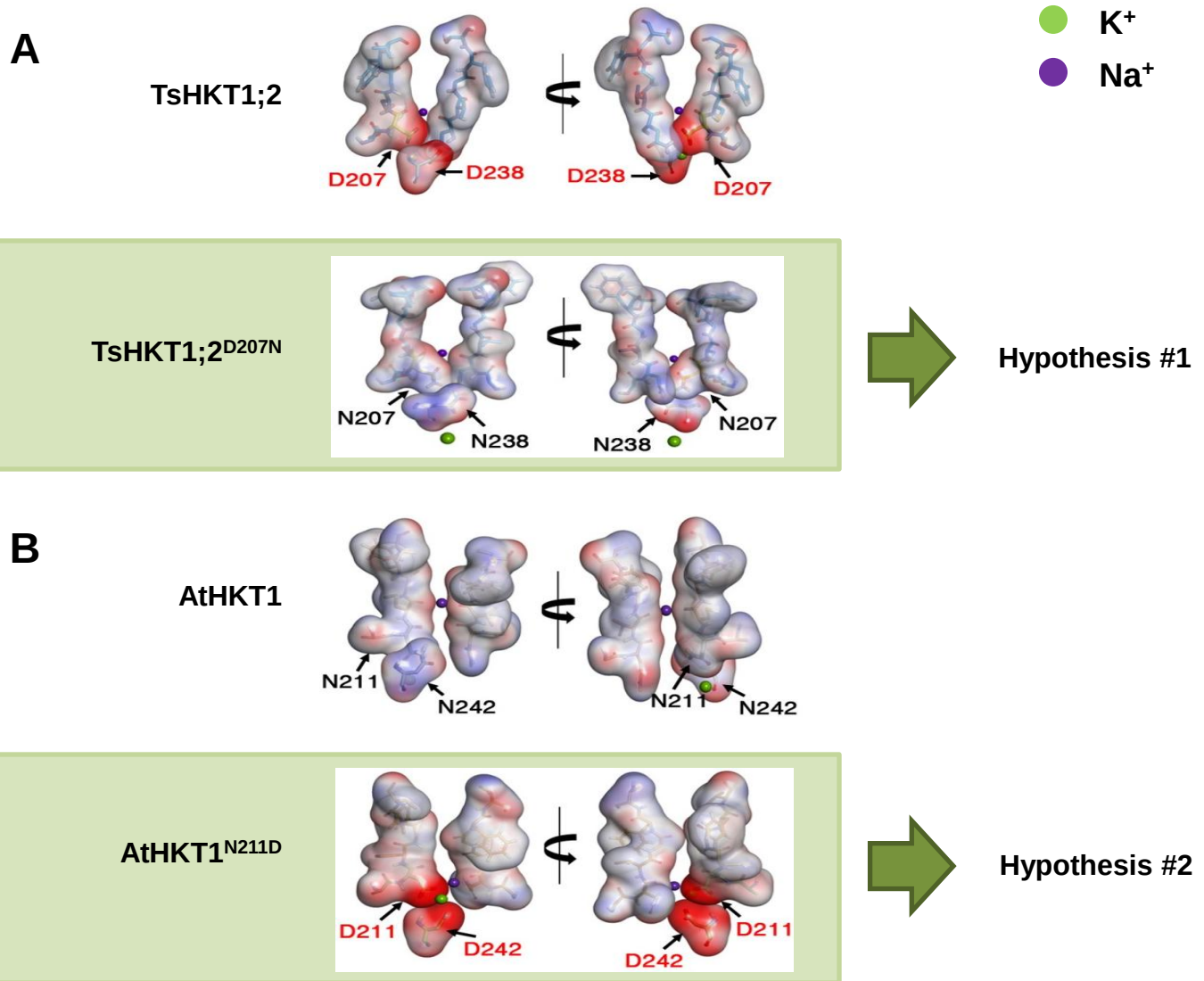


TsHKT1;2 contains Asp (D) at the ion selectivity pore-loop, while at the same position AtHKT1 contain Asn (N) residue. Presence of these conserved residues might responsible for ion selectivity.

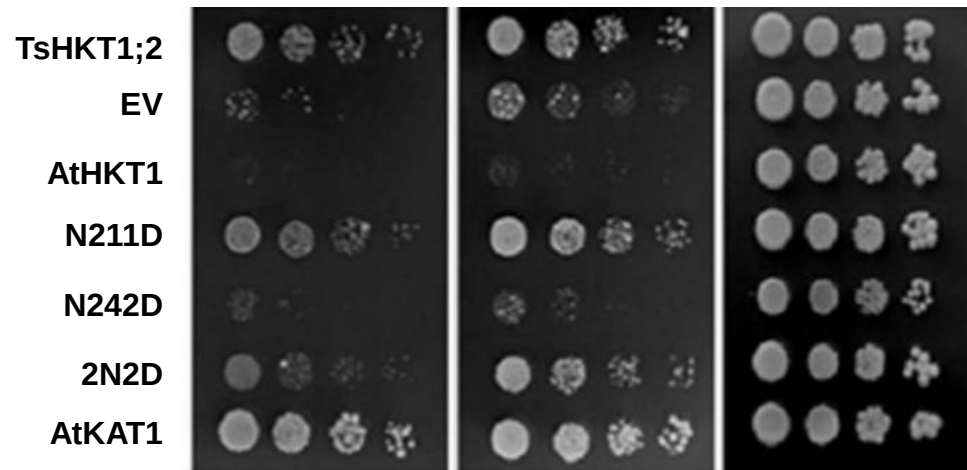
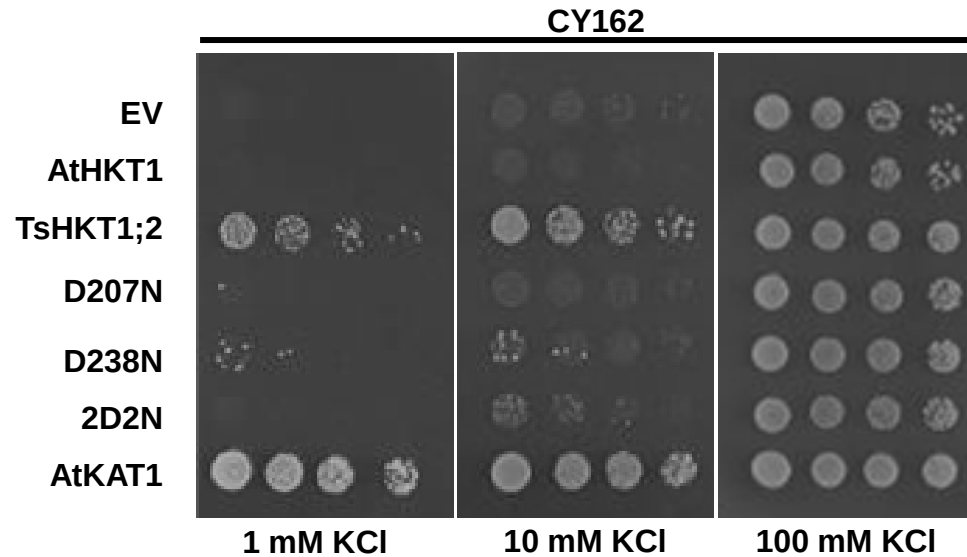
Comparison of HKT1 homologs from different plant species

			↓	↓	
TsHKT1;2	193	PLTFSVFTAVSTLSDCGFVPTNENMIIFRKNSGLLWLLIPQVFMGDTLFPCLVLAIWGL			
AtHKT1	197	PLTFSVFTTVSTFANCGFVPTNENMIIFRKNSGLIWLLIPQVLMGNTLFPCLVLLIWGL			
AIHKT1	192	PLTFSVFTTVSTFANCGFVPTNENMIIFRKNSGLIWLLIPQVLMGNTLFPCLALLIWGL			
SIHKT1;1	250	TYIFTIFTTISSEFVSCGFVPTNENMMVFSSKNSGLLWLLIPLLLGNALYPMCLRFSIRLM			
SIHKT1;2	202	LHTFSLFTTVSTFANCGFLPTNENMMIFKNSGLLLILIPQVLLGNILFAPCLRIVIMFL			
HvHKT1;5	216	MHTFSVFTIVSTFANCGFMPNNEGMAFSRFPGLLLLVMPhVLLGNLTFPVFLRLAIWAL			
HvHKT2;1	238	IVLFSLSVTVASIANGGLVPTNENMVIFSKNSGLLLLLSGQILAGNLLFPLFLRLVWFL			
OsHKT1;1	259	MYTFCIFTAVSSFANCGFTPLNSNMQPFKKNWVLLLLVIPQILAGNTLFSPLLRLCVWL			
OsHKT1;3	232	IIVFSVFTAISSEVNCGFTPVNENMIIFQKNSILLLLIPQILAGNTLFPCLRLMVWSL			
OsHKT1;4	198	TWTFVFTTVSTFANCGFMPTNENMVVFKRDAPLQLLLVPQVLGNLTFAPLLAACVWAA			
OsHKT1;5	248	AHTFAIFTTVSTFANCGFVPTNEGMSFRSFPGLLLLVMPhVLLGNLTFPVFLRLAIAAL			
OsHKT2;1	228	IALFSFSVTVSSFANGLVPTNENMAIFSKNPGLLLLFIGQILAGNTLYPLFLRLLIWFL			
OsHKT2;2	228	IALFSFSVTVSSFANGGLVPTNENMAIFSKNPGLLLLFIGQILAGNTLYPLFLRLIWIWFL			
OsHKT2;3	205	KALFSISVTVSSFTNGGLLPTNESMVVSSNGLLLLLIGQILAGSTLFPVFLRLVIWAL			
OsHKT2;4	205	KALFSISVTVSSFTNGGLLPTNESMAVSSNGLLLLLIGQILAGSTLLPMFLRLVIWAL			
PhaHKT2;1	228	VALFSVSVTVSSFANGGLVPTNENMAIFSKNAGLLLLTGQVLAGNTLFPFLRLLIWFL			
PtHKT1;1	203	IQTFSVFTTVSTFANCGFVPTNENMVAFFKNSGLLLILIPQILLGNLTPSCLRFLIWIWFL			
PutHKT2;1	227	IVLFSISITVSSCANAGLVPTNENMAIFSKHSGLLLLSGMILAGNTLFPFLRLVWFL			
SbHKT1;3	233	FVIFSFTAVSSVANCGFTPVNENMIIFQKRSILLLLIPQILAGNTLFPCLRFLVWSL			
SbHKT1;4	263	VWTFVFTTVSTFSSCGFMPTNENMMVFSRDVPLQLLLVPQALVGNLTFPPLAACVWAA			
SbHKT1;5	176	LSTFSVFTTVSTFANCGFVPTNEGMAFSKFPGLLLLVMPhILLGNLTFPVFLRLSITAL			
SbHKT2;1	221	AALFSVFATVSSFTNGGLLPTNESMAVFAPNRGLLLLLAAQILAGSTLLPVFLRLVVSAT			
TaHKT1;5-B1	219	LHTFSVFTTVSTFANGGFVPNNEGMMVFRSFPGLLLLVMPhVLLGNLTFPVFLRLAIWAL			
TaHKT1;5-B2	215	LHTFSVFTTVSTFANGGFMPNNEEMVAFRSFPGLLLLVMPhVLLGNLTFPVFLRLAIWAL			
TaHKT1;5-D	217	LHTFSVFTTVSTFANCGFVPNNEGMIAFRSFPGLLLLVMPhVLLGNLTFPVFLRLAIWAL			
TaHKT2;1	230	IVLFSLSVTVASCANAGLVPTNENMVIFSKNSGLLLLLSGQMLAGNTLFPFLRLVWFL			
TmHKT1;4-A2	254	VWTFVFTTVSTFSSCGFMPTNENMAVFQRDTGLQLLLVPQVLGNLTFAPLLAACVRAA			
TmHKT1;5-A	212	LHTFSVFTTVSTFANCGFVPNNEGMMVAFRSFPGLLLLVMPhVLLGNLTFPVFLRLAIWAL			
ZmHKT1;5	170	LSTFSVFTTVSSFANCGFVPTNEGMSFSPGMLLLLVMPhILLGNLTFPIFLRLSITAL			
ZmHKT2;1	233	AALFSVSATVSSFTNGGLLPTNESMAVFAANRGLLLLLAGQILAGCTLLPVFLRLAVGAT			
ScTRK1	666	PTWGWFTAMSAFNDLGLTLTPNSMMSFNKAVYPLIVMIWFIIGNTGFPILLRCIWIWFL			
			P_B	$M2_B$	

Molecular modeling of TsHKT1;2, AtHKT1 and their mutants versions



Presence of Asp (D) is essential for K⁺-uptake in HKTs



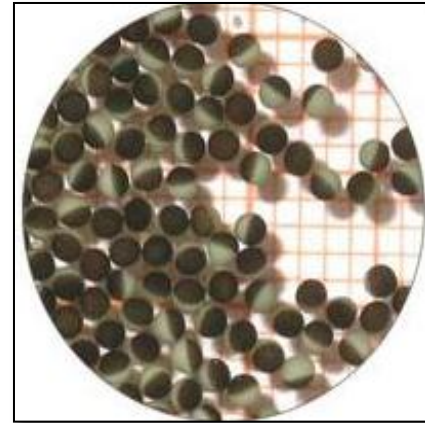
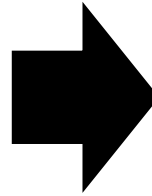
TsHKT1;2 → K⁺ IF D207N → AtHKT1

AtHKT1 → Na⁺ IF N211D → TsHKT1;2

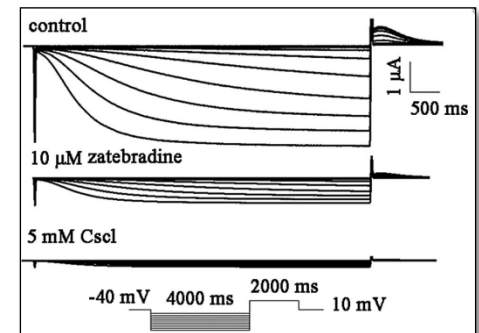
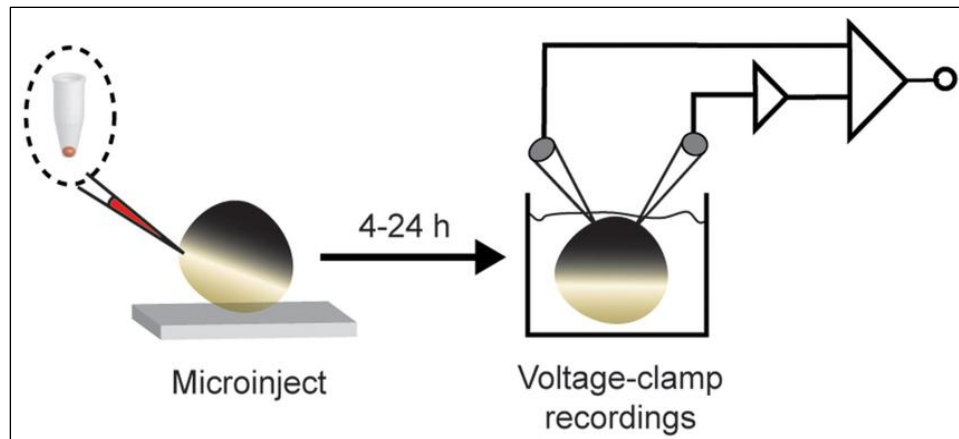
Expression of AtHKT1, TsHKT1;2 and Their mutants in *Xenopus* oocytes



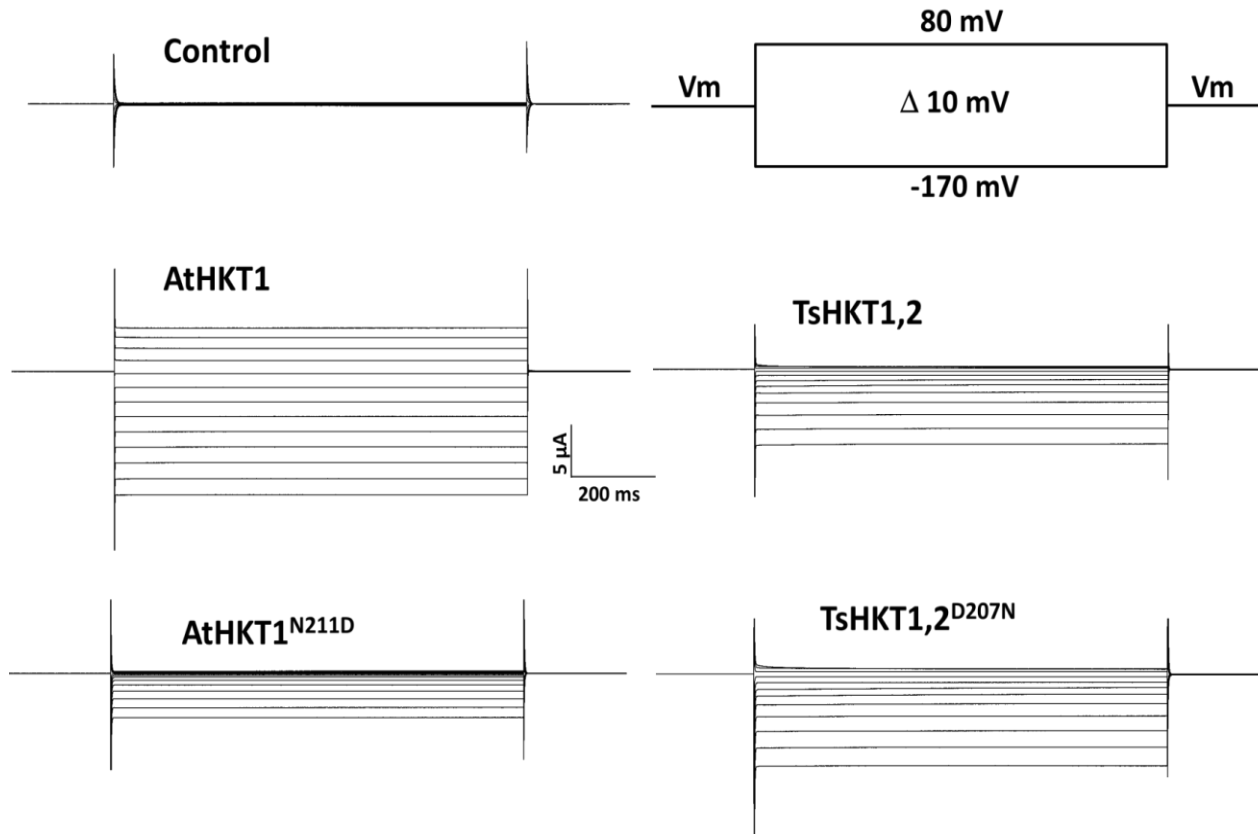
Xenopus



Xenopus oocytes

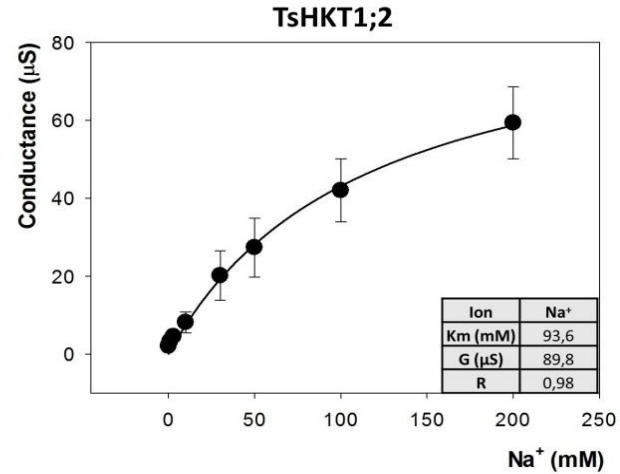
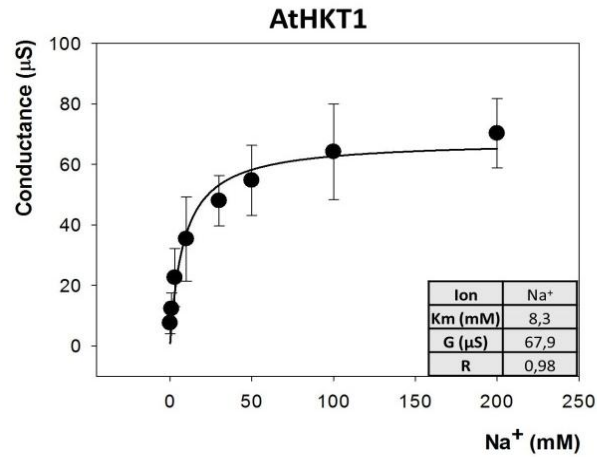


Na⁺-dependent currents generated by HKT1 transporters compared to control *X. laevis* oocytes

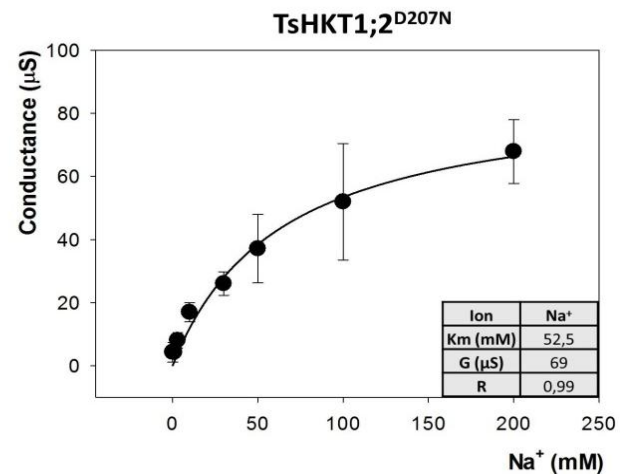
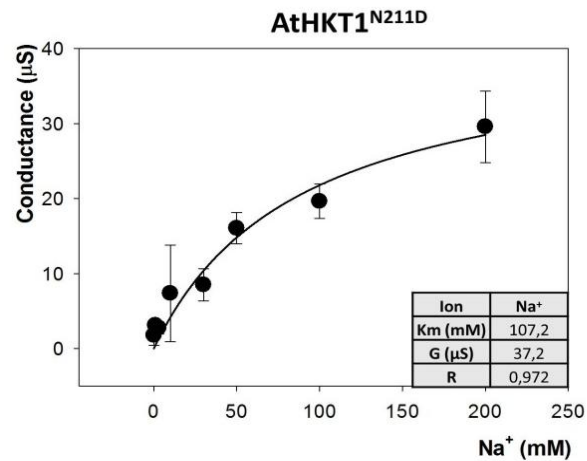


Na⁺-dependent conductivity of AtHKT1 were 4-times higher than TsHKT1;2

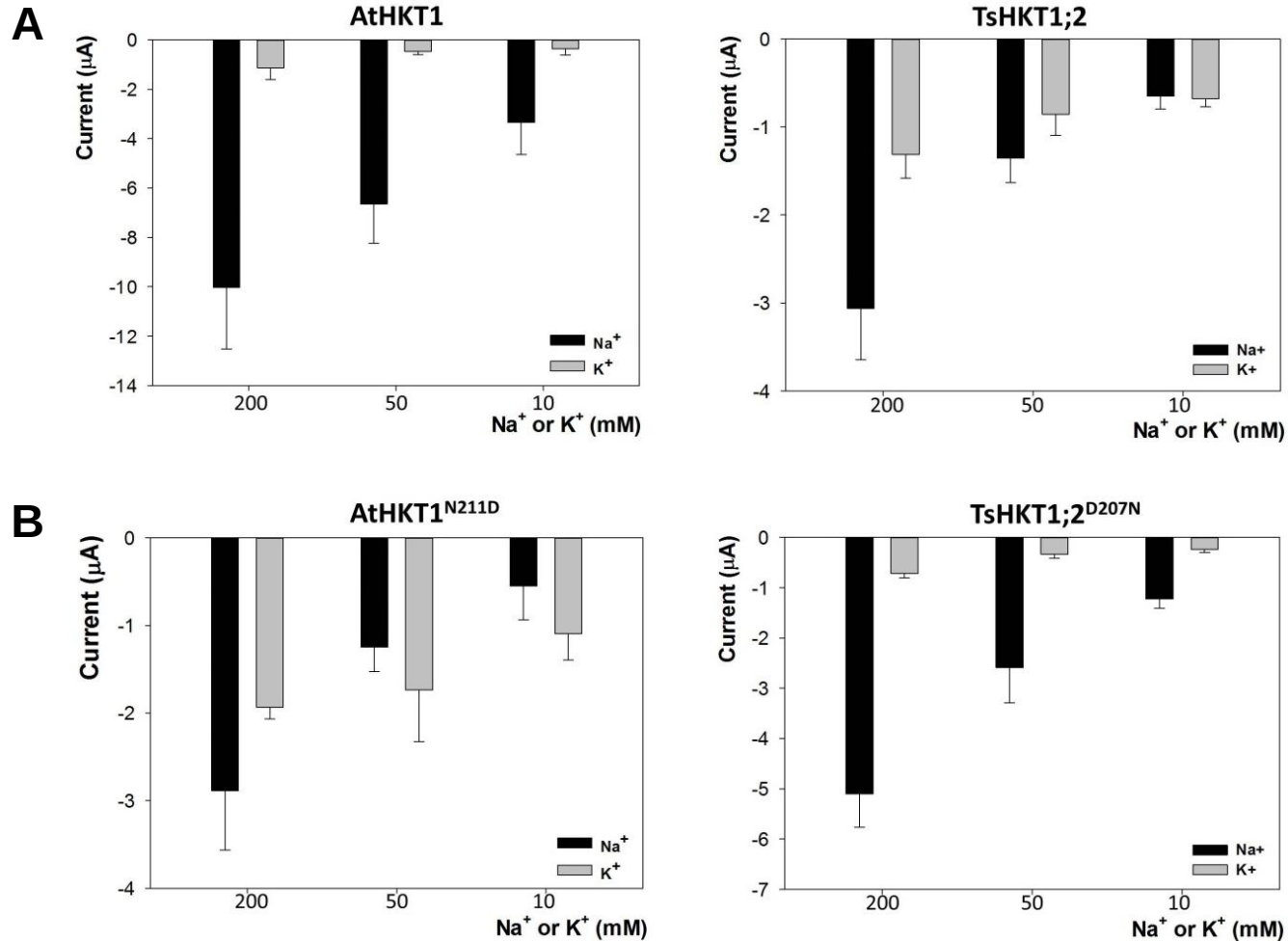
A



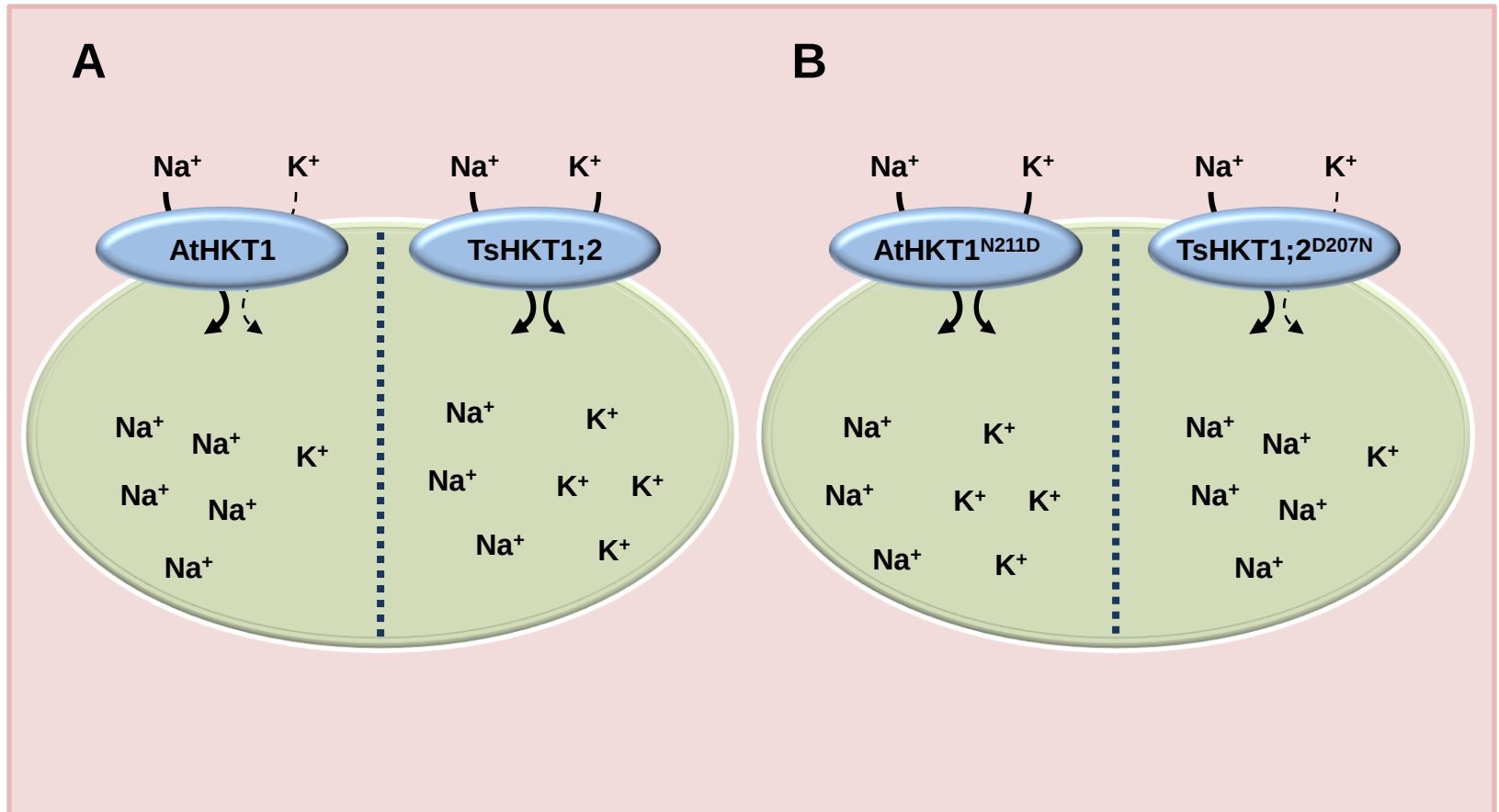
B



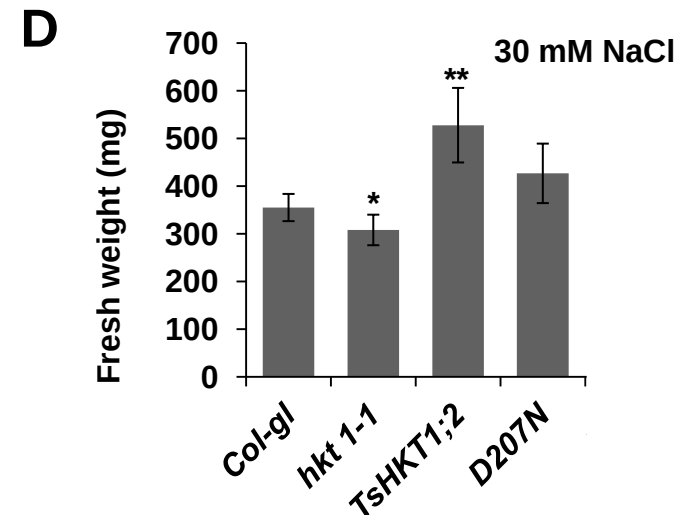
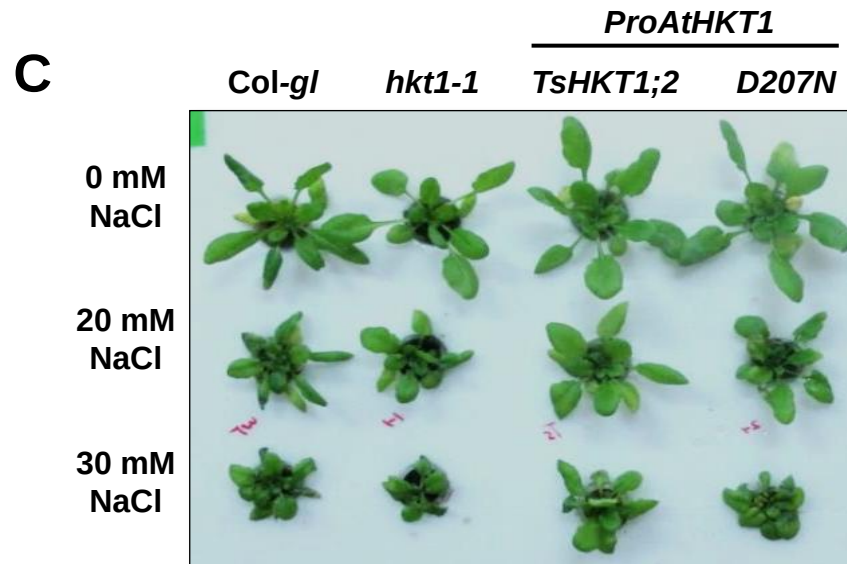
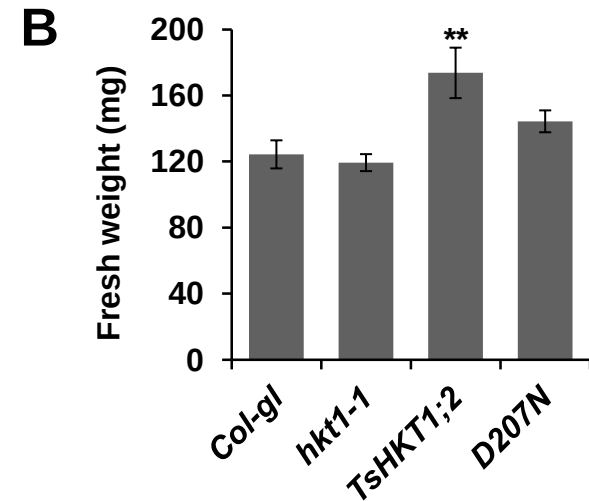
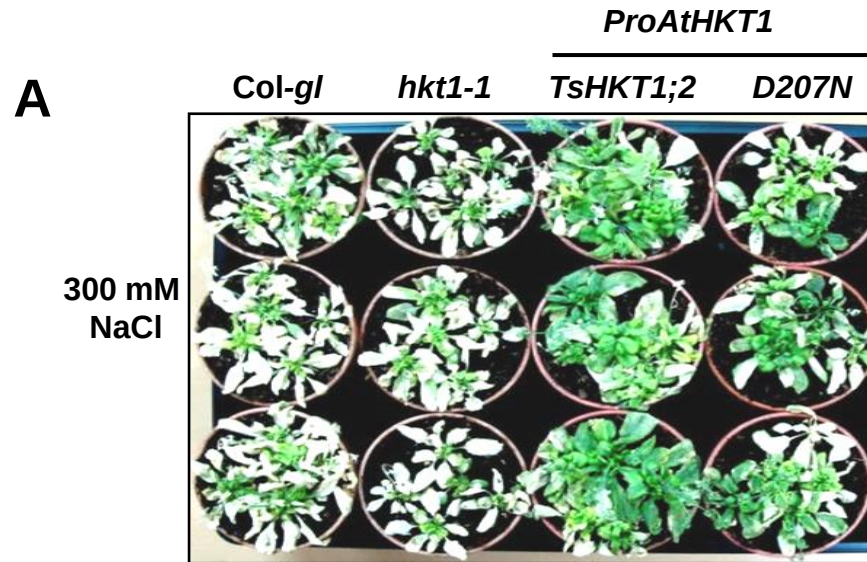
K⁺ dependent currents were 10 times lower than the Na⁺ in AtHKT1 which is only 2.4-times lower in TsHKT1;2



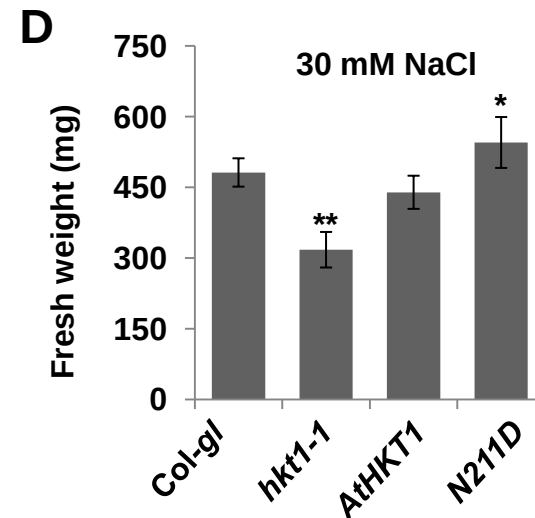
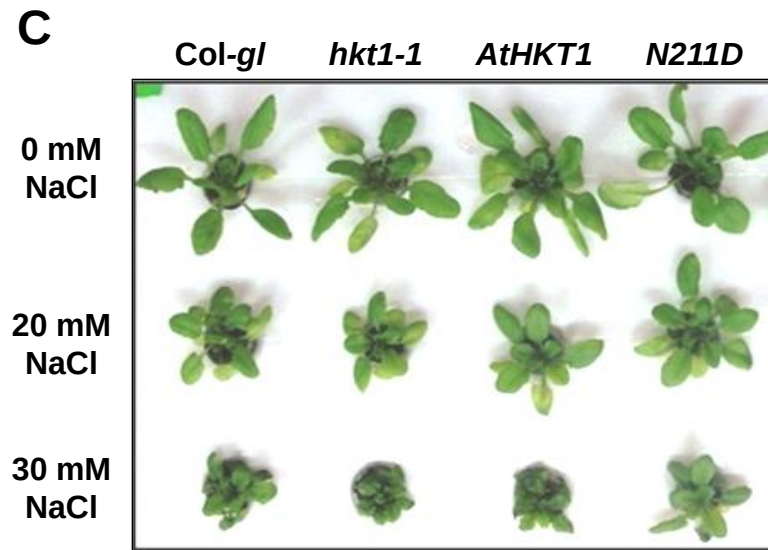
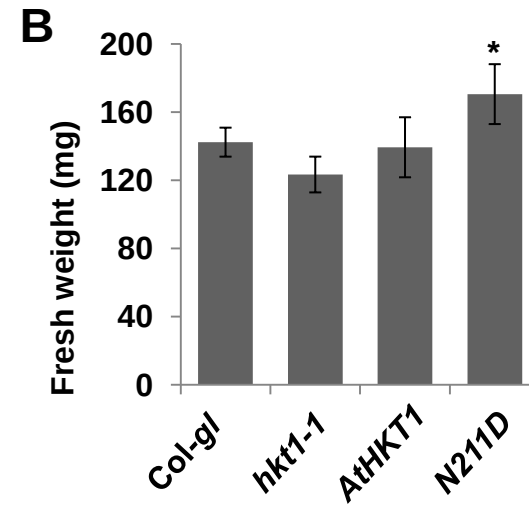
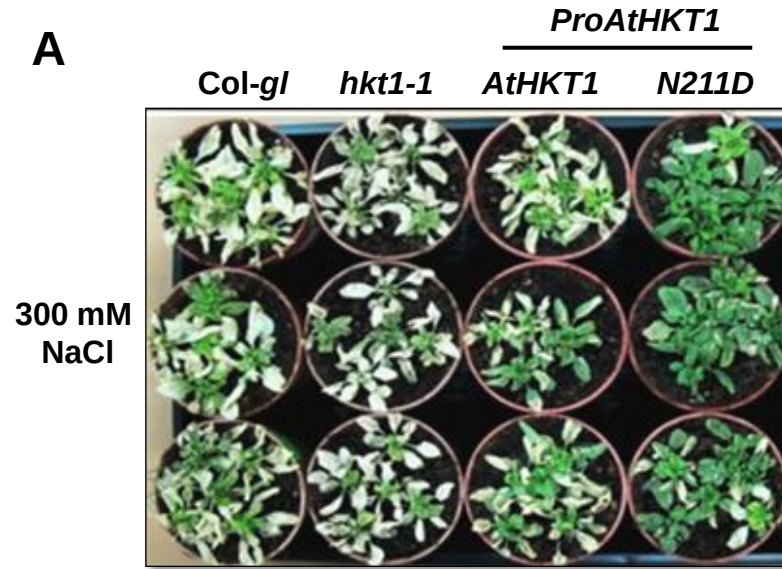
Substitution of N211D and D207N completely change the cation selectivity of AtHKT1 and TsHKT1;2 respectively



TsHKT1;2^{D211N} expressing plants are salt sensitive

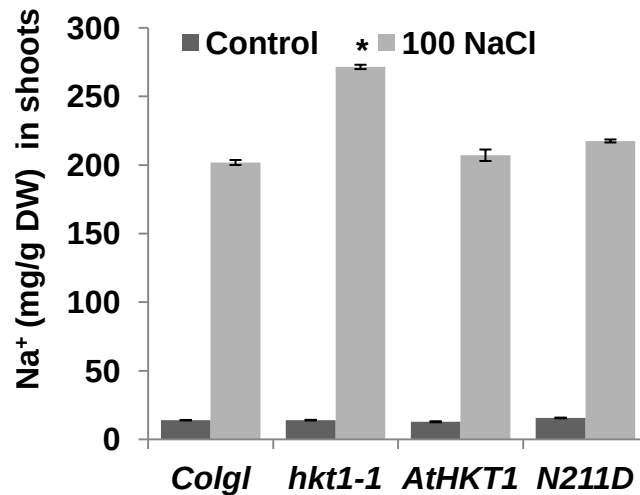


AtHKT1^{N211D} expressing plants tolerate salt stress

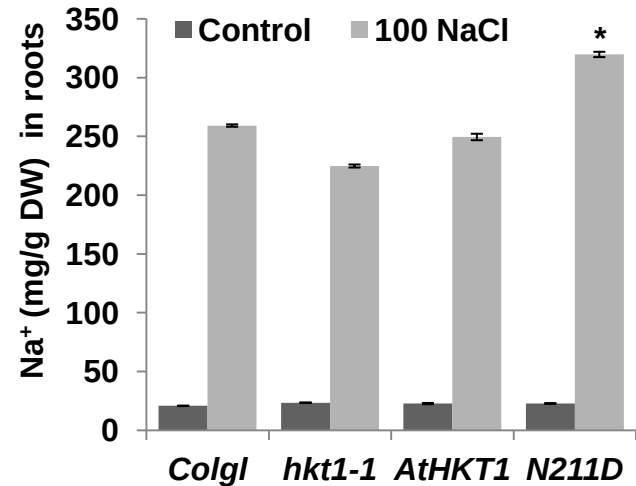


AtHKT1^{N211D} expressing plants accumulate less Na⁺ in the shoots than roots

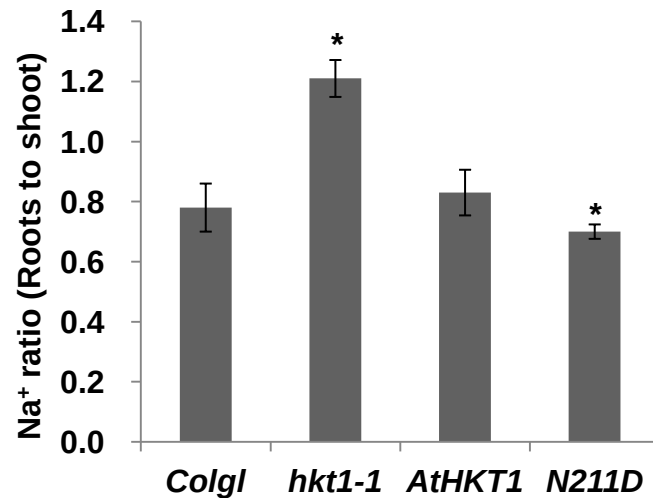
A



B



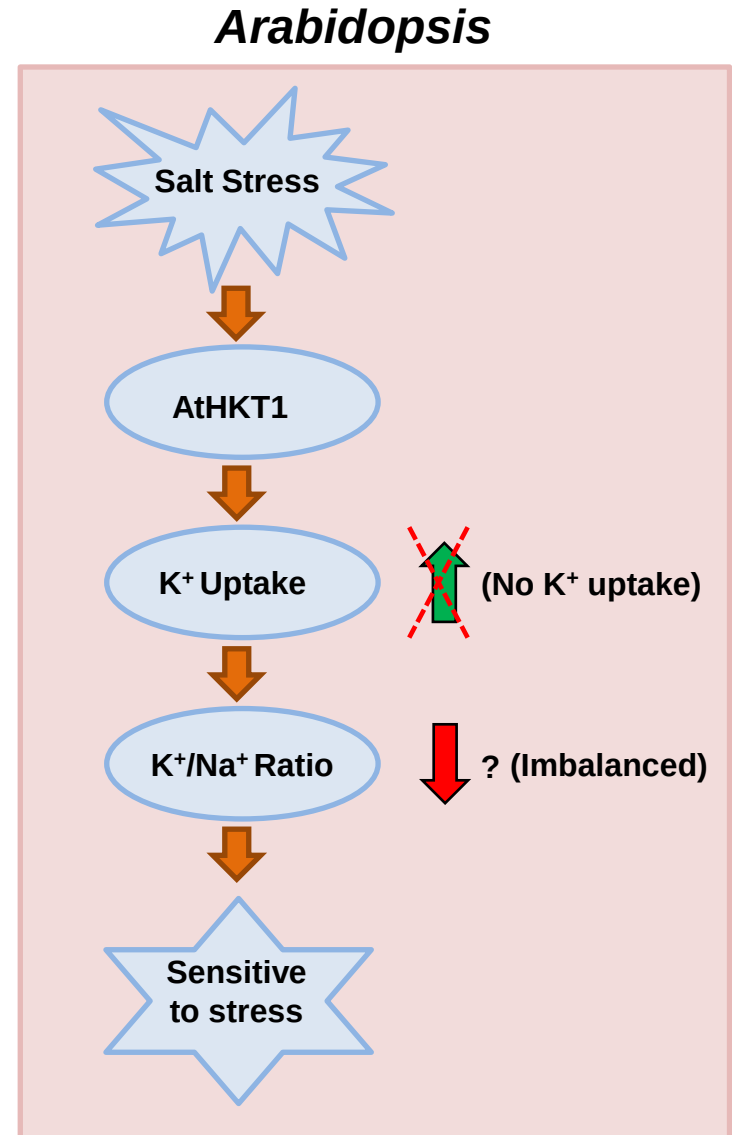
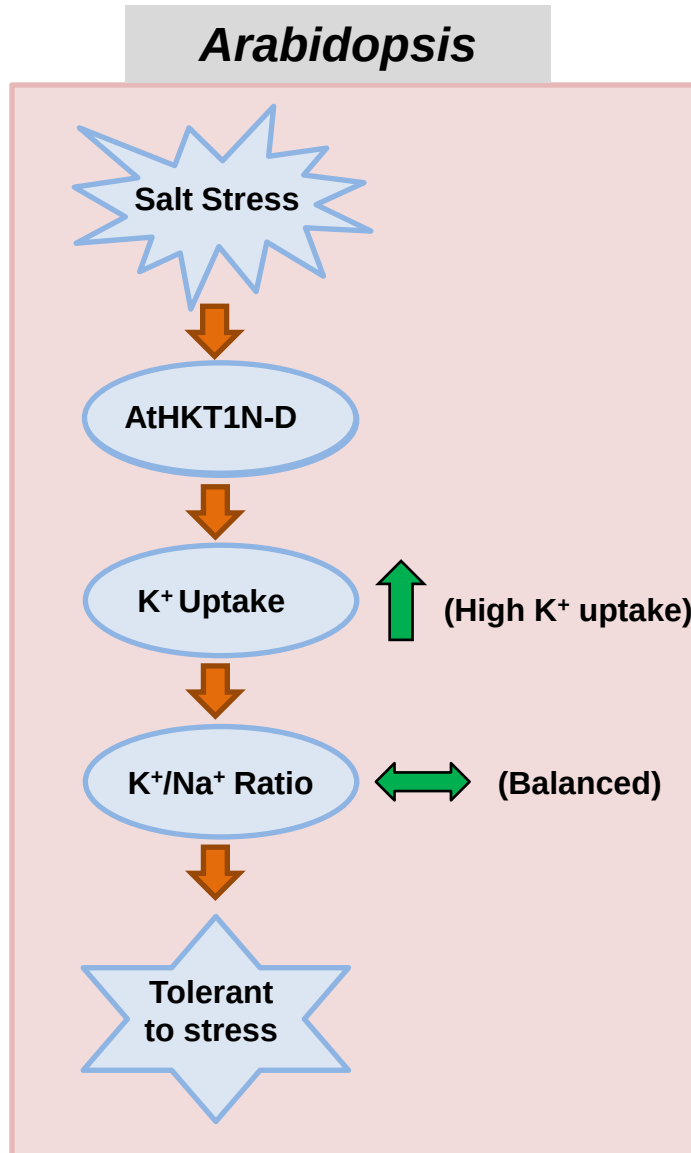
C



Summary

In summary, expressing **TsHKT1;2** (**AtHKT1**^{N211D}), in the cells and tissues that normally express the **AtHKT1** protein leads to an increase in potassium ions such that the deleterious effect of Na⁺ is counteracted and tolerate salt stress.

Hypothetical model of HKT1 in *Arabidopsis* and *Thellungiella*



Thanks