
Cell type-specific manipulation of salt tolerance genes in wheat and barley

Mahima Krishnan

B.Sc. (Hons)

A dissertation submitted to the University of Adelaide in accordance with the requirements of
the degree of PhD in the Faculty of Science, School of Agriculture, Food and Wine

April 2013

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. I acknowledge that copyright of published works contained within this thesis as listed below resides with the copyright holders of those works.

Signed:.....Date:.....

Acknowledgements

I thank my supervisors Dr. Andrew Jacobs and Prof. Mark Tester for their enduring support, positivity and encouragement throughout my PhD.

I thank Alex Kovalchuk, Dr. Andrew Harvey, Angus Syme, Dr. Bettina Berger, Dr. Boris Parent, Dr. Caitlin Byrt, Grace Zurawska, Gwenda Mayo, Jan Nield, Jessica Smith, Jodie Kretschmer, Lidia Mischis, Dr. Margie Pallotta, Dr. Matthew Gilliam, Melissa Pickering, Dr. Rachel Burton, Dr. Rana Munns, Robin Hosking, Prof. Scott Tingey, Dr. Stuart Roy, Dr. Trevor Garnett and Ursula Langridge for their timely help and advice during my PhD studies.

A special thank you to Prof. Karen Gibb who welcomed me into Gibblab and provided me support in various ways during my time in Darwin.

I thank my dear comrades and friends Ash, Dr. Lily, Jess, Jubes, Bards, Machi, Sundars and Julia for their geological patience, faith, mentorship and encouragement.

Finally, I thank my family for everything.

To my dear Karachurs and Krishnans

“I love you to pieces

I dedicate to you, this thesis”

-Jorge Cham

List of Publications

Conference posters

Mahima Krishnan, Caitlin Byrt, Alexander Johnson, Rana Munns, Mark Tester and Andrew Jacobs “*TaHKT1;5-D* is important in controlling shoot Na^+ accumulation in bread wheat.” IWPMB 2010 Adelaide, Australia.

Mahima Krishnan, Caitlin Byrt, Alexander Johnson, Rana Munns, Mark Tester and Andrew Jacobs “*TaHKT1;5-D* is important in controlling shoot Na^+ accumulation in bread wheat.” Gordon Research Conference on Salt and Water Stress in Plants 2010 Les Diablerets, Switzerland.

Mahima Krishnan, Caitlin Byrt, Alexander Johnson, Rana Munns, Mark Tester and Andrew Jacobs “Is *TaHKT1;5-D* important for salt tolerance in bread wheat?” ACPFG Genomics Symposium, The Genomics of Salinity 2009, Adelaide.

Mahima Krishnan, Andrew Jacobs, Alexander Johnson and Mark Tester. “Promot(er)ing salt tolerance in plants.” The University of Adelaide, School of Agriculture, Food and Wine Research Day 2008 Adelaide, Australia.

Mahima, Krishnan, Andrew Jacobs, Alexander Johnson and Mark Tester. “Engineering salt tolerant barley.” ACPFG Genomics Symposium, The Genomics of Drought 2007 Adelaide, Australia.

Oral presentations

Mahima Krishnan, Andrew Jacobs and Mark Tester “Salinity Tolerance in cereals”. Final PhD seminar 2011. University of Adelaide, Australia.

Mahima Krishnan, Alexander Johnson, Mark Tester and Andrew Jacobs. “Building a de-sal plant”. CPIB, University of Nottingham, Nottingham UK. 2010.

Mahima Krishnan, Alexander Johnson, Mark Tester and Andrew Jacobs. “Building a de-sal plant”. INRA, Montpellier, France. 2010.

Mahima Krishnan, Andrew Jacobs, Alexander Johnson and Mark Tester. “Cell type-specific expression of salt tolerance genes in barley.” The University of Adelaide, School of Agriculture, Food and Wine Postgraduate Symposium 2000 Adelaide, Australia.

Mahima Krishnan, Andrew Jacobs, Alexander Johnson and Mark Tester. “Cell type-specific expression of *HvHKT1;5* and *HvHVPI* in Barley (*Hordeum vulgare*)”. ACPFG Joint Research Meeting 2008 Adelaide, Australia.

Table of Contents

Declaration.....	2
Acknowledgements.....	3
List of Publications.....	5
List of Figures.....	14
List of Tables.....	16
List of Appendices.....	17
List of Abbreviations.....	18
Abstract.....	21
1 General introduction.....	23
1.1 Soil salinity and its impacts on Agriculture with a focus on Australia.....	23
1.2 Osmotic stress in plants.....	26
1.3 Ionic stress in plants.....	26
1.4 How do plants cope with high salt levels?.....	27
1.4.1 Na ⁺ exclusion.....	28
1.4.2 Na ⁺ tolerance.....	28
1.5 Genes important in controlling root to shoot translocation of Na ⁺	31
1.5.1 The exclusion mechanism.....	31
1.5.1.1 <i>The high-affinity K⁺ transporter (HKT) gene family</i>	31
1.5.1.2 <i>AtHKT1;1 and its role in salinity tolerance</i>	31
1.5.1.3 <i>HKT1;5 in barley and wheat and its role in salinity tolerance</i>	32
1.5.1.4 <i>Salt overly sensitive (SOS) pathway</i>	33
1.5.2 Tissue tolerance mechanism.....	35
1.5.2.1 <i>Vacuolar H⁺ translocating pyrophosphatase (H⁺PPase)</i>	35

1.5.2.2	<i>Na⁺/H⁺ antiporter (NHX1)</i>	37
1.6	The role of different cell types in a plant's salinity tolerance.....	39
1.7	Increasing salt tolerance of plants.....	40
1.7.1	Strategy for increasing salt tolerance employed by this study.....	41
1.7.1.1	<i>Aim- Enhance Na⁺ exclusion through the unloading mechanism through cell type-specific overexpression of HvHKT1;5</i>	41
1.7.1.2	<i>Aim- Study the importance of HKT1;5 for salt tolerance in bread wheat through employing a gene knockdown approach through RNAi</i>	41
1.7.1.3	<i>Aim- Enhance Na⁺ exclusion through the tissue tolerance mechanism through cell type-specific overexpression of HvHVP1</i>	42
1.8	Thesis outline.....	42
2	Identification and isolation of putative root cortex- and stelar- specific promoters from maize and rice.....	44
2.1	Introduction.....	44
2.2	Materials and methods.....	45
2.2.1	MPSS database searches.....	45
2.2.1.1	<i>MPSS candidate naming system</i>	46
2.2.2	EST sequence retrieval and primer design for semi-quantitative PCR.....	47
2.2.3	Tissue isolation.....	47
2.2.3.1	<i>Root-cortex and -stelar tissue isolation</i>	47
2.2.3.2	<i>Maize reproductive tissue isolation</i>	50
2.2.4	RNA extraction of maize tissue.....	50
2.2.5	Maize PCR to validate MPSS data.....	51
2.2.6	Identification of EST/gene orthologues in rice and isolation of promoters.....	53
2.2.7	Generation of promoter- <i>uidA/GFP</i> fusion lines.....	55
2.2.7.1	<i>Isolation of putative cell type-specific promoters</i>	55
2.2.7.2	<i>Generating entry vectors</i>	57

2.2.7.3	<i>Generating destination vectors</i>	58
2.2.8	Assaying for reporter gene activity.....	60
2.2.8.1	<i>Green fluorescent protein (GFP) detection</i>	60
2.2.8.2	<i>Beta-glucuronidase (GUS) assay</i>	60
2.3	Results.....	61
2.3.1	MPSS signature tags	61
2.3.2	Maize EST sequences corresponding to the candidate MPSS signature tags	63
2.3.3	Validation of spatial patterns of mRNA in MPSS data with PCR on maize tissue series	65
2.3.3.1	<i>Rice orthologues</i>	77
2.3.3.2	<i>GFP assay</i>	77
2.3.3.3	<i>GUS assay</i>	77
2.4	Discussion	77
2.4.1	Confirmation of MPSS transcript patterns	78
2.4.2	Rice promoters.....	80
2.5	Conclusion	82
3	Cell type-specific overexpression of <i>HvHKT1;5</i> and <i>HvHVP1</i> in barley as a strategy to increase its salt tolerance	84
3.1	Introduction.....	84
3.2	Materials and methods	87
3.2.1	Isolation of <i>HvHKT1;5</i> and <i>HvHVP1</i>	87
3.2.1.1	<i>Primer design</i>	87
3.2.1.2	<i>Growing Hordeum vulgare (cv. Golden Promise) for RNA extraction</i>	89
3.2.1.3	<i>RNA extracted from shoot and root</i>	91
3.2.1.4	<i>Generating cDNA</i>	91
3.2.1.5	<i>PCR conditions</i>	91

3.2.2	Generating constructs	91
3.2.2.1	<i>Entry vectors</i>	91
3.2.2.2	<i>Destination vectors</i>	92
3.2.2.3	<i>Promoter insertion into destination vectors</i>	94
3.2.3	Transgenic plants	94
3.2.4	Assaying transgenic plants.....	94
3.2.4.1	<i>Identifying single insert lines</i>	94
3.2.4.2	<i>Salt stress study</i>	97
3.2.4.2.1	<i>Growth of transgenic barley plants</i>	98
3.2.4.2.2	<i>Genotyping barley individuals in salt stress study</i>	99
3.2.4.2.3	<i>Measuring leaf sodium</i>	99
3.2.4.2.4	<i>Management of data</i>	99
3.2.4.3	<i>Confirming the transcription of the transgene</i>	100
3.3	Results.....	101
3.3.1	Identification of single insert line	101
3.3.2	Accumulation of Na ⁺ and K ⁺ in 4 th leaf of transgenic barley	103
3.3.2.1	<i>Control treatments</i>	103
3.3.2.1.1	<i>Transgenic plants containing HvHKT1;5 or HvHVP1 under the control of C34</i>	103
3.3.2.1.2	<i>Transgenic plants containing HvHKT1;5 or HvHVP1 under control of C257</i>	107
3.3.2.1.3	<i>Transgenic plants containing HvHKT1;5 under control of S147 and Ta...</i>	109
3.3.2.2	<i>Salt stress treatment</i>	111
3.3.2.2.1	<i>Transgenic plants containing HvHKT1;5 or HvHVP1 under control of C34</i>	111

3.3.2.2.2	<i>Transgenic plants containing HvHKT1;5 or HvHVP1 under control of C257</i>	113
3.3.2.2.3	<i>Transgenic plants containing HvHKT1;5 under control of S147 and Ta...</i>	115
3.3.3	Transgene mRNA	117
3.3.3.1	<i>Salt stress experiment 1: transgene mRNA</i>	117
3.3.3.2	<i>Salt stress experiment 2: transgene mRNA</i>	120
3.3.3.3	<i>Salt stress experiment 3: transgene mRNA</i>	123
3.4	Discussion	126
3.4.1	Plants containing stelar-specific S147 promoter upstream of <i>HvHKT1;5</i>	126
3.4.2	Why isn't accumulation of shoot Na ⁺ reduced in plants transformed with <i>HvHKT1;5</i> under the control of Ta, putative stelar-specific promoter isolated from upstream of wheat <i>TaHKT1;5-D</i> ?	128
3.4.3	Cortex-specific promoters driving transgenic <i>HvHKT1;5</i> and <i>HvHVP1</i>	129
3.4.3.1	<i>Putative cortex-specific promoters driving HvHKT1;5 do not appear to reduce shoot Na⁺</i>	129
3.4.3.2	<i>Putative cortex-specific promoters driving HvHVP1 do not appear to reduce shoot Na⁺</i>	130
3.4.3.3	<i>Limitations of performing experiments with T₁ plant lines</i>	131
3.4.4	Na ⁺ exclusion and salt tolerance	131
3.4.5	Transgenic gene activity	132
3.5	Conclusion	132
4	Role of <i>TaHKT1;5-D</i> as a salt tolerance determinant in bread wheat	134
4.1	Introduction	134
4.2	Materials and methods	135
4.2.1	Assaying the effect of the RNAi on T ₂ lines of <i>T. aestivum</i> cv. Bobwhite	135
4.2.1.1	<i>Growth of transgenic T. aestivum cv. Bobwhite plants</i>	138
4.2.1.2	<i>Leaf and root sampling of plants</i>	138

4.2.1.3	<i>Genotyping transgenic T. aestivum cv. Bobwhite plants</i>	139
4.2.1.3.1	DNA extraction	139
4.2.1.3.2	PCR determination of plants as positive or null for construct.....	139
4.2.1.4	<i>RNA extraction of root tissue</i>	139
4.2.1.5	<i>Generating cDNA</i>	140
4.2.1.6	<i>Quantitative PCR Primer design</i>	140
4.2.1.7	<i>Quantitative PCR</i>	140
4.2.1.8	<i>Leaf Na⁺ and K⁺ measurements</i>	141
4.2.2	<i>TaHKT1;5-D, TaSOS1, TaNHX1</i> transcript analysis of wildtype <i>T.aestivum</i> cv. Bobwhite plants	141
4.2.3	High-throughput phenotyping of T ₃ plants containing RNAi transgene in The Plant Accelerator	142
4.2.3.1	<i>Preparation of soil for growth of TaHKT1;5-D knockdown lines</i>	142
4.2.3.2	<i>Growth of T.aestivum TaHKT1;5-D knockdown lines</i>	142
4.2.3.3	<i>Genotyping T₃ RNAi plants grown in the APPF Plant Accelerator</i>	143
4.2.3.4	<i>Studying changes in phenotype as a result of the RNAi construct in T. aestivum grown in soil</i>	143
4.2.3.4.1	Fourth leaf Na ⁺ and K ⁺	143
4.2.3.4.2	Non-destructive imaging of plant biomass changes over time.....	144
4.3	Results	144
4.3.1	Native gene repression in RNAi knockdown <i>T.aestivum</i> lines	144
4.3.2	Na ⁺ and K ⁺ accumulation in 4 th leaf sap in RNAi knockdown <i>T. aestivum</i> lines	147
4.3.3	Transcript patterns of <i>TaHKT1;5-D, TaSOS1</i> and <i>TaNHX1</i> in wild type <i>T. aestivum</i> cv. Bobwhite plants	149
4.3.4	Salt tolerance of <i>T.aestivum</i> lines containing RNAi construct	151
4.3.5	Yield component analysis	157

4.3.6	Changes in plant biomass	159
4.3.6.1	<i>Biomass over time and relative growth rate of RNAi plants grown in control conditions s.....</i>	<i>159</i>
4.3.6.2	<i>Biomass over time and relative growth rate of RNAi plants grown in salt stress conditions.....</i>	<i>159</i>
4.4	Discussion	162
4.4.1	Effect of the RNAi on endogenous <i>TaHKT1;5-D</i> expression	162
4.4.2	Issue of RNAi specificity.....	163
4.4.3	Dosage effect of RNAi	163
4.4.4	Role of <i>TaHKT1;5-D</i> in K^+/Na^+ discrimination	164
4.4.5	Is <i>TaHKT1;5-D</i> a salt tolerance determinant?	165
4.5	Conclusion	166
5	General discussion of the results	168
5.1	Genetically engineering Na^+ exclusion in barley plants	168
5.1.1	Conservation of promoter control between different species- lessons learned	168
5.1.2	Unintended consequences- promoter control in barley was different to what was expected but still generated a result where Na^+ exclusion was improved.....	169
5.1.2.1	<i>Na^+ exclusion through HvHKT1;5 overexpression</i>	<i>169</i>
5.1.2.2	<i>Possible enhancement of tissue Na^+ tolerance mechanism through HvHVP1 overexpression.....</i>	<i>169</i>
5.1.3	Spatial location of transgene activity- strategies to resolve the black box	170
5.2	How important is shoot Na^+ exclusion to whole plant salinity tolerance?	171
5.2.1	In barley	171
5.2.2	In bread wheat.....	171
5.3	Future directions	172
6	References	174
	Appendix.....	184

Appendix 2.1: Table containing putative promoter sequences for C34, C257, S147 and Ta.	184
Appendix 3.1: Amino acid sequence alignment of TaHKT1;5-D and HvHKT1;5.....	187
Appendix 4.1: Sequences for RNAi 1 and RNAi 2.....	188
Appendix 4.2: Regions RNAi 1 and RNAi 2 corresponding to <i>TaHKT1;5-D</i>	189
Appendix 4.3: <i>TaSOS1</i> , <i>TaNHX1</i> and <i>TaHKT1;5-D</i> target regions for qPCR	190

List of Figures

Figure 1.1: A map showing the prevalence of soil salinity in Australian Soil with groundwater associated salinity (GAS)..	25
Figure 1.2: A schematic view of the different pathways of Na ⁺ fluxes and possible strategies employed by plants to limit net Na ⁺ accumulation in the shoot.	30
Figure 2.1 Ten day old B73 maize seedlings in two aerated growth treatments.	49
Figure 2.2 Vector maps of reporter constructs used in plant transformation containing promoters and downstream reporters <i>mgfp6</i> or <i>uidA</i> .	59
Figure 2.3a Agarose gel showing transcript patterns of cortex-specific candidate, C5, in root-cortical, root-stelar, shoot tissues and root tips under control and 3 hr treatment with 100 μM ABA and young/mature tassel, young/mature silk and ear at milk and dough stage.	66
Figure 3.1: Growth of barley plants in hydroponics.	90
Figure 3.2: Destination vector pTOOL36 containing promoter upstream of the gene of interest.	93
Figure 3.3: Concentrations of Na ⁺ (a), K ⁺ (b) in 4th leaf sap and K ⁺ /Na ⁺ ratio (c). Raw values are indicated above the respective bars.	106
Figure 3.4 Concentrations of Na ⁺ (a), K ⁺ (b) in 4th leaf sap and K ⁺ /Na ⁺ ratio(c). Raw values are indicated above the respective bars.	108
Figure 3.5 Concentrations of Na ⁺ (a), K ⁺ (b) in 4th leaf sap and K ⁺ /Na ⁺ ratio(c). Raw values are indicated above the respective bars.	110
Figure 3.6 Concentrations of Na ⁺ (a), K ⁺ (b) in 4th leaf sap and K ⁺ /Na ⁺ ratio (c) of plants grown in 100 mM NaCl + 3 mM CaCl ₂ . Raw values are indicated above the respective bars.	112
Figure 3.7 Concentrations of Na ⁺ (a), K ⁺ (b) in 4 th leaf sap and K ⁺ /Na ⁺ ratio (c) of plants grown in 100 mM NaCl + 3 mM CaCl ₂ . Raw values are indicated above the respective bars.	114
Figure 3.8: Concentrations of Na ⁺ (a), K ⁺ (b) in 4th leaf sap and K ⁺ /Na ⁺ ratio (c) of plants grown in 100 mM NaCl + 3 mM CaCl ₂ .	116
Figure 4.1: Graphs showing native gene transcript patterns in RNAi lines and respective null segregants.	146
Figure 4.2: Graphs showing Na ⁺ and K ⁺ concentrations in the 4th leaf sap of RNAi lines and respective null segregants grown under 50 mM salt stress.	148
Figure 4.3: Gene transcript patterns in wildtype <i>T.aestivum</i> cv. Bobwhite plants in control and 50 mM salt stress conditions.	150

Figure 4.4: Graphs showing Na⁺ concentrations in the 4th leaf sap of RNAi lines and respective null segregants grown under control and 75 mM salt stress conditions.152

Figure 4.5: Graphs showing K⁺ concentrations in the 4th leaf sap of RNAi lines and respective null segregants grown under control and 75 mM salt stress conditions.....154

Figure 4.6: Graphs showing K⁺/Na⁺ ratio in the 4th leaf sap of RNAi lines and respective null segregants grown under control and 75 mM salt stress conditions.156

Figure 4.7: Graphs showing 100 seed weight (gm) of RNAi 1 and RNAi 2 compared with their respective controls grown in control treatment and salt stress..158

Figure 4.8: Plots showing changes in biomass over time of RNAi 1 and respective null segregants in control and salt stress (75 mM NaCl) treatment..160

Figure 4.9: Plots showing changes in biomass over time of RNAi 2 and respective null segregants in control and salt stress (75 mM NaCl) treatment..161

List of Tables

Table 2.1 MPSS candidates, primer sequences used for amplifying maize EST sequences and PCR conditions employed for PCR of maize mRNA.....	52
Table 2.2 Tissues and treatments used to generate rice MPSS data.....	54
Table 2.3 Origin of promoter sequences and respective primer sequences used in PCR to amplify promoters.....	56
Table 2.4 Cell type-specific maize MPSS candidates with signature tag and corresponding transcript levels.....	62
Table 2.5 Matching sequence ID and corresponding rice probe set ID for maize MPSS candidates.....	64
Table 3.1: <i>HvHKT1;5</i> and <i>HvHVPI</i> PCR primers and cycling conditions.....	88
Table 3.2: Independent transformants containing a single insert of the transgene.....	102
Table 4.1: Sibling lines used in experiments with T ₂ and T ₃ generation.....	137

List of Appendices

Appendix 2.1: Putative promoter sequences for C34, C257, S147 and Ta.	184
Appendix 3.1: Amino acid sequence alignment of TaHKT1;5-D and HvHKT1;5..	187
Appendix 4.1: Sequences for RNAi 1 and RNAi 2	188
Appendix 4.2: Regions RNAi 1 and RNAi 2 correspond in <i>TaHKT1;5-D</i>	189
Appendix 4.3: <i>TaSOS1</i> , <i>TaNHX1</i> and <i>TaHKT1;5-D</i> target regions for qPCR.....	190

List of Abbreviations

ABA	abscisic acid
ACPFG	Australian Centre for Plant Functional Genomics
At	<i>Arabidopsis thaliana</i>
AtAVP1	VP1 from <i>Arabidopsis thaliana</i>
ATP	adenosine triphosphate
AUD	Australian dollar
BLAST	Basic local alignment search tool
bp	base pairs
BSA	bovine serum albumin
CBL4	calcineurin B-like 4 protein
ccdB	cytotoxic coupled cell division
cDNA	complementary DNA
CIPK24	CBL-interacting protein kinase 24
CPA	cation/ proton antiporters
Cps	counts per second
DNA	deoxyribonucleic acid
EDTA	ethylene diamine tetraacetate
EST	expressed sequence tags
FACS	fluorescence-activated cell sorting
GAPdh	glyceraldehydes- 3- phosphate dehydrogenase
GAS	groundwater-associated salinity
GFP	Green fluorescent protein
GSS	genome survey sequence
GUS	β -glucuronidase
H^+ -ATPase	proton translocating ATPase
H^+ -PPase	proton translocating pyrophosphatase
HA	haemagglutinin

HKT	high- affinity K ⁺ transporter
Hv	<i>Hordeum vulgare</i>
<i>HvHVPI</i>	VP1 from <i>Hordeum vulgare</i>
Hyg	Hygromycin
KAc	Potassium acetate
LB	Luria Bertani
MCS	multiple cloning site
MgAc	Magnesium acetate
MPSS	Massively parallel signature sequence
<i>NHX1</i>	Na ⁺ /H ⁺ exchanger
Nos	Nopaline synthase
Os	<i>Oryza sativa</i>
<i>OsOVPI</i>	VP1 from <i>Oryza sativa</i>
PEG	polyethylene glycol
PPi	pyrophosphate
PTGS	post-transcriptional gene silencing
qPCR	quantitative PCR
RNA	ribonucleic acid
RNAi	RNA interference
RO	reverse osmosis
PCR	reverse transcriptase PCR
S.E.M	standard error of the mean
SDS	sodium dodecyl sulphate
SKOR	stelar K ⁺ outward rectifying channel
SOS	salt overly sensitive
SSC	salt and sodium citrate solution
Ta	<i>Triticum aestivum</i>
<i>TaCycl</i>	gene encoding cyclophilin in <i>T.aestivum</i>
<i>TaEFa</i>	gene encoding elongation factor a in <i>T.aestivum</i>

TE	tris EDTA
TPM	transcripts per million
uidA	β -glucuronidase
USDA	U.S. Department of Agriculture
UTR	untranslated region
v/v	volume per volume
VP	vacuolar H ⁺ pyrophosphatase
w/v	weight per volume
WEA	Wheat Exports Australia

Abstract

More than 67% of Australian cropping land is at risk of becoming saline and agriculture is increasingly utilising salt affected land (Rengasamy, 2002). Salinity has a significant impact on crop yield, and the identification and manipulation of genes that help to ameliorate yield penalties resulting from salinity can enhance agricultural production.

Bread wheat, a hexaploid with AABBDD genome, has been long considered more salt tolerant than the tetraploid durum wheat with an AABB genome. The D genome, originally from *Aegilops tauschii*, contains a locus important for maintaining high K^+/Na^+ , *Kna1*, on chromosome 4, which contains the *HKT1;5* gene encoding a Na^+ specific transporter, *TaHKT1;5-D*. The transcript of this gene was knocked down through RNAi. Plants containing the RNAi construct were found to accumulate higher levels of Na^+ in the 4th leaf regardless of whether they were grown under control or mild salt stress conditions (75mM). This result supports previous findings that orthologues of *HKT1;5* in other plants influence Na^+ translocation from root to shoot (Ren *et al.*, 2005; Davenport *et al.*, 2007). The impact of *TaHKT1;5* on salt tolerance was studied by subjecting transgenic plants to control or salt stress (75mM) conditions. Changes in phenotype were measured through non-destructive plant imaging (LemnaTec[®] Scanalyzer), but no phenotypic variation was observed as a result of the salt stress that was applied, suggesting the stress may have been too mild.

In parallel with the knockdown approach, the *HvHKT1;5* gene, an orthologue of the bread wheat Na^+ transporter (*TaHKT1;5-D*), and a barley inorganic proton pyrophosphatase, *HvHVP1*, were overexpressed in barley through use of promoters thought to control cell type-specific expression. Promoters were identified through an MPSS database search for genes with low to moderate transcript levels and specificity for root-cortex or root-stele. The promoters controlling these genes were then isolated to drive *HvHKT1;5* in root cortex and stele and *HvHVP1* in root cortex. Four promoters were found to be promising: two stelar-specific and two cortex-specific and were placed upstream of *HvHKT1;5* and *HvHVP1*. These constructs were then transformed into barley (cv. Golden Promise). Transgenic plants were grown in 100mM salt stress with two independent lines for each promoter:gene construct.

Independent lines which included a stelar-specific promoter controlling *HvHKT1;5* transcription showed reduced Na⁺ accumulation and increased K⁺ accumulation in 4th leaf xylem sap. Transgene mRNA was detected in both shoots and roots of the plant.

In conclusion, while lowering levels of *HKT1;5* transcript in wheat were not found to impact whole plant salinity tolerance, it did increase Na⁺ accumulation in the shoot. This was supported by the results in barley where overexpression of *HvHKT1;5* resulted in lower Na⁺ levels and a concomitant increase in K⁺ levels in the shoot. Further study on whether this result impacts barley salt tolerance is currently underway.