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

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Declaration

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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.

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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.

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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 *HvHVP1* in Barley (*Hordeum vulgare*)". ACPFG Joint Research Meeting 2008 Adelaide, Australia.

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ABA	abscisic acid
ACPFG	Australian Centre for Plant Functional Genomics
At	*Arabidopsis thaliana*
AtAVP1	VP1 from *Arabidopsis thaliana*
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	*Hordeum vulgare*
HvHVP1	VP1 from *Hordeum vulgare*
Hyg	Hygromycin
KAc	Potassium acetate
LB	Luria Bertani
MCS	multiple cloning site
MgAc	Magnesium acetate
MPSS	Massively parallel signature sequence
NHX1	Na^+/H^+ exchanger
Nos	Nopaline synthase
Os	*Oryza sativa*
OsOVP1	VP1 from *Oryza sativa*
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	*Triticum aestivum*
TaCycl	gene encoding cyclophillin in *T.aestivum*
TaEFa	gene encoding elongation factor a in *T.aestivum*

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 effected 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.