

Role and control of *HKT* in *Oryza sativa* & *Arabidopsis thaliana*

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Abstract

Salinity is a major abiotic stress influencing agricultural production in Australia and around the world. Plants grown in saline conditions are affected by both osmotic and ionic stress components. The focus of this thesis is on the ionic stress component of salinity stress and in particular the build up of sodium ions (Na^+) in the leaf cytoplasm, one of the main components of salinity toxicity.

In this thesis, genes encoding the high affinity potassium transporter family of proteins (HKTs) are studied in the plants *Oryza sativa* (rice) and *Arabidopsis thaliana* (Arabidopsis). These HKT transporters encode Na^+ permeable membrane proteins and transport either Na^+ selectively or co-transport Na^+ and K^+ . HKT transporters have been identified in a number of plant species and to date have mainly been shown to be involved in reducing Na^+ stress.

A family of nine *HKT* genes have been identified in rice. Published reports to date have mainly investigated the function of these *OsHKT* genes in heterologous systems or have focused on one or two *OsHKT* genes *in planta*. During this PhD, tissue specific expression profiles were determined for each of the nine *OsHKT* genes, in ten rice varieties, exposed to a NaCl stress. Of most interest was *OsHKT1;3* which showed very high levels of expression in leaf blades and sheath and higher levels of expression in NaCl treated roots compared to controls, across rice varieties. A range of experiments were designed to obtain further information regarding *OsHKT1;3*, as little was known about this gene or the encoded protein at the time of these experiments. A notable discovery was the identification of a novel *OsHKT1;3* splice variant.

In contrast to rice, Arabidopsis has only one *HKT* gene, *AtHKT1;1*. *AtHKT1;1* is located in cells surrounding the xylem and is likely to be involved in the retrieval of Na^+ from the xylem, thereby minimising shoot Na^+ accumulation. To investigate the regulation of *AtHKT1;1* gene expression two Arabidopsis ecotypes, Columbia-0 (Col-0) and C24, shown by QRT-PCR to have different *AtHKT1;1* root expression levels, were studied. Sequencing of the C24 *AtHKT1;1* promoter revealed substantial sequence differences between the Col-0 and C24 promoters, particularly 150 to 200 bp upstream of the *AtHKT1;1* ATG start codon. It was hypothesised that these sequence differences were responsible for the lack of *AtHKT1;1* root specific expression in C24 plants, due to a lack of transcription factor binding motif(s) or particular root specific transcription factor(s). To test this hypothesis a series of *AtHKT1;1* promoter::*GFP* and *AtHKT1;1* promoter::*AtHKT1;1* cDNA constructs, with different combinations of Col-0 and C24 sequences, were tested. Preliminary results suggest that both the Col-0 and C24 *AtHKT1;1* promoters are able to drive expression of the downstream transgene and therefore the sequence differences between the promoters is not the cause of the lack of C24 *AtHKT1;1* root expression. A 1.6 kb insertion, identified in the second intron of the C24 *AtHKT1;1* gene, is now proposed to disrupt C24 *AtHKT1;1* root specific expression.

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 to Joanna Faye Sundstrom 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.

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.....
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.....
Date

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Abbreviations & Symbols - General

3'	three prime, of nucleic acid sequence
5'	five prime, of nucleic acid sequence
~	approximately
°C	degrees Celsius
#	number
%	percent
x	times
aa	amino acid
amiRNA	artificial micro ribonucleic acid
BAC	bacterial artificial chromosome
bp	base pairs, of nucleic acid
BSA	bovine serum albumin
cDNA	complimentary deoxyribonucleic acid
Ca ²⁺	calcium ion
CaCl ₂	calcium chloride
CaMV	cauliflower mosaic virus
Cat. #	catalogue number
Cl ⁻	chloride ion
cm	centimetre(s)
cps	counts per second
Col-0	Columbia-0
cRNA	complimentary ribonucleic acid
d	day(s)
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
dCT ³² P	deoxycytidine labelled with phosphorus-32
dGTP	deoxyguanosine triphosphate
dH ₂ O	deionised water
dTTP	deoxythymidine triphosphate
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
dS	deciSiemens
DTT	dithiothreitol
ECe	electrical conductivity
EDTA	ethylenediaminetetraacetic acid
EST	expressed sequence tag
<i>g</i>	gravity
g	gram(s)
gDNA	genomic deoxyribonucleic acid
GFP	green fluorescent protein
GOI	gene of interest

GUS	β -glucuronidase protein
H ⁺	hydrogen ion
H ₂ O	water
ha	hectare
HCl	hydrochloric acid
hr	hour(s)
IDSE	intron dependent spatial expression
IME	intron mediated enhancement
IPTG	isopropyl- β -D-thiogalactopyranoside
K ⁺	potassium ion
kb	kilo base pairs, of nucleic acid
KAc	potassium acetate
KCl	potassium chloride
kg	kilogram(s)
km	kilometre(s)
KOH	potassium hydroxide
L	litre(s)
LB	left border, of T-DNA sequence
LB media	luria betani media
LBharvest	youngest fully emerged leaf blade at the time of tissue harvest
LSalt	youngest fully emerged leaf blade at the time of salt application
M	molar
m	metre(s)
MBq	mega Bequerel
mg	milligram(s)
Mg ²⁺	magnesium ion
MgCl ₂	magnesium chloride
min	minute(s)
miRNA	micro ribonucleic acid
mL	millilitre(s)
mm	millimetre(s)
mM	millimolar
mol	mole
MPa	mega Pascals
MPM	transmembrane segment, pore, transmembrane segment domain
MS media	Murashige and Skoog media
N ₂	nitrogen
N/A	not applicable
Na ⁺	sodium ion
NaCl	sodium chloride
NaOH	sodium hydroxide
ng	nanogram(s)
nm	nanometre(s)
nM	nanomolar

nosT	bacterial nopaline synthase terminator sequence
OD ₂₆₀	optical density measured at 260 nm
o/n	overnight
PCR	polymerase chain reaction
pg	picogram(s)
PI	propidium iodide
pmol	picomoles
PVC	polyvinyl chloride
QRT-PCR	quantitative reverse transcription polymerase chain reaction
QTL	quantitative trait loci
RACE	rapid amplification of cDNA ends
RB	right border, of T-DNA sequence
RbCl	rubidium chloride
RIL	recombinant inbred line
RNA	ribonucleic acid
RO	reverse osmosis
rpm	revolutions per minute
RT	room temperature
RT-PCR	reverse transcription polymerase chain reaction
SAM	S-adenosylmethionine
siRNA	short interfering ribonucleic acid
SDS	sodium dodecyl sulfate
sec	second(s)
SNP	single nucleotide polymorphism(s)
SSC	saline sodium citrate
ssp	subspecies
t	tonnes
T ₀	primary rice transformant containing T-DNA
T ₁	primary Arabidopsis transformant containing T-DNA or progeny of T ₀ rice plant
T ₂	progeny of T ₁ plant
TAE	tris-acetate-EDTA
T-DNA	transfer deoxyribonucleic acid
TE	transposable element
TE	tris-EDTA
TF	transcription factor
T _m	melting temperature, of primers
U	units
UAS	upstream activation sequence
µg	microgram(s)
µL	microlitre(s)
µm	micrometre(s)
µM	micromolar
µmol	micromole(s)

UTR	untranslated region
UV	ultraviolet
v/v	volume per volume
w/v	weight per volume
X-Gal	5-bromo-4-chloro-3-indoyl- β -D-galactopyranoside
X-Gluc	5-bromo-4-chloro-3-indoyl-glucuronide

Abbreviations - Organisations

ABARE	Australian Bureau of Agricultural and Resource Economics
ABS	Australian Bureau of Statistics
ACPFPG	Australian Centre for Plant Functional Genomics
AGRF	Australian Genome Research Facility
CSIRO	Commonwealth Scientific & Industrial Research Organisation
FAO	Food and Agricultural Organization of the United Nations
IMVS	Institute of Medical & Veterinary Science
IRRI	International Rice Research Institute
NCBI	National Center for Biotechnology Information
NLWRA	National Land & Water Resource Audit
PMSEIC	Prime Ministers Science, Engineering & Innovation Council
SARDI	South Australian Research & Development Institute