Salinity detection and control of sodium transport in *Arabidopsis thaliana*

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3′	three prime, of nucleic acid sequence
5′	five prime, of nucleic acid sequence number
%	percent
~	approximately
×	times
°C	degrees Celsius
aa	amino acid
ACPFG	Australian Centre for Plant Functional Genomics
AGRF	Australian Genome Research Facility
ANOVA	analysis of variance
Arabidopsis	*Arabidopsis thaliana*
At	*Arabidopsis thaliana*
AtHKT1;1-C24	*AtHKT1;1* allele from C24
AtHKT1;1-Col-0	*AtHKT1;1* allele from Col-0
AtHKT1;1-C24	AtHKT1;1 protein form C24
AtHKT1;1-Col-0	AtHKT1;1 protein from Col-0
AVP	Arabidopsis vacuolar pyrophosphatase
BAC	bacterial artificial chromosome
BLAST	basic local alignment search tool
bp	base pairs, of nucleic acid
BSA	bovine serum albumin
C24	Arabidopsis ecotype C24
Ca^{2+}	calcium ion
$[Ca^{2+}]_{cyt}$	cytosolic free calcium concentration
$CaCl_2$	calcium chloride
CaMV	cauliflower mosaic virus
Cat. #	catalogue number
CBL	calcineurin like-B protein
cDNA	complimentary deoxyribonucleic acid
Cl^-	chloride ion
CIPK	calcineurin like-B interacting protein kinase
cm	centimetre(s)
Col-0	Arabidopsis ecotype Columbia-0

Col-0×C24	mapping population with Col-0 and C24 as parents
cRNA	complimentary ribonucleic acid
d	day(s)
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
dGTP	deoxyguanosine triphosphate
dH_20	deionised water
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
dS	deciSiemens
DTT	dithiothreitol
dTTP	deoxythymidine triphosphate
ECe	electrical conductivity
EDTA	ethylenediaminetetraacetic acid
E_{rev}	reversal potential
FAO	Food and Agricultural Organization of the United Nations
g	gram(s)
G	conductance
g	gravity
gDNA	genomic deoxyribonucleic acid
GFP	green fluorescent protein
GUS	β -glucuronidase protein
H^+	hydrogen ion
H_20	water
HCl	hydrochloric acid
HKT	high affinity potassium transport
hr	hour(s)
I	current
IDSE	intron dependent spatial expression
IME	intron mediated enhancement
K^+	potassium ion
kb	kilo base pairs, of nucleic acid
KCl	potassium chloride
kg	kilogram(s)
KOH	potassium hydroxide

L	litre(s)
L*er*	Arabidopsis ecotype Landsberg *erecta*
LB	left border, of T-DNA sequence
LB	media luria betani media
M	molar
mg	milligram(s)
Mg^{2+}	magnesium ion
$MgCl_2$	magnesium chloride
min	minute(s)
mRNA	messenger ribonucleic acid
miRNA	micro ribonucleic acid
mL	millilitre(s)
mm	millimetre(s)
mM	millimolar
mol	mole
mOsm	milliosmole
MS-media	media, Murashige and Skoog media
mV	millivolt
n	sample size
N/A	not applicable
N_2	nitrogen
mA	milliampere
Na^+	sodium ion
NaCl	sodium chloride
NaOH	sodium hydroxide
NCBI	National Center for Biotechnology Information
ng	nanogram(s)
NHX	Na+/H+ exchanger
nm	nanometre(s)
nM	nanomolar
nosT	bacterial nopaline synthase terminator sequence
NPK	ratio of nitrogen, phosphate and potassium in fertilizer
o/n	overnight
OD_{600}	optical density measured at 600 nm
pC24	2.7 kb promoter region upstream of *AtHKT1;1* from C24

pCol-0	2.7 kb promoter region upstream of *AtHKT1;1* from Col-0
PCR	polymerase chain reaction
pCR8	entry vector pCR™8/GW/TOPO Gateway®
PI	propidium iodide
qRT-PCR	quantitative reverse transcription polymerase chain reaction
QTL	quantitative trait loci
RB	right border, of T-DNA sequence
RdDM	RNA mediated DNA methylation
RIL	recombinant inbred line
RNA	ribonucleic acid
RO	reverse osmosis
rpm	rounds per minute
RT	room temperature
RT-PCR	reverse transcription polymerase chain reaction
s	second(s)
Salty	Salt water crocodile, mascot
Sc	Saccharomyces cerevisiae
SDS	sodium dodecyl sulfate
Semi-qPCR	semi-quantitative polymerase chain reaction
siRNA	short interfering ribonucleic acid
SNP	single nucleotide polymorphism(s)
SOS	salt overly sensitive
T1	primary Arabidopsis transformant containing T-DNA
T2	progeny of T1 plant
TAE	tris-acetate-EDTA
T-DNA	transfer deoxyribonucleic acid TE transposable element
TE	transposable element
TF	transcription factor
T_m	melting temperature, of primers
U	units
UAS	upstream activation sequence
uidA	β-glucuronidase gene
UTR	untranslated region
UV	ultraviolet
V	voltage

v/v	volume per volume
wk	week(s)
w/v	weight per volume
Xenopus	*Xenopus laevis*
µg	microgram(s)
µL	microlitre(s)
µm	micrometre(s)
µM	micromolar
µmol	micromole(s)

Abstract of thesis

Soil salinity is a major abiotic stress, reducing crop yields and endangering global food security. With salt affected areas increasing, understanding the molecular mechanisms of salinity stress is of great importance. Plant salinity stress can be categorised into two phases, the initial shoot ion independent osmotic stress and the later ionic stress. Osmotic stress occurs as soon as the plant encounters salt in the soil and results in an immediate reduction in the shoot growth rate. Ionic stress is caused by the accumulation of ions such as Na^+ and Cl^- in the cytosol of cells in the shoot and results in the inhibition of cellular processes and induces premature leaf senescence.

The two *Arabidopsis thaliana* ecotypes Col-0 and C24 have previously been identified as interesting candidates to study plant salinity tolerance. The Col-0 ecotype is less salt tolerant than the C24 ecotype, based on its reduction in dry weight under stressed conditions. This is despite C24 accumulating significantly more Na^+ in the shoot than Col-0. Interestingly, C24 also appeared to be less responsive to salt stress, as transcript levels of several key salt responsive genes are not substantially altered in response to salt stress. The *AtHKT1;1* gene is one key gene found to be not up-regulated in C24 during salt stress. *AtHKT1;1* encodes a protein likely to be involved in the retrieval of Na^+ from the xylem thereby reducing the amount of Na^+ translocating to the shoot. In this thesis the C24 and Col-0 *HKTs* are compared at the protein and transcriptional levels. Electrophysiological analysis in Xenopus oocytes and a functional assay in yeast confirm Na^+ transport properties of both AtHKT1;1 proteins and, interestingly, indicated AtHKT1;1 from both ecotypes had the ability to transport K^+. To determine the difference in expression profile between the two ecotypes, a series of *AtHKT1;1promoter::GFP* and *AtHKT1;1promoter::AtHKT1;1cDNA* constructs were tested in Arabidopsis. Results suggest that both the Col-0 and C24 *AtHKT1;1* promoters are able to drive expression of the downstream genes, suggesting that differences in the promoter region are not responsible for the lack of *AtHKT1;1* expression in C24. A transposable element identified in the second intron of the C24 *AtHKT1;1* genomic sequence may be important in causing the lack of *AtHKT1;1* expression in roots.

Furthermore, the reduced responsiveness of C24 to salt stress is investigated in relation to how salt is initially perceived by the plant. An assay using aequorin bioluminescence was used to compare the responses in the salt stress inducible Ca^{2+}-signatures of Col-0 and C24 seedlings. Excitingly, C24 appears to be missing part of the Ca^{2+} signature observed in the salt responsive plant Col-0, suggesting that C24 may not detect the ion component of salt stress. This potentially provides a suitable screening methodology for the identification of as yet unknown components in the early stages of the salt signalling pathway. An attempt is made to develop a screening assay suitable for performing QTL analysis on an available Col-0 × C24 mapping population, based on measuring changes in transcript levels of salt responsive genes.

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 Sandra Manuela Schmöckel 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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Date

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