Evaluation of the effects of AtCIPK16 expression on the salt tolerance of barley and wheat

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List of Abbreviations

%	percentage
#	number
×	times
°C	degrees Celsius
®	registered trademark
$^{-1}$	per
-ve	negative
+ve	positive
µL	microliter(s)
µmoles	micromole(s)
µS	microSiemens
3'	three prime, of nucleic acid sequence
35S	promoter of cauliflower mosaic virus 35S
3D	three dimensional
5'	five prime, of nucleic acid sequence
aa	amino acid
ABA	abscisic acid
ABARES	Australian Bureau of Agricultural and Resource Economics and Sciences
ACPFG	Australian Centre for Plant Functional Genomics
AGRF	Australian Genome Research Facility
Agrobacterium	*Agrobacterium tumefaciens*
AKT	*Arabidopsis* potassium transporter
At	*Arabidopsis thaliana*
ANOVA	analysis of variance
AVP1	*Arabidopsis* vacuolar pyrophosphatase
Bay-0	*Arabidopsis* ecotype Bayreuth-0
BLAST	basic local alignment search tool
bp	base pairs, of nucleic acid
C-terminal	carboxyl (COOH)-terminal, of protein
Ca^{2+}	calcium ion
$CaCl_2$	calcium chloride
CaM	calmodulin
$CaSO_4$	calcium sulphate
Cat. No.	catalogue number
CBL	calcineurin B-like protein
cDNA	complimentary deoxyribonucleic acid

CDPK	calcium-dependent protein kinase
CIMMYT	International Maize and Wheat Improvement Centre (Centro Internacional de Mejoramiento de Maíz y Trigo)
CIPK	calcineurin B-like (CBL) interacting protein kinase
Cl^-	chloride ion
cm	centimetre
CML	calmodulin-like protein
CO_2	carbon dioxide
Col-0	*Arabidopsis* ecotype Columbia-0
CRCSLM	Cooperative Research Centre for Soil & Land Management
CRISPR/Cas	clustered regularly interspersed short palindromic repeats/CRISPR-associated
cv.	cultivar
DNA	deoxyribonucleic acid
dNTPs	deoxynucleotide triphosphates
DREB	dehydration-responsive element-binding
dS	deciSiemens
DTT	dithiothreitol
DW	dry weight
E.coli	*Escherichia coli*
EC	electrical conductivity
$EC_{1:5}$	electrical conductivity of a 1:5 soil to water solution
EC_a	apparent electrical conductivity
EC_e	electrical conductivity of a soil extract
EDTA	ethylenediaminetetraacetic acid
EF	elongation factor
EM	electromagnetic
ESP	exchangeable sodium percentage
FAO	Food and Agricultural Organization of the United Nations
FISH	fluorescence in situ hybridization
FW	fresh weight
g	grams(s)
g	gravity
GC	guanine-cytosine, nucleic acid content
gDNA	genomic deoxyribonucleic acid
GFP	green fluorescent protein
GM	genetically modified
GP	Golden Promise
GS	growth stage, of plant
H^+	hydrogen ion
H_2O	water
ha	hectare

HCL	hydrochloric acid
HF	high fidelity
HKT	high affinity potassium channel
hr	hour(s)
Hv	*Hordeum vulgare*
K	potassium
K^+	potassium ion
kb	kilobase pairs, of nucleic acid
kg	kilogram(s)
km	kilometre
km^2	square kilometre
L	litre
LB	luria betani (media or agar)
m	metre(s)
M	molar
min(s)	minute(s)
Mg	magnesium
$MgCl_2$	magnesium chloride
mL	millilitre(s)
mm	millimetre(s)
mM	millimolar
mRNA	messenger ribonucleic acid
mS	milliSiemens
n	sample size
N	nitrogen
N_2	nitrogen, gas
N-terminal	amino (NH_2)-terminal, of protein
Na^+	sodium ion
NaCl	sodium chloride
NAF	asparagine-alanine-phenylalanine motif (NAF in single amino acid code)
nd	not determined
ng	nanograms
NHX	Na^+/H^+ exchanger
NLWRA	National Land & Water Resources Audit
NSCC	non-selective cation channel
nt	line is not transgenic based on genotyping
OGTR	Office of the Gene Technology Regulator
Os	*Oryza sativa*
P	phosphorus
PIC	pre-initiation complex
PCR	polymerase chain reaction

PPC2	protein phosphatase 2C-type
PPI	protein-phosphate interaction
PVC	polyvinyl chloride
QTL	quantitative trait loci
RNA	ribonucleic acid
ROS	reactive oxygen species
RT-PCR	reverse transcription polymerase chain reaction
S	sulphur
s.e.m	standard error of the mean
SDS	sodium dodecyl sulfate
s	second(s)
SnRK	SNF1 (sucrose non-fermenting 1)-related kinase subgroup
SOS	salt overly sensitive
T_1	progeny of the primary transformant containing transgene
T_2	progeny of T_1
T_3	progeny of T_2
T_4	progeny of T_3
T_5	progeny of T_4
Ta	*Triticum aestivum*
TBP	TATA-box binding protein(s)
TE	tris-EDTA
T_m	melting temperature, of primers
™	unregistered trademark
TGS	transgene silencing
TSS	transcription start site
U	unit(s)
Ubi	promoter of maize *Ubiquitin-1*
UTR	untranslated region, of nucleic acid
UV	ultraviolet
v/v	volume per volume
WA	Western Australia

Abstract

Soil salinity is a major constraint to crop production in Australia. This has prompted the need to produce salt tolerant cereal cultivars, through the understanding of genes involved in salt tolerance mechanisms and manipulating their expression levels. *Arabidopsis thaliana Calcineurin B-like Interacting Protein Kinase 16* (*AtCIPK16*) has been identified as a gene involved in sodium (Na^+) exclusion. Analysis of *AtCIPK16* alleles from Arabidopsis ecotypes suggests variances in expression are due to differences in the promoters. Experiments in Arabidopsis, barley and wheat (preliminary) have illustrated that *AtCIPK16* overexpression can enhance biomass production through increased Na^+ exclusion, although its full effect in barley and wheat has yet to be properly characterised in both greenhouse and field environments.

The first focus of this project evaluated the salt tolerance of *35S:AtCIPK16* barley (cv. Golden Promise) grown under low and high salinity field conditions in 2013 and 2014 at Kunjin, Western Australia. Comparisons between years were difficult due to waterlogging of the 2013 high salt site and the increased variability in plot establishment in 2014. *35S:AtCIPK16* barley lines had varying responses to high salt conditions depending on the annual rainfall. Results showed Na^+ and Cl^- exclusion in certain lines, although this correlated with decreased biomass and yield in high rainfall years. *AtCIPK16* expression also increased Na^+ and Cl^- exclusion in 2012 (a low rainfall year) which instead lead to increasing plant growth and yield.

The second focus of this project aimed to fully characterised the effects of the constitutive expression of *Ubi:AtCIPK16* in wheat (cv. Gladius). Despite conducting three hydroponic experiments, no definitive conclusions about the effects of *AtCIPK16* expression on wheat salt tolerance could be drawn. Although, one sibling transgenic line showed increased Na^+ and Cl^- exclusion from both root and shoot tissue accompanied by larger biomass under 200 mM salt stress. Despite this finding several factors hinder the analysis of data including the high number of null segregants, considerable variability between siblings of the same transformation event and minimal transgene expression.

The third focus of this project aimed to investigate expression differences between two *AtCIPK16* alleles from the Arabidopsis ecotypes Bay-0 and Shahdara. Since the only differences between the two alleles was a 10 base pair deletion in the Bay-0 promoter, it was hypothesised this deletion was the reason for the increased expression of *AtCIPK16* in Bay-0 as it forms a TATA box (TATATAA). The aim of this project was to alter the expression of each allele by: mutating the last A to a T, removing the TATA box in Bay-0, and mutating the T after the TATATA sequence to an A in Shahdara, forming a TATA box without the deletion. Through PCR mutagenesis the required point mutations were introduced into portions of the two promoter alleles, however due to technical difficulties and time constraints the point mutations were not introduced back into the full promoter constructs driving GFP. It was therefore unable to be determined if the point mutations to the TATA box would indeed affect *AtCIPK16* expression.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, 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.

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