

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

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Table of Contents

Table of Contents	i
List of Figures	iii
List of Tables	v
List of Abbreviations	vi
Abstract	x
Declaration	xi
Acknowledgments	xii
Chapter 1: Literature review	1
1.1 A global problem.....	1
1.2 Salinity.....	1
1.2.1 Salt-affected soils.....	1
1.2.2 Global salinity.....	2
1.2.3 Salt-affected Australia.....	2
1.3 How salt affects plants.....	3
1.3.1 Sodium toxicity.....	4
1.4 Salt tolerance mechanisms.....	4
1.4.1 Osmotic tolerance.....	4
1.4.2 Ionic tolerance.....	4
1.5 CBLs and CIPKs.....	6
1.5.1 Ca ²⁺ signalling in plants.....	6
1.5.2 Calcineurin B-like proteins (CBLs).....	7
1.5.3 Calcineurin B-like Interacting Proteins Kinases (CIPKs).....	8
1.5.4 CBL-CIPK signalling pathways.....	10
1.5.5 Examples of CBL-CIPK pathways.....	11
1.6 AtCIPK16.....	12
1.6.1 <i>Arabidopsis thaliana</i> Calcineurin B-like Interacting Protein Kinase 16.....	12
1.6.2 Other CIPK16s.....	14
1.7 Research Aims.....	15
Chapter 2: Evaluation of 35S:AtCIPK16 Golden Promise barley lines under field conditions in 2013 & 2014	16
2.1 Introduction.....	16
2.2 Materials and Methods.....	17
2.2.1 Environmental characterisation of field trial site.....	17
2.2.2 Plant material.....	18
2.2.3 Field trial of transgenic barley.....	18
2.2.4 DNA extraction and genotyping analysis.....	20
2.2.5 Soil analysis of field trial plots.....	21
2.2.6 Ion analysis of leaf tissue.....	21
2.3 Results.....	22
2.3.1 Environmental characterisation of field trial site.....	22
2.3.2 Transgenic <i>AtCIPK16</i> barley show variations in plant growth.....	23
2.3.3 Transgenic <i>AtCIPK16</i> expressing barley lines show possible Na ⁺ exclusion.....	25
2.3.4 Expression of <i>AtCIPK16</i> in barley does not improve yield.....	27
2.4 Discussion.....	30
2.4.1 Transgenic <i>AtCIPK16</i> barley has increased Na ⁺ and Cl ⁻ exclusion.....	30
2.4.2 Na ⁺ and Cl ⁻ exclusion does not translate to improved biomass or yield in transgenic <i>AtCIPK16</i> lines.....	31
2.4.3 Variation in results between years linked to environmental factors.....	33
2.5 Conclusions & Future directions.....	35
Chapter 3: Characterisation of Ubi:AtCIPK16 wheat lines in hydroponic experiments	36
3.1 Introduction.....	36
3.2 Materials and Methods.....	37

3.2.1 Plant material.....	37
3.2.2 Growth conditions	37
3.2.3 DNA extraction and genotyping analysis	38
3.2.4 RNA extraction and gene expression analysis.....	39
3.2.5 Ion analysis of leaf and root tissue.....	40
3.3 Results.....	42
3.3.1 Gene presence and expression analysis of <i>AtCIPK16</i> transgenic lines.....	42
3.3.2 Transgenic <i>AtCIPK16</i> lines have varied biomass production	44
3.3.3 Transgenic <i>AtCIPK16</i> lines have varying responses in leaf ion accumulation	46
3.3.4 Transgenic <i>AtCIPK16</i> lines show varied root ion accumulation trends	50
3.4 Discussion.....	53
3.4.1 Response of Gladius wheat to NaCl treatment	53
3.4.2 One transgenic line, CIPK16-2-2, demonstrates a Na ⁺ and Cl ⁻ exclusion phenotype.....	55
3.4.3 Disruption of transgene expression: hypothesised reason for lack of phenotype.....	57
3.5 Conclusions & Future directions	60
Chapter 4: Determination of whether the presence/absence of TATA-box in the <i>AtCIPK16</i> promoter is responsible for the <i>AtCIPK16</i> expression differences observed between <i>Arabidopsis</i> ecotypes.....	61
4.1 Introduction	61
4.2 Materials and Methods.....	63
4.2.1 Analysis of promoter regions to identify mutation sites.....	63
4.2.2 Introducing point mutations by PCR mutagenesis.....	63
4.2.3 Restriction digest and DNA ligation reactions	66
4.2.4 Generation of amplicon C – pCR8 Gateway [®] vectors.....	66
4.2.5 Further steps needed to transform final destination vectors into <i>Arabidopsis</i>	67
4.3 Results.....	69
4.3.1 Analysis of <i>AtCIPK16</i> promoters to introduce point mutations and design primers	69
4.3.2. Successful creation of amplicons A, B and C containing the desired point mutation for both alleles.....	72
4.3.3 Creation of pCR8 vector with full <i>AtCIPK16</i> promoter with point mutation	73
4.4 Discussion.....	77
4.4.1 Difficulties in plasmid construction	77
4.5 Future work.....	78
Chapter 5: General Discussion	80
5.1 Review of thesis aims.....	80
5.2 Summary of main findings	81
5.3 Implications of thesis findings	82
5.3.1 Benefits of <i>AtCIPK16</i> expression in barley and wheat may depend on environment	82
5.3.2 Role of CIPK16 in salt tolerance.....	83
5.3.3 Is exclusion the best mechanism to pursue in these crops?	84
5.4 Future Research	85
5.4.1 GM field trials of transgenic <i>AtCIPK16</i> barley in Australia.....	85
5.4.2 Further characterisation of transgenic <i>AtCIPK16</i> wheat lines.....	87
5.4.3 What is the <i>AtCIPK16</i> network pathway in wheat and barley?	88
5.4.4 <i>AtCIPK16</i> expression: which promoter to use?.....	90
5.5 Concluding Remarks	91
Chapter 6: Appendices	93
Appendix 1.....	93
Appendix 2.....	103
Appendix 3.....	108
References.....	109

List of Figures

Figure 1.1: Map showing the regions of Australia affected or potentially affected by transient (yellow) and dryland (red) salinity.....	3
Figure 1.2: General structure of a calcineurin B-like protein (CBL).....	7
Figure 1.3: Overall structure of a CIPK showing the N-terminus serine/threonine kinase domain, with the activation loop (horizontal lines) and the C-terminus regulatory domain.....	9
Figure 1.4: Sequence alignment of the region of interest of the <i>AtCIPK16</i> promoter and gene.....	13
Figure 2.1: EM38 map of the field trial site in Kunjin, WA (83 m length × 32 m wide) showing the apparent electrical conductivity (EC _a).....	17
Figure 2.2: Average rainfall (mm) and maximum temperature (°C) at Corrigin, Western Australia for the year 2013 and 2014.....	23
Figure 2.3: Electrophoresis gel showing presence of the native <i>HvVRT2</i> gene and the <i>AtCIPK16</i> transgene in extracted gDNA from wildtype, null segregant and three <i>AtCIPK16</i> expressing barley lines grown at Kunjin, WA.....	23
Figure 2.4: Digital images of wildtype and transgenic <i>AtCIPK16</i> expressing barley plots displaying the range of plant densities in both low and high salt trial sites at Kunjin, Western Australia in 2014.....	24
Figure 2.5: Shoot biomass and tiller number of wildtype, null segregant and transgenic <i>AtCIPK16</i> expressing barley grown at Kunjin, Western Australia.....	25
Figure 2.6: Na ⁺ , K ⁺ and Cl ⁻ concentration and Na ⁺ /K ⁺ ratio of wildtype, null segregant and transgenic <i>AtCIPK16</i> barley grown at Kunjin, WA.....	26
Figure 2.7: Grain yield per plants parameters of wildtype and transgenic <i>AtCIPK16</i> expressing barley grown at Kunjin, Western Australia.....	28
Figure 2.8: Grain yield per plot for wildtype and transgenic <i>AtCIPK16</i> expressing barley lines grown at Kunjin, Western Australia.....	29
Figure 3.1: Electrophoresis gel showing representative results of genotyping and expression for null segregants and three transgenic <i>AtCIPK16</i> wheat lines.....	42
Figure 3.2: Photographs of null segregant and three transgenic <i>AtCIPK16</i> wheat lines at 24 days grown in 80 L flood-drain hydroponic systems under different salt treatments.....	43
Figure 3.3: Whole plant biomass measurements and tiller number of null segregant and three transgenic <i>AtCIPK16</i> wheat lines grown in hydroponic experiments.....	45
Figure 3.4: Relative salt tolerance of null segregant and three transgenic <i>AtCIPK16</i> wheat lines grown under hydroponic experiments.....	46
Figure 3.5: Leaf Na ⁺ and Cl ⁻ concentration of null segregant and three transgenic <i>AtCIPK16</i> wheat lines grown in hydroponic experiments.....	48
Figure 3.6: Leaf K ⁺ concentration of null segregant and three transgenic <i>AtCIPK16</i> wheat lines grown in hydroponic experiments.....	49
Figure 3.7: Root Na ⁺ , Cl ⁻ and K ⁺ concentration of null segregant and three transgenic <i>AtCIPK16</i> wheat lines grown in hydroponic experiments.....	51
Figure 4.1: Flow diagram outlining the methods undertaken to perform site directed mutagenesis by PCR on a reporter construct plasmid.....	65
Figure 4.2: Sequence of the region of the <i>AtCIPK16</i> promoter in the pCR8 vector and the primers involved in the site directed mutagenesis.....	71
Figure 4.3: Electrophoresis gel and chromatograph with sequence alignment of amplicons A and B from both Shahdara and Bay-0 alleles containing the desired point mutations.....	72

Figure 4.4: Electrophoresis gel and chromatograph with sequence alignment of amplicon C from both Shahdara and Bay-0 alleles containing the desired point mutations 73

Figure 4.5: Electrophoresis gel of failed double restriction enzyme digest of Bay-0 and Shahdara amplicon Cs..... 73

Figure 4.6: Electrophoresis gel and chromatograph with sequence alignment of amplicon C in pCR8 vector for both Shahdara and Bay-0 alleles containing the desired point mutations..... 75

Figure 4.7: Electrophoresis gels of double restriction enzyme digests and results of gel purification of bands excised from the gel of amplicon Cs in pCR8 vectors and original promoters in pCR8.. . 76

List of Tables

Table 2.1: Fertilisers applied during 2013 and 2014 field at Kunjin, WA.	19
Table 2.2: Herbicides, fungicides and insecticides applied during 2013 and 2014 field trials at Kunjin, WA.	19
Table 3.1: Components and final concentrations in 80 L hydroponic systems of the standard ACPFG growth solution	38
Table 3.2: Details of gene specific primers and PCR conditions used for the amplification of gDNA and/or cDNA from leaf tissue samples of null segregant and three independent <i>AtCIPK16</i> transgenic wheat lines.....	41
Table 3.3: Comparison of mean results for biomass and leaf ion concentration for each sibling transgenic line grown in all three hydroponic experiments to the respective null segregants in the same experiment.....	52
Table 3.4: Comparison of mean results for root ion concentration for each sibling transgenic line grown in all three hydroponic experiments to the respective null segregants in the same experiment.	52
Table 4.1: Description of primers designed for site directed mutagenesis of the <i>AtCIPK16</i> promoter by PCR and details of the amplicons created.	70

List of Abbreviations

%	percentage
#	number
x	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
<i>Agrobacterium</i>	<i>Agrobacterium tumefaciens</i>
AKT	<i>Arabidopsis</i> potassium transporter
<i>At</i>	<i>Arabidopsis thaliana</i>
ANOVA	analysis of variance
AVP1	<i>Arabidopsis</i> vacuolar pyrophosphatase
Bay-0	<i>Arabidopsis</i> 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	<i>Arabidopsis</i> 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
<i>E.coli</i>	<i>Escherichia coli</i>
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)
<i>g</i>	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 ₂ O	water
ha	hectare

HCL	hydrochloric acid
HF	high fidelity
HKT	high affinity potassium channel
hr	hour(s)
<i>Hv</i>	<i>Hordeum vulgare</i>
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	<i>Oryza sativa</i>
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
<i>Ta</i>	<i>Triticum aestivum</i>
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)
<i>Ubi</i>	promoter of maize <i>Ubiquitin-1</i>
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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