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

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A thesis submitted for the degree of
Master of Philosophy
School of Agriculture, Food and Wine
Faculty of Sciences

THE UNIVERSITY of ADELAIDE
May 2016
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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
Ca²⁺  calcium ion
CaCl₂  calcium chloride
CaM  calmodulin
CaSO₄  calcium sulphate
Cat. No.  catalogue number
CBL  calcineurin B-like protein
cDNA  complimentary deoxyribonucleic acid
<table>
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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>CDPK</td>
<td>calcium-dependent protein kinase</td>
</tr>
<tr>
<td>CIMMYT</td>
<td>International Maize and Wheat Improvement Centre (Centro Internacional de Mejoramiento de Maíz y Trigo)</td>
</tr>
<tr>
<td>CIPK</td>
<td>calcineurin B-like (CBL) interacting protein kinase</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>chloride ion</td>
</tr>
<tr>
<td>cm</td>
<td>centimetre</td>
</tr>
<tr>
<td>CML</td>
<td>calmodulin-like protein</td>
</tr>
<tr>
<td>CO₂</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>Col-0</td>
<td><em>Arabidopsis</em> ecotype Columbia-0</td>
</tr>
<tr>
<td>CRCSLM</td>
<td>Cooperative Research Centre for Soil &amp; Land Management</td>
</tr>
<tr>
<td>CRISPR/Cas</td>
<td>clustered regularly interspersed short palindromic repeats/CRISPR-associated</td>
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<tr>
<td>cv.</td>
<td>cultivar</td>
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<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<td>deoxynucleotide triphosphates</td>
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<td>DREB</td>
<td>dehydration-responsive element-binding</td>
</tr>
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<td>deciSiemens</td>
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<td>dithiothreitol</td>
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<td>dry weight</td>
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<td><em>Escherichia coli</em></td>
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<td>EC</td>
<td>electrical conductivity</td>
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<tr>
<td>EC&lt;sub&gt;1:5&lt;/sub&gt;</td>
<td>electrical conductivity of a 1:5 soil to water solution</td>
</tr>
<tr>
<td>EC&lt;sub&gt;a&lt;/sub&gt;</td>
<td>apparent electrical conductivity</td>
</tr>
<tr>
<td>EC&lt;sub&gt;e&lt;/sub&gt;</td>
<td>electrical conductivity of a soil extract</td>
</tr>
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<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EF</td>
<td>elongation factor</td>
</tr>
<tr>
<td>EM</td>
<td>electromagnetic</td>
</tr>
<tr>
<td>ESP</td>
<td>exchangeable sodium percentage</td>
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<tr>
<td>FAO</td>
<td>Food and Agricultural Organization of the United Nations</td>
</tr>
<tr>
<td>FISH</td>
<td>fluorescence in situ hybridization</td>
</tr>
<tr>
<td>FW</td>
<td>fresh weight</td>
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<td>grams(s)</td>
</tr>
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<td>gravity</td>
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<td>GC</td>
<td>guanine-cytosine, nucleic acid content</td>
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<td>gDNA</td>
<td>genomic deoxyribonucleic acid</td>
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<tr>
<td>GFP</td>
<td>green fluorescent protein</td>
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<tr>
<td>GM</td>
<td>genetically modified</td>
</tr>
<tr>
<td>GP</td>
<td>Golden Promise</td>
</tr>
<tr>
<td>GS</td>
<td>growth stage, of plant</td>
</tr>
<tr>
<td>H⁺</td>
<td>hydrogen ion</td>
</tr>
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<td>H₂O</td>
<td>water</td>
</tr>
<tr>
<td>ha</td>
<td>hectare</td>
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<tr>
<td>Term</td>
<td>Definition</td>
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<td>---------------------------------------------------------------------------</td>
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<tr>
<td>PPC2</td>
<td>protein phosphatase 2C-type</td>
</tr>
<tr>
<td>PPI</td>
<td>protein-phosphate interaction</td>
</tr>
<tr>
<td>PVC</td>
<td>polyvinyl chloride</td>
</tr>
<tr>
<td>QTL</td>
<td>quantitative trait loci</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>reverse transcription polymerase chain reaction</td>
</tr>
<tr>
<td>S</td>
<td>sulphur</td>
</tr>
<tr>
<td>s.e.m.</td>
<td>standard error of the mean</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulfate</td>
</tr>
<tr>
<td>s</td>
<td>second(s)</td>
</tr>
<tr>
<td>SnRK</td>
<td>SNF1 (sucrose non-fermenting 1)-related kinase subgroup</td>
</tr>
<tr>
<td>SOS</td>
<td>salt overly sensitive</td>
</tr>
<tr>
<td>T1</td>
<td>progeny of the primary transformant containing transgene</td>
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<td>T5</td>
<td>progeny of T4</td>
</tr>
<tr>
<td>Ta</td>
<td><em>Triticum aestivum</em></td>
</tr>
<tr>
<td>TBP</td>
<td>TATA-box binding protein(s)</td>
</tr>
<tr>
<td>TE</td>
<td>tris-EDTA</td>
</tr>
<tr>
<td>Tm</td>
<td>melting temperature, of primers</td>
</tr>
<tr>
<td>™</td>
<td>unregistered trademark</td>
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<tr>
<td>TGS</td>
<td>transgene silencing</td>
</tr>
<tr>
<td>TSS</td>
<td>transcription start site</td>
</tr>
<tr>
<td>U</td>
<td>unit(s)</td>
</tr>
<tr>
<td>Ubi</td>
<td>promoter of maize <em>Ubiquitin-1</em></td>
</tr>
<tr>
<td>UTR</td>
<td>untranslated region, of nucleic acid</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>v/v</td>
<td>volume per volume</td>
</tr>
<tr>
<td>WA</td>
<td>Western Australia</td>
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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 TATA 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.

In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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I also give permission for the digital version of my thesis to be made available on the web, via the University’s digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

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Acknowledgments

I would like to acknowledge and thank my supervisors Dr. Stuart Roy and Dr. Andrew Jacobs for the guidance and support they have offered throughout my Masters project. It has been an honour and a privilege to have worked with and learnt from you, and I thank you for the patience and understanding you have always shown in your encouragement of my learning.

I am also grateful to the University of Adelaide and the Australian Centre for Plant Functional Genomics (ACPFG) for providing scholarships for the duration of my degree. I would also like to thank the ACPFG and USAID for providing the resources and facilities necessary to undertake my Masters.

I am also grateful to the many people who have helped me during my experiments, especially in conducting field trials. I would like to thank Kalyx Australia (Perth, WA), particularly Dr. Peter Carlton, Mrs. Caris Smith and Mr. Peter Burgess, for their assistance in conducting the GM field trials at Kunjin, WA. I would like also like to acknowledge the work of the ACPFG barley transformation group, ACPFG wheat transformation group and Dr. Parvis Ehsanzadeh for the creation and initial characterisation of the lines used in this project.

I am grateful to for the considerable time and efforts of Ms. Jan Nield who ensured the GM field trials and GM material were compliant to all OGTR licence conditions. I would like to thank Mrs. Ursula Langridge and her glasshouse team as well as The Plant Accelerator for their assistance with the hire of PC2 glasshouses, growth chambers and hydroponic systems. I would like to once again thank my supervisor Dr. Stuart Roy for providing previous years’ field data and braving the heat in 2014 to help harvest. I am unendingly grateful to Dr. Rhiannon Schilling for her friendship as well as technical support and for the field material/data provided. Thanks also to Mr. William Heaslip and Ms. Melissa Pickering for their help in harvesting. Considerable thanks to Ms. Melissa Pickering and Ms. Jodie Kretschmer for their never-ending technical support. A final unendingly thank you to Mr. Daniel Menadue for his friendship and support in attempting to keep me sane, especially during long harvest. To all the other members of the ACPFG Salt Focus Group thank you for your advice and support.

Finally I wish to thank my family, particularly my father Paul, step-father Matthew and sister Amethyst, but especially my mother Pam, thank you for your unceasing encouragement and support in believing in me even when I could not.