

# **Role of AtCIPK16 in Arabidopsis abiotic tolerance**

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THE UNIVERSITY  
*of* ADELAIDE

May 2015



# Table of Contents

<b>Table of Contents</b> .....	<b>i</b>
<b>List of Figures</b> .....	<b>vii</b>
<b>List of Tables</b> .....	<b>xi</b>
<b>List of Abbreviations</b> .....	<b>xiii</b>
<b>Abstract</b> .....	<b>xvii</b>
<b>Declaration</b> .....	<b>xix</b>
<b>Acknowledgments</b> .....	<b>xxi</b>
<b>Chapter 1: Literature Review and Research Aims</b> .....	<b>1</b>
1.1 Salinity.....	1
1.1.1 Impacts of salinity.....	1
1.1.2 Effects of salinity stress on plants.....	1
1.1.3 The plants' tolerance mechanism to salt stress.....	2
1.2 Calcium signalling pathways.....	7
1.2.1 Structural characterisation of CBL.....	8
1.2.2 Structural characterisation of CIPK.....	9
1.2.3 Specificity of the CBL-CIPK signalling pathway.....	12
1.2.4 Function of the CBL-CIPK signalling pathway.....	13
1.3 AtCIPK16.....	18
1.3.1 Potential role of AtCIPK16 in salinity tolerance.....	19
1.4 Research aims.....	20
<b>Chapter 2: General materials and methods</b> .....	<b>21</b>
2.1 Plant materials.....	21
2.2 Plant growth facilities.....	21
2.3 Plant growth in soil.....	21
2.4 Plant growth in hydroponics.....	23
2.5 Plant growth on plates containing Murashige and Skoog media.....	25
2.6 DNA extractions.....	25
2.6.1 Phenol/chloroform/iso-amyl alcohol method.....	25
2.6.2 Edwards DNA extraction method.....	26
2.7 Agarose gel electrophoresis - DNA.....	27
2.8 DNA extraction from agarose gels.....	27
2.9 DNA sequencing.....	27
2.10 RNA extractions and agarose gel electrophoresis.....	28
2.11 cDNA synthesis.....	29
2.12 Polymerase chain reaction (PCR).....	30
2.12.1 Routine gDNA/cDNA PCR.....	30

2.12.2	High-fidelity PCR.....	31
2.12.3	Colony PCR.....	32
2.13	Cloning PCR products into entry vectors.....	33
2.14	Preparation of competent cells ( <i>Escherichia coli</i> ).....	36
2.15	Transformation of plasmid DNA into <i>E.coli</i> cells.....	36
2.16	Isolation of plasmid DNA from <i>E.coli</i> cells.....	37
2.17	Restriction enzyme digestion of plasmid DNA.....	37
2.18	LR reactions.....	38
2.19	Agrobacterium-mediated stable transformation of Arabidopsis.....	40
2.19.1	Preparation of competent <i>A. tumefaciens</i> AGL1 cells.....	40
2.19.2	Transformation of plasmid DNA into <i>A.tumefaciens</i> AGL1 cells.....	40
2.19.3	Transformation by floral dipping.....	41
2.20	Selection of transformants.....	41
2.20.1	Selection in soil.....	41
2.20.2	Selection on MS plate.....	41
2.21	Statistical analysis.....	42
<b>Chapter 3: Identification of upstream regulators of AtCIPK16.....</b>		<b>43</b>
3.1	Introduction.....	43
3.2	Chapter aims.....	46
3.3	Materials and methods.....	46
3.3.1	Yeast two hybrid assays.....	46
3.3.1.1	Cloning for yeast two hybrid assays.....	46
3.3.1.2	Preparation of yeast strain AH109 from stock.....	51
3.3.1.3	Transformation of constructs into <i>S. cerevisiae</i> .....	51
3.3.1.4	Yeast two-hybrid assay.....	52
3.3.1.5	Isolation of plasmid DNA from <i>S. cerevisiae</i> .....	53
3.3.2	Bimolecular fluorescence complementation (BiFC) assay using both transient expression and stable expression.....	53
3.3.2.1	Cloning of <i>AtCBLs</i> and <i>AtCIPK16</i> into BiFC assay vector for transient expression in mesophyll protoplast.....	54
3.3.2.2	Cloning of <i>AtCBLs</i> and <i>AtCIPK16</i> into BiFC assay vector for Agrobacterium-infiltration in Arabidopsis leaves, tobacco leaves and stable constitutive over-expression in Arabidopsis plants.....	58
3.3.2.3	Transient expression of <i>AtCBLs-AtCIPK16</i> in Arabidopsis mesophyll protoplasts..	60
3.3.2.4	Transient expression of <i>AtCBLs-AtCIPK16</i> in Arabidopsis leaves using Agro-infiltration.....	61
3.3.2.5	Transient expression of <i>AtCBLs-AtCIPK16</i> in tobacco leaves ( <i>Nicotiana benthamiana</i> ) using Agro-infiltration.....	62
3.3.2.6	Stable constitutive over-expression of <i>AtCBLs-AtCIPK16</i> in Arabidopsis ecotype	

Col-0.....	63
3.3.2.7 Fluorescence imaging by confocal microscopy.....	64
3.4 Results.....	65
3.4.1 Vector construction for a yeast two hybrid assay.....	65
3.4.2 Yeast two hybrid assay shows AtCIPK16 interacts with 6 AtCBL proteins.....	67
3.4.3 Vector construction for Bimolecular Fluorescence Complementation (BiFC) assay in Arabidopsis mesophyll protoplast.....	68
3.4.4 Bimolecular fluorescent complementation (BiFC) assay in Arabidopsis mesophyll protoplast.....	71
3.4.5 Vector construction for a Bimolecular Fluorescence Complementation (BiFC) assay using either Agro-infiltration of Arabidopsis and tobacco leaves, or stable expression in Col-0.....	75
3.4.6 Subcellular localization using Agro-infiltration in Arabidopsis leaves.....	78
3.4.7 Subcellular localization using Agro-infiltration in tobacco leaves.....	88
3.4.8 Localization of AtCBLs-AtCIPK16 complexes using stable expression in Arabidopsis ecotype Col-0.....	91
3.5 Discussion.....	93
3.5.1 Interacting partners of AtCIPK16 in yeast two hybrid assays.....	93
3.5.2 Interactions and localizations of AtCBL-AtCIPK16 in BiFC assays.....	95
3.6 Summary.....	102
<b>Chapter 4: Identification of downstream targets of AtCIPK16.....</b>	<b>103</b>
4.1 Introduction.....	103
4.2 Materials and methods.....	105
4.2.1 Pull-down assay.....	105
4.2.1.1 Peptide antigen design.....	105
4.2.1.2 Generation of a specific rabbit IgG antibody.....	106
4.2.1.3 Production of recombinant protein.....	107
4.2.1.4 SDS Polyacrylamide Gel Electrophoresis.....	110
4.2.1.5 Western blot for identification of the expected band on the gel.....	111
4.2.1.6 Optimization of recombinant protein synthesis.....	112
4.2.1.7 Purification of denatured protein.....	113
4.2.1.8 Refolding of purified denatured protein.....	114
4.2.2 Yeast two hybrid assay.....	115
4.2.2.1 Cloning for yeast two hybrid assay .....	115
4.2.2.2 Analysis of the protein sequences of AtHKT1;1, AtSOS1 and AtAKT1 .....	115
4.4 Results.....	117
4.4.1 Alignment of the protein sequences of AtCIPK16 with 26 AtCIPKs in Arabidopsis and peptide antigen design.....	117
4.4.2 Construction of plasmid for protein synthesis.....	121

4.4.3	Recombinant His-AtCIPK16 was obtain from <i>E.coli</i> and recognized by anti-AtCIPK16 antibody in Western blot.....	121
4.4.4	Optimization of recombinant protein synthesis.....	123
4.4.4.1	Expression of recombinant His-AtCIPK16 in two codon bias-adjusted <i>E. coli</i> strains showed no improvement in protein yield.....	124
4.4.4.2	Low temperature induction shows no improvement on soluble protein yield.....	126
4.4.4.3	Induction of His-AtCIPK16 using 0.2 % L-arabinose resulted in the maximum yield of insoluble recombinant protein.....	126
4.4.5	Recombinant His-AtCIPK16 was successfully denatured by Guanidine-HCl and purified by using cobalt chelating resin.....	128
4.4.6	Purified denatured His-AtCIPK16 was refolded using gradual dialysis.....	128
4.4.7	Construction of vector for yeast two hybrid assay.....	129
4.5	Discussion.....	133
4.5.1	Expression of recombinant protein His-AtCIPK16.....	133
4.5.2	Potential downstream targets of AtCIPK16.....	134
4.5.3	The alignment of 26 AtCIPKs shows unique regions of AtCIPK16 in functional motifs.....	135
4.5.4	Future work.....	140
4.6	Summary.....	142
<b>Chapter 5: Dissecting the role of AtCIPK16 in salinity tolerance.....</b>		<b>143</b>
5.1	Introduction.....	143
5.2	Chapter aims.....	145
5.3	Methods.....	145
5.3.1	Plant materials for complementary function analysis.....	145
5.3.2	Cloning of <i>AtCIPK16</i> into constitutive expression vector.....	145
5.3.3	Transformation of plasmid DNA into <i>A.tumefaciens</i> AGL1 competent cells.....	146
5.3.4	Stable constitutive over-expression of <i>AtCIPK16</i> in <i>sos2</i> knockout lines.....	146
5.3.5	Selection of transformants of <i>AtCIPK16-sos2</i> .....	146
5.3.6	Phenotyping of T <sub>2</sub> transgenic lines that constitutively over-expresses <i>AtCIPK16</i> in <i>sos2</i> knockout lines under salt stress.....	147
5.3.7	Biomass and flame photometry measurements.....	148
5.3.8	Genotyping.....	148
5.3.9	RT-PCR.....	149
5.3.10	Radioactive Tracer Experiments.....	149
5.4	Result.....	152
5.4.1	Vector constructed for stable constitutive expression of <i>AtCIPK16</i> in <i>sos2</i> knockout lines.....	152
5.4.2	Analysis of the expression level of <i>SOS2</i> , <i>AtCIPK16</i> in <i>sos2</i> knockout lines and complimentary lines.....	153

5.4.3	Constitutive expression of <i>AtCIPK16</i> fails to complement the salt sensitivity phenotype of <i>sos2</i> knockout lines.....	154
5.4.4	Movement of <sup>22</sup> Na <sup>+</sup> through <i>35S:AtCIPK16</i> expressing Arabidopsis.....	158
5.5	Discussion.....	161
5.5.1	<i>AtCIPK16</i> and <i>AtCIPK24</i> could have no functional redundancy.....	161
5.5.2	<i>AtCIPK16</i> may alter net Na <sup>+</sup> influx in root.....	163
5.5.3	Future work.....	164
5.6	Summary.....	165
<b>Chapter 6: Characterization of <i>AtCIPK16</i> under various abiotic stresses.....</b>		<b>166</b>
6.1	Introduction.....	166
6.2	Chapter aims.....	168
6.3	Materials and methods.....	168
6.3.1	<i>In silico</i> analysis of <i>AtCIPK16</i> .....	168
6.3.2	Selection of homozygous transgenic lines that constitutively over-expresses <i>AtCIPK16</i> .....	168
6.3.3	Phenotyping transgenic lines constitutively over-expressing <i>AtCIPK16</i> under ABA treatment.....	17
1		
6.3.4	Characterization of the phenotype of homozygous transgenic lines that constitutively over-expresses <i>AtCIPK16</i> under low potassium stress.....	171
6.3.5	Characterization of the phenotype of homozygous transgenic lines that constitutively over-expresses <i>AtCIPK16</i> when exposed to additional KCl.....	172
6.3.6	Characterization of the phenotype of homozygous transgenic lines that constitutively over-expresses <i>AtCIPK16</i> under drought stress.....	173
6.3.7	Characterization of the phenotype of homozygous transgenic lines that constitutively over-expresses <i>AtCIPK16</i> under osmotic stress.....	174
6.3.8	Characterization of the phenotype of homozygous transgenic lines that constitutively over-expresses <i>AtCIPK16</i> under cold stress.....	174
6.3.9	Proline content measurements.....	175
6.3.10	Chlorophyll content measurements.....	176
6.3.11	Flame photometry measurements.....	176
6.4	Result.....	177
6.4.1	<i>In silico</i> expression profile of <i>AtCIPK16</i> .....	177
6.4.2	Constitutive expression of <i>AtCIPK16</i> resulted in lower germination rate with increasing ABA treatments.....	179
6.4.3	Under low potassium stress constitutively over-expressing <i>AtCIPK16</i> lines have improved root K <sup>+</sup> accumulation compared with Col-0 .....	186
6.4.4	Col-0 and <i>AtCIPK16</i> over-expression lines behave similarly under high KCl stress.....	189

6.4.5	Col-0 and <i>AtCIPK16</i> over-expression lines behave similarly under drought stress.....	192
6.4.6	Col-0 and <i>AtCIPK16</i> over-expression lines behave similarly during osmotic stresses.....	194
6.4.7	Col-0 and <i>AtCIPK16</i> over-expression lines behave similarly during cold stresses..	197
6.5	Discussion.....	199
6.5.1	<i>AtCIPK16</i> exhibits ABA-related characteristics.....	199
6.5.2	<i>AtCIPK16</i> exhibits K <sup>+</sup> transport characteristics.....	202
6.5.3	<i>AtCIPK16</i> exhibits no characteristics for drought, osmotic and cold stresses.....	205
6.5.4	Limitations of experimental techniques.....	206
6.6	Summary.....	208
<b>Chapter 7: General Discussion.....</b>		<b>209</b>
7.1	Summary of accomplished work.....	209
7.1.1	AtCBL interacting partners and localizations of <i>AtCIPK16</i> .....	209
7.1.2	Function of <i>AtCIPK16</i> in Na <sup>+</sup> , K <sup>+</sup> transport and response to ABA in Arabidopsis plants.....	210
7.2	Future work.....	211
7.2.1	Future directions for functionally characterising <i>AtCIPK16</i> in ion transport.....	211
7.2.2	Future directions for identifying <i>AtCIPK16</i> equivalents in different species.....	212
7.2.3	Future directions for identifying downstream targets of <i>AtCIPK16</i> .....	213
7.3	Conclusion.....	215
<b>References.....</b>		<b>217</b>
<b>Appendix.....</b>		<b>241</b>



## List of Figures

Figure 1.1: The main mechanisms of salt tolerance in plants.....	6
Figure 1.2: The general structure of CBL proteins contains four EF-hands. Black numbered boxes representing each EF-hand in CBLs.....	9
Figure 1.3: The general structure of CIPKs.....	10
Figure 1.4: Sequence of the activation loops motif in all AtCIPKs.....	10
Figure 1.5: Diagram showing different abiotic stresses triggering a variety of CBL-CIPK signalling pathways in Arabidopsis.....	13
Figure 2.1: Schematic diagram of pCR8/GW/TOPO TA Gateway® entry vector.....	35
Figure 3.1: The bait vector pTOOL27 was used to express <i>AtCIPK16</i> fused to GAL4 DNA binding domain for the yeast two hybrid assay.....	49
Figure 3.2: The prey vector pTOOL28 was used to express one of the 10 <i>AtCBL</i> genes fused to GAL4 activation domain for yeast two hybrid assay.....	50
Figure 3.3: The vector <i>pUC-SPYNE/GW</i> was used to express <i>AtCIPK16</i> fused to the N-terminal split eYFP fragment for BiFC assay.....	56
Figure 3.4: The vector <i>pUC-SPYNE/GW</i> was used to express 10 <i>AtCBLs</i> fused to the C-terminal split eYFP fragment for BiFC assay.....	57
Figure 3.5: The vector <i>pGPTVII</i> was used to express 10 <i>AtCBLs/AtCIPK16</i> fused to N-/C-terminal split eYFP fragment for Agrobacterium-infiltration in Arabidopsis leaves, tobacco leaves and stable constitutive over-expression in Arabidopsis plants.....	59
Figure 3.6: <i>pTOOL28 + AtCBL1 to 10</i> and <i>pTOOL27 + AtCIPK16</i> .....	66
Figure 3.7: Yeast two hybrid assay showing AtCIPK16 interacts with 6 AtCBL proteins.....	67
Figure 3.8: The <i>pUC-SPYCE/GW+ AtCBL1 to 10</i> and <i>pUC-SPYNE/GW+ AtCIPK16</i> plasmids used for subcellular localization in a mesophyll protoplast expression system.....	70
Figure 3.9: Subcellular localization of AtCBLs::YC and AtCIPK16::YN interactions in Arabidopsis mesophyll protoplasts.....	73
Figure 3.10: <i>pGPTVII.Hyg. AtCBL1 (to 10)::YC</i> , <i>pGPTVII.Hyg.YC::AtCBL1 (to 10)</i> , <i>pGPTVII.Bar.AtCIPK16::YN</i> and <i>pGPTVII.Bar. YN::AtCIPK16</i> plasmids used for Agro-infiltration and stable expression in Col-0.....	77
Figure 3.11: YN::AtCIPK16 and AtCBLs::YC interactions in Arabidopsis leaves.....	81
Figure 3.12: YN::AtCIPK16 and YC::AtCBLs interactions in Arabidopsis leaves.....	83
Figure 3.13: AtCIPK16::YN and YC::AtCBLs interactions in Arabidopsis leaves.....	85
Figure 3.14: AtCIPK16::YN and AtCBLs::YC interactions in Arabidopsis leaves.....	87
Figure 3.15: YN::AtCIPK16 and AtCBLs::YC interactions in tobacco leaves ( <i>Nicotiana benthamiana</i> ).....	90
Figure 3.16: Co-expression of different <i>AtCBLs / AtCIPK16</i> split YFP constructs in	

Arabidopsis ecotype Col-0.....	92
Figure 4.1: The vector pDEST17 was used to express <i>AtCIPK16</i> and <i>AtCBL4</i> in <i>E.coli</i> .....	108
Figure 4.2: Diagram of Western blot setup.....	111
Figure 4.3: Alignment of 26 AtCIPKs protein sequence using Vector NTI version 11.0.....	119
Figure 4.4: Hydrophilicity plot of <i>AtCIPK16</i> using ExPASy ProtScale program.....	120
Figure 4.5: <i>pDEST17-AtCIPK16</i> and <i>pDEST17-AtCBL4</i> used for production of His-tagged <i>AtCIPK16</i> and His-tagged <i>AtCBL4</i> (negative control) in <i>E.coli</i> .....	121
Figure 4.6: Concentration of His- <i>AtCIPK16</i> in <i>E.coli</i> strain BL21-AI after 0-4 h induction period.....	123
Figure 4.7: SDS-PAGE analysis of protein samples from <i>E.coli</i> after either a 2 h or 4 h induction.....	124
Figure 4.8: Rare <i>E.coli</i> Codon analysis of the DNA sequence of <i>AtCIPK16</i> using Rare Codon Calculator.....	125
Figure 4.9: SDS-PAGE analysis of protein samples extracted from various <i>E.coli</i> strains: BL21-CodonPlus(DE3)-RIL, BL21-CodonPlus(DE3)-RP and BL21-AI.....	126
Figure 4.10: SDS-PAGE analysis of protein samples from cultures induced at different temperatures and concentrations of L-arabinose.....	127
Figure 4.11: SDS-PAGE gel of purified denatured His- <i>AtCIPK16</i> extracted from <i>E. coli</i> and refolded His- <i>AtCIPK16</i> .....	128
Figure 4.12: Topology model of AtSOS1, AtHKT1;1 and AtAKT1 with predicted phosphorylation sites.....	131
Figure 4.13: Alignment of 26 AtCIPKs protein sequence using Vector NTI version 11.0...	139
Figure 4.14: The vector <i>pMDC83</i> will be used to express <i>AtCIPK16</i> fused to GFP tag.....	141
Figure 5.1: <i>pMDC32-35S:AtCIPK16</i> for constitutive over-expression of <i>AtCIPK16</i> in <i>sos2</i> knockout lines .....	152
Figure 5.2: Expression analysis of <i>sos2</i> knockout lines and <i>AtCIPK16-sos2</i> complimentary lines.....	153
Figure 5.3: Characterisation of <i>sos2</i> knockout and <i>sos2-AtCIPK16</i> complimentary lines on plates .....	156
Figure 5.4: Characterisation of <i>sos2</i> knockout and <i>sos2-AtCIPK16</i> complimentary lines on soil.....	157
Figure 5.5: The expression levels of <i>AtCIPK16</i> in transgenic lines were confirmed by RT-PCR using cDNA which was synthesised from RNA extracted from root tissue.....	159
Figure 5.6: Measurement of Na <sup>+</sup> content in <i>35S:AtCIPK16</i> over-expressing Col-0 and nulls after 5 days 50 mM NaCl treatment in the preliminary experiments.....	159
Figure 5.7: Measurement of <sup>22</sup> Na <sup>+</sup> content in <i>35S:AtCIPK16</i> over-expressing Col-0 and nulls using radioactive tracer <sup>22</sup> Na <sup>+</sup> .....	160
Figure 6.1: <i>pTOOL2-35S:AtCIPK16</i> for constitutively over-expressing <i>AtCIPK16</i> in Arabidopsis.....	170

Figure 6.2: The transcriptional response of <i>AtCIPK16</i> to various stimuli (e.g. biotic, chemical, hormone, nutrient, photoperiod, stresses and others) in 152 microarray studies stored in Genevestigator.....	178
Figure 6.3: Effect of ABA on seedling growth.....	180
Figure 6.4: Effect of increasing ABA concentrations on germination rate of <i>AtCIPK16</i> over-expression lines day 3 to day 10 after vernalization.....	185
Figure 6.5: <i>AtCIPK16</i> over-expressing lines have improved K <sup>+</sup> uptake under K <sup>+</sup> deficient conditions.....	188
Figure 6.6: <i>AtCIPK16</i> over-expressing Arabidopsis were not more tolerant to high concentrations of K <sup>+</sup> .....	191
Figure 6.7: Over-expression of <i>AtCIPK16</i> does not improve drought tolerance.....	193
Figure 6.8: <i>AtCIPK16</i> over-expressing lines have no significant difference in osmotic stress tolerance when compared to Col-0.....	195
Figure 6.9: Over-expression of <i>AtCIPK16</i> does not improve cold tolerance.....	198



## List of Tables

Table 2.1: Nutrient solution for soil grown Arabidopsis.....	22
Table 2.2: Germination solution for hydroponics Arabidopsis.....	24
Table 2.3: Basal nutrient solution for hydroponics Arabidopsis.....	24
Table 2.4: Primers for sequencing the entry vectors and destination vectors constructed in this project.....	28
Table 2.5: Platinum <i>Taq</i> polymerase PCR solution and program used for routine PCR.....	31
Table 2.6: Platinum <i>Taq</i> polymerase high fidelity and Elongase PCR solution and program used for the routine PCR.....	32
Table 2.7: Platinum <i>Taq</i> polymerase PCR solution and program used for the colony PCR.....	33
Table 2.8: Summary of destination vectors used in this thesis.....	39
Table 3.1: Primers used to the clone coding sequences of 10 <i>AtCBLs</i> , <i>AtCIPK16</i> , <i>AtCIPK16Nt</i> and <i>AtAKT1</i> from Arabidopsis cDNA.....	48
Table 3.2: Primers used to clone coding sequences (without the stop codon) of 10 <i>AtCBLs</i> and <i>AtCIPK16</i> from Arabidopsis cDNA.....	55
Table 3.3: Primers for genotyping the BiFC stable expressed Arabidopsis.....	64
Table 3.4: Summary of entry vectors and destination vectors constructed for yeast two hybrid assay.....	65
Table 3.5: Summary of entry vectors and destination vectors constructed for transient expression of <i>CIPK16</i> and <i>CBL</i> genes in mesophyll protoplast using BiFC assay.....	69
Table 3.6: Summary of entry vectors and destination vectors constructed for transient expression in Arabidopsis and tobacco leaves or for stable expression in Col-0.....	76
Table 3.7: Summary of <i>AtCIPK16</i> – <i>AtCBL</i> interactions and their cellular location in Arabidopsis leaves using Agro-infiltrations with various vector pairs.....	79
Table 3.8: Summary of <i>AtCIPK16/AtCBL</i> interactions as measured using yeast two hybrids and transient expression in Arabidopsis protoplasts, Arabidopsis leaves and tobacco ( <i>N.benthamiana</i> ) leaves.....	99
Table 4.1: Names and accession numbers of 26 <i>AtCIPKs</i> aligned for antibody design.....	106
Table 4.2: Four elution buffers used to dissociate and elute purified protein from resin.....	114
Table 4.3: Prediction of His- <i>AtCIPK16</i> solubility in <i>E.coli</i> .....	124
Table 5.1: <i>sos2</i> knockout lines obtained from NASC.....	145
Table 5.2: Primers for genotyping <i>AtCIPK16-sos2</i> lines.....	147
Table 5.3: Primers for examining the expression levels of <i>AtCIPK24</i> , <i>AtActin2</i> , <i>AtHKT1;1</i> and <i>AtCIPK16</i> .....	149
Table 5.4: Tissue concentrations of <sup>22</sup> Na <sup>+</sup> and % translocated from root to shoot.....	160

Table 6.1: Primers used for identifying homozygous transgenic lines over-expressing <i>AtCIPK16</i> .....	169
Table 6.2: Medium for low stress treatment.....	172
Table 6.3: Programme used for cold treatment.....	175

## List of Abbreviations

<b>Abbreviation</b>	<b>Full term</b>
3'	Three prime, of nucleic acid sequence
5'	Five prime, of nucleic acid sequence
#	Number
%	Percent
±	Plus and minus
×	Times
°C	Degree Celsius
aa	Amino acid
ABA	Abscisic acid
ACPFPG	Australian Centre for Plant Functional Genomics
AGRF	Australian Genome Research Facility
<i>Agrobacterium</i>	<i>Agrobacterium tumefaciens</i>
AKT	Arabidopsis potassium channel
amiRNA	Artificial micro ribonucleic acid
ANOVA	Analysis of variance
Arg	Arginine
Asp	Aspartic acid
At	<i>Arabidopsis thaliana</i>
AVP	Vacuolar H <sup>+</sup> -pyrophosphatase
BLAST	Basic Local Alignment Search Tool
BNS	Basal nutrient solution
bp	Base pairs, of nucleic acid
BSA	Bovine serum albumin
C-terminal	Carboxyl terminal
C-terminus	Carboxyl terminus
Ca <sup>2+</sup>	Calcium ion
Ca(NO <sub>3</sub> ) <sub>2</sub>	Calcium nitrate
CaCl <sub>2</sub>	Calcium chloride
cAMP	Adenosine 3', 5'-cyclic monphosphate
CaMV	Cauliflower mosaic virus
Cat.#	Catalogue number
CBL	Calcineurin B-like proteins
cDNA	Complementary deoxyribonucleic acid
CHX	Cation/H <sup>+</sup> exchangers
CIPK	CBL-interacting protein kinases
Cl <sup>-</sup>	Chloride ion
cm	Centimetre(s)
CoCl <sub>2</sub>	Cobalt chloride
Col-0	Columbia-0

CuSO <sub>4</sub>	Cupric sulfate
d	Day(s)
Da	Dalton
DEPC	Diethylpyrocarbonate
dH <sub>2</sub> O	Deionised water
DNA	Deoxyribonucleic acid
dNTPs	Mixture of equal equivalents of dATP, dTTP, dCTP and dGTP
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agricultural Organization of the United Nations
FW	Fresh weight
g	Gram(s)
g	Gravity
gDNA	Genomic deoxyribonucleic acid
GFP	Green fluorescent protein
H <sub>2</sub> O	water
H <sub>3</sub> BO <sub>3</sub>	Boric acid
HCl	Hydrochloric acid
His	Polyhistidine tag
h	Hour(s)
H <sup>+</sup>	Proton
K <sup>+</sup>	Potassium ion
KAT	Potassium Arabidopsis transporter
kb	Kilo base pairs, of nucleic acid
KCl	Potassium chloride
KDa	Kilo Dalton
KH <sub>2</sub> PO <sub>4</sub>	Monopotassium phosphate
KNO <sub>3</sub>	Potassium nitrate
LB	Luria and Bertani medium
Leu	Leucine
LR	Ligation reaction
Lys	Lysine
M	Molar
MES	2-(N-Morpholino) ethanesulfonic acid, 4-morpholineethanesulfonic acid
Met	Methionine
mg	Miligram(s)
Mg <sup>2+</sup>	Magnesium ion
MgSO <sub>4</sub>	Magnesium sulphate
min	Minute(s)
mL	Millilitre(s)
mm	Millimetre(s)
mM	Millimolar
Mn <sup>2+</sup>	Manganese ion
MnCl <sub>2</sub>	Manganese choride



mRNA	Messenger RNA
MS media	Murashige and Skoog media
mV	millivolt
n	Sample size
N-terminal	Amine terminal
N-terminus	Amine terminus
N/A	Not applicable
Na <sup>+</sup>	Sodium ion
Na <sub>2</sub> HPO <sub>4</sub>	Sodium phosphate dibasic
Na <sub>2</sub> MoO <sub>4</sub>	Sodium molybdate
NaCl	Sodium chloride
NaFe(III)EDTA	Sodium iron EDTA
NaOH	Sodium hydroxide
NCBI	National Centre for Biotechnology Information
NH <sub>4</sub> NO <sub>3</sub>	Ammonium nitrate
NHX	Na <sup>+</sup> /H <sup>+</sup> antiporter
NiCl <sub>2</sub>	Nickel chloride
nM	Nanomolar
No.	Number
NO <sup>3-</sup>	Nitrate ion
nosT	Bacterial nopaline synthase terminator sequence
ng	Nanogram(s)
OD <sub>600</sub>	Optical density measured at 600 nm
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
pI	Isoelectric point
PI	Proidium iodide
PO <sub>4</sub> <sup>3-</sup>	Phosphate ion
Q-PCR	Quantitative real time polymerase chain reaction
QTL	Quantitative trait loci
RNA	Ribonucleic acid
RO	Reverse osmosis
ROS	Reactive oxygen species
sec	Second(s)
SEM	Standard error of the mean
Ser	Serine
SDS	Sodium dodecyl sulphate
SKOR	Stelar K <sup>+</sup> outward rectifier
SOS	Salt overly sensitive
T-DNA	Transfer deoxyribonucleic acid
T <sub>1</sub>	Primary Arabidopsis transformant
T <sub>2</sub>	Progeny of T <sub>1</sub> plant

TAE	Tris base, acetic acid and EDTA buffer
TE	Tris-EDTA
Thr	Threonine
Tm	Melting temperature, of primers
Tris-HCl	Tris (hydroxymethyl) aminomethane hydrochloride
Triton X-100	Toctylphenoxypolyethoxyethanol
Trp	Tryptophan
Tyr	Tyrosine
U	Units
UTR	Untranslated region
V	voltage
v/v	Volume per volume
w/v	Weight per volume
Xenopus	<i>Xenopus laevis</i>
YFP	Yellow fluorescent protein
Zn <sup>2+</sup>	Zinc ion
ZnSO <sub>4</sub>	Zinc sulfate

## Abstract

Soil salinity is a significant environmental problem affecting agriculture around the world leading to reduced crop yield. High concentrations of  $\text{Na}^+$  affect cell metabolism and compete with  $\text{K}^+$  for the binding sites of enzymes which play important roles in cellular function. One mechanism for improving salinity tolerance of crop plants is to minimise the accumulation of  $\text{Na}^+$  in the shoot. *AtCIPK16* (Calcineurin B-like-interacting protein kinase 16) has been identified as a novel candidate gene important in increasing salinity tolerance (Roy *et al.* 2013). Over-expression of *AtCIPK16* has been shown to reduce the shoot sodium in a number of species. In both hydroponic and soil culture, Arabidopsis with constitutive over-expression of *AtCIPK16* show significant reductions in  $\text{Na}^+$  concentration in shoot, compared with wild type and nulls, while Arabidopsis with amiRNA knockdown of *AtCIPK16* exhibit an increase of  $\text{Na}^+$  concentration in shoot (Roy *et al.* 2013). While it can be clearly seen that alterations in the expression of *AtCIPK16* result in increased salinity tolerance, little is known, however, about the role the protein plays in tolerance mechanisms. It is therefore important to identify its cellular location, upstream and downstream targets, and which abiotic stresses it is involved in to elucidate its function in plants.

Yeast two hybrid systems were used to identify the potential upstream CBL partners of *AtCIPK16*. The assay revealed 6 *AtCBLs* (*AtCBL1*, *AtCBL2*, *AtCBL4*, *AtCBL5*, *AtCBL9* and *AtCBL10*) could interact with *AtCIPK16*. Bimolecular Fluorescence Complementation (BiFC) assays were then employed to confirm the result from Y2H and showed one more interacting *AtCBL* partner, *AtCBL3*. Additionally, BiFC demonstrated possible plasma membrane localization of the complexes of *AtCBL1-AtCIPK16*, *AtCBL4-AtCIPK16*, *AtCBL5-AtCIPK16* and *AtCBL9-AtCIPK16*; and cytoplasm localization of the complexes of *AtCBL2-AtCIPK16*, *AtCBL3-AtCIPK16* and *AtCBL10-AtCIPK16* using transient co-expression in *Nicotiana benthamiana* leaves. Moreover, a pull-down assay was planned to identify downstream target proteins of *AtCIPK16*.

The radioactive tracer  $^{22}\text{Na}^+$  was used to quantify net  $\text{Na}^+$  accumulation in the different part of transgenic Arabidopsis overexpressing *AtCIPK16* and nulls to determine if this gene can alter  $\text{Na}^+$  influx or  $\text{Na}^+$  translocation in plants. Only one transgenic line showed lower  $\text{Na}^+$  accumulation in root compare to nulls under salt stress, while all three transgenic lines demonstrated slightly lower but not significant  $\text{Na}^+$  translocation rate and shoot  $\text{Na}^+$  accumulation compare to nulls under 50 mM NaCl treatment. Furthermore, to examine the function redundancy of *AtCIPK24* and *AtCIPK16* in salt stress, complementary lines of constitutively expressing *AtCIPK16* in the *atcipk24/sos2* knockout lines background were

generated and analysed with plate assay and soil assay. The study revealed constitutive expression of *AtCIPK16* could not complement the salt sensitivity phenotype of *atcipk24/sos2* knockout mutants, suggest their different functions which are non-complementary in each other's signalling pathway.

The phenotypes of *35S:AtCIPK16* were characterized under osmotic, drought, cold, low K<sup>+</sup> stresses and ABA treatment to examine the potential function of AtCIPK16 in other stresses. This study revealed that over-expressing *AtCIPK16* plants were more sensitive to ABA and had increased K<sup>+</sup> root accumulation when grown under low K<sup>+</sup> stress, it appears that AtCIPK16 is involved with processes involving the transport of monovalent cations. No significant phenotypic variation was observed in cold, drought, osmotic and high KCl stresses, suggesting AtCIPK16 could be not involved in other stresses which typically require the production of compatible solutes or enzymes which mop up reactive oxygen species. However, the function of AtCIPK16 in salinity tolerance and in the response to other abiotic stresses still requires further characterization.

## Declaration

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Wenmian Huang

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Date



## Acknowledgments

Firstly I would like to thank my supervisors Dr Stuart Roy and Prof. Mark Tester for their time, support, patience, understanding, encouragement and excellent guidance throughout my candidature.

I gratefully acknowledge the financial support provided from The University of Adelaide through provision of Adelaide Graduate Research Scholarship and The Australian Centre for Plant Functional Genomics during my PhD, Grain Research and Development Corporation for a travel grant to attend the IWPMB2013.

I would like to thank all people who have provided advices and technical support during my study. A special thanks to Prof. Joerg Kudla for providing advice and plasmid vectors, Dr Bettina Berger for providing advice on the project, Mr Nadim Shadiac for a lot of advice and assistance in protein experiment, Dr Matt Gilliham for providing advice and support on radioactive tracer experiments, Dr Sam Henderson for designing the experiment and providing assistance in radioactive flux assay, Dr Andrew Jacobs and Ms Jodie Kretschmer for instruction on cloning and providing plasmid vectors, Ms Natasha Bazanova for providing vectors, strains and assistance in yeast two hybrid assays, Dr Yuri Shavrukov for assistance with the flame photometer assay, Mr George Dimitroff for providing tobacco leaves in BiFC assays, Dr Gwen Mayo and Ms Lynette Waterhouse for the help with microscopy, Ms Jan Nield for assistance throughout the vectors import process, Ms. Ruth Harris for assistance and advice in English writing.

Huge thanks to all the members of the ACPFG Salt Focus Group, both past and present, for their support and help: Ms Melissa Pickering, Dr Rhiannon Schilling, Mr Gordon Wellman, Dr Sandra Schmoeckel, Dr Bo Li, Dr Aris Hairsmann, Dr Monique Shearer, Dr Nawar Shamaya, Dr Aurelie Evrard, Ms Jessica Bovill, Dr Joanne Tillbrook. Many thanks to the members of Plant Research Centre for their kind help: Ms Jiaen Qiu, Dr Bo Xu and Dr Jin Zhang.

Finally, I would like to thank my friends and parents for their support and encouragements throughout all of my studies.