Transcription Factors Important in the Regulation of Salinity Tolerance

by

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Table of contents

List of Figures viii
List of Tables xiv
List of Abbreviations xv
Abstract xviii
Declaration xix
Acknowledgements xx
Chapter 1. Literature review 1
1.1. Importance of cereals 2
1.2. The problem of salinity 2
1.3. The effect of salt on plants 3
1.3.1. Sodium entry into plant cells 4
1.4. How plants deal with salinity 5
1.5. Signalling molecules in salt stress response 7
1.5.1. ABA 7
1.5.2. Calcium 9
1.5.3. High affinity K^+ transporter (HKT) 10
1.5.4. SOS 13
1.5.5. NHX 15
1.6. Improving salinity tolerance in plants 17
1.6.1. Plant breeding 17
1.6.2. Plant transformation 18
1.7. Transcription factors regulating abiotic stress responses 19
1.7.1. Myb transcription factors 20
1.7.1.1. Background 20
1.7.1.2. Role in plants 20
1.7.1.3. Role in abiotic stress responses in *A. thaliana* 21
1.7.1.3.1. *AtMYB2* 23
1.7.1.3.2. *AtMYB15* 25

1.7.1.3.3. *AtMYB41* 26
1.7.1.3.4. *AtMYB44* 27
1.7.1.3.5. *AtMYBC1* 27
1.7.1.3.6. *AtMYBL* 28
1.7.1.3.7. *AtFLP and AtMYB88* 28
1.7.1.4. Role in abiotic stress responses in cereals 30
1.7.1.4.1. *OsMYB4* 30
1.7.1.4.2. *OsMYB2R-2* 33
1.7.1.4.3. *OsMYBS-3* 34
1.7.1.4.4. *TaMYB1* 34
1.7.1.4.5. *TaMYB32* 35
1.7.1.4.6. *TaMYBsdu1* 35
1.7.2. AP2/EREBP/ERF transcription factors 36
1.7.2.1. Background 36
1.7.2.2. Role of AP2/ERF genes in plants 37
1.7.2.3. Role AP2/ERF genes in abiotic stress responses in *A. thaliana* 38
1.7.2.3.1. *ERF group* 38
1.7.2.3.2. *DREB group* 39
1.7.2.4. Role of AP2/ERF genes in abiotic stress response in cereals 44
1.7.2.4.1. AP2/ERF genes in wheat 44
1.7.2.4.2. AP2/ERF genes in rice 47
1.7.3. bHLH transcription factors 51
1.7.3.1. Background 51
1.7.3.2. Role of *bHLH* genes in plants 51
1.7.3.3. Role of *bHLH* genes in abiotic stress responses in *A. thaliana* 53
1.7.3.3.1. *AtICE1* 53
1.7.3.3.2. *AtAIB* 53
1.7.3.3.3. *AtNIG1* 53
1.7.3.3.4. *AtbHLH92* 54

1.7.3.3.5. *AtMYC2* 55
1.7.3.4. Role of *bHLH* genes in abiotic stress responses in cereals 60
1.7.3.4.1. *OrbHLH2* 60
1.7.3.4.2. *OrbHLH001* 61
1.7.3.4.3. *TaICE41 and TaICE87* 61
1.7.3.4.4. *OsbHLH1* 62
1.7.3.4.5. *OsbHLH148* 62
1.7.4. The circadian clock and abiotic stress 64
Chapter 2. Gene expression analysis of salt responsive rice transcription factors 68
2.1. Introduction 69
2.2. Materials and methods 69
2.2.1. Materials 69
2.2.2. Bioinformatic analysis of microarray data 70
2.2.3. Quantitative RT-PCR analysis of transcription factor mRNA accumulation 71
2.2.3.1. Primer design 71
2.2.3.2. RNA extraction 71
2.2.3.3. Removal of contaminating DNA 72
2.2.3.4. cDNA synthesis 72
2.2.3.5. PCR check of primer specificity 72
2.2.3.6. Agarose gel electrophoresis 73
2.2.3.7. Confirmation of specific product formation 73
2.2.3.8. Sequencing PCR products 73
2.2.3.9. qRT-PCR 74
2.2.4. Bioinformatic analysis of candidate transcription factors 74
2.3. Results 75
2.3.1. Comparison of microarray and qRT-PCR data 75
2.3.2. Transcription factors selected for further study 83
2.3.2.1. Further characterisation of selected transcription factors 84

2.3.2.1.1. OsOrphan19 84
2.3.2.1.2. OsEREB67 87
2.3.2.1.3. OsMYBR63 89
2.3.2.1.4. OsbHLH17 93
2.3.2.1.5. OsLUX 99
2.3.2.1.6. OsMYB54 102
2.4. Discussion 105
2.4.1. OsOrphan19 105
2.4.2. OsEREB67 107
2.4.3. OsMYBR63 109
2.4.4. OsbHLH17 111
2.4.5. OsLUX 113
2.4.6. OsMYB54 115
Chapter 3. Generation of transgenic rice 116
3.1. Introduction 117
3.2. Materials and methods 117
3.2.1. Materials 117
3.2.2. Cloning transcription factor coding sequences 118
3.2.2.1. Nested PCR to amplify transcription factor coding sequences 118
3.2.2.2. Gel purification of transcription factor coding sequences 118
3.2.2.3. Cloning transcription factor coding sequences 118
3.2.2.4. Transformation of Mach1 Competent Cells 119
3.2.2.5. Colony PCR to screen for entry clones 120
3.2.2.6. Alkaline lysis DNA mini-prep 120
3.2.2.7. Restriction endonuclease digests 121
3.2.2.8. Glycerol culture stocks 121
3.2.2.9. Sequencing of entry vectors 121
3.2.3. Construction of expression vectors 121
3.2.3.1. 35S constitutive overexpression constructs 121

3.2.3.1.1. LR recombination reaction for the construction of expression vectors 121

3.2.3.1.2. Transformation of DH5α competent cells 124

3.2.3.1.3. Restriction endonuclease digests to screen expression clones ...124

3.2.3.2. Salt inducible expression constructs 124

3.2.3.2.1. Restriction endonuclease digests 124

3.2.3.2.2. Gel purification.....124

3.2.3.2.3. Ligation.....125

3.2.3.2.4. Transformation of competent cells 125

3.2.3.2.5. Restriction endonuclease digests 125

3.2.3.2.6. Glycerol culture stocks 125

3.2.3.2.7. DNA sequencing.....125

3.2.3.3. JRC0470 reporter constructs.....125

3.2.3.4. Artificial microRNA knockdown constructs 126

3.2.4. *Agrobacterium*-mediated transformation of japonica rice.....129

3.2.4.1. Transformation of *Agrobacterium tumefaciens* 129

3.2.4.2. Rice transformation.....129

3.3. Results 132

3.3.1. Expression vector construction.....132

3.3.1.1. Cloning transcription factor coding sequences 132

3.3.1.2. 2x35S overexpression vectors.....132

3.3.1.3. Salt inducible expression vectors and reporter constructs 139

3.3.1.4. Artificial microRNA knockdown expression vectors.....147

3.3.2. *Agrobacterium*-mediated transformation of japonica rice.....153

3.3.2.1. Rice plants with constitutive overexpression of transcription factor coding sequences displayed abnormal phenotypes 153

3.3.2.2. Rice plants with salt inducible overexpression of transcription factor coding sequences displayed abnormal phenotypes 153

3.3.2.3. Rice plants with constitutive overexpression of artificial microRNA constructs displayed a stay-green phenotype 158

3.4. Discussion 160
Chapter 4. Genotypic and phenotypic analysis of transgenic rice 162
4.1. Introduction 163
4.2. Materials and methods 163
4.2.1. Materials 163
4.2.2. Southern analysis of T0 rice plants 164
4.2.2.1. Isolation of rice genomic DNA 164
4.2.2.2. Restriction digests of rice genomic DNA 164
4.2.2.3. Gel electrophoresis of digested rice genomic DNA 164
4.2.2.4. DNA transfer to nylon membranes 164
4.2.2.5. Oligo labelling of DNA probes 165
4.2.2.6. Hybridisation 165
4.2.2.7. Autoradiography 166
4.2.3. Analysis of reporter plants 166
4.2.3.1. GUS staining and imaging of reporter plants 166
4.2.4. Salinity tolerance assays of transgenic plants with altered transcription factor expression 167
4.2.5. Analysis of sodium and potassium accumulation in transgenic plants 168
4.2.6. Semi-quantitative RT-PCR analysis of transgenic rice plants 170
4.2.7. Yield 170
4.3. Results 171
4.3.1. Southern analysis of T0 rice plants 171
4.3.2. Analysis of the JRC0470 salt inducible promoter 178
4.3.3. Salinity tolerance assay of T1 plants 183
4.3.4. Analysis of sodium and potassium accumulation in T1 plants 198
4.3.4.1. OsOrphan19 201
4.3.4.2. OsEREB67 209
4.3.4.3. OsMYBR63 215
4.3.4.4. OsbHLH17 221

4.3.4.5. OsLUX 226
4.3.4.6. OsMYB54 234
4.4. Discussion 242
4.4.1. OsOrphan19 245
4.4.2. OsEREB67 249
4.4.3. OsMYBR63 251
4.4.4. OsbHLH17 253
4.4.5. OsLUX 255
4.4.6. OsMYB54 256
4.4.7. Drawback, strengths and future directions 257
4.4.7.1. Silicate affects Na^+ accumulation 257
4.4.7.2. Cultivar selection 257
4.4.7.3. Effectiveness of the approach 258
4.4.8. Concluding remarks 258
Chapter 5. Appendices 260
5.1. Oligonucleotides used in PCR 261
5.2. Media 266
5.3. Statistical tests 268
5.3.1.1. OsOrphan19 268
References 272

List of Figures

Figure 2.1 Graph comparing microarray and qRT-PCR data for Os.3397.1.S1_at 76

Figure 2.2 Graph comparing microarray and qRT-PCR data for Os.11094.1.A1_at 77

Figure 2.3 Graph comparing microarray and qRT-PCR data for Os.18955.1.S1_at 78

Figure 2.4 Graph comparing microarray and qRT-PCR data Os.46956.1.S1_at....80

Figure 2.5 Graph comparing microarray and qRT-PCR data for Os.49746.1.S1_at 81

Figure 2.6 Graph comparing microarray and qRT-PCR data for Os.56210.1.S1_at 82

Figure 2.7 Schematic diagram representing the zinc finger B-box-type domains in OsOrphan19 84

Figure 2.8 Comparison of multiple amino acid sequence of OsOrphan19 and its homologs 86

Figure 2.9 Schematic diagram representing the AP2 domain in OsEREB67 87

Figure 2.10 Schematic diagram representing the Myb-type HTH domain in OsMYBR63 90

Figure 2.11 Pairwise sequence alignment of splice forms of OsMYBR63 91

Figure 2.12 Coiled-coil domain prediction in OsMYBR63.....92

Figure 2.13 Schematic diagram representing the myc-type HLH domain in OsbHLH17 93

Figure 2.14 Coiled-coil domain prediction in OsbHLH17 94

Figure 2.15 Multiple sequence alignment of OsbHLH17 and bHLH consensus sequences 97

Figure 2.16 Schematic diagram representing the Myb-type HTH DNA-binding domain 99

Figure 2.17 Multiple sequence alignment of the myb-type DNA binding domain and B motif 101

Figure 2.18 Myb-type HTH DNA-binding domains.....102

Figure 2.19 Coiled-coil domain prediction in OsMYB54 104

Figure 3.1 Schematic diagram representing the pCR®8/GW/TOPO® entry vector 119

Figure 3.2 Schematic diagram representing the pMDC32 expression vector.....123

Figure 3.3 Schematic diagram representing the artificial microRNA construction using PCR 126
Figure 3.4 Schematic diagram representing the pMDC32/OsOrphan19 expression vector 133
Figure 3.5 Schematic diagram representing the pMDC32/OsEREB67 expression vector 134
Figure 3.6 Schematic diagram representing the pMDC32/OsMYBR63 expression vector 135
Figure 3.7 Schematic diagram representing the pMDC32/OsbHLH17 expression vector 136
Figure 3.8 Schematic diagram representing the pMDC32/OsLUX expression vector 137
Figure 3.9 Schematic diagram representing the pMDC32/OsMYB54 expression vector 138
Figure 3.10 Schematic diagram representing the pMDC164/JRC0470 expression vector 139
Figure 3.11 Schematic diagram representing the pMDC107/JRC0470 expression vector 140
Figure 3.12 Schematic diagram representing the pMDC/JRC0470/OsOrphan19 expression vector.....141
Figure 3.13 Schematic diagram representing the pMDC/JRC0470/OsEREB67 expression vector.....142
Figure 3.14 Schematic diagram representing the pMDC/JRC0470/OsMYBR63 expression vector.....143
Figure 3.15 Schematic diagram representing the pMDC/JRC0470/OsbHLH17 expression vector.....144
Figure 3.16 Schematic diagram representing the pMDC/JRC0470/OsLUX expression vector.....145
Figure 3.17 Schematic diagram representing the pMDC/JRC0470/OsMYB54 expression vector.....146
Figure 3.18 Schematic diagram representing the pMDC32/Orphan19 amiRNA expression vector.....147
Figure 3.19 Schematic diagram representing the pMDC32/OsEREB67 amiRNA expression vector.....148
Figure 3.20 Schematic diagram representing the pMDC32/OsMYBR63 amiRNA expression vector.....149

Figure 3.21 Schematic diagram representing the pMDC32/OsbHLH17 amiRNA expression vector.....150
Figure 3.22 Schematic diagram representing the pMDC32/OsLUX amiRNA expression vector.....151
Figure 3.23 Schematic diagram representing the pMDC32/OsMYB54 amiRNA expression vector.....152
Figure 3.24 Photograph depicting the fan shaped phenotype of transgenic plants.155
Figure 3.25 Photograph depicting the drought sensitive phenotype of *JRC0470:OsMYB54* rice 156
Figure 3.26 Photograph depicting the drought sensitive phenotype of *JRC0470:OsMYB54* rice 157
Figure 3.27 Photograph depicting the stay-green phenotype of transgenic plants .159
Figure 4.1 Photograph of hydroponics tanks used for salinity tolerance assays...168
Figure 4.2 Image of an autoradiograph of *Hind*III digested T0 rice genomic DNA for the transgenic lines containing the pMDC32 empty vector and *2x35S:OsOrphan19*... 172
Figure 4.3 Image of an autoradiograph of *Hind*III digested T0 rice genomic DNA for the transgenic lines containing the constructs *2x35S:OsEREB67* and *2x35S:OsMYBR63* 173
Figure 4.4 Image of an autoradiograph of *Hind*III digested T0 rice genomic DNA for the transgenic line containing the construct *2x35S:OsLUX* 174
Figure 4.5 Image of an autoradiograph of *Hind*III digested T0 rice genomic DNA for the transgenic line containing the construct *2x35S:OsMYB54* 175
Figure 4.6 Image of an autoradiograph of *Hind*III digested T0 rice genomic DNA for the transgenic line containing the constructs *JRC0470:OsOrphan19*, *JRC0470:OsEREB67* and *JRC0470:OsMYBR63*.....176
Figure 4.7 Image of an autoradiograph of *Hind*III digested T0 rice genomic DNA for the transgenic line containing the constructs *JRC0470:OsbHLH17*, *JRC0470:OsLUX* and *JRC0470:OsMYB54*. 177
Figure 4.8 GUS staining in JRC0470:uidA reporter lines – 0 hours, 0mM NaCl 179
Figure 4.9 GUS staining in JRC0470:uidA reporter lines – 24 hours, 25mM NaCl 180
Figure 4.10 GUS staining in JRC0470:uidA reporter lines – 48 hours, 50mM NaCl 181
Figure 4.11 GUS staining in JRC0470:uidA reporter lines – 72 hours, 50mM NaCl 182

Figure 4.12 Graph depicting plant health scores over time in salt stressed *O. sativa* cv. Nipponbare plants to determine on which day the average health score was 5. 184
Figure 4.13 Graph of plant heights and health scores comparing the rice line *2x35S:OsOrphan19* 8A and wild type rice plants.....185
Figure 4.14 Graph of plant heights and health scores comparing the rice line *2x35S:OsOrphan19* 8C and wild type rice plants.....186
Figure 4.15 Graph of plant heights and health scores comparing the rice line *2x35S:OsOrphan19* 9B and wild type rice plants.....187
Figure 4.16 Graph of plant heights and health scores comparing the rice line *2x35S:OsOrphan19* 9D and wild type rice plants.....188
Figure 4.17 Graph of plant heights and health scores comparing the rice line *2x35S:OsEREB67* 10B and wild type rice plants.....189
Figure 4.18 Graph of plant heights and health scores comparing the rice line *2x35S:OsMYBR63* 11A and wild type rice plants.....190
Figure 4.19 Graph of plant heights and health scores comparing the rice line *2x35S:OsMYBR63* 11C and wild type rice plants.....191
Figure 4.20 Graph of plant heights and health scores comparing the rice line *2x35S:OsLUX* 17C and wild type rice plants.....192
Figure 4.21 Graph of plant heights and health scores comparing the rice line *2x35S:OsLUX* 19C and wild type rice plants.....193
Figure 4.22 Graph of plant heights and health scores comparing the rice line *2x35S:OsMYB54* 22C and wild type rice plants 194
Figure 4.23 Graph of plant heights and health scores comparing the rice line *2x35S:OsMYB54* 22E and wild type rice plants.....195
Figure 4.24 Graph of plant heights and health scores comparing the rice line *2x35S:OsMYB54* 23E and wild type rice plants.....196
Figure 4.25 Graph of plant heights and health scores comparing the rice line *2x35S:OsMYB54* 25A and wild type rice plants 197
Figure 4.26 Semi quantitative RT-PCR analysis of transgene transcript levels in *2x35S:OsOrphan19* rice plants 203
Figure 4.27 Semi quantitative RT-PCR analysis of transgene transcript levels in *JRC0470:OsOrphan19* rice plants 204
Figure 4.28 Semi quantitative RT-PCR analysis of endogenous transcript levels in *2x35S:OsOrphan19 amiRNA* rice plants.....205
Figure 4.29 Graph of sodium accumulation in rice plants with altered expression of *OsOrphan19* stressed with 50mM NaCl 206

Figure 4.30 Graph of K^+:Na^+ ratio in rice plants with altered expression of *OsOrphan19* stressed with 50mM NaCl 207
Figure 4.31 Graph of fresh weight in rice plants with altered expression of *OsOrphan19* stressed with 50mM NaCl 208
Figure 4.32 Semi quantitative RT-PCR analysis of transgene transcript levels in 2x35S:OsEREB67 rice plants 210
Figure 4.33 Semi quantitative RT-PCR analysis of endogenous transcript levels in 2x35S:OsEREB67 amiRNA rice plants 211
Figure 4.34 Graph of sodium accumulation in rice plants with altered expression of *OsEREB67* stressed with 50mM NaCl 212
Figure 4.35 Graph of K^+:Na^+ ratio in rice plants with altered expression of *OsEREB67* stressed with 50mM NaCl 213
Figure 4.36 Graph of fresh weight in rice plants with altered expression of *OsEREB67* stressed with 50mM NaCl 214
Figure 4.37 Semi quantitative RT-PCR analysis of transgene transcript levels in 2x35S:OsMYBR63 rice plants 216
Figure 4.38 Semi quantitative RT-PCR analysis of endogenous transcript levels in 2x35S:OsMYBR63 amiRNA rice plants 217
Figure 4.39 Graph of sodium accumulation in rice plants with altered expression of *OsMYBR63* stressed with 50mM NaCl 218
Figure 4.40 Graph of K^+:Na^+ ratio in rice plants with altered expression of *OsMYBR63* stressed with 50mM NaCl 219
Figure 4.41 Graph of fresh weight in rice plants with altered expression of *OsMYBR63* stressed with 50mM NaCl 220
Figure 4.42 Semi-quantitative RT-PCR analysis of endogenous transcript levels in 2x35S:OsbHLH17 amiRNA rice plants 222
Figure 4.43 Graph of sodium accumulation in rice plants with altered expression of *OsbHLH17* stressed with 50mM NaCl 223
Figure 4.44 Graph of fresh weight in rice plants with altered expression of *OsbHLH17* stressed with 50mM NaCl 224
Figure 4.45 Graph of K^+:Na^+ ratio in rice plants with altered expression of *OsbHLH17* stressed with 50mM NaCl 225
Figure 4.46 Semi quantitative RT-PCR analysis of transgene transcript levels in 2x35S:OsLUX rice plants 228
Figure 4.47 Semi quantitative RT-PCR analysis of transgene transcript levels in JRC0470:OsLUX rice plants 229

Figure 4.48 Semi quantitative RT-PCR analysis of endogenous transcript levels in 2x35S:OsLUX amiRNA rice plants 230
Figure 4.49 Graph of sodium accumulation in rice plants with altered expression of *OsLUX* stressed with 50mM NaCl 231
Figure 4.50 Graph of K^+:Na^+ ratio in rice plants with altered expression of *OsLUX* stressed with 50mM NaCl 232
Figure 4.51 Graph of fresh weight in rice plants with altered expression of *OsLUX* stressed with 50mM NaCl 233
Figure 4.52 Semi quantitative RT-PCR analysis of transgene expression in 2x35S:OsMYB54 rice plants 236
Figure 4.53 Semi quantitative RT-PCR analysis of endogenous gene expression in JRC0470:OsMYB54 rice plants 237
Figure 4.54 Graph of sodium accumulation in rice plants with altered expression of *OsMYB54* stressed with 50mM NaCl 238
Figure 4.55 Graph of sodium accumulation in rice plants with altered expression of *OsMYB54* stressed with 50mM NaCl (line 23E removed) 239
Figure 4.56 Graph of K^+:Na^+ ratio in rice plants with altered expression of *OsLUX* stressed with 50mM NaCl 240
Figure 4.57 Graph of fresh weight in rice plants with altered expression of *OsMYB54* stressed with 50mM NaCl 241

List of Tables

Table 1.1 MYB transcription factors involved in abiotic stress response in *A. thaliana* 22

Table 2.1 Motif consensus sequences from *A. thaliana*, Poplar, Rice, Moss and Algae 95

Table 2.2 The conserved AA motif 7 from bHLH subfamily III(d+e) and various plant species 98

Table 4.1 Experimental conditions for sodium and potassium accumulation experiments 200

Table 5.1 qRT-PCR primers 261

Table 5.2 Primers targeting Affymetrix probeset:cDNA combinations 262

Table 5.3 Subcellular locations were predicted using TargetP 262

Table 5.4 Nested primer sets used to amplify transcription factor coding sequences 263

Table 5.5 Primers used in the construction of artificial microRNA expression vectors 264

Table 5.6 Primers used in analysis of transgene expression 264

Table 5.7 Miscellaneous primers 265

Table 5.8 ACPFG plant nutrient solution 267

Table 5.9 Artificial microRNA expressing rice plant information 269

Table 5.10 Transcription factor coding sequence overexpression rice plant information 270

Table 5.11 Salt inducible transcription factor coding sequence rice plant information 271

List of Abbreviations

%	Percent
[α-32P]dCTP	Radioactive deoxycytosine triphosphate
A. thaliana	*Arabidopsis thaliana*
AA	Amino acid
ABA	Abscisic acid
ABF	ABA responsive element-binding factor
ABREL	ABA response element-like
AGRF	Australian genome research facility
amiRNA	Artificial micro RNA
ANOVA	Analysis of variance
AP2	APETALA2
Arabidopsis	*Arabidopsis thaliana*
AREB	ABA responsive element binding protein
AtCBL	*Arabidopsis thaliana* calcineurin B-like Ca^{2+} binding protein
ATHK1	A. thaliana histidine kinase 1
bHLH	Basic helix-loop-helix
BLAST	Basic Local Alignment Search Tool
BLASTN	Basic Local Alignment Search Tool Nucleotide
BLASTP	Basic Local Alignment Search Tool Protein
bp	Base pair
BSA	Bovine serum albumin
bZIP	Basic-domain leucine-zipper
CaCl2	Calcium chloride
CaM	Calmodulin
CBF	C-repeat binding factor
cDNA	Complementary deoxyribonucleic acid
CDS	Coding sequence
CE1	Controlled environment 1
CIPK	CBL-interacting protein kinase 1
CM	Conserved DNA motifs
CPA1	Cation proton antiporter 1
CRT	C-repeat
cv.	Cultivar
DEPC	Diethylpyrocarbonate
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphate mix
DRE	Dehydration responsive element
DREB	Dehydration responsive element binding protein
EC_e	Electrical conductivity
EDTA	Ethylenediaminetetraacetic acid
EE	Evening element
EMSA	Electrophoretic mobility shift assay

ERE	Ethylene responsive element
EREBP	Ethylene responsive element binding protein
ERF	Ethylene responsive factor
EST	Expressed sequence tag
GA	Gibberellic acid
GAP	Glyceraldehyde 3-phosphate
GFP	Green fluorescent protein
GTE	Glucose TE
GUS	Beta-glucuronidase
HKT	High affinity K^+ transporter
HOG1	High osmolarity glycerol response 1
HPLC	High performance liquid chromatography
HTH	Helix-turn-helix
IG	Indole-glucosinate
JRE	Autonomous jasmonate-response element
K+	Potassium ion
LB	Lysogeny broth
LEA	Late embryogenesis abundant
MAFFT	Multiple sequence alignment program
MAPK	Mitogen-activated protein kinase
$MgCl^2$	Magnesium chloride
$MgSO_4$	Magnesium sulphate
mRNA	Messenger ribonucleic acid
MTIDK	A matrix derived from myosins, paramyosins, tropomyosins, intermediate filaments type I - V, desmosomal proteins and kinesins
Myb	Myeloblastosis
Na+	Sodium ion
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NCBI	National Center for Biotechnology Information
O. sativa	Oryza sativa
PCR	Polymerase chain reaction
PP2C	2C protein phosphatase
qRT-PCR	Quantitative Reverse Transcription Polymerase Chain Reaction
QTL	Quantitative trait loci
R40	RNaseA diluted to 40µg/mL
RCD1	Radical induced cell death
RNA	Ribonucleic acid
RT	Room temperature
SDS	Sodium dodecyl sulphate
SIP	SOS3-interacting
Sln1	Synthetic lethal of N-end rule 1
SNRK2	Snf1-related protein kinase 2
SOS	Salt overly sensitive
SSC	Saline-sodium citrate
ssp.	Sub species
TAE	Tris-acetate-EDTA

T-DNA	Transfer DNA
TE	Tris-EDTA
TPA	The Plant Accelerator
uidA	β-glucuronidase
UV	Ultra violet
X-gluc	5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid cyclohexylammonium salt
ZT	Zeitgeber time

Abstract

Salt tolerant plants are able to survive in saline soils by the virtue of an array of channels and pumps that minimise sodium entry into roots and loading into the xylem, as well as the sequestration of sodium in the vacuole of the cells of both root and shoot. Regulation of genes involved in conferring salt tolerance is thought to occur *via* a network of transcription factors. In this project, the aim is to identify transcription factors that are important in regulating genes involved in salinity tolerance.

Affymetrix Rice 57K GeneChip data from a previous project were used to analyse gene expression with and without salt stress in the shoots and roots of the salt sensitive *Oryza sativa* cultivar IR29 and the salt tolerant cultivars FL478, IR63731 and Pokkali. Transcription factors showing differential expression between the salt sensitive and salt tolerant cultivars were identified and confirmed by qRT-PCR. Six transcription factors with confirmed expression patterns were selected and transgenic rice plants were generated either constitutively or salt inducibly over-expressing each of the transcription factor coding sequences. Plants were also made expressing artificial microRNAs designed to reduce levels of transcripts of each transcription factor.

The altered expression of five transcription factors, *OsOrphan19*, *OsEREB67*, *OsbHLH17*, *OsLUX* and *OsMYB54* affected plant salinity tolerance, as evidenced by changes in Na^+ and K^+ accumulation and plant fresh weight. These five transcription factors show significant homology to other previously known stress responsive genes thus suggesting their involvement in plant stress responses. Further experiments such as chromatin immunoprecipitation sequencing and RNA-sequencing of transgenic plants need to be performed to identify the target promoters and downstream genes, respectively, to determine the precise role of these transcription factors in plant responses to salt stress.

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 Michael Dow and, 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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