Role and control of $HKT$ in
$Oryza$ sativa & $Arabidopsis$ thaliana

Joanna Faye Sundstrom
B. Biotech (Hons)

A thesis submitted for the degree of
Doctor of Philosophy
School of Agriculture, Food & Wine
Faculty of Sciences
The University of Adelaide

February, 2011
Abstract

Salinity is a major abiotic stress influencing agricultural production in Australia and around the world. Plants grown in saline conditions are affected by both osmotic and ionic stress components. The focus of this thesis is on the ionic stress component of salinity stress and in particular the build up of sodium ions (Na\(^+\)) in the leaf cytoplasm, one of the main components of salinity toxicity.

In this thesis, genes encoding the high affinity potassium transporter family of proteins (HKTs) are studied in the plants *Oryza sativa* (rice) and *Arabidopsis thaliana* (Arabidopsis). These HKT transporters encode Na\(^+\) permeable membrane proteins and transport either Na\(^+\) selectively or co-transport Na\(^+\) and K\(^+\). HKT transporters have been identified in a number of plant species and to date have mainly been shown to be involved in reducing Na\(^+\) stress.

A family of nine HKT genes have been identified in rice. Published reports to date have mainly investigated the function of these OsHKT genes in heterologous systems or have focused on one or two OsHKT genes in planta. During this PhD, tissue specific expression profiles were determined for each of the nine OsHKT genes, in ten rice varieties, exposed to a NaCl stress. Of most interest was OsHKT1;3 which showed very high levels of expression in leaf blades and sheath and higher levels of expression in NaCl treated roots compared to controls, across rice varieties. A range of experiments were designed to obtain further information regarding OsHKT1;3, as little was known about this gene or the encoded protein at the time of these experiments. A notable discovery was the identification of a novel OsHKT1;3 splice variant.

In contrast to rice, Arabidopsis has only one HKT gene, AtHKT1;1. AtHKT1;1 is located in cells surrounding the xylem and is likely to be involved in the retrieval of Na\(^+\) from the xylem, thereby minimising shoot Na\(^+\) accumulation. To investigate the regulation of AtHKT1;1 gene expression two Arabidopsis ecotypes, Columbia-0 (Col-0) and C24, shown by QRT-PCR to have different AtHKT1;1 root expression levels, were studied. Sequencing of the C24 AtHKT1;1 promoter revealed substantial sequence differences between the Col-0 and C24 promoters, particularly 150 to 200 bp upstream of the AtHKT1;1 ATG start codon. It was hypothesised that these sequence differences were responsible for the lack of AtHKT1;1 root specific expression in C24 plants, due to a lack of transcription factor binding motif(s) or particular root specific transcription factor(s). To test this hypothesis a series of AtHKT1;1 promoter::GFP and AtHKT1;1 promoter::AtHKT1;1 cDNA constructs, with different combinations of Col-0 and C24 sequences, were tested. Preliminary results suggest that both the Col-0 and C24 AtHKT1;1 promoters are able to drive expression of the downstream transgene and therefore the sequence differences between the promoters is not the cause of the lack of C24 AtHKT1;1 root expression. A 1.6 kb insertion, identified in the second intron of the C24 AtHKT1;1 gene, is now proposed to disrupt C24 AtHKT1;1 root specific expression.
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 Joanna Faye Sundstrom 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.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

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 catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

………………………………………
J. F. Sundstrom

………………………………………
Date
Acknowledgements

I wish to thank my supervisors Professor Mark Tester, Dr. Stuart Roy and Dr. Olivier Cotsaftis for the time and effort each have provided during my research candidature. Thank you for all of your support during the good and the not so good times, it has been much appreciated.

I gratefully acknowledge the financial support provided during my candidature from The University of Adelaide, through provision of an Australian Postgraduate Award and The Australian Centre for Plant Functional Genomics for research and financial support. Further thanks go to both the International Society for Plant Molecular Biology, for provision of an ISPMB2006 Award and the Australian Society of Plant Scientists, for financial support to fund attendance at interstate and overseas conferences during my candidature.

Sincere thanks to the many people who have provided advice and technical support throughout this project. While there are too many to thank individually, I would particularly like to acknowledge Dr. Neil Shirley who performed the QRT-PCR analysis, Ms. Zahra Shoaei for assistance with BAC library screening, Ms Melissa Pickering for instruction on Agrobacterium transformation of Arabidopsis and Mr. Michael Dow for instruction on Agrobacterium transformation of rice. I would also like to thank Professor June Nasrallah from Cornell University, USA, for providing the Arabidopsis C24 BAC library filter and BAC clones.

The nature of science means that many colleagues come and go over a relatively short period of time. Thank you to past and present members of the Salt Group and many in the broader Australian Centre for Plant Functional Genomics community, who I have had the pleasure of working with. I look forward to keeping in touch with many of you in the future. I would also like to thank those still in touch from previous studies in the Genetics Department, University of Adelaide. It is wonderful to be able to keep in touch with a network of friends beginning to spread all over the world!

Finally, I would like to thank my Mum and Dad who have supported me throughout all of my studies. While you may not have always understood the specifics, you’ve always been there to listen, thank you!
# Table of Contents

Abstract .......................................................................................................................... i
Declaration ....................................................................................................................... iii
Acknowledgements ........................................................................................................ iv
Table of Contents ........................................................................................................... v
List of Figures .................................................................................................................. x
List of Tables .................................................................................................................... xiii
Abbreviations & Symbols - General .............................................................................. xv
Abbreviations - Organisations ...................................................................................... xviii

## Chapter 1: Review of the literature & research aims .................................................. 1

1.1 Soil salinity .................................................................................................................. 1
1.1.1 Seepage salinity ...................................................................................................... 1
1.1.2 Transient salinity ................................................................................................... 2
1.1.3 Irrigation salinity .................................................................................................. 2
1.2 Salinity in Australia and its impact on agriculture .................................................. 2
1.3 Effects of salt stress on plant growth and development .......................................... 5
1.3.1 Osmotic stress ...................................................................................................... 5
1.3.2 Ionic stress .......................................................................................................... 5
1.4 Plant salinity tolerance ............................................................................................ 7
1.4.1 Mechanisms of salinity tolerance ........................................................................ 8
1.5 The HKT gene family .............................................................................................. 8
1.5.1 HKT structure and function ................................................................................ 9
1.5.2 Two subfamilies of HKT transporters .................................................................. 11
1.5.3 Wheat HKT genes ............................................................................................... 13
1.5.4 Arabidopsis AtHKT1;1 ......................................................................................... 14
1.5.5 Rice HKT gene family ......................................................................................... 15
1.6 HKT summary .......................................................................................................... 18
1.7 Research aims .......................................................................................................... 19

## Chapter 2: General materials & methods ................................................................. 21

2.1 Plant material ............................................................................................................ 21
2.1.1 Arabidopsis thaliana (Arabidopsis) seed ................................................................. 21
2.1.2 Oryza sativa (rice) seed ....................................................................................... 21
2.2 Plant growth facilities .............................................................................................. 21
2.3 Plant growth media and preparation ....................................................................... 22
2.3.1 Soil mixes ............................................................................................................ 22
2.3.2 Growth of Arabidopsis in soil .............................................................................. 23
2.3.3 Growth of Arabidopsis on solid MS media ......................................................... 23
2.4 Genomic DNA extraction ....................................................................................... 25
2.4.1 Phenol/chloroform/iso-amyl alcohol method ..................................................... 25
2.4.2 DNA extraction buffer method .......................................................................... 25
2.5 General PCR method ............................................................................................ 26
2.6 Agarose gel electrophoresis - DNA ................................................................. 27
2.7 DNA extraction from agarose gels ........................................................................ 27
Chapter 3: Transcriptional profiling of the HKT gene family in Oryza sativa (rice)..... 36

3.1 Introduction ........................................................................................................... 36
3.1.1 Rice varieties ................................................................................................. 36
3.2 Materials & Methods ......................................................................................... 38
3.2.1 Plant material ............................................................................................... 38
3.2.2 Rice growth conditions ................................................................................. 38
3.2.3 Rice seed sterilisation and germination .......................................................... 39
3.2.4 Rice seedling transplantation to hydroponic system .................................... 40
3.2.5 Rice tissue harvesting .................................................................................. 40
3.2.6 Determining tissue Na\(^+\) and K\(^+\) concentrations by flame photometry .... 40
3.2.7 Quantitative RT-PCR of the OsHKT gene family ........................................ 41
3.3 Results ................................................................................................................ 43
3.3.1 Na\(^+\) accumulation .................................................................................... 46
3.3.2 K\(^+\) accumulation ...................................................................................... 46
3.3.3 Na\(^+\) to K\(^+\) ratio ....................................................................................... 47
3.3.4 Expression of OsHKT1;1 was most abundant in aerial tissues .................... 51
3.3.5 Expression of OsHKT1;2 was most abundant in sheath tissue ...................... 51
3.3.6 OsHKT1;3 was expressed at high levels in aerial tissues and up regulated in NaCl treated roots ........................................................................................................ 51
3.3.7 Expression of OsHKT1;4 was most abundant in sheath tissue .................... 52
3.3.8 Expression of OsHKT1;5 was most abundant in the roots, but at very low levels 53
3.3.9 OsHKT2;1 expression was up regulated in NaCl treated roots .................... 53
3.3.10 OsHKT2;2 expression was only present in roots ........................................ 53
3.3.11 OsHKT2;3 and OsHKT2;4 expression was most abundant in leaf blades .... 54
<table>
<thead>
<tr>
<th>3.4 Discussion</th>
<th>64</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4.1 Rice grown in 30 mM NaCl were not visibly salt stressed</td>
<td>64</td>
</tr>
<tr>
<td>3.4.2 Salinity tolerance mechanisms in different rice varieties</td>
<td>66</td>
</tr>
<tr>
<td>3.4.3 Na+ accumulation and OsHKT1;5 expression</td>
<td>69</td>
</tr>
<tr>
<td>3.4.4 Sheath Na+ accumulation and OsHKT1;4</td>
<td>72</td>
</tr>
<tr>
<td>3.4.5 Does OsHKT1;3 have a function in both roots and shoots?</td>
<td>74</td>
</tr>
<tr>
<td>3.4.6 OsHKT gene family members not yet discussed</td>
<td>76</td>
</tr>
<tr>
<td>3.4.7 Improvements to experimental design</td>
<td>80</td>
</tr>
<tr>
<td>3.4.8 Summary</td>
<td>83</td>
</tr>
</tbody>
</table>

**Chapter 4: Characterisation of Nipponbare OsHKT1;3** ........................................... 84

<table>
<thead>
<tr>
<th>4.1 Introduction</th>
<th>84</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1.1 OsHKT1;3 cell type specific expression profiling</td>
<td>84</td>
</tr>
<tr>
<td>4.1.2 OsHKT1;3 membrane localisation</td>
<td>84</td>
</tr>
<tr>
<td>4.1.3 Effect of knocking down OsHKT1;3 expression</td>
<td>85</td>
</tr>
<tr>
<td>4.2 Materials &amp; Methods</td>
<td>85</td>
</tr>
<tr>
<td>4.2.1 PCR amplification of OsHKT1;3 from cDNA</td>
<td>85</td>
</tr>
<tr>
<td>4.2.2 QRT-PCR of 1.1 kb and 1.6 kb OsHKT1;3 cDNA splice variants</td>
<td>89</td>
</tr>
<tr>
<td>4.2.3 PCR amplification of a 2.0 kb OsHKT1;3 promoter sequence</td>
<td>90</td>
</tr>
<tr>
<td>4.2.4 Cloning of artificial OsHKT1;3 amiRNA precursors</td>
<td>92</td>
</tr>
<tr>
<td>4.2.5 Construction of expression vectors for rice transformation</td>
<td>95</td>
</tr>
<tr>
<td>4.2.6 A. tumefaciens mediated transformation of Nipponbare rice</td>
<td>96</td>
</tr>
<tr>
<td>4.2.7 Analysis of OsHKT1;3 promoter::GFP by confocal microscopy</td>
<td>99</td>
</tr>
<tr>
<td>4.3 Results</td>
<td>100</td>
</tr>
<tr>
<td>4.3.1 Multiple OsHKT1;3 cDNA splice variants identified</td>
<td>100</td>
</tr>
<tr>
<td>4.3.2 OsHKT1;3 promoter activity</td>
<td>108</td>
</tr>
<tr>
<td>4.3.3 OsHKT1;3 amiRNA precursors to knockdown OsHKT1;3 expression</td>
<td>111</td>
</tr>
<tr>
<td>4.3.4 OsHKT1;3-GFP fusion proteins to determine membrane localisation</td>
<td>112</td>
</tr>
<tr>
<td>4.4 Discussion</td>
<td>112</td>
</tr>
<tr>
<td>4.4.1 Multiple OsHKT1;3 cDNA splice variants identified</td>
<td>112</td>
</tr>
<tr>
<td>4.4.2 Repeat QRT-PCR shows novel OsHKT1;3 splice variant in other rice varieties</td>
<td>114</td>
</tr>
<tr>
<td>4.4.3 What is the role of OsHKT1;3 in planta?</td>
<td>116</td>
</tr>
</tbody>
</table>

**Chapter 5: Comparison of AtHKT1;1 in Arabidopsis ecotypes Col-0 & C24** ............... 119

<table>
<thead>
<tr>
<th>5.1 Introduction</th>
<th>119</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.2 Materials &amp; Methods</td>
<td>120</td>
</tr>
<tr>
<td>5.2.1 AtHKT1;1 gene expression in Col-0 and C24 Arabidopsis</td>
<td>120</td>
</tr>
<tr>
<td>5.2.2 PCR amplification of Col-0 and C24 AtHKT1;1 promoter and AtHKT1;1 gene</td>
<td>123</td>
</tr>
<tr>
<td>5.2.3 PCR amplification of Col-0 and C24 AtHKT1;1 gene from gDNA and cDNA</td>
<td>124</td>
</tr>
<tr>
<td>5.2.4 Sequencing the C24 AtHKT1;1 promoter and AtHKT1;1 gene</td>
<td>125</td>
</tr>
<tr>
<td>5.2.5 AtHKT1;1 promoter motif analysis</td>
<td>130</td>
</tr>
<tr>
<td>5.3 Results</td>
<td>130</td>
</tr>
<tr>
<td>5.3.1 AtHKT1;1 expression is absent in C24 roots compared to Col-0 roots</td>
<td>130</td>
</tr>
<tr>
<td>5.3.2 PCR amplification of Col-0 and C24 AtHKT1;1 promoters and AtHKT1;1 genes</td>
<td>134</td>
</tr>
</tbody>
</table>

vii
Chapter 6: Regulation of \textit{AtHKT1;1} gene expression ......................................................... 150
  6.1 Introduction ............................................................................................................ 150
  6.1.1 \textit{AtHKT1;1} promoter::GFP expression vectors ................................................ 150
  6.1.2 \textit{AtHKT1;1} promoter::\textit{AtHKT1;1} cDNA expression vectors ....................... 153
  6.2 Materials & Methods .......................................................................................... 155
  6.2.1 Cloning of \textit{AtHKT1;1} promoter and \textit{AtHKT1;1} gene from Col-0 and C24 ......... 155
  6.2.2 Col-0/C24 and C24/Col-0 mixed \textit{AtHKT1;1} promoters cloned into pCR8/GW/TOPO TA Gateway® entry vectors ................................................................. 160
  6.2.3 Combining \textit{AtHKT1;1} promoters with \textit{AtHKT1;1} cDNA sequences in pCR8/GW/TOPO TA Gateway® entry vectors ................................................................. 166
  6.2.4 Construction of expression vectors for plant transformation ......................... 172
  6.2.5 \textit{A. tumefaciens} mediated transformation of Arabidopsis ................................. 174
  6.2.6 Analysis of Arabidopsis transformants ............................................................. 176
  6.3 Results ................................................................................................................... 179
  6.3.1 Expression vectors for plant transformation ..................................................... 179
  6.3.2 Arabidopsis T\textsubscript{1} transformants ............................................................ 182
  6.4 Discussion ............................................................................................................. 194
  6.4.1 The C24 \textit{AtHKT1;1} promoter is not the cause of the lack of \textit{AtHKT1;1} root expression in C24 wild type plants ................................................................. 194
  6.4.2 Alternative hypotheses for the lack of \textit{AtHKT1;1} root expression in C24 wild type plants 195
  6.4.3 Experimental problems and future improvements ............................................. 197
  6.4.4 Molecular strategies used to obtain expression vectors for Arabidopsis transformation ........................................................................................................ 199
  6.4.5 Conclusion ....................................................................................................... 201

Chapter 7: General discussion & future directions ...................................................... 203
  7.1 Review of thesis aims ......................................................................................... 203
  7.1.1 Summary of \textit{OsHKT} gene expression profiling ........................................ 203
  7.1.2 Summary of regulation of \textit{AtHKT1;1} gene expression ................................. 204
7.2 Future investigation - HKT gene family .................................................................205
7.3 Future investigation - Improving the salinity tolerance of crop plants ..................208
7.4 Conclusion .............................................................................................................210

Appendix 1: Media & stock solutions for A. tumefaciens mediated transformation of rice ..................................................................................................................212

Media............................. .........................................................................................212
NB medium (1 L).......................................................................................................212
AB medium (1 L) ......................................................................................................212
R2-CL liquid medium (1 L) .......................................................................................213
R2-CS medium (1 L) .................................................................................................213
R2-S medium (1 L) ...................................................................................................214
NBS medium (1 L) ....................................................................................................215
PR-AG medium (1 L) ...............................................................................................216
RN medium (1 L) .......................................................................................................217
P medium (1 L) ..........................................................................................................217

Stock solutions ...........................................................................................................218
N6 macroelements (1 L) ............................................................................................218
FeEDTA (Base NB) (1 L) ........................................................................................218
B5 microelements (1 L) ............................................................................................218
B5 vitamins (1 L) .......................................................................................................219
AB medium stock (1 L) ............................................................................................219
AB salts stock (1 L) ...................................................................................................219
Macroelements R2-I (1 L) ........................................................................................219
Macroelements R2-II (1 L) .......................................................................................219
FeEDTA (Base R2) (1 L) ........................................................................................220
Microelements R2 (1 L) ............................................................................................220
Vitamins R2 (1 L) .......................................................................................................220
2, 4-D (1 mg/mL stock) ..............................................................................................220
Acetosyringone (100 mM stock) ..............................................................................220
Cefotaxime (400 mg/mL stock) ................................................................................220
Vancomycin (100 mg/mL stock) ..............................................................................221
Hygromycin (50 mg/mL stock) ................................................................................221
ABA (Abscisic acid) (5 mg/mL stock) .....................................................................221
BAP (6-Benzylaminopurine) (2 mg/mL stock) .......................................................221
NAA (Naphthaleneacetic acid) (1mg/mL stock) .......................................................221

Appendix 2: Col-0 & C24 AthKT1;1 promoter alignment .............................................222
Appendix 3: Col-0 & C24 AthKT1;1 gene alignment ....................................................227
Appendix 4: Col-0 AthKT1;1 promoter sequence scan ..............................................231
Appendix 5: C24 AthKT1;1 promoter sequence scan ................................................237
References ....................................................................................................................244
# List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Australian land affected or at risk of dryland salinity</td>
<td>4</td>
</tr>
<tr>
<td>1.2</td>
<td>The effect of salt stress on the growth rate of a crop plant</td>
<td>6</td>
</tr>
<tr>
<td>1.3</td>
<td>Variation in salinity tolerance of different plant species</td>
<td>7</td>
</tr>
<tr>
<td>1.4</td>
<td>Predicted structural model of HKT proteins</td>
<td>10</td>
</tr>
<tr>
<td>1.5</td>
<td>Phylogenetic tree of known full-length protein sequences encoded by HKT genes from higher plants</td>
<td>12</td>
</tr>
<tr>
<td>3.1</td>
<td>Pedigree of the <em>indica</em> rice varieties IR28, IR29, Sal208 and FL478, developed at the International Rice Research Institute</td>
<td>38</td>
</tr>
<tr>
<td>3.2</td>
<td>Supported hydroponic system used for growth of rice plants</td>
<td>39</td>
</tr>
<tr>
<td>3.3</td>
<td>Ten varieties of 37 day old rice grown in a supported hydroponic system with control nutrient solution ± 30 mM NaCl</td>
<td>44-45</td>
</tr>
<tr>
<td>3.4</td>
<td>Na⁺ accumulation in ten varieties of rice grown in a supported hydroponic system with control nutrient solution ± 30 mM NaCl</td>
<td>48</td>
</tr>
<tr>
<td>3.5</td>
<td>K⁺ accumulation in ten varieties of rice grown in a supported hydroponic system with control nutrient solution ± 30 mM NaCl</td>
<td>49</td>
</tr>
<tr>
<td>3.6</td>
<td>Na⁺:K⁺ ratio in ten varieties of rice grown in a supported hydroponic system with control nutrient solution ± 30 mM NaCl</td>
<td>50</td>
</tr>
<tr>
<td>3.7</td>
<td>QRT-PCR analysis of OsHKT1;1 expression in ten varieties of rice grown in a supported hydroponic system with control nutrient solution ± 30 mM NaCl</td>
<td>55</td>
</tr>
<tr>
<td>3.8</td>
<td>QRT-PCR analysis of OsHKT1;2 expression in ten varieties of rice grown in a supported hydroponic system with control nutrient solution ± 30 mM NaCl</td>
<td>56</td>
</tr>
<tr>
<td>3.9</td>
<td>QRT-PCR analysis of OsHKT1;3 expression in ten varieties of rice grown in a supported hydroponic system with control nutrient solution ± 30 mM NaCl</td>
<td>57</td>
</tr>
<tr>
<td>3.10</td>
<td>QRT-PCR analysis of OsHKT1;4 expression in ten varieties of rice grown in a supported hydroponic system with control nutrient solution ± 30 mM NaCl</td>
<td>58</td>
</tr>
<tr>
<td>3.11</td>
<td>QRT-PCR analysis of OsHKT1;5 expression in ten varieties of rice grown in a supported hydroponic system with control nutrient solution ± 30 mM NaCl</td>
<td>59</td>
</tr>
<tr>
<td>3.12</td>
<td>QRT-PCR analysis of OsHKT2;1 expression in ten varieties of rice grown in a supported hydroponic system with control nutrient solution ± 30 mM NaCl</td>
<td>60</td>
</tr>
<tr>
<td>3.13</td>
<td>QRT-PCR analysis of OsHKT2;2 expression in ten varieties of rice grown in a supported hydroponic system with control nutrient solution ± 30 mM NaCl</td>
<td>61</td>
</tr>
<tr>
<td>3.14</td>
<td>QRT-PCR analysis of OsHKT2;3 expression in ten varieties of rice grown in a supported hydroponic system with control nutrient solution ± 30 mM NaCl</td>
<td>62</td>
</tr>
<tr>
<td>3.15</td>
<td>QRT-PCR analysis of OsHKT2;4 expression in ten varieties of rice grown in a supported hydroponic system with control nutrient solution ± 30 mM NaCl</td>
<td>63</td>
</tr>
<tr>
<td>4.1</td>
<td>Vector diagram of pCR8/GW/TOPO TA Gateway® entry vector</td>
<td>86</td>
</tr>
<tr>
<td>4.2</td>
<td>Schematic diagram showing the 1.1 kb and 1.6 kb OsHKT1;3 cDNA splice variants in pCR8/GW/TOPO TA Gateway® entry vectors</td>
<td>88</td>
</tr>
<tr>
<td>4.3</td>
<td>Three new OsHKT1;3 QRT-PCR primer pairs designed to differentiate between two OsHKT1;3 cDNA splice variants</td>
<td>89</td>
</tr>
</tbody>
</table>
Figure 6.1 Four *AtHKT1;1* promoters used to drive expression of either GFP, Col-0 *AtHKT1;1* cDNA or C24 *AtHKT1;1* cDNA.............................................................. 151
Figure 6.2 Vector diagram of pCR8/GW/TOPO TA Gateway® entry vector....................... 157
Figure 6.3 Schematic diagram showing the 2.7 kb *AtHKT1;1* promoter in the pCR8/GW/TOPO TA Gateway® entry vector................................................................. 159
Figure 6.4 Fusion PCR strategy to obtain 610 bp mixed partial *AtHKT1;1* promoters with the first 360 bp from one ecotype and the last 250 bp from the other ecotype........... 161
Figure 6.5 Restriction enzyme digestion and ligation strategy used to obtain 2.7 kb *AtHKT1;1* mixed promoters in the pCR8/GW/TOPO TA Gateway® entry vector............. 164-165
Figure 6.6 Restriction enzyme digestion and ligation strategy used to obtain 2.7 kb *AtHKT1;1* promoter::*AtHKT1;1* cDNA constructs in the pCR8/GW/TOPO TA Gateway® entry vector................................................................. 168-170
Figure 6.7 Vector diagram of destination vector pMDC100+nosT used for expression of transgenes in plants.............................................................. 173
Figure 6.8 Vector diagram of an expression vector containing a 2.7 kb *AtHKT1;1* promoter upstream of the GFP reporter gene to investigate *AtHKT1;1* promoter activity in planta.............................................................. 180
Figure 6.9 Vector diagram of an expression vector containing a 2.7 *AtHKT1;1* promoter::*AtHKT1;1* cDNA to investigate *AtHKT1;1* transgene expression patterns in planta.............................................................. 181
Figure 6.10 Analysis of root specific GFP fluorescence in *AtHKT1;1* promoter::GFP T1 transformants............................................................................. 185
Figure 6.11 Analysis of root specific GFP fluorescence in T2 *AtHKT1;1* promoter::GFP transformants in the Col-0 background......................................................... 187
Figure 6.12 Analysis of root specific GFP fluorescence in T2 *AtHKT1;1* promoter::GFP transformants in the C24 background......................................................... 189
Figure 6.13 Analysis of *AtHKT1;1* expression in T2 *AtHKT1;1* promoter::*AtHKT1;1* cDNA lines (C24 background) grown on either control (0 mM) or NaCl supplemented (100 mM) solid 0.5 × MS media.............................................................. 193
List of Tables

Table 1.1 Revised nomenclature for HKT family members.................................13
Table 2.1 Rice varieties.........................................................................................21
Table 2.2 Summary of growth facility conditions.....................................................22
Table 2.3 Comparison of DNA polymerases routinely used including reaction composition and cycling conditions for a 25 µL reaction.................................................26
Table 3.1 Primers used for QRT-PCR analysis of OsHKT genes...............................42
Table 4.1 Primers used to amplify OsHKT1;3 from Nipponbare cDNA.....................85
Table 4.2 Primers used to sequence the OsHKT1;3 cDNA PCR products cloned into the pCR8/GW/TOPO TA Gateway® entry vectors..................................................86
Table 4.3 Primers used for QRT-PCR of OsHKT1;3 cDNA splice variants...............90
Table 4.4 Primers used to amplify a 2.0 kb OsHKT1;3 promoter.............................90
Table 4.5 Primers used to sequence the 2.0 kb OsHKT1;3 promoter in the pCR8/GW/TOPO TA Gateway® entry vector ..............................................................91
Table 4.6 Primers used for site directed mutagenesis of pNW55 to produce OsHKT1;3 amiRNA precursor sequences.................................................................94
Table 4.7 Primers used to confirm A. tumefaciens strain AGL-1 cultures contained expression vectors OsHKT1;3 promoter::GAL4 UAS::GFP in pTOOL31a or 35S::OsHKT1;3 amiRNA in pMDC32b.........................................................97
Table 5.1 Primers used for semi QRT-PCR of the AtHKT1;1 gene in Col-0 and C24 root cDNA from soil grown plants.................................................................121
Table 5.2 Primers used for QRT-PCR on Col-0 and C24, root and shoot cDNA of plate grown Arabidopsis.................................................................123
Table 5.3 Primers used for PCR amplification of AtHKT1;1 promoter and AtHKT1;1 gene...124
Table 5.4 Primers used for PCR amplification of AtHKT1;1....................................125
Table 5.5 Primers used to confirm BAC clones contained both the AtHKT1;1 genea and the AtHKT1;1 promoter plus AtHKT1;1 genea....................................................127
Table 5.6 Primers used to sequence directly from BAC clone AT24 016 B6 to obtain C24 AtHKT1;1 promoter and AtHKT1;1 gene sequences........................................128-129
Table 5.7 Comparison of nucleotide and amino acid differences in the AtHKT1;1 coding region of Col-0 and C24.................................................................................140
Table 6.1 AtHKT1;1 promoter::GFP expression vectors transformed into Col-0 or C24 to examine if differences in AtHKT1;1 promoter sequences result in different AtHKT1;1 promoter activity.........................................................152
Table 6.2 AtHKT1;1 promoter::AtHKT1;1 cDNA expression vectors transformed into the C24 background or Col-0 gl1 hkt1-4 mutants to determine which promoter::cDNA combinations activate AtHKT1;1 root expression from the transgene resulting in reduced shoot Na+...............................................154
Table 6.3 Primers used to amplify the AtHKT1;1 gene from Col-0 and C24 shoot cDNA......155
Table 6.4 Primers used to amplify the AtHKT1;1 promoters from Col-0 and C24 gDNA......156
Table 6.5 Primers used for colony PCR to check presence of either AtHKT1;1 promoter\textsuperscript{a} or AtHKT1;1 cDNA\textsuperscript{b} ........................................................................................................157
Table 6.6 Primers used to sequence the AtHKT1;1 promoter\textsuperscript{a} and AtHKT1;1 cDNA\textsuperscript{b} PCR products cloned into the pCR8/GW/TOPO TA Gateway\textregistered entry vector .................158
Table 6.7 Primers used to sequence Col-0 and C24 AtHKT1;1 promoters in the pCR8/GW/TOPO TA Gateway\textregistered entry vector after digestion and ligation of different clones..................................................................................................................159
Table 6.8 Primers used to amplify fragment A\textsuperscript{a}, fragment B\textsuperscript{b} and the final mixed partial promoter\textsuperscript{c} by fusion PCR..................................................................................................................161
Table 6.9 Primers used to sequence mixed partial AtHKT1;1 promoter products in pGEMT Easy vectors..................................................................................................................................................162
Table 6.10 Combination of Bsa I Xho I restriction enzyme digestion fragments ligated together to obtain 2.7 kb AtHKT1;1 mixed promoters in pCR8/GW/TOPO TA Gateway\textregistered entry vectors ........................................................................................................................................166
Table 6.11 Ligation combinations to obtain 2.7 kb AtHKT1;1 promoter::AtHKT1;1 cDNA sequences in pCR8/GW/TOPO TA Gateway\textregistered entry vectors..............................................................171
Table 6.12 Primers used to confirm A. tumefaciens AGL-1 cultures contained expression vectors AtHKT1;1 promoter::GFP in pMDC107\textsuperscript{a} or AtHKT1;1 promoter::AtHKT1;1 cDNA in pMDC100+nosT\textsuperscript{b} ........................................................................................................174
Table 6.13 Primers used to confirm T\textsubscript{1} status of plants transformed with either AtHKT1;1 promoter::GFP\textsuperscript{a} or AtHKT1;1 promoter::AtHKT1;1 cDNA\textsuperscript{b} T-DNA sequences ......176
Table 6.14 T\textsubscript{2} AtHKT1;1 promoter::GFP lines grown to determine AtHKT1;1 promoter activity .....................................................................................................................................................177
Table 6.15 T\textsubscript{2} AtHKT1;1 promoter::AtHKT1;1 cDNA lines analysed for transgene specific AtHKT1;1 expression ..............................................................................................................................................178
Table 6.16 Primers used for semi QRT-PCR analysis of AtHKT1;1 expression from T\textsubscript{2} AtHKT1;1 promoter::AtHKT1;1 cDNA lines .............................................................................................................................................179
Table 6.17 Four expression vectors each consisting of a different 2.7 kb AtHKT1;1 promoter driving expression of GFP in pMDC107 ...........................................................................................................................................179
Table 6.18 Eight expression vectors each consisting of a different 2.7 kb AtHKT1;1 promoter::AtHKT1;1 cDNA sequence in pMDC100+nosT..............................................................................................................181
Table 6.19 Summary of T\textsubscript{1} transformants obtained for each expression vector in each Arabidopsis background .................................................................................................................................................183
Table 6.20 Analysis of GFP fluorescence in T\textsubscript{2} AtHKT1;1 promoter::GFP lines ........................................................................................................................................................................................191
### Abbreviations & Symbols - General

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3′</td>
<td>three prime, of nucleic acid sequence</td>
</tr>
<tr>
<td>5′</td>
<td>five prime, of nucleic acid sequence</td>
</tr>
<tr>
<td>~</td>
<td>approximately</td>
</tr>
<tr>
<td>°C</td>
<td>degrees Celsius</td>
</tr>
<tr>
<td>#</td>
<td>number</td>
</tr>
<tr>
<td>%</td>
<td>percent</td>
</tr>
<tr>
<td>x</td>
<td>times</td>
</tr>
<tr>
<td>aa</td>
<td>amino acid</td>
</tr>
<tr>
<td>amiRNA</td>
<td>artificial micro ribonucleic acid</td>
</tr>
<tr>
<td>BAC</td>
<td>bacterial artificial chromosome</td>
</tr>
<tr>
<td>bp</td>
<td>base pairs, of nucleic acid</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>cDNA</td>
<td>complimentary deoxyribonucleic acid</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>calcium ion</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>calcium chloride</td>
</tr>
<tr>
<td>CaMV</td>
<td>cauliflower mosaic virus</td>
</tr>
<tr>
<td>Cat. #</td>
<td>catalogue number</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>chloride ion</td>
</tr>
<tr>
<td>cm</td>
<td>centimetre(s)</td>
</tr>
<tr>
<td>cps</td>
<td>counts per second</td>
</tr>
<tr>
<td>Col-0</td>
<td>Columbia-0</td>
</tr>
<tr>
<td>cRNA</td>
<td>complimentary ribonucleic acid</td>
</tr>
<tr>
<td>d</td>
<td>day(s)</td>
</tr>
<tr>
<td>dATP</td>
<td>deoxyadenosine triphosphate</td>
</tr>
<tr>
<td>dCTP</td>
<td>deoxycytidine triphosphate</td>
</tr>
<tr>
<td>dCT³²P</td>
<td>deoxycytidine labelled with phosphorus-32</td>
</tr>
<tr>
<td>dGTP</td>
<td>deoxyguanosine triphosphate</td>
</tr>
<tr>
<td>dH₂O</td>
<td>deionised water</td>
</tr>
<tr>
<td>dTTP</td>
<td>deoxythymidine triphosphate</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>dNTP</td>
<td>deoxynucleotide triphosphate</td>
</tr>
<tr>
<td>dS</td>
<td>deciSiemens</td>
</tr>
<tr>
<td>DTT</td>
<td>dithiothreitol</td>
</tr>
<tr>
<td>ECe</td>
<td>electrical conductivity</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EST</td>
<td>expressed sequence tag</td>
</tr>
<tr>
<td>g</td>
<td>gravity</td>
</tr>
<tr>
<td>g</td>
<td>gram(s)</td>
</tr>
<tr>
<td>gDNA</td>
<td>genomic deoxyribonucleic acid</td>
</tr>
<tr>
<td>GFP</td>
<td>green fluorescent protein</td>
</tr>
<tr>
<td>GOI</td>
<td>gene of interest</td>
</tr>
</tbody>
</table>
nosT bacterial nopaline synthase terminator sequence
OD$_{260}$ optical density measured at 260 nm
o/n overnight
PCR polymerase chain reaction
pg picogram(s)
PI propidium iodide
pmol picomoles
PVC polyvinyl chloride
QRT-PCR quantitative reverse transcription polymerase chain reaction
QTL quantitative trait loci
RACE rapid amplification of cDNA ends
RB right border, of T-DNA sequence
RbCl rubidium chloride
RIL recombinant inbred line
RNA ribonucleic acid
RO reverse osmosis
rpm revolutions per minute
RT room temperature
RT-PCR reverse transcription polymerase chain reaction
SAM S-adenosylmethionine
siRNA short interfering ribonucleic acid
SDS sodium dodecyl sulfate
sec second(s)
SNP single nucleotide polymorphism(s)
SSC saline sodium citrate
ssp subspecies
t tonnes
T$_0$ primary rice transformant containing T-DNA
T$_1$ primary Arabidopsis transformant containing T-DNA or progeny of T$_0$ rice plant
T$_2$ progeny of T$_1$ plant
TAE tris-acetate-EDTA
T-DNA transfer deoxyribonucleic acid
TE transposable element
TE tris-EDTA
TF transcription factor
T$_m$ melting temperature, of primers
U units
UAS upstream activation sequence
µg microgram(s)
µL microlitre(s)
µm micrometre(s)
µM micromolar
µmol micromole(s)
UTR          untranslated region
UV           ultraviolet
v/v          volume per volume
w/v          weight per volume
X-Gal        5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside
X-Gluc       5-bromo-4-chloro-3-indoyl-glucuronide

Abbreviations - Organisations

ABARE        Australian Bureau of Agricultural and Resource Economics
ABS          Australian Bureau of Statistics
ACPFG        Australian Centre for Plant Functional Genomics
AGRF         Australian Genome Research Facility
CSIRO        Commonwealth Scientific & Industrial Research Organisation
FAO          Food and Agricultural Organization of the United Nations
IMVS         Institute of Medical & Veterinary Science
IRRI         International Rice Research Institute
NCBI         National Center for Biotechnology Information
NLWRA        National Land & Water Resource Audit
PMSEIC       Prime Ministers Science, Engineering & Innovation Council
SARDI        South Australian Research & Development Institute