POT proteins are important for chloride transport in Arabidopsis

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Chloride (Cl\(^-\)) is an essential plant micronutrient, but is toxic when accumulated to high concentrations within the cytoplasm, especially in the shoot. Exclusion of Cl\(^-\) from the shoot is an important trait contributing to salinity tolerance of plants, particularly for Cl\(^-\) sensitive woody perennials (e.g. grapevine, citrus and avocado) and legumes (e.g. soybean and lotus), where Cl\(^-\) is considered to be more toxic than the sodium ion (Na\(^+\)). To enhance plant salinity tolerance, it is necessary to understand the mechanisms of Cl\(^-\) transport through the plant and how it is regulated in response to salinity stress. However, when compared with Na\(^+\), much less is known about the transport processes involved in controlling Cl\(^-\) accumulation in the shoot.

Two candidate genes encoding putative Cl\(^-\) transporters in Arabidopsis, *proton dependent oligo-peptide transporter 1* (*AtPOT1*) and *AtPOT2* were investigated to examine their role in controlling the loading of Cl\(^-\) into the apoplastic vessels of root xylem, and therefore Cl\(^-\) accumulation in the shoot. Transient expression of yellow fluorescent protein (*YFP*:::*AtPOT1* or *YFP*:::*AtPOT2*) in Arabidopsis mesophyll protoplasts, along with stable expression of green fluorescent protein (*GFP*:::*AtPOT1* or *GFP*:::*AtPOT2*) determined that both *AtPOT1* and *AtPOT2* are targeted to the plasma membrane, a location necessary for both POTs to be involved in facilitating Cl\(^-\) efflux from a cell. Promoter:*UidA* fusions showed that *pAtPOT1* drives expression of the *AtPOT1* predominantly in the root stelar cells, suggesting the involvement of AtPOT1 in long distance transport in vasculature tissue. In contrast, *AtPOT2* was shown to be located in the cortex of the mature root.

Use of quantitative real-time PCR to determine the levels of mRNA transcripts in response to salt stress demonstrated that *AtPOT1* transcripts are significantly reduced by both salt and
ABA treatments, whereas AtPOT2 transcripts are increased by salt stress. As AtPOT1 transcripts are reduced by ABA and as AtPOT1 encodes an anion transporter located at the plasma membrane of the cells bordering root xylem vessels, it is hypothesised that AtPOT1 is responsible, at least partially, for loading of Cl⁻ into the conductive cells of xylem in roots. Electrophysiological characterisation of AtPOT1 in Xenopus laevis oocytes showed that AtPOT1 is able to facilitate Cl⁻ efflux across the cell membrane at negative membrane potentials, suggesting a role of AtPOT1 in the efflux of Cl⁻ across the plasma membrane of xylem parenchyma cells into the apoplastic xylem transpiration stream. This flux was not affected by the changes in external pH, consistent with the Cl⁻ transport being a uniport, independent of the movement of H⁺.

There were no knockout mutants of AtPOT1 available. Therefore, in order to test the effect of alterations of AtPOT1 expression on Cl⁻ accumulation in the shoot, artificial microRNA knockdown constructs were designed and used to transform Arabidopsis Col-0 plants. AtPOT1 transcripts were shown to be reduced by up to 80% in the knockdown lines when compared with nulls, which resulted in a reduction in shoot Cl⁻ concentration by up to 60%. AtPOT1 expression was found to be negatively correlated with shoot Cl⁻ concentration ($R^2 = 0.77$). Conversely, constitutive over expression of AtPOT1 increased shoot Cl⁻ accumulation, indicating the important role that AtPOT1 plays in facilitating Cl⁻ xylem loading in Arabidopsis.

It is concluded that AtPOT1 mediates Cl⁻ flux into the conductive cells of root xylem in Arabidopsis and the expression of the AtPOT1 is down-regulated during salinity stress. Manipulations of AtPOT1 transcript levels altered shoot Cl⁻ concentrations, which could be utilised for enhancing shoot exclusion of Cl⁻, and hence plant salinity tolerance. Although more functional data is required, AtPOT2 might be involved in the efflux of Cl⁻ from the root to the soil. Therefore, reducing AtPOT1 expression and increasing AtPOT2 expression, may be two strategies for excluding Cl⁻ from the shoot under saline conditions.
Declaration

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# Table of contents

Abstract ........................................................................................................................................... I

Declaration ..................................................................................................................................... III

Acknowledgement ......................................................................................................................... IV

Table of contents ........................................................................................................................... VI

List of figures ................................................................................................................................ X VI

List of tables ................................................................................................................................ XXI

Abbreviations and symbols .......................................................................................................... XXIII

Chapter 1 Introduction, literature review and research aims ................................................... 1

1.1 Salinity ........................................................................................................................................ 1

1.1.1 Global issue of soil salinity ................................................................................................ 1

1.1.2 Soil salinity .......................................................................................................................... 2

1.1.3 Soil salinity in Australia ..................................................................................................... 3

1.1.3.1 Agriculture ................................................................................................................... 3

1.1.3.2 Viticulture .................................................................................................................... 5

1.2 Salinity stress and Cl\(^{-}\) toxicity to plants ............................................................................ 6

1.2.1 Salinity stress of plants ....................................................................................................... 6

1.2.1.1 Osmotic stress .............................................................................................................. 6

1.2.1.2 Ionic stress .................................................................................................................. 6

1.2.2 Plant sensitivity to Cl\(^{-}\) .................................................................................................. 8
1.2.3 Cl⁻ toxicity, plant salinity tolerance and crop yield ........................................ ........................................ 10
1.2.4 Cl⁻ and Na⁺ in the contest of plant salinity stress ......................................................... 12
1.3 Environmental Cl⁻ ............................................................................................................ 13
1.4 Cl⁻ as a micronutrient for plants ...................................................................................... 14
  1.4.1 Cl⁻ as a micronutrient and an osmotic pressure regulator ........................................... 14
    1.4.1.1 Osmotic and turgor balance ................................................................................... 14
    1.4.1.2 Photosynthesis ........................................................................................................ 15
    1.4.1.3 Enzyme activity ........................................................................................................ 15
  1.4.2 Distribution of Cl⁻ in plants .......................................................................................... 15
  1.4.3 The antagonism between Cl⁻ and nitrate (NO₃⁻) in plants ......................................... 16
1.5 Cl⁻ transport in plants ...................................................................................................... 17
  1.5.1 Cl⁻ transport in plants ................................................................................................ 17
    1.5.1.1 A brief history of the study on plant Cl⁻ transport .................................................. 17
    1.5.1.2 Thermodynamics ..................................................................................................... 18
    1.5.1.3 Cl⁻ transport on the plasma membrane .................................................................. 19
      1.5.1.3.1 ATPase and membrane potential ............................................................... 19
      1.5.1.3.2 Active uptake of Cl⁻ ..................................................................................... 19
      1.5.1.3.3 Passive transport of Cl⁻ ................................................................................... 20
    1.5.1.4 Cl⁻ transport on the tonoplast ................................................................................. 20
  1.5.2 Cl⁻ flux in plants regarding to plant Cl⁻ tolerance ....................................................... 21
    1.5.2.1 Cl⁻ flows in plants .................................................................................................... 21
    1.5.2.2 Uptake of Cl⁻ by plant roots from external environment .......................................... 22
    1.5.2.3 Compartmentation .................................................................................................. 23
      1.5.2.3.1 Cellular level compartmentation of Cl⁻ ......................................................... 23
      1.5.2.3.2 Tissue level compartmentation ........................................................................... 23
    1.5.2.4 Long distance transport of Cl⁻ in vasculature bundle ........................................... 24
      1.5.2.4.1 Xylem transport plays an important role in long distance transport of Cl⁻ ............. 24
    1.5.2.4.2 Phloem transport of Cl⁻ .................................................................................... 25
1.6 Cl⁻ xylem transport ........................................................................................................ 25
  1.6.1 Biophysics of Cl⁻ xylem transport ................................................................................. 25
  1.6.2 Cl⁻ xylem loading is inhibited by Abscisic acid (ABA) ................................................ 26
  1.6.3 Whole cell clamping technique to measure ion transport ........................................... 28
  1.6.4 Xylem loading of cations ............................................................................................. 29
1.6.4.1 Xylem loading of $K^+$ ................................................................. 30
1.6.4.2 Xylem loading of $Na^+$ .............................................................. 30
1.6.4.3 Xylem loading of $Cl^-$ ............................................................... 31

1.7 Known genes involved in plant $Cl^-$ transport ......................................... 35
1.7.1 AtSLAC/AtSLAHs ............................................................................ 35
1.7.2 AtCLCs ............................................................................................. 36
1.7.3 AtCCC ............................................................................................... 37
1.7.4 Other candidate that may have an indirect effect $Cl^-$ on in plants .............. 38

1.8 Research aims ....................................................................................... 38

Chapter 2 General materials and methods .................................................. 40

2.1 Plant growth ............................................................................................ 40
2.1.1 Plant grown in soil ............................................................................ 40
2.1.2 Plants grown in hydroponics ............................................................. 42
2.1.3 Plants grown on Murashige and Skoog (MS) plates ............................. 44

2.2 Transgenic plant selection ...................................................................... 45
2.2.1 Selection in soil .................................................................................. 45
2.2.1 Transgenic plant selection on MS plate .............................................. 46

2.3 DNA extractions ...................................................................................... 46
2.3.1 Phenol/chloroform/iso-amyl alcohol method ...................................... 46
2.3.2 Edwards’s buffer method .................................................................. 47
2.3.3 Freeze dry method ............................................................................ 47

2.4 RNA extractions and cDNA synthesis .................................................... 48
2.4.1 RNA extractions ............................................................................... 48
2.4.2 cDNA synthesis ................................................................................ 49

2.5 DNA sequencing ...................................................................................... 49

2.6 Polymerase chain reaction (PCR) ............................................................ 50
2.6.1 Quantitative Real-Time RT-PCR ....................................................... 50
2.6.2 Amplification PCR ............................................................................ 51
2.6.2.1 Elongase ...................................................................................... 51
2.6.2.2 Platinum Taq polymerase PCR ...................................................... 52
2.6.3 Colony PCR ...................................................................................... 53

2.7 E. coli transformation .............................................................................. 54
2.7.1 Preparation of competent cells .......................................................... 54
Chapter 3 Identification of a candidate gene encoding a putative anion transporter important for Cl⁻ xylem loading in Arabidopsis

3.1 Introduction ........................................................................................................................................ 57
  3.1.1 Arabidopsis thaliana, a model plant for gene discovery .......................................................... 59
  3.1.2 GAL-4 enhancer trap system in Arabidopsis ............................................................................ 59
  3.1.3 Isolation of specific cell populations from the GAL-4 enhancer trap lines ......................... 62
  3.1.4 Mining of public databases ....................................................................................................... 65
    3.1.4.1 GENEVESTIGATOR ............................................................................................................ 65
    3.1.4.2 ARAMEMNON .................................................................................................................. 65
3.2 Material and methods ....................................................................................................................... 66
  3.2.1 Plant hydroponics ....................................................................................................................... 66
    3.2.1.1 Seed preparation ............................................................................................................... 66
    3.2.1.2 Plant growth condition ..................................................................................................... 66
    3.2.1.3 A ramped salinity treatment ............................................................................................ 67
  3.2.2 Cell sorting ..................................................................................................................................... 67
    3.2.2.1 Isolation of protoplasts .................................................................................................... 67
    3.2.2.2 FACS .................................................................................................................................. 67
  3.2.3 Microarray analysis ..................................................................................................................... 68
3.2.4 Public database search .................................................................................................................. 68
  3.2.5 AtPOT1 sequence analysis .......................................................................................................... 68
    3.2.5.1 Phylogenetic analysis ........................................................................................................ 68
    3.2.5.2 Trans-membrane domain (TMD) prediction ..................................................................... 69
    3.2.5.3 Pair-wise alignment ............................................................................................................ 69
3.3 Results ................................................................................................................................................ 69
  3.3.1 Candidate genes identified from microarray ............................................................................ 69
  3.3.2 Public microarray database mining ............................................................................................. 72
  3.3.3 AtPOT1 in NRT/PTR1 family .................................................................................................... 74
3.3.4 Phylogenetic analysis of AtPOT1 ................................................................. 76
3.4 Discussion ................................................................................................. 78
  3.4.1 Candidate genes identified from Arabidopsis ........................................ 78
  3.4.2 AtPOT1, a candidate for Cl xylem loading .......................................... 79
  3.4.3 AtPOT1 belongs to the NRT1/PTR transporter family .......................... 80
  3.4.4 Cell type is important for studying cell type specific process of plant salinity adaptive responses ................................................................. 82
3.5 Conclusion................................................................................................. 83
Chapter 4 In planta profiling of AtPOT1 ............................................................. 84
  4.1 Introduction ............................................................................................... 84
    4.1.1 Quantitative RT-PCR ........................................................................ 85
    4.1.2 Gene and promoter fusions ................................................................. 85
      4.1.2.1 Fluorescent proteins ..................................................................... 86
      4.1.2.2 Promoter:GUS fusion ................................................................. 86
  4.2 Material and methods ............................................................................. 87
    4.2.1 Quantitative RT-PCR ......................................................................... 87
    4.2.2 Amplification of AtPOT1 CDS and putative AtPOT1 promoter .............. 88
    4.2.3 Cloning of AtPOT1 CDS and pAtPOT1 into pCR8/GW/TOPO/TA Gateway® entry vector .................................................................................. 90
    4.2.4 Generation of destination vectors ....................................................... 91
    4.2.5 Agrobacterium transformation and Agrobacterium mediated plant transformation .......................................................... 92
    4.2.6 Transient plant transformation in Arabidopsis mesophyll protoplast ......... 93
      Arabidopsis mesophyll protoplasts preparation ....................................... 93
    4.2.7 Confocal microscope imaging of GFP and YFP fusions ....................... 95
    4.2.8 GUS staining and stereo microscope imaging ...................................... 95
    4.2.9 Cross section of pAtPOT1:UidA plants .............................................. 96
    4.2.10 Salt treatments on pAtPOT1:UidA plants and MUG assay .................... 96
  4.3 Results ....................................................................................................... 97
    4.3.1 Clone of AtPOT1 ............................................................................... 97
      4.3.1.1 PCR amplification of AtPOT1 ....................................................... 97
      4.3.1.2 Cloning of AtPOT1 CDS and pAtPOT1 into entry vector pCR8 in sense orientation .......................................................... 98
      4.3.1.3 Clone AtPOT1 into destination vectors .................................... 101
4.3.2 Transformation of destination vectors to plants and plant selections ............... 104
4.3.3 Subcellular localisations ................................................................................. 104
  4.3.3.1 GFP fusions-stable transformation in Arabidopsis root cells .................. 105
    4.3.3.1.1 GFP::AtPOT1 fusion ........................................................................ 105
    4.3.3.1.2 AtPOT1::GFP fusion ...................................................................... 106
  4.3.3.2 YFP::AtPOT1 fusion in Arabidopsis mesophyll protoplasts ..................... 107
4.3.4 Tissue level localisation ................................................................................. 108
  4.3.4.1 pAtPOT1 driven GUS activity predominantly detected in the root vascular
        cells ........................................................................................................... 108
  4.3.4.2 pAtPOT1 driven GUS activity in the root stelar cells ............................. 110
4.3.5 Down-regulated GUS activity of pAtPOT1:UidA plants in response to salt stress
        ................................................................................................................. 111
  4.3.5.1 Salt treatments ....................................................................................... 111
  4.3.5.2 MUG assay ............................................................................................ 113
  4.3.5.3 AtPOT1 promoter analysis ..................................................................... 114
4.3.6 Quantitative RT-PCR .................................................................................... 116
  4.3.6.1 AtPOT1 mRNA level in the roots was decreased after both NaCl and ABA
        treatments .................................................................................................... 116
  4.3.6.2 AtPOT1 transcript levels in root and shoot of different ecotypes .......... 118
  4.3.6.3 Salt or ABA treatments on, AtNRT1.5, AtNRT1.8, AtSLAH1 and AtCCC.... 119
4.4 Discussion ......................................................................................................... 127
  4.4.1 AtPOT1 is a plasma membrane targeted protein ........................................ 127
  4.4.2 AtPOT1 is predominantly expressed in the root stele ............................... 128
  4.4.3 AtPOT1 is down-regulated by both salt and ABA ..................................... 129
  4.4.4 Putative promoter of AtPOT1 .................................................................... 130
  4.4.5 The possible involvement of AtSLAH1, AtNRT1.5, AtNRT1.8 and AtCCC in Cl
        transport ........................................................................................................ 131
4.5 Conclusion ......................................................................................................... 132

Chapter 5 Exploring AtPOT1 function in planta ...................................................... 133

5.1 Introduction ....................................................................................................... 133
5.2 Material and methods ....................................................................................... 134
  5.2.1 T-DNA knockouts ...................................................................................... 134
  5.2.2 Constitutive over-expression of AtPOT1 .................................................... 135
    5.2.2.1 Generation of AtPOT1 over-expression lines ...................................... 136
5.2.2.1 Southern-blotting ................................................................. 136
5.2.2.1.1 Restriction enzyme digestion of DNA ................................... 136
5.2.2.1.2 Gel electrophoresis ............................................................ 137
5.2.2.1.3 DNA transfer to nylon membranes ....................................... 137
5.2.2.1.4 Oligo-labelling of DNA probes ............................................ 138
5.2.2.1.5 Hybridisation of DNA probes to DNA bound to membranes .... 138
5.2.2.1.6 Autoradiography .............................................................. 139
5.2.2.2 Genotyping ........................................................................ 140
5.2.2.3 Phenotyping ....................................................................... 140
5.2.2.3.1 Cl− analysis .................................................................... 140
5.2.2.3.2 NO3− assay ..................................................................... 141
5.2.3 Artificial microRNA knockdown .................................................. 141
5.2.3.1 Molecular clone of artificial microRNAs (amiRNAs) ................. 142
5.2.3.2 Generation of AtPOT1 amiRNA knockdown lines .................... 146
5.2.3.3 Genotyping the AtPOT1 knockdowns ...................................... 146
5.2.3.4 Phenotyping the AtPOT1 knockdowns .................................... 146
5.3 Results ..................................................................................... 147
5.3.1 Unsuccessful disruption of AtPOT1 expression in the SALK lines .... 147
5.3.2 AtPOT1 over-expression resulted in higher Cl− accumulation in the shoot .... 149
5.3.2.1 Generation of destination vector and transgenic lines .............. 149
5.3.2.2 The isolation of two single-copy AtPOT1 over-expression lines .... 150
5.3.2.3 AtPOT1 over-expression lines accumulated significant higher Cl− in the shoot when compared with nulls ........................................ 151
5.3.2.4 Identification of two homozygous T3 AtPOT1 over-expression lines .... 152
5.3.2.5 AtPOT1 over-expression lines (T3) accumulated higher Cl−, but not NO3−, in the shoot ........................................................................ 153
5.3.3 AtPOT1 amiRNA knockdown resulted in lower Cl− accumulation in the shoot ... 156
5.3.3.1 Generation of destination vector and transgenic lines .............. 156
5.3.3.2 Shoot Cl− concentrations in the T2 AtPOT1 knockdown lines under low salt condition (2 mM NaCl) ........................................ 158
5.3.3.3 T2 AtPOT1 knockdown lines under high salt condition (75 mM NaCl) .... 161
5.4 Discussion ............................................................................. 163
5.4.1 Cl− accumulation in the shoot is regulated by AtPOT1 ....... 163
5.4.2 Shoot accumulation of NO3− in the transgenic lines ............... 164

XII
5.4.3 Contribution of AtPOT1 to plant salinity tolerance ........................................... 165
5.5 Conclusion .................................................................................................................. 165

Chapter 6 Characterisation of AtPOT1 protein function .............................................. 166

6.1 Introduction ................................................................................................................. 166
6.1.1 Structural modelling of AtPOT1 ......................................................................... 167
6.1.2 Heterologous systems .......................................................................................... 167
  6.1.2.1 Yeast .............................................................................................................. 168
  6.1.2.2 Electrophysiological characterisation of a plant transporter/channel in Xenopus oocytes ................................................................................................................. 168
    6.1.2.2.1 Xenopus oocytes ...................................................................................... 168
    6.1.2.2.2 Two-Electrode Voltage Clamp (TEVC) ......................................................... 169
6.2 Materials and methods ............................................................................................... 170
  6.2.1 Protein structure 3-D modelling of AtPOT1 ......................................................... 170
  6.2.2 AtPOT1 expression in yeast .............................................................................. 171
    6.2.2.1 Destination vector construction ................................................................. 171
    6.2.2.2 Yeast transformation .................................................................................. 172
    6.2.2.3 Yeast growth inhibition assay ................................................................... 174
      6.2.2.3.1 Growth assay on agar plates ................................................................. 174
  6.2.3 AtPOT1 expression in Xenopus oocytes ........................................................... 174
  6.2.4 Radioactive $^{36}$Cl$^-$ uptake assay in Xenopus oocytes ..................................... 175
6.3 Results ......................................................................................................................... 176
  6.3.1 AtPOT1 protein structure modelling ................................................................. 176
  6.3.2 AtPOT1 function in yeast .................................................................................. 178
    6.3.2.1 Constructed destination vector for expressing AtPOT1 in yeast .................. 178
    6.3.2.2 Growth inhibition assay of yeast strains using Cl$^-$ and Br$^-$ ...................... 179
  6.3.3 AtPOT1 characterisation in Xenopus oocytes ..................................................... 181
    6.3.3.1 Generation of destination vector for expressing AtPOT1 in Xenopus oocytes and the production of AtPOT1 cRNA ................................................................. 181
    6.3.3.2 TEVC performed on Xenopus oocytes ......................................................... 182
      6.3.3.2.1 Na$^+$ salts .......................................................................................... 182
      6.3.3.2.2 N-Methyl-D-glucamine (NMDG$^+$) solutions ....................................... 185
      6.3.3.2.3 Relative permeability of AtPOT1 .......................................................... 187
    6.3.3.3 Radioactive $^{36}$Cl$^-$ uptake assay in Xenopus oocytes ............................... 189
6.4 Discussion .................................................................................................................. 190
  6.4.1 AtPOT1 3-D modelling ......................................................................................... 190
  6.4.2 AtPOT1 in yeast .................................................................................................. 191
  6.4.3 TEVC and $^{36}$Cl flux assay in Xenopus oocytes ................................................. 192
6.5 Conclusion .................................................................................................................. 194

Chapter 7 AtPOT2, the homologue of AtPOT1 in Arabidopsis ....................................... 195

7.1 Introduction .............................................................................................................. 195
7.2 Material and methods .............................................................................................. 196
  7.2.1 Bioinformatics ..................................................................................................... 196
  7.2.2 Plant growth and qRT-PCR ................................................................................ 197
  7.2.3 Cloning of AtPOT2 and destination vector construction ..................................... 197
  7.2.4 Plant transformation and the production of transgenic lines ................................. 198
  7.2.5 amiRNA/AtPOT2 ................................................................................................. 199
  7.2.6 Elemental analysis ............................................................................................... 200
  7.2.7 Microscopic analysis ......................................................................................... 201
  7.2.8 AtPOT2 expression in Yeast .............................................................................. 201
7.3 Results ....................................................................................................................... 203
  7.3.1 Sequence analysis of AtPOT2 ............................................................................. 203
    7.3.1.1 Phylogenetic analysis .................................................................................... 203
    7.3.1.2 Sequence alignment of AtPOT2 ................................................................... 204
    7.3.1.3 TMD prediction of AtPOT2 ....................................................................... 206
  7.3.2 Gene expression profiles ...................................................................................... 207
    7.3.2.1 Gene expression specificity of AtPOT2 between root and shoot ................... 207
    7.3.2.2 Gene expression response of AtPOT2 to salt or ABA .................................... 208
    7.3.2.3 Sub-cellular localisations .......................................................................... 209
    7.3.2.4 Tissue level localisations of AtPOT2 .......................................................... 211
  7.3.3 AtPOT2 function in planta ................................................................................... 213
    7.3.3.1 AtPOT2 knockout ...................................................................................... 213
    7.3.3.2 AtPOT2 constitutive over-expression ......................................................... 215
    7.3.3.3 AtPOT2 knockdown lines ......................................................................... 217
  7.3.4 AtPOT2 transport activity in yeast ...................................................................... 219
7.4 Discussion ................................................................................................................... 221
  7.4.1 AtPOT2 transport activity in yeast ...................................................................... 221

XIV
7.4.2 AtPOT2 function in planta ................................................................. 222
7.5 Conclusion and future directions .......................................................... 224

Chapter 8 General discussion ..................................................................... 225

8.1 Aims of the thesis .................................................................................... 225
8.2 POT transporters are important in Cl⁻ transport in Arabidopsis ................. 226
8.3 AtPOT1 for Cl⁻ exclusion and plant salinity tolerance ............................ 227
8.4 Selectivity of AtPOT1 to Cl⁻ and NO₃⁻ ..................................................... 229
8.5 Cell type specific and salt responsive promoters .................................... 230
  8.5.1 Cell type specific response in salt exclusion ....................................... 230
  8.5.2 Salt inducible features ..................................................................... 231
  8.5.3 The potential identification of cis-acting elements in pAtPOT1 and pAtPOT2 .... 232
8.6 Potential posttranslational modifications on AtPOT1 ............................... 232
8.7 The NRT1/PTR transport family ............................................................. 233
8.8 NAXT subfamily and the renaming of AtPOT1 and AtPOT2 .................... 234
8.9 Concluding remarks ............................................................................. 236

References ................................................................................................. 237

Appendix ...................................................................................................... 263
List of figures

Figure 1. 1 Salt affected and potential salt affected land areas overlapping with the wheat grown zone in Australia. ................................................................. 4
Figure 1. 2 The schematic diagram showing the rate of plant growth in response to two phases of salt stress over time................................................................. 7
Figure 1. 3 The growth response of different plant species to Cl\(^-\) toxicity. ......................... 8
Figure 1. 4 The shoot Cl\(^-\) concentration was shown negatively correlated with plant salt tolerance ................................................................. 11
Figure 1. 5 A negative relationship was shown between citrus leaf Cl\(^-\) concentration and its fruit yield ................................................................. 12
Figure 1. 6 The proposed mechanisms of Cl\(^-\) transport on the root-soil inter-surface and cell-xylem vessels inter-surface in plant roots ................................................................. 18
Figure 1. 7 Two pathways of Cl\(^-\) movement in the plant root. ........................................ 22
Figure 1. 8 Examples of whole cell clamping in revealing the characteristics of plant ion transport ................................................................. 29
Figure 1. 9 Representative current/voltage (I/V) curves of the conductances for anion xylem loading in barley: X-QUAC, X-SLAC, and X-IRAC ................................................................. 32
Figure 1. 10 ABA, water stress and high cytosolic Ca\(^{2+}\) level decreased the occurrence and current density of Zm-X-QUAC ................................................................. 34

Figure 2. 1 The germination-hydroponic setup used to germinate Arabidopsis seeds .......... 43
Figure 2. 2 The growing-hydroponic setup used to grow Arabidopsis plants for elemental analysis ................................................................. 43
Figure 2. 3 The selection of transgenic plants using BASTA in soil .................................... 45

Figure 3. 1 An enhancer trap element (T-DNA insertion) capable of expressing GFP driven by an endogenous enhancer ................................................................. 60
Figure 3. 2 The enhancer trap line J2371\(^*\) and J1551 having mGFP-ER expression in root pericycle cells and root cortical cells, respectively ................................................................. 61
Figure 3. 3 AtNRT1.5 and AtSKOR are preferentially expressed in root pericycle cells........62
Figure 3. 4 A flow chart showing Fluorescent Activating Cell Sorting (FACS) technique........64
Figure 3. 5 The expression of candidate genes AT3G45700 and AT1G32450 (AtNRT1.5) are
likely down-regulated by ABA as revealed by ARAMEMNON.................................72
Figure 3. 6 AT3G45700 (AtPOT1) was suggested to be preferentially expressed in the stelar
and endodermis cells.................................................................73
Figure 3. 7 The predicted trans-membrane domains of AtPOT1.................................74
Figure 3. 8 AtPOT1 and AtNAXT1 protein sequences pair-wise aligned.........................75
Figure 3. 9 A subset of the maxi-likelihood tree of NRT1/PTRs from Arabidopsis, rice
and barley..........................................................77

Figure 4. 1 The PCR amplifications of AtPOT1 CDS and pAtPOT1 visualized on a gel. .....97
Figure 4. 2 The vector diagrams of AtPOT1 CDS or pAtPOT1 cloned into pCR8/GW/TOPO
TA Gateway® entry vector in sense orientation........................................................99
Figure 4. 3 The results of restriction enzyme digestions performed on pCR8/AtPOT1 and
pCR8/pAtPOT1 to obtain clones with fragments in sense orientation..........................100
Figure 4. 4 The Vector diagrams of AtPOT1 in the destination vectors of pMDC44, pMDC83
and pattR-YFP, respectively, and pAtPOT1 in destination vector pMDC162.............103
Figure 4. 5 The confocal image of root cells of GFP::AtPOT1 plants before and after
plasmolysis..........................................................................................105
Figure 4. 6 The Confocal image of root cells of AtPOT1::GFP plants before and after
plasmolysis..........................................................................................106
Figure 4. 7 The AtPOT1 protein was shown targeted to the plasma membrane when
expressed in Arabidopsis mesophyll protoplasts.........................................................107
Figure 4. 8 AtPOT1 is predominantly expressed in the root stelar cells in Arabidopsis...109
Figure 4. 9 AtPOT1 is also expressed in lower amounts in reproductive organs in Arabidopsis
when compared to that in the roots.................................................................110
Figure 4. 10 Cross sections of pAtPOT1:UidA plants root showing stelar specific expression
of AtPOT1 in the root..................................................................................111
Figure 4. 11 The GUS activity driven by the putative promoter of AtPOT1 was down-
regulated by salt treatments..................................................................................112
Figure 4. 12 The GUS activity in the roots of pAtPOT1:UidA plants was down-regulated by
salt stress............................................................................................................113
Figure 4. 13 The expression of AtPOT1 is down-regulated by salt and ABA..................117
Figure 4. 14 The expression of AtPOT1 varies in four Arabidopsis ecotypes.................118
Figure 4. 15 The expression of AtNRT1.5, AtNRT1.8, AtSLAH1, and AtCCC was examined in
response to a 3-h salt treatment..............................................................................120
Figure 4. 16 The expression of AtNRT1.5 in response to salt or ABA.........................121
Figure 4. 17 The expression of AtNRT1.8 in response to salt or ABA.........................123
Figure 4. 18 The expression of AtSLAH1 in response to salt or ABA.........................125
Figure 4. 19 The expressions of AtCCC in response to salt or ABA.............................126

XVII
Figure 5. 1 A schematic map showing the predicted positions of the T-DNA insertions in the two SALK lines (S_111056 and S_111071). .................................................................................. 134
Figure 5. 2 The setup of DNA membrane-transfer for Southern-blotting ........................................... 138
Figure 5. 3 Schematic process showing the generation and function of an artificial microRNA to silence the mRNA of a gene. ............................................................................................................. 142
Figure 5. 4 The overlapping PCRs to replace the native miRNA in the MIR319a (miRNA precursor) with a designed amiRNAs. .................................................................................................................. 143
Figure 5. 5 The genotyping PCRs to confirm the presence of T-DNA insertion in both the S_111056 and S_111071 lines .................................................................................................................. 147
Figure 5. 6 Southern-blotting performed to confirm the presence of the T-DNA insertion in both S_111056 and S_111071 lines .................................................................................................................. 148
Figure 5. 7 The transcript levels of AtPOT1 in SALK line S_111056 and S_111071 indicating an unsuccessful disruption of AtPOT1 expression in both lines ........................................................................... 149
Figure 5. 8 The vector diagram showing AtPOT1 CDS in the pTOOL2 destination vector. 150
Figure 5. 9 Southern-blotting performed to identify AtPOT1 over-expression lines with a single-copy insertion of T-DNA. ............................................................................................................ 151
Figure 5. 10 AtPOT1 over-expression lines (T_2) accumulated higher Cl\(^{-}\) in the shoot when compared with nulls .............................................................................................................................. 152
Figure 5. 11 The survival test of T_3 AtPOT1 over-expression lines under the selection of glufosinate ...................................................................................................................................................... 153
Figure 5. 12 AtPOT1 over-expression lines (T_3) accumulated slightly higher levels of Cl\(^{-}\) in the shoot after a low salt treatment (2 mM NaCl). ..................................................................................... 154
Figure 5. 13 T_3 AtPOT1 over-expression lines accumulated slightly higher levels of Cl\(^{-}\) in the shoot after a high salt treatment (75 mM NaCl). ..................................................................................... 155
Figure 5. 14 The NO\(_3\)\(^{-}\) accumulation in the shoot of all tested plants was inhibited by the high salt treatment ........................................................................................................................................ 156
Figure 5. 15 The vector diagram of amiRNA precursors in the destination vector pTOOL2. .................................................. 157
Figure 5. 16 AtPOT1 knockdown lines (T_2) accumulated less Cl\(^{-}\) in the shoot under low salt condition (2 mM NaCl) ............................................................................................................................... 158
Figure 5. 17 A positive correlation between Cl\(^{-}\) concentration in the shoot and AtPOT1 transcript levels in the root ............................................................................................................................................. 159
Figure 5. 18 A repeat experiment showing that amiRNA AtPOT1 knockdown lines (T_2) accumulated significant lower Cl\(^{-}\) in the shoot when compared with nulls under low salt (2 mM NaCl) ........................................................................................................ 160
Figure 5. 19 The amiRNA AtPOT1 knockdown lines (T_2) under high salt (75 mM NaCl) . 162

Figure 6. 1 The vector diagrams of pYES2-DEST2 empty vector ....................................................... 172
Figure 6. 2 A 3-D molecular model of the AtPOT1 protein structure in two orthogonal orientations ........................................................................................................................................ 177
Figure 6. 3 The vector diagrams of AtPOT1 in the destination vector pYES2-DEST2 for heterologous expression in yeast ............................................................................................................. 178
Figure 6. 4 The growth of AtPOT1 transformed yeast on high concentrations of external Cl− and Br− ................................................................. 180
Figure 6. 5 The vector diagram of AtPOT1 in the destination vector pGEM-HE-DEST constructed for heterologous expression in Xenopus oocytes............................... 181
Figure 6. 6 The quality and the size of AtPOT1 cRNA was examined on a gel with RNA ladders................................. 182
Figure 6. 7 TEVC performed to characterize AtPOT1 in Xenopus oocytes using Na+ solutions.......................................................... 184
Figure 6. 8 TEVC performed to characterize AtPOT1 in Xenopus oocytes using NMDG+ solutions.......................................................... 186
Figure 6. 9 The reversal potential shift and anion conductance derived from the results of TEVC performed .......................................................... 188
Figure 6. 10 The uptake of 36Cl− by Xenopus oocytes micro-injected with AtPOT1 cRNA .190

Figure 7. 1 The vector diagram of the pYES2-DEST52 for expressing AtPOT2 in yeast. ...202
Figure 7. 2 A subset of the maximum likehood phylogenetic tree of NRT1s from Arabidopsis, rice and barley.......................................................... 204
Figure 7. 3 The protein sequence alignment of AtPOT2, AtPOT1 and AtNAXT1. ..........206
Figure 7. 4 The AtPOT2 protein is predicted to have 12 trans-membrane domains and a large hydrophobic loop in the middle................................. 207
Figure 7. 5 The transcript level of AtPOT2 was shown much greater in the root than in the shoot.......................................................... 208
Figure 7. 6 The expression of AtPOT2 is up-regulated by salt stress while is down-regulated by ABA treatments. .......................................................... 209
Figure 7. 7 The AtPOT2 protein was localized to the plasma membrane in Arabidopsis mesophyll protoplasts.......................................................... 210
Figure 7. 8 The confocal image of Arabidopsis root cells of T2 GFP::AtPOT2 plant showing plasma membrane localization of AtPOT2........................................ 211
Figure 7. 9 AtPOT2 is preferentially expressed in the outer part of the root................................. 212
Figure 7. 10 The cross sections of pAtPOT2:GUS plant root showing the cortical specific expression of AtPOT2 in the root.......................................................... 213
Figure 7. 11 A schematic map of the AtPOT2 showing the SM_3.31001 line carrying a single copy of transposable T-DNA insertion in the fourth exon ........................................ 214
Figure 7. 12 The transcript levels of AtPOT2 detected in the SM_3.31001 sibling plants. ..214
Figure 7. 13 Knocking out AtPOT2 may lead to higher Cl− accumulation in the shoot in Arabidopsis.......................................................... 215
Figure 7. 14 The AtPOT2 over-expression lines under low salt condition (2 mM NaCl). ....216
Figure 7. 15 The AtPOT2 over-expression lines under high salt condition (75 mM) .........217
Figure 7. 16 The AtPOT2 knockdown lines under low salt condition (2 mM) ..................218
Figure 7. 17 The AtPOT2 knockdown lines under high salt condition (75 mM NaCl) ......219
Figure 7. 18 The growth of AtPOT2 transformed yeast was slightly inhibited by high levels of external Br− ......................................................... 220
Figure 7. 19 AtNAXT1 in Arabidopsis is predominantly expressed in the root cortex. ....222

XIX
List of tables

Table 1. 1 A summary of Cl\(^-\) accumulation and toxic threshold for different crop species. .....9
Table 1. 2 The CLC transporter family in Arabidopsis. .........................................................37

Table 2. 1 The composition of nutrient solution for watering soil grown Arabidopsis plants.41
Table 2. 2 The composition of germination solution for germination Arabidopsis seeds for hydroponic experiments..........................................................42
Table 2. 3 The composition of basal nutrient solution for plants grew in hydroponic tanks...44
Table 2. 4 PCR solution and program of Elongase-PCR used for amplifying long fragments with high fidelity..........................................................51
Table 2. 5 PCR solution and program used for general PCRs using Platinum taq polymerase. ..........................................................52
Table 2. 6 PCR solution and program used of colony PCRs. ..............................................53

Table 3. 1 A list of 16 candidate genes identified maybe involved in Cl\(^-\) xylem loading. ......71
Table 3. 2 A summary of function-known NRT1/PTRs in Arabidopsis. .........................81

Table 4. 1 Primers designed for quantitative PCRs of AtPOT1 and other candidates.........88
Table 4. 2 Primers used for amplifying and sequencing AtPOT1 CDS and putative promoter of AtPOT1. .........................................................................................................90
Table 4. 3 Primers used for sequencing AtPOT1 CDS and pAtPOT1 in the pCR8 vector......91
Table 4. 4 A summary of destination vectors used for characterizing AtPOT1 expression profiles. ..............................................................................................................92
Table 4. 5 The stock solutions for Arabidopsis mesophyll protoplast preparation. ..........93
Table 4. 6 The ABA signaling pathway associated cis-acting elements identified in the putative promoter region of AtPOT1. .................................................................115
Table 5. 1 Primer sequences and expected results of two genotyping PCRs performed on SALK lines (S_111056 and S_111071)........................................................................................................... 135
Table 5. 2 The composition of the restriction enzyme digestion for Southern blotting...... 137
Table 5. 3 Solutions used for pre-hybridisation and hybridisation in Southern blotting...... 139
Table 5. 4 Construct-specific primers used to genotype the AtPOT1 over-expression lines. 140
Table 5. 5 Primers and templates used in the overlapping PCRs to replace the miRNA in the MIR319a (miRNA precursor) with designed amiRNAs. ................................................................. 143
Table 5. 6 Solutions and programs used in the overlapping PCRs to replace the miRNA in MIR319a (miRNA precursor) with two designed amiRNAs that are specific to AtPOT1.... 144
Table 5. 7 Sequence of primers used in the overlapping PCRs to replace the miRNA in MIR319a (miRNA precursor) with two designed amiRNAs that are specific to AtPOT1.... 145

Table 6. 1 The components of SD media for liquid yeast culture. ................................. 173
Table 6. 2 The components of transformation mix for yeast transformation. ................. 173
Table 6. 3 Primers used in the colony PCR to confirm the presence of the construct (pYES2-DEST52/AtPOT1) in the transformed yeast. ................................................................. 174

Table 7. 1 Descriptions of the destination vectors used for the functional characterizations of AtPOT2 in planta.................................................................................................................. 198
Table 7. 2 Primers used for overlapping PCR to replace miRs in MIR319a with amiRs that is specific to knockdown AtPOT2 expression in Arabidopsis..................................................... 200
Table 7. 3 Construct specific primers used in colony PCRs to check the presence of pYES2-DEST52/AtPOT2 in yeast. ........................................................................................................ 203

Table 8. 1 The protein sequence pairwise alignments of AtNAXT1 with other 6 NAXT members................................................................. 235
## Abbreviations and symbols

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<thead>
<tr>
<th>Symbol</th>
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<tr>
<td>3’</td>
<td>three prime end</td>
</tr>
<tr>
<td>3-D</td>
<td>three dimensional</td>
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<td>5’</td>
<td>five prime end</td>
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<td>Arabidopsis Biological Resource Centre</td>
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<td>Australian Bureau of Statistics</td>
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<td>AGRF</td>
<td>Australian Genome Research Facility</td>
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<tr>
<td>amiRNA</td>
<td>artificial micro ribonucleic acid</td>
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<td><em>Arabidopsis thaliana</em></td>
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<td>BLAST</td>
<td>basic local alignment search tool</td>
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<td>base pair</td>
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<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
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<tr>
<td>DNA</td>
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<tr>
<td>dNTP</td>
<td>mixture of equal equivalents of dATP, dTTP, dCTP and dGTP</td>
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<td>DTT</td>
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<tr>
<td>ECe</td>
<td>electrical conductivity</td>
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<td><em>E.coli</em></td>
<td><em>Escherichia coli</em></td>
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Os  
*Oryza sativa*

PBS  
phosphate buffered saline

PCR  
polymerase chain reaction

PI  
propidium iodide

PLACE  
A Database of Plant Cis-acting Regulatory DNA Elements

PM  
plasma membrane

POT  
Proton dependent oligo-peptide transporter

QTL  
quantitative trait loci

QRT-PCR  
quantitative reverse transcription polymerase chain reaction

RGAP  
Rice Genome Annotation Project

RNA  
ribonucleic acid

RT-PCR  
reverse transcription polymerase chain reaction

rpm  
rotation per minute

s  
second(s)

SDS  
sodium dodecyl sulfate

SSC  
saline sodium citrate

T<sub>x</sub>  
transgenic plants of generation x

TAE  
tris-acetate-EDTA

TAIR  
The Arabidopsis Information Resource

*Taq*  
polymerase identified from *T. aquaticus*

T-DNA  
transfer deoxyribonucleic acid

Tm  
melting temperature

TMD  
trans-membrane domain

U  
unite(s)

UAS  
upstream activation sequence

UTR  
untranslated region

WT  
wild type

Ws  
Wassilewskija

w/v  
weight per volume

x-gluc  
5-bromo-4-chloro-3-indoly-glucuronide

X-KORC  
xylem- K<sup>+</sup> outward rectifying channel

X-IRAC  
xylem- inwardly rectifying anion conductance

X-QUAC  
xylem- quickly activating anion conductance

X-SLAC  
xylem- slowly activating anion conductance

YFP  
yellow fluorescent protein

Zm  
*Zea mays*