

**Characterisation of AtPQL1, AtPQL2 and  
AtPQL3 as Candidate Voltage  
Insensitive Non-Selective Cation  
Channels (vi-NSCCs)**

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B. Biotech (Hons)

A thesis submitted for the degree of  
Doctor of Philosophy  
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The University of Adelaide



THE UNIVERSITY  
*of* ADELAIDE

**February 2013**

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## List of Abbreviations

3'	three prime, of nucleic acid sequence
5'	five prime, of nucleic acid sequence
#	number
%	percent
~	approximately
x	times
°C	degrees Celsius
aa	amino acid
ACPFG	Australian Centre for Plant Functional Genomics
AGRF	Australian Genome Research Facility
<i>Agrobacterium</i>	<i>Agrobacterium tumefaciens</i>
amiRNA	artificial micro ribonucleic acid
ANOVA	analysis of variance
Arabidopsis	<i>Arabidopsis thaliana</i>
At	<i>Arabidopsis thaliana</i>
BLAST	basic local alignment search tool
bp	base pairs, of nucleic acid
BSA	bovine serum albumin
Ca <sup>2+</sup>	calcium ion
CaCl <sub>2</sub>	calcium chloride
CaMV	cauliflower mosaic virus
Cat. #	catalogue number
cDNA	complimentary deoxyribonucleic acid
Cl <sup>-</sup>	chloride ion
cm	centimetre(s)
CNGCs	cyclic nucleotide-gated channels
Col-0	Columbia-0
cRNA	complimentary ribonucleic acid
d	day(s)
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
dGTP	deoxyguanosine triphosphate
dH <sub>2</sub> O	deionised water
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
DTT	dithiothreitol
dTTP	deoxythymidine triphosphate
ECe	electrical conductivity
EDTA	ethylenediaminetetraacetic acid
E <sub>rev</sub>	reversal potential
EST	expressed sequence tag

FAO	Food and Agricultural Organization of the United Nations
g	gram(s)
G	conductance
<i>g</i>	gravity
gDNA	genomic deoxyribonucleic acid
GFP	green fluorescent protein
GLRs	glutamate receptor-like channels
GUS	$\beta$ -glucuronidase protein
H <sup>+</sup>	hydrogen ion
H <sub>2</sub> O	water
HCl	hydrochloric acid
HKT	high affinity potassium transport
hr	hour(s)
I	current
K <sup>+</sup>	potassium ion
kb	kilo base pairs, of nucleic acid
KCl	potassium chloride
kg	kilogram(s)
KOH	potassium hydroxide
L	litre(s)
LB	left border, of T-DNA sequence
LB	media luria betani media
LCT1	low affinity cation transporter
LSM	laser scanning microscope
M	molar
MA <sup>+</sup>	methyl ammonium
mg	milligram(s)
Mg <sup>2+</sup>	magnesium ion
MgCl <sub>2</sub>	magnesium chloride
min	minute(s)
miRNA	micro ribonucleic acid
ml	millilitre(s)
mm	millimetre(s)
mM	millimolar
mol	mole
mOsm	milliosmole
MQ	MoniQ
MS	media Murashige and Skoog media
mV	millivolt
n	sample size
N/A	not applicable
N <sub>2</sub>	nitrogen
nA	nanoampere

Na <sup>+</sup>	sodium ion
NaCl	sodium chloride
NaOH	sodium hydroxide
NCBI	National Center for Biotechnology Information
ng	nanogram(s)
NH <sub>4</sub>	ammonium
NHX	Na <sup>+</sup> /H <sup>+</sup> exchanger
nm	nanometre(s)
nM	nanomolar
nosT	bacterial nopaline synthase terminator sequence
o/n	overnight
OD <sub>600</sub>	optical density measured at 600 nm
PCR	polymerase chain reaction
PI	propidium iodide
PQL	PQ loop
qRT-PCR	quantitative reverse transcription polymerase chain reaction
QTL	quantitative trait loci
RB	right border, of T-DNA sequence
RNA	ribonucleic acid
RO	reverse osmosis
RT	room temperature
RT-PCR	reverse transcription polymerase chain reaction
SARDI	South Australian Research & Development Institute
s	second(s)
Sc	<i>Saccharomyces cerevisiae</i>
SDS	sodium dodecyl sulfate
SNP	single nucleotide polymorphism(s)
SOS	salt overly sensitive
T <sub>1</sub>	primary Arabidopsis transformant containing T-DNA
T <sub>2</sub>	progeny of T1 plant
TAE	tris-acetate-EDTA
T-DNA	transfer deoxyribonucleic acid
TE	transposable element
TE	tris-EDTA
TF	transcription factor
T <sub>m</sub>	melting temperature, of primers
U	units
UAS	upstream activation sequence
<i>uidA</i>	β-glucuronidase gene
UTR	untranslated region
UV	ultraviolet
V	voltage
v/v	volume per volume

vi-NSCC	voltage-insensitive non-selective cation channel
wk	week(s)
w/v	weight per volume
X-Gluc	5-bromo-4-chloro-3-indoyl-glucuronide
YFP	yellow florescent protein
µg	microgram(s)
µL	microlitre(s)
µm	micrometre(s)
µM	micromolar
µmol	micromole(s)

## Abstract

Soil salinity is responsible for significant reductions in crop yield. The salinity tolerance of crops can be improved by minimising the amount of sodium ions ( $\text{Na}^+$ ) accumulating in the shoot. One hypothesis for reducing shoot  $\text{Na}^+$  accumulation is to minimise  $\text{Na}^+$  entering the plant via the root. Previous studies indicate that in most plants, the majority of  $\text{Na}^+$  entry into root cells is through voltage-insensitive non-selective cation channels (vi-NSCCs), however, the molecular identities of these channels are unclear. Recently two genes that belong to the PQL family were identified as putative vi-NSCCs in yeast. This project aims to functionally characterise three orthologous PQL genes from *Arabidopsis thaliana* (*AtPQL1*, *AtPQL2* and *AtPQL3*) and investigate their role in  $\text{Na}^+$  entry into cells and into roots.

Bioinformatic tools and *in planta* techniques were used to determine gene expression profiles, analyse protein sequences and determine the cellular and subcellular localisations of *AtPQL1-3*. The plasma membrane localisation of *AtPQL1* and 2 agrees with the proposed function of vi-NSCCs as ion transport channels. Furthermore, the suggested role of vi-NSCCs in facilitating initial  $\text{Na}^+$  entry into the roots was supported by *in silico* expression profiles of *AtPQL2* and 3 and by observations of reporter proteins driven by *PQL* promoters in root tissues.

Heterologous expression of *AtPQL1* in yeast resulted in yeast which were more salt sensitive than controls, suggesting a role in  $\text{Na}^+$  influx into cells. Furthermore, this sensitivity could be ameliorated by the addition of  $\text{CaCl}_2$ , (indicating  $\text{Ca}^{2+}$  inhibited the movement of  $\text{Na}^+$ ), an attribute which corresponds with known properties of vi-NSCCs.

A number of transgenic *Arabidopsis* lines were generated to have altered expression of *AtPQL1* to 3 and were then phenotypically analysed in hydroponics under a range of salt treatments. Results of these experiments proved largely inconclusive primarily because individual plants with significantly altered expression of *AtPQL1*, 2 and/or 3 could not be obtained.

## 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 Monique Kirsten Shearer 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.

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M. Shearer

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Date

## Acknowledgements

Firstly I would like to thank my supervisors Prof. Mark Tester, Dr. Stuart Roy and Dr. Darren Plett for their support and guidance throughout my candidature. They have helped make my time here enjoyable and have always been around for advice when needed.

I gratefully acknowledge the financial support provided during my PhD from The University of Adelaide, through provision of an Australian Postgraduate Award and The Australian Centre for Plant Functional Genomics (ACPFG). For financial support provided to assist attendance of international and interstate conferences I would like to thank the Australian Federation of University Women (AFUW) and the Australian Society of Plant Scientists (ASPS).

I would like to thank Dr. Anna Amtmann and Dr. Richard Pattison from The University of Glasgow for the constructs and seed material provided for use in this project. I would also like to thank all members of Dr. Anna Amtmann's lab for their help during the time I spent in Glasgow. From The University of Adelaide I would like to thank Prof. Steve Tyerman, Dr. Sunita Ramesh, Dr. Matt Gilliam, and Mr. Bo Xu for their help with *Xenopus* oocyte electrophysiology. I would also like to acknowledge Mrs. Yuan Li who performed the qRT-PCR analysis and Dr. Julie Hayes for her guidance on heterologous expression in yeast.

A huge thank you goes to all the members of the ACPFG Salt Focus Group, both past and present, for their support and all round awesomeness over the past few years. In particular I would like to thank Ms. Sandra Schmöckel for giving many hours of her time to help harvest my plants and for getting me to work every day with enthusiasm. I would also like to thank Mr. Li Bo for his expert advice and assistance in many areas of my project. For general lab support and laughs I would like to thank Mrs. Jessica Bovill, Ms. Rhiannon Schilling, Dr. Joanna Sundstrom, Mr. Gordon Wellman, Ms. Melissa Pickering, Dr. Aurelie Evrard, Mr. Nawar Shmaya, Dr. Damien Lightfoot and Salty Croc.

Finally, I would like to thank my friends and family for their support and attempts at distraction. I have been lucky enough to take some time off to travel at various points throughout my candidature and I would like to thank everyone I have met along the way for giving me some perspective.