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

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List of Abbreviations

3′	three prime, of nucleic acid sequence
5′	five prime, of nucleic acid sequence
#	number
%	percent
~	approximately
×	times
°C	degrees Celsius
aa	amino acid
ACPFG	Australian Centre for Plant Functional Genomics
AGRF	Australian Genome Research Facility
Agrobacterium	*Agrobacterium tumefaciens*
amiRNA	artificial micro ribonucleic acid
ANOVA	analysis of variance
Arabidopsis	*Arabidopsis thaliana*
At	*Arabidopsis thaliana*
BLAST	basic local alignment search tool
bp	base pairs, of nucleic acid
BSA	bovine serum albumin
Ca^{2+}	calcium ion
$CaCl_2$	calcium chloride
CaMV	cauliflower mosaic virus
Cat. #	catalogue number
cDNA	complimentary deoxyribonucleic acid
Cl^-	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_20	deionised water
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
DTT	dithiothreitol
dTTP	deoxythymidine triphosphate
ECe	electrical conductivity
EDTA	ethylenediaminetetraacetic acid
E_{rev}	reversal potential
EST	expressed sequence tag

FAO	Food and Agricultural Organization of the United Nations
g	gram(s)
G	conductance
g	gravity
gDNA	genomic deoxyribonucleic acid
GFP	green fluorescent protein
GLRs	glutamate receptor-like channels
GUS	β-glucuronidase protein
H^+	hydrogen ion
H_20	water
HCl	hydrochloric acid
HKT	high affinity potassium transport
hr	hour(s)
I	current
K^+	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^+	methyl ammonium
mg	milligram(s)
Mg^{2+}	magnesium ion
$MgCl_2$	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_2	nitrogen
nA	nanoampere

Na^+	sodium ion
NaCl	sodium chloride
NaOH	sodium hydroxide
NCBI	National Center for Biotechnology Information
ng	nanogram(s)
NH_4	ammonium
NHX	Na^+/H^+ exchanger
nm	nanometre(s)
nM	nanomolar
nosT	bacterial nopaline synthase terminator sequence
o/n	overnight
OD_{600}	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	*Saccharomyces cerevisiae*
SDS	sodium dodecyl sulfate
SNP	single nucleotide polymorphism(s)
SOS	salt overly sensitive
T_1	primary Arabidopsis transformant containing T-DNA
T_2	progeny of T1 plant
TAE	tris-acetate-EDTA
T-DNA	transfer deoxyribonucleic acid
TE	transposable element
TE	tris-EDTA
TF	transcription factor
Tm	melting temperature, of primers
U	units
UAS	upstream activation sequence
uidA	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 (Na^+) accumulating in the shoot. One hypothesis for reducing shoot Na^+ accumulation is to minimise Na^+ entering the plant via the root. Previous studies indicate that in most plants, the majority of 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 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 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 Na^+ influx into cells. Furthermore, this sensitivity could be ameliorated by the addition of $CaCl_2$, (indicating Ca^{2+} inhibited the movement of 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

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