Investigation of chloride transport mechanisms in *Arabidopsis thaliana* root

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A dissertation submitted for the degree of
Doctor of Philosophy
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2015

Declaration

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Jiaen Qiu **Date**

Acknowledgements

I wish to thank my supervisors Assoc Prof. Matthew Gilliham and Dr. Stuart Roy for your guidance, inspiration and support. Your knowledge, patience and encouragement have helped me throughout my PhD. Matt and Stuart are always available for discussions on my interesting data and enthusiastic about my project. I really enjoyed my PhD, it is a wonderful and memorable time.

I am also grateful to my co-supervisor Prof. Mark Tester for your contributions. Your ideas and guidance have been vital for me to complete my project. Thank you for your support.

I would like to thank the financial support provided by the University of Adelaide during my PhD through the provision of Adelaide Graduate Research Scholarship, and further thank Grain Research and Development Corporation and IWPMB2013 organizing committee for provision of the financial support to attend IWPMB2013 in Japan.

I also would like to thank those people that have contributed to my research. Dr Aurelie Evrard and Dr Ute Baumann from ACPFG for providing microarray data, Dr Maroru Okamoto and Ms Karen Francis for teaching nitrate concentration analysis, Ms Jodie Kretschmer from ACPFG for kindly donating expression vectors, Ms Asmini Athman for assisting CO_2 measurement, Dr Yuan Li from ACPFG for assisting qRT-PCR analysis, Dr Zhengyu Wen for teaching me radioactive flux assay and all the lab crew, Dr Sam Henderdon, Dr Sunita Ramesh, Dr Caitlin Byrt, Dr Bo Li, Dr Bo Xu, Dr Caitlin Byrt, Mr Maclin Dayod, Mr Qu Yue, Dr Brad Hocking and my friend Dr Jin Zhang and Dr Wenmian Huang.

Finally, I would like to thank my parents for their support throughout all of my studies as well as my partner Mr Jia Jiang for helping me get through the tough times.

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Abbreviations and symbols

Abbreviation	Full term
#	Number
%	Percentage
±	Plus and minus
×	Times
°C	Degree celsius
µg	Microgram(s)
µl	Microliter(s)
µM	Micromolar
µmol	Micromole(s)
β	Beta
3´	Three prime end
3-D	Three dimensional
5´	Five prime end
A-9-C	Anthracene-9-carboxylic acid
ABA	Abscisic acid
ABARE	Australian Bureau of Agricultural and Resource Economics
ABRC	Arabidopsis Biological Resource Centre
ABS	Australian Bureau of Statistics
ACPFG	Australian Centre for Plant Functional Genomics
AGRF	Australian Genome Research Facility
amiRNA	Artificial micro ribonucleic acid
At	Arabidopsis thaliana
BLAST	Basic Local Alignment Search Tool
BNS	Basal Nutrient Solution
bp	Base pair
BSA	Bovine serum albumin
Ca^{2+}	Calcium ion
CaMV	Cauliflower mosaic virus
CCC	Cation chloride co-transporter
cDNA	Complimentary deoxyribonucleic acid
CFP	Cyan florescent protein
Cl^{-}	Chloride ion
CLCs	Chloride channel proteins
cm	Centimeter
Col-0	Columbia-0
cRNA	Capped ribonucleic acid
dH_2O	Deionised water

DIDS	4,4'-Diisothiocyano-2,2'-stilbenedisulfonic acid
DNA	Deoxyribonucleic acid
dNTP	Mixture of equal equivalents of dATP, dTTP, dCTP and dGTP
dS	Decisiemens
DTT	Dithiothreitol
E.coli	Escherichiacoli
ECe	Electrical conductivity
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agriculture Organization
FW	Fresh weigh
g	Gravity
g	Gram(s)
gDNA	Genomic deoxyribonucleic acid
GFP	Green fluorescent protein
GOI	Gene of interest
GUS	β-glucuronidase protein
H^+	Hydrogen ion
H^+- ATPase	Proton-ATPase
ha	Hectare
HCl	Hydrochloric acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HKT	High-affinity K+ transporter
hr	Hour(s)
Hv	Hordeum vulgare
IBSC	International Barley Genome Sequencing Consortium
ICP-AES	Inductively Coupled Plasma Optical Emission Spectrometry
K^+	Potassium ion
kb	Kilo base pairs
KBr	Potassium bromide
KCl	Potassium chloride
KD	Knock down
kD	Kilo dalton
KF	Potassium fluoride
KOH	Potassium hydroxide
LB	Left border (of T-DNA)
LBmedia	Luria betanimedia
Ler	Lands bergerecta
M	Molar
MES	2-(N-morpholino) ethanesulfonic acid
mg	Milligram (s)
$MgCl_2$	Magnesium chloride
Min	Minute (s)
ml	Millilitre (s)
mol	Mole

MS	Murashige and Skoog media
mV	Micro voltage
MYTH	Membrane Yeast Two-Hybrid
nA	Nanomolar
Na^+	Sodium ion
NaBr	Sodium bromide
NaF	Sodium fluoride
$NaNO_3$	Sodium nitrate
NaOH	Sodium hydroxide
NASC	European Arabidopsis Stock Centre
NCBI	National Centre for Biotechnology Information
ng	Nanogram (s)
nl	Nanolitre
NLWRA	National Land & Water Resource Audit
nM	Nanomolar
nm	Nanometer
NMDG	N-Methyl-D-glucamine
NO_3^-	Nitrate ion
NRTs	Nitrate transporters
NUE	Nitrogen use efficiency
OD	Optical density
OEX	Over- expression
OR	Outward rectifying
Os	Oryzasativa
P/B	Peak/background ratio
PEG	Polyethylene glycol
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PM	Plasma membrane
POT	Protodependent oligo-peptide transporter
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
QTL	Quantitative trait loci
RIL	Recombinant inbred lines
RNA	Ribonucleic acid
RO	Reverse osmosis treated
rpm	Rotation per minute
RT-PCR	Reverse transcription polymerase chain reaction
SDS	Sodium dodecyl sulfate
SEM	Standard error of the mean
SKOR	Stelar k^+ outwardly-rectifying channel
SLAC	Slowly activated anion conductance
SSC	Saline sodium citrate
TAE	Tris-acetate-EDTA
TAIR	The Arabidopsis Information Resource

Taq	Polymerase identified from T. aquaticus
T-DNA	Transfer deoxyribonucleic acid
TEVC	Two electrode voltage clamp
Tm	Melting temperature
TMD	Trans-membrane domain
Tris	tris(hydroxymethyl)aminomethane
Tx	Transgenic plants of generation x
U	Unite(s)
UAS	Upstream activation sequence
UTR	Untranslated region
w/v	Weight per volume
Ws	Wassilewskija
WT	Wildtype
X-IRAC	Xylem-inwardly rectifying anion conductance
X-KORC	Xylem-K+outward rectifying channel
X-QUAC	Xylem-quickly activating anion conductance
X-SLAC	Xylem-slow activating anion conductance
YFP	Yellow fluorescent protein

Abstract

Salinity tolerance is correlated with shoot chloride (Cl^-) exclusion in many horticultural and crop species (e.g. grapevine, soybean). It is hypothesized that the key regulatory step in root-to-shoot transfer of Cl^- is conferred by plasma membrane-localised anion transporters associated within the root vasculature. Reducing long-distance Cl^- transport by manipulating the regulation of anion transporters in the root vasculature is therefore a strategy that promises to increase plant tolerance to saline environments. However, the information of which candidate genes are responsible for this process is limited. To gain a greater knowledge of the long distance Cl^- movement from a molecular aspect, a number of candidate anion transporters from *Arabidopsis thaliana* were identified from a preliminary microarray study. Quantitative PCR was used to indicate transcriptional levels of candidate anion transporters that decreased upon NaCl and ABA treatment. Based on this analysis, *AtSLAH1*, *AtSLAH3* and *AtNRT1.5* were selected as genes of interest (GOI) that were likely to be involved in the Cl^- movement between the root stele symplast and the xylem vessels.

To functionally characterize the transport properties of all GOIs at a protein level, various heterologous systems were used to investigate the anion (Cl^- and NO_3^-) transport capacity. Two-electrode voltage clamp electrophysiology was used to measure the currents that were generated by the target anions crossing oocyte membranes. A yeast expression system was also used to further study the anion transport properties *in vitro*.

AtSLAH1 cRNA injected oocytes were not able to produce significant anion currents. Also, no evident anion currents were generated from a site-directed mutant of AtSLAH1 in a putative phosphorylation site injected into oocytes. Although there was evidence that anion currents were elicited from *AtSLAH1* and *AtSnRk2.3* co-injected oocytes, due to difficulties in the ability to reproduce these results, it is uncertain whether AtSLAH1 can function as an anion transporter in the conditions tested. Both wild type and site-mutated *AtSLAH1* was also separately transformed into yeast for further examination without an observable phenotype.

In order to examine the effect of altered *AtSLAH1* expression on shoot anion accumulation, *AtSLAH1* amiRNA knockdown and constitutive over expression of *AtSLAH1* mutant plants were generated. *AtSLAH1* knockdown lines (T_2) exhibited strong repression in transcript abundance in low salt environments and resulted in a significant reduction in shoot Cl^- when compared to nulls. Constitutive over expression of *AtSLAH1* showed increased shoot Cl^- contents under high salt stress. These results indicated the potential role of AtSLAH1 in Cl^- transport in plants.

Electrophysiological characterization of AtSLAH3 in oocytes showed that AtSLAH3 was able to produce significant NO_3^- but not Cl^- currents suggesting a role in the efflux of NO_3^- out of cells in most of circumstances. Similar results were gained in *AtSLAH3*- transformed yeast. However, *AtSLAH3* over-expression lines showed a decreased shoot Cl^- without an effect on shoot NO_3^- under high salt stress compared to null plants. The potential reasons for this are discussed and further experiments are proposed to test these hypotheses.

Although AtNRT1.5 has been reported to transport NO_3^-, electrophysiological characterization of AtNRT1.5 in *X. Laevis oocytes* was not able to detect any anion currents induced by the gene. Interestingly, AtNRT1.5 transformed yeast showed a significant inhibited phenotype (grow less well than empty vector control) when challenged with high concentration of Cl^- and NO_3^- within the growth media, indicating a role the transport of both anions. Constitutive over- expression lines showed a potent shoot Cl^- reduction under high salt stress compared to nulls. Interestingly, no significant NO_3^- accumulation in shoot was identified. These results might suggest that AtNRT1.5 was able to regulate both Cl^- and NO_3^- transport from root to shoot; however, the mechanism by which this occurs is unclear.

Previous findings indicated the possibilities that Cl^- and NO_3^- can be transported through the same anion channel/transporter. To further study the regulation of Cl^- and NO_3^- uptake, an anion blocker (DIDS) was used to test the anion shoot accumulation under different salt conditions. Under high salt stress, DIDS was able to reduce the Cl^- accumulation and increase

the NO_3^- contents in shoots. Further experiments are required at both a physiological and molecular level to further understand how plants recognize and respond to this blocker, as the molecular targets of this blocker are a potential way to improve the plant salt tolerance and nitrogen use efficiency under high salt stress.

In summary, new information was revealed on several candidates that affect root-to-shoot loading of chloride and new research avenues have been proposed based on the findings of this study.