Root Cell-Type Specific Expression of Multiple Salinity Tolerance Genes to Alter Plant Shoot Sodium Accumulation

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Abstract

Increasing soil salinity of agricultural land is of growing concern world-wide as excessive soil salinity has a detrimental effect on growth and yield of many plant species of agricultural importance. The accumulation of sodium ions (Na^+) from saline soils into the shoots of crop plants contributes to the negative effect salinity has on plant growth in cereals. In recent years, many molecular targets involved in Na^+ transport in plants have been identified in a number of species. Genetic modification (GM) utilising these genes may enable manipulation of Na^+ transport with an aim of reducing Na^+ accumulation in the shoot. Constitutive and/or tissue-specific over-expression (OX) of such genes in transgenic plants can prove beneficial in reducing Na^+ shoot accumulation and improve plant salinity tolerance in some cases. However, further reductions could be made by fine tuning Na^+ transport through the plant by co-expressing multiple salinity tolerance associated genes of interest (GOI) in specific root-cell types. To date, this has proved difficult.

Previously generated barley (*Hordeum vulgare* c.v Golden Promise) lines with putative cell-type specific OX of salinity tolerance associated GOIs, *High Affinity K^+-Transporter 1;5* (*HvHKT1;5*) and *vacuolar H^+-pyrophosphatase 1* (*HvHVP1*), were screened in saline hydroponics to assess for improvements in salinity tolerance. Lines with the simultaneous root-cell-type specific OX of both *HvHKT1;5* and *HvHVP1* were developed through hybridisation and assessed for improved salinity tolerance. Although no significant improvements were identified in both the single- or dual-GOI transgenic lines, this approach could be used for other transgenic lines with cell-type specific OX of other GOIs combinations.

The role of *vacuolar H^+-pyrophosphatase 1* (*AtAVP1*) was re-examined when over-expressed in the root-epidermal and –cortical cell types in the model plant species *Arabidopsis thaliana*. OX of *AtAVP1* in these cell-types was thought to improve Na^+ sequestration and there-by improve salinity tolerance. However, saline hydroponics assays of lines with root-epidermal and/or –cortical OX of *AtAVP1* failed to identify improvements in plant salt tolerance or Na^+ uptake, suggesting that *AtAVP1* contributes little to Na^+ sequestration in these cell-types.

Finally, a system that would allow the cell-type specific over-expression of different GOIs in different root cell-types was developed. Such a system would allow the trialling

different gene combinations to identify combinations that would allow more targeted manipulation of Na^+ transport throughout a plant and alter salinity tolerance. This work was carried out in the model plant species, *Arabidopsis thaliana*, and cell-type expression was enabled through the use of dual *GAL4* and *HAP1* enhancer-trap systems and trans-activation constructs. Lines and constructs were developed to allow the cell-type specific OX of selected GOIs, however testing of dual salinity tolerance GOI lines was not achievable during the timeframe of this project.

Declaration

I certify that 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 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. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Signed

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Gordon B. Wellman

Date

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List of Publications, Presentations and Conference Posters

General Publications

Gordon Wellman, (2013, March 30) Stranger than fiction: Food crops for a future, *Adelaide Advertiser.*

Radio Interviews

Gordon Wellman (Guest) & Sarah Martin (Presenter) (2015, October, 21), Taking Plant Genomics from Adelaide to Saudi Arabia [Radio broadcast]. In Sarah Martin (Producer), *The Sound of Science.* Adelaide, Australia, Radio Adelaide

Conference Posters

Gordon Wellman, Mahima Krishnan, Stuart Roy and Andrew Jacobs "*Cell-type Specific Expression of Multiple Salt Tolerance Genes to Improve Plant Salinity Tolerance*" International Workshop in Plant Membrane Biology (IWPMB) 2013, Kurashiki, Japan, March 2013

Gordon Wellman, Mahima Krishnan, Stuart Roy & Andrew Jacobs "*Cell-type Specific Expression of Multiple Salt Tolerance Genes to Improve Plant Salinity Tolerance*" ComBio 2012, Adelaide, Australia, September 2012

Oral Presentations

Gordon Wellman, Mahima Krishnan, Stuart Roy and Andrew Jacobs "*Cell-type Specific Expression of Multiple Salt Tolerance Genes to Improve Plant Salinity Tolerance*", ACPFG Joint Research meeting, Adelaide, Australia, November 2014

Gordon Wellman, Mahima Krishnan, Stuart Roy and Andrew Jacobs "*Cell-type Specific Expression of Multiple Salt Tolerance Genes to Improve Plant Salinity Tolerance*", Shinozaki Lab, RIKEN, Tsukuba, Japan, March 2013

Gordon Wellman, Mahima Krishnan, Stuart Roy and Andrew Jacobs "*Cell-type Specific Expression of Multiple Salt Tolerance Genes to Improve Plant Salinity Tolerance*" ACPFG Joint Research meeting, Adelaide, Australia, November 2012

Gordon Wellman, Mahima Krishnan, Stuart Roy and Andrew Jacobs "*Cell-type Specific Expression of Multiple Salt Tolerance Genes to Improve Plant Salinity Tolerance*" ACPFG Joint Research meeting, Adelaide, Australia, November 2012

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Abbreviations

#	number
%	percentage
[element]	concentration of element. e.g. [Na^+], [K^+]
≈	approximately
×	times
°C	degrees Celsius
µg	microgram(s)
µL	microlitre(s)
µm	micrometre(s)
µM	micromolar
µmol	micromole(s)
3′-	three prime, of nucleic acid sequence. End of a coding sequence
5′-	five prime, of nucleic acid sequence. Start of a coding sequence
A	adenine
aa	amino acid
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
Agrobacterium	*Agrobacterium tumefaciens*
ANOVA	Analysis of variance
Arabidopsis	*Arabidopsis thaliana*
At	*Arabidopsis thaliana*. Prefix for Arabidopsis genes
AtAVP1	*Vacuolar pyrophatase 1* from *Arabidopsis thaliana*
ATP	adenosine 5′-triphosphate
ATPase	adenosine 5′-triphosphatase
Barley	*Hordeum vulgare*
BLAST	Basic local alignment search tool
bp	base pairs, of nucleic acid
BSA	bovine serum albumin
C	cytosine
C-	carboxyl (COOH) - terminus of a peptide
C24	Arabidopsis ecotype C24
Ca / Ca^{2+}	calcium / calcium cation
$CaCl_2$	calcium chloride
CaMV	cauliflower mosaic virus
Cat. No:	catalogue number
ccdB	cytotoxic coupled cell division
cDNA	complimentary deoxyribonucleic acid
CFP	Cyan Flourescent Protein
Cl / Cl^-	chloride / chloride anion
cm	centimetre(s) (1 cm = 1×10^{-2} m)
Col-0	Arabidopsis ecotype Columbia-0
CSIRO	Commonwealth Scientific and Industrial Research Organisation
cv.	cultivar
d	day(s)
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate

dGTP	deoxyguanosine triphosphate
dH_2O	deionised/distilled water
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
dNTPs	mixture of equal equivalents of deoxynucleotide triphosphates (dATP, dTTP, dCTP and dGTP)
dTTP	deoxythymine triphosphate
dS	deciSiemens
DW	dry weight (of plant material)
EDTA	ethylene diamine tetraacetate acid
ER	endoplasmic reticulum
EST	expressed sequence tag
FACS	fluorescence-activated cell sorting
FAO	Food and Agricultural Organization of the United Nations
FW	fresh weight (of plant material)
F_y	Progeny resulting from a hybridisation event - y refers to generation from hybridisation F_1 – plants resulting from hybridisation, F_2 – progeny of F_1 plants… etc.
g	gravity
g	gram(s)
G	guanine
GAPdh	*glyceraldehydes- 3- phosphate dehydrogenase*
gDNA	genomic deoxyribonucleic acid
GFP	green fluorescent protein
GOI	gene of interest
GSS	genome survey sequence
GUS	β-glucuronidase protein/assay
H^+	hydrogen ion/proton
H^+-ATPase	proton translocating ATPase
H^+-PPase	proton translocating pyrophosphatase
H_2O	dihydrogen monoxide
H2B	Histone 2B
Ha	hectare (1 ha = 1×10^4 m^2)
HA	haemagglutinin
HCl	hydrochloric acid
HKT	High-affinity potassium transport
Hr	hour(s)
Hv	*Hordeum vulgare,* Barley. Prefix for barley genes
HvHVP1	*Vacuolar H^+-Pryrophosphatase 1* from *Hordeum vulgare*
Hyg	hygromycin B
ICP-AES	inductively coupled plasma atomic emission spectrometry
IMVS	Institute of Medical & Veterinary Science
IPTG	isopropyl-β-D-thiogalactopyranoside
IRRI	International Rice Research Institute
K / K^+	potassium / potassium cation
KAc	potassium acetate
Kan	kanamycin
kbp	kilo base pairs, of nucleic acid
KCl	potassium chloride

kg	kilogram(s)
km	kilometre(s)
KOH	potassium hydroxide
L	litre(s)
LB	left border, of T-DNA sequence
LB media	Luria and Bertani medium
M	molar
m	metre(s)
max.	maximum
mg	milligram(s)
mL	millilitre(s)
mm	millimetre(s)
mM	millimolar
Mha	megahectares (1 ha = 1×10^4 m^2)
MPa	megapascal
Mg / Mg^{2+}	magnesium / magnesium cation
$MgCl_2$	magnesium chloride
Milli-Q H_2O	ultra-pure water
min	minute(s)
miRNA	micro ribonucleic acid
Mn	manganese
Mol	mole(s)
MPM	transmembrane segment, pore, transmembrane segment domain
MPSS	massively parallel signature sequence
mRNA	messenger ribonucleic acid
MS	Murashige and Skoog medium
n	sample size
ng	nanogram(s)
nL	nanolitre(s)
nm	nanometre(s)
nM	nanomolar
nA	nanoampere
N-	amino (NH_2) - terminus of a peptide
N/A	not applicable
N_2	nitrogen
Na / Na^+	sodium / sodium cation
NaCl	sodium chloride
NaOH	sodium hydroxide
NCBI	National Center for Biotechnology Information
NH_4	ammonium
NHX1	Na^+/H^+ exchanger 1
No.	number
nos	nopaline synthase gene
nosT	*nopaline synthase* polyA terminator sequence
NSCC	non-selective cation channel
O/N	overnight
Os	*Oryza sativa*. Prefix for rice (*Oryza sativa*) genes
P	Probability
P	phosphorus
pg	picogram(s)

pmol	picomole(s)
pat	*phosphinotricin acetyl transferase* conferring BASTA resistance.
PCR	polymerase chain reaction
pCR8	entry vector pCR™8/GW/TOPO Gateway®
P_i	inorganic orthophosphate
PI	propidium iodide
PP_i	inorganic pyrophosphate
pro	promoter
qPCR	quantitative real-time polymerase chain reaction
qRT-PCR	quantitative reverse transcription polymerase chain reaction
RACE	rapid amplification of cDNA ends
RB	right border, of T-DNA sequence
Rice	*Oryza sativa*
RNA	ribonucleic acid
RNAi	RNA interference
RO	reverse osmosis
rpm	revolutions per minute
RT	room temperature
RT-PCR	reverse transcription polymerase chain reaction
S	sulphur
S	second(s)
S.E.M	standard error of the mean
SARDI	South Australian Research & Development Institute
Sc	*Saccharomyces cerevisiae* (yeast). Prefix for yeast genes
SDS	sodium dodecyl sulphate
Sec	second(s)
siRNA	short interfering ribonucleic acid
SNP	single nucleotide polymorphism(s)
SOB	Super Optimal Broth
SOS1	Salt Overly Sensitive 1
T	thymine
Ta	*Triticum aestivum* (wheat). Prefix for wheat genes.
TAE	tris base, acetic acid and EDTA
TAIL-PCR	thermal asymmetric interlaced polymerase chain reaction
TAIR	The Arabidopsis Information Resource
T-DNA	transfer deoxyribonucleic acid
TE	tris-EDTA
TF	transcription factor
T_m	melting temperature, of primers
TPM	transcripts per million
Tris	tris(hydroxymethyl)aminomethane
T_x	Progeny of transformation event - x refers to generation from transformation Barley: T_0 – initial transformants, T_1 – progeny of T_0 plants… etc. Arabidopsis: T_1 – initial transformants, T_2 – progeny of T_1 plants… etc.
T_xF_y	Progeny resulting from hybridised transformants. See T_x and F_y. T_3F_1 – plants resulting from hybridisation of T_2 lines T_4F_2 – progeny of T_3F_1 plants… etc.
U	units

UAS	upstream activation sequence
UAS_{GAL4}	upstream activating sequence of *GAL4*
UAS_{HAP1}	upstream activating sequence of *HAP1*
uidA	β-glucuronidase gene
USDA	U.S. Department of Agriculture
UTR	untranslated region
UV	ultraviolet
V	Volts
v/v	volume per volume
w/w	weight per weight
wk	week(s)
X-Gal	5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside
X-Gluc	5-bromo-4-chloro-3-indolyl β-D-glucuronide
Xp	xylem parenchyma
Xy	Xylem