SPATIAL AND TEMPORAL ALTERATIONS OF GENE EXPRESSION IN RICE

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TABLE OF CONTENTS

ABSTRACT

Two problems hampering efforts to produce salt-tolerant plants through constitutive expression of transgenes include:

1. Spatial control. Particular cell-types must respond specifically to salt stress to minimise the amount of Na^+ delivered to the shoot; and,
2. Temporal control. Transgenes are typically expressed in plants at similar levels through time, irrespective of the stress encountered by the plant, which may exacerbate pleiotropic effects and means that, particularly in low-stress conditions, costly and/or detrimental metabolic processes may be active, thus reducing yield.

To address these issues, Gateway® destination vector constructs were developed combining the GAL4 UAS (upstream activating sequence) with the ethanol-inducible gene expression system to drive inducible cell-specific expression of Na^+ transporter transgenes (or to silence salt transporter transgenes inducibly and cell-specifically). Rice (*Oryza sativa* L. cv. Nipponbare) GAL4-GFP enhancer trap lines (Johnson *et al.*, 2005: *Plant J.* **41**, 779-789) that express *GAL4* and *GFP* specifically in either the root epidermis or xylem parenchyma (and therefore 'trap' cell-type specific enhancer elements) were transformed with this GAL4 UAS – ethanol switch construct, thereby allowing both spatial and temporal control of transgenes. In preliminary experiments, the expression system successfully limited the expression of *RFP* to specific cell-types after induction with ethanol. Other genes expressed using this system include *PpENA1*, a Na^+-extruding ATPase from the moss, *Physcomitrella patens*, and *AtHKT1;1*, a Na^+ transporter from *Arabidopsis thaliana*.

The two enhancer trap rice lines were also transformed with the GAL4 UAS driving stable expression of *AtHKT1;1* and *PpENA1* specifically in root epidermal or xylem parenchyma cells. Expression of *AtHKT1;1* in root epidermal cells reduced Na^+ accumulation in the shoots, while expression in the root xylem parenchyma appeared to have little effect on shoot Na^+ accumulation. Using cryo-scanning electron microscopy (SEM) X-ray microanalysis, the outer cells of the roots of the line expressing *AtHKT1;1* in the epidermal cells were found to accumulate higher levels of Na^+ than the parental enhancer trap line. Additionally, this line had decreased unidirectional $^{22}Na^+$ influx. Similar results were observed for plants expressing *AtHKT1;1* driven by the CaMV 35S promoter, but these plants were stunted, presumably from expressing *AtHKT1;1* at increased levels.

STATEMENT

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.

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Date

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LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

$(NH_4)SO_4$	ammonium sulfate
°C	degrees Celsius
µCi	microCurie(s)
µg	microgram(s)
µl	microliter(s)
µM	micromolar
10-yeb	youngest emerged blade after 10 mM Na^+ treatment
2,4-D	2,4-Dichlorophenoxyacetic acid
$^{22}Na^+$	$^{22}Na^+$ radiotracer
2X35S	dually-enhanced CaMV 35S promoter
3'	three prime end of a nucleic acid
35S	CaMV 35S promoter
35Sx2	dually-enhanced CaMV 35S promoter
5'	five prime end of a nucleic acid
50-old	older leaf blade after 50 mM Na^+ treatment
50-yeb	youngest emerged blade after 50 mM Na^+ treatment
AB	AB medium
ABA	abscisic acid
ABRE	ABA-responsive element
Ac/Ds	activator/dissociator transposon system
ace1	activating copper-MT expression transcription factor
ACPFG	Australian Centre for Plant Functional Genomics
ACX	acyl-CoA oxidase
AGRF	Australian Genome Research Facility
AKT	Arabidopsis potassium channel
Al^{3+}	aluminum ion
alcA	alcA promoter
alcR	alcR transcription factor
AleI	*AleI* restriction enzyme
amiRNA	artificial microRNA
amp	ampicillin
AscI	*AscI* restriction enzyme
AtHKT1;1	Arabidopsis thaliana HKT
ATP	adenosine triphosphate
ATPase	enzyme utilising ATP
attL1	L1 Gateway® recombination site
attL2	L2 Gateway® recombination site
attR1	R1 Gateway® recombination site
attR2	R2 Gateway® recombination site
AVP	Arabidopsis vacuolar pyrophosphatase
B	boron
B.C.	Before Christ
BAP	benzylaminopurine
Basta (R)	basta resistance gene

Basta	basta herbicide
Bla (amp)	ampicillin resistance gene
BlpI	*BlpI* restriction enzyme
BOR	high boron requiring
bp	base pair(s)
C_4	C_4 carbon fixation
Ca	calcium
Ca^{2+}	calcium ion
$Ca(NO_3)_2$	calcium nitrate
$Ca(NO_3)_2*4H_2O$	calcium nitrate
$CaCl_2$	calcium chloride
CaMV35S polyA	CaMV 35S 3' UTR poly A signal
CaMV35S	CaMV 35S promoter
CaMV35Sx2	dually-enhanced CaMV 35S promoter
CAT	chloramphenicol acyltransferase
CBL	calcinuerin B-like protein
ccdB	cytotoxic ccdB gene
cDNA	complementary DNA
CF	cortical fiber
Chloramphenicol (R)	chloramphenicol resistance gene
CHX	cation/hydrogen exchanger
CIPK	CBL-interacting protein kinase
Cl^-	chloride ion
cm	centimeter(s)
CNGC	cyclic nucleotide-gated channel
$CoCl_2*6H_2O$	cobalt chloride
ColE1	ColE1 replication origin
CSIRO	Commonwealth Scientific and Industrial Research Organisation
Cu	copper
$CuSO_4$	copper sulfate
$CuSO_4*5H_2O$	copper sulfate
d	day(s)
dH_2O	deionised water
DHHC	DHHC domain
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
DRE	dehydration-responsive element
dS/m	deciSiemens per meter
dsRED	*Discosoma sp*. RFP gene
dsRNAi	double-stranded RNA interference
DT-A	diphtheria toxin A
E. coli	*Escherichia coli*
EBC	epidermal bladder cell
EC	electrical conductivity

EcoRI	*EcoRI* restriction enzyme
EcoRV	*EcoRV* restriction enzyme
EcR	ecdysone receptor
EDAX	energy dispersive spectroscopy
EDTA	ethylenediaminetetraacetic acid
EEO	electroendosmosis
EN	endodermis
ENA	*exitus natru*
EP	epidermis
EX	exodermis
ER	estrogen receptor
ESP	exchangeable sodium percentage
EtOH	ethanol
F_2	F_2 generation
FACS	fluorescently-activated cell sorting
FAO	Food and Agriculture Organization
Fe	iron
Fe^{3+}	iron ion
FeEDTA	iron EDTA
$FeSO_4*7H_2O$	iron sulfate
FST	flanking sequence tag
FW	fresh weight
g	acceleration of gravity
g	gram(s)
GAL4	GAL4 transcription factor
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GFP	green fluorescent protein
GLR	glutamate receptor
GOI	gene-of-interest
Gos2	Gos2 promoter
GPI	glycosylphosphatidylinositol
GR	glucocorticoid receptor
GSK	glycogen synthase kinase
GUS	β-glucoronidase
GVG	GVG chimeric transcription factor
h	hour(s)
H^+	hydrogen ion
H_3BO_3	boric acid
ha	hectare(s)
HAK	high-affinity potassium transporter
HCl	hydrochloric acid
HKT	high-affinity potassium transporter
HVP	barley vacuolar pyrophosphatase
HVT	nucleic acid helicase
hyg	hygromycin
Hygromycin (R)	hyromycin resistance gene

IC	inner cortex
ICPAES	inductively coupled plasma-atomic emission spectroscopy
In2-2	In2-2 promoter
IRRI	International Rice Research Institute
K	potassium
K^+	potassium ion
kan	kanamycin
kanamycin (R)	kanamycin resistance gene
KCl	potassium chloride
kg	kilogram(s)
KH_2PO_4	potassium phosphate
KI	potassium iodide
KNO3	potassium nitrate
KpnI	*KpnI* restriction enzyme
KUP	potassium uptake transporter
kV	kilovolt(s)
L	liter
LacZ	β-galactosidase
LB	left border sequence
LCT	low-affinity cation transporter
LEA	late embryogenesis abundant
LexA	LexA bacterial repressor
LhG4	LhG4 chimeric transcription factor
LiCl	lithium chloride
LR	LR Gateway® recombination
luc	firefly luciferase
m	meter
MAP	mitogen activated protein
Mbp	mega base pairs
Mg	magnesium
Mg^{2+}	magnesium ion
$MgSO_4*7H_2O$	magnesium sulfate
min	minute(s)
miRNA	microRNA
mL	milliliter(s)
mm	millimeter(s)
mM	millimolar
Mn	manganese
$MnCl_2*4H_2O$	manganese chloride
$MnSO_4*H_2O$	manganese sulfate
mRNA	messenger RNA
mV	millivolt(s)
MX	metaxylem
N_2	molecular nitrogen
Na	sodium

Na^+	sodium ion
Na_2EDTA	sodium EDTA
Na_2MoO_3	sodium molybdate
NAA	napthaleneacetic acid
NAC	NAM, ATAF and CUC transcription factors
NaCl	sodium chloride
NaFe(III)EDTA	sodium iron EDTA
NaH_2PO_4	sodium dihydrogen phosphate
$NaH_2PO_4*H_2O$	sodium dihydrogen phosphate
Nax	sodium excluding
NB	NB medium
NBS	NBS medium
ng	nanogram(s)
NG	not germinated
NH_4^+	ammonium ion
NH_4Cl	ammonium chloride
NH_4NO_3	ammonium nitrate
NHA	sodium/hydrogen antiporter
NHX	sodium/hydrogen exchanger
nm	nanometer(s)
nM	nanomolar
NO_3^-	nitrate
Nos	nopaline synthase
nosT	nopaline syntase terminator
nptII	neomycin phosphotransferase II protein
NSCC	non-selective cation channel
OC	outer cortex
OCS term	octopine synthase terminator
OD_{600}	optical density at 600 nm
OEX	overexpression
P	P media
P	phosphorus
P/B	peak/background
P35S	CaMV 35S promoter
pA35S	CaMV 35S promoter
palcA	alcA promoter
pAnos	nopaline synthase promoter
Pat (basta)	basta resistance gene
pBR322 bom	pBR322 basis of mobility
pBR322 ori	pBR322 origin of replication
PCR	polymerase chain reaction
PDK	pyruvate dehydrogenase kinase intron
pH	per hydrogen
PmeI	*PmeI* restriction enzyme
Pnos	nopaline synthase promoter
pOp	pOp artificial promoter

PpENA1	*Physcomitrella patens* ENA
PP_i	pyrophosphate
PR	pericycle
PR-1a	PR-1a promoter
PR-AG	PR-AG medium
pVS1 rep	pVS1 replication function
pVS1 sta	pVS1 stability function
Q-PCR	quantitative PCR
QTL	quantitative trait locus
R^2	coefficient of determination
R2-CL	R2-CL medium
R2-CS	R2-CS medium
R2-S	R2-S medium
RB	right border sequence
RCD	radical-induced cell death
RFP	red fluorescent protein
RK2 ori	RK2 origin of replication
RN	RN medium
RNA	ribonucleic acid
RNAi	RNA interference
RO	reverse osmosis
ROS	reactive oxygen species
rpm	revolutions per minute
RT	room temperature
s	second(s)
SacI	*SacI* restriction enzyme
SARDI	South Australian Research and Development Initiative
SAS	sodium overaccumulation in shoots
SCABP	SOS3-like calcium-binding proteins
SDS	sodium dodecyl sulfate
SE	standard error
siRNA	short interfering RNA
SKC	small conductance calcium-activated potassium channel
SKOR	stelar potassium outwardly rectifying channel
SOS	salt overly sensitive
Spec prom	spectinomycin promoter
spec	spectinomycin
Spectinomycin R	spectinomycin resistance gene
t	tonne(s)
T_0	T_0 generation
T_1	T_1 generation
T_2	T_2 generation
T_3	T_3 generation
TATA	TATA box DNA sequence
T-Border (left)	left border sequence
T-Border (right)	right border sequence

T-DNA	transferred DNA
TetR	tetracycline repressor gene
TGV	TGV transcriptional activator
TIGR	The Institute for Genomic Research
T_m	melting temperature
Tris-EDTA	trishydroxymethylaminomethane-EDTA
tRNA	transfer RNA
TYNG	TYNG medium
UAS	upstream activation sequence
uidA	β-glucoronidase enzyme
UN	United Nations
US	United States
USSL	United States Salinity Lab
UTR	untranslated region
UV	ultraviolet
VP16	herpes simplex virus transcriptional activator
WT	wild type
XP	xylem parenchyma
XRMA	x-ray microanalysis
XVE	XVE chimeric transcription activator
YEB	youngest fully emerged blade
Zn	zinc
$ZnSO_4*7H_2O$	zinc sulfate