

# **SPATIAL AND TEMPORAL ALTERATIONS OF GENE EXPRESSION IN RICE**

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## 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<sup>+</sup> 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<sup>®</sup> 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<sup>+</sup> 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 *PpENAI*, a Na<sup>+</sup>-extruding ATPase from the moss, *Physcomitrella patens*, and *AtHKT1;1*, a Na<sup>+</sup> transporter from *Arabidopsis thaliana*.

The two enhancer trap rice lines were also transformed with the GAL4 UAS driving stable expression of *AtHKT1;1* and *PpENAI* specifically in root epidermal or xylem parenchyma cells. Expression of *AtHKT1;1* in root epidermal cells reduced Na<sup>+</sup> accumulation in the shoots, while expression in the root xylem parenchyma appeared to have little effect on shoot Na<sup>+</sup> 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<sup>+</sup> than the parental enhancer trap line. Additionally, this line had decreased unidirectional <sup>22</sup>Na<sup>+</sup> 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.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

Signed

Date

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## LIST OF ABBREVIATIONS

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

<b>Basta</b>	basta herbicide
<b>Bla (amp)</b>	ampicillin resistance gene
<b><i>BlpI</i></b>	<i>BlpI</i> restriction enzyme
<b>BOR</b>	high boron requiring
<b>bp</b>	base pair(s)
<b>C<sub>4</sub></b>	C <sub>4</sub> carbon fixation
<b>Ca</b>	calcium
<b>Ca<sup>2+</sup></b>	calcium ion
<b>Ca(NO<sub>3</sub>)<sub>2</sub></b>	calcium nitrate
<b>Ca(NO<sub>3</sub>)<sub>2</sub>*4H<sub>2</sub>O</b>	calcium nitrate
<b>CaCl<sub>2</sub></b>	calcium chloride
<b>CaMV35S polyA</b>	CaMV 35S 3' UTR poly A signal
<b>CaMV35S</b>	CaMV 35S promoter
<b>CaMV35Sx2</b>	dually-enhanced CaMV 35S promoter
<b>CAT</b>	chloramphenicol acyltransferase
<b>CBL</b>	calcinuerin B-like protein
<b><i>ccdB</i></b>	cytotoxic <i>ccdB</i> gene
<b>cDNA</b>	complementary DNA
<b>CF</b>	cortical fiber
<b>Chloramphenicol (R)</b>	chloramphenicol resistance gene
<b>CHX</b>	cation/hydrogen exchanger
<b>CIPK</b>	CBL-interacting protein kinase
<b>Cl<sup>-</sup></b>	chloride ion
<b>cm</b>	centimeter(s)
<b>CNGC</b>	cyclic nucleotide-gated channel
<b>CoCl<sub>2</sub>*6H<sub>2</sub>O</b>	cobalt chloride
<b>ColE1</b>	ColE1 replication origin
<b>CSIRO</b>	Commonwealth Scientific and Industrial Research Organisation
<b>Cu</b>	copper
<b>CuSO<sub>4</sub></b>	copper sulfate
<b>CuSO<sub>4</sub>*5H<sub>2</sub>O</b>	copper sulfate
<b>d</b>	day(s)
<b>dH<sub>2</sub>O</b>	deionised water
<b>DHHC</b>	DHHC domain
<b>DMSO</b>	dimethyl sulfoxide
<b>DNA</b>	deoxyribonucleic acid
<b>dNTP</b>	deoxyribonucleotide triphosphate
<b>DRE</b>	dehydration-responsive element
<b>dS/m</b>	deciSiemens per meter
<b>dsRED</b>	<i>Discosoma sp.</i> RFP gene
<b>dsRNAi</b>	double-stranded RNA interference
<b>DT-A</b>	diphtheria toxin A
<b><i>E. coli</i></b>	<i>Escherichia coli</i>
<b>EBC</b>	epidermal bladder cell
<b>EC</b>	electrical conductivity

<b><i>EcoRI</i></b>	<i>EcoRI</i> restriction enzyme
<b><i>EcoRV</i></b>	<i>EcoRV</i> restriction enzyme
<b>EcR</b>	ecdysone receptor
<b>EDAX</b>	energy dispersive spectroscopy
<b>EDTA</b>	ethylenediaminetetraacetic acid
<b>EEO</b>	electroendosmosis
<b>EN</b>	endodermis
<b>ENA</b>	<i>exitus natru</i>
<b>EP</b>	epidermis
<b>EX</b>	exodermis
<b>ER</b>	estrogen receptor
<b>ESP</b>	exchangeable sodium percentage
<b>EtOH</b>	ethanol
<b>F<sub>2</sub></b>	F <sub>2</sub> generation
<b>FACS</b>	fluorescently-activated cell sorting
<b>FAO</b>	Food and Agriculture Organization
<b>Fe</b>	iron
<b>Fe<sup>3+</sup></b>	iron ion
<b>FeEDTA</b>	iron EDTA
<b>FeSO<sub>4</sub>*7H<sub>2</sub>O</b>	iron sulfate
<b>FST</b>	flanking sequence tag
<b>FW</b>	fresh weight
<b>g</b>	acceleration of gravity
<b>g</b>	gram(s)
<b>GAL4</b>	GAL4 transcription factor
<b>GAPDH</b>	glyceraldehyde 3-phosphate dehydrogenase
<b>GFP</b>	green fluorescent protein
<b>GLR</b>	glutamate receptor
<b>GOI</b>	gene-of-interest
<b>Gos2</b>	Gos2 promoter
<b>GPI</b>	glycosylphosphatidylinositol
<b>GR</b>	glucocorticoid receptor
<b>GSK</b>	glycogen synthase kinase
<b>GUS</b>	β-glucoronidase
<b>GVG</b>	GVG chimeric transcription factor
<b>h</b>	hour(s)
<b>H<sup>+</sup></b>	hydrogen ion
<b>H<sub>3</sub>BO<sub>3</sub></b>	boric acid
<b>ha</b>	hectare(s)
<b>HAK</b>	high-affinity potassium transporter
<b>HCl</b>	hydrochloric acid
<b>HKT</b>	high-affinity potassium transporter
<b>HVP</b>	barley vacuolar pyrophosphatase
<b>HVT</b>	nucleic acid helicase
<b>hyg</b>	hygromycin
<b>Hygromycin (R)</b>	hyromycin resistance gene

<b>IC</b>	inner cortex
<b>ICPAES</b>	inductively coupled plasma-atomic emission spectroscopy
<b>In2-2</b>	In2-2 promoter
<b>IRRI</b>	International Rice Research Institute
<b>K</b>	potassium
<b>K<sup>+</sup></b>	potassium ion
<b>kan</b>	kanamycin
<b>kanamycin (R)</b>	kanamycin resistance gene
<b>KCl</b>	potassium chloride
<b>kg</b>	kilogram(s)
<b>KH<sub>2</sub>PO<sub>4</sub></b>	potassium phosphate
<b>KI</b>	potassium iodide
<b>KNO<sub>3</sub></b>	potassium nitrate
<b><i>KpnI</i></b>	<i>KpnI</i> restriction enzyme
<b>KUP</b>	potassium uptake transporter
<b>kV</b>	kilovolt(s)
<b>L</b>	liter
<b>LacZ</b>	β-galactosidase
<b>LB</b>	left border sequence
<b>LCT</b>	low-affinity cation transporter
<b>LEA</b>	late embryogenesis abundant
<b>LexA</b>	LexA bacterial repressor
<b>LhG4</b>	LhG4 chimeric transcription factor
<b>LiCl</b>	lithium chloride
<b>LR</b>	LR Gateway <sup>®</sup> recombination
<b>luc</b>	firefly luciferase
<b>m</b>	meter
<b>MAP</b>	mitogen activated protein
<b>Mbp</b>	mega base pairs
<b>Mg</b>	magnesium
<b>Mg<sup>2+</sup></b>	magnesium ion
<b>MgSO<sub>4</sub>*7H<sub>2</sub>O</b>	magnesium sulfate
<b>min</b>	minute(s)
<b>miRNA</b>	microRNA
<b>mL</b>	milliliter(s)
<b>mm</b>	millimeter(s)
<b>mM</b>	millimolar
<b>Mn</b>	manganese
<b>MnCl<sub>2</sub>*4H<sub>2</sub>O</b>	manganese chloride
<b>MnSO<sub>4</sub>*H<sub>2</sub>O</b>	manganese sulfate
<b>mRNA</b>	messenger RNA
<b>mV</b>	millivolt(s)
<b>MX</b>	metaxylem
<b>N<sub>2</sub></b>	molecular nitrogen
<b>Na</b>	sodium

<b>Na<sup>+</sup></b>	sodium ion
<b>Na<sub>2</sub>EDTA</b>	sodium EDTA
<b>Na<sub>2</sub>MoO<sub>3</sub></b>	sodium molybdate
<b>NAA</b>	naphthaleneacetic acid
<b>NAC</b>	NAM, ATAF and CUC transcription factors
<b>NaCl</b>	sodium chloride
<b>NaFe(III)EDTA</b>	sodium iron EDTA
<b>NaH<sub>2</sub>PO<sub>4</sub></b>	sodium dihydrogen phosphate
<b>NaH<sub>2</sub>PO<sub>4</sub>*H<sub>2</sub>O</b>	sodium dihydrogen phosphate
<b>Nax</b>	sodium excluding
<b>NB</b>	NB medium
<b>NBS</b>	NBS medium
<b>ng</b>	nanogram(s)
<b>NG</b>	not germinated
<b>NH<sub>4</sub><sup>+</sup></b>	ammonium ion
<b>NH<sub>4</sub>Cl</b>	ammonium chloride
<b>NH<sub>4</sub>NO<sub>3</sub></b>	ammonium nitrate
<b>NHA</b>	sodium/hydrogen antiporter
<b>NHX</b>	sodium/hydrogen exchanger
<b>nm</b>	nanometer(s)
<b>nM</b>	nanomolar
<b>NO<sub>3</sub><sup>-</sup></b>	nitrate
<b>Nos</b>	nopaline synthase
<b>nosT</b>	nopaline syntase terminator
<b>nptII</b>	neomycin phosphotransferase II protein
<b>NSCC</b>	non-selective cation channel
<b>OC</b>	outer cortex
<b>OCS term</b>	octopine synthase terminator
<b>OD<sub>600</sub></b>	optical density at 600 nm
<b>OEX</b>	overexpression
<b>P</b>	P media
<b>P</b>	phosphorus
<b>P/B</b>	peak/background
<b>P35S</b>	CaMV 35S promoter
<b>pA35S</b>	CaMV 35S promoter
<b>palcA</b>	alcA promoter
<b>pAnos</b>	nopaline synthase promoter
<b>Pat (basta)</b>	basta resistance gene
<b>pBR322 bom</b>	pBR322 basis of mobility
<b>pBR322 ori</b>	pBR322 origin of replication
<b>PCR</b>	polymerase chain reaction
<b>PDK</b>	pyruvate dehydrogenase kinase intron
<b>pH</b>	per hydrogen
<b><i>PmeI</i></b>	<i>PmeI</i> restriction enzyme
<b>Pnos</b>	nopaline synthase promoter
<b>pOp</b>	pOp artificial promoter

<b>PpENA1</b>	<i>Physcomitrella patens</i> ENA
<b>PP<sub>i</sub></b>	pyrophosphate
<b>PR</b>	pericycle
<b>PR-1a</b>	PR-1a promoter
<b>PR-AG</b>	PR-AG medium
<b>pVS1 rep</b>	pVS1 replication function
<b>pVS1 sta</b>	pVS1 stability function
<b>Q-PCR</b>	quantitative PCR
<b>QTL</b>	quantitative trait locus
<b>R<sup>2</sup></b>	coefficient of determination
<b>R2-CL</b>	R2-CL medium
<b>R2-CS</b>	R2-CS medium
<b>R2-S</b>	R2-S medium
<b>RB</b>	right border sequence
<b>RCD</b>	radical-induced cell death
<b>RFP</b>	red fluorescent protein
<b>RK2 ori</b>	RK2 origin of replication
<b>RN</b>	RN medium
<b>RNA</b>	ribonucleic acid
<b>RNAi</b>	RNA interference
<b>RO</b>	reverse osmosis
<b>ROS</b>	reactive oxygen species
<b>rpm</b>	revolutions per minute
<b>RT</b>	room temperature
<b>s</b>	second(s)
<b><i>SacI</i></b>	<i>SacI</i> restriction enzyme
<b>SARDI</b>	South Australian Research and Development Initiative
<b>SAS</b>	sodium overaccumulation in shoots
<b>SCABP</b>	SOS3-like calcium-binding proteins
<b>SDS</b>	sodium dodecyl sulfate
<b>SE</b>	standard error
<b>siRNA</b>	short interfering RNA
<b>SKC</b>	small conductance calcium-activated potassium channel
<b>SKOR</b>	stelar potassium outwardly rectifying channel
<b>SOS</b>	salt overly sensitive
<b>Spec prom</b>	spectinomycin promoter
<b>spec</b>	spectinomycin
<b>Spectinomycin R</b>	spectinomycin resistance gene
<b>t</b>	tonne(s)
<b>T<sub>0</sub></b>	T <sub>0</sub> generation
<b>T<sub>1</sub></b>	T <sub>1</sub> generation
<b>T<sub>2</sub></b>	T <sub>2</sub> generation
<b>T<sub>3</sub></b>	T <sub>3</sub> generation
<b>TATA</b>	TATA box DNA sequence
<b>T-Border (left)</b>	left border sequence
<b>T-Border (right)</b>	right border sequence

<b>T-DNA</b>	transferred DNA
<b>TetR</b>	tetracycline repressor gene
<b>TGV</b>	TGV transcriptional activator
<b>TIGR</b>	The Institute for Genomic Research
<b>T<sub>m</sub></b>	melting temperature
<b>Tris-EDTA</b>	tris(hydroxymethyl)aminomethane-EDTA
<b>tRNA</b>	transfer RNA
<b>TYNG</b>	TYNG medium
<b>UAS</b>	upstream activation sequence
<b>uidA</b>	β-glucuronidase enzyme
<b>UN</b>	United Nations
<b>US</b>	United States
<b>USSL</b>	United States Salinity Lab
<b>UTR</b>	untranslated region
<b>UV</b>	ultraviolet
<b>VP16</b>	herpes simplex virus transcriptional activator
<b>WT</b>	wild type
<b>XP</b>	xylem parenchyma
<b>XRMA</b>	x-ray microanalysis
<b>XVE</b>	XVE chimeric transcription activator
<b>YEB</b>	youngest fully emerged blade
<b>Zn</b>	zinc
<b>ZnSO<sub>4</sub>*7H<sub>2</sub>O</b>	zinc sulfate