Modifying sodium transport to improve salinity tolerance of commercial rice cultivars (Oryza sativa L.)

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List of Abbreviations

%	percent
~	approximately
×	times
°C	degrees Celsius
3′	three prime, of nucleic acid sequence
35S	promoter of cauliflower mosaic virus 35S
5′	five prime, of nucleic acid sequence number
ABA	abscisic acid
ACPFG	Australian Centre for Plant Functional Genomics
AGRF	Australian Genome Research Facility
ANOVA	analysis of variance
At	*Arabidopsis thaliana*
AtAVP	Arabidopsis vacuolar pyrophosphatase
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
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. No	catalogue number
CBL	calcineurin like-B protein
cDNA	complimentary deoxyribonucleic acid
Chu N6	basal salt mixture Chu N6
CIPK	calcineurin like-B interacting protein kinase
Cl^-	chloride ion
cm	centimetre(s)
Col-0	*Arabidopsis* ecotype Columbia-0
d	day(s)
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
dGTP	deoxyguanosine triphosphate
dH_20	deionised water
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
dS	deciSiemens
ECe	electrical conductivity
EDTA	ethylenediaminetetraacetic acid
FAO	Food and Agricultural Organization of the United Nations
g	gram(s)
g	gravity
gDNA	genomic deoxyribonucleic acid
GFP	green fluorescent protein
GRiSP	Global Rice Science Partnership
GUS	β-glucuronidase protein
h	hour(s)

H^+	hydrogen ion
H_20	water
HCl	hydrochloric acid
HKT	high affinity potassium transport
ICRR	Indonesian Centre for Rice Research
IRRI	International Rice Research Institute
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
M	molar
mg	milligram(s)
Mg^{2+}	magnesium ion
$MgCl_2$	magnesium chloride
min	minute(s)
mL	millilitre(s)
mm	millimetre(s)
mM	millimolar
mol	mole
mRNA	messenger ribonucleic acid
MS	Murashige and Skoog media
n	sample size
N_2	nitrogen
NA	not available
Na^+	sodium ion
NaCl	sodium chloride
NaOH	sodium hydroxide
NCBI	National Center for Biotechnology Information
ng	nanogram(s)
NHX	Na^+/H^+ exchanger
NO_3^-	nitrate
Nos	bacterial nopaline synthase terminator sequence
OD_{600}	optical density measured at 600 nm
Os	*Oryza sativa*
OsOVP	rice vacuolar pyrophosphatase
PBS	phosphate buffered saline
PCR	polymerase chain reaction
Pi	phosphate
PPase	pyrohosphatase
PpENA	Na^+ pumping ATPase from *Physcomitrella patens*
PPi	pyrophosphate
qRT-PCR	quantitative reverse transcription polymerase chain reaction
QTL	quantitative trait loci
RB	right border, of T-DNA sequence
RIL	recombinant inbred line
RNA	ribonucleic acid
RO	reverse osmosis
RT	room temperature

RT-PCR	reverse transcription polymerase chain reaction
sec	second(s)
SOS	salt overly sensitive
SSC	saline sodium citrate
T_0	primary rice transformant containing T-DNA
T_1	progeny of T_0 plant
T_2	progeny of T_1
TAE	tris-acetate-EDTA
T-DNA	transfer deoxyribonucleic acid
Tm	melting temperature, of primers
U	units
UAS	upstream activation sequence
Ubi	promoter of maize *Ubiquitin-1*
UC mix	University California soil mix
UidA	β-glucuronidase gene
UV	ultraviolet
V	voltage
v/v	volume per volume
w/v	weight per volume
WT	wild type
X-Gluc	5-bromo-4-chloro-3-indoyl-glucuronide
µL	microlitre(s)
µm	micrometre
µM	micromolar
µmol	micromole(s)

Abstract

Salinity tolerance in rice is negatively correlated with sodium accumulation in the shoot. Therefore, one approach to improve rice salinity tolerance is through the modification of sodium transport pathways within the plant, either by constitutive or cell type-specific expression of genes encoding proteins important for sodium homeostasis. In rice, work so far has predominately been limited to poorly adapted cultivars or has used technologies incompatible with future breeding programs. It is therefore important to transfer the knowledge obtained from the modification of Na^+ transport processes in other plants and test the validity of this approach in commercially relevant rice cultivars, using compatible technologies for further application of the approach in the field.

Five candidate commercial rice cultivars were selected from Indonesia. The salt tolerance of these rice cultivars were studied in hydroponics. Variation existed in the salinity tolerance mechanisms among the rice cultivars, offering the potential to use different approaches for improving salinity tolerance. *Agrobacterium*-mediated transformation efficiency of the cultivars was evaluated using calli derived from the scutellum of mature seeds. The study revealed only two cultivars, Fatmawati and IR64, could regenerate transgenics.

A non-destructive image based phenotyping protocol was developed for screening rice undergoing salt stress and was used to further examine the salinity tolerance of Fatmawati and IR64. The two cultivars showed differences in both their salinity tolerance and in the salinity tolerance mechanisms they used. Due to the differences in their salinity tolerance and due to their amenability for *Agrobacterium*-mediated transformation, Fatmawati and IR64 were selected for transformation with salinity tolerance genes using constitutive and cell type-specific promoters.

The maize *Ubiquitin-1* and cauliflower mosaic virus *35S* promoters were used as constitutive promoters. Cell type-specific promoters were identified from either the literature or rice databases and used to drive the genes in specific cells in the root. The cell type-specific alterations are targeted to minimize net sodium influx into the root from the soil, maximise sodium retrieval from the xylem, or increase sodium compartmentalization in the root tissue.

Rice lines were generated which constitutively expressed the genes encoding the vacuolar H^+-pyrophosphatases *AtAVP1* and *OsOVP4* and the protein kinase *AtCIPK16.* Transgenic rice lines were also developed which expressed Na^+ transporter *OsHKT1;5* driven by a stelar specific promoter and Na^+ pumping ATPase from *Physcomitrella patens* (*PpENA1)* driven by an epidermal specific promoter. The salinity tolerance of the transgenic rice lines was characterized in the T_1 generation using either non-destructive image based phenotyping or destructive analysis in hydroponic experiments. Results from this study showed that constitutive expression of *AtAVP1* lead to increased biomass of transgenic rice both under salt stress and non-stress conditions. The present study demonstrated the expression of *OsHKT1;5* in the root stele reduced shoot Na^+ accumulation, while the expression of *PpENA1* in the root epidermis reduced root Na^+ concentration. However, the effect of the alteration on the whole plant salinity tolerance of the transgenic rice still requires further characterization. Further assessment of these transgenic lines in later generations is necessary.

Declaration

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*Hairmansis A, Berger B, Tester M, Roy SJ. 2014. Image-based phenotyping for non-destructive screening of different salinity tolerance traits in rice. Manuscript submitted to Rice journal

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