

Evaluating the abiotic stress tolerance of transgenic barley
expressing an *Arabidopsis* vacuolar proton-pumping
pyrophosphatase gene (AVP1)

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List of Abbreviations

3'	three prime, of nucleic acid sequence
5'	five prime, of nucleic acid sequence
~	approximately
°C	degrees Celsius
Ψ_m	matric potential
(-)	negative control (water)
ACPFPG	Australian Centre for Plant Functional Genomics
ANOVA	analysis of variance
AVP1	type- I <i>Arabidopsis</i> vacuolar H ⁺ -pyrophosphatase
AVP1D	gain-of-function <i>AVP1</i> allele
bp	base pairs, of nucleic acid
bv	between vein
CaCl ₂	calcium chloride
CaMV	cauliflower mosaic virus
Ca-P	calcium phosphate
cDNA	complementary deoxyribonucleic acid
Cl ⁻	chloride ion
cm	centimetre(s)
cv.	cultivar
d	day(s)
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
dS	deciSiemens
DHA	dehydroascorbic acid
dpi	dots per inch
DW	dry weight
EC _{1:5}	electrical conductivity of 1:5 (soil:water) extract
EC _a	apparent soil electrical conductivity
EM	electromagnetic
FAO	Food and Agricultural Organization of the United Nations
FW	fresh weight
g	gram(s)
gDNA	genomic deoxyribonucleic acid
GM	genetically modified
GRDC	Grains Research and Development Corporation
GUS	β -glucuronidase protein
h	hour(s)
H ⁺	hydrogen ion
H ⁺ -PPase	proton-pumping pyrophosphatase
H ⁺ -ATPase	proton-pumping adenosine 5'-triphosphatase
ha	hectare
H ₂ O	water
HCl	hydrochloric acid
ICP-OES	Inductive Couple Plasma Optical Emission Spectrometry
K ⁺	potassium ion
kg	kilogram(s)

kPa	kiloPascal(s)
L	litre(s)
M	molar
mg	milligram(s)
Mg ²⁺	magnesium ion
min	minute(s)
mL	millilitre(s)
mm	millimetre(s)
mM	milliMolar
MPa	megaPascal(s)
n	sample size
Na ⁺	sodium ion
NaCl	sodium chloride
NO ₃ ⁻	nitrate
nulls	null segregants
P	phosphorus
PCR	polymerase chain reaction
pH	power of hydrogen
P _i	orthophosphate
PM	plasma membrane
PO ₄ ³⁻	phosphate
PP _i	inorganic pyrophosphate
ppm	parts per million
RNA	ribonucleic acid
RO	reverse osmosis
RT-PCR	reverse transcription polymerase chain reaction
SA	South Australia
s	seconds
SWP	soil water potential
T ₁	1 st progeny of primary transformant
T ₂ to T ₅	2 nd , 3 rd , 4 th and 5 th progeny of T ₁ plant
<i>uidA</i>	β-glucuronidase gene
UV	ultraviolet light
VRT	vernalisation
v/v	volume per volume
WA	Western Australia
WABC	Western Australia Biogeochemistry Centre
WAS	Waite Analytical Services
wk	week(s)
WHC	water holding capacity
WT	wild-type
w/v	weight per volume
X-Gluc	5-bromo-4-chloro-3-indoyl-glucuronide
µg	microgram(s)
µL	microlitre(s)
µm	micrometre(s)
µM	micromolar
µmol	micromole(s)
µS	microSiemens

Abstract

Commercially relevant barley varieties with improved abiotic stress tolerance are needed to increase crop productivity. Previously, transgenic barley with constitutive *CaMV 35S* expression of *AVP1*, a gene encoding the type I *Arabidopsis* vacuolar proton-pumping pyrophosphatase (H⁺-PPase), had a larger shoot biomass in non-saline and saline conditions compared to null segregants. However, the growth and grain yield of the transgenic *AVP1* barley was yet to be evaluated in a saline field. It was also yet to be investigated whether the larger shoot biomass of transgenic *AVP1* barley in both non-saline and saline conditions arose from a change in tissue solute accumulation, water use, plant nutrition, carbohydrate metabolism, heterotrophic growth or a combination of these traits. In addition, for this *AVP1* technology to be applicable for barley grain growers, a commercially relevant transgenic *AVP1* barley cultivar with well-regulated control of *AVP1* expression was needed.

The first focus of this project evaluated the growth and grain yield of *35S:AVP1* barley (cv. Golden Promise) in a low and high salinity field near Kunjin, Western Australia. Field trial results validated greenhouse-based findings of improved shoot biomass in transgenic *AVP1* barley compared to wild-type. Furthermore, results demonstrated for the first time that transgenic *AVP1* barley had increased grain yield per plant compared to wild-type in a field with high salinity. These findings suggest that transgenic *AVP1* barley is a promising option to help increase the grain yield of cereal crops in a saline field.

The second focus of this project investigated the abiotic stress tolerance and potential factors contributing to the larger shoot biomass of *35S:AVP1* barley. At low phosphorus (P) supply, *35S:AVP1* barley had a larger shoot biomass, greater root P uptake and increased rhizosphere acidification compared to wild-type. At low nitrate (NO₃⁻) supply, two *35S:AVP1* barley lines had increased shoot biomass but with no difference in NO₃⁻ uptake capacity compared to null segregants. The shoot biomass of *35S:AVP1* barley was also increased compared to null segregants under low water availability and low water availability concurrent with salinity. Furthermore, an increase in plant biomass from 6 days after seed imbibition, thus seedling vigour, was detectable in *35S:AVP1* barley compared to null segregants. Leaf metabolites involved in ascorbic acid synthesis were also significantly altered in the *35S:AVP1* barley compared to null segregants. Collectively, these findings suggest that a combination of traits is contributing to the improved growth of transgenic *AVP1* barley.

The third focus of this project evaluated the salt stress inducibility of the *ZmRab17* promoter and investigated the salinity tolerance of commercially relevant barley (cv. WI4330) expressing *AVP1* via the *ZmRab17* and the constitutive *ZmUbi1* promoter. The *ZmRab17* promoter was salt-stress inducible in barley root stelar cells with basal transgene expression in non-saline conditions. However, the shoot and root biomass of *ZmRab17:AVP1* and *ZmUbi1:AVP1* barley did not differ to wild-type and null segregants in saline conditions. These findings suggest that the type of promoter driving *AVP1* expression in transgenic barley is an important factor.

Declaration

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 to Rhiannon Kate Schilling 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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List of Publications

Research Articles

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List of Awards

The following prizes were awarded to Rhiannon K. Schilling during her PhD candidature:

The AgPOGS Prize (2012)

Awarded for the best oral presentation by audience choice at the University of Adelaide, School of Agriculture, Food and Wine Postgraduate Symposium by the Waite Agriculture Postgraduate Society.

The K.P. Barley Prize (2012)

Awarded by the Faculty of Sciences to a postgraduate student within the School of Agriculture, Food & Wine or the School of Earth & Environmental Sciences at The University of Adelaide on the basis of academic merit and research performance.

The Max Tate Prize (2012)

Awarded for the best oral presentation at the University of Adelaide, School of Agriculture, Food and Wine Postgraduate Symposium.

The Royal Society of South Australia Postgraduate Student Prize (2nd)

Awarded runner-up for the best oral presentation by a South Australian postgraduate student delivered at the Royal Society of South Australia meeting in 2012.

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