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

Rhiannon Kate Schilling

B. Sc. Ag. Sc. (Hons)

A thesis submitted for the degree of
Doctor of Philosophy

Faculty of Sciences
School of Agriculture, Food & Wine
The University of Adelaide


June 2014

# Table of Contents

# List of Figures

# List of Tables

# List of Abbreviations

| | |
|---|---|
| 3’ | three prime, of nucleic acid sequence |
| 5’ | five prime, of nucleic acid sequence |
| ~ | approximately |
| ºC | degrees Celsius |
| $\Psi_m$ | matric potential |
| (-) | negative control (water) |
| ACPFG | Australian Centre for Plant Functional Genomics |
| ANOVA | analysis of variance |
| AVP1 | type- I *Arabidopsis* vacuolar $H^+$-pyrophosphatase |
| AVP1D | gain-of-function *AVP1* 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 |
| $H_2O$ | 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 |
| $T_1$ | 1st progeny of primary transformant |
| $T_2$ to $T_5$ | 2nd, 3rd, 4th and 5th progeny of $T_1$ plant |
| *uidA* | *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 ($NO_3^-$) supply, two *35S*:*AVP1* barley lines had increased shoot biomass but with no difference in $NO_3^-$ 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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*Schilling, R. K., Marschner, P., Shavrukov, Y., Berger, B., Tester, M., Roy, S.J., and Plett, D.C. (2014). "Expression of the *Arabidopsis* vacuolar $H^+$-pyrophosphatase gene (*AVP1*) improves the shoot biomass of transgenic barley and increases grain yield in a saline field" Plant Biotechnology Journal **12**(3): 378-386.

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R. K. Schilling

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Date

# List of Publications

### Research Articles

Schilling, R. K., Marschner, P., Shavrukov, Y., Berger, B., Tester, M., Roy, S.J., and Plett, D.C. (2014). "Expression of the *Arabidopsis* vacuolar $H^+$-pyrophosphatase gene (*AVP1*) improves the shoot biomass of transgenic barley and increases grain yield in a saline field" Plant Biotechnology Journal **12**(3): 378-386. (5-year IF: 6.247)

# 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.

# Acknowledgements

I would like to acknowledge and thank my PhD supervisors: Dr. Stuart Roy, Dr. Darren Plett, Prof. Petra Marschner and Prof. Mark Tester. I am fortunate to have had the opportunity to work with you all and greatly appreciate all your input into this project. You were all excellent supervisors who helped to guide me in the right direction, whilst giving me the freedom to research the questions that were of interest to me. Thank you all for the helpful advice and for always encouraging me to achieve my best.

I would also like to thank my independent advisor, Dr. Julie Hayes, for providing valuable input into my experimental designs and for helping to ensure my PhD project had tangible outcomes.

I am also grateful to the many people who have helped me during my PhD experiments. At the end of each chapter, I have included an acknowledgments section to thank those who have contributed to specific aspects of my PhD. I would also like to thank Kalyx Australia (Perth, WA), particularly Dr. Peter Carlton, Mrs. Caris Smith and Mr. Peter Burgess, for their assistance in conducting the GM field trials near Kunjin, WA. I am also grateful for the time and effort of Mrs. Jan Nield who completed the necessary reporting procedures to ensure the GM field trials in this project were compliant to all OGTR licence conditions; Dr. Andrew Jacobs for his help in organising the logistics of GM field trials; and the ACPFG administration team for their help in organising my frequent travel across to WA. I would also like to thank The Plant Accelerator team for their assistance during the hire of a PC2 greenhouse and growth chamber. I would like to acknowledge the financial support of a Grains Industry Research Scholarship from the Grains Research and Development Corporation (GRDC) and an Australian Postgraduate Award (APA). In addition, I would like to thank the Australian Centre for Plant Functional Genomics (ACPFG) for providing the resources necessary to undertake my PhD at The University of Adelaide, Waite campus.

To all the lab members of the ACPFG Salt Focus Group both past and present, particularly Dr. Monique Shearer, Mrs. Jessica Bovill, Dr. Aurelie Evard, Dr. Bo Li, Dr. Sandra Schmöckel, Mr. Gordon Wellman, Dr. Nawar Shayma, Dr. Joanne Tillbrook, Ms. Wenmian Huang, Ms. Jiaen Qiu, Dr. Aris Hairmansis, Ms. Melissa Pickering and Ms. Jodie Kretschmer, thank you all for helping to make daily life as a PhD student enjoyable. To all the lab members both past and present of the Soil Biology Group at the University of Adelaide thank you also for your support and friendship throughout my PhD.

Finally, to all my family and friends, particularly my parents Nigel and Wendy, thank you for your endless support and for always encouraging me to follow my interests.