



THE UNIVERSITY
of ADELAIDE

Direct and indirect influences of water deficit on salt uptake, ion accumulation
and root-shoot interactions of grapevines.

By

Kerry Anne DeGaris

Thesis submitted to School of Agriculture, Food and Wine of the University of
Adelaide in fulfilment of the requirements for the degree of

Doctor of Philosophy

Copyright © Kerry Anne DeGaris 2015

Direct and indirect influences of water deficit on salt uptake, ion accumulation and root-shoot interactions of grapevines.

By:

Kerry Anne DeGaris

Supervised by:

Professor Stephen Tyerman
Head, Plant Physiology, Viticulture and Horticulture Research Group
The University of Adelaide

Dr. Rob Walker
Chief Research Scientist,
CSIRO Agriculture flagship

Dr. Brian Loveys
Retired
Formerly CSIRO Agriculture flagship

Thesis submitted in fulfillment of the requirements for the degree of
Doctorate of Philosophy

School of Agriculture, Food and Wine
Faculty of Science
The University of Adelaide
Waite Research Institute, Glen Osmond, SA 5064
Email: Kerry.degaris@adelaide.edu.au

Copyright © Kerry DeGaris 2015

Contents

List of Tables and Figures (excluding journal articles).....	iii
Abstract	v
Declaration.....	vii
Journal of Papers Published as part of this Research:	viii
Acknowledgements	ix
Conference Proceedings & Industry Publications	x
Abbreviations.....	xi
Chapter 1 : Literature Review	11
1.1 Introduction.....	11
1.2 Response of grapevines to salinity and water stress	12
1.2.1 Osmotic effect (non-specific).....	14
1.2.2 Toxic effect (non-specific).....	15
1.3 Effect on grapevine productivity	15
1.3.1 Salinity.....	15
1.3.2 Water Stress	16
1.4 Effect on yield	16
1.4.1 Salinity.....	16
1.4.2 Water Stress	17
1.5 Effect on mineral uptake.....	17
1.5.1 Salinity.....	17
1.5.2 Water stress.....	18
1.6 Effect of salinity on juice and wine Cl ⁻ and Na ⁺ concentrations	18
1.7 Movement of Na ⁺ and Cl ⁻ ions.....	19
1.7.1 Sodium transport	19
1.7.2 Chloride Transport	20
1.8 Root to shoot signaling.....	21
1.8.1 Hydraulic Signals	22
1.8.2 Chemical signals	23
1.8.2.1 Salinity	24
1.8.2.2 Water Stress.....	24
1.9. Forms of deficit irrigation	25
1.10 Combined effect of salinity and deficit irrigation	26

1.11 Summary	29
1.12 Objectives of the Research & linking statement	29
References.....	31
Chapter 2. Published Article: Impact of deficit irrigation strategies in a saline environment on Shiraz yield, physiology, water use and tissue ion concentration.....	54
Chapter 3. Accepted Article: Comparative effects of deficit and partial root-zone drying irrigation techniques using moderately saline water on ion partitioning in Shiraz and Grenache grapevines.	67
Chapter 4. Submitted Article: Exogenous application of ABA to root systems of grapevines with or without salinity influences water relations and ion allocation.....	95
Chapter 5: Differential responses of PIP and TIP aquaporins and their role in altering root hydraulic conductance in Shiraz grapevines exposed to salt stress and exogenously applied ABA	141
Chapter 6. General Discussion.....	160

List of Tables and Figures (excluding journal articles)

Chapter 1

Tables:

Table 1. Plant response to salinity at different time scales.

Table 2. Summary of experiments demonstrating the physiological effects from using a PRD irrigation technique.

Figures:

Figure 1. Signaling mechanisms triggered in the presence of salinity and/or water stress

Figure 2. Agriculturally important stress combinations demonstrating potential interactions that can have important implications for agriculture.

Chapter 5

Tables:

Table 1. Accession numbers of aquaporin genes and sequences of primer pairs used for quantitative PCR

Table 2. Pearsons correlation coefficients between aquaporin expression and hydraulic conductivity (L_o), transpiration (E), stomatal conductance (g_s) and leaf water potential (Ψ_l)

Figures:

Figure 1. The effect of ABA in the presence and absence of 75 mM Cl^- on root hydraulic conductance (L_o) at termination of experiment (day 15). Each bar represents the average of 6 plants. Each error bar is SEM. Different letters above each bar indicate a significant difference between treatments ($P < 0.001$).

Figure 2. Relationship between leaf transpiration rate (E) (a), stomatal conductance (g_s) (b), leaf water potential (Ψ_{leaf}) (c) and root hydraulic conductance normalized to root dry weight (L_o) after 15 days for Shiraz for Control (■), ABA 50 μM (▲), ABA 100 μM (▼), Salt 75mM (◆), ABA 50 μM + salt (○) and ABA 100 μM + salt (□). Values are means, $n=6$. Regression equations: (a) Control: $y=2.81*x-2.65$, combined treatments: $y=4.90*x-0.40$ (b) Control: $y=48.3*x-0.62$, combined treatments: $y=146.9*x+0.52$ (c) Salt: $y=11.88*x+15.90$, combined treatments: $6.39*x+10.87$

Figure 3. Relative gene expression of aquaporins *Vv PIP1;1* (a), *VvPIP2;1* (b), *VvPIP2;2* (c), *VvPIP2;3* (d), *VvPIP2;4* (e), *VvTIP1;1* (f) and *VvTIP2;1* (g) in the roots of Shiraz at the completion of the experiment. Relative gene expression is the ratio of the starting quantity of the target gene and the starting ratio of the reference gene, normalised to the mean of the control sample group. Columns with different letters indicate significant differences ($P < 0.001$), $n=30$. Each error bar is SEM

Figure 4. Relative gene expression of (a) *VvPIP 1;1*, (b) *VvTIP 1;1* and (c) *VvPIP 2;3* in response to time of day after 15 days of applied treatments for Control (■), ABA 50 μM (▲), ABA 100 μM (▼), Salt 75 mM (◆), ABA 50 μM + salt (○) and ABA 100 μM + salt (□). n=5.

Figure 5. Regression plot for the prediction of L_o using *VvPIP2;3* expression and LWP. The regression equation used: $L_o = 8.921 \times 10^{-6} + 1.14 \times 10^{-7} \text{ PIP2;3} + 5.716 \times 10^{-6} \times \text{LWP}$.

Abstract

The area affected by salinity in Australian grape production regions is increasing, predominantly due to reliance in some regions on poorer quality water for irrigation and to changes in rainfall patterns resulting in reduced leaching of soil borne salts. Combined with an increased requirement to improve water use efficiency the implementation of deficit irrigation techniques has become common practice. The aim of this research was to assess the effect of saline irrigation water and deficit irrigation techniques on the performance of own-rooted grapevines as well as test the hypothesis that PRD reduces the salt transport to the shoot.

A field experiment was established in Padthaway on own-rooted Shiraz vines in seasons 2009-2011. Three irrigation treatments were applied using moderately saline irrigation water (2.3dS/m): control (1.0-2.3ML/ha), reduced control (RC) and partial rootzone drying (PRD) (both approximately 50% of control). This study found that grape juice Cl^- and Na^+ concentrations were not affected significantly by irrigation treatment. Seasonal variation in rainfall and total irrigation applied had a greater effect on altering grape juice Cl^- and Na^+ concentrations than the application of irrigation water with the same moderate salinity but with the different irrigation treatments.

A pot trial was established to replicate the treatments mentioned above in conjunction with slightly increased saline irrigation water (2.46dS/m) from the field trial for the 2011-2012. At the end of the second year the vines were destructively harvested and growth and ion concentrations for different vine organs assessed. PRD was found to have higher concentrations of Cl^- , Na^+ , K^+ and Ca^{++} present on a whole vine basis. Although Cl^- concentration was elevated in leaves for PRD, it was partitioned away from the leaves on a total content basis relative to both control and RC. This research highlighted that ion partitioning within grapevines will depend on the type of deficit applied and that the higher total root dry weights observed in the PRD treatment could possibly be responsible for the higher whole plant concentrations of Cl^- , Na^+ , K^+ and Ca^{++} that were observed.

To gain a better understanding of the role Abscisic acid (ABA) plays in modulating the effect of salinity a glasshouse study was undertaken in 2012-2014. The aim was to evaluate the effect of exogenously applied ABA to grapevine root systems, with or without saline irrigation water, on water relations, ion allocation, root hydraulic conductance normalized to root dry weight (L_o) and aquaporin expression. Exogenously applied ABA was found to increase L_o and decrease water use in ABA-only treatments, while in the presence of excess Cl^- salts, it also reduced Cl^- transport to the shoot. This reduction could not be accounted for by reduced transpiration. Strong positive correlations were observed between L_o and E and L_o and g_s with a slope of the relationships increasing with both ABA and salt treatments.

Aquaporin gene expression was not significantly different between treatments an interesting finding that warrants further investigation. However in a linear combination with leaf water potential, the expression of one aquaporin gene *VvPIP2;3*, could explain more than 50% of the variation in L_o independent of the salt and ABA treatments. The expression of the tonoplast aquaporin *VvTIP1;1* was also correlated to the expression of *Vv PIP2;1*.

This study has led to a greater understanding of the implications for growers when irrigating with moderately saline irrigation water in conjunction with some form of deficit irrigation technique. Although the initial hypothesis was negated in both the field and pot trial with Cl^- concentrations in the shoot remaining similar to the control, the glasshouse study proved that ABA has the ability to reduce salt transport to the shoots independently of its effects on stomatal conductance and root water transport. Further research to probe the mechanism of the effect of ABA on Cl^- transport will require the membrane transporters responsible for Cl^- transport to be identified and their possible transcriptional and post-translational control by ABA determined.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, 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. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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.

The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

14/10/2015

Kerry Anne DeGaris

Date

Journal of Papers Published as part of this Research:

Presented in Chapter 2:

DeGaris, K.A., Walker, R.R., Loveys, B.R. and Tyerman, S.D. (2015)

Impact of deficit irrigation strategies on Shiraz yield, physiology, water use and tissue ion concentration in a saline environment.

Australian Journal of Grape and Wine Research Vol **21** (3): 468-478

Presented in Chapter 3:

DeGaris, K.A., Walker, R.R., Loveys, B.R. and Tyerman, S.D. (2015)

Comparative effects of deficit and partial root-zone drying irrigation techniques using moderately saline water on ion partitioning in Shiraz and Grenache grapevines.

Australian Journal of Grape and Wine Research (Accepted)

Presented in Chapter 4:

DeGaris, K.A., Walker, R.R., Loveys, B.R. and Tyerman, S.D. (2015)

Exogenous application of ABA to root systems of grapevines with or without salinity influences water relations and ion allocation.

Australian Journal of Grape and Wine Research (Submitted)

Each of these manuscripts is displayed in the thesis in either published or submitted for according to the instructions to author of the specific journal

This thesis has been prepared according to the University of Adelaide's specifications for 'PhD by publications' format

Acknowledgements

I would like to thank the patience of my supervisors Steve, Rob and Brian who have supported me on this very long journey! Thanks to Wendy Sullivan and Deidre Blackmore for their help and support during the many hours of measurements and analysis in both the glasshouse and laboratory. Additionally the assistance of Kara Levin and Rebecca Vandeleur who assisted with the QPCR measurements as part of the results in Chapter 5 of this thesis. To the staff at Accolade wines in particular Sarah Millard who helped with trial set up and measurements as well as the company for allowing me to use their vineyards and staff to complete various tasks. I gratefully acknowledge the funding of the project by Wine Australia (previously Grape and Wine Research and Development Corporation) and ARC in Plant Energy Biology.

To my friend Campbel Giles, who edited my first paper and reignited a friendship during this 7 year PhD process. Unfortunately she was unable to see the completion of my thesis before passing away in February of 2015.

Thanks to Mum and Dad who have supported me throughout this entire process, without their guidance and help it would not have been possible. Words cannot say how much of this PhD is due to your love and support.

I would also like to thank Suzanne McLoughlin for her friendship and advice as well as providing me with somewhere to live on the many trips to Adelaide over the past 5 years.

There are really too many people to thank individually over this PhD/thesis writing process, but I want to thank the many people who have contributed no matter how small – those people will know who they are.

Conference Proceedings & Industry Publications

DeGaris, K.A., Tyerman, S.D., Walker, R. R, & Loveys, B. (2010)

The effect of water deficit and salinity on root-shoot interactions in grapevines

In poster proceedings 14th Australian Wine Industry Technical Conference, 3-8th July, Adelaide, Australia

DeGaris, K.A., Tyerman, S.D., Walker, R. R, & Loveys, B. (2013)

Irrigation strategies can change the allocation of chloride in Shiraz grapevines subjected to saline irrigation

In poster proceedings 15th Australian Wine Industry Technical Conference, 13-18th July, Sydney, Australia

DeGaris, K.A., Tyerman, S.D., Walker, R. R, & Loveys, B. (2013)

Chloride and sodium levels present in grape juice and leaf laminae are influenced by seasonal rainfall and irrigation applied.

In poster proceedings 15th Australian Wine Industry Technical Conference, 13-18th July, Sydney, Australia

DeGaris, K.A. (2015)

Salinity Management Strategies

ASVO Seminar – ‘Vineyard longevity – maintaining the asset’, July 22-23, Mildura, Australia.

Abbreviations

Ψ_l	Leaf Water Potential
Ψ_m	Midday leaf water potential
Ψ_{pd}	Pre-dawn leaf water potential
A	Assimilation
ABA	Absciscic Acid
ANOVA	Analysis of Variance
Ca ⁺⁺	Calcium
Cl ⁻	Chloride (nominal Cl ⁻ , ³⁵ Cl ⁻)
DI	Deficit Irrigation
dS	Deci-seimen
E	Transpiration
EC	Electrical Conductivity
EC _e	Electrical conductivity, saturated paste
ET _c	Crop evapotranspiration
FAO	Food and Agriculture Organisation
g _s	Stomatal Conductance
H ⁺	Hydrogen
Ha	Hectare
K ⁺	Potassium
L _o	Root hydraulic conductance normalized to root dry weight
Mg ⁺⁺	Magnesium
mg	milligram
N	Nitrogen
Na ⁺	Sodium
NaCl	Sodium Chloride
NO ₃ ⁻	Nitrate
PRD	Partial Rootzone Drying
RDI	Regulated Deficit Irrigation
VPD	Vapour pressure deficit