IDENTIFICATION OF ANION TRANSPORTERS THAT CONFER CHLORIDE EXCLUSION AND SALT TOLERANCE TO GRAPEVINE ROOTSTOCKS.

FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **GWT 1201**

Principal Investigator: **SAM HENDERSON**

Research Organisation: **THE UNIVERSITY OF ADELAIDE**

Date: **09/10/2012**

Contents

Abstract

Sam Henderson, a GWRDC funded PhD candidate at the University of Adelaide presented a research poster at the Gordon Research Conference Salt and Water Stress in Plants at the Chinese University of Hong Kong from June 24th-29th 2012. The cost of attending this conference was supported by a GWRDC travel grant. The purpose of the travel was to gain exposure to world class research in the field of plant salt stress with the aim of using this knowledge to better improve research into salt tolerance of grapevine back in Adelaide. Since returning to Adelaide this travel has assisted in the preparation of a manuscript. This report summarises the activities and key outcomes arising from this travel, in particular it summarises the conference presentations found most valuable to the author.

Executive Summary

- Sam Henderson is a PhD candidate at The University of Adelaide studying genes involved in salt tolerance of grapevine.

- The GWRDC supported Sam Henderson to attend the Gordon Research Conference Salt and Water Stress in Plants in Hong Kong, one of the leading conferences in the field of plant salt stress.

- The diverse group of conferees presented mostly unpublished work about plant abiotic stress tolerance mechanisms.

- By attending, valuable insight was gained from leading researchers in the field, which has assisted in the preparation of a scientific paper and will aid future manuscript preparation.

- Attendance has helped the author focus his research and improve his presentation skills which was demonstrated by Henderson being awarded an ASPS prize at Combio 2012.

- Australian audiences have heard about this research through local conferences, and further exposure was gained through an article in the GWRDC newsletter R&D@Work which was published in the December 2012 edition.

- Sam Henderson would like to thank the GWRDC for supporting this project.

Background

The possibility exists for greater future reliance on saline irrigation water for viticulture, especially with predicted future water shortages, water restrictions and climate change. Fortunately some rootstocks are available that confer salt tolerance to scions (Walker and Clingeleffer, 2009). To expedite future breeding efforts to incorporate salt tolerance and other desirable traits into a single rootstock, molecular markers for chloride exclusion are needed.

The chloride ion can accumulate to toxic levels in grapevine when irrigated with saline water (Shani and Ben-Gal, 2005; Stevens *et al.*, 2011). The GWRDC funded PhD project GWR Ph1001 "Identifying the mechanism of chloride exclusion in grapevines" has used molecular techniques to identify novel genes involved in chloride movement in grapevines. In particular this project has used microarray hybridisation to compare the transcriptome of rootstocks K51-40 and 140-Ruggeri, which are salt sensitive and tolerant respectively. Additionally, a number of putative chloride transporter genes have been cloned and functionally characterised from Cabernet Sauvignon.

To communicate these findings and gain insight from leading researchers in the field of plant salt tolerance, a poster was presented at the Gordon Research Conference (GRC) "Salt and Water Stress in Plants". GRCs are leading conferences in their fields, with informal and interactive sessions encouraging the sharing of ideas and development of collaborations. The GRC is a non-for-profit organisation that has been running conferences for over 75 years. Salt and Water Stress in Plants is held biennially therefore this represented a single opportunity to attend this high calibre conference during the timeframe of a PhD.

Project objectives and performance targets

The project had two key objectives:

1. To present research findings from project GWR Ph001 at the GRC "Salt and Water Stress in Plants", Hong Kong (24^{th}-29^{th} June 2012). The following poster was presented: "Identification of anion transporters that confer chloride exclusion and salt tolerance to grapevine rootstocks".

2. To generate collaboration with, and obtain feedback from, leading researchers in plant salt tolerance.

The planned output targets for this project are detailed in Table 1.

Table 1: Planned output targets for GWRDC project GWT 1201 in 2012

Output	Performance Targets	Date
1. Submit abstract to the proceedings of Salt and Water Stress in Plants 2012	Abstract accepted	April 2012
2. Presentation of data at Salt and Water Stress in Plants 2012	Present findings to peers at Salt and Water Stress in Plants via poster. Obtain feedback to aid further experimentation, manuscript writing, and data analysis.	June 2012
3. Dissemination of knowledge generated through PhD project to date.	Preparation of article by Lauren Jones for R&D@work on PhD project outcomes.	July 2012
4. Presentation of conference highlights to research group at University of Adelaide school of Agriculture, Food and Wine.	Oral presentation of conference highlights and findings to researchers at the fortnightly Viticultural Group Meeting at the University of Adelaide, Waite Campus.	August 2012
5. Dissemination of knowledge gained from the conference via GWRDC travel report and industry article.	Preparation of GWRDC final report. Preparation of short article suitable for publication in an industry technical journal overviewing conference highlights (if applicable to wine industry).	August 2012
6. Dissemination of knowledge gained, and additional data obtained as a result of travel, at Combio conference in Adelaide.	Presentation of research findings to Australian audience of broad scientific background via poster or seminar.	September 2012
7. Publication of findings regarding anion exclusion pathways in *Vitis vinifera* and grapevine rootstocks.	Prepare and submit manuscript to journal Plant Physiology, impact factor 7.016	December 2012

Gordon Research Conference "Salt and Water Stress in Plants" Hong Kong

From 24th to 29th of June 2012, Sam Henderson attended the GRC conference "Salt and Water Stress in Plants". This conference was held at the Chinese University of Hong Kong (CUHK), Hong Kong. The conference brought together 165 participants from diverse backgrounds including academia and industry (e.g. Syngenta, Bayer, BASF) from all parts of the world (Figure 1). Conference proceedings were not printed to encourage participants to present their latest unpublished work and this led to some fantastic posters and oral presentations.

Of particular interest and relevance to the author were presentations that highlighted the function of channels and transport proteins that facilitate the movement of anions across plant cell membranes.

Within this theme multiple presenters informed us about advances being made in the regulation of guard cell closure under abiotic stress. Rainer Hedrich (Germany) spoke about advances that his research group has made in understanding drought stress signalling in guard cells. It is well known that the phytohormone abscisic acid (ABA) is synthesised under stress conditions and induces stomatal closure which prevents water loss by transpiration. But only recently has this pathway been characterised. The mechanism involves anion efflux from guard cells by anion channels. These channels are regulated by phosphorylation by specific kinases. Shintaro Munemasa (USA) spoke about the reconstitution of this entire pathway in oocytes from the African clawed toad *Xenopus laevis*. This work was also presented as a poster by Benjamin Brandt (USA) which summarised these recently published findings (Brandt *et al.*, 2012). While this impressive body of work confirms the involvement of specific kinases in the regulation of guard cell anion channels, it is also interesting to see an advanced application of this expression system which is used routinely in the Adelaide laboratory at the Waite Campus for understanding ion movement.

An interesting phenomenon is that plant roots cells express anion channels belonging to the same family as those found in guard cells. These channels could be involved in root to shoot movement of chloride in plants under salt stress. Knowledge about the possible regulation

of this pathway is therefore highly valuable. Indeed a homolog of this channel from the salt tolerant rootstock 140-Ruggeri has been cloned in Adelaide. Therefore attending these presentations has provided valuable insight into the possible future direction of this work.

Another class of plant protein that is interesting for grapevine salt tolerance is the nitrate transporter family. Many plant nitrate transporters have been shown to also transport chloride, and therefore might have a role in rootstock tolerance to sodium chloride. A poster by Christelle Taochy (France) highlighted findings about a plant nitrate excretion transporter (NAXT). She showed that this transporter is found in plant roots, and also used some unique methods to characterise this protein such as expression in *Lactococcus lacti*. This presentation was valuable as our microarray data has indicated differential expression of many nitrate transporters, including a NAXT homolog, in root tissue between salt tolerant and salt sensitive grapevine rootstocks. Observing and discussing the many different ways to further understand this class of plant protein has provided many ideas about the further direction of the current project.

For a protein to facilitate the movement of toxic Cl^- in or out of a grapevine cell it must be located on the outer cell (plasma) membrane. For some grapevine proteins it has proven difficult to achieve this experimentally in Adelaide. Manuel Nieves-Cordones (France) presented his findings on an Arabidopsis potassium channel and the importance of the c-linker region for correct targeting to the plasma membrane of tobacco cells. This presentation provided helpful ideas for characterising grapevine membrane proteins.

Selective xylem loading of ions in the grapevine root is thought to be the key factor that determines whether a rootstock can restrict the movement of toxic Cl^- (or other ions) to the shoot. Sergey Shabala (Australia) presented “Xylem Loading and Salinity Tolerance in Plants: Strategies and Mechanisms”. This was highly informative talk that explained the electrochemical driving force for cation movement and how excessive NaCl can interfere with normal plant xylem loading.

Although the presentations mentioned above were conference highlights every presentation was excellent in its own right. I was privileged to be able to attend such a high quality scientific meeting and thank the GWRDC for supporting my attendance.

Figure 1: Attendees at GRC "salt and Water Stress in Plants" 2012 at the CUHK.

Communication to Australian audiences

Conferences

To ensure that the research findings were presented to Australian audiences (outputs 4 & 6, Table 1) the following seminars were presented upon returning from Hong Kong:

1. Postgraduate symposium 2012, School of Agriculture Food and Wine, University of Adelaide

This symposium is held annually in the School of Agriculture Food and Wine and highlights research undertaken by postgraduate students. In 2012, over 40 presentations were given. My talk received many positive responses from my peers and senior academics in the school. The quality of my presentation and the research it contained was improved by having attended the GRC.

2. Combio 2012, Adelaide

The annual Combio conference is a national conference held by the Australian Society for Biochemistry and Molecular Biology, and was held in Adelaide during September 2012. I was given the opportunity to present my research in the colloquium for plant science. I received much positive feedback about the research, some valuable comments that will aid publication, and received a prize from the Australian Society of Plant Scientists.

Journal Articles

Since returning from Hong Kong a manuscript has been prepared that describes findings from a gene expression study of salt stressed grapevine and two rootstocks. This manuscript is currently in the advanced stage of preparation, and will be submitted slightly later than originally anticipated for output 7 (Table 1). The GWRDC will be acknowledged in this paper.

Industry Articles

In collaboration with Lauren Jones, an article for the GWRDC publication R&D@work was prepared for the December 2012 issue (output 3, Table 1).

Conclusion

Sam Henderson from the University of Adelaide attended the GRC Salt and Water Stress in Plants in Hong Kong and was supported financially by GWRDC. The author gained valuable suggestions from leading plant researchers which has significantly aided the current research into salt tolerant, “chloride excluding” rootstocks with a manuscript prepared and a prize awarded for presentation at an Australian Conference. Attendance at GRC Salt and Water Stress in Plants will, in the coming twelve months, aid the preparation of additional manuscripts of interest to Australian grape growers, and also help to ensure the timely completion of the PhD project.

Acknowledgements

The activities detailed in this report were supported financially by Australia’s grape growers and wine makers through their investment body the Grape and Wine Research and Development Corporation (GWRDC), with matching funds from the Australian Government. Sam Henderson wishes to thank the GWRDC and the University of Adelaide for supporting this project.

Appendices

Poster presented at GRC Hong Kong:

The role of *VvNRT1.5* in chloride exclusion and salt tolerance of grapevine rootstocks

Sam Henderson[1], Mandy Walker[2], Rob Walker[2], Steve Tyerman[1], Matthew Gilliham[1]
1 The University of Adelaide, School of Agriculture, Food and Wine. 2 CSIRO Plant Industry, Adelaide

Background

Symptoms of grapevine salt stress include leaf burn, reduced yield and reduced vigour[1]. These effects are due to chloride (Cl^-) accumulation in leaves[2]. Cl^- in berries can also spoil wine flavour and aroma.

A *Vitis* spp. rootstock has been identified (140-Ruggeri) that has reduced xylem loading of Cl^- due to restricted efflux of Cl^- through anion transport proteins across xylem parenchyma cells, compared to *Vitis vinifera* and rootstock K51-40.

Reverse (gene characterisation) and forward genetics (microarray/sequencing) are being used to find genes that may confer this trait. Findings could be used to assist future rootstock breeding efforts.

Results

VvNRT1.5 clusters with members of the Arabidopsis Nitrate Transporter (NRT) family[3] (figure 1).

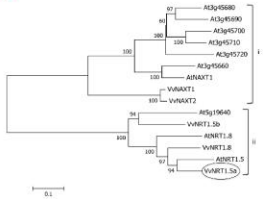

Figure 1: Identification of* Vitis vinifera *NRT1.5. *Vitis vinifera NRT amino acid sequences were identified by BlastP searches of Arabidopsis orthologues. Sequence alignment was performed by ClustalW2. Neighbour joining tree was constructed using MEGA5.*

Results

VvNRT1.5 is oppositely regulated to *VvNRT1.8* under salt stress (figure 2).

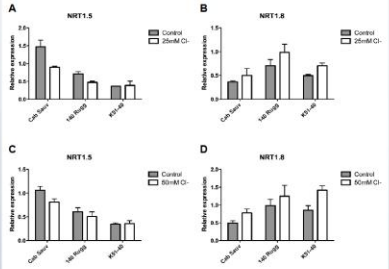

Figure 2: Exression analysis of VvNRT1.5 and VvNRT1.8 in Cabernet Sauvignon and two rootstocks of contrasting salt tolerance. *Grapevine rooted leaves were grown hydroponically and treated with 0mM, 25mM and 50mM Cl-. Relative expression was measured in total root tissue.* ***(A + C)*** *VvNRT1.5.* ***(B + D)*** *VvNRT1.8. error bars represent SEM. n = 3 - 4. Each biological replicate consisted of 3 pooled individuals.*

VvNRT1.5 transcript is expressed in the root stele, close to the xylem (figure 3).

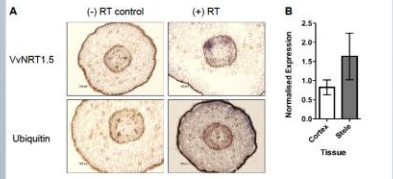

Figure 3: Localisation of* VvNRT1.5 *transcript in grapevine root tissue. ***(A)*** *In Situ PCR of grapevine roots. Reverse transcription and PCR was carried out directly on thin cross sections. DIG-11-dUTP was incorporated into the PCR product and probed with anti-DIG antibody.* ***(B)*** *qPCR of stele and cortex enriched root fractions. The stele and cortex were separated by hand. Bars represent SEM. N = 3.*

Results

VvNRT1.5 does not localise to the plasma membrane *in vivo* in a heterologous system (figure 4).

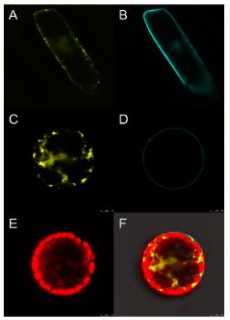

Figure 4: Localisation of* VvNRT1.5:YFP *in vivo. *VvNRT1.5:YFP does not localise to the plasma membrane of onion epidermal cells* ***(A)****, unlike the ROP11:CFP PM marker* ***(B)****. Similarly, VvNRT:YFP* ***(C)*** *does not co-localise with ROP11:CFP PM marker* ***(D)*** *in Arabidopsis mesophyll protoplasts. Instead, localisation appears cytoplasmic.* ***(E)*** *Chlorophyll autofluoresence.* ***(F)*** *Merged image of C, D and E plus DIC. Bars = 10 µM.*

VvNRT1.5 transports nitrate in Xenopus oocytes (figure 5).

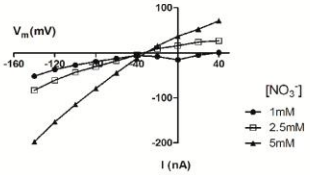

Figure 5: Current to voltage relationship of* VvNRT1.5 *injected Xenopus laevis oocytes. *The curve shown was recorded from a single VvNRT1.5 injected oocyte treated with CsNO3 (pH 5.5) at varying concentrations. Currents from water injected controls were subtracted for each voltage.*

Conclusions

VvNRT1.5 is a member of the nitrate transporter family in plants.

VvNRT1.5 is down-regulated by salt stress and oppositely regulated to VvNRT1.8. This is consistent with findings in Arabidopsis[4].

VvNRT1.5 is expressed in the root stele close to the xylem, suggesting a potential role in xylem loading and/or retrieval of nitrate (and possibly chloride).

VvNRT1.5 shows characteristics of a low affinity nitrate transporter in Xenopus oocytes.

Future work

In planta localisation of *VvNRT1.5*

Characterisation of *VvNRT1.5* chloride transport kinetics.

Characterisation of other membrane transporters involved in chloride transport in grapevine identified through microarray and sequencing.

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Certificate of attendance

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CERTIFICATE OF PARTICIPATION

June 26, 2012

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This letter certifies your participation as a(n) Poster Presenter at the Gordon Research Conference on Salt & Water Stress in Plants held June 24, 2012 - June 29, 2012 at The Chinese University of Hong Kong in Hong Kong China.

Poster Entitled: Identification of anion transporters that confer chloride exclusion and salt tolerance to grapevine rootstocks

For Dr. Nancy Ryan Gray, Director

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