FINAL REPORT

Conference trip to Davis, California, for the 5th Grape Research Coordination Network Conference 2013 (GRCN2013)

Project Number: GWT 1309

PROTEOMIC ANALYSIS OF ABIOTIC STRESSES IN GRAPEVINE

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Abstract:

The purpose of the travel was present my work at the 5th Annual Grape Research Coordination Network Conference (GRCN2013) held in at UC Davis, California, USA from July 9 to July 12, 2013. During the conference, I presented a poster titled 'Shotgun Proteomic Analysis of Grape Buds Exposed to Different Photoperiods'. The poster highlighted the proteomic changes seen in two grape genotypes; photoperiod responsive and photoperiod nonresponsive siblings caused due to the impact of short day and long day photoperiods, 42 days after imposition of differential photoperiod treatments. Poster sessions were well patronized and I received helpful feedback and comments from the delegates who attended the conference. The oral sessions provided very useful insights into my current research. The potential for collaborative research work was discussed with new and existing contacts. My principal supervisor, Prof. Paul Haynes was an invited speaker to the 5th Annual Grape Research Coordination Network Conference and this was an added advantage of my participation in the same conference. Attending the conference gave me an opportunity to meet and discuss my work with several researchers from all over the world who work on grape research, thus exposing my research project to an international forum.

Executive Summary:

- The 5th Annual GRCN Conference was held on the UC Davis campus in California, USA from the evening of July 9, 2013 until mid-day July 12, 2013.
- It had more than 50 participants spanning American, European and Asian countries some of which were Australia, United States of America, Belgium, Chile, Spain, Italy, France, Israel, China, Nepal and India.
- The goal of the GRCN2013 conference was to stimulate discussion towards developing collaborations and resources for grape functional genomics with a broad systems biology approach.
- The keynote opening talk was delivered by Jean-Mari Peltier, President, National Grape and Wine Initiative, Sacramento, CA, USA.
- The seven topics covered in the GRCN2013 conference included: Transcriptomics; Proteomics; Metabolomics; Systems Biology; Genome Sequencing; Genome annotation, prediction and functional annotations; and Grape Breeding.
- For each of the topics, three or four speakers presented a 30 minute research presentation on the work done in their research groups. The future aims of each topic were discussed to aid improvement within the grape research community. The talks were highly dynamic, enlightening and informative.
- Each research presentation concluded with a ten minute time slot for questions and open discussion.
- The poster session was held in the Silverado Vineyards Sensory Theatre, UC Davis on July 11, 2013.
- The conference ended with breakout sessions of group discussions to facilitate strategic planning for the future. A tour of several wineries in the Sonoma Valley was organized after the conference.

Conference details:

GRCN2013 is an internationally renowned conference bringing together grape researchers from many fields and countries, to share their research and establish collaborations of that benefit the grape research and the wine industry. Given below is the list of invited speakers to the GRCN2013 and their topic of lecture:

- Dr. Mario Pezzotti; University of Verona; Moving forward from microarray to RNA-seq keeping in mind grapevine biology and genetics.
- Dr. Laurent Deluc; Oregon State University; Use of transcriptomic resources to map gene regulatory networks in grapevine.
- Dr. Chris Davies; CSIRO, Australia; The control of berry ripening.
- Dr. Dennis Gray; University of Florida; Grape Promoters.
- Dr. Paul Haynes; Macquarie University; Quantitative proteomic analysis of temperature and water stress in grapes.
- Dr. Fulvio Mattivi; Foundation Edmund Mach; The grapevine metabolome via complementary analytical techniques on many genotypes
- Dr. Flavia Guzzo; University of Verona; Towards a scientific interpretation of the terroir concept.
- Dr. Grant Cramer; University Nevada; Current Progress in Plant Systems Biology.
- Dr. Anne Fennell; South Dakota State University; Photoperiod signaling in grapevine.
- Dr. Dario Cantu; UC Davis; Systems biology of the vineyard: the interaction between the grapevine and its microbial inhabitants.
- Dr. Sushma Naithani; Oregon State University; VitisCyc: a Metabolic Pathways Database for Grape (*Vitis vinifera*).
- Dr. Anne Françoise Adam-Blondon; INRA, Versailles; Latest news from the grapevine reference genome sequence.
- Dr. Massimo Delledonne; University of Verona; Genome reconstruction of the *Vitis vinifera* cv. Tannat, the red wine variety with the highest polyphenol content and remarkable antioxidant properties.
- Dr. Patricio Hinrichsen; Instituto de Investigaciones Agropecuarias, Santiago; How different are the wine and table grape genomes? Implications for table grape breeding.
- Dr. Stephane Rombauts; Ghent University; Finishing and annotating genomes in the NGS era.
- Dr. Ian Korf; UC Davis Genome Center; You can also make wine from grapes.
- Dr. Jerome Grimplet; Universidad de La Rioja, Instituto de Ciencias de la Vid y del Vino; Annotation of the Grapevine Genome.
- Dr. Andy Walker; UC Davis; Using marker assisted selection to optimize grape breeding.
- Dr. Allen Van Deynze; UC Davis; Deciphering the heat behind peppers.
- Dr. Gabriele di Gaspero; University of Udine; Revisiting the achievements of historical breeding for mildew resistance: insights from selective sweeps.
- Dr. Serge Delrot; ISVV, Bordeaux; Phenotyping grapevine in the context of climate change.

Key Activities:

The travel fund from GWRDC enabled me to travel from Macquarie University, Sydney, Australia to UC Davis, California, USA and present a poster at the 5th Annual Grape Research Coordination Network Conference 2013. This conference gave me a unique opportunity to present the results of my research project to an international community which works exclusively in grape and wine research. The talks gave me insights to look at different perspectives of my research project. All sessions were relevant to my current PhD work but of particular significance were sessions on proteomics, genome annotation, prediction and functional annotations and systems biology. I was able to share my ideas and gain knowledge from the cohort of scientists and wine researchers from all over the world, especially during the poster session. I was able to meet some of my collaborators, Prof. Grant Cramer, University of Nevada, Reno, who was also the organiser of the GRCN2013, and Prof. Anne Fennell of South Dakota State University. I was able to discuss my research with them and obtain feedback. I was able to strengthen my network with existing contacts and meet people who could provide opportunities for new collaborations in grape research in future.

Collaborations and Aims:

I was able to make new contacts and interact personally with my existing contacts during the GRCN2013. One outcome of the conference is that members of the Haynes Lab, Macquarie University and the Cramer lab, University of Nevada have decided to work together in order release a review paper on grape proteomics to inform the research community on the strides being made in this field. We also aim to publish a list of current protocols being used for protein extraction on different grape tissues. This would benefit the grape research community. The possibility to reach out to other labs that are doing grape proteomic research was discussed. Grape proteomics, which is my area of research, can assist in developing markers for marker assisted selection which is beneficial for grape breeders. Aims and strategies to improve the grape genome annotations were discussed with collaborators. This conference aims to enhance the research in the grape and wine sector in order to make it profitable, competitive and sustainable through innovation.

Acknowledgements:

I thank GWRDC for the travel grant GWT 1309 and the Post Graduate Research Fund from the Faculty of Science, Macquarie University for supporting my travel and participation in the 5th Annual Grape Research Coordination Network Conference.

Budgets:

I was awarded $2100 from GWRDC. I won a Post Graduate Research Fund from the Faculty of Science, Macquarie University to cover the shortfall between what I have been awarded from GWRDC and what the actual cost was.

Flight tickets from Sydney to Sacramento =	$3209
Accommodation $84 for six nights =	$500
Per diem $50 for six days =	$300
Total expenses =	$4009

Funding:

Grape and Wine Research and Development Travel Award funded	$2100
Post Graduate Research Fund from the Faculty of Science funded	$1900
Own money	$9
Total expenses =	$4009



Photo: Participants of the 5th Annual Grape Research Coordination Network Conference 2013 at UC Davis, CA, USA.

Shotgun Proteomic Analysis of Grape Buds Exposed to Different Photoperiods

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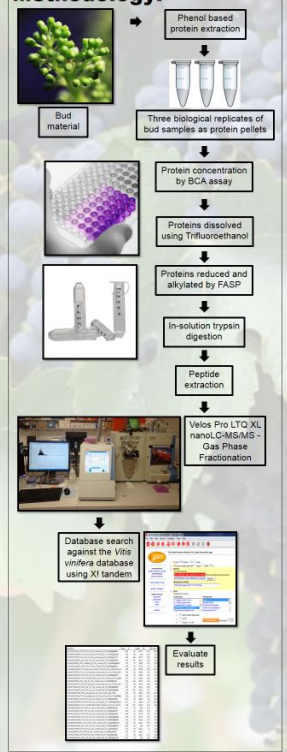
Introduction:

Grapes are an important economic crop in Australia where wine production is a significant industry. Daylength can trigger significant complex plant responses in grapevines and is an important environmental cue for synchronizing growth, flowering, and dormancy with seasonality. We are investigating the influence of photoperiod on floral pathway related protein expression, with particular reference to the proteomic changes induced by impact of long day (LD, 14 h) and short day (SD, 13 h) photoperiods in two genotypes, *Vitis riparia* (F2 110) and V. spp. 'Seyval' (F2 40). In this study, photoperiod treatments were imposed 35 days after bud break, and buds were harvested after 42 days of bud development after exposure to long day and short day photoperiods. For proteomic analysis, protein extraction was performed by the phenol based extraction protocol. Proteins were denatured using trifluoroethanol (TFE) followed by Lys-C and trypsin digestion by the Filter Aided Sample Preparation (FASP) method using Amicon Ultra 0.5 mL 10K ultrafiltration devices. Proteins were identified using nanoLC-MS/MS spectra that were acquired by gas phase fractionation (GPF) on a Velos Pro LTQ XL mass spectrometer. Tandem mass spectra were searched against the Uniprot *Vitis vinifera* protein database in the Global Proteome Machine (GPM) containing 65,328 entries.

Objectives:

1. To investigate the proteomic responses of grape buds of the two genotypes, *Vitis riparia* (F2 110) and V. spp. 'Seyval' (F2 40), exposed to different photoperiods.
2. To further characterize the proteins identified in understanding their function at a molecular level.
3. To correlate the effects with comparison to transcriptomic responses of grape buds exposed to the same stress.

Methodology:



Results:

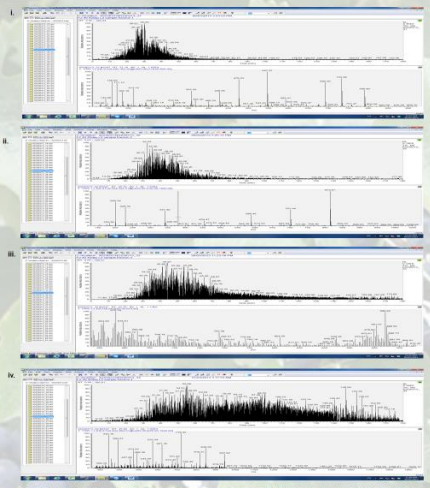


Figure 1: The m/z ranges for four gas phase fractions (i-iv) were calculated by running the *Vitis vinifera* FASTA file in an online tryptic GPF calculator thus creating four GPF fractions of i. 400-506, ii. 501-658, iii. 653-913 and iv. 908-1600 m/z ranges. Spectra was acquired for 180 seconds per fraction. Dynamic exclusion duration was 90s. Chromatography: Reverse phase column (8 cm; C18 Magic; 0.5 x 100 micron).

Sample	No. of proteins identified			Redundant count of peptides			No. of proteins common to all three replicates	Protein FDR %
	Rep1	Rep2	Rep3	Rep1	Rep2	Rep3		
F2 110 42d LD	1977	2141	2171	11051	11158	11216	1011	0.3%
F2 110 42d SD	2185	2119	1939	14754	14386	11053	1031	0.4%
F2 40 42d LD	2289	2257	2186	13654	13713	12121	1128	0.4%
F2 40 42d SD	2019	2053	2067	10371	10296	11528	977	0.4%

Table 1: Table shows the number of proteins identified in each photoperiodic treatment in the respective genotype. A protein had to be present in all three biological replicates and the total number of spectral counts in triplicates had to be a minimum of 5, for a protein to be accepted as a valid hit. Data were filtered using false discovery rates and we aimed to keep the protein false discovery rate (FDR) below 1%.



Figure 2: The Heat Map is a colour map of protein abundance for the selected proteins plotted based on the Normalized Spectral Abundance Factor (NSAF) values; lighter bands represent lower abundance and darker bands represent higher abundance proteins. The rows represent the expressed proteins and the columns represent the triplicates of each different treatment.

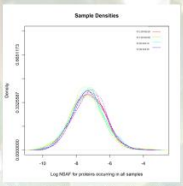


Figure 3: A spectral fraction of 0.5 was added to all spectral counts. Plot shows log NSAF values for proteins occurring in all samples.

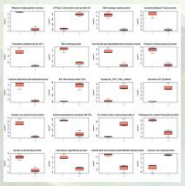


Figure 4: Mean abundance patterns of the top 20 most differentially expressed proteins which were variably expressed in 110 LD vs. 40 LD.

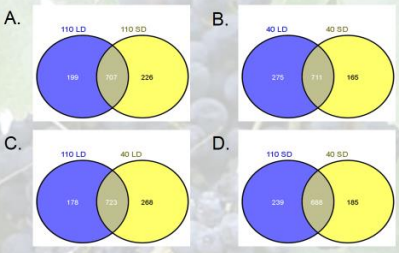


Figure 5: Venn Diagrams showing number of proteins which are unique to respective genotypes and photoperiods. LD = Long day, 14 hours; SD = Short day; 13 hours.

COMPARISON	UP REGULATED	DOWN REGULATED
A. F2 110 LD vs. F2 110 SD	95	58
B. F2 40 LD vs. F2 40 SD	67	68
C. F2 110 LD vs. F2 40 LD	85	90
D. F2 110 SD vs. F2 40 SD	66	111

Table 2: Number of differentially expressed proteins which were up-regulated and down-regulated.
Protein expression data is described for LD buds relative to SD buds of the same age (i.e. up-regulated in LD = down-regulated in SD and down-regulated in LD = up-regulated in SD).
Protein expression data is described for F2 110 genotype relative to F2 40 genotype buds of the same age (i.e. up-regulated in F2 110 = down-regulated in F2 40 and down-regulated in F2 110 = up-regulated in F2 40).

Conclusion:

A. F2 110 *Vitis riparia* Long day vs. Short day

- Proteins involved in carbohydrate metabolic process, response to light and heat shock proteins were up-regulated in 110 SD.
- Pathogenesis-related proteins were down-regulated in 110 LD and fruit development was up-regulated in 110 LD.

B. F2 40 Vitis spp. 'Seyval' Long day vs. Short day

- General photoperiod effects were observed, proteins involved in photosynthesis, light harvesting and heat shock proteins were up-regulated in 40 LD.
- Ripening related proteins and aquaporins were up-regulated in 40 LD.

C. F2 110 *Vitis riparia* Long day vs. F2 40 Vitis spp. 'Seyval' Long day

- Photosynthesis, protein biosynthesis and cell division up-regulated in 40 LD compared to 110 LD.
- Aquaporins were up-regulated in 40 LD while protein folding was down-regulated in 40 LD.
- Genotype specific development effects observed.

D. F2 110 *Vitis riparia* Short day vs. F2 40 Vitis spp. 'Seyval' Short day

- Glycolysis, protein metabolic processes, signals that trigger flowering were up-regulated in 110 SD.
- Proteolysis, oxidoreductase activity and tubulin family proteins were down-regulated in 110 SD.
- Up-regulation of fruit-specific, thaumatin-like protein of 24 kDa that accumulates at very high levels during berry softening in grapes observed in 110 SD.
- Dormancy specific effects observed.

Proteins with a potential dual role in floral development and dormancy transitioning were identified using photoperiod induced differences in floral development between LD and SD buds. *Vitis riparia* (F2 110) is more photoperiod responsive compared to V. spp. 'Seyval'. Many floral development signals are photoperiod regulated and they could have a regulatory role in bud endodormancy. In this study, SD delayed inflorescence development in the grape bud and this could be related to trends in protein expression patterns.

References:

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Acknowledgements:
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Poster: Figure of the poster presented at the 5th Annual Grape Research Coordination Network Conference 2013 at UC Davis, CA, USA.