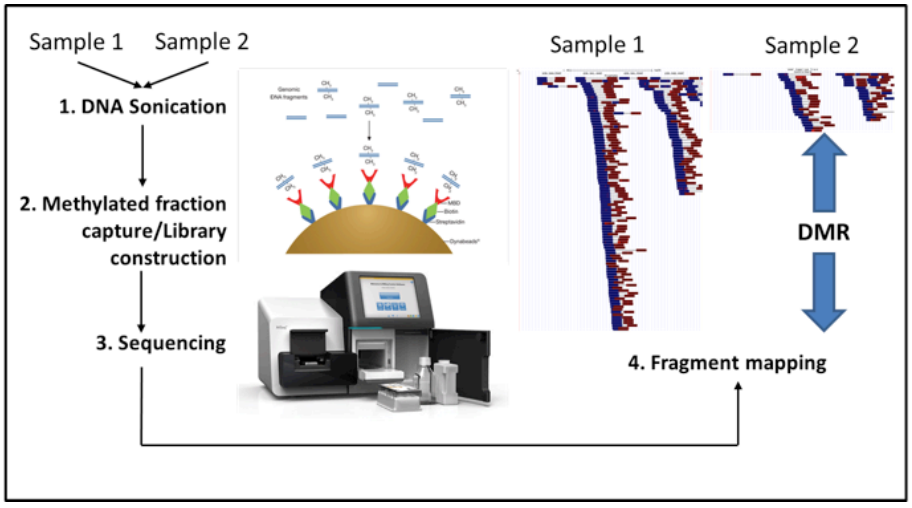
Australian Government

Australian Grape and Wine Authority

THE UNIVERSITY of ADELAIDE

Breeding drought and heat tolerant grapevines using epigenetic tools



FINAL REPORT to

AUSTRALIAN GRAPE AND WINE AUTHORITY

Project Number: UA 1402

Principal Investigators: Dr Roberta De Bei and Dr Carlos Rodríguez Lopéz

Research Organisation: THE UNIVERSITY OF ADELAIDE

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1. Abstract

Plants have acquired the ability to prime their genomes after environmental stress, enabling a faster and stronger response to future environmental cues. This is most immediately tangible for long-lived crops such as grapevine. DNA methylation is an epigenetic mechanism by which plants control their responses to stress. Such epigenetic memory could be used for breeding purposes. We used MC-Seq to determine the genomic regions showing DNA methylation changes in grapevines during/after heat, drought and combined heat/drought stress. A total of 13,968 genes were identified as differentially methylated between control and stressed plants. The methylation status of 233 of these genes was maintained after physiological recovery and was deemed to reflect epigenetic memory of stress.

2. Executive Summary

The Australian wine industry is under pressure to sustain its profitability due to challenges such as climatic anomalies, heatwaves, irregular precipitation and rising input costs.

Vitis vinifera is known to be a species of high phenotypic plasticity and hence it adapts to climate and different growing conditions (Sadras et al., 2009).

Successful management of such plasticity, to make it adaptable to a changing environment, would help to maintain the leading position of the Australian wine industry.

An organism's cells from different tissues, developmental stages or grown under different environments are generally genetically identical but may present widespread differences in their DNA methylation profiles. Characterisation of such differences may prove suitable for the identification of epigenetic markers linked to useful characters.

This project was needed to understand whether DNA methylation and epigenetics could help understand the mechanisms of grapevine plasticity and its potential use for breeding stress tolerance into the vine.

The aims of the project were:

- Phenotypic characterisation of a grapevine glasshouse population with induced methylation changes through a targeted stress (heat and drought) intervention.
- Identification of epigenetic markers associated with heat and drought stress in grapevine
- Validation of epigenetic markers controlling drought and heat resistance in grapevine by interrogating gene expression and methyl-capture DNA libraries derived from the intervened population
- Generation of a database of potential stress tolerance epialleles for future research
- Publication of the results in a high impact factor journal

To fulfil the project objectives a collaboration with Dr Everard Edwards from CSIRO was initiated to collect samples from a glasshouse experiment where grapevines were subjected to water stress, heat stress and a combination of the two.

Samples were selected based on plant physiology measurements in order to choose water and heat stress extremes.

The results showed that approximately 14,000 genes were differentially methylated in response to water and/or heat stress. Most importantly, more than 200 differentially methylated genes were maintained after stress and recovery. This is an important finding since the methylation differences which are kept after recovery could mean that a ‘memory’ of the stress is retained in the plant and thus could be exploited, for example, to induce stress resistance in the plant through targeted breeding programs, by priming mother vines before propagation in nurseries.

This work has served as a proof of concept for what we believe could become a larger project aimed at exploiting epigenetics and the memory of stress as tools to prime grapevine varieties for a changing environment.

We would like to acknowledge the Australian Grape and Wine Authority for supporting this project. Dr Everard Edwards (CSIRO Plant Industry) has provided the samples needed to carry out the study and guidance throughout the project. Dr Ute Baumann (ACPFG) has carried out the bioinformatics and statistical analysis.

3. Background

The Australian wine industry produced 1.67 million tonnes of wine grapes in the 2015 vintage. This is a vital industry, which is however vulnerable due to climate change, climate anomalies (heatwaves), increased water scarcity and rising input costs. It has been predicted that by 2050 there could be a 44% reduction in land suitable for growing vines (Anderson et al., 2008; Garnaut, 2008). Successful exploitation of grapevine plasticity, to make it adaptable to a changing environment, would help to maintain the leading position of the Australian wine industry.

An organism's cells from different tissues, developmental stages or grown under different environments are generally genetically identical but may present widespread differences in their DNA methylation profiles (Rodríguez López et al, 2010; Geyer, Rodríguez López et al, 2011; López et al, 2012). DNA methylation is a major mechanism by which gene expression is controlled in response to changing environments (Tricker, López et al, 2012; Tricker, López et al, 2013a and b). Characterisation of such differences may prove suitable for the identification of epigenetic markers linked to useful characters.

4. Project Aims and Performance targets

4.1. Aims

- Phenotypic characterisation of a grapevine glasshouse population with induced methylation changes through a targeted stress (heat and drought) intervention.
- Identification of epigenetic markers associated to heat and drought stress in grapevine
- Validation of epigenetic markers controlling drought and heat resistance in grapevine by interrogating gene expression and methyl-capture DNA libraries derived from the intervened population
- Generation of a database of potential stress tolerance epialleles for future research
- Publication of the results in a high impact factor journal

4.2. Outputs

Year 1	Output	Target date	Activities
a	Data identifying heat/drought induced epigenetic modification in grapevines	30/06/2015	• Identify heat/drought induced epigenetic modification in grapevines by interrogating at least 40 samples from RNA-seq and methyl-captured DNA libraries from vines subjected to different temperature / drought treatments
b	Dissemination of knowledge on epigenetic modification in grapevines through at least two extension mechanisms	30/06/2015	• Prepare report on project findings • Prepare material for presentation at workshops and seminars

The Australian Grape and Wine Authority (AGWA) approved a variation to the project and allowed an extension of the Project End Date until 30 September 2015.

The allocated budget allowed the processing and sequencing of 24 rather than 40 samples as indicated in the Activities. Also, the methyl-capture sequencing of recovery samples was carried out instead of RNA-Seq. This was believed to be of higher value for the purpose of the study in order to verify whether a 'memory of the stress' is maintained in the vines upon recovery from stress. However RNA has been extracted and will be sequenced if more funding becomes available.

5. Materials and methods

5.1 Plant Material

Plant material to conduct the project was provided by Dr Everard Edwards from CSIRO Agriculture. Dr Edwards provided leaf samples from a glasshouse heat x drought experiment on own rooted Cabernet Sauvignon cuttings, from which DNA and RNA were extracted for subsequent analysis.

5.2 Experimental conditions

A brief description of the experiment conducted by CSIRO personnel is described below.

Three-year-old Cabernet Sauvignon vines were grown in a glasshouse and two treatments were applied: i) well-watered control (T0) with the soil moisture kept over 19%, ii) stressed (T4) with soil moisture ranging from 6 to 9%. Soil moisture was measured with soil moisture probes (Decagon 10HS sensors attached to ICT International SMM logger: ICT International, Armidale, NSW).

When the percentage of soil moisture reached the desired range for both T0 and T4, the plants were allocated to two additional treatments: i) ambient temperature (not exceeding 32°C) and ii) high temperature (maximum of 45°C). The temperature in the two glasshouses was measured using Tinytag Loggers (Hastings Data Loggers, Port Macquarie, New South Wales, Australia). The heat treatment was applied for two days during which physiological measurements were performed: i) stomatal conductance, ii) midday leaf and stem water potential and iii) leaf temperature.

5.3 Physiological measurements

Stomatal conductance was measured on one leaf per vine using a Delta-T AP4 Porometer at day 1 and 2 of heat stress and on day 1, 3 and 14 of recovery (temperature returned to ambient). Similarly, water potential was also measured using a Scholander-type pressure chamber. Leaf temperature was measured using an infrared thermometer on all leaves that were used for both stomatal conductance and water potential readings.

Samples for gene expression analysis consisted of the leaves on which the non-destructive physiological measurements were taken.

5.4 Analysis of stress-induced-changes on DNA methylation

Leaf samples from three plants of each of the four treatments were randomly selected for DNA methylation analysis. All selected plants were sampled the day after exposure to stress and after physiological recovery. In brief, DNA was isolated from all samples, then sheared to 200-400 bp using a Covaris S220 sonicator and enriched for the methylated DNA fraction using the MethylMiner Methylated DNA Enrichment Kit (Invitrogen), prior to Illumina DNA library construction. The captured fraction of the genome was then used to create sequencing libraries for each plant at both time points (i.e. stress and recovery) using the NEBNext Ultra DNA Library Prep Kit (New England Biolabs) according to the manufacturer's instructions and using NEBNext Multiplex Oligos for Illumina (1-12). Library yield was determined by Qubit dsDNA High Sensitivity Assay Kit. Agilent 2100 Bioanalyzer High-Sensitivity DNA Chip was used to assess library quality and determine average insert size. Libraries were then mixed in two pools and sequenced using two Illumina HiSeq 2000 lanes (Illumina Inc., San Diego, CA) using 125bp paired end V4 chemistry by QBI Centre for Brain Genomics.

5.5 Bioinformatics analysis

Differentially Methylated Regions between treatments were calculated by simple subtraction of each (normalised) sample count. Differential methylation was deemed significant when the calculated difference in methylation between two treatments was 2 standard deviations away from the mean ($P<0.05$). Loci counts were calculated based on 1,857,793 500bp non-overlapping sliding windows covering the entire aligned genome. Read counts were normalised to window size (reads per base) to account for windows smaller than 500bp. Windows with no counts for either sample were discarded (Figure 1). Normalised counts for both samples were plotted to identify the correlation between the loci count from each sample.

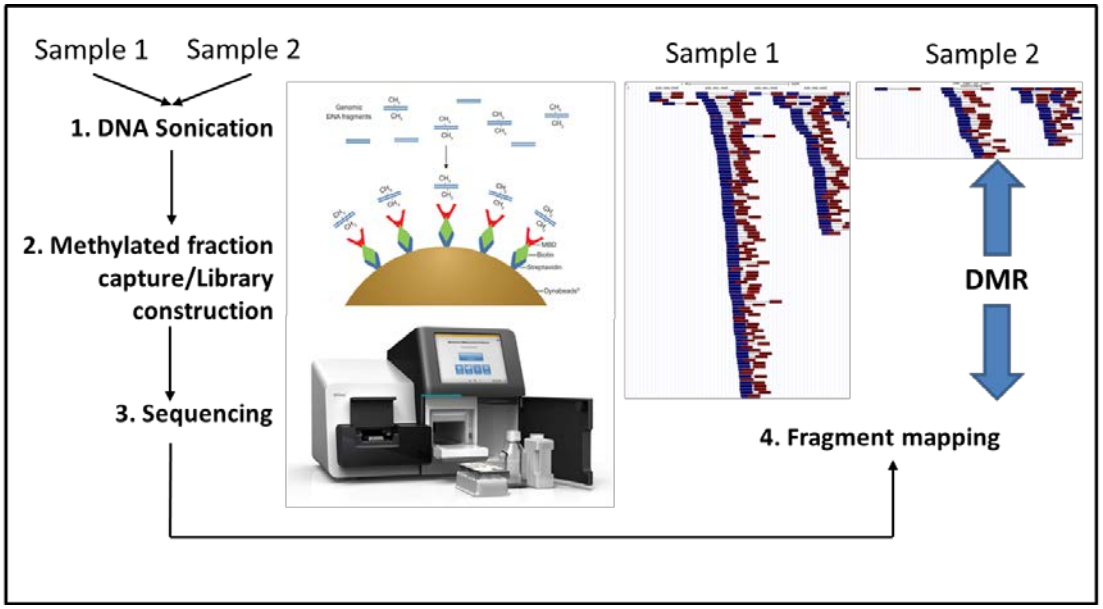


Figure 1: Schematic representation of the MethylCapture protocol for the identification of Differentially Methylated Regions (DMRs). (1) Genomic DNA samples are first sonicated to an average size of 200-400bp. **(2)** The methylated fraction of the genomes is captured using antibodies or MBD protein. Individual sequencing libraries are prepared and barcoded for each sample. **(3)** Next Generation Sequencing (NGS) is carried out and individual sequences are demultiplexed. **(4)** Resultant NGS reads are mapped against the reference genome of the organism and DMRs are statistically inferred by locus specific differences in number of reads (i.e. higher number of reads equals higher level of methylation).

6 Results and discussion

6.1 Physiological measurements

Physiological data provided by Dr Edwards (i.e. stomatal conductance and water potential) was used as indicator of vine stress and recovery. T4 vines were considered recovered from stress when their stomatal conductance values reached those observed for T0 on the same day.

As expected, stomatal conductance (g_s) decreased as soil moisture decreased during the water-stress treatments, regardless of heat treatment (Figure 2). In control plants, stomatal conductance was higher in the heated treatment than in the ambient treatment. Water potential was lower overall on the second day of the heat treatment (Figure 3). On the first heat treatment day, hot T0 vines had lower water potential than T4. On the second day, both heated and ambient T0 vines were lower than other treatments.

6.2 Analysis of stress induced changes on DNA methylation

Measurement of stomatal conductance and water potential was used to select the most appropriate samples for DNA methylation analysis. Twenty four sequenced samples (12 plants sampled during stress and after physiological recovery) generated a total of 912 million reads with an average number of reads of 38 million per sample. Reads with a sequencing quality higher than 30 were mapped to the *Vitis vinifera* available genome allowing for a maximum number of SNPs of 3% of the read. Average mapping efficiency was 52.5% (Table 1).

A 500bp sliding window approach was then used to determine the level of methylation across the genomes of all samples sequenced. The total number of reads per window in each sample was normalised against the total number of reads of that same sample to account for differences in sequencing depth between samples. Differentially Methylated Regions (DMRs) between treatments were calculated by subtraction of each (normalised) sample count. Differential methylation was deemed significant when the calculated difference in methylation between two treatments was 2 standard deviations away from the mean ($p<0.05$) (Figure 4). Loci counts were calculated based on

1,857,793 500bp non-overlapping sliding windows covering the entire aligned genome. Read counts were normalised to window size (reads per base) to account for windows smaller than 500bp. Windows with no counts for either sample were discarded. Normalised counts for both samples were plotted to identify the correlation between the loci count from each sample. Using this approach a total of 13,968 genomic regions within 1.5 Kb of a gene were identified as differentially methylated between stress control and stressed plants (Table 2).

The methylation data across the genomes of all 12 plants at the first time point (one day after stress) was then used to generate a presence/absence binary matrix (1/0) representing the methylation status of all the resulting genomic windows generated using the MethylCapture sequencing data (described above). The resultant matrix was then used to generate a cluster dendrogram based on the epigenetic distance between plants (Figure 5). Four clear epigenetic clusters were detected each containing the samples from each of the experimental treatments (control, drought, heat, drought-heat). This indicates that otherwise genetically identical plants that should group in a single cluster, present significantly different DNA methylation profiles depending on the environmental conditions (i.e. stress) to which they were exposed for only two days. More importantly, 233 genes maintained their stress-induced methylation status after physiological recovery and were deemed to reflect epigenetic memory of stress (Table 2). This supports our hypothesis that stress-induced DNA methylation can be maintained long after the removal of the causing stress. Potentially, these observed epigenetic changes could have a long-term effect on the plant transcriptome and phenotype, opening the enticing possibility of using such epigenetic memory for epigenetic breeding of stress tolerance in perennial/vegetatively propagated plants.

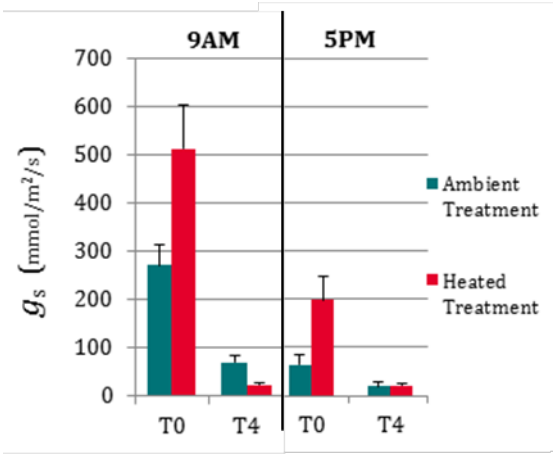


Figure 2: Stomatal conductance on day 1 of temperature treatments at 9am and 5pm. T0 shows the average stomatal conductance measured from well irrigated plants (soil moisture kept over 19%). T4 shows average stomatal conductance for stressed plants (soil moisture 6-9%). Day ambient temperature did not exceed 32°C. Heated treatment reached a maximum of 45°C. (Modified from Bronwyn Smithies, "How do grapevines survive when it is hot and dry?" CSIRO Food, Health and Life Sciences, Summer student program 2012-13)

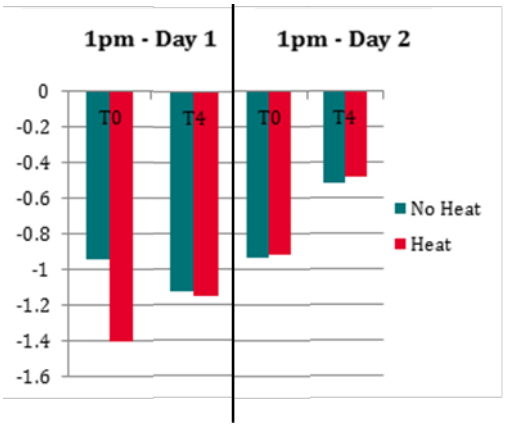


Figure 3: Comparison of water potential on day 1 and day 2 of the temperature treatment. T0 shows the average water potential measured from well irrigated plants (moisture kept over 19%). T4 shows average water potential for stressed plants (soil moisture 6-9%). Day ambient temperature did not exceed 32°C. Heated treatment reached a maximum of 45°C. (Modified from Bronwyn Smithies, "How do grapevines survive when it is hot and dry?" CSIRO Food, Health and Life Sciences, Summer student program 2012-13)

Table 1: Sequencing statistics

Plant number	Watering	Temperature	Collection	Pair End Reads	Single End Reads	Sum	Mapped Reads	%Mapping Rate
1	Control	Control	Recovery	35,939,700	641,185	36,580,885	20,160,210	55.11
5	Control	Control	Recovery	35,439,518	461,033	35,900,551	18,461,458	51.42
8	Control	Control	Recovery	27,691,806	304,424	27,996,230	15,886,479	56.75
2	Control	Heat	Recovery	26,121,206	452,310	26,573,516	15,331,329	57.69
6	Control	Heat	Recovery	29,282,456	570,742	29,853,198	16,150,360	54.10
10	Control	Heat	Recovery	28,454,626	435,390	28,890,016	16,046,800	55.54
3	Drought	Control	Recovery	27,738,990	635,538	28,374,528	16,568,554	58.39
7	Drought	Control	Recovery	44,797,578	521,436	45,319,014	22,959,044	50.66
9	Drought	Control	Recovery	29,287,046	477,943	29,764,989	16,805,898	56.46
4	Drought	Heat	Recovery	29,765,056	572,445	30,337,501	17,109,746	56.40
11	Drought	Heat	Recovery	25,526,586	408,224	25,934,810	14,552,434	56.11
12	Drought	Heat	Recovery	33,815,714	695,205	34,510,919	20,526,061	59.48
1	Control	Control	Stress	43,687,466	1,251,475	44,938,941	23,933,786	53.26
5	Control	Control	Stress	44,821,574	1,222,286	46,043,860	21,857,923	47.47
8	Control	Control	Stress	47,330,266	7,836,264	55,166,530	24,262,631	43.98
2	Control	Heat	Stress	37,947,834	1,207,286	39,155,120	16,607,109	42.41
6	Control	Heat	Stress	44,079,600	2,581,483	46,661,083	23,851,039	51.12
10	Control	Heat	Stress	41,305,966	2,238,156	43,544,122	17,535,488	40.27
3	Drought	Control	Stress	36,386,704	950,041	37,336,745	20,998,349	56.24
7	Drought	Control	Stress	38,896,474	925,653	39,822,127	19,031,100	47.79
9	Drought	Control	Stress	34,393,450	1,771,495	36,164,945	16,238,737	44.90
4	Drought	Heat	Stress	49,624,162	2,008,660	51,632,822	29,953,203	58.01
11	Drought	Heat	Stress	56,401,670	1,351,483	57,753,153	31,962,396	55.34
12	Drought	Heat	Stress	36,233,872	942,975	37,176,847	19,287,804	51.88

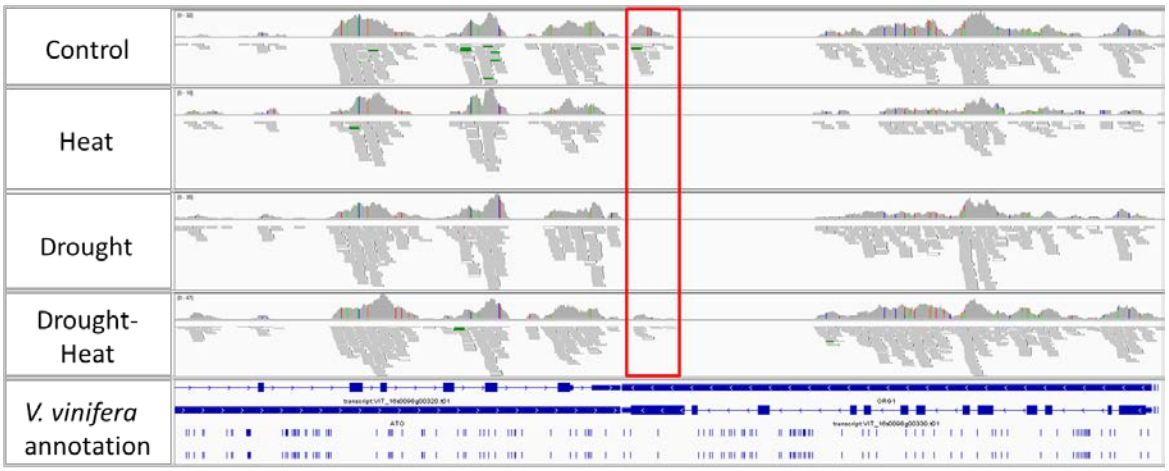


Figure 5: Detection of stress induced Differentially Methylated Regions (DMRs) in the grapevine genome. Estimation of global methylation level was achieved by comparison of the normalised number of reads per 500bp window. Red box shows a 500bp window showing a significant difference in the number of reads between control and all stressed (Heat, Drought and Drought-Heat) samples.

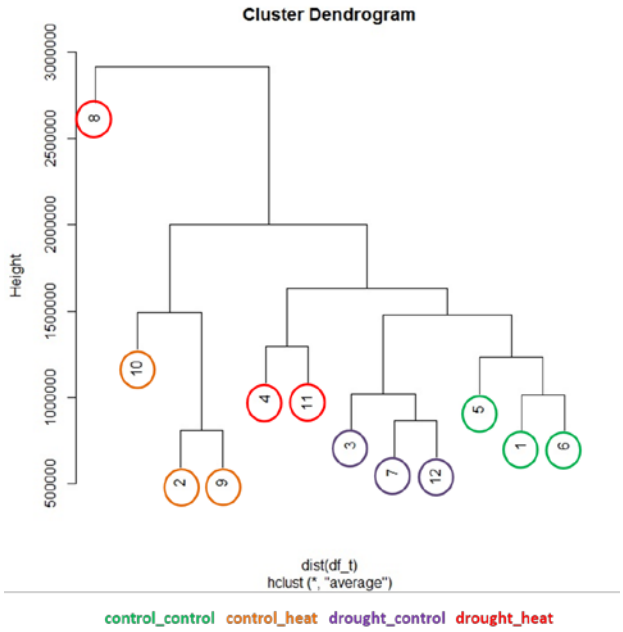


Figure 5: Analysis of stress induced epigenetic variability in grapevine. Cluster analysis was built using the epigenetic distance between 12 genetically identical grapevine plants grown under four different conditions: (Control (green): **32°C/soil moisture over 19%; Heat**(orange): **45°C/soil moisture over 19%; Drought** (purple): **32°C/soil moisture over 6-9%; Drought-Heat** (red): **45°C/soil moisture over 6-9%).** Grapevine plants estimation of pairwise epigenetic distances were obtained from individual DNA methylation profiles **generated by methylcapture sequencing**.

Table 2: Summary of Differentially Methylated Regions between control and stress plants: Figures indicate the number of significantly DMRs (P<0.05) between control and stressed plants (Drought, Heat and Drought-heat combined) plants. Methylation levels were measured on the day after stress (Stressed) and 14 day after stress (Recovery). Genomic regions presenting the same DMRs during stress and after recovery were deemed to reflect epigenetic memory of stress.

	Stress	Recovery	Epigenetic memory
Drought	2509	1473	68
Heat	588	1836	14
Drought-Heat	4750	4521	151

7 Outcome/Conclusion

The project performed well and achieved the proposed outputs stated in the original application. In particular output (a): '**Data identifying heat/drought induced epigenetic modification in grapevines'** was completed and more than 13,000 genes were identified as differentially methylated in response to either water or heat stress. 233 of these differences were also maintained after physiological recovery of the plant from the stress. The methylation that is maintained after recovery could be exploited to induce stress resistance to the plant through targeted breeding programs however, more research is needed in this regard.

Results from this study have already been discussed with Prof. Mario Pezzotti from the University of Verona. Prof Pezzotti is the organiser of the IX Grapevine Physiology and Biotechnology conference that will be held in Verona (Italy) in July 2016 and he has encouraged the Chief Investigators of this project to present at least one paper for the conference. As a consequence two abstracts have been submitted and are awaiting acceptance.

A paper is also in preparation and it will be submitted to a high impact factor journal (New Phytologist).

A further positive outcome of this project has been the creation of strong collaborative links with the research groups led by Dr Everard Edwards (CSIRO) and Dr Ute Baumann (ACPFG). As a consequence a joint project proposal was submitted for consideration to AGWA.

This work has served as a proof of concept for what we believe could become a larger project aimed at exploiting epigenetics and the 'memory of stress' as tools to prime grapevine varieties for a changing environment. Considering the high dependence of grapevine production on the climatic conditions and the susceptibility of the Australian wine industry to climate change, findings from this and consequent projects could aid adaptation of vines to a changing environment. These findings could be applied in nurseries for the

production of plant material with improved water use efficiency (WUE). An enhanced WUE of cuttings can lead to costs reductions during planting (fewer losses) and plant establishment. The identification of epigenetic markers for stress might lead to methods for early stress diagnosis thus ensuring more timely and targeted interventions. This could become a major commercial advantage for Australian nurseries and growers.

Our findings could lead to the production of cuttings that are more tolerant to water and heat stresses, thus reducing costs of irrigation and water applied after planting and plant establishment and so lowering the environmental impact of the Australian wine industry.

8 Recommendations

This project has proved that grapevines, if subjected to stress, carry a ‘memory’ of the stress. It is yet to be studied whether this could make the vines less susceptible to subsequent exposures to the same stress. The project has yielded a large list of genes, which were differentially methylated from the controls, in response to the applied stress treatments. The findings can be applied to further this research into new projects on epi-breeding. Successful epi-breeding will increase the competitiveness of the Australian wine industry (2015-2020 AGWA strategic plan Priority area 2) by facilitating the adoption of innovative breeding and nursery propagation technologies with the potential to generate improved varieties with induced stress resilience and plasticity to respond to the changing climate. Equally, enhanced water use efficiency will improve vineyard water use efficiency and irrigation sustainability addressing:

Strategy 4: Improving resource management and sustainability - Climate adaptability - Sustainable resource management,

Strategy 5: Improving vineyard performance - Enhancing grapevine and rootstock performance -Efficient and sustainable vineyard management

Strategy 8: Building capability - Developing people by training the new researchers in the field of epi-breeding.

This study should be extended into a PhD project as outlined below:

Project 1: Epi-breeding for heat and drought tolerance in grapevine

Epigenetic marks (epialleles) superimposed on the DNA sequence provide agility and plasticity to the organisms in terms of response to the environment. Important agronomical traits such as water use efficiency (WUE), disease resistance and yield can be regulated by such marks. Modern sequencing technologies allow the mapping and characterisation of the genome regions regulated by epigenetic marks in response to environmental cues. These marks can be artificially induced on elite cultivars and selected (epi-breeding) to generate

stress tolerance without changing their genetic background. Although this has been tested on model plants (*Arabidopsis*) and on crops (Canola), there have been no systematic attempts to explore the use of epi-breeding for grapevine improvement.

During this study we will search for epialleles linked to drought/heat stress, induced by targeted intervention in a grapevine glasshouse population. We expect that data generated in this study will open future research avenues for the use of epialleles associated to water/heat stress as a tool for breeding drought resistant grapevine varieties.

9. Communication

Results from this project will be disseminated through presentation of a seminar at the IX Grapevine physiology and biotechnology conference which will be held in Verona in 2016.

An article to be submitted to a peer-reviewed journal is in preparation.

This report will also serve as a means of dissemination of the results to the Australian wine industry and scientific community.

10. Intellectual Property

Two patents were filed on the method for selecting the methylation markers to diagnose environmental stress and on the method to produce Stress Tolerant Plant or Precursor with priority dates of 12/05/2010. The patents have subsequently entered national phase in US, Japan, Australia, Europe, India, and China (granted).

The potential markers could be filed as new patents. The new patents will provide protection over the application of the markers for the diagnosis and potential tolerance to specified stress in grapevine propagules.

The development and patent filing on both methods occurred while the two inventors (Prof Mike Wilkinson and Dr Carlos Rodriguez Lopez) were employees of Aberystwyth University. Upon moving to the University of Adelaide the patents were assigned to the University of Adelaide.

The marker validation will be conducted at the University of Adelaide by Dr Carlos Rodriguez Lopez if successful in subsequent grant applications.

11. References

Anderson, K, Findlay C, Fuentes S and Tyerman S (2008). Viticulture, wine and climate change. Garnaut Climate Change Review. Accessed at http://www.garnautreview.org.au/CA25734E0016A131/WebObj/01-HViticulture/$File/01-H%20Viticulture.pdf

Garnaut. Climate change review. Accessed at: http://www.garnautreview.org.au/

Geyer, K.K., Rodríguez López, C.M., Heald, J., Wilkinson, M.J., Hoffmann K.F. (2011) DNA methylation regulates egg production in the pathogenic blood fluke *Schistosoma mansoni*. Nature Communications.

Rodríguez López, C.M., Beckham, M., Consuegra, S., Lago, F., Espiñeira, M., Morán P. (2012). Detection and Quantification of Tissue of Origin in Salmon and Veal Products using Methylation Sensitive AFLPs. Food Chemistry.

Rodríguez López, C.M., Wetten, A.C., Wilkinson, M.J. (2010) Progressive erosion of genetic and epigenetic variation in callus-derived cocoa (Theobroma cacao) plants. New Phytologist.

Sadras, V.O., Reynolds, M.P., de la Vega,A.J., Petrie, P.R. and Robinson, R. (2009) Phenotypic plasticity of yield and phenology in wheat, sunflower and grapevine. Field Crops Research. 110 (3): 242-250.

Soar CJ, Collins MJ, Sadras VO (2009). Irrigated Shiraz vines (*Vitis vinifera*) upregulate gas exchange and maintain berry growth in response to short spells of high maximum temperature in the field. Functional Plant Biology 36, 801-814.

Tricker P., Gibbings G., Rodríguez López, C.M., Hadley P., Wilkinson, M.J. (2012) Low relative humidity triggers RNA-directed de novo DNA methylation and suppression of genes controlling stomatal development. Journal of Experimental Botany

Tricker P., Rodríguez López, C.M., Gibbings G., Hadley P., Wilkinson, M.J. (2013) Transgenerational, dynamic methylation of stomata genes in response to low relative humidity. International Journal of Molecular Science.

Tricker P., Rodríguez López, C.M., Hadley P., Wagstaff, C., Wilkinson, M.J. (2013) Pre-conditioning the epigenetic response to high vapour pressure deficit increases the drought tolerance in *Arabidopsis thaliana.* Plant Signaling & Behavior.

12. Staff

The following staff contributed to the project:

Dr Carlos Rodriguez Lopez (Chief Investigator)

Dr Roberta De Bei (Project Supervisor)

Collaborators:

Dr Everard Edwards - CSIRO Plant Industry (research team leader)

Dr Ute Baumann – ACPFG (group leader: transcriptomics, bioinformatics and abiotic stress)