Eighth International Symposium on Grapevine Physiology and Biotechnology

Philippa Pattison Australian Society of Viticulture and Oenology

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TG08006 (28th February 2009)

Eighth International Symposium on Grapevine Physiology and Biotechnology

Debra Robinson et al.

Australian Society of Viticulture and Oenology

TG08006

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This document reports on the achievements and outcomes of the 8th International Symposium on Grapevine Physiology and Biotechnology held 23-28 November in Adelaide.

Thursday, 26 February 2009

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Media Summary

Climate change and fruit quality were areas of key interest when Adelaide played host to some of the world's top grapevine scientists at the 8th International Symposium on Grapevine Physiology and Biotechnology - held at The National Wine Centre from 24th-28th November, 2008. The conference attracted 196 delegates, surpassing previous conferences in this series. There were 83 overseas delegates from: Brazil, Canada, Chile, France, Germany, Hungary, India, Israel, Italy, Japan, Spain, New Zealand, Portugal, South Africa, Spain, Switzerland, Turkey, USA. The aim of the conference was to discuss the latest biotechnology techniques that will help the table-grape. dried fruit and wine industries. It showcased the latest research with a focus on where new techniques in biotechnology are being used to advance our understanding of vine physiology. With the recent sequencing of the grapevine genome, this opened many opportunities in grapevine biotechnology to improve berry quality, and to help understand the consequences and mitigate the impacts of climate change, such as heat waves, drought and salinity. The areas discussed at the conference were: Plant growth and development, Fruit development and composition, Grapevines and water usage, Environment and plant responses to climate change, Pathogens and disease resistance, New advances in genomics and functional genomics, and New advances in plant physiology. A special issue of the Australian Journal of Grape and Wine Research will contain reviews from keynote speakers, and abstracts oral and posters presentations. These reviews will capture the essence of the symposium and will be of great benefit to researchers and practitioners alike. As an indication of the outcomes of the symposium some of the titles of the reviews received to date are: The use of genetic transformation for functional genomics in grapevine. Conventional and biotechnological approaches for the improvement of table grapes: a review. Managing grapevines to optimise fruit development in a challenging environment: a viticultural climate change primer. Improving water-use-efficiency in grapevines: potential physiological targets for biotechnological improvement. Molecular strategies to enhance the genetic resistance of grapevines to powdery mildew. Several areas of R&D focus were identified in the presentations and discussions. Of primary importance was the desire to co-ordinate effort internationally, particular for large projects relating to climate change and grapevine systems biology and genomics.

Evaluation of effectiveness

Feedback from delegates: only verbal feedback was obtained from delegates. This was generally very positive. Delegates were especially impressed with the integration of the molecular and biotechnology areas with the physiology and biology of grapevines. Positive comment was also obtained for: the venue (and seating arrangements that fostered interactions); the fact that all presentations (oral and posters) will have abstracts published in a special issue of Australian Journal of Grape and Wine Research; our consideration of student participation and student prizes awarded for best posters. We had one negative comment regarding a missing delegate name in the conference proceedings. We also had comment from some of the European delegates about not having hot full meals (with wine) for lunch, but we deliberately did not do this in the interests of full attendance at the afternoon sessions.

As another indicator of effectiveness: **Media coverage:** ABC SA Country Hour, (see below), CSIRO Times, Australian Wine 2030 Newsletter, South Australian Wine Industry (Ass. Inc.) Newsletter, Riverlink, GWRDC Newsletter, Wine Business Magazine, Australian Wine Industry Journal.

Grapes on the menu (ABC SA Country Hour) http://www.abc.net.au/rural/sa/content/2006/s2432142.htm By Annabelle Homer

Friday, 28/11/2008

If you're into the scientific makeup of the viticultural world - Adelaide is the place to be this week as the International Symposium on Grapevine Physiology and Biotechnology is being held at the National Wine Centre. Over 150 delegates from around the world are here to find out more behind the humble grape and it's genetic makeup. One of the big topics of discussion is how grapes cope with stress which is of particular interest considering the current climate conditions. Two professionals in the science in the industry are Chris Ford, senior lecturer wine and horticulture Waite Campus, University of Adelaide and Professor Grant Cramer, from the University of Nevada. Chris Ford says he's looking at how the plant grows in the vineyard and how to better improve its productivity. Mr Ford has been impressed by recent breakthroughs. "From our research perspective in the last couple of years the availability of an entire genetic sequence of the grapevine." he says. "To put that in simple terms, as scientists, we've arrived at grape vine city and we've now been given this very detailed street atlas. Within that street atlas there is the structure of the city, how it's all laid out and then there all the instructions on how you'd get from one part of the city to another." This means scientists now understand how the grape vine makes it leaves, takes up water and how the berries are made and the qualities in the berries. It also means that scientists can measure how grapes can cope in stressful climate conditions - such as drought. Professor Cramer specialises in environmental stress in the wine industry. "Normally when the grape vine is growing it's going to put is photosynthetic energy into different parts of the plant including the shoot tips which compete with the fruit." he says. "So when you put a water stress on the vine the shoot tips stop growing and that energy can now be directed more fully to the fruit." In this report: Chris Ford, senior lecturer wine and horticulture Waite Campus, University of Adelaide; Professor Grant Cramer, University of Nevada



Program

National Wine Centre of Australia Corner of Botanic and Hackney Roads, Adelaide November 2008

Sunday 23rd November 2008

Time	Session
17.30 - 19.30	Cocktail Reception - Welcome Mixer
	Venue: National Wine Centre of Australia - Pod 3
	Welcome: Professor Steve Tyerman – University of Adelaide (Australia)

Monday 24th November 2008

Venues

Registration: Entrance to Hickinbotham Hall, National Wine Centre of Australia

Sessions: All sessions will be held in Hickinbotham Hall

Poster Presentations: Refer to the 'Poster Program' at registration desk to confirm your allocated time to display your poster. Posters will be displayed in "The Vines"

Time	Session
7.30 - 8.30	Delegate Registration
8.30 - 10.30	Session 1
	Chair: Dr Paul Petrie – Foster's Group Limited (Australia)
	Key note speakers:
	1.1 Mr Peter Hayes - International Organisation of Vine and Wine (OIV) Scene setting: using the genotype and management to cope with environmental challenges
	1.2 Professor Hans Schultz - Forschungsanstalt Geisenheim (Germany)Plant-Environment research status, what it can offer to address the challenges and limitations
	1.3 Mr Peter Clingeleffer - CSIRO Plant Industry (Australia)Plant management research-status, what it can offer to address challenges and limitations
	1.4 Professor Martinez Zapater - Dpto. de Genética Molecular de Plantas (Spain)Plant Genetic research - status, what it can offer to address the challenges and limitations
	10.30 – 11.00 ~ Morning tea
11.00 - 12.30	Session 2
	Chair: Professor Greg Dunn – University of Melbourne (Australia)
	Key note speaker: 2.1 Professor Peter Dry – AWRI, University of Adelaide (Australia) Reproductive performance of the grapevine: effect of site, season and cultural practices
	2.2 Dr Suzy Rogiers - NSW Department of Primary Industries (Australia) The contribution of photoassimilates and carbohydrate reserves to fruit set in Chardonnay vines
	Dr Lucie Fernandez - CNB (Spain) The Reiterative Reproductive Meristems (RRM) phenotype of a Carignan somatic variant is associated to a transposon insertion in the VvTFL1A gene promoter
	2.4 Dr Cassandra Collins - University of Adelaide (Australia) Polyamines in molybdenum-deficient Merlot
	12.30 – 14.00 ~ Lunch and poster viewing

14.00 - 15.30	Session 3
	Chair: Dr Mark Krstic – Grape and Wine Research and Development Corporation (Australia)
	Key note speaker: 3.1 Dr Markus Keller – Washington State University (USA) Managing vines to optimise fruit development in a challenging environment
	3.2 Ms Vanessa Melino - University of Adelaide (Australia) Ascorbate metabolism during grape berry development
	3.3 Mr Kazuya Koyama - National Research Institute of Brewing (Japan) Effect of bunch shading during different developmental stages on the flavonoid biosynthesis in berry skins of Cabernet Sauvignon grape
	3.4 Dr Philippe Vivin - INRA (France) Simulating berry growth and sugar concentration in a ripening grape with a process-based fruit model
	$15.30 - 16.00 \sim \text{Afternoon tea}$
16.00 - 17.30	Session 4
	Chair: Professor Snow Barlow – University of Melbourne (Australia)
	Key note speaker: 4.1 Professor Grant Cramer – University of Nevada (USA) Abiotic stress & plant responses – from the whole vine to the genes
	Dr Chris Soar - South Australian Research and Development Institute (Australia) Resilience of Shiraz exposed to short episodes of heat stress in the field
	4.3 Dr Victor Sadras - SARDI (Australia)Phenotypic plasticity of yield and phenology in grapevine
	Dr Anuradha Upadhyay - National Research Centre for Grapes Biotechnology (India) Changes in gene expression in response to heat stress during berry development in grapevine
1	

Tuesday 25th November 2008

Time	Session
9.00 - 10.30	Session 5
	Chair: Professor Rob Walker – CSIRO Plant Industry (Australia)
	Key note speaker: 5.1 Professor Alain Carbonneau - Montpellier SupAgro (France) A review of canopy management research: From field observation to modeling, and back to vineyard and beyond
	5.2 Dr Jeff Bennett - Marlborough Wine Research Centre (New Zealand) The influence of vine management on Sauvignon blanc performance and carbohydrate reserves
	5.3 Dr Bruno Holzapfel - Charles Sturt University (Australia) The relationship between carbohydrate reserve dynamics and vine productivity
	Professor Alan Lakso - Cornell University (USA) "VitiSim", a simplified model of grapevine dry matter production, partitioning and fruit abscission
	10.30 – 11.00 ~ Morning Tea

11.00 - 12.30	Session 6
	Chair: Dr Amanda Walker – CSIRO Plant Industry (Australia)
	Key note speaker: 6.1 Professor Brian Smith-White - National Center for Biotechnology Information (USA) Genomics & bioinformatics – The role of NCBI
	6.2 Dr Jérôme Grimplet - South Dakota State University (USA) Systems Biology of the Grapevine
	 6.3 Mr Álvaro Cuadros-Inostroza - Max Planck Institute of Molecular Plant Physiology (Germany) Characterization of the grapevine development and ripening by integrated analysis of transcriptome and metabolome
	Ms Camila Gomez - INRA (France) Two <i>Vitis vinifera</i> MATE proteins act as tonoplast acylated anthocyanin/H ⁺ antiporters
	12.30 – 14.00 \sim Lunch and poster viewing
14.00 - 15.30	Session 7
	Chair: Dr Chris Ford – University of Adelaide (Australia)
	Key note speaker: 7.1 Dr Steven Lund - University of British Columbia (Canada) Expression profiling and kinetic characterization of a flavonol- and anthocyanin-3'5'-O- methyltransferase (FAOMT) from grapevine - implications for anthocyanin stability
	7.2 Dr Chris Winefield- Lincoln University (New Zealand) Characterisation of the LOX-HPL biochemical pathway as a potential source of distinct flavour and aroma characteristics of Sauvignon blanc wine
	Dr Mark Downey - Department of Primary Industries (Australia) Influence of bunch exposure on flavonol accumulation in Shiraz and Cabernet Sauvignon grape skin
	7.4 Dr Bhaskar Bondada - Washington State University (USA) Physio-anatomical and compositional characterization of berry shrivel - a ripening disorder of grapevine
	15.30 – 16.00 ~ Afternoon tea
16.00 - 17.30	Session 8
	Chair: Professor Chris Steel – National Wine & Grape Industry Centre (Australia)
	Key note speaker: 8.1 Dr Ian Dry – CSIRO Plant Industry (Australia) Molecular strategies to enhance the genetic resistance of grapevines to fungal pathogens
	8.2 Professor Melane Vivier - Stellenbosch University (South Africa)Functional analysis of Polygalacturonase-inhibiting proteins (PGIPs) from <i>Vitis</i> species:Identification of an additional role in plant defense
	Dr Wenping Qiu - Missouri State University (USA) Functional analyses of a grapevine mitogen-activated protein kinase kinase gene
	Professor Jean-Marc Neuhaus - University of Neuchatel (Switzerland) Beta-aminobutyric acid-induced resistance in grapevine against downy mildew and abiotic stress
	17.30 onwards \sim Poster viewing and social networking

Time	Session
8.15 - 17.30	McLaren Vale Wine Region Tour – See separate "Tour Program" Buses depart promptly from 'The National Wine Centre' at 8.30am (lunch provided)
18.00 - 19.00	Poster viewing and social networking
19 00 - 23 30	Symnosium Dinner

Wednesday 26th November 2008

Thursday 27th November 2008

Time	Session
9.00 - 10.30	Session 9 Chair: Dr Nicola Cooley – University of Melbourne (Australia)
	Key note speaker: 9.1 Dr Jaume Flexas – Universitat de les Illes Balears (Spain) Improving water-use-efficiency in grapevines: physiological and molecular approaches
	Dr Rebecca Vandeleur – University of Adelaide (Australia) The role of aquaporins in controlling root hydraulic conductance in grapevine
	Dr Felicidad de Herralde - Institut de Recerca i Tecnologia (Spain) Influence of rootstock and water availability on young 'Grenache' vines growth, water use efficiency and yield
	9.4 Dr Marisa Collins – CSIRO Plant Industry (Australia) Characterising water-use strategies of major winegrape varieties grown in Australia
	10.30 – 11.00 ~ Morning Tea
11.00 - 12.30	Session 10
11.00 - 12.30	Session 10 Chair: Dr Ian Dry – CSIRO Plant Industry (Australia)
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11.00 – 12.30	 Session 10 Chair: Dr Ian Dry – CSIRO Plant Industry (Australia) Key note speaker: 10.1 Dr Gabriele di Gaspero - University of Udine (Italy) Application of genomics to grapevine improvement 10.2 Dr Laura Costantini - Fondazione Edmund Mach-Istituto Agrario di San Michele all'Adige (Italy) Characterization of major quantitative trait loci controlling berry and phenology – related traits
11.00 – 12.30	 Session 10 Chair: Dr Ian Dry – CSIRO Plant Industry (Australia) Key note speaker: 10.1 Dr Gabriele di Gaspero - University of Udine (Italy) Application of genomics to grapevine improvement 10.2 Dr Laura Costantini - Fondazione Edmund Mach-Istituto Agrario di San Michele all'Adige (Italy) Characterization of major quantitative trait loci controlling berry and phenology – related traits 10.3 Dr Reinhard Töpfer - Julius Kühn-Institute (Germany) Alleles of anthocyanin 5-glucosyltransferase of <i>Vitis vinifera</i> are non-functional
11.00 – 12.30	 Session 10 Chair: Dr Ian Dry – CSIRO Plant Industry (Australia) Key note speaker: 10.1 Dr Gabriele di Gaspero - University of Udine (Italy) Application of genomics to grapevine improvement 10.2 Dr Laura Costantini - Fondazione Edmund Mach-Istituto Agrario di San Michele all'Adige (Italy) Characterization of major quantitative trait loci controlling berry and phenology – related traits 10.3 Dr Reinhard Töpfer - Julius Kühn-Institute (Germany) Alleles of anthocyanin 5-glucosyltransferase of <i>Vitis vinifera</i> are non-functional Dr Etti Or - Volcanu center, ARO (Israel) A lesson from a large-scale comparative analysis of bud response to different artificial dormancy stimuli

14.00 - 15.30	Session 11 Chaine De Dennis Crean - Charles Sturt University (Australia)
	Key note speaker : 11.1 Dr Louise Comas – Penn State University (USA)
	Biological and environmental factors controlling root dynamics and functioning: consequences of root aging affects and soil moisture
	11.2 Mr Stewart Field - Charles Sturt University (Australia) Seasonal carbohydrate, nitrogen and cytokinin dynamics and their impact on shoot and root growth
	11.3 Mr David Oag - Department of Primary Industries & Fisheries Horticulture & Forestry Australia)Timing of 15N-depleted ammonium nitrate uptake by grapevines in a subtropical environment
	11.4 Dr Everard Edwards - CSIRO Plant Industry (Australia)Above and below-ground effects of six years of deficit irrigation in Cabernet Sauvignon grapevines
	15.30 – 16.00 ~ Afternoon tea
16.00 - 17.30	Session 12
	Chair: Dr Mark Downey – Department of Primary Industries, Victoria (Australia)
	Key note speaker: 12.1 Dr Laurent Torregrosa - Montpellier SupAgro (France) Grapevine functional genomics and transgenic technology
	12.2 Dr Anne Fennell - South Dakota State University (USA) Functional genomics of dormancy induction in grapevines
	12.3 Dr Maria Cruz Cutanda - UCLM (Spain)Over-expression of VvHB13 gene changes the size of different organs in transgenic tobacco plants
	Dr Amanda Walker - CSIRO Plant Industry (Australia) Altering flavonoid composition in grapevine
	Mr Peter Hayes - International Organisation of Vine and Wine (OIV) Nomination and selection of the host for the "9 th International Symposium on Grapevine Physiology and Biotechnology"
	17.30 onwards ~ Poster viewing and social networking

Friday 28th November 2008

Time	Session
9.00 - 10.30	Session 13
	Chair: Dr Simon Robinson - CSIRO (Australia)
	Key note speaker: 13.1 Dr Avi Perl - Colcani Center (Israel) Conventional and biotechnological approaches for the improvement of table grapes
	13.2 Dr Claudio Moser - E. Mach Foundation-IASMA (Italy) A molecular study of the regulation of grape berry ripening
	13.3 Dr Violeta (Tsolova) Colova - Florida A & M University (USA) Comparative analyses of differentially expressed genes involved in flavonoid biosynthesis in North American native grapes: 'Noble' and 'Ison' muscadines vars., and 'Cynthiana' aestivalis var
	 Professor Mario Pezzotti – University of Verona (Italy) Systems biology of berry ripening and postharvest withering processes
	10.30 – 11.00 ~ Morning Tea

11.00 - 12.30	 Session 14 Chair: Dr Mark Thomas – CSIRO Plant Industry (Australia) Key note speaker: 14.1 Professor Serge Delrot - UMR Ecophysiologie et Genomique (France) Understanding the biology: Moving away from observation based science to predictive science
	14.2 Dr Claudio D'Onofrio - University of Pisa (Italy) Molecular characterization of aroma genes in <i>Vitis vinifera</i> var. moscato bianco
	3 Dr Manuel Pinto - INIA (Chile) Characterization of early light inducible proteins (ELIPs) in <i>Vitis vinifera</i> L.
	14.4 Dr Nathalie Ollat - INRA (France) Differential gene expression in <i>Vitis</i> genotypes submitted to iron deficiency
12.30 - 12.45	Closing symposium presentation Chair: Professor Steve Tyerman Farewell with lunch provided

First Name	Last Name	Organization Name	Country
Phil	Abel	Hilltop Wines	NEW ZEALAND
Patricio	Arce-Johnson	Pontificia Universidad Catolica De Chile	CHILE
Nardia	Baker	DPI - Victoria	AUSTRALIA Vic
Snow	Barlow	The University of Melbourne	AUSTRALIA Vic
Shay	Bayly	Rural Press Limited	AUSTRALIA SA
Sally-Jean	Bell	The Australian Wine Research Institute	AUSTRALIA SA
Jeff	Bennett	Hort Research	NEW ZEALAND
Bhaskar	Bondada	Washington State University Tri-Cities	USA
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Gabriele	Di Gaspero	University of Udine	ITALY
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Joy	Dick	Orlando Wines	AUSTRALIA SA
Nieves	Diestro Sanchez		SPAIN
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Troy	Fischer	GWRDC	AUSTRALIA SA
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Vittorino	Novello	University of Turin	ITALY
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Nathalie	Ollat	INRA	FRANCE
Mercy	Olmstead	Washington State University	USA
Etti	Or	Volcani Center ARO	ISRAEL
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Manuel	Pinto	Instituto de Investigaciones Agropecuarias	CHILE
Andriy	Podolyan	Lincoln University	NEW ZEALAND
Wenping	Qiu	Missouri State University	USA
Mary	Retallack	Scholefield Robinson Horticultural Services	AUSTRALIA SA
Amy	Richards	McLaren Vale Grape, Wine & Tourism Association	AUSTRALIA SA
Simon	Robinson	CSIRO	AUSTRALIA SA
Suzy	Rogiers	National Wine and Grape Industry Centre	AUSTRALIA NSW
Ariel	Rotman		ISRAEL
Ernst	Ruhl	Geisenheim Research Centre	GERMANY
Victor	Sadras	SARDI	AUSTRALIA SA
Manuel	Sanchez-Diaz		SPAIN
Beverley	Sandow		AUSTRALIA
Alex	Sas	Constellation Wines Australia	AUSTRALIA SA
Hans	Schultz	Forschungsanstalt Geisenheim	GERMANY
	Senanz	i ofsendingsanstart Gersennenn	OLIGINITY

Brian	Smith-White	National Center for Biotechnology Information	USA
Chris	Soar	South Austalian Research and Development Institute	AUSTRALIA SA
Magna	Soelma	BR 428 Km 152 CP23zONE Rural	BRAZIL
Anthony	Somers	DPI - NSW	AUSTRALIA NSW
Kathleen	Soole	Flinders University of South Australia	AUSTRALIA SA
Jim	Speirs	CSIRO	AUSTRALIA SA
Chris	Steel	National Wine & Grape Industry Centre	AUSTRALIA NSW
Manfred	Stoll	Geisenheim Research Centre	GERMANY
Crystal	Sweetman	Flinders University of South Australia	AUSTRALIA SA
Toby	Tennent		AUSTRALIA SA
Marc	Thomas	Charles Sturt University - NWGIC	AUSTRALIA SA
Mark	Thomas	C.S.I.R.O. Plant Industry	AUSTRALIA SA
Reinhard	Topfer	Julius Kühn-Institute for Grapevine Breeding	GERMANY
Laurent	Torregrosa	UMR BEPC, Campus Agro-M/INRA, 2	FRANCE
Steve	Tyerman	The University of Adelaide	AUSTRALIA SA
Anuradha	Upadhayay	National Research Centre for Grapes	INDIA
Rebecca	Vandeleur	The University of Adelaide	AUSTRALIA SA
Carmo	Vasconcelos	HortResearch	NEW ZEALAND
Andrea	Vega	Pontificia Universidad Catolica de Chile	CHILE
Jose	Vidal	Universidad de Santiago de Compostela	SPAIN
Melane	Viver	Institute for Wine Biotechnology	SOUTH AFRICA
Philippe	Vivien	ISVV Bordeaux	FRANCE
Rob	Walker	CSIRO Plant Industry	AUSTRALIA Vic
Amanda	Walker	CSIRO Plant Industry	AUSTRALIA SA
Andrea	Watt	University of Melbourne	AUSTRALIA VIC
Ashley	Wheaton	University of Melbourne	AUSTRALIA VIC
Karen	Wilson		AUSTRALIA
Chris	Winefield	Lincoln University	NEW ZEALAND
Ayalsew	Zerihun	Curtin University of Technology	AUSTRALIA WA
Ying	Zhu	CSIRO Plant Industry	AUSTRALIA

Key Outcomes

- Willingness to co-ordinate large R&D projects internationally in the areas of climate change, grapevine genomics, and systems biology.
- Decision to hold another conference in the series for 2012 in Santiago, Chile.
- Publishing of the special issue of the Australian Journal of Grape and Wine Research containing major reviews from keynote speakers on important topics related to climate change, vine physiology, flowering and fruit-set, table grape breeding, and grapevine functional genomics.
- Showcasing Australian grapevine R&D to major international groups and fostering of future international collaborations.
- Allowing Australian researchers to benchmark their research against the best internationally.
- Good media coverage giving opportunity for the general public and industry to find out about the conference and the issues being discussed.
- Opportunity for delegates from industry sponsoring organisations to attend and contribute, and directly apply the results of research in their vineyards.
- Sponsored keynote speakers stayed on in Australia, and in several cases visited the sponsoring organisations for more detailed discussions and further seminars.
- There have since been at least two funding applications from over seas delegates to come to Australia to further their research efforts with Australian scientists.

Recommendations

It was clear from this conference that we face major challenges in grape production world wide. All the problems that have been recently evident in Australia (drought, water quality, resources, climate change (carbon dioxide and temperature, pests and diseases, use of GMOs) are clearly foremost in all grape growing nations. Many R&D projects are similar from one nation to another and there is a real need to consolidate this effort for faster advancement in the face of the challenges listed above. It is recommended that funding providers consider mechanisms to allow greater international collaboration of R&D effort.

Acknowledgments

We wish to acknowledge: Phillippa Paterson (previous executive officer of ASVO) for her help and efforts to secure sponsorship and the ASVO staff: Mary Andrews, Sue Milnes, and Christina Ursini for their strong commitment and effort.

Many people helped make the conference a success and we acknowledge their help and support:

Co-chairs

Professor Steve Tyerman – University of Adelaide Dr Paul Petrie – Foster's Group Ltd.

Secretary

Mr Peter Hayes

Committee Members

Dr Mark Thomas – CSIRO Plant Industry, Professor Snow Barlow – University of Melbourne, Dr Robert Walker – CSIRO Plant Industry.

Scientific Committee

Co-chairs Associate Professor Greg Dunn – University of Melbourne,

Dr Mark Thomas - CSIRO Plant Industry

Committee Members: Dr Nicola Cooley (University of Melbourne), Dr Ian Dry (CSIRO Plant Industry), Dr Steve Swain (CSIRO Plant Industry), Dr Mandy Walker (CSIRO Adelaide), Prof. Steve Tyerman (University of Adelaide), Dr Christopher Ford (University of Adelaide), Dr Cassandra Collins (University of Adelaide), Dr Chris Soar (South Australian Research and Development Institute — SARDI), Dr Mark Downey (Department of Primary Industries, Victoria), Dr Mark Krstic (GWRDC), Dr Dennis Greer (NWGIC, Charles Sturt University).

International Scientific Committee

Dr Cecilia Agüero (Argentina), Dr Steven Lund (Canada), Dr Anne-Francoise Adam-Blondon (France), Dr Laurent Torregrosa (France), Prof. Hans Schultz (Germany), Prof. Kalliopi A. Roubelakis-Angelakis (Greece), Prof Avichai Perl (Israel), Prof. Raffaele Testolin (Italy), Prof. José Miguel Martinez Zapater (Spain), Prof. Jaume Flexas (Spain), Prof. Jean-Marc Neuhaus (Switzerland), Prof. Markus Keller (USA), Prof. Larry Williams (USA), Prof. Grant Cramer (USA), Prof. Mark Matthews (USA), Prof. Serge Delrot (France), Prof. Alain Carbonneau (France), Prof. Riccardo Velasco (Italy), Dr Nami Goto-Yamamoto (Japan).

University of Melbourne pre-conference tour organisers

Professor Snow Barlow, Associate Professor Gregory Dunn, Ashley Wheaton, Sonja Needs, Dr Nicola Cooley, Andrea Watt, Dr Leanne Webb.

McClaren Vale Conference Tour

Dr Cassandra Collins and Vanessa Melino for organising and catering; Paxtons (Toby Bekkers) and Rosemount Estate Fosters Group McClaren Vale (David Hansen) for hosting the conference tour, and Amy Richards (Viticulture Officer McLaren Vale Grape, Wine & Tourism) for her help and hosting.

Amy Russell, Director - Natural Resources Winemakers' Federation of Australia, for her excellent address at the conference dinner.

Staff of the Adelaide National Wine Centre.

Appendix 1 Accounts

8ISGPB

GPO Box 582 ADELAIDE SA 5001

Profit & Loss Statement

July 2008 through June 2009

26/02/2009 4:53:36 PM

Income Registrations Pre-conf bus tour Sponsorship Total Income	\$70,208.58 \$11,800.00 \$71,681.14 \$153,689.72
Cost of Sales	
Gross Profit	\$153,689.72
Expenses Venue hire & catering Pre-conf bus tour Bus tour Bank Charges HAL Project funding Seed funding repayments Equipment Rental Expense Speaker Accommodation/meals Speaker expenses reimbursement Student poster prizes Insurance Secretariat fees Total Expenses	\$68,130.37 \$11,800.00 \$3,177.50 \$60.79 \$19,499.95 \$10,000.00 \$2,319.02 \$18,560.82 \$32,615.77 \$1,000.00 \$850.50 \$16,964.15 \$184,978.87
Operating Profit	-\$31,289.15
Other Income	
Net Surplus / (Deficit)	-\$31,289.15

Appendix 2

Proceedings

8th International Symposium on Grapevine Physiology and Biotechnology

Book of Abstracts

Adelaide, Australia 23rd to 28th November 2008 Dear Delegate,

On behalf of the organising committee and our sponsors it is a pleasure to welcome you to the 8th International Symposium on Grapevine Physiology and Biotechnology and to the city of Adelaide in the State of South Australia.

The goal of this symposium is to showcase the latest research across the spectrum of disciplines from applied physiology through to cutting edge molecular biology with a focus on where new techniques in biotechnology are being used to advance our understanding of vine physiology.

The main theme of the Symposium is:

21st Century Grape Research: Challenges and Opportunities

Research articles and reviews from plenary and selected speakers from the symposium will be published in a special issue of the Australian Journal of Grape and Wine Research. The Australian Journal of Grape and Wine Research has the highest impact factor for journals specialising in viticulture and oenology.

The National Wine Centre of Australia, which is managed by the University of Adelaide, makes an excellent venue for the symposium, and is a showcase for the Australian Wine Industry. Please take the "Wine Discovery Journey" which is a state-of-the-art, award-winning interactive wine experience.

Within an hours drive of the city of Adelaide are the premium wine regions of the Barossa Valley, Adelaide Hills and McLaren Vale. You will have the opportunity to visit McLaren Vale as part of the symposium tour on Wednesday. We encouraged you to make the most of your visit and partake in the diversity of fine food and wine on offer, not only in South Australia but also in other regions and States of Australia.

There are over 150 delegates registered, with a similar number of poster and oral presentations planned. Due to the large numbers of posters submitted we have split the poster viewing into two sessions. Please take every opportunity to view the posters during the lunch breaks, the "poster mixers" and prior to the conference dinner.

Once again welcome to Adelaide and the 8thISGPB

Paul Petrie

Paul Petrie

I teve Tyerman

Steve Tyerman

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Sunday 23rd November 2008

Time Session

17.30 - 19.30 COCKTAIL RECEPTION – Welcome Mixer

Venue: National Wine Centre of Australia - Pod 3

Welcome: Professor Steve Tyerman - University of Adelaide (Australia)

Monday 24th November 2008

VENUES

Registration: Entrance to Hickinbotham Hall, National Wine Centre of Australia

Sessions: All sessions will be held in Hickinbotham Hall

Poster Presentations: Refer to the 'Poster Program' at registration desk to confirm your allocated time to display your poster. Posters will be displayed in "The Vines"

Time	Session	
7.30 - 8.30	DELEGATE REGISTRATION	
8.30 - 10.30	SESSION 1	
	Chair: Dr Paul Petrie – Foster's Group Limited (Australia)	
	Key note speakers:	
	1.1 Mr Peter Hayes - International Organisation of Vine and Wine (OIV)	
	Scene setting: using the genotype and management to cope with environmental challenges	
	1.2 Professor Hans Schultz - Forschungsanstalt Geisenheim (Germany) Plant-Environment research status, what it can offer to address the challenges and limitations	
	 Mr Peter Clingeleffer - CSIRO Plant Industry (Australia) Plant management research-staus, what it can offer to address challenges and limitations 	
	1.4 Professor Martinez Zapater - Dpto. de Genética Molecular de Plantas (Spain) Plant Genetic research - status, what it can offer to address the challenges and limitations	
	10.30 – 11.00 ~ Morning tea	
11.00 – 12.30	SESSION 2	

Chair: Professor Greg Dunn – University of Melbourne (Australia)

Key note speaker:

- 2.1 Professor Peter Dry AWRI, University of Adelaide (Australia) Reproductive performance of the grapevine: effect of site, season and cultural practices
- 2.2 Dr Suzy Rogiers NSW Department of Primary Industries (Australia) The contribution of photoassimilates and carbohydrate reserves to fruit set in Chardonnay vines

Monday 24th November 2008 (cont) Time Session 2.3 Dr Lucie Fernandez – CNB (Spain) The Reiterative Reproductive Meristems (RRM) phenotype of a Carignan somatic variant is associated to a transposon insertion in the VvTFL1A gene promoter Dr Cassandra Collins - University of Adelaide (Australia) 2.4 Polyamines in molybdenum-deficient Merlot 12.30 – 14.00 ~ Lunch and poster viewing 14.00 - 15.30 **SESSION 3** Chair: Dr Mark Krstic - Grape and Wine Research and Development Corporation (Australia) Key note speaker: 3.1 Dr Markus Keller - Washington State University (USA) Managing vines to optimise fruit development in a challenging environment 3.2 Ms Vanessa Melino - University of Adelaide (Australia) Ascorbate metabolism during grape berry development 3.3 Mr Kazuya Koyama - National Research Institute of Brewing (Japan) Effect of bunch shading during different developmental stages on the flavonoid biosynthesis in berry skins of Cabernet Sauvignon grape Dr Philippe Vivin - INRA (France) 3.4

Simulating berry growth and sugar concentration in a ripening grape with a processbased fruit model

15.30 - 16.00 ~ Afternoon tea

16.00 – 17.30 SESSION 4

Chair: Professor Snow Barlow – University of Melbourne (Australia)

Key note speaker:

- 4.1 Professor Grant Cramer University of Nevada (USA)Abiotic stress & plant responses from the whole vine to the genes
- 4.2 Dr Chris Soar South Australian Research and Development Institute (Australia) Resilience of Shiraz exposed to short episodes of heat stress in the field
- 4.3 Dr Victor Sadras SARDI (Australia) Phenotypic plasticity of yield and phenology in grapevine
- 4.4 Dr Anuradha Upadhyay National Research Centre for Grapes Biotechnology (India) Changes in gene expression in response to heat stress during berry development in grapevine

17.30 onwards ~ Poster viewing and social networking

Tuesday 25 th I	November 2008	
Time	Session	
9.00 - 10.30	SESSION 5	
	Chair: Professor Rob Walker – CSIRO Plant Industry (Australia)	
	 Key note speaker: 5.1 Professor Alain Carbonneau – Montpellier SupAgro (France) A review of canopy management research: From field observation to modeling, and back to vineyard and beyond 	
	5.2 Dr Jeff Bennett – Marlborough Wine Research Centre (New Zealand) The influence of vine management on Sauvignon blanc performance and carbohydrate reserves	
	 5.3 Dr Bruno Holzapfel – Charles Sturt University (Australia) The relationship between carbohydrate reserve dynamics and vine productivity 	
	 5.4 Professor Alan Lakso – Cornell University (USA) "VitiSim", a simplified model of grapevine dry matter production, partitioning and fruit abscission 	
	10.30 – 11.00 ~ Morning Tea	
11.00 - 12.30	SESSION 6	
	Chair: Dr Amanda Walker – CSIRO Plant Industry (Australia)	
	 Key note speaker: 6.1 Professor Brian Smith-White - National Center for Biotechnology Information (USA) Genomics & bioinformatics – The role of NCBI 	
	6.2 Dr Jérôme Grimplet – South Dakota State University (USA) Systems Biology of the Grapevine	
	 6.3 Mr Álvaro Cuadros-Inostroza – Max Planck Institute of Molecular Plant Physiology (Germany) Characterization of the grapevine development and ripening by integrated analysis of transcriptome and metabolome 	
	6.4 Ms Camila Gomez – INRA (France) Two Vitis vinifera MATE proteins act as tonoplast acylated anthocyanin/H+ antiporters	

12.30 – 14.00 ~ Lunch and poster viewing

14.00 – 15.30 SESSION 7

Chair: Dr Chris Ford – University of Adelaide (Australia)

Key note speaker:

- 7.1 Dr Steven Lund University of British Columbia (Canada)
 Expression profiling and kinetic characterization of a flavonol- and anthocyanin-3'5' O-methyltransferase (FAOMT) from grapevine implications for anthocyanin stability
- 7.2 Dr Chris Winefield Lincoln University (New Zealand) Characterisation of the LOX-HPL biochemical pathway as a potential source of distinct flavour and aroma characteristics of Sauvignon blanc wine

Tuesday 25th November 2008 (cont)

Time	Session
	7.3 Dr Mark Downey - Department of Primary Industries, Victoria (Australia)
	Influence of bunch exposure on flavonol accumulation in Shiraz and Cabernet
	Sauvignon grape skin
	7.4 Dr Bhaskar Bondada – Washington State University (USA)
	Physio-anatomical and compositional characterization of berry shrivel - a ripening
	disorder of grapevine
	15.30 – 16.00 ~ Afternoon tea
16.00 – 17.30	SESSION 8
	Chair: Professor Chris Steel – National Wine & Grape Industry Centre (Australia)
	Key note speaker:
	8.1 Dr Ian Dry – CSIRO Plant Industry (Australia)
	Molecular strategies to enhance the genetic resistance of grapevines to fungal pathogens
	8.2 Professor Melane Vivier – Stellenbosch University (South Africa)
	Functional analysis of Polygalacturonase-inhibiting proteins (PGIPs) from Vitis
	species: Identification of an additional role in plant defense
	8.3 Dr Wenping Qiu – Missouri State University (USA)
	Functional analyses of a grapevine mitogen-activated protein kinase kinase gene
	8.4 Professor Jean-Marc Neuhaus – University of Neuchatel (Switzerland)
	Beta-aminobutyric acid-induced resistance in grapevine against downy mildew and abiotic stress

Wednesday 26th November 2008

,	
Time	Session
8.15 – 17.30	MCLAREN VALE WINE REGION TOUR
	See separate "Tour Program"
	Buses depart promptly from 'The National Wine Centre' at 8.30am (lunch provided)
18.00 – 19.00	Poster viewing and social networking
19.00 – 23.30	SYMPOSIUM DINNER
	Venue: National Wine Centre of Australia - Hickinbotham Hall

Thursday 27 th	November 2008	
Time	Session	
9.00 – 10.30	SESSION 9	
	Chair: Dr Nicola Cooley – University of Melbourne (Australia)	
	 Key note speaker: 9.1 Dr Jaume Flexas – Universitat de les Illes Balears (Spain) Improving water-use-efficiency in grapevines: physiological and molecular approaches 	
	9.2 Dr Rebecca Vandeleur – University of Adelaide (Australia) The role of aquaporins in controlling root hydraulic conductance in grapevine	
	9.3 Dr Felicidad de Herralde – Institut de Recerca i Tecnologia (Spain) Influence of rootstock and water availability on young 'Grenache' vines growth, water use efficiency and yield	
	9.4 Dr Marisa Collins – CSIRO Plant Industry (Australia) Characterising water-use strategies of major winegrape varieties grown in Australia	
	10.30 – 11.00 ~ Morning Tea	
11.00 – 12.30	SESSION 10	
	Chair: Dr Ian Dry – CSIRO Plant Industry (Australia)	
	Key note speaker:	
	10.1 Dr Gabriele di Gaspero - University of Udine (Italy) Application of genomics to grapevine improvement	
	10.2 Dr Laura Costantini – Fondazione Edmund Mach-Istituto Agrario di San Michele all'Adige (Italy) Characterization of major quantitative trait loci controlling berry and phenology – related traits	
	10.3 Dr Reinhard Töpfer - Julius Kühn-Institute (Germany) Alleles of anthocyanin 5-glucosyltransferase of <i>Vitis vinifera</i> are non-functional	
	10.4 Dr Etti Or – Volcanu center, ARO (Israel) A lesson from a large-scale comparative analysis of bud response to different artificial dormancy stimuli	
	12.30 – 14.00 ~ Lunch and poster viewing	
14.00 – 15.30	SESSION 11	

Chair: Dr Dennis Greer – Charles Sturt University (Australia)

Key note speaker:

- 11.1 Dr Louise Comas Penn State University (USA)
 Biological and environmental factors controlling root dynamics and functioning: consequences of root aging affects and soil moisture
- 11.2 Mr Stewart Field Charles Sturt University (Australia) Seasonal carbohydrate, nitrogen and cytokinin dynamics and their impact on shoot and root growth

Thursday 27th November 2008 (cont) Time Session 11.3 Mr David Oag - Department of Primary Industries & Fisheries, Horticulture & Forestry Australia) Timing of ¹⁵N-depleted ammonium nitrate uptake by grapevines in a subtropical environment 11.4 Dr Everard Edwards - CSIRO Plant Industry (Australia) Above and below-ground effects of six years of deficit irrigation in Cabernet Sauvignon grapevines 15.30 - 16.00 ~ Afternoon tea 16.00 - 17.30 **SESSION 12** Chair: Dr Mark Downey - Department of Primary Industries, Victoria (Australia) Key note speaker: 12.1 Dr Laurent Torregrosa – Montpellier SupAgro (France) Grapevine functional genomics and transgenic technology 12.2 Dr Anne Fennell – South Dakota State University (USA) Functional genomics of dormancy induction in grapevines 12.3 Dr Maria Cruz Cutanda – UCLM (Spain) Over-expression of VvHB13 gene changes the size of different organs in transgenic tobacco plants 12.4 Dr Amanda Walker – CSIRO Plant Industry (Australia) Altering flavonoid composition in grapevine 12.5 Mr Peter Hayes - International Organisation of Vine and Wine (OIV) Nomination and selection of the host for the "9th International Symposium on Grapevine Physiology and Biotechnology"

17.30 onwards ~ Poster viewing and social networking

Friday 28" November 2008		
Time	Session	
9.00 - 10.30	SESSION 13	
	Chair: Dr Simon Robinson – CSIRO, Plant Industry (Australia)	
	 Key note speaker: 13.1 Dr Avi Perl – Colcani Center (Israel) Conventional and biotechnological approaches for the improvement of table grapes 	
	13.2 Dr Claudio Moser – E. Mach Foundation-IASMA (Italy) A molecular study of the regulation of grape berry ripening	
	13.3 Dr Violeta (Tsolova) Colova – Florida A & M University (USA) Comparative analyses of differentially expressed genes involved in flavonoid biosynthesis in North American native grapes: 'Noble' and 'Ison' muscadines vars., and 'Cynthiana' aestivalis var	
	13.4 Professor Mario Pezzotti – University of Verona (Italy) Systems biology of berry ripening and postharvest withering processes	
	10.30 – 11.00 ~ Morning Tea	
11.00 – 12.30	SESSION 14	
	Chair: Dr Mark Thomas – CSIRO Plant Industry (Australia)	
	 Key note speaker: 14.1 Professor Serge Delrot – UMR Ecophysiologie et Genomique (France) Understanding the biology: Moving away from observation based science to predictive science 	
	14.2 Dr Claudio D'Onofrio – University of Pisa (Italy) Molecular characterization of aroma genes in <i>Vitis vinifera</i> var. moscato bianco	
	14.3 Dr Manuel Pinto – INIA (Chile) Characterization of early light inducible proteins (ELIPs) in Vitis vinifera L.	

14.4 Dr Nathalie Ollat – INRA (France) Differential gene expression in Vitis genotypes submitted to iron deficiency

12.30 - 12.45 **CLOSING SYMPOSIUM PRESENTATION**

Chair: Professor Steve Tyerman

Farewell with lunch provided
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Oral Presentations

Scene setting: using the genotype and management to cope with environmental challenges

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The theme of this symposium, 21st Century Grape Research: Challenges and Opportunities suggests that management of "environment", in its assorted forms will be a most significant focus for research and development, innovation and adaptation in the viticultural sector in the immediate term and over the remainder of the century.

Given the heightened recognition of emerging climatic and resource pressures, global economic uncertainties and accelerated population growth, it is timely that the *Vitus spp*, its management and genotype be the focus of this symposium.

Expectations of science and industry have been sharpened not least from these pressures but also from the promise and "hype" surrounding science and technology in general, and more specifically surrounding genetics and its derivative technologies. For researchers in viticulture, perhaps the most cultivated (and culturally significant) of perennial crops, the challenges spread across aspects of productivity, tolerance to environmental stresses and acute environmental shocks, the application of diagnostic and predictive tools and systems and manipulation of sensory qualities of the grape.

Use of gene-technologies in vine improvement and breeding programs and the integration of biotechnologies into viticultural R&D and vineyard management will demand considerable multidisciplinary planning and effort, paralleled by actions directed to defining and demonstrating potential benefits and problems for environment and consumers, not simply for producers.

It is clear that interaction and interchange between the disciplines of vine physiology, genetechnology and vine selection and breeding is improving but perhaps the time is now right for a deliberate mapping of the fields of interaction and development of a mid-term strategy for delivering significant results for environment, management and society.

Development of a broadly supported, results focussed strategy will provide a basis for more fundamental breakthroughs in systems research for the benefit of both the R&D sector, industry and society; perhaps as important for R&D practitioners, it will considerably assist in maintaining understanding, acceptance and ongoing funding for both established and newer approaches to research.



Plant-Environment research status, what it can offer to address the challenges and limitations

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The key issues of future research will be the rapidly increasing world population and the scarcity of suitable land for agricultural food production together with a changing climate. The first will ultimately put pressure on grape producing areas for the use of land and the input of resources into grape production. The second will have a pronounced impact on grapevine physiology, biochemistry and ultimately production methods. For most grape producing areas the predicted developments in climate will be identical to becoming more marginal for quality production and/or to be forced to improve resource management. The entire area of stress physiology, from the gene to the whole plant and vineyard level (including soils) will need to be expanded to give suitable answers to questions such as:

Vineyard management – environmental interaction

Which varieties/rootstocks will tolerate increased temperatures, have the highest water use efficiency and salinity tolerance, require a minimum input of fertilizers and produce the quality the consumer wants? Research tools to address this include genetic, biochemical, isotopic and physiological methods. Limit and challenge alike is the combination of many methods to achieve the ultimate goal.

Global CO2 enrichment, greenhouse gases

How will rising CO_2 affect the physiology and biochemistry of grapevines, how gene expression? FACE (free air carbon dioxide enrichment) systems are needed which would allow studies in combination with other predicted environmental parameters to change (temperature and precipitation). Limits are a lack of international co-operation to solve the financial constraints.

How do soils contribute to the carbon footprint of a vineyard, how can we minimize the release of nitrous oxide through soil management, how can we maintain biodiversity in vineyard ecosystems? There is a lack of basic data on these topics, and modelling efforts are needed to develop decision support systems for sustainable production.

Fruit quality

What can be done to optimise fruit composition? The genetics and physiology of berry development together with associated functional problems (wilting, necrosis a. s. o.) needs further studies. Limits and challenges are the generation of these problems under controlled- or semi-controlled conditions to investigate them.

Pests and diseases

Plant-disease interaction on the molecular and physiological level, will they be modified by changes in the environment? The challenge is the development of sustainable strategies for disease control.



Plant management research - status, what it can offer to address challenges and limitations

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Background and Aims

Seasonal fluctuations in yield, grape composition and wine attributes, largely driven by variable climatic conditions, are major challenges for the wine industry aiming to meet consumer expectations for consistent supply, wine style and product quality. This presentation will address known causes for this variability and identify management techniques, together with their limitations, that offer potential to modulate these responses. Potential solutions offered from adoption of new rootstocks and varieties (ie. enhanced germplasm) will also be discussed.

Methods and Results

Results will be presented from research studies, conducted over a number of seasons, which link vineyard management practices with fruit composition and wine assessments. They show that there is potential to develop integrated systems to stabilise yield, fruit composition and wine quality attributes across seasons. These techniques include lighter pruning, deficit irrigation techniques and adoption of low-moderate vigour rootstocks which lead to reduced shoot vigour and the development of open canopies and small bunches with small berries, with enhanced colour, phenolics and sensory appeal; application of mechanical and chemical crop thinning techniques for yield stabilisation and promotion of early maturity, colour and flavour development.

However, the results also show that variability between seasons in many cases is much larger than can be achieved by modifying management practices. Hence, opportunities to use management practices to completely reduce the seasonal variability, particularly with respect to grape composition and wine attributes, factors largely affected by climatic conditions during berry development, may be limited. There exists significant potential in the longer term to use new varieties and rootstocks better adapted to variable and changing climatic conditions.

Conclusions

It can be concluded that vineyard practices can be modified to stabilise yield, grape composition and wine attributes to varying degrees and hence, minimise the impact of variability in climatic conditions from season to season. In the longer term, the adoption of new varieties together with new rootstocks adapted to higher temperatures and limited water supply will assist the wine industry to cope with impacts of climate variability and change and address ever changing consumer expectations.

Significance of Study

A total systems approach to vineyard management offers potential to modulate seasonal fluctuations in yield, grape composition and wine quality attributes with significant benefit for an industry. Adoption of new rootstocks and varieties (ie. enhanced genetics) will also play a pivotal key role in meeting the future challenges of climate variability and change.



Plant Genetic research - status, what it can offer to address the challenges and limitations

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Background and Aims

Natural genetic variation constitute the major resource for genetic improvement in grapevine considering both as the development of new varieties and the clonal selection performed in established cultivars. Publications of the PN40024 and Pinot Noir genome sequences have opened the way to functional genomics in grapevine that can be addressed towards understanding the genetic and molecular basis of natural genetic variation. This major objective requires the development of additional resources and information at molecular, genetic and phenotypic levels.

Methods and Results

The high level of sequence variation uncovered when comparing the two genomes of a heterozygous genotype like Pinot prompts to sequence additional genomes in *Vitis vinifera* to develop a comprehensive vision of nucleotide and genome size variation within the species boundary. Furthermore, sequencing genomes belonging to the interfertile species within the genus *Vitis* will provide a view of what could be considered as the *Vitis* pan-genome. At the genetic and phenotypic levels the available grapevine natural genetic variation in grapevine is reduced to a few thousand genotypes. This variation needs to be increased by collecting, cataloging and characterizing all available germplasm in cultivated and wild forms. Further genetic resources uncovering cryptic genetic variation and permanent mutant collections should also be generated. This will require the development of new genetic tools and procedures to facilitate management, storage and distribution of genetic variation.

Conclusions and Significance

Genomic tools and strategies successfully developed in grapevine can now be directed to understand and exploit the species genetic variation what constitutes the basis of all production applications and their wide diversity. Undertaking this challenge requires re-focusing on the plant with a genetic variation experimental framework and harnessed with a complete set of genomic and genetic tools.



Reproductive performance of the grapevine: effect of site, season and cultural practices

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Background and Aims

Flowering and fruitset are principal determinants of grapevine yield. Morphological, physiological and environmental factors interact to determine the number of flowers borne on the inflorescence and the percentage of flowers that develop into berries. Poor fruitset is said to limit the yield of many varieties in most regions in Australia—however, there is a lack of knowledge of the reproductive performance of most varieties and the relationship with climatic and other environmental factors under Australian conditions.

Methods and Results

The reproductive performance of 10 winegrape varieties, that are considered anecdotally to have poor fruitset, was studied in 4 consecutive growing seasons (commencing in 2004/05). Vineyard sites were selected across a range of climatic regions from cool (Adelaide Hills) to warm (McLaren Vale). Reproductive performance was measured in terms of flower number per inflorescence, berry number per bunch, coulure index (CI) and millerandage index (MI)—the latter two are novel indices for the quantification of reproductive performance which were developed during the course of this project. Differences between varieties and between sites were greater than the differences between seasons for most variety x site combinations. When averaged over all sites, there was little effect of season on fruitset. The varieties have been grouped on the basis of their reproductive performance and range from those with low flower number, moderate set, low to moderate berry number such as Pinot Noir to those with moderate flower number, low set, low berry number such as Sangiovese. Cultural practices, namely shoot topping and CCC foliar application, applied before or during the flowering period, increased fruitset and yield.

Conclusions

The high variability of berry number for many varieties may be as much a consequence of variation in flower number as variation in fruitset. Certain varieties have a reputation for 'poor fruitset' that has been inferred from relatively low berry number per bunch; however, it seems that fruitset is not the limiting factor in these cases. We have shown that cultural practices such as shoot topping, CCC application and molybdenum foliar application can exert an effect on fruit set via an influence on pollen tube growth, ovule fertilisation or ovule cell morphology.

Significance of Study

An improved understanding of the reproductive performance of winegrape varieties has been achieved.



The contribution of photoassimilates and carbohydrate reserves to fruit set in Chardonnay vines

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Background and Aims

Fruit set in *Vitis vinifera* L. can be reduced by cool temperatures or adverse weather conditions leading up to, or during flowering. A reduction in carbohydrate supply is commonly thought to be underlying this response, but the capacity for carbon mobilised from storage tissue to compensate for reduced availability of current assimilate remains unclear. The objective of this work was to assess fruit set of vines with depleted carbohydrate reserves in combination with low or high photosynthesis rates.

Methods and Results

Chardonnay vines were pre-treated with warm (25°C) and cool (16°C) root-zone temperatures after budburst to manipulate the rate of root starch utilization and thus whole vine carbohydrate reserves. Just prior to flowering these vines were moved into growth cabinets with ambient (336ppm) or low (94ppm) CO₂ concentrations to assess the interactive effects of carbohydrate reserve status and photosynthesis on fruit-set independently of temperature effects. The warm root temperature pretreatment reduced starch reserves relative to the cool treatment, and stimulated both shoot growth rates and inflorescence development. Transfer of these vines to ambient [CO₂] resulted in 20% lower fruit set than vines which had received the cold root pre-treatment, but maintained higher root starch concentrations. Those vines that were transferred to low [CO₂] had limited leaf photosynthesis rates. The duration of flowering (0 to 100% cap-fall) was dramatically slowed relative to those in ambient [CO₂], and percentage fruit set was more than halved. Vines from the cool temperature pre-treatment with high carbohydrate reserves had more inflorescences reach 100% cap-fall, but this was not sufficient to offset the effects of low photosynthesis. The carbohydrate reserves status of the vine did not change percent fruit-set under low [CO₂].

Conclusions

Under conditions of low carbon assimilation, high carbohydrate reserves may improve the progression of inflorescences through flowering. However, photosynthesis, rather than carbohydrate reserve status, appears to have the predominate influence on absolute fruit set percentage in Chardonnay vines.

Significance of Study

Vines with low reserves can still have good fruit set if conditions for photosynthesis are favourable. However, for optimal set, and possibly uniformity of berry development, high carbohydrate reserves may be advantageous.



The Reiterative Reproductive Meristems (RRM) phenotype of a Carignan somatic variant is associated to a transposon insertion in the *VvTFL1A* gene promoter

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Background and Aims

Grapevine reproductive development determines quantity and quality of fruit production. However, little is known about the molecular mechanisms controlling early stages of inflorescence and flower development. Somatic variants altered in reproductive development represent an unique material to identify the genetic mechanisms involved in these processes. The aim of this work was to characterize the RRM phenotype to shed light on the process of flower initiation.

Methods and Results

Morphological characterization of RRM variant indicated that the mutation affects both vegetative and reproductive development by delaying differentiation of flower and tendril meristems. Transcriptional profiling along early stages of inflorescence development showed association of the RRM phenotype to overexpression of a few regulatory genes including *VvTFL1A*. Further genetic and molecular comparison analyses of *VvTFL1A* in RRM versus Carignan (wt) associated the insertion of a transposable element in the *VvTFL1A* promoter to its up-regulation in the apex.

Conclusions

The effect of *VvTFL1A* over-expression supports a role for this gene in the positive regulation of cell proliferation delaying developmental program transitions in the shoot apex as has been proposed in other species such as *Arabidopsis*.

Significance of Study

The biological function identified for *VvTFL1A* in grapevine suggests is involvement in the determination of cluster size and branching structure. For the first time, we identified an active class II transposable element which was found to induce critical changes in reproductive organs. Based on this observation, it can be assumed that these elements could have a large contribution to natural variation of grapevine.



Polyamines in molybdenum-deficient Merlot

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Background and Aims

Previous studies have shown that molybdenum-deficient vines suffer from reduced fruitset. In plants, polyamine metabolism responds to external stresses, especially to mineral nutrient deficiencies. Polyamines, an integral part of N metabolism, have been shown to participate in the regulation of flower induction and fruitset. A synergistic relationship between polyamine biosynthesis and ethylene biosynthesis has also been observed. The aim of this study was to investigate the effect of molybdenum deficiency in Merlot on polyamine synthesis in developing flowers and berries.

Methods and Results

Molybdenum-deficient Merlot vines at a McLaren Vale vineyard, South Australia, were treated with a pre-flowering foliar spray of sodium molybdate. Flowers and berries were collected at specific developmental stages and analysed for free polyamine levels. Also, Real-Time PCR analysis was carried out to determine the expression of genes involved in polyamine and ethylene biosynthesis. The application of molybdenum to deficient vines increased fruitset and bunch weight. Relative to molybdenum-deficient vines, molybdenum-treated had lower levels of the ethylene biosynthesis gene ACC synthase and higher levels of expression of the polyamine biosynthesis genes arginine decarboxylase and spermine synthase in developing flowers and berries. Greater expression of the polyamine biosynthesis gene resulted in increased levels of the free polyamines spermine and spermidine in these tissues.

Conclusions

A higher level of ACC synthase in Mo-deficient flowers, at the beginning of flowering, suggests an increase in the synthesis of ethylene in these flowers. This, in conjunction with the higher levels of expression of ADC in the Mo-treated flowers at the same time point, as well as higher levels of spermine synthase at all the time points during the season, may indicate a higher level of polyamine biosynthesis gene expression, and an alteration in the polyamine:ethylene synergy. This increase in the expression of a gene in the ethylene biosynthetic pathway is a potential indicator of the mechanism which is responsible for the abscission of flowers in Mo-deficient Merlot vines. Also, the corresponding decrease in ACC synthase expression in the Mo- treated vines is strong evidence for the involvement of both polyamine and ethylene in successful berry development.

Significance of Study

Previous researchers have shown that that the exogenous application of polyamines increased the percentage of fertilization in grape flowers; however, this study is the first to show a relationship between molybdenum deficiency and the ethylene:polyamine balance.



Managing vines to optimise fruit development in a challenging environment

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Background and Aims

Grapevine reproductive development extends over two seasons, and the genotypic expression of yield potential and fruit composition is subject to environmental impacts, including viticultural manipulations, throughout this period. This paper reviews current knowledge and attempts to identify challenges, opportunities and priorities for research and practice.

Methods and Results

The present analysis of published information gives a critical appraisal of recent advances in research and practice concerning variables influencing yield formation and fruit composition at harvest. Exciting discoveries in fundamental research on the one hand and an increasing focus on outcomes and knowledge transfer on the other is enabling the development and implementation of practical recommendations that will impact grape production in the future.

Conclusion

Future research should aim to minimise seasonal variation and optimise the profitable and sustainable production of high-quality fruit for specific uses in the face of climate change, water and labour shortages, shifting consumer preferences and global competition.

Significance of Study

Better control of product quantity and quality, and differentiation to meet consumer demands and market preferences will enhance the competitiveness and sustainability of the global grape and wine industries.



Ascorbate metabolism during grape berry development

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Background and Aims

Ascorbate (Asc) is a well characterised anti-oxidant and redox regulator within plant cells. The Asc system modulates processes associated with fruit development. In grapevines (*Vitis vinifera*), Asc has another role as the precursor of tartaric (TA) and oxalic acids (OA). Tartaric acid is the predominant acid found in grape berries, and is responsible for maintaining berry and wine acidity. Our research investigates the developmental co-ordination of Asc and TA biosynthesis.

Methods and Results

HPLC measurement of Asc and TA in berries of *V. vinifera* c.v. Shiraz showed that both compounds rapidly accumulate during the early stage of development. We show by *in-planta* radiotracer results that the Smirnoff-Wheeler biosynthetic pathway for Asc is active in young grape berries. Identification of genes encoding *V.vinifera* Asc biosynthetic homologs enabled us to study their developmental expression. The molecular data shows increased transcript levels of genes associated with the Smirnoff-Wheeler pathway in young berries, and that an alternative Asc biosynthetic pathway is up-regulated in mature berries.

Conclusions and Significance of Study

These results lead us to hypothesise that the pre-veraison accumulation of Asc is mediated by the Smirnoff-Wheeler pathway, which supports the synthesis of TA and OA. These results provide a basis to study TA metabolism and the potential to modify or manage grape berry acidity.



Effect of bunch shading during different developmental stages on the flavonoid biosynthesis in berry skins of *Cabernet Sauvignon* grape

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Background and Aims

The aim of this study was to examine the effect of bunch shading during early development (stages I and II) and/or during ripening (stage III) on the composition of flavonoid, i.e., proanthocyanidin (PA), anthocyanin, and flavonol, in the berry skins of *Cabernet Sauvignon* grape, as well as on the mRNA levels of these biosynthetic pathway.

Methods and Results

The berries of field-grown Cabernet Sauvignon grape was shaded using the shade cloth from one week after anthesis. Four treatments of shading during different developmental stages: light exposure throughout fruit development; light exposure during stages I and II and shading during stage III; shading throughout fruit development were applied to bunches on each vine. Shading decreased the PA concentrations during stages I and II. The PA concentrations decreased during stage III, and the decrease of the concentrations was lower in berries shaded during stages I and II than that in the control. Thus, no significant effect of shading during stages I and II was observed at harvest. Shading during stage III did not influence this decline in the PAs. On the other hand, shading during stages I and II induced changes in the composition, such as a decrease of the trihydroxylated subunits within PAs, which agreed with the relative decrease of *VvF3'5'H* expression. The anthocyanin concentrations were remarkably reduced by shading during stage III, which was in accordance with the decreased transcription of several anthocyanin biosynthetic genes and transcriptional factors. Shading during stages I and II did not influence the anthocyanin concentrations at harvest; however, it decreased the proportion of tri-hydroxylated anthocyanins.

Conclusions

Bunch shading during different developmental stages influenced the composition of the flavonoids produced during the same period through transcriptional change of the related genes on their biosynthetic pathways. In addition, shading during stages I and II had a prolonged influence on the flavonoid composition during stage III.

Significance of Study

Detailed information of the change in the flavonoid composition and related gene expression in the berry skins shaded during specific stages shown in this study will be important for research of plant secondary metabolism and also practical viticulture.



Simulating berry growth and sugar concentration in a ripening grape with a process-based fruit model

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Background and Aims

Modelling is a powerful approach to describe the main processes involved in the build-up of fruit quality. A biophysical model of grape berry growth was developped from two existing processbased models that simulate seasonal and diurnal dry mass growth, water and sugar accumulation in peach fruit (Fishman and Génard 1998).

Methods and Results

The model represents a virtual mean berry during the post-veraison developmental stage, which is assumed to behave as a single cell separated by a composite membrane from the parent vine and the outside environment. At each run step, water accumulation was calculated through the water balance between xylem and phloem water influx and transpirational water loss, controlled by water potential gradient between the berry and the parent vine. Meanwhile, dry mass accumulation was simulated with the balance between phloem sugar import and respired carbon depletion. The imported carbon into the berry was then used as an input in a sugar sub-model to calculate total sugar amount, and finally combined with berry fresh mass to obtain sugar concentration which in turn has feed-back effects on water and sugar influx in the next step. In addition, the underlying effects of climatic factors on berry growth and sugar concentration were also incorporated in the model. Experimental data were acquired on Vitis vinifera cv. Cabernet Sauvignon fruiting cuttings grown under two leaf-to-fruit to calibrate and validate the model. Using sugar concentration in phloem, climatic data and initial berry weight as inputs, the model satisfactorily reproduced the observed data in terms of berry dry weight, fresh weight and sugar concentration for both leaf-tofruit ratios treatments. Furthermore, the within-cluster variations in berry fresh weigh, dry mass, and sugar concentration was also properly simulated when different initial fresh and dry masses were considered.

Conclusions

The relative contributions of assimilate supply, metabolism, and dilution which may potentially cause changes in sugar concentration in response to leaf-to-fruit ratios were assessed with the model. The data showed that the decreased sugar concentration under low leaf-to-fruit ratio is mainly due to the decreased assimilate supply, while the effects of metabolism and dilution are almost not visible. Since the model worked in a one-hour step, its capability of simulating the diurnal fluctuations in fresh mass and berry water balance were also demonstrated and discussed.

Significance of Study

This virtual berry model provides a useful tool to enhance our understanding of mechanisms regulating berry growth and quality built-up in response to viticultural managements and environment conditions.



Abiotic stress and plant responses - from the whole vine to the genes

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Background and Aims

Drought, salinity and extreme temperatures significantly limit the distribution of grapes around the world. All three stresses reduce crop yields, but only water deficits have been used in a positive way to enhance flavor and quality characteristics of the berries. This paper will review the literature of grape responses to abiotic stress with particular reference to whole plant and molecular responses observed in studies performed by my research group.

Methods and Results

We have conducted a number of short-term and long-term studies on grapevine shoots and berries using a systems biology approach. Transcript, proteins and metabolites were profiled using genome arrays, two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and gas chromatographymass spectrometry (GC-MS). Water deficit, salinity and chilling altered the steady-state abundance of a large number of transcripts. Common responses to these stresses included changes in hormone metabolism, particularly abscisic acid (ABA), photosynthesis, growth, transcription, protein synthesis, signaling and cellular defenses. Some of the transcriptional changes induced by stress were confirmed by proteomic and metabolomic analyses. More than 2,000 genes were identified whose transcript abundance was altered by both water deficit and ABA. Different gene sets were used to map molecular pathways regulated by ABA, water deficit, salinity and chilling in grapevine.

Conclusion

ABA is a central regulator of abiotic stress tolerance mechanisms. ABA affects signaling pathways that trigger important molecular activities involving metabolism, transcription, protein synthesis, and cellular defense and also regulates important physiological responses such as stomatal conductance, photoprotection and growth.

Significance of Study

Systems biology approaches are providing more comprehensive understanding of the complex plant responses to abiotic stress. The molecular sets generated from mapping the ABA-inducible stress responses provide numerous targets for genetic and cultural manipulation for improved plant protection and grape quality.



Resilience of Shiraz exposed to short episodes of heat stress in the field

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Background and Aims

Heat events (> 35 °C) are part of the current seasonal weather variation in warm regions, and the frequency of these events is likely to increase in the next decades. Anecdotal evidence indicates severe impairment of vine physiology and berry development in response to heat stress but this effect has rarely been directly studied in the field. This study aimed to assess the impacts of short duration heat stress events at different developmental stages on the physiology and berry development of Shiraz vines in the field.

Methods and Results

In the 2006/07 and 2007/08 seasons heating chambers were deployed in an experimental block of drip-irrigated Shiraz at the SARDI Research Station in South Australia's Barossa Valley. We compared untreated controls (maximum air temperature average: 32.6°C, range 24.5 to 39.8) with heat-stress treatments (maximum air temperature average: 39.4°C, range 35.3 to 42.6). Heat stress was applied for three days at one of four developmental stages: post fruit set, pre-veraison, during veraison and pre-harvest. We assessed the response of the vines in terms of leaf, canopy and bunch temperature, stomatal conductance, leaf photosynthetic rate, berry growth, sugar accumulation and quality attributes. None of the treatments had a significant effect on yield, berry growth, or accumulation of sugars. Heated vines had consistently higher leaf stomatal conductance rates than control vines.

Conclusions

Contrary to expectations, we found that weekly-irrigated Shiraz exhibited significant buffering capacity against short episodes of heat stress with increased transpiration providing enhanced evaporative cooling in treated vines.

Significance of Study

Increased frequency of extreme temperatures will not necessarily have disastrous impacts on viticultural production in warm growing regions. Some grape varieties have significant capacity to cope with high temperatures provided there is adequate water. What defines an adequate water supply will depend on multiple factors including but not limited to variety, rootstock, soil properties and desired wine attributes


Phenotypic plasticity of yield and phenology in grapevine

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Background and Aim

Phenotypic plasticity is "the amount by which the expressions of individual characteristics of a genotype are changed by different environments" (Bradshaw, 1965). In this paper we review Bradshaw's seminal principles of phenotypic plasticity in the light of recent developments in crop physiology and genetics. A framework with four principles is proposed: (i) plasticity is a trait of its own, under its own genetic control; hence plasticity evolves and can be a target of breeding and selection; (ii) plasticity is specific for a trait and is specific in relation to particular environmental drivers; plasticities of related traits can be (iii) negatively or (iv) positively related. Our aim was to explore associations between plasticity of yield and plasticity of phenology (principles iii and iv).

Methods and Results

We calculated coefficients of phenotypic plasticity (Finlay and Wilkinson 1963, Calò *et al* 1975) for yield, and for timing of budburst, flowering, and veraison in seven varieties, viz. Cabernet Franc, Cabernet Sauvignon, Chardonnay, Merlot, Riesling, Semillon and Shiraz grown in 14-19 environments of South Australia. Yield ranged from 1.2 to 18.7 t/ha; all varieties produced similar yields in low-yielding environments, but there were large (P < 0.0001) varietal differences in high-yielding environments that where captured quantitatively by the coefficients of yield plasticity (range 0.72 to 1.29). Hence, for this collection of varieties and environments high plasticity of yield was a desirable trait. Yield plasticity was unrelated (P \ge 0.16) to mean date of budburst, mean date of anthesis and mean date of veraison. Yield plasticity was positively associated (r \ge 0.79, P< 0.05) with both plasticity of budburst and plasticity of flowering (principle iv).

Conclusion

The variety-dependent capacity to capture the benefits of favourable growing conditions was partially explained by the capacity to accommodate pre-veraison development to environmental conditions. Improved matching of varieties and environments needs to account not only for the average pattern but also for the phenotypic plasticity of phenological development. Understanding the genetic control of phenological plasticity would contribute to crop adaptation.



Changes in gene expression in response to heat stress during berry development in grapevine

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Background and Aims

Berry development in grape is a complex process and involves distinct phases of growth and metabolic activities. Environmental factors are key determinants of metabolite composition and subsequent berry and wine attributes. Grapevines experience fluctuating temperatures during berry development that influence berry attributes at harvest as well as harvest date. This study was conducted to better understand the response of the berry to heat stress by investigating gene expression.

Methods and Results

The changes in gene expression of heat responsive genes during heat stress were studied under growth room conditions. The L1 Pinot Meunier mutant was chosen as the test plant due to its small stature and continuous flowering. The vines were maintained at 25°C with a 16 hr photoperiodic light in an environment controlled growth chamber. Unripe early stage berries and ripening berries were used. The temperature of berries was increased to 40°C for 4 hours. Gene expression patterns of four putative transcription regulators were analyzed using real-time RT-PCR. The expression of one gene was found to be up-regulated in response to heat both in unripe and ripening berries by a mean factor of approximately 8 with the expression of another gene up-regulated by a factor of approximately 2 only in ripening berries. Another gene was down-regulated by a factor of 0.25 only in unripe berries with its expression unchanged in ripening berries. The remaining gene was unaffected by heat treatment at both developmental stages. PCR products from genomic DNA and mRNA were cloned and sequenced for *in silico* and genetic analysis. For one gene, *in silico* analysis revealed strong homology between its putative protein product and the transcriptional co-activator MBF1 suggested to be a regulator of thermal tolerance in *Arabidopsis*.

Conclusions

A grapevine model system for investigating berry heat stress under controlled conditions was successfully used to identify gene expression changes of transcription regulators. The results indicate that the gene expression response of grapevine berries to heat stress is a complex process influenced by the developmental stage. The described system is expected to prove useful for further elucidation of genes and processes that are affected by heat stress.

Significance of Study

This study has shown that transcription regulators in the berry show either a negative or positive change in gene expression in response to heat stress.



A Review of Canopy Management Research - From field observation to modelling, and back to vineyard and beyond

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This presentation summarizes 50 years of canopy management related research, insisting on the recent evolutions.

Research usefulness

Practical problems and field observations concerning canopy management: from technical efficiency, to quality control, up to environment and ethics.

Research Backgroung

Diversity of canopy architectures and training systems: historical, available or mostly used, innovations.

Research Deepening

Main physiological functions affected by canopy management and the state of corresponding modelling:

- through canopy microclimate (SFE/Exposed Leaf Area, growth, water regime, photosynthesis),
- through berry microclimate,
- through plant source-sink relationships.
- Research integration: understanding the integrated whole plant physiology:
- the main general functions of the whole plant,
- the dominating regulating factor: water limitation,
- the concept of 'Biological triptych' applied to SFE/Production/Vigour.

Research Questioning

Considering plant physiological responses to environment as:

- series of continuous mean functions inside a coherent model, or variations around mean functions (example of stress effects),
- a possible introduction to a theory of grape berry maturation 'unfolfing fruity typicity' and 'derivatives series',
- an opportunity to make links between genomics and ecophysiology?



The influence of vine management on Sauvignon Blanc performance and carbohydrate reserves

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Background and Aims

Much of Marlborough's Sauvignon Blanc grape crop is produced using the high-yielding, pruning intensive, 4-cane Vertical Shoot Positioned (VSP) pruning system. In cool, late or high-cropping seasons achieving desired fruit ripeness levels by commercial harvest time using this system can prove unattainable. Altering yields through cropload manipulation by means of pruning regime is one practical way of influencing ripeness levels. To test this approach a field trial was initiated with the primary aim of altering cropload by adjusting the pruning regime used on Sauvignon blanc grapevines.

Methods and Results

Established grapevines previously trained to 4 canes were either left as is or pruned back to 2 canes per vine in July 2003. These pruning regimes were maintained on the same vines in successive seasons. Trunk over-wintering carbohydrates reserves were assessed towards the end of each dormant winter season.

Pruning back to 2 canes in 2004 halved vine yield, increased harvest fruit ripeness (brix level), pruning weight and reduced the yield prune ratio compared with 4 cane vines. Carbohydrate reserves responded with starch and total CHO levels being significantly higher in 2 cane vines. Over successive seasons the yield of 2 cane vines partially compensated reaching a maximum of 77-80% of 4 cane yield in 2006-2007. Pruning weight, yield prune ratios as well as carbohydrates reserves also showed signs of compensation and full recovery respectively. The partial yield compensation displayed by 2 cane vines was primarily the result of increases in non-count shoots per vine, increases in cane thickness (diameter) and associated increases in node fruitfulness.

Conclusions

Pruning to only 2 canes per vine to down regulate yield and improve fruit ripeness was successful. However, the effectiveness diminished with time due cropping compensation. The ability of the grapevines to adjust to the pruning regimes with time illustrated the capacity of the vines to reestablish their natural balance as influenced by vineyard site environment in which they grew.

Significance of Study

The study highlights the relative ease to down regulate yield of Sauvignon blanc grapevines and improve fruit ripeness, without negatively impacting on the over-wintrering CHO reserves. These outcomes have two major benefits for the current Marlborough wine industry; Firstly; yield and crop volumes can be controlled to avoid increasing issues with grape oversupply, and secondly; overall risk to fruit condition and quality can be reduced by increasing early crop ripeness and hence brining forward harvest date.



The relationship between carbohydrate reserve dynamics and vine productivity

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Background and Aims

Carbohydrate source-sink relationships are complex and dynamic, and the allocation of carbohydrates to the different vine parts during the season changes with developmental stage and activity of the various sinks. Stored carbohydrate reserves support early shoot growth and there is also evidence that the availability of reserves may influence reproductive development. The accumulation and dynamics of reserves is influenced by climatic factors, variety, rootstock and vineyard management. The productivity level determines the allocation assimilates into storage due to the amount of sugars required to mature the crop. The time vines have to replenish reserves after harvest to leaf-fall is consequentially more important in highly productive vineyards. This work aimed to determine the factors impacting on winter carbohydrate reserve and seasonal dynamics and the relationship of reserves to vine productivity.

Methods and Results

The length of the post-harvest period was altered by early crop removal and leaf removal after harvest in Semillon. These treatments induced large differences in starch levels in wood (4.5-10.6% DW), root tissue (13.8-32.1% DW) and yields by about two fold. Post-harvest water stress has been implemented in Shiraz vines in the Riverina reducing starch concentrations by about 1/4 in the roots, while wood reserves levels and yields were unchanged. Similarly, winter carbohydrate levels are lowered by the mid season water deficit (RDI), which also reduced yields. Frequent seasonal root and wood carbohydrate determinations in Shiraz showed periods of high carbohydrate demand (bud-burst to flowering, during grape maturation) while surplus carbohydrate is present between flowering and veraison and after harvest.

Conclusions

The results show that the amount of reserves stored prior to leaf-fall can be influenced by management practice, and that this may alter yield in the following season. The two main periods in a season of carbohydrate reserve demands are during early shoot development and maturation, the multitude of these changes are most likely influenced by productivity levels. Higher reserves in the perennial structure are present during periods of high root growth and nutrient uptake. More information on how environmental factors and crop load influence whole vine carbon balance is required to properly understand the seasonal dynamics of reserve accumulation.

Significance of Study

The research will provide a better understanding of the relationships between reserve status and yield and overall source-sink relationships. Further, it will assist to comprehend how vineyards can be managed to avoid or reduce the extent of seasonal yield fluctuations.



'VitiSim' – a simplified Model of Grapevine Dry Matter Production, Partitioning and Fruit Abscission

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Background and Aims

Crop load is an important, but qualitative concept of supply to demand, typically for carbon or energy. It also is normally expressed as one value for a season though we know supply and demand are dynamic. To better understand these dynamics a simplified dynamic seasonal grape dry matter production, partitioning, fruit growth and abscission model, called VitiSim, has been developed and partially validated. The model is based on experimental results but emphasizes mechanistic bases.

Methods and Results

The model uses a daily time step, a big-leaf daily canopy photosynthesis light response, respiration of organs based on mass and specific respiration rates, and temperature-driven leaf area development. Weather inputs are daily max and min temperatures and radiation. Partitioning is based on the balance of total supply to total demand with relative sink strength partitioning coefficients if the carbon supply is less than total demand. Fine root growth and respiration submodels have been recently developed based on recent root studies. A new fruit growth and abscission submodel has been developed based on abscission of berries that fall below critical growth rates. Validation studies indicate that simulated total dry matter production and seasonal dry matter patterns are very realistic in pattern and amount. Seasonal dynamics of simulated carbon supply to demand suggests that the periods of greatest carbon deficit are around or shortly after bloom and immediately after veraison. The period of greatest positive carbon balance appears to be just before veraison when the canopy is complete, but the crop growth is reduced. The model has been used to elucidate patterns of the seasonal variation of carbon supply versus demand as affected by weather, climate, pruning and training systems. An example is the difference in simulated carbon balance patterns of minimally versus normally pruned vines. Simulations indicate that compared to normally-pruned vines, minimally-pruned vines have significantly better earlyseason carbon balance during set and bud development that allows high crop set and also sustained cropping, but have worse carbon balance after veraison leading to delayed maturation.

Conclusions

The main dry matter production model appears to be quite sound. Partitioning and fruit abscission simulations produce realistic behavior but need further validation.

Significance of Study

Overall the dynamic nature of the model allows clearer understanding of shifting patterns of carbon supply to demand and helps elucidate how environment and culture affects crop load dynamics.



Genomics and bioinformatics – The role of NCBI

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Plant genomics is a simple expansion of the scope of genomics at the National Center for Biotechnology Information (NCBI). In addition to the tools for storage of and analysis of nucleotide sequence such as, respectively, GenBank and BLAST, genomics at NCBI includes databases that enable 1) monitoring the progress of genome sequencing projects (Entrez Genome Projects), 2) datamining of probes (Entrez Probes), 3) datamining of gene information (Entrez Gene) and 4) viewing genome units (MapViewer). These standalone tools are enhanced at NCBI by the capability to move among these and other databases as the data associations dictate. The pan-organism resources are supplemented by plant-specific resources: plant text search, PlantBLAST, and plant-EST BLAST. PlantBLAST provides organism-specific databases composed solely of the accessions associated with mapped loci visible through MapViewer. EST-BLAST provides plant-specific databases composed solely of the ESTs from those plants with more than 40,000 ESTs.

This expanded scope of data will be used in examples of the developing capabilities of the genomic resources for plants at NCBI.



Systems Biology of the Grapevine

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Background and Aims

During recent years, an increasing amount of genomics data has been released within the grapevine community, including a significant share from our laboratories. The next critical challenge is to annotate the recently released grapevine genome and adapt existing interpretive tools of model species to the specificities of the grapevine genome. Our goal is to develop and validate a database of the molecular networks occurring in the grapevine.

Methods and Results

A beta version of the molecular networks will be displayed online through the METNET database. This database allows expert users to curate the pathways through internet. The sequences from the genome sequencing project and EST data have been matched for determining unique sequences, leading to 39,423 unique sequences. Amongst them, 8,608 genes have been assigned to 120 pathways. So far, the pathways set include 90 metabolic pathways, 3 transporter pathways, 15 genetic information processing pathways, and 12 signal pathways mainly focused around hormones signaling. In addition to pathways, more than a thousand transporters have been categorized according to the transport classification database and added to the pathways. The visualization of "omics" data is performed with Cytoscape, which allow input of quantitative data.

Conclusion

This tool will allows the visualization of the changes of the transcriptome, proteome and metabolome within molecular networks (for example, metabolic or signal pathways), during a given experiment.

Significance of Study

Having grapevine specific tools will increase the power and speed of genomic and systems biology data analysis.



Characterisation of the grapevine development and ripening by integrated analysis of transcriptome and metabolome

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Background and Aims

Different physiological and biochemical processes are involved in fruit setting, development and ripening of grapevine berries. Diverse efforts using varied omics technologies are being applied in order to explain certain key biological stages of this fruit which could allow, in a near future, to improve the quality of grapevine berries and consequently the quality of wine. To gain information concerning the genes and metabolites involved in such processes, we measured primary and secondary metabolites together with transcript levels of three Vitis vinifera cultivar berries during their growth period.

Methods and Results

Samples of cultivars Carmenère, Merlot and Cabernet Sauvignon were collected every three days and during two growth seasons, starting with flowers and finishing with mature berries. We established a qRT-PCR platform to investigate the transcriptional changes during growth development and setting that allows us to determine the expression levels of around 800 grapevine genes. We measured levels of 121 primary metabolites by using GC-TOF-MS. Metabolite profiling of secondary metabolites by using high mass accuracy FT-ICR-MS is currently in progress. Since huge amount of data is generated, we use a network analysis approach based on correlations as a tool to elucidate the relationship between transcripts and metabolite changes and their role in regulation of metabolic pathways during ripening.

Conclusions

We developed a system biology approach combining transcripts, metabolites and bioinformatics tools to elucidate gene-metabolite networks that allows a better understanding of the key factors involved in grape development.

Significance of Study

The knowledge acquired can be used to improve agricultural techniques in order to obtain better quality fruits for better wines.



Two *Vitis vinifera* MATE proteins act as tonoplast acylated anthocyanin/H+ antiporters

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Background and Aims

In cells, anthocyanins are synthesised in the cytoplasm and accumulated inside the vacuole. However, little is known about the transport of these compounds through the tonoplast. Two genes were identified as anthocyanin transporter candidates and named anthoMATE1 and anthoMATE3 since they contain conserved domains of the Multidrug and Toxic Compound Extrusion protein family (MATE).

Methods and Results

The expression of both genes was evaluated in grapevine tissues in relation with anthocyanin accumulation. *AnthoMATE1* and *anthoMATE3* expression is essentially fruit-specific and concomitant with the increase of anthocyanin during berry development. Subcellular homologous localization assays revealed that anthoMATE1 and anthoMATE3 are localized at the tonoplast. Yeast vesicles expressing anthoMATE proteins transported a mixture of acylated anthocyanins in the presence of MgATP. Inhibitor studies demonstrated that anthoMATE1 and anthoMATE3 proteins act in vitro as acylated anthocyanin /H+ antiporters. In a range of cultivars showing variable anthocyanin composition the expression of *anthoMATE3* is well correlated with acylated anthocyanins berry content. This is not the case with *anthoMATE1*, suggesting that this protein may have additional function to anthocyanin transport.

Conclusions

Taking advantage of available genomic resources as a result of the sequencing of *Vitis vinifera*, two MATE genes in grape berries were identified, characterized and demonstrated the involvement of the encoding proteins in the transport of acylated anthocyanins inside the vacuole.

Significance of Study

Anthocyanin pigments are particularly important for red wine quality. Acylated anthocyanins are known to be exceptionally resistant to colour loss. Recently, the access to *Vitis* sequence speed up the identification of genes underlying traits of interest. Our work demonstrated the role of MATE proteins n the transport of acylated anthocyanin.



Biochemical Genomics and Kinetic Characterization of a Flavonol- and Anthocyanin- 3'5'-O-Methyltransferase (FAOMT) from Grapevine

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Background and Aims

At veraison in *Vitis vinifera* berries, the exocarp turns color from green to red and then to purple, which is due to the accumulation of anthocyanins and the extent of methylation of these compounds. We were interested in determining the *O*-methyltransferase family member responsible for this latter activity.

Methods and Results

We determined via quantitative transcript and protein profiling that a striking up-regulation in expression of a putative O-methyltransferase family member occurred at veraison and was concurrent with the accumulation of the polyhydroxylated glucosylated and methylated anthocyanins. The putative O-methyltransferase was heterologously expressed and purified to near homogeneity based on SDS-PAGE and MALDI-TOF data. Kinetic enzyme assays were carried out using a broad array of anthocyanin and flavonoid substrates. We demonstrated that this enzyme, which we named FAOMT, carries out 3'- and 3',5'-O-methylation of a multitude of anthocyanin and other flavonoid compounds in grape berries with a preference for glycosylated substrates.

Conclusion

FAOMT activity is a key final step in anthocyanin and other glycosylated flavonoid metabolism in grapevine, and potentially other plant species.

Significance of Study

By reporting on a full length functional FAOMT cDNA from *V. vinifera* and further demonstrating for the first time the biochemical properties of its product, we tie together and conclusively advance data from previous related publications and also extend results to suggest a potential biological significance of FAOMT activity for thermal stability of anthocyanins related to UV radiation and heat exposure in field conditions.



Characterisation of the LOX-HPL biochemical pathway as a potential source of distinct flavour and aroma characteristics of Sauvignon blanc wine

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Background and Aims

New Zealand's Sauvignon blanc wine is internationally recognized for its distinctive aroma and flavour characteristics. The presence in wine of short-chain aldehydes, ketones and alcohols has been found to contribute greatly to the wine quality and style. Moreover, these compounds have also been implicated as precursors of important volatile thiols, which in wine correspond to box tree, citrus zest and passion fruit aromas. The current research is focused on characterisation of a biochemical pathway which contributes to the formation of short-chain aldehydes, ketones and alcohols in grape berries.

These short-chain volatiles are most likely the derivatives of a lipoxygenase-hydroperoxide lyase (LOX-HPL) pathway. The lipoxygenase enzyme (LOX) catalyses hydroperoxidation of fatty acids. Further cleavage of the resulting hydroperoxides, by hydroperoxide lyase (HPL), releases volatile aldehydes and oxoacids. The LOX-HPL pathways from other plant species are involved in a wide range of physiological processes including, growth, development and plant defence response to biotic and abiotic stresses.

Methods

Grape berries of Sauvignon blanc have been selected from different developmental stages. Standard molecular biological techniques have been used to clone the LOX-HPL genes, express proteins from them and biochemical assays carried out to characterise their activity.

Results

Using a candidate gene approach we have cloned representative LOX and HPL members expressed in S. blanc berries. Real-time qPCR data demonstrates differential expression of the isolated genes in the berry over development and upon treatment with various elicitors. The clones have been successfully expressed in *E. coli* as recombinant HIS-tag fusions. Biochemical activity of the recombinant enzymes has been tested against several substrates and the identity of the reaction products identified. Determination of the spatial localisation of the candidate genes and study of their functions *in vivo* is currently underway.

Conclusion, Significance of Study

This study should add significantly to the knowledge about the processes involved in regulating the formation of those green leaf volatiles and their derivatives that represent important flavour and aroma compounds in grapes.



Influence of Bunch Exposure on Flavonol Accumulation in Shiraz and Cabernet Sauvignon Grape Skin

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Background and Aims

Flavonols are products of the flavonoid biosynthetic pathway and play a role in colour stability in red wines through the process of co-pigmentation. Co-pigmentation is the association between flavonols and anthocyanins, the pigments in red winegrapes, resulting in enhanced colour intensity or stability. Therefore, the processes that influence flavonol content and composition in grapes may impact on wine quality. Flavonol research has largely focussed on fruit at harvest with some work also examining flavonols at veraison. Where flavonol accumulation during berry development has been studied it has been limited to Shiraz from a cool production area. The aim of the current study was to determine the effect of bunch exposure on flavonol accumulation throughout berry development in Shiraz and Cabernet Sauvignon from warm irrigated vineyards.

Methods and Results

Viticultural canopy management practices were employed to alter bunch exposure for three successive vintages, 2003, 2004, and 2005. The treatments used to modify bunch exposure varied between vintages and included a foliage wire that lifted the canopy above the existing trellis (Exposed), in addition to the foliage wire extensive leaf removal in the fruit zone was applied (Highly Exposed), and a treatment where lifting wire and leaf removal was employed and finally 70% shade cloth applied to both sides of the canopy (Shaded). The Control was current commercial practice in the vineyard comprising a two wire vertical trellis. In addition to canopy manipulation, a light exclusion treatment was applied to some bunches on the control vines. Grapes were collected at weekly intervals throughout berry development and skins removed and frozen for analysis. Skin were extracted in a 50% methanol in water solution and analysed by reversed-phase high-performance liquid chromatography. The flavonols quantified included myricetin-3-*O*-glucoside, quercetin-3-*O*-glucoside, larictrin-3-*O*-rhamnose-7-*O*-trihydroxycinnamic acid, kaempferol-3-*O*-caffeoylate, isorhamnetin-3-*O*-glucoside, and syringetin-3-*O*-galactoside. Flavonols generally increased with increasing bunch exposure, not all flavonols responded similarly to the same treatments.

Conclusions

Bunch Exposure influences the accumulation of flavonols in both Shiraz and Cabernet Sauvignon grape skin. Flavonol content and composition was affected by varying bunch exposure. The impact on wine quality and wine colour stability is yet to be determined.

Significance of Study

Canopy management can be used to influence flavonol content and composition. This may contribute to improved wine quality. The role of individual flavonols in copigmentation requires further investigation.



Physio-anatomical and compositional characterization of berry shrivel - a ripening disorder of grapevine

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Background and Aims

Berry Shrivel (BS) is a recurring ripening disorder that renders clusters unsuitable for winemaking in both red and white varieties. The objective of this study was to characterize physiological, compositional, and ultrastructural changes of Semillon and Cabernet Sauvignon berries afflicted with BS.

Methods and Results

Clusters exhibiting symptoms of BS were harvested after veraison and the berries were analyzed for composition, nutrients, and changes in anatomy. In afflicted berries, the juice was watery and tasted very sour due to low sugar accumulation (8-10 °Brix) compared to the healthy ones (20–25 °Brix). Shriveling inhibited color development and the cuticles appeared to be thinner than the ones in the healthy berries. Furthermore, they had low concentrations of oxalic and citric acids and no succinic acid; however, in general, they were highly acidic when compared with healthy berries. The concentrations of P, K, Ca, and Mg were higher in the shriveled berries than in the healthy berries. Most of the cells were viable in the healthy berries as opposed to predominantly dead cells in the afflicted berries. The xylem pathways in canes of healthy and afflicted vines were functional with no pluggings in the vessel elements. The sieve tube members (STM) in the peduncles of healthy cluster were functional with unobstructed sieve plate. The STM in the peduncles of Berry Shrivel cluster was healthy; whether or not its sieve plates are plugged will be examined in future studies.

Conclusions

Berries afflicted with BS were flaccid and appeared like a deflated soccer ball, less colored, the juice was watery and very sour with no perceptible sugar, and very often the berries developed an off-flavor.

Significance of Study

Precisely what causes BS is not known at this time. An understanding of symptoms arising from alterations in cellular, physiological, and biochemical machinery will perhaps guide us to pinpoint its causal factors.



Molecular strategies to enhance the genetic resistance of grapevines to fungal pathogens

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Background and Aims

The European winegrape *Vitis vinifera* has little or no genetic resistance to the major fungal pathogens powdery mildew (*Erysiphe necator*) and downy mildew (*Plasmopora viticola*). These pathogens were first introduced into French vineyards from North America in the 1800's before spreading to all major grape producing regions of the world. As a result, grape production is highly dependent on the use of fungicides. With the increasing financial and environmental costs of chemical application and the emergence of fungicide-resistant strains, the introduction of natural genetic resistance against these fungal pathogens is a high priority for viticultural industries worldwide.

Methods and Results

We are utilizing a number of different molecular-genetic approaches to increase our understanding of the basis of resistance to these important major fungal pathogens and to identify potential new sources of genetic resistance. These approaches include: (a) map-based cloning of powdery mildew and downy mildew resistance genes from the wild North American grapevine *Muscadinia rotundifolia* (b) identification and functional characterisation of powdery mildew susceptibility genes in *V. vinifera* and (c) characterisation of basal immune responses to powdery mildew infection in the Vitaceae family.

Conclusion

This presentation will outline the progress and the potential of each of these different molecular strategies to the generation of fungal-resistant grapevine germplasm.

Significance of Study

Genetic manipulation approaches based on the introduction of resistance genes from wild grape species or the manipulation of expression of host genes offers the possibility will generate grapevines with enhanced genetic resistance to fungal pathogens.



Functional Analysis of Polygalacturonase-inhibiting proteins (PGIPs) from *Vitis* species: Identification of an additional role in plant defence

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Background and Aims

Polygalacturonase-inhibiting proteins (PGIPs) in plant cell walls inhibit fungal endopolygalacturonases (ePGs). This inhibition interaction directly limits the effective ingress of the pathogen relying on functional ePGs to breach the plant cell wall and is hypothesised to facilitate the prolonged presence of elicitor active molecules to upregulate the plant's defense response. Recently, PGIP activity, ePG inhibition and decreased *Botrytis* susceptibility could be correlated in tobacco plants overexpressing a grapevine PGIP-encoding gene (Joubert et al. 2006; 2007). In this study, transgenic lines with resistance phenotypes, overexpressing PGIPs isolated from a range of *Vitis* species were used for in depth functional analyses to understand *how* PGIPs influence plant defense systems.

Methods and Results

PGIPs isolated from *V. vinifera* and other *Vitis* species were stably overexpressed, leading to transgenics with a PGIP-specific resistance phenotype. The non-vinifera PGIPs specifically lead to excellent resistance phenotypes in the whole-plant infection assays. Microarray analysis showed that several genes involved in cell wall metabolism were affected by the presence of constitutive copies of PGIP. Real-Time quantitative PCR confirmed the data from the microarray analysis regarding the genes involved in cell wall metabolism, as did a biochemical analysis of the transgenic lines. The observed cell wall strengthening was further evaluated by functional analysis of some of the implicated genes to determine their contribution to the observed resistance phenotypes.

Conclusions

The PGIP-specific resistance phenotypes could be linked to one of the most basic mechanisms plants use to defend themselves, namely strengthening of cell walls. The fact that this analysis was done under uninducing conditions, points to an additional role for PGIP beyond the direct inhibition of ePGs and defense signaling that is currently accepted.

Significance of Study

PGIP expression is typically upregulated by infection; having constitutive levels of this known defense protein might directly activate general defense responses such as strengthening of cell walls, even in the absence of infection. Linking this "priming" phenomena with the PGIP-specific resistance phenotypes under uninfecting conditions does significantly contribute to understanding *how* PGIPs might improve disease resistance and also provide insight into the *in planta* roles of PGIPs.



Functional analyses of a grapevine mitogen-activated protein kinase kinase gene

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Background and Aims

A common pathway for organisms to perceive biotic and abiotic stimuli is the activation of mitogen-activated protein kinase (MAPK) cascade, which consists of MAPKKK, MAPKK, and MAPK. A grapevine MAPKK ortholog was found to be induced in the powdery mildew (PM)-infected leaf tissues in PM-susceptible *Vitis vinifera* 'Cabernet Sauvignon' and transcribed at higher level in PM-resistant *Vitis aestivalis* 'Norton' (Fung et al. 2008) . This study was to investigate the role of the grapevine MAPKK in stress responses and to identify genes that are regulated by the MAPK signaling module.

Methods and Results

We isolated a *VaMAPKK* gene from *V. aestivalis* 'Norton', and transferred it into inter-specific hybrid grape variety 'Freedom'. We compared the transcript levels of genes in two independent *VaMAPKK*-overexpressing 'Freedom' lines and in two control lines using the Affymetrix *Vitis* GeneChip®. Statistical analysis of the data revealed 1,313 transcripts whose levels were significantly different between the *VaMAPKK*-transgenic and control lines. Out of the 1,313 transcripts, 523 were increased and 790 suppressed in the presence of *VaMAPKK*. In addition, we discovered that one of the *VaMAPKK*-transgenic lines became more susceptible to the PM fungus and overexpression of *VaMAPKK* in model tobacco plants has no impact on drought tolerance.

Conclusions

Overexpression of *VaMAPKK* is linked to the up-regulation of two WRKY transcription factors and the down-regulation of defense-related PR1, Defective Induced Resistance 1 and Germin-like protein genes. *VaMAPKK* likely is a negative regulator of the grapevine defense response.

Significance of Study

The discovery of *VaMAPKK*-regulated grapevine genes provides new insights into cross-talk between grapevine's abiotic stress tolerance and defense response regulatory circuits.



Beta-aminobutyric acid-induced resistance in grapevine against downy mildew and abiotic stress

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Background and Aims

Grapevine (*Vitis vinifera* L.) is a major fruit crop worldwide and is affected by many diseases. Downy mildew, caused by the oomycete *Plasmopara viticola* is one of the most serious diseases in vineyards worldwide. Both susceptible and resistant cultivars can be colonised by *P. viticola* zoospores, but in resistant ones, the development of the parasite is rapidly inhibited. The majority of the traditional cultivars that are cultivated are susceptible to this disease, necessitating the intensive use of chemicals to limit the damage in vineyards. One possible solution would be the activation of the plants own defence system, known as induced resistance.

Methods and Results

β-Aminobutyric acid (BABA), a non-protein amino acid, has previously been shown to induce resistance against many oomycetes and to be effective in inducing resistance against various downy mildews. It was observed that the protective effect of BABA in *Arabidopsis* was due to the potentiation of natural defence mechanisms, a phenomenon referred to as priming. Priming is the capacity of a plant to express a faster and stronger basal defence response upon pathogen infection. Recently, in grapevine it has been shown that callose deposition as well as defence mechanisms depending on the phenylpropanoid and the jasmonic acid (JA) pathways all contributed to BABA-IR in the susceptible cultivar Chasselas. Microarray analysis was performed to compare gene expression in BABA-and water-treated infected Chasselas (susceptible cultivar) as well as to compare Chasselas with Solaris, a resistant cultivar.

BABA can also prime resistance of grapevine to abiotic stress. BABA-treated leaves close their stomata faster upon drought stress, probably via an increased ABA production. Microarray experiments also reveal a number of induced and repressed genes that could contribute to this better adaptation.

Conclusions

The expression of a small number of genes is modified by BABA but a higher number is primed both in pathogen-defence and in drought adaptation

Significance of Study

Susceptible cultivars have a potential of improved resistance to pathogens and abiotic stress that could be used to reduce pesticide applications.


Improving water-use-efficiency in grapevines: physiological and molecular approaches

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Improving water-use-efficiency (WUE) in grapevines is a necessary requisite for vineyard sustainability under conditions of increasing aridity, which occur due to global climate change, particularly in Mediterranean climates (Schultz, 2000).

From a physiological perspective, WUE reflects the ratio between the amount of carbon assimilated by leaf photosynthesis and water transpired. Under water stress, stomata close and WUE increases (Flexas et al., 2002), although at the expense of decreased photosynthesis rates and decreased grape yield (Medrano et al., 2003). Still, keeping stomata partially closed, by means of controlled deficit irrigation (CDI), represents an opportunity to increase WUE. We have recently reviewed physiological tools based on the indirect estimation of stomatal conductance, which potentially can be used for CDI in grapevines (Cifre et al., 2005).

It may be even more interesting – although not so readily available – to achieve increases in WUE by means of increasing photosynthesis without increasing water loses. This could result in simultaneously increased WUE and yield. Theoretically, at the leaf level there are two ways to achieve this goal: (1) to improve CO_2 diffusion to the sites of carboxylation without increasing stomatal conductance, and (2) improving the carboxylation efficiency of Rubisco. Ecophysiological studies show that the first way would be feasible by means of increasing the mesophyll conductance to CO_2 which, in species other than grapevines, depends on aquaporins (Flexas et al., 2006) and, hence, these could be regarded as potential targets for genetic engineering to increase grapevine WUE. The second way could be achieved if grapevine Rubisco, whose specificity for CO_2 over O_2 is 100 (Bota et al., 2002), could be replaced by Rubiscos from other C3 species reaching a specificity of 110 (Galmés et al., 2005). Future prospects to improve grapevine WUE by using these physiological features are discussed.



The role of aquaporins in controlling root hydraulic conductance in grapevine

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Our knowledge of aquaporin structure and function in plants has rapidly advanced in the last few years. They are gated channels that sense a variety of signals important in cell metabolism and signalling. This enables rapid responses to sudden environmental changes. Transcriptional regulation and membrane targeting occurs over diurnal time scales and may also be rapid. Interactions occur between different aquaporin isoforms indicating that function may not be elucidated by functional expression of one isoform individually. Combined there are many avenues by which aquaporins may be regulated in the plant making interpretation of the large and often rapid changes in hydraulic conductance in roots and leaves more difficult at the molecular level. Signalling between root and shoot in both directions is important to balance conductances under differing transpirational demand. Root conductance, which is linked with the expression of a PIP aquaporin in grapevine, is correlated with leaf transpiration indicating that long distance signalling is occurring. This ultimately allows regulation to achieve maximum extraction of water from the soil. Understanding these signals will be as important as is our understanding of the long distance signalling involved in stomatal regulation. The differences between cultivars in their water relations physiology (root, leaf and berry) present an opportunity to correlate the physiology with differences in gene expression or even single nucleotide polymorphisms within aquaporin genes.



Influence of rootstock and water availability on young Grenache vines growth, water use efficiency and yield

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Background and Aims

Under the context of global change, any decrease in water use by crops is essential in Mediterranean areas, where grapevines are widely grown, because water is becoming a more and more scarce resource in that region. Rootstock characteristics confer different properties to the varieties grafted onto, basically due to the different hydraulic characteristics and root biomass and distribution.

Methods and Results

A study was carried out under Mediterranean weather conditions in Caldes de Montbui (Spain), in a 2-year-old vineyard of Black Grenache grafted onto three different rootstocks: 110-R, 41-B and 161-49C. Plants were Non-Irrigated (NI) or Irrigated (I) (40% ET0). At the end of vegetative growth period (berry at pea size), veraison and post harvest, biomass and its allocation were measured. Root and trunk hydraulic conductance were determined. Soil cores were extracted to measure soil water content and root distribution. Sap flow was measured by the heat balance method form April to September. Carbon isotope composition (δ^{13} C) in leaf dry matter, grape yield and quality were also determined. Vine biomass was significantly higher in I than in NI plants, being root/shoot ratio only higher in 41-B I plants. Root and trunk hydraulics showed differences between rootstocks and dates, but not between irrigation treatments. In roots hydraulic resistance was higher in 110-R grafted and in NI plants. Biomass was dependant on water supply, whereas water transport was more related to genotype. δ^{13} C pointed out differences in WUE related to water availability. Yield and pruning weight and total must acidity were increased by the irrigation applied. Quality parameters, measured as TPI, extractability and protoantocyanidins showed differences among treatments and rootstocks.

Conclusion

Biomass is highly dependant on water supply, whereas the water transport is more related to genotype. Water use in the vines is related with leaf area and hydraulic conductivity, but further research is needed in the relationship root biomass and hydraulic conductivity, especially with whole root systems instead of excised segments. Yield and quality showed some differences but those can change with vineyard ageing.

Significance of Study

Rootstock selection should be seriously regarded for new vineyards to reduce or avoid the potential negative effects of global change in grape yield and quality



Characterising water-use strategies of major winegrape varieties grown in Australia

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Background and Aims

In Australia the majority of winegrapes are grown with irrigation to supplement rainfall and stored soil water. In recent years sustained dry conditions and reductions in the amount of water available for irrigation have highlighted the need to improve the efficiency of water use. This project aims to provide information about the relative water requirements of the major winegrape varieties grown in Australia through an understanding of the expression of their isohydric (drought avoiding) or anisohydric (drought enduring) characteristics.

Methods and Results

To assess differences between varieties stomatal behaviour and water relations were examined in vines grown under commercial conditions, driven by natural environmental variation. Nine varieties were examined in a variety block at the SARDI Research Station, Barossa Valley. Stomatal conductance (g_s), leaf water potential (Ψ_L) and xylem sap abscisic acid (X-ABA) were measured in the morning and afternoon on 8-12 days in seasons 2006/07 and 2007/08. The effect of seasonal variation on the stomatal behaviour of all varieties was significant, with 06/07 being severely affected by late frosts and low winter and spring rainfall, resulting in small canopies and low yield. Slightly more favourable conditions in 07/08 ensured good sized canopies and average yields. Varieties that controlled water loss via stomatal closure in response to increasing VPD included Grenache, Riesling, Shiraz, Cabernet Sauvignon and Sangiovese. Varieties that showed poor stomatal control included Merlot, Semillon, Chardonnay and Verdelho. In season 06/07 Ψ_L was correlated with g_s in most varieties however in 07/08 the relationship was less clear though most varieties maintained Ψ_L in a narrow range throughout the season. Increased X-ABA was associated with stomatal closure in varieties that decreased g_s in response to VPD.

Conclusions

The effect of seasonal differences on stomatal / plant water relations suggests that ranking cultivars as isohydric or anisohydric may have limited use. A more useful measure may be to rank varieties based on a scale ranging from drought tolerance to drought intolerance according to interactions between VPD, g_s , Ψ_L and ABA.

Significance of Study

As water becomes an increasingly scarce and valuable commodity for the Australian wine industry scientific knowledge about water use strategies of the different varieties will assist managers in making resource allocation decisions.



Application of genomics to grapevine improvement

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Imagine a breeder browsing a grape chromosome nucleotide-by-nucleotide around a trait locus, scrolling down the ordered list of catalogued genes across a genetic interval, resequencing for a few thousand dollars the superior grapevine he has selected. In the past couple of years, this vision has become a reality. The availability of the reference genome sequence has provided significant assistance in the saturation of trait loci with targeted genetic markers. Grape breeders are now offered unprecedented possibilities for selecting plants using DNA sequences within or intimately near to the gene that controls a desirable trait, rather than handling their phenotypes. Current technologies in marker assisted selection are revolutionizing the grape projects and apply to thousands of seedlings every year, as previously feasible only for cereals and annuals. The expected outcome of this new era of genomics assisted breeding is a renewal of the varietal platform, with brand new genotypes that meet the growing demand for disease-free vines and flavourful grapes. How far we go in exploring and characterising germplasm is crucial for translating the extant natural diversity into newly bred cultivars, which could grow beyond the fence line of the experimental vineyards and gain substantial market share.



Characterization of Major Quantitative Trait Loci Controlling Berry and Phenology-Related Traits

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Background and Aims

Most traits of interest for viticulturalists and winemakers have a complex nature, being attributable to the interactions of multiple genes and their environment. Statistical genetics using QTL (Quantitative Trait Locus) mapping is a powerful tool to determine the genetic architecture of phenotypic traits and provides a basis for the identification of candidate genes underlying trait variation. The aim of our work was to investigate the genetic determinism of relevant traits related to berry composition and development.

Methods and Results

Target features were evaluated in two segregating progenies in three growing seasons. To this purpose we recorded flowering, veraison and ripening dates, we measured berry size, seed number and weight, and we quantified the main monoterpenes responsible for Muscat flavour through highresolution gas chromatography-mass spectrometry (HRGC-MS). A number of QTLs were found with reproducible effects over years, which in some cases clustered in genomic regions controlling different characters. Interesting findings came out from the molecular characterization of the main QTLs for phenology and monoterpene content based on the whole grapevine genomic sequence. Candidate genes playing a potential regulatory role in trait variation were suggested according to their predicted function and their observed co-localization with QTLs. The functional significance of these associations is currently being evaluated through expression and association analysis. Focusing on the most promising candidate gene for Muscat flavour, we investigated the quantitative and temporal relationship existing between transcript level (RT-PCR) and monoterpene accumulation (HRGC-MS) in a small set of aromatic and non-aromatic grapevine varieties collected from pre-veraison to over-ripening. Moreover, we exploited the natural variation of a grape germplasm collection including aromatic and non-aromatic varieties (150 accessions) in order to test the existence of correlations between specific polymorphisms and aroma degree.

Conclusions

In this work we located the genomic determinants of berry and phenology-related traits. We observed interesting associations between candidate genes and QTLs, which provided the basis for functional studies testing their significance.

Significance of Study

This research revealed new insights into the genetic control of relevant grapevine features. Testing the role of specific genes in trait variation will contribute not only to the understanding of plant biology but also to crop improvement by breeding.



Alleles of anthocyanin 5-glucosyltransferase of Vitis vinifera are non-functional

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Background and Aims

Since a long time it is known that traditional red and black cultivars of *Vitis vinifera* produce only anthocyanin 3-monoglucosides in the berry skins after véraison but no or only traces of anthocyanin 3,5-diglucosides. In contrast, many other *Vitis* species synthesize significant or even huge amounts of diglucosylated anthocyanins. To elucidate the genetic basis of this phenomenon the gene coding for anthocyanin 5-glucosyltransferase (5-gt) catalyzing the formation of anthocyanin 3-glucosides to anthocyanin 3,5-diglucosides, was isolated from grapevine cultivar 'Regent'. This new *V. vinifera* cultivar descends from a cross of 'Diana' (traditional *V. vinifera* cv.) x 'Chambourcin' and produces anthocyanin 3,5-diglucosides.

Methods and Results

Using a PCR approach with degenerated primers deduced from 5-gt genes of different species five 5-gt homologous gene fragments have been obtained from 'Regent'. The expression pattern of theses five genes was investigated in berry skins of various genotypes, either with or without the ability to form anthocyanin diglucosides, using the 3' RACE technique. One gene, 5-gt7, showed a clear correlation of gene activity and anthocyanin diglucoside formation. Both alleles of the 5-gt gene were obtained after screening a BAC library of 'Regent' and sequencing two different BAC clones. Southern blot analysis confirmed the allelic state of the single copy 5-gt gene. The most striking difference between the two alleles is that among nine point mutations found one is leading to a premature stop resulting in a truncated open reading frame and probably inactive enzyme. A molecular marker was developed capable to differentiate the two alleles and applied on a 'Regent' x 'Lemberger' population segregating for the dominant trait 'anthocyanin diglucosides'. A complete correlation was observed between the full length 5-gt allele to 'diglucosides' and the truncated allele to 'no diglucosides', respectively. Thus, in the case of cv. 'Regent' the formation of anthocyanin 3,5-diglucosides is the result of a functional 5-gt coming most probably from a nonvinifera species. Moreover, both 5-gt alleles of cv. 'Lemberger' are truncated due to different deletions leading to frame shifts. In addition the 5-gt alleles from cv. 'Pinot noir', recently published, also show mutations leading to truncated proteins.

Conclusions

A functional 5-GT synthesizes anthocyanin 3,5-diglucosides in grapes. Mutated alleles of 5-GT in traditional *V. viniferas* are probably responsible for the absence of anthocyanin 3,5-diglucosides.

Significance of Study

The data deliver an explanation for the old observation why anthocyanin 3,5- diglucosides are missing in grapevine generally.



A lesson from a large-scale comparative analysis of bud response to different artificial dormancy stimuli – A station in the ongoing voyage dedicated to exploration of the mechanism responsable for bud dormancy release

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Background and Aims

Hydrogen cyanamide (HC) provides controlled, evident, synchronized and relatively rapid induction of bud dormancy release within a well-characterized time frame, thereby creating a traceable and reliable system that facilitate the identification of various biochemical components possibly involved in mechanism of bud dormancy release. Using this system, we previously showed that catalase expression and activity are inhibited shortly after HC application, and hydrogen peroxide level is increased. We also detected transcriptional up regulation of pyruvate decarboxylase, alcohol dehydrogenase, thioredoxin h, glutathion S transferase, ascorbate peroxidase, glutathion reductase and sucrose synthase. Overall, these changes suggested the development of oxidative and respiratory stress in the bud, and may hint that redox signaling is involved in dormancy release.

Identification of similar changes following the application of different stimuli that induce dormancy release may support the relevance of these changes to the dormancy release process. It may also become a potent tool to identify additional factors that may play a central role in dormancy release, independent on the nature of stimulus. Accordingly, we compared the effects of HC and heat shock (HS) on the expression patterns of the genes mentioned above and showed that they are similarly induced by both stimuli, also in different timing and intensity. These findings suggest that similar mechanisms might be triggered by different stimuli, and support involvement of the above genes in the process of dormancy release. In the current study the comparative approach was used in a large scale attempt to expose factors and pathways involved in dormancy release.

Methods and Results

Large-scale comparative analysis of bud response to HS and HC was performed, using custom grape-bud cDNA microarrays. Detailed analysis of the microarray data and further biochemical analyses will be presented.

Conclusions

A working hypothesis is suggested, where temporary inhibition of oxidative phosphorylation may lead to respiratory and oxidative stress, expressed as decreased production of ATP and a temporary increase in H_2O_2 level. In response, glycolysis and anaerobic respiration are induced to face the energy crisis, and respiration is enhanced, as reflected by CO_2 evolution. The antioxidative machinery and related pathways are induced in parallel to face the oxidative burst. Potential candidates that may stimulate the subsequent events in the pathway, which appear to involve cellwall loosening and induction of the cell cycle, will be discussed.

Significance of Study

This study considerably improves our knowledge about factors involved in the process of bud dormancy release.



Biological and environmental factors controlling root dynamics and functioning: consequences of root aging effects and soil moisture

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Background and Aims

Understanding factors controlling root dynamics and functioning can lead to more efficient and profitable vineyard management. Internal carbon demands in plants (endogenous factors) interact with environmental factors (exogenous factors) to regulate seasonal changes in root growth and mortality. However, our current understanding of how endogenous or exogenous factors regulate root dynamics is limited, particularly under field conditions. We will present our current understanding of grape root dynamics from long-term field data and supporting greenhouse studies. We will focus on data collected with minirhizotron cameras that allow for direct assessment of dynamics, and experiments with roots of known age that allow for linkages between root phenology and function.

Generalizations and Conclusions

Timing of grape root production varies widely among different regions, as well as varying among rootstocks and canopy management in the same region. Timing of production can be responsive to differences in soil moisture. Grape root lifespan, however, appears less affected by soil moisture due to nocturnal hydraulic redistribution. Root function, such as capacity for P and N uptake, declines rapidly with root age. Differences in timing of root production can lead to differences in the age structure of root populations and effect aboveground growth. Because young roots are more susceptible to herbivory and pathogens, attacks shift the age structure of affected vines and can lead to greater or lesser vine susceptibility to root pests depending on the timing of pest attack.

Significance of Study

Improving our understanding of when roots grow and are functionally active in agricultural systems can lead to improved water and fertilizer applications and more precise vineyard management. Because both environmental and biological factors affect root dynamics, simple predictions of timing of root production or standing populations with shoot development are unlikely to be achieved. However, with multi-year data on root dynamics and environmental and biological factors, regionally specific models of root populations and their functioning may be possible to develop.



Seasonal carbohydrate, nitrogen and cytokinin dynamics and their impact on shoot and root growth

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Background and Aims

The growth of grapevines is determined, in part, by the extent to which water and nutrient supply enables maximum photosynthesis rates to be maintained throughout the season. To establish this photosynthetic and nutrient uptake capacity, growth is initially supported by reserve carbohydrates and nitrogen. Root-synthesised cytokinins are also translocated in the xylem to the shoot tips where they are involved in regulating growth. In other plant species a close relationship between cytokinin concentration and nitrogen has been shown (Sakakibara, 2006). The aim of this experiment was to determine if seasonal carbohydrate, nitrogen and cytokinin dynamics influence grapevine growth and development.

Method and Results

Carbohydrate, nitrogen and cytokinin concentrations in the root, wood, leaf and xylem sap were measured throughout the growing season on field-grown Shiraz vines. Wood and root carbohydrate and nitrogen reserves declined following bud-break. Shoot length growth rates during this period were correlated with cytokinin concentrations in the pre-dawn xylem sap, suggesting involvement of this plant hormone in early vine growth. Carbohydrate reserves partially recovered between flowering and veraison, but then decreased again during ripening, consistent with a high crop load demand. Carbohydrates then increased after harvest to the high levels occurring just prior to budbreak. Periods of carbohydrate recovery (carbon surplus) were associated with increased root growth activity. In addition, considerable variation in root cytokinin concentration occurred throughout the growing season and may be used to control seasonal root growth rates.

Conclusions

Results show that carbohydrate and nitrogen reserve dynamics were consistent with vine growth and productivity throughout the season. Cytokinins also apparently played a key role in regulating early shoot growth. In addition, cytokinin concentrations in the root would appear to regulate timing of root growth, when surplus carbon was available.

Significance of Study

The research enhances the understanding of how seasonal carbohydrate and nitrogen reserve dynamics influenced cytokinin production to alter grapevine growth and development. Such results will ultimately contribute to better canopy management.



Timing of ¹⁵N-depleted ammonium nitrate uptake by grapevines in a subtropical environment.

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Background and Aims

Nitrogen fertilization practices in tablegrape vineyards of subtropical Queensland have largely resemble programmes used in temperate areas of Australia. This is despite the shorter growing season from budburst to harvest and longer postharvest period in the subtropics. The relatively small amount of white root on vines between budburst and veraison, and absence of a spring root growth flush highlights the need to confirm the appropriateness of applying N fertilizer during the spring period. The objective of this study was to quantify nitrogen uptake by grapevine roots during the spring and postharvest periods in a subtropical environment.

Methods and Results

Isotopic ammonium nitrate (¹⁵N-depleted) was applied between budburst and flowering or after harvest, to 11 year old vines of Thompson Seedless in a vineyard on a Chessborough red earth at Mundubbera (151°18'E, 25°35'S). Single leaf tissue samples of the youngest fully expanded leaf were collected at 2 and 3 day intervals over 87 days (spring) and 70 days (postharvest) following application and analysed for total nitrogen and ¹⁵N.

Nitrogen uptake as indicated by declining atom% ¹⁵N in leaves, continued over a longer interval in the postharvest period (47 days) than in the spring period (26 days). The percent recovery of labelled fertilizer was greater after harvest (62%) than during spring (41%).

Conclusions

Uptake of nitrogen by vines growing in a subtropical environment occurs in spring and after harvest, irrespective of the disparity in amount of white root on the vine at each time of the season. The faster rate of decrease in atom% ¹⁵N and therefore absorption of fertilizer N in spring suggests N uptake is demand driven by the rapidly growing shoots, inflorescences and subsequently the newly set berries.

Significance of Study

The relative contribution of white roots and mature woody roots to N uptake during each period was not determined and requires further field research. Increasing the amount of white root present on the vine in spring may be beneficial for berry growth and fruit ripening in subtropical environments, however the ability to achieve this goal through vine management also requires further study.



Below ground effects of five years of deficit irrigation in Cabernet Sauvignon

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Background and Aims

Due to the difficulties involved with below ground study, knowledge of grapevine root responses to their environment is limited, particularly under Australian conditions. As 83% of Australia's 174,000 ha of vineyards are irrigated and water scarcity is an increasing problem, deficit irrigation is likely to become more common. Below ground effects of irrigation strategy may be significant in determining the long-term survival of the vine, both in terms of efficient water uptake and provision of nutrients. This study has examined the effect of six seasons of deficit irrigation on root biomass and basic soil properties.

Methods and Results

In 2002 a trial was set up in a commercial vineyard using 36 rows of mature Cabernet Sauvignon vines and consisting of twelve replicates of three irrigation treatments: regulated deficit irrigation (RDI), prolonged deficit (PD - an extension of RDI with no irrigation until early veraison) and a well watered control, which received double irrigation in the years 2005-7. Prior to budburst in the 2007-8 season 1 m deep, 50 mm diameter soil cores were taken, adjacent to a dripper, from each of the 36 replicates. These were split into four depths, the roots extracted and soil conductivity and pH examined. Finally, a grid of nine 0.75 m deep cores was taken from two replicates for each treatment. The grid stretched from under the vine to the mid-point between rows and from the trunk to the mid-point between vines. In terms of soil chemistry there was little effect of the PD treatment, whereas conductivity and pH were reduced by the additional irrigation in the control treatment. Approximately 75% of total root biomass was recovered in the top 50 cm of soil in all three treatments and there was no evidence of an irrigation effect on fine roots adjacent to the drip line. However, biomass from the grid of cores indicated a clear difference between treatments, with control vines having 18% more and PD vines 32% less root biomass than RDI.

Conclusions

There was no evidence that whole vineyard root biomass increased in response to deficit irrigation, but it did match irrigation effects on aboveground growth in the previous season.

Significance of Study

There are few reports of the effect of irrigation strategy on the root systems of mature field grown vines. This work indicates that deficit irrigation can have a major effect on root biomass.



Grapevine functional genomics and transgenic technology

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Background and Aims

The understanding of the genetic determinism of plant phenotypes requires the functional annotation of genes governing specific traits including the characterization of their regulatory networks. A striking feature of the grapevine genome and proteome lies in the existence of large families related to wine attributes (Jaillon *et al.*, 2007), that have a higher gene copy number than in other sequenced plants. During speciation, the appearance of new adaptive functions is often based on the evolution of orthologous genes eventually associated with duplication (paralogous sequences) leading to new proteins and expression profiles. The presence of many original features in grapevine, including perennial status, vegetative architecture and ramification, formation of inflorescence/tendril, flower organization (corolla), fleshly fruit of considerable acidity, astringency and sugar content makes compulsory the development of functional genomic research in an homologous context. Because of the current limitation of high throughput reverse genetic techniques (mutant populations) and the difficulties of genetic mapping (high level of allele diversity, chimerism, long generation times), genetic transformation is a critical technology for characterizing the function of genes.

Material and Results

The first success in transforming grapevine was reported by M.G. Mullins (1990) and since then other groups have reported introducing genes for pathogen resistance or berry quality. During the last 15 years, transformation technology has also been optimized to allow the transformation of cells, tissues, organs and whole plants from a broad spectrum of *Vitis* genotypes, including *V. vinifera*. The main advances in grapevine transformation technology will be reviewed.

Conclusions

Though grapevine transformation is still not "a long and quiet river", different techniques are available to gain functional information about genes. The choice of a particular transformation approach depends of the type of process investigated (metabolism, developmental process, etc) and experimental purpose (e.g. induction of ectopic functions, promoter studies, subcellular localization).

Significance of the Study

The recent achievement of a draft genome sequence offers new perspectives to better understand the link between phenotype and genotype. It is crucial for people contemplating or involved in a grapevine functional genomics program to know the current state-of-art, the potential and the limits of transformation technology.



Functional Genomics of Dormancy Induction in Grapevines

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Background and Aims

Bud dormancy in grapevines is an adaptive strategy for the survival of drought, high temperature, low temperature and freeze dehydration stress; however, it may also limit the range of cultivar adaptation. Development of a comprehensive understanding of the biological mechanisms involved in bud dormancy is needed to promote advances in selection and breeding, as well as developing improved cultural practices for dormancy management in existing grape cultivars.

Methods and Results

An integrated functional genomic approach using transcriptomic, proteomic, and metabolomic profiling is being used to identify mechanisms regulating bud endodormancy. Short (SD) and long (LD) photoperiods were used to induce endodormancy or maintain paradormancy, respectively, in photoperiod responsive (*V. riparia*) and non-responsive (V. spp. 'Seyval') genotypes. Analysis of age-matched buds at 7 time points during endodormancy induction or paradormancy maintenance allows separation of endodormancy regulation from bud maturation responses. The transcriptomic, proteomic and metabolomic data is being mapped onto *Vitis* molecular networks and visualized using OmicsViz plugin in Cytoscape. Mapping 3892 significantly expressed transcripts provided visualization of 36% of these and showed dynamic time x treatment interactions.

Conclusion

Genotype-specific differential response to dormancy induction treatments allows identification and mapping of endodormancy specific gene expression.

Significance of Study

This information will be used to further the understanding of molecular mechanisms that promote and maintain bud dormancy and to identify markers for breeding and mapping programs.



Over-expression of *VvHB13* gene changes the size of different organs in transgenic tobacco plants

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Background and Aims

In grapevine, VvHB13 gene has been initially identified in different microarrays and cDNA suppression subtractive hybridization experiences, and associated with berry flesh development. This gene shows a strong induction just after fertilization, when cell division in the mesocarp is most active, but it is also expressed in other organs like roots, stems, leaves and inflorescences. *VvHB13* gene encodes a protein which share homology with the homeodomain leucine zipper (HD-Zip) protein family of *Arabidopsis* and it is closely related to the member of the subfamily I *ATHB13*. The role as transcription factors of the members of this subfamily has been related to developmental events in response to environmental conditions. In this study, we have taken a reverse genetic approach to clarify the biological function of *VvHB13*. Although grapevine presents many specific characteristics, the over-expression in a model plant like tobacco can elucidate conserved functions.

Methods and Results

Fifty independent transgenic lines with different levels of *VvHB13* expression were obtained by *Agrobacterium-mediated* genetic transformation. Wild type *N. tabacum* cv. SR1 plants and three different homozygous transgenic lines were grown under controlled conditions and phenotyped at different development stages. The size of cotyledons, leaves and internodes is severely reduced in transgenic plants during vegetative growth. Conversely, inflorescences branches and internodes elongate more in transgenic plants, equalizing the size of wild type control plants at the end of reproductive phase. Finally, flowers and capsules size were also modified in transgenic plants. Sepals are smaller and capsules are rounder and bulkier at the base. All modifications are mainly related with a change in the number of cellules.

Conclusion

Over-expression of *VvHB13* modifies the size of different organs in a negative or in a positive sense, depending on the tissue and the development stage of the plant. However, modifications in transgenic tobacco capsules does not seem to provide a strong evidence in support of the relationship between the maximal *VvHB13* expression and the intense cell division observed in specific tissues of the grapevine berry, so further confirmation in a fleshy fruit plant model is still required.

Significance of Study

This study supports a function of the *VvHB13* gene in the integration of development signals to cell division in different organs of grapevine.



Altering flavonoid composition in grapevine

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Background and Aims

Flavonoids are secondary metabolites, comprising three classes in grape berries and two of them contribute greatly to wine quality. Anthocyanins are red coloured compounds, present in the skin of red and black grapes. Condensed tannins or proanthocyanidins are polymers found in the skin and the seed of grape berries and they contribute to mouthfeel and astringency of fruit and wine.

These compounds are synthesised via a well-characterized biochemical pathway which branches to form the three major classes of compounds. The pathway is tightly regulated to produce specific compounds in different tissues of the plant and at different times in the development of the vine and particularly the berry. For example tannins are synthesised early in berry development and expression of ANR (encoding anthocyanidin reductase which is thought to synthesise epicatechin) correlates with tannin synthesis. MYB transcription factors provide specificity for control of the pathway branches and therefore regulate the class of compounds synthesised. Recently MYB regulators VvMYBA and VvMYBPA1 have been described that control anthocyanin and tannin synthesis, respectively (1-3) through control of the relevant branches of the pathway.

Methods and Results

Two sets of transgenic grapevines have been developed with reduced expression of ANR and overexpression of VvMYBA1. These vines have an altered tannin composition and produce anthocyanin in young berries. In transgenic grapevines with constitutive MYBA1 expression leaves, stems and tendrils have a deep purple colour due to the presence of large quantities of malvidin-3-glucoside. Roots are red too but contain cyanidin compounds. These plants also have increased flavonol concentration and altered tannin composition in some tissues compared to Chardonnay. Some of these changes can be related directly to alterations in gene expression.

Conclusions

These results will be discussed with relation to our understanding of the control of flavonoid synthesis and how proanthocyanidins are synthesised.

Significance of Study

The results of these experiments indicate the opportunities to utilize regulators to modify flavonoid composition for improved wine quality. The MYBA gene also has proved to be a valuable marker for transformation due to its ability to regulate anthocyanin synthesis effectively.



Conventional and biotechnological approaches for the improvement of table grapes

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Seedless grapes are generally preferred by consumers for fresh consumption. Approximately 80% of the table grapes sold is seedless. Most commercial seedless cultivars are stenospermocarpic and require normal fertilization for fruit set. The pollen of such cultivars has generally been found to be viable and has been used in controlled crosses to produce seedless types in several breeding programs in both research institutes and commercial companies worldwide.

The general objectives in most breeding programs for table grape can be summarized as follows: Better eating quality; Extension of the season by creating early maturing types and late maturing types; Seedlessness; Attractive appearance; Large berry size; Bright colors; Unusual shape; Long shelf life; Firm flesh; Naturally loose bunches; Uniform berries; Novel tastes and aromas.

Improved embryo rescue protocols were the main tool that enabled recovery of sufficient populations of F1 hybrids to facilitate selection. Plant growth regulators are one of the important factors affecting success of embryo rescue and have been widely used to improve the efficiency of embryo germination following crosses. The role of growth regulators applied in the field before the in- vitro step will be discussed. Examples for new and promising selections developed recently in different breeding project worldwide will be presented.

Genetic transformation for the improvement of table grape had been long discussed as an important tool to develop new products. It allows incorporating of single or few traits into popular varieties. Potentially, no changes will occur in the other existing desirable characteristics of a variety. It allows to circumvent problems encountered in conventional breeding and may provide rapid screening of the engineered trait. The presentation will review potential application of genetic transformation in table grapes for bacterial, fungal and viral disease resistance. Abiotic stress such as tolerance to cold, salt, drought and heavy metals and quality traits such as: seedlessness, flavor, reduced browning and ecological characteristics.

Several limitations were reported during the years while trying to implement transformation for creating novel products for the table grape industry. These limitations are of two kinds: 1. Social, legal and public acceptance issues. 2. Scientific issues such as reduction in the embryogenic potential and subsequent germination rates, necrotic responses to Agrobacterium and low transformation efficiencies. Studies trying to understand the molecular and physiological background of these limitations and the way to improve transformation efficiencies will be presented.



A Molecular Study of the Regulation of Grape Berry Ripening

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Background and Aims

We recently identified a core-set of 1861 genes significantly and specifically modulated during Pinot Noir berry ripening suggesting a tight regulation of this process at transcriptional level. Within this set a relevant fraction of genes encode transcription factors, proteins involved in signal transduction or hormone metabolism. We also observed the strong accumulation of hydrogen peroxide around veraison, suggesting a possible signalling role for the reactive oxygen species (ROS) in this developmental process. This paper reports the study of ethylene responsive factors (ERFs) and reactive oxygen species as possible players within the regulatory network which governs berry ripening.

Methods and Results

ERF domain-containing sequences have been searched in the Pinot Noir genome and grouped according to their sequence similarity in order to obtain a phylogenetic tree and compare them to the homologous proteins in other species. Gene expression studies on some representative members showed large differences regarding tissue specificity and transcript's profile along berry development. In silico analysis using publicly available gene expression data and promoter analysis identified a group of ERFs modulated putative target genes during ripening. ROS accumulation and ROS metabolism have been studied at the biochemical level by measuring enzymatic activities (e.g. enzymes of the ascorbic acid/glutathione cycle, catalase) and oxidative stress markers (e.g. lipid peroxidation and hydrogen peroxide intracellular levels). A general burst of oxidative power at véraison and a possibly consequent activation of the scavenging enzymes have been observed.

Conclusions

Some members of the ERF family are modulated along berry ripening and two of them are induced at véraison in correspondence of a reported peak of endogenous ethylene. A burst of ROS and of ROS scavenging activity is also observed starting from véraison.

Significance of Study

There is several data suggesting that ethylene plays an important role during grapevine berry ripening, although this fruit is classified as non-climateric. In this study some transcription factors modulated during ripening and possibly regulated by this hormone have been characterized.


Comparative analyses of differentially expressed genes involved in flavonoid biosynthesis in North American native grapes: 'Noble' and 'Ison' muscadines vars., and 'Cynthiana' aestivalis var

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Background and Aims

The common *Muscadinia* possesses one of the highest flavonoid levels among fruits. Due to the different biological activities of plant secondary metabolites, their regular consumption may have significant consequences for human health. Study of differentially expressed genes in the flavonoid biosynthesis was carried out to generate knowledge of the production of these compounds in *muscadinia* and *aestivalis* and identify candidate genes for further functional analysis in the North American grape species.

Methods and Results

Key developmental stages – "véraison" and "physiological maturity" – were studied by the use of high throughput 70-mers microarrays in the *muscadinia* 'Noble' var. The expression profiles of 13 differentially expressed genes involved in critical steps of the flavonoid pathway were further validated in the 'Ison' var. and 'Cynthiana' *aestivalis* var. by real-time PCR. Six transcripts revealed similar expression patterns across the two species. From the remaining 7 genes, 5 showed significantly similar expression patterns in 'Noble' vs. 'Ison' compared to 'Noble' vs. 'Cynthiana', and for 2, the expression patterns were different in each of the varieties.

Conclusion

Out of the 13 analyzed genes involved in the flavonoid biosynthesis, 11 revealed similar expression patterns in the two *muscadinia* varieties and only 5 when 'Noble' was compared to the *aestivalis* variety. A high-resolution picture of the transcriptome dynamics that occur during the final developmental stages – "véraison" and "physiological maturity" of berry ripening in 'Noble' and 'Ison' provided a unique and comprehensive view of key genes involved in the flavonoid biosynthesis of the muscadines.

Significance of Study

This is the first step towards understanding the genetical enhancement of the expression of flavanoid compounds in the North American grape species during two important grape developmental stages.



Systems Biology Of Berry Ripening And Postharvest Withering Processes

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Background and Aims

Postharvest withering of grape berries is used in the production of dessert and fortified wines to alter must quality characteristics and increase the concentration of simple sugars. The molecular processes that occur during withering are poorly understood. The identification of the driving molecular events characterizing the ripening and withering of the *Vitis vinifera* cv. Corvina grapes is matter of interest for the production of Amarone and Reciotto wines.

Methods and Results

A grapevine 25471-gene chip, Combinatrix technology, allowed characterizing modulation of berry gene expression during the development and postharvest withering processes. Changes in the proteome and in the metabolome of Corvina berry during the same processes were also studied by DIGE and HPLC-MS analyses

Conclusions

This experiment has made a significant contribution to understanding the molecular basis of grape berry ripening and withering to identifying useful molecular, biochemical and metabolic markers.

Significance of Study

Integration of the three data sets in order to describe the systems biology of grape berry ripening and post harvest withering



Understanding the Biology: Moving away from Observation Based Science to Predictive Science

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Due to the pressure of the consumers, of the policy makers, and of the climate, several major challenges must be faced by viticulture : (a) reduce the phytochemical treatments on vineyards (b) limit the alcohol content of the wine (c) maintain selected grapevine varieties in specific areas in spite of global warming and limited water supply.

In order to face these challenges, it is necessary to predict the physiological response of the plant, and the final metabolic response of the berries to a wide range of abiotic and abiotic stresses. Significant progresses in our understanding of the physiological and metabolic processes that contribute to the final berry content under a changing environment has been achieved both by high throughput approaches and by studies on candidate genes. The major problems of high throughput approaches is that they generate extensive amounts of data that are not yet easy to store, to analyze and to interconnect for data mining. The major problem of candidate gene approaches is the lack of an easily and efficiently transformed short cycling genotype, and the lack of mutant collections.

Important issues for further progress in the control of berry quality are a better chemical and sensorial characterization of the compounds affecting the organoleptic quality of the berries, and of the impact of diseases and climate on their biosynthesis.

A perfect predictive system should be able to model the effect of any parameter involved in grape growth and development (genotypes of rootstock and variety, climate, viticultural practices, diseases, phytochemical treatments and alternative strategies) and in wine-making (biological, physico-chemical and technological aspects of fermentation, assembly, ageing) on the final composition of the wine. This would allow to adapt the composition of the wine to the wide variety of preferred tastes and flavors found among the wine consumers over the world.

Some aspects of these ideas will be illustrated by recent work and efforts made in our lab and in our institute on various topics.



Molecular Characterization of Aroma Genes in *Vitis Vinifera* var. Moscato Bianco

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Background and Aims

Grape-derived flavour compounds and some flavour precursors modified during fermentation, wine evolution and ageing, are fundamental in determining the organoleptic parameters used to define wine quality as the aromatic quality, persistency and complexity are wine characteristics that may influence consumer choice. Although hundreds of secondary metabolites that potentially contribute to wine flavour have been detected in grapes, the knowledge of the biosynthesis of many of these compounds is not well understood and only a few genes involved in flavour pathways have been discovered and characterised. The flavour and aroma of certain *Vitis vinifera* grape varieties is dominated by volatile terpenes and small volatile aldehydes. Typical monoterpenol components of Muscat cultivars, a group of *Vitis vinifera* aromatic varieties, are linalool, geraniol, nerol, citronellol, and alpha-terpineol. The aim of this research is the discovery and characterization of genes involved in terpenoid biosynthesis of "Moscato Bianco", one of the most important Italian Muscat varieties.

Methods and Results

Samples of flowers and berry at different developmental stages, from fruit set to technological ripening, have been analysed for their aroma content and the expression of both previously characterised genes and some that are predicted to be involved in grape flavour pathways. The aroma compounds have been extracted by SPE and SPME procedures and analysed by GS-MS, while the expression of characterised grape aroma genes and some candidate genes have been analysed by real-time RT-PCR.

Among the genes we analysed, some showed a peak of expression at berry set, while others are expressed at veraison or during the berry ripening. In particular, some candidate genes showed a peak of expression in flowers (3 putative geranyl or geranylgeranyl diphosphate synthase, alphaterpineol synthase, valencene and germacrene D synthase, and 3 other additional putative terpene synthase genes), another during fruit set (HMG-CoA reductase), one at veraison (carotenoid cleavage dioxygenase 1) and others during ripening (1 putative geranyl or geranylgeranyl diphosphate synthase, 3 putative terpene synthases).

Conclusions

The analysis of correlations between aroma accumulation and gene expression has allowed the identification of candidate genes potentially involved in grape aroma compound biosynthesis and these will be functionally characterised.

Significance of Study

The discover and characterization of genes that encode the enzymes from grapevine flavour pathways and analysis of their activity during berry development would support decisions on management of genotype, environment and viticultural practices for improving grape flavour and aroma potential.



Characterization of early light inducible proteins (ELIPs) in Vitis vinifera L.

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Background and Aims

ELIPs are proteins induced by light (Meyer and Kloppstech, 1984). Its induction by high light intensities has suggested a protective role against photoinhibition (Hutin et al 2003). However, after recent reports this assumption seems to be controversial (Rossini et al, 2006). Induction of ELIPs by UV radiation has been also reported (Savenstrand, H et al 2004). In contrast with what happens in annual plants, studies of ELIPs in grapevine are scarce. As grapevine is normaly grown in sites with high radiation, we liked to characterize its expression under natural light.

Methods and Results

ELIPs expression was studied in young and mature leaves as a function of different light intensities obtained using neutral filters. Leaf temperature was kept at 28°C (\pm 2). ELIPs were detected by immunoblotting. In young leaves expression started at 300 µmol PAR m⁻²s⁻¹ reaching a maximum at 1000 µmol PAR m⁻²s⁻¹. Independently of the light intensity and the time of induction, no ELIPs were detected in mature leaves. A positive correlation was observed between the level of ELIPs and the degree of photoinhibition. Using cut off filters, natural UV-B radiation resulted more effective than UV-A in inducing ELIPs. An EST database analysis, showed that in grapevine Elips gene has a sequence of 597 bp, with 3 exons and 2 introns. This gene would codify for a protein with 199 amino acids with three trans-membrane domains and a signal peptide for chloroplast destination.

Conclusions

In grapevine, expression of ELIPs occurred only in young leaves. Expression starts at 300 μ mol PAR m²s⁻¹ which is an intensity lower than those reported for annual species. In grapevine UV-B resulted more effective in inducing ELIPs than UV-A and in no case the presence of this protein resulted in a decrease of photoinhibition. The predicted sequence of the *elip* gene presents a great homology with the *elip* gene described for pea and arabidopsis.



Differential gene expression in *Vitis* genotypes submitted to iron deficiency

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Background and Aims

The molecular bases of plant responses to iron deficiency are well described in model plants such as *Arabidopsis* and tomato. Upon iron shortage, grapevine displays some of the classical modifications involved in "strategy I" to cope with this situation: induction of root ferric chelate reductase, acidification of external medium, and accumulation of organic acids in root tips. The aim of this work was to compare gene expression in roots of two grapevine genotypes, one tolerant and one susceptible to iron deficiency.

Methods and Results

Using the cDNA-AFLPs technique, differentially expressed fragments were isolated from roots of Vitis riparia cv Gloire de Montpellier (RGM) and Vitis vinifera cv Cabernet Sauvignon (CS) plants growing in vitro with or without iron supply to the medium. Fragments were re-amplified, cloned and sequenced. Validation of gene expression was performed using Quantitative Real Time-PCR, on root samples collected on hardwood cuttings of the same genotypes after one day and one week of culture under hydroponic conditions with or without 90 µM of Fe-EDTA, or 0.5µM of iron and 5 mM of bicarbonate. Quantitative Real Time-PCR was carried out with SYBR® Green in an i-Cycler (BIO-RAD) according to protocols set up in the laboratory. A set of 104 differentially expressed fragments (64 from RGM and 40 from CS) were sequenced. The comparison of sequences in the NCBI database revealed that more than 58% in RGM and 78% in CS showed high homologies with known functions related to energetic metabolism (phosphate dehydrogenase, cyanase hydrolase...), iron metabolism (metal transport protein, copper transporting P-type ATPase), stress related genes and cellular signal homology. Out of the 104 sequences, 28 were selected to validate differential gene expression pattern. Differential expression among treatments and genotypes was validated for more than 50% of the sequences. Specific sequences with known functions such as the Glyceraldehyde 3-P dehydrogenase showed higher expression in samples grown without iron (between 2,33 and 2,77 folds), but no difference was found between genotypes. Several genes related to metal transport showed 26 fold more expression in the genotype sensitive to iron limitation.

Conclusions

Gene expression in roots of two grapevine genotypes was modified by iron nutrition conditions. Genes related to carbon metabolism and divalent metal uptake were up-regulated under stress conditions, depending on the genotypes, in agreement with results obtained for model species.

Significance of Study

These data may contribute to develop molecular assisted selection of grapevine genotypes, mainly rootstocks, well adapted to iron limiting conditions.



Poster Presentations

Thermal imagery for pre-symptomatic diagnosis of biotic and abiotic stress

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Background and Aims

Leaf temperature can be a surrogate for estimating stomatal conductance. Thermal imagery reveals the temperature distribution on surfaces by detecting long wave infrared radiation. Leaf temperature varies with evaporation and hence becomes a function of stomatal aperture. The aim of this study was to implement thermal imagery for contemporaneously monitoring leaf temperature of confined, infected versus non-infected areas upon the attack by *Plasmopara viticola* and of water stressed versus well watered grapevines under greenhouse conditions.

Methods and Results

Contrasting thermal effects due to the pathogen attack were found between measurements on well irrigated and water stressed plants. With irrigated vines pathogen development caused an increase in leaf temperature at the point of infection. In contrast, under severe water stress the inoculated plants showed a lower temperature at the sites of inoculation compared to the rest of the leaf.

Conclusions

Analysis of the spatial and temporal sensitivity of the temperature profile, obtained from the deviation of individual pixels from the mean along a straight line, successfully distinguished between healthy and infected positions on the leaf irrespective of the plant water status.

Significance of Study

Under greenhouse conditions and for predefined areas of the leaf surface evidence was also acquired for characteristic thermal responses to be apparent not later than four days past inoculation that is at least three days before visible symptoms appeared. Thus, early and remote detection using thermal imagery has the potential for pre-symptomatic diagnosis of biotic stress.

Variation for chloride exclusion in a family from a cross between rootstocks K 51-40 and 140 Ruggeri

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Background and Aims

Grapevine rootstock 140 Ruggeri is known for its ability as a chloride excluder, whereas rootstock K 51-40 is poor in this regard and behaves more like a 'chloride accumulator.' Previous studies have shown that chloride exclusion in 140 Ruggeri, when used as a rootstock, is regulated mainly at the root level, with reduced transport of chloride to the scion. This study investigated the variation for chloride exclusion between individuals within a family of 60 hybrids obtained by crossing K 51-40 (seed parent) with 140 Ruggeri (pollen parent), the aims being to determine whether a single or multiple genes were involved and to quantify heritable versus non-heritable variation in chloride exclusion within the hybrid family.

Methods and Results

Controlled crosses were made between K 51-40 and 140 Ruggeri from which 60 hybrid progeny were obtained. Seedlings of the hybrids were planted in the field and established to the stage of mature vines. Cuttings were then propagated to obtain six uniform vines of each hybrid, their parents and two standard rootstocks (Ramsey and 1103 Paulsen). The vines were trained to single shoots in pots under glasshouse conditions and subjected to a salinity treatment of 50 mM chloride with cations Na: Ca: Mg in the ratio 6:1:1 for 27 days. They were then harvested with all laminae and petioles in the middle 60% of each vine sampled for chloride analysis. A root sample was also taken from each vine of 4 blocks.

There were significant differences between hybrids for mean chloride concentrations in laminae, petioles and roots, and this variation was continuous, indicating multiple rather than single gene control for chloride exclusion within the family. There was no evidence for transgressive segregation, with this especially evident in petiole samples where parents K 51-40 and 140 Ruggeri were the worst and best of the chloride excluders, respectively.

Conclusions

Chloride exclusion in a hybrid family from a cross between K 51-40 and 140 Ruggeri was controlled by more than one gene. An upper limit of about 40% of the total phenotypic variation in chloride exclusion was attributable to heritable sources.

Significance of Study

The observation that more than one gene was involved is important and may help to explain previous observations with 140 Ruggeri (Tregeagle et al. 2007), which demonstrated the complexity of this trait and how it can be modified by root morphology.

Rootstock and rootzone salinity effects on partitioning of inorganic ions and organic acids in Shiraz and Chardonnay grape berries

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Background and Aims

Salt tolerant rootstocks offer a means of coping with increased soil salinity. Rootstocks also influence the organic acid composition of grapes of the scion variety. A high tartaric acid to malic acid ratio, for example, can confer advantages in wine quality. This study investigated the partitioning of inorganic ions and organic acids/anions in the skin and pulp of Chardonnay and Shiraz grape berries on own roots and grafted to Ramsey, 1103 Paulsen and 140 Ruggeri rootstocks at a cooler coastal site (Padthaway, SA) and hot inland site (Merbein, Vic).

Methods and Results

Vines in randomised complete block designs were drip irrigated with water containing predominantly sodium and chloride and having electrical conductivities of 2.1 dS/m (Merbein) and 1.6 - 1.8 dS/m (Padthaway). Fifteen mature berries per vine were peeled and skin and pulp (seeds removed) were separated. Pulp samples were gently crushed to obtain a liquid sample and skin samples were extracted in de-ionised water in an autoclave at 15 psi for 15 min. Chloride was measured by silver ion titration and cations by ICP. Organic acids were measured by HPLC.

Chloride, sodium, potassium, malic and tartaric acid concentrations were higher in skin than in pulp. Chloride concentrations in skin ranged from 3.8 fold higher (Shiraz on 1103 Paulsen at Merbein) to 26.3 fold higher (Chardonnay on 1103 Paulsen at Merbein) than in pulp. A significant negative linear regression existed between malic acid and chloride concentrations in skin (but not pulp) of Chardonnay berries sampled from Merbein ($r^2 = 0.63$) and Padthaway ($r^2 = 0.29$). Skin and pulp tartaric acid to malic acid ratios were higher in own rooted relative to grafted Shiraz (on Ramsey, 1103 Paulsen and 140 Ruggeri) and Chardonnay (on Ramsey and 1103 Paulsen) at Merbein.

Conclusions

Concentration differences between skin and pulp were highest for chloride. The significant negative linear regression between malic acid and chloride concentrations in skin from Chardonnay berries from Merbein may indicate competition for similar transporter proteins involved in malate and chloride loading into skins or alternatively the higher salt concentrations in skins may have accelerated malic acid catabolism.

Significance of Study

Presence or absence of skin contact during winemaking determines final concentrations of inorganic ions and organic acids in wines. While rootstocks reduced the concentration of chloride and sodium in skins (except for Chardonnay at Padthaway), concentrations of potassium, malic and tartaric acids were either not reduced or were higher in skins of grafted vines, with potential to significantly impact on resultant concentrations of these ions and acids in resultant wines.

Semillon grape and wine composition influenced by vine resources under different environmental conditions

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Background and Aims

Vine reserves vary during the growing season, the magnitude of these changes depends on nutrient uptake, assimilate production and utilisation. Grapes are a major sink for both sugars and nutrients and therefore can depend on these reserves. It has been shown that timing of nutrient supply and irrigation management can substantially impact on grape composition. The winter nutrient and carbohydrate reserves can influence vine productivity and basic grape composition in the following season. Stress on the vine or nutrient and irrigation management might give the accumulated reserves a critical role during grape maturation, influencing berry composition and ultimately wine quality. The aim of this project is to clarify the link between vine nutrient reserve, fruit and wine composition in Semillon by investigating the vine resource distribution during grape maturation under different environmental conditions.

Methods and Results

A Semillon field trial has been established and site conditions are monitored by an on-site weather station and plant water status is also measured. Nitrogen (N) has been applied in different levels (0kg N/ha and 50kg N/ha) and at different stages during the growing season (post harvest or bloom). These N treatments are combined with two different water supply regimes, one simulating water stress during the early ripening period. Vine samples have been collected throughout the seasons at key stages (bud-burst, bloom, veraison, harvest, leaf-fall) and berry samples during berry maturation. The accumulation of nitrogenous compounds such as amino acids in the fruit has been investigated together with other compositional changes during berry ripening. Water stress results in a yield reduction of 28% and in total soluble solids increase of 1.5°brix. Treatments without N application showed lower yeast availavble N levels than fertilized treatments. Small lots of wine have been made to determine the effects on sensory attributes. N deficient must led to a lag phase and slower fermentation rates. These findings were also observed when all must were adjusted to the same yeast available N level.

Conclusions

The differences in berry N concentrations in response to the N and irrigation treatments are reflected in the fermentation kinetics. The determinations of fruit and wine composition will further clarify the relationship to vine reserves.

Significance of Study

The work will lead to an improved understanding of the role of carbohydrate and nutrient reserves during grape maturation under different environmental conditions, particularly with regard to fruit and wine composition.

Transcriptional pofile changes in grapevine (Vitis vinifera L.) cv. Malbec induced by UV-B radiation

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Background and Aims

Ultraviolet-B radiation (UV-B) is a natural component of the solar radiation that affects plant growth and development. In Mendoza (Argentina), vineyards with top reputation for premium wines are those located at altitudes of 1500 m, where UV-B reaches high values, with consequences on berry growth and quality. An analysis of the transcriptional profile changes produced by different UV-B treatments on grapevine plants was undertaken.

Methods and Results

Grapevine cv. Malbec plants were grown in vitro 45 d under PAR (100 μ mol m⁻² s⁻¹) and then subjected for one day to an UV-B dose similar to the radiation registered at 1500 m of altitude but with two different distributions. The treatments were: a) 16 h PAR without UV-B (control); b) 16 h PAR + 16 h UV-B (7,2 μ W cm⁻²); c) 16 h PAR + 4 h UV-B (28,5 μ W cm⁻²). Total RNA was isolated from apical leaves and used to hybridize grapevine custom Affymetrix GeneChips (developed by the GrapeGen project) containing 23000 probe-sets. For the "4 h" treatment, a total of 4029 genes were differentially expressed (p < 0.05), while 1909 genes reached this threshold for the "16 h" treatment. Functional analysis of those genes was performed by means of the FatiGO web-tool (Al-Shahrour et al., 2008) and the MapMan software (Thimm et al., 2004). Up-regulation of genes responsible for the biosynthesis of flavonoids, phytoalexins and those involved in light and biotic stress response was common in both treatments, while a different sub-set of genes were exclusively up-regulated at "4 h" (biotic stress) and at "16 h" (water stress and ATPases). A different scenario was observed for the down-regulated genes, since just the auxin class was jointly repressed. Chromatin packaging and remodelling, cell growth and death and DNA metabolism classes were solely down-regulated in the "4 h" treatment, while genes for cell wall metabolism were down-regulated in the "16 h" treatment.

Conclusions

Our preliminary results suggest the existence of common and specific pathways in grapevine defence responses to high and low UV-B intensity treatments. While protective responses are evoked by both treatments, DNA damage and repair mechanisms are particularly affected by the high intensity UV-B application.

Significance of Study

This is the first study to analyze the global genome transcriptional changes in grapevine under UV-B radiation. The information generated here will help to identify genes and pathways involved in grapevine defence and acclimation mechanisms triggered by UV-B stress.

Ecophysiological response to environmental stress of three winegrape genotypes

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Background and Aims

Many studies pointed out that the Earth's climate is subjected to a rapid raise of air temperature and decrement of rains: in this frame, the genotype response to warm and drought conditions come back into a great interest. Plant genotypes have different "strategies" to face these environmental stresses. They involve either stomatal or non stomatal mechanisms able to lower plant transpiration. Nevertheless, this mechanisms reduce the plant CO_2 assimilation. Hence, the relationship between the rate of leaf net carbohydrate fixation and the rate of stomatal conductance assume great importance.

Methods and Results

In the semi-arid environment of Southern Italy, physiological behaviours of three grapevine varieties were studied: the local cv. Nero di Troia, cv. Cabernet Sauvignon and the cross breeding Albarossa.

Pre-dawn and stem water potential, air VPD and leaf gas exchange were measured at vine flowering, post-veraison, pre-harvest, stages which naturally match different environmental stress levels. The light Red:Far-red ratio at the foliage basal-interior part was measured, as an indicator of the canopy expansion. Measurements showed maximum stress at post-veraison.

At flowering all cultivars showed good physiological performances. However, R:Fr ratio indicated a lower canopy expansion of Albarossa.

At post-veraison Nero di Troia had the highest pre-dawn and stem water potentials, possibly due to a better ability for osmotic adjustment. Cabernet and Albarossa had same water status. Leaf gas exchanges were active at early morning: stomatal conductance progressively decreased in Nero di Troia, Cabernet and Albarossa, but the first two cultivars had same photosynthetic rates, thus Cabernet seemed to have a higher carboxilation efficiency under severe stress conditions. During warm days, stem water potentials felt down and were as negative as at post-veraison. Albarossa maintained higher values than Cabernet, possibly due to its lower canopy expansion. Leaf gas exchange were still active at early morning, but less than at post-veraison possibly due to the leaf aging or to a down-regulation caused by the persisting stress conditions.

Conclusions

The local variety showed a tendency for a higher physiological activity, while Albarossa did the opposite. Most of physiological behaviours of Cabernet were sometimes close to Albarossa and sometimes close to Nero di Troia.

Significance of Study

The "intermediate" behaviour of Cabernet S. could explain the high adaptability of this "international" cultivar to different environmental and growing conditions maintaining a good quality standard.

Effects of water deficit and vintage temperature characteristics on heat sensitivity of grapevine photosynthesis

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Background and Aims

Heat sensitivity of grapevine (*Vitis vinifera* L. cv. Kékfrankos) chlorophyll *a* fluorescence was studied in two vineyards (Eger-Kőlyuktető flat, Eger-Nagyeged hill steep slope) with different mesoclimates and water supply conditions in two climatically different years.

Methods and Results

Seasonal changes of pre-dawn water potential and gas-exchange were measured in 2005 and 2007 at both vineyards. *In vivo* chlorophyll fluorescence was measured in intact, dark-adapted leaves with pulse amplitude modulation fluorometers (PAM 101-103 and PAM 2000 Walz, Germany). Samples for the measurements were collected two times during the growing season of 2005, at the beginning of July and in the middle of September. In 2007, measurements were taken in the middle of September. For the determination of the breakpoints (T_c) of F_0 vs. T or F_s vs. T curves the method of heat induction of fluorescence was applied as described by Schreiber and Berry (1977).

2007 was drier and warmer with higher vapour pressure deficit (VPD) than 2005. In situ gasexchange and pre-dawn water potential measurements indicated water deficit at the steep-sloped vineyard in each year. In July 2005, mild water deficit enhanced the thermostability of grapevine photosynthesis, as reflected in the temperature dependence of optimal quantum yield $(F_{\sqrt{F_m}})$ and in the critical temperature of initial fluorescence (F_0T_c) . Decreased $F_{\sqrt{F_m}}$ and actual quantum yield $(\Delta F/F_m)$ was recorded at most temperatures in September at the water-stressed site. This time F_0T_c s were also lower due to early leaf senescence. In September 2007 heat sensitivity of $F_{\sqrt{F_m}}$ was similar to 2005. In 2007 $\Delta F/F_m$ ' indicated higher thermostability at both sites while keeping the consistent difference between the vineyards. The critical points of steady-state fluorescence (F_sT_c) were higher by 3-6°C at both vineyards in 2007 than in 2005. Higher xanthophyll cycle pigment pool size (V+A+Z)/(Chl a+b) was measured at the stressed site in each year. However, enhanced pool size was detected at Eger-Kőlyuktető in 2007 than in 2005.

Conclusion

The higher V+A+Z in 2007 and at the stressed vineyard suggests that water deficit, high temperature and VPD play a role in changing (V+A+Z)/(Chl a+b), and results in higher thermostability under high light conditions.

Significance of Study

Moderate water deficit and higher temperature characteristics of the growing season enhances the heat tolerance of grapevine chlorophyll *a* fluorescence.

Temperature, light and humidity effects on the flowering biology of *Vitis vinifera* L. Pinot Noir

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Background and Aims

In spite of the optimism in Tasmania's wine industry, the threat of poor fruitset and in turn poor yields remains a significant concern and limit to profitability for growers, winemakers and investors alike.

A large body of information has been accumulated over time on the phenomena of fruitset, but to date, no reliable method has yet been devised to overcome the detrimental effect of unseasonable weather conditions during flowering. This study set out to investigate several aspects of flowering and fruit development in response to variations in light, temperature and humidity.

Methods and Results

Potted Pinot Noir (clone D5V12) grapevines were placed in four different environments; outside, a shade house, a glasshouse held at temperature 20-25°C and a glasshouse held at 30-35°C. Capfall, pollen viability, pollen tube growth, stigma receptivity and fruitset were all measured. Stigma receptivity appeared not to be effected by treatments, whereas shade and high temperatures (30-35°C) resulted in both lower pollen viability and pollen tube growth and in turn a lower per cent fruitset.

The experiment also revealed a previously unreported diurnal pattern for capfall in Pinot Noir. Results suggest that the trigger for capfall may be related to a change in water potential.

Conclusions

Pollen viability and pollen tube growth were impaired by the shading treatment and hence fruitset was negatively affected. The same occurred when inflorescences were subjected to high temperatures (30-35°C). The mean rate of capfall was greatest between 7am and 12noon, with a greatly reduced rate of capfall at other times during the day.

Significance of Study

This study has gone some way to advancing the understanding of flowering and fruitset in cool climates. Findings will lead to further field trials on management of fruitset and flowering.

Genome-Wide Analysis of MIKC^C-Type MADS-Box Genes in Grapevine

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Background and Aims

MIKC^C-type MADS-box genes encode transcription factors that play crucial roles in plant growth and development. Analysis of the grapevine genome revealed up to 38 MIKC^C-type genes. We report here a complete analysis of this gene family regarding their phylogenetic relationships with homologous genes identified in other sequenced dicot genomes, their genome location, and gene structure and expression.

Methods and Results

Grapevine MIKC^C-type MADS-box genes cluster with their *Arabidopsis thaliana* and *Populus trichocarpa* counterparts in 13 subfamilies. The lack of recent whole genome duplications in grapevine helps to assign the gene diversification processes observed within each subfamily to either an ancestral poliploidization event predating divergence of those three species or to later duplication events within each lineage. Quantitative RT-PCR expression profiles of MIKC^C-type genes in vegetative and reproductive organs show conserved expression domains for specific subfamilies but also reflect characteristic features of grapevine development. Furthermore, expression analyses along latent buds development reveal common features previously described in other plant system as well as the possibility of new roles for members of some subfamilies during flowering transition.

Conclusions

Over a general pattern of conservation in the number of gene subfamilies and their expression patterns, variations in gene number and expression are found in specific subfamilies suggesting the existence of subfunctionalization / neofunctionalization processes. Some of these variations in expression profiles reflect characteristic features of grapevine development. For example, the expression of AP1/FUL subfamily in tendril and the detection of SEP and AP3/PI subfamily members along fruit development and ripening; that could reflect the developmental differences between dry silique fruits and fleshy berry fruits.

Significance of Study

The analysis of MIKC^C-type genes in grapevine helps understanding the origin of gene diversification within each subfamily and provides the basis for functional analyses to uncover the biological role of these MADS-box genes in grapevine development.

A proteomic approach to unravel bud dormancy mechanisms in grapevine

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Background and Aims

Dormancy regulation in vegetative buds is a complex process crucial for plant survival of abiotic stress (drought, low temperature, frost). In grapes, a better understanding of these processes will lead to improve the yield of this species but also cultural practices in order to promote a better cultivar selection.

Methods and Results

Protein from bud samples (Vitis riparia and "Seyval") harvested at 1, 3, 7, 14, 21, 28 and 42 days of dormancy induction were extracted using a phenol protein extraction protocol (Vincent et al., 2005). Protein extracts from all time points were pooled for each genotype. Two series of gel runs (24 gels in total) were performed in order to choose the best gels for further analysis. 2D gels were analyzed using PDQuest Software (8.0.1). The match set experiment revealed 868 spots in common between the two genotypes. Comparison of these data with another virtual gel generated from Vitis vinifera shoot data indicated that only 272 proteins were found common between the two experiments and identified by appropriate MALDI-MS-MS analysis. The major functional categories of these common proteins belong to the Metabolism category for 30% of the annotated proteins while proteins related to Energy represented 20% of the total identified proteins. Surprisingly, 20% of the total annotated proteins belong to a non-usual category (Protein fate). Of the 596 bud specific proteins detected, 260 have been identified. The partitioning of the functional categories from this 260 spots set was similar to that observed for the set of proteins common to bud and shoot. Identification of the other 336 spots is in progress to have a global survey of a protein map on buds. Comparisons between the bud protein map and other protein maps will be performed.

Conclusion

This approach enables us to generate the first protein map of vegetative buds from grapevine with the identification of some cultivar-specific proteins.

Significance of Study

This information will be useful to better understand of molecular mechanisms that trigger bud dormancy in grapevines.

The effect of defoliation and bunch thinning on fruit set, bunch morphology, grape berry composition and sensory attributes of Semillon

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Background and Aims

Leaf removal and bunch thinning are viticultural techniques frequently used in vineyards to reduce yield and to improve fruit quality. Bunch microclimate is improved by increased exposure of bunches to light and air which in turn reduces the incidence of rot and potentially improves flavour development of fruit in cool climate vineyards. The aim of this study was to examine the effect of timing of severe basal leaf removal on fruit set, bunch compactness, yield, berry composition, berry sensory attributes and bud development of Semillon over one growing season and to compare it to a bunch thinning treatment two weeks after fruit set.

Methods and Results

Two experimental sites, one the Barossa Valley and the other at the Waite Campus, Glen Osmond, South Australia were chosen for this study. Own-rooted Semillon vines were trained to a bilateral cordon and received irrigation at both sites. Treatments consisted of control (C), bunch thinning with 30% of total bunches removed two weeks after fruit set (BT), leaf removal one week before flowering (LB), leaf removal at start of flowering (LS) and leaf removal two weeks after fruit set (LA). The first eight basal leaves (or 60% of leaves on shoots with less than 14 leaves) were removed from all shoots on a vine. The effect of leaf removal and bunch thinning on parameters measured was more significant at the Waite Coombe vineyard. Even though vegetative parameters were generally found to be unaffected by treatments, reproductive parameters such as fruit set, berry number, bunch weight, yield and bunch compactness decreased with early season leaf removal. Most of the berry compositional measurements were found to be affected by all treatments apart from leaf removal after flowering. Sensory attributes such as berry colour, skin pulp flavour and acidity, skin tannins and astringency and seed colour, flavour and astringency were altered by leaf removal and bunch thinning treatments.

Conclusion

Early season defoliation is effective in reducing yield and bunch compactness and has potential to alter berry composition and sensory attributes. Berry composition and sensory attributes were also altered by bunch thinning treatments.

Significance of the Study

This project has shown that yield and wine quality can be manipulated from the application of these cultural practices. This will benefit the wine industry when trying to meet target yields and quality specifications.

Investigating the mechanisms involved in different cluster structure of different Pinot noir and Chardonnay clones

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Background and Aims

Cluster structure is a major factor in bunch rot resistance of grapes. Loose open bunches are less susceptible to infections than tight ones, due to less moisture in the bunch and less berries pushed off by others. Various Pinot noir and Chardonnay clones show different cluster structure. The aim of the study was to follow the stem and pedicel growth in two distinctive Pinot noir and Chardonnay clones with significantly different cluster structure to identify the relevant mechanisms.

Methods and Results

Cluster stem and pedicel elongation of the Pinot noir clones 18 Gm and 1-84 Gm and the Chardonnay clones 1-45 Gm and 54 Gm were recorded starting at flowering. From the number of flowers and berries per cluster the degree of fruit set was calculated.

While the loose cluster of Pinot noir clone 1-84 Gm originated from both longer clusters and longer pedicels, the loose cluster of Chardonnay clone 1-45 Gm was only caused by longer clusters.

Conclusion

Depending on the variety, loose clusters may originate from longer main stems and longer pedicels or longer stems alone.

Significance of Study

Results help the understanding in the mechanism relevant for clones with loose cluster structure.

The impact of bearer length on inflorescence size and yield components of *Vitis vinifera* L. Cabernet Sauvignon grown in hedge pruned system

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Background and Aims

Pruning is the easiest and less expensive means of yield regulation in grapevines, especially as when it is combined with bud fertility measurements, to regulate yield to a similar target every year. However very little is known about how pruning intensity, as modulated by varying bearer length, impacts on yield in machine pruned systems. The effect of bearer length within the canopy on the yield components of Vitis vinifera L. Cabernet Sauvignon in Coonawarra, South Australia were investigated during the 2005/06 season.

Methods and Results

Bearers ranging in length from 1 to 5 nodes were selected from within the machine pruned canopy prior to bud bust. Yield components (on a per shoot basis, where one shoot arose from each node) were analysed according to the node position on the bearer at which the shoot arose. Both budburst and inflorescence number per node were highest at the distal node positions on each length bearer. This occurred even if nodes that were at the same positions from the base of the bearer were compared. Shoots growing from nodes at the same position would otherwise be expected to have similar fertility. Shoots that arose from the two most distal node positions had the highest flower number per inflorescence and berry number per bunch. Flower number per inflorescence was significantly higher on two-inflorescence shoots then single-inflorescence shoots. Although yield was highest from the bearer with the highest node number (five nodes), there was no significant difference in yield per bearer for the bearers between three and five nodes in length.

Conclusions

Budburst appeared to act by modifying inflorescence number per node based on the relative location of each node from the apex of the bearer. The relationship between bunch size and node position, unlike that between inflorescence number and node position, was dependent on bearer length. There was a positive, non-linear relationship between average fruit yield per bearer and bearer length.

Significance of Study

These results will assist growers to understand how their pruning regimes may are affect yield and how these pruning regimes may be modified to achieve a target yield — particularly when growers are faced with seasons of varying bud fertility.

Developmentally and UV induced defence responses in grapevine flowers

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Background and Aims

Although number of studies characterized grapevine defences to various forms of environmental (biotic or abiotic) fluctuations, the capability of inflorescences to respond to stresses was only poorly investigated.

Methods and Results

Defence reactions were followed in inflorescences during development and in response to UV-C stress, measuring (i) expression of defence-related genes encoding pathogenesis-related proteins such as chitinases (CH3), β -1,3-glucanase (GLUC), phenylalanine ammonia-lyase (PAL) and stilbene synthase (STS), (ii) variations of chitinase activity and (iii) accumulation of resveratrol phytoalexin. In inflorescences of non-treated plants, defence-related genes exhibited basal level of expression which fluctuated both in stalks and flowers/berries from separated floral buds to groatsized berries, and was generally higher in flowers/berries. Considering GLUC, basal level of expression increased gradually in flowers, whereas it does not fluctuate in stalks. Interestingly the basal level of GLUC expression still increased in berries at fruit set and then suddenly dropped in groat-sized berries. Following UV-C treatment defence responses were induced in stalks of both inflorescences and clusters, as revealed by (i) the stimulation of PR proteins encoding gene expression resulting in augmented chitinase activity and (ii) the increase of PAL and STS expression in association with resveratrol accumulation. Amazingly, no defence mechanism was triggered in flowers following UV-C exposure, whatever the stage. Similarly, in berries at fruit set, defence mechanisms were poorly induced. However, in groat-sized berries, responsiveness to UV-C suddenly increased, as revealed by the induction of CH3, PAL or STS expression and the accumulation of resveratrol.

Conclusions

Opposite to vegetative organs such as stalks or leaves, defence responses in grapevine flowers appear to be developmentally regulated but poorly inducible by abiotic stress. Beside defence function, the expression of defence genes such as *GLUC* may also be involved in the reproduction process facilitating pollen tube extension by their hydrolyzing activities in the female tissue when pollen tube grows.

Significance of Study

The poor inductibility of defence in flowers may be correlated with its greater sensitivity to both biotic and abiotic stresses.

Reproductive development and morphometric analysis of inflorescence primordia in latent buds of *Vitis vinifera* L. cv. Sauvignon Blanc grown in Marlborough, New Zealand

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Background and Aims

Sauvignon Blanc is the most important winegrape cultivar to the New Zealand wine industry contributing 50% of the total economic return from wine. However, annual yield fluctuations create problems for harvest intake scheduling, fruit ripening and matching supply to demand. Quantitative studies elucidating environmental influences on bunch primordia morphology and morphometry in the early stages of development may provide a fuller understanding of factors such as bunch structure and factors controlling yield.

Methods and Results

A regional study in 2004/2005 undertaken by the Marlborough Wine Research Centre (MWRC) investigated variations in yield and vigour across a range of Marlborough locations. This resulted in a collaborative study between MWRC and The University of Melbourne that focused on the analysis of yield components from two locations used in the original trial, the Booker vineyard in the Wairau valley and Seaview vineyard located in the Awatere valley (south of the Wairau valley), in order to determine causes behind yield differences. Also, a study of inflorescence morphology and bunch architecture was undertaken in the following seasons, 2005/2006 and 2006/2007. Compound buds were collected from season 2005/2006 at anthesis and véraison and analysed using scanning electron microscope (SEM) to more accurately describe the timing of initiation and differentiation of inflorescence primordia. The lower yielding Seaview vineyard was found to have less inflorescence primordia per node and they were smaller and less developed than the Booker vineyard at both anthesis and véraison. This resulted in smaller bunches, lower inflorescence number per shoot and lower potential flower number in the following season for the Seaview vineyard. Bunch morphology was found to be significantly different in a number of parameters but the main driver of yield differences was found to be the number of inflorescences produced per shoot.

Conclusions

The stage of differentiation of inflorescence primordia at each node position along the same shoot was described for the first time. The two vineyards had significantly different yield components such as flower number and inflorescence number resulting in different inflorescence sizes and yields

Significance of Study

Differentiation of morphological structures within latent compound buds has not been documented before in this Sauvignon Blanc. This study provided new insights into the fundamental developmental stages of Sauvignon Blanc, its response to environmental variables and the influence of these on yield.

Estimation of Phenolic Compounds in Tropical Red Wines elaborated in Northeast Brazil

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Background and Aims

Vitiviniculture has been developed since 1980's in an area between 8° and 9 ° S latitude of Northeast Brazil. This area presents an intra-annual climate variability, with an annual average temperature of 26.4°C, located at 350 m above sea level. The rainy season occurs from December to March, with about 567 mm of rainfall. The heliothermical availability is about 3000 hours of luminosity.year⁻¹ and allows a continuous crop vegetative development. The total area cultivated for winemaking is about 700 ha and cultivars used for tropical red wines are Syrah and Cabernet Sauvignon. The objective of this study was to estimate total anthocyanins and tannins according to spectrophotometric methods in four red wines, elaborated from cultivars recently introduced in the region.

Methods and Results

Tempranillo, Alfrocheiro, Petit Verdot and Barbera were introduced in December/2004 and grafted on IAC-572 (*Vitis caribaea* x 101-14 Mgt), cultivated on pergola trellis system and irrigated by drip. The grapes were harvested according to total sugars and acidity estimation. Wines were elaborated by traditional methods in 500 L inox tanks. The results showed that the responses of each cultivar to the edaphoclimatic conditions were different. Total tannins varied between 2.7 (Barbera) and 4.8 g.L⁻¹ (Tempranillo), while total anthocyanins varied between 262.1 (Tempranillo) and 868.5 mg.L⁻¹ (Petit Verdot).

Conclusions

These results show that the cultivars have different enological potential and the winemaking process need to be specifically adapted according to each cultivar.

Significance of Study

New studies will be carried out to evaluate the influence of harvest date on grape and wine phenolic compound profiles.

Enological Potential of Grapes produced in different periods in a Tropical Region of Northeast Brazil

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Background and Aims

The Lower-Middle São Francisco river Valley is a new vitivinicultural region located in northeast Brazil, between 8° and 9° S latitude. In this region it's possible to have two-three harvests a year, mainly due to an annual average temperature of 26.4°C, with about 567 mm of rainfall between January and April, altitude of 350 m above sea level and use of drip irrigation. There is a continuous vegetative development and grapevine growth occurs throughout the whole year. Grape composition can vary strongly according to harvest time of the year due to different climatic conditions. Wineries harvest grapes for winemaking between May and December. The aim of this study was to compare Tempranillo grapes composition harvested in two periods: June and December 2007, to best understand the influence of harvest date on grape quality.

Methods and Results

Vines were introduced in December/2004 and grafted on vigorous rootstock (*Vitis caribaea* x 101-14 Mgt), cultivated on pergola trellis system. The analyses carried out on grapes at harvest were berry weight, total soluble sugars, pH and total acidity. The results showed that grapes harvested in June presented similar weights, but very high acidity, very low sugar content and pH as compared to grapes harvested in December.

Conclusions

These results suggest that winemaking process has to be adapted for each harvest season and the wine potential can vary according to month of production.

Significance of Study

New studies need to be made in order to better understand the grape characteristics and tropical wines potential from semi-arid region of Brazil.

Influence of dehydration temperatures on polyphenols, volatiles compounds, and internal structure of Aleatico red wine grape

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Background and Aims

Temperature is an important parameter for grape drying but it must be known its effect on quality characteristics of grape. In this paper we report the results of an experimental work to see the effect of drying temperature on Aleatico red grape.

Methods and Results

10, 20, and 30°C drying temperatures were used with 40% RH and 1.5 m/sec of air flow. Drying was performed up to a weight loss (wl) of 40%. Samplings were done at 10, 20, 30, and 40% wl. Polyphenols were analysed by HPLC and volatiles compounds by GC-SPME. ADH (alcohol dehydrogenase) was also measured. Moreover, MRI (magnetic resonance image) was used to study the water movement and NIR-AOTF and electric nose as tools to discriminate berry quality during drying. 40% of weight loss was reached in 7, 15, and 26 d respectively at 30, 20, and 10°C and SSC were 36, 32, and 30°Brix. At 20°C we observed the highest increase in caftaric acid, catechin, resveratrol and total polyphenols at 10 and 20% of weight loss and successively declined. Anthocyanins declined in all samples. At 20°C the volatiles fraction was the richest but with high volatile acidity. ADH activity rose greatly at 10% of wl in 30°C sample. Terpenols fraction was maintained at 10°C the event occurred at 30% wl. NIR was able already at 10% wl to discriminate samples, and at 10°C the PCA separation among sampling times was clearer than at 20 and 30°C.

Conclusions

20°C is good temperature to increase useful compounds for wine up to 20-30% wl; 10°C is good temperature to maintain primary volatile compounds and control volatile acidity. At 30°C grape dries fast but the berry quality is lower.

Significance of Study

This paper is useful for operators who are practicing grape drying for making wine because they can choose the temperature and percentage of weight loss, in order to obtain what they like to have in wine.

Comparison of three different methods of monoterpene extraction

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Background and Aims

Monoterpenes are major flavour components in many wine grape varieties. A reliable method is essential to study the aroma potential of varieties and clones. In the past aroma compounds in grape juice and wine e.g. monoterpenes were isolated by a liquid-liquid-extraction with trichlorofluoromethane (Rapp *et al.* 1985). Nowadays chlorofluorocarbons are banned in most countries because they destroy the ozone layer. Consequently this elegant method cannot be applied any longer and suitable alternatives are required.

Methods, Results and Significance of Study

In this study three methods of aroma extraction were compared: A high vacuum distillation with a solvent assisted flavour evaporation-system (SAFE), a rapid steam distillation (RSD) and a solid-phase extraction (SPE). An aqueous solution with known contents of the monoterpenes Linalool, Geraniol, Nerol, α -Terpineol, Linalool oxide (cis + trans), sugar (glucose and fructose) and tartaric acid was used to simulate the matrix of grape juice. Each procedure was repeated five times and the extracts analysed by GC-MS.

All three methods have their advantages and disadvantages. The SAFE method is comparably fast, yields 'clean' chromatograms (low co-elutions), but has low recovery rates. The RSD is even faster (actual distillation takes less than 5 minutes), gives 'clean' chromatograms and sufficient recovery rate, but is restricted to volatile components and requires a stringent distillation protocol. The SPE method is the most time and money consuming option. Its advantages are a wide spectrum of extracted substances, the procedure is basically available in most chemical laboratories. On the other hand, the larger number of extracted substances may cause co-elutions and confuse peak detection and quantification. The recovery rates rely not only on the extraction method applied, but also on the specific monoterpene.

Conclusion

Consequently, the selection of the preferred method depends on the laboratory procedures available and the monoterpene present in the studied variety.

Isolation and characterisation of winegrape tannin by diol phase separation and phloroglucinol analysis

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Background and Aims

Condensed tannins (proanthocyanidins) are polymers composed of flavan-3-ol subunits. Sources of tannin include grape, wine, apple and cacao. Tannins are differentiated by their subunit chemistry, interflavan bond linkage, degree of polymerisation and degree of galloylation. Prodelphinidin, procyanidins and galloylated species are particular to grape. Differences in tannin structure can influence the organoleptic properties of wine. The range of possible tannin structures also makes characterising tannin a complex and difficult task. In this study, diol preparative high performance liquid chromatography (HPLC) was used to isolate fractions of varying polymer length. Tannin fractions were then characterised by subunit composition and polymer length using acid-catalysed cleavage in the presence of phloroglucinol.

Methods and Results

Tannin was extracted from grape seed, grape skin and raw cacao. Extracted tannin was then separated by preparative liquid chromatography using a diol column. Tannin extracts were independently fractionated by diol preparative high performance liquid chromatography (HPLC) and characterised by phloroglucinolysis. Grape seed, grape skin and cacao tannin gave polymer fractions of varying composition and length with the degree of polymerisation ranging between 1 and 8 subunits.

Conclusions

This study characterised tannin polymer fractions from grape seed, skin and cocao material by polymer length and composition. This approach proved valuable for furthering our understanding of tannin composition in winegrapes.

Significance of the Study

Characterising individual tannins by composition and polymer length contributes to determining the types of winegrape tannin polymers that exist. The diol separation also provides a method for isolation of smaller polymeric material that might be used for purification of standards for tannin research and analysis, which will lead to a more comprehensive understanding of the organoleptic properties in wine such as astringency and mouthfeel derived from grape tannins.

Extraction of condensed tannin from grape skin into acetone and ethanol mixtures

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Background and Aims

Winegrape tannin plays a significant role to wine quality contributing to its organoleptic properties. Determining the content of tannin in the winegrape at harvest provides an indication of how much tannin might be extracted into wine during the winemaking process. A number of published methods, which precipitate tannin with bovine-albumin serum (BSA) protein and methyl cellulose have been suggested for routine analysis of grape tannin in the winery. However, recent research has demonstrated discrepancies between the results generated by these two methods. It is thought the extraction solvents employed by these methods may be one of the reasons for this discrepancy, as tannin is extracted by a 70% acetone mixture in water for the BSA protein method while the methylcellulose method utilises a mixture of 50% ethanol in water.

Previous research has indicated that 70% acetone extracts the largest amounts of tannin compared to absolute and 75% ethanol. However, there does not appear to be a direct comparison of tannin extraction between two solvents across a range of mixtures. A study was conducted to compare the extraction of tannin across a series of acetone and ethanol mixtures and determine the most effective extraction solvent.

Methods and Results

Condensed tannins were extracted from the skins of Shiraz grape berries using a series of acetone and ethanol mixtures. Tannin content and composition was determined by acid-catalysed cleavage in the presence of phloroglucinol with quantification by high performance liquid chromatography (HPLC). The amount of tannin extracted was also determined across the series of extraction solvents by the published BSA protein and methylcellulose precipitation methods. Measured by HPLC, tannin extraction was consistent for a range of acetone mixtures, while ethanol extraction varied.

Conclusions

Comparisons were made on tannin extraction and composition for two extraction solvents using three analytical methods to determine the most effective extraction solvent for tannin analysis. In this study, acetone was found to be a more effective extraction solvent than ethanol for analysing grape skin tannin.

Significance of the Study

Determining the most effective extraction solvent is a fundamental step for providing consistent and comparable results for analysing tannin in the vineyard and making winery decisions. This study provides information that can be employed for practical and scientific tannin analysis.
Manipulation of nitrogen application in the vineyard to optimise the secondary metabolite profile of red wines to meet consumer demand

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Background and Aims

Little research has been made on the effects of nitrogen application in the vineyard on the secondary metabolites in grapes and wine, especially in red wine. The aim of this study was to investigate the impact of nitrogen supplementation in the vineyard on the secondary metabolite profile of red grapes and wine.

Methods and Results

In season one (2006/2007) different rates of nitrogen fertiliser were applied to Shiraz vines on own roots grown in Langhorne Creek, South Australia. In the first and second season it was confirmed that this site was low in nitrogen and thus responded to the application of nitrogen. To date nitrogen has been observed to influence the tannin content of grapes which was reflected in the newly pressed wine. In contrast, nitrogen had little effect on anthocyanins in the grape but there was a significant impact on wine colour density. The flavour profile analysis and the sensory analysis component of this study are in progress.

Conclusions

Although in its infancy, this study has shown that nitrogen supplementation in a low fertility vineyard can have an impact on the quantity of important secondary metabolites in red grapes and wine. The sensory analysis of these wines will reveal whether these changes are of significance to have an impact on the sensory attributes of the final wine.

Significance of Study

Managing vine nitrogen status in the vineyard has the potential to modify wine quality as defined by sensory analysis. Understanding how nitrogen influences the grape and wine secondary metabolite profile can aid the grape grower and winemaker to achieve the optimal balance between vineyard and winery nitrogen to produce a wine that meets consumer demand.

Aroma levels of different White Riesling clones

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Background and Aims

White Riesling is a variety with fruity flavours. Peach, apricot, apple or lemon are typical wine descriptions. The aim of the study was to investigate the terpenes and some C6 components in different Riesling clones at different vineyard sites in Germany.

Methods and Results

Berry samples were collected at three different ripening levels from seven clones at the same location. In another trial the effect of the location was assessed by comparing identical clones at several locations. White Riesling clones had different types of terpenes and different terpene levels. Terpenes increased with ripening, but not in all clones to the same extend. Clones 24 Gm and 94 Gm contained almost no terpenes, while 198 Gm and 239 Gm had high levels. Similar effects were obtained with C6 components like hexanal, 1-hexanol or hexan acid. The vineyard site also had a significant effect on terpene levels and concentration of C6 components.

Conclusions

White Riesling clones differ significant in terpene and some other flavour compounds (C6).

Significance of Study

The large clonal variation in flavour provides the option to develop clones with different flavour types suited to different vineyard sites.

Linking grape and wine composition to wine sensory attributes

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Background and Aims

Wine aroma arises from a complex mixture of volatile compounds originating from grapes and wine-making processes. Wine flavours vary according to the grape variety as well as between fruit of the same variety sourced from different geographic regions or experiencing different management techniques or environmental conditions. This indicates that grape composition must play a significant role in the final wine aroma. However, the influence of the environment and vineyard management on the development of grape-derived flavour and aroma in wine is not well understood. The aim of this work is to relate grape composition to wine chemical and sensory attributes to find correlations that may be used to explore the impact of vineyard variables on wine composition.

Methods and Results

We have analysed the chemical composition of Cabernet Sauvignon grapes sourced from different viticultural regions and small scale wines made from the same parcels of grapes. A total of 20 grape/wine samples were obtained across three growing seasons (2003/04, 2004/05 and 2005/06). Fifty volatile and 178 glycosides were quantified in the grape samples and 114 volatiles were quantified in the corresponding wines using solid phase microextraction and gas chromatographymass spectrometry. Principal component analysis was used to identify patterns amongst the wine samples and vintage and regional effects were observed. Associations between chemical variates and sensory variates were identified using non-orthogonal analysis of variance and a forward selection regression procedure.

Conclusions

Although there are vintage and regional effects, using suitable statistical treatments we have been able to identify correlations between wine components and sensory attributes. These will be experimentally tested to assess their use in predictive models. We will also extend these analyses to include grape chemical composition with the aim of identifying links between grape composition and wine composition and sensory attributes.

Significance of Study

We will apply the results of this work to issues of vineyard management, climate and variety/cultivar to build an understanding of how inputs in the vineyard can affect grape and wine composition and thus manipulate wine sensory quality.

Crop thinning of Merlot in Queensland - influence of timing of thinning on grape quality

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Background and Aims

Timing of crop thinning has been shown to influence grape quality (Filippetti *et al.*, 2007). This investigation was carried out into the influence of pea size and véraison crop thinning on yield and fruit quality of Merlot in vineyards in Queensland's Granite Belt and South Burnett in the 2008 season.

Methods and Results

At both sites approximately half the crop was removed from randomly allocated panels at pea size and at véraison. Vine measures were recorded at véraison and fruit samples from all treatments were taken at harvest and analysed for various fruit quality parameters.

Treatments thinned at pea size were significantly retarded in ripening, as was fruit from the véraison thinned treatment in the northern site. Although thinning did not significantly affect berry malic acid concentration, significant reductions were noted at both sites in TSS, pH and tannins, and at the southern site increased TA and reduced anthocyanins and total phenolics.

Leaf area to yield ratio (LA/Y) was significantly increased by all crop thinning treatments leading to thinned treatments having a LA/Y greater than recommended as optimal (Dry et al. 2004).

Conclusions

It can be concluded that under the seasonal conditions of this study, reducing yield by thinning did not result in an increase in fruit quality and that crop thinning at pea size may adversely influence quality of Merlot. Other authors have shown seasonal influences to override the effects of crop thinning (Keller *et al.* 2005), a factor believed to also impact on this study. Thinning also resulted in vines no longer having optimal LA/Y thus the findings may reflect vines being undercropped.

Significance of Study

This study indicates that the common practice of crop thinning may not enhance fruit quality and may even be detrimental. It is recommended that growers should be cautious only to thin if crop loads are exceptionally high.

Crop thinning of Merlot in Queensland – seasonal influence on quality of grapes from crop thinned vines

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Background and Aims

Crop thinning is a common practice in wine grape vineyards, believed to improve fruit quality (Jackson and Lombard, 1993). This trial was carried out in a Queensland vineyard in 2007 and 2008 in order to investigate seasonal variation on effect of crop thinning on Merlot fruit quality.

Methods and Results

In both seasons half the crop was removed from randomly allocated panels at véraison. Vine measures were recorded at véraison and fruit samples from all treatments analysed at harvest for various fruit quality parameters.

In 2007 fruit from crop thinned vines ripened more rapidly, attained a higher pH and was significantly higher in anthocyanin concentration than fruit from unthinned control vines. Crop thinning in 2008 had no influence on TSS however this fruit had significantly lower anthocyanin and phenolic concentrations compared to fruit from unthinned controls. Leaf area to yield ratio (LA/Y) was significantly increased by all crop thinning treatments resulting in thinned treatments having LA/Y values greater than those recommended as optimal (Dry et al. 2004).

Conclusions

Crop thinning in 2007 improved Merlot TSS and anthocyanin accumulation. In 2008 no significant difference in TSS was observed in fruit from crop thinned vines, however this fruit was significantly lower in anthocyanin and phenolic concentration. Other authors have shown seasonal influences to override the effects of crop thinning (Keller *et al.* 2005), a factor believed to also impact on this study. A cool growing season in 2008 may have caused vines to be unable to accumulate high levels of secondary metabolites, thus masking positive effects of crop thinning. Crop thinned vines being out of balance may also have impacted on results.

Significance of Study

This study indicates that the common practice of crop thinning may be beneficial to fruit quality in some seasons, however seasonal factors play a significant role in ripening of quality grapes.

Using a high resolution LC-MS metabolic profiling technology for the discovery and characterization of biomarkers related to wine quality

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Background and Aims

The different steps that form the chain of wine production, from growing grapes to wine production and aging, going to the final consumer, needs to be monitored to control possible frauds and to quantify the level of some components critical for the final quality of the product. Wine contains numerous metabolites coming from grape berries and from yeast, covering both a large number of primary metabolites (sugars, organic acids, amino acids) and secondary metabolites (flavonoids, anthocyanins and other pigments). Liquid-chromatography-mass-spectrometry (LC-MS) can be adapted to a wider array of molecules, including a range of secondary metabolites such as alkaloids, flavonoids, glucosinolates, isoprenes, oxylipins, phenylpropanoids, pigments and saponins. Considering the advantages provided by using LC-MS, we are applying this technology in order to establish a procedure to discover biomarkers related to wine quality.

Methods and Results

Previously published data proves that metabolic profiling (with a limited numbers of specific metabolites) applied to wine allows the differentiation of wine with respect to grape variety and its origin. Based on this general proof of concept, we started metabolically profile commercial red wines (different varieties, provenance and quality) by using LC-FT-MS technology.

Conclusions

In this work, we analysed a large set of Chilean wine samples and preliminary results clearly demonstrating the validity of our LC-MS analysis to discriminate different quality levels of Chilean wines.

Significance of Study

This can provide the bases to identify putative biomarkers associated to wine quality.

Effects of various soil colours on fruit ripening

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Background and Aims

One fundamental factor to grape quality is climate and in particularly solar radiation. Due to its influence on berry development and composition, the radiation microclimate within the bunch zone is of particular interest. The aim of the present study was to assess how soil material of different colour can influence soil radiative properties and hence vineyard soil surface temperature and how this would affect fruit composition.

Methods and Results

After flowering, (BBCH 79) a loess-type soil (control) was covered with a thin layer of three different materials: a) black coarse slate, b) red clay brick, and c) white pumice. The vines (*Vitis vinifera* L. cvs. Riesling) were trained to a vertical shoot positioning (VSP) system. Surface colour had significant effects on the quantity and quality of reflected radiation into the fruiting zone. The pumice covered soil showed the highest amount of reflected - and the highest ratio of red-to far red light.

Conclusions

The different soil materials influenced the microclimate through at least two mechanisms: temperature and spectral composition of reflected radiation. Large thermal effects on soil surface temperature and on berry skin temperature were found. By varying the distance of clusters to the ground, the temperature of berry skins declined rapidly within the first 0.3 m when fruit was exposed to the red, white or natural coloured soil. In contrast, over coarse ground slate the absolute berry surface temperature was higher and remained constant over the same distances.

Significance of Study

Berry ripening was affected by surface colour and preliminary results indicate that altered vineyard microclimate has effects on berry composition. Small effects on fruit phenolic composition were found but it remains to be determined whether these changes will ultimately affect wine quality.

Involvement of abscisic acid in the control of tanin biosynthesis in skin berry: new elements regarding the regulation of leucoanthocyanidin reductase (LAR) and anthocyanidin reductase (ANR) activities and expression

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Background and Aims

Proanthocyanidins, or condensed tannins, are crucial polyphenolic compounds for grape and wine quality. Recently, significant advances were achieved in understanding the biosynthesis of their main subunits : (+)-catechin and (-)-epicatechin, produced by catalysis of leucoanthocyanidin reductase (LAR) and anthocyanidin reductase (ANR), respectively. Expression studies and enzyme activities have been published but no data were available on their regulation by hormonal status. The present work aimed to determine if ABA application on green berries affects the tannin contents, tannin composition, LAR and ANR activities and their genes expression *(VvANR, VvLAR1, VvLAR2)* in skin berry during ripening.

Methods and Results

Cabernet-sauvignon small green berries were treated with a 200ppm solution of ABA and collected at different time course between beginning of veraison and harvest. Tannin contents, tannin composition, LAR and ANR activities and their genes expression (*VvANR, VvLAR1, VvLAR2*) in skin berry were analyzed. Our results show that ABA affects berry maturity status and is involved in tannin metabolism in skin berry by decreasing of LAR and ANR activities and by repressing the expression of their genes few days after application.

Conclusions

ABA treatment decreased tannin contents in green berries without modifying their composition suggesting that ANR and LAR were co-regulated by ABA.

Significance of Study

These informations will permit to establish the important role of hormonal status in green berry to the control of tannins biosynthesis.

Possible links between cell-wall polysaccharides and tannins in ripening grape skins

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Backgrounds and Aims

A visual and gustative quality of red wines needs a polyphenol extraction from grapes as complete as possible. This extraction is modulated by their localization and their ability to be bounded with the different skin compounds. In this way, proanthocyanidin localization was determined over the time course of ripening in isolate skins (*Vitis vinifera* L. cv. Cabernet sauvignon).

Methods and Results

Our results show that, at the harvest, tannins associated with cell-walls are predominant, influencing then on their extraction. To better understand the relation of these conjugate tannins with cell-wall constituents, a pre-treatment of cell-walls with different classes of pectolytic enzymes (polygalacturonase, pectin methyl esterase, cellulose, xylanase) was performed. Samples were taken at time interval and the tannin composition was analyzed. The action of pectolyases increases tannin extractibility, pectinases allowing the better capacity of tannin extraction. These results suggest a close interaction between pectic polysaccharides and tannins. A sequential chemical extraction of skin cell-wall polysaccharides shows a large amount of tannins in the strong bounded cell-wall material, fraction enriched for covalent type bounds.

Conclusions

The fine composition of these bounded tannins was finally analysed and the relation of this composition is discussed in relation with skin cell-wall integrity.

Significance of Study

Cell-wall integrity will be related to changes in polysaccharide and polyphenol composition.

Possible methods to evaluate grape skin texture in relation with ripening process

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Backgrounds and Aims

Grape skins are crucial tissues for berry quality. The grape ripening process is a complex development program in which senescing tissues undergo programmed changes in firmness, texture, coloration, flavour and susceptibility to microbial infection. Many studies used mechanical properties (skin hardness and thickness, pulp firmness) to evaluate grape ripeness.

Methods and Results

In this work, sensory profiling, penetrometry and water avaibility measurement were applied to evaluate skin texture in relation with grape skin maturity. *Vitis vinifera* cv. Cabernet Sauvignon grapes ripeness was compared between two vineyards located in Bordeaux region. All methods succeed in discrimating ripening stages and sensory analysis correlates with physical measurement like water avaibility and penetrometry. In correlation with physical tests, the most interesting sensory parameter for measuring firmness is shredding.

Conclusions

These results are validated by comparison with chemical analysis and allow to differentiate berries according to their texture and degree of maturity.

Significance of Study

The possible prediction of firmness attributes by physical methods was discussed.

Identification and Characterisation of the enzymes involved in the biosynthetic pathway of tartaric acid in *Vitis vinifera*

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Background and Aims

Tartaric acid (TA) is a relatively rare metabolite in higher plants. In *Vitis vinifera* L. however, TA (along with malic acid) accounts for 90% of the total acid found in developing grape berries. Ascorbic acid (Asc, Vitamin C) is the principle precursor of this acid, with additional evidence for a minor synthetic route via D-gluconic acid. The focus of our work is to define, with the use of molecular and biochemical techniques, the enzymes responsible for the synthesis of TA in *V. vinifera*. L-idonate dehydrogenase (DeBolt *et al.* 2006) is the only TA biosynthetic enzyme that has been isolated from plants and it was shown to be active in the perceived rate-limiting step of the Asc-pathway.

Methods and Results

Candidate genes were identified in the grapevine genome by homology to bacterial enzymes that mediate identical reactions to those proposed in TA synthesis. Candidates were limited by the presence of functional domains (NAD catalytic and binding domains), conserved regions relating to the proteins active sites and expression in pre-veraison grape berry EST libraries. Grapevine homologues of enzymes from *Erwinia* spp (Truesdell *et al*, 1991) and *Escherichia coli* (Yum *et al*, 1998) were cloned from cDNA samples of pre-verasion berries and recombinant proteins expressed in *E. coli*.

Conclusions

Currently, assays are being conducted to identify the activity of these grapevine enzymes *in vitro*. The progress of these reactions are monitored spectrophotometrically via the consumption of the cofactors NAD(P)H.

Significance of Study

By understanding the process involved in TA production, cultivars with high levels of TA synthesis can be identified or engineered by molecular means, lowering the level of acid required for addition to wine musts to maintain a low pH during fermentation. Alternatively, a disablement of the major pathway could lead to an increase in Asc in berries.

Physiological and Molecular Characterization of VvMADS1 Transgenic Grapes

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Background and Aims

VvMADS1 is a grapevine transcription factor that was shown to be expressed in the later stages of flower development and throughout berry growth. In order to have a better understanding of the role of VvMADS1 in fruit development, transgenic Sultana and Chardonnay vines were produced and berry growth characterized at the molecular and physiological level.

Methods and Results

Cultivars were transformed with the VvMADS1 gene under the control of the 35S promoter and plants grown under field conditions at an OGTR approved field site. Berry development was followed and samples collected during fruit development from 4 weeks post-anthesis until the ripe stage at harvest. VvMADS1 gene expression analysis by RT-PCR revealed different expression profiles between transgenic vines and their respective controls. Berry weight, total soluble solids content and fruit firmness were measured allowing the identification, in both genetic backgrounds, of two classes of berry phenotypes. An extreme berry phenotype was observed with fleshless berries and a disturbed ripening profile and an intermediate phenotype with smaller but ripening berries.

Conclusions

VvMADS1 is known to be an important transcription factor involved in flower development. Evaluation of transgenic grapevines indicates that it is also involved in berry growth and appears to have a role in fruit ripening with similar transgene phenotypes observed in two different cultivars. Further investigation of these transgenic vines may provide a unique insight into the control of berry development and how the VvMADS1 transcription factor is regulating other berry genes and processes.

Significance of Study

This study is the first report of the involvement of VvMADS1 in berry ripening.

Analysis of the flavonoid biosynthetic pathway in grapevines using a hairy root transformation system

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Background and Aims

The flavonoid biosynthetic pathway leads to the production of important secondary metabolites such as anthocyanins, proanthocyanidins and flavonols in plants. These compounds are synthesised in grapes and are important for providing colour and mouth feel in wine. Many of the genes encoding the biosynthetic enzymes as well as several transcription factors regulating this pathway have been identified in grapevine. Further characterisation of these genes requires forward and reverse genetic studies. However, as grapevine is a woody perennial crop, it is difficult and time-consuming to generate transgenic plants. Hairy root culture technique is a valuable tool for secondary metabolites, their rapid growth, and the ease of generation of transgenic hairy root cultures. In this study, a hairy root transformation system was used for further characterisation of the genes of flavonoid biosynthetic pathway.

Methods and Results

A liquid hairy root culture system was used for the constitutive expression and silencing of several biosynthetic genes of the grape flavonoid biosynthetic pathway. The flavonoid profile and gene expression pattern of this pathway in transgenic hairy roots were then analysed. Transgenic hairy roots with reduced expression of anthocyanidin reductase (ANR) and leucoanthocyanidin reductase (LAR) genes, which are responsible for the production of the extension units required for proanthocyanidin biosynthesis, have been used to analyse the contribution of the different extension units to the total proanthocyanidin profile. The composition of the proanthocyanidins was significantly changed by reducing the expression of both ANR and LAR genes. The total proanthocyanidin levels were reduced in the LAR silenced hairy roots indicating the importance of the LAR for tannin synthesis in grapevine.

Conclusions

Expression of both ANR and LAR genes were shown to be important for maintaining the regular tannin profile in grapevine. LAR expression was also shown to be a major contributor to the total proanthocyanidin levels. This is different from the Arabidopsis model system where only ANR contributes to proanthocyanidin synthesis.

Significance of Study

In the present work, a novel method of a liquid hairy root culture system was used to further characterise the genes of the flavonoid biosynthetic pathway, indicating the utility of this transformation system for rapid investigation of secondary metabolism pathways.

The effects of UV radiation on grape berry chemistry and molecular biology: Implications for wine making and vineyard management

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Background and Aims

New Zealand and Australia have high levels of UV radiation compared to similar latitudes in the Northern Hemisphere. This UV radiation is potentially a very significant environmental stress and regulatory factor for grape growth and development. The aim of this study was to investigate the effect of UV radiation (UV-A and UV-B) on grape berry development, the chemistry and molecular biology of grape vines under field trial conditions.

Methods

The fruit zone of Sauvignon blanc vines was screened from all UV, or from UV-B, or was exposed to UV-transmitting and ambient treatments. Instrumental chromatography techniques such as high performance liquid chromatography (HPLC) was used to examine UV effects on grape quality.

Results

Physical symptoms, such as "brown sunburn spotting", were clearly seen under UV-transmitting conditions, but not in UV screened berries. These changes were complemented by changes to phenolic composition, particularly flavonoid accumulation. Analysis of gene expression is taking place to assess the induction of biosynthetic genes for the phenylpropanoid pathway. Changes in gene activity are also being determined for analysis of genes of the lipoxygenase pathway. This is a major biosynthetic pathway leading to the production of aroma compounds, signal transduction and molecules associated with defence against biotic and abiotic stress. In addition, changes in amino acid composition have been determined.

Conclusions, Significance of Study

This study revealed a number of important UV effects on grape quality parameters. These changes in chemical composition will be discussed in the context of their implications for wine making and vineyard management.

Functional characterisation of volatile organic compound biochemical pathways using *Vitis vinifera* suspension cell cultures as a model system.

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Background and Aims

Functional characterisation of candidate genes isolated from grapevine offers a number of significant challenges. The long generational time and large size of this plant precludes many of the functional genomic approaches utilised in other important crop species. These biological constraints coupled to regulatory and public resistance to the use of genetically modified organisms also restricts the usefulness of using fully regenerated transgenic vines to analyse gene function. Therefore we have sought to develop a model system based on the elicitation of grape suspension cell cultures to answer functional questions. Specifically we are using suspension cell culture to elucidate the involvement of various enzyme families involved in the formation of volatile organic carbon compounds (VOCs) in grapes and in particular grape berries.

Methods

We have generated suspension cell cultures from Sauvignon blanc leaves and meristems and have begun to determine elicitation strategies that activate the formation of VOCs in these cultures. A solid phase microextraction-gas chromatography-mass spectroscopy (SPME-GCMS) method has been adapted to measure and identify compounds evolved from the suspension cell cultures. Additionally, biolistic and *Agrobacterium* mediated transformation methods are being trialled to manipulate candidate gene expression and therefore test their function with respect to VOC production in grapes.

Results

Results will be presented on the effects of elicitation of cell cultures with hyphal extracts of *Botrytis, Botryosphaeria* and *Cylindrocarpon.* Results will also be presented on attempts to stably transform these cultures with genetic constructs aimed at silencing a range of lipoxygenase genes expressed upon elicitation and thought to be involved in the formation of short chain aldehydes, ketones and alcohols in grape berries.

Conclusion, Significance of Study

Through this model system, gene activity and important biochemical pathways can be studied to provide significant insight into grape tissue responses to biotic and abiotic stresses.

Variation in winegrape tannin, anthocyanin and total phenolic levels and their seasonal differences between varieties

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Background and Aims

Winegrape colour assessment has been extensively adopted by wine industry both in Australia and overseas to increase the quality of fruit taken into various wine programs. In many cases this has lead to reward-for-quality based payment systems. In addition to anthocyanins, which are responsible for grape and wine colour, tannins directly contribute to wine quality in terms of long-term colour stability and mouthfeel. While there are many methods available to measure tannin, they are yet to be utilised widely by industry as either a quality assessment or a management tool. Before tannin measurement can be applied to routine analysis of winegrapes, some knowledge of the range variation in tannin levels in the major winemaking varieties is needed. This study aimed to develop an understanding of the variation of tannin, anthocyanin and phenolic content in a wide range of winegrape varieties. Variation within varieties, between varieties, and between seasons was examined.

Methods and Results

Grape were collected from vineyards across California during the 2005, 2006 and 2007 vintages. Samples included Merlot, Rubired, Cabernet Sauvignon, Shiraz, Zinfandel, Ruby Cabernet, Grenache, Barbera, Durif, Carignane, Carnelian and Chardonnay. Tannins, iron reactive phenolics and anthocyanins were extracted from whole berry homogenates using 50% ethanol in water. Tannin concentration was determined by protein precipitation quantified with ferric chloride. Total phenolics were also determined by reaction with ferric chloride. Anthocyanin concentration was determined from absorbance at 520nm. The results from this study indicate considerable variation in tannin, anthocyanin, and phenolics concentration between samples of the same variety and between varieties. There were also seasonal differences in grape composition observed, with an overall increase in tannin concentration observed between vintages. The differences observed were more pronounced in some varieties. Regression of tannin, anthocyanin and phenolic levels showed no obvious trends.

Conclusions

Tannin, anthocyanin and phenolic concentration is highly variable between grape varieties and between seasons. Tannin, anthocyanin and iron reactive phenolic levels are also highly variable within grape varieties suggesting a high level of plasticity in flavonoid biosynthesis between varieties. The lack of any relationship between tannin, anthocyanin and phenolic levels suggests that biosynthesis of these flavonoids is under differential regulatory control.

Significance of Study

Variability in tannin, anthocyanins and phenolic levels within varieties and seasons suggests the potential to manage winegrape flavonoids in the vineyards. Lack of a strong relationship between these parameters suggests different management techniques will be required for each flavonoid class.

Characterisation of two methyltransferase genes from *Vitis vinifera* L. cv. Cabernet Sauvignon capable of the final step in methoxypyrazine biosynthesis

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Background and Aims

3-Isobutyl-2-methoxypyrazine (IBMP) and 3-isopropyl-2-methoxypyrazine (IPMP) are volatile grape derived aroma compounds that are important contributors to the distinct herbaceous character of Cabernet Sauvignon and Sauvignon blanc wines. The presence of an enzyme in Cabernet Sauvignon berries with the ability to methylate 3-isobutyl-2-hydroxypyrazine (IBHP) and 3-isopropyl-2-hydroxypyrazine (IPHP) to produce IBMP and IPMP has been shown previously (Hashizume *et al.* 2001). The aim of this study was to characterise methyltransferase genes responsible for the production of IBMP and IPMP in grapevines.

Methods and Results

Two similar grapevine gene sequences were identified showing homology to the N-terminal sequence of the grapevine methyltransferase protein with hydroxypyrazine methylating activity. The two genes, named *VvHPMT1* and *VvHPMT2* (*Vitis vinifera hydroxypyrazine methyltransferase*), were cloned from Cabernet Sauvignon cDNA and the encoded proteins produced using a HIS-tagged bacterial expression system. Functional enzyme assays using synthesised hydroxypyrazine substrates and the recombinant proteins confirmed that the gene products were capable of producing methoxypyrazines. Both VvHPMT1 and VvHPMT2 were found to have the ability to methylate both IBHP and IPHP. Quantitative PCR analysis of a Cabernet Sauvignon developmental berry series showed that the expression of *VvHPMT1* and *VvHPMT2* preceded IBMP and IPMP accumulation. *VvHPMT1/2* expression was highest early in berry development peaking at four weeks post flowering and methoxypyrazines accumulated from four to eight weeks post-flowering after which levels declined until harvest. Analysis of other grapevine tissues also revealed a correlation between *VvHPMT1/2* gene expression and methoxypyrazines than other tissues yet had a similar level of *VvHPMT1/2* gene expression to berries.

Conclusions

This study shows that two similar methyltransferase genes, *VvHPMT1* and *VvHPMT2* encode enzymes that can perform the final step of IBMP and IPMP biosynthesis in grape berries. The levels of methoxypyrazines in berries appear to be dependent on the level HPMT expression suggesting that the methylation of hydroxypyrazine to methoxypyrazine is a rate limiting step in the biosynthetic pathway in these tissues. However, in roots, the high levels of methoxypyrazines does not seem to be a result of increased *VvHPMT1/2* expression and may be due to a greater level of hydroxypyrazine substrate availability.

Significance of Study

The identification of genes responsible for methoxypyrazine production will help in understanding the effect of different viticultural and environmental factors on methoxypyrazine accumulation in berries as well as having potential biotechnological applications for altering berry composition.

Developmental changes in cell vitality, osmotic water uptake and susceptibility to splitting in the post-veraison grape berry

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Background and Aims

Much research has been conducted on factors contributing to cultivar differences in susceptibility to splitting but how the propensity for grape berries to split changes during ripening is poorly known. Our aim was to examine the interaction between berry osmotic uptake of water, susceptibility to splitting and cell viability from veraison to maximum fresh weight and beyond in berries of *Vitis vinifera* (L.) cv. Shiraz (clone 1654).

Methods and Results

Cell vitality was assessed on berry cross-sections using nitro-blue tetrazolium (NBT). Osmosis was quantified by the rate at which berries bathed in water gained weight and susceptibility to splitting was gauged by the length of time it took for berries bathed in water to split. The rate of osmosis increased weight by $3 - 6 \% d^{-1}$ shortly after veraison and this had declined to $1 - 4 \% d^{-1}$ by 90 DAA, eventually reaching rates of $0.5 - 2 \% d^{-1}$ by the time shrivelling was evident. The area of individual berry cross-sections NBT stained remained relatively constant (60 - 100 %) from veraison until 90 DAA, after which it began to decline. Typically 30 % or less of the cross-sectional area of shrivelling berries was stained. Prior to 90 DAA berries bathed in water split in six days or less, whereas after 100 DAA the number of days until splitting was as long as 17 days and some sampled on or after 110 DAA failed to split.

Conclusion

The decline in the rate of water uptake by berries bathed in water early in the post-veraison period in spite of increasing °Brix values is likely to be due to the accumulation of apoplastic solutes which have the effect of reducing the osmotic gradient controlling water movement into berry cells. The decline in susceptibility to splitting > 100 DAA is accompanied by a decline in NBT staining. Thus, assuming that the decline in cell vitality is indicative of a decline in the integrity of semi-permeable cell membranes, a combination of apoplastic solutes and lack of osmotically competent cells are responsible for the decrease in the susceptibility of berries to splitting in the latter stages of ripening.

Significance of Study

The susceptibility of Shiraz berries to splitting declines dramatically once maximum berry fresh weight has been attained but remains relatively high prior to this.

Two-dimensional imaging of metastable-technetium xylem transport for the study of grapevine physiology

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Background and Aims

Metastable-technetium (^{99m}Tc) is an isotope whose gamma radiation can be detected nondestructively using scintigraphy, enabling technetium concentrations to be quantified in two- and three-dimensional space. Sodium pertechnetate is a nutrient analogue that is readily absorbed by grapevine roots and rapidly transported in the xylem. These attributes make technetium a highly effective tracer for the study of whole-vine transport of xylem-mobile nutrients. We have used this approach to better understand xylem flows in grape bunches and the transport of nutrients in xylem vessels.

Methods and Results

Pertechnetate was introduced to pre- and post-veraison grape bunches through the end of cut shoots or using a cotton wick threaded through the rachis and after four or 24 hours, respectively, the fruit and shoots were imaged scintigraphically to quantify technetium distribution. Technetium was present throughout pre-veraison berries but only in the brush of post-veraison berries. The wick results indicated that there is a component of xylem flow back towards the shoot in grape bunches and the presence of technetium in berries proximal to the wick and in leaves indicated that these organs were responsible for this flow.

Feeding pertechnetate to individual roots for 24 h labelled leaves on both sides of the shoot but only one shoot of two-bud cuttings. These results indicate ready exchange of nutrients between vascular bundles within current season growth but sectoriality within one-year old structures. Predictably, pertechnetate introduced to vine roots or cut shoots accumulated most rapidly in those leaves with the highest transpiration rates. Unexpectedly, technetium concentrations were higher in nodes than internodes. Microscopic observations of the dye sulphorhodamine G indicated that water exits xylem vessels in nodes and internodes, thus the high concentrations of technetium are not simply due to inter-vessel movement of water within nodes.

Conclusion

Xylem flow through grapevines is complex, involving bi-directional flows through organs such as the rachis. This research has also revealed that unloading mechanisms exist in nodes that concentrate xylem-mobile compounds within this region.

Significance of Study

While many studies have examined the uptake of technetium by plants, this study is the first to use the scintigraphic detection of technetium as a means of better understanding plant physiology.

A novel morphometric analysis method demonstrates variety dependent mesocarp cell death and berry shrivel

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Background and Aims

Berry shrinkage before harvest in varieties such as Shiraz can result in yield reductions of 25%. Varietal differences in mesocarp cell vitality through berry development have been found (Tilbrook and Tyerman, 2008). The onset of cell death was contemporaneous with weight loss and berry shriveling in Shiraz. The aim was to quantify and characterize berry cell vitality and shrivel in different grape varieties. Novel morphometric and fluorescent imaging techniques were developed to achieve this.

Methods and Results

Berry samples from 22 grape vine varieties were obtained at similar ^oBrix. Each berry was sliced in half; one half used to obtain total soluble solids (^oBrix) and the other stained with fluorescein diacetate to assess living tissue (LT) across the mesocarp. Berry LT and morphological data were analysed semi-automatically (MATLAB® R2008a). A Shrivel Index (ShI) was developed from the berry morphological data. A Principal Component Analysis (PCA) was used to develop a hierarchy of variables to find patterns between mesocarp cell death and morphological changes in berries. Four variety clusters were identified. Red wine varieties presented similar cell death patterns and shrinkage indices. Table grape varieties had more living tissue across the mesocarp close to harvest. White wine varieties presented medium to high mesocarp cell death, but did not show shrivel. We found that cell death in the mesocarp is relatively common in a number of varieties before usual harvest maturity.

Conclusions

Our results show that our indices and the PCA separated the varieties into four distinct clusters. The clusters reflected the varietal differences observed in water relations, shrivelling and cell vitality in the mesocarp of ripening berries. This confirms that grapes have variety dependent strategies that may be identified and predicted using this method of analysis.

Significance of Study

Cell death before harvest is likely to impact on flavor development in wine grape varieties. Tools to identify the onset of cell death and berry shrinkage may allow more refined measures of quality and maturity assessment.

Reference

Tilbrook J, Tyerman SD (2008) Cell death in grape berries: varietal differences linked to xylem pressure and berry weight loss. Functional Plant Biology 35: 173-184

Aroma levels and development during berry ripening in different Pinot noir clones

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Background and Aims

Wines of various Pinot clones are often described with different taste and flavour. The aim of the study was to identify typical flavour components in different Pinot noir clones.

Methods and Results

Berry samples were collected at up to seven different ripening stages from four Pinot noir clones and one Pinot Madeleine clone, which in Germany is regarded as a distinct variety due to 2 week earlier ripening. All vines were growing at the same location and under similar management systems. A number of substances could be detected like 1-Hexanol, 3-Hexen-1-ol or Benzaldehyd, but only Hexanal, Phenylacetaldehyd, Diethyldisulfid and Limonen reached levels above their odour thresholds. The concentration of most components varied with clone and ripening time.

Conclusions

Pinot noir clones differ substantially in their berry flavour compounds.

Significance of Study

The clonal variation in flavour provides the option to identify and develop clones with different flavour types suited to different vineyard sites and winemakers' ideas.

Identification of organic acid carriers

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Background and Aims

Tartaric and malic acids are the predominant organic acids found in grapes. They are essential in winemaking to keep juice pH low during fermentation to prevent spoilage, while in wine they augment flavour and aroma and contribute to bottle stability. These acids are synthesised preveraison and subsequently stored in the vacuoles of berry pulp cells. Vacuolar compartmentation prevents the metabolism of these acids, but only transiently in the case of malic acid because its concentration declines rapidly post-veraison. Previous studies in our laboratory identified the first tartaric acid biosynthetic enzyme, L-Idonate dehydrogenase (DeBolt et al 2006). Identification of additional biosynthetic enzymes is ongoing, however, recently our studies have expanded to identify proteins that mediate vacuolar compartmentation of organic acids.

Methods and Results

We are studying five putative transport proteins we identified in the grapevine genome that are highly homologous to organic acid transporters recently described in the experimental plant *Arabidopsis thaliana*. Subcellular localisation using GFP-fusion proteins and transient expression in plant cells has confirmed that these proteins are targeted to the vacuolar membrane. Quantitative RT-PCR on a grape berry developmental series showed that all these genes are expressed in berries and that gene expression of each is developmentally regulated. Functional analysis of these proteins to determine transport kinetics and substrate range will be performed in *Arabidopsis thaliana* knockout mutants and in *Xenopus laevis* oocytes.

Conclusions

Our analysis to date has shown that these putative organic acid transporters are localised to the vacuolar membrane, and are expressed in berries at developmental stages when metabolic and physiological events of great importance to organic acids are occurring. We await results from the functional dissection of these proteins with great excitement and expect to identify transporters responsible for vacuolar compartmentation of malic and tartaric acid in grape berries.

Significance of Study

This project aims to pinpoint key determinants of grape berry acidity and hasten the identification or engineering of 'high acid' grapevine cultivars that will enable the optimisation and control of organic acid composition in the face of changing environmental and management practices.

Hydraulic connection of grape berries to the vine: varietal differences in hydraulic conductance and occurrence of backflow

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Background and Aims

It has been shown that a hydraulic connection is maintained between post-veraison berries and the vine. Here we compare Chardonnay and Shiraz in terms of the degree of hydraulic connectivity of berries with the vine taking into account the directions of flow. The two varieties contrast in the degree of berry weight loss that is normally observed: Chardonnay berries reach a maximum weight that is generally maintained until harvest, while Shiraz berries reach a maximum weight and then lose up to 30% before harvest maturity.

Methods and Results

Using a flow meter, flow rate into detached bunches at a constant applied pressure decreased similarly in the two varieties up until 90-95 days after anthesis (daa), thereafter Shiraz bunches maintained similar inflow rate until harvest maturity while in Chardonnay inflow was reduced close to zero. Flow measurements using a pressure probe attached to individual berries via the pedicel showed that from 105 daa (the point where Shiraz berries began to lose weight) hydraulic conductance for flow inwards to Shiraz berries was significantly greater than that for Chardonnay. This difference between varieties was also observed for flow outwards from the berry simulating backflow, but the conductances were smaller particularly for Chardonnay berries. Backflow from the berries to the vine was visualised in the two varieties using a fluorescent xylem mobile dye introduced into the stylar ends of berries still attached to bunches on potted vines. At later stages of development the dye travelled a greater distance in the xylem network of the vine in Shiraz compared to Chardonnay.

Conclusions

The degree of berry hydraulic connection with the vine after veraison is variety dependent. The xylem hydraulic conductance of individual berries for inflow is significantly higher than for outflow. The quantitative difference in hydraulic conductance between the two varieties reflects the degree of back flow that occurs between the varieties, which may account for differences in weight loss at advanced stages of ripening.

Significance of Study

An explanation is now provided for the mechanism of weight loss in Shiraz berries. This knowledge can now be used to apply techniques to limit weight loss.

Responses of malate metabolising enzymes throughout grape berry development

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Background and Aims

The predominant organic acids in grape berries are malic acid and tartaric acid. While tartaric acid remains relatively inert, malic acid is intimately involved in berry metabolism, and provides carbon intermediates that are required for ripening. Degradation of malic acid is largely responsible for the observed increase in pH and decrease in titratable acidity seen during ripening, and can therefore influence characteristics of the juice at harvest. According to the pattern of malic acid content throughout development of grape berries, a metabolic switch occurs prior to veraison (onset of ripening), whereby net synthesis of malic acid gives way to net degradation. Therefore a shift in the activity of malate-metabolising enzymes can be expected. The ripening-related degradation of malic acid can occur through multiple complex pathways involving numerous enzymes.

Methods and Results

Samples were taken at critical time-points throughout the development of Shiraz berries and assayed for malic acid content, and for specific enzyme activities involved in pathways potentially responsible for malate degradation. Pathways thus represented include the TCA cycle, gluconeogenesis, fermentation and pyruvate metabolism. Results show increased activities of NADP-dependent malic enzyme, PEP carboxykinase, pyruvate kinase and alcohol dehydrogenase simultaneous with malate degradation. Malate dehydrogenase activity was maintained throughout development.

Conclusions

The post-veraison up-regulation of numerous enzymes involved in pyruvate metabolism suggest that malate may play a critical role in supplying pyruvate to the cells of ripening berries. This pyruvate may feed into many pathways of biochemical importance in the ripening berry, including the TCA cycle and alcohol fermentation.

Significance of Study

Metabolism of grape berry organic acids is of central importance in supporting the fruit ripening process, and in creating a suitable juice for wine production. While numerous pathways are implicated in the metabolism of malic acid during ripening, the degree of involvement from each pathway is unknown, and may be dependent on environmental influences. Such information could help target specific enzymes as regulators of malic acid degradation.

Abscisic acid application at particular developmental stages can modify the timing and synchronicity of berry ripening

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Background and Aims

Fruit have traditionally been divided by physiologists into two groups, either climacteric or nonclimacteric. Grapes are considered to be non-climacteric as they do not show increased respiration at ripening and do not respond dramatically to ethylene as do climacteric fruit such as bananas. We are investigating the role of plant growth regulators during berry development and testing their ability to manipulate ripening. Abscisic acid (ABA) is of particular interest as its levels in the berry increase dramatically at the commencement of ripening consistent with it having a controlling influence.

Methods and Results

(+)ABA at a concentration of 400 mg/L and the ethylene releasing compound Ethrel (145 mg/L) were applied to field grown *Vitis vinifera* L. berries at various times prior to veraison. Compared to the controls, ripening of the ABA-treated fruit was advanced as measured by changes in berry weight, skin colour and sugar accumulation (measured °Brix). This effect was consistent over three seasons. Treatments with ABA earlier than two weeks, or less than one week, before veraison did not advance ripening. Treatments with Ethrel at any stage did not appear to enhance ripening. The developmental stages of the control berry population became more synchronised as measured by °Brix as maturation continues. Treatment with ABA makes the fruit more synchronous.

Conclusions

We have demonstrated over a number of seasons that ABA application to berries approximately two weeks prior to veraison can advance ripening as measured by enhanced sugar levels, berry size and skin colouration. In contrast, ethylene did not seem to significantly alter ripening which is characteristic of a non-climacteric fruit rather than a climacteric fruit. The increased synchronisation of the ABA-treated fruit was most probably due to the advancement of ripening. The stimulation of ripening by ABA is dependent on berry developmental stage and there is a relatively small window when ABA treatment is effective. We are now investigating the mechanism of ABA action during ripening in treated and untreated berries.

Significance of Study

These studies suggest that it is possible to reproducibly manipulate the timing of berry ripening and ripening synchronicity through the manipulation of the levels of ABA.

Transcriptional Regulation of Grape Cytochrome P450 Monooxygenase Gene CYP736B Expression in Response to *Xylella fastidiosa* Infection

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Background and Aims

Plant cytochrome P450 monooxygenases are a group of versatile redox proteins that mediate the biosynthesis of lignins, terpenes, alkaloids, and a variety of other secondary compounds which act as plant defense agents. The aim of the study was to investigate if the transcriptional and posttranscriptional modification patterns of a cytochrome P450 monooxygenase gene CYP736B are involved in defense response to *Xylella fastidiosa (Xf)* infection.

Methods and Results

Pierce's disease susceptible (9621-94) and resistant genotypes (9621-67) selected from a segregating population of *V. rupestris x V. arizonica* were used for this study. Expression experiment was conducted in the greenhouse where the treatment group was mechanically inoculated with *Xf.* RNA was collected from Leaf and stem tissues at one week, six week and ten week post inoculation. Cloning of genomic DNA and cDNA revealed that the CYP736B gene was composed of two exons and one intron with GT as a donor site and AG as an acceptor site. It was up-regulated in resistant plants and down-regulated in susceptible plants at 6 weeks after *Xf* infection. However, its expression was almost completely suppressed in stem tissues at all evaluated times. 5'RACE analysis found five major upstream transcriptional initiation regions (TIR) used in leaf tissues, TIR-I through TIR-V, consisting of 38 transcription initiation sites. The TIR-III and TIR-III regions played important roles in regulating CYP736B gene expression in infected grapevines. 3'RACE analysis found three major and five sub-polyadenylation regions (PAR), PAR-Ia, PAR-Ib, PAR-IIa, PAR-IIb and PAR-III, consisting of 31 transcription termination / polyadenylation sites. The usage of the PAR-IB and PAR-II sites was greatly affected when resistant plants were inoculated with *Xf*.

Conclusions

These results demonstrate that the expression of the cytochrome P450 monooxygenase CYP736B gene is regulated differentially and in response to Xf infection at both transcriptional and post-transcriptional levels.

Significance of Study

This study demonstrates that the selective usage and coordination of both transcription initiation and termination/polyadenylation sites may play important role in PD resistant grapevines against Xf infection by determining the transcriptional efficiency, pre-mRNA splicing, and mRNA maturation.

Characterization of some *Vitis Vinifera* Cv. Touriga Nacional pip and tip Aquaporins using *Saccharomyces Cerevisae* Heterologous Expression System

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Background and Aims

Water availability is of fundamental importance for all living organisms. To cope with environmental and physiological stresses, plants must be able of a rapid cellular adaptation for survival and growth. Depending on the environmental conditions and the plant water balance, plants can modify the relative contribution of apoplastic and cell-to-cell water-flow pathways across the tissues to adjust the overall hydraulic conductivity. Aquaporins are essential in the cell-to-cell pathway as their presence allows water or solutes transport across membranes by facilitated diffusion. Since the first aquaporin gene from plants was cloned and functionally expressed in 1993, an increasing number of aquaporin genes classified in four subfamilies have been reported, including in *Vitis*. For improving the understanding of water stress fluxes mechanism of grapevine aquaporins, our aim is to identify some plasma membrane and tonoplastic aquaporins (PIPs and TIPs) of a Portuguese grapevine cultivar and to express each identified *Vitis vinifera* aquaporin gene in the heterologous system *Saccharomyces cerevisae* lacking native aquaporins.

Methods and Results

Heterologous expression system together with stopped-flow experiments allow us to characterize aquaporins separately and accurately. Currently, we identified and sequenced six putative genes from *Vitis* callus cells. By comparing their sequences with the available database sequences of *Vitis vinifera*, we found homologies with the plasma intrinsic proteins (PIP2;2 and PIP1;1) and the tonoplastic intrinsic protein (TIP2;1). Moreover, different isoforms of PIP2;2 and TIP2;1 have been detected. Each of them was cloned in *Saccharomyces cerevisae*. The expression and localization of theses aquaporins are being investigated using a green fluorescent protein GFP-fusion aquaporin system and their properties evaluated in walled cells of the yeast transformant strains by fluorescence in a stop flow device.

Conclusions

This study allow us to identify 6 putative *Vitis vinifera* aquaporins (PIP and TIP) from a Portuguese grapevine cultivar. Preliminary results associating this two techniques demonstrated a plasma membrane localization in yeast and putative water channel transport function was being investigated.

Significance of Study

The study described plasma membrane and tonoplastic aquaporin sequences from a portuguese *Vitis vinifera* cultivar (Touriga nacional) and focused in utilization of a yeast heterologous system to evaluate putative aquaporin properties in a stop flow device.

Phylogentics Analysis of *Aestivales* (Planchon), American Native Grapes by Nuclear Microsatellite Profiling

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Background and Aims

The close proximity of related species and many variants to *Vitis aestivalis* has created confusion among taxonomists. This research study was conducted to define the phylogenetic relations among the grape species and subspecies listed under *Aestivales* Planchon group via data mining in the existing North American grape germplasm collections, and specifically expressed in the members of the group DNA microsatellites for using them in breeding programs and grape improvement.

Methods and Results

DNA isolation and quantification of nine *Aestivales* accessions, evenly distributed throughout their area of natural habitat was completed. Microsatellite specific PCR amplification products were obtained with nine out of ten microsatellite markers originating from *V. riparia*, which were previously and successfully used for grape identification. A series of dendrograms was generated using the software STATISTICA version 4.5.

Conclusion

The use of DNA identification methods and more specifically the use of microsatellite markers proved that there is a clear delineation between origin of the accession and proximity in the dendrogram. By tracing the phylogentic relations of the *Aestivales* group, a gene pool for the development of new and improved cultivars for Florida and the Southeastern United States may be developed yielding a better quality and stable red color for wines.

Significance of Study

This study was designed to help reconstruct evolutionary relationships among selected North American native grape species yet having qualities of red color stability for wine making, and can be a useful gene pool for grape improvement.

The use of the induced site-specific DNA excision technique for an efficient marker gene removal in grape: potentials and constraints

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Background and Aims

During gene transfer, the *nptII* gene conferring kanamycin resistance is commonly used for removing from the cultures the cells that have not inserted the gene of interest. Marker genes may be undesirable and the site-specific DNA excision seems to be a promising technique for the removal. We are exploiting this strategy in grape where its application has not yet been reported.

Methods and Results

Vitis vinifera cv. Brachetto plants were obtained after co-culture of embryogenic calli with *Agrobacterium* LBA4404 carrying the chemical-inducible site-specific *cre/loxP* pX6 vector (Zuo *et al.*, 2001, Nature Biotechnol. 19, 157-161) with the *nptII* gene, where *gfp* gene was replaced with a coat protein sequence of the Grapevine Virus A coding for a hairpin RNA (pX6-pKcpGVA, Turturo *et al.*, Proc. 14th ICVG Conference, Locorotondo, Italy 12-17 Sept. 2003). The *cre* recombinase is regulated by the 17- β -estradiol.

The *nptII* removal was induced on buds during micropropagation, assessing hormone concentration (10 or 20 μ l), supply strategies (solid *versus* liquid) and application time. The efficiency of *nptII* removal was evaluated on plantlets cloned from a mother plant containing 0.55 *nptII* mean copy number.

A degree of exogene removal was quantified in the samples when the whole plantlets were analysed (0.26 mean copy number), and statistical analysis proved no significant differences between hormone concentrations nor between supply strategies. However, when separately testing 5 different regions of the plantlets, the analysis of variance showed significant differences among the tissues.

Conclusions

Gene removal occurred with different efficiencies along the plant tissue, with the highest level in the roots, according to the lowest mean copy numbers quantified (0.01 and 0.13 respectively for liquid and solid supply).

Since different concentration, supply strategies and application time of the hormone did not result significant when buds induced to microrpopagate were used, we believe that morphogenic *status* of the explants is a key point of the overall strategy. Accordingly, assays during secondary regeneration are in progress.

Significance of Study

Marker gene removal is a promising technique for grape gene transfer, and our research is a first attempt to exploit the site-specific DNA excision strategy in *Vitis*.

cDNA Cloning and Analysis of UDP Glucose-Flavonoid 3-O-Glucosyltransferase (3GT) in *Vitis amurensis Rupr*

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Background and Aims

According to the research results of anthocyanin biosynthesis and regulation in Zea mays, Petunia hybridia,etc. The relevant research of anthocyanin in grapes has also made considerable progress. UDP glucose:flavonoid 3-o-glucoseyl transferase (3GT) is the key enzyme in anthocyanin biosynthetic pathway, which catalyzes the transfer of the glucosyl moiety from UDP-glucose to the 3-hydroxyl group of anthocyanidins. We first cloned the 3GT cDNA sequence from grape skin of Vitis amurensis Rupr.

Methods and Results

A full-length cDNA of UDP glucose-flavonoid 3-o-glucosyl transferase (3GT) from Vitis amurensis Rupr was cloned with RT-PCR and SMART RACE. The full-length cDNA of 3GT was 1,477 bp in size, containing a 1,371 bp open reading frame (ORF) which corresponds to a protein of 456 amino acids with a predicted molecular mass of 50.185 kDa and an isoelectric point of 5.98, containing a 24 bp 5'untranslated region and 52 bp 3'untranslated region. The 3GT is unstable protein, is UDPGT super-gene family, and includes signal peptide. The predicted amino acid sequence exhibited 98%, 97%, 59%, 51% and 42% homology to the UDP glucose-flavonoid 3-o-glucosyltransferase of Vitis vinifera, Vitis labrusca, Arabidopsis thaliana, Petunia and Zea mays, respectively.

Conclusions and Significance of Study

We cloned the full-length cDNA sequence of 3GT in Vitis amurensis Rupr., and analyzed the gene characteristics. Cloning and characterization of 3GT in Vitis amurensis Rupr., will provide more theoretical basis for molecular breeding of improving different color grape varieties and production of different anthocyanin using cell culture technology.

Functional analysis of grapevine carotenoid cleavage dioxygenase (*VvCCD1*) using over-expression and silencing strategies

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Background and Aims

The *Vitis vinifera* carotenoid cleavage dioxygenase (*VvCCD1*) belongs to a family of enzymes that catalyse the cleavage of carotenoids at specific double bonds to form apocarotenoid products. These products are important flavour/aroma compounds and have been implicated in plant signalling pathways. VvCCD1 has previously been shown *in vitro* to cleave zeaxanthin and lutein forming 3-hydroxy- β -ionone and a C14 dialdehyde. By over-expressing or silencing *VvCCD1* in grapevine, this study aims to elucidate the *in planta* function of the gene.

Methods and Results

A cDNA copy of *VvCCD1* was isolated from *V. vinifera* L. cv Pinotage and over-expressed in *V. vinifera* L. cv Sultana. A hairpin construct, targeting the 3'-UTR of *VvCCD1*, was used to generate silenced Sultana lines. Transformation was confirmed via Southern hybridisation and the levels of *VvCCD1* transcripts in the lines were monitored using Real-Time PCR. Methods were developed to analyse pigment profiles and the formation of volatile flavour/aroma compounds in grapevine leaf tissue using High performance liquid chromatography (HPLC) and Head-space Solid Phase Micro Extraction Gas chromatography-mass spectrometry (HS-SPME-GC/MS), respectively. These transgenic resources coupled with the analysis methods developed are providing insight into the functional role of *VvCCD1* in grapevine.

Conclusions

In order to elucidate the functional role of *VvCCD1*, transgenic grapevine lines have been generated, genetically characterised and the levels of selected metabolites analysed. The profiling methods developed allow for the identification and quantification of a number of compounds of specific importance in grapevine that include: carotenoids, chlorophylls, terpenes and norisoprenoids.

Significance of Study

The generation of transgenic grapevine with altered *VvCCD1* transcript levels provides a useful tool for the functional analysis of this gene and its products. The analytical techniques developed collectively allow for the metabolic profiling of grapevine.

Functional analysis of a putative MYB60 orthologue: a candidate gene to increase drought tolerance in grapevine (*Vitis vinifera*)

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Background and Aims

Under drought conditions, plants accumulate the signalling hormone abscisic acid (ABA), which induces a rapid stomatal closure to prevent water loss. This event is generated by a series of signals produced inside guard cells which finally reduce their turgor. The Arabidopsis thaliana MYB60 gene codifies for a R2R3 MYB transcription factor which is specifically expressed in guard cells, and its expression is negatively modulated during drought. A null mutation in AtMYB60 results in the constitutive reduction of stomatal opening and in decreased wilting under water stress conditions (Cominelli et al., 2005). In order to engineer stomatal responses as an approach to reduce water loss and enhance drought tolerance in grapevines, we have isolated and partially characterised a grape AtMYB60 homologue, isolated from *Vitis vinifera* cv. Pinot noir leaves.

Methods and Results

A grape MYB60 homologue was previously identified in a genome-wide analysis of the MYB family in the grape genome sequence (Matus et al., 2008). This gene was isolated by means of RT-PCR and functionally characterised in terms of organ and cell-specific gene expression, sub-cellular localisation using GFP translational fusions and analysis of promoter-GUS transcriptional fusion activity. Mutant complementation it's being conducted.

Conclusions

We have isolated and partially characterised a putative MYB60 orthologue in Vitis. Ongoing experiments are being developed to produce a MYB60 loss of function in grapevines.

Significance of Study

Since this gene modulates the physiological response of guard cells, it opens new possibilities for engineering stomatal activity and help plants survive desiccation.

References

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A New GeneChip for Grapevine Transcriptomic Analyses

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Background and Aims

Due to its economic importance, grape berry development has been a major target for expression profiling analyses during the last years. Crucial information has been generated through several studies regarding tissue specific expression in (Grimplet et al., 2007) and expression profiles either around veraison (Pilati et al., 2007) or during several stages of berry development (Deluc et al., 2007). All these works were performed with the commercial 14K Affymetrix GeneChip and obtained sorted Unigene catalogues according to the classically studied biochemical events taking place during berry development. In this work we develop a new and more complete GeneChip together with additional tools designed to facilitate functional analysis of the hybridization results. These tools have been used to follow berry development and ripening in a more comprehensive approach.

Methods and Results

A 23K custom Affymetrix GeneChip was developed as a result of the Spanish-Canadian GRAPEGEN project. All probe-sets were manually re-annotated and classified in order to perform functional analyses with state-of-the-art tools like MapMan (Thimm et al., 2004) or Babelomics (Al-Shahrour et al., 2008). Additionally, a web-based application was developed to display all the information associated with a probe-set list (including external dbase links). This GeneChip was used to analyze ten different berry development stages of the cultivar Muscat Hamburg during two seasons and dissecting skin and flesh from the pre-veraison stage. Analyses were sub-divided in green stages, veraison (skin/flesh) and ripening (skin/flesh). Differentially expressed genes were clustered using k-means and significant functional groups were identified within each cluster using Babelomics and graphically represented by means of MapMan.

Conclusions

The results of these experiments validate previous indications regarding the involvement of functional groups like stress responses, photosynthesis, cell wall metabolism and plant hormones metabolism and response during berry development and ripening. Furthermore, there are additional hints on the start of oxidative stress at the green stages, the down-regulation of the phenylpropanoid metabolism and auxins before ripening or the involvement of ethylene during ripening,. These phenomena were examined both in skin and flesh.

Significance of Study

We present and validate a new custom made Affymetrix GeneChip (representing a 70% probe-set increment respect to the commercial one) together with a suite of functional analysis tools. These tools allowed us to perform an unbiased functional classification of the genes involved in grape berry development, veraison and ripening.

Modulation of Leucoanthocyanidin Dioxygenase (LDOX) activity by 14-3-3 Proteins in *Vitis Vinifera (*cv. Cabernet Sauvignon)

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Background and Aims

Leucoanthocyanidin Dioxygenase (LDOX) is one of the enzymes of anthocyanidin biosynthesis. In this work, a Vitis LDOX was identified and characterized. We then investigated whether this enzyme is regulated by 14-3-3 proteins.

Methods and Results

Vitis LDOX was functionally characterized after heterologous expression in *E. coli* and purification by affinity chromatography from bacterial extracts. This enzyme converted (+)-catechin to its dimer identified by LC-MS. The kinetic parameters, K_M for (+)-catechin and Vmax were determined and magnesium ions were showed to stimulate LDOX activity. Overlay experiments and pull-down assays were in favour of an interaction between LDOX and recombinant 14-3-3 proteins. Furthermore, phosphorylation of LDOX was required for this interaction. The potential role of 14-3-3 proteins in the modulation of LDOX activity then was investigated. The results showed that 14-3-3 proteins decreased significantly LDOX activity.

Conclusions

The observed modulation of LDOX activity by 14-3-3 proteins corroborated the apparent interaction between LDOX and *Vitis* 14-3-3 proteins *in vitro*. Thus, LDOX could be a target for 14-3-3 proteins *in vivo*.

Significance of the Study

These results suggest that 14-3-3 proteins are part of post-translational mechanisms mediating modulation of LDOX activity. In consequence, this may imply a regulation of anthocyanin production in grapeberries at the level of LDOX activity.

Vineyard Cover Crop Effects on Leaf Water Use Efficiency and Grape Production and Quality Under Mediterranean Conditions

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Background and Aims

Cover crops under temperate climates are useful to reduce excess water availability for grapevines, which otherwise limit grape quality. Under Mediterranean conditions, the usefulness of those cover crops is matter of controversy, because of limited water availability. However, they could be useful to limit soil erosion and to reduce excess vegetative vigour. In this sense, early senescent species can be interesting in order to restrict water competition to early phases of grapevine development. The objective of this experiment was to study the effects of self reseeding herbaceous cover crops in Mediterranean vineyards, particularly water use efficiency, grapevine production and grape quality.

Methods and Results

The experiment was carried out along three years in an organic vineyard of *Vitis vinifera* L. cv. Manto Negro in central Mallorca (Spain). Three intercropping strips of 200 m long x 2.5 m wide per treatment were considered. Plants were at a planting distance of 1.2 m. Three different treatments were established: perennial grasses and legumes mixture, no tillage and traditional tillage (ploughed soil). The grapevines were rain fed until veraison, when drip irrigation was applied in order to ensure grape production. The pre-dawn leaf water potential and gas exchange parameters were measured at: anthesis, "pea size", veraison, fruit maturity and post-harvest. Cover crops reduced the leaf transpiration, total leaf area and plant vigour at early growth phases. Nevertheless, stomatal conductance and photosynthesis were higher in the cover crop treatment during veraison and ripening phases, what could be a consequence of leaf area reduction. Intrinsic water use efficiency increased from flowering until veraison-maturity in all treatments. The final grape production was lower in the cover crop treatments (both, permanent mixture and no tillage) than in the traditional tillage in all the years. Nevertheless, grape quality parameters were slightly better in the permanent mixture treatment.

Conclusions

The use of cover crops led to a decrease of grape production that can be partially counteracted by the increase of grape quality. In addition, the leaf area reduction induced by the cover crops allowed grapevines to show greater stomatal conductance rates at mid summer, what can be of interest in very dry years to ensure grape production.

Significance of Study

This study shows the interest of using cover crops in Mediterranean vineyards to reduce vegetative vigour, which is usually excessive under the modern training systems, as well as their effects on grape production and quality.
Optimizing Grapevine Water Use: Limits For Water Use Efficiency Improvement

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Background and Aims

Water availability is an important factor for grapevine productivity and grape quality. Now, in Mediterranean areas it is a scarce resource and, according to the last predictions of climate change it will be even scarcer in near future, thus, improving water use efficiency (WUE) is becoming crucial for a sustainable viticulture. In grapevines, the plant production (harvest) dependency on water availability is clearly reflected in the relationship between net photosynthesis and stomatal conductance (A_N vs. g_s) which clearly shows two main targets for improving WUE: 1: control of water loss (transpiration) irrigating to maintain a certain soil water deficit 2: Search for more efficient varieties.

Methods and Results

A critical review of agronomic ways to improve WUE via control of irrigation schedule and dosage and other crop practices is shown. A wide range of variation of WUE (A_N/g_s values from 0 to 230 mmol/mol was present along different soil water stress experiments including recovery with a certain correspondence with respect to A_N/E (2 to 6 mmol mol⁻¹), This range was also present for diurnal time courses and among different leaf positions in the canopy. WUE increase with water stress but steeply decreased for g_s values below 0,05 mmol m⁻² s⁻¹. The correspondence among those instantaneous leaf values and the biomass gain/water expenses ones showed also a clear decline in WUE (biomass) for extreme drought conditions showing the limits of WUE improvement by water availability reductions. Field and pot experiments on 22 grape varieties showed a wide genetic variability in grapevine WUE (estimated as A_N/g_s) stressing the interest of a better assessment on this parameter for future qualifications of the market varieties.

Conclusions

Different agronomic ways can lead to an optimization of the grapevine water use enabling to expect a clear improvement the WUE in near future.

Significance of Study

Data on range of variation of grapevine water use efficiency in pot and field conditions shows the limits of water use for a sustainable crop in near future.

Measurement of Trellis Tension in Vineyards

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Background and Aims

Monitoring trellis wire tension offers an innovative method of tracking grapevine growth and fruit development throughout the season.

Methods and Results

Load cells were installed along the cordon wire and monitored continuously for up to five years. The Trellis Tension Monitor (TTM) system indicated the expected growth curve of vine and fruit, demonstrated sensitivity to management practices like overhead irrigation, crop thinning, and dormant pruning. A simple empirical model showed mixed results for yield estimation, with some estimates within 5% of the harvested weight of fruit within the vicinity of the load cell vines.

Conclusions

The Trellis Tension Monitor provides accurate, real-time data for dynamic yield prediction and monitoring vine growth throughout the season.

Significance of the Study

Growth patterns of the vines and fruit can be inferred from the tension trace, which can be used in decision aid support systems to refine traditional sampling efforts. Yield estimation can be automated and provide continuous, dynamic information to the grower or winery.

The effect of shade on growth, yield and berry composition in *Vitis vinifera* cv. Semillon grapevines

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Background and Aims

Extreme heat events can impact negatively on productivity and quality of fruit in grapevines. There is also an expectation that climate change will increase the frequency of heat events. The aim of this work was to evaluate overhead shade as a means of ameliorating the canopy microclimate and to assess the impact on growth, productivity and berry and wine composition in Semillon grapevines.

Methods and Results

Shadecloth (30 % transmission) was placed over whole panels of Semillon grapevines and canopy temperatures monitored. Shoot growth, fruit development and berry composition were measured. Shade reduced maximum canopy temperatures by 3°C but also the frequency of temperatures above 35°C. Final shoot length, leaf area and total dry matter production were reduced by shade. Dry matter allocation shifted slightly towards stem and fruit at the expense of leaf biomass. Yield was reduced 18% and a marked reduction in soluble solids and a slight shift in TA and pH indicated that juice quality was also affected. The effect on wine quality is currently being assessed.

Conclusions

Overhead shade was an effective means of ameliorating canopy temperatures but significantly reduced available light to the vines. Consequently, growth, dry matter allocation and yield were all affected. The impact on wine quality remains to be determined.

Significance of Study

High temperature events can cause crop losses in vineyards and poor wine quality. Practical methods of reducing heat extremes are desirable but, as this study has shown, may have unintended consequences on yield and fruit composition.

Maintenance of grafted vine health and productivity under forced 0 irrigation

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Background and Aims

Water availability for many Australian vineyards has become increasingly scarce due to both consecutive low rainfall events and current water allocations; in some regions as little as 6% of allocated water. For this reason, grapegrowers need strategies to cope with reduced water availability from season to season in order to produce a viable crop. In 2007-2008 a pilot trial was conducted to assess rootstock response to a zero irrigation regime in a region with 500mm annual rainfall. The aim was to confirm the reported drought tolerance of rootstocks in the current season and performance in the following season, as measured by an ability to maintain canopy, berry weight, Total Soluble Solids and bud fertility under a reduced irrigation regime.

Methods and Results

Shiraz on its own roots and grafted to rootstocks Ramsey (*V. champini*), 140 Ruggeri, 1103 Paulsen, 99 Richter, 110 Richter (*V berlandieri x V. rupestris*), and Schwarzmann (*V. riparia x V. rupestris*) were assessed for one season in the Barossa Valley, South Australia for their ability to maintain growth and produce a crop when subjected to a zero irrigation regime. Treated vines were deprived any irrigation while control vines received 11 irrigations during December 2007 to February 2008, totalling 0.5ML per ha. Berry weight decreased for all treatments in response to zero irrigation. Rootstocks Schwarzmann and 140 Ruggeri experienced a greater reduction in berry weight than Ramsey or 1103 Paulsen when not irrigated. 140 Ruggeri recorded the highest ^oBrix reading and lowest berry weight at harvest inferring a low tolerance to water stress. Ramsey and 1103 Paulsen reported similar ^oBrix and berry weights, comparable with their irrigated controls, which demonstrated an ability to cope with water stress.

Conclusions

Preliminary evidence suggests that the level of drought tolerance of rootstocks is not necessarily correlated with rootstock parentage. Additionally, the reported drought-intolerant rootstock Schwarzmann did not perform as well as the *V berlandieri x V. rupestris* hybrid rootstocks (with the exception of 140 Ruggeri), thereby confirming previous reports. Bud fertility measurements during 2008-09 will be used to assess other long-term impacts of water stress.

Significance of Study

The study demonstrates the need to determine the individual limits of drought tolerant rootstocks when grown under a forced zero irrigation regime. More detailed studies are underway to investigate the effects of water stress on the reproductive performance of rootstocks.

Interactive effects of irrigation and crop level on Tempranillo grapes. Water relations, vine performance and fruit and wine composition

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Background and Aims

The effects of crop level and irrigation on water relations, yield, grape and wine quality were studied during two consecutive seasons in a Tempranillo vineyard in Spain.

Methods and Results

Vines were trained via vertical shoot positioning. Irrigation was applied at two levels: R2 (at 100% of estimated crop evapotranspiration, ETc from flowering to harvest) and R1 (at 50% ETc from flowering to veraison and at 100% ETc thereafter) and compared to non-irrigated. Crop levels studied were Low, Medium and High (11, 20 and 27 clusters per vine, respectively), and were regulated by shoot and cluster thinning. Over all treatments, yield and leaf area to yield ratio were very different between years: 4.4 and 16.3 t ha-1 and 1.72 and 0.88 m2 kg-1 in 2005 and 2006, respectively. Crop level did not enhance gas exchange and practically had not effect on midday stem water potential. Irrigation, instead, improved plant water status, increased leaf assimilation rates and vine growth; consequently yield increased in proportion to the amount of water application. In 2005 large differences in grape and wine quality occurred between non-irrigated and irrigated treatments, but not between R1 and R2 treatments. Wines from non-irrigated vines were more acid, had higher total anthocyanins, phenols content and higher colour intensity. In 2006, when yield was much larger, irrigation had no effect on grape and wine quality. Differences in wine anthocyanins content among irrigation treatments could not be entirely explained by the larger berry size of the irrigated vines (dilution effect). In fact, skin concentration of anthocyanins was also larger in the non-irrigated vines, while no significant differences among treatments were observed in the anthocyanins extractability. On the other hand the effect of crop level on wine composition was different between seasons because the very different leaf area to yield values among years.

Conclusions

The irrigation supplied had a detrimental effect on grape quality that was less pronounced under a larger crop demand. Grape quality was negatively affected by high crop level only for values of leaf area to yield lower than $1.3 \text{ m}^2 \text{ kg}^{-1}$.

Significance of Study

For premium wine production irrigation should be used with caution and preferentially employed with high crop levels. The leaf area to yield ratio can be successfully used to manage vine crop load level, accordingly to vine water status.

Relationship between Soil Water Content and Vine Water Status: Characterization of the rootstock effect on the response of vine transpiration during a drought cycle

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Background and Aims

Water is the main limiting factor for yield in viticulture. Vine water status also strongly impacts grape quality. Studies concerning vine water deficit stress are numerous but the level of water stress is seldom rigorously controlled. Drying cycles used in other woody species allow neither to know exactly the intensity of water stress nor vigour differences. The aim of this work was first to define a method for applying the same gradual water stress to all the individuals of a pot experiment, whatever their leaf area. Second, the response of transpiration induced by different rootstocks was characterized.

Methods and Results

Young grafted vines of Cabernet Sauvignon were grown in 7L pots filled with a known amount of sandy-loamy soil. The rootstocks studied were *V. riparia* "Gloire de Montpellier", *Vitis* hybrid 110 Richter and several hybrids *V. vinifera* Cabernet Sauvignon * *V. riparia* Gloire de Montpellier. Water retention properties of the substrate were primarily determined. The amount of water in the substrate was used to monitor soil water status. Transpiration was evaluated daily by weighing each pot individually. Irrigation was applied in the mid morning in order to compensate exactly the difference between the daily water loss due to transpiration in a particular pot and the loss of water of the least transpiring plant. Leaf area measurements were performed regularly in order to calculate the daily transpiration per units of leaf area.

A progressive water limitation occurred. Daily water status of the substrate was expressed as the amount of water still present in the pot. Transpiration was stopped within 40 days. Normalized daily transpiration data per unit of leaf area were plotted with percentage of water retention capacity. Some specific parameters of these relationships were used to compare various rootstock genotypes. The threshold of water content corresponding to the onset of regulation for vines was calculated to characterize the various rootstocks. This threshold presented significant differences between the studied rootstocks. 110R presented a significantly lower threshold than the others in two different experiments run in 2006 and 2007.

Conclusions

Thanks to this method, every plant faced the same water stress at a daily scale. This control allows more accurate comparisons of the water extraction capacities for different rootstocks.

Significance of Study

This method may be useful to compare vine rootstock performances related to water availability.

Prioritizing the use of irrigation water; effects of crop load and late season irrigation on vine physiology, total yield and fruit composition

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Background and Aims

The amount of irrigation water required to grow quality grapevines depends upon site, the stage of vine growth, row spacing, size of the vine's canopy and amount of rainfall during the previous and present growing season (Williams, 2001). The effects of water stress on vine physiology, total yield and fruit and wine composition are largely dependant upon timing and severity. Early season water stress accounts mainly for vegetative growth control, reduction on berry size, and increase in fruit and wine quality if moderate (Shellie, 2006; Salon, 2005; McCharty, 1997). Moderate water stress around veraison increases fruit color (Ojeda, 2002), while late season water stress promotes yield losses due to berry dehydration (Sivilotti, 2005).

Methods and Results

An experiment was conducted during the 2007 season in a commercial Merlot vineyard grafted to 5C rootstock located in the Central Valley of California. All vines in the study were irrigated at the standard deficit irrigation level (0.7 ETc) until the fruit reached approximately 20 Brix. Irrigation was increased to 1.2 ETc only in one set of vines afterwards. Two crop load treatments were also imposed in each irrigation treatment, with vines either unthinned (control) or thinned to one cluster per shoot, at veraison.

Irrigation during the latter part of the season significantly decreased yield losses, especially in the unthinned vines. Vines receiving 1.2 ETc during the final stages of ripening had greater levels of chlorophyll, higher carbon assimilation rates and reduced mid-day leaf water potential compared to vines at 0.7 ETc. These differences were smaller in the unthinnned vines. Overall, irrigation had little impact on fruit composition, but high crop load had a significant and negative effect on fruit color and aroma precursors. Pruning weights showed no differences among the treatments, suggesting that late season irrigation and crop thinning at veraison did not impact canopy growth.

Conclusions

Vine water status during ripening is influenced by both irrigation and crop load. Yield losses due to berry dehydration are mitigated by irrigating during the latter part of the season.

Significance of Study

Grape growers suffering irrigation water shortage should reserve a quote of their allotment to be used during ripening.

Stomatal control of transpiration in irrigated and non-irrigated Semillon (*Vitis vinifera* L.)

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Background and Aims

Leaf burn is not uncommon in Semillon vineyards of NSW where soaring leaf-to-air vapour pressure deficits (VPDs) can occur during ripening. The dehydration originates at the margin of the leaf and can spread through entire leaf lobes after a heat event or during periods of low soil moisture. The aim of this work was to understand why this occurs more readily in Semillon compared to other varieties grown in similar conditions.

Methods and Results

Vine sap flow, leaf gas exchange plant water status and soil water balance were investigated for Semillon field vines grown under a range of irrigation strategies across two grape growing regions in NSW. Semillon water relations were also compared to other varieties in a replicated variety block and within controlled environment chambers. Leaf burn occurred after severe heat events in vineyards of both the MIA and the Hunter Valley. The leaf burn was more severe in those vineyards that experienced higher temperatures and on vines grown in soils with relatively low rootzone water availability. Predawn leaf water potentials (Ψ) indicated only partial recovery of vine water status in low soil moisture conditions and during warm, windy nights. Sap flow through field vines was responsive to leaf VPD but maximum rates were dependent on soil moisture. When compared to nine other varieties grown under similar soil moisture levels, leaf burn occurred only in Semillon and Ψ_{predawn} and Ψ_{midday} were most negative for this variety. A comparison to Grenache, a near-isohydric variety, in a deficit irrigation treatment indicated that throughout the drying down phase of an irrigation cycle leaf transpiration rates were relatively high for Semillon, as was stomatal conductance (g_s) . Higher transpiration rates, however, were not due to higher stomatal density. Shoot:root dry weight ratios were similar for the two varieties indicating similar canopy water demands for a given root biomass. Ψ_{midday} reached -1.2 MPa in Semillon but only to -0.8 MPa in Grenache at similar soil moisture levels. Ψ_{Root} correlated well to Ψ_{leaf} for both varieties. In Semillon, high g_s persisted despite midday increases in abscisic acid concentrations within the xylem sap.

Conclusions

Semillon is not an efficient user of water and vines lack tight control over stomatal aperture, exhibiting anisohydric stomatal behaviour. This leads to low vine water status and results in leaf burn when available soil moisture is limited and high VPD's prevail.

Significance of Study

The unpredictable rainfall patterns and warmer temperatures that are associated with climate change are already having a negative impact on Semillon growth and production in NSW. In the warm regions of Australia, it is recommended that Semillon is grown with a reliable source of water and with soil moisture monitoring.

Vine vigour influence on leaf gas exchange and recovery from water stress

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Background and Aims

In viticultural production systems there is often considerable variation in vine vigour. Here we report on a study tat was aimed monitoring effects of variation in vigour on vine physiological functions, in particular on leaf gas exchange and recovery from water stress.

Methods and Results

The monitoring study was carried out on a commercially run vineyard using 10 year old *Vitis vinifera* cv Shiraz. The study vineyard was subdivided into different zones based on aerially determined plant cell density metric. For four vigour classes, gas exchange (photosynthesis and stomatal conductance) and fluorescence measurements were carried out several times between flowering and harvest, often before and after application of low volume drip irrigation. The results clearly demonstrated that high vigour vines had markedly higher photosynthesis and stomatal conductance rates than low vigour vines during the entire monitoring period. This was also generally the case prior to- and post-irrigation application. High vigour vines also used a greater proportion of the absorbed light energy in photochemistry than the low vigour vines. There was also evidence of a higher non-photochemical quenching in low vigour vines than in high vigour vines.

Conclusion and Significance of Study

Variation in vigour has a corresponding effect on vine physiology. The observations that there are vigour-dependent gas exchange and recovery responses are relevant to decision making regarding irrigation management in a spatially variable vineyard.

Identification and Characterization of Water Stress-Induced Proteins and Transcripts in Muscadine and Florida Hybrid Bunch Grapes

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Background and Aims

Water deficit stress has major impact on yield and wine quality of grape. Water stress delays ripening, alters berry composition, and introduces undesirable flavors in wine. The aim of this research is to determine genetic variation in water use efficiency among muscadine and Florida hybrid bunch grape and to identify genotypes with low water requirement. We also hope to identify genes and proteins related to drought tolerance in muscadine and hybrid bunch grapes in order to develop grape genotypes with high water use efficiency suitable to Florida.

Methods and Results

Two-year old container grown grape plants (Muscadine cv. Carlos, and Florida Hybrid bunch cv. Suwannee) maintained in a greenhouse were subjected to water stress by withholding irrigation for 5, 10, 15 and 20 days. Leaf samples were collected from both irrigated and stressed plants. Proteins were extracted and separated by 2D-PAGE and further sequenced using LC/MS. Total RNA from leaf was extracted and cDNA transcripts were isolated using Differential Display RT PCR.

The results showed that short periods (<5 days) of water stress did not cause major changes in grape leaf protein composition. However, longer periods of stress (10 days) caused suppression of several low molecular weight (10 to 60 kDa) polypeptides in Florida hybrid bunch grape compared to muscadines. Most of the suppressed proteins are major photosynthetic enzymes such as Rubisco, ultra violet–B repressive rubisco activase, glyceraldehyde-3-phosphate dehydrogenase and phosphoribulokinase. Several cDNA transcripts are differentially expressed in response to water stress (5 to 15 day) indicating changes at initial stress levels. Of these, 24 transcripts were over expressed and 13 suppressed in muscadine; while 18 transcripts were over expressed and 22 suppressed in hybrid bunch.

Conclusions

Genetic variation in protein composition was observed in leaf tissue of Muscadine and Florida Hybrid Bunch grape genotypes. Several photosynthesis regulated enzymes are suppressed due to water stress. More number of transcripts was found to be up-regulated in Muscadine grape cultivar than the hybrids indicating their high tolerance to water stress. These transcripts exhibited high homology with drought responsive genes such as CBF like transcription factor, geramin like protein and dehydrin.

Significance of Study

The detailed proteomic and transcriptome studies would help explain specific pathways disconcerted with water deficit stress. These studies would also help determine the effect of water stress on muscadine and hybrid berry composition which has a major impact on their enological and nutraceutical traits.

Functional Genomics of Cold Hardiness in Vitis Amurensis Rupr. Grape

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Background and Aims

Vitis amurensis Rupr. is the most cold-hardy grape species. It has been used for development and improvement of both grape scion and rootstock cold tolerance. Vitis amurensis cv. 'Zuoshanyi', one of the most cold-hardy commercial grape varieties in China, was chosen for the functional genomics of cold hardiness study.

Methods and Results

One year old 'Zuoshanyi' vines in one gallon pots were treated at 4C or ABA for two days, while the controlled vines were maintained in a growth chamber at $28 \Box$ under a 16h light /8h dark photo period. mRNAs were isolated from the two-day cold-acclimated and ABA treated vines as well as the non treated control vines. A subtractive hybridization method was used to identify cold induced and regulated genes. Two forward subtracted cDNA libraries, from 4C and ABA treatments, respectively, were constructed. About 400 randomly picked clones from the subtractive libraries were sequenced, assembled and functionally annotated. Majority of these clones were homologous to genes released from Vitis vinifera genome sequences, while small proportion of these clones was found not matching any of the V. vinifera genome. Functional analysis revealed that some of these genes have been previously characterized as environmental stress responding genes while the others have unknown function. While comparing genes obtained from the two subtract libraries, some of the genes were found common in both.

Conclusions

A set of cold stress related genes were identified by cold and ABA treatment in V. amurensis "Zuoshanyi" grape.

Significance of Study

Identification of genes regulated by cold and ABA treatments would lead towards characterization genes associated with cold tolerance in grapevines.

Combining cover cropping with deficit irrigation strategies in a Mediterranean low vigour vineyard

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Background and Aims

The aim of this research was to test the effects of vineyard floor management practices combined with deficit irrigation strategies on weed dynamics, soil and plant water status, vine vegetative growth and its consequences on yield and berry composition of the variety Aragonez.

Methods and Results

In a split-plot factorial design two floor management practices (soil tillage in interrows - ST - and permanent resident vegetation - RV) were combined with three deficit irrigation - 50% ET_0 - strategies (RDI, PRD and control of PRD - DI). The annual broad-leaved species comprised the majority of the weed species surveyed in both floor management practices. After the mowing and soil cultivation carried out at the end of April, RV treatment showed a significantly higher total amount of above-ground dry matter than ST until the end of spring. Compared to ST the resident vegetative growth berry weight and yield. RDI showed a significant reduction on vine vegetative growth berry weight and yield when compared to the other two irrigation treatments. With the exception of the lower titratable acidity present in RDI, no other significant differences were observed in berry composition either for the two floor management practices or for the three irrigation strategies.

Conclusions

Compared to ST the resident vegetation was effective in reducing soil water content during the spring. By withholding irrigation during the first two weeks after full bloom period RDI, as compared to PRD and DI, induced the aimed reduction in berry weight. However, in this low vigour vineyard, those effects were not strong enough to improved berry composition.

Significance of Study

The effects of the control of vine vegetative growth and berry development promoted either by the RV or RDI on berry composition were not beneficial in this "terroir" and season. In dry areas and low vigour vineyards the combination of resident vegetation with deficit irrigation strategies should be looked with care as it can reduces yield without any benefits to grape quality.

Canopy temperature as an indicator of water stress in Shiraz grapevines

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Background and Aims

Canopy temperature (T_c) of well watered plants is lower than air temperature (T_a) because of the cooling effect of the transpiring leaves. Stomatal closure in response to soil water deficits can result in increased leaf temperature due to lower transpiration rates. As transpiration (ET_a) decreases, T_c increases to above that of the air. The aim of this work was to investigate the application of inexpensive, fixed-position infrared temperature sensors to monitor T_c for irrigation scheduling purposes.

Methods and Results

The relationship between grapevine ET_a and T_c - T_a was investigated during the 2006/2007 season in a Shiraz vineyard near Tatura, Victoria, Australia. ET_a and T_c were measured on five vines at half hourly intervals during January and February 2007. ET_a was measured by sap flow using the T_{max} method (Green et al. 2003). Infrared sensors (RAYMID10LTCB8, Raytek Corporation, Santa Cruz, California, USA) were used to measure T_c . Weather data, including T_a and reference crop evapotranspiration (ET_o) were measured using an on-site weather station. Vines were usually irrigated twice a week in the evenings or at night. On days immediately following irrigation events (I_{t+1}), ET_a increased rapidly from dawn until early morning, reached a peak mid-afternoon and then declined rapidly later in the day. T_c - T_a on these days was relatively low. The time since irrigation events, and hence soil water availability, affected sap flow. On days post irrigation ($I_{t>1}$), transpiration rates were considerably lower, relative to ET_o , and remained constant from early morning until late afternoon. T_c - T_a on days $I_{t>1}$ was high compared to T_c - T_a on days I_{t+1} . A negative correlation existed between daily ET_a , adjusted for ET_o , and T_c - T_a between 1200 and 1500 h.

Conclusions

Decreases in ET_a , associated with soil water deficits, were coupled with increases in T_c-T_a of grapevines. Further work is required to establish threshold levels of T_c-T_a for irrigation purposes.

Significance of Study

The observed responses could be used to detect the onset of water stress and aid irrigation scheduling decisions using relatively inexpensive equipment and wireless networks.

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Root-to-shoot communication in Cabernet Sauvignon during drought: the role of root-derived abscisic acid

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Background and Aims

Vineyard irrigation strategies are becoming increasingly important both as a method for optimizing grape quality and for minimizing water use. The plant hormone abscisic acid appears to play an important part in the plant's response to variations in available root water but the mechanism of the response is still not clearly understood. The aim of the work presented here was to examine the effects of different irrigation strategies on field grown Cabernet Sauvignon to determine in particular the synthesis, transport and catabolism of ABA in the roots, leaves and xylem of the plants and the effects on leaf water potential and stomatal conductance.

Methods and Results

A field trial was established on Cabernet Sauvignon grapes in a commercial vineyard in the Riverland and irrigation was applied throughout the growing season at the following rates: 6.6 ML/ha, 3.3 ML/ha and 1.3 ML/ha. Leaf water potentials, stomatal conductances and levels of xylem sap ABA, measured at weekly intervals, were found to be strongly influenced by the irrigation strategy both in terms of water applied and periodicity of application . Stomatal conductance was correlated with levels of ABA in the xylem sap and with leaf water potential. Levels of ABA in the xylem sap were related to the expression of the principal gene associated with ABA synthesis, *NCED1*, in the roots but not in the leaves. Expression of the genes associated with catabolism of ABA in the xylem sap. However, the expression of one of the catabolic genes, 8'-hydroxylase (*VvHyd-OH1*), was seen to increase in the leaves in the two drier irrigation treatments when there was a significant drop in ambient VPD suggesting a role for catabolism in the regulation of ABA in the leaf.

Conclusions

Cabernet Sauvignon grapevine adjusts to reduced levels of irrigation by regulating transpiration via stomatal conductance. The level of ABA in the xylem sap is correlated with changes in stomatal conductance and is, in turn, regulated by synthesis predominantly in the roots. Catabolism of ABA in the leaves in response to changes in microclimate in the canopy may play an important short-term role in the regulation of water use.

Significance of Study

This and similar studies on different grapevine cultivars and under different environments will help to define the parameters of most importance in planning irrigation strategies for optimal water use in irrigated viticulture.

Near infrared reflectance spectroscopy as a technique to measure vine water status

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Background and Aims

With increased demand on water resources there is a requirement for rapid and sensitive measurements of vine water status to enable more efficient irrigation. In the near infrared (NIR) absorbance spectrum there are several wavelengths that are strongly influenced by the presence of water in the measured sample, suggesting that NIR could be used as a method for the assessment of grapevine water status. In this study, a multivariate analysis of the NIR absorption spectrum and physiological measures of leaf and stem water potential in field and pot grown Shiraz and Cabernet Sauvignon grapevines is described.

Methods and Results

Field grown Cabernet Sauvignon and Shiraz vines were measured for stem and leaf water potential (ψ_S, ψ_L) and stomatal conductance. The NIR spectrum was obtained on the same leaves (upper surface) where ψ_L was subsequently measured. Volumetric soil water content was measured with a portable capacitance probe. Results highlighted that it was possible to determine clear trends in the spectra that would allow a rapid detection of the onset of water stress. NIR calibrations yielded good correlation coefficients between predicted and measured ψ_S and ψ_L . A variety effect was also evident since R² values were higher for Cabernet sauvignon (0.87) compared to Shiraz (0.67). The best calibrations were obtained for ψ_S rather than ψ_L . To examine this further, laboratory experiments were performed under conditions to induce rapid water stress. Measurements of ψ_L and the NIR spectrum were collected from attached leaves (adaxial side). Good calibrations were obtained for Shiraz leaves was used rather than the upper surface.

Conclusions

The results obtained in this study demonstrate the potential of NIR spectroscopy to provide a rapid and non-destructive method to assess grapevine water status. However the calibrations are variety dependent and also dependent on the surface of the leaf used to obtain the NIR spectrum.

Significance of Study

NIR may be used to measure relative changes in leaf water status within a variety. Quantitative measures will require further detailed calibrations taking into account variety and leaf surface.

Physiological response of red wine grape varieties to sustained deficit irrigation

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Background and Aims

Current irrigation practices do not always take into account the different responses that grapevine varieties display towards water deficit. Various water deficit studies on grapevine physiology have shown there are differences in water relations and photosynthetic responses between *Vitis vinifera* cultivars. Accordingly, grapevines have been broadly classified into two ecological categories based on their stomatal response to water deficit as being either isohydric (pessimistic-drought avoiding) or anisohydric (optimistic-drought tolerant). This study attempted to determine whether certain grapevine cultivars are better suited to an induced water stress than others, and how this may ultimately affect grape and wine composition.

Methods and Results

Field trials were established in north-west Victoria, Australia on *Vitis vinfera* cvs. Cabernet Sauvignon and Shiraz grafted to 140 Ruggeri (*V. berlandieri* x *V. rupestris*) rootstock. The vines were drip irrigated with full irrigation equivalent to 100% field capacity (control) or sustained deficit irrigation (approximately 44% volume of the control). Diurnal measures of stomatal conductance (g_s), leaf water potential (Ψ_1) and abscisic acid (ABA) were made from veraison to harvest. Leaf water potential and stomatal conductance levels tended to be significantly reduced in the field grown Cabernet Sauvignon and Shiraz when exposed to a sustained water deficit. Compared to Cabernet Sauvignon, the Shiraz tended to produce higher leaf water potentials in the afternoon as well as having higher stomatal conductance, which potentially indicates a difference in hydraulic conductivity between these varieties. While xylem sap [ABA] levels were significantly higher for those vines exposed to a soil water deficit, the pattern of xylem sap ABA production during the day for both varieties was different.

Conclusion

The differences in xylem sap [ABA] between Cabernet Sauvignon and Shiraz may be due to the balance between hydraulic and hormonal signals. Under field conditions, Cabernet Sauvignon appeared to display physiological responses typical of an isohydric-like vine, compared to the anisohydric-like responses of the Shiraz.

Significance of Study

An understanding of the differential responses of grapevine cultivars to water deficit could be used to enhance water use efficiency or preserving vineyard viability during periods of reduced water allocations or severe drought respectively.

Intra- and inter-seasonal effects of short-term water deficit on grapevine physiology

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Background and Aims

Irrigated agriculture accounts for approximately 90% of fresh water consumed globally. Furthermore, 83% of Australia's 174,000 ha of vineyards are irrigated. Deficit irrigation represents a mechanism whereby the water use efficiency of a crop may be improved, although in grapevines it is primarily used to control growth and provide a more reliable crop. However, even relatively brief periods of water stress can have effects on a perennial plant that last for a whole growing season or longer. This study has examined the effect of six seasons of deficit irrigation on leaf physiology, vine water use, carbohydrate reserves and yield.

Methods and Results

In 2002 a trial was set up in a commercial vineyard using 36 rows of Cabernet Sauvignon vines, originally planted in 1996. The trial used a randomised block design consisting of twelve replicates of three irrigation treatments; regulated deficit irrigation (RDI), prolonged deficit (PD - an extension of RDI with no irrigation until early veraison) and a well watered control, which received double irrigation in the years 2005-8. Measurements were made in the 2006-7 and 2007-8 seasons.

Intra-seasonal effects were at the leaf level. Prior to the PD treatment stomatal conductance and assimilation rates of RDI vines were only slightly below those of controls and there were no differences between RDI and PD leaves. During the PD treatment period conductance and assimilation were reduced in the PD vines, but only partially recovered after soil moisture was returned to match the RDI treatment. Differences between PD and RDI treatments continued until the onset of senescence. Inter-seasonal effects were at the whole vine level. Although bud and bunch counts were highest in the PD vines, leaf area index (LAI) was lower than in RDI vines. This was true even from early in a growing season i.e. prior to that season's PD treatment. LAI was highest in the control vines which continued to grow after maximum LAI had been reached in the other treatments.

Conclusions

A short period of water deficit had both inter- and intra-seasonal effects that lasted beyond the period of water deficit itself. The extent of these effects were seasonally dependent.

Significance of Study

This work has shown that the use of an extended deficit irrigation can affect vine physiology and carbon gain beyond the recovery of soil and vine water status and that multi-year effects can lead to reduced vine growth and productivity.

Sources of yield variability in a Pinot noir vineyard

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Background and Aims

The impact of vine size and location within the vine on phenological development and ripening dynamics were evaluated in a mature vineyard characterized by heterogeneous vine size and associated vigour.

Methods and Results

Guyot-trained Pinot noir vines were assigned into three vigour categories, small (S), medium (M) and large (L), averaging 20, 21.5, and 23 cm² trunk cross-section, respectively. Phenological development was followed in different locations within the vines. Small vines initiated growth four and two days earlier than L and M vines, respectively. Vine size did not affect flowering time. Clusters on distal shoots flowered earlier than those close to the vine trunk. Time elapsed from bud burst to bloom was 60, 62 and 63 days for L, M, and S vines, respectively. There was a big impact of cluster position on the shoot on flowering and fruit set. Basal clusters had more flowers, flowered two days earlier, and had a lower fruit set percentage than clusters on position two. When three clusters were on the shoot, the apical cluster flowered five days after the basal cluster. Although fruit set was improved in apical clusters, the resulting clusters were less compact. When comparing only clusters on basal positions, flowering was faster when the shoot had more than one cluster. Small vines reached véraison one day earlier than M and L vines. Clusters in basal positions on the shoot reached véraison one day and three days earlier than those on position two and three, respectively. Time elapsed between flowering and véraison was not affected by vine size. Clusters in positions that had delayed flowering, such as those on proximal shoots and in apical positions within the shoot required less time between flowering and véraison. Overall, L vines required 164 days between budburst and 23° Brix while M rand S required 166 and 168, respectively. Vine size did not have an impact on the rate of sugar accumulation but L vines took two days longer for acidity to decline to the same level than did M and S vines.

Conclusions

Differences in development rate observed earlier became smaller as the season progressed, however, differences between vine vigour categories still persisted by harvest time

Significance of Study

We found evidence that differences in vine vigour within a vineyard may be a significant source of variability in fruit composition in New Zealand.

Soil Water Repellency Mitigation - Effects on Irrigation Efficiency and Grape Productivity

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Background and Aims

Soil water repellency (SWR) reduces soil affinity to water thus affecting diverse hydrological processes that influence irrigation efficiency and crop productivity. While SWR is recognized in Australian soils, little information exists on effects to productivity and irrigation efficiency in high value horticultural crops. With ongoing drought affecting water quantity in the Murray-Darling basin and the expansion of production of high value orchard and vine crops, the sustainability of production requires development of novel strategies to optimize the efficient delivery and use of water. The objective of this study was to utilize surfactant treatments as a means of mitigating SWR effects in grapes (Vitis vinifera L.) so as to elucidate differences in soil water and crop yield.

Materials and Results

Three replicated trials were conducted in Victoria, AU on table grapes ('Black Muscat') or wine grapes ('Shiraz') growing under drip irrigation in clay loam or loam soils. A blend of alkylpolyglycoside (APG) and ethylene oxide/propylene oxide (EO/PO) block copolymer surfactants (Aquatrols Corporation, Paulsboro, NJ, USA) was applied at initial rates of 0 or 5 L ha⁻¹ in the spring, then at 0 or 2.5 L ha⁻¹ monthly for up to four months to mitigate SWR. Soil volumetric water content (VWC) was monitored at 10 cm or 25 cm using a Theta probe (Delta-T Devices, Cambridge, UK). At harvest, fruit weights were measured and used for crop yield estimations. Over the course of the growing season, VWC was consistently lower (p = 0.05) in untreated soils than in the surfactant treatments regardless of soil type. Due to thinning bunch numbers were similar in both treatments. Bunch weights were significantly lower and heat stress damage higher in the untreated controls (p = 0.05). SWR induced yield differences of 2.4–2.5 Mg ha⁻¹ (13%) were observed between the two treatments (p = 0.05).

Conclusions

This study demonstrates that SWR depressed grape productivity by 13%. A SWR mitigation strategy improved soil hydrological status resulting in net difference in financial return of $2053 - 4922 \text{ ha}^{-1}$.

Significance of Study

SWR deleteriously reduced effective rootzone delivery of applied water in clay loam or loam soils and suppressed grape productivity in Victoria.

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Performance of the Cultivar Merlot under different training systems

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Background and Aims

Merlot is considered a first preference for new plantations in the Veneto region, located in North Eastern Italy. Information on trellis system performance is critical to achieve optimal yield, and grape and wine quality in new plantations. The aim of this work was to evaluate the effect of different training systems on the performance of Merlot.

Methods and Results

Guyot, spur pruned cordon and free cordon were compared on the basis of bud fertility, vegetative growth, water status, berry composition, yield and wine quality during two seasons (2006 and 2007). Bud fertility was not influenced by the training system; however, there was a season effect. The total leaf surface (TLS) per vine was strongly influenced by the trellis system. The vines TLS, on spur pruned cordon at veraison, was 45 % higher than that of Guyot. Measurements of the shoots dry matter partitioning confirmed a larger accumulation in the leaf for the spur pruned cordon. On the contrary, free cordon allocated a higher percentage of dry matter in clusters. Stem water potential (ψ S) was strongly related to TLS, Guyot presented the higher ψ S during both seasons. An effect of the shoot position on the vines water status was also noticed, free cordon had the lower ψ S compared to the other trellis systems with upwardly oriented shoots. Yield was strongly affected by the trellising; vines on Guyot presented 40% lower yield compared to the spur pruned cordon vines, even though pruning weight was similar, thus resulting in a 2 year mean crop load of only 3.4. Soluble solids content was higher for vines on Guyot. Despite differences in yield, titratable acidity was not affected. The anthocyanins concentration in the skin of berries was higher in the vines on Guyot; therefore, the wines made from these berries were characterized by the highest colour. Wines from Guyot had also the highest fruity and floral flavour.

Conclusions

Results indicated that high wine quality in the plane area of the Veneto region may be obtained using the Guyot training system. The free cordon despite large crop size and high crop loads, may be adopted considering its high adaptability to mechanization.

Significance of Study

The study has shown that different training systems can give very different outcomes of berry quality and yield in Merlot, and that an optimum exists for a particular climate and region.

Effects of training, pruning severity and limited irrigation before verasion on growth, yield and quality of kalecik karasi clones grown in Central North of Anatolia

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Background and Aims

Kalecik Karası is the most popular local red wine variety of Turkey and its name comes from a small town "Kalecik" located to 70 km east of Ankara. This experiment was planned to determine the effects of training and pruning level on growth, yield and quality of three leading clones (9,12,16) of Kalecik Karası grown under non and limited irrigated conditions of central north of Anatolia between 2004 and 2007.

Methods and Results

Experimental vineyard were established in 1999 with 1,5 m x 2,5 m planting density on 1103 P (Clone 113) and grapevines were trained as bilateral cordon and Guyot, and pruned at three levels in non (12,15,18 buds/vine) and limited irrigated (15, 18, 21 buds / vine) at berry set, pea size and vèrasion.

Limited irrigation till verasion had no marked effect on crop quality, however berry size and yield were increased significantly. Although both training systems were found to be convenient for all three clones, Guyot had some slight favors to cordon.

Despite, minimum loading (12 buds / vine) in non irrigated vines decreased yield markedly, crop quality was improved. Whereas, maximum loading (21 buds / vine) for limited irrigation resulted in opposite effect.

Conclusions

As a conclusion, medium loading (15 buds for nonirrigation, 18 buds for limited irrigation till verasion) for both training systems can be recommended for all three clones of Kalecik Karası in semi-arid conditions of central north of Anatolia.

Significance of Study

This is a unique study to find out how the agronomic performances of three leading clones of Kalecik Karası were influenced by the interactions of the training systems and pruning levels for non and limited irrigated growing conditions in semi-arid climate of central north of Anatolia.

Influence of climate on the susceptibility of grape berries to bunch rot fungi

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Background and Aims

Bunch rot of grapes is typically caused by the fungus *Botrytis cinerea* (grey mould) however a range of other micro-organisms have been implicated in the rotting of grape berries. The aim of this work was to investigate the effect of climate on bunch rot organism predominance.

Methods and Results

Mature detached berries of *Vitis vinifera* (Cabernet Sauvignon) (22.4°Brix) were inoculated with two bunch rot pathogens; *B. cinerea* or *Greeneria uvicola* (bitter rot) either singularly or in combination at one of two temperatures, either 20°C or 27°C. In co-inoculation studies *B. cinerea* out-competed *G. uvicola* at 20°C. Conversely *G. uvicola* out-competed *B. cinerea* at 27°C. In field trials conducted in the Hunter Valley in 2005 and 2006 non-Botrytis bunch rot incidence and severity was found to be greater in westerly facing canopies suggesting that the likely higher temperatures on the western-side of the row predisposed fruit to bunch rots such as *G. uvicola*. Bunch rot incidence and severity was further investigated over two growing seasons, 2006/7 and 2007/8 and data examined with respect to climatic records for the November to February growing period. The 2006/7 season was hotter (62 days >30°C) and drier (176 mm rain) than the long term average. In this season *B. cinerea* was absent from the vineyards examined. The predominant bunch rotting organisms recorded were *G. uvicola* and *Botryosphaeria* spp. This contrasted with the 2007/8 season which was cooler (19 days >30°C) and wetter (579.2 mm rain) than the long term average. In this season *B. cinerea* was recorded at the six sites examined and was the predominant bunch rot organism.

Conclusions

Bunch rot incidence and type is influenced by climatic conditions. In warm and dry years bunch rots such as bitter rot are likely to predominate in the Hunter Valley while cooler and wetter years are likely to lead to a higher occurrence of grey mould.

Significance of Study

Our findings support the hypothesis that heat stress can pre-dispose grape berries to infection by non-Botrytis bunch rots such as *G. uvicola*. It is hoped this work will lead to improved management practices and a greater understanding of bunch rot epidemiology and will form the basis of disease forecasting models in response to climate change.

Identification of Genes Associated with Anthracnose Resistance in Florida Hybrid Bunch Grapes

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Background and Aims

Anthracnose is a major foliar disease of Florida hybrid bunch grape caused by the fungus, *Elsinoe ampelina*. The scope of this study is to isolate differentially expressed genes upon *Elsinoe* infection in anthracnose-tolerant grape genotypes. Spraying with fungicides although provides some disease control, but is expensive and not environmental friendly.

Methods and Results

Differentially expressed genes were isolated using Differential Display RT-PCR and Subtractive Hybridization techniques upon *Elsinoe* inoculation of grape leaves. DDRT-PCR results revealed significant up-regulation of several cDNA transcripts in tolerant genotype compared to susceptible genotype studied. Subtractive hybridization also yielded several partial cDNAs uniquely expressed in tolerant genotypes. These uniquely expressed transcripts were isolated, sequenced and identified as chalcone synthase, stilbene synthase, PR proteins, chitinase, protein/sugar kinase and transcription factor. Expressions of these genes were confirmed through real time PCR. Induction of these novel genes upon *Elsinoe* infection in tolerant genotypes indicates their adaptation mechanism for pathogen infestation.

Conclusion

This result clearly suggests that anthracnose-tolerant genotypes were able to express a series of genes upon *Elsinoe* infection to suppress pathogen growth, where as anthracnose-susceptible cultivar failed to do so. These genes appear to play a role in inducing anthracnose tolerance in Florida hybrid bunch grape genotypes.

Significance of Study

Identification and characterization of gene/s responsible for anthracnose tolerance would help develop anthracnose-tolerant grape cultivars to increase grape production and farmer's profit.

Induced systemic resistance in grapevine against *Botrytis cinerea* by *Pseudomonas* spp. rhizobacteria

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Background and Aims

Gray mould (Botrytis cinerea) is among the most serious fungal constraints in grapevine. This disease is generally controlled with chemical fungicides. However, because of the environmental impacts due to pesticide use considerable interest has been devoted to alternative strategies including activation of plant resistance by non-pathogenic microorganisms. The rhizobacteria-induced systemic resistance (ISR) is based on multiple mechanisms, including enhancement of the capacity of plant to mobilize defense responses. In this study, we assessed different Pseudomonas spp, including those originating from vineyard, for their capacity to elicit defense responses and systemic resistance to B. cinerea in grapevine. We also investigated the bacterial factors operative in triggering ISR in grapevine.

Methods and Results

ISR was assayed on in vitro plantlets by inoculating roots with live or boiled grapevine-associated Pseudomonas fluorescens PTA-CT2 and PTA-268 before challenge with Botrytis cinerea. The capacity of P. fluorescens CHA0 and P. aeruginosa 7NSK2 to induce ISR in grapevine was compared with that of different mutants on siderophores and 2,4-diacetylphloroglucinol (DAPG). We show that root inoculation of plantlets with PTA-CT2 and PTA-268 resulted in a significant disease control. Both strains triggered oxidative burst and phytoalexins (i.e. resveratrol and viniferin) in grape cells and leaves. Treatment with boiled bacteria strongly enhanced these responses and resistance to B. cinerea. Both facts support the production and release of signaling molecules from bacteria serving as inducers of systemic resistance. Results also show that mutants P. fluorescens WCS417 (pyochelin-negative), P. fluorescens Q2-87 (DAPG -positive) and P. putida WCS358 (SA-negative) induced resistance to an extent similar to that induced by the wild type P. fluorescens CHA0. Similarly, both 7NSK2 and mutant KMPCH (pyochelin-negative, SA-positive) induced defense responses and resistance to B. cinerea. However, the mutant KMPCH-567 (pyoverdin-negative, pyochelin-negative, SA-positive) did not triggered ISR against B. cinerea, but induced similar defense responses.

Conclusions

Grapevine-associated Pseudomonas spp. induced ISR towards B. cinerea. These bacteria also triggered oxidative burst and phytoalexin production. Responses to boiled bacteria indicate that ISR could occur without the need of metabolically active bacteria. The use of mutants suggests that a synergistic interaction of pyoverdin and pyochelin could be effective in triggering ISR against B. cinerea.

Significance of Study

The study demonstrates that grapevine resistance could be induced by rhizobacteria or signaling molecules released from these bacteria. The use of both bacteria and molecules may contribute to sustainable viticulture.

Powdery mildew and salicylic acid-induced gene regulation in a susceptible grapevine

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Background and Aims

Obligate biotrophic pathogens, such as powdery mildews (PMs), are thought to trigger a defense response via the salicylic acid (SA)-mediated signal transduction pathway. The grape PM fungus, *Erysiphe necator*, was previously shown to trigger an increase in SA levels in *V. vinifera* (Fung et al. 2008). We compared gene expression changes induced by *E. necator* infection or by external application of methyl-salicylate (MeSA) in *V. vinifera*.

Methods and Results

Gene expression levels were measured using the Affymetrix Vitis GeneChip in leaves of the PMsusceptible grapevine Cabernet Sauvignon. GeneChip assays were performed in plants infected by *E. necator*, treated with MeSA, or in healthy control plants. Internal SA levels in leaf tissues were quantified using HPLC. Many, but not all, of *E. necator*-responsive genes were stimulated in a similar manner by MeSA and PM. Among the genes that responded differently to the two treatments were the stilbene synthases and a NAC-type transcription factor. These were upregulated by *E. necator*, but remained weakly or non-induced by SA. Genes that were suppressed by MeSA or PM, but remained non-affected by the alternative treatment, also were identified. Expression patterns of key genes were validated by qRT-PCR.

Conclusions

Activation of the SA-mediated central defense pathway is either not required, or required but insufficient, for the regulation of certain *E. necator*-responsive genes. This suggests that the *E. necator*-triggered defense reaction is complex, and likely involves multiple signaling pathways in grapevine.

Significance of Study

These results provided new insights into the PM-induced defense response in *V. vinifera*. The promoters of the differentially regulated grapevine genes will serve as tools to dissect the *E. necator*-triggered signaling network.

Reference

Fung, R.W.M., Gonzalo, M., Schachtman, D.P., McIntyre, L., Fekete, C., Kovacs, L.G. and Qiu W. (2008) Powdery Mildew Induces Defense-Oriented Reprogramming of the Transcriptome in a Susceptible But Not in a Resistant Grapevine. Plant Physiology 146. 236-249.

Viral infections induce transcriptional changes that affect ripening in grapevine berries

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Background and Aims

Viral infections in grapevine cause deformations in leaves, alterations in color and irregular ripening. These infections are compatible interactions, in which pathogens spread through all plant tissues without inducing a resistance response. However, susceptible hosts are not passive against viruses and they can set up a defense response that is not enough to stop viral replication and dissemination. Molecular, cellular and physiological changes induced by viruses affects vegetative growth, decline photosynthesis and induce senescence. With the aim to investigate the effect of viral infections during berry grape ripening, transcript profile of the red wine cultivar Cabernet Sauvignon naturally infected with GLRaV-3 was compared with virus-free grapevine plants.

Methods and Results

Total RNA was extracted from berries of healthy and GLRaV-3 infected plants at two developmental stages: veraison and maturation. These RNA samples were evaluated using *Vitis vinifera* GeneChip® from Affymetrix. The most relevant changes in gene expression occurred at maturation stage with 514 genes induced and 393 genes repressed in viral-infected grapevines berries. These genes were associated with several biological functions including processes of biosynthesis of primary and secondary metabolites, developmental processes, senescence and cell defense. Considering cellular localization, an important group of affected genes is related to membrane systems and also with the chloroplast. The expression of sugar transport and anthocyanin biosynthesis genes was further evaluated using real time RT-PCR during ripening in healthy and infected berries.

Conclusions

The physiological changes observed in virus-infected grapevine berries like delayed ripening, low sugar content, and the reduced color observed in red cultivars may be associated to important changes in gene expression induced by viral infections in grapes. Since most repressed genes are involved in anthocyanin biosynthesis, the possible effect of virus in this process is discussed and also the effect of virus infection on the expression of transcription factors genes.

Significance of Study

This is the first study of gene expression in virus-infected grapevine berries. It contributes to understand the viruses-triggered changes on grape physiology and berry maturation.

Transcriptome analysis of P. viticola infected grapevines

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Background and Aims

Plasmopara viticola is an obligate oomycete plant pathogen and the agent downy mildew of grape. Interaction with the plant is supposed to involve and exchange of molecules and signal, which is almost completely unknown to-date, both in susceptible and in resistant genotypes.

Methods and results

Plants of *Vitis vinifera* cv. Pinot Noir (susceptible) and *Vitis riparia* cv. Gloire de Montpellier (resistant) were grown *in vitro*. Leaves were infected with *P. viticola* or treated with distilled water as a control, and collected at 12 and 24 hours post-inoculation. A comprehensive microarray analysis of transcriptional changes has been undertaken on a Combimatrix *Vitis* gene chip, carrying 24562 specific probes in triplicates. Differentially expressed genes were selected using by a SAM analysis, and clustered by Genesis software. Results showed that Gloire de Montepellier strongly responds to *P. viticola* infection as early as 12 hpi, while a massive repression of gene expression is observed in Pinot at 24hpi. Different classes of genes modulated by infection in one or the other genotype are presented, with special emphasis on components of signal transduction cascades.

Conclusions

Results suggest that response to infection can be activated earlier in the resistant genotype and that susceptibility is associated to an early downregulation of gene expression.

Significance of study

The presented data provide hypotheses about the physiological events underlying compatibility in susceptible *V. vinifera*, as well as about the signals and pathways involved in resistance in *V. riparia*, in the first steps of the infection process.

Characterisation of resistance strategies to powdery mildew infection within the Vitaceae (grape) family

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Background and Aims

The biotrophic fungal pathogen, powdery mildew, penetrates plant epidermal cells to obtain nutrients in order to complete its life cycle. Plants have evolved various mechanisms to restrict infection. One of the first lines of defence is penetration resistance, which involves the polarised secretion of cell wall materials and defence compounds. However, some fungal pathogens have adapted to overcome this. In response, certain plant hosts have developed pathogen resistance genes (R-genes) that recognise pathogen attack and induce a hypersensitive response (HR) to limit pathogen spread.

The European cultivated grapevine, *Vitis vinifera*, is a host for the powdery mildew pathogen *Erisyphe necator*. Several American *Vitis* species have been reported with resistance to *E. necator* but little is known about the basis of this resistance. Therefore we investigated and characterised sources of resistance to *E. necator*, in members of the *Vitaceae* family.

Methods and Results

Members of the *Vitaceae* family including the genera *Vitis*, *Muscadinia*, *Cissus*, *Ampelopsis* and *Parthenocissus* were infected with both the adapted host powdery mildew, *E. necator* and the non-adapted mildew *Erisyphe chicoracearum*, levels of penetration resistance and HR induction were subsequently scored. *V. vinifera* lacks an R-gene to recognise *E. necator* and therefore induces no HR. However several other members of the *Vitaceae* family including species from *Muscadinia* and *Cissus* were found to induce HR-like responses to *E. necator*. Penetration resistance to *E. necator* and *E. chicoracearum* was found to vary in members of the *Vitaceae*. Interestingly, the wine grape *V. vinifera* showed the lowest penetration resistance to both pathogens while *Parthenocissus* members showed the highest. Studies using cell machinery inhibitors suggest that this penetration resistance is not dependent on polarised secretion.

Conclusion

Our results indicate that the different genera of the *Vitaceae* contain genetic sources of resistance to powdery mildew, including both penetration resistance and R-gene mediated resistance.

Significance of Study

Powdery mildew infection of cultivated grapevine by *E. necator* is the most economically important fungal disease of viticulture worldwide. Therefore studies to elucidate resistance mechanisms to this pathogen may lead to the control of this disease.

Identification of grapevine *MLO* gene candidates involved in susceptibility to powdery mildew

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Background and Aims

All winegrape (*V.* vinifera) varieties are highly susceptible to the powdery mildew pathogen *Erisyphe necator* and controlling fungal outbreaks is currently achieved by spraying fungicides at critical times during grapevine development. Specific members of the *MLO* gene family have been shown to mediate powdery mildew susceptibility in *Arabidopsis*, tomato and barley. In grapevine, seventeen *VvMLO* genes have been identified falling into 6 distinct phylogenetic clades. The aim of this study was to identify *VvMLO* genes involved in mediating susceptibility to *E. necator*.

Methods and Results

The expression of MLOs representing each clade were analysed by real time PCR in a range of grapevine tissues and in response to abiotic and biotic factors. The *VvMLO* genes were found to be expressed in all of the grape tissue types examined, but varied widely in the level of expression. None of the *VvMLO* genes examined appeared to be tissue-specific and there were no clade-specific expression patterns. A significant increase (12-40 fold) in the transcript levels of *VvMLO17*, *VvMLO3* and *VvMLO4* was observed in grape leaves within 8 hours of *E. necator* inoculation. *VvMLO9* was also induced following inoculation, but the timing was delayed relative to the other induced *VvMLO* genes. None of the other 8 *VvMLO* genes examined were significantly induced in response to powdery mildew inoculation. Protein sequence alignment reveals that *VvMLO17*, *VvMLO3* and *VvMLO4* are most closely related to the *Arabidopsis* and tomato MLO genes required for powdery mildew susceptibility.

Conclusion

Our research has identified four possible candidates linked to powdery mildew susceptibility. The next step is to test whether grapevines with reduced expression of one or more of these *VvMlo* gene candidates show reduced susceptibility to powdery mildew.

Significance of Study

The existence of fungicide-resistant strains and mounting pressure to reduce the use of agrochemicals has increased the interest in the development of new grapevine cultivars with enhanced genetic resistance to powdery mildew. This research has lead to the identification of a number of *VvMLO* genes are promising candidates for manipulation to generate powdery mildew resistant germplasm.

Osmotic stress-induced change in polyamine metabolism can modulate grapevine defense responses and resistance to Botrytis cinerea

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Background and Aims

Polyamines have been shown to play several roles in plant adaptation to abiotic stresses. They also act as mediators in plant defense signaling against pathogens. Nevertheless, the functional significance of polyamines in abiotic stress and in priming defense responses in grapevine remains to be elucidated. In the present work, we investigate if the osmotic stress-induced change in polyamine metabolism can modulate grapevine defense responses and resistance to Botrytis cinerea.

Methods and Results

Grapevine leaf discs (GLD) were excised from mature leaves on rooted plants and incubated on a reference buffer alone, or in the presence of metabolic inhibitors of polyamines before they submitted to osmotic stress induced by PEG-6000 or mannitol (-1.5 MPa). We show that osmotic stress enhances free polyamine amounts in GLD. The effects of α -DL-difluoromethylarginine (DFMA) and α -DL-difluoromethylornithine (DFMO), specific irreversible inhibitors of arginine decarboxylase (ADC) and ornithine decarboxylase (ODC), and of aminoguanidine (AG), an inhibitor of diamine-oxidases (DAO), indicate that osmotic stress did not block polyamine biosynthesis through ODC, but induced a stimulation of ADC and polyamine oxidation pathways. We further examined the effect of osmotic stress and metabolic inhibitors on phytoalexin amount, photosynthetic efficiency (Q_{PSII}) and disease resistance of GLD. Osmotic stress resulted in large amounts of phytolaexins (resveratrol and ϵ -viniferin), but in a inhibition of Q_{PSII} capacity and a strong susceptibility of GLD to *B. cinerea* infection.

When the osmotically-stressed discs were pre-treated with DFMA or DFMO, the osmo-induced phytoalexin accumulation and Q_{PSII} capacity were repressed. However, GLD showed similar and severe disease symptoms to those of stressed tissues. The DAO inhibitor (AG) resulted in a strong reduction of ε -viniferin production and Φ_{PSII} activity, and enhancement of necrotic lesions caused by *B. cinerea*.

Conclusions

We propose that accumulation and further oxidation of free polyamines under osmotic stress can affect defense reactions and cell homeostasis in grapevine, but only polyamine oxidation seems required for basal resistance against B. cinerea.

Significance of Study

Abiotic stress can modify defense responses and basal resistance of grapevine to fungal pathogens. Change in polyamine content could interfere with defense signaling pathways and may lead directly or through polyamine oxidation products to significant changes in basal host resistance to pathogen.

The characterisation of virus-based vectors for functional genomic studies in grapevine

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Background and Aims

Recently, the complete grapevine genome has been sequenced and this leads to an increasing demand for functional genomic studies in grapevine. *Grapevine virus A* (GVA, genus *Vitivirus*, family *Flexiviridae*) naturally infects grapevine and the herbaceous host *Nicotiana benthamiana*. This study aims to characterise GVA-based vectors as functional genomic tools in grapevine.

Methods and Results

Two infectious T7-promoter driven cDNA full-length clones of GVA (T7-GVA118 and T7-GR5, provided by M. Mawassi, Israel; Haviv et al. 2006) were brought under control of a CaMV 35S-promoter (35S-GVA118, 35S-GR5). This allows the infection of host plants by the method of agroinoculation. Systemic GVA symptoms were detectable 4 days post agroinoculation. Sequences of the tobacco phytoene desaturase gene (PDS) and the green fluorescent protein (GFP) were integrated into 35S-GVA118 and after agroinfiltration, led to virus-induced gene silencing (VIGS) phenotypes in *N. benthamiana* or GFP-transgenic *N. benthamiana* (line 16c; Brigneti et al. 1998), respectively. To test the GVA VIGS-system in grapevine *in vitro* plantlets of the cultivar Sultana were vacuum-agroinfiltrated with 35S-GVA118 carrying a grapevine PDS. Systemic defined photobleaching symptoms associated to leaf veins were observed on some grapevine leaves suggesting that PDS is silenced. Furthermore, ORF 2 and ORF 5 of 35S-GR5 were replaced by GFP and GUS sequences, respectively. The expression of marker genes in *N. benthamiana* and grapevine were assessed.

Conclusions

It was shown that agroinfiltration of 35S-GVA constructs led to virus infection in *N. benthamiana* and grapevine. In contrast to *N. benthamiana*, GVA is restricted to the phloem tissue in grapevine. This could explain why the observed PDS VIGS phenotype was confined to major phloem tissues like leaf veins in grapevine. In future experiments and by insertion of additional grapevine genes to be silenced it has to be elucidated if GVA-based VIGS vectors are efficient for broad range screenings of genes of interest.

Significance of Study

In this study, two T7 promoter driven GVA infectious cDNA clones (Haviv et al. 2006) were brought under control of a CaMV 35S-promoter. We report the successful 35S-GVA118 agroinfiltration of grapevine which led to PDS silencing phenotypes in grapevine and N. *benthamiana*.

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Shoot growth dynamics and the net carbon balance of *Vitis vinifera* cv. Semillon grapevines

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Background and Aims

Assessing the impacts of environmental stresses and climate change on grapevine growth and productivity require an understanding of growth processes and the carbon economy that underpins this growth. The aim of this work was to use quantitative modeling to describe shoot growth dynamics and determine the carbon balance of fruiting vines.

Methods and Results

Potted Semillon grapevines were grown in a controlled environment and budbreak, leaf appearance and expansion, stem and bunch growth and photosynthesis were measured at intervals. Dry matter allocation was assessed by destructive harvests and with photosynthesis, used to determine the shoot carbon economy. Quantitative modelling was conducted using the Boltzmann sigmoid function enabling mathematically based, physiological descriptions of shoot growth patterns to be derived.

Conclusions

Leaf appearance and time of leaf expansion occurred in three distinct zones along the shoot with differentiation in expansion rates, leaf area, internode length and photosynthetic capacity. A negative carbon balance occurred early after budbreak but the vines maintained a positive carbon balance throughout bunch growth. Differentiation in shoot growth dynamics appears to be a physiological adaptation in Semillon vines to establish a canopy rapidly and achieve a positive carbon balance early in spring.

Significance of Study

Shoot growth dynamics conform to the architectural patterns of Semillon vines in relation to leaf number and shoot length. However, the zonal pattern of leaf appearance is not consistent with the published structure of dormant buds of grapevines in numbers of preformed leaf primordia and warrants further investigation.

Rootstock effects on carbohydrate and root growth dynamics

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Background and Aims

Rootstocks are known to have an influence on the vegetative growth and yield of the scion, and also can influence fruit quality in grafted vines with direct effects on the uptake of ions, or more indirectly by affecting the balance between fruit and shoot growth. Recently, seasonal root growth dynamics has been shown to vary from season to season and linked to vine carbohydrate supply (Comas *et al.*, 2005). The aim of the study was to further understand rootstock effects on resource allocation and seasonal root growth dynamics.

Method and Results

A study on pot-grown Shiraz on own-roots and six rootstocks (Ramsey, 140 Ruggeri, Schwarzmann, 5BB Kober, 101-14, 420A) was undertaken to study the effect of rootstock and water stress on whole vine growth. Rootstocks altered biomass partitioning by varying the allocation between roots, shoots, and the fruit. Rootstocks that carried high crop loads showed reduced carbohydrate reserve accumulation. In addition, reduced irrigation favoured the biomass allocation to the shoots away from the roots, resulting in lower starch concentrations and amounts in these roots. This suggests a low priority for root growth and carbohydrate reserve allocation compared to the fruit under limited water supply.

A field-grown study was then undertaken on rootstocks that showed the biggest biomass differences from the pot trial (Own-roots, Ramsey, 140 Ruggeri, Schwarzmann). Minirhizotron techniques were used to study the root development of each rootstock over the season. Preliminary results from the first season of measurements showed a main peak of fine root growth around flowering for all rootstocks. However, a significant rootstock effect on the post-harvest fine root growth was observed. Differences in crop load and the associated rate of carbohydrate root reserve replenishment during fruit ripening was linked to the differences in post-harvest root growth.

Conclusions

Rootstocks can have implications on biomass partitioning, by varying the allocation between roots, shoots and the fruit. In addition, the carbohydrate supply and competing carbon sinks is an issue for root dynamics, and consequentially water and nutrient uptake.

Significance of Study

The impacts on vine performance of the different rootstock varieties on the scion can become important selection criteria when deciding which rootstock is best for certain situations. The understanding of root dynamics will assist in optimising vine water and nutrient supply for enhancing grape production.

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Periodicity of root growth of grapevines in a subtropical environment - Grapevines in a subtropical environment have a single major root growth flush each year.

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Background and Aims

The growing seasons of tablegrape vines in subtropical areas of Queensland is characterised by a relatively short period from budburst to harvest and long postharvest period (5-6 months). Knowledge of the timing of new root growth flushes is important for effective management of vine nutrition because of the prominent role of white roots in nutrient uptake, but has not been described for grapevines grown in a subtropical environment.

Methods and Results

The annual cycle of root growth was studied over $2\frac{1}{2}$ years using rhizotrons installed alongside six vines at Mundubbera $151^{\circ}18^{\circ}E$, $25^{\circ}35^{\circ}S$). The total length of white root visible on the rhizotron window (575 mm x 575 mm) was recorded at fortnightly or monthly intervals.

A postharvest flush of root growth commencing soon after fruit harvest was the only substantial root flush observed throughout the year and continued for one to three months. A spring root growth flush did not occur between budburst and flowering. The minimum total length of white root coincided with flowering. A small flush of new root growth was observed around veraison but was not consistent for all rhizotrons or across all years.

Conclusion

This pattern of root growth distinguishes vines grown in the subtropics from those in temperate environments, where two distinct periods of new root growth occur, one in spring and one after harvest.

Significance of Study

It is highly probable the suberised and woody roots are responsible for water uptake at certain times during the season, and especially from budburst to harvest when there is little white root on the vine but massive growth of the aerial parts of the vine. The lack of substantial new root growth in spring is most probably associated with the strong demand for water and nutrients by the actively growing annual parts of the vine, and subsequent lack of surplus photosynthates to sustain root growth. The postharvest root flush follows the removal of a major sink on the vine at fruit harvest. Most of the roots grown after harvest were retained through winter into the flowing growing season. The postharvest period is therefore an important time for replenishing and expanding the root system of the vine.
Biomass allocation control within rootstock-scion combinations in *Vitis*

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Background and Aims

Despite the fact that approximatively 80% of the vines worldwide are grafted, the mechanisms of rootstock-scion interactions remain unexplained. The aim of this work was to analyse the development capacities of the root system for various rootstock varieties. Considering the role of aquaporins in root development, we also investigated the expression of aquaporin genes in these varieties.

Methods and Results

A double graft system was developed based on a single scion variety supported by two different root systems. Different *Vitis* varieties were used either as scion or rootstocks. Some plants were submitted to various treatments limiting leaf area. After one growing season, dry matter partitioning between organs was determined and the expression of ten aquaporin genes was investigated in roots according the methodology described by Fouquet *et al.* (2008).

110 Richter root system displayed significantly higher biomass accumulation than Riparia Gloire (RGM). Limiting leaf area by leaf removal or topping did not affect clearly dry matter partitioning between these two genotypes when grafted with a single scion. The association of leaf removal and topping seemed to decrease further dry matter allocation to RGM root system. Dry matter partitioning to the root system was strongly affected by scion genotypes. When RGM was used as scion, 110R and RGM as rootstocks, biomass accumulation in RGM root was boosted and became significantly higher than the 110R one. The same phenomena occurred with 101-14Mgt despite the fact that 110R and 101-14Mgt root systems had similar size when associated to CS or 110R. The expression of the aquaporin genes was very different in roots compared to berries. One PIP and one TIP gene were highly expressed in roots of all the studied genotypes, but not in berries. 110R was also characterized by a high expression of two additional aquaporin genes.

Conclusions

Even if root development appears to be genotype-dependent in grapevine, scion variety, rather than carbon supply, seems to affect deeply this trait in grafted plants. Although this study did not aim to establish a causal link between the development of the root system and aquaporins, the hybrid 110R was characterized by active accumulation of dry matter in roots and a strong expression of specific aquaporin genes.

Significance of Study

This work provides information about scion-rootstock interactions in grapevine. Associated to its influence on root development, the effects of scion variety on gene expression in roots now need to be further investigated.

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Phenological and thermal demand (degree-days) characterization of four varieties of white grapevine cultivated in Sao Francisco River Valley, Brazil

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Background and Aims

The aim of this research was to characterize the phenological behavior and thermal demand (degrees-day) of four varieties of white grapevine cultivated in Sao Francisco River Valley, Northeast Brazil.

Significance of Study

this is considered as a new wine making region and one of the most important in tropical zones, where there is not enough information about phenological and thermal demand of wine grape cultivars (Moura et. al, 2007). The climate is unique and classified as semi-arid, where the mean of total annual precipitation is 540mm, the corresponding mean pan evaporation is about 2700mm and the mean air temperature is 26.5°C.

Methods: The experimental area was established in a vineyard at Experimental Field of Mandacaru, Embrapa Tropical Semi-Arid, located in the Municipio of Juazeiro, Bahia State, Brazil. Four varieties of white grapevine were evaluated: Chenin Blanc, Riesling Italico (Welch Riesling), Sauvignon Blanc and Semillon. The evaluations started at the pruning on the first and second semester from 2003 to 2007.

Results: It was observed the number of the days from the pruning to harvest. The thermal demand of the four white grapevine varieties was determined by the degree-day sum from pruning to harvest. The base temperature of 10°C was considered, and the air temperature was measured by the weather station located in the experimental area. There were not statistical differences by *Tukey's test (5%)* to degree-day sum and productive cycle duration among the varieties.

Conclusions

the mean of the duration's cycle were 107, 111, 119 and 120 days, respectively to Semillon, Sauvignon Blanc, Chenin Blanc, and Riesling Italico. Their thermal demand was, respectively, 1804, 1895, 2025, and 1995 degree-days.

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Degree-days and phenological characterization of five red grapevines cultivated in a Tropical Semi-Arid region of Brazil

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Background and Aims

The Tropical Semi-Arid of Northeast Brazil has one important area cultivated with irrigated fruits. In this area, the mean air temperature is 26.5°C, the total annual precipitation is 540mm, and mean pan evaporation is about 2700mm, related to a negative soil water balance. Grapes are irrigated by drip, for example, using water from the Sao Francisco River, allowing a continuous vegetative development of grapevines throughout the whole year.

Significance of Study

As this new tropical zone is starting to produce young wines, and the information about thermal demand and phenological characterization of red grapevines are required to allow an increase of irrigated vineyards area (MOURA et al, 2007). This work aimed to determine degree-days and phenological characterization of five red grapevines cultivated in Northeast Brazil. Methods: Data were obtained in an experimental vineyard located at Embrapa Tropical Semi-Arid, Bahia State. It was evaluated the number of days from pruning to harvest starting in the first and second semester of 2003 to 2007, for five red grapevines: Cabernet Sauvignon, Grenache, Petit Syrah, Petit Verdot and Tempranillo. Degree-days sum of the grapevines was determined using 10°C as base temperature. The air temperature was measured in an Agrometeorology Weather Station located in the experimental area. Results: The results evaluated through *Tukey's Test (5%)* showed that there were not statistical differences for degree-day sum and productive cycle duration between the studied varieties.

Conclusions

The mean sums were 1815, 1950, 1963, 2154 and 2164 degree-days, related to 109, 112, 115, 129 and 117 days from the pruning to harvest, respectively for Tempranillo, Petit Verdot, Petit Syrah, Cabernet Sauvignon, and Grenache.

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Mapping of Photoperiod-Induced Growth Cessation in the Wild Grape *Vitis riparia* Michx

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Background and Aims

In grapevines, the timing of growth cessation in the fall is an important aspect of adaptation and a key objective in breeding new grape varieties suitable for cold climates. Growth cessation is a complex biological process that is initiated by environmental cues such as day length and temperature as well as water and nutrient availability.

Methods and Results

The genetic control of growth cessation in grapevines was studied by mapping quantitative trait loci (QTL) in a hybrid grape population. An F2 mapping population was developed by selfing a single F1 plant derived from a cross between an accession of the North American species Vitis riparia Michx. and the wine cultivar 'Seyval'. A linkage map was constructed using 115 SSR markers and 6 candidate genes in a population of 119 F2 progeny. The markers provided coverage of the 19 Vitis linkage groups with an average distance between markers of 8.6 cM. The critical photoperiod for growth cessation in lateral buds for the parents and F2 progeny was determined in a replicated field trial in 2001 and 2002 and under controlled photoperiod treatments in a greenhouse in 2002, 2003, and 2004. QTL analysis using Composite Interval Mapping identified a single major QTL for critical photoperiod in field and greenhouse trials.

Conclusion

The field and greenhouse QTLs mapped to different linkage groups in the two different environments, suggesting the presence of non-photoperiodic cues for induction of growth cessation in the field.

Significance of Study

We constructed a map for an F2 population from the cross V. riparia x Seyval and identified a QTL responsible for the segregation for photoperiod-induced growth cessation as well as a field QTL for growth cessation unrelated to photoperiod. This map will be useful for mapping additional traits such as cold hardiness and chilling requirements for bud break.

Dry matter and carbohydrate partitioning in Cabernet-Sauvignon vines grafted onto four different vigor-controlling rootstocks

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Background and Aims

Much of the world's viticulture is based on grafting. The rootstock interacts strongly with the scion to modify the developmental traits of the whole plant, but the underlying mechanisms are still unclear. In the present study, we compared the dry matter and carbohydrate partitioning of different rootstocks grafted with the same scion. Our objectives were to determine if vigourous rootstocks increased root production and activity, especially under low irradiance, compared with weaker rootstocks.

Methods and Results

One-year-old grapevine plants (*Vitis vinifera* L. cv Cabernet-Sauvignon) grafted on four vigorcontrolling rootstocks (*Vitis riparia* L. cv Gloire de Montpellier, 101-14 MGt, 1103 P and 110 R) were grown aeroponically under three irradiance level. Shoot growth parameters (number of leaves, stem length, leaf area), as well as nitrate reductase activity, total non-structural carbohydrates (TNC) and amino acids (FAA) in leaves and roots were measured 0, 4, 12 and 25 days after light treatment exposure. Regardless to irradiance level, vines grafted on 1103 P had higher leaf canopy development (leaf production and expansion) and whole plant dry matter accumulation than vines grafted on 110R, whereas RGM and 101-14MGt rootstocks exhibited an intermediate behaviour. In addition, '1103 P' favored dry matter accumulation in shoots while '110R' favored accumulation in roots. Finally, a opposite relationship between root-to-shoot ratio and root carbohydrates contents was observed.

Conclusions

Grafting a scion such as Cabernet Sauvignon to different rootstocks does have the potential to significantly alter overall grapevine performance and root/shoot balance. This differential response is exacerbated under reduced light growth conditions.

Significance of Study

The present study provides additional and consistent information about the complex control of grapevine scion vigour by the rootstock.

Developing carbon partitioning and fruit abscission models for Concord grapes

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Background and Aims

The balance between vine supply and crop demand, the crop load, is a major determinant of vine performance and fruit quality. Many physiological indicators of the vine balance can be used. However there is a need for a more dynamic, model-type approach, which takes into account the supply and the demand of the vines but also the climate. This paper reports about some field experiments that were carried out in order to develop carbon partitioning and fruit growth/abscission components for our Concord grape model.

Methods and Results

We first assessed whether the growth of fruit sinks behave as independent units or if the sink development responds to the whole plant source-sink status. In vines thinned to lower and higher yields, different numbers of clusters per shoot were retained after cluster thinning one week after set. Results showed that the seasonal accumulation of berry dry weight, fruit fresh weight, and the berry total soluble solids at harvest were only affected by the whole vine crop level. A second experiment was performed to asses shoot and berry relative sink strength by quantifying the organ dry mass accumulation in response to different vine sink-source status. It was found that, during the period between fruit set and veraison, shoot dry weight growth was reduced more by shading than berry dry weight growth. Overall these results allowed us to better simulate vine partitioning with the "VitSim" carbon balance model as presented in a companion paper. Finally in vines in the field under standard conditions, individual florets were tagged shortly after flowering and their growth and abscission was followed until final set. As found in apples, berry abscission was related to a critical reduction in its growth rate. Research is now in course to validate the fruit set model component with an independent 25-year fruit set database available for Concord.

Conclusions

Crop carbon demand and growth can be modeled on a whole vine basis by considering the organ potential growth rate and their relative sink-strength. Between set and veraison the fruit had somewhat more priority than the shoot in the carbon partitioning under carbon-limited conditions.

Significance of Study

The results intend to provide some experimental support to modeling carbon partitioning and fruit set and growth in grapevines. It is provided the first evidence than in grapevine, as in other fruit crops, berry abscission can be related to its growth rate.

Determinants of soluble solids yield in commercial Shiraz vineyards

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Background and Aims

Yield of soluble solids is determined by the capacity of a grapevine to produce and utilise assimilate. The production of assimilate (source strength) is in turn a function of the ability of the vine to convert radiation energy into sugar. Assimilate utilisation (sink strength) is due to the number of bunches, berry size and berry number per bunch. This study aimed to investigate source and sink determinants of soluble solids yield in commercial Shiraz vineyards.

Methods and Results

Data were collected from 100 individual vines in five commercial vineyards across viticultural regions in Australia (Griffith, Tatura, Mildura, Great Southern and Langhorne Creek) over a three year period. Total fresh weight yield, bunch number, berry fresh weight, soluble solids concentration, photosythetically active radiation interception and incoming solar radiation were measured. Soluble solids yield per vine varied by greater than 10-fold within each site. Bunch number had the greatest influence on soluble solids yield compared with berry fresh weight and berry number per bunch suggesting a sink limitation to soluble solids yield. There was a positive linear relationship between soluble solids yield and intercepted radiation at each site. However, the relationship varied between sites due to differences in radiation use efficiency and/or harvest index.

Conclusions

Substantial spatial variation in soluble solids yield in commercial Shiraz vineyards was attributed to number of bunches per vine and radiation interception. Soluble solids yield appeared to be sink limited.

Significance of Study

Crop load needs to be considered in terms of canopy size and incoming solar radiation in order to maximise soluble solids yield. Generally, bunch number per vine in this study was insufficient to maximise soluble solids yield.

Characterization of the Nitrate and Ammonium transport mechanisms operating in *Vitis vinifera*

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Background and Aims

The uptake of nitrate and ammonium by plant roots involves two main mechanisms involving high (HATS) and low (LATS) affinity transport systems. In many plant systems the physiological and genetic control of these two pathways have been characterised with the intention to better understand plant N acquisition systems and how best to manage this essential element required for plant growth. We are currently exploring the inorganic nitrogen transport systems operating in roots of *Vitis vinifera* with the aim in identifying N related phenotypes including transport capacity, specificity and regulation.

Methods and Results

Dormant 1-2 node hardwood cuttings of *Vitis vinifera* (cvs Chardonnay, Merlot, Shiraz) were propagated in a mistbed chamber to initiate callus and root development. Rooted cuttings were then transferred to defined hydroponic nutrient solutions and cultured in the glasshouse for 3-4 weeks to a standardized root and shoot size (3-4 leaves). Plants were then exposed to various pre nitrogen treatments to induce or repress nitrogen transport systems and then exposed to external nitrogen solutions containing the stable isotope ¹⁵N-nitrate or ¹⁵N-ammonium at varying concentrations and time periods. ¹⁵N/¹⁴N ratios were measured in plant tissues using isotope ratio mass spectrometry. The uptake of both nitrate and ammonium was typical of that found in other plant systems where both the HATS and LATS systems were present for both inorganic ions. When supplied together at equal molar concentrations, ammonium uptake was significantly greater than that of nitrate and was clearly the preferred nitrogen ion accumulated by grapevine roots.

Conclusions

A robust hydroponic system has been established to investigate nitrogen uptake in *V. vinifera*. Both the nitrate and ammonium uptake transport systems were found to be active in grapevines. Ion selectivity was evident between ammonium and nitrate, where ammonium was found to be the preferred nitrogen form accumulated by the root system.

Significance of Study

Understanding the mechanisms controlling N uptake in grapevines will allow for careful dissection of the N cycling pathways over the annual growing season and allow for the fine tuning of N provision to support growth and fruit development.

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