XIth International Symposium on

GRAPEVINE PHYSIOLOGY AND BIOTECHNOLOGY | 2021

31 Oct - 5 Nov | Stellenbosch, South Africa

Book of Abstracts

Oral & Poster Abstracts

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International Society for Horticultural Science

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WELCOME

A warm welcome to the symposium!

This 11th instalment of this symposium series follows on the highly successful event that was held in Verona during 2016. The global COVID-19 pandemic has led to a one-year postponement to the original date of the event, with the hope that by October 2021, we would be able to come together for a physical meeting in Stellenbosch, South Africa. The ongoing pandemic has unfortunately necessitated us to resort to an Online format, and now we will "make history" in convening the first fully online ISGPB symposium series.

We are grateful that the international grapevine community showed their interest and support to the symposium by registering, submitting Abstracts and making their time available to participate in the symposium. The theme of the symposium is "grapevines in interaction with their environments" and based on the Abstracts submitted, there is currently a very strong focus placed on the disruptive potential of climate change, specifically in terms of water constraints. It is clear that this aspect is under scientific "attack" from a range of angles. It is also obvious that the previously emerging theme of precision viticulture is growing rapidly with a significant number of Abstracts submitted on the testing and optimising of sensor-based technologies to monitor grapevines and their environments. There is also part of a session dedicated to the advances in gene editing in grapevine – a topic that was not reflected yet in the previous symposia in this series. The symposium is organised under five themes and, in addition, will host a workshop to outline the important initiatives and progress of the Integrape COST action that aims to bring the international grapevine community closer to the important topic of interoperability of data and data-integration.

We want to express our sincere gratitude to the local committee, as well as the excellent support of the scientific committee that participated in the organisation of the symposium. Without their encouragement, insights and just plain hard work of so many colleagues from around the world, the symposium would not have come together. We also want to thank the sponsors of the symposium for their generous support and also the team of Conferences et al, specifically Deidre Cloete and Kurt Johnson, for their invaluable assistance to the local organising committee.

We sincerely hope it will be an invaluable event and we look forward to the 10 plenaries, 46 orals, 99 posters (of which 18 will also be presented as flash poster presentations) that will be presented over the five days of the symposium. It was an honour to work with all of you in organising this event and now we place our trust in our collective interest in progressing the scientific advances in grapevine physiology and biotechnology to make this a successful symposium.

Professors Melané Vivier and Johan Burger
Conveners of the ISGPB 2021 Organising Committee

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SCIENTIFIC COMMITTEE

NAME	COUNTRY	AFFILIATION
Melané Vivier	South Africa	Stellenbosch University
Lizel Mostert	South Africa	Stellenbosch University
Johan Burger	South Africa	Stellenbosch University
Carlos Poblete-Echeverria	South Africa	Stellenbosch University
Erna Blancquaert	South Africa	Stellenbosch University
Kobus Hunter	South Africa	Agricultural Research Council
Huiqin Ma	China	China Agricultural University
Aaron Fait	Israel	Ben-Gurion University
Etti Or	Israel	Agricultural Research Organization ARO
Ian Dry	Australia	Commonwealth Scientific and Industrial Research Organisation (CSIRO)
Sigfredo Fuentes	Australia	University of Melbourne
Suzy Rogiers	Australia	New South Wales Department of Primary Industries
Chris Davies	Australia	Commonwealth Scientific and Industrial Research Organisation
Chris Winefield	New Zealand	Lincoln University New Zealand
Patrice This	France	Montpellier SupAgro
Laurent Torregrosa	France	Montpellier SupAgro
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Hipólito Medrano	Spain	University of the Balearic Islands
Javier Tardaguila	Spain	University of La Rioja

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NAME	COUNTRY	AFFILIATION
José Tomás Matus	Spain	University of Valencia
Markus Rienth	Switzerland	Changins, University of Applied Sciences and Arts of Western Switzerland
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Samuel Ortega	Chile	University of Talca
Patricio Hinrichsen	Chile	Agricultural Research Institute
Claudio Pastenes	Chile	University of Chile

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LOC COMMITTEE

NAME	AFFILIATION
Talitha Venter	South African Grape and Wine Research Institute/Dept Viticulture & Oenology, Stellenbosch University
Manuela Campa	Dept Genetics, Stellenbosch University
Johan Burger (Co-convener)	Dept Genetics, Stellenbosch University
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SCIENTIFIC PROGRAM

DAY 01

SUNDAY 31TH OCTOBER 2021

TIME	Opening event	Presenter	Presentation Title	Live/Pre-recorded
16:15 - 16:30	Welcome and general info	Melane Vivier and Johan Burger (Conveners); Nick Dokoozlian (E&J Gallo)		Live event
Session moderators: Nick Dokoozlian & Bruce Reisch				
16:30 - 17:20	Opening lecture + Q&A	Jill Farrant	L1 - Resurrection grapevines: Can understanding vegetative desiccation produce this phenotype?	Live event
17:20 - 18:00	Plenary lecture + Q&A	Dario Cantu	L2 - Grape research in the age of pangenomes	Live event
18:00-18:10	ISHS presentation	Alexander Levin	ISHS Overview	

DAY 02

MONDAY 01TH NOVEMBER 2021

TIME	Topic A: Environmental Interaction	Presenter	Presentation Title	Live/Pre-recorded
Session A1		Session moderators: Ian Dry, Lizel Mostert & John Moore		
Poster presentations will be available to view on the dedicated ISGPB2021 microsite throughout the symposium days.				
8:55-9:00	Introduction of moderators/session: Melane Vivier			Live event
9:00-9:30	Plenary lecture	Jan van Kan	L3 - How to prosper on grape berries: the tricks and treats of Botrytis infection processes	Live event
9:30-9:45	Presentation	Suzanne Balacey	L4 - The role of volatile signalling in grapevine responses to drought	Live event
9:45-10:00	Presentation	Patrice This	L5 - What are the main drivers of grapevine's phyllosphere microbiome structuring ?	Live event
10:00-10:15	Presentation	Ana Margarida Fortes	L6 - Transcriptional, hormonal, and metabolic changes in susceptible grape berries under powdery mildew infection	Live event
10:15-10:45	Q&A			Live event
10:45-10:50	Flash Presentation	Elyashiv Drori	P1 - Water availability and vegetation density constrain the dispersion of wild grapevines populations at the southern distribution edge	Pre-recorded
10:50-10:55	Flash Presentation	Romain Darriaut	P2 - Dysbiosis in soil microbial diversity is differently managed by the grapevine rootstock genotype	Pre-recorded
10:55-11:00	Flash Presentation	Jose Escalona	P3 - Intra-cultivar genetic diversity in improving water use efficiency in grapevine	Pre-recorded

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DAY 02

MONDAY 01TH NOVEMBER 2021

TIME	Topic A: Environmental Interaction	Presenter	Presentation Title	Live/Pre-recorded
	Session A2	Session moderators: Aaron Fait and Philip Young		
11:55-12:00	Introduction of moderators/session: Melane Vivier			Live event
12:00-12:15	Pre-recorded presentation	Vinay Pagay	L7 - A comparison of drought tolerance between pre-clonal and clonal Cabernet Sauvignon grapevines under field and glasshouse conditions	Pre-recorded
12:15-12:30	Pre-recorded presentation	Joanah Midzi	L8 - Abiotic stress-induced inter-vine signalling via plant volatiles	Pre-recorded
12:30-12:45	Pre-recorded presentation	Esther Hernández Montes	L9 - Combined effects of heat and water stress on grapevine growth and physiology	Pre-recorded
12:45-13:00	Q&A			Live event
	Session A3	Session moderators: Suzy Rogiers & Manfred Stoll		
15:55-16:00	Introduction of moderators/session: Johan Burger			Live event
16:00-16:30	Plenary lecture	Joerg Bohlmann	L10 - Montbretin A: Secondary Metabolite Pathway Discovery and Applications for Human Health	Live event
16:30-16:45	Presentation	Claudio Pastenes	L11 - Energy conversion responses to water deficit in 4 grapevine varieties with contrasting stomatal sensitivity	Live event
16:45-17:00	Presentation	Evodia Setati	L12 - Vintage and geography surpass farming practice as key factors shaping the grape mycobiome	Live event
17:00-17:30	Q&A			Live event

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DAY 03

TUESDAY 02ND NOVEMBER 2021

TIME	Topic B: Grapevine Development	Presenter	Presentation Title	Live/Pre-recorded
		Session sponsored by Villa Crop		WINFIELD UNITED South Africa
Session B1		Session moderators: Etti Or & Chris Davies		
Poster presentations will be available to view on the dedicated ISGPB2021 microsite throughout the symposium days.				
08:55-9:00	Introduction of moderators/session: Johan Burger			Live event
9:00-9:30	Plenary lecture	Nathalie Ollat	L13 - Rootstocks and scions: how these two players interact in response to environmental conditions	Live event
9:30-9:45	Presentation	Anne Janoueix	L14 - Imaging xylem connections between the scion and rootstock of grafted grapevine with X-ray microtomography	Live event
9:45-10:00	Presentation	Giovanni B. Tornielli	L15 - A novel berry phenological scale based on gene expression	Live event
10:00 - 10:30	Q&A			Live event
10:30-10:35	Flash Presentation	Julia Gouot	P34 - Grape berry acclimation to high and extreme high temperatures	Pre-recorded
10:35-10:40	Flash Presentation	Grégoire Loupit	P35 - Understanding stilbene accumulation at the graft interface of grapevine and their potential use for predicting graft incompatibility	Pre-recorded
10:40-10:45	Flash Presentation	Estefanía Uberegui	P36 - Footprint of rootstock on Vitis vinifera response to drought	Pre-recorded
10:45-10:50	Flash Presentation	Silvina Dayer	P38 - Grapevine drought tolerant ideotypes to adapt viticulture to climate change	Pre-recorded
Session B2		Session moderators: Chris Winefield & Justin Lashbrooke		
11:55-12:00	Introduction of moderators/session: Melane Vivier			Live event
12:00-12:15	Pre-recorded presentation	Markus Rienth	L16 - Individual berry sampling reveals novel transcriptomic modifications induced by leafroll virus infection in grapevine (V. vinifera) berries	Pre-recorded
12:15-12:30	Pre-recorded presentation	Ignacio Buesa	L17 - Rootstock effects on physiological response of Tempranillo cultivar	Pre-recorded
12:30-12:45	Pre-recorded presentation	Philippe Vivin	L18 - Unravelling the genetic and environmental control of root system architecture traits in grapevine rootstocks using the structural root model, Archisimple	Pre-recorded
12:45-13:00	Pre-recorded presentation	Steve Tyerman	L19 - Cell death in grape berries	Pre-recorded
13:00-13:15	Q&A			Live event

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DAY 03

TUESDAY 02ND NOVEMBER 2021

TIME	Topic C: Fruit Composition	Presenter	Presentation Title	Live/Pre-recorded
Session B3		Session moderators: Simone Diego Castellarin & Eunice Avenant		
15:55-16:00	Introduction of moderators/session: Melane Vivier			Live event
16:00-16:30	Plenary lecture	Patricio Hinrichsen	L20 - Genetics and markers for quality traits in table grapes: From the heaven of single traits to the deepness of polygenics	Live event
16:30-16:45	Presentation	Laurent Deluc	L21 - Influence of seed development on the timing of ripening initiation in grape berry	Live event
16:45-17:00	Presentation	Erica D'Incà	L22 - CARPO-NAC regulator controls the initiation of organ maturation in grapevine	Live event
17:00-17:30	Q&A			Live event

DAY 04

WEDNESDAY 03RD NOVEMBER 2021

TIME	Topic D: Computational Resources and Data Integration	Presenter	Presentation Title	Live/Pre-recorded
Session C1		Session moderators: Jerome Grimplet and Erna Blancquaert		
Poster presentations will be available to view on the dedicated ISGPB2021 microsite throughout the symposium days.				
8:55-9:00	Introduction of moderators/session: Johan Burger			Live event
9:00-9:30	Plenary lecture	Zhanwu Dai	L23 - Modeling berry growth and quality formation at multiple levels	Live event
9:30-9:45	Presentation	Pietro Previtali	L24 - Delayed ripening affects the formation of berry quality traits in Cabernet Sauvignon grapes	Live event
9:45-10:00	Presentation	Franco Röckel	L25 - High-density genetic mapping and aroma compound-related QTL analysis in a white wine F1 population	Live event
10:00-10:15	Presentation	Michaela Griesser	L26 - Grape Berry Shrivel Disorder ' Lessons learnt and Knowledge Gaps	Live event
10:15-10:30	Presentation	Lina Wang	L27 - Combining leaf-to-fruit ratio manipulations with abscisic acid application adjusts sugar and anthocyanin concentrations in ripening berries	Live event
10:30-11:00	Q&A			Live event
11:00-11:05	Flash Presentation	Claudia Muñoz-Espinoza	P60 -Towards the identification of the genetic determinants of harvest date in table grapes	Pre-recorded
11:05-11:10	Flash Presentation	Ron Shmuleviz	P61 - Temperature affects organic acid, terpene and stilbene metabolisms in wine grapes during post-harvest dehydration	Pre-recorded

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DAY 04
WEDNESDAY 03RD NOVEMBER 2021

TIME	Topic D: Computational Resources and Data Integration	Presenter	Presentation Title	Live/Pre-recorded
Session C2		Session moderators: Javier Ibanez & Manuela Campa		
11:55-12:00	Introduction of moderators/session: Johan Burger			Live event
12:00-12:15	Pre-recorded presentation	Kamal Tyagi	L28 - The consequences of plant growth regulators application and girdling on phenolic and volatiles composition of grapes	Pre-recorded
12:15-12:30	Pre-recorded presentation	Josh VanderWeide	L29 - Terpene biosynthesis is uncoupled from berry maturity and predominately regulated by solar radiation in Riesling (Vitis vinifera L.)	Pre-recorded
12:30-12:45	Pre-recorded presentation	Sebastián Vargas	L30 - Regulated deficit irrigation on cv. cabernet sauvignon grapes: impact on aroma and phenolic composition	Pre-recorded
12:45-13:00	Pre-recorded presentation	Ghislaine Hilbert	L31 - Increased temperature, elevated atmospheric CO2 and water deficit modify grape composition of different Tempranillo (Vitis vinifera L.) clones	Pre-recorded
13:00-13:15	Q&A			Live event
Session C3		Session moderators: Luis Sanchez & Claudio Pastenes		
15:55-16:00	Introduction of moderators/session: Melane Vivier			Live event
16:00-16:30	Plenary lecture	Phillipe Gallusci	L32 - Methylome dynamics in grapevine: from fruit development to stress responses and memories	Live event
16:30-16:45	Presentation	Simone Diego Castellarin	L33 - Impact of ripening-related hormones on terpene biosynthesis in Gewürztraminer grapes (Vitis vinifera L.)	Live event
16:45-17:15	Q&A			Live event

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DAY 05

THURSDAY 04TH NOVEMBER 2021

TIME	Topic E: Technological Advances	Presenter	Presentation Title	Live/Pre-recorded
Session D1		Session moderators: Maria Stella Grando & Ana Margarida Fortes		
Poster presentations will be available to view on the dedicated ISGPB2021 microsite throughout the symposium days.				
8:55-9:00	Introduction of moderators/session: Melane Vivier			Live event
9:00-9:30	Plenary lecture	Sigfredo Fuentes	L34- Artificial Intelligence advances for Digital Viticulture and Oenology	Live event
9:30-9:45	Presentation	David Navarro-Payá	L35 - Genome-wide exploration of transcription factor targets involved in grape specialized metabolism and visualization through DAPBrowse: a centralized genome-browser for DAP-seq data	Live event
9:45-10:00	Presentation	Stefania Pilati	L36 - Vitis OneGenE: a causality-based method for gene network analysis in grapevine. Characterization of the laccase and dirigent protein gene families	Live event
10:00 - 10:30	Q&A			Live event
10:30-10:35	Flash Presentation	Marco Moretto	P71 - A COMPASS for VESPUCCI: a FAIR way to explore the grapevine transcriptomic landscape	Pre-recorded
10:35-10:40	Flash Presentation	Jessica Vervalle	P72 - The construction of a high-density consensus map based on three mapping populations using the Vitis18K SNP chip and a reference genome sequence	Pre-recorded
Session D2		Session moderators: Patrice This & Talitha Venter		
11:55-12:00	Introduction of moderators/session			Live event
	Topics D and E			
12:00-12:15	Pre-recorded presentation	Bernadette Rubio	L37 - Small RNA populations reflect the complex dialogue established between heterograft partners in grapevine	Pre-recorded
12:15-12:30	Pre-recorded presentation	Philip Gauthier	L38 - Using Geolocated Proxy Sensor Systems to Assess Spatiotemporal Stability of Seasonal Wood Biomass in Northern California Vineyards	Pre-recorded
12:30-12:45	Pre-recorded presentation	Nele Bendel	L39 - Climate protection based on increased humus amounts in vineyard soils: Sensor-based characterization of grapevine vitality	Pre-recorded
12:45-13:00	Pre-recorded presentation	Anke Berry	L40 - Estimation of grapevine lateral leaf area (LLA) from RGB imagery using colour thresholds and automatic k-means image segmentation	Pre-recorded
13:00-13:15	Pre-recorded presentation	Diego Lijavetzky	L41 - Whole genome resequencing and custom genotyping unveil clonal lineages in `Malbec´ grapevines (Vitis vinifera L.)	Pre-recorded
13:15-13:35	Q&A			Live event

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DAY 05

THURSDAY 04TH NOVEMBER 2021

INTEGRAPE WORKSHOP (COST Action CA17111)
Introdiction of session and moderator: Anscha Zietsman

COST Action
CA17111
INTEGRAPE

Data Integration, The Power of Omics & Grape Improvement

		Presenter	Moderator:Daniela Holtgräwe	
14:30-14:40		Mario Pezzotti	Introduction of the INTEGRAPE COST ACTION	Live event
14:40-14:50		Eric Duchene	Phenotyping guidelines	Live event
14:50-15:00		José Tomás Matus	Gene Nomenclature and Gene Reference Catalogue	Live event
15:00-15:10		Jérôme Grimplet	White cook book- guidelines for genomics/ transcriptomics data	Live event
15:10-15:20		Fulvio Mattivi	Metabolomics guideline	Live event
15:20-15:30		Camille Rustenholz	The new PN40024 reference genome assembly	Live event
15:30-15:40		J. Grimplet/T. Matus	Training Schools	Live event
15:40-16:05		Ana Margarida Fortes	The STSM/ITC program. PechaKucha of student STSM experiences	Live event
16:05-16:15			Showcasing the new website	Live event
16:15-16:30	Round Table		Round Table/Forum for discussion	Live event

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DAY 06

FRIDAY 05TH NOVEMBER 2021

TIME	" Topic E: Technological Advances"	Presenter/Moderator	Presentation Title	Live/Pre-recorded
	Session E1	Session moderators: Carlos Poblete and Markus Rienth		
Poster presentations will be available to view on the dedicated ISGPB2021 microsite throughout the symposium days.				
8:55-9:00	Introduction of moderators/session: Melane Vivier			Live event
9:00-9:30	Plenary lecture	Anna Kircherer	L42 - High-throughput field phenotyping in vineyards: objectives, approaches, goals	Live event
9:30-9:45	Presentation	Giovanni Caruso	L43 - Monitoring grapevine water status by unmanned aerial vehicle (UAV) and plant based sensors	Live event
9:45-10:00	Presentation	Pablo Carbonell-Bejerano	L44 - Clonal improvement-associated somatic mutations identified from diploid genome assembly in Tempranillo grapevine cultivar	Live event
10:00-10:30	Q&A			Live event
10:30-10:35	Flash Presentation	Jaco Luus	P77 - Automatic image filtering for determining representative canopy temperatures with thermography in vineyards	Pre-recorded
10:35-10:40	Flash Presentation	Elsa Chedid	P78 - Genetic variability of grapevine pruning wood parameters as described with LiDAR data and associated quantitative trait loci	Pre-recorded
10:40-10:45	Flash Presentation	Andrej Svyantek	P79 - The Vines -They Are A-Changin': QTL in Grapevine Acclimation Across Environments	Pre-recorded
SHORT BREAK (30min)				
	Closing session: Progress with Grapevine CRISPR Technologies	Session moderators: Johan Burger, Reinhard Töpfer & Riccardo Velasco		
11:15-11:30	Introduction of moderators/session: John Burger			
11:30-11:45	Presentation	Lisa Giacomelli	L45 - Generation of non-transgenic mildew-resistant grapevine clones via gene-editing: potentials and hurdles	Live event
11:45-12:00	Presentation	Lorenza Dalla Costa	L46 - Edited grapevine knocked-out for VvEPFL9-1 showed reduced stomatal density	Live event
12:00-12:05	Flash Presentation	Britt Eubanks	P80 - Developing a Spray-Induced Gene Silencing (SIGS) methodology for the control of grape Powdery Mildew (Erysiphe necator)	Pre-recorded
12:05-12:10	Flash Presentation	Samaneh Najafi	P81 - Establishments of an efficient platform for genome editing in grapevine	Pre-recorded
12:15-12:20	Flash Presentation	Katarina Spencer	P82 - CRISPR-based genome editing tools for virus resistance in grapevine	Pre-recorded
12:20-12:25	Flash Presentation	Satyanarayana Gouthu	P83 - Evaluation of cell penetrating peptide-mediated delivery of CRISPR Ribonucleoproteins in microvine embryogenic cells	Pre-recorded
12:25-12:50	Panel discussion		Panel of Scientific members to lead discussion	Live event
12:50-13:00	ISHS Business meeting	Johan Burger on behalf of A Levin		Live event
13:00-13:10	Closing remarks			Live event

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OPENING EVENT
L1 – L2

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L1

Resurrection grapevines: Can understanding vegetative desiccation produce this phenotype?

JM Farrant*

DSI-NRF SARChI, Department of Molecular and Cell Biology, University of Cape Town, Private Bag X3, Rondebosch, 7701, South Africa.

***Presenting author:** jill.farrant@uct.ac.za

Drought is the greatest threat to world agriculture and due to global warming, increased aridification is predicted in most current food producing areas. To safeguard food security, it is essential to radically improve drought tolerance in crops and fodder. This is particularly significant for Africa, where 95% of agriculture is rain fed. All current crops are intolerant of extreme water loss and while improved resistance to water loss has been achieved, such mechanisms fail under severe and prolonged drought. Resurrection plants, so called because of their ability to survive and revive from the extreme water loss of 95% cellular water content, have increasingly become models for understanding this phenomenon, with the view of using such understanding to produce extremely drought tolerant crops. Such vegetative desiccation tolerance is rare in angiosperms, with only 115 species reported to date. Resurrection angiosperms occurring in Southern Africa also survive extreme heat; conditions which severely limit current agricultural practices. My group has systematically investigated the mechanisms whereby several different resurrection plants, each as a model for a crop to be transformed, tolerate these extreme conditions. A multidisciplinary approach is used, with studies embracing ecological, physiological, biochemical and molecular techniques utilized to interrogate the complex mechanisms of desiccation tolerance, and biotechnological tools utilized to functionally interrogate derived data and the ultimate produce a more drought tolerant phenotype in select crops. In this presentation an overview of molecular physiological processes associated with DT in a range of resurrection plants will be given, and current and future applied outputs discussed.

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L2

Grape research in the era of pangenomes

Dario Cantù*

Department of Viticulture and Enology, University of California, One Shields Avenue, Davis, USA

***Presenting author:** dacantu@ucdavis.edu

Combined improvements in sequencing technologies and assembly algorithms have led to staggering improvements in the quality of grape genome assemblies. The value of developing and using genome references that contain the genes and alleles underlying traits of interest will be discussed. Examples will be provided that show how the availability of multiple genome references allows dissecting the genetic basis of aroma, flavor, and disease resistance and why a single genome reference is insufficient. User-friendly web platforms, like www.grapegenomics.com, rapidly and broadly share genomic datasets and foster multidisciplinary collaborations and progress in grape research. Significant advancements have also been made in accurately and completely representing both haplotypes. Completely phased haplotypes have been instrumental to advances in grape research due to high heterozygosity, structural variation, and gene content variability across homologous chromosomes. The identification of candidate sex determination genes using phased genomes representing cultivated hermaphrodites and wild dioecious grapes will be presented to illustrate the value of diploid genomes for studying complex genetic loci and allele-specific expression.

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TOPIC A: ENVIRONMENTAL INTERACTIONS
L3 – L12

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L3

How to prosper on grape berries: the tricks and treats of *Botrytis* infection processes

Jan van Kan*

Wageningen University, Laboratory of Phytopathology, Wageningen, Netherlands

***Presenting author:** jan.vankan@wur.nl

The fungus *Botrytis cinerea* is an important pathogen, causing significant economic damage in dozens of crops worldwide. Despite chemical control and the development of biological control agents, the yield and quality losses remain substantial, both in the vegetative phase and in harvested produce. In vineyards, the infection by *Botrytis* on vines and leaves is generally negligible, however, the fungus can readily infect floral tissues and especially invade and colonize the berries beyond the bunch closure stage. In grape berries, the fungus can cause both spoilage ("grey mould") as well as "noble rot" which can be beneficial for the production of sweet dessert wines. Studies on *Botrytis* have been boosted in the past decade by the availability of a high quality genome assembly with manually curated gene models, as well as the development of a CRISPR-Cas9 system for targeted mutagenesis. In my presentation I will describe some molecular aspects of the fungal infection mechanisms that contribute to development of grey mould. I will specifically elaborate on the importance of plant cell death induction (by fungal proteins and metabolites) in the infection and on the degradation of plant cell walls by pectinolytic enzymes that results in tissue rot, especially in berries. I will also provide evolutionary arguments to explain why resistance to *Botrytis* in grape berries is a tricky trait to obtain.

Keywords: infection mechanism; programmed cell death; pectin degradation

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L4

The role of volatile signalling in grapevine responses to drought

Steve Tyerman, Suzanne Balacey*, Dimitra Capone

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Volatiles released by plants are becoming important to understand how plants may exchange information. With a wide chemical variety, plant derived volatiles have been shown to be used by plants for pollination and defence against biotic stress. In a drought stress situation, where stomata play a central role in tolerance, plants were observed to have their emission of volatiles associated with stomatal closure decreased, while emission of other volatiles increased. However, the specific functions of the volatiles remain obscure. In many studies on plant responses to drought and rehydration, but without volatile analysis, a particular phenomenon can be observed where the well-watered plants displayed a drought-like response similar to the water-stressed plants when co-located in the same environment. Indeed, while a reduction of stomatal conductance (gs) of plants under water deficit is an expected response, it is not for plants with continuous adequate watering. Thus, the main hypothesis to be tested is that volatiles are released by water stressed plants that induce stomatal closure in nearby well-watered plants. Supposedly, the water-stressed plants would emit volatiles triggering a closure of stomata of the nearby plants in order to preserve water in the likely event of further reduced water availability. To test the hypothesis, potted plants of *Vitis vinifera* were examined in three configurations of drought/rehydration experiments: i) having well-watered (WW) and water-stressed (WS) treatments together, ii) separating the treatments with custom-made individual plastic chambers and, iii) having both treatments together and a separate growth cabinet for controls. For each configuration volatiles were sampled with solid-phase micro-extraction (SPME) and DVB/CAR/PDMS fibres desorbed and analysed with GC-MS. All results combined tend to support the hypothesis of the gs of WW plants not being stable during the severe stress phase applied on the WS group, and supported by multilinear regression analysis showing a stronger effect of WS gs on WW gs than light or VPD. When WS grapevines were enclosed in chambers this interaction was not evident. The volatile samples revealed a change in the emission profile during the drought stress phase and some volatiles showed strong significant correlations with WW and WS gs. Especially, 1,2,3-trimethylbenzene was significantly negatively correlated with gs for both WW and WS plants.

Keywords: volatile emission, stomatal conductance, drought stress

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L5

What are the main drivers of grapevine's phyllosphere microbiome structuring?

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The aerial surface of the plant (phyllosphere) is the habitat of complex microbial communities. This phyllosphere microbiome may have profound effects on host plant health and its performance traits. The interaction of the vine with this very close biotic environment has, until very recently, been mostly ignored. In particular, the question of its inclusion as a selection target (extended ideotype) can be raised.

To begin to answer this question, we have analysed several factors influencing the phyllosphere microbial community structuring. Using amplicon sequencing of the 16S rRNA gene and of the internal transcribed spacer (ITS), we explored the microbial diversity at genus level for both bacteria and fungi present in the phyllosphere of leaves and grape berries. We analysed it on different grape taxonomic level (between 5 Vitis species ora set of *Vitis vinifera* cultivars chosen to represent the 3 genetic pool of the species), for different years and on five commercially important varieties of *Vitis vinifera* that were sampled from three different French terroirs.

Our results indicated the presence of complex microbial diversity and assemblages in the phyllosphere and the presentation will describe the observed diversity. A significant effect of several factors (organ, grape species, growing year and terroir) on taxa abundance was observed with varying degrees of effect. At a given location, genotypes have an impact on microbial assemblage in the phyllosphere of leaf and berries, most pronounced on fruits but the effect of terroir was much stronger than the cultivar identity when the leaf phyllosphere of five grapevine varieties grown in different agro-climatic zones was compared. Limitations of the study as well as implied consequences of this work will be discussed.

Keywords: Biotic interactions, Phyllosphere microbiome, amplicon sequencing, extended ideotype

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L6

Transcriptional, hormonal, and metabolic changes in susceptible grape berries under powdery mildew infection

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Vitis vinifera berries are extremely sensitive to infection by the biotrophic pathogen *Erysiphe necator* causing powdery mildew disease and deleterious effects on grape and wine quality. The combined analysis of the transcriptome and metabolome associated with this common fungal infection has not been previously carried out in any fruit. In order to identify the molecular, hormonal and metabolic mechanisms associated with infection, healthy and naturally infected Carignan berries were collected at two developmental stages: late green (EL33) and early véraison (EL35). RNA sequencing combined with GC-EI/TOF-MS, GC-EI/QUAD-MS and LC-MS/MS analyses revealed that powdery mildew-susceptible grape berries were able to activate defensive mechanisms with the involvement of salicylic acid and jasmonates and to accumulate defense-associated metabolites (e.g. phenylpropanoids, fatty acids). The defensive strategies also indicated organ-specific responses namely the activation of fatty acid biosynthesis. However, defense responses were not enough to restrict fungal growth. The fungal metabolic program during infection involves secretion of effectors related to effector-triggered susceptibility, carbohydrate-active enzymes and activation of sugar, fatty acid and nitrogen uptake and could be under epigenetic regulation. This study also identified potential metabolic biomarkers such as gallic, eicosanoic and docosanoic acids, and resveratrol, that can be used to monitor early stages of infection.

Keywords: Biotic stress, *Erysiphe necator*, grapevine, hormonal profiling, metabolome, plant defense, powdery mildew, transcriptome, susceptibility, *Vitis vinifera*.

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L7

A comparison of drought tolerance between pre-clonal and clonal Cabernet Sauvignon grapevines under field and glasshouse conditions

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Resilience to drought stress is an important crop selection and breeding target, particularly where there is an increase in freshwater scarcity as a consequence of reduced precipitation and higher evapotranspiration rates. Identifying new genetic material and clones with superior drought tolerance would increase available genetic resources for both grapevine breeders and propagators. Genetic material with improved water use efficiency would reduce reliance on supplemental irrigation. We explored the long-term drought adaptation of dry-grown Cabernet Sauvignon grapevines of pre-clonal origin (from shallow (PS) and deep (PD) soils, representing low and high soil water availability, respectively) in the field. In a glasshouse study, the same pre-clonal vines were propagated and compared to three commercial clones that received abundant irrigation (WP). Under field conditions, although PS had lower soil moisture availability than PD, both groups had similar vine water status, based on measurements of midday stem water potential (Ψs) and leaf stomatal conductance (gs). PS had higher intrinsic water use efficiency (WUEi) than PD, however the carbon isotope ratio (δ13C) of the fruit at harvest was similar between the two groups. A glasshouse study of propagated field-selections of PS and PD exposed to multiple cycles of drought stress indicated that PS group had significantly higher gs and net photosynthesis compared to vines in the PD and WP groups. The higher gas exchange rates observed in the PS clones could be explained by their higher chlorophyll fluorescence parameters and mesophyll conductance (gm) rather than hydraulic (Ψs, leaf aquaporins) or chemical (xylem sap abscisic acid) traits. Our results also indicate a “priming effect” in PS vines based on an improved WUE in the second drought cycle compared to the first cycle. Overall, we demonstrate that pre-clonal Cabernet Sauvignon grapevines dry-grown in shallow soils have superior drought tolerance compared to dry-grown vines in deep soils as well as well-watered commercial clones, and that this trait is potentially inheritable.

Keywords: water stress, irrigation, water use efficiency, grapevine clones, gas exchange, chlorophyll fluorescence

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L8

Abiotic stress-induced inter-vine signalling via plant volatiles

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The survival of the sessile plant in the 'rough and tumble' of its natural environment relies on the efficiency of its highly evolved and sophisticated surveillance system. A plant's ability to recognise the wide range of endogenous and exogenous cues from associated environmental assaults and its subsequent initiation of rapid and effective defense responses to each, guarantees its ultimate survival and productivity. Recent studies have shown that non-stressed plants are able to prime themselves for defence against impending danger by eavesdropping on certain chemical cues being emitted from their stressed neighbours. Plant volatile organic compounds (VOCs) are signalling chemical which have gained significant attention for their role in plant-plant communication. However, the signalling pathways and mechanisms involved in volatile-mediated plant-plant interactions have been vaguely understood and sparsely studied under abiotic stress conditions. The aim of the study is to investigate plant-plant volatile-mediated signalling under drought and mechanical stress conditions; integrating physiological, metabolome and transcriptome analyses. Preliminary studies in grapevine using gas chromatography mass spectrometry analyses, coupled with targeted gene expression analyses using quantitative polymerase chain reaction, provided evidence of possible entrainment between co-located stressed emitter vines and control receiver vines. Significant differences between isolated control vines and co-located receiver vines in their VOC concentrations and gene expression levels under both drought stress and mechanical damage conditions were also noted. Findings in this study will contribute towards understanding of the underlying mechanisms involved in airborne signalling, offering an opportunity for a wider application of VOCs in agricultural systems as an eco-sustainable plant protection strategy against abiotic stress.

Keywords: Abiotic stress, plant-plant interactions, priming, stress signalling, VOCs, volatile-mediated signalling

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L9

Combined effects of heat and water stress on grapevine growth and physiology

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Extreme events such as heat waves and drought periods are predicted to increase in frequency and magnitude over the coming decades. The effects of water stress on grapevine physiology and growth during heat waves requires a better understanding of the interaction of these effects and their variability among genotypes and phenological stages. The aim of this study was to evaluate the response of two grape varieties to heat and drought stress and subsequent recovery at different phenological stages. Pot-grown Cabernet Sauvignon and Riesling were studied under environmentally controlled conditions at bloom, pre-veraison and veraison in 2017 and 2018. After 7 days of acclimation in environmentally-controlled growth chambers, different treatments were imposed: control (no stress), water stress, heat stress (10°C above control), and combined water and heat stress. Plant growth, leaf gas exchange, and leaf water potential were measured in 2017 and 2018 before the stress episode, during 7 days of stress, and through 7 days of recovery. Additionally, chlorophyll fluorescence was measured in young and mature leaves during 2018. Water stress and combined stress decreased shoot length, number of main leaves, lateral leaves, and total leaf area in both varieties. At bloom, water stress decreased gas exchange parameters in both varieties. Combined stress decreased gas exchange only in Riesling. During pre-veraison, heat stress reduced gas exchange and chlorophyll fluorescence, both in young and mature leaves in 2018 but not in 2017. Combined stress drastically decreased most of the parameters in both varieties during both years. During veraison, drought was the dominant factor that affected most parameters in 2017 and 2018. Additionally, heat stress exacerbated the drought stress effect on the physiological parameters. During the recovery periods, no significant differences were found among treatments in any parameter, indicating that both varieties were able to recover fully from the imposed stresses.

Keywords: high temperature, irrigation, gas exchange, leaf area, leaf age

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L10

Montbretin A: Secondary Metabolite Pathway Discovery and Applications for Human Health

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Plants and their diverse specialised metabolites have been used by humans for centuries in traditional and modern medicine. They remain an important source for the discovery of new pharmaceuticals and nutraceuticals. Montbretin A (MbA) is a complex flavonoid metabolite; a highly potent and selective inhibitor of the human pancreatic α-amylase (HPA); and a potential new treatment option for Type-II diabetes and obesity.

The only known source for MbA are the below-ground storage organs of montbretia (*Crocosmia*) species, which are native to the grasslands of southern and eastern Africa and commonly grown in gardens around the world. Due to the low abundance of MbA in montbretia and due its complex chemical structure, natural product extraction and chemical synthesis are insufficient for scalable MbA production. Our goal is to develop an improved bio-production system for MbA using genes, enzymes and regulating factors of MbA biosynthesis in montbretia.

In recent work, we discovered the complete biosynthetic pathway of MbA using an approach that combined knowledge of montbretia biology, metabolite profiling, differential transcriptome analysis, cDNA cloning, heterologous gene expression, and enzyme biochemistry. This includes the discovery of genes encoding UDP-sugar dependent glycosyltransferase and acyltransferase enzymes, which catalyze the assembly of MbA from its different building blocks. We are using these genes to bioengineer the production of MbA in yeast and plants.

This presentation will discuss challenges and opportunities of exploring plant biosynthetic systems for the development of new drugs, and bioproducts in general.

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L11

Energy conversion responses to water deficit in four grapevine varieties with contrasting stomatal sensitivity

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Absorbed energy in excess of that used by photosynthesis has been thought to induce photoinhibition, a common case in water deficit conditions resulting in reductions in stomatal conductance. In grapevines, controlled water deficit is a common field practice, but little is known about the impact of a given water shortage on the energy transduction processes at the leaf level, in relation to contrasting stomatal sensitivities to drought. Here, we assessed the effect of a nearly similar water deficit condition on four grapevine varieties: Cabernet Sauvignon (CS), Sauvignon blanc (SB), Chardonnay (CH) and Carmenere (CM), grown in 20L pots, outdoors. Plants were maintained to nearly 94% of field capacity (WW) and 83% FC (WD). We have assessed plant water status, photosynthesis (A_N), photorespiration, A_N vs. PAR, AC_i curves, photochemical (qP) and non-photochemical (NPQ) fluorescence quenching vs. PAR, the photoprotective effectiveness of NPQ (qPd), dry and fresh mass in root and aerial plant parts and, finally, light interception by leaves. Contrasting stomatal sensitivities were observed, where CS is the most sensitive, opposed to CH. Photorespiration is important under WD, but to a different extent between varieties, related to stomatal sensitivity, maintaining a safe proportion of PSII reaction centres in an open state. Also, the capacity for carboxylation is affected by WD, but up to a higher extent in more sensitive varieties. As for NPQ, in WD it saturates at 750 mmol PAR $m^{-2}s^{-1}$, irrespective of the variety, which coincides with PAR from which NPQ photoprotective effectiveness declines and qP reaches a value of 0.5, indicated as a threshold for long term photoinhibition. Also, that same PAR intensity is intercepted by WD leaves from highly stomatal sensitive varieties, due to a modification of the leaf angle in those plants. Results will be discussed in relation to light interception, photosynthetic capacity and photorespiration, associated to stomatal sensitivity in grapevines.

Keywords: Stomatal sensitivity, Water Deficit, Photosynthesis, Photorespiration

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L12

Vintage and geography surpass farming practice as key factors shaping the grape mycobiome

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Wine grape berries harbour a complex microbiome comprising fungi and bacteria that affect grape quality and the winemaking process. However, grapes are seasonally ephemeral, and their microbial assemblages are variable. The density and diversity of the microbial species is influenced by several factors including grape ripening, the sanitary state of the grapes, climatic conditions, farming practice and geographical location. However, each of these factors may differentially affect the relative abundance of the species. We determined the fungal community composition in *Vitis vinifera* L cv. Cabernet Sauvignon grape must across consecutive years, farming practices and locations. A checkerboard sampling design was applied to collect 30 kg of grapes from each vineyard. Fungal community composition of freshly crushed grape must by amplicon sequencing of the ITS-5.8S rRNA region. A core group of genera present in the vineyards across both years was identified, however, the overall fungal community composition differed between sites and between the two years (ANOSIM $R = 0.5$, $p < 0.05$), while it was more similar when comparing farming practices (ANOSIM $R = 0.25$, $p < 0.05$). The data suggest that the minor species as well as the relative abundances of the major species could be the main drivers of temporal and spatial variation in fungal community structure. Rain, relative humidity, temperature as well as radiation were key climatic factors that contributed to temporal and spatial variations in fungal community structures.

Keywords: amplicon sequencing; microbial diversity; wine fermentation; non-Saccharomyces yeasts; microbiome

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TOPIC B: GRAPEVINE DEVELOPMENT
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L13

Rootstocks and scions: how these two players interact in response to environmental conditions

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Recent studies predict that viticulture may be endangered in some traditional wine growing areas because of ongoing and future changes in climate. Increasing temperatures may reduce the suitability of currently grown varieties because of changes in phenology and fruit composition. Water scarcity and limited access to freshwater for irrigation may also affect both yield and fruit quality. In many regions, the maintenance of wine production will rely on modifications of cultural practices to adapt grapevines to new climatic conditions. As the vast majority of vineyards worldwide are grown grafted, rootstocks are a key component of the adaptive potential of these agrosystems. Although there are methodological difficulties in analysing root systems *in situ* and scion x rootstock interactions in a woody perennial crop like grapevine, there is an increasing scientific interest in advancing our understanding of how rootstocks communicate with scions (and vice versa) and modulate grapevine functioning. In the last 20 years, we have been studying scion/rootstock interactions in grapevine at the agronomical, physiological, molecular and (epi)genetic levels, with the view to determine the underlying mechanisms. This work has shown that interactions between rootstock and scion genotypes are huge and that they are difficult to predict from individual partner performances. Mobilizing genome-based approaches combined to advanced phenotyping studies should improve our understanding of this complex issue. Our overall objective is to provide knowledge for breeding new rootstocks and helping growers to choose the best genotype combinations to adapt their vineyard to limiting environments. This talk will provide an overview of the most recent outputs of this research.

Keywords: Rootstocks, genotype x environment interactions, signalling, root development, genetic, omics

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L14

Imaging xylem connections between the scion and rootstock of grafted grapevine with X-ray microtomography

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Grafting *Vitis vinifera* on to Phylloxera resistant rootstocks is necessary in most wine growing regions of the world, as such; most of viticulture relies upon successful graft union formation between the scion and the rootstock. The process of graft union formation begins with formation of a necrotic layer, followed by adhesion of the two grafted partners, callus cell proliferation and the establishment of a functional vascular system. Initially two-dimensional (2D) classical microscopy studies were performed to describe graft union formation. However, the establishment of graft union takes place in 3D and the lack of data in the third dimension makes it difficult to understand the process. In order to overcome this lack of information, 3D imaging techniques have been used, but with limited resolution. Here we will present methods to characterize the 3D morphological developments at the graft interface of grapevine using X-ray microtomography (micro-CT). This technique provides information on tissue architecture in relation with X-ray tissue density and was used to visualize the 3D organization of tissues such as callus and vascular tissues. In addition, we used iohexol, a contrast agent, to visualize functional xylem vessels connecting the scion and rootstock. Images were compared before and 24 h after feeding iohexol to cut roots, which moved to the shoot and labelled xylem vessels connected between the scion and the rootstock. These techniques were used to characterize xylem connections between the scion and rootstock of grafts made with different grafting techniques.

Keywords: Wood, stem, anatomy, canes, wounding, 3D imaging

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L15

A novel berry phenological scale based on gene expression

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Phenology scale systems widely adopted by viticulturists define stages of the annual development of the vine based on the visual description of well recognisable traits related to organ growth and morphology, including grape features from fruit set to maturity. However, although some stages can be easily described (e.g., fruit set, veraison), defining a comparable developmental stage for grapes of the same cultivar when grown in different conditions or for grapes of different cultivars can be challenging, in particular after the onset of ripening. By analysing transcriptomic data collected over berry development, it was shown that the variations of a portion of the transcriptome exhibited conserved dynamics across cultivars and growing condition of grapevines, and thus may be used to describe the developmental stage of berry development. In this work, we used the transcriptomic data generated from grape berries weekly sampled from Cabernet Sauvignon and Pinot noir vines grown in the same location over three consecutive vintages, focusing on conserved annual dynamics rather than on the biological significance of the expression program inferred by gene function. By interpolating the transcriptomic samples dispersed in a 3D space of a PCA we built a 30-stage Transcriptional Phenology Scale (TPhS) precisely defining the progression of development from berry formation to full ripening. The performance of the scale was assessed projecting onto the TPhS both RNA-seq and microarray transcriptomic samples from the same dataset used to elaborate the scale, and from several other public datasets. The results allowed to align samples on the new phenological scale and to highlight differences related to variables like the grape variety, the cultivation site, the vintage, or the applied treatment such as cluster thinning, defoliation, water limitation and temperature regimes. In some cases, the phenological re-scaling of sample collections from previous studies provided valuable hints to re-interpret the experimental results. Overall, we show that the transcriptomic information can be accessed to precisely define a transcriptional phenology scale that can be used to map the ontogenetic development of the fruit with high precision and to align the stage of berry development of different grapes.

Keywords: grapevine, phenology, berry development, gene expression

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L16

Individual berry sampling led to the discovery of novel transcriptomic modifications induced by leafroll virus infection in grapevine (V. vinifera) berries

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Infections with grapevine leafroll associated virus (GLRaV) deteriorate vine physiological performance and cause high losses of yield and fruit quality. Studies on GLRaV are relatively scare and molecular mechanisms responsible for decreased berry quality are not well understood so far. GLRaV infections delay ripening and reduce anthocyanin and sugar accumulation in grapes, which has hitherto been linked to a repression of anthocyanin biosynthesis and sugar metabolism genes. However, previous molecular studies did never account for berry heterogeneity and virus induced phenological shifts which could have induced potential biases in gene expression.

Our study investigated the effect of GLRaV on vine and grape physiology. Pinot noir vines were infected with either GLRaV-1 or co-infected with GLRaV-1 and GLRaV-3. Physiological data was collected from 2015 to 2018 and RNA-seq was performed on 2 reconstituted berry ripening stages. Therefore 245 berries were individually sampled and analyzed for sugar and organic acids to re-constitute 2 homogenous ripening stages with similar acid and sugar content. This way it was possible to circumvent intracluster berry heterogeneity and to compensate for phenological shifts induced by virus infections. RNA of reconstituted samples was extracted and sequenced by single end sequencing and analyzed for differentially expressed genes (DEGs).

A total of 2136 DEGs was detected comprising several transcription factors related to abiotic and biotic stress, which showed a high variation in dependence of ripening stage and infection severity. Strikingly, the previously reported repression of anthocyanin biosynthesis and sugar metabolism could not be confirmed in the present study. This illustrates that the negative effect on fruit quality of GLRaV infections is rather related to a phenological shift than to a direct impact on metabolism. Our results provide novel insight in the mechanism of leafroll infection and emphasize the importance of the sampling protocol of molecular studies investigating berry metabolism.

Keywords: Grapevine leafroll virus, GLRaV-1, GLRaV-3, RNA-seq, berry metabolism, grapevine

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L17

NO TITLE

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Rootstock choice plays a major role in the vineyard response to soil water deficit. This work assessed the effect of 13 rootstocks on water status, photosynthetic capacity and stomatal regulation under water deficit conditions in the Tempranillo cultivar during three seasons. The rootstocks evaluated included 5 widely used rootstocks (1103 Paulsen, 110 Richter, 140 Ruggeri and 420A) and 8 rootstocks recently bred by Vitis Navarra (RG2, RG3, RG4, RG6, RG7, RG8, RG9 and RG10). Results showed that rootstock significantly affected vine water status (determined by assessing both stem water potential and stomatal conductance) leading to differences in net photosynthesis rates. In addition, differences in intrinsic water use efficiency of up to 28% were detected due to rootstock effect. Nevertheless, it was the estimated hydraulic conductivity of the root-stem which was the physiological parameter that best explained water use efficiency in terms of biomass and yield. This suggests that it is the hydraulic conductivity conferred by the rootstock that largely modulated the response in photosynthetic capacity and stomatal regulation of the scion under moderate water stress conditions. Furthermore, this ultimately resulted in differences in vigour and productivity. These results confirm the potential of grapevine rootstocks for improving water use efficiency and provide the physiological basis for future studies and breeding processes.

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L18

Unravelling the genetic and environmental control of root system architecture traits in grapevine rootstocks using the structural root model, Archisimple

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Root system architecture (RSA) defines the spatial distribution of roots in the soil over time. It is shaped by genetically driven growth and developmental processes, which are highly modulated by internal and exogenous cues. Since RSA largely determines the ability of a plant to access water and nutrients, it represents a promising target for breeding plants more tolerant to the future adverse environments. In perennial grafted plants, rootstock genetic variation in RSA and their consequences for soil resource capture has not been studied a lot mainly because of the difficulty to phenotype large root systems in situ. In grapevine rootstocks only few robust quantitative trait loci (QTLs) have been identified for RSA traits (i.e. total root number, length, or biomass) and in general they explain a low percentage of the phenotypic variance. A better understanding of the genetic determinants of RSA as well as its plasticity is important in any grapevine rootstock breeding program. By dissecting RSA into its underpinning components (*e.g.* root emission, axial elongation, radial growth, branching, or tropism) and identifying the relationships between them, functional-structural 3D root models are promising tools for analysing the diversity and complexity of RSA phenotypes with Genotype × Environment interactions. The model parameters are assumed to be simpler traits, less influenced by the environment, and consequently with less polygenic architectures than the integrative RSA traits they drive. Root models can serve as a basis for the development *in silico* of root ideotypes by highlighting the physiological mechanisms and parameters that most likely influence the RSA fitness. Based on this principle, we provide here an overview of our original work on RSA phenotyping and model-assisted trait dissection in grapevine. First, we calibrated the root model, Archisimple (Pagès et al., 2014) on a set of rootstocks grown in containers and assess its value to simulate several RSA traits measured at the root system level. Secondly, using the parameters of Archisimple as phenotyping traits, we aimed to identify the relevant traits underlying genotypic diversity among the 138 genotypes and the 2 parents of a mapping progeny derived from an inter-specific cross between *Vitis vinifera* cv. Cabernet-Sauvignon × V. riparia cv. Gloire de Montpellier grown in the field for 2 years. Broad-sense heritability and QTLs analyses were carried out for model parameters and variables outputs with a consensus map, and compared with other QTLs obtained on classical used RSA descriptors. The preliminary results give new insights on the genetic control of RSA in grapevine rootstocks.

Keywords: Root system architecture, functional-structural root modelling, QTL, Archisimple, rootstock, Vitis

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L19

Cell death in grape berries

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In some winegrape cultivars (e.g. Syrah) loss of mass occurs late in ripening. This reduces yield, and with the corresponding further concentration of sugars, can have negative impacts on wine quality. Loss of mass in the berry correlates with cell death in the mesocarp, which is exacerbated by high temperatures and water stress. Cell death, measured by vital stains or electrical impedance, generally becomes obvious around 90 to 100 days after anthesis, prior to flavour development and harvest. Cell death in the mesocarp was found to be related to a greater degree of hypoxia in the berry mesocarp. The hypoxia is related to increased respiratory demand of the berry and increased resistance for oxygen diffusion into the berry. The skin appears to have a very high diffusion resistance to oxygen while lenticels in the berry pedicel appear to be the primary path for oxygen ingress. Hypoxia in the berry results in fermentation leading to the production of ethanol. This can be monitored either enzymatically of by the use of volatile ethanol sensors. The hypoxic regions also correlate with increased production of reactive oxygen species. There are important metabolites that are triggered by hypoxia and by membrane breakdown that are likely to impact on yeast fermentation and flavour profiles in wine. The impact of bunch and canopy shading and rootstocks on berry development and cell death are being investigated.

Keywords: berry development, hypoxia, cell death, ethanol, shiraz, syrah

L20

Genetics and markers for quality traits in table grapes: From the heaven of single traits to the deepness of polygenics

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Over the last years, our group has been advocated to search for the magic stones that could represent markers able to assist at key selection steps during the breeding process in table grapes. The focus was on different traits related to berry quality, such as seedlessness, berry size, and sugar/organic acids balance, which is mostly a concern for consumers. Thereafter, agronomical traits such as rachis architecture and the response and metabolism of the berry growth regulator GA_3 have been addressed. Finally, we have been involved in berry firmness and berry drop (berry-pedicel tightness and other sub-traits) studies, postharvest issues that are of special concern for exporters to remote markets. To explore the genetics of all these traits, an instrumental tool has been the search for QTLs using biparental populations and germplasm collections, followed by the analysis of the expression level of candidate genes found in the corresponding confidence intervals for each case, coupled to the search for sequence variants (SNPs, SSRs, InDels) that can be associated to the phenotypic variance. In addition, several transcriptomic experiments have also been developed, always carefully choosing the key phenological stages. All these platforms have led to the identification of genomic regions that show higher probabilities of harboring the genes behind the traits, as well as to detect individual genes that can be associated to target traits. In doing this, we have gone from successfully designing a selection marker for seedlessness that behaves as a pseudo-monogenic trait (the first we studied because of its relevance for varietal acceptance), to a very complex and polygenic trait such as berry size, which appears to reside on a quite large number of genetic determinants. In this lecture, a compilation of these findings will be presented, emphasising the most recent work, which are based on transcriptomic studies of berry drop and berry size. In the latter case, the efforts to design a breeding-friendly SSR markers set will be discussed.

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L21

Influence of seed development on the timing of ripening initiation in grape berry

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The ripening initiation is a transitional developmental phase characterized by a complete reprogramming to primarily facilitate the dispersal of the seeds with significant implications on the final fruit composition. Several signalling pathways will integrate changes in the environmental and development cues to ensure the fruits' transition to the ripening phase until maturity. While environmental cues have been extensively studied to examine the ripening onset, the influence of any developmental signal associated with the timing of ripening initiation has yet to be characterized. The uneven ripening of a cluster reflects the implication of a developmental program that controls the entry of each berry into the ripening onset. Recent evidence suggests that uneven ripening poorly correlates with differential anthesis time. Instead, a High Seed Index (seed mass relative to the berry mass) was observed in most lagging fruits at véraison in conjunction with a more significant accumulation of auxin in the pericarp tissues of these fruits at pre-véraison time. Seeds are a reservoir of many plant hormones that are particularly active until pre-véraison. Therefore, we hypothesized that a differential decline of seed-derived auxin associated with the seed index would explain the differential entry of individual berries in the ripening phase resulting in significant molecular changes in the pericarp tissues. Using an integrated approach combining genome-wide transcript profiles and metabolite signatures, we found that the developmental divergence observed between two véraison berry populations with contrasted seed indices (High and Low Seed Index) is a discrete and dynamic process during pre-véraison times responsible for significant transcriptional and metabolite changes. This developmental divergence will reach its maximum at mid-véraison. Still, a developmental convergence between the two berry classes will follow for the remaining berry ripening, which will result in mitigating the previous differences. Comparing the gene expression networks between the two classes (High and Low Seed Index) suggests a complex but coordinated transcriptional program across the three tissues that involves auxin, sugar, and ABA signalling pathways during both the developmental divergence and convergence.

Keywords: ripening initiation, seed development, auxin, uneven ripening

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L22

CARPO-NAC regulator controls the initiation of organ maturation in grapevine

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In the current contest of extensive climate changes which widely affect agriculture, grapevine cultivation is assuming a notable attention due to its high sensibility to global warming effects. To maintain a sustainable viticulture with a stable production of high-quality grape, an increase of the knowledge on the overall regulation of the developmental program occurring in grapevine organs and of the transition to the mature phase is required. This transition is featured also by grape berries during the seasonal development and marks the onset of ripening. This work was focused on the functional characterization of the NAC family member VviNAC60, which we renamed VviCARPO (Controlled Adjustment of Ripening and maturation in Plant Organs), identified as a switch gene of the deep transcriptome reprogramming occurring during organ phase transition to mature growth. We used the DAP-seq approach combined to transcriptomic data to identify high confidence VviCARPO targets, revealing its role as positive regulator of genes involved in secondary metabolism and leaf senescence. In particular, we found that VviCARPO could induce anthocyanin accumulation and chlorophyll degradation through the up regulation of VviMBA1 and VviSGR1, respectively. Moreover, we demonstrated the VviNAC60 capability to interact with itself as homodimer and the heterodimers formation of VviCARPO with two other maturation-related NAC genes, VviNAC33 and VviNAC03, through BiFC analysis in protoplasts. Grapevine stable transgenic lines overexpressing VviCARPO and transgenic chimeric repressors of VviCARPO showed a clear alteration of senescence process. In addition, the VviCARPO overexpression in the tomato nor mutant showed the same level of restoring than the NAC-NOR, supporting again its role as a positive regulator in fruit ripening. Taken together, these results led us to propose a regulatory network model of the VviCARPO mechanisms of action in which it emerges as a master regulator of the grapevine vegetative-to-mature phase organ transition by controlling different ripening- and senescence-related processes.

Keywords: NAC transcription factors, master regulators, grapevine functional analysis, grapevine transformation, tomato NOR mutant

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TOPIC C: FRUIT COMPOSITION
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L23

Modeling berry growth and quality formation at multiple levels

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Grape quality is a complex trait that mainly refers to berry chemical composition, including sugars, organic acids, phenolics, aroma and aroma precursor compounds. It is known that the composition and concentration of chemical compounds dynamically change along berry development and can be affected by genotypes (rootstock and scion), environment (light, temperature and water) and nutrient status (carbon and nitrogen). Moreover, the ongoing climate change is affecting the physiology of grapevine and ultimately wine quality and typicity. Therefore, a better understanding of the mechanisms controlling the accumulation of quality-related metabolites (both primary and secondary) in grape berry is essential to choose grapevine cultivars and viticultural practices best adapted to a given growth region. Process-based models can mechanistically integrate various processes involved in fruit growth and composition and simulate the plant responses to weather and management practices, making them a promising tool to study the response of berry quality to those factors.

We developed process-based models for sugar accumulation and anthocyanin composition in grape berries, which allowed us to determine the key processes responsible for these two important quality components. A 3D structure-functional model was developed to simulate water transport, leaf gas exchanges, carbon allocation, and berry growth in various genotype x environment scenarios. The interactions among the different scales of regulation will be further modelled to offer a model toolkit that allows more accurate predictions of grapevine growth and berry quality elaboration under changing environments and paving a way towards model-assisted breeding. Recent advances in fruit modelling will also be comprehensively introduced.

Keywords: Environmental adaptation, Vitis vinifera, berry quality, modeling.

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L24

Delayed ripening affects the formation of berry quality traits in Cabernet Sauvignon grapes

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In response to extremes of heat and drought, grape berry sugar accumulation has been shown to accelerate, resulting in the desynchronization between primary and secondary berry metabolites. The development of cultural practices to reduce the rate of sugar accumulation during ripening has been advocated in order to re-synchronize sugar accumulation with the accumulation of berry constituents responsible for wine aroma, mouthfeel and color. Experimental treatments were used to obtain fruit ripening rates that were faster and slower than the control. The effect on the accumulation of primary and secondary metabolites was characterized through a sequential sampling approach. Delayed fruit ripening, as measured by reduced sugar accumulation rates, had negligible effects on the concentrations of organic acids, which were mainly responsive to the maturity stage. The phenolic composition of grapes was either unchanged or positively impacted by delayed ripening, suggesting benefits to wine color and mouthfeel. Slowing down ripening also improved grape aroma composition, decreasing the concentration of compounds responsible for undesirable green aromas while increasing desired fruit aromas, volatiles and glycosides. These data further demonstrate the importance of controlling berry ripening rate in order to increase the value and winemaking potential of grapes grown under the warmer and drier conditions associated with a changing climate.

Keywords: delayed ripening, fruit composition, grape quality, organic acids, phenolic compounds

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L25

High-density genetic mapping and aroma compound-related QTL analysis in a white wine F1 population

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A modern grapevine variety is selected for three major branches: (1) resistances against biotic (major pathogens) and abiotic stress, (2) general viticultural suitability (e.g. vine vigor, yield and phenological adaptation) and (3) the wine quality potential as the most important trait. Markers for marker-assisted selection (MAS) of pathogen resistances are available, while markers or prediction tools for the wine quality potential are developing slowly due to the complexity of this trait with a strong environmental variation. Hence, breeders highly demand the development of genetic markers regarding wine quality potential, as they will increase grapevine breeding efficiency considerably. In a first step, validated and reliable tools bear the potential to an early removal of poor quality genotypes in the beginning (negative selection) and could lead to an early selection of high quality genotypes in an advanced stage (positive selection). The project »SelWineQ« aims therefore at the development of novel and robust quality prediction tools by combining genomic, metabolomic and sensory approaches. The backbone of the project is an intensively phenotyped and genotyped white wine F1 population (150 F1 plants; 'Calardis Musqué' x 'Villard Blanc') cultivated at two locations (Geilweilerhof; Neustadt/Weinstraße), showing a multitude of segregating traits.

Genotyping by sequencing (GBS) data was used and an innovative bioinformatic pipeline developed that extracts adjacent biallelic SNPs as local haplotype information. The resulting haplophased markers were used to construct a high-density genetic map with more than 110 highly-informative co-dominant markers almost equally distributed along each of the 19 grapevine chromosomes (2260 markers in total). As promising results, wine quality-related aroma compounds (monoterpenes, C13-norisoprenoids) determined in the wines led to several promising QTL present in consecutive years providing the basis for the development of new quality-trait linked genetic markers.

Keywords: GBS, genetic mapping, QTL, terpenes, norisoprenoids, genetic marker

L26

Grape berry shrivel disorder: lessons learnt and knowledge gaps

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Grapevine ripening physiological disorders affect considerably the yield of the vine and the quality of the grape berries. Among them the sugar accumulation disorder, known as berry shrivel (BS), sugar accumulation disorder (SAD) or suppression of uniform ripening (SOUR), is a grapevine ripening disorder with still unclear physiological mechanisms. Recognizable symptoms of BS are: a suppression of sugar accumulation shortly after veraison, high contents of organic acids, low pH values, low amounts of anthocyanin in red grape varieties and finally shrinking berries. Shrinking berries are a common feature for all shriveling disorders in grapevine like sunburn, late-season dehydration, bunch stem necrosis and BS, but time of symptom emergence, anatomical changes and metabolic shifts provide clear distinctions. The current knowledge on biochemical, morphological and molecular processes affected by BS increased substantially the last years, but this information has to be validated, cross-linked with each other and related to the enormous amount of available information on grape berry ripening control in general. Identifying similarities, dissimilarities and knowledge gaps may provide us with the data basis to develop specific hypothesis about BS induction. The presented study aims to extract the most recent developments in BS research, highlight major steps forward in understanding BS symptom developments, identify overlaps with other ripening disorders and discuss obvious knowledge gaps. Thereby we focus on the process of grape berry ripening control, as BS sugar accumulation stops short after veraison and recent transcriptomic results identified a lack or delay in “switch” gene induction in affected berries. A key question is, if there is a common regulation of this set of genes and phytohormones are realistic candidates. Additionally, it would be tempting to know, which process(es) keeps BS berries metabolically active during the ripening phase, as shedding or abscission of such berries would be resourceful. By combining phytohormone analyses with RNASeq results, we propose two distinct photohormone profiles before and after veraison. A pre-verasion ACC peak was determined which possibly could interact with the complex ethylene-auxin relationship in ripening control, while during the ripening process other differences in the phytohormone profiles were observed, maybe associated with withering processes and sink activity in berries. Many of the targeted processes are not yet fully understood, therefor we need to develop sophisticated approaches and defined experiments in order to scale down the complex phenomenon of ripening disorders in grapevine. By merging and analysing existing knowledge from different approaches, we can deduce more precise hypothesis of BS induction and thereafter successful prevention strategies.

Keywords: Berry Shrivel, grape berry ripening, phytohormones

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L27

Combining leaf-to-fruit ratio manipulations with abscisic acid application adjusts sugar and anthocyanin concentrations in ripening berries

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Climate change is causing extreme weather events to increase both in frequency and intensity, significantly affecting vine physiology and grape berry composition. Elevated temperatures (ET) typically result in increased berry sugar concentrations, while reducing organic acids and anthocyanin levels, modifying wine quality and typicality. Viticultural practices such as leaf-to-fruit ratio manipulation, combined with application of abscisic acid (ABA) can potentially be used to mitigate ET effects by adjusting berry composition. To explore the biological mechanisms underlying the effects of leaf-to-fruit ratio manipulations and application of ABA on berry composition, transcript abundance of genes related to sugar, anthocyanins and ABA metabolism were studied on greenhouse-grown Cabernet-Sauvignon fruiting cuttings.

The results confirmed that reducing the leaf-to-fruit ratio had a significant effect on berry composition, with a reduction of sugar and anthocyanin content, a slight increase in total organic acids and modification of the free amino acid composition. ABA application increased sugar and anthocyanin concentration, and partially restored the coupling between sugar and anthocyanin accumulation under low leaf-to-fruit ratios, without affecting the amino acid/sugar/organic acid ratios. Transcript abundance analysis revealed that several anthocyanin biosynthetic genes (CHS2, CHS3, CHI, F3H, DFR, LODX, UFGT, MybA1 and MybA2) were decreased under low leaf-to-fruit ratio, whereas some genes (CHI, F3H, F3’5’H, LODX, UFGT, MybA1 and MybA2) were up-regulated after ABA treatment. ABA treatment had little effect on the transcript abundance of sugar accumulation related genes, and leaf-to-fruit ratio also had little effect on ABA biosynthetic genes.

In conclusion, our results showed that ABA and sugar signaling synergistically interact to regulate the expression of anthocyanin biosynthetic genes and increase anthocyanin accumulation during berry ripening. Thus, ABA application was able to increase the ratio of anthocyanins to sugar under low leaf-to-fruit ratio at harvest, and combining leaf-to-fruit ratio manipulation with ABA applications may offer a fine-tuned way to reduce sugar concentration, while maintaining anthocyanin concentrations in grape berry, potentially offering a way to partially alleviate climate change related warming effects.

Keywords: Berry composition, climate change, abscisic acid, leaf-to-fruit ratio, sugar, anthocyanins

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L27

Combining leaf-to-fruit ratio manipulations with abscisic acid application adjusts sugar and anthocyanin concentrations in ripening berries

Michaela Griesser[a]*, Stefania Savoi[b], Radomira Vankova[c], Astrid Forneck[d]
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Keywords: Berry composition, climate change, abscisic acid, leaf-to-fruit ratio, sugar, anthocyanins

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L28

The consequences of plant growth regulators application and girdling on phenolic and volatiles composition of grapes

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Berry enlargement in seedless grapes is carried out by the plant growth regulators (PGRs) gibberellic acid (GA3), synthetic cytokinin forchlorfenuron (CPPU) and by trunk girdling and it is of interest to understand what the effects of those treatments on berry composition are. GA3, CPPU (and their combination) and trunk girdling were applied to 6 mm diameter fruitlets of 'Sable Seedless', and berries were sampled 51 and 70 days (d) following application. PGR treatments increased berry size and delayed sugar accumulation and acid degradation with a stronger effect of CPPU. Similarly, trunk girdling resulted in a consistent increase in berry size with a minor effect on sugar content. While CPPU reduced the levels of anthocyanins by 50%, the GA+CPPU and girdling reduced the levels by about 25% (51 d) and 22% (70 d) respectively. The CPPU treatment had minor effects on flavonol content but increased the levels of monomeric flavan-3-ols and proanthocyanidins (PAs). Similarly, girdling increased the levels of monomeric flavan-3-ols, flavonol and PAs. The mean degree of polymerization (mDP) was reduced from 26 to 21 and 19 in girdling and CPPU, respectively. Volatile analysis by GC-MS showed changes in composition with CPPU, GA_3 and girdling treatment with potential impact on flavour. RNA-seq analysis showed that GA_3 had a minor overall effect on the transcriptome whereas CPPU had pronounced effects on gene expression at both 51 and 70 d. Comparing the control and CPPU at similar Brix of ca. 19.7°, a reduced expression of stilbene synthases (STSs) including their regulators VvMYB14 and VvMYB15, and other phenylpropanoid-related genes was observed in CPPU-treated grapes. Overall, our study shows that CPPU and girdling had a major influence on the phenylpropanoid pathway and aroma related compounds whereas GA_3 has no effect on the phenylpropanoid pathway.

Keywords: Vitis vinifera; seedless; table grapes; tannins; proanthocyanidin; CPPU; GAs; Girdling; Phenolics

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L29

Terpene biosynthesis is uncoupled from berry maturity and predominately regulated by solar radiation in Riesling (*Vitis vinifera* L.)

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Certain volatile organic compounds, particularly terpenes and C13 norisoprenoids, increase markedly during grape maturation. Likewise, solar radiation also impacts the biosynthesis of grape volatiles. Despite this knowledge, no information currently exists which suggests whether berry maturity or the environment play a larger role in grape terpene biosynthesis. To investigate this, a 2x2 factorial experiment was established using greenhouse potted Riesling (*Vitis vinifera* L.) vines. The first treatment involved altering berry maturation at 25% veraison (~8 °Brix) by girdling shoots above and below clusters to estimate the effect of halting berry maturity on terpene production. Second, clusters were covered with light-impenetrable bags at the same point, halting solar radiation effects on terpene production. 'Girdling' and 'bagging' treatments were compared to vines subjected to a combination of both treatments, as well as an untreated control. In addition to measuring gas exchange and basic fruit quality parameters, free and glycosylated terpenes were profiled from veraison to harvest maturity stages, including at two (~9 °Brix), ten (~14 °Brix), and 30 days (~20 °Brix) post-treatment to gauge short-, mid-, and long-term responses to treatments. In 'bagging' treatments, solar radiation was reduced from ~250 µmol m-2 s-1 to < 2 µmol m-2 s-1 without altering berry temperature. Photosynthetic assimilation was significantly decreased by 'girdling' from one-week post-treatment until harvest due to feedback inhibition of photosynthesis. Total soluble solids accumulation was unaffected by 'bagging,' but was significantly inhibited by 'girdling'. Terpenes increased from mid-ripening to harvest maturity, and were significantly decreased by 'bagging,' but unaffected by 'girdling'. Individual terpenes involved in Riesling varietal character were largely reduced (> 90%) by 'bagging', while only linalool oxides (I and II) were decreased (77%, 100%) by 'girdling'. In conclusion, this data suggests that terpene biosynthesis is "uncoupled" from berry maturity and predominately regulated by solar radiation. Additionally, the oxidation of linalool may be related to berry maturity.

Keywords: Grape, terpenes, maturation, radiation, photosynthesis

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L30

Regulated deficit irrigation on cv. Cabernet Sauvignon grapes: impact on aroma and phenolic composition

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Regulated deficit irrigation (RDI) is a technique aiming to address two main objectives: water resource savings and wine composition modification. Even though there is a large body of information that has been produced regarding the impact of RDI on grape and wine composition, there are still gaps to fill regarding site-specific optimization strategies. This study aims to understand the impacts of different levels of deficit irrigation on a large number of metabolites and other chemical parameters in grapes, from aroma to non-volatile compounds, in an industrial-scale trial. A three-year RDI experimental trial was implemented in a commercial vineyard of Cabernet Sauvignon in the Maule Valley in Central Chile. Four RDI treatments were employed to replenish different portions of evapotranspiration (ET) from pea-size until harvest. These irrigation treatments were conceived as 100% ET, 70% ET, 50-100% ET (50% ET before veraison and 100% ET afterwards) and 35-100% ET (35% ET before veraison and 100% ET afterwards). The following parameters were measured: midday stem water potential (Ψstem), stomatal conductance (gs), grape growth, yield and must composition. Chemical analysis included GS-MS/MS for C13-norisoprenoids, monoterpenes, C6 alcohols and aldehydes, and methoxypyrazines; and HPLC for anthocyanin and low molecular weight phenols.
RDI significantly affected yield, mainly due to reductions in cluster weight, berry size, and berry number. °Brix were found to be similar with no clear effect of the most restrictive RDI against the control. Significant differences were found in several secondary metabolites including higher concentrations of anthocyanin, tannins, and some specific flavonoids like catechin, quercetin, and myricetin in the most water-restricted treatments. Regarding aromas, RDI treatments increased the concentration of isobutyl-2-methoxypyrazine (IBMP), hexanal, 1,4 and 1,8-cineole, and linalool. Indicating that in the long run, RDI will concentrate most of the metabolites, either positive like anthocyanins and quercetins or potentially negative such as IBMP and hexanal.

Keywords: regulated deficit irrigation, Cabernet Sauvignon, stem water potential, stomatal conductance, yield components, IBMP, low molecular weight phenols

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L31

Increased temperature, elevated atmospheric CO_2 and water deficit modify grape composition of different Tempranillo (*Vitis vinifera* L.) clones

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Grape composition is strongly influenced by climate growing conditions and their modification, as expected in the coming years (increased temperature, elevated atmospheric CO_2 levels and water scarcity) could significantly modify the biochemical composition of berries at harvest, and thus wine typicity. The rich intra-varietal diversity opens the possibility to select better adapted plant material in order to mitigate the potential negative effects of climate change on grape quality. The effects of increased temperature, elevated atmospheric CO_2 levels and water deficit on grape composition of Tempranillo clones, which differ in the length of their reproductive cycle, were studied with the aim of detecting potential interesting traits among them. Fruit-bearing cuttings were grown in temperature gradient greenhouses and in growth chamber greenhouses under two temperatures (ambient temperature versus ambient temperature + 4ºC), two CO_2 levels (400 ppm versus 700 ppm) and two water regimes (well-watered versus water deficit), both in combination or independently, in order to simulate future climate change scenarios. The concentration of sugars, malic acid, amino acids and anthocyanins in grapes was measured. Elevated temperature favoured hastened sugar accumulation in grapes, both individually and combined with high CO_2. However, this effect was mitigated by water deficit. On the other hand, elevated temperature increased malic acid degradation, notably when combined with elevated CO_2 level and water deficit. Total anthocyanin levels at maturity were not strongly modified by the increase of temperature and CO_2 levels but the combination of elevated temperature, high CO_2 and water deficit led to a strong decrease of anthocyanin levels and to the imbalance between anthocyanins and sugars. The magnitude of these alterations was different among clones, which was not always solely explained by differences in the length of reproductive cycle, thus indicating intra-varietal variability in the response of Tempranillo to climate conditions. These results highlight the importance of multi-stress approaches to provide insights into the response of clones to optimize the adaptation of traditional cultivars in their growing regions.

Keywords: berry composition, climate change, CO_2 level, drought, heat stress

Keywords: regulated deficit irrigation, Cabernet Sauvignon, stem water potential, stomatal conductance, yield components, IBMP, low molecular weight phenols

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L32

Regulated deficit irrigation on cv. Cabernet Sauvignon grapes: impact on aroma and phenolic composition

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Epigenetics corresponds to the complement of genetic information carried in chromatin beyond the DNA sequence. It includes the post translational modification of histones, DNA methylation and small RNAs, all involved in the control of gene expression and transposon mobility. Studies in *Arabidopsis*, tomato and many other plants, have demonstrated the relevance of epigenetic mechanisms in the control of development, their impact on agronomic traits and on genome stability, as well as their role in the responses of plants to various stresses. However, most epigenetic studies so far were performed on annual plants, whereas epigenetic studies in perennials are still in their infancy. Hence as a woody perennial, grafted and clonally propagated plant, grapevine presents unique features that make it an interesting model for epigenetic studies.

In this context we have investigated the role of epigenetic mechanisms, focusing on DNA methylation, in grapevine berries, demonstrating tissue specific modifications of DNA methylation during berry development and ripening. Additional works have analyzed the impact of grafting on both scion and rootstock small RNA populations and epigenetic landscapes as well as the response and adaptation of grapevine to stresses (drought stress, pesticide, carbon deprivation). More particularly we have investigated the possible role of DNA methylation in the epigenetic memory of stresses over years. Various aspects of the data we have obtained will be discussed in light of the current knowledge of epigenetic regulations in plants.

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L33

Impact of ripening-related hormones on terpene biosynthesis in Gewürztraminer grapes (*Vitis vinifera* L.)

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Terpenes dictate the grape flavour of several wine grape varieties and the aroma of the derived wines. In these varieties, terpene biosynthesis is strongly promoted during ripening and terpene levels peak around harvest time. Terpenes can be accumulated in the berry as free (volatile) or glycosylated (non-volatile) forms. Despite their importance, little is known about the endogenous factors controlling terpene biosynthesis in grape. In this study, abscisic acid, ethylene, indoleacetic acid, naphthalene acetic acid, jasmonic acid, and methyl jasmonate, that either promote or repress ripening, were applied to Gewürztraminer clusters at véraison to assess their effect on ripening and terpene biosynthesis. Ethylene and methyl jasmonate uncoupled fruit technological maturity from terpene biosynthesis indicating that terpene production is not strictly controlled by sugar levels. Additionally, jasmonic acid, and methyl jasmonate induced the biosynthesis of both free and glycosylated terpenes through the upregulation of terpene genes. This result was achieved without significant crosstalk from other ripening related hormones.

Keywords: aroma, gene expression, hormones, ripening, terpenes

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TOPIC D: COMPUTATIONAL RESOURCES AND DATA INTEGRATION
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L34

Artificial Intelligence advances for Digital Viticulture and Oenology

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Climate change has threatened the status quo within the viticultural and wine industry due to increased ambient temperatures, the variability of precipitation, and increased climatic risks. These main threats are specifically related to the compression of phenological stages, earlier harvests, many of these within the hottest months producing a dual warming effect. Furthermore, the increase of climatic anomalies, such as floods, frosts and bushfires, in number, intensity and window of opportunity within the growing season directly impacts yield and grape and wine quality. New and emerging technologies could play a critical role in the viticulture and winemaking of the future. The viticulture and winemaking of the future need to have a transformational process to be more predictive rather than only reactive by implementing disruptive technology based on artificial intelligence (AI).

There is currently enough knowledge related to digital viticulture and winemaking that could be readily integrated into the grape production, winemaking and commercialization chain. This integration will allow stakeholders to share information to maintain wine styles, increase quality, and respond better to environmental hazards and climate change. Precision and digital viticulture has been recognized as the best approach to maintain and increase the competitiveness of the wine industry in a changing climate and in a market where consumers are demanding higher quality and production standards.

Technologies to be discussed are related to viticulture using unmanned aerial vehicles and remote sensing, short-range remote sensing using infrared thermal imagery, near-infrared spectroscopy. For winemaking, it will cover the development of new integrated electronic nose sensor technology to assess grapevine biotic and abiotic stresses, smoke contamination in grapes, and smoke taint in wines. All these technologies are coupled with machine learning and AI applications for the viticultural and winemaking industries.

Keywords: Machine learning, computer vision, sensor technology

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L35

Genome-wide exploration of transcription factor targets involved in grape specialized metabolism and visualization through DAPBrowse: a centralized genome-browser for DAP-seq data

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Transcription factors (TFs) act on cis-regulatory DNA sequences that determine when, where, and how genes are expressed. Several studies in plant model species have shown that development-related TFs are able to bind thousands of genes and directly regulate the expression of a considerable fraction of them. However, the extent of this massive regulation (from now on called 'orchestration') has not been explored for specialized metabolism-related regulators. In grapevine, many TFs controlling specialised metabolism have been characterised to date but just a few targets are known for most of them, representing an ideal case for addressing the extent of their binding landscapes (cistromes). Accordingly, we have examined the cistromes of previously characterized R^2R^3-MYB regulators using the *in vitro* genome-wide approach DNA affinity purification sequencing (DAP-Seq). For all of them, we obtained thousands of binding events assigned to genes, with around 30% of peaks falling within -5kb from transcription start sites. Bound genes were overlapped with condition-dependent aggregate gene centred co-expression networks and in some cases with the transcriptomes of TF-overexpressing plants to predict and validate high confidence targets. Our results suggest that specialized metabolism TF orchestrators may also regulate several primary metabolic or hormone pathways and responses to the environment. We demonstrate how this approach can also be used to identify novel pathway genes which can later be characterized. To visualise the cistromes of different TFs we have created a Jbrowse based platform called DAPBrowse which is currently hosted for the grapevine community. This platform provides a way to explore DAP-Seq data for specialised metabolism TFs in the context of the 12XV2 PN40024 genome assembly and it's being updated with the new PN40024.v4 genome assembly.

Keywords: DAPBrowse, MYB, cistrome, anthocyanins, stilbenes

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L36

Vitis OneGenE: a causality-based method for gene network analysis in grapevine. Characterization of the laccase and dirigent protein gene families

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The availability of transcriptomic data organized in coherent databases and the development of causal inference methods have paved the way to gene network analysis in grapevine. The identification and visualization of gene-gene interactions as networks can be supportive to several aims, such as the identification of hub genes or modules, the prediction of gene function, the integration of different -omics data and the comparison of the found networks across species. OneGenE, an *in-house* developed causality-based data mining tool, has been applied to the *Vitis Vespucci* dataset to produce a list of directly interacting genes, for each gene of the grapevine genome. Due to the computational power requirement of the OneGenE algorithm, it has been run as part of the gene@home project, a distributed computation project relying on thousands of volunteers' computers by means of the TN-Grid, an infrastructure based on BOINC system. Finally, bioinformatics tools have been built *in-house* to reconstruct association gene networks using OneGenE generated lists. All this information and analysis tools are now available to the community through the website vitis.onegenexp.eu. Here, we present the integration between OneGeneE approach and a phylogenetic analysis to *in silico* characterize the two gene families of laccases and dirigent proteins. These enzymes are considered to be involved in the final steps of lignin biosynthesis, but we found that in grapevine a diverging group of them seemed rather related to the biosynthesis of oligomeric stilbenoids, important plant phytoalexins with antifungal properties. Co-expression analyses performed in dedicated datasets supported this hypothesis. Although experimental validation is undoubtedly required to confirm these findings, they represent an interesting example of how the causality-based OneGenE approach can drive knowledge discovery in grapevine.

Keywords: OneGenE, gene network, laccase, dirigent protein, causal inference, stilbenoids, co-expression analysis

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L37

Small RNA populations reflect the complex dialogue established between heterograft partners in grapevine

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Grafting is an ancient method that has been intensively used for the clonal propagation of vegetables and woody trees. Despite its importance in agriculture the physiological and molecular mechanisms underlying phenotypic changes of plants following grafting are still poorly understood. In the present study, we analyse the populations of small RNAs in homo- and heterografts and take advantage of the sequence differences in the genomes of heterograft partners to analyse the possible exchange of small RNAs. We demonstrate that the type of grafting per se dramatically influences the small RNA populations independently of genotypes but also show genotype specific effects. In addition, we demonstrate that bilateral exchanges of small RNAs, mainly short interfering RNAs, may occur in heterograft with the preferential transfer of small RNAs from the scion to the rootstock. Altogether, the results suggest that small RNAs may have an important role in the phenotype modifications observed in heterografts.

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L38

Using geolocated proxy sensor systems to assess spatiotemporal stability of seasonal wood biomass in northern California vineyards

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Previous research has investigated the temporal stability of spatial variation of vineyard wood biomass as a tool for optimizing crop management practices. These studies have typically used manual pruning weight measurements to obtain data over blocks of a single variety in small geographic areas. Recent advances in proxy sensor systems have allowed for the rapid collection of vine wood biomass data across hundreds of acres of vineyards within a single dormant season. This project seeks to build on previous research efforts to better understand spatial variation in vine size throughout vineyards and determine to what extent this variation is stable over multiple years. Data collected via a Physiocap wood biomass mapping system will be used to analyze the spatial and temporal stability in annual wood biomass in the context of block-specific variables of California vineyards. Physiocap's raw shoot diameter data points will be processed into raster layers of wood biomass before the layers are analyzed to assess the spatial and temporal variation between and within vineyard blocks. Values will be examined in a block-specific context along with parameters like grape variety, management style, or local weather conditions. It is anticipated that this project will yield similar results as previous studies, showing that spatial patterns of wood biomass are generally preserved while individual vine size measurements vary significantly both spatially within the field and temporally between vintages. It is hypothesized that this variance or stability of wood biomass is stronger in specific grape varieties, or in those with different growing terroir.

Keywords: biomass, dormancy, geospatial, geolocation, GIS, proxy sensors, pruning, sensors, spatial, spatial analysis, wood

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TOPIC E: TECHNOLOGICAL ADVANCES
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L39

Climate protection based on increased humus amounts in vineyard soils: Sensor-based characterization of grapevine vitality

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Humus rich soils form one of the largest terrestrial carbon repositories and, thus, contribute significantly to climate protection. In the context of climate change mitigation, the incorporation of organic amendments into soils may further contribute to reduce carbon from the atmosphere. In vineyards, deep cultivation is typically performed only once before planting, followed by decades of rest of the subsoil. Slower humus degradation can therefore be expected, which is why vineyard soils are particularly suitable for long-term carbon storage. In order to investigate the effect of this novel approach on soil characteristics, greenhouse gas emissions and plant behaviour, two substrates (greenwaste compost and biochar compost substrate) were incorporated into the subsoil of vineyards. By planting the fungus-resistant grapevine cultivar 'Calardis Musqué', fewer plant protection applications are required leading to less tractor traffic which subsequently reduces both soil consolidation and further CO_2 emissions.

The effect of these organic amendments on grapevine health and vitality is evaluated using innovative sensor technologies. Data were assessed during the most important phenological stages (flowering, véraison, harvest) over three consecutive years using two non-imaging optical sensors – Dualex Scientific and FieldSpec 4 Hi-Res. Thereby, chlorophyll content was the focus of these analyses, as it is an important stress indicator. High correlation of sensor data with ground truth chlorophyll data was observed. In addition, grape health and must quality were examined in detail. Besides using FTIR analyses to determine ripening parameters (e.g. sugar and acid contents), the BI sensor was applied in order to phenotype the berry impedance Zrel. Thereby, Zrel indicates permeability and thickness of the cuticle and its epicuticular wax layers, which can provide information on *Botrytis* resilience. Preliminary results already indicate differences in plant vitality depending on the respective organic material incorporated, but also on grapevines' position within the vineyard.

Keywords: sensor-based phenotyping, organic soil amendments, grapevine vitality, grape quality

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L40

Estimation of grapevine lateral leaf area (LLA) from RGB imagery using colour thresholds and automatic k-means image segmentation

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Lateral leaf area (LLA) is an alternative method to evaluate canopy size and leaf area index (LAI). LLA estimation requires accurate image segmentation since conditions such as shadows, light, or soil interference need to be excluded to increase accuracy. In this study, a field experiment was set up to evaluate LLA determinations using different colour spaces transformations together with manual colour threshold and automated k-means segmentation. In the field, RGB images were obtained of the east side of the canopies at mid-morning using a white panel background to avoid the interface of subsequent rows. Four colour spaces: RGB (red, green, blue), HSV (hue, saturation, value), YCbCr (brightness, Cb, Cr) and CIELab (luminance, green to red, blue to yellow) were evaluated for a colour thresholding process. Also, an automatic method using k-means classification was evaluated. Each image was first subjected to a detailed manual image segmentation process to create a reference LLA-image which represented the true LLA value where only the leaves of the canopy are present. Manual and automatic methods were compared against this reference LLA-image using a MATLAB script to obtain the accuracy of the methods (% of agreement). Results show that the automatic k-means method presented good performance for low and medium vigour vines. Among the colour spaces tested, HSV was the most promising for LLA estimation, regardless of the density of the canopy with a consistent agreement of around 80 to 90%. The methodology presented is accurate, inexpensive and can be applied to seasonal LLA-image data to evaluate spatial and temporal variability of canopy size.

Keywords: Lateral leaf area, colour spaces, RGB-image, colour threshold, image segmentation

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L41

Whole genome resequencing and custom genotyping unveil clonal lineages in `Malbec´ grapevines (*Vitis vinifera* L.)

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Grapevine cultivars are clonally propagated to preserve their varietal attributes. However, genetic variations accumulate due to the occurrence of somatic mutations. This process is anthropically influenced through plant transportation, clonal propagation and selection. Malbec is a cultivar that is well-appreciated for the elaboration of red wine. It originated in Southwestern France and was introduced in Argentina during the 1850s. In order to study the clonal genetic diversity of Malbec grapevines, we generated whole-genome resequencing data for four accessions with different clonal propagation records. A stringent variant calling procedure was established to identify reliable polymorphisms among the analyzed accessions. The latter procedure retrieved 941 single nucleotide variants (SNVs). A reduced set of the detected SNVs was corroborated through Sanger sequencing and employed to custom-design a genotyping experiment. We successfully genotyped 214 Malbec accessions using 41 SNVs and identified 14 genotypes that clustered in two genetically divergent clonal lineages. These lineages were associated with the time span of clonal propagation of the analyzed accessions in Argentina and Europe. Our results show the usefulness of this approach for the study of the scarce intra-cultivar genetic diversity in grapevines. We also provide evidence on how human actions might have driven the accumulation of different somatic mutations, ultimately shaping the Malbec genetic diversity pattern.

Keywords: Malbec, genetic diversity, clonal lineages, propagation history, whole genome resequencing

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L42

High-throughput field phenotyping in vineyards: demand, approaches, objectives

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Phenotyping in perennials, like grapevine, needs to be done directly in the field. Phenotyping methods and tools are very useful for genetic studies, breeding, and to develop new precision viticulture applications. Standard methodologies encompass visual scoring of phenotypic traits (e.g. ampelographic traits, yield, and disease resistance) and phenology (e.g. bud burst, flowering, veraison) as well as the use of physiological measurements of rate of photosynthesis and of water status or destructive biochemical measurements for quality assessment, as well as molecular genetic procedures.

In recent years, however, there have been significant advances in the development and application of non-destructive and sensor-based phenotyping technologies suitable for use on a vineyard scale and designed for high throughput in breeding programs or even precision applications in viticulture.

The state-of-the-art of research for application of phenotyping in grapevine management and breeding will be discussed such as:

- the needs and technical requirements (field research, traits, throughput) from the
- perspective of agronomic management practices and breeding programs;
- the current non-invasive technologies (imaging methods, data analysis pipelines and
- automation), that have significant chance of application in grapevine, based on the needs and traits;
- finally, a critical evaluation of the most promising research directions within and some future avenues for solutions for specific use cases.

Keywords: automation, phenotyping bottleneck, digitalization, precision viticulture, disease detection, phenotyping platform, sensor-based approaches

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L43

Monitoring grapevine water status by unmanned aerial vehicle (UAV) and plant based sensors

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In the last decade unmanned aerial vehicles (UAV) have been proposed for the monitoring of plant water status due to their flexibility in flight scheduling and the increasing availability of dedicated miniaturized sensors. Similarly, plant based sensors (PBS) are becoming of common use because they allow to continuously monitor different parameters linked to plant water status. We tested UAV and PBS to monitor vine water status in a mature vineyard (cv. Sangiovese) in a sandy clay loam soil, subjected to three irrigation regimes: full irrigation (FI, 100% of reference evapotranspiration), sustained deficit irrigation (DI, 50% of FI) and rain fed (RF). Grapevine water status was monitored by measuring the stem water potential (SWP) throughout the season. Multispectral and thermal images were acquired from an altitude of 50 m above ground level. Three vines for each irrigation treatment were equipped with trunk dendrometers in order to measure continuously the daily trunk diameter fluctuations. Differences in SWP between irrigation treatments were evident starting from 14 days from the beginning of the different irrigation regimes and throughout the irrigation period. The daily water stress integral values of FI, DI and RF vines were -0.47, -0.79 and -1.03 MPa, respectively. The crop water stress index (CWSI) derived from aerial thermal images was well correlated (R^2=0.8) with SWP measured concomitantly. The maximum daily shrinkage (MDS), derived from the daily course of trunk fluctuation, increased as the SWP decreased until approximately -1.0 MPa and then decreased as water deficit increased according to a curvilinear quadratic equation (R^2= 0.65).

Keywords: crop water stress index; dendrometer; maximum daily shrinkage; stem water potential; thermal images

L44

Clonal improvement-associated somatic mutations identified from diploid genome assembly in Tempranillo grapevine cultivar

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Tempranillo is the third most widely grown red wine cultivar worldwide and the most relevant in the Iberian Peninsula. Here we produced a diploid genome assembly of Tempranillo to study the origin of clonal improvement in this cultivar. After PacBio and Oxford Nanopore sequencing, a trio binning approach produced chromosome-arm level and complete assemblies for the two haplo-phases. Based on the *de novo* assembly, we searched for genetic variation in ten re-sequenced Tempranillo clones selected for variation in agronomically relevant traits including fruit colour, low fruit sugar accumulation adaptable to warmer climates or loose bunches that are less prone to fungal diseases. Thousands of somatic mutations were detected in the ten clones, which, combined with gene annotations based on PacBio Iso-seq, identified candidate dominant missense variants related with selected phenotypes. We could also phase inter-chromosomal structural variation (SV) in the origin of white gape and loose cluster phenotypes. Our results provide a quality genome assembly for inter- and intra-cultivar comparisons and release candidate missense and SV mutations that could be responsible for clonal diversification and improvement.

Keywords: somatic variation, clonal improvement, genome assembly, variant calling, structural variation, candidate mutations

L45

Generation of non-transgenic mildew-resistant grapevine clones via gene-editing: potentials and hurdles

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Grapevine (*Vitis vinifera*) is among the most valuable crops cultivated in Europe for both economic and cultural reasons, but it is also highly susceptible to fungal diseases such as powdery mildew (PM) and downy mildew (DM). The control of these diseases' accounts for over 50% of the fungicides that are used every year in Europe, raising the urgent need for sustainable viticulture practices with a lower impact on human health and the environment. Such practices include, but are not limited to, the introduction of PM- and DM-resistant varieties. The recent advent of New Breeding Techniques (NBTs), and in particular of gene-editing, offered a great opportunity to obtain resistant plants either by the introduction of known resistance genes, or by knocking out susceptibility genes in commercial cultivars. As compared to traditional breeding, this approach has the advantage of maintaining the integrity of the parental variety by introducing very specific and small mutations, and of being faster because it doesn't require several cycles of backcrossing. Some hurdles need to be overcome before the creation of non-transgenic grape cultivars by NBTs becomes of practice: i) the identification of appropriate target genes to generate resistant cultivars, ii) the development of efficient protocols to deliver the CRISPR-Cas machinery as protein/RNA complex into single cells, and iii) plant regeneration. In addition to these technical problems, the products of NBTs are considered GMOs under the current European regulation. In the last years, we tackled these challenges by acting along two lines. On one hand, we characterized susceptibility genes of the MLO and DMR6 gene families by generation of knock-out mutants, in order to identify which genes are required for the establishment of the DM- and PM-diseases. Embryogenic calli were transformed via *Agrobacterium tumefaciens* with CRISPR/Cas9 vectors designed to specifically edit candidate susceptibility genes. Highly efficient targeted-mutagenesis in one or two genes was obtained, and several edited lines were regenerated from transformed calli. Edited plants grown in soil were challenged with the DM and PM pathogens, and preliminary results highlighted a role of these genes in grapevine susceptibility. In parallel, we also developed a new DNA-free methodology to obtain non-transgenic edited grapevine plants regenerated from protoplasts derived from embryogenic calli. These plants were regenerated from a single edited-cell, and therefore do not show chimerism.

Keywords: *Vitis vinifera*, downy mildew, powdery mildew, genome editing, resistance

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Edited grapevine knocked-out for *VvEPFL9-1* showed reduced stomatal density

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Stomatal density and distribution are under the control of small cysteine-rich peptides called epidermal patterning factors (EPFs), highly conserved in a wide range of higher plants. In *Arabidopsis* and cereals, three members of this family play a key role in the formation of stomata: EPF1, EPF2 and EPFL9. EPF2 and EPF1 prevent stomata formation while EPFL9 is a positive regulator, acting antagonistically to the other two. In this work, CRISPR/Cas9 technology was applied to investigate the role of *VvEPFL9-1* gene in grapevine via knockout (KO) mutants. Gene transfer experiments were carried out co-culturing embryogenic calli of a table grape and a rootstock with *Agrobacterium tumefaciens* carrying the binary vector with a specific sgRNA. Edited lines were phenotypically characterized under well-watered and water-stressed conditions using a full suite of physiological parameters. Vvepfl-1 KO plants showed a reduction in stomatal density compared to the wild type (WT) followed by an improved water-use efficiency both under optimal and water stress conditions. In addition, ad-hoc experiments, carried out to test micrografted combinations of KO and WT lines, showed a moderate gain in water-use efficiency when micrografts were formed by an edited scion or rootstock. Our results highlight the possibility to optimize water-use efficiency by manipulating stomatal density via *VvEPFL9-1* knockout and suggests stomatal anatomical features as a promising target for climate change resilient grapevine varieties.

Keywords: Genome editing, stomata, climate change, water use efficiency

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TOPIC A: ENVIRONMENTAL INTERACTIONS
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P1

Water availability and vegetation density constrain the dispersion of wild grapevines populations at the southern distribution edge

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The distribution of plants in space is constrained by eco-geographic factors which determine the range and abundance of the species. Wild grapevine is distributed from Switzerland in the north to the Levant in the south. Yet, little is known on the eco-geographic constrains of this species, especially at the edge of its distribution range. Here, we present a multi-layer characterization of a wide V.v. *Sylvestris* collection representing the southern part of the Levant. A niche modelling analysis of potential habitats featured the relative contribution of environmental factors affecting the distribution of wild grapevine. We show that proximity to flowing water sources and the medium-high vegetation density are the main indicators of wild grapevine distribution. A population structure analysis using 22 SSR standard markers indicated that the wild grapevine population in the southern Levant can be divided into two distinguished clusters in accordance with geographic distribution. Finally, analysis of the wild grapevine accessions phenotypes in the two distinctive genetic populations indicated that leaf hairiness significantly differentiates the two populations while the genetic differentiation between populations was mild. As these two populations originate from environmentally distinct habitats, these observations imply an environmental pressure possibly leading to the development of unique phenotypic traits at the edge of wild grapevine distribution range.

Keywords: Levant, MaxEnt, Population structure, Phenotypic diversity, SSR markers, *Vitis vinifera* ssp. *sylvestris*, Wild grapevine

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P2

Dysbiosis in soil microbial diversity is differently managed by the grapevine rootstock genotype

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The microbiome at the interface with the root system, referred to as the rhizobiome, influences vine fitness and its adaptation with its environment. Grapevine decline, which is depicted as a multiannual yield downsize caused by the sudden or progressive vine death, is not always associated with identified diseases or mineral deficiency or excess. Herein, the role of the soil and root-associated microbiome diversity and functioning to explain such grapevine decline has been explored in vineyard and in a pot experiment.

A plot was selected in a Bordeaux vineyard that contains both a localized area with declining (Symptomatic S) and non-declining vines (asymptomatic AS). Soil analyses confirmed that the decline (loss of vigour, yield and berry quality) was not associated with an identified disease or mineral deficiencies, but with differences in microbial composition and activities between S and AS areas from the same plot.

Soil was collected from the inter-rows nearby S and AS vines, to perform a greenhouse experiment. One-year old plants of Cabernet Sauvignon (CS) grafted onto Riparia Gloire de Montpellier (RGM) and 1103 Paulsen (1103P) were planted in pots filled with the soil collected in S and AS zones. A metagenomic analysis was performed five months after planting on the rhizospheric, roots, and bulk soil compartments to explore the bacterial and fungal diversity in both AS and S soils for RGM and 1103P rootstocks using 16S rRNA gene and ITS high-throughput amplicon sequencing.

The same bacterial and fungal richness has been found in the two soils for both rootstocks, but with a lower microbial diversity in the S soil compared to the AS one. It appears that the rootstock genotype impacts differently the β-diversity, and the abundance of some keystone taxa. Results suggested that S soils display initially a dysbiosis in microbial diversity, that was afterwards differently managed depending on the rootstock genotype.

Keywords: Soil microbiome, microbiome dysbiosis, grapevine decline, rootstock genotype

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P3

Intra-cultivar genetic diversity in improving water use efficiency in grapevine

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Water scarcity is an increasing threat in many wine growing regions worldwide. Given the predicted water scarcity scenarios, optimizing water management in viticulture has become critical to its sustainability and profitability. Improvement of crop water use efficiency (WUE) by selecting the best adapted genotypes has been identified as a possible strategy to address these challenges. The identification of genotypes with the highest WUE within a cultivar would be particularly interesting given the strong support for local cultivars. With this aim, this work assessed the responses to water stress by means of water status parameters, leaf gas exchange and carbon isotopic discrimination in three widespread Spanish red varieties (Tempranillo, Grenache and Mourvedre). Experiments were conducted both in the field and in pots over a period of 3 to 5 years. The results showed that there was a high degree of variability in WUE within each cultivar. The Tempranillo and Grenache showed differences of up to 30% among clones under moderate water stress conditions, while Mourvedre showed an intra-cultivar variability lower than 10%. These results confirm the potential of selecting clones with higher WUE and provide the physiological basis for genetic breeding in grapevine.

Keywords: Clone, *Vitis vinifera*, water stress, breeding

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P4

Grapevine ability to withstand drought stress: comparing the cv. `Touriga Nacional´ with `Tempranillo´ under deficit irrigation at the Portuguese Alentejo winegrowing region

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The aim of this work was to compare physiological responses of the two red grapevine cvs. 'Touriga Nacional' (TN) and Aragonez (AR; syn. 'Tempranillo') cultivated side by side under deficit irrigation during three growing seasons in a commercial vineyard, located at the Portuguese Alentejo winegrowing region. In each varietal plot, a randomized complete block design was established with four replications and two irrigation treatments: DI1 - sustained deficit irrigation (0.28 to 0.36 of crop evapotranspiration) and DI2 (similar irrigation strategy but with lower water volumes: 52 to 72% of DI1). Predawn (ψ_{pd}) and midday (ψ_m) leaf water potential and mid-morning and midday stomatal conductance (g_s) and net CO_2 assimilation (A_n) were periodically assessed. At mid-morning, the leaf to air temperature difference presented a significantly negative correlation with gs for both cvs., however the slope of the regression line was significantly lower for TN. Regarding the relationships between A_n or g_s and ψ, no significant correlations were detected with Ψ_m but ψpd was positively correlated with An and gs. While at mid-morning the slope of regression lines of the relationships between A_n or g_s and ψ_{pd} (independent variable) were similar for both cvs., at midday AR regression lines presented significantly higher slopes than those of TN. The differences observed in the slopes of these regression lines indicates that the two cvs. have different stomatal regulation, with a more "optimistic" behaviour in TN. Furthermore, the higher slope of the regression line between leaf to air temperature difference and gs presented by AR at mid-morning, suggest that this cultivar has more difficulties in heat dissipation than TN, differences that can be due to the higher leaf size of AR. This knowledge will provide information for the choice of the cultivars and management practices to face the challenges of the climate change expected scenarios.

Keywords: leaf water potential, stomatal conductance, photosynthesis, stomatal regulation, Touriga Nacional, Tempranillo

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P5

Suitability of abscisic acid and proline as markers for heat, drought, and combined stress in grapevines

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Heat and drought are among the most limiting abiotic factors in crop production. In view of changing climatic conditions, heat and drought stress will gain relevance in the coming years. Viticulture will be particularly affected by these changes, as above-average climatic changes are predicted for wine-growing regions. There are several reports on the responses of grapevines to simple heat or simple drought stress, but little is known so far about the effect of combined stress on grapevines. In studying heat, drought, and combined heat and drought stress, it is of particular interest to find traits that indicate stress before symptoms become apparent or yield declines. Therefore, we investigated whether the two commonly used traits proline and abscisic acid (ABA) biosynthesis are appropriate markers of heat, drought, or combined stress and whether gene expression of key ABA biosynthesis enzymes is regulated in leaves of two grapevine cultivars under these stress conditions. Plant growth, leaf gas exchange, and photosynthesis were examined to evaluate plant responses to elevated temperature and water deficit. Our findings reveal an interaction between heat and drought stress for gas exchange as well as for proline and ABA biosynthesis. ABA concentration was a suitable marker of heat, drought, and combined stress whereas proline was only good marker for combined stress. Gene expression of P5CS showed the same pattern as proline concentration. Gene expression of NCED1 in leaves was an appropriate marker for drought and combined stress, while NCED2 was not.

Keywords: *Vitis vinifera*; grapevine; heat and drought stress; abscisic acid; proline; P5CS; NCED

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P6

Fungal microbiota associated to the "Matriarca", a pre-phylloxera white *Vitis vinifera* cv Treixadura in NW Spain

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At the end of the 19th century most of the vineyards were devastated by Phylloxera worldwide. Since then, all vines are grafted with American rootstocks resistant to the plague and the use of cultivars without grafting is forbidden. Nevertheless, there are a few ancient vines which survive these days as the "Matriarca", a Treixadura cultivar from 1813 located in Arbo belonging to the Marqués de Vizhoja Cellar (NW Spain). With the focus on biocontrol, the aim of this study was to isolate and identify fungi from different compartments of this vine and test them as potential biocontrol agents. Samples of leaves, scions, roots, and soil were harvested and carried to the laboratory. Tissue samples were disinfected by rinsing in sodium hypochlorite solution and distilled water, dried, cut into small sections, plated on Malt Extract Agar amended with Streptomycin (MEAs) and incubated in darkness at 20-25ºC. Soil samples were dried, sieved and plated on MEAs. After incubation, colonies were subcultured onto Potato Dextrose Agar and pure cultures were firstly identified up to genus level by morphological characteristics and secondly to level species by molecular technics. A total of 22 fungal isolates of 15 different species were selected which many of them are commonly used as biocontrol agents as *Trichoderma* species. To evaluate their antagonist capacity, each isolate was grown in dual cultural test with Neofusicoccum parvum, considered one of the most aggressive fungi involved in grapevine trunk diseases. Further research is needed to understand the roll of these microorganisms in the plant defence system.

Keywords: fungal microbiota, biocontrol, grapevine, Treixadura, *Trichoderma*

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P7

Water stress affects the structural composition of proanthocyanidins in Cabernet Volos skins, with main effects on the biosynthesis of prodelphinidines

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Modern viticulture aims to produce high quality wines with low environmental impact, while being hindered by the challenges posed by climate change. This study would contemplate all these three topics, studying the modifications on the proanthocyanidin composition on berry skins of Cabernet Volos (a new grapevine variety resistant to fungal diseases), under drought conditions. The influence of water stress on proanthocyanidins is still under debate. During the seasons 2018 and 2019, a water stress experiment was carried out at the experimental farm of the University of Udine on Cabernet Volos grown in field conditions, comparing a well-water with a non-irrigated treatment. Berry samples were collected from veraison to harvest, skins were separated under liquid nitrogen and analysed for the structural characteristics of proanthocyanidins (mDP, mean degree of polymerisation; %G, % of galloylation; and %P, % of prodelphinidines). In parallel the expression of target related genes was ascertained through qPCR analysis. In both seasons, on the stressed skin samples mDP and %G were tendentially lower and higher, respectively, while significantly higher values were found for %P. With regard to gene expression, while there was no clear trend for GAT genes (putative genes responsible for the galloylation of proanthocyanidins), there was a clear upregulation of four F3’5’H homologue genes, which correlated well with the higher percentage of prodelphinidines in berry skin proanthocyanidin composition.

Keywords: proanthocyanidins, galloylation, prodelphinidines, water stress, gene expression

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P8

Effects of salicylic acid on leaf stomatal conductance and leaf temperature in grapevines exposed to heat stress

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Salicylic acid (SA) has been reported to improve photosynthetic performance by regulating stomatal conductance (gs) in various crops subjected to heat stress. However, its effects have been inconsistent. In this study, one vinifera grape cultivar, 'Riesling'(RS), and two interspecific hybrids, 'Golden Muscat'(GM) and 'Black Queen'(BQ), were conditioned in an unshaded greenhouse and subjected to foliar spray of 0 (control) or 100 µM SA prior three regimes of 5d ambient maximal temperature (Tmax), long-term heat stress (LH) with an avg. of > 3 hr. of daily T_{max} > 40°C, short-term heat stress (SH) with an avg. of 1 hr. of daily T_{max} > 40°C, and normal temperature (NT) with daily Tmax < 40°C through the 5d period. In LH, leaf stomatal conductance (gs) in GM and RS were significantly increased after the SA spay. On the other hand, SA did not affect gs in BQ exposed to LH or in all three cultivars exposed to SH or NT condition. In SA treatment, significant elevations in gs were recorded at air temperature (T_{air}) > 40oC in GM and RS. Consequently, greater differences in leaf to air temperatures ($T_{leaf-air}$) were observed in RS and GM but not in BQ when exposed to high $T_{air.}$ The results indicated that the effect of foliar SA spray in grapevines was cultivar dependent and only significant when the vine was exposed to prolonged heat stress with daily T_{max} > 40°C.

Keywords: interspecific hybrid, leaf to air temperature difference, Riesling, Golden Muscat, Black Queen

P9

Performance in a semi-arid climate of MS49 and MS104: new genotypes obtained from crosses of Monastrell and Syrah

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Viticulture in Mediterranean climatic areas will have to face an increase in radiation, periods of drought, and a reduction in water resources in the future. Accepting these climatic forecasts, viticulture cannot be sustained only through the application of deficit irrigation techniques and it will be necessary to adopt other complementary measures such as the development of new varieties that are better adapted to drought and high temperatures. This will allow the development of sustainable and high-quality viticulture in such areas. This work presents the results obtained from the study under deficit irrigation and rainfed conditions of two new genotypes from 'Monastrell' x 'Syrah' crosses, MS49 and M104, selected within the improvement program developed at IMIDA and cultivated in the semi-arid Region of Murcia (southeast of Spain). The behavior of these genotypes under conditions of water scarcity and high temperatures was studied by the evaluation of phenological, productive, and grape quality parameters during three seasons. The results obtained show that, under deficit irrigation conditions, the two genotypes gave an adequate and similar yield, but it was decreased by 35% for MS104 and by 56% for MS49 under rainfed conditions. Both genotypes exhibited good enological quality; in particular, MS104 reached physiological maturity with a low value of ºBaumé, under both deficit irrigation (10.9) and rainfed conditions (10.6), which is very interesting for the production of wines with low alcohol contents in hot climates.

Keywords: Deficit irrigation, semi-arid climate, grape quality, productivity, *Vitis vinifera*

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P10

Downy mildew resistance evaluation in 27 new genotypes obtained by breeding programs in Spain

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Downy mildew (caused by *Plasmopara viticola*) is some of the most important diseases of grapevines worldwide, reducing the crop yields and quality and causing economic losses in the wine sector. The need to develop a more sustainable and environmentally friendly form of viticulture has led to numerous grapevine improvement programmes aiming to increase resistance to downy mildew. The success of such programmes relies on the great variation generated by cross-breeding programs that allows the selection of new genotypes, on the availability of protocols that can quantify the resistance/susceptibility of these new genotypes, and on the existence of molecular markers of resistance loci that can aid in the selection process. The present work assesses the presence of *Rpv3* locus, and the degree of phenotypic resistance/susceptibility to downy mildew of 27 new genotypes obtained from crosses between 'Monastrell' and 'Regent'. In general, laboratory assessments of resistance at the phenotype level agreed with the resistance expected from the presence of resistance-associated alleles of simple sequence repeat (SSR) markers for the locus *Rpv3* (inherited from 'Regent'), confirming their usefulness as indicators of likely resistance to downy mildew. It is relevant to underline that 12 genotypes were transgressive with respect to the resistance donor 'Regent' exhibiting a higher level of resistance. The very resistant lines identified here are considered valuable material to be used in further experiments to obtain durable resistant genotypes and to characterize the molecular basis of resistance to downy mildew.

Keywords: Downy mildew, resistance, grapevine, marker-assisted breeding, *Vitis*

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P11

Phenotyping and polyphenolic discrimination of Croatian native grapevine varieties inoculated with *Plasmopara viticola*

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Grapevine-downy mildew interaction is one of the most challenging biotic stresses. In order to avoid undesirable features of non-vinifera species, usually used for resistance breeding, the resistance of cultivated (V. vinifera) varieties to the main diseases is in the spotlight again. Aiming to find differences among Croatian native grapevine varieties in susceptibility to downy mildew, whose causal agent is *Plasmopara viticola*, an experiment has been conducted. Young leaves of 14 Croatian native grapevine varieties, together with Chardonnay (susceptible control variety), Solaris (partially resistant variety) and *Vitis riparia* (resistant genotype) were sampled and used in this research. A leaf disc bioassay was applied according to the OIV descriptor 452-1 whereby the genotypes were ascribed to corresponding OIV classes 1, 3, 5, 7, and 9 from the most susceptible to the totally resistant ones. Polyphenolic compounds were quantified by high-performance liquid chromatography (HPLC). The majority of evaluated native varieties (≈80 %) are highly susceptible or susceptible to P. viticola belonging to OIV classes 1 or 3, respectively. However, scarce sporulation (21-40 %) developed on leaf discs of the following varieties Malvazija istarska, Ranfol and Teran placed them to the OIV class 5. PCA clearly distinguished diverse classes of susceptibility among evaluated *V. vinifera* varieties based on their polyphenolic composition. Nonetheless, their polyphenolic composition in terms upon inoculation was not significantly different. Methods applied in this research proved to be reliable and complement one to another. Early changes in polyphenolic profiles upon inoculation were not found, although they served as a decisive feature for distinguishing classes of susceptibility which were previously obtained by artificial inoculation. Less susceptible grapevine varieties that belong to class 5 could be of interest to use in breeding programs.

Keywords: *Vitis vinifera*, downy mildew, susceptibility, leaf discs, HPLC analysis

P12

Transcriptome analysis of grapevine under drought conditions and identification of key genes responsible for drought tolerance

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Grapevine is one of the most important crops in the world. However, the effect of climatic changes on *Vitis vinifera* L. could affect dramatically to their productivity, causing a lot of economic and ecological damages associated to them. One of the most important factor in their effects is the drought and water availability, and their sensitivity is more pronounced in commercial varieties like Merlot than local varieties like Callet. In order to better understand their adaptive differences between the two varieties previously named we have used the transcriptomic, metabolic and hormonal analysis, in three different levels of drought stress (mild, high and extreme), using Richter 110 as rootstock. The differential expression genes (DEG) analysis was performed using RNA-seq data for each condition in root and leaf. DEG results shown that the adaptation started in roots, expressing more DEGs at mild drought stress in the two varieties. However, more DEGs were found out in Merlot than Callet, showing a principal adaptation in Merlot to mild drought. Nevertheless, in high and extreme drought conditions, DEGs analysis in leaves shown a high increase of DEG in the two varieties, being more pronounced in Callet than Merlot, differencing the strategy of adaptation among them. Hormonal and metabolic results shown two different pathways in drought adaptation. While Merlot destinated more resources to maintain cellular turgor increasing sugar concentration and alditols into the cells, Callet produced more content in secondary metabolites like mono or sesqui-terpenes, helping the sugar transport to the fruit and increasing quality berry. Furthermore, a higher photosynthetic activity was found in Callet than Merlot, showing higher water used efficiency and photosynthesis activity

Keywords: Drought, Grapevine, Transcriptome, Merlot, Callet

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P13

Comparative transcriptome analysis of wild grapevine (*Vitis vinifera* L. ssp *sylvestris*) and commercial rootstock in response to salinity

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Climatic change affects drastically the viticulture causing several abiotic stresses in plants including changes in water availability and salinity in soil. Previous studies have shown that the genotype called AS1B, an accession of wild grapevine (*Vitis vinifera* L ssp *sylvestris*) found in a coastal area of Asturias (Spain), could have better survival rates than Richter 110, a commercial variety commonly used as rootstock, under high salinity conditions in substrate. To found out the genetic basis involving in their different response to this abiotic stress, a transcriptomic profile of these varieties was built using RNA-sequencing. It was made a functional characterisation of the most expressed genes on different times and concentration of NaCl. The results shown that AS1B increased gene expression for ion transportation and post-translational modifications, in comparison to Richter 110, whose gene expression is more focused in pathways involved in hydric regulation and secondary metabolism. In consequence, AS1B profile of gene expression was focusing in ion transport, while Richter 110 had more diffuse response. Moreover, these results show that, in high salinity conditions, Richter 110 cannot respond with the same efficiency and specificity than AS1B, because Richter 110 approach salinity conditions from a water stress perspective, having more proficiency in hydric regulation, but not against ion toxicity. This regulation would imply higher oxidative damage in Richter 110 than AS1B, relying on secondary metabolism expression genes to solve it. These results showed the potential suitability of AS1B as a good alternative to use as rootstock resistant to high salinity concentrations in soil, improving viticulture adaptation as consequence of climatic change.

Keywords: Salinity, *Sylvestris*, R110, Transcriptomic

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P14

Rootstocks modulate the physiology of the scion responses to water deficit

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Rootstocks have been pointed as an option to cope with drought, one of the main effects of global warming. However, the rootstock effects on the scion and at the stomatal level remain unclear.

Aiming to clarify the rootstock impact under drought conditions, the same scion (cv. Tempranillo) growing on five rootstocks: 1103P, R-110, 161-49C, 41B and 140Ru were analyzed in the semiarid winegrowing region of D.O.Ca. Rioja, North Eastern Spain. For this purpose, physiological parameters such as stomatal conductance (g_s), photosynthesis (A_N), and mid-day (ΨMD) and pre-dawn water potentials (ΨPD) as well as stomatal area and density were measured. Then, intrinsic water use efficiency (WUEi) and whole-plant water conductivity ($Kh_{whole\text{-}plant}$) were calculated.

Differences between drought and irrigation treatments were observed in all rootstocks. Individually, 1103P, 41B and 140Ru showed the highest differences in AN and gs between drought and irrigated plants, while 161-49C and R110 were the less responsive to water withholding. Consequently, 1103P and R110 were also the rootstocks with the highest and the lowest $Kh_{whole\text{-}plant}$, respectively. Regarding the individual comparison of the water potentials of each rootstock, 41B and 1103P showed the greatest response to drought, both in the Ψ_{PD} and in the Ψ_{MD}. Water use efficiency (A_N/g_s) showed differences between 140Ru (with the lowest efficiency) and the others in the irrigated plants, being 140Ru and 41B the most responsive to drought stress. Finally, stomatal areas were higher in the irrigation treatment, although there was no association with A_N or with g_s. No differences were found in stomatal density between treatments or between rootstocks.

These results suggested a near-isohydric behavior of the scion with the rootstocks 161-49C and R110 and a near-anisohydric behavior with the rootstocks 1103P, 41B and 140Ru. The former kept their stomata open in response to water deficit while the latter close them. This study emphasizes the importance of choosing the appropriate rootstock based on the specific characteristics of the place.

Keywords: Rootstock, water stress, stomatal regulation

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P15

Small-RNA and methylation landscape of Red Blotch virus genome during the early phase of infection in grapevine (*Vitis vinifera* L.)

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Grapevine red blotch virus (GRBV) is a single-stranded DNA geminivirus that has been identified as the etiological agent of the Grapevine Red Blotch disease. In the early phases of infection, viral-derived small interfering RNAs (vsiRNAs) are generated via the plant RNA interference (RNAi) machinery, which represents one of the first antiviral responses activated by plants. This results in either viral mRNA degradation, known as Post-Transcriptional Gene Silencing (PTGS), or DNA methylation of the viral genome, known as Transcriptional Gene Silencing (TGS). To characterize these early responses we have adapted an Agro-infiltration protocol for monitoring the activity of GRBV every three days. Real-Time PCR assays on infected plants revealed that the exponential phase of viral replication occurred as early as two weeks post-inoculation. We are currently extracting small RNAs from infected plants to identify vsiRNAs through small-RNA sequencing. Short reads will be mapped to the GRBV-NY358 genome using Bowtie2. The resulting alignments will be processed through MISIS2 for the visualization of vsiRNAs across the viral genome. For the evaluation of the viral methylome, a bisulfite sequencing (BS-seq) experiment will be conducted. The resulting reads will be processed through Bismark software for BS-seq reads mapping and methylation calling, and the output will be processed through ggplot2 R-package for graphic representation of the viral methylome. The generation of these two layers of information will contribute to the identification of “hotspots” targeted by grapevine in response to GRBV infection. This information will be essential in the further design of double-stranded RNA sequences aiming to be used to produce a new generation of RNAi-based biopesticide for GRBV. This could represent in the long-term a reliable and economically sustainable alternative to intense labor management and/or removal of infected plants, which are currently the only options available in grapevine production following GRBV infection.

Keywords: Post-Transcriptional Gene Silencing, Transcriptional Gene Silencing, Grapevine Red Blotch Virus

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P16

Dissecting the genetic and physiological mechanisms of grapevine resilience to heat stress

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Grapevine (*Vitis* spp.) is one of the most widely cultivated perennial fruit crops in the world and its economic relevance is mainly related to wine production. In recent years, the increased frequency of extreme phenomena such as heat waves has been acknowledged as one of the most significant climate variables negatively affecting grape yield and berry composition, with consequences also on wine quality. Thus, studying the physiological, metabolic and genetic factors that are involved in grapevine response to high temperatures is essential to improve the knowledge of mechanisms underlying thermotolerance, aiming to support plant breeding innovation and the development of new management strategies in viticulture. Here, we report some preliminary results from the genetic and phenotypic characterization of a segregating population obtained by crossing 'Rhine Riesling' and 'Cabernet Sauvignon'. After genotyping 139 F1 individuals with the *Vitis*18K SNP chip and microsatellites, a high-density linkage map was developed that contained 3 459 representative markers with an average inter-locus gap of 0.78 cM. The progeny was evaluated in the field by investigating heat-responsive traits (such as phenology, berry ripening indexes and physiological parameters), for which wide variability was observed among genotypes and also between temperature regimes in the case, for instance, of chlorophyll fluorescence parameters. This information, together with the data that will be obtained from the metabolomic analysis of leaves collected under control/heat stress conditions, will finally be used to identify QTLs (Quantitative Trait Loci) for thermotolerance. Selected individuals with contrasting response to high temperatures will be further investigated in controlled conditions, where the analysis of volatile organic compounds (VOCs) emitted from leaves will additionally be included. To this purpose, preliminary assays were conducted to optimize the experimental settings.

Keywords: Grapevine; Thermotolerance; Linkage map; Phenotyping; QTLs;

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P17

Comparison of microclimate conditions in a grapevine canopy with a field weather station

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Due to the growing environmental awareness as well as new regulations, it is one of the most important aims to reduce pesticides. Especially fungicides take the biggest part in the viticulture sector. Therefore, to control the occurrence of *Plasmopara viticola* nowadays it is common to use weather forecasting models. These models help reducing sprayings that were only preventive actions. The data for these models relay on several field weather stations, which were located mostly far from the vineyards. Due to the complex structure of vines, the measured data from these weather stations may not have seen as appropriate for representing the climatic conditions inside the canopy. Which leads to the hypothesis that the prediction models cannot precisely represent the situation inside a grapevine canopy, while using data from outside the canopy. This study was set-up from June to September of 2021 in a 34-year-old vineyard (Pinot Meunier). It determined the similarity and dissimilarity of the air temperature (t), humidity (h) and leaf wetness (lw) from three different heights inside the canopy. It also shows the correlation between the measured parameters inside the canopy and those parameters, measured in the vineyard by a field weather station. The sensors for t, h, and lw inside the canopy were attached to heights of 100 cm, 130 cm and 160 cm from ground. The weather station was in a distance of 75 m beside the vineyard and had its measurement instruments at a height of 200 cm. It turns out, that during the day the measured temperatures from inside the canopy varied up to more than +3 °C compared with the temperature measured outside by the weather station. Furthermore, the temperatures inside the canopy had consistent increasing from the lowest temperature at 100 cm to the highest temperature at 160 cm. In accordance the measured humidity's in all three heights inside the canopy were always lower (up to -5 %) than the humidity measured outside the canopy from the weather station. An increase of humidity in the different heights, was also detected, but with lowest humidity at 160 cm and highest humidity at 100 cm. It also turned out that the measured leaf wetness inside the canopy is highly dependent on the temperature, which expresses that high temperatures show higher leaf wetness in the centre at 130 cm and lower temperatures show higher values at the top of the canopy at 160 cm. Moreover, the measured leaf wetness inside the canopy from all three heights was nearly stable and not that much influenced from single weather events like heavy rain that was in discrepancy with the measured data from the outside leaf wetness sensor. All these data embody, that the measurements from the field weather stations vary a lot in comparison to the measurements inside the canopy, which may give the opportunity to more precise predicting models by using data measured inside the canopy.

Keywords: *Plasmopara viticola*, microclimate, temperature, humidity, leaf wetness

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The effect of homozygosity of locus Rpv12 on downy mildew resistance

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The downy mildew pathogen, *Plasmopara viticola*, causes considerable yield losses under appropriate climatic conditions, e. g. dramatic in summer 2021 in parts of Germany (palatinate region). Therefore, large quantities of protective chemicals are applied in viticulture to prevent yield loss. Reduction of fungicide sprays is a major aim of modern grapevine breeding activities. To achieve this goal resistance loci need to be identified and introgressed into elite cultivars. Development of molecular markers flanking the genomic resistance regions allowed to identify more than 20 resistance loci against *Plasmopara viticola*. Newly bred resistant cultivars contain some preferentially used loci: Rpv1, Rpv3, Rpv10 and Rpv12. However, the causative resistance genes within these loci are known only in a few cases. For optimal combination of loci, it is important to understand the function of the loci and their resistance-mediating genes. Rpv12, seems a quite strong resistance locus, and was transferred into breeding material starting from the 1970s. It is derived from the Asian wild species *Vitis amurensis* that originates from eastern China and Russia, where cooler climates prevail. Rpv12 is located on chromosome 14 and has an additive effect to Rpv3. Microscopy on several different carriers of Rpv12 was undertaken to study its cellular characteristics. It revealed a strong reduction of mycelial growth in Rpv12-carrying genotypes. Pathogen challenge leads to production of H2O2 and a rapid hypersensitive response. This inhibition of mycelial development was enhanced in genotypes carrying Rpv12 in the homozygous state. Initially, comparison of two genotypes from an open pollination called Hozy01 (Rpv12/Rpv12) and Hozy10 (Rpv3.1/Rpv3.1 and Rpv12/Rpv12) was performed. They both showed nearly no mycelial growth. To verify these results 30 genotypes from a \`Kunbarat´ (Rpv12) open pollination were investigated. Results indicate that the homozygosity of Rpv12 may influence the degree of resistance. Microscopy of genotypes homozygous for the Rpv12 locus showed a similar reduction of mycelial growth as a combination of *Rpv3* and Rpv12 in heterozygous state. This new observation offers new possibilities in grapevine breeding. Transmission of the locus is passed on to progeny if homozygous Rpv12 carriers are used, superseding the necessity of marker analysis in selection. In addition, the expression of the resistance trait can be reinforced by the generation of homozygous Rpv12 carriers of newly bred resistant grapevine cultivars.

Keywords: downy mildew, *Plasmopara viticola*, homozygosity, Rpv12, resistance

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Contrasting hydromechanic adjustments of two grapevine cultivars to different levels of water supply and crop load

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Crop plants cope with water stress through different strategies, such as the controversial isohydric or anisohydric classification proposes. However, the extent to which such behavior is modified by demand for photosynthetic carbon by crop load has not been fully addressed. Our previous results showed that high crop load reduces plant water status, suggesting that water and photosynthetic carbon pathways share regulation mechanisms. We aim to evaluate the interactive effect of water supply and crop load on the water-carbon dynamics of two contrasting grapevine genotypes. We designed a factorial experiment in a commercial vineyard located in central Chile during two seasons. Twenty-four Sauvignon blanc (more vigorous) and twenty-four Chardonnay (less vigorous) grapevines were assessed under two irrigation regimes (100% and 50% water reposition from Penman-Monteith evapotranspiration) either with full (100%) or no crop load (0%). Results indicate that Sauvignon blanc water-stressed plants maintain tissue hydration by reducing gas exchange (i.e., stomatal conductance decreased from 0,140 to 0,060 mol m^{-2} s^{-1}) and maintaining liquid water fluxes; the latter associated with reduced leaf water potential (from -0.85 to -1.12 MPa) and leaf capacitance (from 1.1 to 0.8 mol m^{-2} MPa^{-1}). On the other hand, Chardonnay stressed plants reduce their bulk elastic modulus (from 6.8 to 2.4 MPa) and increase leaf capacitance (from 1.0 to 1.5 mol m^{-2} MPa^{-1}) to maintain similar gas exchange rates and water status levels to the observed in well-watered plants. Currently, we are studying how these strategies might change under high and low crop load, and how water and CO_2 flux pathways interact under these contrasting conditions of photosynthetic carbon demand. These results will improve understanding physiological aspects on productivity context in a climate change scenery.

Keywords: water deficit, crop load, water relations, gas exchange, water use efficiency, isohydric, anisohydric

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Studying the adaptability and resilience of grapevine cultivars and rootstocks to water constraints in a model, 'fit-for-purpose' vineyard

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In South Africa, consistent and more extreme droughts is one of the major climate change impacts predicted and grapevine production could be significantly affected, specifically since most vineyards are dependent on irrigation. There are excellent existing and emerging knowledge that confirm that grapevines intrinsically monitor and respond to stressful conditions, but it is also clear that those responses are influenced by a multitude of interconnected factors related to the specific scion and rootstock combination, the site, and the management practices. This is typically referred to as the Genotype X Environment X Management Interactions that underlie the appearance/performance (or the phenotype) of the plants. Exciting breakthroughs are emerging, showing that the plant material itself (the genotype) in these interactions can adapt (to a certain extent) to the environments (site specific and/or through management practices) over the course of the lifetime of the plants, using epigenetic changes that lead to phenotypic plasticity to the stresses experienced. Moreover, the modulating environment is also no longer described as only the physical environment of the plant, but also includes the microbial environment, since we now know that there is an active exchange between a plant and all its microorganisms (microbiomes) and that these microorganisms can contribute to stress resilience of the plants. These aspects will be included in an integrated study that aims to evaluate and compare the adaptability and resilience of current commercial scion/rootstock combinations, established, and maintained under both optimal and sub-optimal water conditions using a holistic approach and novel techniques. The study will make use of a model vineyard that has been established in 2020 and is currently being validated for the study objectives, particularly to test the effectiveness of the different irrigation treatments for their ability to effectively induce stress in the vines using a variety of climatic, phenological and physiological measurements.

Keywords: water stress, genotype, environment, microbiome, adaptability

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Functional characterisation confirms that defensin peptides protect grapevine plants against pests and diseases in vivo

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Plant defensins are small, cationic, cysteine rich antimicrobial peptides. Plant defensins have been extensively analysed for their *in vitro* antifungal activity, but little is known about their in vivo functions, especially in *Vitis vinifera*. The goal of this study was to evaluate the in vivo functions of plant defensin peptides in grapevine in terms of growth, as well as biotic stress impacts. Four transgenic populations of two *Vitis vinifera* cultivars (Sultana and Red Globe), overexpressing two different plant defensin peptides (Rs-AFP2 and Hc-AFP1) were used as the plant resources in this study and subjected to functional characterisation studies that included analyses of potential growth impacts, as well as profiling analysis for potential antifungal, and anti-insect phenotypes. Our data shows that the overexpression of the peptides did not lead to prominent growth phenotypes, although slight changes to root formation were observed, with the genetic background (cultivar) demonstrated to be a major driver in the observed phenotypic diversity. Although the peptides were previously shown to strongly inhibit *Botrytis cinerea in vitro*, no enhanced protection against two strains of this necrotrophic pathogen was seen in the transgenic population. The plants overexpressing the peptides however showed strong enhanced resistance against the powdery mildew pathogen, *Erysiphe necator*, with increases in penetration resistance and a stronger localised hypersensitive response at the infection sites. Subsequent experiments, using synthetic Rs-AFP2 peptides applied to control (non-transgenic) grapevines, confirmed that the peptides, when applied, also protect the plants against E. necator. Moreover, survival assays with *Planococcus ficus* (mealybugs) confirmed significantly reduced mealybug infestation on all the transgenic lines, with the mealybugs having the highest mortality rates on the Sultana Hc-AFP1 overexpressing lines. Our results confirm that defensins have multiple functions within grapevines and contribute to several stress protection strategies.

Keywords: Plant defensin peptides; Biotic stress; Rs-AFP2; Hc-AFP1; mealybug; powdery mildew fungus

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Studying the adaptability and resilience of grapevine cultivars and rootstocks to water constraints in a model, 'fit-for-purpose' vineyard

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The cultivation of grapevine in semi-arid areas with seasonal drought poses a major threat, especially in areas identified as climate change hotspots. Therefore, it is vital to understand the responses of grapevine cultivars and rootstocks to drought stress and abiotic stress mitigation in general. Plant defensin peptides are known for their role in biotic stress mitigation but limited information is available for their roles in abiotic stress. No functional characterisation data is available for defensin peptides in grapevine and potential drought resistance phenotypes. Previous *in silico* analysis of transcriptomic data indicated the upregulation of Defensin-like (DEFL) genes in response to water deficit stress and several abiotic stress responsive genes were co-expressed with the grapevine plant defensin, Vvi-AMP1. In this study, seven transgenic *Vitis vinifera* populations, over-expressing three defensin peptides (Hc-AFP1, Rs-AFP2 and Vvi-AMP1) in two grapevine cultivars (Sultana and Red Globe) were analysed to evaluate the defensins' potential role in drought stress. The transgenic lines were subjected to active drying experiments and evaluated in terms of their intrinsic water use efficiency (iWUE). The results indicated that the genotype had a strong influence in the behaviour of the different transgenic lines as the Sultana and Red Globe populations did not show the same responses to the active drying treatment for the different constructs. However, the Sultana Hc-AFP1 and Rs-AFP2, as well as the Red Globe Hc-AFP1 transgenic lines, all demonstrated increased iWUE under the induced drought stress conditions. The over-expression of the peptides seems to positively impact on grapevine stomatal behaviour under water stress conditions, resulting in improved hydration status and improvement of the iWUE of plant lines expressing the defensins. These promising results provide motivation to explore grapevine defensins for roles beyond the traditional biotic defence roles and point to more general stress responsive roles.

Keywords: Plant defensin peptides; drought stress; abiotic stress; active drying; Intrinsic water use efficiency; Rs-AFP2; Hc-AFP1; Vvi-AMP1

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Functional Study of Lipoxygenase-mediated Resistance against *Erysiphe necator* in Grapevine

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Grapevine powdery mildew, caused by the fungus *Erysiphe necator*, is a devastating disease with a worldwide occurrence. Commercial *Vitis vinifera* cultivars are highly susceptible to the pathogen, while several wild *Vitis* spp. demonstrate a high level of resistance. Understanding the resistance mechanism will contribute to breeding programs aiming at introducing resistance factors to commercial varieties. Lipoxygenase (LOX) genes play important role in plant defence through peroxidation of lipids leading to the production of many compounds, including volatiles, involved in signalling and response or with antifungal activities. In this work the role of LOX in host resistance against *E. necator* was investigated *in silico and in planta*. Phylogenetic analysis with a maximum likelihood estimation approach of grapevine and well-characterized *Arabidopsis* homologs showed the separation of 9-*LOX* and 13-*LOX* and several paralogous grapevine LOXs were found tandemly localized. Moreover, several putative chloroplast transit peptides were predicted. In addition, gene network analysis performed with OneGenE identified many defence-related genes as associated with LOX isoforms. A controlled *E. necator* infection experiment was carried out using *V. vinifera* cultivar Teroldego (S) and NY_39 (R) genotype and LOXs gene expression patterns were analysed. Microscopy observation of infected leaves showed successful penetration and hyphal growth of the pathogen in the (S) variety at 24 hours post inoculation (hpi) while extensive hyphal growth was observed at 72 hpi. Conversely, pathogen establishment and growth were hampered in the (R) genotype with an evident accumulation of reactive oxygen species at the pathogen penetration site. Grapevine *VviLOX2* and *VviLOX12* were significantly upregulated at 12hpi in the infected leaves of (R) genotype, while upregulation of *VviLOX7* and *VviLOX9* occurred at 48hpi; *VviLOX13* was upregulated in infected leaves of the (S) genotype at 24 hpi. Efforts are underway to over-express and knock-out (CRISPR/Cas9) candidate LOXs in the (S) background for in-depth functional characterization.

Keywords: *Vitis vinifera*, resistance, lipoxygenase, *Erysiphe necator*, CRISPR/Cas9

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Screening for powdery mildew resistance in germplasm from Spain

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Grape powdery mildew (PM) is an economically important disease worldwide, and the most damaging fungal disease in "Mediterranean" (dry and warm) viticulture. In addition, PM has the potential to spread to other areas as a consequence of climate change. It needs to be controlled by a high number of chemical sprays along grapevine growth cycle. Reducing chemical inputs to manage grapevine diseases is an essential requirement for the sustainability of future viticulture. The development and deployment of novel mildew resistant varieties is considered one of the most promising strategies towards a sustainable viticulture. In order to obtain durable resistance in the field, breeding cultivars bearing resistance from multiple backgrounds is needed. Screening for new sources of PM resistance in exotic germplasm is therefore a relevant approach. We have conducted a screening of a nuclear collection of *Vitis vinifera sylvestris* from Spain (64 accessions) in order to identify potential new sources of resistance to mildew diseases. *In vitro* assays were carried out on detached leaves with 4 replicates and the degree of infection was evaluated following a 1-5 scale at 7 and 14 days post inoculation. Results will be presented regarding the seven accessions presenting reduced mycelium growth and reproduction, and their genetic characterization..

Keywords: *Erysiphe necator* , *Vitis vinifera sylvestris*, host-pathogen interaction

P25

Identification of the grapevine pectin methylesterases and characterization of their role in Botrytis bunch rot

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Plant Pectin Methylesterases (PMEs) are tissue-specific and developmentally regulated proteins, encoded by a large gene family. PMEs are involved in plant Cell Wall (CW) remodelling by modifying the degree of pectin methyl esterification and therefore affecting the properties of the CW matrix. Pectin methylesterification impacts on fruit susceptibility to pathogens as *Botrytis cinerea (Bc)*, a necrotrophic fungus, which in grapevine causes *Botrytis* bunch rot. A sequence homology search using the recent revision of the *Vitis vinifera* genome assembly and the new annotation available allowed us to identify 63 putative *V. vinifera* PME (*VvPME*) isoforms, 16 more than those previously reported. Co-expression analysis of the *VvPME* genes, performed using the *in-house* developed *Vitis* gene expression database VESPUCCI, identified six different co-expression modules. Interestingly, two of them showed very similar modulation upon pathogen infection and one of them included eight *VvPME* genes co-expressed and modulated in response to *Bc* infection. To assess the contribution of the basal expression of the selected *VvPME* genes on *Bc* susceptibility, we are firstly investigating their expression profiles in a panel of organs from grapevine genotypes with different levels of susceptibility to bunch rot. In a second step, berries of the same genotypes will be artificially inoculated with *Bc* and analysed both for the expression of the selected genes and for the CW biochemical composition over a time course of the infection.

The results obtained in this work will contribute to better understand the role of the *VvPME* gene family in the grapevine-*Bc* interaction and represent the basis for the functional characterization of selected *VvPME* genes in *V. vinifera* plants and for future breeding applications.

Keywords: Grapevine, Pectin Methylesterase, Cell wall, *Botrytis* cinerea, Bunch rot, Gene expression

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Detection of host defensive proteins in vine roots under biotic stress

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Biotic stresses may significantly affect plant growth. Root-feeding grape phylloxera (*D. vitifoliae*) manipulates the root tips of grapevines (*Vitis* spp.) to induce hook-shaped galls so called nodosities. Recent studies show that the compatible interaction (if limited to nodosities) may have positive and negative effects on the grapevine and that the resources allocated for host defence may play a major role. Here we aim to investigate the accumulation of host defensive proteins during root gall (nodosity) formation on a tolerant rootstock cultivar. Teleki 5C (*V. berlandieri x V. riparia*) cuttings were vegetatively propagated, infested with a single founder phylloxera lineage and cultivated in a climate chamber (25 °C, 60% rH and 16 h pp). Root tips of non-infested plants (control), developing and matured root galls were sampled and analysed using proteomic mass spectrometry. The overall analysis showed an enrichment of proteins functionally associated to starch accumulation, phytohormone regulations and a decrease of proteins linked to cell wall degradation in root galls as compared to control tips. Regarding host defence proteins, our data showed a significant enrichment of biosynthetic enzymes of the flavonoid pathway (*VviCHS, VviCHI, VvSTS, VviLAR, VviANR* and *VviUFGT*) and an enrichment of pathogenesis related protein *VviPR4* and four members of the *VviPR10* family, known to exhibit antibacterial and antifungal activities. Interestingly the PR-protein VIT_218s0001g15650, an anti-apoptosis inhibitor (VIT_201s0010g01180), two disease resistance-responsive proteins (VIT_208s0007g07000, VIT_200s0317g00070) and a stress responsive barrel domain protein (VIT_211s0016g01340) were decreased in root galls compared to control root tips, indicating physiological hurdles for phylloxera's gall formation. In conclusion, the results supports previous studies reporting physiological modifications root gall formation and give insights into the defence proteins involved in vine roots affected by biotic stresses such as the root-parasite interaction belowground.

Keywords: host-parasite interaction, root gall physiology, gall formation, nodosity mass spectrometry, PR proteins, host defence pathways

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Evaluation of grapevine responses to variable water conditions using reference methods and proximal sensing tools

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Most viticultural activities occur in Mediterranean and semi-arid areas that are characterised by hot and dry summers. As a result, vines are exposed to potential water stress conditions during their active growth stage. Reliable water status monitoring, sustainable irrigation practices and drought tolerant cultivars have become increasingly important, especially considering the increased drought incidences and higher demand for water resources. Most commercial grapevine cultivars are grafted, and the choice of rootstock significantly influences drought tolerance and water use efficiency. The interactions of scions and rootstocks and their respective influences on modulating the water responses are still not fully elucidated. This study forms part of a field pot trial where five scion cultivars and four rootstocks (on their own roots), as well as all possible grafting possibilities are being analysed for their water stress responses and the scion/rootstock interactions. The reference methods to determine plant water status are destructive and therefore one of the associated aims is to evaluate and validate non-destructive proximal sensing tools for their suitability to evaluate the water stress responses. Here we present the physiological responses of *V.champinii ‘Ramsey’, V. berlandieri x V. rupestris ‘Richter 110’,V. riparia x V. rupestris* ‘Mgt 101-14’and *V. berlandieri x V. rupestris x Jacquez* ‘US Vit 8 7’rootstocks as being assessed using conventional and proximal sensing techniques. The experimental layout included six replicate vines per cultivar that were arranged in a randomized design in a field pot trial. Measurements were recorded at non-stress water levels and after inducing water deficit through active drying. The midday stem water potential (Ψstem) and stomatal conductance (g_s) measurements were taken on each replicate vine. Canopy temperature and canopy spectral reflectance values were recorded using ground-based imaging sensors. The relationship between the midday Ψstem and g_s reference methods and information derived from proximal sensing data (vegetation and thermal indices) was used to evaluate the sensitivity of such tools to fluctuations in grapevine water status and the rootstock cultivars were compared for their responses undergoing active drying.

Keywords: Water status monitoring, sensing tools, genotype x environment, rootstock, scion

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Physiological and biochemical responses to biofungicides application in *Vitis vinifera* cv. Touriga Franca

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Portugal viticulture has a huge impact on the country's economy, especially in the Douro Demarcated Region (DDR, Northeast Portugal). This area has peculiar edaphoclimatic characteristics providing a suitable "terroir" for premium wine production, where cv. Touriga Franca is widely use. Climate change should influence grapevine pathogens development, survival, and host susceptibility, which may increase the use of fungicides and simultaneously affect the physiological and biochemical adaptation and response of grapevine. The fungicides available are diminishing continuously, mainly due to their toxicity and ecotoxicity effects, becoming necessary to find other solutions, namely the use of more friendly products. This study aimed to verify the effect of alternative products on physiological and biochemical parameters. A trial was installed in a vineyard located in DDR, Vila Real. Five different foliar treatments were tested: i) nettle extract (*Urtica* sp.); ii) *Japanese knotweed* extract (*Reynoutria japonica*); iii) homeopathic product; iv) conventional treatment and v) water. Ten applications were done between pea-size berries and ripening development stages. Biochemical and physiological analyses at harvest were performed, namely, photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoids), soluble sugars, starch, leaf gas exchange measurements (using IRGA) and yield. Statistical analyses were performed using SPSS Statistics. Significant differences ($P \leq 0.05$) were observed between treatments, with exception in some leaf gas exchange parameters and in photosynthetic pigments concentration. *Japanese knotweed* extract seems to improve the performance of grapevine, even in water stress. Nettle extracts and the homeopathic product application increased photosynthetic pigment contents. Conventional treatment was expected to be the most effective in all the parameters analysed, however it only revealed the best results in some of leaf gas exchange parameters and yield. In the next years these experiments will be repeated to confirm the influence of these alternative products in grape quality and yield.

Keywords: Grapevine, bioproducts, climate change, sustainable production

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The grapevine LysM receptor-like kinase VvLYK5-1 mediates chitin-triggered immunity

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The establishment of defence reactions to protect plants against invading pathogens first requires the recognition of Microbe-Associated Molecular Patterns (MAMPs), detected by plasma membrane-bound Pattern Recognition Receptors (PRRs). These MAMPs, also termed elicitors, are used in several biocontrol products that are gradually developing to reduce the use of chemical pesticides in agriculture. Chitin, the main component of fungal cell walls, as well as its deacetylated derivative, chitosan, are two chitooligosaccharides (COS) that can be found in some of these products. Unfortunately, the mechanism allowing the perception of these molecules is still poorly understood in *Vitis vinifera*, sometimes hampering the improvement and the generalization of these emerging crop protection tools. On the contrary, chitin perception in the model plant *Arabidopsis thaliana* is well described and relies on a tripartite complex formed by three membrane-bound LysM Receptor-Like Kinases named AtLYK1/CERK1, AtLYK4 & AtLYK5, the latter having the strongest affinity for chitin. In grapevine, COS perception has for the moment only been demonstrated to rely on VvLYK1-1 & VvLYK1-2, two constitutively expressed genes that did not specifically respond to pathogens according to genes expression analysis. In this context, we performed experiments to complement the *Arabidopsis* lyk5 simple mutant and the lyk4/5 double mutant, which showed altered response to COS, by overexpressing their grapevine orthologous genes (VvLYK5-1/2). Our results revealed that MAPK activation and defence gene expression were partially restored after chitin treatment by the complementation with VvLYK5-1 but not with VvLYK5-2. These preliminary results seem to indicate that VvLYK5-1 participates in the chitin recognition in grapevine, together with VvLYK1-1 previously identified. Furthermore, as the perception of chitosan does not seem altered in the Atlyk5 mutant, it would also appear that the perception of chitin and chitosan does not involve the same receptors. FRET-FLIM experiments initiated with these co-receptors will soon provide additional information to better decipher the mechanism of COS perception in grapevine.

Keywords: Pathogen-Associated Molecular Patterns (PAMPs), Pattern Recognition Receptors (PRRs), induced immunity, chitooligosaccharides (COS), LysM receptor-like kinase (LYK)

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Esca complex grapevine trunk disease leads to cell wall changes in asymptomatic grape berries

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Grapevine Trunk Diseases causes destructive wood rot that result in loss of vigour and productivity, as well as a shortened lifespan of the infected grapevines. No effective treatment and no resistant cultivars are available. The Esca complex is associated with pathogenic fungi that degrade the woody parts of grapevines. Symptoms include discoloured foliar “tiger stripe” patterns and dark wood streaking and infections leads to eventual plant death. Symptoms on berries are light/mild only visible as irregular dark spots occurring along the surface of the berry epidermis of white cultivars. The effects of Esca in berries are poorly understood. There is no data linking Esca disease to direct effects on grape berry development and the resulting wine.

The berry cell wall is an important defensive barrier against infection as it acts as both a physical barrier and an active defence matrix against necrotrophic and biotrophic invasive pathogens. However, considering that the Esca fungi spread through xylem vessels within the plant, we asked the question whether Esca triggers cell wall changes without a visible berry breach site.

The cultivars Aragonez was used in this study and ripe berries from grapevines with Esca symptoms and from grapevines without symptoms were harvested and analysed using GC-MS and CoMPP. The analysis of monosaccharides that constitute the main cell wall polymers showed an increase of glucose upon symptomatic infection, and a decrease of arabinose, galacturonic acid, rhamnose, fucose, and mannose. These changes may be associated with de-pectination of the cell wall, a common outcome in fungal infections resulting from enzymes secreted by the pathogens. These results point to an alteration in the cell wall matrix that might have consequences on the quality of the berry fruit and resulting wine.

Keywords: cell wall, trunk disease, Esca, berry, GC-MS, CoMPP

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Clonal selection in *Vitis vinifera* cv. Carignan Noir for improved powdery mildew tolerance

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Vitis vinifera cv. Carignan Noir likely originated in Aragon region (Spain), close to the town of Cariñena. Its late budding and ripening, high acidity and high anthocyanin content are characteristics which make this cultivar suitable to grow under conditions of global warming. However, Carignan Noir is losing popularity because of its high susceptibility to *Erysiphe necator* (powdery mildew, PM). The main objective of this study was the selection of Carignan Noir clones with increased tolerance to PM as well as identifying the genetic variation underlying their tolerance. Carignan Noir prospection from vineyards at the Cariñena designation of origin (DO) area resulted in 1119 true-to-type virus-free accessions. We selected three individuals showing different susceptibility to PM in the field. We confirmed their differential susceptibility in *in vitro* inoculated leaves. The most extreme susceptible and tolerant lines were compared in an RNA-seq transcriptome analysis in healthy apices and PM-infected adult leaves. The tolerant line showed upregulation of wax and lipid synthesis genes and specific pathogen response genes in both healthy and infected samples. Wax synthesis and a polygalacturonase gene upregulation could provide a physical barrier to powdery mildew, as well as a pathogen-related protein precursor (PRP1). On the other hand, the sensitive line presented upregulation of phenylpropanoid and flavanol synthesis genes. These results provide candidate genes for which to study their possible involvement in conferring tolerance to PM.

Keywords: powdery mildew, pathogen tolerance, grapevine, clonal selection, RNA-seq, Carignan Noir

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Botrytis cinerea infection of grape berries analysed from the cell wall point of view

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Vitis vinifera grapevine cultivars are severely affected by the necrotrophic fungi *Botrytis cinerea* with damage to berries negatively impacting both the wine and table grape industries. To successfully infect plant organs, B. cinerea has to breach the plant cell wall – a complex matrix of polysaccharides and proteins. Despite its obvious importance in the infection strategy, knowledge of cell wall changes during grape berry infection with B. cinerea is still limited.

In this study, B. cinerea infection experiments were conducted on grapevine berries under controlled conditions and combined with detailed cell wall analysis using comprehensive microarray polymer profiling in combination with monosaccharide analysis. Scanning electron microscopy and X-ray computed tomography were used to follow the infection progression. The cell wall responses of grape berries at three ripening stages between véraison and harvest (ripe) from the wine grape cultivars Sauvignon Blanc and Cabernet Sauvignon, and the table grape cultivars Dauphine and Barlinka, were followed and compared.

At véraison, no visible infection could be observed, whereas at post-véraison and ripe stages, tissue degradation and maceration were prevalent with pectin epitope signals decrease, indicative of pectin polymer degradation. Hemicellulose and arabinogalactan proteins epitopes were mostly unaffected while glucan epitope accumulated with infection progression and extensin epitope seemed to be deposited in the table grapes at the site of infection. These results emphasise the role of the cell wall network composition during plant-pathogen interaction and particularly pectin degradation as well as extensin deposition.

Keywords: cell wall, *Botrytis* cinerea, pectin, extensin

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P33

Variability of Spanish minority varieties to *Erysiphe necator* infection

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Erysiphe necator is the causal agent of powdery mildew on grapevines, being the most damaging fungal disease in European vineyards since its appearance in the 19th century. Although most of *Vitis vinifera* varieties are very susceptible to this pathogen, reaching high or very high levels of disease, tolerant varieties have also been described. In the current climatic conditions, which are favouring the development of this disease, tolerant varieties may be an alternative to reduce the number of spray applications to control the fungus. The objective of this work was to evaluate the response to fungal infection of 32 Spanish minority varieties from different wine-growing areas. Young leaves, previously sterilized, were inoculated using a vacuum tower. Fungal development was evaluated on a scale from 1 to 8, seven and 14 days after inoculation. Although mycelium growth was observed in all varieties at 7 days, significant differences were found at 14 days. Five varieties showed infection levels lower than level 4 (development of the fungus with conidiophores in the first stages of development), 15 were in a medium degree (levels 4 to 6), the rest were highly susceptible, showing highly developed conidiophores and a high degree of leaf surface colonization. Minority varieties from the Catalonia region showed the lowest fungal development. Results reported differences in grapevine response to powdery mildew attack, and allowed the identification of germplasm useful in the analysis of the interactions between these species that can contribute to design sustainable strategies for viticulture.

Keywords: berry anatomy, vascular tissues, xylem, phloem, imaging

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Grape berry acclimation to high and extreme high temperatures

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Understanding the adaptive response of grapevines and berries to abiotic stresses may be key to alleviate increasing climate variability. In Australia, a large proportion of the vineyard area is planted in already warm and hot regions, which, with more frequent heatwaves, could be exposed to abnormally high temperatures in the early stages of the growing season. The aim of this study was to test several parameters, such as frequency, timing of heatwaves and berry acclimation, as well as measuring berry flavonoid response to temperature.

Two potted-vine experiments, with well-irrigated Shiraz vines, were conducted inside a UV-transparent glasshouse during the 2016-17 and 2018-19 seasons, using fans blowing hot air onto individual vines or bunches, without affecting light exposure. These experiments examined short spells of high day temperature at two phenological stages at the vine level (E-L 31 and/or E-L 32/33) or at the bunch level (E-L 31 and/or E-L 34/35). Using a factorial design, a range of treatments was applied to test the effect of a single heat event (HE), and the additive effect of repeated HEs. Berries were sampled from fruit set until maturity at regular intervals and berry flavonoid composition was analysed by LC-MS/MS.

During Experiment 1, HEs were applied for three days and three nights (+6 °C) at a two-week interval, with maximum day temperature reaching 45 °C at the hottest of the experiment and the first HE being more intense than the second. Interactions between the two HEs were found for several parameters. Skin tannin composition was significantly impacted by the first HE, which was imposed during the main biosynthesis period, but not by the second. Seed physiology and tannins were also affected by both HEs. A small decrease in total soluble solids and total anthocyanins at the end of véraison suggested that ripening was slightly delayed when two HEs were applied. During Experiment 2, following a first HE (3 days, maximum of 40 °C), berry weight was significantly lower, but skin and total seed tannins were only slightly affected. Bunches solely exposed to the second HE (3 days, maximum of 45 °C) at the onset of véraison exhibited significant berry damage (desiccation) and a decrease in skin tannins, with changes in composition. Bunches exposed to both HEs did not show any sign of acclimation and were the most damaged. Finally, most impacts on physiology and composition observed at some sampling dates, for non-damaged berries, were no longer evident by harvest in both experiments.

Further research is required around the notion of cumulative effect and acclimation. In this study, the effect of more than one heatwave within the same growing season was assessed, which was, to our knowledge, studied for the first time in grapevines. Additional assessment of seeds from heat-stressed berries could also bring to light new adaptive strategies as seeds could 'memorise' heat exposure from one season to the next.

Keywords: Berry composition, Berry development, Cumulative events, Frequency, High temperature, Phenolics, Phenological stage, Tannins

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Understanding stilbene accumulation at the graft interface of grapevine and their potential use for predicting graft incompatibility

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Grapevine plants are, most of the time, formed from two genotypes (scion and rootstock) to improve tolerance to Phylloxera, but also to combine different shoot and root traits together in one plant. The mechanisms underlying graft union formation are complex and remain poorly understood despite at least a hundred years of scientific study. In grapevine, some scion/rootstock combinations are considered as incompatible, i.e. they are unable to form and maintain a functioning graft union, and subsequently die, which implies an economic loss for the wine nursery sector. The early identification of incompatibility in grapevine would allow the selection of non-viable plants before planting, would improve the quality of produced plants, and would have a beneficial impact on the wine nursery sector.

The aim of this study was 1.) to identify early metabolite markers (from 24 primary and 49 secondary metabolites) quantified at the graft interface and in both scion and rootstock tissues that could be used to predict grafting success in grapevine, and 2.) to characterise the spatial and temporal changes in metabolites occurring during graft union formation.

Correlations between secondary metabolite concentration, especially stilbenes, and grafting success were identified and could be used as indicators of grafting success in different scion/rootstock combinations. Also, knowledge of the spatial and temporal changes in metabolite concentration furthers our understanding of the events preceding the accumulation of these marker metabolites and identifies genotype-specific differences.

In viticulture there is much interest in using new rootstocks to adapt grapevine to climate change, but graft compatibility is an essential prerequisite but laborious to assess. The identification of early metabolite markers of grafting success could be used to phenotype plants for genetic research and in breeding programs.

Keywords: grafting, polyphenol, compatibility

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Footprint of rootstock on *Vitis vinifera* response to drought

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Drought stress affects yield and berry quality in grapevine. Different vine species and varieties are capable to cope with water stress to different degrees, from extremely sensitive to more tolerant. The use of interspecific hybrids as rootstocks of valuable varieties can significantly determine the response to water stress. Particularly, the rootstock "Paulsen 1103" has been widely used to confer drought tolerance. In contrast, the rootstock "SO4" has been described to be moderately susceptible to water stress deficit.

We aim to characterise the mechanisms underlying water deficit response, thus we preliminary focused to determine the impact of rootstocks in grapevine response to drought stress. Castelão (*Vitis vinifera*) was used as the scion for both grafts, and Paulsen 1103 (*Vitis berlandieri x Vitis rupestris*) and SO4 (*Vitis berlandieri x Vitis riparia*) as the rootstocks. Plants were grown in the greenhouse in well-watered conditions and, after two months, plants were subjected to drought treatment by withholding water for 23 days. Leaves and roots samples were collected at four different leaf water potential points: well-watered (-0.15 to -0.20 MPa), mild (-0.35 to -0.50), moderate (-0.55 to -0.60 MPa), and severe (-0.75 to -0.85 MPa) drought stress conditions. Here, we present the biochemical, molecular, and physiological responses to water deficit conditions in leaves and roots of the two grafted grapevines. RNA-based regulatory mechanisms that could explain such effects are under study.

Keywords: grapevine, grafting, plant tolerance, water deficit, molecular mechanisms

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Grapevine drought tolerant ideotypes to adapt viticulture to climate change

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Water scarcity is a main environmental limitation that threatens grape production in dry growing regions, especially in most traditional non-irrigated viticultural areas. Changing climatic conditions could exacerbate this situation as more intense and prolonged drought events are predicted for many regions. Adapting viticulture to these challenges will require an improved understanding of how grapevines behave under drought to enable sustainable management strategies and develop new varieties and rootstocks. In this study, we use a multi-trait phenotyping approach and model-assisted analyses to identify grapevine drought tolerant ideotypes. We first characterized a wide range of hydraulic traits in nine *Vitis* genotypes, across and within species. We then create in silico new trait recombinants ("or trait cocktails") by varying and rearranging these quantified traits randomly. Finally, by modelling the performance of existing cultivars along with these novel trait cocktails we identified elite ideotypes with increased drought tolerance. The results of our study demonstrated that elite ideotypes have the potential to outperform existing varieties across important wine regions and under future climate scenarios. Furthermore, our study reveals the traits and trait combinations that can be leveraged to guide breeding programs aimed at increasing grapevine drought tolerance.

Keywords: stomata, drought tolerance, genotype, *Vitis*, loss of hydraulic conductance, traits, climate change

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How grapevine grafting for hybrids can affect wine under cold climatic conditions of Quebec, Canada?

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Grapevine production is relatively recent in Quebec, Canada, and several challenges restrict quality grape production. Quebec's rigorous climate and short growing season are just a couple of limiting factors in grape production and varietal selection. Rootstocks adapted to growing conditions allow producers to plant varieties that are better adapted and more efficient in specific soil and climatic conditions. Selected scion/rootstock combinations could be better suited to growing conditions found in Quebec vineyards, thereby homogenizing vegetative growth for all vines, reducing costs associated with management and help to reach maturity and optimum berry quality. The main objective of this project was to evaluate the use of grafting as a technique to adapt hybrid vines to cold climate growing conditions found in Quebec, Canada. Several combinations were produced using Frontenac, Frontenac blanc and Marquette cultivars along with 4 rootstocks (101-14 MGT, 3309 R, Riparia Gloire de Montpellier, SO4), as well as own-rooted vines. The experimental plot was implanted in 2013 in gravelly-loam soil. Several parameters were observed, such as yield, berry chemistry and wine sensory analysis. Rootstock effect showed little impact on yield, but a significant impact on berry chemistry, mainly on total soluble solids and on titratable acidity. Moreover, a significant effect on wine appreciation was noticed, where the use of rootstocks generally increased wine quality. In Quebec, grafting hybrid cultivars is not a common practice, but it could be profitable to the producer to select rootstocks adapted to their soil and climate conditions in order to improve profitability.

Keywords: rootstock, cold-hardy hybrids, cold climate

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Amino nitrogen content in grapes: the impact of crop load

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As an essential element for grapevine development and yield, nitrogen is also involved in the winemaking process and largely affects wine composition. Grape must amino nitrogen deficiency affects the alcoholic fermentation kinetics and alters the development of wine aroma precursors. It is therefore essential to improve nitrogen use efficiency by the plant to guarantee a sufficient grape nitrogen content at harvest. Understanding the impact of environmental conditions and cultural practices on nitrogen use efficiency in plants would allow us to better orientate our technical choices with the objective of quality and sustainability.

This trial focuses on the impact of a common viticultural practice in Europe – i.e., crop limitation – on nitrogen distribution in the plant and on grape nitrogen composition. A wide gradient of crop load was set up in a homogeneous plot of Chasselas (*Vitis vinifera*) in the experimental vineyard of Agroscope, Switzerland. Dry weight and nitrogen dynamics were monitored in the roots, trunk, canopy and grapes, during two consecutive years. Grape amino nitrogen content was assessed in both years, at veraison and at harvest.

The close relationship between fruits and roots in the maintenance of plant nitrogen balance was highlighted. Grape nitrogen concentration remained unchanged regardless of crop load to the detriment of the growth and the nitrogen content of the roots. Meanwhile, the size and the nitrogen concentration of the canopy were not affected. Leaf gas exchange rates were reduced in response to lower yield conditions, reducing carbon and nitrogen assimilation and increasing intrinsic water use efficiency. The must amino nitrogen profiles could be discriminated as a function of both the year and the crop load. These findings demonstrate the impact of plant balance on grape nitrogen composition and contribute to the improvement of predictive models and sustainable cultural practices in perennial crops.

Keywords: crop thinning, nitrogen use efficiency, yeast assimilable nitrogen, amino acids, partitioning, reserve mobilization

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Amino nitrogen content in grapes: the impact of crop load

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Keywords: crop thinning, nitrogen use efficiency, yeast assimilable nitrogen, amino acids, partitioning, reserve mobilization

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Understanding growth and developmental genetic variations of Concord (*Vitis labruscana*) and wine grapes (*Vitis vinifera*) for differences in yield, fruit quality, and productivity

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The interspecific cultivated variety, Concord (*Vitis labruscana*) is a cross between *Vitis vinifera* and *Vitis labrusca*. Consequently, its growth and developmental characteristics resemble that of both species. However, these features are yet to be fully characterized in these closely related species. To fill this knowledge gap, we compared vegetative and reproductive structures and fruit quality attributes, including seed morphology of Merlot and Concord grapes. The vegetative growth entailing shoot growth, development, morphology and canopy development, and the reproductive structures entailing inflorescence architecture, flower development, pollination and fertilization, and berry growth and development, and fruit composition are compared between the two species to characterize the genetic diversity, and how environmental conditions impact them.

Keywords: Acids, grapevines, leaves, morphology, seeds, sugars

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Evaluation of rootstock characteristics on graft performance

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Grafting success in grapevine nurseries varies considerably from year to year and between different batches of wood, yet we have little understanding of the causes of this variation. In order to identify factors explaining this variation in grafting success, rootstock wood batches (*Vitis berlandieri x V. rupestris* cv. 110 Richter) of different origins and growing conditions were characterised and these batches were used to determine grafting success rate. Ten different batches from different mother vineyards were analysed: six batches from Spain and 4 batches from France. To characterise the wood, the presence of viruses (GFkV, GFLV, GLRaV1 and GLRaV3) and pathogens associated with fungal trunk diseases (Petri disease, Black foot and Botryosphaeria dieback) was tested; the only pathogen detected was *Cadophora luteo-olivacea* in two batches. Furthermore, wood was histologically characterized by measuring tissue size (pith, xylem and phloem), the number of xylem vessels and xylem conductivity. Main differences between batches were the presence of large vessels, which ranged in size between 240 and 320 µm diameter, what resulted in a significantly higher xylem specific conductivity. Also, the content of metabolites (starch, sucrose, glucose, fructose and proteins), macronutrients (N, Na, K, Ca, S, Mg and P), micronutrients (Bo, Zn, Mn, Fe, Cu and Al) and isotopes (δ13C and δ15N) was determined. All these factors show some significant differences in individual batches. Finally, grafting success rate was recorded after up-rooting and was studied in relation to all the parameters analysed in the wood used for grafting. A positive correlation of grafting success with the parameters of water stress (δ13C), nitrogen assimilation (δ15N), the percentage of large vessels (200-280 µm) and starch content was observed.

Keywords: Graft, rootstock, success rate, conductivity, xylem, water stress

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Vine response to spur and cane pruning criteria differing in their respect of sap flow paths is affected by plant material and growth conditions

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The longevity of vineyards, and consequently, the sustainability of the sector, is challenged by the trunk diseases whose incidence might be increased by an inappropriate pruning management. Vineyards are pruned in order to decrease the number and length of shoots, reducing the amount of clusters and increasing their quality. However, good pruning practices are being forgotten leading to more aggressive pruning techniques that increase the damage over the plants, causing a detrimental economic impact on the winegrowing sector. Although the study of vineyard longevity is difficult to address, we aimed to characterize the response of the grapevine to different pruning managements for obtaining long-term healthy vineyards. During the experiments, four adult vineyards were monitored. Two planted in Tempranillo trained in a double cordon Royat in North Spain (SP) and two in Cabernet franc trained in simple Guyot system in South France (FR). Three alternative pruning systems to compare with the winegrower´s were applied: i) PW (with protection wood); ii) PW + SF (with protection wood and respecting the sap flow); and iii) AGGR (aggressive pruning, without protection wood or respecting the sap flow). In Tempranillo trained in a double cordon Royat system, AGGR increased the pruning weight and the shoot weight, and tended to decrease the green pruning. In Cabernet franc trained in simple Guyot, SF decreased the shoot number and pruning weight compared to AGGR and PW. However, green pruning was affected differently in the two monitored FR vineyards. Results showed that pruning effects on grapevine growth are highly dependent on the vegetal material, growth conditions, and trellis system. Therefore, we observed a lower effect of AGGR pruning on FR vineyards comparing to SP, where AGGR presumably decreased the bud number, diminishing the shoot competition and consequently, enhancing growth compared to PW and PW+SF pruning systems.

Keywords: Alternative pruning, sap flow, sustainability, vineyard longevity

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P45

Vine response to spur and cane pruning criteria differing in their respect of sap flow paths is affected by plant material and growth conditions

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Keywords: Alternative pruning, sap flow, sustainability, vineyard longevity

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Using plant biostimulants as an alternative to hydrogen cyanamide treatment for dormancy release in table grapes *Vitis vinifera* Crimson Seedless

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Vitis vinifera grapevine is a perennial crop which is globally cultivated, surviving cold winters in temperate zones by entering a state of dormancy. In spring, bud dormancy is released by increasing day lengths and warmer temperatures. Rapid, even bud break is vital to ensure optimal crop yield and carry out effective management practices. High and uniform bud break is dependent on the accumulation of sufficient chill units during winter. Regions with warm climates with mild winters, such as in South African table grape growing regions, rarely reach required chilling accumulation. In such regions, hydrogen cyanamide (HC) is commonly applied to ensure optimal and uniform bud break. Due to the toxicity of HC, alternative environmentally-friendly treatments are sought by the industry. The molecular mode of action of HC has been well studied over the last 20 years. Several models detailing mode-of-action exist in the literature. HC inhibits catalase and induces hypoxia, activating a complex molecular cascade within bud tissues. The levels of reactive oxygen species (ROS) increase significantly, carbon flux is modulated, and phytohormone levels change. These events lead to the activation of transcription factors associated with growth resumption and cell expansion. This project aims to combine candidate biochemical agents (e.g., nitric oxide donor molecules with plant biostimulants to enhance bud break in eco-dormant buds. Plant biostimulants are defined as natural substances (excluding nutrients & pesticides) which have the capacity to modify physiological processes of plants. Two plant biostimulants, BC204 (citrus-based plant extract) and Lumichrome (riboflavin derivative), are the focus of this study. These products are being evaluated in a similar approach in the table grape Crimson Seedless V. vinifera, buds, combining phenotypic and molecular data. Glasshouse & field trials are being executed over two growth seasons.

Keywords: bud break, hypoxia, Dormex®, grapevine, antioxidant response, molecular cascade, insufficient chill units

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Early molecular response of cold-deacclimated grapevine buds to late frost occurrences

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Despite the observed increase of average global surface temperatures, spring frosts are expected to remain a threat to grapevine production in several areas of the world. Due to higher vulnerability in proximity to budbreak, late freezing events can greatly damage bud vegetative and reproductive tissues alike thus affecting multiple years of development. Therefore, improving our understanding of the mechanisms underlying tolerance to unpredictable freezing events during cold deacclimation is of paramount importance to enhance grapevine's resilience in a changing climate. A spring frost was registered, on April 7th 2021, in the Italian region Friuli Venezia Giulia, allowing the field study of the early molecular responses of cold-deacclimated buds in the hybrid Sauvignon Nepis (Sauvignon Blanc × Bianca), showing a good tolerance to this event. Differential thermal analysis (DTA) was adopted to monitor bud cold hardiness levels at 15-days intervals throughout the 2020/2021 winter season and gene expression analyses were performed to investigate bud reactivity to the spring frost event. Selected genes linked to winter freezing resistance and abiotic stress perception were examined. Results highlighted differential responses to late frost exposure depending on bud developmental stage, with the BBCH 00 stage (winter bud) being the most reactive. In particular, freezing tolerance-related genes (*VvCBF3, VvDHN1/2*) and raffinose synthase encoding gene (*VvRS*) were upregulated in response to freezing shock. *VvNCED1*, involved in abscisic acid biosynthesis, and ethylene receptor VvEIN3 also appeared involved in this process. This work outlines several molecular pathways activated in cold-deacclimating buds in response to sudden frost occurrence and draws attention to the varying adaptation capabilities in relation to bud phenological stage. These results can be a starting point for future investigations concerning grapevine's adaptive potential in coping with late frost events.

Keywords: bud break, hypoxia, Dormex®, grapevine, antioxidant response, molecular cascade, insufficient chill units

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P48

Early molecular response of cold-deacclimated grapevine buds to late frost occurrences

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Keywords: bud break, hypoxia, Dormex®, grapevine, antioxidant response, molecular cascade, insufficient chill units

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Pre-flowering leaf removal affects gamete viability and reduces cluster compactness in *Vitis vinifera* L. Grüner Veltliner

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Pre-flowering leaf removal (PFLR) is a management practice that involves the removal of basal leaves from selected nodes of the vine before the onset of flowering. Numerous works indicate the beneficial effect of this practice to mitigate yield loss derived from cluster rot diseases (such as gray and sour rots), particularly in grapevine cultivars with highly compact clusters. However, few of them indicate the effect of PFLR on the reproductive performance of the vine. Here, we tested the effect of two PFLR treatments applied at two different moments (one or two weeks before flowering time) on the cluster yield parameters of Grüner Veltliner, one of the most important cultivars for the Austrian wine industry. Our results indicate that PFLR treatments affect male gamete development and viability, probably due to the detrimental effect of pre-flowering carbohydrate source limitation on gametogenesis processes. This limitation affected fruit set processes, which in turn decreased the number of berries per cluster of defoliated vines. As it did not generate changes in rachis architecture or berry dimensions, the reduction in berry number had a loosening effect on Grüner Veltliner clusters. Interestingly, no effects on female gamete development were observed, as defoliated and no-defoliated plants showed a similar number of seeds per berry, suggesting some asynchrony between male and female gametes development and/or different degree of susceptibility to PFLR treatments. The lower number of berries per cluster observed in defoliated plants led to lower cluster weight compared to the undefoliated ones. However, this lower yield could be considered as an acceptable trade-off for the wine industry, as the significantly lower cluster compactness might contribute to a better sanitary status of Grüner Veltliner grapes for high-quality winemaking.

Keywords: Cluster weight; Early defoliation; Grapevine yield; Pollen viability; Reproductive performance

P50

Characterization of a cv. Tempranillo Tinto variant exhibiting a male-like flower phenotype

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Domesticated grapevine (*Vitis vinifera* L.) is used for wine, fresh fruit, raisins and juice production. Two subspecies can be identified within this species: *V. vinifera* ssp. vinifera, the cultivated form comprising mostly hermaphrodite and some female cultivars and *V. vinifera* ssp. *sylvestris*, the suggested wild dioecious ancestor. Studies dealing with this trait identified a major QTL on chromosome 2 as the grapevine Sex Determining Region (SDR), which harbours several proposed candidate genes. The aim of this work is the genetic and molecular characterization of a Tempranillo Tinto somatic variant that shows an androgenized flower phenotype. Whilst flowers in this somatic variant develop normal stamens, they present a reduced gynoecium that, unlike canonical male flowers of *V. vinifera* ssp. *sylvestris*, still enable fruit setting and ripening. Phenotyping results of a self-cross progeny of this variant line (more than 100 offspring) indicated that the mutant flower phenotype is inheritable. Furthermore, genotyping results of the microsatellite marker VVIB23, linked to the SDR, showed that the putative mutation co-localizes with this locus. One of the proposed female development inhibitor genes underlying the SDR locus is VviAPT3, which encodes an adenine phosphoribosyl transferase that may inactivate cytokinins by using them as substrate. The inactivation of these hormones, which promote gynoecium development in wild male vines if applied exogenously, could explain the mutant phenotype. RT-qPCR and RNA-seq expression analyses during flower development demonstrated the overexpression of VviAPT3 in the mutant line compared to a normal flower Tempranillo Tinto line used as control. Several experiments are ongoing to identify the genetic variation that causes this male-like phenotype, such as the comparison of the whole genome sequences of the variant and a control Tempranillo line, or the genotyping of VviAPT3 and other candidate genes through Sanger sequencing.

Keywords: Grapevine, flower mutant, sex determination

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Grape Berry Shrivel Disorder ' Transcriptional Changes in Rachis Tissue

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Berry shrivel (BS) is a physiological ripening disorder with significant economic impact in viticulture with 'Zweigelt' being the most-afflicted cultivar in Austria. The symptoms are well characterized and comprise deficient sugar accumulation and high acidity in berries, low anthocyanin biosynthesis in skins, and shriveled berries. The actual causes and triggers of BS are unknown. In this study we followed the hypothesis that BS could be the consequence of a disturbed assimilate transport in the rachis' vascular system. Previous microarray analyses based on pooled healthy and BS-affected rachis samples were utilized to identify relevant pathways and important genes which were further analysed by qPCR. In rachis samples collected from 30 through 72 days after anthesis, twelve candidate transcripts were identified and analysed by qPCR. They originated from the GO pathways 'carbohydrate metabolism', 'amino acid metabolism', 'RNA biosynthesis and transcription', 'cell wall organisation', and 'solute transport'. The interpretation of qPCR results revealed that the time course of expression of the analysed transcripts followed three distinct expression patterns. The first pattern showed at veraison a peak of expression of cell wall organisation- and transcription-related genes which was followed by a phase of down-regulation. The second pattern displayed a peak of expression during the ripening phase around 65 days after anthesis and comprised cell wall organisation- and solute transport-related genes. The third pattern showed a peak of expression around 65 days after anthesis which was sustained until the end of the ripening period. Relevant genes were carbohydrate metabolism- and amino acid metabolism-related genes. From these results we conclude that cell wall modification pathways and solute transport pathways are affected in the pedicel of BS-affected Zweigelt clusters. Critical for future studies will be to elucidate the timescale of events in different tissue like rachis, pedicels, and berries in order to distinguish causes from consequences.

Keywords: Grapevine; Berry Shrivel; Rachis; qPCR; Cell Wall

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Somatic embryogenesis as tool to generate improved white-berried grapevine lines from red-berried periclinal chimeras in Tempranillo cultivar

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The grapevine meristem contains at least two distinct cell layers, L1 and L2, which can bear different genotypes and give rise to chimeric tissues and organs. Grape skin epidermal and internal tissues derive from L1 and L2 cell layers, respectively. Tempranillo Gris, a periclinal chimera of Tempranillo Tinto, display berries with grey colour: This colour is due to the hemizygous deletion in the L2 meristem cell layer of VviMybA genes responsible for the regulation of anthocyanin biosynthesis and pigmentation of the berry. We carried out direct somatic embryogenesis methods to obtain embryos of unicellular origin from Tempranillo Gris. In this way, embryos from L1 layer would give rise to plants generating black berries (functional VviMybA locus), while embryos from L2 layer would develop into plants producing white berries (null VviMybA locus). Somatic embryogenesis was induced from stamen filaments using different combinations of growth regulators, thidiazuron (TDZ) and 2,4-dichlorophenoxyacetic acid (2,4-D), in a basal medium containing Nitsch and Nitsch salts and Murashige and Skoog vitamins. A total of 52 somatic embryo-derived plantlets were obtained and genotyped through an assay based on single nucleotide variant (SNV), which discriminates for functionality of the VviMybA locus. All somaclones came from single cell-originated somatic embryos with genotype only attributable to L1 origin meristem cells. These results indicate that L2-derived cells do not have competence to regenerate somatic embryos. As the most frequent source of somaclonal variation through *in vitro* culture techniques is change in ploidy levels, these were determined by flow cytometry in the regenerated plants. The results showed absence of somaclonal variation, having all tested plantlets a nuclear diploid level. We show that direct somatic embryogenesis could be considered as a powerful tool for grapevine biotechnological improvement by rescuing genotypes of interest present in L1 meristem cells.

Keywords: Somatic embryogenesis, Tempranillo, grape color, somaclonal variation

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Adapting perennial crops for climate change: Graft transmissible effects of rootstocks on grapevine shoots

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A fundamental goal in plant biology is an understanding of the genomic and environmental basis of phenotypic variation. Typically, adaptation and plasticity are concepts describing the ecological fit of plants to varied climate conditions. Few crops allow for the specific partitioning of the above- and below-ground organ systems of plant plasticity. Grafted grapevines are such constructs, in that they represent the physical chimerization of the shoot (scion) and root (rootstock) genotypes from different species. For the last 5 years, we have been pursuing a number of large-scale experiments in the United States, examining the impact of rootstocks and climate on shoot phenotypes. Multi-year studies in research vineyards in Missouri (Aim 1), and commercial production vineyards in California (Aim 2) have concluded, shedding light on the ionomic, transcriptomic, and phenomic variation in grapevine. Ongoing research in a 200-genotype rootstock mapping family population (Aim 3) replicated in New York, Missouri, and South Dakota demonstrate the potential for expanded scion phenotypic optimization in the varied climates of Eastern North America. Through pursuing these aims, the project has successfully developed cross-disciplinary student training and collaboration with our industry partner, E. & J. Gallo Winery (Aim 4).

Keywords: Graft, Rootstock, Climate, Genetic Mapping, Ionomics, Transcriptomics

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Functional characterisation of cuticle-related grapevine genes

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The epidermal layer of aerial plant tissues is covered by a hydrophobic structure known as the cuticle. This cuticular layer plays a major role in protecting the plant against abiotic and biotic stress factors, namely water loss and pathogen infection, as well as contributing towards postharvest fruit quality traits such as colour, texture, firmness, and shelf-life. Despite its economic significance, there are still considerable knowledge gaps concerning cuticle biosynthesis and its regulation in grapevine. The aim of this study is to identify and functionally characterise cuticle-related grapevine genes. To this end, putative cuticle regulators were selected from RNAseq data generated from an *in vitro* VviERF045 overexpressing grapevine line displaying impaired cuticle development. The selected genes were cloned and then overexpressed in grapevine (VviERF045) and tomato (VviSOC1a, VviAG1, VviMYB141 and VviSHN3), as well as knocked out in grapevine (VviMYB141). Preliminary analysis of the T0 tomato populations showed potential involvement of these genes in cuticle accumulation, trichome development, conical cell formation, floral organ identity and fruit ripening. In addition, evaluation of the acclimated overexpressing and knockout grapevine lines exhibited observable alterations to the stomatal and trichome densities of leaves. Promoter transactivation assessment, using a dual luciferase assay, confirmed the association of all selected genes within the greater regulatory pathway of VviERF045.

Keywords: VviERF045, cuticle, trichome, floral identity, ripening, conical cell, transcriptional regulation

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Vascular connections into the grape berry

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Grapevine vascular bundles transport water, nutrients and photo-assimilates and therefore play a critical role in berry growth and composition. The receptacle/berry junction is a critical branching point for vascular tissues that supply either the peripheral network under the berry skin or the central bundles traversing the core of the berry. Differential staining of transverse sections of berries and receptacles followed by fluorescent microscopy was carried out for examining the vascular connection between berries and pedicles. Morphometric and vascular characteristic analyses incorporated with plant hydraulic modelling, within the brush zone of four commercial cultivars including seeded (i.e., Shiraz and Sauvignon Blanc) and seedless (i.e., Ruby Seedless and Flame Seedless) cultivars, were carried out. This study revealed variation in vascular growth between grape pedicels and berries was independent of seededness but differences in receptacle xylem vessel size and the distribution of vessels with various sizes could contribute to cultivar-dependent xylem backflow constraint.

Keywords: berry anatomy, vascular tissues, xylem, phloem, imaging

P56

Understanding the genetic basis of grapevine phenological traits through QTL mapping in a segregating population

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Long-term studies on grapevine phenology have clearly demonstrated that global warming is affecting phenological events, leading to an anticipation in their timing and increasing the impacts of temperature on grape quality. Adaptation of local varieties to changing climatic conditions is thus a major breeding target, which includes the selection of late ripening varieties/clones, whose bunches may escape the warmer summer condition by postponing the ripening period. However, assessment of the genetic basis of phenology and quality related traits is a prerequisite to develop breeding programs for grapevine varieties adapted for the cultivation in specific viticultural areas and to identify the candidate genes for the new breeding technology approaches. For this purpose, we have evaluated a population from the cross between the locally (Verona province – Italy) grown grapevine variety "Corvina" and the variety "Solaris", highly divergent for their phenology and fruit ripening traits. Seedlings were developed, propagated and grown in field conditions to be evaluated for mapping of genetic traits. High throughput SNP genotyping of the cross population was applied through hybridization to an Illumina Vitis18KSNP chip. Field phenotyping, based on data collected over two seasons included the determination of the main phenological stages (budbreak, flowering, veraison) together with the assessment of some morphological and quality traits at harvest on all progenies with the final purpose of QTL mapping. Preliminary results about genotypic and phenotypic characterization are presented. These data will be used for QTL mapping to allow the identification of genetic determinants for these traits. New markers derived through the described characterization of the Corvina x Solaris cross are expected to assist future selections. Altogether, the described approaches will finally allow to improve our current understanding of the genetic control of phenology and berry quality traits in grape, thus helping and assisting breeding.

Keywords: Grapevine, Phenology, diverse climates, QTL mapping

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Is late winter pruning an agronomic management to encourage the adaptation of viticulture to climate change in Chile?

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The quality and production of the vine depends on the climate and climate change can therefore affect the sustainability of the grapevine. In Chile, a temperature increases of at least 1°C has been projected for the central zone. The temperature increase can affect development and growth of the vine, and this is noticeable as changes in the budbreak and harvest dates, shortening of the phenological periods as well as changes in quality. Considering this, it is relevant to seek agronomic management adaptations to achieve sustainability of viticulture in the face of a climate change scenario. A suggestion is to modify the pruning dates, allowing better conditions to development the maturity of the grapes. This can be done by performing late pruning (after budbreak), rather than a delay in the development of the grapevine, due to the elimination of the reserves already mobilized in the plant.
During the 2020-2021 season, three pruning dates (traditional, budbreak and 2-4 cm shoot length) were evaluated in the Central Valley of Chile, in cv. Cabernet Sauvignon, under conditions of increased temperature.
Preliminary results showed that the increase in temperature decreased the acidity and the anthocyanin concentration and shows an imbalance between sugars and phenolic compounds in the control treatments. In high temperature conditions, pruning during sprouting delays the flowering date by 9 days, the fruit setting by 7 days and the harvest by 6 days. This phenological delay had no effects at the photosynthetic level (efficiency of the photosystem and photosynthetic pigments), or on the number of inflorescences. Furthermore, pruning in sprouting showed greater numbers of inflorescences than in the other two evaluated treatments. Technological maturity did not present changes in pH or acidity (considering a differentiated harvest at the same amount of total soluble solids). In relation to phenolic maturity, pruning in bearing increases total anthocyanins, and partially the easily extractable anthocyanins.

Keywords: late pruning, grapevine, climate change, budbreak

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Effects of temperature increase on photosynthesis and grape quality in cv. Syrah in Central Valley of Chile using Open Top Chambers

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Climate change involves modifications in the annual average temperature and precipitation scheme. The temperature increase is especially alarming because it affects several mechanisms in the grape physiology, which determines yield and quality.

To evaluate the effects of temperature increase on the physiology and quality of a cv. Syrah in Central Valley of Chile under commercial conditions in the field, open-top chambers (OTC) were used. Two temperature treatments (high temperature and ambient temperature) were evaluated. The experiments were carried out over three seasons. The effects of OTC were evaluated by measuring microclimate and physiological parameters at key phenological stages. At harvest time, yield and quality were determined and measurement included anthocyanin contents and total soluble sugars (TSS).

OTC increased the temperature of the grapevine leaves. Under these conditions, plants decreased their photosynthesis and stomatal conductance. Although the increase in leaf temperature in the OTC could cause this phenomenon, it is relevant to emphasize that these temperatures did not exceed 30°C. This drop in photosynthesis may be related to the interaction between temperature and reduced water availability. Effects on gas exchange were not the only changes observed as the interaction between the increase in temperature produced by the OTC and the water deficit was found to decrease quality and production. Finally, the TSS values at harvest were higher and the concentration of anthocyanins lower under the OTC conditions compared to ambient conditions. Therefore, we could suggest that the interaction between the increase in temperature and the water deficit would produce a decoupling in the ripening of the berries. This may cause a change in the ripening conditions which could influence the harvest date and novel and improved agronomic managements.

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Molecular control of grapevine bud fruitfulness and its interaction with environmental factors

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Bud fruitfulness is a key component of reproductive performance of grapevine and it plays a significant role in annual production of vineyards. Environmental factors such as light and temperature are important parts of terroir and can have a strong impact on the formation of inflorescence primordia in latent buds, which determines the potential yield for the coming season. This study aims to investigate the effects of different levels of temperature and light intensity on grapevine vegetative growth and bud fruitfulness, and the interactions of the temperature and light with the transcription on the bud level. Semillon vines were propagated in growth rooms under six controlled environments, including two temperature regimes and three levels of light intensities within each temperature regime. Vegetative growth, including leaf area, shoot weight and length, number of nodes, and internode length were recorded at E-L Stage 16. Bud fruitfulness was assessed by bud dissection analysis at three stages according to the development of shoots. Results demonstrated that vegetative development was accelerated by higher temperature but was negatively correlated with light intensity. Moreover, shoot leaf area, the weight and length of shoots and internode length were higher under the lower temperatures and lower light intensities. There is a positive linear relationship between bud fruitfulness and both temperature and light, with more and larger inflorescence primordia under higher temperature and higher light intensity. RNA-seq of buds from different treatments was conducted at the same three stages with bud dissection. Results showed that the transcription was significantly distinct among the groups, with substantial gene expression differences due to growth stage, temperature and light. This study clarifies the mechanism underlying the bud development response to temperature and light and provides a better understanding of the regulatory mechanisms for IP initiation and differentiation.

Keywords: Bud fertility, vegetative growth, inflorescence primordia, grapevine bud transcription

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How effective are plant biostimulants to alleviate drought stress in grapevine?

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There is currently an increasing demand for innovative agronomic tools able to enhance crop resilience against environmental stresses caused by climate change. Even though plant biostimulants (PBs) have been found able to increase water stress tolerance of several crops under different growing conditions, research on the use of these natural compounds in viticulture is still incomplete and results are often inconsistent. The aim of this study was therefore to assess the ability of different commercial PBs to mitigate the adverse effects of water limitation in grapevine. PBs, including arbuscular-mycorrhizal fungi (AMF), protein-hydrolysate (PH), seaweed extracts (SEA) and silicon (SI) were tested on potted cv. Sauvignon Blanc grown in controlled conditions under two water regimes: well-watered (WW) and water-stress (WS). Vine growth performances, during and after the drought period, as affected by the different PBs applications were monitored every other day by measuring several physiological and biometric parameters. During WS, midday stem water potential of AMF and SEA-treated plants showed a slower decrease (-0.2 MPa, corresponding to 36% less) than the untreated-WS vines. Moreover, the PBs pre-stress application accelerated the leaf gas exchange recovery to WW values. Upon rewatering, SI-WS-treated plants showed increments of leaf gas exchange rates that were around 30% higher than those of the control-WS vines. As for the seasonal biomass accumulation, it resulted lower in WS-vines, independently of the PBs. Overall, the application of selected PBs before the water stress allowed to slow down the build-up of the drought stress in grapevine, therefore enhancing the possibility for the crop to overcome the negative physiological consequences of periods of water scarcity. Further evidence of PBs effectiveness is nevertheless needed especially under vineyard (open field) conditions.

Keywords: drought tolerance, seaweed extracts, protein hydrolysates, AMF, silicon, vine physiology

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TOPIC C: FRUIT COMPOSITION
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Towards the identification of the genetic determinants of harvest date in table grapes

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Grapevine (Vitis vinifera L.) is one of the most economically important fruit crops in the world, and its development is highly dependent on environmental conditions. Moreover, climate change has a direct implication in grapevine phenology, affecting growth and fruit maturation. Therefore, the characterization of the genetic architecture of harvest date trait, and based on that, to identify genetic markers that could be used as selection tools in table grape breeding are key points for the development of new cultivars better adapted to new climatic conditions. To approach this issue, a genotyping-by-sequencing (GBS) experiment was performed considering 120 grapes genotypes exhibiting contrasting phenotypes for harvest date, resulting in a diverse pool containing table, wine and mixed-use grapes, as well as segregant lines that belong to INIA's breeding program. Subsequently, biallelic polymorphisms were selected and filtered by MAF (> 5%) and missing data (< 5%) using a VCF pipeline, identifying 255,313 high-quality SNPs which were annotated using SnpEff. In parallel, the phenotypic diversity for harvest date in table grape genotypes was determined, as well as other relevant phenological stages such as budbreak, flowering, fruit set and véraison time. In addition, phenotypic evaluations were performed at harvest including effective leaf area index (LAIe), chlorophyll fluorescence, internode number, cluster weight, among others. Subsequently, phenotypical and phenological data from two seasons were analyzed, and considered for genome-wide association studies (GWAS) using TASSEL software and GLM model. Our preliminary results allowed the identification of chromosomic regions associated to harvest date at chromosomes 3, 4, 5, 7, 8, 13, 14, 15, 16 and 18. Further studies applied to harvest date-related traits are currently under analysis. Our ultimate goal is the selection of a set of markers with association to harvest date which will be validated using amplicon sequencing in order to evaluate their informativeness.

Keywords: Harvest date, SNPs, GWAS, molecular markers, table grapes, plant breeding.

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Temperature affects organic acid, terpene and stilbene metabolisms in wine grapes during post-harvest dehydration

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The partial dehydration of grapes after harvesting (also called withering) is a traditional practice in several wine producing regions to obtain high quality wines. Postharvest dehydration has a significant impact on general berry metabolism and physiology, yielding a final product that is richer in sugars, solutes, and aroma compounds. These changes are controlled at least in part at the transcriptional level and are highly dependent on grape water loss kinetic and the environmental parameters, especially temperature, inside the facility where grapes are stored. However, it is difficult to separate the effects driven by each single environmental factor from the ones associated to the dehydration rate, being the latter also affected by the environment. To define the temperature effect on wine grape physiology and composition during postharvest dehydration, bunches of the red-skin cv. Corvina and Corvinone (*Vitis vinifera*) were withered at two distinct temperatures, 8 ± 0.5 °C and 11.6 ± 0.6 °C, while maintaining the same water loss rate by adjusting the relative humidity to 59.5 ± 1.6 % and 70.4 ± 2.7 %, respectively. Following 84 days of withering, significantly higher levels of organic acid were found in berries dehydrated at the cooler temperature. Instead, the content of sugars, total polyphenols and anthocyanins were similar under both conditions. A GC-MS aromatic profile analysis showed higher concentrations of terpenes such as glycosidically-bound linalool and geraniol in grapes dehydrated at cooler temperature. Moreover, targeted gene expression analysis revealed a lower expression of malate dehydrogenase (VvMDH) and a higher expression of terpene synthase (VvTPS) and stilbene synthase (VvSTS) in Corvina grapes dehydrated at cooler temperature. In conclusion, the temperature during post-harvest withering can be confirmed as an important factor affecting the metabolism of wine grape, with potential impact on the quality of the produced wine.

Keywords: Wine grapes, post-harvest dehydration, withering temperature, GC-MS aromatic profile analysis, gene expression analysis, Amarone

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Searching for the Drivers that Regulate Fruit Acidity

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Acidity plays an important role in the production of quality wines. The accumulated malate in grapes is consumed by respiration and gluconeogenesis processes throughout the fruit maturation phase. Respiration losses in malate can be compensated in part by CO_2 fixation in the fruit. The adequate exposure of the fruit to sunlight will be crucial in the current scenario of climate change with high temperatures causing a general reduction of malate in grapes during ripening and potentially negative consequences for wine quality. However, many physiological changes occur at different times and rates in different berries on the same cluster or vine, as exemplified by the phenological stage of veraison. The main objective of this work was to study the variability of titratable acidity (TA), organic acids, respiration and photosynthesis in sun exposed and non-exposed berries at veraison, and to investigate the relationship between these parameters in this short period of time. An experiment was carried out in 2016 using field-grown Merlot vines. The changes in soluble solids, TA, organic acids, respiration and photosynthesis were measured in berries at seven stages: hard green, soft green, pink, red, blue, ripe and overripe. Temperature and light intensity were recorded in sun-exposed and non-exposed clusters. The results showed the massive and rapid changes that occur in grape berries at the beginning of ripening. Sun-exposed berries had higher TA than the non-exposed berries at the stages of pink and red. At pink stage, malic acid per berry was higher in the sun-exposed clusters than in the non-exposed clusters. The variability of the measured parameters and the relationship between them in two sun exposure levels showed the importance of understanding “veraison” considering each one of the stages that compose it.

Keywords: light exposure, temperature, malic acid, tartaric acid, titratable acidity, fruit gas exchange

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Towards grape vineyards rich in bioactive compounds

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Phenolic compounds of grapes are mainly found in the skin and seeds while in the pulp only a low amount is reported. However, teinturier wine grapes with coloured pulp and potential for intense colour, have high levels of polyphenols not only in the skin and seeds but also in the pulp. The aim of this work was to study the Total Phenolic Compounds (TPC) and Total Antioxidant Capacity (TAC) of several grape cultivars (*Vitis vinifera* L.) in their edible parts (skin and pulp) in order to screen for new varieties. We have compared grape samples from teinturier grapevines Morrastell Bouschet (MB), Petit Bouschet (PB) and Alicante Bouschet (AB), with grape samples that came from a cross between these cultivars and the table grape cultivars, Itumone and Sugraone. Grape samples of PF1008 (Alicante Bouschet x Itumone) showed higher values of TPC, with 12.0 g GAE kg-1 dw, and TAC from 17.4 to 24.8 g TE kg-1 dw. PF1008 were 41-82% higher in TPC and 38-113% higher in TAC than the teinturier varieties. High values of bioactive compounds suggest an interesting usage as non-alcoholic, vitivinicultural products for human consumption such as musts and juices, table grapes and dried grapes.

Keywords: coloured pulp, teinturier grapes, phenolic profile, antioxidant activity, *Vitis vinifera* L.

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Adaptation of an *in vitro* grape berry growth culture system to study the development and ripening of Pinot noir berries

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Pinot noir grape production now ranks second only to Sauvignon blanc as the most widely grown grape varietal by area in New Zealand, and now makes an important contribution to the reputation of the New Zealand wine industry. Part of the success of Pinot noir can be attributed to expression of regionality within New Zealand, resulting in premium and ultra-premium wine. However, this regional expression and the variety's relative sensitivity means Pinot noir is subject to a commercially significant amount of variation in production and fruit quality impacting both the consistency of product and winery revenue. As part of the Bragato Research Institute Pinot noir programme, we are engaged in research to understand the fundamental mechanisms underpinning the influences of region and yield on quality. Grape berry development is a highly complex process influenced by an array of biotic and abiotic factors. To study any one of these factors in situ is challenging because of the interactive and/or confounding effect of multiple influences in a vineyard environment. An experimental system to test factors in a controlled environment would advance our understanding of the direct effects on berry development and quality. Here we report on the adaptation of an *in vitro* berry culture system for Pinot noir. Although other wine grape cultivars have been reported to be amenable to *in vitro* culture, there are no examples in the literature on the performance of Pinot noir berry in a culture system. Following three seasons of trials we demonstrate, through the optimisation of culture conditions, successful in vitro culture of Pinot noir berries for up to 60 days over the pre-post véraison period. We also illustrate that increasing the supply of sugar in culture media increases berry sugar concentration and is strongly associated with the production of berry anthocyanins.

Keywords: Pinot noir, *in vitro* culture, berry ripening, sugar, anthocyanins

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Influence of production level on the characteristics of the grape and wine of the cv. Tempranillo

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Recently, vineyard yields have shown an increasing trend, mainly due to the greater technification in crop management, leading to a loss of grape quality in many cases. In Spain, many vineyards are located within an Appellation of Origin, limiting the maximum productive level of the vineyards. This limit is variable, is not usually defined using agronomic criteria, and, for a fixed maximum quality level, each vineyard's maximum yield can differ from the one defined by the Appellation of Origin. This work aims to study the influence of three theoretical yield levels on grape and wine quality. For this purpose, a method of early yield estimation (fruit set) and subsequent production adjustment at the beginning of the veraison phenological stage at 5000 kg.ha-1, 7000 kg.ha-1 and 9000 kg.ha-1 was used in a Tempranillo vineyard within the Ribera del Duero Appellation of Origin. The results showed very similar values for the grape quality parameters between yield levels. However, some differences were detected in the phenolic composition and colour of the wines regarding wine quality. Similarly, the organoleptic analysis has shown different wine profiles.

Keywords: polyphenols, yield, production, crop load, organoleptic analysis, sensory analysis

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Genomic analysis of QTLs associated with monoterpene biosynthesis in grapevine

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Monoterpenes are a class of specialized metabolites in grapevine (*Vitis vinifera*) that play a vital role in plant-environment interactions such as pollinator attraction and pathogen/herbivore defence. In the case of agricultural cultivation their significant contributions to both the flavour and aroma profiles of table grapes and wine are particularly important. The monoterpene synthase (mTPS) gene family is extensive in grapevine and recent progress in TPS characterization studies and whole-genome sequencing of several grapevine cultivars indicate the existence of unique cultivar-specific mTPSs. Due to the high variability of mTPSs between cultivars, there exists great potential to identify and characterise novel TPSs in grapevine. In this study two genomic regions identified via prior QTL analysis were investigated for their contribution to monoterpene content in a grapevine mapping population. One region on linkage group 12 associated with geraniol accumulation while the other region on linkage group 13 associated with the accumulation of cyclic monoterpene. Genomic screening identified several putative monoterpene synthase genes in each region. These were then further investigated via a range of molecular techniques and *in silico* approaches, including gene expression analysis, molecular cloning, gene sequencing, molecular phylogenic analysis, and gene copy number determination. Results highlight the role extensive gene duplication events have played in the diversity mTPS gene function in grapevine.

Keywords: Grapevine, terpene synthase, monoterpenes, *Vitis vinifera*

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Meta-QTL analysis of fruit and bunch quality traits in grapevine

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Fruit and bunch quality traits are key targets for improvement in grapevine breeding programs. Bunch compactness is an important grapevine disease resistance and fruit quality trait that affects both wine and table grapes as compact bunches are more susceptible to fungal infections and uneven berry ripening. Compactness is a complex polygenic trait that is associated with multiple cluster architecture traits. Understanding the underlying genetics of cluster architecture is important for identifying DNA markers for marker-assisted selection, increasing the selection efficiency in breeding programs, and potentially leading to faster novel cultivar development. Quantitative trait loci (QTL) studies have been used previously to determine the genetic control of fruit quality traits; however, QTL effects are strongly influenced by genetic backgrounds and environments, and detected QTLs need to be validated before associated markers can be considered for marker-assisted selection. To validate and refine QTL positions, a meta-QTL analysis was performed for 15 grapevine fruit and bunch quality traits by integrating 203 QTLs from previously published mapping studies. Meta-QTL regions were identified for bunch, rachis, and berry morphology traits. Genomic analysis of these regions may lead to the identification of genes functionally linked to these traits. While markers significantly associated with these meta-QTLs can be used as possible candidates for marker-assisted selection in future work, addressing the lack of markers available for bunch traits.

Keywords: Fruit quality, QTLs, meta-QTLs, bunch compactness, cluster architecture traits

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Gene Expression Validation in Berry Shrivel Affected Berries of cultivar Blauer Zweigelt

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A number of physiological disorders can disrupt the ripening process of *Vitis vinifera* berries. The berry shrivel (BS) ripening disorder in the Austrian grapevine cultivar Blauer Zweigelt causes low sugar content, low anthocyanin content and high acidity levels in berries. No proposed trigger or cause could be affirmed to this date. This study aims to provide additional information of the expression of selected candidate genes during the process of BS symptom development determined with a qPCR approach. The samples were collected on five dates between 44DAA and 67DAA in a Zweigelt vineyard in Herrnbaumgarten, Lower Austria. The intents of this trial are (1) to find differences in the genetic expressions of BS affected berries, (2) to validate the only other available dataset of the development of the genetic expressions in BS berries and (3) to show that a new approach of repeatedly sampling of BS clusters is possible. The findings display the disturbed anthocyanin metabolism on a genetic level as well as a disturbed ethylene and ABA metabolism. Although in the latter case it can be expected that the expression of significant different genes is rather a symptom than a cause of the disorder, since the differences appear only after veraison. Also, cell wall modifying genes like xyloglucan endotransglucosylase/hydrolase and expansin were observed to be downregulated in berry shrivel affected clusters. A not further identified cupin gene displayed the highest difference. To compare the expression development of the performed qPCR (relative expression) and the dataset of the RNAseq (counts) both datasets were combined in the same graphics. The trends in both studies showed parallels. Further it was possible to show that a repeated sampling of one cluster, did not disturb the development of the disorder.

Keywords: qPCr, ripening disorder, anthocyanin

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Albariño cv. (*Vitis vinifera* L.) berry composition is influenced by topo climate in Uruguay oceanic conditions

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Albariño is a cultivar recently planted in Uruguay because its agronomy behaviour that promise a well acclimatation in Uruguay. Nevertheless, local knowledge of their behaviour is needed for our conditions to provide cultural techniques for their adaptation. The aim of this work is to assess final berry composition of Albariño in relation to topo climate. For this purpose, ten plots in a commercial vineyard in Eastern Uruguay were selected according to their topography (slope, altitude, and exposition). Temperature sensors were installed in their trellis system and temperature database of three growing seasons (2019, 2020 and 2021) was analyzed through bioclimatic indices adapted to viticulture. Berries metabolites were determined (sugars, total acidity, and pH) through weekly samples during the ripening period for each studied plot. Univariate (ANAVA) and multivariate (CPA) statistical analyses were carried out to assess the relationships between temperature, topography, and Albariño berry primary composition. The three studied growing seasons showed differences in climate, where 2020 was the hotter season and 2021 the wettest during the ripening. The region showed a high spatial variability of temperature explained by the topographic plots' conditions. During the growing season, plots situated at higher altitude showed less thermal amplitude compared to those in lower positions. In terms of berry composition, only final sugar content reached in each plot was statistical different between years (mean 210.8 g/l). However, total acidity showed the highest spatial variability, associated with plots' slope (2.6 g/l the highest difference between plots in 2021). No differences were reached through the relation between malic or tartaric acid composition at harvest (Mean value=0.43). Those results are of interest of our conditions, because demonstrate differences of berry final composition of a new white cultivar associated with topography. This information could be useful to differentiate potential wine styles at a plot scale.

Keywords: Grapevine, Albariño, Primary metabolites, Climate variability, South America

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Effect of vineyard fertilisation on *Vitis vinifera* cv. Ugni blanc berry skin characteristics, must and wine composition

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Yeast Assimilable Nitrogen (YAN) is one of the key parameters for alcoholic fermentation. It is occasionally measured during grape maturation and more commonly in the must just before yeast addition and fermentation. Base wine used for distillation are usually harvested between 13 & 21 °Brix, which is lower than for classical wine. YAN in Ugni blanc, the most planted variety in the Cognac appellation, averaged 94 mg/L over the last decade, which is too low for ideal fermentations. YAN of 120 mg/L is the minimum advised level for Cognac base wine, and it is mostly adjusted by adding inorganic nitrogen during winemaking. This practice is simpler and cheaper than improving must YAN using viticultural manipulation, which is challenging to achieve without increasing the vigour and therefore also the risk of *Botrytis cinerea* infection. However, anecdotal evidence suggested that the minimal YAN level is still required in the must since adjustments in the winery could not completely correct very low YAN, and excessive levels can lead to unbalanced aromatic profiles.

The experiment was conducted during the 2020 and 2021 seasons in the Protected Designation of Origin of the Cognac region. The parcel was split into two halves leading to a simple experimental design for a commercial vineyard with two plots: one control (C) and one treated (N). Nitrogen solution (ammonitrate) was applied at the beginning of flowering to the N plot. YAN was measured via an enzymatic method for both the control and treatment. This was conducted from the onset of véraison until harvest as well as in the resulting musts. Leaf area, yield and grey mould infection rate were estimated in both seasons. Additional measurements of leaf nitrogen and berry sensitivity to *Botrytis* were performed in 2021. Finally, C and N wines were made under controlled conditions with YAN adjustment to the same level using diammonium phosphate and the wine aroma compounds analysed by GC-FID.

In 2020, the N application was successful to significantly improve YAN (+54%) without impacting any other parameters, including yield. Vintage conditions were not favourable to *Botrytis* development. However, in 2021, which was cold and wet, YAN was only slightly increased (+20%) and N berries seemed to be more sensitive to *Botrytis* just after véraison. Berry skin characteristics (thickness and porosity) were also evaluated but did not show any differences between the control and treatment. Lower natural YAN slightly slowed down fermentation and led to lower concentrations of esters in C while in N isoamyl acetate was increased right at the end of the fermentation in 2020.

Keywords: berry, *Botrytis*, fertilisation, fermentation, esters, Yeast Assimilable Nitrogen

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TOPIC D: COMPUTATIONAL RESOURCES AND DATA INTEGRATION
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A COMPASS for VESPUCCI: a FAIR way to explore the grapevine transcriptomic landscape

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Successfully integrating transcriptomic experiments is a challenging task with the ultimate goal of analysing gene expression data in the broader context of all available measurements, all from a single point of access. In its second major release VESPUCCI, the integrated database of gene expression data for grapevine, has been updated to be FAIR-compliant, employing standards and created with open-source technologies. It includes all public grapevine gene expression experiments from both microarray and RNA-seq platforms. Transcriptomic data can be accessed in multiple ways through the newly developed COMPASS GraphQL interface, while the expression values are normalized using different methodologies to flexibly satisfy different analysis requirements. Sample annotations are manually curated and use standard formats and ontologies. The updated version of VESPUCCI provides easy querying and analysing of integrated grapevine gene expression (meta)data and can be seamlessly embedded in any analysis workflow or tools. VESPUCCI complete documentation can be found at https://vespucci.readthedocs.io/.

Keywords: grapevine, gene expression, data integration, transcriptomics, fair, database, compendium, manual curation, standard, ontologies

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The construction of a high-density consensus map based on three mapping populations using the Vitis18K SNP chip and a reference genome sequence

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The improvement of grapevine through biotechnology requires the understanding and identification of molecular bases of traits of interest. These determinants can be identified through studying the association between molecular markers, such as SNPs, and target traits. The Vitis18K SNP chip provides an inexpensive genotyping tool that allows for genome-wide scale marker analysis across populations. The majority of linkage maps are based on a single mapping population, but a consensus map obtained from multiple mapping populations can increase marker density and provide insight into marker order conservation. Here we present a consensus map based on three mapping populations. The genetic parents consist of well-known wine cultivars such as 'Cabernet Sauvignon', 'Corvina' and 'Riesling' often used for constructing linkage maps, but also include lesser known varieties like 'Deckrot' and a table grape selection, G1-7720. Data analysis in linkage map construction for large datasets can be time consuming or requires extensive coding skills. Different algorithms can allow for faster analyses, but quite often at the cost of number of markers mapped, or accuracy. In this study, we used a workflow which combines the genetic data of a mapping population with a reference genome sequence to construct three high-density population maps with an average inter-locus gap distance ranging between 0.74 and 0.99 cM. These maps show high correlation (0.9797 – 0.9997) with the 'PN40024 12X.2' reference assembly, whilst still allowing to report on approximately 100 markers with large order discrepancies, of which a third is consistent across multiple populations. Finally, we present a consensus map containing 6 895 molecular markers with an inter-locus gap distance of 0.53 cM, which shows even higher collinearity to the reference assembly compared to the single population maps. The presented workflow helps to construct linkage maps faster and more accurately, whilst the consensus map provides a promising tool for the identification of molecular determinants associated with traits of interest. This information is useful for researchers who want to use the Vitis18K SNP chip, but also showcase the integration of data which allows us to further our understanding of the grapevine genome.

Keywords: Vitis18K SNP chip, high-density consensus map, collinearity, reference assembly, data integration

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Identification of grapevine varieties using Deep Learning (CNN) and Keras

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Ampelography is the science that studies the identification and classification of grapevines (Vitis spp.). Today, although much has evolved on the DNA and laboratory side, it is a laborious science and ampelography is carried out manually through visual surveys usually performed by agronomists, which requires an enormous amount of time. Image processing and computer vision based on machine learning methods can enable agronomists to minimise the time spent on variety identification. This work aims to classify grapevine varieties using Deep Learning. A CNN (Convolutional Neural Network) to classify grapevine varieties was built and trained using a deep learning framework Keras/TensorFlow written in R and a dataset of 1200 leaf images of cv. Tinta del País (Tempranillo) and cv. Verdejo, divided into 90% of images for training and 10% of images for testing. CNNs can efficiently learn increasingly complex visual concepts by identifying spatial hierarchies of patterns and reprocessing the results of convolution layers. The CNN architecture consists of 3 components. A convolution, consisting of a filter to extract features from the input; pooling, to reduce dimensionality; and fully connected layers with neurons full connected to all activations in the previous layer. The goal of the trained CNN was to extract important features from the input leaf images in order to classify new leaf images. The detection model achieved a particularly good accuracy rate of 94.68%.

Keywords: deep learning, Keras, CNN, Convolutional neural network, Tensorflow, leaf recognition, variety recognition

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Genomics characterization of Carmenère clonal selections cultivated in Chile at Concha y Toro Vineyards

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The domestication process in *Vitis vinifera* has originated new cultivars and the vegetative propagation of cultivars has originated a variety of clonal selections, which are currently cultivated for wine production at Concha y Toro Vineyards, in Chile. Vegetative propagation trigger mutations which accrue in the genome of somatic cells. Most of them are deleterious, however, also recessive in heterozygous alleles. Hence, they do not affect the fitness of the plant.

In order to do a genomics characterization of Carmenère clonal selections, we sequenced 6 samples, discovered the Structural Variants (SVs), Single Nucleotide Polymorphism (SNPs), and short InDels in them, and performed a preliminary analysis of the effects of these mutations. Then, we associated the mutated genes with metabolic pathways.

We found 1.8 million SNPs and 308,610 short InDels; 97% of the effects produced by these mutations are modifiers, affecting non-coding regions; 0.096% are high impact, affecting coding regions, mainly being frameshift variants; 2,513 genes have at least one high impact variation. On the other hand, 740 SVs were found. 62% of the effects predicted are high impact impacting 711 genes. These effects are mainly deletions of sequences that include a transcript segment. Using KEGG Pathway Database, we associated 594 genes with 99 pathways. The top pathways are Starch and sucrose metabolism, Glutathione metabolism and, Amino sugar and nucleotide sugar metabolism participating respectively 11, 9, and 8 genes.

Altogether, these results suggest that clonal selections have few deleterious mutations but the majority of which are related with metabolic pathways relevant in grapes. Despite this, the characteristics of the plant, apparently, are not affected.

As future work, we propose to study the zygosity of the mutations to see if occur mainly in heterozygous alleles, and for this reason, they do not affect the plant's characteristic

Keywords: Structural variants, Carmenère, whole-genome sequencing, genomics analysis

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Technological advances for resistant grapevine varieties in a French private company

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Since some years, the French wine sector faces strategical challenges, all linked to climate changes. Multiple issues have been observed like disease development, early frost, hailstorms, drought, change in the precocity and maturity of grapes, each one resulting in loss of productivity and yield. In France, the varieties proposed today by nurseries are historical varieties that are not well adapted to those changes. Therefore, Mercier Frères, the first French and second world leader grapevine nursery, have decided to start its own research programs, with the help of its laboratory Novatech, to answer the growing demand for new grapevine varieties. Two different approaches will be presented, one creating new varieties and one improving cultivated varieties.

The NATHY program consists in traditional breeding improvement. In partnership with breeders around the world, the aim is to develop and propose resistant varieties first to the most harmful fungi: downy and powdery mildew, and black rot. Traditional breeding of perennial species as grapevine can take 25 to 30 years. The challenge for the company is to reach a breeding cycle of 8 to 10 years from the seed to the registration of the variety. Advances in production method, genotyping and phenotyping could be the way to reach this goal.

The NEXT program consists in bringing new resistance traits through genetic engineering. The aim is to find target genes for various biotics and abiotics stresses to improve elite cultivated varieties. The laboratory Novatech have started a massive production of transformable material of wine's varieties, the embryogenic callus. From the embryogenic callus to the transformed plant, Novatech try to open technical barriers and optimize protocols to obtain rapidly edited plants. The collaboration with research teams around the world makes it possible to work with known genes to incorporate efficiently many resistance traits.

Keywords: climate change, diseases, resistant varieties, breeding, genetic engineering

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Pan Genome Resources for Grapevine

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Capturing and characterizing the gene space within a species and linking to agronomic traits will be critical to improving agricultural practices and accelerating crop improvement. Recent advances in genome sequencing and assembly technologies are now making it possible to generate many accurate high quality reference genome assemblies for each species. The Grapevine community is no longer limited to the single PN20004 reference assembly, but has access to many high-quality reference assemblies for wine and table grapes. With this increase in the number of genomes, there is a need to integrate the information to support the transition from single reference to pan-genome research approaches. More recent work at Gramene has focused on the development of species pan-genome sites providing access to reference assemblies for multiple accessions, facilitating the identification and characterization of common and variable regions. Our first horticultural species features grapevine (https://vitis.gramene.org) and includes 11 grape reference genomes, including two versions of the partially inbred Pinot Noir reference (clone PN40024) and six outgroup species, Japonica rice, maize, *Arabidopsis thaliana*, a vascular plant, a single-celled green algae, and Drosophila melanogaster. These genomes were used to build a powerful phylogenetic framework, constructed with 594,645 input proteins from 553,999 individual genes, resulting in 24,050 protein-coding gene family trees of which 8,881 are grapevine specific. Complementing the protein comparisons are whole genome pairwise DNA alignments to *Arabidopsis*. These resources allow exploration of gene gain and loss between different accessions of grapevine reference assemblies. We have also included the newest reference assembly and annotations of Pinot noir PN40024 V4 and continue to host V3, used as reference for gene expression and orthology-based pathway projections. We gratefully acknowledge funding from USDA-ARS (1907-21000-030-00D).

Keywords: pangenome

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P77

Automatic image filtering for determining representative canopy temperatures with thermography in vineyards

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Thermography has been proven to be an accurate indicator of plant water stress in vineyards by using the crop water stress index (CWSI). The process of calculating representative canopy temperatures from thermal images is important as there are several other elements in the images such as soil, sky, and other non-leaf materials. In this study, six automatic methods of determining a representative canopy temperature were evaluated. The methods include simple and weighted averages, temperature histogram analyses, colour thresholding with the RGB images and reference temperature thresholding with CWSI Twet and Tdry measurements. Thermal images taken in a commercial vineyard on seven days, with varying climatic conditions, were used to evaluate the methods. The automatic methods were evaluated against accurate manually analysed thermal images in which only leaf material was carefully selected. The simple average method does not provide accurate temperatures and the weighted average method performs slightly better. Colour thresholding does not perform as well as expected. The results indicate that the reference temperature thresholding technique and histogram analyses can calculate canopy temperatures within 1°C of the manually analysed images. The reference temperature thresholding technique does require accurate measurements of CWWSI Twet and Tdry.

Keywords: Thermography; Crop water stress index; Temperature thresholding; Image analysis

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Genetic variability of grapevine pruning wood parameters as described with LiDAR data and associated quantitative trait loci

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Estimating growth traits of grapevine plants is common practice to describe the effects of cultural practices, training systems, environmental conditions, or genotypic effects, both for rootstock and scion varieties. Among these traits, the simple pruning weight is one of the most popular, while being time-consuming when hundreds of genotypes are to be characterized. To face the challenge of adaptation to climate change and the global demand for grapevine varieties resistant to disease, there is an increasing need for high throughput phenotyping methods. We studied in the vineyard the variability of plant vigor in the progeny of the cross between two genotypes carrying resistance genes for powdery and downy mildew. The pruning weight was measured for more than 200 genotypes and, in parallel, we used a newly developed high throughput LiDAR acquisition system to characterize the plants in winter. We will present the LiDAR system and the comparison between LiDAR data and pruning weights. Using “genotyping by sequencing” technology to describe the genetic heritage of the offspring, we performed quantitative trait loci (QTL) detection and identified several genomic regions associated with the genetic variability both for pruning weights and LiDAR data.

Keywords: grapevine, pruning wood, LiDAR, QTL

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The vines -they are a-changin': QTL in grapevine acclimation across environments

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Grapevine (Vitis spp.) dormancy acclimation is defined by a suite of critical traits essential for winter preparation, survival through extreme climatic events, and continued productivity in challenging environments. An association panel composed of ten sub-populations was created to examine dormancy acclimation in the context of applied grapevine breeding. Five distinct parents (E.S. 10-18-58, 'Frontenac', 'King of the North', 'Marquette', and 'Sabrevois') were intercrossed to form a half-diallel without selfing or reciprocal crossing. Seedlings were maintained in a growth chamber simulating the progression from the active growing season to the end of the year via a fluctuating temperature regime in conjunction with a photoperiod reduced by 0.5 hr each week. Visual phenotyping was conducted for five traits, and from these traits multiple metrics were calculated including a new trait, area under acclimation progress curve, based upon the commonly used metric, area under disease progress curve. Additionally, low temperature exotherms (LTEs) for buds were examined at the conclusion of growth chamber phenotyping. From these cumulative phenotypic responses, a total of 284 distinct QTL were identified across all linkage groups at a LOD threshold of 3.0; this number was refined to 113 QTL after adjusting the LOD threshold to 4.59 to account for marker density. Significant QTL occurred on 13 linkage groups. The influence of parentage was observed for LTE values, yet no significant QTL were identified for LTE traits. The breadth of QTL identified across multiple phenotyping environments emphasize the quantitative nature of grapevine environmental responses. These populations were subsequently field planted to allow for continued field- and growth chamber-based phenotyping of dormancy acclimation responses in conjunction with future planned work towards understanding fruit and wine composition in the context of cold-hardy grapevine breeding populations for the purposes of GWAS and refining methods for future genomic selection.

Keywords: dormancy acclimation, low temperature exotherms, cold hardy grape cultivars

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Developing a Spray-Induced Gene Silencing (SIGS) methodology for the control of Grape Powdery Mildew (*Erysiphe necator*)

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The emergence of fungicide resistance for Grape Powdery Mildew (*Erysiphe necator*) suggests the need to develop new strategies for control. Recent research demonstrates that agricultural pests can be controlled through the exogenous application of RNA molecules to trigger RNA interference (RNAi). The primary goal of this project is to develop a Spray-Induced Gene Silencing (SIGS) program to induce RNA interference mechanisms targeting both the grapevine and the fungus. The plant genes targeted for RNAi belong to a susceptibility-gene family to *Erysiphe necator* (Mildew Locus O: MLO genes). Additionally, we will target three pathogen-regulated genes critical to the life cycle of the fungus. One objective of the project is to identify stretches of RNA molecules with the maximum interference activity to repress several members of clade V MLO genes (VitviMLO 3,4,6,9,13,17). In addition, three fungal genes (Dicer-like protein 1 and 2 and CYP51) involved in the fungal life cycle will be tested. *In silico* analyses of the MLO amplified regions showed these regions will likely be processed to generate small interfering RNAs. Initially, we aim to develop an *in vitro* model to test the silencing effect of the dsRNA species employed. Previous works on other models have shown the ability for uptake of dsRNA via root soaking. We developed an experiment using *in vitro* microvine plantlets growing in a solid medium as plant material. They were supplemented with a solution of dsRNA at 20 ng/mL for VitviMLO 13 and 17. Leaf and root samples were collected at 0-, 3-, 7- and 14-days post-treatment. Real-Time PCR experiments are being conducted. Additionally, we prepared a Cy3-labelled dsRNA solution for both targeted genes to examine the systemic movements of the dsRNA species using confocal microscopy on cross sections of roots and petioles. We aim to demonstrate the ability for silencing and movement of dsRNA molecules.

Keywords: MLO, *Erysiphe necator*, double-stranded RNA, Spray

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Establishments of an efficient platform for genome editing in grapevine

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Grapevine is an important agricultural fruit crop plant consumed worldwide and with a key role in the global economy. Grapevine is strongly affected by both biotic and abiotic stresses, which impact grape growth at different stages during plant and berry development and pre-and post-harvest, consequently causing significant economic losses. Recently global warming has propelled the anticipation of the onset of berry ripening determining the reduction of a grape color and increased volatilization of aroma compounds. Climate change could negatively alter the physiological characteristics of the grape and affect the berry and wine quality. Modern plant breeding can provide tools such as genome editing for improving grape resilience traits while maintaining intact the viticultural and oenological quality characteristics of the genotype. This study aims at developing a platform for genome editing application in grapevine plants with the final goal to improve berry quality, biotic and abiotic resilience traits. We chose to directly deliver ribonucleoproteins (RNP, preassembled Cas protein, and guide RNA) into plant protoplasts, and, from these cell structures, regenerate grapevine plants edited in specifically selected genes controlling traits of interest. Edited plants regenerated by somatic embryogenesis from protoplasts will then be sequenced and molecularly characterized. Embryogenic calli of Sultana and Shiraz cultivars were initiated from unopened leaves of in-vitro shoot tip cultures and from stamens, respectively. Leaves were placed on NB2 medium while stamens on PIV was medium and incubated in the dark at 28 °C for three months. Viable protoplasts, tested by FDA staining, isolated from embryogenic calli were cultured by disc method at 1*105 protoplasts/ml. Mature well-shaped somatic embryos developed directly in the protoplast culture medium two months later and were transferred in the light into to shooting medium for further growth. Regenerated plants were then transferred to the greenhouse, no phenotypic alterations were observed when compared to non-in-vitro cultured plants. The performed experiments allowed to establish, an efficient protocol of embryogenic calli production, protoplast isolation, and regeneration of the whole plant through somatic embryogenesis, in both Sultana and Shiraz. Regenerated plants, through direct somatic embryogenesis deriving from a single cell, avoid the risk of chimerism during the regeneration process, therefore, improving the genome editing process. As a pre-requisite of genome editing an efficient method for transfection of protoplast by YFP marker genes was also established and experiments of the direct delivery of CRISPR–Cas9 ribonucleoproteins (RNPs) in protoplasts to achieve efficient DNA-free targeted mutations are in progress.

Keywords: *Vitis vinifera*, CRISPR-Cas9, Protoplast isolation, Plant Regeneration

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CRISPR-based genome editing tools for virus resistance in grapevine

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Clustered regularly interspaced short palindromic repeat (CRISPR), along with its CRISPR-associated (Cas) proteins, is a system which has been harnessed from the prokaryotic immune system and adapted for genome editing technologies. A recent extension to the CRISPR toolbox is the Cas13 endonuclease, which exclusively targets single-strand RNA. Grapevine (*Vitis vinifera* L.) is an important global fruit crop that is susceptible to over 70 different viruses. Grapevine viruses impact both the yield and quality of the fruit crop, and therefore a sustainable method to control these viruses is of importance . The majority of plant viruses have RNA genomes, and thus the CRISPR-Cas13 system can be a helpful tool in implementing virus resistance in plants, by directly cleaving the RNA virus genome. This study aimed to develop an efficient genome editing system for virus resistance in grapevine using two Cas13 systems in Nicotiana benthamiana, namely LwaCas13a and CasRx. Grapevine virus A (GVA) infects V. vinifera, but can infect the model plant N. benthamiana, making it a helpful model to study virus infection in grapevine. Vectors containing either LwaCas13a or CasRx genes were assembled, and used to transiently and stably-transform N. benthamiana. Multiple gRNAs were designed which targeted the GVA genome, and were delivered to transgenic N. benthamiana plants overexpressing LwaCas13a or CasRx, using a tobacco rattle virus (TRV) vector. The TRV vector delivery system allowed for transient and systemic expression of the gRNAs, and along with the transfection of a GVA infectious clone, the virus interference of both CRISPR-Cas13 systems was evaluated by comparing and analysing the virus titre using real-time quantitative PCR (RT-qPCR). By evaluating the CRISPR/Cas13-mediated RNA targeting of both systems, a robust and efficient virus interference system may be developed for applications in N. benthamiana and ultimately V. vinifera.

Keywords: CRISPR Grapevine CRISPR/Cas13 RNA-interference Virus-resistance GVA

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Evaluation of cell penetrating peptide-mediated delivery of CRISPR Ribonucleoproteins in microvine embryogenic cells

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Techniques to obtain transgene-free plants from genome edited plants either through direct DNA-free genome editing or through elimination of transgenes following conventional transformation methods are not established in many clonally propagated perineal crop models including grapevine. Direct DNA-free genome editing approach by delivering CRISPR components as RiboNucleoProteins (RNP) uses protoplast material, regeneration of which is not easily achieved in grapevine. The objective of this study is to establish a CRISPR delivery system into intact embryogenic cells, after which the edited cells can be regenerated into plants. For visual detection, we expressed the GFP reporter gene, via Agro-mediated transformation method, in the microvine, which is a fast-growing mutant bred for rapid genetic studies in grapevine. To facilitate the entry of the Cas9/RNP through cell and plasma membrane, we used Cell Penetrating Peptide (CPP). The cellular internalization of this arginine rich CPP (4G9R4L) in the embryogenic cells and the callus stage for optimum internalization was evaluated using a TAMRA-tagged 4G9R4L peptide. The TAMRA dye internalization in the cells was confirmed. The conjugated CPP-Cas9-sgRNA designed to disrupt the expression of GFP gene in the transgenic microvine was delivered through co-incubation of the embryogenic callus. Gene editing rate was assessed through High-Resolution Melting analysis (HRM) by comparing the differences in melting temperature profiles of the amplified target GFP gene region between non-treated and the embryogenic calli treated with the CPP-conjugated RNP.

Keywords: transgene-free, genome editing, Ribonucleoproteins, Cell Penetrating Peptide

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High-density linkage mapping and QTL identification of black rot resistance towards marker-assisted breeding in grapevine

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Today sustainability is a pivotal objective for viticulture, but it also presents us with new challenges. The decrease in treatments has in fact changed grapevine-pathogen interactions and dynamics causing the reaffirmation of diseases previously considered secondary. This is the case of black rot (BR, caused by Phyllosticta ampelicida), which is spreading with increasing pressure in warm-humid regions causing the loss of entire crops. The interinstitutional DAMAGE project between Edmund Mach Foundation (FEM) and the Institute for Grapevine Breeding (JKI-Geilweilerhof) aims to characterize BR resistance and to develop a toolkit of molecular markers (MM) to be routinely used in marker-assisted breeding for the introgression of this trait into mildew resistant backgrounds. The first fungal strain was isolated from infected leaves collected in Trentino-Italy and mixed with a second strain isolated in southwestern Germany. This inoculum has been used to screen a segregating population derived from 'Merzling' (V. rupestris × V. lincecumii, mid-resistant) × 'Teroldego' (V. vinifera, susceptible). BR resistance phenotyping consisted of a newly developed inoculation protocol on potted plants. The F1 individuals were genotyped with the Vitis18KSNP chip and a high-density genetic map has been constructed, following the integration of 190 informative SSRs. Moreover, explorative QTL analyzes have been conducted on phenotypic data of 2020 and 2021 seasons. Finally, upon the genomic interval characterization, new MM will be designed, tested and validated on various segregating populations with different genetic backgrounds.

Keywords: Vitis spp., Guignardia bidwellii, marker-assisted selection, disease resistance, plant phenotyping

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Real-time PCR, droplet digital PCR and Next Generation Sequencing: comparison of techniques for quantitative assessments in the development of transgenic grapevine lines

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Functional genomics studies in plants are crucial to prove the function of a candidate gene. The main strategies adopted to this aim are gene over-expression and gene inactivation in a homologous transgenic plant system. *Agrobacterium tumefaciens* transformation, actually the most used method to engineer plants, leads to T-DNA integration copy numbers (CN) ranging from one to multiple copies as well as to chimeric tissues with modified cells mixed to wt cells which resulted in not integer CN or in values lower than 1. Both multiple copies and chimerism should be avoided in vegetatively propagated plants, thus, an accurate and precise CN measurement is critical for lines selection, being single or low CN generally desired. Nowadays, several techniques are available to quantify CN. In this study, we compared and evaluated three methods based on (i) Real-time Polymerase Chain Reaction (qPCR), (ii) droplet digital PCR (ddPCR) and (iii) next generation sequencing (NGS), to carry out a molecular characterization of grapevine edited lines obtained with CRISPR/Cas9 technology, which contain inserted T-DNA cassette carrying the Cas9 gene, the sgRNA and the marker gene nptII. According to our results, as for accuracy, qPCR and ddPCR outputs are largely in agreement, especially for low CN values. Regarding precision, ddPCR resulted more precise than qPCR. On the contrary, CN detected with the NGS method were often not consistent with those calculated by qPCR and ddPCR and NGS was not able to discriminate the integration points in 3 out of 10 lines. On the other hand, NGS method can positively identify T-DNA truncations or the presence of tandem/inverted repeats, providing extensive information about the transgene integration asset. Our work, by reporting a case-study on grapevine edited lines, may offer useful information about the most advanced diagnostic techniques available in order to precociously select the proper transgenic material for research studies or commercial purposes.

Keywords: transgenic plants, *Agrobacterium tumefaciens*, T-DNA integration, copy number quantification

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Genome-wide epigenetic changes during and following grapevine somatic embryogenesis

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For winegrowers for whom varietal identity is an important market signal, novel clones are an attractive route for grapevine improvement. Somaclonal variation is a proven source of genetic diversity, and we are using sequencing approaches to enhance this approach by enabling the rapid characterisation mutations in large populations of vines regenerated from tissue culture. However, the stress of tissue culture has been shown to alter the epigenetic state of plant cells in a way that may persist in regenerated plants. To study the epigenetic changes induced by grapevine somatic embryogenesis, we performed bisulphite sequencing on embryogenic callus (EC) and plants regenerated from EC. Compared with leaf material, we found that EC had increased cytosine methylation, particularly in the asymmetrical CHH context, which is indicative of de novo methylation. However, genes and transposable elements (TEs) within genes showed reduced TE-specific CHG methylation. Plants regenerated from EC showed hypermethylation across all cytosine contexts three years after tissue culture. Interestingly, when EC were treated with the demethylating compound 5-Aza-cytidine, regenerated plants showed higher methylation than those from untreated callus. These data suggest that demethylation of TEs during somatic embryogenesis triggers de novo hypermethylation that is stabilised across symmetric cytosine contexts, and which persists years into the future. As a result, future nanopore-based genotyping efforts are being adapted to include methylation calling, so that we may assess the functional impact of these epigenetic changes in our somaclonal population.

Keywords: grapevine, somaclonal mutation, epigenetics, transposable element

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Heat transfer method (HTM): New automatic method for calculating crop water stress index (CWSI) reference temperatures in grapevines

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The use of temperature as an indicator of water stress is gaining attention. However, the need for reference temperature measurements in the field for the calculation of the crop water stress index (CWSI) impedes the practical implementation of this plant water stress measurement technique. Therefore, in this study a new comprehensive model, the Heat transfer method (HTM), based on physical heat transfer principles, is presented and compared with the standard method of natural references. The HTM does not require field measurements and uses only input data obtained from a conventional weather station to determine reference temperatures. To develop and evaluate the new model, physical measurements of the reference temperatures were taken in a commercial vineyard cv. Cabernet Sauvignon under different levels of water stress at two times on seven days. These physical measurements were used to optimize unknown parameters in the new method by using the Particle Swarm Optimization procedure. Input data for the model was collected by a conventional weather station located nearby the experimental vineyard block. In the validation process, it was found that the HTM can accurately predict the reference temperatures to within 0.5°C and 1°C for Twet and Tdry respectively, reacting to environmental conditions as expected. The new method can provide reference temperatures that do not require physical measurements which can make the use of CWSI more practical and easier to implement for determining plant water stress in vineyards.

Keywords: Water management; Climatic data; Crop water stress index; Particle swarm optimization

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Selection of grapevine hybrids resistant to fungal diseases

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Powdery and downy mildew are two fungal grapevine diseases caused by the pathogens *Erysiphe necator* (syn. Uncinula necator) and Plasmopora viticola, respectively. The control of these diseases entails a high economic cost and a great environmental impact. The identification of wild species of Vitis spp with different degrees of natural resistance to these diseases allows the introduction of these genes into new varieties of grapevine. The use of molecular markers facilitates the selection of those hybrids that carry alleles of resistance at an early stage, speeding up the breeding process. It is highly desirable to combine as many resistance genes as possible into one enhanced line, because the presence of one resistance locus may not be sufficient for robust resistance. Our study consisted of marker-assisted selection of a progeny obtained by a cross between Regent (a variety obtained in Germany) and a hybrid, It 12-3-73 (a hybrid obtained in our breeding program), carrying the Ren3 and *Rpv3* genes that confer resistance to powdery and downy mildew respectively. In total, 184 hybrids with different molecular markers linked to the Ren3 and *Rpv3* genes have been analysed. Of the 184 hybrids analysed, 60 (33%) have inherited the alleles of resistance to powdery and downy mildew, 72 (39%) only to powdery mildew and 42 (23%) to downy mildew.

Keywords: Powdery and downy mildew, resistance genes, molecular markers, plant breeding, Vitis.

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Relationship between vegetation indices calculated from Sentinel-2 imagery and ground-truth data measured in the vineyard

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Remote sensing is a tool that has been increasingly used to obtain important information about crops. This information is captured by sensors mounted on different platforms, which can be land based or air vehicles or even satellites, among others. Each platform has its advantages and disadvantages and the use of satellites has several advantages, such as their free use in the specific case of the Sentinel-2 satellites, managed by the European Space Agency. In addition, the use of images obtained from Sentinel-2 satellites has proven to be useful in viticulture, including several applications such as sub-unit management, classification of vineyards according to vigour, evaluation of the water status of the vineyard or monitoring the quality of table grapes. A practical way to use the information obtained from satellites is the calculation of vegetation indices, which are algebraic combinations designed to highlight the contrast of certain properties or plant characteristics. The most commonly used index is the NDVI (Normalized Difference Vegetation Index), although there are other indices that may be used in vineyards with good results, such as SAVI, CTVI or CLG.

The aim of this paper was to analyze the relationship between the most commonly used vegetation indices, calculated from Sentinel-2 images, and the ground-truth data measured in the field; in order to check which indices have the greatest relationship with the ground-truth data and at what time of year it would be most interesting to use the spectral information.

Keywords: vegetation indices, precision viticulture, NDVI, Sentinel

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Thermoregulation capacity of the leaf canopy according to vine variety

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The thermal regulation capacity of plants is directly related to their transpiration capacity, which depends, among other factors, on the amount of vegetation on the plant, its capacity for stomatal regulation, the ambient humidity and the water available in the soil. In addition, for certain levels of ambient temperature, this capacity is conditioned by a good physiological state of the plant. Knowledge of the relationship between the plant's thermal regulation capacity and its vegetative development is key to improving vineyard management.

In this experiment, a drone flight was carried out over a trellised vineyard plot planted with 46 different minority grape varieties, with the aim of analysing the relationship between the vegetative development of the plant and its thermal regulation capacity, for the same level of irrigation and without altering the natural phenological cycle of each variety. A Micasense ALTUM camera with a multispectral sensor and a thermal sensor was used to obtain the spectral information. The results show that there can be differences in the plant canopy of the same plant of up to 10˚C between the side that receives solar radiation and the shaded side and that the greater the vegetative development, the greater the plant's capacity for thermal regulation, with a high correlation between the vegetative development of the plant and the average temperature values of the plant canopy.

Keywords: LWIR, minority varieties, *Vitis vinifera*, UAS, chlorophyll, remote sensing, phenology.

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Spatio-Temporal Analysis using Sentinel 2 and Landsat 8 satellite imagery to identify vineyard yeast differences according to biogeographic origin

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Biogeography is a key concept which characterizes the wines and associates them with microbial terroir responsible for the differentiation and uniqueness of the wines. One of the factors influencing this microbial terroir is the vegetation, which in turn is influenced by other factors such as climate, soil or cultural practices. Remote Sensing instruments can detect variations in vegetation; therefore, it can be interesting to use remote sensing tools to identify and provide useful information about vegetation and then, about microbial terroir and the interrelationships between these. In this study, a spatio-temporal analysis during 2015 was developed using NDVI calculated from high-resolution satellite imagery, among several vineyards belonging to four different AOPs (Appellations of Origin) in organic and conventional grapes and musts from Galicia (Spain). The satellite imagery included Sentinel2 (10m pixel) and Landsat8 (30m pixel) images. The results of this study are in line with previous studies, confirming the higher the NDVI, the higher the yeast species richness in musts and grapes, but also showing that a higher NDVI was associated with the yeast biogeographical patterns of the AOPs with higher species richness consisting of weakly fermenting yeasts such as Hanseniaspora uvarum, Starmerella bacillaris, Pichia spp. and Zygosaccharomyces spp. Moreover, this study shows that Sentinel 2 images can establish differences in microbial terroir even better than Landsat8.

Keywords: Denomination of Origin; Appellation of Origin; NDVI; Vegetation Index; precision viticulture; yeast; biogeography; terroir; *Vitis vinifera* L.; Remote Sensing

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Phenotyping of virus-infected grapevine leaves through hyperspectral imaging and machine learning

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This research explores the use of hyperspectral imaging and machine learning techniques for the detection and classification of grapevine viruses, namely grapevine leafroll-associated viruses(GLRaVs) and grapevine red blotch virus (GRBV). Due to the incurable nature of viral diseases of grapevine, accurate detection and subsequent removal of infected plants is vital to maintaining a healthy vineyard. Because many current methods of virus detection may be costly, time-consuming, and unable to scale, this work seeks to develop machine learning methods to detect infection and classify the causal virus from hyperspectral images within the near-visible light spectrum (500-700nm). Hyperspectral imaging was used to capture about 440 leaf images from both healthy and infected black grapevine cultivars. Leaves were sampled four times, one month apart in two different vineyards during fruit ripening. Leaves were separated from the petioles and imaged in a dark environment under controlled led lightning that did not emit in the infrared region. Viral infection was assessed by PCR-based assays using petioles. Images were preprocessed by accessing raw image radiance data, segmented from the background, and converted to reflectance values through the use of a Spectralon standard included within each frame. Reflectance data were used as input in two machine learning methods, random forests and convolutional neural networks (CNNs), and were applied to image data for prediction of grapevine infection status. As assessed with a 5-fold cross-validation scheme, the highest performing random forest model produces an accuracy of 68% while the CNN model achieved an accuracy of 70% (averaged across both healthy and infected classes). While differentiation between plants infected with GLRaVs and GRBV proved to be relatively challenging, both models showed promising accuracies across classes and further investigation is needed to improve virus prediction of infected vines in the vineyard.

Keywords: spectroscopy, high-throughput phenotyping, deep-learning, convolutional neural networks, random forest

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Comparing the yield and viability of protoplasts isolated from three grapevine tissues with regenerative capacity

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The recent technique of delivering genome editing technologies directly into protoplasts for the purpose of CRISPR-Cas9 ribonucleoprotein-based genome editing has re-drawn the attention of the grapevine biotechnology community to the use of protoplast. Irrespective of the plant species in question, the process of regenerating plant tissue from protoplasts is universally considered recalcitrant. Protoplasting efforts in grapevine have been reported from 1985 onwards, and somatic embryogenic callus (SEC) is currently the explant of choice for this purpose. Given the technical expertise required, the long time-frames and non-routine nature of grapevine SEC generation, there is value in evaluating alternative explants for grapevine protoplast isolation and regeneration. The aim of this study was therefore to compare grapevine meristems, zygotic embryos and anthers-derived SEC cultures as potential sources of regenerative protoplasts. Alongside the isolation of protoplasts from Chardonnay and Pinotage anther-derived SEC cultures, we tested two alternative explants for potential use as efficient and viable protoplast sources, namely zygotic embryos, obtained from using embryo rescue techniques, and meristematic bulks, formed from shoot growth tips manipulated to form meristematic bulks in culture. Our results show that meristematic bulks serve as a promising explant for high yielding, viable protoplasts, whereas zygotic embryos are a labour-intensive source of low-yielding protoplasts. Explant-specific optimisations would still be required on the alternative explants, because the yields from SEC were still 5 times and 3.7 times higher than from zygotic embryos and meristematic bulks respectively. The yield and viability of the protoplasts isolated using the respective methods are compared, as well as a detailed description of the cellulose fraction that remains in solution after isolation is discussed. Preliminary analyses of protoplasts from somatic embryogenic cultures and meristematic bulks, subjected to culturing, confirmed that cell divisions occurred and the appearance of microcalli was evident, but embryos regeneration has not yet been achieved. Lastly, we also showcase the successful isolation of protoplasts from the SEC of a commercially important South African cultivar, Pinotage.

Keywords: protoplasts, *Vitis vinifera*, meristematic bulks, zygotic embryos, Pinotage

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Comparing the yield and viability of protoplasts isolated from three grapevine tissues with regenerative capacity

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Keywords: protoplasts, *Vitis vinifera*, meristematic bulks, zygotic embryos, Pinotage

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Increasing the clonal diversity of the South African bred cultivar Pinotage through gamma irradiance

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Pinotage is a cross between Pinot Noir and Cinsault Noir and was first commercially released in 1959, but now ranks amongst the top 100 most planted grape varieties. Given the relatively young age of the cultivar, the clonal diversity is severely limited, and the three most widely planted Pinotage clones display very similar characteristics. In this project, these three clones were targeted for induced somatic mutagenesis through gamma irradiance in an effort to increase the clonal diversity of this flagship South African cultivar. The clones were propagated *in vitro* and subjected to gamma irradiance at 0, 10, 20, 30 and 40 Gy, after which nodal segments were excised and grown to establish a population of plantlets that were hardened off for the subsequent analysis. Irradiance levels at and above 30 Gy led to low survival rates and all subsequent irradiance doses were limited to 0 (controls)-20 Gy. A population of 1416 plants were subjected to first rounds of characterisation under controlled growth conditions, with scoring for attributes such as growth habit, ampelographic markers and distinct phenotypes. Based on these analyses, a core group of plants where selected for further analyses under field-grown conditions. The field-trial, comprising 500 own rooted plants, planted in a randomised block design and are being analysed over the next 2-3 years. The first season will focus on vegetative characteristics using both quantitative as well as qualitative methods. Higher-throughput analyses methods will also be used for example the use of LiDAR remote sensing technology to determine the leaf area index of each vine as well as the use of morphometric analysis to compare leaf size and shape. The initial vegetative description of the population will be followed by reproductive trait analyses as well as potential genetic screening, particularly for potentially useful mutations in the colour locus.

Keywords: Somatic mutation, artificial mutagenesis, grapevine

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Utilisation of phased diploid genomes to study the grapevine terpene synthase (VviTPS) family

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The PN40024 reference genome has been the lone workhorse for genomic studies for more than a decade. However, recent advances in sequencing technologies have resulted in the release of numerous new Vitis spp. genomes that utilise long read sequencing to generate very long scaffolds. These additional genomes allow for the study of complex gene families where expansions are prevalent, for example, the grapevine terpene synthase family (VviTPS). Extensive duplications within the VviTPS family resulted in fragmented scaffolds for the reference genome and therefore a number of these genes are not assigned to a chromosome. Available phased-diploid genomes allowed us to study haplotype variations and the cultivar-specific landscape of the VviTPS family. The PN40024 reference genome was found to inadequately represent the variation and size of the VviTPS family when compared to three phased diploid genomes. For example, we putatively annotated >28 VviTPS genes to chr. 10, which was previously annotated with a single VviTPS gene on the reference genome. Furthermore, comparative genomics was combined with functional predictions and phylogenomics around the VviTPS active site to generate a novel resource for studying the VviTPS family. This resource is easily accessible and allows for the identification of putative VviTPS duplications, allelic variants and genotype specific VviTPS genes. The data is available in the form of Cytoscape networks that can be queried through BLAST, allowing for mining of this resource. Our analysis provides a novel and valuable resource to the grapevine community that could serve as a blueprint for studying expanded gene families and their genotypic variations.

Keywords: terpene synthase, genomics, bioinformatics

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Organelle engineering to modulate grapevine terpene production: validation of a hairy root culturing system

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Terpenes are plant secondary metabolites that are produced in response to developmental cues or stimulation by the environment. Terpenes play a crucial role in plant defense, have high nutritional value and unique characteristics that have led to their exploitation in several industries. In viticulture and oenology, terpenes contribute to grape flavor and wine bouquet. The robust industrial applications of terpenes in cosmetics, the food industry, agriculture, and pharmaceuticals has propelled research on ways in which its biosynthesis and accumulation *in planta* can be improved. This study forms part of a collaborative project that will use elicitation of grapevine hairy root cultures using novel chemical compounds that target the endoplasmic reticulum, a key organelle in the terpene biosynthetic pathway to increase its size as well as its metabolic activity to improve the grapevine terpene production. Proof of principle that endoplasmic reticulum enhancement favors terpene production has been shown in yeast (Arendt et al, 2017). Here we outline the planned activities to create hairy root cultures of several grapevine scion and rootstock cultivars by transformation using Agrobacterium rhizogenes and their validation as a suitable experimental system to study terpene production and endoplasmic reticulum stimulation.

Keywords: Grapevine hairy roots, Agrobacterium rhizogenes, Organelle engineering, ER, terpenes

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The effect of variety on the conversion of grape pixels into yield at grapevine level

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Vineyard yield estimation is a challenge that has been subject to research for several years. Common practices are invasive, labor intensive and often inaccurate, as vineyard yield varies both spatially and temporally. Non-invasive image-based grapevine yield estimation methods focus on automatically identifying yield components in digital images which are then converted into yield. Such methodologies are dependent on the accuracy of yield components detection in the images and corresponding conversion into mass. Several works mention the potential of bunch pixels (projected area; BA) as estimator of bunch weight. However, most relationships have been studied in laboratory conditions and/or on single bunches. At plant level, from a 2D perspective, such relationships depend on occlusions by leaves and by other bunches, as well as bunch morphology. The present work aims at comparing the relationships between actual grapevine total bunch weight and corresponding BA, in order to estimate grapevine yield from 2D images taken in field conditions. The study included four varieties (Arinto, Encruzado, Castelão, Syrah) from two commercial vineyards, trained on a vertical shoot positioning system, located at two sites within the Lisbon winegrowing region, Portugal. Images were collected from 100 vines defoliated at bunch zone and BA was segmented manually from each image. The regression analysis between BA and actual yield presented high and significant R^2 for all cases (> 0.90). However, despite the statistically similar slope of the regression lines for pooled data of the two sites, some significant differences were detected among the slopes of the fitted lines of the four cultivars, indicating cultivar dependency. Our work corroborates recent research regarding the use of BA as a good estimator of bunch weight, however in a multi-cultivar scenario, this relationship should be considered with caution. Further research is ongoing aiming at creating a generalized model using other image-based complementary variables.

Keywords: Image analysis, yield estimation, proximal sensing, bunch projected area, *Vitis vinifera* L.

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3D visualisation of voids in grapevine flowers and berries using X-ray micro computed tomography

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X-ray micro computed tomography (micro-CT) is a non-destructive 3D imaging technique particularly useful for imaging voids in the delicate flowers and soft berries of *Vitis vinifera*. The characterisation of gas spaces and connections can offer insights into the process of tissue aeration and this may have implications on cell function and vitality. In this study, the internal structures of flowers and berries were captured through rapid micro-CT scanning and subsequently were recreated in 3D using image processing. Low density regions and porous tissue were visualized within the pedicel and the berry. Voids were present in the proximal mesocarp of berries forming a 'detachment zone' in both seeded and seedless cultivars. Voids permeated the mesocarp of mature seedless grape cultivars, but not seeded grapes. Micro-CT offers new insights regarding the distribution of voids on the morphology and compositional heterogeneity of organs that are difficult to dissect and/or view with light microscopy.

Keywords: 3D visualisation, flower, grape morphology, micro-CT

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The design of a model vineyard established for long-term water research using an integrated approach and advanced technologies

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Here we describe the design of a model vineyard in support of an integrated study that aims to evaluate and compare the adaptability and resilience of current commercial rootstock-scion combinations to scenarios of (severe) water limitation over the lifespan of the plants. Novel techniques will be used to monitor water availability and water stress, as well as plant physical characteristics such as leaf area index. The model vineyard (0.41ha) site is located on the Welgevallen experimental farm, Stellenbosch, and was selected considering soil conditions (homogeneity) and topography.

Since cultivars and rootstocks differ in their sensitivity and response to dryer conditions, this vineyard was planted with six different rootstock-scion combinations in order to determine their future value for the South African wine industry under increasingly water scarce conditions. To simulate various water availability scenarios, the irrigation system in the block has been adapted to allow for three different irrigation regimes to be applied to specific plots throughout the vineyard. The trellis system has been modified slightly to facilitate the extraction of data from aerial images of specific target areas without the interference of adjacent vines from other treatments or control areas.

Several advanced technologies such as thermal cameras, high-definition cameras, multispectral cameras and sensors, among others, were validated for the use in determining plant water stress in an established commercial vineyard in the Stellenbosch region. These technologies will be applied in the model vineyard. In this poster, we present the characteristics of the model vineyard and some preliminary results related to vine growth obtained during the establishment period.

Keywords: water stress, rootstock-scion, precision viticulture, sensor technology

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XIth International Symposium on
GRAPEVINE PHYSIOLOGY
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INTEGRAPE WORKSHOP (COST ACTION CA17111)

DATA INTEGRATION, THE POWER OF OMICS AND GRAPE IMPROVEMENT

COST Action
CA17111
INTEGRAPE

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INTEGRAPE Workshop (COST ACTION CA17111)
Data integration, the power of omics and grape improvement

Matus JT, Grimplet J, Rustenholz C, Holtgräwe D, Duchêne E, Mattivi F, Fortes AM, Navarro D, Savoi S, Frommer B, Rodríguez-Izquierdo A, Margaryan K, Royo-Brun C, Adam-Blondon AF, Pezzotti M.

The European network INTEGRAPE ("data integration to maximize the power of omics for grapevine improvement"; https://integrape.eu/) seeks to integrate all information regarding one of the world's oldest crops, the grapevine. Omics and large-scale phenotyping techniques allow the study of thousands of grape characteristics at once, generating huge datasets manageable only with the support of dedicated informatic tools. However, these datasets are often generated in different and non-standardized formats across research laboratories and many times stored only locally, which makes them poorly accessible for further use. INTEGRAPE is developing and promoting tools that are dedicated to harnessing and exploiting all available datasets collected by grapevine scientists in the fields of physiology, molecular biology, genetics, biochemistry and systems biology. Since officially starting in September 2018, we have established an open, international, and representative network that integrates data from existing resources in a cost-effective manner, as well as generating interoperable grapevine datasets and tools available in a secure and standardized format. Our main goal is to offer and make this data available and usable beyond the original experiments, interoperable in a secure and standardized format, and made available to public and private research communities. We are actively collaborating with world-wide research teams, promoting networking and collaboration and offering training schools and short-term scientific missions (STSMs) for students and researchers. Among the most recent achievements of our network, those that will be showcased in this workshop, we present:

i. *Guideline 'cookbooks'* and *Dictionary of unified grape-sample ontologies:* We are providing recommendations for genomic and metabolomic data submission, extraction and use, compliant to FAIR principles, together with recommended repositories and standard formats for organ naming.
ii. *PN40024 fourth genome assembly:* we have served as a platform for researchers to discuss and promote the release of the fourth grape genome reference assembly, allowing group meetings to manually curate the v4 annotation.
iii. *Gene Reference Catalogue:* We have built a centralized repository for the standard annotation of grapevine genes and their functions, based on past and current gene/gene-family characterizations. Recommendations for gene identifiers and standardization of functional validation levels are also given in this context.
iv. *Hub of repositories and tools:* We have recently released a new website, enlisting all available resources for the community, in terms of data exploration and visualization, including several omics and systems biology approaches.

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In full visibility of our network deliverables, we present this workshop to provide an update of the general objectives of INTEGRAPE, to disseminate to the entire grapevine community and formally invite all researchers to form part of this network, and to introduce and advertise ongoing and future opportunities in terms of collaborative networks, grants, training and meetings. In particular, we expect that in future years we can all contribute to make INTEGRAPE the largest network hub for the community. The world's grape and wine industries will have full access to up-to-date information to meet their major challenges: to improve grape berry quality and produce better wines.

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