

**ICGBG**

XII International Conference on  
GRAPEVINE BREEDING and GENETICS

July 15-20, 2018  
Bordeaux FRANCE

# ABSTRACT BOOK

## **GBG 2018 – Bordeaux, France**

### 15 – 20 July



RÉGION  
**Nouvelle-Aquitaine**







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- **Marcela Lilian Martínez**, University of Cordoba, Argentina
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- **Didier Merdinoglu**, INRA, Colmar, France
- **Nathalie Ollat**, INRA, ISVV, Bordeaux, France
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- **Andy Walker**, University of California Davis, USA
- **José Miguel Martínez Zapater**, CSIC, Spain
- **Eva Zyprian**, JKI, Geilweilerhof, Germany

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- **Elisa Marguerit**, Bordeaux Sciences Agro, ISVV (Co-convener)
- **Alexia Bâiri**, University of Bordeaux, ISVV
- **Emmanuel Baugier**, University of Bordeaux, ISVV
- **Catherine Chabirand**, INRA Bordeaux, ISVV
- **Sarah Cookson**, INRA Bordeaux, ISVV
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- **Claudine Trossat-Magnin**, University of Bordeaux, ISVV
- **Cornelis Van Leeuwen**, Bordeaux Sciences Agro, ISVV
- **Philippe Vivin**, INRA Bordeaux, ISVV

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**University of Bordeaux**  
351 Cours de la Libération,  
33400 Talence  
France

Website: <https://www.u-bordeaux.fr/>



**Bordeaux Sciences Agro**  
1 cours du Général de Gaulle  
33175 Gradignan  
France

Website: <http://www.agro-bordeaux.fr/>



**International Society for Horticultural Science**  
Corbeekhoeve - Pastoriestraat 2  
3360 Korbeek-Lo  
Belgium

Website: <https://www.ishs.org/>



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## **FR Biologie Intégrative et Ecologie**

Campus INRA Bordeaux, 71 avenue Edouard Bourlaux  
33882 Villenave d'Ornon  
France

Website: <https://bie.u-bordeaux.fr/La-FR-BIE>



## **LabEx COTE**

Laboratoires d' Excellence - Université de Bordeaux  
166 cours de l'Argonne  
33000 Bordeaux  
France

Website: <http://cote.labex.u-bordeaux.fr/>



## **IdEx Bordeaux**

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33000 Bordeaux  
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## **Investissement d'Avenir**

Commissariat général à l'investissement  
Hôtel de Cassini, 32 rue de Babylone  
75007 Paris

Website: <http://investissement-avenir.gouvernement.fr>



## **Institut Français de la Vigne et du Vin**

39 Rue Michel de Montaigne  
33290 Blanquefort  
France

Website: <http://www.vignevin.com/>



## **ENTAV-INRA**

3430 Route de l'Espiguette  
30240 Le Grau-du-Roi  
France

Website: <http://www.entav-inra.fr/>



**Institute of Botany – Chinese Academy of Science**

No.20 Nanxincun, Xiangshan  
Beijing 100093  
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Website: <http://english.ib.cas.cn/>



**Organisation Internationale de la Vigne et du Vin**

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France

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**Conseil Interprofessionnel du Vin de Bordeaux**

1, cours du 30 Juillet  
33000 Bordeaux  
France

Website: <http://www.bordeaux.com>



**Pépinières Mercier**

16 Rue de la Chaignée  
85770 Vix  
France

Website: <http://www.mercier-groupe.com/>



**Bureau National Interprofessionnel du Cognac**

Cité Mondiale, 23 Parvis des Chartrons  
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France

Website: <http://www.cognac.fr>



**Conseil des Grands Crus Classés en 1855**

1, cours du 30 Juillet  
33000 Bordeaux  
France

Website: [www.grand-cru-classe-1855.com](http://www.grand-cru-classe-1855.com)



**Château Giscours**

10 Route de Giscours  
33460 Labarde  
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Website: <http://chateau-giscours.com/>



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**Bordeaux Métropole**

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Website: <http://www.bordeaux-metropole.fr/>



**La Cité du Vin**

Esplanade de Pontac, 134 Quai de Bacalan  
33300 Bordeaux  
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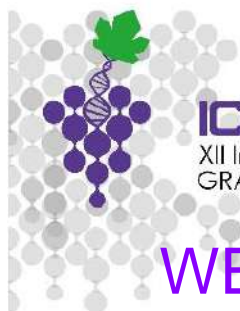
Website: <https://www.laciteduvin.com/fr>



**ENSEIRB-MATMECA**

1 avenue du Dr Albert Schweitzer  
33402 Talence  
France

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GRAPEVINE BREEDING and GENETICS

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# WELCOME MESSAGE

Grapevine is cultivated since millenia for various purposes and is part of our cultural heritage, even though this heritage must constantly adapt. Sustainable viticulture relies on the achievement of an economical balance between the cost of production and a market price accepted by the consumer. On the production side, the cost depends on the planting, on the yield, and of all the steps needed to reach harvest (such as irrigation, fertilization, pesticide treatment and cost of labour). On the market side, this price depends on the consumer's taste, culture and priorities.

While the diversity of tastes and cultures is very large, one common priority found all around the world nowadays is the need for viticultural practices to avoid or strongly limit the use of pesticides. Reduction of pesticide treatments may be obtained by improving the physiological status of the plant, both under normal or stress conditions, and by better understanding the basis of the plant/pathogen interactions. Another common constraint all over the world is the need to adapt to climate change, which will bring reduced rainfall and higher temperatures.

Strategies allowing viticulture to adapt to these constraints are thus needed. On one hand, changes in viticultural practices (clonal and varietal diversity, manipulation of sink/source ratio, light exposure, etc..) provide one set of strategies with short term effects. Genetic improvement through breeding is a longer term goal, which should result in wider and more integrated adaptation strategies.

Four years after the outstanding XI<sup>th</sup> Grapevine Breeding and Genetics Conference organized in Yanqing (China), GBG2018 will provide an excellent opportunity to have an overview of the progress made in breeding strategies, and in all the scientific domains directly or indirectly related to breeding. This covers the maintenance and extension of genetic resources, the genetic, phenotypic and physiological characterization of grapevine populations, the functional characterization of genes involved in the control of development, berry ripening and composition, and adaptation to biotic and abiotic stress. Particularly interesting are the appearance of varieties with pyramided resistance, genome editing strategies, and tools for high-throughput phenotyping. All these approaches generate an enormous flow of data that must be organized and exploited. Viticulture, like many other scientific and societal issues, thus enters the era of big data. The congress will thus host a presentation of the Integrape COST action that is presently starting (Mario Pezzotti), and a workshop dedicated to high throughput phenotyping (Reinhard Töpfer, Ullrich Schurr and coworkers).

The organization of such an event is obviously a collective effort. I wish to express my deepest thanks to the International Scientific Committee who helped to set up the programme, as well as the local organizing committee, and beyond all members of the laboratory Ecophysiology and Functional Genomics of Grapevine who handled all the aspects of logistics. Special thanks to our secretaries, Catherine Chabirand and Catherine Thioulouse, to Elisa Marguerit who coordinated the local Organizing Committee, to Nathalie Ollat who handled our contacts with the wine industry and organized the tribute to Alain Bouquet.

We thank very much our academic and private sponsors for their generous support, and Château Giscours for welcoming the gala dinner. Without their contribution, this event could not have been organized.

We are very happy and honoured to welcome you in Bordeaux, and we hope that you will enjoy an excellent conference. This abstract book gathers almost 270 contributions that will be displayed during the conference. It has been edited and prepared by Zhanwu Dai, Sabine Guillaumie, Ghislaine Hilbert, Nathalie Ollat, Azalée Rombaut, Claudine Trossat-Magnin, Philippe Vivin and myself.

**Professor Serge Delrot**  
**Convener for the GBG2018**  
**Organizing committee**

# CONFERENCE PROGRAM

## July 15, Sunday

17:00-20:30	Registration and welcome reception at <b>Cité du Vin (Bordeaux)</b>
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## July 16, Monday

8:00-9:00	Registration and poster installation	
9:00-10:00	<b>Opening ceremony</b>	
10:00-10:40	<b>Opening lecture</b> : Antoine Kremer, INRA Bordeaux, France Adaptation to environment: lessons from tree genetics	
<b>Session 1 : Breeding, consumers and markets</b> Chair : Mark Krstic		
10:40-11:10	<b>Keynote lecture</b> : Hervé Hannin, SupAgro Montpellier, France Breeding, consumers and market issues : main evolutions in the vine and wine industry	<b>O1</b>
11:10-11:25	Yann Raineau Resistant varieties and market receptiveness: An assessment using experimental auctions	<b>O2</b>
11:25-11:40	Luigi Bavaresco Impact of grapevine breeding for disease resistance in world wine industry	<b>O3</b>
11:40-11:55	François Delmotte OSCAR, A national observatory to support the deployment of new grapevine disease-resistant varieties in France	<b>O4</b>
11:55-14:00	Lunch + Poster session	
<b>Session 2 : Genetic resources and breeding</b> Chair : Erika Maul		
14:00-14:40	<b>Keynote lecture</b> : Bruce Reisch (Cornell University, USA) Genetic resources and breeding: current status and shifting paradigms	<b>O5</b>
14:40-14:55	Goran Zdunic Inventory and descriptions of wild grapevine ( <i>Vitis vinifera</i> subsp. <i>sylvestris</i> ) from Slovenia, Croatia and Bosnia and Herzegovina	<b>O6</b>
14:55-15:10	Pilar Gago Successfully amplification of ancient DNA from Clemente's herbarium (1803-1804)	<b>O7</b>
15:10-15:25	Erika Maul Preservation via utilization: minor grape varieties on-farm	<b>O8</b>
15:25-15:40	Patricia Leao Genetic resources for table grape breeding in the Brazilian tropical semi-arid region	<b>O9</b>
15:40-15:55	Carmina Gisbert Recovering ancient grapevines varieties in the Spanish provinces of Alicante and Valencia	<b>O10</b>
15:55-16:10	Silvia Vezzulli The FEM grapevine breeding program for downy and powdery mildew resistances: Towards a green viticulture	<b>O11</b>
16:10-16:25	Ross Bicknell Exploring the use of transposon mobilisation to produce a gene-tagged population for grapevine	<b>O12</b>
16:30-19:00	Coffee break + Poster session	
17:00-19:00	<b>IGGP steering committee</b>	p294

**July 17, Tuesday**

Session 2 (continued) : Genetic resources and breeding		
Chair : Elisa Marguerit		
8:30-8:45	Darko Preiner Synthesis of grapevine chimeras	O13
8:45-9:00	Carolina Royo Characterization of deletions causing berry color variation in Garnacha and Tempranillo	O14
9:00-9:15	Jinggui Fang A successful molecular design breeding practice for grape coloring trait based on MYB haplotypes	O15
9:15-9:30	Dalbo Marco Rootstocks breeding for resistance to grapevine decline and dieback in southern Brazil	O16
9:30-9:45	Guido Cipriani Pyramidizing resistance genes in grape: a breeding program for the selection of `elite` cultivars	O17
9:45-10:00	Laurent Audeguin Breeding programs : the new role of IFV and its department Geno-Vigne® as a national technical institute	O18
10:00-10:30 Coffee break		
Session 3 : Classical breeding and NBT		
Chair : Bruno Mezzetti		
10:30-11:10	<b>Keynote lecture</b> : Zhenchang Liang (Institute Botany Beijing, China) The future of grape breeding: theory and technology	O19
11:10-11:25	Christophe Schneider Inra-ResDur : the French grapevine breeding program for durable resistance to downy and powdery mildew	O20
11:25-11:40	Soon-Chun Jeong Identification of haplotypes controlling seedless by genome resequencing of grape	O21
11:40-11:55	Irene Perrone Molecular mechanisms behind the somatic embryogenesis process in grapevine: from key transcripts to epigenetic signature	O22
11:55-12:10	Lisa Giacomelli Generation of mildew-resistant grapevine clones via genome editing	O23
12:10-14:00 Lunch + Poster session		
Session 4 : Genomics and data handling		
Chair : Mario Pezzotti		
14:00-14:40	<b>Keynote lecture</b> : Dario Cantu (UC Davis, USA) Uncovering the wealth of grapevine genetic diversity through whole genome sequencing and assembly	O24
14:40-14:55	Diana Bellin An integrated meta-QTL and transcriptomic data mining approach to select candidates controlling veraison time in grapevine	O25
14:55-15:10	Islam El-Sharkawy The first version of the Whole-Genome Sequencing (WGS) and assembly of the Muscadine grape, <i>Muscadinia rotundifolia</i> cv. Noble	O26

15:10-15:25	Timothée Flutre Genome-wide association study of a diverse grapevine panel to uncover the genetic architecture of numerous traits of interest	O27
15:25-15:40	Marianna Fasoli Unraveling the key molecular events of grape berry ripening under varying crop loads	O28
15:40-15:55	Yann Dussert Adaptation of downy mildew to grapevine partial resistance	O29
15:55-16:10	Alessandro Vannozzi The combined role of WRKY and MYB TFs in the regulation of stilbene synthase genes in grapevine ( <i>Vitis vinifera</i> L.)	O30
16:10-16:30	Mario Pezzotti The COST Integrape Data integration to maximise the power of omics for grapevine improvement	p295
16:30-19:00	Coffee break + Poster session	
17:00-19:00	<b>Phenotyping workshop (Reinhard Töpfer)</b>	p296

### July 18, Wednesday

Session 5 : Phenotyping and genotyping Chair : Melane Vivier		
08:30-09:10	<b>Keynote lecture</b> : Reinhard Töpfer (JKI Siebeldingen, Germany) Sensor based phenotyping for grapevine breeding and genetic analyses	O31
09:10-09:25	Manna Crespan Extensive genotyping of a large collection of rootstocks, population structure analysis and core collection extrapolation for new breeding programs	O32
09:25-09:40	Anna Schneider Few main parents contributed to traditional variety assortment in north western Italy as revealed by microsatellites and SNPs	O33
09:40-09:55	Chin-Feng Hwang QTL mapping of downy mildew and botrytis bunch rot resistance in a <i>Vitis aestivalis</i> - derived 'Norton'-based population	O34
09:55-10:10	Didier Merdinoglu Variation of recombination rate along the genome in <i>Vitis vinifera</i> x <i>Vitis rotundifolia</i> interspecific hybrids	O35
10:10-10:40	Coffee break	
10:40-10:55	Anna Kicherer Phenoliner: a multi-sensor field phenotyping platform	O36
10:55-11:10	Paola Barba Combining high throughput genotyping and phenotyping for the genetic improvement of table grapes in Chile	O37
11:10-12:10	<b>A tribute to Alain Bouquet</b>	
12:10-13:30	Lunch + Poster session	
13:30	Bus transfer to ISVV Bordeaux-Aquitaine (Villenave d'Ornon)	
14:00-14:20	<b>GBG 2018 official photo</b>	
14:30-19:00	<b>Field trip and visit of wine estates</b>	
19:00	Bus back to ENSEIRB-MATMECA (Talence)	



**July 19, Thursday**

<b>Session 6 : Vine growth and development</b> Chair: Anne Fennell		
9:00-9:40	<b>Keynote lecture :</b> José Miguel Martínez-Zapater (ICVV Logrono, Spain) Genetic variation for grapevine reproductive development	O38
9:40-9:55	Anne Fennell Mapping the genetic architecture of grapevine bud dormancy and chilling fulfillment traits	O39
9:55-10:10	Etti Or Ethylene-induced macromolecule catabolism - the switch required for bud meristem growth resumption?	O40
10:10-10:25	Sabine Guillaumie Dissecting the control of shoot growth in grapevine : genetics and genomics identify potential regulators	O41
10:25-10:55	Coffee break	
10:55-11:10	Anna Schwandner Determination of genetic loci in the control network of grapevine flowering	O42
11:10-11:25	Javier Ibanez Characterization of the reproductive performance of a collection of grapevine varieties	O43
11:25-11:40	Eva Zyprian Molecular analysis of bunch architecture in grapevine	O44
11:40-11:55	Sarah Jane Cookson Understanding scion/rootstock interactions at the graft interface of grapevine	O45
11:55-12:10	Grant Cramer A transcriptomic comparison of late-ripening Cabernet Sauvignon berry skins from Bordeaux and Reno	O46
12:10-14:00	Lunch + Poster session	
<b>Session 7 : Berry yield and composition</b> Chair : Jeff Bennett		
14:00-14:40	<b>Keynote lecture :</b> Sara Zenoni (University of Verona, Italy) Genetic dissection of grape berry ripening and composition	O47
14:40-14:55	Johan Burger Can transcriptomics shed light on the "old-vine" character of wines?	O48
14:55-15:10	Charles Romieu Diversity of condensed tannins in a large collection of Vitaceae	O49
15:10-15:25	Rosa Arroyo-Garcia Berry skin development in wild grapevine ( <i>Vitis vinifera</i> L ssp <i>sylvestris</i> ): Distinct patterns of gene expression	O50
15:25-15:40	Chiara Pagliarani Clone-specific transcript profiling of 'Nebbiolo' grape berries unveils environmental responses mediated by sugar and secondary metabolite signalling	O51
15:40-16:10	Coffee break	
16:10-16:25	Pablo Carbonell-Bejerano Grape color variation involves genetic and micro-environmental changes that alter berry phenolic and aromatic composition	O52



16:25-16:40	Gan-Yuan Zhong Genomic and transcriptomic analyses of the 'Concord' grape revealed a novel molecular mechanism in the regulation of a 'foxy' flavor gene in <i>Vitis</i> species	O53
16:40-16:55	Thuy-Thanh Truong Genetic analysis of grapevine secondary metabolism using non-targeted metabolomics	O54
17:30	Bus departure to gala dinner	
19:30	<b>Gala dinner at Château Giscours</b>	
23:00	Bus back to Bordeaux downtown	

## July 20, Friday

Session 8 : Breeding and adaptation to abiotic stress Chair : Grant Cramer		
9:00-9:40	<b>Keynote lecture</b> : Nathalie Ollat (INRA Bordeaux, France) Grapevine adaptation to abiotic stresses: an overview	O55
9:40-9:55	Jack Dunlevy Identification of loci and genes responsible for sodium and chloride ion exclusion in grapevine rootstocks for use in marker assisted selection	O56
9:55-10:10	Noé Cochetel In grafted grapevines, physiological, transcriptional and hormonal responses to nutrient availability are strongly influenced by the rootstock genetic background	O57
10:10-10:25	Jason Londo Phenotypic deconstruction of dormant bud winter hardiness in grapevine	O58
10:25-10:55	Coffee break	
10:55-11:10	Lijun Wang Global proteome analyses of phosphorylation and lysine acetylation reveal new insight into alternative splicing, photosynthesis and HSPs in grape response to heat	O59
Session 9 : Breeding and adaptation to biotic stress Chair : Eva Zyprian		
11:10-11:50	<b>Keynote lecture</b> : Lance Cadle-Davidson (USDA-ARS, Geneva, USA) A perspective on breeding and implementing durable powdery mildew resistance	O60
11:50-12:05	Ian Dry Investigations into the mechanisms of activation of the MrRUN1 and MrRPV1 resistance proteins and the signal transduction pathways leading to resistance to grapevine powdery and downy mildew	O61
12:05-12:20	Guillaume Barnabé Organization, diversity, expression and evolutionary dynamics of the NB resistance gene family in grapevine and related species	O62
12:20-14:00	Lunch + Poster session + Poster removal	
14:00-14:15	Summaira Riaz Durable powdery mildew resistance in grapevines: myth or reality?	O63
14:15-14:30	Giulia Malacarne The Rpv3-3 locus and stilbenoid induction mediate downy mildew resistance in a grapevine inter-specific population	O64
14:30-14:45	Jiang Lu The <i>Plasmopara viticola</i> candidate effector PvrXLR131 interacts with a plant Brassinosteroid and ERECTA receptor kinases	O65

14:45-15:00	Annalisa Polverari Differential responsiveness of ATL156 promoters from <i>Vitis riparia</i> and <i>Vitis vinifera</i> towards defense-related stimuli and transcription factors	O66
15:00-15:15	Andreia Figueiredo Subtilisin-like proteins and lipid signaling events: the missing links in grapevine resistance to <i>P. viticola</i>	O67
15:15-15:30	Claudio Moser The molecular dialogue between grapevine inflorescence/berry and <i>Botrytis cinerea</i> during initial, quiescent, and egression infection stages	O68
15:30-16:00	Coffee break	
16:00-16:15	Sandrine Eveillard Contrasting susceptibilities to Flavescence dorée in wild <i>Vitis</i> species, <i>Vitis vinifera</i> cultivars and progenies suggest segregation of genetic traits involved in disease response	O69
16:15-16:30	Mickael Malnoy Control of the grapevine moth <i>Lobesia botrana</i> through the genetic engineering manipulation of the host plant's volatiles	O70
16:30-16:45	Daniel Pap Living on the edge: the narrow genetic base of the rootstocks is a serious threat	O71
16:45-18:00	<b>Poster awards, conclusions and GBG 2022</b>	
18:00	End of the conference	

# POSTER PRESENTATIONS

Poster	Session	Title	Presenting author	Page
P1	7	Understanding methoxypyrazine production in Marlborough Sauvignon blanc grapes	Jeff Bennett	106
P2	7	Grape bunch heating alters the accumulation and regulation of anthocyanin production in Pinot noir berry skin	Jeff Bennett	107
P3	7	Modification of transcriptome and main sensory compounds in grape berry adaption to varied ripening initiation timing	He Lei	108
P4	8	An Investigation on natural ice-wine, harvest freezing method and training-pruning techniques in cool climates region of China	Wen-Dong Zhao	109
P5	5	Whole genome sequencing and gene annotation of Georgian grape cultivars	Vazha Tabidze	110
P6	9	Growth and development of Pierce's disease tolerant hybrid bunch grapes in southeastern U.S	Elina Coneva	111
P7	5	Development of an efficient DNA marker system for predicting skin color in grape	Akifumi Azuma	112
P8	2	Identification of downy mildew resistance genes Rpv10 and Rpv3 by DNA-marker analysis in Russian grapevine germplasm collection	Elena Ilitskaya	113
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## **Abstracts for oral presentations**

**Adaptation of forest trees to climate – How much can we learn from the past to address the future ?**

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A pivotal question in forest research and management is the adaptation of trees to ongoing environmental changes. In my presentation, I will address this issue by providing a historical perspective reviewing past evolutionary changes under documented environmental changes. Trees have experienced during the late Pleistocene recurrent environmental changes over long time periods during the glacial-interglacial sequences. There is a growing body of evidence stemming from different sources of information (evolutionary history; observations from population and species transfers, common garden experiments) showing that trees responded and adapted rapidly to these changes. Taking oaks as a study case, the review shows that rapid migration, extensive gene flow and hybridization were the main processes that permitted oaks to track climatic warming. In a second part I will examine major evolutionary trends over shorter time spans stemming from a diachronic approach and based on genetic monitoring of oak populations that underwent recent environmental changes (since the Little Ice Age and during current climatic change). Overall this review suggests that substantial future evolutionary shifts can be expected in response to ongoing climate change due to the high level of genetic diversity existing in forest trees, and that gene flow will be an important driver of adaptive evolution.

**Keywords:** adaptation, climate change, environment, evolution, tree

## **Session 1: Breeding, consumers and markets**

## Breeding, consumers and market issues; main evolutions in the vine and wine industry

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This paper tries to display the main topics presently focussed by social sciences regarding the wine markets and consumers. It aims to give researchers a better vision of the economic wine context and the way it has been explored by these sciences; present research led and their results soon offered will have to meet a real demand and the expectations of the vine growers; this aspect is even reinforced due to the normal delay between such laboratory works in genetics and the concrete marketing of wines that will result of them. Thus it has been also necessary to approach the way they could evolve in the near future: this part takes advantage of recent and continuous work in foresight sciences, particularly in France. Firstly the paper shows the diversity of disciplines as far as the subjects involved. Secondly, it enlightens the evolutions of the topics and their links with the development of the specific vine and wine history, since the middle of the nineteenth century. Thus, a retrospective analysis leads to separate 5 great periods in which the different disciplines have successively appeared and have fostered particular topics, linked with the major issues of these times : the first period focussed to the historic and economic conditions of development for vineyards in various - and sometimes hard - contexts like phylloxera ; the beginning of the XXth century, as it was still traumatized by the overproduction crisis in 1907 has produced economic works on market equilibriums and prices, regulation and public policies as well as collective strategies (cooperatives) ; after WWII, many economic works were achieved regarding improvements of quality – generally linked with « appellation of origin » labelization - and production optimisation, as well as sociological aspects of the consumption ; in the meantime, marketing sciences were born offering to approach the consumers and their behaviour, their changing habits ; but paradoxically though the consumption was already clearly declining, such marketing issues have had difficulty to get accepted in traditional producing countries ; the next period has helped, taking advantage of the « new world countries » breakthrough, offering new models of management, marketing and strategic issues and methods, rapidly extended in the whole world. Globalization of the markets, changes in the world wine governance and new research networks helped the wine sector to take new topics in charge like business strategies, marketing-mix and export policies; they have been spread in a new environment with opportunities like big data and digital transition, but also threats, due to global competition, product acceptability by new consumers, societal expectations of citizens - on health and environment- and climate change. In this context, plant breeding has become essential because it can provide relevant answers for these adaptations. Thus, many articles recently explored the chain of innovation, the obstacles and boosters for the adoption of improvements and the international challenges in terms of competition. In the context of geographical indications, the compatibility of new varieties with old specifications and with consumer expectations calls for analyzes and socio-economic responses. It is time to use foresight methods, able to design potential scenarios and help public policies and major stakeholders to make choices for the future markets, consumers and globally for the whole vine and wine industry.

**Keywords :** breeding, consumers, economy, market

## Resistant varieties and market receptiveness: An assessment using experimental auctions

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Vine varieties resistant to cryptogamic diseases are a major innovation aiming to reduce the use of phytosanitary products (Merdinoglu et al., 2009). Several scientific tracks are currently debated in order to identify the most effective ways to perpetuate this resistance (Bouquet 1980, Merdinoglu et al., 2009) and to improve the quality of the final products from these varieties (Salmon, Ojeda and Escudier, 2017). On our part, we propose an economic analysis based on the consumers' responses to these "new wines", in order to evaluate the real possibilities of introducing them on the market. Focusing on the *Occitanie* white wines, we give the results of an experimental market conducted in Paris in June 2017, with a representative panel of French consumers. The new wines are compared with conventional and organic wines from the same region of production and the same vintage (2015). We elicit the different consumers' willingness to pay (WTP) for one bottle of each wine after tasting them and get increasing information about environmental performances, certifications, and pesticides residues. The methodology employed to make the WTP credible is based on the experimental auctions theory taking the 'surplus comparison mechanism' (Combris, Giraud-Héraud, Seabra Pinto, 2015). We can then measure (i) the different consumers' products valuations (via the WTPs) and (ii) the different possible market shares (using also the selling prices available at the moment of the experiment). The results highlight the need for a communication strongly oriented towards the environmental and sanitary performances to guarantee a good valorisation by the consumers for resistant varieties wines. However, we show that increasing the quality of conventional wines can help to contain their losses in market share.

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**Acknowledgments:** We thank Céline Franc, Gilles de Revel, Isaac Rodriguez Pereiro and Rafael Cela for carrying out the pesticide residue analyzes of the wines presented in this experiment. We also thank Anne-Sophie Masure for her precious help in setting up the experimental market.

## Impact of grapevine breeding for disease resistance in world wine industry

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*Vitis vinifera* L. is the most cultivated species in the world for grape production, covering about 95% of the commercial vineyard surface. The majority of the grapes is utilized for wine making, followed by fresh consumption, raisins, juices, jellies and marmalades. Due to its disease susceptibility, *V. vinifera* has to be protected by spray treatments, with environmental, economic and societal impacts. Wild grapevine, on the other hand, are disease resistant but of poor grape quality. A way to combine disease resistance with grape quality is breeding which aims at obtaining new varieties. Breeding programs were developed from the 19th century on, in both the old (Europe) and new world, as a way for viticulture to be sustainable. A survey on the main breeding results and the impact on the production of commercial grapes is done, ranging from the first direct production hybrids to the most recent varieties. Productive, legislative and commercial aspects are considered, especially for wine production, which is a sector strongly controlled in European Union. The perspectives of breeding for disease resistance are discussed, including the new techniques (cis-genesis and genome editing). The importance to interact with the society so that these innovations (by both traditional and new methods) can be accepted, is emphasized. While less acceptance problems are expected with table grapes, raisins or rootstocks, more concerns can arise with wine grapes, that are a cultural produce, especially in Europe. The role of science is to give the legislator tools to cope with sustainability and to educate the society (from grape grower to wine consumer) to a correct understanding. Only if innovations are accepted by all the actors of the wine chain, they can produce a real advantage.

**Keywords:** disease resistance, hybrids, innovation, science, society

## **OSCAR, a national observatory to support the deployment of new grapevine disease-resistant varieties in France**

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New high quality/high resistance varieties have been registered in France and it is expected that this offer will greatly increase in the coming years. The cultivation of disease-resistant varieties makes it possible to reduce drastically the number of sprays used in viticulture. Their recent but increasing deployment raises several issues that need to be addressed. The first issue concerns the qualitative potential of the varieties and their marketing. The second issue, more collective, concerns the management of the durability of resistance. Several cases of erosion or resistance breakdown have indeed already been described in Europe (Delmotte et al. 2014; Delmas et al. 2016). The monitoring of the evolution pathogens populations targeted by the resistance is therefore required to maintain the long-term efficacy of grapevine resistance. The third issue is the design of cropping systems adapted to resistant varieties, i.e. that maintain production objectives, promote the durability of resistance while using as little as possible phytosanitary products. To meet these challenges, INRA have set up the National Observatory for the Deployment of Resistant Cultivars (OSCAR's Website: <http://observatoire-cepages-resistants.fr>). OSCAR is a participative network based on the plots in production situations planted by growers. The participative dimension of the network promotes the sharing of experiences on the agronomic behavior, the potential for mechanization, the ease of driving and the quality of wines. The observatory also allows monitoring the emergence of new diseases or of virulent strains. Powdery and downy mildew (targeted by resistance) isolates are collected and tested in laboratory conditions to follow the evolution of population aggressiveness. Data from OSCAR will feed mathematical models to understand how the epidemiological dynamics of erosion of resistance are affected by deployment strategies and landscapes features.

Delmas CE et al. 2016. *Evolutionary Applications*, 9, 709-725.

Delmotte F et al. 2014. *Infection, Genetics and Evolution*, 27, 500–508.

**Keywords:** cropping system, deployment of disease-resistant grapevine varieties, participatory science, resistance durability

## **Session 2: Genetic resources and breeding**



## Genetic resources and breeding: current status and shifting paradigms

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For grapevine breeding and genetics to meet future challenges requires not just a crystal ball, but also multidisciplinary and cooperative work. Essential to the development of new wine, table, raisin, juice and rootstock cultivars, is the availability of germplasm resources. With three major centers of *Vitis* diversity (North America, Asia, and Europe), germplasm preservation and characterization are the foundations for grapevine improvement to meet the growing challenges of a changing climate, biotic stress, and the need for superior fruit quality. Though there are numerous germplasm collections around the world, not all have stable, long-term funding, and there is a continuing need to both analyze the amount of diversity preserved, and to add to the gaps thereby identified. Effective studies of grape germplasm have led to the direct use of alleles for disease resistance from all three gene pools, and have provided illuminating genealogical / pedigree information. Grape breeding efforts have been highly successful, and examples of success will be discussed. Breeders utilize diverse germplasm resources, innovative phenotyping technology, cost-effective genotyping, and a resourceful, collegial community. The wine industry has been most recalcitrant to change, with many countries planting more than 70% of total area with the same 12 cultivars that represent about 1% of the total genetic diversity. Yet changes are taking place, even in western Europe, possibly due to environmental concerns, coupled with the close proximity of vineyards to homes. On the other hand, the spectrum of table and raisin grapes undergoes continual change due to a competitive marketplace and the accomplishments of both public and private breeders. Many breeders now employ the tools of marker-assisted selection, and the list of marker-trait associations available to breeders continues to grow. Large community-based efforts, such as VitisGen and Innovine, provide the foundations for future success.

**Keywords:** breeding, diversity, genetics, germplasm, *Vitis*

## Inventory and descriptions of wild grapevine (*Vitis vinifera* subsp. *sylvestris*) from Slovenia, Croatia and Bosnia and Herzegovina

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The wild grapevine (*Vitis vinifera* subsp. *sylvestris* Gmel. Hegi) is a dioecious subspecies considered as the ancestor of the cultivated grapevine (*Vitis vinifera* L.). Due to high human pressure for natural plant resources in the last hundred years, accompanied with pest, diseases and global climate change, the wild grapevine is highly threatened. The diversity of wild grapevine is poorly represented in gene banks while their habitats constantly decline. The wild grapevines in Slovenia, Croatia and Bosnia and Herzegovina are poorly known. Therefore, current study inventoried the remaining populations of wild grapevines in these territories and evaluated their morphological and genetic traits. The inventory started in 2015 confirming several historical populations in eastern Adriatic regions but also finding some new ones not recorded up to now. During prospecting, wild grapevines were found at eight geographically distant locations in this territory. Each observed individual was considered to meet truly morphological criteria (dioecism and leaf morphology) for *sylvestris*. Ninety-eight *sylvestris* individuals belonging to 8 populations have been genotyped at 20 nuclear simple sequence repeats (SSR) loci to detect genetic diversity. Distance- and model-based cluster analysis differentiated among samples highlighting at least three different groups. The results showed the existence of considerable level of genetic diversity of wild grapevine but also hidden alleles that could be of interest for breeding programs. The study provides necessary information for conservation management and further characterization of wild grapevine.

**Keywords:** ampelography, conservation, SSR markers, wild grapevine

### Successfully amplification of ancient DNA from Clemente's herbarium (1803-1804)

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Simón de Rojas Clemente y Rubio is considered to be the father of modern ampelography. He developed the first scientific method for describing grapevine varieties through the examination of material he collected himself in Andalusia between 1803 and 1804. The Royal Botanical Garden in Madrid conserves the material he herborised (leaves and shoots) for each of the varieties he studied. This herbarium, the oldest of all grapevine variety herbaria, is a treasure for ampelographic studies among others and provides a unique insight into pre-phyllloxeric grapevine cultivation in early 19th century. In the present work it has been exploited as a source of samples for molecular analyses based on a set of microsatellite markers that nowadays are routinely used in grapevine characterization. We have developed a method that enabled us to successfully extract and amplify ancient DNA from leaf samples herborized more than 200 years ago, allowing the identification of four specific grapevine varieties. These four identified varieties in Clemente's herbarium correspond to varieties still cultivated nowadays. Finally, this work reflects how ancient herbaria collections are a source of plant material for molecular and other studies.

**Keywords:** ancient herbarium specimens, grapevine variety, Madrid Royal Botanical Garden, SSR markers

**Preservation via utilization: minor grape varieties on-farm**

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In the last 20 years, minor grape varieties reputation increased considerably. Growers' magazines and wine journals reported with enthusiasm about the renaissance of neglected and even threatened grape varieties. Thus, they assisted to raise awareness and promoted their on-farm cultivation. Wine growers and consumers are profiting and orient their interest accordingly. These minor varieties represent a huge value with respect to cultural heritage, adaptation to ecological conditions, historical aspects comprising knowledge on origin, spread, migration route and synonymy, diversification of products, marketing of specialties and niche products with a higher added value and creation of typicality. Recognizing the heritage and value of traditional crop genetic diversity maintained on farms, ECPGR published a "Concept for on-farm conservation and management of plant genetic resources for food and agriculture" in 2017. This paper describes a future strategy for the preservation of genetic resources. Most priority actions defined in the document are transmissible to Vitis. They encompass the development of descriptors, an inventory of on-farm vineyards, the definition of threat categories and overcoming of legal obstacles insuring that obsolete varieties can be grown. Focus on these four issues was laid in the scope of the ECPGR Grant Scheme Activity "On-farm inventory of minor grape varieties in the European Vitis Database", running in 2017. Twelve participants from 10 countries participated in the activity. First step was the dissemination of the information that a database is going to be established, listing minor grape varieties cultivated on-farm. Descriptors were selected for the grower (12 descriptors), variety preserved (17 descriptors), vineyard description (17 descriptors) and commitments (6 descriptors). During the project 151 vineyards were registered: Spain (97), Albania (16), Montenegro (14), Croatia (13) and Germany (13) France (11), Serbia (5) and Austria (3). Criteria for 4 threat categories were defined and used to initiate a process for legal inclusion of rare historical varieties in national variety catalogues. The on-farm preservation of traditional grape varieties disburdens grapevine collections. A further positive aspect is that cultivation under practice conditions provides deeper knowledge about agronomic features and wine quality.

**Keywords:** cultural heritage, diversity, documentation, European Vitis Database, marketing, niche products, on-farm preservation

## Genetic resources for table grape breeding in the Brazilian tropical semi-arid region

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The Grape Germplasm Bank of Embrapa Semiarid is the only one present in the Northeast of Brazil, in a semi-arid tropical climate and represents a strategic resource for the sustainability of the tropical viticulture. The first vines were planted in 1965 at the Mandacaru Experimental Station in Juazeiro, BA (9°24 "S, 40°26" W and 365.5 m altitude). Currently, the collection includes 268 genotypes: 54% are table grapes and raisins cultivars, 34% are grapes for wine and juice, 4.8% are of unknown origin, 5.2% are rootstocks and 1.9% are wild American species. Regards to the botanical classification, 168 are *Vitis vinifera* L., 8 are *V. labrusca* L., 73 are interspecific hybrids and 8 are American species of *Vitis* spp. (*V. rupestris*, *V. riparia*, *V. champinã*, *V. cinerea*, *V. gigas* Fennel, *V. candicans* Engelman, *V. doaniana* Munson, *V. shuttleworthi* House), in addition to 10 genotypes for which no information on origin, species or pedigree was found in the literature. Therefore, 62.6% belong to the species *V. vinifera* L., the interspecific hybrids being the second group with the highest number of genotypes (27.2%). There are two harvests per year for agronomic characterization according to IPGRI/UPOV/OIV (1997) descriptors. The Grape Germplasm Bank of Embrapa Semiarid has been used as the source of germplasm for the grape breeding program aiming to develop new seedless table grapes cultivars adapted to the semiarid tropical environment of Northeast Brazil, in addition to being used in other studies like genetic divergence, screening for diseases resistance genotypes, characterization for bioactive compound. The results have been important for the strengthening and sustainability of the Brazilian viticulture industry.

**Keywords:** genetic resources, grapevine germplasm, tropical viticulture

## Recovering ancient grapevines varieties in the Spanish provinces of Alicante and Valencia

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The provinces of Alicante and Valencia, located in the Mediterranean coast of Spain are important viticulture areas with three Denominations of origin (DO) for wine production, DO Alicante, DO Valencia and DO Utiel-Requena and one for table grape, DO Uva de mesa del Vinalopó. The richness in grapevine cultivars before the arrival of the phylloxera pest in both provinces is well documented (i.e. in 1889, more than 150 varieties were grown in these areas). In the context of the research project CGL2015-70843-R, we initiated at UPV different approaches in order to contribute to recovering of ancient varieties in risk of disappearance: 1) prospections in ancient or neglected vineyards; 2) identification of varieties by SSRs; 3) analysis of grapevine germplasm diversity using different approaches and, 4) development of protocols for virus sanitation and *in vitro* conservation. Synonymies and homonymies which are very common in grapevine have been also detected in our study. Among the historic varieties, several accessions of 'Valenci Blanc', Valenci Negre, Grumer, Muskat of Alexandria, Planta Mula, or the endangered varieties 'Esclafagerres' (or 'Esclafacherres/is'), Gateta, or 'Raïm del Clotet' have been analyzed. Different protocols for *in vitro* sanitation and germplasm storage have been developed. At UMH, a survey was carried out covering the main area of Monastrell cultivation in the Alicante DO. This ancient variety, also known as Mourvèdre, is cultivated mainly in the southeast of Spain and it is highly adapted to the dry and warm climate of this area. Genotyping by Sequencing (GBS) was used in order to estimate the genetic diversity of this variety and high variability was found. In addition, this analysis will provide a high number of high quality SNPs well distributed across the genome, suitable for clone genotyping, which will allow the design of strategies to optimize its conservation and use.

**Keywords:** Esclafagerres, Gateta, Grumer, Monastrell, Muskat of Alexandria, Planta Mula, Raïm del Clotet, Valenci

**Acknowledgment:** This research was funded by the MINECO project CGL2015-70843-R cofunded with FEDER funds.

**The FEM grapevine breeding program for downy and powdery mildew resistances: towards a green viticulture**

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During the last decade, besides the emerging need of innovation raised by grapevine growers, the quest for sustainable viticulture has been addressed to the development of new varieties (mid-) resistant to the major pathogens challenging grapes in temperate-humid climates. In order to achieve this goal, a FEM double-step breeding program has been undertaken. During the initial scouting phase, a total of 264 accessions acquired from (non-)European breeding programs, an Italian private breeding platform and wild-collected in north-eastern America were studied. Most individuals were phenotyped for downy and powdery mildew resistance, while all were genetically characterized. Firstly, 9 reference microsatellite (SSR) markers were used for the true-to-type identification through international and private databases, where feasible. Secondly, in order to validate the available pedigree information and to infer new relationships, 50 informative SSRs were analyzed and employed for parentage analysis. Moreover, all studied accessions were screened at 12 exploitable disease resistance-associated (R) loci derived from *Vitis* spp. and described in literature; this novel “all vs all” approach allowed the discovery of unanticipated R-loci combination in traditionally bred material. Moreover, the distribution of loci related to downy (Rpv) and powdery (Run/Ren) mildew resistance and the field response unveiled potentially novel and exclusive genetic resources. During the following operational phase, the Marker-Assisted Breeding program took off taking advantage of the preparatory information. Nowadays, 32% of the selected genotypes is pyramided for 2 Rpv and 2 Run/Ren loci, while 6% conveys 3 Rpv and 3 Run/Ren loci. Currently, several genotypes carrying up to 7 R-loci are under selection.

**Keywords:** marker-assisted selection, pedigree analysis, resistant varieties, R-loci

## Exploring the use of transposon mobilisation to produce a gene-tagged population for grapevine

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The grape genome is estimated to contain approximately 240,000 transposable elements (TEs), representing 50% of the genetic material within the nucleus. Building on our observation that the transcription of several common retrotransposon classes can be stimulated by stress, we are conducting a feasibility study on the use of TE mobilisation to create a gene-tagged population in this plant. Stress treatments are applied to embryogenic callus to reduce the influence of chimerism. Plants are then regenerated and raised through to field planted vines to assess the influence of the treatments at both the genotypic and phenotypic levels. Reduced representation fragment libraries are being sequenced and novel software has been developed to quantify and locate any new insertion sites within the genome of each regenerant. The talk will describe the results of our studies to optimise somatic embryogenesis, the impact of different stress treatments on TE transcription, the establishment of the reduced representation sequencing approach and the development and results obtained using our novel bioinformatics pipeline.

**Keywords:** gene-tagging, somatic embryogenesis, transposable elements



## Synthesis of grapevine chimeras

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Chimeras are plants that contain tissues of at least two genotypes in different cell layers, as well as in organs resulting from these layers. Many grapevine cultivars and clones important for viticulture production today are periclinal chimeras which evolved spontaneously because of the mutations in one layer of apical meristem (Pinot gris, Pinot meunier etc.). In comparison with chimeras that have evolved from mutations, there are also so-called intra- and interspecific chimeras, which consist from two different genotypes originating from different cultivars or species. Synthetic chimeras could have huge potential as an alternative breeding method for grapevine since it is vegetatively propagated, but also to investigate cell autonomous vs non-cell autonomous developmental programs and track the movement of non-cell autonomous molecular information.

Research on the possible development and detection of synthetic chimeras was conducted using three different grapevine cultivars: Cabernet sauvignon (CS), Chardonnay (C) and Babić (B) using meristematic bulk tissue (MBT) culture. First, conditions for successful MBT development and organogenesis were determined for individual cultivars. After this, organogenesis was induced from mixed MBT developed after contact growth of two pairs of genotypes (CSxC i CSxB), and in this way 260 plants were obtained and acclimatized. After visual evaluation of all plants, seven of them showed some level of tissue (leaves) heterogeneity, while others had a uniform phenotype. All plants were analysed using microsatellite (SSR) markers for grapevine and results for 8 plants suggest that they could be composed of 2 different genotypes, ie. chimeras. Research on stability and type of chimerism in vegetatively propagated chimeras' progeny is in progress, using combination of phenotyping and genotyping.

**Keywords:** grapevine breeding, microsatellite markers, synthetic chimeras, tissue culture

## Characterization of deletions causing berry color variation in Garnacha and Tempranillo

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Gray and white somatic variants that can be the base of new cultivars occasionally appear in some black-berried grapevine cultivars. Genetic and molecular studies have associated color loss to the emergence of deletions at the grape color locus located on chromosome 2 in heterozygous colored cultivars carrying a functional and a null allele. Depending on the size of the deletions, side-effects adding to the loss of pigmentation capability may appear in these variants. In this study, we developed a SNP-based chip to evaluate along chromosome 2 the extension of hemizygous deletions in grape color variants through loss of heterozygosity analysis. These markers were used to characterize white and gray isolates originated from Garnacha Tinta and Tempranillo Tinto and collected along the Ebro valley (NE Spain). Two main deletion classes were detected in Garnacha Blanca correlating with the geographical origin of the accessions, while Tempranillo gray berry variants showed higher variation. Comparative genomics of Garnacha variants after whole-genome re-sequencing was addressed to understand the mutational mechanisms generating color variation. The results show that these deletions are generally associated with more complex genome rearrangements. Additional structural variation between independent lines of Garnacha Blanca likely emerged in different ancestral clonal lines of Garnacha Tinta. These results can be exploited for the selection of color variants best suited for the development of new cultivars and can help to detect rearrangement hotspots in the grapevine genome.

**Keywords:** berry color, color locus, deletions, genome structural variation, heterozygosity loss, somatic variation

## A successful molecular design breeding practice for grape coloring trait based on MYB haplotypes

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MYBA1 and MYBA2 haplotype composition (A, B, C-N, C-Rs, E1, E2) at the color locus is a major genetic determinant of anthocyanin diversity and grape skin color. The white grape cultivars are characterized by a homozygous non-functional HapA, whereas colored grape cultivars contain at least one allele of the remaining functional haplotypes. By investigating the color density and the MYB haplotypes of 213 grape cultivars, our studies have shown that dark-skinned cultivars tended to contain HapC-N and HapE2, whereas HapB, HapC-Rs and HapE1 appeared in high frequencies in the red-skinned varieties. When the ploidity was concerned, the more functional alleles it contained, the darker the skin color tended to be. A proportion of 46.23% investigated *V. vinifera* cultivars are allodiploid of A/C-Rs at the color locus. Allodiploid of A/E1 and allotriploid A/E1/E2 were the dominant genotypes in the hybrid cultivars between *V. vinifera* and *V. labrusca*. The above results shed light on the potential strategy in the early prediction of color diversification during the classical cross-breeding. To further verify this assumption, two hybrid populations, ‘Muscat Hamburg’ (A/C-Rs) × ‘Crimson seedless’ (A/C-Rs) and ‘Cuibao seedless’ (A/A) × ‘Qihongbao’ (A/C-Rs) were developed in 2015. The berry skin color of the progeny was early predicted according to haplotype compositions using the seedlings’ genomic DNA. Subsequent color observation indicated that the presence of berry color segregated at the Mendel’s ratio with the predicted haplotype compositions. Overall, the present study is of both scientific and practice interests in assisting in breeding high-quality cultivars with favorable coloration.

**Keywords:** anthocyanin, berry color

## Rootstocks breeding for resistance to grapevine decline and dieback in southern Brazil

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Grapevine decline and dieback is a serious problem for grape production in southern Brazil. It is characterized by a set of symptoms that lead to the weakening and death of affected plants. The main causes are a root scale, the ground-pearl (*Eurhizococcus brasiliensis*), associated to soil fungi (*Cylindrocarpon*, *Fusarium*, *Phaeacrimonium* and others), and high clay content soils. Apparently, the root wounds caused by ground-pearl in poorly aerated soil conditions allow the entrance of root decay fungi, resulting in plant deaths. The creation of grape rootstocks resistant to root decay fungi is one objective of the grape breeding program at Epagri, the Agricultural Research Enterprise of Santa Catarina State, Brazil. The first step was the identification of resistance sources. In rootstock trials carried out in areas where high mortality rates were observed previously, all traditional rootstocks were highly susceptible. *Vitis caribaea* hybrids showed a high level of resistance but also some defects, such as excessive vigor and absence of winter dormancy. Some genotypes of *V. shuttleworthii* and *V. palmata*, originated from waterlogged soil conditions of Everglades (Florida, USA) also showed a high level of resistance. However, the performance of these genotypes and their descendants in dry soil conditions was very poor and more generations of crossing will be necessary to eliminate undesirable characters. The most promising breeding lines resulted from self-pollination of the rootstock IAC 572 (*Vitis caribaea* x 101-14 Mgt). The selection process started from 840 seedlings and resulted in 23 selections that showed no symptoms of vine decline in three different sites. These selections are being evaluated now for productivity and fruit quality in rootstock trials with different scion cultivars.

**Keywords:** *Eurhizococcus brasiliensis*, *Cylindrocarpon*, margarodes, viticulture

## Pyramidizing resistance genes in grape: a breeding program for the selection of 'elite' cultivars

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Grape is one of the most important fruit crops cultivated worldwide both for fresh consumption and as hedonistic beverage in form of wine. Grape is also one of the crops less friendly for the environment. In Europe, where viticulture developed since the ancient Greek and Roman dominations, all the cultivars of *Vitis vinifera* are susceptible to several pathogens. In the northern more humid climate, downy mildew (*Plasmopara viticola*) is the most dangerous disease, causing heavy defoliation and crop losses. In the southern Mediterranean countries, powdery mildew (*Uncinula necator*) is the major concern. Pesticides are largely used against pathogens in all crops but the grape cultivation requires approx. 60% of all the chemicals sprayed in Europe. An alternative is to move viticulture to the cultivation of resistant varieties. Several genotypes of wild American and Asian *Vitis* species evolved resistances to powdery and downy mildews. These resistances were recognized since the early years of the last century and several cross selections were developed mainly in France and ex-USSR. The first generations of grape hybrids were usually of very poor enological quality. Recently, new cultivars were developed with a much better quality, comparable with that of the traditional ones. The plant pathogen and its host struggle for each own survival. A possible strategy to avoid that pathogens overcome the genetic resistances is to introduce multiple resistances in each individual selection. Here, we present a breeding program, developed at University of Udine, aiming to pyramidize genes of resistance to powdery and downy mildew into a high quality genetic background for the selection of new wine and table grape cultivars. Almost 6,000 seedlings were grown, resulting from 71 different crosses done during 2010 – 2013. The parents were selected from a panel of elite wine or table cultivars and resistant genotypes. Parents with pyramidized resistance genes were selected when possible. Offsprings with pyramidized genes were obtained from 64 of the initial crosses. The seedlings were selected for the resistance to powdery and downy mildew. Field natural infections were recorded during the first year of growing. Eight-hundred and twenty seedlings were also genotyped using molecular markers linked to four recognized resistance genes to downy mildew (*Rpv1*, *Rpv3*, *Rpv10*, *Rpv12*) and four resistance genes to powdery mildew (*Run1*, *Ren1*, *Ren3*, *Ren4*). After the phenotypic and genotypic selection, 1,128 progenies were grafted onto SO4 rootstock and grown in open field. The following traits were observed for at least two years of production: berry color, type of cluster (sparse vs. compact), production (high, medium, low), vegetative habitus (weak vs. vigorous), susceptibility to diseases other than powdery and downy mildews (mainly *Botrytis cinerea* and black rot). A general overview of genotypes with pyramidized resistances is presented.

**Keywords:** downy mildew, microsatellite, molecular markers, organic production, powdery mildew

## Breeding programs : the new role of IFV and its department Geno-Vigne® as a national technical institute

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How a Technical Institute is investing new technique, new challenges. The change of paradigm at IFV ! IFV has been playing a major role in the improvement of plant material through clonal selection for over than 50 years. Since 1971, this led to the registration of 1200+ certified clones representing 350+ cultivars. Getting approved plant material for sanitary and technological standards remains the main mission of the Plant Material Department of IFV. However, this procedure is reaching its limits and facing several new challenges. Among them, the diminution of pesticides inputs is now taken into consideration by the industry and the scientific community. In this context, the creation of the UMT Géno-Vigne® in 2008 can be considered as the first step. Géno-Vigne®, regrouping INRA Montpellier, Montpellier SupAgro and IFV, has progressively invested the fields of breeding. This collaboration led to the production of a set of genotypes carrying two QTLs of resistance to downy mildew and two to powdery mildew, according to the national strategy of INRA. In 2013, in close collaboration with the INRA Colmar Unit, breeding programs became more important. Nowadays, several 15 years-long partnerships are underway with most of the French wine regions. And more are in preparation. In this purpose, breeding programs are organized as follows:

With the local wine industry:

- Definition of ideotype(s) based on what would be the ideal new cultivars for the considered wine area ; Designation of 2 or 3 emblematic varieties whose qualities are widely appreciated; Selection of 4 to 6 resistant genotypes (as genitors), with appropriate phenotypical characteristics.

At Geno-Vigne® and INRA: breeding in order to obtain ~ 4000 seedlings ; Marker-assisted selection to guarantee the proper parents, the presence of the desired QTLs of resistance, and the hermaphroditism ;

At IFV: maintenance of a sub-population of 150+ individuals (=stage 1); Grafting and production of a minimum of 5 plants per genotype

In facilities of the local wine industry: planting of the sub-population into the wine region; Monitoring with any appropriate tool to sort out the most promising genotypes (=stage 2); Selection of 20 to 30 genotypes, planting of VCU, validation of cultivation and use (=stage 3).

Finally: registration of new variety (ies)...

**Keywords:** breeding, Geno-Vigne, IFV, innovation, resistance

## **Session 3: Classical breeding and NBT**

## The future of grape breeding: theory and technology

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In the past decades, traditional breeding strategies based on the development of molecular markers were dominant in grape breeding. Intraspecific cross is preferred due to the industry requirement in grape. With the development of next generation sequencing technologies, the 'Grape World' data platform was built, and the platform included massive genomic, transcriptomic, metabolic and phenotypic data. The big data analysis indicated that it could be difficult to breed new cultivars which could suit to the climate challenges if crosses were made within *V. vinifera* species.

The grape population structure showed that the genetic variation inside *V. vinifera* cultivars were tiny, and relative big in wild species and interspecific hybrids. The transcriptome data of grape berries showed that the wild species and interspecific hybrids had more abundant gene expression types, alternative splicing events, and *de novo* genes than *V. vinifera*. Sugar contents were surveyed in more than 18 populations in the last sixty years; due to the heterosis phenomenon, the sugar content in interspecific cross populations were significantly higher than in intraspecific cross populations. These results indicated that interspecific cross should be an important breeding tendency in the future.

The GMO products are likely to cause concern to consumers in spite of the slow development of grape transgenesis. The new gene editing technologies (CRISPR/Cas9/Cpf1) have been confirmed in grape, and they might be accepted by customers and industry. So they should be important technologies in grape improvement in the future.

**Keywords:** breeding, CRISPR, heterosis, sugar



## **Inra-ResDur: the French grapevine breeding program for durable resistance to downy and powdery mildew**

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The current strategy to control grapevine downy and powdery mildew relies on chemical treatments. The most promising option to reduce the needs of fungicides in viticulture is the use of resistant varieties. This is why a new breeding program called INRA-ResDur was launched in 2000 to create varieties with a durable resistance to downy and powdery mildew and with a berry quality suitable for the production of high quality wines. Various American and Asian resistance sources have been described for a long time. During the last decade, intense genetic analyses of some of them have unveiled several resistance loci. However, resistance breakdown has already been observed for the locus Rpv3 (resistance to *Plasmopara viticola* derived from the resistant variety Bianca) and for the locus Run1 (resistance to *Uncinula necator* derived from *Vitis rotundifolia*). To ensure the durability of resistance, we used in the INRA-ResDur program marker-assisted selection (MAS) to stack resistance factors derived from multiple sources. Thus, MAS allowed us to follow six resistance alleles, Rpv1, Rpv3 and Rpv10 for downy mildew and, Run1, Ren3 and Ren3.2 for powdery mildew. This strategy led to the development of candidate varieties bearing not only one but 2 or 3 genes to control each disease. Four new resistant varieties, Artaban, Floreal, Vidoc and Voltis, were already registered in 2018 and a set of ca 20 additional ones will be released by 2024. This project is a result of collaborations between INRA and IFV, the French Vine and Wine Institute, and other European breeding institutes.

**Keywords:** breeding, disease resistance, durability, new varieties

## Identification of haplotypes controlling seedlessness by genome resequencing of grape

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Seedlessness is one of the most prized traits in table or raisin grapes. Most of seedless table grape cultivars with known pedigrees derive from the stenospermocarpic variety Sultanina, also known as Sultanine or Thompson Seedless. The most accepted hypothesis proposed for inheritance of stenospermocarpic seedlessness in grapevine is that the expression of three independently inherited recessive genes is controlled by a dominant regulator gene. The dominant locus was later named SDI for “seed development inhibitor”. The MADS-box gene *VvAGL11* has been proposed as a candidate for SDI because its Arabidopsis homologous genes are involved in ovule and seed development and its female flower carpel-specific expression in Arabidopsis. However, the molecular nature of the recessive genes is unknown. In this study, we obtained dense variation data based on an analysis of high-depth resequencing data of a diverse group of 8 seeded and 15 seedless grape genomes sequenced to > 45× mean depth. The data were first used to examine the genetic population structure and relationships among the seeded and seedless grapes. We are analyzing a set of single-nucleotide polymorphisms (SNPs) to understand the patterns of nucleotide diversity and linkage disequilibrium (LD) surrounding SDI locus as well as to predict loci of the minor recessive gene. The results of this study will be of value to soybean breeders that are striving to more effectively harness haplotype variation at the SDI locus to develop superior seedless grape cultivars.

**Keywords:** haplotype, resequencing, seedlessness

## Molecular mechanisms behind the somatic embryogenesis process in grapevine: from key transcripts to epigenetic signature

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Somatic embryogenesis (SE) is the initiation of embryos from plant somatic tissues and in grapevine it is induced by means of tissue culture. SE represents one of the most suitable tools for the application of *in vitro* manipulation in the *Vitis* genus. Moreover, it is pivotal for functional genomics, since somatic embryogenesis is an essential step in genetic engineering studies applied to grapevine. Although SE protocols are available for some grapevine cultivars, this process has still some limitations that hamper a wider use in genetic improvement applications based on cisgenesis and genome editing approaches. For these reasons, a deeper knowledge and a better mastery of SE would greatly help. Grapevine somatic embryogenesis is affected by many factors, such as explant type, composition of culture media and genotype. Interestingly, different *Vitis* genotypes show different regenerative embryogenic competence, making fundamental the understanding of recalcitrance mechanisms behind the regenerative aptitude.

Our previous studies showed that Sangiovese and Cabernet Sauvignon are two cultivars characterized by an opposite aptitude for somatic embryogenesis. In this work, the behaviour of these grapevine genotypes during *in vitro* somatic embryogenesis has been investigated by profiling mRNAs, small RNAs and methylated DNA by high-throughput sequencing technologies. Embryogenic tissues were induced from immature stamens excised from field-collected flower clusters of both cultivars and cultured on a callus induction medium. Starting explants and calli with and without embryogenic competence were analysed by the deep sequencing techniques cited above.

This study allowed us to explore the reorganization of gene expression changes characterizing the process of embryogenesis, pointing out the key role of gene functional categories such as nucleotide, carbohydrate and secondary metabolism. Moreover interesting findings about the epigenetic control of the SE process emerged, further confirmed by *in vitro* assays on explants treated with compounds able to modify the DNA methylation level.

**Keywords:** *in vitro* culture, methylome, somatic embryogenesis, transcriptome

## Generation of mildew-resistant grapevine clones via genome editing

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Pesticides-mostly fungicides- are massively used in viticulture to contain spreading of fungal and fungal-like diseases such as powdery mildew (PM) and downy mildew (DM), towards which cultivated grapevine (*Vitis vinifera*) is highly susceptible. Such consumption of fungicides is costly and deleterious both for human health and the environment.

Alternatively, the successful control of such diseases was obtained by inactivation of plant susceptibility genes in other crops: in fact, knocking out of DMR6 genes was demonstrated very effective in controlling DM in *Arabidopsis thaliana* and other species. However their efficacy toward DM resistance has yet to be demonstrated in grapevine. In addition, silencing of a group of MLO genes resulted in resistance to PM in grapevine.

Selection of non-functional copies of susceptibility genes can be achieved via traditional breeding; however crossing is not always desirable in grapevine, since maintenance of clonal genetic integrity is commercially important (i.e. in wine grapes).

The revolutionary advent of genome editing now offers tools to edit and completely knock out susceptibility genes in many crops while maintaining their cultivar and clonal genetic backgrounds. CRISPR /Cas 9 technology was used in this work to edit DM and PM susceptibility genes in different grapevine clones. Several plants edited in DMR6 and MLO genes were obtained and are currently being screened for DM and PM resistance. This work will establish whether DMR6 is determinant in controlling resistance to DM in grapevine, whether a full knock-out of DMR6 and MLO genes provides resistance to DM and PM respectively, and will hopefully deliver mildew resistant grapevine plants.

**Keywords:** CRISPR/Cas9, DMR6, downy mildew, MLO, powdery mildew, susceptibility genes

## **Session 4: Genomics and data handling**

## Uncovering the wealth of grapevine genetic diversity through whole genome sequencing and assembly

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There is substantial unshared gene content between grape cultivars. Between 5 and 10 percent of protein-coding genes are not shared between pairs of cultivars from the grape germplasm. Importantly, a seminal study of Tannat showed that cultivar-specific genes can contribute considerably to phenotypic differences between cultivars. This structural diversity makes using a single one-size-fits-all reference genome inadequate for studying the function of non-reference cultivar genomes. Furthermore, the heterozygosity throughout the grape genome poses a challenge to typical assemblers, resulting in highly fragmented assemblies and ambiguity at heterozygous loci. We recently showed that accurate and contiguous assemblies of grape genomes can be built rapidly by assembling long, Single-Molecule Real-Time (SMRT) sequencing reads using FALCON-Unzip, a diploid-aware assembler. These megabase-scale contigs can be further combined using optical mapping and Hi-C technology into phased chromosome-scale scaffolds. This approach was used to reconstruct the Cabernet Sauvignon genome; its primary phased assembly was 443Mb in 56 sequences (N50=16.5Mb) and its secondary, phased assembly, representing all alternative haplotypes was 330Mb in 33 sequences (N50=6.9Mb). The phased diploid assembly captured heterozygous loci, haplotype structure, and heterozygous structural variations within coding sequences. Long SMRT reads also capture full length transcripts used to refine predicted gene models (including untranslated regions), to identify cultivar-specific genes and to characterize multiple splice variants for most genes in the genome. Based on our experience with Cabernet Sauvignon, we optimized the FALCON-unzip pipeline to assemble additional wine grape and wild grape genomes. These rapidly assembled, high-quality resources are better suited for transcriptome studies and mapping traits than reference genomes of cultivars other than that being studied.

**Keywords:** cDNA sequencing, comparative genomics, full length alternative splicing, haplotype phasing, heterozygosity, structural genomics

## **An integrated meta-QTL and transcriptomic data mining approach to select candidates controlling veraison time in grapevine**

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High temperature impairs the quality of grapes and wines. Understanding the genetic control of phenology-related processes is crucial to breed successfully grapevine varieties more adapted to a changing climate. Veraison time, in particular, is a key factor for determining climatic conditions during ripening. Quantitative trait loci (QTL) studies attempting to elucidate the genetic determinism of developmental stages in grapevine have identified genomic regions including large numbers of genes. Broad scale transcriptomic studies, by identifying sets of genes modulated along berry development and ripening, have also highlighted a huge number of putative candidates. With the final aim of providing a functional and integrated genomic overview for the genetic control of grapevine veraison time, and of prioritizing possible main genetic regulators, we have applied a meta-QTL analysis for grapevine phenology-related traits and checked for co-localization of transcriptional candidates. Twelve QTL studies were considered, including 174 QTLs related to phenology. By using the software BioMercator v4.2 a consensus genetic map including 3130 markers was compiled. QTLs were projected onto the consensus map and clustered into meta-QTLs. Anchoring to the grapevine genome assembly 12X.v2 allowed us to select positional candidates. We generated 18 meta-QTLs from 69 QTLs for the traits flowering, veraison and ripening among which 4 specifically related to veraison time. Moreover, 11 meta-QTLs for genomic regions generically affecting phenology were revealed. This approach allowed us to reduce the number of positional candidates by almost 4-fold. Expression data generated by transcriptomic studies along berry development performed on several grape varieties were mined with different approaches to select, among positional candidates, genes significantly modulated at veraison time. The polymorphisms diversity of these genes will be tested for association to the veraison time phenotypic diversity of an Italian grapevine germplasm collection. A complementary GWA (Genome-Wide Association) strategy will be also applied on a reduced core collection.

**Keywords:** climate change, QTLs, transcriptomics, veraison

**The first version of the whole-genome sequencing (WGS) and assembly of the muscadine grape, *Muscadinia rotundifolia* cv. Noble**

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American native *Muscadinia* grape holds distinctive traits and it is a dream source of valuable germplasm that can enable us to understand the molecular dialogues underlying diverse economically important traits, including resistance to most of diseases and pests that limit the worldwide production of *V. vinifera*, tolerance to heat and humidity, adaption to poor soil, unique spectrum of beneficial phytochemicals, and pleasant fruit quality traits, in which all are of great commercial interest. In this work, we capitalize on the latest achievements of genomic technologies to deliver long needed critical knowledge on the muscadine WGS for the benefit of the viticulture industry at large. The aim is to develop a breeding platform to meet the industry demands under challenging climate and increased pathogen pressures. Here, we report the 1<sup>st</sup> version of the whole-genome sequencing and assembly of the muscadine grape, *Muscadinia rotundifolia* cv. Noble. PCR-free libraries of total genomic DNA were generated and used to produce 200 million paired-end reads (~100 Gb). A K-mer analysis of the sequences revealed an estimated genome size of 414 Mb, a heterozygosity of 1.5%, and approximately 100 Mb (24%) of the genome is represented by repetitive sequences. Using Discovar De Novo for primary assembly construction and Redundans for consolidating redundant contigs due to heterozygous regions, we were able to generate an assembly representing 400 Mb of the estimated 414 Mb genome (~97%). Half of the genome is represented in a thousand of the largest scaffolds, which are at least 107 Kb in size (N50). Additional scaffolding of the assembly using in-situ and in-solution proximity ligation (Hi-C) is currently being performed in partnership with Dovetail Genomics, which is expected to significantly improve the assembly and yield near-chromosome-level scaffolds.

**Keywords:** breeding, functional genomics, genetics, genetic variability, genomics, *Vitis*



## Genome-wide association study of a diverse grapevine panel to uncover the genetic architecture of numerous traits of interest

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A major challenge facing viticulture consists in decreasing inputs, especially synthesized fungicides, and adapting to climate change, while maintaining berry quality and differentiated wine styles, as well as reasonable profits to the winegrowers. In this endeavor, breeding new varieties is an important lever. However, before selecting which varieties to cross, it is necessary to assess how much additive genetic variance is present in the population as a whole for the traits of interest, and to understand their genetic architecture. Therefore, to go beyond QTL mapping in bi-parental crosses, we exploit the panel of 279 cultivars capturing most of the genetic and phenotypic diversity existing within the French collection of genetic resources (INRA Vassal). The panel was planted in the vineyard in five blocks, and numerous traits (berry and cluster weight,  $\delta^{13}C$ , organic acids, polyphenols) were phenotyped over several years. With such an experimental design, broad-sense heritability ranges from 58% to 96%, depending on the trait. In parallel, the 18k Illumina genotyping microarray provided a set of 12000 filtered, uniformly distributed SNPs. Using these SNPs to compute additive genetic relationships allowed us to estimate narrow-sense heritability, ranging from 22% to 82%. Then, we conducted a genome-wide association study for each trait, either testing each SNP separately and controlling for relatedness, or all SNPs jointly to handle linkage disequilibrium. For traits having an oligogenic architecture, several significant associations were detected, some being new compared to the already-known QTLs. Putative candidate genes will be presented based on the annotation of the reference genome. Future prospects include analyzing more already-available traits and genotyping-by-sequencing data to densify the markers, hence better handling the short linkage disequilibrium in this panel, as well as performing multivariate analyses to account for genetic correlations among traits, hereby increasing power to detect more QTLs.

**Keywords:** berry weight, delta 13C, gwas, organic acids, polyphenols, quantitative genetics

## Unraveling the key molecular events of grape berry ripening under varying crop loads

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Despite the economic importance of grape berries and their processed products, the molecular complexity of berry development and ripening are poorly understood. In addition, the impact of yield on fruit quality has been studied extensively, but responsible mechanisms have not been characterized at the level of genetic regulation. To identify the key molecular events controlling berry ripening we created a highly detailed transcriptomic and metabolomic map of berry development from fruit-set to maturity using Pinot noir and Cabernet Sauvignon grapevines for three consecutive years. Coordinated waves of gene expression were observed at early development, veraison/mid-ripening and late-ripening stages and were consistent across vintages. Co-expression analysis identified a core network of transcripts, as well as, variations representing varietal differences. By focusing on transcriptomic rearrangements close to veraison, we identified two rapid and successive transitions involving genes with expression profiles linked to the molecular reprogramming of berry development. To examine the impact of crop load on transcriptomic and metabolomic changes during grape berry maturation, Pinot noir and Cabernet Sauvignon grapevines with 50% or 75% cluster removal following fruit set were compared to unthinned vines for three consecutive vintages. We identified genes modulated by crop load around veraison, representing putative transcriptional key triggers responding to crop load. This study allowed us to progress towards the construction of robust models describing the molecular network that characterizes berry development and the impact of crop load on its genetic regulation.

**Keywords:** berry development, crop load, metabolomics, ripening, transcriptomics

## Adaptation of downy mildew to grapevine partial resistance

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Invasions by plant pathogens are responsible for tremendous damage in crops and are increasing in frequency. Consequently, population genetics studies of pathogens are of great interest to understand invasive population dynamics and adaptation to new hosts (e.g. resistant cultivars) and environments. *Plasmopara viticola* is a heterothallic oomycete responsible for grapevine downy mildew, a major and costly disease worldwide. It has been introduced very recently (around 150 years ago) from North America in Europe and has subsequently invaded European vineyards in a few years. In the last two decades, resistant grapevine cultivars have been used to control the disease, but *P. viticola* populations quickly adapted to this resistance.

The genome of *P. viticola* has been sequenced with PacBio long reads, resulting in a high quality assembly covering 85% of the estimated genome size (BUSCO pipeline: 95.7% completeness). A total of 63 European isolates collected on sensible and resistant grapevines (harboring the Rpv3 QTL) have been resequenced and phenotyped for aggressiveness traits. While isolates collected on sensible grapevines were not structured geographically, isolates from resistant cultivars, in addition to being more aggressive, were genetically differentiated in three separated groups. This suggested the existence of multiple independent adaptations to grapevine resistance. Detection of genomic regions linked to adaptation to cultivated grapevine and resistant cultivars, as well as genes responsible for the determination of mating type, are currently on-going.

**Keywords:** adaptation, downy mildew, population genomics, resistant cultivars

## The combined role of WRKY and MYB TFs in the regulation of stilbene synthase genes in grapevine (*Vitis vinifera* L.)

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Grapevine embodies one of the most representative examples of how next generation sequencing technology (NGS) massively impacted plant genomics, as well as molecular and functional biology in these days. The rapidity and reliability of NGS technologies, the quantity and quality of sequencing data that can be produced, and the striking reduction in costs, led to a huge increase in genomic and transcriptomic data available to the scientific community, which represent per se an extraordinary tool for research. All these factors, combined with the availability of datasets shared by the grapevine research community in public repositories, led to increased attention in the use of correlation analyses and gene co-expression networks (CGNs) for many proposes providing support for targeted functional studies. The present study is an example of how these large transcriptional datasets, together with gene co-expression network analysis can be used for inferring gene function and to draw putative regulatory networks. The topic is the transcriptional regulation of the stilbene synthase (VvSTS) genes. These genes, which in grapevine are organized in a multigenic family encompassing up to 48 members, encode for the key enzyme leading to the biosynthesis of resveratrol, which is the basic unit in the biosynthesis of plant stilbenes. Plant stilbenes are a small class of phytoalexins which are accumulated in grapevine and a few other plant species in response to environmental stimuli as well as biotic and abiotic stresses. Based on the notion that genes involved in similar or related processes may exhibit similar expression patterns over a range of experimental conditions, we performed large-scale condition-independent co-expression analysis screening two separate transcriptome compendia based on microarray and RNA-Seq data and identifying candidate TFs belonging to different gene families. Among these are TFs belonging to the R2R3-MYB, including VviMYB14 and VviMYB15, which were already demonstrated by our group to be involved in the transcriptional regulation of several VvSTS members. Together with these TFs, CGN analyses highlighted the existence of other candidate regulators belonging to other families than R2R3-MYB one, including the WRKY multigenic family. Functional analyses on candidate WRKYs confirmed a direct or indirect role for these TF in the regulation of the stilbene pathway with some members acting in combination with previously mentioned MYB regulators.

**Keywords:** gene co-expression, network, resveratrol

## **Session 5: Phenotyping and genotyping**

## Sensor based phenotyping for grapevine breeding and genetic analyses

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For decades, practical grapevine breeding required skilled people for evaluation and selection of breeding lines. Around 2005, marker assisted selection (MAS) for resistances was introduced into the breeding workflow permitting faster and more efficient selection opening unprecedented possibilities for selecting resistant genotypes. Today MAS is very efficient for stacking of resistance loci, ideally 3 loci for major diseases, but other traits remain to be addressed by MAS. Thus, breeding is demanding markers suitable for early selection in a breeding program of other traits than resistances. One of the most limiting steps for marker development of complex traits is the lack of highly efficient and high throughput phenotyping of mapping populations or varieties for association studies. Many traits like, phenology, yield, or metabolome analysis require fruiting plants and consequently cultivation in the field. Sensor based phenotyping techniques have been developed and are currently tested. A robust phenotyping platform called Phenoliner was recently set up. Using this platform geo-referenced sensor data are collected and first automatic evaluation tools are tested to extract trait relevant information.

**Keywords:** breeding, field phenotyping

**Extensive genotyping of a large collection of rootstocks, population structure analysis and core collection extrapolation for new breeding programs**

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A large repository of 441 rootstocks and other non-vinifera varieties was established beginning from 2013 by the University of Milan with the aim to collect most of the genetic variability of *Vitis* spp. useful for rootstock genetic improvement. This kind of collection is one of the largest in Italy, with accessions coming from different donor institutes in California, Spain and Italy, and encompasses progenies obtained by University of Milan breeding programs. A preliminary genetic characterization was performed as usually done for better management of grape germplasm. Eighty-six percent of the accessions were evaluated by genotyping with SSR markers and by phenotyping the sex of flowers. Twenty-two carefully selected and highly polymorphic SSR markers were used out of 41 taken into consideration, including the nine internationally applied for *V. vinifera* genotyping and 26 VChr SSRs, having a longer core tandem repeat. However, 5 out of the 22 markers showed to be critical when applied on a large-scale for genotyping non-vinifera plants and were subsequently discarded. SSR profiles comparison showed that more than one-third accessions were redundant. Less than half were identified as true-to-type, more than one third remained anonymous. These anonymous, still uncharacterized genotypes represent a precious reservoir of genetic diversity. The analysis of the genetic structure revealed 4 main groups, with more than one-third genotypes classified as admixed. A genetic core collection able to capture the whole allelic variation was set up, as a valuable starting point for both rootstock breeding and genome-wide association studies.

**Keywords:** genetic improvement, SSR, VChr markers, *Vitis* genetic resources

**Few main parents contributed to traditional variety assortment in north western Italy as revealed by microsatellites and SNPs**

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Studies on parentage and kinship of traditional grape varieties allow shedding light into the likely natural breeding at the basis of the assortment evolution of cultivated grapevines in a certain area during several centuries. They therefore contribute to understand the process of domestication and local development of viticulture through farmer selection and to draw the origin and the history of traditional grape varieties. For wine grapes, these evidences add significant marketing value to wines and their terroirs, being highly appealing for wine consumers and wine lovers. Around 300 grape varieties, traditional from north and north western Italy, and 700 unique cultivars mainly from other Italian regions and Central Europe were examined for genetic kinship through microsatellite markers (SSR). More than 100 genotypes showed parent/offspring or kinship relationships as revealed by 32 SSR loci. Twenty-four trios of parents and descendants were disclosed, among which the pedigree of Dolcetto, the third most planted variety in Piedmont. For samples analyzed also by the 20K Illumina SNPs chip, the parental relationships were confirmed. The results, confirming a pattern shown by other studies, indicate closer kinship among varieties from the same geographical area, and a complex network of pedigrees with very few major founders showing from 12 to 20 links with other cultivars. These main genitors are often varieties no longer cultivated and/or threatened for extinction today. This fact highlights on one hand the urgency of rescuing and preserving these genetic key links before they get missed, and on the other hand, it explains the puzzle still unsolved about the origin of many renowned varieties. Also, their ampelographic features provide significant clues for linking their identity with ancient varieties depicted or described centuries ago, contributing to shed light on the history of descendant cultivars of high economic value today

**Keywords:** breeding, cultivar parentage, genetic resources, molecular markers, pedigree



## QTL mapping of downy mildew and botrytis bunch rot resistance in a *Vitis aestivalis*-derived 'Norton'-based population

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*Vitis aestivalis*-derived 'Norton' is the official grape of the State of Missouri grown in regions with high disease pressure and cold winter temperatures where *V. vinifera* is not adapted. It reportedly offers an abundance of traits, including resistance to powdery and downy mildew as well as Botrytis bunch rot, which can be used to naturally improve existing *V. vinifera* germplasm. To identify genetic determinants for resistance to downy mildew caused by *Plasmopara viticola* and Botrytis bunch rot caused by *Botrytis cinerea* in *V. aestivalis*-derived 'Norton', a mapping population of 182 individuals was constructed from a cross between 'Norton' and *V. vinifera* 'Cabernet Sauvignon'. A consensus genetic map was constructed with 411 simple sequence repeat (SSR) markers. In collaboration with VitisGen ([www.vitisgen.org](http://www.vitisgen.org)), approximately 43,320 single nucleotide polymorphism (SNP) markers generated by genotyping-by-sequencing (GBS) were identified, and a consensus map of 3,825 SNPs was developed. Of these, 1,665 SNP and 407 SSR markers were clustered into 19 linkage groups for a total of 2,072 markers spanning a genetic distance of 2,203.5 cM. In preparation for placing traits on this integrated high-resolution map, disease progression and resistance reaction in response to *P. viticola* and *B. cinerea* were evaluated in this population for two years. The quantitative trait loci (QTL) analysis indicated a major resistance locus on linkage group 18 for downy mildew and linkage group 2 for Botrytis bunch rot, respectively. This data will be further presented and discussed. The ultimate goal of this program is to use genetic markers to rapidly deploy favorable alleles and accelerate breeding cycles for new cultivar releases.

**Keywords:** *Botrytis cinerea*, marker-assisted selection, *Plasmopara viticola*, QTL mapping, *Vitis aestivalis*-derived 'Norton', *Vitis vinifera* 'Cabernet Sauvignon'

**Acknowledgements:** this project was supported by Agriculture and Food Research Initiative Competitive Grant, Award No. 2013-67014-21360, and Specialty Crop Research Initiative Competitive Grant, Award No. 2011-51181-30635, of the USDA National Institute of Food and Agriculture.

**Variation of recombination rate along the genome in *Vitis vinifera* x *Vitis rotundifolia* interspecific hybrids**

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Wild plant species related to crops provide plant breeders with a wide range of genetic resources. But breeders often face the challenge of obtaining viable and fertile interspecific hybrids. *Vitis rotundifolia*, an American species related to the cultivated grapevine *V. vinifera*, displays resistance to several major grapevine pathogens. These traits makes *V. rotundifolia* particularly interesting for grape breeding. Unfortunately, *V. rotundifolia* is difficult to cross with cultivated grapevine varieties, crosses between both species most often leading to sterile hybrids and developmental abnormalities in the offspring. In order to understand the incompatibility between both genomes, we studied the recombination rate between homeologous chromosome pairs in *V. vinifera* x *V. rotundifolia* interspecific hybrids. To this end, three mapping populations were generated by pseudo-backcrosses using *V. rotundifolia* as the donor parent and several *V. vinifera* cultivars as the recurrent parents. Genotyping-by-sequencing was used to establish high-density genetic linkage maps and to determine the genetic composition of each chromosome of the analysed individuals. We showed that recombination distribution along the chromosomes is similar to grapevine in interspecific hybrids, when the chromosome pair is composed by one entire chromosome of each species. Interestingly, when in a chromosome pair one chromosome is derived from a crossover between *V. vinifera* and *V. rotundifolia*, recombination rate increases in homologous regions, whereas it dramatically decreases in hybrid regions. Altogether, these results provide new insight to improve the use of *V. rotundifolia* in grape breeding, notably to optimize the introgression process.

**Keywords:** breeding, high-density genetic mapping, interspecific cross, recombination

## **Phenoliner: a multi-sensor field phenotyping platform**

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Due to its perennial nature and size, the acquisition of phenotypic data in grapevine research is for the most part restricted to the field. Techniques to assess phenology and morphology traits are mostly based on visual scoring. Some traits like biotic and abiotic stress and especially quality traits are evaluated by invasive measurements. The new arising sensor technologies make non-destructive evaluations of phenotypic traits available for grapevine research by using different sensors and sensor platforms. Varying light conditions and background showed the biggest environmental impact and challenge for field phenotyping of grapevines.

Facing these problems the presented Phenoliner is a new type of ground based, robust field phenotyping platform. Following the concept of a movable tunnel, the vehicle is based on a grape harvester. It is equipped with different sensor systems within the tunnel (multi-camera system, hyperspectral cameras) and above (RTK-GPS, orientation and speed sensors). Through an artificial light source in the tunnel, it is independent from external light conditions. In combination with the artificial background the Phenoliner allows standardised acquisition of high-quality, geo-referenced sensor data. The multi-camera system is used for the automated acquisition of colored 3D data of multiple vine rows for the automated calculation of yield parameters (number of grape bunches and berries, berry size) to be used for yield prediction. The hyperspectral cameras are used to detect spectral data in a broad range of spectral bands covering a spectrum from 400 nm to 2,500 nm to evaluate e.g the health status.

The Phenoliner can be used for a high-throughput, automatic and non-invasive acquisition of phenotypic data directly in the field. It allows a fast, robust and precise screening of grapevines for several traits. The platform can be extended through further sensors at any given time.

**Keywords:** field phenotyping platform, hyperspectral imaging, phenotyping, yield parameters

## Combining high throughput genotyping and phenotyping for the genetic improvement of table grapes in Chile

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Although Chile leads table grape exportations by volume, the renewal of table grape varieties has just recently taken off. Among those, Maylen (Iniagrape-one cv.) a mid-season black seedless variety from the Chilean Breeding Program at INIA stands out for its flavor and excellent post-harvest life. In order to accelerate the development of more new table grape varieties in local conditions, we have improved the phenotyping of fruit quality characteristics through the development of apps and tools to measure traits such as berry size, shape and color, rachis architecture and colour, both in field and laboratory conditions, at harvest and after cold storage. For instance, Vine Tracker is a mobile app which replaces the use of field books. It combines a cloud-located database of the breeding program with the mobile network capacity to allow simultaneous evaluation of vine or fruit phenotypes, reducing the cost, time, involuntary error, and allowing to filter and consult data on real time. Berry Analyzer is another tool developed for fruit phenotyping. It consist of a series of scripts to quickly retrieve information from berries, such as polar and equatorial diameters, shape and color from images, in a small fraction of the time needed with standard scientific or design software that requires human intervention. Information obtained with these tools during season 2018 has been combined with high throughput genotyping by sequencing of more than 500 accessions of the breeding program to characterize our elite material. Measures of heritability, as well as association with molecular markers on a diverse genetic background have been determined.

**Keywords:** breeding, genotyping by sequencing, phenotyping, table grape

## **Session 6: Vine growth and development**

## Genetic variation for grapevine reproductive development

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Reproductive development has been targeted during domestication and subsequent breeding of crop species to improve the production and suitability of fruits and seeds for human profit. In cultivated grapevine, variation in flower induction and initiation, inflorescence and flower development, flower sex, or gamete and seed development have direct implications on production traits such as fertility, cluster weight or berry weight but also on quality traits such as cluster compactness, berry skin to pulp ratio or seedlessness. At the same time, functional sexual reproduction has not been under positive selection in grapevine due to the extensive vegetative multiplication of cultivars, which has both positive and negative consequences for the crop. Mutations that would not be transmitted through sexual generations in a natural environment can provide interesting quality traits to grapevine cultivars. In this way, genetic variation in gamete viability can lead to decreased seed number and berry size as well as reduced cluster compactness, current goals in clonal selection of wine cultivars. Nevertheless, this trait can also have negative consequences on yield and quality depending on environmental conditions. Complete lack of gamete viability can be behind some forms of parthenocarpic development and it is indeed in the origin of Corinto raisins. Genetic variation affecting ovule and seed development is behind stenospermocarpic seedlessness, a main quality trait in table grape breeding. All these examples point out how understanding grapevine reproductive development and particularly the existent genetic variation for these processes can help developing more suitable and adapted cultivars for both wine and table grape production.

**Keywords:** fruit set, gamete viability, genetic variation, reproductive development, seedlessness, yield

## Mapping the genetic architecture of grapevine bud dormancy and chilling fulfillment traits

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Dormancy is an adaptive winter survival strategy for the grapevine. In a changing climate, timing of dormancy induction and release are critical for sustainable production. The underlying genetic architecture of dormancy and chilling fulfillment was determined using a F2 wine grape mapping population (n =100) and quantitative trait analyses. QTL analyses were conducted on photoperiod and temperature response phenotypes collected in multiple years using a genetic map with 1449 SNP markers across 19 linkage groups. QTLs were identified for individual traits with phenotypes mapping repeatedly to linkage groups 11, 13, and 18 in multiple years. The QTLs identified typically accounted for 10-20% of the phenotypic variation. Clarifying the genetic relationship in these complex dormancy induction and release traits provides information needed to identify key genes and generate markers for marker assisted selection for sustainable cultivars under changing climatic conditions.

**Keywords:** bud break, chilling fulfilment, dormancy, SNP marker

**Ethylene- induced macromolecule catabolism - the switch required for bud meristem growth resumption?**

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The molecular mechanism regulating dormancy release in grapevine buds in response to either natural or artificial stimuli is as yet unclear, and this limits its manipulation to optimize grape production. We formerly suggested that (i) abscisic acid (ABA) represses bud–meristem activity; (ii) anaerobic respiration, which is initiated in response to perturbation of the cytochrome pathway activity, induces an interplay between ethylene and ABA metabolism, which leads to removal of repression; and (iii) gibberellin (GA)-mediated growth is resumed.

We will present transcriptomic, metabolomic and transgenic experimental data that (1) support the proposed function of ABA, (2) question the proposed role of GA during dormancy release and (3) propose a refined and innovative model in which ethylene- induced macromolecules catabolism is activated in response to changes in sugar metabolism, and is mandatory to trigger growth resumption after the dormancy cycle.

**Keywords:** bud, development, dormancy, hormone, sugar signaling



## Dissecting the control of shoot growth in grapevine: genetics and genomics identify potential regulators

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Sarah Cookson and Eric Gomès contributed equally to the present work.

Grapevine is one of the most important horticultural crops, yet little is known about the regulation of shoot development in grapevine or other perennial fruits crops. Shoot growth in *Vitis vinifera* x *Vitis riparia* hybrids is known to be highly variable. In this work we combined genetics and genomics tools to identify candidate genes of shoot growth control in grapevine.

An F2 population of 337 genotypes from a cross between *V. vinifera* and *V. riparia* was used and a genetic map based on 156 microsatellite markers was made. The population was phenotyped for internode length and cane pruning weight, and Quantitative Trait Loci (QTLs) identified. The genes differentially expressed between the normal sized and dwarf individuals of this F2 population were studied using whole genome microarrays.

Shoot development was highly variable in this population; almost 25 % of the individuals exhibited a dwarf phenotype. Three QTLs were identified on linkage groups 7, 14 and 18 explaining 40.7, 14.7, 10.5 % of the phenotypic variation, respectively. The gene expression study revealed the differential expression of 52 genes between the normal and dwarf individuals. Among them, four candidate genes located within the interval confidence of the QTL on LG7 were strongly down-regulated. These four genes included CURLY LEAF (CLF) and a Caffeoyle CoA O-methyltransferase both known as potentially involved in regulation of shoot development. Sequencing of BAC DNA suggested the presence of a deletion leading of these four genes in the genomes of dwarf genotypes. We have developed a PCR-based marker to detect the presence of this deletion responsible of the dwarf phenotype. This DNA marker can be used to improve early genotyping in future grapevine breeding programs.

Finally, the phenotype of the dwarf individuals was similar to that of *clf* mutants of *Arabidopsis thaliana* and the known targets of CLF in *A. thaliana* were mis-regulated in the dwarf plants. This suggests that CLF, a major developmental regulator in *A. thaliana*, controls shoot development in grapevine.

**Keywords:** CURLY LEAF, dwarf phenotype, F2 population, shoot growth

## Determination of genetic loci in the control network of grapevine flowering

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Winegrowers observe considerable changes in phenology of grapevines due to climatic changes. Hence, understanding the genetic regulation of phenological traits, like timing of bud break or flowering is becoming more and more important for adjustment of viticulture to future environmental conditions. Even though homologs of flowering time control genes were already identified and characterized in model plants, our overall knowledge about which genetic factors are involved in the complex flowering time control network of *Vitis* remains very limited. Quantitative trait locus (QTL) analyses within a biparental mapping population of GF.GA-47-42 (early flowering) x 'Villard Blanc' (late flowering), consisting of 151 F1 individuals, revealed a consistently recurring QTL on chromosome 14 as well as QTL on 6 additional chromosomes with few of them covering rather large chromosomal segments. Two approaches for validation and refinement of the QTL regions were followed: (i) an extension of the mapping population by 1000 additional F1 individuals, (ii) high resolution genetic map based on SNPs derived from RAD-Seq. Using sequence information of model organisms and the grapevine reference genome PN40024, novel flowering time candidate genes were identified genome-wide. Association studies with amplicon sequences of selected candidate genes within and outside of QTL regions showed a significant correlation with a specific flowering time phenotype. Moreover, season-spanning RNA-Seq experiments revealed differential expression for several candidate genes. Findings of all adopted approaches support a potential role of novel candidate genes in the flowering time control network of grapevine.

**Keywords:** climatic change, flowering time, QTL, RNA-Seq

## Characterization of the reproductive performance of a collection of grapevine varieties

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Genetic, physiological and environmental factors interact in grapevine to determine the flower number in the inflorescence and the fruitset rate. Abnormal behavior in the reproductive performance may cause coulure (excessive fall of flowers) or millerandage (excessive presence of seedless berries and/or live green ovaries (LGOs)), which may affect yield. In this work, the reproductive performance of a set of 129 wine grape and table grape cultivars was studied in two consecutive growing seasons. Measures of reproductive performance were taken in ten inflorescences/bunches per cultivar and included flower number per inflorescence, berry number per bunch, fruitset (%), coulure and millerandage indices (0-10). The flower number per inflorescence was estimated from the number of flower caps collected in fine mesh bags set before bloom. After flowering, flower caps from each individual bag were scanned and the digital images were used for manual or automatic counting, with a dedicated tool developed in ImageJ. Those clusters used for flower counting were collected at harvest time and characterized, including counts of seeded berries, seedless berries and LGOs. Different methods were used to estimate fruit set, millerandage and coulure indices. Fruitset average values among all the cultivars was 46-48% in 2016 and 41-43% in 2017. The whole range of variation for fruitset among cultivars was very high, more than 90% every year. Within cultivars, differences in fruitset between years ranged from 0 to 40%, with an average difference around 9-10%. Coulure and millerandage indices also showed great variability among cultivars, reaching the whole range of variation, while they were stable between seasons (difference average: 1 unit). The results allow establishing a preliminary classification of a large number of cultivars according to their fruitset rate. Besides, the huge variability found constitutes a very suitable base for the study of the genetic processes involved in the grapevine reproductive performance.

**Keywords:** berry number, coulure, flower number, fruit set, image analysis, millerandage, phenotyping, reproductive development

## Molecular analysis of bunch architecture in grapevine

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A loose grape cluster is a desired trait in grapevine breeding, since it reduces the abundance and severity of fungal infections. This is partly due to a better coverage of the grape bunch with antifungal spraying agents but also mediated by a better air exchange within the grape cluster. The reduced exposure to high humidity acts as a physical barrier against pathogens which are in need of high moisture to proliferate, e.g. *Botrytis cinerea*. The aim of this study was to identify genes influencing bunch architecture and to deduce molecular markers to accelerate the selection process in grapevine breeding. The compactness of the bunch was characterized by phenotyping several subtraits. The experiment comprised plants of a mapping population (GF.GA-47-42 x 'Villard blanc') and a set of 'Pinot noir' clones with loose as well as compact clusters. The latter were sampled from three different wine-growing regions in Germany. A genetic mapping and QTL analysis approach based on the trait-segregating population of 150 F1 individuals yielded quite many reproducible QTL distributed around the genome. Some of them are found in accumulation at specific genomic regions. Further statistical evaluation revealed 8 such QTL clusters to be of major relevance and identified flanking molecular markers for marker-assisted selection. In a transcriptional profiling approach 2 loosely and 2 compactly clustered clones of 'Pinot noir' were compared to each other. RNA from dormant winter buds and compound buds harvested during the growing period were used in differential gene expression experiments. RNA sequencing was performed at three different stages of development. Candidate genes found in these experiments were selected and re-analyzed in a more comprehensive set of 'Pinot noir' clones from the three different German growing locations. Finally two genes appeared differentially expressed in comparison of the 2 clone groups over the 3 locations.

**Keywords:** bunch architecture, gene expression analysis, molecular markers

## Understanding scion/rootstock interactions at the graft interface of grapevine

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In viticulture, grafting is used to facilitate grapevine cultivation in soils infected with the Phylloxera, a soil-dwelling insect pest introduced to Europe from America at the end of the 19th century. Successful grafting of plants is a complex biochemical and structural process that begins with an initial wound response, followed by callus formation and the establishment of a functional vascular system between the two grafting partners. Despite the importance of the scion/rootstock interface in viticulture, we know little of the processes involved in forming a successful graft union.

The developments at the scion/rootstock interface of grapevine have been studied using a variety of techniques. Morphological developments have been studied using microscopy techniques and high resolution computed tomography, and xylem connectivity has been assessed using a high pressure flow meter. Microarrays have been used to identify the genes differentially expressed between the wood and graft interface tissues of homo-grafts (the same genotype grafted together) and at the graft interface between different scion/rootstock combinations (hetero-grafts). Primary and secondary metabolite profiling has been done as well as the quantification of hormone concentrations.

An overview of the interdisciplinary approaches currently being used to piece together the puzzle of graft union formation in an important woody, perennial crop will be presented.

**Keywords:** imaging, gene expression, grafting, rootstock

## **A transcriptomic comparison of late-ripening Cabernet Sauvignon berry skins from Bordeaux and Reno**

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Grape berry ripening is strongly influenced by the climate or “terroir” of a place. Temperature and day length are major factors that affect the rate of berry development and metabolite composition. To better understand the effect of “place” on berry ripening, transcriptomic analyses of the skins of Cabernet Sauvignon grown in Bordeaux (BOD), France were compared to those in Reno (RNO), Nevada, USA during the late stages of berry development and at similar sugar levels (19 to 26 °Brix). Day lengths were about the same in both locations, but day temperatures were warmer and night temperatures were cooler in RNO. Sugar levels were lower in berries harvested in BOD compared to RNO at maturity levels considered optimum for harvest in their local regions. RNA-seq analysis revealed that many genes had similar expression levels at the same level of sugar, but there were approximately 6,000 differentially expressed genes (DEGs) between BOD and RNO grape skins at 22°Brix. The top GO categories of the DEGs were involved in response to stimulus (1745 genes, corrected p-value = 6.89E-19), response to stress (1075 genes, corrected p-value = 1.17E-14), and alcoholic metabolic process (320 genes, corrected p-value = 3.11E-12). Some DEGs between BOD and RNO included genes encoding terpene synthases, cell wall enzymes, kinases, transporters, transcription factors, photoreceptors and the core circadian clock. Gene expression in the berry skins at maturity was highly dynamic in both BOD and RNO. The dynamic changes in gene expression in the late ripening stages occurred in BOD berry skins at lower levels of sugars as compared to RNO berry skins indicating that BOD berries matured at lower sugar levels than RNO berries. While many factors may influence gene expression and berry development, it is proposed that temperature and the plant temperature sensor, phytochrome B, may play a major role.

**Keywords:** berry ripening, Cabernet Sauvignon, transcriptomics

## **Session 7: Berry yield and composition**

## Genetic dissection of grape berry ripening and composition

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Berry development is a complex process involving profound physiological and metabolic changes. Quality traits of a ripe berry rely mainly on sugars, organic acids and secondary metabolites such as tannins, flavonols, anthocyanins, aroma precursors, and volatile compounds that accumulate during berry development. Genotype and environment, as well as their specific interactions (GxE), strongly affect the final berry composition. Viticulture practices also impact the final berry quality, in particular, irrigation, canopy management and cropping levels, are normally used to guide maturation. However, the incomplete knowledge of the molecular mechanisms that control berry ripening, hinders our ability to precisely predict the impact of varietal attributes, climate, soil, viticulture practices and all the possible interactions among them, on the berry traits at harvest. The availability of the grapevine genome sequence and the ability to perform genome-wide studies has allowed the characterization of berry development at the levels of the transcriptome. Thanks to this information the core transcriptomic changes distinguished from those variety-dependent, has been profiled during berry development. Classes of genes with waves of expression at the pre-veraison, veraison/mid-ripening and late-ripening phase have been described during different vintages and in different varieties. Genes whose expression could be associated with a particular viticulture practice, were also identified, as well as genes with expression profile related to specific GxE interaction, allowing to better understand how grapevine respond to diverse environments. Furthermore, different transcriptomic data mining approaches revealed that the striking metabolic transition characterizing the onset of ripening, is associated with a profound transcriptome rearrangement and that a small set of genes, so-called switch genes, probably encode key regulators of this transition. Various classes of transcriptional regulators (NAC, WRKY, bHLH and MADS-box families) were found to be the most important effectors of the switch to ripening. An overview of the diverse putative regulators will be presented, with some examples of preliminary results of their functional characterization.

**Keywords:** berry ripening, key regulators, NAC genes, quality, transcriptome



## Can transcriptomics shed light on the "old-vine" character of wines ?

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In South Africa there is a newfound interest in old vines and vineyards, and the exceptional wines produced from them. These wines are generally accepted as having more depth and complexity than young-vineyard wines, thus the term “old vine” tends to be used on wine labels as an indication of a superior, high-quality wine. However, there is only anecdotal evidence that these wines are truly of higher standard. This study is the first scientific research into the so-called “old-vine” wine character, aiming to determine any significant differences in gene expression in leaves and berries of young and old clonal vines, at the time of harvest. Gene expression of 40-year old and 7-year old vines, growing in a commercial Pinotage vineyard, and used for the production of such premium wines, were analysed as the first steps to elucidate the origins of the old-vine character. RNA-seq allowed for the identification of 925 genes differentially expressed between young and old vines. Many of these genes are involved in metabolic pathways active during fruit ripening. A general trend was observed towards delayed berry ripening in old vines. Berries of these vines also had a lower sugar concentration and higher titratable acids at the time of harvest compared to young-vine berries. Collectively, these results would suggest that berries of old vines take longer to ripen, possibly allowing for the accumulation of volatile aromas that influence berry flavour.

**Keywords:** "old-vine" character, ripening, transcriptome sequencing

## Diversity of condensed tannins in a large collection of Vitaceae

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Condensed tannins or proanthocyanidins (PA) are important oenological traits of berries, owing to their role on colloidal stability, oxydo-reduction reactions and astringency in wine. It can therefore be hypothesized that their composition underwent selection during both domestication and modern breeding.

The present study aims to analyze the diversity of PA composition within Vitaceae and particularly within *Vitis vinifera* L. Thanks to international collaborations and the contribution of the Vassal-Montpellier Grapevine Biological Resource Center (INRA, France), pericarps were collected on 249 genotypes (of which 112 belonged to *V. v. subsp. sativa* and 66 to *V. v. subsp. sylvestris*) at the pre-veraison stage, once tannin accumulation is complete. After elimination of seeds, condensed tannins were depolymerized by mercaptolysis and their sub-unit composition determined by UPLC. All usual flavan-3-ol constitutive units described in Tracheophyta were found with large relative variation within and among Vitaceae, with *Cyphostemma* exhibiting the simplest structure (homopolymers of non-galloylated epicatechin [EC]), and *Nekemias* the highest trihydroxylation and galloylation ratios. Many Asian species do not synthesize epigallocatechin [EGC] and its galloylated form [EGCG], but produce both forms of EC.

Modern cultivated grapes clustered in a tight group with little variation around 20% of EGC+EGCG, while this percentage was higher in *V. v. subsp. sylvestris*. For this trait, some old traditional cultivars could not be distinguished from *V. v. subsp. sylvestris*, suggesting that the trihydroxylation ratio could have been counter-selected for oenological and taste reasons.

The diversity of PA composition displayed an East-West gradient, with more diversity in the East-Mediterranean and Caucasus region, similarly to what observed for genetic diversity. The analysis of phenotype-genotype relationships is under way with the objective to explore the heritability of tannins and their potential links with domestication patterns.

**Keywords:** diversity, domestication, genetics, proanthocyanidin subunits composition

**Berry skin development in wild grapevine (*Vitis vinifera* L ssp *sylvestris*): distinct patterns of gene expression**

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Dynamic changes in *Vitis vinifera* L ssp *sativa* during grape berry development are well characterized but little is known about it in *Vitis vinifera* ssp *sylvestris*. We analyzed 126 wild accessions in order to measure their phenolic compounds contents (anthocyanins and flavonols). Following data analysis, samples of two berry developmental stages (véraison and full ripening) with extreme profiles were collected from wild and the cultivated grapevine “Monastrell”. We performed RNAseq analysis to evaluate their expression patterns. Some functional gene categories were differentially regulated in wild berries vs cultivated one. The most statistically significant categories were related with photosynthesis, cellular wall development, phenylpropanoid pathway and stress-related transcriptional factors. Differential expression in secondary metabolisms between wild and cultivar berries were confirmed by qPCR including 5 cultivar and 5 wild accessions selected according their anthocyanins and flavonols profiles. Samples were obtained from 15 days after anthesis to 60 days after véraison. The qPCR data confirm the transcriptional patterns obtained in RNAseq analysis and showed that *MYBA1*, *MYBA2*, *MYBF*, *F3'H*, *F3'5'H*, *UFGT*, *OMT*, and *FLS* transcripts are in some cases correlated with the characteristic anthocyanin and flavonol profiles in wild berry skin. These results reveal a unique pattern of transcription and biosynthesis pathways regulation underlying the enological characteristics of wild grape. In addition, the expression profiles of stress-related genes showed a specific dynamic modulation during berry development in wild berries. These results provide new knowledge on the distinct chemistry and characteristics of wild berry development.

**Key words:** berry skin, wild grapevine

## Clone-specific transcript profiling of 'Nebbiolo' grape berries unveils environmental responses mediated by sugar and secondary metabolite signalling

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During the last years, several research works aimed at uncovering how different grapevine cultivars respond to environmental conditions, but the molecular mechanisms tuning this phenomenon need to be further deepened. In particular, little is known on the molecular processes underlying the interplay between clones of the same cultivar and the environment.

In order to investigate this point, we analyzed the transcriptome of berries collected over the ripening period from 3 'Nebbiolo' clones (CVT 71, CVT 423, CVT 185, whose respective genome sequences have recently been released) grown in three different vineyards of the Langhe area (Piedmont, North-West Italy), in two vegetative seasons. Transcriptomic data obtained through RNA-sequencing analysis were integrated by: i) real-time RT-qPCR of candidate genes; ii) HPLC quantification of abscisic acid, flavonoid and stilbenoid contents; iii) agronomical parameters; and iv) climatic conditions. This multidisciplinary approach was instrumental for exploring the whole complexity of the genotype-environment interaction and identifying the molecular changes controlled by clone, vineyard, phenological phase or a combination of them.

The results showed that transcript categories associated to sugar mediated-signalling, anthocyanin biosynthesis and transport can be differently modulated among diverse clones of the same cultivar, thus influencing berry quality and agronomical features. Conversely, genes involved in the production of secondary metabolites, typically playing a function in stress response, such as stilbene synthase encoding-genes, were significantly affected by the vineyard location, consistently with the accumulation patterns of different stilbenoids.

We thus demonstrated that clone-specific molecular responses exert a key role in determining the agronomic performances of a grapevine variety in different environments. These results provide valuable indications also for directing viticulture practices aimed at enhancing the typicality of grapevine productions in light of both the cultivation area and clone choice.

**Keywords:** candidate gene expression, Nebbiolo clones, RNA-seq, secondary metabolism, sugar metabolism

**Grape color variation involves genetic and micro-environmental changes that alter berry phenolic and aromatic composition**

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Black- and white-berried grapevine cultivars are distinguished by their ability to accumulate anthocyanins in the berry skin. To assess possible side-effects of color variation in berry development and composition in a near-isogenic background we compared white-berried somatic variants (WV) to their black berry ancestors in Garnacha and Tempranillo cultivars. Absence of anthocyanins correlated with lower berry temperature at daytimes in WV. At transcriptome level, besides genes related to anthocyanin accumulation, transcripts encoding enzymes involved in the biosynthesis and modification of flavonoid backbone were down-regulated from veraison in WV skin. Genes mapping on hemizygous genome regions in WV were down-regulated as well irrespective of berry tissue or developmental stage. Light-responsive genes including flavonol and monoterpenoid biosynthesis genes were up-regulated in WV from veraison. In agreement, flavonol partitioning was altered and tri-hydroxylated forms were practically absent in WV, whereas higher levels of specific volatile monoterpenoids and their soluble precursors were detected in WV pericarp. Interestingly, levels of the stress-related GABA were lower in WV skin, whereas Phe and Tyr precursors of phenolic compounds tended to be higher in WV pericarp. Greater differences were observed in Tempranillo than in Garnacha evidencing genetic background-dependent effects. These results indicate that the grape color locus directly controls the metabolism of colorless flavonoids, whereas additional alterations in grape quality compounds are established in a cultivar-dependent manner in response to alteration of the berry microclimate caused by the absence of anthocyanins.

**Keywords:** berry composition, berry microclimate, grape color, somatic variation, transcriptome

**Genomic and transcriptomic analyses of the 'Concord' grape revealed a novel molecular mechanism in the regulation of a 'foxy' flavor gene in *Vitis* species**

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The 'Concord' grape, derived from the species *Vitis labrusca* is the leading grape cultivar for juice production with more than 450.000 tons/year produced in the United States, due to its unique aromatic flavor, excellent productivity and good nutritional properties. Methyl anthranilate is one of the main compounds contributing to the characteristic 'foxy' aroma of 'Concord' berries, and its accumulation is catalyzed by an AMAT gene. To understand the genetic and molecular mechanisms controlling this and other important traits in *Vitis*, we have developed a draft genome sequence for 'Concord' and compared genomic and transcriptomic variation between 'Concord' and the reference genome of *V. vinifera* 'PN40024', especially focusing on fruit-enriched genes including AMAT. Further, we investigated genomic and transcriptomic variation of the AMAT gene for 46 *Vitis* genotypes, including *V. labrusca*, *V. vinifera* and other species and identified a novel transposon-in-transposon-based mechanism likely responsible for the regulation of the AMAT gene expression in *Vitis* species.

**Keywords:** AMAT, Concord, 'foxy' flavor, genome sequencing, transposon

## Genetic analysis of grapevine secondary metabolism using non-targeted metabolomics

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Grapevine secondary metabolism plays major roles in many important traits in viticulture, especially disease resistance and wine quality. However, knowledge about the genetic determinism of grape secondary metabolism is still scarce.

In this work, we have used a non-targeted metabolomic approach to perform a global metabolic quantitative trait loci (mQTL) analysis with a progeny from a cross between Riesling (RI) and Gewurztraminer (GW).

High performance liquid chromatography coupled to mass spectrometry was performed both on berries and leaves samples, showing genetic variability for 4216 ions corresponding to molecules from different families such as phenolic and isoprenoid compounds.

High-density genetic maps, based on single nucleotide polymorphism (SNPs), allowed the detection of 3992 significant mQTL for berries samples and 706 mQTL for leaves samples. Beside known QTL for the synthesis of terpenols, this approach allowed the detection of new QTLs for glycosylated terpenols and norisoprenoids, as well as the identification of relevant candidate genes.

This work illustrates how non-targeted metabolomics can be applied to global mQTL analysis and we will present some of the newly detected QTL. Characterization of major genes involved in grapevine secondary metabolism will allow the development of molecular markers for a more efficient selection for metabolic traits in grapevine breeding programs

**Keywords :** QTL, secondary metabolism

## **Session 8: Breeding and adaptation to abiotic stress**



## Grapevine adaptation to abiotic stresses: an overview

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Adaptation to abiotic stresses is a complex and challenging biological issue for a geneticist, especially for perennial plants such as grapevine. According to Copper and Hammer (1996), adaptation is both a “status” and a “process”. The “status” characterizes a genotype with a specific combination of alleles which allows the plant to survive and perform well in a specific environment. The adapted phenotype may result from a constitutive expression of the genotype or from genotype x environment interactions, in other words from the plasticity of traits. This modification of phenotype is referred to as acclimation. Physiological changes underlying acclimation occur over the short-term or over the life-cycle length of the individual. The “process” of adaptation will result from the new combination of alleles over generations to obtain a phenotype better able to survive or grow under abiotic constraints. By definition, the “process” time-scale is the reproductive cycle length. In addition for a crop, an adapted phenotype will include survival, yield maintenance, and especially for grapevine, optimal fruit composition. Considering the economic importance of grapevine, the ongoing and expected climate changes make the issue of adaptation even more challenging. Given the numerous environments where this plant can be found and the huge intra and interspecific diversity, we can assume that the grapevine genome bears many alleles which could be mobilized to “adapt” this crop and maintain its sustainability. The challenge is to identify these alleles and understand how they can be leveraged in manipulating the phenotype. The diversity of abiotic constraints (thermal stress, drought, salinity, mineral deficiency, etc...) and their characteristics in terms of their timing, duration, and intensity need to be taken into account. On the plant side, the traits underlying adaptation and the stage of sensitivity should be clearly defined. Targeted traits are often complex and under the control of various genetic mechanisms. Over the past ten years, there have been numerous achievements in grapevine in genome sequencing, phenotyping, genetic architecture analyses, gene identification, and modeling. Thanks to this new knowledge and technologies, our understanding of adaptation to abiotic stresses has improved and can now be used to screen for particular behaviors in existing germplasms or to breed new ones. An overview of the work performed in France over the past years aimed at adaptation to temperature and drought will be presented.

**Keywords:** abiotic stress, berry composition, drought, heat stress, phenology, rootstocks

**Identification of loci and genes responsible for sodium and chloride ion exclusion in grapevine rootstocks for use in marker assisted selection**

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Soil salinity is an important issue to the Australian wine industry, particularly in viticultural areas that face a diminishing and/or expensive supply of high quality irrigation water. When grown on saline soils traditional *Vitis vinifera* winegrape cultivars can suffer from decreased growth and yield, and reduced berry quality due to the high accumulation of chloride ions in particular and to a lesser degree sodium ions. Fortunately, some rootstocks derived from North American *Vitis* species are capable of enhanced ion exclusion, whereby the translocation of these ions from the roots to the leaves and berries is reduced, resulting in increased salt tolerance. A current aim of CSIRO's rootstock breeding program is to identify genes responsible for these key salt tolerance traits and to incorporate this knowledge into a marker-assisted selection breeding program.

This presentation will focus on recent work that has led to the identification of a major locus and underlying gene associated with 70% of the variation of sodium exclusion in a complex interspecific rootstock family. The characterization and genetic nature of four unique alleles, their species of origin, and implications for breeding work will be discussed.

The development of a rapid seedling screen and its use in the discovery and fine mapping of a locus linked with chloride exclusion in a segregating *V. berlandieri* x *V. vinifera* backcross F2 family will also be shared.

**Keywords:** ion exclusion, marker-assisted selection, rootstock, salt tolerance

# **In grafted grapevines, physiological, transcriptional and hormonal responses to nutrient availability are strongly influenced by the rootstock genetic background**

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Grafting is widely used in fruit species and vegetable crops to facilitate asexual production of plants, to enhance disease resistance, abiotic stress tolerance or increase yield. In Europe, grapevine is mainly cultivated grafted to facilitate growth in soil infected with the Phylloxera. In addition, it is well known that the rootstock has a profound impact on several other agronomic traits such as scion growth and fruit quality. For grape growers, to select a rootstock adapted to the pedoclimatic conditions of its vineyard and respect the equilibrium between vegetative development and fruit yield are critical parameters regarding the economic importance of the production of high quality berries. In this context, it is crucial to understand the interactions established between the scion and the rootstock and particularly, how different rootstock genetic backgrounds can modulate the development of a grafted plant. Since it is one of the most important factors ensuring plant growth and fruit production, nutrient availability was studied by modifying nitrogen (N) or phosphorus (P) availability. To study the response to N supply modifications, a transcriptomic analysis (RNAseq) was performed on grapevine combinations cultivated in a split-root system. Important differences depending on the rootstock were highlighted as well as the importance of the hormone signaling through the strigolactone pathway. The effect of P supply modifications was also monitored through RNAseq and revealed, as expected, interesting differences in the transcriptional response. In both cases, the choice of the rootstock led to striking differences in the plant adaptive strategies. Altogether, these results confirmed that the intrinsic properties of each rootstock are able to refine the whole plant behaviour in response to N or P availability. Further work is needed to better understand scion-rootstock interactions in response to nutrient availability and the context of the climate change highlight the importance of such investigations.

**Keywords:** grafting, grapevine, nitrogen, phosphorus, strigolactones, transcriptomics

## Phenotypic deconstruction of dormant bud winter hardiness in grapevine

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Low winter temperatures and spring frosts threaten the sustainability of grapevine production in many cool climate regions. Grapevine winter survival is a set of complex and interconnected traits incorporating temperature and daylength perception, acclimation to low temperature, dormancy, and deacclimation in response to warm temperatures. Unambiguous phenotypes to describe cold hardiness are essential to begin determining the genetic architecture of these traits and to uncover genetic markers for breeding and improvement. Through field studies of wild and cultivated grapevines and growth chamber experiments, we have begun to elucidate the various phenotypes that encompass winter survival. Statistical modeling of lethal temperature curves across multiple years reveals significant differences in the temperature responsiveness among wild species. Screening the northern wild grapevine, *Vitis riparia*, for variation in cold hardiness suggests that cold hardiness is determined long before freezing temperatures occur but is modulated by environmental variation each year. Additionally, use of low temperature exotherms to measure loss of cold hardiness (supercooling) in controlled conditions uncovered a link between deacclimation rates and chilling requirement. Measuring the loss of supercooling ability as a function of temperature and chilling hour exposure produces a specific deacclimation potential during winter. This relationship is genotype specific for *V. vinifera* and wild grapevine species and suggests that deacclimation, chilling, and budburst synchronicity are linked and predictable. These genotype specific parameters can now be used to accelerate phenotyping of mapping families, marker development, and model prediction.

**Keywords:** acclimation, cold hardiness, deacclimation, dormancy, phenotype

## Global proteome analyses of phosphorylation and lysine acetylation reveal new insight into alternative splicing, photosynthesis and HSPs in grape response to heat

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Heat stress represents one of the most serious environmental factors that significantly limit the growth and productivity of plants. Grape is one of the most important crops worldwide, its quality and yield are often constrained by heat stress. Based on analyzing proteomic and transcriptomic changes in grape leaf under four different temperature regimes (25°C, 35°C, 40°C and 45°C) in a previous study (Jiang et al., 2017), we further conducted phosphoproteome and acetylproteome analysis on the same materials. The result showed that 1124 peptides with a significantly changed phosphorylation levels and 241 peptides with a significantly changed lysine acetylation levels were screened out under 35°C, 40°C and 45°C compared with 25°C. Moreover, these changes mainly appeared under 40°C and 45°C. After functional classification and enrichment analysis, we found that phosphorylation rather than acetylation of spliceosome components and ser/arg (SR) -rich splicing factors was involved in up-regulation of alternative splicing of genes, which explains to some extent why alternative splicing events occurred more frequently during grape leaf response to high temperatures in the previous study. Moreover, these results suggested that acetylation modification modulated more photosynthesis proteins and was more sensitive to high temperatures than phosphorylation modification. Especially, a large set of heat shock proteins (HSPs) underwent phosphorylation and acetylation modification, which indicated that HSPs are involved in heat tolerance in grape leaf not only through transcriptional and protein level but also phosphorylation and acetylation modifications. Integration of the two omic data showed 18 proteins overlapped between significantly changed phosphorylation and acetylation levels, indicating that the crosstalk was very important in grape leaf response to high temperatures, and acetylation may be a balance pathway to phosphorylation for protein activity. Therefore, for the proteins involved in different biology pathways in grape leaf, phosphorylation and acetylation modification plays a role at different levels. To our knowledge, the results provide the first evidence for plant leaf responses to high temperature in terms of phosphorylation and acetylation modifications.

**Keywords:** acetylation, grape, heat, phosphorylation

## **Session 9: Breeding and adaptation to biotic stress**

## A perspective on breeding and implementing durable powdery mildew resistance

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A strong international effort has resulted in the discovery of a dozen powdery mildew resistance loci in grapevine, and their introgression into cultivated grapevines could have multi-billion Euro economic impact. However, some of the resistance loci have minor effects and most have been shown to be race-specific. One approach to combat virulent pathogen strains overcoming race-specific resistance is to stack multiple resistance genes in a single cultivar, but this creates challenges for selecting which genes to use, tracking them with DNA markers, and validating the added value of stacked genes. To identify complementary sets of resistance loci, breeders and pathologists on the U.S. VitisGen grape breeding project developed a strategy to evaluate various resistance gene stacks against phenotypically diverse isolates of powdery mildew (*Erysiphe necator*) collected from their center of origin. Leveraging the pathogen genetic diversity provides a crystal ball to predict evolution and selection of virulence after commercialization. To develop these data, we track resistance loci using AmpSeq, which uses highly multiplexed Illumina sequencing of PCR amplicons. We phenotype leaf disc samples using automated imaging and quantification of severity after controlled inoculation, and such methods have good power to detect genetic contributions. However, even if this approach successfully identifies complementary stacked resistance genes and these become widely adopted in commercial cultivars, the pathogen will eventually adapt and win. Thus, we must consider complementary disease management tactics that should be mandated or strongly encouraged to protect this new generation of disease resistant cultivars. With a coordinated effort, we aim to make powdery mildew resistance breeding easy, efficient, and effective, and to provide the viticultural strategies to protect this investment for future generations of grape growers around the world.

**Keywords:** durability, host resistance, marker-assisted breeding, oidium, powdery mildew

**Investigations into the mechanisms of activation of the MrRUN1 and MrRPV1 resistance proteins and the signal transduction pathways leading to resistance to grapevine powdery and downy mildew**

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The two most important pathogens of cultivated grapevines are powdery mildew (*Erysiphe necator*) and downy mildew (*Plasmopora viticola*). Since the appearance of these pathogens on *V. vinifera* winegrape varieties in Europe in the 1800s, grapegrowers all over the world have relied on the frequent application of fungicides to minimize the impact of these pathogens on grape yield and quality. Over the last 10-15 years, a number of different research groups have embarked on marker-assisted breeding programs to develop new powdery and downy mildew-resistant wine and table grape varieties. An important component of the resistance within these new varieties is that conferred by the RUN1/RPV1 locus from the North American grape species *Muscadinia rotundifolia* because not only do the MrRUN1 and MrRPV1 genes at this locus offer strong resistance against their respective pathogens, their close physical proximity means they are inherited as a single dominant locus which offers great advantages from a breeding perspective. However, despite the widespread use of the RUN1/RPV1 locus, we still have limited knowledge about the way these two highly homologous TIR-NB-LRR resistance proteins function in terms of pathogen effector recognition, activation and signal transduction leading to the inhibition of pathogen development. This talk will summarise recent results from a multi-institutional investigation into (a) defining and characterising the ‘effectorome’ of *E. necator* and *P. viticola* as a step towards identifying potential avirulence effectors (b) structural and functional analysis of the TIR signalling domains of MrRUN1 and MrRPV1 and (c) identifying signal transduction pathways involved in mediating effector-triggered immunity following R protein activation.

**Keywords:** downy mildew, effectors, *Erysiphe necator*, *Plasmopora viticola*, powdery mildew



**Organization, diversity, expression and evolutionary dynamics of the NB resistance gene family in grapevine and related species**

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While the cultivated grapevine is susceptible to numerous diseases causing important economic loss in viticulture, breeding of varieties based on introgression of resistance genes derived from wild *Vitis* species in an alternative to pesticides. Most of these genetic factors are located in regions rich in resistance gene analogs. Among them, the NB genes, which encode for proteins containing a nucleotide binding (NB) domain, constitute the largest group. In grapevine, the organization, the diversity and the evolution dynamics of resistance genes is still incompletely described, even if a reference sequence of the genome is available. Our study aimed at producing an exhaustive annotation of the NB resistant genes in the reference grapevine genome. Eight hundred and twenty-nine genes were predicted, out of them 450 displaying a canonical structure. The NB genes are unevenly distributed along the 19 grapevine chromosomes, the majority of them being organized into 122 physical clusters. The expression analysis revealed that NB genes belonging to the same cluster are preferentially co-expressed. The analysis of diversity of the NB gene family through presence/absence variations and CNV (Copy Number Variation) revealed a high conservation of the gene clusters in the whole *Vitis* genus. More, according to the CNV detection, the majority of the NB genes displayed a strong divergence at the sequence level putatively due to mutation accumulations, truncation or hemizyosity. Altogether, these results suggest that the evolution of the NB gene family mainly occurs through tandem duplications leading to co-expressed genes grouped in physical clusters, which are ideal structures to trigger efficient responses against pathogen attacks. Nevertheless, TIR-NB-LRR and CC-NB-LRR sub-families exhibit two different evolutionary histories putatively leading to contrasted roles.

**Keywords:** CNV, evolution, NB genes, resistance genes

## Durable powdery mildew resistance in grapevines: myth or reality

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Grape powdery mildew is an economically important disease worldwide that necessitates a high input of fungicides for successful grape production. Development of varieties with resistance from multiple backgrounds is an important alternative strategy to control this disease. This strategy requires (a) the identification of sources of resistance and genetic marker development, (b) characterization of resistance mechanisms, (c) marker assisted breeding to introduce the selected loci into elite varieties, and (d) an understanding of pathogen biology and response to different resistance sources. In this study, we have identified and tagged novel sources of resistance from eight genetic backgrounds including *M. rotundifolia*, two Chinese species (*V. piasezkii* and *V. romanetii*), and a wide array of *V. vinifera* cultivars and *V. sylvestris* from Eurasia. We use tightly linked genetic markers to different loci within weeks of germination to select seedlings with the resistance loci. Powdery mildew resistance from two or more genetic backgrounds is combined in single or multiple steps, while maintaining high fruit quality attributes and other important horticultural traits. Resistance is confirmed by careful evaluation for disease development in the field or in the greenhouse and confirmed by an *in vitro* assay. A multi-faceted resistance screen of seedling plants allows for a better assessment of pathogen strains and their interaction with different resistance loci. Results will be presented in regard to the mechanism of resistances from different backgrounds, specificity to different isolates and effectiveness of molecular markers in the breeding program.

**Keywords:** *Erysiphe necator*, gene pyramiding, marker-assisted selection

## The Rpv3-3 locus and stilbenoid induction mediate downy mildew resistance in a grapevine inter-specific population

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The cultivated Eurasian grapevine (*Vitis vinifera* L.) is highly susceptible to downy mildew (DM) – caused by the biotrophic oomycete *Plasmopara viticola* (Berk. & Curt) – the major disease of temperate-humid climates among various pathogen treats. DM control mainly relies on the massive use of fungicides leading to environmental pollution, development of resistance and residual toxicity. The exploitation of DM-resistant wild genetic resources for the development of new resistant varieties represents a promising alternative. Taking advantage of a segregating population derived from Merzling (M, a mid-resistant hybrid) and Teroldego (T, a susceptible landrace), recent studies highlighted the importance of stilbenoids among phenolic compounds in conferring resistance to this oomycete. In order to elucidate the genetic bases of DM resistance and polyphenol biosynthesis upon *P. viticola* infection, 136 M×T F1 individuals were characterized by an integrative approach combining genetic, phenotypic and gene expression data. An improved M×T linkage map was obtained by scoring 192 microsatellite markers. The progeny was further screened for degree of resistance and production of 42 phenolic compounds (including 18 different stilbenoids). QTL mapping showed that DM resistance is associated to a specific haplotype at the Rpv3 locus – herein named Rpv3-3, derived from the French hybrid Seyval – and identified 46 novel metabolic (m)QTLs linked to 30 polyphenol-related parameters. A list of the 76 most relevant candidate genes was generated by specifically exploring the genomic regions underlying the mQTLs associated to the stilbenoids induced by the infection. Finally, the expression analysis of 13 genes in Rpv3-3+/- genotypes, displaying divergent DM resistance and stilbenoid accumulation, revealed significant candidates for the genetic control of stilbenoid biosynthesis and oligomerization. These overall findings emphasized that DM resistance can be mediated by the major Rpv3-3 locus, stilbenoid induction, and their combined protective action.

**Keywords:** marker-assisted breeding, Merzling, *Plasmopara viticola*, polyphenols, QTL analysis, *Vitis* spp.

**The *Plasmopara viticola* candidate effector PvRXLR131 interacts with a plant Brassinosteroid and ERECTA receptor kinases**

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Downy mildew is the most destructive disease of grapevine, causing tremendous economic loss in cultivation and wine industry every year. The causal agent, *Plasmopara viticola*, is an obligate biotrophic oomycete. Oomycete pathogens deploy RXLR effectors as weapons to modulate plant cell processes and conquer immunity for infection. We have predicted 100 RXLR effectors from a *P. viticola* genome, but their biological functions are largely unknown. We report here that one effector PvRXLR131 is a virulence factor that suppresses plant innate immunity to promote pathogen infection. It targets plant BR and ER receptor kinases co-inhibitor, BKI1, which is also the only reported inhibitor of receptor kinases in plants. C-terminal effector domain of PvRXLR131 is responsible for association with BKI1 and phosphorylation of BKI1 in a critical site disrupts their interaction. PvRXLR131-transgenic Arabidopsis displayed a BKI1-overexpression dwarf phenotype, caused by suppression of BR and ER signaling. In addition, BKI1 was essential for PvRXLR131 to fulfill virulence function. We first report that oomycete effector interacts with receptor kinases inhibitor, enhances its inhibitory activity on the molecular switches of BR and ER signaling, leading to altered defense-related signaling pathways, thereby facilitated infection.

**Keywords:** *Plasmopara viticola*, receptor kinases, RXLR effectors

## Differential responsiveness of ATL156 promoters from *Vitis riparia* and *Vitis vinifera* towards defense-related stimuli and transcription factors

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We have previously selected the *Vitis riparia* gene *ATL156* as specifically upregulated in the resistant *V. riparia* upon downy mildew infection [1] and generated stably transformed *V. vinifera* (cv. Shiraz) constitutively expressing *ATL156*. *VviATL156* encodes an E3-ubiquitin ligase, belonging to the ATL gene family [2] and is the closest homolog of Arabidopsis ATL2, which is highly responsive to elicitors and hormones [3]. Accordingly, transgenic grapevines are more resistant towards downy mildew. Genetic engineering of grapevine premium varieties to improve disease resistance can be performed through a cis-genic approach by transferring defence-related genes from resistant wild relatives in their native form. However, to that purpose, the analysis of native promoter responsiveness and of their specific regulation is crucial for the development of efficient resistant traits. In this context, the *VviATL156* regulative regions from both the resistant and the susceptible species were cloned and sequenced. Bioinformatic analyses of core promoter structures and cis-acting element composition revealed some over-represented cis-acting elements in the *V. riparia* promoter, likely related to disease resistance. The promoters were then functionally characterized in stably transformed *A. thaliana* plants, under physiological conditions and in response to hormones and pathogen infection. Moreover, promoter transactivation by specific transcription factors was evaluated in a Dual Luciferase Assay experiment in transiently transformed *Nicotiana benthamiana*. Results showed a stronger transactivation of the *V. riparia* promoter by selected transcription factors belonging to the WRKY family, in comparison to the *V. vinifera* promoter. The link between ATL156 and WRKY TFs in grapevine was also supported by gene co-expression network built across a number of defense-related transcriptomic experiments [4].

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**Keywords:** *Arabidopsis thaliana*, *Plasmopara viticola*, resistance, RING-H2 zinc fingers, ubiquitination

## Subtilisin-like proteins and lipid signaling events: the missing links in grapevine resistance to *P. viticola*

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The reduction of phytochemicals used to control pests and diseases is one of modern agriculture's demands. Grapevine is one of the most important crops grown in temperate climates where Europe's wine industry represents 40% of the world production. The cultivated grapevine, *Vitis vinifera* is prone to several diseases, with downy mildew being one of the most devastating. Preventive fungicide applications are used during each growing season to control disease incidence with major environmental and economic constraints. A deeper knowledge on the grapevine-*P. viticola* interaction is needed to define alternative disease control strategies.

A systems biology approach, based on 'OMIC technologies, allowed the identification of lipid-associated signaling mechanisms and subtilisin-like proteases as key players for the establishment of the incompatible interaction. We have shown that during the first h of interaction with *P. viticola*, the modulation of chloroplast associated lipids is important for photosynthetic machinery protection and jasmonic acid (JA) biosynthesis. We have also identified subtilisin-like proteases as strong resistance-associated candidates. We have performed a characterization of the grapevine subtilase gene family and shown that some subtilases increase their expression following *P. viticola* inoculation in the incompatible interaction. Moreover, one particular subtilase *VviSBT4.19* presents sequence homology to tomato and Arabidopsis subtilases that participate in innate immunity activation. Our results also suggest a link between the activation of these subtilases and JA. A deeper knowledge on these players may give clues that allow developing new breeding strategies leading to a pesticide-free sustainable viticulture.

**Keywords:** grapevine resistance, immune priming, jasmonic acid, lipid signaling, *P. viticola*, subtilases

**Acknowledgments:** This work was supported by projects PEst-OE/BIA/UI4046/2014, PEst-OE/QUI/UI0612/2013, UID/MULTI/00612/2013, investigator FCT program (IF/00819/2015) from Fundao para a Cincia e Tecnologia (FCT/MCTES/PIDDAC, Portugal).

## The molecular dialogue between grapevine inflorescence/berry and *Botrytis cinerea* during initial, quiescent, and egression infection stages

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Grapes quality and yield are affected by bunch rot disease caused by the necrotrophic fungus *Botrytis cinerea*. Primary infections by airborne conidia often occur at blooming, although the fungus remains quiescent until maturity and egresses at ripening, causing bunch rot. The molecular dialogue between *B. cinerea* and grapevine inflorescence/berry from bloom till maturity is not completely elucidated although its comprehension is vital to implement proper management limiting consequent yield losses.

In this study, a molecular characterization of *B. cinerea*-flower/berry interaction was achieved using confocal microscopy and integrating transcriptomic and metabolic analysis of the host and the pathogen. Open flowers from fruiting cuttings of cv. Pinot Noir were infected with GFP labelled *B. cinerea* and samples collected at 24 and 96 h post inoculation (hpi), at 4 weeks post inoculation (wpi), and at 12 wpi were studied. Our results indicated that penetration of the flower epidermis by *B. cinerea* at 24 hpi induced genes encoding virulence factors, representing the effort of the pathogen to invade the host. On the other hand, grapevine flowers responded rapidly involving genes associated with the accumulation of PR proteins, stilbenoids, reactive oxygen species and cell wall reinforcement. In particular, specific pectin methylesterases (PMEs) and PME inhibitors were identified as putative mediators of cell wall integrity maintenance. At 96 hpi the transcriptional reaction appeared largely diminished both in the host and in the pathogen. Afterwards, infected berries continued their developmental program without any visible symptom, although the presence of *B. cinerea* could be ascertained. Nonetheless, both the fungus and the hard-green berries displayed to be transcriptionally active. At 12 wpi, the egressed *B. cinerea* expressed almost all virulence and growth related genes to enable the pathogen to colonize the berries. In response to egression, ripe berries reprogram different defense responses, though futilely.

**Keywords:** *Botrytis cinerea*, berry, cell wall, egression, flower, RNAseq, quiescence

## Contrasting susceptibilities to Flavescence dorée in wild *Vitis* species, *Vitis vinifera* cultivars and progenies suggest segregation of genetic traits involved in disease response

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Flavescence dorée (FD) is a severe epidemic disease of grapevine in Europe caused by FD-phytoplasma (FDp): a small wall-less bacteria transmitted by the leafhopper vector *Scaphoidenus titanus* and classified as a quarantine organism. The mandatory control of the disease consists in eliminating infected grapevines, spraying insecticides against the vector and planting healthy material. Given the economic, environmental and social impacts of such measures, viticulture is awaiting alternatives. After extensive surveys in vineyards, we showed that Cabernet Sauvignon is highly susceptible, with a high proportion of symptomatic branches and high phytoplasma titers, in contrast to Merlot. Localized insect transmissions and grafting experiments showed that phytoplasma circulate in the whole plant in the Cabernet-Sauvignon cultivar, whereas in Merlot they are restricted to the transmission point. Analysis in parentage of Merlot and Cabernet Sauvignon suggested that Merlot inherited its low susceptibility from its maternal genitor, the Magdeleine Noire des Charentes (Mag). This hypothesis was reinforced by preliminary analysis of a reconstructed progeny between the Merlot's parents, Mag and Cabernet Franc. Furthermore, we developed an insect-mediated transmission under high confinement mimicking natural conditions. This allowed the classification of 28 *Vitis* accessions into 3 distinct categories, according to the percentage of infected plants and their phytoplasma titers. In the *Vitis vinifera* cultivars, reduced symptoms, low phytoplasma titers, and low percentages of infected plants were found to be associated. Rootstocks and their *Vitis* sp. parents, although displaying high percentages of infected plants and intermediate to high phytoplasma titers, shared a symptomless response. Thus, rootstocks in mother plants parcels, or when they grow wild nearby vineyards, are a potential and silent reservoir of contamination endangering vineyards. Altogether, our data suggest distribution of genetic traits within the *Vitis* genus involved in insect-mediated phytoplasma transmission, multiplication, circulation and symptom development (Eveillard et al., 2016, Contrasting susceptibilities to Flavescence dorée in *Vitis vinifera*, rootstocks and wild *Vitis* species, *Frontiers in plant science*)

**Keywords:** genetic traits, leafhopper vector, phenotyping, phytoplasmas, quantification, symptoms, transmission



## Control of the grapevine moth *Lobesia botrana* through the genetic engineering manipulation of the host plant's volatiles

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The European grapevine moth *Lobesia botrana* is one of the key pests of grape. The caterpillar feeding activity leads to a direct damage on reproductive plant tissues (flower buds and berries) but also to an indirect damage by promoting secondary infections of microorganisms. Current control systems are based on the use of insecticides or on mating disruption: while the first is not environmentally friendly, the second is not particularly suitable for non-delimited areas, or areas where pest population is high. Previous studies have showed that a synthetic blend of the three terpenoids (E)- $\beta$ -caryophyllene, (E)- $\beta$ -farnesene and (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) was as attractive for the moth as the complete grape odour profile in laboratory conditions. The same studies also showed that the specific ratio of these compounds in the grape bouquet was crucial, because a percentage variation in any of the three volatiles resulted in almost complete inhibition of the blend's attractiveness. Here we report on the creation of stable grapevine transgenic lines, with modified (E)- $\beta$ -caryophyllene and (E)- $\beta$ -farnesene emission and thus with an altered ratio compared to the original plants. When headspace collections from these plants were tested in wind tunnel behavioural assays, they were less attractive than control extracts. This result was confirmed by testing synthetic blends imitating the ratio found on natural and transformed plants, as well as by testing the plants themselves. With this evidence, we suggest that a strategy based on volatile ratio modification may also interfere with the host-finding behaviour of *L. botrana* in the field, creating avenues for new pest control methods

**Keywords:** (E)- $\beta$ -caryophyllene, (E)- $\beta$ -farnesene, host selection, *Lobesia botrana*, sesquiterpenes, transgenic

## Living on the edge: the narrow genetic base of the rootstocks is a serious threat

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Rootstocks have been the foundation of viticulture since the French first noticed phylloxera crawling and feeding upon the root of a *Vitis vinifera* grapevine in the mid 1800s. The phylloxera mediated economic loss to the French and then the European wine economy promoted an extensive amount of research into resistant American *Vitis* species, and quickly led to the introduction of *V. berlandieri*, *V. rupestris* and *V. riparia*. The latter two species were used directly as rootstocks and they were all used as parents to integrate their various attributes and create the rootstocks we use today. There have been limited efforts to develop new rootstocks with alternative genetic backgrounds, and the rootstocks we use today mostly derive from a very narrow genetic base. Multiple outbreaks of soil-borne pests have been reported in the literature including new biotypes of phylloxera and virulent root-knot nematode pathotypes. Such outbreaks are serious threats since today's rootstocks originated from very similar genetic backgrounds and may not provide the genetic diversity necessary to combat evolving soil-borne pests. We are investigating resistance to phylloxera and root-knot nematodes in classic rootstocks, a genetically diverse collection of accessions within the species used to breed these rootstocks, and a wide range of American grape species. In this study we are examining the diversity among resistance sources and are creating genetic maps for phylloxera and root-knot nematode resistance. The outcome of this project will be the utilization of novel resistance sources capable of slowing or preventing the adaptation of pests to their rootstock hosts, and thus contributing to more sustainable viticultural practices.

**Keywords:** phylloxera, root-knot nematode, rootstocks

## **Abstracts for posters**

## Understanding methoxypyrazine production in Marlborough Sauvignon blanc grapes

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Methoxypyrazines (MPs) with their signature herbaceous and vegetative aroma play a pivotal role in determining the distinctive flavour and aroma style of Marlborough Sauvignon blanc wine. Despite their stylistic importance, relatively little is known about MP accumulation in Sauvignon blanc grapes and what influences their production. Several years of vineyard experimentation illustrate that MP accumulation in Sauvignon blanc grapes commences as early as 5 weeks before véraison when berries are the size of “small peas”, and peaks in concentration 2-3 weeks later before declining during and after véraison. Determination of MP content illustrates that concentrations of MPs at harvest are, depending on the circumstances, a function of dilution rather than of net degradation of MPs. Partitioning of MP in the berry shows that although skin has up to 5 times the concentration of MP than found in the pulp, the pulp contains 60% of the MP present in a berry. Berries growing on the outside of grapevine canopy (exposed to sunlight) exhibit a significantly reduced ability to accumulate MPs compared with shaded berries on the inside of the canopy. By harvest time exposed berries have up to 80% less MP. The exposure effect appears to manifest during early berry development (fruitset - pea-size) and occurs independently of grape sugar maturity. Temperature may moderate the accumulation of MP during the véraison period, but changes in light environment during this period appear to have no influence. A family of methyltransferases, regulated by the expression of OMT genes, are responsible for conversion of odourless hydroxypyrazines into aromatic methoxypyrazines. Expression analysis shows that OMT 1 and 2 are not consistently up-regulated in shaded berries, whereas OMT3 is up-regulated in shaded berries before véraison. A lag phase of 1-2 weeks is seen between maximum OMT expression and peak MP concentration.

**Keywords:** accumulation, berry, exposure, methoxypyrazines, methyltransferases, Sauvignon blanc

## Grape bunch heating alters the accumulation and regulation of anthocyanin production in Pinot noir berry skin

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New Zealand's cool climate wine regions, like many others around the world, face the threat of climate change and increasing temperatures. Since colour and phenolic formation in grapes are predominately *in situ* processes, determining the direct effect of heat on bunch phenolic chemistry may be of more relevance than the effects of heat on the vine. Using a modified air delivery system with an in-line heater, Pinot noir bunches were heated for 7 days during véraison to temperatures of 22–40°C, while unheated control bunches on the same vines experienced ambient temperatures of 11–31°C. The elevated temperature range resulted in small increases in berry sugars, reduced berry acidity faster and increased the rate of amino acid accumulation. Total anthocyanin concentration was 25% lower in heated grapes at harvest. The accumulation of malvidin, petunidin and delphinidin-3 glucosides was totally arrested by heating, while peonidin and cyanidin-3 glucosides continued to accumulate, albeit at a lower rate. In a second year of heating only the accumulation of petunidin and delphinidin-3 glucosides was inhibited by heating. The expression of a subset of key anthocyanin structural genes and pathway regulators, including dihydroflavonol-4-reductase (DFR), anthocyanin repressor transcription factor (MYB4) and proanthocyanidin-activating transcription factor (MYB5a), were altered by heating, with some responding quickly (days) and others responding over a longer period of time (weeks). The study predicts that high bunch temperature, even for short periods (7 days), is likely to alter the ripening of Pinot noir grapes and, in the context still table wine production, compromise the phenolic profile of the grape skin.

**Keywords:** anthocyanins, berry, climate change, heat, Pinot noir, ripening

**Acknowledgements:** this work is part of the PFR Wine Research programme, funded by the MBIE Strategic Science Investment Fund (SSIF), delivered by PFR in consultation with New Zealand Winegrowers Inc.

## Modification of transcriptome and main sensory compounds in grape berry adaption to varied ripening initiation timing

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The initiation and duration of grape ripening are very important traits in viticulture as they dramatically affect the quality of harvest-ripe grape berries. To dissect the mechanism underlying the influence of ripening duration on berry quality-related metabolisms, we used abscisic acid (ABA) or  $\alpha$ -naphthaleneacetic acid (NAA) to shorten or extend the time interval between berry phenological periods, and compared the difference in transcriptome and metabolites based on the same phenological stage. The grapes treated with 1000 mg/L ABA (ABA1000) expectedly exhibited shorter intervals from E-L 33 to E-L 35 stage and from E-L 35 to E-L 36 stage compared with the control, whereas the 200 mg/L NAA treatment (NAA200) significantly increased the intervals. Transcriptomic comparisons between the E-L 35 and E-L 33 stage showed that a short ripening duration was associated with the largely up-regulated expression of genes related to berry softening, hydrolysis and transport of sucrose, ABA biosynthesis and signaling, and SA (salicylic acid) signaling in grapes. By contrast, a long ripening duration was related to the down-regulated expression of genes involved in berry softening, sucrose synthase, and degradation of malate, as well as an up-regulated expression of genes related to auxin signaling. As a result of the adaption to varied ripening initiation timing, the grape berries with hormone treatments and control showed a big difference in anthocyanin and volatile compounds biosynthetic metabolisms when they reached the same E-L 35 and E-L 36 stages. *VvF3'H* expression was up-regulated in both ABA1000-treated and NAA200-treated grape berries, accompanied by an increased accumulation of the 3'-substituted anthocyanins. But the 3'5'-substituted anthocyanins were largely reduced in the grapes treated with NAA200, and hardly affected in the ABA1000 grapes when compared to the control. Concerning the biosynthesis of volatile compounds, except that *VvCCD4a* and *VvCCD4b* were up-regulated and the C13-norisopenoids were correspondingly increased in the NAA200 grape berries, other volatile metabolites such as terpenes and C6/ C9 compounds appeared to be independent upon the timing of ripening initiation.

**Keywords:** anthocyanins, grape berry, ripening duration, volatile compounds

**An investigation on natural ice-wine, harvest freezing method and training-pruning techniques in cool climates region of China**

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The trellis, training system, renewing pruning and standardized management technology were systematically studied on ice grapes with regard to fruit falling from trees (isolated freezing) in production caused by soil covering on trees during winter in Huairan, China. The results of a five-year study showed that fruit of ice grape should adopt natural freezing of conjoined fruit in the north region of winter, should adopt trellis cultivation methods, and the implementation of alternate bearing of double line or double vine. Preparatory vine for bearing next year after Renewing pruning is the key technology for natural freezing of conjoined fruit. It is better to keep another alternate vine with short cutting apart from a preparatory vine during soil covering, which can sprout 4-5 d earlier than latent bud from old vines next season. Preserving two preparatory vines during renewing pruning can reduce the thickness of vine shoots and length of internodes. At the same time, two-vine trellis can increase the rate of fruiting-branch, and improve the yield. Double arm hedgerow or double "V" type canopy adopted for natural freezing of conjoined fruit in cultivation of ice grape, can yield 669.7 kg/667m<sup>2</sup> with little effect on the main components of fruit juice and original wine. The comprehensive technology studied for natural freezing of conjoined fruit strictly implement international ice grape production standards.

**Keywords:** ice grape, natural freezing of conjoined fruit, production standards

## Whole genome sequencing and gene annotation of Georgian grape cultivars

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The genomes of four Georgian grape cultivars—Chkhaveri, Saperavi, Meskhetian green, and Rkatsiteli, belonging to different haplogroups, were resequenced. The shotgun genomic libraries of grape cultivars were sequenced on an Illumina HiSeq. Pinot Noir nuclear, mitochondrial, and chloroplast DNA were used as reference. Mitochondrial DNA of Chkhaveri closely matches that of Pinot noir mitochondrial DNA, which is a member of the same haplogroup. Phylogenetic relationship among the grape cultivars obtained by mtDNA sequence analysis is in good agreement with our previous results, which were observed by complete plastid DNA sequencing, where chloroplast DNA sequence of *Vitis vinifera* cultivars revealed a high level of identity among the members of the same haplogroup. Unlike mtDNA, Pinot noir chromosomal DNA is closer to the Meskhetian green than to other cultivars. Substantial differences in the number of SNPs in mitochondrial and nuclear DNA of Chkhaveri and Pinot noir cultivars are explained by backcrossing or introgression of their wild predecessors before or during the process of domestication. Annotation of chromosomal DNA of Georgian grape cultivars by MEGANTE, a web-based annotation system, shows 66,745 predicted genes (Chkhaveri—17,409; Saperavi—17,021; Meskhetian green—18,355; and Rkatsiteli—13,960). Among them, 106 predicted genes and 43 pseudogenes of terpene synthase genes were found in chromosomes 12, 18 random (18R), and 19. Four novel TPS genes not present in reference Pinot noir DNA were detected. This work performs the first attempt of comparative whole genome analysis in different haplogroups of *Vitis vinifera* cultivars. Based on complete nuclear and mitochondrial DNA sequence analysis, hypothetical phylogeny scheme of formation of grape cultivars is presented.

**Keywords:** chloroplast DNA, Illumina, mitochondrial DNA, nuclear DNA, sequencing, SNP



## Growth and development of Pierce's disease tolerant hybrid bunch grapes in southeastern U.S

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In the humid subtropical climate of southeastern region of U.S., commercial cultivation of European (*V. vinifera* L.) and hybrid bunchgrapes has largely been prevented by the presence of Pierce's Disease (PD), a bacterial infection caused by *Xylella fastidiosa*, a gram negative bacterium endemic to the region. Consequently, only PD resistant or tolerant grape cultivars could be currently sustainably grown in the region. An experimental vineyard was established at the Sand Mountain Research and Extension Center, Crossville, AL in 2007 with a main objective to evaluate the vegetative characteristics and the cropping potential of 10 PD tolerant hybrid bunch grape cultivars. The experiment was a randomized complete block design with 4 replications. Data collection was generated to evaluate vine vigor, yield potential, fruit quality and foliar disease resistance of selected PD tolerant American and French-American hybrid bunch grape cultivars including 'Black Spanish', 'Blanc du Bois', 'Champanel', 'Conquistador', 'Cynthiana', 'Favorite', 'Lake Emerald', 'Seyval Blanc', 'Stover', and 'Villard Blanc'. Based on 2011-2017 results for cumulative yield per vine it was found out the most productive cultivars in our environment were 'Villard Blanc', 'Favorite', and 'Black Spanish' (yielding 104.3, 96.2, and 79.8 kg/vine respectively), while 'Conquistador' was the least productive with 34 kg/vine. 'Blanc du Bois' also produced high yields (12.4, 12.8 and 15.4 kg/vine) during the last three seasons. 'Villard Blanc' had the largest fruit clusters throughout the study period (194.6 g), while 'Champanel' consistently produced the largest berries (4.2 g), followed by 'Blanc du Bois', 'Villard Blanc', and 'Stover'. 'Champanel' and 'Cynthiana' were highly resistant to berry rot diseases. Based on their overall performance 'Villard Blanc', 'Black Spanish' and 'Blanc du Bois' are considered suitable for sustainable commercial grape production in Alabama and the Southeast.

**Keywords:** biotic stress, plant growth, subtropical

## Development of an efficient DNA marker system for predicting skin color in grape

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Recently, poor coloration of grape berry skin has become a common problem caused by high temperatures during the maturation stages in regions with a warm climate. Because of the commercial importance of grape, it is relevant to understand how grape coloration is affected by genetic factors, as this knowledge will contribute to more stable production of well-pigmented grapes despite global atmospheric warming. Our recent genetic studies of grape skin color have revealed that the MYB haplotype composition at the color locus is the major genetic determinant of the anthocyanin content and composition in grape berry skin. Using this knowledge, we developed an effective DNA marker linked to skin color in grape. It enabled us to predict the skin color of grapes from very young seedlings by examining the MYB haplotype composition. For example, identifying seedlings that are homozygous in functional MYB haplotypes by means of PCR let us select accessions that will contain a high quantity of anthocyanins in the grape berry skin. In addition, identifying seedlings that contain Hap B or Hap C-Rs let us select accessions that have an attractive red color and that predominantly contain di-hydroxylated non-methylated anthocyanins in the skin. Detailed process of our DNA marker system will be demonstrated in this study.

**Keywords:** anthocyanins, genotype, global warming, marker-assisted selection, skin color

# **Identification of downy mildew resistance genes *Rpv10* and *Rpv3* by DNA-marker analysis in Russian grapevine germplasm collection**

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The largest Russian grapevine germplasm collection contains over 4000 accessions and belongs to the North-Caucasian federal scientific center of horticulture, viticulture and winemaking. More than 70% of samples are local cultivars from different regions of viticulture. Interspecific cultivars compose 23.4% of the collection, mostly hybrids *V. vinifera* L. x *V. amurensis* Rupr., and there are series of genotypes, combining high fruit quality with resistance to diseases, pests and freezing, which were bred using interspecific hybrids Seyve Villard. Study of cultivars and search for donors of valuable traits are conducted, including the use of molecular genetic methods. Downy mildew is one of the most common fungal diseases of the vine, caused by *Plasmopara viticola*. Current work is focused on the search for donors of genes of resistance to downy mildew *Rpv10* (inherited from *V. amurensis* Rupr.) and *Rpv3* (originating from the North American species) according to DNA marker analysis data. Most of the studied genotypes are inter-species varieties of Russian breeding. Cultivars with known identified allelic status of the studied genes have been included in the study as reference genotypes. To determine *Rpv10* gene we used a closely linked microsatellite marker GF 09-46 (Schwander et al., 2012). The gene *Rpv10* was found in 9 out of 41 analyzed genotypes. Gene *Rpv3* has 7 haplotypes of resistance, which can be identified by DNA-markers UDV305, UDV737 (Di Gaspero et al., 2012). To identify the gene *Rpv3*, 55 grapevine genotypes were analyzed by these markers. *Rpv3* was detected in 19 cultivars; haplotypes *Rpv3*299-279, *Rpv3*321-312 and *Rpv3*null-271 were found.

**Keywords:** DNA-markers, resistance to downy mildew, *Rpv10*, *Rpv3*

**Acknowledgements:** the Russian Foundation for basic research and administration of Krasnodar region financially supported the study (grant № 16-44-230314 r\_a).

## **Influence of rootstocks on maturation and productivity of interspecific hybrids of white wine grapes**

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Knowledge on the evolution of grape maturation is essential to harvest in the ideal time for winemaking. Several factors influence the maturation such as climate, vintage, canopy genotypes and the interaction between canopy variety and rootstock. Thus, the present work aimed to evaluate the influence of rootstocks on maturation evolution and productivity of five interspecific hybrids of white grape for winemaking. The experiment was carried out in Jundiaí, SP (23° 06 'S and 46° 55' O, with in altitude of 745 m) and the treatments consisted of the combination of SR 0.501-17, IAC 21-14 Madalena, Moscatel de Jundiaí, Moscato Embrapa and BRS Lorena grafted onto rootstocks 'IAC 766 Campinas' and 'IAC 572 Jales'. The experimental design applied was random blocks with five repetitions and each experimental plot was composed of five plants. The evolution of grape maturation was monitored from 'veraison', when the soluble solids content (SS), pH, titratable acidity (AT) were determined weekly and the maturation indexes (IM) were calculated, and at the time of harvest, the productivity in ton.ha<sup>-1</sup> was estimated. Statistical analyzes were performed using the SISVAR software and the comparison of means for productivity and regression adjustment for the maturation variables were performed. In conclusion, the rootstocks did not influence the chemical variables evaluated in maturation evolution for the genotypes studied. In addition, for productivity, there were no significant differences among the evaluated hybrids, but when grafted on IAC 766 the plants were more productive than on IAC 572.

**Keywords:** brazilian grape cultivars, maturation index, productivity

## Molecular characterization of the downy mildew resistance in the table grape Lasta

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Nowadays, the value of the table grape production is increasing and Italy is one of the main producers in Europe and in the world. Despite this, there are not many high quality cultivars with resistance to powdery and/or downy mildews. 'Lasta' is a table grape cultivar characterized by a medium dense bunch and oval green yellow berries. It was obtained in Serbia by crossing Muscat de St. Vallier and Lyana, which are both table grapes, and does not seem susceptible to *Plasmopora viticola*. The source of this resistance is still unclear: the pedigree of 'Lasta' shows that it could derive from *Vitis rupestris* and 'Seibel 4614'; the screening with the SSRs markers usually used for the detection of the known resistance genes did not give the allelic profile expected but one similar to some *V. vinifera* cultivars. For this reason, other markers, available for the locus Rpv3, were used to analyze more strictly the region of interest and verify the presence of a different haplotype of the gene. Furthermore, the progeny of the controlled cross 'Picolit x Lasta' was obtained to generate a map using different types of markers, such as microsatellites, already known to map the 19 linkage groups of grape, and SNPs, detected by ddRAD technique. The progenies were also tested in a greenhouse experiment for resistance/susceptibility using leaf discs sprayed with a suspension of the pathogen. This phenotypic data were used to detect possible QTL associated to the resistance trait.

**Keywords:** breeding, genetic mapping, molecular markers, *Plasmopara viticola*

## Evaluation of new grape selections with introgressed resistance genes

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*Vitis vinifera* grape varieties are used for the production of high quality wines and table grapes, but they are susceptible to a number of diseases caused by pathogens, mainly fungi and viruses. Early attempts to introduce resistant cultivars, derived from hybrids between European and American genotypes, provided an alternative to chemical protection but failed in the wine market. In the last decades, new resistant cultivars entered the market responding to the request of customers for more environmental friendly agricultural products without compromising on wine quality. Different genes have been recognized to protect grapes against the two major pathogens, the large part of them in American or Asian species. Downy and powdery mildews are the two most common diseases in European countries. Since 1998 at University of Udine a breeding program started with the purpose of introgressing resistance genes into elite wine cultivars. The first ten varieties were released in collaboration with the Institute of Applied Genomics and introduced into the market by Vivai Cooperativi Rauscedo, one of the leading grape nurseries. Here we present the results of a second generation of cultivars, in particular varieties producing Pinot-like wine styles. Crosses were made in 2005 and 2007, and more than one thousand seedlings were selected in the following decade for resistance to mildews, berry sensory attributes and wine sensory attributes based on single-seedling vinification, resulting into seven genotypes for large-scale trials. Evaluation plots with 40 graftings per selection were planted in 2015, and data were collected since their second vegetative season. Phenological (bud break) and agronomical characteristics (yield per plant, vigor, field resistance, cluster weight), and must parameters (brix°, acidity) have been determined in two years. Wines were produced in 2016 and 2017. Several enological traits were determined: alcohol (%), pH, total acidity, tartaric and malic acid content and non-reducing extract. We compare and comment the data obtained during the first two years of trial cultivation at the experimental farm of the Vivai Cooperativi Rauscedo located in Northeastern Italy.

**Keywords:** downy mildew, *Plasmopara viticola*, wine evaluation

**Genome editing of Pinotage, a South African grapevine cultivar**

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In the last few years genome editing with CRISPR/Cas9 opened an immense number of opportunities in science, from medicine to agriculture. The technology is precise, efficient and flexible. Grapevine is one of the most important fruit crops in the world, but the cultivars of *Vitis vinifera*, the grapevine species mostly planted, are not well adapted to abiotic and biotic stresses. Genome editing hold great potential advantages as one of the tools that can be used in grapevine improvement strategies, but still requires optimised workflows and will benefit from a growing list of success stories. The cultivar Pinotage originated in South Africa from the crossing of Pinot Noir and Cinsaut (Hermitage) and is an interesting cultivar that displays excellent adaptability to a range of climatic factors, while producing significant phenotypic plasticity from the few clones that are used for all the Pinotage plantings. The aim of this study is to develop genome editing tools and workflows for Pinotage. The Pinotage genome has recently been sequenced, facilitating the preparation of accurate gene editing constructs. Moreover, several genes from this cultivar had already been genetically and functionally characterised. One such gene is the *V. vinifera* lycopene beta cyclase (VviLBCY) encoding gene, which will be edited (knocked out) in Pinotage, by using a multiplexing approach. The editing will result in phenotypes that would be readily detected by profiling for pigment content/composition. Four targets for this gene were identified and cloned in the vector pDIRECT\_22C. Grapevine protoplast transformation will be performed to confirm the functionality of the targets. Pinotage embryogenic calli will be subjected to *Agrobacterium tumefaciens* transformation, selection and the regenerated plants will be analysed molecularly, phenotypically and by analytical chemistry. This study will provide a proof of concept for the genome editing of Pinotage using a multiplexing approach, as well as further information on the *in planta* functions of VviLBCY, specifically in context of abiotic stress mitigation.

**Keywords:** Crispr/Cas9, genome editing, multiplexing, Pinotage

## Evidence for sexual reproduction and production of fertile oospores of *Plasmopara viticola* on partially-resistant grapevine varieties

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The use of quantitative resistance (i.e. incomplete resistance) to control pathogens is increasingly seen as a valuable and durable approach to crop protection. Downy mildew, caused by *Plasmopara viticola*, is a highly destructive disease of grapevine in all vine-growing areas. Over the past decades, European breeding programs for disease resistance have led to the creation of new cultivars that are partially resistant to downy mildew. Recent studies have demonstrated that *P. viticola* can erode partial resistance even in conditions of limited deployment of disease varieties in vineyards. Pathogen isolates from these cultivars were indeed more aggressive than isolates from susceptible hosts. They indeed had a shorter latency period and higher levels of spore production during the asexual phase. However, we still lack biological insights into the effects of partial host resistance on the survival of the pathogen during the sexual phase of its life-cycle. Here, we present the results of a two years experiment in which we assessed the survival of *P. viticola* during the sexual phase (oospores) and the success of the following infections on cultivars having Rpv1 or/and Rpv3 resistance. Briefly, infected leaf discs from resistant cultivars containing oospores were maintained in the field during winter for maturation. At spring, leaf discs were put in control conditions for macrosporocyst germination. Macrosporocyst (sporangia resulting from oospore germination) pathogenicity was tested by propagation of offspring on uninfected sensitive *V. vinifera* leaves. Our results bring evidence for the production of viable oospores on all grapevine resistant genotypes tested (Rpv1, Rpv3 alone and Rpv1/Rpv3). This demonstrates that *P. viticola* is able to complete its life cycle (asexual and sexual) on partially-resistant grapevine varieties. The fact that populations of downy mildew can maintain and evolve on partially-resistant grapevines in natural conditions is a fairly significant challenge for the durability of resistance.

**Keywords:** disease-resistant variety, oospore, *Plasmopara viticola*, Rpv1, Rpv3



## Inheritance of monoterpenes in grape (*Vitis vinifera*) populations of Muscat Hamburg X Crimson Seedless

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Muscat flavor is one of the most important characters for the selection of new table grape cultivars. It is generally acknowledged that the existence of a series of terpenes lead to this aromatic sensory experience. Terpenes and their contents were analyzed in mature berries of populations of 'Muscat Hamburg' X 'Crimson Seedless' during two successive years. Headspace solid phase micro-extraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS) combined with Automated Mass Spectral Deconvolution and Identification System (AMDIS) were employed to qualify and quantify the free terpenes. Identification of terpene was based on retention indices of reference standards and mass spectra matching using the standard NIST 14 library. For quantifications, calibration curves were obtained by standards with their regression coefficients all above 98%. Compounds without calibration curves were estimated with those standards that had the same functional group and/or similar numbers of carbon atoms. A total of 27 monoterpene were identified, among them, cis rose oxide, trans-rose oxide,  $\alpha$ -muurolene, cis-isogeraniol, and trans-isogeraniol showed 1:1 segregation in the progenies, geraniol, geranic acid,  $\gamma$ -geraniol, nerol, nerol oxide showed 15:1 segregation by chi-square test. 17 compounds including  $\beta$ -myrcene, limonene, phellandrene,  $\beta$ -trans-ocimene,  $\gamma$ -Terpinene,  $\beta$ -cis-ocimene, terpinolene, allo-ocimene, (E,Z)-allo-ocimene, cis-furan linalool oxide, citronellal, linalool, 4-terpineol, neral,  $\alpha$ -terpineol, geranial,  $\beta$ -citronellol showed continuous variation in the progenies and skewed to the low parents, but transgressive inheritance was noted among some of the progenies. Broad sense heritability of nerol, geranic acid, geraniol, neral, cis rose oxide, nerol oxide, geranial were higher than 0.8. The correlation coefficients of limonene, phellandrene, terpinolene, 4-terpineol, cis-furan-linalool,  $\beta$ -myrcene, allo-ocimene,  $\beta$ -cis-ocimene, E,Z-allo-ocimene,  $\beta$ -trans-ocimene,  $\gamma$ -Terpinene, linalool,  $\alpha$ -terpineol were higher than 0.8. The results will be helpful for breeding of good Muscat cultivars in the future.

**Keywords:** genetics, monoterpene

## A plant regeneration platform to apply New Breeding Techniques for improving disease resistance in grapevine rootstocks and cultivars

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Worldwide grapevine cultivation is based on the use of elite cultivars, in many cases strictly linked to local important wine brands. Most of *Vitis vinifera* cultivars have high susceptibility to fungal and viral diseases therefore, new breeding techniques (eg. Cisgenesis, RNAi and gene editing) offer the possibility to introduce new clones of the main cultivars with increased diseases resistance, in order to reduce environmental impact and improve quality in the intensive wine grape industry. This study is finalized to develop efficient *in vitro* regeneration and transformation protocols to extend the application of these technologies in wine grape rootstocks and high-quality clones of local interest. With this aim, *in vitro* regeneration protocols based on the production of meristematic bulks (Mezzetti et al, 2002) were optimized for different grapevine rootstocks and clones of local interest. The meristematic bulks were then used as explants for Agrobacterium mediated genetic transformation protocols, by comparing the use of NPTII and e-GFP as marker genes. Results confirmed the efficiency of meristematic bulks as regenerating tissue to produce new modified plants in all genotypes. Some genotypes showing higher regeneration efficiency allowed the selection of stable modified lines with only the use of e-GFP marker gene. This protocol can be applied to modify important grape wine rootstocks and clones without the use of antibiotic selectable marker genes, so to reduce food safety risks and increase public acceptance of the new plants.

**Keywords:** genetic transformation, *in vitro* regeneration, organogenesis, selectable marker genes

## The Pinotage genome: stress response genes are a major source of inter-cultivar genetic diversity in grapevine

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Grapevine (*Vitis vinifera*) is one of the most important crop plants in the world, especially considering the vast economic impact of the wine industry. Grapevine displays a great level of inter-cultivar phenotypic diversity, both in viticultural and oenological traits. Understanding the genetic diversity is an important step towards developing improved grapevine cultivars, but also the conservation of the important traditional cultivars. Pinotage is a red-wine cultivar resulting from a viticultural Pinot Noir/Cinsaut cross, created 1925, with the South African climate and growing conditions in mind. This study focused on the sequencing and bioinformatic analysis of the Pinotage genome and transcriptome using high-throughput sequencing technologies. A de novo assembly strategy was performed to produce the first Pinotage draft genome sequence. Sequencing data were also aligned to the reference Pinot Noir genome (PN40024), and, from this alignment, the Pinotage/Pinot Noir variant density was determined. The Pinotage genome and transcriptome data were combined to identify genes present in the Pinotage genome but absent in both Pinot Noir PN40024 and ENTAV115 genome assemblies. These were classified as both structural and regulatory genes. Genes involved in the stress response network(s) are a major gene class contributing to the genetic differences between Pinotage and Pinot Noir. This and other information generated in this study will aid in grapevine breeding programs for sustainable production of high quality wine in a changing environment.

**Keywords:** next-generation sequencing, Pinotage genome, stress network

## Investigations into nematode resistance of grapevine rootstocks within the joint project "MureViU"

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Plant parasitic nematodes are an underestimated source of crop loss in viticulture each year. Not only aggressive root feeding causes plant damage, but also a possible transmission of so-called nepoviruses. The ectoparasitic dagger nematode *Xiphinema index* vectors grapevine fanleaf virus (GFLV). Among others, this virus is responsible for fanleaf degeneration, one of the most severe viral disease in viticulture. The lack of treatment possibilities shifts research interest to resistance breeding programs of rootstocks. Within the nationwide joint BMEL/BLE project "multiresistant Vitis rootstocks" (MureViU), the institute of plant protection at the DLR Rheinpfalz participates in the identification of novel *X. index* resistances in rootstocks. Therefore, available genetic resources of all project partners are screened for preferably lowest nematode reproduction rates by a so-called glass tube test. First results show strong variations in *X. index* reproduction on multiple wild Vitis species and their F1 crosses, and thus indicate variant host suitabilities of potential rootstocks. Those identified candidates are analyzed for a concomitant virus resistance. Furthermore, gene expression analyses of known plant resistance genes are performed to identify potential marker genes for the development of faster screening methods.

**Keywords:** dagger nematode, grapevine fanleaf virus, nematode resistance, virus-transmitting nematode, *Xiphinema index*

## Cycle, physicochemical characterization and climate adaptation of a white hybrid grape onto different rootstocks

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Genetic breeding programs in search of new varieties of grapes study canopy/rootstock interactions in order to recommend specific genotypes to different wine-growing regions. However, the final product, related to the fruit, is one of the main factors analyzed before recommending the best combination for the wine region. This study aimed to evaluate the duration of the cycle, chemical and physical characteristics of the fruits and climate adaptation of the hybrid 'SR 501-17' for the production of white wine, grafted on four rootstocks. The experiment was conducted in case blocks and the treatments were comprised of four rootstocks 'IAC 766 Campinas', 'IAC 572 Jales', 'IAC 571-6 Jundiaí' and 'IAC 313 Tropical' planted in two climatic regions in São Paulo state, CWA and AW, in two years of cultivation, 2014 and 2015. The evaluations were carried out for cycle duration, weight of the clusters, weight, length and width of berries, number of berries per bunch, soluble solids content, titratable acidity and the maturation index was also estimated. Statistical analyzes were performed using the SAS software and the comparison of means was realized by Tukey test to evaluate the interaction canopies/rootstocks. The characterization of the cultivar for the two regions, climates and different years, was made through the analysis of main components. The rootstocks did not influence the behavior of the 'SR 501-17' hybrid. In order, the CWA climate resulted in higher concentrations of soluble solids content in must and largest cycle in the year 2014. On the other hand, SR-501-17 cultivation in AW climate resulted in shorter production cycle, weight and width of berries, mainly in 2015. Thus, the 'SR 501-17' presented longer cycle and better chemical characteristics of the must in CWA conditions regardless of the rootstock used.

**Keywords:** canopy/rootstock interaction, principal component analysis

## **BRS Vitoria: new seedless table grape cultivar for the São Francisco Valley, Northeast of Brazil**

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BRS Vitoria is a seedless table grape released by the grape breeding program of Embrapa in 2012. It attracts great interest of grape growers because of its high fertility of buds, exotic and pleasant taste and tolerance to downy mildew (*Plasmopara viticola*). The aim of this study was to characterize the agronomic and productive behavior of the grape BRS Vitoria in tropical conditions of the São Francisco Valley. The study was carried out over four seasons (2014-2015) in a commercial vineyard in Petrolina, Pernambuco state, Brazil. Five plants were evaluated for the following variables: sprouting (%), fertility rate of buds, production and number of bunches per plant, biometric measurements of clusters and berries, total soluble solids content (SS) and titratable acidity (TA). 'BRS Vitoria' presented a phenological cycle from pruning to harvest dates of 104 days, being characterized as an early cultivar. Average sprouting was 60.1% and 61.0% for the 1st and 2nd semesters of 2015, respectively, with high bud fertility, more than one bunch per shoot. Average production was 14.64 kg per plant, corresponding to an estimated yield of 36.6 ton/ha year. It was obtained an average of 92 bunches per plant with a mass of 220 g, measuring 15.43 cm long and 7.51 cm wide. The berries have median size, round shaped, black color, with a mass of 3.70 g and measure 22.5 mm long and 16.8 mm in diameter. The SS content ranged from 18.56 to 21.7 ° Brix while berries showed TA ranged from 0.56 to 0.72%. The average value of SS/TA ratio was 27.47. The new cultivar BRS Vitoria proved well adapted to produce two crops a year, attracting a great interest of the table grape growers and the cultivated area reaches about 1000 ha in the region of the São Francisco Valley, Northeast of Brazil.

**Keywords:** new cultivars, table grape, tropical viticulture

## Rootstock for table grape cultivar BRS Clara in the São Francisco Valley, Northeast Brazil

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The present work aimed to evaluate the influence of rootstocks on yield and fruit characteristics of table grape 'BRS Clara' in tropical conditions of São Francisco Valley, Northeast of Brazil. An experiment was carried out during six growing seasons between 2014 and 2017 in Petrolina, Pernambuco State, Brazil (9°09'S, 40°22 'O and average altitude of 365.5 m). 'BRS Clara' is a white seedless table grape released by Genetic Grape Breeding Program of Embrapa. It was grafted on six rootstocks: IAC 313, IAC 766, IAC 572, SO4, Harmony and Paulsen 1103. The experimental design consisted in randomized blocks with 3 replicates and 2 useful plants per plot. The data were submitted to variance analysis and comparison of means by the Tukey test at the 5% probability level. The results were expressed as means of the six harvests and showed that yield were influenced by rootstock. Highest yields representing an increment of 39.7% were obtained with rootstock Paulsen 1103 (13.7 ton/ha) compared to 5.4 ton/ha with rootstock IAC 572. The number of bunches per vine and mass of the bunch were also higher in vines grafted on 'Paulsen 1103' rootstock and differed significantly from 'IAC 572'. The physical and physico-chemical characteristics of the grapes, such as length and width of the bunch, mass and diameter of the berry, soluble solids content, titratable acidity and ratio were not influenced by the rootstocks. The vines grafted on 'Paulsen 1103' presented mean values of 21.1°Brix for soluble solids content, 0.56 g tartaric acid/100 mL for titratable acidity and 39.8 for ratio which corresponds to grapes of very good quality to meet the requirements for consumption of different markets. In conclusion, the rootstock Paulsen 1103 is recommended for table grape 'BRS Clara' growth in the São Francisco Valley, Northeast of Brazil, for increasing yield without affecting other quality characteristics of the grape.

**Keywords:** table grape, tropical viticulture, rootstock

## Synonyms and homonyms in grapevine varieties of Bosnia and Herzegovina

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Today's grapevine assortment in Bosnia and Herzegovina is the result of several evolutionary, agroecological and historical factors. One of them is the proximity of Dalmatia as a wine-growing area, which was source of varieties introduction. They were adapted to the breeding conditions of Bosnia and Herzegovina. Over time, varieties have migrated from one place to another inside the country. Some of them retained the same name in different areas of growth while others were renamed. Within the program of collection and maintenance of autochthonous grape varieties in Bosnia and Herzegovina at the Faculty of Agriculture and Food Technology University of Mostar, more than 35 genotypes were collected. For reliable grapevine germplasm characterization and identification, genetic analysis of 9 standard microsatellite loci was performed. Based on the microsatellite profiles obtained, cluster analysis was carried out and mutual relations were represented by the UPGMA dendrogram. Several synonyms and homonyms have been identified among the genotypes analyzed. Comparison of genetic profiles of grapevine varieties from Bosnia and Herzegovina and Croatia was also done. Synonyms and homonyms were identified within these two groups and strong connections with the Dalmatian wine growing region was confirmed.

**Keywords:** Bosnia and Herzegovina, homonyms, microsatellites, synonyms



# Effects of spraying gibberellic acid before anthesis on rachis elongation and berry flavonoid content in *Vitis vinifera* L

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Gibberellic acid (GA3) is a plant growth regulator commonly used for flowering and fruit-set in grapes. In this study, the effectiveness of different concentrations of GA3 sprayed 2-3weeks before anthesis on field-grown *Vitis vinifera* Cabernet Sauvignon (CS) and Cabernet Franc (CF) grapevines, was investigated in Shandong Peninsula of China as a tool for rachis elongation and induction of clusters compactness that are less susceptible to rot. For both cultivars, the most concentrated solutions (50 mg/L and 100 mg/L) of GA3 enhanced the elongation of the rachis significantly while the growth trend of inflorescence, fruit-setting ratio and berry seed number were not affected. A time-dependent study suggests that the 50 mg/L and 100 mg/L GA3 treatments may decrease the berry diameter and weight, but have no significant effect on the total soluble solid, pH and titratable acid of the both cultivar berry juice. For the ripen berry, neither the concentrations nor the contents of anthocyanin, flavonol and flavan-3-ol were significantly influenced by GA3. Thus these findings indicate that GA3 application could elongate inflorescence and cluster length of CS and CF, but not significantly impact the berry flavonoids which may influence the colour and flavor of wine. GA3 spraying at concentrations higher than 50 mg/L on cluster can achieve an optimal effect.

**Keywords:** flavonoid, gibberellic acid, rachis elongation

**Key genitors of Croatian grapevine germplasm - ubiquitous but almost forgotten**Maja Zulj Mihaljevic<sup>a,\*</sup>, Darko Preiner<sup>a</sup>, Goran Zdunic<sup>b</sup>, Edi Maletic<sup>a</sup>, Marijan Bubola<sup>c</sup>, Ivan Pejic<sup>a</sup><sup>a</sup> *Svetosimunska cesta 25, Zagreb, Croatia*<sup>b</sup> *Put Duilova 11, Split, Croatia*<sup>c</sup> *Karla Huguesa 8, Porec, Croatia*\* **Presenting author:** mzulj@agr.hr

Due to the significance of grapevine as a crop plant and a cultural heritage, it is very desirable to understand the genetic and hybridization events which led to today's cultivars development and distribution. The knowledge about pedigree can reveal its geographical origin and genetic composition and help to establish and understand cultivars migration routes and historical importance. Based upon previous research, it was expected that genitors of most Croatian varieties were traditional local varieties. To examine their origin, all presumable Croatian varieties preserved in national grape collections were submitted to parentage reconstruction via microsatellite markers. This was carried out using 20 SSR markers common to INRA's Vassal collection, and further confirmed with a set of 14 tri- and tetra-nucleotide SSR markers. In addition, to dissect maternal origin, chlorotypes were defined also via cpSSR markers. Unlike presumed, in many cases one of the parents was a foreign variety - that is a genotype that was not recorded in any of the Croatian grape collections. This refers the most to Bombino bianco, a high-yielding variety from Italian region Apuglia which turned out to be the mother of several Dalmatian varieties. It is also the case for cultivars rarely found *in situ* nowadays like Blank blauer (syn. Vulpea) and Alba imputotato. The reconstructed kinship group of Gouais blanc, consisting of varieties grown in northwestern part of Croatia, but also in Primorje and Istria (coastal area) gives support to the alternative hypothesis about its Pannonian origin.

**Keywords:** croatian varieties, key genitors, microsatellites, parentage

**The genetic dissection of Natural Dry-on-Vine (NDOV) trait in grapevine**Jonathan Fresnedo Ramírez<sup>a\*</sup>, Steven A. Lee<sup>b</sup>, Craig A. Ledbetter<sup>b</sup><sup>a</sup> *The Ohio State University, Department of Horticulture and Crop Science, Wooster, OH 44691, USA*<sup>b</sup> *USDA-ARS, SJVASC, Crops, Disease, and Pest Genetics, Parlier, CA 93648, USA***\* Presenting author:** fresnedoramirez.1@osu.edu

The impact of mechanized raisin harvesting has been positive in terms of reducing crop losses thanks to the development of varieties exhibiting the natural dry-on-vine trait (NDOV) in grape germplasm. However, while drying rates of specific genotypes are being determined and cultivars are being released, the genetic components influencing the NDOV trait are yet unknown. A deeper understanding on the genetic mechanisms influencing NDOV will enable scientists to develop tools for breeding applications, such as molecular markers for seedling selection, and formulate strategies for the further study of NDOV and its biochemical basis, which may provide insights on agricultural management practices. Previous genetic mapping attempts of the NDOV trait using distinct phenotypic data were unsuccessful. A study in tomato demonstrated that the gene *cpw1* affects fruit cuticle leading to fruit dehydration on the plant. Then, *cpw1* was selected as a potential candidate underlining the NDOV phenotype in grapevine. By using the grapevine genome reference sequence, two loci were identified as having high homology to tomato *cpw1*, one on chromosome 3 and another on chromosome 18, here referred to as *cpw1vv3* and *cpw1vv18*, respectively. Sequences of these loci were retrieved from the genome sequences of ‘Thompson Seedless’, ‘Pinot Noir’, ‘Flame Seedless’, ‘Cabernet Sauvignon’ and *Vitis cinerea* B9, for multiple sequence alignments for homology quantification and identification of putative polymorphisms. The DNA sequences of *cpw1vv3* and *cpw1vv18* have been cloned from the NDOV raisin cultivar ‘Sunpreme’ and introduced in tomato to verify function. In addition, screening for polymorphisms in raisin-related germplasm with and without the NDOV trait is underway using AmpSeq, while obtaining supporting phenotypic data from NDOV-segregating germplasm. Studies on cuticle features are also envisioned. The results from this study will enable the development of tools and a body of knowledge for the further understanding of the NDOV trait in grapevine.

**Keywords:** cuticle, marker-assisted selection, raisin breeding, polymorphisms

## AmpSeq as a tool for genetics and breeding of grapevine

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AmpSeq is a novel, practical, and intuitive strategy with a semi-automated computational pipeline that analyzes single nucleotide polymorphisms (SNPs), haplotypes or sequences associated with traits through amplicon sequencing. The flexibility of AmpSeq's wet lab methods makes it a tool of broad interest for diverse species, and AmpSeq excels in flexibility, speed, high-throughput, and low-cost. AmpSeq has been successfully implemented to assist in breeding for powdery mildew resistance considering introgression and tracking of five resistance loci from wild species into domesticated backgrounds, with the goal of pyramiding multiple resistance genes, to provide durable disease resistance to breeding selections and ultimately cultivars. In addition, AmpSeq has been used to approach flower sex segregation considering a mixture of genomic backgrounds in research and breeding populations including several genomic backgrounds from North American species, showing that AmpSeq facilitates the scrutiny of high genetic diversity backgrounds. Also, through AmpSeq the evaluation of transferability of molecular markers across populations and breeding programs has been assessed. In summary, AmpSeq provides a high-throughput, cost-effective tool successfully applied to integrate divergent technologies for marker-assisted selection and genetic analysis of a variety of traits in grapevine.

**Keywords:** disease resistance, flower sex, gene-pyramiding, marker-assisted breeding, marker-assisted seedling selection

**Downy mildew resistant QTLs in *Vitis amurens* "Shuang Hong" grapevine**Shiren Song<sup>a</sup>, Peining Fu<sup>a</sup>, Jiang Lu<sup>a,\*</sup><sup>a</sup> Center for Viticulture and Enology, Shanghai JiaoTong University, 800 Dongchuan Rd., Minhang District, Shanghai, 200240, China\* **Presenting author:** jiang.lu@sjtu.edu.cn

Downy mildew, caused by the pathogen *Plasmopara viticola*, is a major disease in grapevine. In order to identify *P. viticola*-resistance (Rpv) loci in *Vitis amurens*, we investigated 91 F1 progeny from a cross between the downy mildew-resistant *Vitis amurens* 'Shuang Hong' and the susceptible *V. vinifera* 'Cabernet Sauvignon'. In order to measure the level of Rpv among the F1 hybrids, leaf discs from each individual were inoculated with *P. viticola*, and the degree of resistance to *Plasmopara*, sporulation density, and percentage of leaf disc area yielding sporangiophores were scored from 5 to 7 d after inoculation. The Rpv of the progeny varied continuously and segregated as a quantitative trait, so a genotyping-by-sequencing strategy was used to construct linkage maps. The 'Cabernet Sauvignon' map included 3907 single nucleotide polymorphisms (SNPs) on 19 linkage groups (LGs), covering 2189.50 cM in total and with an average inter-SNP distance of 0.59 cM, whereas the 'Shuang Hong' map included 4584 SNPs on 19 LGs, covering 2418.75 cM in total and with an average inter-SNP distance of 0.55 cM. Meanwhile, the integrated map spanned 3163.16 cM and included 7598 SNPs on 19 LGs. Linkage analysis identified a major QTL for Rpv (Rpv22) on LG 15 and two minor QTLs (Rpv23 and Rpv24) on LGs 02 and 18. Furthermore, Rpv22 explained up to 45.7% of the phenotypic variance in Rpv with spanning a section of 2.4 cM. Comparison to a reference grape genome identified 18 resistance gene analogues, including 11 CC-NBS-LRR genes, three CC-NBS genes, one TIR-NBS-LRR gene, one RPP13-like gene, one PTI6 gene, and one other disease resistance gene.

**Keywords:** downy mildew resistance, genotyping-by-sequencing, quantitative trait locus, *Vitis amurens*

# ***In vitro* embryo rescue in the table grapes breeding for the semi-arid tropical region of Brazil**

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Seedlessness in commercial table grapes cultivars is caused by stenospermocarpy that results in embryo abortion before complete development. The technique of embryo rescue and culture *in vitro*, before abortion, allowed the crosses between seedless cultivars as parents, with the main advantage of increasing the frequency of seedless individuals in the progeny. The present work aimed to evaluate the efficiency of table grape breeding by controlled hybridizations and embryo rescue in the development of new table grapes cultivars for tropical semi-arid conditions in Northeast of Brazil. The crosses were carried out in the Experimental Fields of Embrapa Semiarido in Petrolina, PE and Juazeiro, BA, in the São Francisco River Valley between 2011 and 2017. In this period, 938 inflorescences were pollinated, obtaining a formation of 527 clusters (56.2%), of which 16,469 seed traces were obtained and inoculated *in vitro*. This resulted in 7,532 (45.7%) immature embryos rescued, obtaining success in the germination of 2922 seedlings (38.8%). A great variability of responses was observed in function of the genotypes used in the crosses. The genotypes 'Thompson Seedless', 'Superior Seedless', 'CG 351', 'Maroo Seedless' used as female parents and 'BRS Linda', 'Jupiter' and 'CG38049' as male parents presented the best results for fruitfulness and the formation and germination of embryos. The number of seedlings obtained by the embryo rescue technique is correlated with the genotype used as parent, being higher in the crosses between *V. vinifera* cultivars when compared to those involving hybrid cultivars. Also noteworthy is the progress in the results obtained in the years 2015 and 2016 in relation to the period 2011-2014, when 'Maroo Seedless' was distinguished as a female parent in crosses using pollen of 'A110'5, 'A Dona', 'BRS Linda', 'BRS Isis', 'BRS Clara', 'BRS Vitória', 'CG 351', 'Feal' and 'Jupiter', obtaining in all combinations high rates of fruitfulness (54 to 100%), embryo rescue (26 to 62%) and germination of seedlings (46 to 97%).

**Keywords:** controlled crossings, embryo rescue, table grape

## Rootstock for table grape cultivar BRS Maria Bonita in the São Francisco Valley, Northeast of Brazil

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'BRS Maria Bonita' is a red seedless table grape cultivar developed by Embrapa's grape breeding program. This work aimed to study the influence of rootstock on production components and physical-chemical characteristics of BRS Maria Bonita grapes in the tropical conditions of the São Francisco Valley. The experiment was carried out in the Experimental Field of Embrapa Semiárido in Petrolina, PE, Brazil, during 5 production cycles, using six rootstocks: 'Harmony', 'SO4', 'Paulsen 1103', 'IAC 572', 'IAC 313' and 'IAC 766'. The experimental design consisted in random blocks with 3 replicates. The averages of 5 production cycles presented statistically significant differences for most variables. Rootstock IAC 766 increased yield and number of bunches compared to 'Harmony' and 'SO4'. An average of 14.8 ton/ha per cycle and 35 bunches per plant was obtained on 'IAC 766'. The size of the bunch and berry was lower in rootstock SO4, but did not differ in the other rootstocks. Soluble solids content was not influenced by rootstock, obtaining mean values of 16.24 °Brix, while the titratable acidity was higher when the grapes were grafted on 'SO4' (0.56 g tartaric acid/100 mL) and 'Harmony' (0.45 g tartaric acid/100 mL) and 'IAC 313' (0.47 g tartaric acid/100 mL). The SS/AT ratio also did not show differences between rootstocks, ranging from 32.4 ('SO4') to 41.8 ('Harmony'). Rootstock IAC 766 should be used to increase yield and number of bunches of the 'BRS Maria Bonita' cultivar grown in the São Francisco Valley, Northeastern of Brazil.

**Keywords:** berry composition, bunch size, rootstock, yield

## Evaluation of physicochemical and storability attributes of 5 new Chinese table grape cultivars

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In recent years, many new table grape cultivars with Muscat flavor have been released in China. But limited information is available on their postharvest storability. In the present study, using 'Muscat hamburg' as control, physiochemical and storability attributes of 5 new table grape cultivars with muscat flavor were measured during storage for 45 days at 2 °C. The results showed that genotypes significantly influenced physiochemical attributes such as total soluble solids (%), titratable acidity (TA) as well as aroma compounds content and storage attributes including the rot berries ratio (%), the drop berries ratio (%), weight loss (%), the browning index (%) and berry retention force (kg). The rot berries ratio and the drop berries ratio of 'Ruidu Hongmei' and 'Ruidu Hongyu' were significantly lower than in the control cultivar. In the process of storage, weight loss of 'Muscat hamburg' was lowest, followed by 'Ruidu Hongmei' and 'Ruidu Hongyu'. Additionally, the browning index of 'Ruidu Hongyu' presented the lowest level, while its berry retention force showed the highest level. Overall, 'Ruidu Hongmei' and 'Ruidu Hongyu' showed better storage attributes. The content of aroma compounds decreased significantly during low temperature storage. The results obtained will provide references for the selection of new grape cultivar by breeders and viticulturists in the future.

**Keywords:** muscat flavor, new cultivar, postharvest, storage attributes



## Effects of different rootstocks on aroma profiles of *Vitis vinifera* L. cv. Cabernet Sauvignon grapes

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This study investigated the effects of 8 rootstocks (101-14, 110R, 5A, 5BB, Ganzin1, Harmony, Riparia5 and SO4) on vine vigour, berry physicochemical parameters and aroma profiles of Cabernet Sauvignon over two consecutive seasons. None of these rootstocks significantly affected yield, pruning weight and vine crop load. 5A and SO4 significantly enhanced berry total soluble solids, and SO4 had negative effect on titratable acidity in berries. K-means clustering analysis was used to identify developmental profiles of berry aromas. C6/C9 compounds, certain carbonyl compounds and benzenic compounds in grafted berries displayed trends similar to those of own-rooted berries during berry development. Specifically, these compounds peaked at pre-harvest and declined at harvest. Some higher alcohols, esters and terpenes decreased from green stage until harvest in both grafted and own-rooted berries, while the decreasing rate of these compounds from green stage to pre-veraison in grafted berries was clearly higher than that in own-rooted berries. Besides, certain higher alcohols, terpenes and C13-isoprenoids in only own-rooted berries showed a clear peak at E-L 35.5 and then decreased until harvest. On the basis of quantitative data matrix of berry aromas at harvest, CS/110R, CS/5A, CS/5BB, CS/Riparia5 and CS/SO4 clearly differed from own-rooted vines using orthogonal partial least squares-discriminant analysis (OPLS-DA) models, respectively. The volatile compounds of these grafted berries in comparison with own-rooted berries were identified. The concentrations of hexanal in grafted berries were lower than that in own-rooted berries. Furthermore, these grafted vines except for CS/5BB imposed negative effects on the accumulation of  $\alpha$ -terpenes and rose oxide in berries. 5BB negatively affected on the accumulation of total norisoprenoids and nerol oxide. Compared to own-rooted vines, CS/SO4 displayed significant higher concentration of 3-isobutyl-2-methoxypyrazine.

**Keywords:** aroma profiles, Cabernet Sauvignon, rootstocks

## Use of marker-assisted selection in Alphonse Lavallee x Regent hybrids for determination of downy mildew resistance

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The use of molecular markers associated with disease-resistance genes in Marker-Assisted Selection (MAS) studies has been gaining popularity in grapevine breeding. Downy mildew and powdery mildew are the main fungal diseases causing significant crop losses in viticulture in Turkey.

Thus, development of a table grape cultivar genetically resistant to these diseases is one of main issues in Turkish grapevine breeding studies. The objective of this study was to employ marker assisted selection in order to identify the genotypes resistant to downy mildew (*Plasmopora viticola*) disease at early stages of the development.

An Alphonse Lavallee (susceptible) x Regent (resistant) population was used to develop a table grape cultivar resistant to downy mildew. A progeny of 200 genotypes were genetically analyzed by two SSR markers of GF18-06 and GF18-08. These markers were previously developed from the downy mildew resistance locus Rpv3 (Regent).

Allele sizes of PCR amplification products were determined in Advanced Analytical Fragment Analyzer. Genotypes with resistance-related alleles were selected. The results will be discussed.

**Keywords:** downy mildew, grapevine breeding, marker-assisted selection, SSRs

## DNA methylation in fleshy fruits: from tomato to grape

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Epigenetic refers to heritable changes in chromatin organization, which may lead to modifications in gene expression while the underlying genomic DNA sequence remains unchanged. DNA methylation occurs on the 5th carbon of cytosine (5mC). This major epigenetic mark is involved in the control of gene expression and transposon mobility. Studies in Arabidopsis and other plants have now demonstrated the relevance of epigenetic mechanisms in the control of plant developmental processes and their potential impact on traits of agronomical interest such as flowering time. Recently the distribution of 5mC over the tomato genome was shown to vary during fruit ripening suggesting that fruit development not only relies on hormones and genetic factors, but also on epigenetic regulations.

We have now shown that the balance between active DNA demethylation and methylation is critically important to tomato fruit development. We have generated various tomato lines that are impaired in DNA methylation (MET) or demethylation (DML). Metabolomics and RNA seq analysis were performed indicating that several aspects of fruit ripening are inhibited in fruits of DML lines. Inhibition of fruit ripening is due to the hypermethylation and repression of the expression of genes encoding ripening transcription factors and rate-limiting enzymes of important metabolic pathway. Inversely MET RNAi lines develop small fruits without clear alteration of fruit ripening, although plant development is severely impaired. Interestingly, whereas active DNA demethylation is critically important to tomato fruit ripening control, analysis of grape fruit methylome is not consistent with a similar role in grape fruits. Hence, although DNA methylation is important in both grape and tomato fruits, their function likely differs.

**Keywords:** DNA methylation, fleshy fruits, grapevine, ripening, tomato

## Genetic gains of selection in ancient grapevine varieties

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The genetic diversity accumulated over centuries within ancient grapevine varieties is the raw material for any genetic/genomic selection. It allows us to achieve high genetic gains of selection and respond to present and future challenges of viticulture. Therefore its evaluation should be efficiently done, which involves the establishment of field trials with powerful experimental designs (such as row-column and alpha designs) and the fitting of appropriate models for data analysis. The key point is to perform a well-planned phenotyping because it is a requisite for any efficient genetic/genomic selection in grapevine.

For quantitative genetics analyses focused on selection, linear mixed models are fitted to large data sets using residual maximum likelihood estimation. In this work, those models are fitted to yield and must quality traits data from initial field trials (with a high number of genotypes, from 100 to 255) of 11 ancient varieties. Polyclonal selection (selection of a superior group of clones) is performed, with prediction of genetic gains for several traits. The predicted genetic gain was computed as the mean of the empirical best linear unbiased predictors of the genotypic effects of the selected genotypes. Prediction intervals at 95% are presented for the genetic gain of those selected groups of clones. The results showed that the higher the intravarietal variability, the heritability, the number of replicates and the efficiency in controlling spatial variation, the lower the standard error of prediction of genotypic effects, and thus, the lower the width of the prediction interval. Consequently, a more precise prediction of genetic gain is achieved. The results reinforce the importance of the selection for the economic success of vine and wine industry and represent a sound support for genomic selection implementation.

**Keywords:** genetic resources, grapevine selection, linear mixed models, quantitative genetics

## Mitigating the effects of climate change on berry composition by canopy management

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Primary and secondary metabolites are major components of grape quality and their balances define wine typicity. Global climate change is modifying vine physiology and especially the biochemical composition of grape berries at harvest, by decoupling the phenolic and aromatic maturity (defined by secondary metabolites) with technical maturity (defined by primary metabolites) [1]. These modifications can be limited through adaptation in the vineyard. One of the rapid and efficient ways to mitigate the climate change effect is to modify vine canopy that modifies the relationship between source and sink [2,3].

To face this challenge, we used cv. Cabernet Sauvignon 1) to analyse the response of yield and biochemical composition in ripening berries, including sugars, organic acids, amino acids, phenolic compounds (anthocyanins, flavonols) and aromatic compounds (including methoxypyrazines, thiols, and precursors of thiols), with UPLC, GC-MS and LC-MS; 2) to link the modified berry composition with wine quality by microvinifications; 3) to study the response of berry transcriptome to canopy manipulation, by RNAseq or qPCR analyses.

The preliminary results showed that metabolites had different sensitivities to the modulation of leaf-to-fruit ratios, demonstrating that it is possible to determine an optimal leaf/fruit ratio to reduce sugar concentration in the berry without much impact on the typicity on Bordeaux wines.

**Keywords:** berry composition, climate change, grape quality, leaf/fruit ratio

**Acknowledgments:** We thank the CIVB for the financial support of the CANOGRAPPE project N° 44233 and FAM for the financial support of the CANABA project N°414.

**Amino acid metabolism as a potential source for unique flavors in table grapes**

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Amino acids are precursors for the production of hundreds of specialized metabolites in plants including volatile compounds important for aroma and flavor. Acetyl esters derived of amino acids metabolism contribute to the full flavor and aroma of many fruits such as banana, strawberry, melon and apple, but are normally absent in grape berries (*Vitis vinifera* L.). Research on amino acids metabolism to volatile compounds in grapes has received little attention. To search for a potential way to confer new flavors to grapes and new genes involved in grape flavor metabolism, berry sections were incubated with exogenous L-amino acids, resulting in the accumulation of 13 new volatile compounds, including aldehydes, alcohols and acetyl esters. The levels of 3-methylbutyl acetate and 2-phenylethyl acetate, acetyl esters displaying potential positive contributions to berry flavor increased. Cell-free extracts derived from grape berries displayed alcohol acetyltransferase (AAT) activities and supported the formation of the esters. *VvAAT2*, a newly characterized gene expressed in the grape berries and was functionally expressed in *E. coli*. *VvAAT2* possesses AAT activity utilizing benzyl alcohol, 2-phenylethanol, hexanol or 3-methylbutanol as substrates. Our study demonstrates that grape berries have a concealed potential to accumulate volatile esters and additional volatile compounds. Transcriptional barriers as well as substrate availability limit ester formation in grape berries. More attention in breeding programs is needed to study volatile compounds metabolism to meet growing market demands for unique flavors.

**Keywords:** flavor, table grapes, volatile compounds

## A novel grapevine microRNAs database and its application to investigate *Vitis vinifera* responses to Flavescence dorée infection

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Micro(mi)RNAs play crucial roles in plant developmental processes, as well as in defence responses to biotic and abiotic stresses, through post-transcriptional control of several biological pathway intermediates. In the last years, many works on small RNAs in grapevine (*Vitis* spp.) were published, and several conserved and putative novel grapevine-specific miRNAs were identified. In order to reorganise the high quantity of available data, we produced 'miRVIT', the first database of all novel grapevine miRNA candidates characterised so far, and still not deposited in miRBase. To this aim, each miRNA accession was renamed, repositioned in the last version of the grapevine genome and compared with all the novel and conserved miRNAs detected in grapevine. Overall, 901 sequences referred to as novel miRNAs were found of which 621 are 20–22 nt long and classified as 469 unique novel miRNAs. However, miRNA\* sequences were identified for only 150 accessions, and 45 of these were found in at least two different works. Only 5% (45 of 901) of the novel miRNAs previously identified in grapevine could be considered robust unique novel miRNAs. Conserved and novel miRNAs catalogued in miRVIT were then used for analyzing *V. vinifera* plants infected by Flavescence dorée (FD). FD is considered one of the most severe phytoplasma diseases affecting grapevine, and the spontaneous, complete and stable remission of symptoms (recovery) is a still poorly understood phenomenon. The analysis of small RNAs from healthy (H), recovered (R) and FD-infected (FD) cv. Barbera grapevines revealed key roles of miRNAs in cell development and photosynthesis (vvi-miR156, vvi-miR166, vvi\_miC137-3p), jasmonate signalling (vvi-miR319, vvi-miR167) and disease resistance response (vvi-miR482, vvi\_miC1031-5p, vvi\_miC64-5p), associated to regulation of FD-infection and recovery processes. The application of miRVIT in a biological context confirmed the effectiveness of the approach, especially for the identification of novel miRNA candidates in grapevine.

**Keywords:** disease resistance, jasmonate, novel miRNAs, phytoplasma, targets, univocal database

## Interplays between *Vitis vinifera* and grapevine virus B (GVB) in field conditions leads to ameliorate berry secondary metabolism

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A plant virus infection results from a complex molecular and physiological interplay with the host. In light of this, the impact of the phloem-limited virus Grapevine virus B (GVB) on the *Vitis vinifera* wine-red cultivar ‘Albarossa’ was analyzed in field conditions. Although not particularly widespread in grapevine, GVB is closely associated with the Corky bark disorder; it is generally considered harmful and it potentially affects graft unions. This study was carried out by combining agronomical, molecular, biochemical and ecophysiological approaches. The data obtained showed that GVB did not induce symptoms on ‘Albarossa’, but it rather affected the ecophysiological performances of vines in terms of assimilation rates, particularly at the end of the season, without compromising yield and vigor. Moreover, GVB infection impaired phloem loading and transport, by callose deposition as a defense response mechanism, resulting in carbohydrate accumulation in leaves and expression profiles of sugar- and photosynthetic-related genes seemed to activate defense responses similar to those observed in plants infected by phytoplasmas, although with lesser extent. Interestingly, several genes (*VvMybA1*, *VvUFGT*, *Vv3AT*, *VvF3'5'H* and *VvF3'H*) involved in anthocyanin biosynthesis, showed higher expression levels in GVB-infected berries over the ripening period reflecting the higher concentration of total anthocyanins, particularly tri-hydroxylated form and acylated anthocyanins in infected mature berries. This resulted in positive sensorial effects on the wine produced from GVB-infected ‘Albarossa’ berries, as attested by the overall judgment of tasters. Noteworthy, GVB presence not only did not cause detrimental phenotypic effects, but it also positively affected anthocyanin profiles in the berry, thus suggesting the existence of an indirect beneficial role on wine quality and stability as well. All together, these results can contribute both to improve current understanding of the multifaceted grapevine-virus interaction in response to environmental condition and to support future approaches of sustainable viticulture.

**Keywords:** anthocyanins, gas exchange, plant-virus interaction, sugar signaling



## Use of "neovigen96" chip to understand the defence status of cultivar or resistant genotypes of *Vitis vinifera*

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A pioneering tool ("NeoViGen96" chip) based on high-throughput qRT-PCR of 85 defence related gene expressions has been developed to assess the defence status of the grapevine and to correlate it with a protection level on plants (Dufour et al., 2016). This new tool is useful to set up alternative or complementary pest management methods with plant defence stimulators, associated or not with other pest management methods such as biological control or plant breeding.

The "NeoVigen96" chip enables to monitor the expression level of a selected-defense gene set which covers widely the various defense ways described in grapevine (SA, JA / ET-dependent signal transduction, PR proteins, phytoalexins production and the cell wall reinforcement). This tool has been used to assess the defences on susceptible cultivars and/on grapevine hybrids partially or totally resistant to downy and powdery mildew, in controlled conditions.

With this tool, it is possible to visualize the defense mechanisms displayed in leaves of different resistant genotypes having powdery and downy mildew resistance QTLs (Rpv1, Rpv2, Rpv1+Rpv2), and/or hybrids such as Solaris, Regent, Rv4 and RV5. This tool can help us to understand which metabolic pathways are important for protection against powdery and downy mildews, or other diseases, and can help us to select resistant genotypes

### Reference:

Dufour M-C, Magnin N., Dumas B., Vergnes S. and Corio-Costet M-F. (2016) High-throughput gene-expression quantification of grapevine defense responses in the field using microfluidic dynamic arrays. BMC genomics, 17: DOI 10.1186/s12864-016-3304-z.

**Keywords:** breeding, defence status, downy mildew, gene expression, powdery mildew, resistance

## Molecular improvement of powdery mildew resistance in grapevine

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Powdery mildew is the most economically important disease of cultivated grapevines worldwide. Currently, viticulture and wine production in the world is mainly based upon the cultivars derived from the Eurasian grape species *Vitis vinifera* on account of its superior aroma and flavor characteristics. However, this species lacks genetic resistance against powdery mildew caused by *Erysiphe necator* Schw. (syn. *Uncinula necator*). Consequently, viticulture and wine production worldwide strongly depend on the frequent use of fungicides. To avoid extensive fungicide applications, utilization of R-genes from grapevine or other plants as transgenes in grapevine would provide an attractive strategy for breeding grapevines with durable powdery mildew resistance. Here, we report that the Arabidopsis broad-spectrum disease resistance gene *RPW8.2* and the gene homologous to Calcium-dependent protein kinases (CDPKs), *VpCDPK9*, isolated from the wild Chinese *Vitis pseudoreticulata* could improve resistance to powdery mildew in *Vitis vinifera* cv. Thompson Seedless. Infection tests with an adapted grapevine powdery mildew isolate EnNAFU1 showed that hyphal growth and sporulation were significantly restricted in *RPW8.2* transgenic grapevines. The resistance appeared to be associated with the onsite accumulation of H<sub>2</sub>O<sub>2</sub>. Transcriptome analysis revealed that ectopic expression of *RPW8.2* in grapevines not only significantly enhanced salicylic acid-dependent defense signaling, but also altered expression of other phytohormone-associated genes. Over-expression of *VpCDPK9* in grapevine promoted powdery mildew-induced necrosis and leaf abscission at the late stage of powdery mildew colonization, and contributed resistance to adaptive powdery mildew pathogen. Taken together, our results indicate that *RPW8.2* and *VpCDPK9* could be utilized as transgenes for improving resistance against powdery mildew in grapevines.

**Keywords:** cell death, ethylene, H<sub>2</sub>O<sub>2</sub> accumulation, powdery mildew, *Vitis pseudoreticulata*

## Into the wood - Potential of non-destructive imaging approaches for phenotyping grapevine tolerance to trunk diseases

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Imaging approaches and image processing have considerably improved plant phenotyping and are nowadays increasingly used for phenotypic trait measurement.  $\mu$ -RMI and X-ray computed micro-tomography are non-destructive imaging techniques respectively based on the magnetization properties of atomic nuclei, and the relative radio-density. They are widely used for medical diagnosis although these techniques remain rare in plant studies. However, they could enable the study of complex plant-pathogen interactions, as observed in grapevine trunk disease (GTD).

Our research aims to evaluate the potential of non-destructive imaging tools for (i) the detection of GTD, (ii) the dynamic and non-destructive monitoring of pathogens propagation into the wood, and (iii) the description of their impact on host tissues.

Using  $\mu$ -RMI and X-ray  $\mu$ CT, we performed a dynamic monitoring of wood colonization by two fungi artificially inoculated under controlled conditions. Interesting results were thereby collected on both the progression of each fungus in the different tissues and their impact on the live plant. In parallel, we were also able to detect, localize and quantify different types of degraded tissues in old grapevine plants collected in vineyards. Altogether, these results proved that non-destructive imaging provides various proper tools to monitor pathogenic fungi progression in the wood. They could lead to the development of new markers for monitoring trunk diseases, and for phenotyping genetic resources for their level of tolerance.

Imaging approaches open new perspectives for increasing our knowledge on GTD. These news tools could also be used for evaluating varietal tolerance in breeding programs, and for measuring the real effect and efficacy of new molecules or biocontrol agents onto the pathogen propagation in the wood. Non-destructive imaging approaches would then benefit both the wine growing industry and the researchers.

**Keywords:** fungus, host-pathogen interaction, magnetic resonance imaging, non-destructive imaging, phenotyping, trunk disease tolerance, X-Ray computed-tomography

## Comparative transcriptomics of eight grapevine powdery mildew-resistance loci

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Powdery mildew is one of the most important fungal diseases affecting cultivated grapes (*Vitis vinifera*) worldwide in terms of crop damage, cost and potential environmental impact. The causal pathogen of this disease, the biotrophic ascomycete *Erysiphe necator*, can infect any green tissue of the plant, triggering a decline of the leaf photosynthetic capacity and a reduction of both berry yield and quality. To control powdery mildew, grape growers apply extensive and frequent prophylactic fungicide treatments throughout the growing season; applications that are costly, labor intensive and potentially harmful for the environment. Multiple sources of genetic resistance have been identified in North American and Asian *Vitis* species and are being used in breeding programs around the world. Little is known about the genome-wide transcriptional dynamics associated with the different sources of resistance. In a previous study, we provided a first exploration of the different functions and defense strategies associated with different levels of partial resistance to powdery mildew in seven Ren 1-like *V. vinifera* accessions from Central Asia (Amrine et al., 2015). In the present study, we extended the genome-wide transcriptional analysis to seven additional resistant loci including: Ren2, Ren3, Ren4, Ren6, Ren7, Run1 and Run2. The leaf transcriptomes of 18 F1 grapevine accessions derived from crosses between a susceptible parent and the different sources of powdery mildew resistance were compared with their sister lines that did not inherit the resistance loci. All transcriptomes were sequenced at two time points after infection with the *E. necator* isolate C-strain in triplicate, representing a total of 384 libraries. The comparative analysis of the transcriptional responses dependent on the different resistance genes will help (i) determine the extent of functional overlap between the resistance genes, (ii) identify unique features associated with one or several groups of genes, (iii) estimate the functional complementarity between resistance genes.

**Keywords:** comparative transcriptomics, powdery mildew, resistance loci

**iTRAQ protein profile differential analysis of berry firmness by GA3 of Summer Black grape**

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Berry firmness is one of the most important quality traits in table grape breeding programs. GA<sub>3</sub> is commonly used to improve crop yield and quality in tree fruit production. To better understand the mechanism by which GA<sub>3</sub> increases berry firmness at the protein level, berries from 5-year-old table grape cv. 'Summer Black' (*Vitis vinifera*-*Vitis labruscana*) plants were treated with GA<sub>3</sub> (25 mg L<sup>-1</sup>) 30 days after anthesis (DAA), and sampled 70 days after treatment for proteomic analysis. The result showed that berries firmness increased significantly from 834 g to 1780 g after GA<sub>3</sub> treatment. Moreover, we identified 6015 proteins, of which 788 were expressed differentially between GA<sub>3</sub>-treated and untreated fruits, 246 proteins were up-regulated, while 542 proteins were down-regulated. Gene Ontology (GO) and KEGG pathway enrichment analyses were carried out. GO results showed that most of the differentially expressed proteins were involved metabolic process, cellular process, response to stimulus, oxidation-reduction process and response to stress. KEGG results showed that there were 123 significant pathways enriched with these interacting proteins, including biosynthesis of secondary metabolites, metabolic pathways, ribosome, starch and sucrose metabolism, and so on. These results provide a better understanding of the proteins and mechanisms involved in the control of grape berry firmness by GA<sub>3</sub>.

**Keywords:** berry firmness, GA<sub>3</sub>, iTRAQ

## Effects of sunlight exclusion on the profiles of monoterpene biosynthesis and accumulation in grape exocarp and mesocarp

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Terpenes are important aroma for table Muscat grape and wine, and their contents in berry can be affected by sunlight. The effects of sunlight exclusion on monoterpene profiles and relevant gene expression profiles in the exocarp and mesocarp of table muscat grape 'Jingxiangyu' in different development stages were thoroughly surveyed by bagging pre-veraison clusters in special opaque boxes. The responses of monoterpenes to sunlight treatments varied in three types, representatively linalool, ocimene and geraniol. Linalool was the most sensitive compound to sunlight, whose biosynthesis was severely inhibited by sunlight exclusion and then was elevated by re-exposure. Ocimene and glycosylated geraniol showed a certain suppressive and stimulative responses to sunlight exclusion respectively. Further transcription analysis revealed that *VvPNLinNer1*, *VvCSbOci*, *VvGT7* and *VvGT14* genes were mainly responsible for monoterpene accumulation and sensitive to sunlight. *VvDXS2* and *VvDXR* genes were partially related to the differential accumulation of total terpenes under different sunlight treatments. The results will guide the optimised cultivation techniques to obtain abundant aroma and provide a scientific basis to further understand the light regulation mechanism of terpenoid synthesis.

**Keywords:** free and bound aroma, monoterpene, Muscat grape, sunlight response, terpene synthase

## A new late-maturing grape cultivar 'Crystal Red'

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'Crystal Red' is a new late-maturing grape variety with good quality and attractive appearance. The original seedling was bred from the cross between 'Manicule Finger' and 'Muscat Hamburg' at the vineyard in the Zhengzhou Fruit Research Institute, Chinese Academy of Agriculture Sciences(CAAS). The characters of 'Crystal Red' are as follows: coniform cluster shape, no pair of ear, big cluster, 18-23 cm long, 15-18 cm wide, average cluster weight 820 g, cluster density medium to tight. The shape of berry is pointed oval and the average weight is 8.3g with the maximum of 10.1g, 2.9-3.3cm in diameter, 1.5-1.7cm long. The fruit color is red. The berries are uniform in shape and color. The thickness of the skin is thin, the flesh is juicy and crisp, the firmness of the berries is medium. The length of peduncle is medium. Soluble solid content is 15.4%, total sugar is 13.2%, and titrable acid is 0.28%. The content of tannin was 644 mg·kg<sup>-1</sup>, the content of Vc was 6.87mg·kg<sup>-1</sup>. The flavour is sweet, the quality is good. In Zhengzhou area, the time of bud-burst is the beginning of April and flowering at mid May, the fruits mature on September 10th. The average rate of fruiting shoot is 40% and the yield is about 22.5 tonnes per hectare.

**Keywords:** Crystal Red, new cultivar

## Breeding for mildew resistance to improve environmental and socio-economic sustainability in hotspot areas of Veneto

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In the European viticulture, the control of mildews typically relies on frequent application of fungicides, which are becoming increasingly prohibitive due to their adverse effects on human health and the environment. This issue is particularly important in humid temperate regions like Veneto (Northern Italy), where the organic management of mildews is also frequently ineffective due to high disease pressure. Furthermore, vineyard-areas in this region are still increasing due to the growing demand of Prosecco, the most worldwide exported wine to this day. Therefore, to reduce the environmental impact of agrochemicals as well as to support the needs of local producers, CREA-VE started in 2012 a breeding program with the aim of selecting new genotypes with durable resistances to downy and powdery mildews, and good agro-enological traits. For this purpose, the elite cv Glera (used for Prosecco production) was crossed with different selections carrying multiple sources of resistance to mildews (*Rpv3.1*, *Rpv3.3*, *Rpv10*, *Rpv12*, *Ren3*, *Ren9*). Early selection of seedlings with inherited resistance loci was made by using molecular markers; the phenotyping of selected plants was performed through artificial inoculation (greenhouse and/or leaf disc assays). About 1000-1200 resistant genotypes have been selected until now. Recently, sensor based phenotyping has been adopted in order to speed up and improve the efficiency of grapevine evaluation programs. In particular, multispectral analysis was performed on resistant and susceptible genotypes for the pre-symptomatic detection of infection. Preliminary results were encouraging, reason why multi- and hyper-spectral analysis will be further developed on breeding progenies.

**Keywords:** downy mildew, Glera, MAS, phenotyping, powdery mildew



## Comparison of multi- and hyperspectral sensors regarding their ability to detect grapevine diseases

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Since grapevines (*Vitis vinifera*) are large perennial plants, their phenotyping is principally bound to the field. However, phenotyping is mostly based on visual ratings, which is time consuming and subjective. For some traits, such as berry quality and disease incidence, predominantly destructive measurements are available. New evolving sensor technologies can help to solve these problems, as they are faster, objective, and most importantly non-destructive. Therefore, they allow the observation of a trait over a longer period of time. Multi- and hyperspectral sensors provide information not only in the visible range of light, but also in the infrared region. Thereby, these sensors are able to detect biochemical and biophysical changes in plants upon an infection before they become visible. In this study, hyperspectral data were acquired using the newly introduced 'Phenoliner' - a robust field phenotyping platform. The ground-based hyperspectral measurements were accompanied by airborne multispectral recordings performed by an unmanned aerial vehicle (UAV). Hyperspectral sensors record a large number of contiguous spectral bands thereby providing a continuous spectrum for every pixel in the image. Multispectral images contain less information in comparison to hyperspectral data since only selected wavelengths are recorded. Nevertheless, applied by using UAVs, multispectral image acquisition is cheaper, faster, and more flexible. Thus, multispectral cameras are more suitable for practical application. Therefore, the aim of this study was the comparison of multi- and hyperspectral imaging. In field tests and under laboratory conditions different plants were recorded by the two sensor systems. The Normalized Difference Vegetation Index (NDVI) was used as standard index to compare and analyze the different spectra. Furthermore, as a practical application their ability to detect foliar symptoms of Esca is in progress.

**Keywords:** disease detection, hyperspectral, multispectral, phenotyping, sensors

## Mechanisms and candidate genes for seed and fruit set in grapevine

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Fruit setting and seedlessness underpin grapevine yield and quality, with the latter being especially appreciated by the table grape consumer. Consequently, their control is a major breeding objective. Most seedless cultivars exhibit the Sultanina-derived stenospermocarpy, for which substantial advances have been made in the comprehension of the underlying molecular mechanisms, whereas different sources of seedlessness have been much less investigated and exploited. With the aim of providing additional insights into the regulation of seed/fruit formation, we explored the germplasm collections at FEM and CNR-IPSP searching for clones with contrasting seed content. In total, we identified nine variant pairs that differ only in those characteristics related to the presence of seeds while showing identical genetic profile at several microsatellite loci. We report their phenotypic and molecular characterization, as well as multi-year observations on fruit and seed set upon different pollination treatments, with special emphasis on the Sangiovese/Corinto Nero pair. Our morphometric data suggest that stenospermocarpy is not restricted to Sultanina-derived cultivars. The seedless phenotype of the false Corinto Nero is potentially driven by pollen and/or embryo sac defects, as supported by microscopic analysis of gametophytes, by genotyping/ploidy analysis of seedlings derived from embryo rescue and by differential gene expression with respect to Sangiovese. Moreover, three genotypes, including Sangiovese/Corinto Nero, were unexpectedly found to develop fruits without pollen contribution and occasionally showed normal-like seeds. In the search for structural variation each seedless mutant was compared to its seeded reference variety by using the GrapeReSeq\_Illumina\_20K\_SNP\_chip and a RNA-Seq dataset. Identified polymorphisms are suitable to be tested as diagnostic markers in clone identification and as functional candidates for the seedless phenotype.

**Keywords:** berry, flower, reproductive development, seed, single-nucleotide polymorphism, somatic variation, transcriptome

## Characterizing the intra-varietal genomic and phenotypic variation in *Vitis vinifera* L. cv. Malbec

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Grapevines for wine production are clonally propagated through asexual cuttings. Nonetheless a notorious phenotypic variability has been reported at the intra-varietal level for several cultivars, which is attributed mainly to the effect of somatic mutations. Here we studied the case of Malbec (synonym name for the French cultivar Cot), which is the main cultivar for Argentine viticulture. We aimed to characterize the existing genomic and phenotypic variation among Malbec clones, with the further objective of developing a molecular-based workflow to classify clones showing phenotypes of interest. To assess the genomic variation of this cultivar we re-sequenced four Malbec clones at a ca. 35x depth. We employed bioinformatic algorithms to align our sequences to the reference genome (PN40024), perform variant calling for single nucleotide variations (SNVs) discovery and apply quality filters to determine a set of reliable SNVs. We discovered ca. 2.6 million SNVs, that distinguish cv. Malbec from the reference genome. We also identified between 22 and 29 thousand SNVs that are unique for each sequenced clone. After assessing the predicted effect on gene expression of the identified clone-specific SNVs, we observed that nearly 60% occurred in non-coding regions. However, 40% of the clone-specific variations occurred both in regulatory and exonic regions, therefore affecting genic expression at some extent. In order to assess the phenotypic variation, we performed biochemical analyses to trace the ripening process and also to quantify berries' polyphenolic content on 27 clones (three plants per clone were analyzed). The latter revealed great variation among clones, at the timing to arrive to the technological maturity (24° Brix for Malbec) and also on the anthocyanin concentration of berries' skins, differences of nearly 50% were found. Therefore, in agreement with the observed for other cultivars, the high degree of phenotypic variation is in concordance with the discovered genome-wide variation.

**Keywords:** clonal variation, genome sequencing, Malbec, NGS

**Energetic aspects of sugar import and malate breakdown in the ripening berry**Charles Romieu<sup>a,\*</sup>, Rezk Shahood<sup>b</sup>, Laurent Torregrosa<sup>c</sup>, Antoine Bigard<sup>a</sup><sup>a</sup> INRA 2 Place Viala, bat. 21, 34060 occitanie Montpellier, France<sup>b</sup> General Commission for Scientific, Agricultural Research, Lattakya, Syria<sup>c</sup> Supagro, 2 Place Viala, DAAV bât 21, 34060 Montpellier cedex, France\* **Presenting author** : charles.romieu@inra.fr

Phloem unloading of sucrose suddenly accelerates during berry ripening, when hexoses replace malic acid as the major vacuolar osmoticum. This acceleration would involve a shift from the symplastic to the apoplastic pathways of phloem unloading and was associated with multifaceted transcriptomic changes regarding water and sugar transporters expressed at the tonoplast and plasma membranes. Exhaustive analysis of thousands berries allowed us to quantify the fluxes of hexoses, malic acid and water inside the individual fruit. These fluxes were compared with O<sub>2</sub> respiratory demand, that was measured in real time on fruits importing sugars on the plant. These approaches showed that (a) water and sugar accumulation stop simultaneously at the end of ripening, and (b) respiration decreases simultaneously with the vacuolar malic acid content, but is unaffected by the actual rate of hexose import. The simplest interpretation is that the huge activation of sugar loading in ripening berries does not involve accelerated turnover of oxidative phosphorylation, which apparently rules out that bulk energization of H<sup>+</sup>/sugar symporters at the plasma membrane is needed in this process. The rate of malic acid breakdown appears quantitatively consistent with the activation of H<sup>+</sup>/sucrose antiporter on the vacuolar membrane, in agreement with transcriptional activation of *VvHT6* (VIT\_18s0122g00850) and the function of its closest ortholog in *Arabidopsis* (5,6). However, another transport mechanism must be activated when sugar entry continues after the completion of malate breakdown. Noticeably, the berry becomes particularly prone to aerobic fermentation during this late ripening period. The sugar accumulation pathway is clearly energetically optimized in grape berry. Sampling conditions must be critically evaluated in order to elucidate the precise interaction between malate breakdown and sugar loading and decipher its plasticity with respect to genotypexenvironment interactions.

**Keywords:** energetics, malate, respiration, ripening, sugar, transport, vacuole

**Breeding for cold-resistant seedless grapes using embryo rescue**

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Most seedless grape cultivars belong to *Vitis vinifera* L. and have small berries and poor resistance to cold and fungal disease. The aim of this study was to breed new seedless grapes with cold-resistance by embryo rescue. Four stenospermocarpic seedless grape cultivars ‘Flame Seedless’, ‘Ruby Seedless’, ‘Qinhong-2’, and ‘Qinhong-10’ (*V. vinifera*) were used as female parent, and one cold-resistant interspecific hybrid ‘00-1-5’ (*V. vinifera* × *V. amurensis*) was used as male parent. Hybridization was carried out between the seedless grape cultivar and ‘00-1-5’, respectively. The time of embryo abortion of different female parents was different, immature berries were collected for several successive weeks after pollination. The berries were surface sterilised with 75% (v/v) ethanol and 1% (v/v) sodium hypochlorite solutions, respectively. Then, they were dissected and the ovules were cultured *in vitro* in ER (Emershad and Ramming) medium, which was improved as a two-phase (solid phase and liquid phase) medium. After ovule dark culture for 8 weeks, solid WPM (Woody Plant Medium) was used for embryo germination. A total of 332 new hybrids progenies from 4 cross combinations were obtained by embryo rescue. In order to ensure the survival of each hybrid, subculture *in vitro* was performed. The stronger plantlets were selected for acclimation and transplantation in greenhouse. Adhering gel was washed away using sterile water, each plantlet was transplanted to a paper cup filled with a synthetic soil mix and watered with distilled water. The plantlets were each covered with a larger transparent plastic cup and kept in a greenhouse with natural daylight for acclimation. Finally, surviving plants were transplanted to the field in spring. Our next job is to identify these seedlings with cold resistance.

**Keywords:** breeding, cold-resistance, embryo rescue, seedless grape

## Searching for black rot resistance in a wide *Vitis* germplasm

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*Guignardia bidwellii*, the causal agent of Black rot disease in grapevine appeared in Europe in 1885, shortly after mildews. European grapevine cultivars are highly susceptible to this fungal pathogen, however until the latest years this disease had only local importance in humid vine growing regions. With the reduction of chemical plant protection which aimed to control mildews but at the same time also combat Black rot and changing weather conditions more favourable for *G. bidwellii*, this pathogen became economically important. In the last decade serious outbreaks of Black rot epidemics have been reported from most European wine growing regions. The ambition to cultivate grapevine in low input systems with reduced chemical plant protection requires varieties with Black rot resistance. However, till nowadays this pathogen was not in the target of European resistance breeding programs. The aim of this work was to screen a large scale of *Vitis* genotypes for Black rot resistance and select appropriate sources for breeding. Altogether 221 accessions, interspecific *Vitis* hybrids originating from Black rot resistant North American *Vitis* spp, *V. amurensis* x *V. vinifera* hybrids, a set of *V. vinifera* varieties indigenous in Georgia and a breeding population created from Black rot resistant hybrid 'BR16', have been evaluated for disease resistance. Parallel tests for leaf and berry resistance concluded that symptoms detected on leaves are not always in agreement with symptoms detected on berries. 'Csillám' the offspring of 'Seibel 4643' and 'Blaufrankish' stand alone with its symptomless Black rot resistance. 4 other accessions: 'Seyval blanc', 'Merzling' and *V. amurensis* x *V. vinifera* F2 hybrids '5-11-6' and '5-10-6' have been selected for high level resistance. Few genotypes had medium level resistance and the majority of tested accessions were evaluated sensitive-very sensitive.

**Keywords:** germplasm screening, grape black rot, grapevine resistance breeding, *Guignardia bidwellii*, sustainable viticulture

## Grape genetic resource and researches at Zhengzhou National Grape Germplasm Repository of China

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China is one of major centers of diversity of *Vitis* species, and therefore hosts one of the most abundant sources of *Vitis* germplasm in the world. Chinese wild grapes possess several important traits resistant to biotic and abiotic stresses, such as cold, drought, pests and diseases, which are valuable for grape breeding. There are 39 species, one subspecies and 14 varieties of wild grapes native to China. Zhengzhou National Grape Germplasm Repository (ZNGGR) is a part of Chinese Crop Germplasm Resource Information System (CGRIS). It aims to collect, preserve, characterize and distribute grape germplasm resource. It is one of the largest domestic grape germplasm repositories in China. More than 1,400 grape accessions including 46 *Vitis* species are collected and preserved, which are from France, USA, Japan and other 33 countries. Passport data and morphological characterization of the accessions and digital imaging have been initiated. Genetic fingerprinting have been carried out using 9 SSR markers following the *Vitis* International Variety Catalogue (VIVC). Genomic and bioinformatic research methods are used to resolve and explore the molecular genetic regulatory mechanism of resistance, quality, yield and other important agronomic traits. Efficient molecular breeding technologies are developed based on the latest achievements of molecular biology. Nine table grapes (Chaobao, Guiyuan, Zhengmei, Hongmei, Qingfeng, Zhengpu No.1, Zhengpu No.2, Brilliant seedless and Zhengyan Seedless) and two rootstocks varieties (Kangzhen No.3 and Kangzhen No.5) were bred. Among them, Kangzhen No.3 and Kangzhen No.5 were highly resistant to phylloxera, root knot nematode, salinity and have been planted in multiple places in China.

**Keywords:** breeding, genetic fingerprinting, genetic resource, repository germplasm

## Search of wild-growing forms of grape in the Crimean mountain forests and their characteristic of diversity using microsatellite loci

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Viticulture in Crimea goes back thousands of years. It is assumed that some of the autochthonous Crimean varieties originate from local wild-growing grape forms *V. vinifera* ssp. *silvestris* Gmel. Forested areas of Crimean mountains were examined in 2015-2017 in order to identifying habitats of wild growing grapes. The total track made 300 km. The discovered vine plants primarily grew at 600 m above sea level, in areas with sufficiently moisturized soil. Places of wild forms detection were described and placed on a map-scheme of routes using a GPS-navigator. A total of 260 wild-growing lianas were detected. Image-documentation of a shoot, leaf, inflorescence and cluster (if available) was created. The composition of wild-growing grapes was for the most part heterogeneous. We identified samples with staminate flowers, as well as different leaf blade form, lobing degree and tomentosity. The analysis of samples using 9 nuclear (nSSR) and 3 chloroplast (cpSSR) microsatellite loci was carried out to characterize the genetic diversity. Separation of microsatellite fragments was performed on ABI 3130 genetic analyzer. The standard genetic parameters were calculated using the POPGENE software (v.1.32). As a result, allelic polymorphism was studied of 41 samples from Yalta (pop1) and Alushta (pop2) regions. In the total sample, 77 alleles were detected, 65 of them in the pop1, 59 in the pop2. The share of polymorphic loci was 100%. The average number of alleles / loci was 8.56 (7.2 and 6.6, respectively), the effective number of alleles (ne) was 3.6 and 3.4, the information index of Shannon (I) was 1.5 and 1.4. A large number of rare alleles was noted (25.4%). The average value of the Wright fixation index  $F_{is}$  = 0.107.

**Keywords:** allelic polymorphism, genetic diversity, SSR, *Vitis vinifera* spp. *silvestris* L.

**Acknowledgements:** this research is being carried out with the support of the RFBR, grant n°15-29-02715.



## Extended diversity analysis of cultivated grapevine *Vitis vinifera* with 10K genome-wide SNPs

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Grapevine is a very important crop species that is mainly cultivated worldwide for fruits, wine and juice. Identification of the genetic bases of performance traits through association mapping studies requires a precise knowledge of the available diversity and how this diversity is structured and varies across the whole genome. An 18k SNP genotyping array was evaluated on a panel of *Vitis vinifera* cultivars and we obtained a data set with no missing values for a total of 10207 SNPs and 783 different genotypes. The average inter-SNP spacing was ~47 kbp, the mean minor allele frequency (MAF) was 0.23 and the genetic diversity in the sample was high ( $H_e = 0.32$ ). Fourteen SNPs, chosen from those with the highest MAF values, were sufficient to identify each genotype in the sample. Parentage analysis revealed 118 full parentages and 490 parent-offspring duos, thus confirming the close pedigree relationships within the cultivated grapevine. Structure analyses also confirmed the main divisions due to an eastern-western gradient and human usage (table vs. wine). Using a multivariate approach, we refined the structure and identified a total of eight clusters. Both the genetic diversity ( $H_e$ ,  $0.26 \pm 0.32$ ) and linkage disequilibrium (LD,  $28.8 \pm 58.2$  kbp) varied between clusters. Despite the short span LD, we also identified some non-recombining haplotype blocks that may complicate association mapping. Finally, we performed a genome wide association study that confirmed previous works and also identified new regions for important performance traits such as acidity. Taken together, all the results contribute to a better knowledge of the genetics of the cultivated grapevine.

**Keywords:** association studies, genetic diversity, genetic linkage disequilibrium, structure, parentage analysis, SNP

**Resistance to *Plasmopara viticola* ' hyperspectral investigations**Rebecca Höfle<sup>a,\*</sup>, Anna Kicherer<sup>a</sup>, Andreas Backhaus<sup>b</sup>, Reinhard Töpfer<sup>a</sup><sup>a</sup> Julius Kühn-Institut, Institute for Grapevine Breeding, Geilweilerhof, 76833 Siebeldingen, Germany<sup>b</sup> Fraunhofer Institute, for Factory Operation and Automation IFF, Biosystems Engineering, Sandtorstr. 22, 39108 Magdeburg, Germany\* **Presenting author:** rebecca.hoefle@julius-kuehn.de

Downy mildew caused by the oomycete *Plasmopara viticola* is a devastating disease in viticulture, which causes high economical losses. Two principal approaches could bring a solution: fungicide applications or resistant cultivars. On the long term, only the combination of both approaches will bring a sustainable solution as currently massive applications of fungicides to avoid crop losses show environmental impact, and fungicide resistance in *P. viticola* populations occurs. Increasing acreage of resistant cultivars similarly is expected to result in the selection of resistant races. As a consequence low fungicide application and resistant cultivars carrying stacked resistance loci should be the future goal for viticultural practice. Therefore, the introgression and combination of different resistance loci is a major task in grapevine breeding programs.

While genotyping methods based on marker assisted selection (MAS) are well established and fast methods to select resistant genotypes in the offspring of crosses exist, the necessary phenotyping is still time-consuming, labor-intensive and subjective. Therefore faster and objective phenotyping methods are needed to try to distinguish the phenotype caused by different resistance loci.

The objective of the present study was the testing of an evaluation method of responses to *P. viticola* based on hyperspectral imaging of (1) susceptible genotypes (2) genotypes carrying one resistance locus and (3) breeding lines with two or three combined resistance loci, respectively.

To achieve this aim, leaf disc bioassays with 28 different genotypes were implemented and hyperspectral images were recorded daily. Hyperspectral data were referenced by visual and microscopic assessments.

Hyperspectral images contain beside spatial information, reflection properties of objects in the spectral range from 400 nm to 2500 nm. For detection of changes in leaf tissue constitution due to an infestation or alternatively a resistance reaction against *P. viticola*, the spectral reflectance in near (NIR) and shortwave infrared (SWIR) is suitable. In ongoing data modeling, phenotypic differences between resistance responses due to different resistance loci should be detected in a reliable and repeatable manner. The results of the present work are the basis for further investigations of sensor-based phenotyping of *P. viticola* responses.

**Keywords:** downy mildew, hyperspectral imaging, phenotyping

# **Over-expression of the grapevine transcription factor, VlbZIP30, in *Arabidopsis thaliana* enhances drought tolerance via the abscisic acid core signaling pathway**

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Drought stress limits the growth and development of grapevines, thereby reducing productivity, but the mechanisms by which grapevines respond to drought stress remain largely uncharacterized. The basic region/leucine zipper (bZIP) transcription factors are known to play key roles in response to abiotic stress. Here, we characterized a group A bZIP gene from 'Kyoho' grapevine, *VlbZIP30*, which was shown to be induced by abscisic acid (ABA) and dehydration stress. Overexpression of *VlbZIP30* in transgenic *Arabidopsis thaliana* enhanced dehydration tolerance during seed germination, and in the seedling stage. Various physiological parameters related to stress responses were analyzed to gain further insight into the role of VlbZIP30 and it was found that osmotic stress caused less damage to the transgenic seedlings than to the corresponding wild type plants. This correlated with an increase in endogenous ABA content as a consequence of the constitutive overexpression of *VlbZIP30*, and the up-regulated expression of stress-inducible target genes associated with tolerance of dehydration stress. Transcriptome analysis revealed that a major proportion of ABA- and/or drought-responsive genes are transcriptionally regulated by VlbZIP30 during ABA or mannitol treatment at the cotyledon greening stage. The RNA-seq results were confirmed by qRT-PCR. We propose that VlbZIP30 functions as a positive regulator of drought-responsive signaling in the ABA core signaling pathway.

**Keywords:** ABA, drought stress, RNA-seq, VlbZIP30

## VITIRAMA: a program to characterise disease susceptibility in French ampelographic collections

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The main objective of VITIRAMA (2018-2020) is to better characterize the genetic resources in the main French ampelographic collections for their exploitation in the short and medium term. The program deals with the tolerance to major diseases directly impacting grape production (*V. vinifera* and interspecific hybrids): downy and powdery mildews, grey-mould and several trunk diseases or causing decline (*Phomopsis* cane and leaf spot, *Eutypa* dieback, Esca disease and Black Dead Arm). The collections involved are those of INRA Vassal-Montpellier, INRA Colmar, IFV Pôle Matériel Végétal and IFV Vinnopôle Sud-Ouest.

The objectives are i) to organise already existing data and to acquire new or complementary information on diversified genetic resources (field phenotyping) and ii) to make available a synthetic knowledge to the grape growers (viticulturists and nurseries), the breeders and the scientific community. The obtained data, compiled, harmonized and analysed would be useful for:

- documenting and advising growers about variety performances,
- helping breeders to design their mating designs taking into account original and complementary germplasm less susceptible to several pathogens,
- establishing study panels, composed of diversified and relevant genetic resources, for fundamental research on the genetic determinisms related of main disease resistance traits.

**Keywords:** genetic resources, phenotyping, phytopathology, resistance, *Vitis*

## The functional analysis of the highly expressed stilbene synthase genes from Chinese wild *Vitis quinquangularis*

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Grapevine ranks the top three of the important fruit trees in the world, due largely to its economic benefits from wines, table grapes, grape juice and raisin. *Vitis vinifera* cultivars yield the main production in grapevine. They have high quality and various utilizations, and resveratrol content plays an important role in human health. However, most of them are susceptible to *Uncinula necator*, and the fungicide cost of preventing powdery mildew exceeds 3 billion dollars every year only in America. Therefore, the key to improve the resistance of these cultivars is to make use of grapevine germplasm resources with high resistance to the disease. This work focused on Chinese wild grapevines with both high resistance to the disease and high resveratrol content, and involved analyses of transcriptome, proteome and metabolome during the grape fruit development. The studies relate to resveratrol accumulation, including the biology study, regulation mechanism, molecular breeding technique and germplasm innovation; and the functional identification and analysis of the important genes related to resveratrol accumulation. The biological process of resveratrol accumulation and gene expression were investigated from blooming to fruit maturity in 13 Chinese wild grapevines and 4 *V. vinifera*. From *V. quinquangularis*, 23377 expressed genes were identified, including 1500 specific genes; 48 stilbene synthases were expressed at veraison and fruit maturity stage; 3751 proteins were also identified, including 578 proteins with differential expression; 203 metabolic substances were investigated, including resveratrol, piceid, pterostilbene and viniferin. Stilbene synthase gene *VqSTS6* with high expression in berry was transformed into *V. vinifera* cv. Thompson Seedless to identify stilbenes and its resistance to the disease. VqDUF642 protein is involved in fruit development and improved resistance to the disease and gray mold. *VqSTS21*, *VqSTS30* and *VqSTS32* were induced by powdery mildew and abiotic stresses. In summary, the disease-resistance genes from Chinese wild grapevines were used to improve the resistance of *V. vinifera*. Therefore, the results obtained are relevant for improving the resistance of cultivars and increasing resveratrol content based on Chinese wild grapevines.

**Keywords:** chinese wild *Vitis* species, disease resistance, genetic transformation, resveratrol, stilbene synthase gene

## Panel-C4, a grapevine core collection designed for climate change studies

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Over the past 20 years, progress in the field of grapevine genetics has led to the identification of a number of QTLs and few genes that are mainly involved in diseases resistance and berry quality. Now, the challenge for the future of climate change has become strategic. However, only few results have made it possible to progress in the determination of climate adaptation characteristics that can be used in breeding. To contribute to develop more relevant research in this field, we present here a set of cultivated varieties (*V. vinifera* subsp. *vinifera*) selected in the large ampelographic collection of Vassal-Montpellier through a multi-factorial approach. The aim is to use them as a basis for genetic research such as GWAS to better understand mechanisms of varieties adaptation to climatic factors (temperature, water use, etc.) and to discover selection indicators that can be used for grape breeding. To be more flexible according to the needs and the means of the different research programs and methods, this “Core Collection for Climate Change” (or Panel-C4) is composed of 3 nested sub-panels of 50, 100 and 200 *V. vinifera* cultivars respectively. To establish and optimize this panel, varieties have been selected taking into account several traits and parameters (phenology, length of growing cycle, temperature reaction, water supply behavior, potential for berry sugar accumulation and acidity, geographical origin, genetic diversity, limited relatedness, bibliographical data and personal expertise) with the aim to maximize the diversity and to only retain the most significant genotypes.

**Keywords:** diversity, germplasm, global warming, sampling, *Vitis vinifera*

## Ancient DNA of grape seeds provides insights into viticulture and cultivation practices in Roman Gaul and Medieval France

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The grapevine (*Vitis vinifera* subsp. *vinifera*) was one of the earliest fruit crops to have been domesticated and has since played an important role as food staple and for wine production. Despite being clonally propagated, present-day grape cultivars hold high levels of morphological and genetic diversity, with thousands of varieties that have been described. Some of these varieties can be traced as far back as the Middle Ages, based on morphological descriptions and historical records. However the true genetic relationship between earlier grapes and modern varieties remains unknown.

Exploiting a newly developed DNA approach, we explored identities and genetic relationships of grape cultivars across times, by assembling a dataset of ancient and modern samples from France, a main grape growing region that played an important role in grape history. Specifically, we performed targeted-high-throughput sequencing of ten thousand Single Nucleotide Polymorphisms, on 28 grape seeds from archaeological sites and layers dated to the Roman and Medieval periods. We compared these ancient samples to a large panel of present-day wild and domesticated varieties, and were able to assign them to a defined geographic cluster of domesticated grapes. One archaeological sample dating to 1050–1200 Current Era, was found to be an identical clone of ‘Savagnin’, a variety cultivated to-day for wine in Northern France. In addition, our analyses identified 16 different ancient cultivars and resolved their genetic relationships with grape varieties modernly used in West Europe for winemaking. We did not find genetic evidence of large-scale use of wild genetic resources by Romans or Medieval people at these sites, supporting an earlier hypothesis that even though many pips look morphologically wild, they in fact originate from domesticated varieties. Furthermore, we show genetic evidence of clonal propagation over at least one thousand years.

**Keywords:** archeogenetics, DNA capture, domestication, history, viticulture

**Acknowledgment:** this work was co-funded by the National Research Agency of France, project ANR-16-CE27-0013 Viniculture.

**QTL-analysis of organic acids**Florian Schwander<sup>a,\*</sup>, Eva Zyprian<sup>a</sup>, Reinhard Töpfer<sup>a</sup><sup>a</sup> *Julius Kühn-Institut, Institute for Grapevine Breeding, Geilweilerhof, 76833 Siebeldingen, Germany*\* **Presenting author:** [florian.schwander@julius-kuehn.de](mailto:florian.schwander@julius-kuehn.de)

Organic acids are key factors for wine quality: they protect berries from spoilage in the vineyard, stabilize the vinification process and play an important role in sensory perception during wine consumption. Breeding of new grapevine varieties with adjusted acidity levels for the different growing regions is becoming one of the major challenges considering the ongoing climate change. The expression of this trait is strongly influenced by environmental factors and its assessment is very time-consuming. This is in particular true for northern wine growing regions with cool and inconsistent climatic conditions. Thus, evaluation to identify the genetic base to develop reliable molecular markers for marker-assisted selection (MAS) needs a detailed data acquisition for several years. A F1-cross population with 150 individuals between the breeding strain GF.GA-47-42 ('Bacchus' x 'Seyval') and 'Villard Blanc' differing in acidity levels and ripening was investigated in detail for seven years (2011-2017). The acidity profile was recorded by FTIR analysis between veraison and harvest under field conditions at Geilweilerhof (Palatinate, Germany). To exclude the differential ripening behavior of the individuals, data were equilibrated by sugar content or temperature sums and subsequently subjected to QTL-analysis in order to identify genetic regions with major involvement in acidity regulation. Investigated traits include total acidity, tartaric and malic acid levels as well as pH values and potassium quantities in musts and wines. Tartaric and malic acid are the two most important organic acids in grapes and have major impact on acidity perception of wine. Potassium regulates the acidity level by precipitation of tartar (potassium hydrogen tartrate) and is relevant for a full-bodied wine perception. QTLs identified in an extensive dataset of seven growing seasons give new insights into the key genomic regions influencing acidity characteristics of grapevine cultivars.

**Keywords:** acidity profile, climate change, genetic mapping, molecular marker, wine quality



## Evaluation of an automated 3D based phenotyping pipeline for grapevine bunches to determine bunch architecture traits

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Grapevine breeders focus on loose bunch architecture as a trait increasing resilience against Botrytis bunch rot. Bunch architecture is highly complex consisting of different sub-traits. Phenotyping of such a complex trait requires trained personnel and is very labor-intensive. Thus, the number of plants evaluated during an ongoing season is limited by working load. In addition, data are restricted by subjectivity with an unpredictable error variation.

To overcome such problems we initiated a study to develop a sensor based, fast and reliable phenotyping pipeline. The optical sensor Artec Spider 3D scanner was used to generate dense three-dimensional (3D) point clouds of grapevine bunches under lab and field conditions. The automated software '3D-Bunch-Tool' was developed in order to extract a set of single 3D bunch traits (e.g. number of berries, berry diameter, total volume of berries, bunch width and bunch length). Precision and reliability of the pipeline was validated on a subset of different grapevine cultivars ('Riesling', 'Calardis Blanc', 'Pinot Noir' and 'Dornfelder') and more than 150 genotypes of a morphological variable mapping population of GF.GA-47-42 ('Bacchus' x 'Seyval') x 'Villard Blanc'. Statistical analysis showed reliable phenotypic precision with highly significant correlations (up to  $r^2 = 0.94$  e.g. for berry number) compared to reference data. In a mapping approach the 3D phenotyping pipeline revealed identical QTL regions comparable to the reference data.

The Artec Spider 3D sensor was further applied directly in the field. The phenotypic data generated showed comparable precision in contrast to the lab application. The non-invasive and non-contact usage in the field provides the basis for fast and high-precision field phenotyping approaches to determine bunch architecture traits of large sets of plants.

**Keywords:** *Botrytis*, bunch compactness, grapevine phenotyping, Organization of Vine and Wine (OIV) descriptor 204, sphere detection

**Pedigree ascertainment of interspecific grapevine cultivars**Thierry Lacombe<sup>a,\*</sup>, Valérie Laucou<sup>a</sup>, Jean-Michel Boursiquot<sup>b</sup><sup>a</sup> INRA, UMR AGAP, Université Montpellier, UMT Géo-Vigne, 34060 Montpellier, France<sup>b</sup> Montpellier SupAgro, UMR AGAP, Université Montpellier, UMT Géo-Vigne, 34060 Montpellier, France\* **Presenting author:** thierry.lacombe@inra.fr

Parentage of *V. vinifera* cultivars has been widely studied, both to discover the origins of traditional cultivars and to check the genitors of recent ones. However, few studies exist for *Vitis* interspecific hybrids (i.e. producer hybrids and rootstocks) although the scientific interest is obvious: following-up the origin of certain genes of resistance through complex pedigrees with the genealogy data. The origin of interspecific hybrids is historically more recent than that of *V. vinifera* cultivars and related to the introduction of viticulture in Northeastern America and especially to the arrival of American diseases in Europe at the end of the 19th century. Thus, in many cases, we know the breeders who made the modern crosses and the names of the parents they said they used. This is why the study of genealogies of hybrids is more to validate (confirm or invalidate) the parents announced by the hybridizers. The objective of our study was to carry out such large-scale verification by analysing the hybrid cultivars from the INRA collection of Vassal-Montpellier (850 cv. producers and 250 cv. rootstocks) previously identified on the basis of morphological and passport data. The methodology used for this validation is the same as the one previously used by our team on *V. vinifera* cultivars: genotyping using 20 nSSR, analyse using FaMoz software and integration of bibliographic data. The results show a variable rate of confirmations according to the periods and the breeders. Invalidations mainly concerns parents used as fathers in crosses. Finally, one of the problems encountered in this work is the absence of the intermediate genitors used in complex mating designs, which have not generally been preserved in ampelographic collections.

**Keywords:** genealogy, hybrids, parentage, rootstocks, varieties, *Vitis*

## Development of genome editing in grape

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The CRISPR/CAS9 technology has become the dominant genome editing technology after recent demonstration of its efficacy in eukaryotic cells. Beyond its obvious interest in biology, recent results have demonstrated its considerable advantages as a new plant breeding tool.

In this context, our objective is to evaluate the efficiency of this technology in grape. In this purpose, we have chosen to knockout the *VvGAI-1* gene in order to produce dwarf grapes. This phenotype facilitates the identification of transformed plants, since it is visible at a very early stage of plant regeneration. Two strategies will be developed. In the first one, we will remove the DELLA domain, by using two sgRNA cloned in a multiplex vector. And in the second, we will introduce a single point mutation, by using a DNA repair sequence to produce a DELHA domain. The optimization of these two strategies will first be conducted in grape protoplasts. Stable and/or transient transformations will then be carried out and analyzed. Beyond the gene targeted in this work, we also hope to collect more general knowledge that may be useful for the routine use of this technology in grape breeding.

**Keywords:** CRISP-CAS9, genome editing, methodology, VvGAI-1

## Prolonged ripening on the vine affects the polyphenolic profile of grapes and wine 'Plavac mali' (*Vitis vinifera* L.)

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Prolonged grape ripening beyond phenolic and technological ripeness is a common practice in Mediterranean vineyards. It is believed to increase overall wine quality, although the chemical composition of those wines is poorly understood. This vineyard practice is common in the Mediterranean part of Croatia where 'Plavac mali' is widely grown to produce premium red wines. The aim of this study was to quantify the compositional differences in polyphenolic profile, and physicochemical parameters of grapes, and corresponding wines of 'Plavac mali' at five different harvest dates. Ethanol concentration was in the range of 9.9 to 14.5 %. Prolonged ripening enhanced anthocyanin, and flavonol concentration in skin tissue, with the exception of 3-O-glucoside of delphinidin, petunidin, quercetin, and 3-O-rutinoside of quercetin. Various trends in skin low molecular weight phenolic compounds were detected: increase in (+)-gallo catechin and (-)-epicatechin, and decrease in the others ((+)-catechin, (-)-epigallocatechin, and proanthocyanidins (B1, B2, B3, B4)). Grape seeds reached stable concentration of the low molecular weight phenolic compounds in 3rd harvest date, while prolonged ripening had a significant influence on (-)-epicatechin, and (-)-epigallocatechin. In contrast, in corresponding wines anthocyanins concentration had two peaks in 3rd and 5th harvest date, without any significant difference between those two, except in peonidin-3-O-glucoside. Prolonged ripening resulted in wines with high concentration of (+)-gallo catechin, (-)-epicatechin, and the lowest concentration of the (-)-epigallocatechin. Various trends were detected for flavonols and phenolic acid compounds with regard to cofactor color activity. Grape reached phenolic maturity in 3rd harvest date. Phenolic composition of 'Plavac mali' can be improved with the practice of prolonged ripening. Negative effects of prolonged grape ripening should also be taken into account such as: decrease in grape yield, primary amino nitrogen, total acidity, increase in sugars and pH. The results indicate good potential of 'Plavac mali' for prolonged grape ripening but negative aspects should also be considered.

**Keywords:** anthocyanins, (+)-catechin, flavonoids, Mediterranean climate, postharvest overripeness, proanthocyanidins

## Integrating spatial variations in the vineyard to enhance QTL detection

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French vineyards are often located on the least fertile lands, generally more heterogeneous than lands devoted to food crops. The detection of quantitative trait loci (QTLs) is all the more precise as the number of genotypes studied is high, but increasing the size of experimental plots means taking the risk of exploring more spatial heterogeneity. Designing randomized blocks experiments is a classical way to overcome this difficulty. However, when several hundred of genotypes are studied, this not only requires more land but is also time consuming. Another approach is to take into account the spatial heterogeneity when searching for QTLs. We will present examples of QTL detection in the progeny of a Riesling (RI) x Gewurztraminer (GW) cross. Three hundred and eighty-three genotypes of this progeny are planted in the INRA experimental vineyard located in Alsace (France). They are planted in elementary plots (EP) of three plants, and are not replicated. The parent genotypes, Riesling and Gewurztraminer, are also planted in EPs of three plants but these EPs are replicated 12 and 13 times, respectively, with a regular spatial distribution. RI and GW are also present in the buffer rows surrounding the experiment. Kriging methods were used with the data obtained for the parent varieties to build the map of the parental expected value for a trait for each EP of the experiment. QTL detection was subsequently performed on raw values recorded for each genotype but also on the differences between the raw values and the parental expected values for each EP. Examples of QTL detection will be presented for  $\delta^{13}\text{C}$  of the berries at harvest, related to stomatal closure and response to drought stress, as well as for characteristics of the canopy evaluated by MULTIPLEX® RESEARCH, a portable field fluorometer providing indices related to anthocyanins, flavonols, chlorophyll and nitrogen balance of the plants.

**Keywords:** grapevine, spatial variations, QTL, vineyard

## A multi-level modelling framework for simulating grape growth and biochemical compositions under genotype x environment interactions

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The growth and quality of grape berries are regulated by both biophysical and metabolic processes, which are influenced by genotype x environment interactions. Growth is mainly determined by the balances of water and carbon fluxes under the control of biophysical laws, while grape quality mainly refers to berry chemical composition, including sugars, organic acids, phenolics, and other aroma compounds that are controlled by various enzymes. Mathematical models can mechanically integrate various processes to reproduce the fruit responses to climatic conditions and management practices, making them a promising tool.

At the fruit level, we developed a biophysical model for berry growth, an enzyme-activity based kinetic model for sugar metabolism and accumulation, a metabolic model for anthocyanin composition, and a thermodynamic model for organic acids. At the whole-plant level, 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. In addition, we initiated the integration of the biophysical growth model with the enzyme-based kinetic model, in order to investigate the regulation and/or coordination between grape biophysical properties and metabolic activities over fruit development. These results will allow the creation of a multi-level modelling framework to better predict grape growth and quality in changing environments.

**Keywords:** fruit quality, grapevine, growth modeling

## Virome status of old Slovenian grapevine varieties as determined by small RNA deep sequencing

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The aim of our project was to identify collections of viruses that affect grapevine production in Slovenia, focusing on old plants of autochthonous Slovenian varieties. As a method of choice, we chose small RNA sequencing technology because it is a highly efficient technology for virus identification and discovery. These small RNAs, which cover frequently the whole genome of the infectious agent, are 21–24 nt long and are known as vsRNAs for viruses and vdRNAs for viroids. Small RNA (sRNA) fraction has been shown to be the most promising template for preparations of NGS libraries. The isolation was done by enrichment procedure using the mirVana™ miRNA Isolation kit, which enables that RNA molecules of ~200 nt and less can be efficiently purified away from the larger RNA species. We analyzed 13 grapevine plants of 6 different cultivars that were infected with viruses as confirmed by routine ELISA tests. *De novo* and reference aided assembly of virus genomes were performed using free, open-source bioinformatics pipeline VirusDetect, that can efficiently analyze sRNA datasets to identify both known and novel viruses. Using a threshold of 0.6 for the ratio between length of consensus and reference sequences, 10 viruses and viroids were identified, namely Arabis mosaic virus large satellite RNA, Grapevine fanleaf virus, Grapevine fleck virus, Grapevine leafroll-associated virus 1, Grapevine leafroll-associated virus 2, Grapevine leafroll-associated virus 3, Grapevine Pinot gris virus, Grapevine satellite virus, Grapevine yellow speckle viroid 1 and Hop stunt viroid. Only Hop stunt viroid was common to all analyzed samples. The NGS analyses were confirmed by Sanger sequencing of RT-PCR products to allow an efficient generation and validation of identified virus and viroid genome sequences. In addition, by applying this strategy we were able to identify differences between Grapevine Pinot gris virus of symptomatic and asymptomatic plants of the cultivar Volovnik. We have shown that NGS analysis of sRNAs is fast, efficient and cost effective tool to determine the virome status of grapevine plants.

**Keywords:** diagnostics, grapevine, NGS, small RNA, *Vitis vinifera*, virus, virome

## Selection for mildew-resistant grape varieties for wine spirit distillation

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Today, it is necessary to reduce the application of phytosanitary products. Alternatives to chemical pesticides need to be explored, particularly concerning protection against yearly vine diseases.

A biological solution was developed and widely used in European vineyards until the 60/70s: resistant vine varieties obtained by crossing *Vitis vinifera* with wild vines. This solution is recently being re-looked at. A. Bouquet (INRA) created and selected (1990-2009) new crossings between *Vitis rotundifolia* (known to be resistant to many aggressors) and *Vitis vinifera*. Thanks to many backcrossings (4 to 6) with *vinifera*, he obtained downy and powdery mildew resistant vine varieties that produce good quality wines. In 2003, the BNIC asked him to cross one of his resistant varieties, chosen for its productivity and high acidity, with Ugni blanc, the main variety used for Cognac production, in order to fit with the defined ideotype of a “vine for wine-based distillation”. This crossing gave 800 grape seeds which were rapidly sorted using recently identified genetic markers. Following the testing for powdery mildew on potted plants growing in the greenhouse, 43 varieties were selected and planted in 2008 in the Cognac vineyard (5 to 10 plants / variety) in order to be phenotyped. A first selection, based on agronomic and viticultural criteria, resulted in 15 varieties being retained. These grapes were then harvested and the musts were microdistilled and analyzed in order to assess precociously their aromatic profile. Microvinification/distillation, analyses and tasting of the wines were then conducted. Finally, 4 varieties were selected using all the collected data, processed with a statistical tool based on the defined characteristics for a distillation wine.

**Keywords:** distillation base wine, ideotype, mildew resistance, phenotyping, quality wine



**Studies on the resistance Rpv10 locus against downy mildew (*Plasmopara viticola*) of grapevine (*Vitis vinifera*)**

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The resistance locus Rpv10 against *Plasmopara viticola* was first described in the cross-population Gf.Ga-52-42 x ‘Solaris’ and is thought to originate from the Asian wild *Vitis* species *Vitis amurensis*. The resistance locus comes from ‘Solaris’. The locus could be fine-mapped by SSR-marker analyses (~84kb). Both the resistant and susceptible allelic variants of this locus were sequenced by chromosome walking and BAC clone screening. Through the present sequence of the two haplotypes comparative bioinformatic analyses were performed. In the Rpv10-locus of both haplotypes, candidate genes and their promoter regions were selected, for amplification and cloning. These will be transformed into susceptible genotypes for functional verification. Comparative RNA-Seq experiments with a selfed progeny of ‘Solaris’ (homozygous Rpv10) and a susceptible F1 individual of the cross-population Gf.Ga-52-42 x ‘Solaris’ were performed. The time point before and 6 hours after inoculation with *P. viticola* were chosen for the analysis. In the evaluation, particular attention was paid to the transcriptional expression of the candidate genes. Microscopic infection studies were carried out in parallel to molecular and bio-informatic studies. These were analyzed using genotypes with the Rpv10-locus compared to susceptible genotypes. After inoculation with the pathogen, different time points were investigated. Thus, the pathogen can be examined in different stages of development during the infection. This allows important conclusions to be drawn about the vine's resistance mechanism to this oomycete.

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

## Genetic and phenotypic diversity of the variety 'Teinturier' and its offspring

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The so-called teinturier grapevine varieties (also dyers) are characterized by a more or less deep red coloration of the complete habitus and especially the berry flesh. Leading to intense dark wines, they were typically used to enhance the color of weaker red wines. Nowadays, teinturier breedings like 'Dakapo', a German cultivar, are also used for making varietal wines. The 'Teinturier' mutant probably originated from the region around Orléans (France) where it was already mentioned in the 17th century. Louis Bouschet, a famous French grapevine breeder, already started breeding of new teinturier varieties with improved viticultural traits in 1824 because of the lower wine quality of the ancestor 'Teinturier'. Based on the breeding efforts of his son Henri, the teinturier variety 'Alicante Henri Bouschet' was selected and shows today with approximately 19.398 hectares the worldwide highest acreage of all teinturier varieties.

The anthocyanin biosynthesis in varieties with colored berries is generally controlled by two adjacent MYB-related transcription factor genes, VvmybA1 and VvmybA2, located on chromosome 2 with indication for the involvement of an ectopic VvmybA1 overexpression as a possible molecular cause of the teinturier phenotype. Hence, the chromosomal region at berry color locus of the teinturier mutation was analyzed in detail. As French ampelographers from the last century described already 'Teinturier' clones differing only in the intensity of the coloration, the influence of clonal variation on the phenotype was evaluated on a large set of different 'Teinturier' accessions and teinturier phenotypic varieties.

**Keywords:** clonal variation, Teinturier, VvmybA

## Phenotyping and genotyping of *Vitis berlandieri* seedling population

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The aim of our study was to characterize and distinguish the individuals of *Vitis berlandieri* Planchon seedling population. Fifty-six seedlings were characterized with 57 OIV descriptors. The 3192 data were analyzed and grouped by principal component analysis. Microsatellite analyses were carried out. Nine nuclear SSR primer pairs VVS2, VVMD5, VVMD7, VVMD25, VVMD27, VMD28, VVMD32, VrZag62 and VrZag79 were chosen based on GenRes081, GrapeGen06 EU projects. Tendril length (OIV 017) was one of the key morphological traits that distinguished the *V. berlandieri* seedlings. In addition, the following descriptors significantly discriminated ( $p = 0.05$ ) the genotypes: intensity of anthocyanin coloration on prostrate hairs of the shoot tip (OIV 003); density of prostrate hairs between main veins on lower side of blade (4th leaf) (OIV 053), number of leaf lobes on mature leaves (OIV 068), blistering on the surface of mature leaves (OIV 075), depth of the lateral sinus on mature leaves (OIV 094); and growth of lateral shoots (OIV 352). Based on the SSR allele sizes, the population of seedlings formed two groups. The VVMD5 and VVMD7 loci were monomorphic. The additional 7 primer pairs generated 30 polymorphic fragments in the 56 samples. We identified the highest allele variation in the VVMD32 and the lowest in the VVS2 and VVMD28 loci. There was no association between the phenotypic and genetic groupings of the seedling population. This lack of association could have been due to strong environmental impacts on morphological differences among the genotypes. Genetic analysis at a finer scale may have been better able to detect these associations.

**Keywords:** diversity, genetic analyses, grape, morphology, microsatellite primers phenotype

**Acknowledgement:** this work/research was supported by the Hungarian Government and the European Union, with the co-funding of the European Regional Development Fund in the frame of Szechenyi 2020 Programme GINOP-2.3.2-15-2016-00054 project.

## Studies on the resistance locus *Rpv12* against downy mildew of grapes (*Plasmopara viticola*)

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*Plasmopara viticola* is the causative agent of grapevine downy mildew, a widespread severe disease. The heterothallic obligate biotrophic oomycete *P. viticola* was imported to Europe in 1878 from North America. Because *P. viticola* causes a high crop loss annually, research and breeding of resistant grape varieties is essential for a sustainable viticulture. Only with precise knowledge of the resistance mechanisms and the genetic location, a targeted breeding is possible to reduce the annual amount of consumed pesticides. In 2013, Venuti et al. identified the resistance locus *Rpv12* using QTL analysis of *Vitis amurensis*. *V. amurensis* is native to the cool climates of the Far East (China and Russia) and shows a resistance against *P. viticola*. In the early 20th century the asiatic *V. amurensis* Ruprecht was crossed with *Vitis vinifera* 'Getsh' ('Michurinets'). Other interesting cultivars are 'Kunbarat' and 'Kunleany'. They possess resistance characteristics due to *Rpv12*. This locus was detected on Chromosome 14 and is inherited independently of other resistance genes. Within the locus *Rpv12* 12 NBS- or NBS-LRR genes (nucleotide binding site – leucine rich repeats) have been identified within the reference genome. An additive effect with *Rpv3* was detected. It confers a foliar resistance to strains that are virulent on *Rpv3* cultivars. For identification of the responsible gene for the resistance, we compare susceptible grapevine with resistant cultivars by leaf disc assay and light microscopy. The aim is to identify physiological responses of the cell. These results should reveal molecular mechanisms and the candidate genes involved, which shall be later evaluated by amplification, comparative sequencing and gene expression analysis.

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

## Role of Auxin-Response Factor 4 (VitviARF4) in the timing of ripening initiation in *Vitis vinifera*

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The timing of the ripening initiation is an important trait for wine grape production. The research project aims to validate the regulatory function of ARF4 in the timing of ripening initiation. The objectives are to: 1) characterize its role by inducing or silencing the gene expression in microvine system and to identify its interacting protein partners during the ripening process, 2) to identify ripening-related genes targeted by ARF4, and 3) to evaluate fruit composition of berries, where the timing of ripening-initiation is altered. Towards this goal, we established the microvines at OSU and optimized different steps of producing embryogenic calli through anther culture, inoculation of calli with agrobacterium harboring the genes of interest, production of transformed embryos, transitioning of these embryos to plantlets, and finally to potting in the green house. We conducted our first trial experiments to engineer the biosynthesis of abscisic acid, an hormone that promotes ripening, using this pipeline and we were successful in obtaining the transformed embryos. Currently, cloning of different plasmid constructs aimed to induce and silence ARF4 in the microvine has been performed. Towards identifying the protein partners of ARF4, we found 170 potential candidates using protein-protein interaction screening assays. They include proteins involved in ABA signaling (VitViPAPA1), sugar sensing (VitviHXXK1), and ethylene signaling (VitviETR4), all of which are known to influence fruit ripening. Finally, we adapted and tested a new analytical method to measure metabolites associated with organic acids, amino acids, phenolics, carbohydrates, polyols, and three classes of flavonoids (anthocyanins, flavonols, and monomer and dimer of tannins), and built an in-house library of 95 analytes including tartrate, malate, glucose, fructose, sucrose, and several polyphenol-related compounds.

**Keywords:** auxin, microvine, ripening initiation

## Developing a model system to identify main mechanisms involved in nitrogen growth responses of grafted grapevines

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The scion growth potential (vigor) in grafted grapevines results from the three-way interactions between environment, scion genotype, and rootstock genotype. Since nitrogen (N) availability is a major driver of grapevine growth, understanding N regulation in scion and rootstock will lead to new insights to control canopy size in vineyards. We are developing a model system to study N regulation by evaluating the N supply responses of 12 scion-rootstock combinations with known differences in scion and rootstock vigor. Our primary objectives are to understand the influence of scions and rootstocks on growth parameters and resource allocation, and to evaluate the role of N uptake regulation in scion growth response. To address the first objective, we measured components of vine water relations and gas exchange, plant biomass, carbon (C), and N allocation in four plants tissues (leaves, stem, trunk and roots). Preliminary results supported the expected vigor behavior of the three Pinot noir scions used in this experiment, but this was not true for the four rootstocks examined. Nitrogen availability altered C and N allocation in all tissues, but scion vigor was not affected. It seems that the N requirement for 1-year old vines was satisfied by our lowest N rate, and the experiment will be repeated under greater N limitation. However, this first trial will allow us to study the role of C and N reserves on scion vigor during the second growing season. We are addressing the second objective by comparing N uptake and N transport among two rootstocks using <sup>15</sup>NO<sub>3</sub>. Several experiments are underway to compare N uptake kinetics over a range of N concentrations and N transport rate in response to plant N status. These analyses will be complemented with gene expression studies targeting the transport and signaling of N in roots and leaves.

**Keywords:** nutrient transport, rootstock scion interactions, vigor

## Identifying potential genetic markers for grapevine salt tolerance

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Salinity is a serious challenge facing viticulture and winemaking, and is caused by high sodium and chloride ( $\text{Cl}^-$ ) concentrations in soils and irrigation water. *Vitis vinifera* is moderately sensitive to salinity, especially to  $\text{Cl}^-$ . Excessive  $\text{Cl}^-$  reduces vine water uptake, causes plant growth inhibition, leaf burn and vine death. High  $\text{Cl}^-$  concentrations in berries lead to wines with salty and soapy tastes, and wines that potentially exceed legal  $[\text{Cl}^-]$  limits. These challenges can be minimised using  $\text{Cl}^-$  excluding rootstocks, but further investigations are required to uncover the genetic mechanisms contributing to this phenotype. Genes in the Aluminium-activated-malate-transporter family *VviALMT2* and *VviALMT8*, and genes in the Nitrate/peptide-transporter-family *VviNPF2.1* and *VviNPF2.2*, were more highly expressed in the good  $\text{Cl}^-$  excluder 140-Ruggeri than in the poor excluder K51-40. Gene expression analyses and *in vitro* functional assays were conducted to evaluate whether these genes have roles in grapevine salt tolerance. RT-qPCR analysis showed that *ALMT2* was more highly expressed in grapevine root stele than cortex under  $\text{Cl}^-$  stress; and when expressed in *Xenopus laevis* oocytes *ALMT2* was most permeable to  $\text{NO}_3^-$ . *NPF2.1* and *NPF2.2* were most highly expressed in roots and leaves, and isotope-flux assays using *Xenopus* oocytes suggested that they potentially transport  $\text{Cl}^-$  and  $\text{NO}_3^-$ . Expression levels of *NPF2.1* and *NPF2.2* in  $\text{NO}_3^-$  starved grapevine roots reduced in response to  $[\text{NO}_3^-]$  resupply. Since *ALMT2* is highly abundant in root stele and transports  $\text{NO}_3^-$ , and *NPF2.1* and *NPF2.2* are likely  $\text{NO}_3^-$  transporters more highly expressed when  $\text{NO}_3^-$  starved, these genes may contribute to maintaining a higher  $\text{NO}_3^-/\text{Cl}^-$  ratio in the xylem, hence contributing to grapevine's  $\text{Cl}^-$  tolerance. Further experiments will functionally characterise these candidate genes *in planta*, and the results may help to develop genetic markers for salt tolerant grapevine breeding.

**Keywords:** gene expression, genetic markers, ion transporters, salt excluding rootstocks, salt stress, salt tolerance

## SelWineQ: Wine quality prediction tools based on a white wine F1 population

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The evaluation of the wine quality potential of new grapevine genotypes is the time limiting factor during the process of breeding and selection. Therefore, the availability of appropriate prediction models for the trait wine quality would accelerate grapevine breeding tremendously. In a first step, a validated and reliable prediction model should give the possibility to early remove genotypes with poor wine quality (negative selection). A training set of a segregating white wine F1 population (150 F1 plants = POP150; GF.GA-47-42 x 'Villard blanc') planted at two locations will be intensively genotyped and phenotyped to gain new insights. The aspects evaluated and relevant for quality along the project are (1) the genetic quality potential (irrespective of the environment), (2) the metabolic quality potential (genotype by environment interaction) of the primary product (juice or must), and (3) the wine quality (analytical and sensory properties). In addition to targeted and non-targeted metabolomics approaches (GC/MS and LC/MS analysis) to evaluate must and wine contents, the wine quality in general is judged by a sensory evaluation of a highly trained and experienced panel. Genotyping and construction of an improved high-density genetic map in combination with the solid phenotypic data of several years are expected to result in tools to predict the quality potential of grapevine seedlings (as a negative selection) and to decrease the time needed for the process of breeding and selection in the future.

**Keywords:** breeding, metabolomics, modelling, negative selection, sensory evaluation, wine quality



**The powdery mildew resistance loci Ren3 and Ren9 from 'Regent' confer a hypersensitive response and Ren3 co-segregates with an NBS-LRR gene cluster**

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Grapevine powdery mildew (PM) caused by the obligate biotrophic pathogen *Erysiphe necator* is one of the most prominent and devastating diseases that viticulture must face. Grapevine growers can avoid crop loss by intense application of fungicides during the vegetation period. This plant protection regime is not only time-consuming and expensive but also harmful to the environment. To reduce the amount of applied fungicides, breeding for new, powdery mildew resistant grapevine cultivars represents a promising strategy for future viticulture. In this process, natural genetic resistances e.g. from wild North American and Asian *Vitis* species are introgressed in the genetic background of elite *Vitis vinifera* cultivars. Here we report the characterization of the widely used PM resistance locus Ren3 from the cultivar 'Regent' on a microscopic and molecular level. Fine-mapping and artificial inoculation experiments of selected recombinant F1-individuals led to the delimitation of this resistance locus to approximately 200 kb on chromosome 15. These analyses allowed simultaneously the identification of a second novel resistance locus called Ren9 upstream of Ren3 on chromosome 15. Subsequent sequencing of the genomic interval of Ren3 identified a cluster of four NBS-LRR genes of which two are actively transcribed. Further comparison of Ren3 with the analogous regions in the reference genome PN40024 12X and in 'Cabernet Sauvignon' revealed an accumulation of LTR-retroelements in the coding region of one of the two transcribed NBS-LRR genes in the two susceptible genotypes.

**Keywords:** genetic mapping, powdery mildew, Regent, Ren3, Ren9, resistance, *Vitis* sp.

## Understanding the establishment of scion/rootstock interaction in grapevine

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The commercial use of grafting depends upon the success of graft union formation i.e. the vascular and symplastic connections formed between the scion and rootstock. It has been shown in hypocotyl grafts of *Arabidopsis thaliana* that the phloem reconnects before the xylem during graft union formation (Melnik et al., 2015), but nothing is known about woody grafts of perennial species. In terms of symplastic connections, plasmodesmata, small membrane channels that pass through the plant cell wall and connect neighboring cells, can be formed across the graft interface (Kollmann and Glockmann, 1985), but whether they are functional and have a role in scion/rootstock communication is unknown.

The aim of this project is to describe (1) the early cellular events taking place at the graft interface, especially the establishment of symplastic connections between the rootstock and the scion, and (2) the three-dimensional cellular organization at the graft interface. The first challenge in this project is to localize precisely the scion/rootstock interface in the disorganized tissues of the grapevine and the *A. thaliana* micro-graft interface. The use of transgenic lines, expressing a trapped endoplasmic reticulum protein fused to fluorescent proteins, allows us to identify precisely the graft interface thanks to fluorescence microscopy.

Plasmodesmata are so small (about 30 nm in diameter) that they can only be observed with electron microscopy, so electron microscopy must be combined with fluorescence microscopy using Correlative Light Electron Microscopy to characterize the plasmodesmata precisely at the graft interface. Thanks to *Arabidopsis* lines with fluorescently labeled plasmodesmata or expressing fluorescent proteins that can freely diffuse through plasmodesmata, the formation and function of symplastic connections has been studied. The three-dimensional organization of the graft interface will be studied by light-sheet microscopy, a special technique that after clearing of samples allows us an in-depth imaging of the whole graft.

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**Keywords:** grafting, microscopy, plasmodesmata

## Next generation sequencing and annotation of whole plastid genomes of wild grapes (*Vitis vinifera s ubsp. sylvestris*) from South Caucasus, Europe and Mediterrean Basin

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*Vitis* L. belongs to Vitaceae, one of the oldest family of flowering plants. Wild grape *Vitis vinifera subsp. sylvestris* is a predecessor of cultivated grape and represents the only species of the genus aboriginal to Eurasia. First appeared app. 65 million years ago, they are predominantly forest climbers and occur in disjunct populations from the Atlantic coast to Tadzhikistan and the western Himalayas. According to many researchers the cultivated grape is believed to have been domesticated around 6000 BC. Recent chemical analyses of ancient organic compounds absorbed into the pottery fabrics from sites in Georgia in the South Caucasus region, dating to the early Neolithic period (ca. 6,000–5,000 BC), provide the earliest biomolecular archaeological evidence for grape wine and viniculture from the Near East, at ca. 6,000–5,800 BC. The discovery of early sixth millennium BC grape wine in this region is crucial to the later history of wine in Europe and the rest of the world. Meanwhile, multiple origins of cultivated grapevine, one in the Near East and another in the Western Mediterranean region are also considered. Over the last years the next-generation plastid DNA genomics has emerged as a powerful and increasingly accessible tool for plant phylogenetics. The main goals of the research presented here were: 1. Assessment of genetic diversity of wild grape samples from South Caucasus, Europe and Mediterranean basin by using complete chloroplast DNA Illumina sequencing; 2. Annotation of sequenced and assembled plastid genomes of wild grape samples from the above-mentioned regions. The obtained results are very important for the understanding of wild grape plastid genomes compositions and for the study of genetic relationships between wild and cultivated grapes from different geographical locations to explain the molecular bases of grape origin and evolution.

**Keywords:** genome annotation, Illumina sequencing, next-generation genomics, plastid DNA, *Vitis vinifera subsp. sylvestris*, wild grapes

## Detection of the disease resistance genes using GWAS in grape vine

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Grapes have cultivated worldwide, especially grown for brewing purposes and accounting for about one-third of the world's fruits production. However, grape ripe rot disease caused by *Colletotrichum spp.* is an economically important disease in grape production. Therefore, we conducted a study to identify the trait associated with grape disease-resistant and performed genome-wide association study (GWAS) for pheno/genomics using grape core collections. Firstly, we conducted phenotypic characterization to prove the pathogenicity of the fungal isolates *Colletotrichum acutatum* and *C. gloeosporioides* which were inoculated onto healthy grape leaves. Results of pathogenicity test from 844 grape cultivars (obtained from RDA Korea) showed 726 (87%) susceptible and 118(13%) resistance, respectively. Secondly, we selected 350 cultivars (118 resistance and 232 susceptible cultivars) and constructed the genotyping-by-Sequencing (GBS) library with 96 barcode sets to find the disease-resistant related (NB-LRR) genes. After that, the experiment was carried out to select the candidate genes, phenotype data converted into analyzed format data and performed GWAS analysis with GBS data (using TASSEL software). As a result of GWAS, we identified 6 resistance-related candidate genes for *C. gloeosporioides* and 7 candidate genes for *C. acutatum* using filtered 77,126 SNPs by their trait. Among them, only 2 candidate genes were included disease resistance LRR family protein. However, it is necessary to confirm the current results which are actually related or not to resistance genes. Therefore, additional studies should be carried out to confirm resistance genes which are related to current candidate genes.

**Keywords:** disease-resistance genes, genome-wide association study, genotyping-by-sequencing, NB-LRR, ripe rot

## Combining genotyping by sequencing and genomic prediction within bi-parental crosses to speed up selection of grapevine cultivars

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A major challenge facing viticulture consists in decreasing inputs, especially synthetic fungicides, and adapting to climate change, while maintaining berry quality and differentiated wine styles, as well as reasonable profits to the winegrowers. In this endeavor, breeding new varieties is an important lever. To speed up this process, genomic selection may be advantageous to quickly test in the field candidates precociously selected for various complex traits. We assess here the feasibility of this approach based on three bi-parental crosses (Syrah x Grenache, Riesling x Gewurztraminer and Cabernet-Sauvignon x Riparia Gloire de Montpellier), and several types of traits (yield components, phenology, terpenols, transpiration) whose phenotypes were already used for QTL mapping. The plant material was genotyped by sequencing (Keygene patents), providing around 18000 filtered, uniformly distributed SNPs per genotype. Depending on genetic architecture and broad-sense heritability, the average accuracy of genomic prediction obtained by cross-validation can go from 0% (transpiration rate), 50% (budbreak) to 80% (berry weight). The impact of the number of offsprings per cross, and the number of SNPs, are also studied. In conclusion, such an integrated approach aims at exploiting the full extent of available phenotypic, genotypic and genealogical data in order to efficiently keep adapting the plant material. In parallel, the approach is also tested in a specific breeding program for the rosé wines, in order to speed up the selection of genotypes resistant to powdery and downy mildew, and adapted to the rosé vinification. Results will be compared to classical selection operated after offsprings are planted in the vineyard.

**Keywords:** berry weight, breeding, genomic selection, phenology, terpenols, transpiration

**New cold tolerant grapevine cultivars for red wines**Elena Ilnitskaya<sup>a,\*</sup>, Tatiana Guguckina<sup>a</sup>, Anna Talash<sup>a</sup><sup>a</sup> *st.40-letiya Pobedy, 39, 350901 Krasnodar, Russian Federation*\* **Presenting author:** ilnitskaya79@mail.ru

Krasnodar Region is the main region of industrial viticulture in the Russian Federation. Natural conditions allow to grow high quality varieties there, however in certain years impact of low-temperature stresses of the winter period, summer droughts and diseases limit the stable high yields production of *Vitis vinifera*. Breeding of grapevine varieties for high quality wine-making and adaptability to local agro-climatic conditions is carried out in the North Caucasian Federal Scientific Center of Horticulture, Viticulture and Winemaking. Interspecific hybrids are used as donors of resistance in cross with high quality *Vitis vinifera* varieties. New hybrids were studied by agrobiological research, wines and must from grapes were studied by organoleptic evaluation, physico-chemical and biological indicators. Highly adaptive cultivars Dmitriy, Kurchanskiy, Vladimir for red wines are selected as the result. They are tolerant to low temperatures of winter period: down to -27 ° C. Cultivars have high level of resistance to the main pathogens (mildew, botrytis) and Dmitriy – to phylloxera. Yields of Kurchanskiy, Vladimir were 12-13 tons per ha, and that of Dmitriy 14-15 tons per ha. These cultivars combine high adaptability to unstable conditions of the south of Russia with high quality of wine production. Cultivar Vladimir distinguishes according to the total number of phenolic substances (average 3603 mg. L<sup>-1</sup>). Samples of wine Kurchanskiy and Dmitriy have high accumulation of anthocyanins (more than 1000 mg. L<sup>-1</sup>). Cultivars are now tested by governmental authorities.

**Keywords:** adaptability, cold tolerance, grapevine breeding, red wines

## True to type confirmation of twenty unknown grapevine accessions at grape germplasm collection in Split, Croatia

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The grape germplasm collection in the Institute for Adriatic Crops and Karst Reclamation (IAC, HRV048) Split is one of core collections in Croatia. In line with the wider endeavours of long-term conservation and utilization of plant genetic resources in Europe, it places special importance on maintaining genetic diversity of predominantly native Dalmatian varieties. Although IAC collection is already being largely genotyped, 20 accessions maintained in the field collection had unknown or unconfident cultivar status and needed verification of trueness to type. These unknown accessions were accessed from different academic and non-academic sources. Morphological validation was done by standard OIV descriptors encompassing shoot, leaf and cluster characteristics. A standard set of 9 simple sequence repeats (SSR) were amplified and genetic diversity indices were calculated. The allelic data of 20 unknown genotypes were compared with reference SSR profiles from the European database Vitis International Variety Catalogue (VIVC, <http://www.vivc.de/>) and grape germplasm collection of the United Kingdom. Three accessions proved to be mislabelled and identified as known European grapevine cultivars (Blaufraenkisch, Chardonnay, Merlot), two unknown accessions showed identical genotypes matching Chasselas blanc and Chasselas rose, while 12 unknown genotypes confirmed their declared status. Finally, 3 unknown genotypes of interspecific crosses (Ljana, Orginal, Nero) were not found in VIVC database nor in published literature revealing novel SSR profiles to our knowledge. This study presents necessary information to establish cultivar status (true to type) of 20 unknown accessions from IAC Split grape collection providing clear identity of accessions that is essential for further collection management and achieving maximum genetic diversity in a limited collection capacity.

**Keywords:** ampelography, grapevine genetic diversity, SSR markers

## Strengthening resistance of grapevine by beneficial bacteria relies on distinct defense pathways in susceptible and resistant genotypes to downy mildew

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Downy mildew caused by the biotrophic oomycete *Plasmopara viticola* and grey mould caused by the necrotroph fungus *Botrytis cinerea* are among the highly threatening diseases in vineyards. Their control relies totally on the use of fungicides, which cause environmental damage and health problems. The major challenge is now to limit phytochemical treatments in viticulture, and one of the promising strategies to control grapevine diseases is the activation of the plants own immune system by non-pathogenic microorganisms, known as induced systemic resistance (ISR). ISR has been linked to a lower fitness-cost priming state that results in accelerated and enhanced activation of cellular defense responses only after pathogen challenge. Multiple bacterial species have been shown to trigger ISR against the necrotrophic fungus *B. cinerea* in grapevine (Verhagen et al., 2011; Gruau et al., 2015; Aziz et al., 2016). Bacteria-mediated ISR in grapevine involves both activation of immune response at root and systemic level and priming state after *B. cinerea* challenge. However, the effectiveness of bacteria against the oomycete *P. viticola* remains unknown. In order to understand what makes bacteria efficient against pathogens with different life-style, we focused on bacteria-ISR in grapevine against *B. cinerea* and *P. viticola* by using two grafted genotypes differing in their susceptibility to downy mildew. We also explored and compared mechanisms underlying induced resistance to both pathogens. Bacteria can induce systemic resistance against both pathogens by reducing the severity of disease symptoms and development of pathogens. ISR efficiency seems to depend not only on bacterial strain and pathogen life-style, but also on the basal resistance of grapevine genotype. Although some bacterial strains seem to be more effective than others in inducing ISR, the efficacy of both relied on the priming of different signaling pathways and accumulation of specific phytoalexins depending on pathogen lifestyle.

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**Keywords:** downy mildew, grey mould, induced systemic resistance



**Metabolomic study to evaluate the resistance against *Plasmopara viticola***

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Downy mildew is a destructive disease of grapevine caused by the biotrophic oomycete *Plasmopara viticola*; it is responsible for extreme damages to vineyards in humid regions for the cultivated species *Vitis vinifera*. The introduction of resistant or tolerant varieties can be the solution to avoid or reduce the massive use of fungicides. Metabolomics can help in exploring the interaction between grapevine and *Plasmopara viticola* and in extending the current knowledge about the perturbations occurring in the plant system after biotic stresses. In our study, we evaluated the metabolic changes in 4 resistant or tolerant genotypes containing different sources of resistance (Bianca, Jasmine, BC4 and Solaris) and one susceptible genotype (Pinot noir). We investigated primary and secondary metabolism identifying and quantifying lipids (LC-MS/MS), phenols (LC-MS/MS), primary compounds (GC-MS), and semi-quantifying volatile compounds (GC-MS) at 0, 12, 48 and 96 h post infection. Jasmine and Bianca show the smallest number of altered metabolites (47 and 48 respectively), while Pinot noir has the greatest number of altered metabolites (82). BC4 and Solaris show less extreme behaviour with 56 and 63 altered metabolites respectively. Finally, we applied a network-based visualization approach to integrate the outcomes of different metabolomics assays and to explore the differences between the varieties.

**Keywords:** biotic stress, downy mildew, metabolomics, *Plasmopara viticola* resistance

## Development of a sensor-based approach for objective characterization grapevine berry cuticles

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The risk for Botrytis bunch rot is seriously increasing in regions with cool climates especially when high humidity or prolonged rain results in persistent moisture on berry surfaces. Botrytis then reduces yield and quality of wines due to off-flavors or reduced wine stability. Besides climatic conditions, susceptibility of grapes against Botrytis is mainly influenced by morphological properties like bunch compactness, canopy structure as well as the thickness and hydrophobic characteristics of the berry cuticle. For the grapevine berry cuticle, classification of phenotypes by traditional visual estimations is only possible for the appearance of wax layer (OIV 227).

In the present study we focused on non-imaging sensor (impedance) which was tested for fast phenotyping of grapevine berry cuticle of different varieties with regard to its thickness, permeability and distribution of the epicuticular wax layer. All of these traits are known to contribute to differences in susceptibility to Botrytis bunch rot. Varieties which differ with regard to their Botrytis infestation (susceptible/resilient), phenology (early/late ripening) and bunch compactness (loose/dense) were selected from the genetic repository at Geilweilerhof in Siebeldingen, Germany. Sensor data were validated regarding the observed Botrytis infestation in the field (natural control) and in the lab (standardized conditions). Thickness and permeability of the cuticle and its epicuticular waxes differ significantly between susceptible and resilient varieties and thus, sensor data were used for preliminary prediction of the risk for Botrytis bunch rot.

Additionally, imaging sensors will be validated in order to enable the investigation of further grapevine berry traits with regard to Botrytis resilience. Hereby, fusion of sensor data and cross validation of multi-year evaluations will be one promising strategy in order to develop reliable prediction models for Botrytis resilience for improved characterization of breeding material.

**Keywords:** *Botrytis* resilience, grapevine berry skin, high-throughput, precision, sensor-based phenotyping

## Identification of grapevine sensitivity molecular markers for *Eutypa lata*

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Grapevine trunk diseases are currently a threat to the sustainability of the wine industry. Esca, BDA and Eutypiosis are the most encountered of these diseases, that are vascular fungal diseases causing wood necrosis and plant death. No chemical treatment currently exists and the use of sensitive varieties result in significant economic loss. The study focuses on the molecular markers of sensitivity to *Eutypa lata* for different grapevine varieties.

First, 3 varieties known for their different levels of sensitivity were analysed with healthy plants and experimentally infected ones (*E. lata* mycelium) in greenhouse: Merlot (tolerant), Ugni Blanc (sensitive) and Cabernet Sauvignon (sensitive). The expression of selected genes was studied by RT-qPCR in order to validate some of these genes as molecular markers of the variety's sensitivity. Unfortunately, these results also depend on culture conditions.

In order to monitor rapidly and efficiently the grapevine response to the infection, a new *in vitro* simplified system of interaction was thus developed. In this system, foliar discs are infected under controlled conditions. Several grape varieties were studied using this system and some markers for *E. lata* sensitivity could be selected.

After validation, these genes might be used by vine breeders to accelerate the selection of less sensitive varieties or clones. Subsequently, a functional study could be implemented on plants to correlate gene expression with physiological mechanisms of tolerance against *E. lata* in order to find a new way to control Eutypiosis development.

**Keywords:** Eutypiosis, markers, vine varieties, sensitivity, *in vitro* test

# **Genetic diversity of a large collection of grapevine cultivars from the island of Crete, Greece assessed by SSR markers**

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Indigenous grape cultivars in the island of Crete, Greece represent one of the oldest populations of the species; nevertheless, very scarce information is available about its genetic structure. In the framework of the present research an effort was undertaken in order to collect and to conduct a preliminary characterization of this material with the help of microsatellite markers(SSR). Following an initial screening employing 13 SSR loci a total of 163 accessions were retained for further analysis. Following cluster analysis twenty-four (24) cultivar-specific clusters were identified as well as fourteen (14) cases of synonyms and ten (10) groups of homonyms. Genetic admixture analysis revealed three genetic groups (putative ancestral populations) while further hierarchical structure analysis revealed additional stratification within each of the three ancestral populations. The molecular characterization of indigenous grape cultivars of Crete contributes to the knowledge of the overall genetic diversity of the species while it provides insights into the genetic structure of a historical germplasm. The Greek and Cretan vineyard is one of the oldest in the world (perhaps dating back 4,000 years) and, despite its small size, it is genetically quite polymorphic with a relatively high number of cultivars. In the future, molecular genetic information combined with ampelographic data will provide an integrated characterization of existing diversity and will allow for its exploitation in breeding efforts in commercial viticulture.

**Keywords:** admixture, cluster, historic, indigenous, molecular fingerprinting

## Transcriptional regulation of fruit development in grape

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Worldwide from 2016 to 2017, 21.94 million metric tons of grapes (wine, table, and raisin) were produced. Exports alone valued at more than \$7 billion in 2016, making grape the most important specialty crop worldwide. For each grape (*Vitis vinifera*) market class (wine, table, and raisin), specific traits such as seed presence, berry texture, and skin thickness have been selected. The genetic regulation underlying these market class differences is not well understood and likely occurs at multiple points during berry development. To test this, berry samples were collected every two to four weeks from anthesis to full color for a single wine, table and natural dry-on-the-vine (NDOV) raisin cultivar. RNAseq data was generated for each cultivar and time point and expression differences are being compared. In addition, metabolite data has been collected and is being analyzed for each cultivar and timepoint to develop a systems-level comparison of fruit development.

**Keywords:** raisin grape, systems biology, table grape, transcriptomics, wine grape

**Physiological impacts of early defoliation on the cold hardiness of grapevine (*Vitis vinifera* L.) cv. Bidaneh Sefid**Hassan Sarikhani<sup>a,\*</sup>, Hooman Delgarm<sup>a</sup><sup>a</sup> *Department of Horticultural Science, Bu-Ali Sina University, Hamedan, Iran*\* **Presenting author:** sarikhanih@yahoo.com

After harvest, defoliation of grapevine is widespread in some regions of the world, because of early burying of canes for winter protection, grazing of the livestock, insect infestation, disease, use of chemicals or in out of season production for reducing chilling requirement. The main purpose of this study was to investigate the effects of early defoliation after harvest on the cold-hardiness of grapevine. The grapevines cv. Bidaneh Sefid were defoliated at two stages (1 and 15 October) and were compared to those of natural abscission (15 November) 2017. Cane samples were collected on 28 December 2017 and 28 February 2018, analyzed for their relative water content, abscisic acid, carbohydrate, proline, and protein concentrations in both buds and cane tissues, then exposed to 3 h freezing treatment between -8°C to -24°C at 3°C intervals to assess their level of cold-hardiness. The results showed that carbohydrate and proline concentrations were generally decreased by early defoliation, while relative water content was increased. The LT50 values of freezing treatment, both estimated through the electrolyte leakage measurements and through the tetrazolium stain test, indicated that early defoliation significantly decreased cold-hardiness. On December samples, the buds LT50 values estimated by electrolyte leakage ranged from -14.28°C in very early defoliation to -19.85°C in natural abscission. Compared to the buds, cane samples showed less susceptibility to winter cold-injury. Similar results with lower cold hardiness were obtained in those samples were taken in February. As a conclusion, early defoliation revealed the capacity to reduce the freezing tolerance of grapevine (cv. Bidaneh Sefid). Maintaining the leaves could be a prophylactic tool to reduce winter injury.

**Keywords:** abscisic acid, cold hardiness, electrolyte leakage, lethal temperature, relative water content

## Field performance of five white PIWI varieties in Southern Brazil

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The southern Brazil is the largest grape producer region in the country. It is characterized by the production of labrusca grapes due to the difficulty of growing *V. vinifera*. This can be explained by the high occurrence of fungal diseases, especially downy mildew, which is favored by its environmental conditions (high temperatures, relative humidity and rainfall). PIWI (Pilzwiderstandsfähige) are a group of wine grape varieties similar to *V. vinifera* but with resistance to downy and powdery mildew. These varieties open the possibility of reducing production cost and the use of agrochemicals for grape growing in Brazil. In order to achieve this objective, studies related to phenological, productive and qualitative characteristics are necessary. A PIWI variety trial was established in 2015, in the city of Videira, Santa Catarina State, Brazil, with five white varieties. The vineyard were trained in VSP and pruned in double cordon. The studied varieties were Felicia, Calardis Blanc, Helios, Bronner and Aromera, grafted on Paulsen 1103. The variables evaluated were phenology, incidence of downy mildew, yield, number of clusters, average cluster weight, total soluble solids, pH and total titratable acidity. In terms of productivity, the outstanding varieties were Felicia and Calardis Blanc, with yields above the traditional *Vitis vinifera* varieties. Felicia was the earliest variety and produced the heaviest clusters. Calardis Blanc presented the highest number of clusters and Aromera was the latest variety. All varieties presented high resistance to downy mildew compared to *Vitis vinifera*, with Felicia being the less resistant. Soluble solids contents, pH and acidity were adequate in all varieties. In general, all tested varieties presented potential for the studied region.

**Keywords:** Brazil, downy mildew resistance, PIWI

## Viticultural performance of disease resistant genotypes (PIWI) in highlands of Southern Brazil

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The viticulture in highlands of Southern Brazil, as a new wine region, show some production risks. One of the main problems that may restrict production are phytosanitary problems, like downy mildew. This disease often occurs in Brazil, particularly in warm and humid climates. The use of resistant varieties could be an alternative to cultivation, helpful to decrease the pesticides level, reducing costs and increasing wine quality. The objective of this study was to characterize the viticultural performance of PIWI genotypes grown in highlands of Southern Brazil. The experiment was conducted at a commercial winery (28°13S, 50°04'W, altitude 1,100m), the vineyard was planted in 2016 and the evaluations occurred in 2018 vintage. The evaluated varieties were Regent, Baron, Prior and Calandro. The phenological stages evaluated were bud break, flowering, veraison and maturity. At harvest were evaluated yield and technological maturity. The average date of bud break was September 3, the average date of flowering was October 19, the average date of veraison was December 21 and the average date of maturity was February 25. The variety Calandro presented the shortest cycle, with 149 days; the growth cycle from the others presented 183 days on average. The varieties Calandro and Regent presented the highest yields above 6 ton/ha. Even in a climate condition more favorable to downy mildew, all PIWI varieties evaluated presented a reduction in disease development with a considerable level of resistance and produced grapes with attributes suitable for production of high quality wines.

**Keywords:** downy mildew, grape quality, phenology, *Vitis vinifera* L.



## Do grape rootstocks have really important role in drought tolerance?

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Drought tolerance is unquestionably one of the most important characteristics of grapevine due to the water scarcity in many growing regions in the world. The searching engine of Google found more than 0.5 million articles for such a term: 'grape drought tolerance'. Closely 100 scientific articles are dealing with grapevine drought tolerance according to the Scopus database since 1998 (Web of Science 121). Drought tolerant and drought sensitive rootstocks are separated in practice and they are identified by research results as well. Our review and study will highlight the complexity of the grape vineyard water stress. The growth of the root system depends on many factors out of the rootstock genotype (soil type, climate, variety...). The signalling system of the plants is much more sophisticated than only depending on rootstocks. The leaf water potential depends on the methods of measurement, time, age of shoot among others. Conditions and methods of measurements need to be standardized, otherwise the differences in water potential caused by rootstock genotypes cannot be interpreted. Seasonal root growth, yield quantity and quality with connections of climatic impact of five rootstocks grafted on 'Cabernet sauvignon' and 'Kékfrankos', leaf water potential of more than 50 rootstocks will be presented which were carried out in nursery and in field conditions. This review and our study will address the question of the grape rootstock role in vineyard's response to soil water deficiency.

**Keywords:** climate, leaf water potential, root growth, water deficiency, *Vitis*

**Acknowledgments:** this research was supported by Hungarian Government and the European Union, with the co-funding of the European Regional Development Fund in the frame of Széchenyi 2020 Programme GINOP-2.3.2-15-2016-00054 project.

## Phylogeny of *Vitis* species based on a VvMybA1 marker analysis

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The Vitaceae family contains about 14 genera and 900 species which are distributed in the north temperate region. East Asia and North America are the main growing areas with 25-30 and approximately 30-40 taxa, respectively. In Europe *Vitis vinifera* (*V. vinifera* subs. *sylvestris*, *V. vinifera* subs. *vinifera*) is the only species which grow in the Mediterranean Basin. Nowadays the increasing interest in the wild grapes is due to their possible utilization in resistance breeding. Several works have attempted to clarify the relationship of taxa within the *Vitis* genus with various DNA markers without unambiguous conclusions. In our preliminary studies with the 20D18CB9 marker - which is linked to VvMybA1 transcription factor gene regulating the anthocyanin biosynthesis - polymorphism between the *V. vinifera* and other *Vitis* species was detected. Accordingly, we assumed that it can be suitable for further examinations to construct a new phylogenetic tree within the Vitaceae family. Based on the sequenced PCR fragments, generated with the 20D18CB9 primer pair, the North American species have a 34-bp deletion, so they form a separate group. The Asian species, which do not contain this deletion, belong to another group. However, some North American accessions also lacked this 34 bp DNA deletion. According to our results a new phylogenetic relationship of *Vitis* species was set up using the VvMybA1 linked marker.

**Keywords:** 20D18CB9 marker, phylogenetic tree, Vitaceae taxonomy

**Acknowledgments:** the work is supported by the EFOP-3.6.3-VEKOP-16-2017-00008 project. The project is co-financed by the European Union and the European Social Fund.

## Construction of a high-density linkage map of a mapping progeny of 'Deckrot' x G1-7720: an important resource in support of both table and wine grape breeding

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In the era of genomics, high-density linkage maps continue to be a valuable tool in grapevine breeding and genetic research. Such maps are used in combination with the available reference genome to fine-map quantitative trait loci (QTL) and identify candidate genes for important agronomic traits, thus enabling subsequent marker-assisted selection for these traits. The construction of high-density linkage maps requires a mapping progeny segregating for the traits of interest. The Agricultural Research Council (ARC) of South Africa has created a segregating mapping progeny using the wine grape 'Deckrot' x the table grape selection G1-7720 to enhance their table and wine grape breeding programme. These parents were chosen for their contrasting phenotypes in traits of interest, such as flesh colour, bunch architecture, aroma and waxy bloom. The mapping progeny has now been genotyped with the Vitis18KSNP chip and 118 microsatellites. A total of 6930 markers (6839 SNPs and 91 microsatellites) were informative and successfully genotyped in at least 90 % of the mapping progeny. Subsequently, 4069 loci was excluded from the linkage analysis, as they were identical in genotype across all individuals, highlighting the need to genotype additional seedlings to resolve the high number of SNP markers. The 'Deckrot' x G1-7720 linkage map consists of 2 008 markers that includes 1952 SNPs and 56 microsatellites with an average marker interval of 0.78 cM, which makes this one of the most dense linkage maps in grapevine. The largest gap between markers is 14.53 cM, whilst the average genome length was estimated at 1403.42 cM. The utility of this linkage map for the grapevine breeding program will be discussed.

**Keywords:** breeding resource, linkage map, SNP chip

## 1,000 new clones per year: uncovering the genetic and epigenetic impact of a transposon burst in grapevine

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Endogenous transposable elements (TEs) can be stimulated, through a combination of tissue culture and stress treatments, to produce novel genetic diversity in plants. Using this approach, we are generating a population of novel grapevine somaclonal mutants as a resource for both the international scientific community (gene function studies) and the wine industry. This talk will discuss molecular and bioinformatic tools that we have developed to enable semi-automated, high throughput genotyping of approximately 60,000 genomic TE insertion sites in each vine, so that a searchable database of genetic variation is established alongside the population. It will also describe the genome-wide epigenetic impact of a loss of TE silencing and the consequent TE mutagenesis, a fundamental process in eukaryotic evolution.

**Keywords:** crop improvement, epigenetics, genotyping, mutation, transposable elements, transposons

## Breeding high quality southern grape cultivars for meeting industry demands in Florida and Southeastern US

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The Florida A&M University (FAMU) Grape Breeding Program is supporting and ensuring the sustainable growth of the southern wine and table grape growing industry for more than four decades. The Center for Viticulture and Small Fruit Research is committed on leveraging the competitiveness of Florida viticulture industry. “Improving the quality of Florida’s grapes and wines” is the key mandate of Florida Viticulture Policy Act, Ch599FS and is the major goal of the Center of Viticulture, Florida A&M University ([www.famuedu/Viticulture](http://www.famuedu/Viticulture)). The Center maintains the major public grapevine germplasm collection for Pierce's Disease (PD) tolerant southern grapes and serves as a regional center for the National Clean Plant Network (NCPN) for grape in the southeastern United States. Recently, we deployed an accelerated 3 years evaluation pipeline for release of novel, disease-resistant varieties with desired wine aroma and flavor characteristics, and attractive large berry appearance for fresh fruit grape consumption. We present the data for wide variety of attractive Pierce's Disease new varieties and advance breeding lines with valuable qualities for commercialization: ‘Majesty’ (US patent PP21, 965 P3), two ongoing new releases: “Floriana” (muscadine red wine variety, and “Onyx” (muscadine red fresh fruit variety) and two high quality white wine grape advance selections.

**Keywords:** breeding, genetics, genetic variability, *Muscadinia*, *Vitis*

## How do grapevine rootstocks modify phosphorus concentration in scion ?

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Crop productivity is often limited by nutrient availability and rootstocks offer a means to increase the sustainability and nutrient efficiency of agriculture. In grapevine, the parentage of rootstocks appears to alter scion nutrient concentration, in particular phosphorus (P). Our objective is to characterise functional processes involved in P nutrition in two rootstock genotypes: *V. riparia* cv. Gloire de Montpellier (RGM), known to confer low P content, and *V. rupestris* × *V. berlandieri* cv. 1103 Paulsen (1103P), known to confer high P content. Phosphorus use/acquisition, allocation and remobilization from perennial tissues were quantified using <sup>32</sup>P labelling. Root system development was phenotyped and modifications of rhizosphere were characterised by quantifying root exudation in hydroponic culture. Differences in shoot P concentrations were not associated with differences in P allocation or P remobilisation from perennial tissues. The root system of 1103P is deeper and more branched than RGM, so potentially able to explore a higher soil volume. In addition to this morphological advantage, the roots of 1103P are more efficient in P uptake and have a greater effect on the rhizosphere (*i.e.* exudation of more protons and acid phosphatases which can increase P availability). The ability of 1103P to confer high scion P is related to efficiency gains in morphological and functional root traits, this is presumably related to the *V. berlandieri* parent, which originates from calcareous soils often deficient in P. This study suggests that selecting rootstock genotypes originating from low nutrient environments could improve the performance of grafted plants in agriculture.

**Keywords:** acquisition, phosphorus, rhizosphere, rootstock, root system architecture

## Disease-resistant grapevine cultivars drastically reduce fungicides use: results of a five years multi-criteria evaluation of two low-fungicide input cropping systems

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Resistant cultivars are an alternative to pesticides with three major assets: cost effectiveness, lack of negative side effects on human health and on the environment and high specificity, which avoids side effects on non-target organisms. As such, resistant cultivars are a cornerstone of any integrated pest management strategy along with others control methods: decision making systems estimating the need for and timing of pesticide treatments, mechanical and agronomic management of weeds, use of biocontrol agents. In France, the treatment frequency index (TFI) for the protection of grapevine against pests and disease was 12.6 in 2010, and fungicide treatments, targeting mainly powdery and downy mildew, represented 80%. In this long term study conducted in Bordeaux, we used a system approach to compare two cropping systems on a randomized large field experiment of 1.8 ha with 3 repetitions. Specifically, we compared a low pesticide system using the grapevine susceptible variety Merlot (cropping system INT) and a system using the fungus resistant variety Artaban (cropping system RES). Artaban is resistant to both powdery mildew and downy mildew. A multi-criteria evaluation of these two systems was performed over 5 years by measuring TFI, pesticide residues, environmental impact with INDIGO® method, yields, pest control levels, costs and working time. Compared to regional references, a reduction of 50% of the TFI was achieved in the cropping system INT using the decision making systems Mildium. This reduction reached 90% for the cropping system RES. RES had the best environmental performances and both systems maintained satisfactory agronomic and economic performances. To our knowledge, this study is the first one demonstrating the major interest of fungus resistant grapevine varieties. It opens new perspectives for a drastic reduction of pesticide use in vineyards.

**Keywords:** cropping system, integrated pest management, multi-criteria evaluation, pesticides alternatives, resistant cultivars

**The study of hormonal metabolism of Trincadeira and Syrah cultivars indicates new roles of salicylic acid, jasmonates, ABA and IAA during grape ripening and upon infection with *Botrytis cinerea***

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Hormones play an important role in fruit ripening and in response to biotic stress. Nevertheless, hormone analyses are scarce in plant development and defense. Infection of grape with the necrotrophic pathogen *Botrytis cinerea* leads to significant economic losses worldwide. In this work, the changes in hormonal metabolism were compared between a susceptible (Trincadeira) and a resistant (Syrah) variety during grape ripening and upon infection with *Botrytis cinerea*. Peppercorn-sized fruits were infected in the field and mock-treated and infected berries were collected at green (EL32), veraison (EL35) and harvest (EL38) stages for hormone analysis and target qPCR analysis of genes involved in hormonal metabolism. The results indicate a substantial reprogramming of hormonal metabolism during grape ripening and in response to fungal attack. Syrah and Trincadeira presented differences in the metabolism of ABA, auxins and jasmonates during grape ripening that may be connected to fruit quality. On the other hand, high basal levels of SA, JA and IAA at an early stage of ripening, together with activated SA, JA and IAA signaling, are likely regulating a fast defense response leading to grape resistance/ tolerance towards *B. cinerea*. Moreover, the interaction of the different hormones seems to depend on the ripening stage and on the intra-specific genetic background and may be fundamental in providing resistance or susceptibility. In addition, this study indicated new roles of SA and IAA in defense against necrotrophic pathogens and gave insights into possible strategies for conventional breeding and/or for manipulation for cross talk engineering aiming at improving grape quality and grape resistance against *Botrytis cinerea*.

**Keywords:** *Botrytis cinerea*, fruit ripening, hormones, pathogen resistance



**Differential monoterpenes accumulation of two table grape varieties between greenhouse and open-field cultivation**

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Greenhouse cultivation is extensively used in horticultural crops production, but its effect on accumulation of monoterpenes in table grapes remains unclear. In the present study, the early ripening table grape varieties 'Xiangfei' and 'Zaomeiguixiang' were grown in the greenhouse and open-field conditions. The berries were sampled from veraison to maturity. Headspace solid phase micro-extraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS) combined with Automated Mass Spectral Deconvolution and Identification System (AMDIS) were employed to analyze the free monoterpenes in the two grape varieties. A total of 29 monoterpenes from mevalonate pathway were identified. The total contents of monoterpenes increased during berry development and 'Xiangfei' grape had higher monoterpenes contents than 'Zaomeiguixiang' grape at maturity. The monoterpenes contents of berries under open-field cultivation were significantly higher than that in greenhouse. 'Zaomeiguixiang' grape in greenhouse could be clearly distinguished from the control by principal component analysis (PCA), but the ripening 'Xiangfei' grape in greenhouse was close to the control. In general, grape berries under open-field cultivation produced higher contents of limonene, (Z)-allo-ocimene, (E, Z)-allo-ocimene, linalool,  $\beta$ -myrcene, terpinolen, (Z)- $\beta$ -ocimene, cis/trans rose oxide,  $\beta$ -citronellol and cis-pyran linalool oxide.

**Keywords:** greenhouse, monoterpene, open-field

## Genetics and mapping of grapevine resistance from a muscadine source to the dagger nematode *Xiphinema index*

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The dagger nematode *Xiphinema index* vectors in the soil from plant to plant, Grapevine fanleaf nepovirus (GFLV), the first grapevine virus worldwide. Resistance to *X. index* ingrape rootstock breeding by arresting or delaying viral transmission is a promising GFLV control alternative to the removal of highly toxic chemical nematicides. In the muscadine *Muscadinia rotundifolia*, accession NC184-4 is today one of the best sources for resistance (R) to *X. index* and can be used through its F1 resistant individual VRH8771 (= *Vitis vinifera* x NC184-4). Inheritance of resistance to *X. index* (non-viruliferous individuals) has been evaluated using a one-year test under greenhouse conditions for the selection of durable R factors. Despite the difficult hybridization between the genera *Vitis* and *Muscadinia*, a backcross (BC1) progeny of 66 individuals between VRH8771 and the susceptible (S) *V. vinifera* cv. Cabernet-Sauvignon (CS) has been phenotyped for resistance. Given that BC1 individuals exhibit highly heterogeneous vigor, a Principal Component Analysis (PCA) approach has first demonstrated that the criteria used for resistance phenotyping and for plant development vary independently. A clear segregation has been observed and, out of the 66 individuals, the 58 R : 8 S ratio (~ 7 R : 1 S) suggests the hypothesis of three dominant and independent R factors. Using a VRH8771 x CS genetic map in progress based on microsatellite and GBS (genotyping by sequencing) markers, a mapping method derived from BSA allowed the detection of markers linked to resistance at three chromosomal locations that fit this hypothesis. Future studies will aim at confirming the monogenic segregation of each factor from new BC2 progenies obtained by backcrossing appropriate BC1 individuals carrying single factors with S (recessive) individuals. Additional experiments will also aim at deciphering the putative effects of the combination/pyramiding of R factors on both the durability of resistance to *X. index* and the correlative resistance (or delayed infection) to GFLV.

**Keywords:** genetic markers, *Muscadinia rotundifolia*, nematode population, rootstock

## Validation of QTLs for muscat flavor in hybrid table grapes

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Muscat flavor is of great importance for table grape breeding. In Japan, the production of the hybrid muscat cultivar 'Shine Muscat' is increasing rapidly, and the cultivar has become the fourth biggest now. However, the production of other muscat varieties is limited, hence one of the biggest breeding objectives in Japanese breeding programs is to breed new muscat varieties. Several QTLs for muscat flavor have been identified from *V. vinifera*. Especially, QTL in LG5 was shown to be important for muscat flavor, and VvDXS was found in the locus and studied in detail. Here, we analyzed 3 QTLs (LG2, 5, 10) based on SSR genotyping data of our selected individuals derived from our breeding program. SSR haplotypes in 3 QTLs were defined by several closely linked SSR markers. Muscat traits were phenotyped by sensory evaluation for 2 years or more at maturity. We identified SSR haplotypes in all three QTLs that muscat individuals harbor more than non-muscat individuals and *vice versa*. SSR markers in the haplotypes could be used for marker assisted selections for breeding of muscat table grapes.

**Keywords:** hybrid grapes, Muscat flavor, QTL, SSR

**Acknowledgments:** this research was supported by grants from the Project of the Bio-oriented Technology Research Advancement Institution, NARO (the special scheme project on advanced research and development for next-generation technology).

## Genetic variability in grapevine clones of Muscat of Alexandria

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Among grapevine varieties, the ‘Muscat’ family includes several widespread types that share a characteristic pronounced floral aroma and a typical ‘Muscat’ flavor. ‘Muscat à Petit Grains blanc’ and ‘Muscat of Alexandria’ are the most representative and ancient varieties. The grapevine variety ‘Muscat of Alexandria’ is of great importance within the “Valencia” and “Alicante” D.O (Designation of Origin, a prestigious Spanish regional product classification). Fruits from this variety are the basis of different appreciated wines, being also consumed as table grapes or used for raisin production. We used a set of selected SSR markers to confirm the identity of different clones of ‘Muscat of Alexandria’ (with differential ampelographic traits). Additionally, using AFLP markers we found intra-varietal genetic variability. Now, a more accurate genotyping has been conducted using GBS (genotyping by sequencing). The GBS generated 2 to 4 million of reads per sample, of which 85% (in average) were mapped (mapping quality > Q20) to the reference genome developed by the French-Italian consortium (*V. vinifera* IGGP 12x). Around 40.000 SNPs were identified, with a coverage greater than 10X, polymorphic between and within the seven analyzed clones. The experimental validations of the identified SNPs will provide markers to accurately fingerprint these clones. They are also suitable for association studies or to develop molecular markers useful in selection programs.

**Keywords:** AFLPs, GBS, SSRs

**Acknowledgments:** this study was partially supported by the MINECO project PRPCGL2015-70843-R co-funded with FEDER Funds.

## Phenotypic, hormonal and genomic variation among Tempranillo clones with different cluster compactness and reproductive performances

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Previous studies on grapevine (*Vitis vinifera*) showed that the number of berries in the cluster is a major component of its compactness level. Variation in the number of fruits is regulated by events occurring in the fruit set, but also before during the flower formation and pollination, affecting factors like the initial number of flowers or the gametic viability. Therefore, the identification of the genetic bases of this variation would provide an invaluable knowledge of the grapevine reproductive development and useful tools for managing yield and cluster compactness. We performed the phenotyping of four clones of the Tempranillo cultivar with reproducible different levels of cluster compactness over seasons: two compact and two loose clones. Measures of reproductive performance included pollen viability, flower number per inflorescence, berry number per bunch, fruit set, coulure and millerandage indices. Besides, contents in several hormones during the inflorescence and flower development were determined, and their transcriptomes were evaluated at critical time points (E-L 18-19 and E-L26). Compared to the compact clones, clones bearing loose clusters showed lower number of berries per cluster and seeds per berry, or higher coulure, but they also differed between them for other reproductive traits like pollen viability or fruit set rate, indicating that they use different ways to produce loose clusters. Variation between clones was observed for ABA and Gibberellins levels at particular development stages which could be related to phenotypic differences. Likewise, various changes between clones were found at the transcriptomic level. Many of the differentially expressed genes between one of the loose clones and the compact clones were known to be over-expressed in pollen. Many of them were related to cell wall modification processes or to the phenylpropanoids metabolism. We also found polymorphisms between clones in candidate genes that could be directly involved in the variation of the compactness level.

**Keywords:** berry number, coulure, flower number, fruit set, hormones, phenotyping, pollen viability, RNAseq, somatic variation, transcriptomics

## Phytohormonal metabolism of Carignan grape berries upon infection with powdery mildew

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Grapevine (*Vitis vinifera* L.) is susceptible to several diseases; one of the most dramatic is powdery mildew (PM) that is caused by the obligate biotrophic fungus *Erysiphe necator*, which affects berry yield and wine quality. Phytohormones have been shown to play an important role in biotic stress response. In this study, regulation of the hormonal metabolism in susceptible grape berries infected with PM is being analyzed. Naturally infected and control 'Carignan' samples were collected at green and véraison stages, and expression of genes involved in phytohormone metabolism was carried out in order to be integrated with future hormone analysis. Genes associated with salicylic acid signaling pathway, namely EDS1, PAD4, and PR1, were up-regulated in infected berries at both developmental stages, whereas in control samples expression of PAD4 and EDS1 decreased significantly during grape development. For ABA biosynthesis and signaling pathway, the expression of NCED showed no significant difference between control and PM infected samples, and a slight increase in ABA receptor PYL4 expression was observed in infected grapes at véraison. Concerning jasmonates, MYC2 expression increased in PM infected berries at both stages, and OPR1 showed a slight increase at the green stage. Regarding auxins, IAA-amido synthetase GH3.2 and AUX1 were highly expressed in infected berries at green stage. IAA-amido synthetase expression decreased from green to véraison stage, with the expression in infected samples decreasing to levels similar to control. These results suggest an extensive hormonal reprogramming upon infection with PM in particular at green stage. Furthermore, the data indicates that both jasmonates and auxins which are hormones not classically associated with response against biotrophic pathogens may be important in defense response against PM in grapevine.

**Keywords:** fruit ripening, hormones, pathogen response, powdery mildew

**‘Clean’ genome editing in grapevine (*Vitis* ssp.)**Lorenza Dalla Costa<sup>a,\*</sup>, Loredana Moffa<sup>a</sup>, Stefano Piazza<sup>a</sup>, Mickael Malnoy<sup>a</sup><sup>a</sup>*Via Edmund Mach 1, 38010 San Michele a Adige (Trento), Italy*\* **Presenting author:** lorenza.dallacosta@fmach.it

In recent years new plant breeding techniques (NPBT), and in particular genome editing via Crispr/Cas9, emerged as breakthrough tools for the genetic improvement of agricultural species, allowing to precisely modify specific genes in shorter time compared to traditional breeding and without altering the genetic heritage of cultivars. Grapevine, the most economical valuable fruit crop in the world, may receive a major benefit from NPBT since viticulture is based on a few elite varieties. However, to date the European Commission (EC) has not yet deliberated on the legal status of the NPBT products, whether they should or should not be covered by GMO legislation (Directive 2001/18). Waiting for the EC decision, we applied the Crispr/Cas9 system in grapevine for the inactivation of the *VvMLO7* gene which plays a key role in susceptibility to powdery mildew. Our “clean” strategy aims at leaving in the plant genome the minimal trace of exogenous DNA. It uses the classical *Agrobacterium tumefaciens* (A.t.) to introgress Cas9, the sgRNA and the selection marker gene *nptII* and allows removing the T-DNA cassette from the grapevine genome once the targeted mutations have been obtained. To this purpose, the FLP recombinase gene under the control of a heat-shock inducible promoter has been integrated in the T-DNA as well as its recognition sites (FRT), placed next to the A.t. left and right borders. NptII- and Cas9-positive lines of ‘Chardonnay’, ‘Thompson Seedless’ and ‘Microvine’ were analyzed by next generation sequencing in order to assess the induced mutations in the target site. Subsequently, the site-specific removal of the T-DNA cassette was evaluated in the heat-treated lines by quantifying *nptII* copy number with a Real-time PCR method. The effect of powdery mildew infection on *VvMLO7*-edited plants is currently under evaluation.

**Keywords:** FLP/FRT system, genome editing, marker-free, powdery mildew, VvMLO

## Investigating the variability of the cultivar Pinot blanc under different aspects

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Pinot blanc is a naturally occurring mutant of Pinot gris. It is mainly spread in Central and Western Europe. In Austria, Pinot blanc is cultivated on ca. 2000ha, which corresponds to 4% of the Austrian wine-growing area and 12% of the world production (15,500 ha). Only in Germany (5000 ha) and in Italy (3000ha) more Pinot blanc is grown. In the production area of Leithaberg a PGO wine (DAC) is made from this grape. The flavor of local wines is usually dominated by aromas of pear and ripe apple, that often appear in combination with floral notes from herbs, acacia flowers or nutty aroma. Wines of Pinot blanc are usually elegant, full-bodied and delicate. They show ageing potential and these matured wines present honey and almond impressions. Pinot blanc wines usually can be sold for an agreeable price for the wineries.

For the experimental field we have chosen 14 different certified clones to compare them under the same conditions. Our study focused on the structure of the bunch, phenological behavior, stability against diseases especially sensitivity to *Botrytis*. It is appreciated to know the strength and advantageous traits of each clone to have a real choice. Also important are the agronomical data as yield per vine and quality of grapes. Finally wines of each clone were made and these wines were sensorial evaluated as well as chemically analyzed with a specific focus on the aromatic compounds.

Ethyl trans-2-cis-4-decadienoate is well known as an impact compound in fresh and processed pear products. This substance had not been noticed in wine until now. In the course of this study, the content of ethyl trans-2-cis-4-decadienoate and other pear aromas (isoamyl acetate, methyl trans-geranoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate) was investigated in the different Pinot blanc wine samples. For detection of these compounds in wine HS-SPME-SIM-MS was used. According to the results the wines contain a relevant quantity of ethyl trans-2-cis-4-decadienoate. The results of the study showed, however, that the perceived pear flavor in Pinot blanc wines is a result of the interference of some of the analyzed aromas. It has already been shown that the pear aroma is important for the typicity and quality of the local Pinot blanc wines.

We have tried, therefore to analyse the clones according to their sensorial behaviour, once in a general modus and otherwise with defined aromatic impressions. Sensorial description was gained by specific evaluations of a tasting panel.

The genetic differences of the clones were searched by using microsatellites, interSSR markers and SNPs. Analysis revealed several polymorphic DNA loci for the identification of some clones. Furthermore, the relationship between the clones was calculated and their differences were demonstrated. Comparing the age of Pinot blanc to other traditional cultivars it could be concluded that the lower variability within these clones is an indication for the youth of the clones or the origin from only few sources.

**Keywords:** ampelographic description, aroma compounds, clones, genetic analysis



**Methyl jasmonate application on *Vitis labrusca* L. grown under subtropical conditions**Laís Moro<sup>a,\*</sup>, Eduardo Purgatto<sup>b</sup>, Niara Tanabe da Silva<sup>b</sup>, Neuza M.A. Hassimotto<sup>b</sup><sup>a</sup> *Via giuseppe verde, 101, 38100 TRENTO, Italy*<sup>b</sup> *Av. Prof. Lineu Prestes 580, bl 14, Butantã, Av. Professor Lineu Prestes, 580 - Bloco 14, 05508000 São Paulo-São Paulo, Brazil*\* **Presenting author:** lais.moro@gmail.com

There has been an increased demand for eco-friendly means improving the bioactive content in fruits. Methyl Jasmonate - MJ, a plant growth regulator, has been the focus of various studies. Field applications seems to induce secondary metabolism on *Vitis vinifera* L. grapes, but there are few studies on *Vitis labrusca* L. species whose varieties represent about 80% of processed grapes in Brazil. Therefore, the aim of our study was to evaluate in *Vitis Labrusca* L. grapes, grown under subtropical conditions, the best period for the field application of MJ and its impact on free volatile compounds. The project was conducted at Rio Grande do Sul state (South Brazil) in two consecutive years. Free volatile compounds were extracted by SPME, analyzed by CG-MS and identified by similarity with NIST library entries and Kovats index. Multivariate analyses were performed in MetaboAnalyst 3.0. The best results were obtained when MJ applications occurred in two periods, during veraison and pre-harvest. Our study showed, on both harvests, an increase on ester and aldehyde levels, without interference on sugar and organic acid content. Increasing volatile compounds on *Vitis labrusca* L. grapes might be interesting since during processing, especially of juice and jellies, there is a decrease on their total content.

**Keywords:** methyl jasmonate, pre-harvest, *V. labrusca*, volatile compounds

## Flavor volatiles profiling in the different species of grapes between *V. vinifera* and *V. labruscana*

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Table grapes have some attractive consumable characters, including flavors. Domestic grapes in Korea are crossed with American grape species (*Vitis labrusca*) or their hybrids (*V. labruscana*) to prevent freeze damage and severe disease due to hard climate. Volatiles were collected from several domestic and imported grape (*V. vinifera*) using GC-MS analysis, and their profiling data were compared with PCA analysis. Imported grape in Korea, ‘Thompson Seedless’ and ‘Crimson Seedless’ from Chile and domestic grapes were differentially grouped in the PCA plot. Featured flavor volatiles separating the two different groups were identified with OPLS-DA analysis. Domestic grapes (*V. labruscana*) have typical volatiles including an ester group; ethyl butanoate, ethyl octanoate, ethyl hexanoate, and ethyl (z)-2-butenate, whose contents were higher than in imported grapes. Otherwise, imported grapes (*V. vinifera*) have groups of flavor volatiles (aldehydes and monoterpenes) differing from those of domestic grapes. Also, scents of storage materials such as hexanal and dibutyl phthalate were also detected in imported grapes. In domestic grape (*V. labruscana*), tetraploid variety has more kinds of flavor volatiles than diploid variety. Ethyl caproate is a typical volatile in tetraploid variety in Korea, whose content is higher than those in diploid variety and imported grapes. Diploid variety has typical volatiles such as 4-terpineol, p-cymene, and  $\beta$ -terpinene.

**Keywords:** flavor, GC-MS, PCA, volatiles

**Scouting downy and powdery mildew susceptibility genes: a diversity study in *Vitis spp***

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World viticulture is continually threatened by both known and emerging pathogens. Until now, the investigation on resistance loci/genes has been the main trend to understand the interaction between grapevine (*Vitis spp.*) and mildew causal agents. Dominantly inherited gene-based resistance has shown to be race-specific in some cases, not to confer total immunity and to be potentially overcome within a few years. Recently, on the footprint of research conducted on Arabidopsis and barley, genes associated to downy (DM) and powdery (PM) mildew susceptibility have been discovered also in the grapevine genome.

In the present work, in order to find new sources of broad-spectrum recessively inherited resistance against pathogens five susceptibility genes were re-sequenced (Illumina, 1000X depth) in 96 grapevine accessions including wild, vinifera and hybrid individuals. The scouted genes were *VvDMR6-1*, *VvDMR6-2*, *VvDLO1*, *VvDLO2* involved in susceptibility to DM and *VvMLO7* associated with susceptibility to PM. These genes were mapped on the reference genome and analysed to identify polymorphisms and haplotypes using dedicated software to study the mutation impact. Preliminary results showed 10 mutations affecting the *VvMLO7* protein structure (high-medium impact) dispersed in 75% of accessions; in particular, one Single Nucleotide Polymorphisms (SNPs) led to premature stop codons. Moreover, 70% of the accessions showed a total of 13 SNPs in *VvDMR6-1* and 11 in *VvDMR6-2* impacting their coding and amino acid sequences. Finally, 12 mutations were detected in the *VvDLO1* coding sequence in 37 individuals, whereas the *VvDLO2* sequence appeared much more conserved with only one SNP identified in four accessions. These findings will be validated taking advantage of several reliable parentages. Prior haplotype function confirmation, the final results will corroborate genomic-assisted breeding programs for resistance to biotic stresses.

**Keywords:** DLO, DMR, MLO, resistance, SNP

**Metabolite profiling at the graft interface of grapevine**

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In viticulture, grafting is used to facilitate grapevine cultivation in soils infected with the Phylloxera, a soil-dwelling insect pest introduced to Europe from America at the end of the 19th century. We have used microarrays to identify the genes differentially expressed between the wood and graft interface tissues of homo-grafts (the same genotype grafted together) and at the graft interface between different scion/rootstock combinations; this previous work has shown that transcripts encoding enzymes of both primary and secondary metabolism are differentially expressed during graft union formation.

Using enzymatic assays, high-performance liquid chromatography and liquid chromatography-mass spectrometry metabolomics approaches, the objective of this study was to profile primary as well as secondary compounds accumulated at the graft interface of homo and hetero-grafts of grapevine one month after grafting. Moreover, the activity of the phenylalanine ammonia lyase (PAL), first and committed enzyme in the phenylpropanoid pathway, was assessed.

The quantification of approximately 70 metabolites revealed that the majority of them were associated with either the different genotypes or tissue types studied. However, some metabolites showed a specific accumulation pattern at the graft interface of the different scion/rootstock combinations, in particular, the branched-chain amino acids leucine, isoleucine and valine, along with the basic amino acids glutamine and asparagine, and some glucosylated stilbenes such as astringin (piceatanol glucoside), trans- and cis-piceid (resveratrol glucosides).

The results also revealed higher PAL activity in the graft interface tissue compared to the surrounding wood, which was accompanied by a decrease of phenylalanine content and the increase of total stilbene contents. Based on these observations, we suggest an increased flow of primary metabolites to the stilbene biosynthesis in this tissue type. This study highlights the complexity of the metabolite changes occurring at the graft interface in grapevine.

**Keywords:** graft interface, HPLC, LC-MS/MS, metabolomics, microarrays

## Uncover the genetic basis of drought response in grapevine rootstocks

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Consequences of global climate change are becoming more evident, since abiotic stresses including drought, flooding and extreme temperatures severely impact viticulture in all wine-producing regions. Breeding grape rootstocks for resilience to water deficit is an achievable strategy; however, little information is available about the genetic control of grape tolerance to drought. A Genome Wide Association Study and a Candidate Gene approach were adopted in our study to investigate the genetic basis of drought response in an *ad hoc* core-collection consisting of different genotypes of *Vitis spp.* and hybrids. Grape accessions were characterized using the 20K SNP genotyping array and by resequencing four candidate genes. The effect of water stress experiment on pot-grown plants was evaluated under semi-climate controlled conditions in the greenhouse and phenotypic differences in stomatal conductance were assessed by means of thermal infrared imaging. A significant genetic association was found for stomata closure under severe drought therefore some representative rootstock genotypes (101.14, SO4, Riparia Gloire de Montpellier and 110R) were chosen for a deeper characterization. Plant transpiration and photosynthesis parameters were evaluated in additional drought stress experiments in greenhouse and in a hydroponic system. Differences in stomatal sensitivities and plant stress index were observed both among studied genotypes and the two experimental settings. These results represent a step forward in the dissection of grapevine rootstocks mechanisms of drought resilience.

**Keywords:** abiotic stress, GWAS, phenotyping, polymorphism, rootstocks, single-nucleotide

## Evaluation of resistance mechanisms of tolerant grapevine genotypes against *Plasmopara viticola* and its implication for crop protection management

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Viticulture worldwide is based on European grapevine cultivars which are highly susceptible to downy and powdery mildew pathogens. Therefore regular and extensive fungicide applications are required. This does not only strain the environment but is also associated with high expenses. To reduce the ecological and economic burden, resistant grapevine cultivars with genetic resistance loci against these pathogens are a promising alternative to reduce fungicide treatments. Several sources of resistance to downy mildew have been described in American and Asian native *Vitis* species and were used in resistance breeding. However, not much is known about the resistance degree of these new cultivars in vineyards, nor the actual fungicide saving potential or the respective resistance mechanisms. Field studies with new fungus resistant grapevine cultivars and reduced fungicide application variants showed on the one hand their high potential concerning fungicide reduction and on the other hand their different resistance degrees against *P. viticola*. Taken together, our results suggest that it is possible to reduce fungicide applications up to 75 % compared to susceptible varieties and depending on the cultivar and climate conditions. To gain new knowledge of the defense mechanisms comparative infection studies and gene expression studies in an Rpv 3.1, Rpv 3.1/Rpv 12 and susceptible cultivar have been performed. Hereby, variations in stilbene accumulation, occurrence of necrosis and differentially expressed genes have been identified between the different genotypes. Ongoing functional studies of these genes will reveal to which extent and in which resistance mechanism they could be involved during the defense against downy mildew. The knowledge about the differences of grapevine resistance mechanisms could be used for grapevine breeding attempts combining different mechanisms to increase the level and durability of grapevine resistance against downy mildew.

**Keywords:** *Plasmopara viticola*, Rpv3, Rpv12, stilbene

## Polyphenols composition of Dalmatian grapevine varieties grown under continental climate

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Much of the data acquired during the last few decades suggest that climate changes are taking place. These climate changes, in particular increasing temperatures, have important consequences on viticulture and different approaches have been proposed for adaptation. Shifting of grape varieties growth from their traditional sites towards a cooler climate is one of them. In Croatia, there are 125 autochthonous grape varieties including 38 red ones. The great majority of red grape varieties are native to the Coastal Croatia region, belonging to the wine growing zone CII and mediterranean climate. The aim of this study was to investigate the content and composition of individual polyphenolic compounds of 11 Dalmatian varieties which were grown in Zone B with continental climate. Since Merlot is used in continental climate for production of high quality red wines, it was used for comparison. Out of 11 cultivars included in this research, highest skin content (based on dry weight) of anthocyanins was determined for 'Nincusa' (63068 mg/kg), 'Trnjak' (29716 mg/kg), 'Dobricic' (24464 mg/kg), 'Ljutun' (23695 mg/kg). The content of anthocyanins of 'Plavac mali', (cv. with very late ripening) was lower (18740 mg/kg) but almost the same as that of 'Merlot' (19334 mg/kg). A similar trend was observed in the case of flavonol-glycosides. The content of flavan-3-ols determined in varieties 'Nincusa' and 'Rudezusa' was lower than that observed in 'Merlot', while the values obtained for 'Plavac mali', 'Dobricic' and 'Ljutun' were higher than for 'Merlot'. These results suggest that these varieties, based on the content and composition of polyphenolic compounds, have a potential for usage in continental climate wine production.

**Keywords:** anthocyanins, autochthonous Croatian grape varieties, flavonol-glycosides, flavan-3-ols

## New leaf-feeding Phylloxera (*D. vitifoliae* Fitch) biotypes collected in commercial vineyards employing a simple bioassay

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Leaf-feeding phylloxera populations are observed in commercial vineyards throughout the world. Feeding occurs not only on interspecific grape hybrids, but also on *Vitis vinifera* varieties. The reasons for this manifestation are still unknown, but the possible impact of climate changes in the environment and viticulture practices are currently studied. Both may pose selection pressure on existing phylloxera populations that, as a consequence, adapt their feeding behaviour, creating new biotypes. Our knowledge on the range of existing biotypes relies on an accumulation of small studies that define these biotypes based on plant symptoms and insect performance, sometimes using assays in fundamentally different settings. Moreover, although the presence and known impacts of phylloxera increased over the last years, there is no standardised or routinely used biotyping bioassay that can function as a reference for leaf-feeding behaviour.

Here we present a first study to biotype leaf-feeding phylloxera strains collected in commercial vineyards in Italy and Germany, employing a simple “bottle” system that allows the screening of isolated plants with phylloxera in replicates under standardized experimental conditions. We hypothesize that the *V. vinifera*-leaf feeding phylloxera strains comply with the biotype G definition (Forneck et al. 2016). The experiments were conducted with single founder lineages: IT1 and IT9: collected on leaves in commercial vineyards in Italy, H1: collected on leaves on rootstocks in Austria, MP1: collected on leaves on rootstock in France, and two standard Biotypes C and A. The experimental set up comprised three host plants (rootstock 5C, *V. vinifera* cv. Riesling and the interspecific hybrid Maréchal Foch) in 5 replications for each experimental lineage tested. Phylloxera feeding was monitored for 40 d, rating life table parameters (insect) and plant symptoms of root- and leaf-feeding on a weekly basis.

Our results first show the differentiation of leaf-feeding phylloxera biotypes corresponding to the classification G: feeding on leaves of *V. vinifera*, rootstocks and interspecific hybrids. We also present a simple and feasible assay and evaluation procedure for leaf-feeding phylloxera.

**Keywords:** biotypes, environment, galls, leaf-feeding, Phylloxera, resistance



## A preliminary screening of grape *Phylloxera* resistance on roots in wild grapevine accessions from different European regions

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Crop wild relatives are considered interesting novel resources of genetic variation in modern genetic programs for the improvement of fruit quality and resistance traits in perennial crops. Regarding the cultivated grapevine (*Vitis vinifera* L. *ssp.* *vinifera*), recent reports indicate that certain accessions of its wild relative (*V. vinifera* L. *ssp.* *sylvestris*) show some level of resistance against relevant grapevine diseases, including powdery and downy mildew, which indicates the interest of further screenings against other diseases or pests. Consequently, the present study aims at testing susceptibility levels of *V. vinifera* L. *ssp.* *sylvestris* accessions originating from different European regions (Italy, Germany and Turkey) towards two different biotypes of grape phylloxera (*Daktulosphaira vitifoliae* fitch). We used Riesling (*V. vinifera* L.) and rootstock Teleki 5C (*V. berlandieri* x *V. riparia*) as susceptible and partially resistant hosts, respectively. In isolated climate chambers dormant grapevine cuttings were rooted and individually inoculated with ten eggs from two root-galling grape phylloxera strains belonging to biotype A (adapted to Riesling) and biotype C (adapted to Teleki 5C). Root gall number and size were evaluated 30 days after inoculation. Although preliminary, a lower number of root galls was observed on the roots of some wild accessions when compared to the control, highlighting the interest of this plant material for further analyses aiming to determine the genetic factors contributing to grape phylloxera resistance mechanisms.

**Keywords:** genetic resources, phylloxera, resistance, resistance gene, root, rootstock, *Vitis sylvestris*

## Regulatory network behind the berry ripening: the role of *Vitis vinifera* NAC60 transcription factor

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Transcriptomic data obtained during berry development and integrated network analyses allowed to identify some members of the *VvNAC* gene family as candidate genes for the regulation of the onset of berry ripening. The NAC TFs family is functionally involved in a large variety of plant growth and development related programs. *VvNAC60*, which expression is very low in the vegetative/green tissues, significantly high in the mature/woody organs and shows a high negative correlation with genes that are down regulated during ripening was identified as “switch” gene and might represent a master regulator for the transition from vegetative-to-mature growth.

With the aim to characterize the function of *VvNAC60*, the Chromatin Immunoprecipitation Sequencing (ChIP-Seq) approach was selected to identify putative targets of this transcription factor. A preliminary structure prediction of the *VvNAC60*, obtained using either comparative modeling and *de novo* structure prediction methods, allowed to design and produce polyclonal antibodies anti-NAC60. Moreover, an efficient protocol for nuclei isolation and chromatin shearing has been optimized and preliminary results are reported.

The ability of *VvNAC60* to induce the expression of other members of NAC family was previously observed in Sultana leaves, transiently overexpressing *VvNAC60*. It was found by microarray experiments that *VvNAC60* activates the expression of *VvNAC03* and *VvNAC18*, the two grapevine NAC members closest to *LeNOR*, a master regulator of ripening in tomato. The ability of *VvNAC60* to activate the regulative region of *VvNAC03* and *VvNAC18* has been analyzed using the Dual-Luciferase reporter assay in *N. benthamiana*.

It is well known that NAC TFs undergo an intensive post-transcriptional regulation that includes microRNA-mediated cleavage. *Vv-miR164* has already been validated as a development-stage specific miRNA regulating *VvNAC33* expression, another “switch” gene. Based on this evidence, an artificial-miRNAs design of *Vv-miR3626*, the putative *VvNAC60* regulator, is ongoing and further berry infiltration experiments, aimed at the study of *VvNAC60* upstream regulation, will be performed.

**Keywords:** ChIP-Seq, Dual-Luciferase reporter assay, miRNAs, *VvNAC60*

# Transcriptomic analysis reveals a higher expression of genes involved in preformed defenses in the American grapevine *Vitis rupestris* compared to Eurasian grapevine *V. vinifera*

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The American grapevine *V. rupestris* proved to be unsuitable for winemaking, but it is commonly used as rootstock or to obtain hybrids with the Eurasian grapevine *V. vinifera*, due to its resistance to several phytopathogens. At present, the knowledge of the mechanisms involved in *V. rupestris* resistance is scarce and fragmentary. It has been hypothesized that metabolic and structural constitutive defences play a central role in *V. rupestris*, differently from *V. vinifera* in which a crucial role of the inducible defenses has been demonstrated. We compared the constitutive gene expression in *in vitro* cell cultures of *V. rupestris* and *V. vinifera* by means of RNA-seq coupled with bioinformatics analysis. In *V. rupestris* several genes involved in chemical defence were over-expressed, e.g. genes regulating the phenylpropanoid pathway as *PAL*, *STS*, *C4H* (stilbene synthesis); *LAR*, *ANR*, *CHI* and *F3H* (flavan-3-ols). Overexpression was also observed in genes involved in the responses to abiotic (*MYB* 4 and 30, *WRKY* 14, 26, 31, 46 and 53) and biotic (*WRKY* 7, 12, 14, 19, 32, 40, 41, 51) stress. In addition, several genes involved in the biosynthesis of PR proteins (*PR* 1, 4 and 10, *MPL*-like 28, *NDR* 1, *MLO*-like, *EDS* 1-like) as well as in the biogenesis of cell wall matrix component (pectins, hemicelluloses and glycoproteins) were differentially expressed. Our results support the hypothesis that in *V. rupestris* the dominant strategy to prevent the access and propagation of pathogens is largely based on preformed chemical (constitutive synthesis of phytoalexins) and structural (strengthening of the cell wall) defences. The obtained data do not exclude that in *V. rupestris*, as in *V. vinifera*, inducible defenses could be also activated, such as neo-synthesis of stilbenic phytoalexins and reinforcement of cell wall. Additional studies will be carried out in planta to extend transcriptomic analysis in both the species treated with elicitors or phytopathogens.

**Keywords:** plant defence, RNA-seq, *Vitis rupestris*

## Differential expression patterns within the grapevine stilbene synthase gene family revealed through their regulatory regions

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The analyses of the grapevine (*Vitis vinifera* L.) genome have revealed an unusually large and closely related stilbene synthase (*VvSTS*) gene family. Interestingly, despite the high sequence similarity among those genes, several studies have observed clear differences between their expression patterns. Here, we studied the transcriptional responses to different elicitors of several *VvSTS*s in cellular suspension cultures. Primarily, we performed the *in silico* analysis of the *VvSTS* regulatory sequences and found the presence of several putative cis-regulatory elements. Then, we evaluated the effect of three treatments—naphtalene acetic acid, methyl jasmonate (MeJA), and ethylene—over the gene expression and found that the genes follow expression patterns probably specific to their sequences. According to this, we focused our study on their regulatory regions and adopted a novel and efficient transient expression assay to determine the activity of these promoters. The results demonstrated that variation in gene expression could be assessed through the analysis of *VvSTS* regulatory sequences under the effect of different stimuli such as MeJA and cyclodextrins. Furthermore, taking advantage of the lower sequence identity at the promoter level, this strategy accomplished a more accurate alternative to differentiate the members of a large multi-gene family such as STS. This work is expected to provide evidence of the specific expression functions of *VvSTS* promoters and may benefit future research in understanding the regulation of gene expression.

**Keywords:** cis-regulatory elements, expression profiles, promoter analysis, resveratrol, transcriptome, transient transformation

**Evaluation of viral sanitary status of three portuguese minority grapevine varieties**

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Grapevine (*Vitis vinifera* L.) is a socioeconomically important crop in Portugal, but it is susceptible to numerous diseases caused by pathogens transmitted and perpetuated by vegetative propagation. According to the International Council for the Study of Virus and Virus-like Diseases of the Grapevine (ICVG), there are about 75 infectious agents, such as viruses, viroids, and phytoplasmas, that may reduce grapevine's vigour and the productive life of vineyards, as well as affect grape's quality. Reliable diagnostic procedures are pivotal to check sanitary conditions of the propagation material, hence, contributing for the growth of the viticulture industry. Therefore, the aim of this work was to evaluate the viral status of plants from three Portuguese minority grapevine varieties, Preto Martinho, Cornifesto and Malvasia Preta, using enzyme-linked immunosorbent assay (ELISA) as a standard diagnostic procedure. Scraping extracts from mature canes of donor plants were used as antigen samples to detect the presence of four viruses: Arabic mosaic virus (ArMV), Grapevine fanleaf virus (GFLV), Grapevine leafroll-associated virus 1 (GLRaV-1) and Grapevine leafroll-associated virus 3 (GLRaV-3). The virus infection of the referred field plants used as source material was confirmed and this preliminary work precedes the need for the implementation of virus eradication techniques in future assays, such as *in vivo* thermotherapy, combined with meristem, shoot-tip or axillary bud culture and somatic embryogenesis, in order to produce quality mother plants for this three Portuguese minority grapevine varieties.

**Keywords:** ArMV, GFLV, GLRaV-1, GLRaV-3, ELISA, *Vitis vinifera*

**Acknowledgments:** This work was supported by the European Regional Development Fund through the North Portugal Regional Operational Programme, under the project VINE AND WINE INNOVATION PLATFORM - INNOVINE&WINE, operation NORTE-01-0145-FEDER-000038.

**Genome-wide association study for crown gall disease resistance in grapevine**

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Crown gall disease, which commonly affects grapevines, is caused by *Agrobacterium vitis*. As eradication of *A. vitis* is difficult without the use of chemicals, the development of *A. vitis*-resistant grape cultivars is urgently needed. In a genome-wide association study (GWAS), green shoot cuttings of 350 grape cultivars were inoculated with *A. vitis* to obtain phenotypic data. Sixty days after inoculation, the pathogenicity of the formed crown galls was evaluated by measuring gall diameter. Gall diameters were found to range from 0 to 9.215 mm. A set of 350 grape cultivars underwent genotyping by sequencing (GBS), yielding 58,635 polymorphic and informative single-nucleotide polymorphisms (SNPs) after data editing. Using GBS data, cultivar phenotypes were converted into formatted data for GWAS analysis using TASSEL software. Six disease-resistant loci, containing 50 significantly associated markers, were identified from 58,635 SNPs. The P-values of the detected associations ranged from  $4.50 \times 10^{-11}$  to  $9.75 \times 10^{-8}$ . Of these markers, the most significant associations were present on chromosome 14, which contained 44 significantly associated markers, including proteins from the alpha/beta-hydrolase superfamily, a protein from the RING/U-box superfamily, a Frigida-like protein, and a NOP56-like precursor RNA processing ribonucleoprotein. Our findings will help future studies of crown gall resistance and the cloning of the genes involved in this process. This study provides insights into the genetics of crown gall resistance in grapevines, which may find utility in science-based crop improvement strategies.

**Keywords:** crown gall disease, GBS, GWAS

## Major characteristics of seedless grape cultivars bred by a grape breeding program in Korea

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‘Cheongsoo,’ ‘Hongju,’ and ‘Shiny Star’ stenospermocarpic seedless grapes are new cultivars (*Vitis* hybrids) developed by the National Institute of Horticultural and Herbal Science, bringing the total number of releases from the program to 17. These releases expand the options for grape growers for local markets in the Republic of Korea. The ‘Cheongsoo’ cultivar is green-fruited with a citrus-flower flavor and ripens in late August in the southern area of Korea. This cultivar is a cold-hardy white wine grape with high productivity and aromatic/flowery wine characteristics. The fruits of ‘Hongju’ and ‘Shiny Star’ have crispy flesh with good skin quality, fruit cracking tolerance, and cold hardiness. ‘Hongju’ is red-fruited with exceptional flesh crispness and Muscat flavor and ripens in mid-September in southern Korea. This cultivar presents large clusters of firm large berries containing seed rudiments. ‘Hongju’ will work well for export markets as it stores well for up to three months under the proper conditions. ‘Shiny Star’ berries are green, rather soft in texture, and have a foxy flavor. These grapes usually ripen in late August in southern Korea at the same time as the ‘Himrod’ cultivar and approximately 5 weeks before the ‘Thompson Seedless’ cultivar. These new cultivars provide for a range of dates of harvest along with choices of fruit color, shape, texture, and flavor. Because these new releases tend to be larger-fruited, crispier, and variable in aborted seed size compared with most commercial cultivars, these new cultivars will benefit the local-market growers in the Korea.

**Keywords:** new cultivar, seedless, stenospermocarpic

**Inner no outer transcription factor and its conserved role in eudicot ovule development**

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Inner no outer gene (*Ino*) is essential for formation and asymmetric growth of the ovule outer integument. It belongs to the YABBY gene family, coding putative transcription factors and encompassing different members whose proposed role in *Arabidopsis* is to specify abaxial cell fate. Mutations leading to failure in seed formation were studied in the model system *Arabidopsis thaliana*, where complete absence of seed was associated with defect in ovule development, which is a seed precursor. Moreover, in the tree species *Annona squamosa*, a spontaneous case of seedlessness associated to a deletion of the *Ino* locus was described. In our study, we investigated the phenotypic effect of two grapevines inner no outer alternative transcripts through genetic complementation experiments in the *Arabidopsis ino* mutant. The fully complemented lines showed wild-type ovules with normal outer integuments that form round or oval shaped ovules, whereas partial complementation showed limited growth of the outer integument with an exposed inner integument, but still with a significant right angle bend not seen in the uncomplemented lines. In grape *Ino* is located on chromosome 1 on a genomic region where a QTL for seed fresh weight was previously reported. A meta-QTL analysis on this QTL and co-localising QTLs involved in berry size allowed to restrict the original QTL to a closer region still including this candidate. Moreover, to further explore a possible functional involvement of *Ino* in modulation of seedlessness trait in grape, we compared the expression levels in seeded/seedless grapes and found reduced expression of this gene in the seedless variety.

**Keywords:** inner no outer, ovule development, yabby



**Aromatic characterization of croatian white autochthonous grapevine cultivars**

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Numerous volatile compounds of grape berries play a major role in the aroma of the future wine. Despite the significant effect of different environmental conditions and management practices, grapevine cultivar has a dominant impact on content and composition of volatile compounds in white wines. The significant attention given to the local grapevine cultivars could present a new wine market trend which would help their promotion and revitalization. Since wine aroma largely affects the quality and value of white wines it is necessary to perform detailed aromatic characterisation of neglected cultivars before the final assessment of their potential for modern wine production.

So far, 125 autochthonous grapevine cultivars were found in Croatia. Dalmatia, the coastal part of Croatia, is especially rich in autochthonous grapevine cultivars. However, only a couple of these have a greater economic value. The aim of this study was to evaluate the volatile compounds in 24 rare white autochthonous grapevine cultivars for the first time. The volatile compounds represented by monoterpenes, norisoprenoids and C6-compounds were detected and quantified using GC-MS method.

According to the investigated compounds, highest content of monoterpenes was determined in cvs. Silbijanac (27,59 µg/kg), Prc (22,63 µg/kg) and Gegic (21,30 µg/kg) compared to the other investigated varieties. The content of the norisoprenoids analyzed was higher in Silbijanac (1,1 µg/kg), Trisnjavac (0,99 µg/kg) and Marastina (0,95 µg/kg) than in other varieties investigated. The highest content of C6-compounds was detected in Malvasija dubrovacka (32,38 µg/kg) and Marastina (22,53 µg/kg).

Based on these results, most varieties showed good potential for white wine production. This study presents a step-in characterization of Croatian autochthonous grapevine varieties. However, these results showed that these rare varieties can become a good alternative to the most prevalent grapevine varieties used in Croatia.

**Keywords:** aromatic profile, autochthonous grapevine cultivars, monoterpenes, norisoprenoids

## Functional characterization of a heat-inducible ethylene response factor and its putative role in the control of the sugar/acid balance in grape berries

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One of the main consequences of global warming in grapevine consists in the rise of berry K<sup>+</sup> and sugar concentrations at harvest. This trend already observed since several decades in Europe (and farther) results in wines with increasing alcohol contents, more flat, and with decreased ageing potential. The control of the sugar/acid balance of the berries would allow maintaining the typicity, the quality and the market value of French wines. This requires a better understanding of the molecular basis of K<sup>+</sup> and sugar accumulations along grape berry development and in response to climate change. To this aim, the characterisation of the molecular repertoire of genes and regulatory networks involved in the fine-tuning of the fruit sugar/acid balance at harvest was initiated through the french ANR SWEETKALIGRAPE program. Differentially expressed genes from flesh cells upon drought and/or high temperature stress were identified through RNA-SEQ analysis. Here, we describe the functional characterization of a heat-inducible transcription factor belonging to the large ERF family (Ethylene Response Factor). VvERFhs is ubiquitously expressed in vine and highly inducible upon heat stress (HS). A RNA-SEQ analysis of transgenic grape cells overexpressing dominant and dominant-negative versions of VvERFhs was conducted. Several putative VvERFhs target genes linked to K<sup>+</sup> or sugar accumulation were identified. Complementary approaches (Electrophoretic mobility shift assays, yeast-one-hybrid, and dual-luciferase assays) are on the way to evaluate the ability of VvERFhs to bind and transactivate the promoters of these different genes. In conclusion, the SWEETKALIGRAPE program should contribute to open new research lines through the identification and characterization of key players involved in the control of berry quality and should provide an improved basis for the selection of vines adapted to our future environment.

**Keywords:** acidity, ethylene response factor, grapevine, heat stress, potassium

## Genetic characterization of grape genotypes from Apulia and synonymies in other Mediterranean regions

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Currently Apulia region accounts about 59 winegrape cultivars authorised for growing, including historical varieties. The existence of autochthonous minor varieties in the region has been recognized and is recently at the centre of a program aiming to their recovery and their further exploitation for commercial use. In the present study, 87 traditional, autochthonous and minor Apulian grapevine accessions were examined for their genetic profiles. Among them, 64 unique genotypes were found. Synonyms among Apulian genotypes and Italian varieties cultivated in Apulia or other Italian regions were identified by searching for genetic matching in different molecular database publicly available and through bibliographic search with published genetic data. The main goal of the current study was to identify, characterise and catalogue local germplasm rich of economical and historical significance.

**Keywords:** grapevine identification, microsatellites, *Vitis vinifera*

**Valorization of autochthonous Apulian grapevine varieties for spumante production**

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Italian spumante has a long tradition, deeply-rooted in the Piedmont region since the 19th century. Acidity plays a fundamental role in the spumante production, since it influences the quality and the taste of spumante. The acidity of the berry is due to the accumulation of H<sup>+</sup> ions in the vacuole of the cell and this process is regulated by several genes able to achieve a certain acidity level. Furthermore, acidification is strongly influenced by environment; in fact, higher temperature is associated to a lower acidity level. As part of a project aimed to evaluate and promote Apulian cultivars for spumante production, the autochthonous variety Maresco was compared with the international variety Pinot Blanc, commonly used for spumante production for the study of factors involved in the vacuolar acidification. An RNA-seq experiment was carried out in order to identify genes potentially involved in this process. The experiment was performed on three different tissues, seed, berry skin and flesh, at two different time-point, pre-veraison and 50% of veraison stages. Two candidate genes were identified and investigated in eight different grapevine varieties, such as the Apulian cultivars: Bianco d'Alessano, Bombino Nero, Maresco, Minutolo, Negramaro, and Uva di Troia, and the international varieties Pinot Blanc and Pinot Noir, through Real Time PCR in order to highlight different expression levels. Then, the molecular data were correlated to the acidity values of the berries.

**Keywords:** RNA-seq, spumante, vacuolar acidity

## **A *Vitis* hybrid 'library': comparing diverse R-loci combinations and mildew resistance levels in field**

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Nowadays, it is almost universally recognized that the viticulture of the future will require the management of grapevine pests and diseases with fewer chemical inputs. The development and the deployment of novel varieties - which are now extremely resistant to mildews as well as hold the potential to display a durable resistance during the coming years - are considered one of the most promising strategy towards an eco-friendly viticulture.

In the frame of the Euregio project VITISANA, a collection of approximately 100 grapevine accessions - including (mid-)resistant genotypes derived from cross pollination between *Vitis* hybrid and vinifera varieties or backcrosses - was studied. Their leaf and cluster level of downy (DM) and powdery (PM) mildew resistance was evaluated in an untreated field (Marlengo, I) at veraison and harvesting time in both 2016 and 2017. In addition to attempting a true-to-type analysis, an exhaustive genetic characterization was carried out at the 12 exploitable loci associated to mildew resistance (R-loci) available in the literature to date. Besides genotypes carrying a single R-locus associated to DM or PM resistance, our findings highlighted the pyramiding of R-loci against DM in 15% and against PM in 35% of the total accessions. In particular, 56 genotypes resulted pyramided for R-loci to both mildews. Finally, combining the R-loci-based characterization of the studied traditionally bred resistant varieties with their pathogen response in untreated field, we will understand the impact of diverse R-loci assets on overcoming disease attacks under the same environmental conditions.

**Keywords:** downy mildew, powdery mildew, natural infection, pyramiding, resistance-associated markers

## New cultivars from the table grape breeding program at the Agricultural Research Council (ARC), South Africa

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Changing consumer preferences necessitate regular revision of aims in table grape breeding programmes. The bulk of the South African table grape crop is exported and mainly to European and UK markets. Therefore, good cold storage ability is paramount for successful commercialization of new cultivars. The ARC Infruitec-Nietvoorbij institute conducts a table grape breeding program and develops new seedless cultivars by *in vitro* embryo rescue techniques and seeded cultivars with unique characteristics through conventional breeding. Joybells, a new red seedless table grape was introduced globally at Fruitlogistica in Berlin on 6 February 2018. Joybells ripens in the early mid-season (shortly after Flame Seedless), has a slightly bell-shaped berry, a vibrant dark red skin colour, crunchy flesh and a neutral flavour, with an excellent sugar/acid balance. Independent storage trials have confirmed very good storability. Queen Ruby with a pending application for Plant Breeders' Rights is next in line for release. Queen Ruby is an early ripening, rosé coloured, seedless cultivar with large natural berries and firm to crunchy flesh. In warmer production areas abscisic acid or ethephon may be needed for good colour. Bunches set naturally loose to very loose. G3-578 is a seeded selection with excellent eating quality. It resembles one of the parents, Red Globe, but has a distinct fruity (passion fruit/ granadilla) aroma. It is envisioned that these new cultivars will contribute to the growth of the South African industry, especially since they were developed under local climate conditions.

**Keywords:** cold storage, fruit quality, new cultivars, seedlessness

## Functional analysis of SCL8/VviPAT6 and orthologous SlGRAS10: role in nonclimacteric and climacteric fruit ripening

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The plant-specific GRAS family is important transcription factors involved in a wide range of functions in plant growth, development and stress response as suggested from functional and genome-wide analyses.

Recently, studies in grapevine and in tomato suggest an involvement of the orthologues SCL8/VviPAT6 and SlGRAS10 in the onset of ripening in nonclimacteric and climacteric species, respectively.

In this study, we generated constructs for targeted knock-out by using CRISPR/Cas9 technology in both fruits. Agroinfiltration assays for transient expression in grapes are undergoing. Regarding SlGRAS10, two single guide RNAs were designed to target different sites of the gene. Through the *A. tumefaciens*-mediated transformation method, we obtained a total of 37 transgenic lines. To precisely calculate the efficiency, the target sequences were sequenced in each transgenic plant. Based on PCR 27 lines were positive for Cas9 endonuclease presence. Mutation rates were the same for the two constructs, namely 50% for Sg1 and Sg2. However, mutation efficiencies were between 88.7% and 7.3% but higher for Sg2. In fact, Sg2 seems to be the best candidate for further analyzes and to develop T1 transgenic lines. Considering guidelines already available, the fact that gRNA2 targets the non-transcribed strand and has an average GC content (55%), makes gRNA2 a better candidate than gRNA1. Furthermore, changes in tomato ripening parameters will be evaluated using next generation RNA sequencing for the transcriptome and GC-MS for the metabolome. Tomato plants overexpressing the SlGRAS10 will be also developed to study the effect of upregulation of SlGRAS10 on fruit ripening. By assessing the phenotype, transcriptome and metabolome of the mutated fruits, the functional role of the GRAS gene in climacteric fruit ripening is expected to be characterized and ascertained.

**Keywords:** characterization, CRISPR, GRAS, maturation, tomato (*Solanum lycopersicum*), transcription factors

## Genetic fingerprint of autochthonous varieties preserved in ancient vineyards of mountain valleys of Argentina

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Native varieties of South America called criollas have been assumed to be a reduced group of few cultivars widely spread in Argentina. However, there is recent evidence that the variability observed within this group of varieties is higher than previously thought. Based on our previous findings in the INTA Vine Collection and on bibliographic data from XIX and early XX centuries, we hypothesized that minor and relict varieties scarcely dispersed may still exist, preserved in the valleys of Los Andes Mountains. For this, we collected vegetal material from ancient vineyards in San Juan and Catamarca provinces. We used 20 nuclear simple sequence repeat markers and parental analysis to identify and propose the pedigree of 25 samples collected in 5 valleys of these provinces. The results were compared with data recorded in large international databases (e.g. VIVC) and our database, focused on autochthonous genotypes. Our results showed that 10 genotypes corresponded to not previously described varieties which could be associated to criollas family according to its parental origin. Furthermore, the ampelographic characteristics of these genotypes were coincident with the description of old varieties that were thought missing. By contrast, others genotypes analyzed, corresponded to already identified varieties currently conserved at the INTA Vine Collection. The maintenance and cultivation of ancient vineyards by small farmers in isolated valleys, play a key role in the conservation of genotypes that otherwise may have been lost. The rescue, identification, and conservation of these genotypes represent a challenge and also an opportunity to characterize the potential use (e.g. breeding programs) of this unexplored genetic variability, generated over five centuries.

**Keywords:** autochthonous genotypes, conservation, cultivar identification.



## Relevant factors contributing to the final size of the berry in two Tempranillo segregant populations

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Berry weight is considered one of the most relevant traits contributing to berry quality both in wine and table grapes. Besides, berry shape may influence phenolic extractability due to the ratio skin/pulp. Berry weight has been associated to flower size and shape and to seed parameters. Two wine grape populations (Garnacha x Tempranillo and Graciano x Tempranillo) have been phenotyped for berry parameters (berry weight, length, diameter and shape) and flower traits (pistil length, ovary length, flower shape and flower sex types) in two different years. In both progenies, segregation of sex hermaphrodite/female adjusted to a 3:1 ratio. Flower shape, measured as the ratio between length and diameter, was significantly different in hermaphrodites and female flowers, being female flowers more rounded. However, no significant differences were observed between berry parameters in relation with sex genotype in either population. Significant differences were found between heterozygote genotypes Hf for the sex locus compared to the homozygotes HH evaluated with the VVIB23 marker, for berry weight and flower shape. Associations between berry and flower traits have been observed in both genetic backgrounds suggesting that berry shape is determined mainly by flower length. Correlations were higher in the Garnacha x Tempranillo progeny. This work provides tools for identifying the main genetic determinants for berry weight and shape and its relationship with sex and flower size and shape. Relationships among sex and quality features in wine grape will be discussed.

**Keywords:** berry weight, flower sex, Garnacha, Graciano, hermaphroditism

## Associations between flower and berry parameters and wine composition of Graciano x Tempranillo hybrids

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Consumers demand for different and high quality wines has been frequently answered by the market by introducing foreign varieties. An alternative strategy could be to obtain cultivars by the genetic improvement of traditional varieties selected for better adaptation to climate change. In this context, the aim of this study was to evaluate 12 genotypes selected from a progeny of Graciano x Tempranillo, and to assess the relation between flower and berry parameters and wine composition. Selection of genotypes was based on data from 3 different vintages including agronomic traits as well as quality traits. Out of the 12 genotypes studied, 7 were considered early-ripening, compared to Tempranillo, and 5 late-ripening. Microvinifications were conducted in 2017 with 3 replications for both set of selections and the parental genotypes. Wines were analyzed by OIV official methods, for the standard parameters. For the analyses of berry parameters data were taken of two different harvests by measuring berry length, diameter and shape. Flower parameters; length, diameter and shape of pistil and ovary, were measured by digital tools. The results suggest that late-ripening genotypes differed significantly from Tempranillo, having lower values of malic acid, pH, Cielab index, berry length, diameter and size. Based on those results, they could be an interesting alternative in the context of climate change, depending on sensorial analysis. Besides, late-ripening genotypes had significantly lower values of IPT and IC in comparison with early-ripening ones, and higher Cielab parameter index. A correlation matrix showed that berry diameter was significantly negative correlated with anthocyanin content, IPT and IC, suggesting that small berries lead to higher quality. Also, a significant positive correlation between ovary and berry shape, and IPT was found, showing associations that would need to be corroborated by genetic analyses.

**Keywords:** berry composition, Cielab index, climate change, IPT

**Characterization of different clones of cv. Criolla chica (Syn. Listán Prieto), a variety long-time cultivated in South America**

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Listan Prieto is an old Spanish variety brought to Peru from the Canary Islands during the XVI century. Thereafter, it was spread to different countries, including Argentina where it was the main cultivated variety under the name of Criolla chica until the middle of the XVIII century. Hence, it has a long history of cultivation (around 5 centuries) in the region. The aim of this work was to characterize different clonal variations collected in vineyards located in Salta, San Juan, and Mendoza provinces (Argentina), and afterwards conserved in the Vine Collection of INTA Mendoza. For this aim, we analyzed 20 SSR loci to determine the genotype identity, and we employed 18 OIV descriptors to characterize their phenotype. In addition, the content of polyphenols in the berry was also analyzed. Our results showed important variations in some phenotypic traits such as time of ripening and maturation, berry color and size, color of petioles, leaf indumentum and flower sex. One of the clones presented male and hermaphrodite flowers with female organs atrophied, leading to poor berry set. The rest of the clones presented hermaphrodite flowers only. We also found differences in polyphenols content which may be interesting in terms of enological potential. By contrast, other ampelographic traits remained unchanged (e.g. shape of blade and shape of teeth in the mature leaf). The selected traits allowed us to describe the intravarietal variation observed in Listán Prieto clones and open the avenue for new research in terms of selection for berry size and wine composition.

**Keywords:** berry composition, berry size, flower sex, intravarietal variability, phenotype

**Field evaluation and salts exclusion of some new nematodes resistant rootstocks**Ashraf El-Kereamy<sup>a,\*</sup>, Jennifer Hashim-Maguire<sup>a</sup>, Matthew Fidelibus<sup>b</sup><sup>a</sup> *University of California, Cooperative Extension Kern County, Bakersfield, CA 93307, USA*<sup>b</sup> *Department of Viticulture and Enology, University of California, Davis, Kearney Agricultural Center, Parlier CA 93648, USA*\* **Presenting author:** aelkereamy@ucanr.edu

Grapevine roots are vulnerable to numerous soil pests, including plant-parasitic nematodes and phylloxera. In the San Joaquin and Coachella valleys of California, areas most suitable for growing table grapes, nematodes are the most critical soil pests. Numerous species of nematodes may feed on grapevine roots and cause severe damage. Rootstock breeding programs in California have released many nematode-resistant stocks in recent years, including 10-17A, 10-23B, RS-3, RS-9 and GRN series (GRN 1-5). These rootstocks have been subjected to rigorous nematode-resistance screening tests in laboratory and greenhouse settings. However, data are lacking to describe their horticultural performance as grafted vines. Therefore, we tested these rootstocks against vines on standard stocks and own-rooted vines in field studies to assess effects of the stocks on vine nutrition, salt uptake, and vine productivity and fruit quality of Autumn King and Scarlet Royal table grapes in commercial vineyards. Bloom nutritional analysis, marketable yield, and fruit quality were determined for three years. Our data showed that most of the rootstocks reduced sodium and chloride accumulation in the petioles at bloom stage compared to vines with *Vitis vinifera* roots. Our data revealed that in addition to the resistance to nematodes, some of the new rootstocks reduced the accumulation of sodium and/or chloride in the scions and therefore might provide some resistance to salinity without affecting vine yield or fruit quality. Some of these rootstocks affected the accumulation of nitrogen, potassium, and zinc in the scions.

**Keywords:** autumn king, rootstocks, salinity, scarlet royal, table grapes

## Screening of *Vitis berlandieri* for Phylloxera response and molecular markers of a major QTL for Phylloxera resistance

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Grape phylloxera (*Daktulosphaira vitifoliae* (Fitch 1855)) is a severe pest threatening commercial vineyards worldwide. The sap-sucking insect is native to North America. The co-evolution with native *Vitis* species led to the development of natural defense mechanisms against this pest. Roots of European grapevines (*Vitis vinifera*) are highly susceptible, thus the only means of controlling phylloxera is to graft *V. vinifera* onto rootstocks which are derived of American species. Different degrees of resistance to phylloxera can be observed in a number of accessions of several wild *Vitis* species including *V. berlandieri*.

A collection of about 700 *V. berlandieri* accessions was visually assessed for foliar reactions to phylloxera in the field. A subset of accessions displaying three different types of foliar reactions (local necrosis also called hypersensitive response (HR); HR and some fertile galls; fertile galls only) were screened additionally for foliar and root reaction as potted plants. A large range of reactions was observed. A correlation analysis was conducted between greenhouse and in field foliar reaction and between leaf and root reactions. In parallel, all ~700 individuals were screened for the presence of markers for a major QTL of root resistance called Rdv1 (Resistance *Daktulosphaira vitifoliae*). Rdv1 had been localized previously in a *V. vinifera* x Börner (*V. riparia* x *V. cinerea*) cross. It is linked to the HR of the rootstock Börner, inherited from *V. cinerea* Arnold. The species *V. berlandieri* appears to be closely related to *V. cinerea*, indeed they are also frequently regarded as a single species. Hence the *V. berlandieri* collection was screened for the presence of Rdv1 resistance marker alleles. In the accessions evaluated, no clear association with Rdv1 marker alleles was detected. The absence of these alleles points towards alternative genetic determinants of phylloxera root resistance that may be utilized in future breeding programs.

**Keywords:** phylloxera, Rdv1, *Vitis berlandieri*

## Molecular characterization of wine grape cultivars from Calabria region

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The wine sector is one of the most active and profitable in Italy, thus classifying Italy as the first largest wine producer in the world followed by Spain and France. For this reason, it is subjected to an extensive legislative discipline. Calabria is the sixteenth Italian region for wine production; however, in the last years many efforts have been made to promote the wine sector in this region. Nine Calabrian wines are designed as PDO (Protected Designation of Origin) and ten wines as PGI (Protected Geographical Indication). Among the PDO wines, “Terre di Cosenza” and “Cirò” are the most famous. The fraud prevention is necessary for the consumer protection and for product valorization and promotion. The microsatellite marker analysis represents a reliable and effective tool for varietal characterization, allowing the detection of adulteration in wine making process. We sampled twenty grapevine cultivars from different areas of Calabria and twenty Italian varieties in order to carry out a molecular characterization. The analysis was performed through 6 simple sequence repeat (SSR) markers proposed by the International Organisation of Vine and Wine (OIV), VVSS2, VVMD5, VVMD7, VVMD27, VrZAG62, and VrZAG79. We obtained a panel with the allele size at each of the six loci analyzed. Some of the cultivars showed the same molecular profile revealing the presence of synonymies and incorrect name assignment.

**Keywords:** Calabria, molecular markers, traceability, wine

**GBS-derived SNP catalogue from a wide grape (*V. vinifera* L.) germplasm collection that includes the most representative Apulian autochthonous varieties**

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Grape (*Vitis vinifera*) is one of the oldest cultivated plants. Its domestication began in the Near East 6000–8000 years ago. Genetic diversification and phenotypic differentiation generated a wide diversity which remains largely under-explored. Apulia has for centuries served as a site for the meeting of different cultures since ancient times. This resulted in a rich heritage of grape genetic resources that best adapt to the region's climate and soils. Information on genome-wide patterns of genetic variation and knowledge on population structure of *V. vinifera* germplasm is essential to define priorities for management and conservation of gene pools, to develop new sustainable cropping systems and to recover alleles left behind by selective breeding. In this study we applied genotyping-by-sequencing (GBS) to assess genetic diversity of a wide grape germplasm collection including 188 cultivars, most of which are represented by Apulian autochthonous varieties. GBS tags were aligned to the Pinot Noir genome (PN40024) and a reference-based SNP calling pipeline generated over 134k unfiltered single nucleotide polymorphisms (SNPs). The starting list of SNPs was then subjected to filtering and pruning in order to remove all the SNPs that are in high linkage disequilibrium. These operations generated a high-quality SNP dataset (~9,200 SNPs) that was fed into different population structure analysis software. In addition, to improve knowledge on genetic diversity in *V. vinifera*, the large panel of SNP markers we generated will be combined with phenotypic data points via genome-wide association studies in order to facilitate the discovery of genetic loci that are associated with key agronomic traits.

**Keywords:** GBS, genetic diversity, *Vitis*

## Recovery from flavesence dorée phytoplasma infection in *Vitis vinifera* cv Barbera: a putative role for sugar metabolism

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Flavesence dorée is an epidemic disease, caused by FD phytoplasma (FDp) infection, which widely affects European grapevine yields in terms of quantity and quality. Symptoms, as leaf veins reddening or floral abortion, occur along the vegetative season following phytoplasma colonization of phloem sieve tubes. Nevertheless, FDp-infected plants can undergo recovery, a natural symptom regression connected to a decreasing phytoplasma titre. The aim of this work was to induce activation of recovery in the FDp-highly susceptible grapevine cultivar Barbera, grown under controlled conditions, in order to investigate in depth physiological and molecular mechanisms involved in this process. As previous results showed that phytoplasmas affect carbohydrate levels in infected tissues, we focused on the regulation of sugar metabolism. Induction of recovery was performed in artificially infected, pot-grown plants, using a girdling treatment. Diagnostic assays and FDp titre quantification, performed from July to October 2016, showed that girdling was effective in inducing recovery. Expression profiles of some target genes, such as sucrose synthase (VvSUS2), and vacuolar invertase (VvGIN2), demonstrated up-regulation in the infected leaf samples, in particular at the early stages of development. Soluble sugar quantification shows an increase of sucrose in recovering plants, suggesting a possible role of this carbohydrate in activating the recovery process.

**Keywords:** Flavesence dorée, girdling, recovery, sugar metabolism, sucrose



## Getting closer to the whole picture: transcriptome and metabolome analyses of Aglianico and Falanghina berry tissues during fruit maturation

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Since the physiological and biochemical features of grape berries at harvest has a profound impact on the wine characteristics, there is great interest in understanding the molecular mechanisms triggered and regulated during berry ripening. Among all the Campania wines, Aglianico and Falanghina are appreciated worldwide for their sensorial features, although the molecular mechanisms responsible of their distinctiveness remain largely unexplored. The aim of this work was to decipher the transcriptional and metabolic regulation in Aglianico e Falanghina during berry formation and ripening. To this end berries were collected during fruit-set, post-fruit set, véraison, ripening and technological ripening, dissected in skin and pulp and used for metabolome profiling through GC-MS and LC-HRMS and genome-wide expression analysis using mRNA-seq. As for metabolic profiling, Aglianico displayed a higher level of branched-chain amino acids (bcAA), phenylpropanoid and C5-lipid volatiles, whereas Falanghina exhibited increased levels of monoterpenoids, norisoprenoids and C6-lipid derived volatiles. Furthermore, we observed metabolites with cultivar- and tissue-specific accumulations. Regarding gene expression analysis, the overall trends were mainly related to temporal dynamics rather than tissue identity. Indeed, during different developmental stages, the magnitude of gene expression at véraison was the highest for most of the genes analysed both in skin and pulp. Genes involved in NADH-dependent cell respiration, electron transport and catabolism of sucrose and starch, displayed a reduction of expression in all tissues and during all stages analysed. In contrast, the genes involved in the metabolism of glutathione and the anabolism of protoporphyrin and chlorophyll showed a statistically significant expression increase from fruit-set on. Similar patterns have been observed for polyphenol biosynthesis genes. In conclusion, the data obtained provide a deeper knowledge on the correlation between genes and related metabolite in Aglianico e Falanghina useful to assist the development of wines of higher quality.

**Keywords:** berry development, Campania wines, GC-MS, LC-HRMS, mRNA-Seq, volatiles

## Overview of genetic loci for traits in grapevine and their integration into the VIVC database

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In 2010, on the 10th International Conference of Grapevine Breeding and Genetics in Geneva, New York, USA, a group of grapevine breeders and geneticists discussed a nomenclature system for genetic loci in grapevine. Especially for resistance loci it was suggested to go on with the naming and spelling type of previously published loci with a consecutive number, e. g: Ren1, Ren2 or Rpv1, Rpv2, etc. In new cases where no resistance locus has been published yet it was recommended to follow the naming system accordingly: Rxy, with R for resistance and xy for the initials of the Latin name from the pathogen. The second aspect was to establish a system avoiding homonymous naming of different loci that would lead to confusion in the literature later on.

To make the current status of published grapevine trait loci or genes more transparent and publically accessible we compiled a table with most of the relevant data available. This 'Table of loci for Traits in Grapevine' has been uploaded as a pdf document on the Vitis International Variety Catalogue (VIVC) web site and can be retrieved under [www.vivc.de/loci](http://www.vivc.de/loci). This table will be updated if there are new data published or when somebody asked to reserve a new trait locus name ahead of publishing. To provide more comfort we are currently linking this information to the data of the VIVC database itself by implementing a search module.

**Keywords:** mildew, resistance loci, trait, VIVC

## Low night temperature enhances anthocyanin accumulation and induces gene expression in cv. Corvina (*Vitis vinifera* L.)

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Compared with day temperature, night temperature has increased faster at global scales and therefore all plants, included grapevine, in the future will be exposed to warmer nights, which could greatly influence plant yield and physiology as well as the ripening of the berries. Following these premises, in the present study we evaluated the effect of night temperatures on grape coloration in the cv. Corvina (*Vitis vinifera* L.). In 2015 and 2016 potted plants were cooled overnight (10-11°C) during two phases of berry ripening, veraison (TV) or post-veraison (TPV), and compared to control vines (C) grown at ambient night temperature (15-20 °C on average). Regardless of the timing of application, no effects of night temperature decrease on berry TSS accumulation and on titratable acidity at harvest were detected in both years, even if climatic conditions in 2015 and 2016 were very different. Cooling treatment around veraison (TV) hastened berry anthocyanin accumulation especially in 2015, when the veraison phase was characterized by higher temperature in comparison to the last part of the season and to the following year (2016). On the other hand, the same treatment applied after veraison (TPV) was ineffective in both the experimental years. Molecular analysis revealed an increased transcription of four key genes involved in anthocyanin biosynthesis (CHS3, F3H1, MYBA1, UFGT) only in TV. This increase was not affected by the seasonal trends and surprisingly, preceded the enhancement of anthocyanin accumulation. These results suggest that the anthocyanin biosynthesis capacity was enhanced under cool nights during veraison. However, since the gene expression was not always temporally correlated to the increase in anthocyanin concentration, we speculate on the presence of post-transcriptional mechanisms that may contribute in regulating the anthocyanin accumulation under low night temperatures.

**Keywords:** anthocyanins, climate change, gene expression, night temperature

## Functional complementation of non-ripening (*nor*) tomato mutant with four NAC transcription factors, putative master regulators of the vegetative-to-mature organ transition in grapevine

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The NAC (NAM/ATAF/CUC) family is one of the largest classes of transcription factors (TFs) in plant kingdom with important functions as components of the regulation of various biological processes. Recently, some grapevine NAC members have been indicated as putative master regulators of the transcriptome shift driving the plant into a maturation program. In particular, VvNAC33 and VvNAC60 were selected as putative master regulators able to promote the immature-to mature transition in the entire plant, VvNAC11 in the berry, whereas VvNAC03 as close homologue gene of tomato NOR. The challenge of this study is an attempt to understand the roles of this four VvNACs in the regulatory network controlling the transcriptomic reprogramming which takes place along plant and berry development. Due to the current lack of grapevine mutant collections and the moderate recalcitrance of this species to stable transformation, a functional complementation analysis on *nor* mutant tomato (*Solanum lycopersicum* cv. Ailsa Craig) overexpressing the four NAC TFs were carried out. Different developmental stages of T2 tomato fruit were deeply analyzed to check the ability of the selected VvNACs to fulfil the tomato NOR function. Regarding the phenotype, some of transgenic fruits among VvNAC11 and VvNAC33 showed a slight pericarp pigmentation after breaker, whereas all VvNAC03 and VvNAC60 transgenic fruits presented pericarps with different degrees of yellowness at the same stage, in comparison to the same-stage *nor/nor* fruits. Moreover, the production of ripening-related ethylene was measured and the expression level of three known ripening-related regulator genes, i.e. ACC synthase (ACS4), polygalacturonase (PG2a), phytoene synthase (PSY1), were performed on the overexpressing fruits. The results supported the phenotypes above-mentioned, suggesting a partial complementation of the *nor* mutation and, thus, the involvement of these four VvNACs in the organ phase transition to mature growth.

**Keywords:** functional analysis, master regulators, NAC TFs, tomato NOR mutant, vegetative-to-mature organ transition

## MYB5 and WRKY transcription factors in grapevine (*Vitis vinifera* L.) co regulate vacuolar acidification through the activation of P-type ATPases

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MYB-bHLH-WD repeat (MBW) transcriptional complexes are highly conserved among plant species and control multiple pathways related to epidermal cell fate. The flexibility of the MBW complex in the transcriptional control of different targets largely relies on the alternative participation of different bHLH and MYB proteins and on interactions with other transcription factors. Here we investigated the function of a MBW complex in grapevine (*Vitis vinifera* L.) involving two MYB proteins and a further layer of regulation represented by the recruitment of the WRKY-type transcription factor VvWRKY26. We found that VvWRKY26 is a target of a VvMYB5a-driven MBW regulatory complex and physically interacts with the WD repeat partner to create an autoregulatory loop similarly to other species. The stable transformation of grapevine plants to alter the expression of each transcription factor generated severe phenotypes featuring abnormal leaf morphology, crude leaf extract pH, and pigment accumulation. Transcriptomic analysis defined a core set of putative targets controlled by the regulatory network involving VvMYB5a, VvMYB5b and VvWRKY26 (MBWW complex). These targets are mainly related to vacuolar transport and membrane remodelling. We demonstrated that VvWRKY26 probably enhances the expression of selected target genes, including those encoding the P-type ATPases VvPH5 and VvPH1, induced by VvMYB5a/b. In addition, VvWRKY26 is also recruited specifically by VvMYB5a, reflecting the functional diversification of VvMYB5a and VvMYB5b.

**Keywords:** MBW complex, transcriptomics, vacuolar transport, WRKY TFs

**Twelve new QTLs for phenological traits in *Vitis vinifera* L.**

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Shifts in phenology are a major response to climate change in currently cultivated grape varieties. It is therefore essential to account for these traits in breeding programs, to maintain yield and quality in the future. We have looked for QTLs of phenological traits in three bi-parental populations (N=139, 174, 191), using already published genetic maps. Budburst, flowering, veraison and ripening dates were recorded in the field, in at least two years for each trait, and heat sums were calculated using the Grapevine Flowering Veraison model. For each date and interval between dates, the linear mixed model best fitting the data was selected, and Best Linear Unbiased Predictors of genetic values were extracted for QTL detection on consensus and parental maps. We found 12 QTLs never been reported before, repeated in at least two years: three for budburst date on chromosomes 7, 13 and 16, explaining 8-24% of total variation; four for veraison date on chromosomes 8, 17 and 18 (2 QTLs), which explained 5-11% of total variance; one for budburst-flowering interval on chromosome 19 (14% variation explained), two for budburst-veraison on chromosomes 1 (8%) and 15 (11%), one for budburst-ripening on LG 17 (20%) and one for flowering-veraison on chromosome 4 (16%). We also found 6 repeated QTLs confirming already published ones. Based on NCBI RefSeq annotations, we looked for candidate genes potentially involved in dormancy or veraison genetic control, in QTL confidence intervals. We found a few particularly relevant ones (e.g. involved in circadian rhythm or CONSTANS-like). The potential interest of these findings for breeding new adapted varieties will be discussed.

**Keywords:** bi-parental population, candidate gene, phenology, QTL

**Identification and functional characterization of master regulators of the onset of berry ripening in grapevine**

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In the last years, numerous studies focused on the formation, growth and ripening of grapevine (*Vitis vinifera* L.) berries have greatly increased the knowledge about the physiological and molecular mechanisms governing changes associated with berry development. It has been clearly shown that a profound transcriptome reprogramming characterizes the onset of ripening (veraison), but the molecular triggers of this crucial event are still poorly known. We have started a program of functional characterization of a set of transcription factors, identified by analyzing large transcriptomic dataset of berry development. These factors are induced as very initial molecular signals at veraison and thus could represent key master regulators of ripening. The transcription factors *VviNAC60*, *VviNAC33*, *VviAGL15*, *VviWRKY19* and *VviBHLH75* have been selected and their functional characterization has been performed by transgenic approaches (stable grapevine transformation) or by transient overexpression in grapevine leaves through agroinfiltration. Preliminary results of gene expression analyses revealed that several genes modulated in transformed leaves take part to the berry ripening program, suggesting they may represent targets of each transcription factor. The exhaustive phenotypic and molecular analysis of transformed plants will allow to better define the functions of these transcription factors and possibly to confirm their role of master regulators of the vegetative-to-mature transition of grape berries.

**Keywords:** berry ripening, functional analysis, master regulators, veraison

## A novel high-density grapevine integrated linkage map using GBS in a multi-parental population: preliminary results

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The development of next-generation sequencing approaches and new sequencing platforms has boosted plant genomics research by allowing the generation of high-density linkage maps with relatively low-cost and robust procedures. Here, we report the application of genotyping-by-sequencing (GBS, Keygene patents) to discover and map single nucleotide polymorphisms (SNPs) in a large grapevine population ( $N \sim 600$ ) derived from a half-diallel crossing design composed of 10 interconnected segregating progenies from 5 common grapevine cultivars (Cabernet Sauvignon, Grenache, Pinot Noir, Syrah and Terret Noir). The GBS approach was useful to identify more than 70,300 markers in the whole population, including biallelic SNPs and multiallelic markers. We obtained an average of 17,300 segregating markers per progeny, including 1,300 fully informative ones. These polymorphisms were used to construct an individual consensus genetic map for each progeny, which were then merged by linear programming to minimize mean absolute error between marker intervals in the individual maps and the integrated map. As a result, we created a high-density integrated genetic map with a high informative content and an acceptable marker order agreement between progenies. This map will be used in further genetic research aimed at unraveling the genetic determinism of yield components, phenology, and berry and must quality traits in grapevine. These genotypic data will also allow us to test genomic prediction in specific configurations.

**Keywords:** genetic map, multiallelic marker, next-generation sequencing, progeny, single nucleotide polymorphism



## Whole plant regeneration from protoplasts obtained from embryogenic calli of two Italian grapevine varieties

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Plant protoplasts represent a useful tool for basic research and biotechnological approaches. Protoplasts can be exploited for physiological, biochemical and molecular studies, from functional analysis of gene and characterization of metabolic pathways to recent applications of genome editing. However, most of these studies require the regeneration of the entire plants from protoplasts. This phase represents the bottleneck of this technology, because, most agronomically important plant species, including grapevine, are recalcitrant to regeneration. Grapevine (*Vitis vinifera* L.) protoplasts were obtained from many sources of plant material (leaves, stems, roots, mesocarp) and used for many studies, but the regeneration of plants was successfully performed only from protoplasts isolated from embryogenic tissue. Here, we report the application of a modified previously reported protocol for protoplasts isolation and plant regeneration of two Italian cultivars, Garganega and Sangiovese. Protoplasts of both varieties were obtained from stamen-derived embryogenic calli. After isolation, protoplasts were cultivated in solid Nitsch's medium, supplemented with sugars, auxin and cytokinin. Within four months from the initiation of culture, well developed protoplasts-derived torpedo somatic embryos were transferred into medium supplemented with cytokinin under light in order to induce germination. Subsequently, germinated somatic embryos were moved in a rooting medium. Regenerated plants were transferred to the greenhouse and showed a normal morphology. Finally, protoplasts PEG-mediated transfection has been tested using a plasmid carrying GFP as marker gene. Fluorescence microscopic analysis showed that the GFP expression was initially low, but it took place after 24 h and continued after 48 and 72 h from the transfection. These results indicate that this system represents a useful tool for numerous applications in grapevine, including the genome editing.

**Keywords:** embryogenic calli, PEG-mediated transfection, plant regeneration, protoplasts

**Calardis blanc ' a new grapevine variety with combined resistances against downy mildew, high resistance against black rot, and high botrytis resilience**

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Threats like downy mildew (*Plasmopara viticola*) and powdery mildew (*Erysiphe necator*) make extensive plant protection necessary as winegrowers depend on healthy grapes for wine production. Resistant varieties are used for quality wine production in Germany since the late 1990s, however, all resistant varieties protected in Germany rely on only one resistance against downy (Rpv3 or Rpv10) and one double locus against powdery mildew (Ren3/Ren9). For a stronger and more durable resistance a combination of different resistance loci is desired. 'Calardis blanc' represents the first variety protected in Germany that has two resistance loci against downy mildew (Rpv3-1 and Rpv3-2).

Climate change and a desired reduction of plant protection might open doors for new diseases that in the past did not show practice relevance due to an unfavourable climate or because of side effects of the extensive plant protection against mildews. One of these diseases is black rot (*Guignardia bidwellii*). A series of cultivars: Felicia, Calardis musque, and Calardis blanc, has inherited a strong resistance against black rot and are therefore well prepared for an increasing black rot pressure and also well suited for areas in which black rot is already a considerable threat. Another disease leading to a high loss of yield every year is gray mold (*Botrytis cinerea*). Especially in wet weather conditions Botrytis can infect bunches if they are not drying efficiently. New techniques and developments in the field of sensors and special algorithms allow us to elucidate and dissect the factors of Botrytis resistance in fast and detailed ways. We could show that Calardis blanc has a sturdy berry skin and loose clusters which allow the grapes to dry quickly, both parameters leading to the strong resilience against Botrytis bunch rot.

Apart from the strong resistances against a wide variety of diseases, 'Calardis blanc' convinces consumers with its quality. The wines are fresh and have a nice, unobtrusive aroma which might cause associations to Muscat a Petits Grains Blancs (syn. Muskateller). The vines furthermore show a very nice upright growth, good yield, and a medium to late harvest time - factors favored by wine growers.

**Keywords:** black rot, botrytis, downy mildew, grapevine breeding, new variety

## Physiological functions of ASR proteins regarding sugar signaling, transport and metabolism of two cell culture models in grapevine

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Sugars are metabolic signals involved in plant development and responses to environmental cues. Sugar transporters are both actors of sugar partitioning and targets of sugar signaling. A Grape ASR (ABA, Stress, Ripening), VvMSA, is identified as a regulatory protein controlling gene expression of the hexose transporter *VvHT1* in grape berry ripening and in response to water deficit. The aim of the present work is to assess the physiological functions of VvMSA by an integrative biology approach. The first objective of our study consisted in the establishment of biological models, embryogenic and non-embryogenic grape cells, sharing the same genetic background but growing on the most appropriate culture media to maintain their phenotypic plasticity and specific cell fate determination. The characterization of the proliferation kinetics and metabolomes of both cell types revealed differences in their sensitivity/tolerance to sugar starvation. The second challenge was focused on the regulation of *VvHT1* expression in both cell types and their mutants overexpressing or silenced for VvMSA. The pharmacological approach using glucose analogues, coupled to the analysis of gene expression and glycolytic enzymes activity, demonstrated that VvMSA affects *VvHT1* expression through the glucose signaling pathway dependent on glycolysis. Eventually, we carried out a quantitative and comparative proteomic analysis of nuclear proteins in embryogenic wild type and VvMSA silenced cells. Proteins whose expression is affected by grape ASR repression suggest a new functional role of VvMSA at the interplay between metabolic responses to stress and epigenetic regulation of gene expression.

**Keywords:** ASR proteins, glucose signaling, glycolysis enzymes activities, grape embryogenic and non-embryogenic cells, metabolic behavior, nuclear proteome, sugar transporters

## Topo-climate effects on phenology and metabolism of wine grapevine berries of a grapevine varietal collection in arid environments

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Climate change is a challenge for most world wine producing regions. Consequently, wine production is anticipated to gradually migrate from traditional areas to new terroirs. Desert conditions are considered beyond the climate frame of traditional wine-producing belts due to water scarcity, high temperatures and excess light/UV intensity, all negatively affecting berry metabolism and wine quality. Nevertheless the semiarid to arid regions are inevitably becoming wine grapevine growing area in Israel, the Negev desert offers diverse topoclimate conditions, with altitudes ranging from 250 to 900 m asl. To identify cultivars with crop quality potentials under desert environments two experimental vineyards were setup in Ramat Negev R&D Center, and Ramon, at 300 and 850 m asl, respectively comprising 10 white and 20 red cultivars. In the first two harvest years (2015 and 2016), Chenin Blanc and French Colombard among the white cultivars, and Petit Verdot and Malbec among the red ones, exhibited promising wine qualities, with some advantages to the relatively cooler region, Ramon. In 2017, a consistent two-week difference in plant and berry phenology between the two vineyards was preserved from bud break to véraison, while the differences among cultivars at each site were small. The ripening period from véraison to harvest (July-August) was on average 50% longer in the significantly warmer Ramat Negev vineyard, where a considerable number of fruit clusters shriveled before reaching the BRIX harvest threshold. Metabolic data of berry skin and pulp are being processed to assess environmental and varietal interaction on primary and secondary metabolism.

**Keywords:** arid environment, berry composition, climate change, high temperature, phenology

## Phenology and thermal requirement of disease resistant genotypes (PIWI) growth in Goethe Grape Valley region (Brazil)

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The Goethe Grape Valley (GGV) is an important and traditional Brazilian region for grape and wine production from hybrid vines, due to the climate conditions, such as high rates of rainfall, making it difficult to grow *Vitis vinifera* varieties susceptible to diseases, especially *Plasmopara viticola*. Thus, new genotypes of *V. vinifera* with resistance to *P. viticola*, are being evaluated for cultivation in the region. In this context, the aim of this work was to characterize the phenology and the thermal requirement of PIWI genotypes grown in GGV. The experiment was carried out at an experimental winery (28°32'S, 49°19'W, altitude 80 m asl), established in 2016 and the evaluations were done in 2018 vintage. The evaluated PIWI varieties were Aromeira, Baron, Calardis blanc and Felicia. Minimum and maximum air temperatures were recorded daily, using 10 °C as the down threshold temperature, 25 °C as the optimum temperature for development, and 35 °C as the upper threshold temperature of development. The evaluated phenological stages were bud break to flowering, flowering to veraison and veraison to maturity. The total thermal requirement mean was 1.239 °C day, being the variety Felicia showed the lower thermal requirement (1.123 °C day), which contrasted with the Aromeira that presented the higher thermal requirement (1.326 °C day). Baron needed 1.245 °C day and Calardis blanc needed 1.263 °C day. Between bud break and flowering the average of the thermal requirement was 146 °C day, between flowering to veraison the mean was 641 °C day and 452 °C day from veraison to maturity. The four evaluated varieties exhibited good development in the field with thermal requirement compatible with the region of Goethe Grape Valley. Noting the lower thermal requirement, and consequently lower cycle the Felicia variety, and with greater thermal requirement the Aromeira variety.

**Keywords:** downy mildew, *Plasmopara viticola*, PIWI variates

## Genetic stability of plants from portuguese minority grapevine varieties regenerated by somatic embryogenesis

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Grapevine is one of the most important fruit crops in the world, and is particularly important in Portugal, due not only to the considerable value of viticulture and winemaking, but also to the importance of genetic diversity and rich heritage in ancient varieties of grapevine still existing. However, this genetic diversity which contributes so much to an environmentally sustainable viticulture is at risk, due to the use of a limited number of cultivars for wine production and to vineyards restructuring with commercially available clones. Furthermore, it is imperative to preserve late-maturing varieties in order to cope with the extreme hot temperatures and precipitation deficits registered in Portugal. Thus, genetic and biotechnological tools must be implemented to enhance the conservation of natural grapevine biodiversity. The aim of this study was two-fold. Firstly, 2983 anthers and 560 gynoecia from immature inflorescences of three Portuguese minority varieties were established *in vitro*. In terms of somatic embryo induction,  $2.00 \pm 14.00\%$ ,  $3.83 \pm 19.19\%$  and  $18.82 \pm 48.87\%$  of explants with somatic embryos of Preto Martinho, Malvasia Preta and Cornifesto were obtained, respectively. In addition,  $6.50 \pm 46.99\%$ ,  $8.75 \pm 83.21\%$  and  $12.00 \pm 58.79\%$  of plantlets for the referred varieties were regenerated, respectively. These results were quite positive when compared to one of the reference grape varieties in Portugal - Touriga Nacional -, considering that only  $0.42 \pm 6.44\%$  of induced explants and  $10.00 \pm 154.60\%$  of plantlets were attained. Secondly, Inter-Simple Sequence Repeat (ISSR) markers were used to evaluate the trueness-to-type of regenerated plants from somatic embryos induction. Somaclonal variation was almost absent in our grapevine plant regeneration system.

**Keywords:** genetic fidelity, *in vitro* conservation, ISSR markers, somatic embryos

**Acknowledgments:** this work was supported by the European Regional Development Fund through the North Portugal Regional Operational Programme, under the project VINE AND WINE INNOVATION PLATFORM - INNOVINE&WINE, operation NORTE-01-0145-FEDER-000038.

## Genetic diversity of autochthonous *Vitis vinifera* L. - contribution for the preservation of grapevine portuguese gene pool

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Grapevine (*Vitis vinifera* L.) is one of the oldest plant species that have been domesticated and it is considered one of the most important crops worldwide. Portugal possesses one of the richest diversities of grapevine varieties, enhanced by the ongoing discovery of new genotypes. Despite these unknown genotypes' potential value, only a limited number of cultivars are used for wine production, which is leading to the gradual disappearance of minor and unknown genotypes. Consequently, grapevine genetic pool is diminishing and crop vulnerability to abiotic and biotic stresses is increasing. Thus, identification of accessions that include a broad representation of rare grapevine varieties from 'Douro' Controlled Designation of Origin (DOC) Region and detection of possible synonymies to other varieties through molecular analysis were the main goals of this study, in order to contribute to the Portuguese grapevine's genetic resources conservation. A total of 139 accessions from four different sampling locations were analysed using the Organisation Internationale de la Vigne et du Vin (OIV) core set of nuclear microsatellites (nSSRs) to access their trueness-to-type. The SSR profiles were also compared to those of SSR databases. Very well-known Portuguese grape cultivars, such as Touriga Nacional, Aragonez and Marufo were identified, as well as minor and neglected varieties, namely Tinta Carvalha, Tinta Cão, Samarrinho, Donzelinho Roxo, and a high percentage of unknown genotypes.

**Keywords:** biodiversity, genotyping, grapevine, SSR markers

**Acknowledgments:** this work was supported by the European Regional Development Fund through the North Portugal Regional Operational Programme, under the project VINE AND WINE INNOVATION PLATFORM - INNOVINE&WINE, operation NORTE-01-0145-FEDER-000038.

**'Cuihongbao': a new seedless late-ripening table grape**

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'Cuihongbao' is a late-ripening and seedless grape with high yield, and medium pathogen resistance. 'Cuihongbao' is the result of hybridization between 'Muscat Hamburg' and 'Crimson seedless' in spring of 2004 in the Pomology Institute, Shanxi Academy of Agricultural Science. 324 single plants were obtained in 2005. These plants began to produce fruits in 2009. After three years observation, 'Cuihongbao' was selected in 2012 for its seedless, good quality, stable fruit bearing and medium pathogen resistance. In November of 2017, it was put on records by the crop variety appraisal committee of Shanxi province and named 'Cuihongbao'. 'Cuihongbao' belongs to *V. vinifera*, has diploid and bisexual flower, has conical cluster with two shoulders. Average cluster length is 16.1 cm, average cluster width is 11.7 cm, average cluster weight is 292.2 g. The fruit grain is ellipsoidal, pericarp color is purplish red, and average grain weight is 4.8 g. The berries are seedless and crisp. The soluble solid content is 18.2%, titratable acid content is 0.37%. In the Jinzhong area, the time of budburst is usually middle April, and flowering at late May. Time elapsing from bud breaking to harvest time is about 150 d, and it is harvested mid-september in Jinzhong, Shanxi province. The juvenile period is short (2 years). This variety can bear fruits, and more than 1 100 kg for 666.7 m<sup>2</sup> during full fruit period. 'Cuihongbao' can be planted in open field or under rain-shelter.

**Keywords:** 'Cuihongbao', grape, late-ripening, new cultivar, seedless



## Characterisation of the pan-genome of *Vitis vinifera* using Next Generation Sequencing

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Analyses of structural variations have shown that a single genome alone does not reflect the complete genomic complement of a plant species, leading to the concept of pan-genome. The latter is composed of a Core Genome (CG), common to all individuals of a species, and a Dispensable Genome (DG), absent from at least one individual. DG appears to be largely the youngest and most dynamic component of the pan-genome. Smaller deletions and insertions, due to recent movement of transposable elements and larger variants referred to as Copy Number Variants (CNVs) contribute to high levels of structural variation. In plants, the dispensable fraction of the genome may be widely influenced by the very active transposable elements. We re-sequenced more than 50 *Vitis vinifera* varieties and two related species and, based on a variety of approaches, we produced a catalogue of Single Nucleotide Polymorphisms (SNPs) and Structural Variants (SVs). SNP markers were used to explore the grapevine population structure, the geographical patterns of diversity, and to assess the genetic relationships between varieties. In order to gain knowledge about DG composition, structural variants of different sizes were detected using paired-end mapping information. We will describe the dispensable fraction of the grapevine pan-genome, its composition and extent, and its phenotypic and epigenetic effects. Using transcriptomic data, we also analysed the effects of gene copy number variation and invasion of transposable elements into the gene space on measures of gene expression. Furthermore, we will explore the mechanisms that generate the dispensable portion. Gaining insights into the composition and function of the DG will contribute to understand the mechanisms that create genetic diversity and phenotypic variation.

**Keywords:** pan-genome, single nucleotide polymorphisms, structural variants

## The intensity of Rpv3-dependent downy mildew resistance varies with the genetic background of the resistant variety

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Rpv3 triggers foliar necrosis in response to the biotrophism of *Plasmopara viticola*. This locus was introgressed from North american grapes into the genetic background of many European and Caucasian winegrapes and table grapes from the Near East, with the aim of creating resistant varieties for a wide range of uses. We used field data from four seasons to show interannual variability in foliar resistance among 76 introgression lines that share the major R gene in diverse genetic backgrounds. All the varieties that carry the most common Rpv3 haplotype showed necrosis underneath foliar lesions in the response to natural infections of *P. viticola*, while *V. vinifera* controls showed unrestricted sporulation. Early backcross generations showed the highest levels of resistance, consistent among years and regardless of the level of disease pressure, while a significant drop in OIV452 values was observed in late backcross generations. We also sorted resistant varieties of late backcrosses into categories, based on the type of *vinifera* parent used in the last cross-combination. The cross-combinations with *occidentalis* winegrapes showed a distribution of OIV452 values skewed towards higher resistance than cross-combinations with *orientalis* table grapes. These data confirmed that Rpv3 is associated with the defense response but other factors, provided by the genetic background, contribute to the level of expression of the resistance phenotype. The wild alleles for these factors, once present in the donor of resistance, may have been replaced by less effective *vinifera* alleles during independent processes of backcrossing, with some degree of variation among *viniferas*, thus explaining the differences in Rpv3 efficacy among introgression lines. These findings have implications for grape breeding, suggesting that the combination between an R gene and an optimal genetic background is critical to obtain high levels of field resistance.

**Keywords:** downy mildew, *Plasmopara viticola*, Rpv3

## Profiling and comparison of flavor compounds in table grape cultivars in Japan

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Flavor is an important trait for breeding of table and wine grapes. Because of high amount of rainfall and disease resistance, many hybrids between *Vitis vinifera* and *V. labrusca* have been bred as table grape cultivars in Japan. Based on sensory evaluation of berries, they have been divided into four groups including muscat flavored cultivars, foxy flavored cultivars, non-flavored cultivars and others. However, information about their flavor compounds is limited. In this study, we analyzed flavor compounds in cultivars including the main table grape cultivars in Japan and muscadine (*Vitis rotundifolia*) varieties. We extracted volatile compounds with a solvent assisted flavor evaporation (SAFE) method and analyzed them by GC-MS. In the berries of 33 cultivars, we detected peaks of 128 volatile compounds. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) showed that the aroma profiles of these cultivars could be classified into 5 types consistent with the groups classified by previous sensory test. The results indicated that C6 sesquiterpenoid compounds were commonly detected in almost cultivars. Group1 cultivars were composed of muscat flavored grapes.  $\beta$ -linalool and its related monoterpenes were mainly contributed. Group2 cultivars were foxy flavored grapes. The grapes were rich in many kinds of ethyl esters such as ethyl phenylacetate, ethyl hexanoate. In group3, each cultivar was characterized by several compounds. In group4 cultivars, we could not detect any specific or abundant compounds. In group5 composed of muscadine grapes, hexyl or butyl were abundant. These results indicated a diversity of flavor compounds in table grapes in Japan. These information will be useful for strategies of breeding of table grapes.

**Keywords:** aroma, foxy, muscat, SAFE

**Acknowledgements:** this research was supported by grants from the Project of the Bio-oriented Technology Research Advancement Institution, NARO (the special scheme project on advanced research and development for next-generation technology).

**Table grape breeding for high quality in Hebei Province, China**

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China is one of the leading countries for grape growing in the world with 847,200 ha vineyard and an annual production 14.6 million tons in 2016. Table grapes account for about 85% of the grape planting areas and total production. Consumers in China generally prefer large berry size, firm flesh, uniform colour and high eating quality grapes. The lack of ideal table grape cultivars with big berries and good quality has become the bottleneck of table grape industry development in China. In order to meet the consumers demand for new table grape cultivars, the table grape breeding program was initiated in the early 1980's in Changli Research Institute of Fruit Trees, HAAFS. The breeding methods are the classical crossing breeding with embryo rescue, molecular mark assisted selection, polyploidy (triploidy and tetraploidy) breeding. Seven new table grape cultivars have been released including 3 triploid seedless cultivar: 'Earlyred Seedless' in 2000, 'Champion Seedless' 2004 and 'Moonlight Seedless' in 2009; and 4 tetraploid cultivars in 2013: 'Spring light', 'Honey light', 'Sapphire light' and 'Peak light'. The main characteristics of seven new cultivars are presented in this paper including the phenology, productivity, ripening time, bunches, berries, disease resistance, viticultural performance, adaptability to local environment conditions. The specific management technologies aiming to protect the vineyard are also introduced. The seven cultivars above are suitable for cultivation in open air or green house conditions.

**Keywords:** breeding, new cultivar, polyploidy, seedless, table grape

## Grapevine plasticity and terroir: a multidisciplinary approach for dissecting the single effect of soil on grape quality

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Phenotypic plasticity, the capacity of a given genotype to render different phenotypes upon different environmental conditions, is a means to cope with environmental heterogeneity that is particularly adequate for sessile organisms such as plants. Many studies have shown that plants are plastic for numerous ecologically important traits, ranging from morphology, physiology and anatomy to developmental and reproductive timing. Grapevine (*V. vinifera* L.) represents a particularly interesting species for what concerns phenotypic plasticity, considering that the terroir, meant as the contribution of geography, geology and climate of a certain place, together with the agronomical practices utilized, may deeply influence the berry phenotype of a certain variety at the physiological, molecular, and biochemical level. This phenomenon can lead to the production of wines that, although produced from the same clonal variety, present totally different enological profiles, and represents an issue of increasing interest not only from a biological but also from an economical point of view. Recently, in a first-of-a-kind study on the grapevine phenotypic plasticity, the berry transcriptome of a single clone of vegetatively-propagated *V. vinifera* cv Corvina was investigated by comparing fruits obtained in 11 different vineyards characterized by diverse environmental and agricultural parameters within the Verona area of Italy. The present project is aimed at deeper understanding the determinism of phenotypic plasticity in grapevine (*Vitis vinifera* L), trying to decompose the concept of terroir in two of its principal components: the soil and the climate factors, and to describe the singular effect that different chemical and microbiological soil profiles, on one hand, and different geo-climatic conditions, on the other, can produce in terms of berry plasticity at the molecular, biochemical physiological and enological level. We here present preliminary data obtained comparing physiological and phenological data at both vegetative and reproductive phases of two grapevine varieties, namely Corvina and Glera, grown in different soils located in the same geographical location and, conversely, in the same soil under different environmental conditions.

**Keywords:** berry, plasticity, soil

**The added value of genome haplotype identification to unravel trait/allele relations**Nabil Girollet<sup>a</sup>, Nathalie Ollat<sup>a</sup>, Timothée Flutre<sup>b</sup>, Pierre-François Bert<sup>a,\*</sup><sup>a</sup> UMR EGFV, Bordeaux Sciences Agro, INRA, University of Bordeaux, ISVV, 210 Chemin de Leyssotte, 33882 Villenave d'Ornon, France<sup>b</sup> INRA, UMR AGAP, 2 place Viala, 34070 Montpellier, France**\* Presenting author:** pierre-francois.bert@inra.fr

The construction of contiguous genome assemblies has allowed for discovery of genes and gene function as well as improved our understanding of genomic elements and structure that regulate biological processes. At the simplest, it allows for association of genetic markers for selection and introgression of traits across germplasm to enable hypothesis-driven crop improvement. At a higher level, phased genomes are important to understand genetic and epigenetic regulation. Haplotype phasing is also critical to assess the functional consequences of genetic variants, and to allow precise definition of haplotype blocks which is useful to understand genotype-phenotype association. Plant geneticists have taken great concern in choosing a highly homozygous plant for sequencing to reduce the number of problematic bubbles during computational assembly generation but the haploid consensus sequence assembly does not accurately portrays both haplotypes. To combat the issue of heterozygosity in grapevine, we performed the whole genome assembly of *Vitis riparia* (cv. Riparia Gloire de Montpellier) with long-read, enabled by Single Molecule, Real-Time (SMRT) Sequencing, with a final genome length of 515Mb (plus 318Mb of haplotigs) in agreement with the estimated genome size and 50% of scaffolds (NG50) being longer than 1.5Mb and the largest scaffold spanning 8.1Mb. We took advantage of the recent release of the *Vitis vinifera* Cabernet Sauvignon genome sequence to reanalyze QTL data for agronomic traits of the F1 population issued from the cross of these two genotypes as parents. Integration of transcriptomic and QTL-seq data from genetic mapping to haplotyped genomes provides more acuity into identification of candidate alleles.

**Keywords:** gene/QTL identification, haplotype assembly, whole genome sequencing

## A first draft of grapevine's physiological and molecular responses to flooding

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During the period 2001-2010 floods have been the most frequent extreme event (IPCC). Studies on model plants have shown that soil flooding exposes roots to hypoxic stress resulting in metabolic re-programming, increased synthesis of stress hormones, and changes in reactive oxygen species (ROS) synthesis and metabolism. Grapevine's physiological and transcriptional responses to flooding are poorly characterized. We have carried out a preliminary survey on the physiological and molecular changes occurring in cv Sauvignon blanc (*Vitis vinifera* L.) potted plants grafted on K5BB rootstock following flooding applied before bud-break. Primary roots were sampled at 2 (T1), 8 (T2), 16 (T3) and 21(T4) days after flooding, and one week after recovery (T5). While the initial phenology was significantly affected by the flooding event, upon recovery, higher and earlier stem internode elongation and leaf expansion rates were observed in plants that had been subjected to flooding compared to control plants. Expression analyses of three early hypoxia markers (VvACO1, VvSusy and VvADH1) by qRT-PCR, pointed out a marked up-regulation at T1, followed by a general down-regulation at following time-points and a full normalization at recovery. RNAseq profiling of the root (K5BB) transcriptome at T1 allowed to identify 850 and 1,365 up- and down- regulated genes, respectively. Enrichment analyses highlighted extensive changes of the root physiology, concerning the categories “response to stress”, “oxygen transport”, “response to phytosteroids”, and “flavonoids/(di)terpenoids metabolism”. At T5 (recovery), only few differentially expressed genes (15 down and 2 up) could be identified in the comparison of flooded vs not-flooded plants. The same experimental set up was replicated in a second season and substantially confirmed the results of the first year. Both the datasets will enable the development of a model of the dynamics of grapevine roots responses to hypoxia.

**Keywords:** growth, hypoxia, roots, transcriptome, waterlogging

## Genotyping by sequencing and genetic mapping of phenology-relevant and berry quality traits from two grapevine mapping populations

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Renewed grapevine breeding efforts are needed to meet the challenge of the ongoing climate change and societal demand of low input viticulture. The NEWVINE program aims to generate new cultivars that will meet these two goals. As a part of this program, the UMR 1287 EGFV has generated two F1 intra-vinifera mapping populations in order to identify molecular markers linked to phenology, berry quality and Bordeaux wine typicity. The first generated F1 mapping population was obtained by the genetic cross of two red cultivars: Petit Verdot (PV) and Cabernet Franc (CF). Cabernet Franc is one of the progenitors of Cabernet-Sauvignon and Merlot, two emblematic premium red cultivars of Bordeaux vineyards. And Petit Verdot is known for the complexity of his wine and his late ripening trait. Five hundred sixty plants were obtained from 1500 seeds. All individuals were genotyped with two microsatellite markers to eliminate self-fertilizations (3.9%) and the remaining individuals were genotyped for the character of skin color on the base of VvMYBA1 gene sequence (This et al. 2017). Sixty-nine percent of the F1\_PV x CF population will be able to produce red berries. A set of 200 individuals were randomly selected on this part of the population for genotyping by sequencing (GBS) analyses and subsequent genetic map construction and quantitative trait loci (QTL) detection. A second F1 population from white grapevines was produced by crossing Ugni Blanc (UB) and Sauvignon Blanc (SB). Four hundred sixty-seven plants were obtained from 1300 seeds. The genotyping based on two microsatellite markers revealed 3.6 % of self-fertilizations. A set of 200 individuals was also randomly selected to realize genotyping by sequencing (GBS) analyses. GBS libraries of these two populations will be prepared with the help of the GPTR genotyping platform (UMR AGAP, Montpellier, France). The sequencing by Illumina HiSeq 3000 will be performed on GET-plage platform (Toulouse, France). After bioinformatics treatments, genome-wide association studies will be performed on the SNPs data sets with the aim to identify QTLs related with traits of interest (phenology, berry quality...). The molecular markers identified in this project will be useful for marker-assisted selection and future breeding programs in grapevine.

**Keywords:** berry quality, genotyping by sequencing, phenology, QTL detection

**Acknowledgments:** We thank the CIVB for the financial support of the NEWVINE project N° 901.



## Genome editing in grapevine: plant regeneration from embryogenic-calli derived protoplasts

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The main bottleneck in applying cisgenesis and genome editing in grapevine is plant transformation and regeneration. Indeed, many genotypes show recalcitrance to tissue culture and transformation, as well as to regeneration from protoplasts, limiting efforts to use biotechnological approaches for grapevine genetic improvement or functional genomics studies. Grapevine embryogenic calli, induced in tissue cultures by means of growth regulators, are used for *Agrobacterium*-mediated transformation, since they are tissues harbouring totipotent cells and able to regenerate transformed plants. Protoplasts obtained from embryogenic calli provide additional advantages for genome editing purposes, including delivery of multiple plasmids for cotransformation and high frequency transformation. Most importantly, by use of polyethylene glycol or electroporation, the protoplast system allows the direct delivery of the genome editing machinery, such as preassembled Cas9-gRNA ribonucleoproteins, rather than plasmids encoding these components, removing the likelihood of inserting recombinant DNA in the host genome. The machinery is needed to trigger DNA repair and incorporate modifications but it is degraded rapidly after transfection, reducing the frequency of off-target effects in regenerated plants. In the present contribution, we describe the setup of a protocol to regenerate *Vitis vinifera* cultivars starting from embryogenic-calli derived protoplasts, first step on the way to the generation of DNA-free genome edited grapevine.

**Keywords:** genome editing, grapevine, protocol, protoplasts

## Role of DNA methylation in graft formation

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Grafting is a widely used technic, which combines the root system from one plant and the shoot of another plant. However, we have little understanding of the biological process leading to a successful graft. One of the essential steps is the re-establishment of a fully connected vascular system, necessary for the transport of water and nutrients between organs. Cells from both rootstock and scion contribute to this process, by producing a callus, from which new vessels differentiate. This initial step requires cell dedifferentiation followed by cell differentiation, which both rely on epigenetic regulation, more specifically on genomic DNA methylation. The role of this major epigenetic modification which is known to impact developmental processes, has been investigated with the aim to determine its possible contribution to the grafting process. The impact of reduced DNA methylation levels on cell differentiation and de-differentiation, as well as grafting efficiency were investigated, using either a pharmacological approach to limit genomic DNA methylation, or transgenic plants affected in CG methylation maintenance (MET-RNAi) or in the demethylation process (DML-RNAi). The comparative analysis of different graft combinations between WT and transgenic partners could not reveal any difference in the kinetic of vascular reconnections irrespective to the partner's genotype. In contrast the adhesion between scions and rootstocks varied depending on the genotypes: graft unions involving a DML-RNAi partner were more cohesive than interaction between two WT partners, whereas graft unions with a MET1-RNAi partner were less cohesive. This suggests that DNA methylation may affect differentially certain aspects of the graft formation. The result obtained from grafting experiment are consistent with *in vitro* experiments, showing that hypo-methylation decreases plant cell *in vitro* callus formation.

**Keywords:** callogenesis, DNA methylation, epigenetic, graft

## Deciphering the molecular and genetic bases of grape tolerance to trunk diseases

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Differences among grape cultivars have been observed regarding the expression of symptoms associated with trunk diseases (i.e. Eutypa dieback, esca, Black Dead Arm). However, due to the complexity of the dieback process, little is known about the molecular and genetic bases of these differences. The TOLEDE program aims to explore this issue using combined approaches and a large diversity both in the plant (12 major cultivars and a highly diverse panel of 93 cultivars in the wine west genetic compartment) and in pioneer fungi: Eutypa lata, Phaeomoniella chlamydospora, Togninia minima, Neofusicoccum parvum (each with collections of several dozen strains). The project first includes an analysis of the differences in tolerance between grape varieties (WP 1) and in fungi aggressiveness (WP2). For the tolerance components, we will analyze with (i) or without (ii) a priori hypotheses: i) plant cell wall biochemical composition, phenolic compounds content, vessels size and the expression profiles of defense genes and ii) disease expression segregation in vineyard-grown bi-parental populations, and we will carry out an association genetics study (on the diversity panel inoculated under controlled conditions). For the aggressiveness components of the four pioneer species studied, we will compare the isolates with (i) or without (ii) a priori hypotheses: i) for the production of wall degrading enzymes and phenolic compounds as well as for the toxic compounds already described and ii) by searching for new toxic compounds (glycoproteins) and conducting association genetics studies. The results of WP 1 and WP2 will be confronted in order to validate the main tolerance components and the genetic or biochemical markers identified will be applied in WP3 to the selection and breeding of grape varieties, to the detection and monitoring of diseased vines in vineyards and as indicators for increasing tolerance through agronomical practices.

**Keywords:** aggressiveness, associated fungi, diversity, grapevine trunk diseases, varietal tolerance

**Acknowledgments:** This work is supported by the Plan National Dépérissement du vignoble (<https://www.plan-deperissement-vigne.fr>) from January 2018 to December 2020.

**DNA methylation regulates anthocyanin accumulation in the grape cell suspension**Junhua Kong<sup>a,\*</sup>, Emeline Teyssier<sup>a</sup>, David Lecourieux<sup>a</sup>, Fatma Lecourieux<sup>a</sup>, Philippe Gallusci<sup>a</sup><sup>a</sup> UMR EGFV, Bordeaux Sciences Agro, INRA, University of Bordeaux, ISVV, 210 Chemin de Leyssotte, 33882 Villenave d'Ornon, France\* **Presenting author:** junhua.kong@u-bordeaux.fr

During ripening, grapes of red wine varieties accumulate large amounts of anthocyanins, which are major determinants of wine color and are also used as natural colorants in the food industry or as human health promoting molecules. Grape cell culture *in vitro* has been used for the production of anthocyanins despite important yield variations related to cell culture conditions, such as light and sugar availability. As DNA methylation was shown to inhibit anthocyanin biosynthesis in apple and pear fruit skin, we have investigated the possible function of DNA methylation in the regulation of anthocyanins biosynthesis in Gamay-Teinturier cell suspensions. Two different drugs (zebularin and RG108) known to interfere with the activity of enzymes responsible for DNA methylation were used. Each drug was added to exponentially growing cells at different concentrations (20 to 100  $\mu$ M) and their impact on cell cultures was analyzed. Both drugs had a positive, dose-dependent effect on anthocyanin accumulation: anthocyanins content of light grown cells was increased and *de novo* anthocyanins biosynthesis was induced in dark grown cells. In order to better characterize the drug effect, different parameters were analyzed: cell growth, cell morphology, and primary metabolites accumulation. Moreover a few genes coding for anthocyanins biosynthesis enzymes and regulators were selected and their expression was analyzed by RT-qPCR. DNA methylation was also estimated at specific genome loci, focusing in particular on MYBA1 gene, which is known to play a role in the regulation of anthocyanin biosynthesis. Taken together, the results suggest that DNA methylation has a negative effect on anthocyanins biosynthesis in Gamay-Teinturier cell suspensions. The drugs, zebularin and RG108, by lowering the DNA methylation level, would alleviate this negative effect and as a consequence, induce anthocyanin biosynthesis.

**Keywords:** anthocyanins, DNA methylation, epigenetic

# **Molecular mechanism of downy mildew resistance mediated by MrRpv1gene in *Muscadinia rotundifolia* grape**

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Grape downy mildew (DM), is an old destructive oomycete disease for viticulture. MrRpv1, a typical TIR-NBS-LRR gene, is the first DM resistance gene cloned from *M. rotundifolia* using a map-based cloning strategy. Functions of MrRPV1 domains have been studied in our previous work. However, the resistance mechanism mediated by MrRPV1 is still unknown. This study aims to investigate the molecular mechanism of MrRPV1 resistance to DM disease by comparing the transcriptome of MrRpv1-transgenic ‘Shiraz’ (resistant) and non-transgenic ‘Shiraz’ (susceptible). A total of 1382 differentially expressed genes (DEGs) were identified from MrRpv1-transgenic Shiraz upon infection with *P. viticola*. The top 3 groups of these DEGs are the genes encoding receptor-like protein kinases and stilbene synthases and transcription factors. The DEGs were significantly enriched in pathways such as plant-pathogen interaction and biosynthesis of secondary metabolites. In the future, DEGs-DEGs interaction network will be constructed and validated by Y2H, BiFC and Co-IP. Finally, a molecular model will be built to explain the molecular mechanism of grapevine downy mildew resistance.

**Keywords:** downy mildew, molecular mechanism, *Muscadinia rotundifolia*, MrRpv1, resistance gene

## Plastochron index of five genotypes disease resistant (PIWI) growth in the Goethe Grape Valley, South Brazil

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The plastochron index (PI) is calculated based on the thermal requirements of a plant for the emission of a new node, and consequently to the emission of new leaves, considered as the photosynthetic unit. This information is important in order to model the development of new genotypes in climates where their cultivation is possible. In the present study, we evaluated the PI of 5 downy mildew resistant PIWI genotypes grown in the Goethe Grape Valley (GGV), Santa Catarina State, South Brazil. The experiment was conducted in an experimental vineyard in the GGV (28° 32'S, 49° 19'W, altitude 80 m asl), in the 2017 vintage. The evaluated genotypes were Gf. 2004-043-0024 and Gf. 2004-043-0015, in addition to the Calardis blanc, Bronner and Regent varieties. Evaluations were performed from bud break to veraison. The daily thermal sum (dTS, °C day) was calculated using the lower, optimum and upper base cardinal temperatures respectively of 10, 25 and 35 °C, the dTS was used to obtain the accumulated thermal sum (aTS, °C day). The PI was estimated based on the inverse of the angular coefficient of the linear regression between the number of nodes per cane and aTS. The genotypes studied presented, on average, the PI of 58.8 °C day. Regent variety showed the lower thermal requirement (39.5 °C day) while Bronner presented the highest requirement (73.5 °C day). Calardis blanc presented PI of 62.5 °C day and Gf. 2004-043-0024 and Gf. 2004-043-0015 genotypes presented PI respectively of 63.6 and 58.1 °C day. The genotypes tested presented different levels of thermal demand, possibly presenting better ecophysiological adaptability in different climatic regions. These results are important for the development of similar studies involving the climatological modeling of these genotypes.

**Keywords:** ecophysiology, new variates, PIWI variates, *Plasmopara viticola* resistance

## Grapevine resistance to *Plasmopara viticola*: the search for metabolic biomarkers

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Nowadays there is an increasing social and political request for a sustainable agriculture in highly important and indispensable crops. One of the most cultivated crops in the world, with a major economic importance, is grapevine (*Vitis vinifera* L.). The domesticated *V. vinifera* cultivars frequently used for wine production are highly susceptible to different diseases, especially downy mildew, caused by *Plasmopara viticola*, one of the most destructive vineyard diseases. Downy mildew affects all the green parts of the vine, causing yield reduction and significant production losses. Thus, if not controlled, it presents serious negative effects in the economy of several countries. To cope with this threat, the application of chemical products is currently the mainly strategy. This is not the most efficient and environmental friendly approach and winegrowers are forced to reduce the use of chemical products ; the development of alternative strategies become highly important. The creation of new cultivars by breeding, as done by the Julius K3hn-Institut (JKI), one of the main institute for grapevine breeding in Europe, is the most sustainable approach. Resistance to *P. viticola* (RPV) in crossing lines was achieved by crossing suitable parent lines or cultivars and the subsequent selection in the offspring to identify desired combinations of traits in particular resistance and quality. However, this process is laborious and takes years to accomplish. The identification of metabolites that discriminate between resistant/susceptible cultivars, either infected with *P. viticola* or elicited with natural based products, allied to a phenotypic discrimination, will allow us to identify novel biomarkers for breeding programs.

**Keywords:** biomarkers, *Plasmopara viticola*, sustainable agriculture

**Greffadapt: a relevant experimental vineyard to speed up grapevine rootstock selection**

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Grapevine is grown as a grafted plant since the end of the 19th century. The large majority of rootstocks were selected at this period. Among the large diversity of existing rootstocks, few of them are commercially used in the vineyard. However, rootstocks could be considered as a relevant way of adaptation to climate change context because they have no major impact on wine typicity unlike the changes of scion varieties. Rootstock selection is a long term process. Consequently, in addition of the selection of new bred genotypes, characterizing existing rootstocks already used in foreign countries or available in germplasm collections, is a complementary strategy to allow a faster enlargement of the rootstock range available for vinegrowers.

Greffadapt is an experimental vineyard created to get and actualize the agronomical characteristics of 55 rootstocks. These rootstocks were grafted with 5 scions in 3 blocks of 5 vines each. Blocks very defined according to soil resistivity measurements and the statistical power of the experimental designed was calculated. Before planting, the genetic identity of each genotype was checked with 20 microsatellites markers and their sanitary status was analyzed with ELISA assays. Planting occurred in 2015, 2016 and completed in 2017. The fresh weight of each plant was determined at grafting and the pruning weight of each vine has been recorded annually since the plantation.

Microsatellite profiles were used to calculate the genetic distances among the 55 rootstock genotypes allowing the classification in different genetic groups. Phenotypic data were analyzed according to these groups and *Vitis berlandieri* parentage was discussed. The weight of each plant at the grafting time and the pruning weight since the plantation were assessed. The significant relation between these variables was discussed taking into account annual data sets. The first results showed that the range of conferred vigour among the rootstock panel was large enough to identify the required diversity, necessary to fit different production objectives in the French vineyard.

Overall, Greffadapt is a very unique experimental facility to speed up the selection of rootstocks and to analyze the relationship between conferred vigour and drought tolerance, two major selection criteria for rootstocks.

**Keywords:** conferred vigour, drought tolerance, rootstock, rootstock × scion interaction, *Vitis berlandieri*



## Media selection is important for embryo rescue efficiency in cold-hardy table grape breeding

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The development of new seedless table grape cultivars is improved through the use of embryo rescue technique. Although this technique has been widely used, the percentage of plants obtained through this method is low, which makes the process of developing seedless table grapes costly. Female parent, ovule extraction sampling time, and growth medium are most crucial to the success of this technique. To increase the efficiency of this method, experiments were conducted to (1) investigate the best time after bloom to harvest ovules from the berries of the cold-hardy mother plant, and (2) to evaluate the effects of the addition of plant growth regulators to the embryo development medium. Open-pollinated seeds were collected at different berry developmental stages from MN1369 at 3 time points and from ‘Somerset Seedless’ at 6 time points. The ovules were cultured on Lloyd & McCown Woody Plant Basal Medium (WPM) for three months before embryos were extracted and transplanted to new media with WPM. For experiment 1, the berry developmental stage at which ovules were harvested did not influence the embryo germination rate or the percentage of plants developed. This suggests that it is suitable to harvest at veraison, when extraction is easier due to softer berry flesh and larger embryos.

For the second experiment, ovules from a ‘Vanessa’ x ‘Louise Swenson’ cross were cultured on 4 media for 3 months. After dissecting the ovules, embryos were transferred to different medium treatments supplemented with plant regulators. Embryo germination was highest (100%) when ovules were cultured on Nitsch & Nitsch medium with  $1.7 \text{ mg} \cdot \text{L}^{-1}$  of indole-3-acetic acid,  $0.5 \text{ mg} \cdot \text{L}^{-1}$  of GA3,  $30 \text{ g} \cdot \text{L}^{-1}$  of sucrose,  $4 \text{ g} \cdot \text{L}^{-1}$  of agar,  $2 \text{ mg} \cdot \text{L}^{-1}$  of activated charcoal, and  $400 \text{ mg} \cdot \text{L}^{-1}$  of casein hydrolysate. However, only 10% of those embryos developed into normal plants. The highest number of normal plants (39%) was obtained from the treatment with  $2.41 \text{ g} \cdot \text{L}^{-1}$  of WPM Medium with vitamins;  $0.1 \text{ g} \cdot \text{L}^{-1}$  of myo-inositol,  $4.4 \mu\text{M}$  6-benzylaminopurine,  $20 \text{ g} \cdot \text{L}^{-1}$  of sucrose,  $3 \text{ g} \cdot \text{L}^{-1}$  of activated charcoal,  $7 \text{ g} \cdot \text{L}^{-1}$  of agar. The results indicated that media selection is important for obtaining normal plants.

**Keywords:** cold-hardy table grape, embryo rescue, seedless grapes

**Graft transmissible effects of rootstocks on grapevine shoot phenotype**

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What is the effect of rootstock on vine physiology, ion concentrations, scion gene expression, flowering, and berry chemistry in grafted grapevines? Grafting dates to the mid 1800's when the root-damaging North American aphid *Phylloxera* was inadvertently introduced into Europe. Subsequently, *V. vinifera* was grafted to phylloxera-resistant North American grapevines including *V. cinerea* ssp. *bellerii*, *V. riparia*, *V. rupestris* and hybrid derivatives. Funded by the US National Science Foundation, our team is carrying out a multi-site, multi-year, three-part study to assess genotype x scion x environment interactions in grafted grapevines. Aim 1 takes places in an experimental vineyard in Mount Vernon, MO, where 'Chambourcin' is growing on its own roots and is grafted to three different rootstocks ('1103P', '3309C', 'SO4'). This set of four combinations is replicated 72 times in a randomized block experimental design with an irrigation treatment. Aim 2 incorporates site and scion effects by studying rootstock/scion combinations in three commercial vineyards in California. Aim 3 develops a rootstock mapping population where F1 offspring of *V. rupestris* x *V. riparia* are currently being grafted to 'Marquette'. This grafted rootstock mapping population will be deployed to four locations in the US in 2019. In all aims we are carrying out comprehensive scion phenotyping including berry chemistry, gene expression in reproductive and vegetative tissues, leaf shape, leaf ion concentration, leaf metabolites, and vine physiology. Analyses of the Aim 1 'Chambourcin' vineyard demonstrates complex interactions among rootstock and irrigation on leaf shape; variation in ion concentration is influenced by rootstock, irrigation, and leaf position. Gene expression patterns reveal effects of rootstock x irrigation x time. This comprehensive, multi-site, multi-year project extends through 2021 and will provide a multi-dimensional perspective on graft transmissible effects of rootstocks on grapevine shoots.

**Keywords:** grafting, high-throughput phenotyping, RNAseq, rootstock scion interaction

**Search for SSRs associated to berry weight in table grapes**

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In the search for a molecular kit to be applied in assisted selection in table grape breeding for berry size, our group had identified 38 polymorphisms of the SNP and InDel types with association to this largely quantitative trait. In addition, 7 candidate genes for berry size had been experimentally validated, including bHLH TFs as well as GDSL esterase/lipase, stilbene synthases and HSP. VvCEB1 and VvNAC26 genes associated to berry size were also recently reported. Our aim was to identify SSR markers located nearby the described SNPs/InDels and candidate genes with association to berry size, in an effort to have a simplified and confident marker system. To approach this search, regions of 1 Mb from the *V. vinifera* reference genome (12X.2) surrounding the 44 markers and genes selected, were analyzed using the SAMTOOLS and MISA softwares, considering five repeats as minimum. A total of 29,173 SSRs were found, with an average density of 0.7 SSRs per Kb. Mono-nucleotidic repeats were the most common, accounting for 69.4%, followed by di-, tri-, tetra-, penta- and hexanucleotide repeats, accounting for 18.2%, 9.7%, 2.0%, 0.6% and 0.1%, respectively. The length of microsatellites varied from 5 to 55 repeats. From the preliminary screening of 17 regions distributed in chromosomes 1, 9, 10, 11, 15, 16, 17 and 18, primers were designed for 125 SSRs, currently under experimental evaluation. A subset of 86 SSRs were evaluated using four table grape varieties ('Autumn Royal', 'Dawn Seedless', 'Beauty Seedless' and 'Sultanina') as first screening; 41 of them (48%) showed polymorphic patterns, while 16% were monomorphic and 36% not amplified or were non-informative. Subsequently, the set of 41 polymorphic SSRs were analyzed in a group of eight table grapes varieties and four segregants from the RxS crossing, with contrasting phenotypes for berry size. Up to now, we have identified 10 SSRs that are associated to berry size.

**Keywords:** berry size, microsatellites, selection markers, SSR

**Acknowledgments:** financed by FONDECYT-Chile, grant 1171378.

**Discovery and validation of SNP and InDel markers associated to berry size**

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Berry size is considered as one of the main selection criteria in table grape breeding programs, due to consumers' preferences. Considering its economical importance, it is relevant to determine the genetic architecture of this trait and, based on that, to identify genetic markers that could be used as selection tools in table grape breeding. To approach this issue, SNPs and INDELs were detected using a transcriptomic approach (RNA-Seq) in a 'Ruby' x 'Sultanina' (RxS) progeny (N=139) with contrasting phenotypes for berry size, using GATK structural variant calling. Biallelic polymorphisms were selected and filtered by thin (100 bp) and MAF (20%) using a VCF pipeline. Of the hundreds of markers initially identified, 30 SNPs and 8 INDELs were selected and experimentally validated. To assess the best combination of candidate polymorphisms as predictor markers, 2 groups of varieties with different genetic backgrounds were considered as well as the RxS progeny. The first group consisted in 31 table grape varieties, corresponding to some extent to the INIA's breeding program crossing block. This group has been phenotyped for berry size, seed content as well as polar and equatorial diameter during three seasons at La Platina Research Station. The second group included 113 *V. vinifera* genotypes belonging to the INRA-Vassal collection (France), selected for their contrasting berry sizes. Preliminary genotype-phenotype associations using Tassel software, unbalanced ANOVA and Random Forest analyses including individuals from the RxS progeny and the table grapes varieties showed several degrees of association of these markers with berry size (10.6% to 34.5%). Thirty-one table grapes varieties and 41 varieties from INRA-Vassal collection, including seedless and seeded genotypes, have been partially genotyped using candidate markers and a qPCR-HRM platform, with good association to berry weight. The ultimate goal is to develop a multi-allelic selection system for berry size feasible to be included in the breeding early stages.

**Keywords:** berry size, Indel, selection markers, SNP, table grape, transcriptomics

**Acknowledgments:** financed by FONDECYT-Chile grants 3150519, 1171378.

**Postharvest evaluation of cold-hardy table grape breeding lines**Laise Moreira<sup>a,\*</sup>, Matthew Clark<sup>b</sup><sup>a</sup> 1080 25th Ave SE, Minneapolis MN 55414, USA<sup>b</sup> 1970 Folwell Ave, St. Paul 55108, USA\* **Presenting author:** desou038@umn.edu

The University of Minnesota grape breeding program has evaluated a small number of cold-hardy varieties and breeding lines for postharvest storage traits. This is the first experiment for comparing breeding lines to commercial varieties for these traits. Ten genotypes were evaluated for berry weight, berry size, cluster weight, fruit chemistry, rachis browning, berry and skin disorders at harvest. Three clusters per genotype were packed in ventilated polyethylene bags and arranged in cartons. Paper pads and SO<sub>2</sub> pads were placed on top of the bunches in each carton. The cartons were stored at 2.2 °C for 2, 4, 6, and 8 weeks. Clusters were destructively sampled at each storage time point and evaluated for stem desiccation, berry splitting, fungal disorders, change in fruit weight, flavor, and an overall rating of acceptability. An advanced selection, MN1296, was the top performing seedless variety for overall acceptability for postharvest traits but has small berries that tend to shatter. ‘Louise Swenson,’ ‘Swenson Red,’ and MN1296 were rated as the best performing cultivars for stem dehydration in the descending order. ‘Swenson Red’ is the largest-fruited Elmer Swenson/UMN variety but has limited commercial planting due to its seeded berries. ‘Swenson Red’ also had no shattering after storage. Berry splitting was worst for ‘Louise Swenson,’ a seeded white grape with multiple uses including wine. MN1369, a newly identified selection, and ‘Louise Swenson’ maintained their highly aromatic character through the storage periods. MN1380 had severe rachis browning, whereas ‘Vanessa’ suffered from shattering due to decay around the pedicel that also lead to mold development. ‘Jupiter’ and ‘Vanessa,’ with the largest clusters and berries, can be grown in Minnesota with marginal success using the J-vine training system. Additional research on bud survival and consumer preference will be needed to determine if any of the advanced selections are suitable for cultivar release.

**Keywords:** cold-hardy grape, fruit quality, postharvest, table grape

## Transcriptional analysis of the early response to GA3-treatment in table grape genotypes with different susceptibility to berry drop

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Gibberellins (GA, GA3) are the most used growth regulator for table grape production, increasing berry size through pericarp cell expansion. However, GA applications also have undesirable effects, such as postharvest berry drop. Previous studies have suggested that molecular changes in the berry pedicel related to the GA treatment would have a role in this disorder. Here we report the transcriptomic analysis of pedicel response to GA treatment in two genotypes with contrasting performance for berry drop. The genotypes are the breeding line L23, which is 10 times more susceptible than cv. Thompson Seedless in terms of berry drop. GA induced several morphological changes in the pedicel, including modifications in lignin content, as well as cell expansion causing enlargement of the pedicel. Differential expression analysis showed upregulation of 1,098 and 1,525 genes by GA treatment on L23 and cv. Thompson Seedless, respectively ( $FDR \leq 0.05$ ,  $\log FC \geq 2$ ). By other side, 493 and 603 downregulated genes were detected for L23 and cv. Thompson Seedless. Gene ontology annotation showed an enrichment in biological processes such as phenylpropanoid, cell wall metabolism and xylem development elicited by GA treatment, evaluated seven days after the hormone application. Validation of DE genes exclusive to the susceptible L23 genotype could be used as response markers, providing also further insight into the basis of differential susceptibility to berry drop. In this context, 20 genes were selected and analyzed by qPCR, confirming the differential expression of genes related to lignin and phenylpropanoid metabolisms, which are essential components of the cell wall and are probably associated to pedicel rigidity. This is the first report of transcriptomic analysis of berry pedicel, a complex woody tissue for which postharvest performance is key to breeding new table grape varieties.

**Keywords:** berry drop, gene expression, gibberellic acid, RNAseq

**Acknowledgments:** financed by Genoma-Chile grant G13I.0003 from FONDEF-CONICYT

## The influence of grapevine rootstocks on scions in response to water stress

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Grafting of *Vitis vinifera* scions onto interspecific rootstocks is the mandatory strategy to manage the phylloxera attack, a homopteran insect (*Daktulosphaira vitifoliae* Fitch) that, feeding on roots, leads ungrafted vines to die. Rootstock can affect vegetative, productive and physiological aspects of the scion, including vigor and yield potential, mineral nutrition and adaptation to limiting soil and climate conditions. The aim of this project is the characterization of the influence of rootstock genotypes (101.14 and 1103P, a susceptible and a tolerant genotype) in the adaptive response of scion (Cabernet Sauvignon) to different water limiting conditions by the physiological and transcriptomic point of view. The rootstocks reduced the transpiration rate of scion and the water use efficiency. The transcriptomic analysis showed an up regulation of genes related to development of root apparatus, capacity of water extraction from the soil and photosynthetic activity. The 1103P genotype exhibited a conservative attitude towards water, while the 101.14 genotype a dissipative attitude.

**Keywords:** drought, RNA-seq, transpiration rate

## A genome editing approach to study the drought stress tolerance in *Vitis* spp

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In the era characterized by great challenges to face climatic changes and to develop sustainable agricultural models based on moderate irrigation and use of pesticides, the selection of new genotypes for ensuring an optimal productivity is mandatory. Among the strategies of molecular breeding, the genome editing approach is a new molecular tool widely used due to the efficiency and reliability to perform targeted genome changes. Grapevine and its rootstocks are relatively tolerant to water deficit. Nevertheless, severe drought can affect crop quality and yield. In the past year, a new drought tolerant genotype (M4), obtained by a conventional breeding program at the University of Milan, was selected. Under severe water conditions, M4 maintains photosynthetic activity, accumulates high levels of resveratrol in roots and the expressions of resveratrol and flavonoid biosynthetic genes are induced. A candidate locus for M4 drought tolerance was identified in *VvSTS* (a stilbene synthase) promoter region. With respect to the current state of the art, the aim of this project will be the functional characterization of *VvSTS* gene, supposed to be related with the tolerance to drought, by CRISPR/Cas9 system technique. To reach this scope a loss of function experimental design has been set up in a model variety (Sultanina) and adapted the protocol to the M4 genotype. In this work, the preliminary results related to the knock-out of *VvSTS*s by agroinfiltration will be discussed.

**Keywords:** rootstock, water stress, stilbene synthase, CRISPR/Cas9



## Screening and modelling the diversity of root system architecture in *Vitis* genotypes : new opportunity for rootstock selection ?

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Root system architecture (RSA), the overall spatial arrangement of individual parts of the root system, determines the capacity of a plant to access soil resources and is important for plant productivity particularly in environments with low or strongly stratified water and nutrient availability. RSA is variable between genotypes and very plastic in response to the environment, so it represents a promising target for breeding plants more tolerant to the predicted increases in water and nutrient limitation. Genetic variations in RSA, and consequent potential efficiencies of resource capture, have received attention for a number of major crops, but these complex traits have been much less studied in perennials such as grapevine because investigations on roots are still restricted by the fact it is laborious to phenotype roots *in situ*. Numerous approaches have been recently developed for non-destructive observations of RSA with the support of advanced imaging techniques. None of them are without shortcomings, but they are a prerequisite to accurately analyze the RSA traits and its heritability on large sets of genotypes. In addition to the emergence of high-throughput phenotyping systems for RSA, breeding approaches would also benefit from the development of novel functional-structural three dimensional root models. They can serve as a basis for the development *in silico* of root ideotypes by highlighting the underlying genetic mechanisms and parameters that are most likely influence RSA. We provide here an overview of our pioneering work in grapevine on RSA phenotyping and modeling, and discuss their opportunities to aid our understanding the genetic factors affecting RSA traits of value for practical rootstock improvement.

**Keywords:** modeling, phenotyping, rootstock, root system architecture

## Multi-omics for secondary metabolite accumulation in grape berry skin and culture cells

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Grape accumulates various metabolites, including anthocyanin, tannin and resveratrol, which are important not only for resistance against biotic and abiotic stresses but also for fruit quality. To clarify the mechanism of secondary metabolite accumulation in grape, we have performed multi-omics of grape berry skin and culture cells. Transcriptome and metabolome analyses showed specific induction of the resveratrol synthetic pathway but not the flavonoid synthetic pathway in the grape berry skin after UV-C irradiation (*Plant Physiol* 2015, 168: 47-). We used two different grape culture cell lines, called VR (Vitis Red) and VW (Vitis White) for proteome and metabolome analyses. Proteome and metabolome analyses showed specific induction of the anthocyanin synthetic pathway in VR after light irradiation, on the other hand, the resveratrol synthetic pathway in VW after the jasmonic acid + elicitor (cyclodextrin) treatment. To identify anthocyanin and resveratrol transporters, we isolated vacuolar membranes from the anthocyanin-induced VR cells or plasma membranes from the resveratrol-induced VW cells and performed proteome analysis. We will introduce these multi-omics for secondary metabolite accumulation of grape in this symposium.

**Keywords:** anthocyanin, metabolomics, proteomics, resveratrol, transcriptomics

**Physiological and biochemical responses of some autochthonous grapevines in Turkey**

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Due to the global climate changes, the effect of drought stress which is one of the important abiotic stress factors has been increased. Therefore breeding of grapevine varieties with resistance to drought stress is an important point to develop. In this context, we investigated leaf water content (RWC) and water level (RWL), membrane stability index (MSI), leaf pigment concentrations (Chl a, Chl b, carotenoids), lipid peroxidation (MDA), proline accumulation, the activities of antioxidant enzymes (catalase, CAT and ascorbate peroxidase, APx) activity in some autochthonous grapevines cultivars (Karabarcık, Manda Gözü, Bilecik İri Karası, Horozkarası, Rumi, Tekirdağ Çekirdeksiz, Çavuş, Trakya İlkeren) in Turkey. Own-rooted autochthonous vines were subjected to drought stress for 7 days and then were irrigated at the field capacity for 2 days. Plant leaf samples were taken at four times (0, 3, 7 and 9. days). In this study, Manda gözü, Rumi, Horozkarası and Bilecik İri Karası exhibited relatively higher tolerance to drought than Trakya İlkeren, Kabarcık, Tekirdağ Çekirdeksiz and Çavuş. In conclusion, this study showed that Manda gözü, Rumi, Horozkarası and Bilecik İri Karası grapevine cultivars must be considered in further selections programs for the tolerance to drought stress condition.

**Keywords:** ascorbate peroxidase, catalase, drought stress, lipid peroxidation, prolin

## Toward deciphering the grape berry extracellular matrix proteome

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The cuticle is a lipidic layer that covers the outer surface of the grape berry. Despite the important functions attributed to the cuticle, little is known about fruit cuticle function and biosynthesis. In grape berries, the cuticle is thought to play a central role as a barrier against pathogen invasion and insect herbivores and to function as a barrier to prevent water loss. Cuticle is composed by cutin and waxes, which precursors are synthesized in the endoplasmic reticulum. However, it is yet unclear how hydrophobic cuticle precursors are transported across the hydrophilic matrix to the cuticle. It has been suggested that cutin monomers and waxes may require additional proteins to increase their solubility in the apoplast. To identify candidate proteins with a role in cuticle extracellular assembly, we proceeded to the extraction of grape skin apoplast proteins at three different phenological stages (pea size, veraison and at full maturation). A vacuum-infiltration-centrifugation method was optimized to collect the apoplastic fluid from grape berry skins. Free-gel LC-MS/MS was used to identify and quantify the most abundant proteins in each phenological stage. The results showed that most of the identified proteins belong to oxidation-reduction, response to stress, transport and carbohydrate metabolic processes. Venn diagram showed that most of proteins were common to the three phenological stages, but the relative abundance differed depending on the stage studied.

**Keywords:** apoplastic fluid, cuticle

## Italian Variety Club, a research and innovation network for the genetic improvement of table grapes

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The ambitious idea for the development of new tablegrape seedless varieties, suitable for growing in the Mediterranean basin and highly competitive on the international markets, has recently led to the foundation (in 2015) of the Italian Variety Club (IVC) a network of 17 enterprises and 2 research Institutions in Southern Italy. This synergic collaboration among private companies and researchers aims to develop and transfer varietal and technical innovation to the growers and to strengthen the Italian table grape supply chain merging high quality products and sustainable agriculture.

The breeding program is organized in different interconnected activities such as: “seed x seedless” varieties and “seedless x seedless” varieties controlled crosses, involving Apulian traditional genotypes and elite cultivars; F<sub>1</sub> seedling production with molecular marker assisted selection (MAS) for an early identification of seedless genotypes; *in vitro* embryo culture; plant evaluation under field conditions. In the coming years the activities of varietal description and official registration, Patent protection, and establishment of stock fields will be initiated.

As result of the 3-year activities, the progenies of 75 “seed x seedless” varieties crosses, 28 “seedless x seedless” varieties crosses and 12 crosses involving germplasm with resistance traits have been obtained and analysed and the field evaluation begun. With regard to progenies derived from “seedless x seedless” varieties crosses, the average embryogenesis percentages (no. of germinated embryos/no. of cultivated ovules) was about 8-9%, varying according to the parents used. Following the extensive use of MAS about 40% of each “seed x seedless” varieties progeny carried allele associated to seedless locus. Actually about 6,000 new genotypes are grown in the first selection field, while further 6,000 seedless plants are going to be planted within 2018.

**Keywords:** seedless varieties progenies, table grape

## A new late-maturing table grape cultivar 'Shenzhou Red'

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'Shenzhou Red' is a new late-maturing table grape variety with good quality and attractive appearance. The original seedling was bred from the cross between '*Vitis vinifera* cv. Christmas Rose ' and '*Vitis vinifera* cv. Muscat Hamburg ' through artificial hybridization pollination and the cross was made in 2002. After 10-years investigation, the character of this new grape variety was found as follows: the shape of cluster was coniform, the number of seeds per berry ranged from 1 to 3, with an average of 1.4; the average weight of fringe was 870 g, the shape of berry was long oval and the average weight was 8.9 g; the quality was high compared to the varieties ripened in the same time. Total sugar was 16.98%, and total acid was 0.29%. The content of tannin was 718mg/kg. 'Shenzhou Red' was ripened on August 15 in Zhengzhou. The flavour is sweet and the color of skin is bright red. The pulp of 'Shenzhou Red' is brittle and can be cut into pieces, so it is suitable for storage and transportation. This variety is resistant to disease and is not susceptible to *Botrytis cinerea* and downy mildew. It is especially important that it is very easy to manage which is no need for complex flower and fruit management techniques, etc.

**Keywords:** selection, new cultivar, Shenzhou Red, table grape

## New table grape varieties IMIDA-ITUM of Spain: protocol for *in vitro* introduction of *Vitis vinifera* var. Itumfifteen & Itumsixteen

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Murcia is the Spanish region that produces and exports the largest amount of table grapes, with some European countries consuming a large proportion of the production. Thirty years ago the vine growers of our region cultivated local varieties with seeds, then the varietal landscape was transformed with the foreign seedless varieties and since 2013 they are cultivating grapes obtained in our own IMIDA-ITUM breeding program. At present, the partner companies have 16 new varieties at their disposal that stand out by their crisp texture, of which 8 are white, 6 are red and two are black varieties, and they already cultivate an area that exceeds 700 ha, equivalent to more than 12% of the regional land devoted to the cultivation of table grapes.

A decade ago, we started a new line of work to obtain disease-tolerant varieties, the result of which is "Itumfifteen", our first registered variety with genes for tolerance to powdery mildew. Growing them, producers will have less harvest losses and will also save phytosanitary treatments; consumers will get grapes with less residues, and there will be a considerably smaller environmental impact, all of which aims at achieving a more sustainable type of agriculture.

The development of the new varieties IMIDA-ITUM was initially promoted to meet the needs of local farmers, but due to the interest shown by producers from the rest of the world, plant material has been sent to Chile, Peru, Argentina, Brazil, South Africa and Australia.

We need to fine-tune the protocols for introducing plant material from field to *in vitro* culture of each variety, to make shipments to these countries, in line with the optimization of the *in vitro* rooting of Itumfifteen and Itumsixteen with the use of two types of auxins, Indoleacetic acid (IAA) and indole butyric acid (IBA), in two concentrations (5 and 10  $\mu$ M), using as a control a basal medium without hormone. Root presence or absence data were taken for 35 days at 7-day intervals. Itumfifteen rooted the best in the presence of 5  $\mu$ M IAA, however, for Itumsixteen the best results were obtained with 10  $\mu$ M of IBA; it depends on the genotype of the plant.

**Keywords:** powdery mildew, resistance, table grape

## IGGP Proposal Call 2018

**Background:** For the past years, the members of International Grape Genome Program have committed efforts to drive the grape community towards an international but federate endeavor to coordinate research initiatives toward the same goal: an imperative need for improving Findability, Accessibility, Interoperability and Reusability (FAIR) of large data sets at any layer of biological information. Recent publications in BMC Plant Biology (Grimplet et al., 2014) and more recently in Nature Horticulture Research (Adam-Blondon et al., 2016) are relevant examples of this commitment. In the light of the recently funded European COST action to Prof. Pezzotti (Italy) the committees of the International Grape Genome Program propose to contribute to the development of the international Working Groups (WG) in partnership with the awarded action. These WGs will serve as building blocks to construct a coordinated federation of information systems holding grapevine metadata information distributed around the world. Proposals emphasizing on, but not limited to, the development of integrated toolkit such as the next generation of data mining and integration tools (sequencing, mass spectral data, phenotyping, network analysis, genetic resources, new breeding techniques), rich and common semantic integration software for appropriate standards for data annotation and formatting, and bioinformatics structures and web services, are strongly encouraged. The development of these groups must be seen as the next step of the IGGP's commitment to coordinate international research efforts. This initiative is considered a major critical driving force of the future work of the IGGP to move forward in the development of an open system of scientific information. It will also enhance innovative international collaborations between researchers, industrial partners and government entities that increasingly seek more substantial transnational funding programs.

**Content of the proposal (1 page):** A short description of the WG will be submitted to the IGGP steering committee that will review it for consistency with the IGGP objectives. To be fully considered, the proposal must contain:

- A title describing the topic that the WG will be in charge of
- The name of the coordinator (Principal Investigator [PI]) and the participants (Co-PIs) of the WG at this stage, with their email addresses. The composition of the WG may evolve and become more inclusive over time. However, it is recommended to inform the IGGP of any change.
- A concise explanation of the listed objectives of the WG.
- A clear description of achievable milestones during the first two years.

**Proposal evaluation:** The proposal will be evaluated on both intellectual merit and broader impact. The IGGP committee will inform the WG about the acceptance of the proposals within one month after the deadline, with opportunities to revise and resubmit once.

Once the proposal is accepted, the members of the WG will provide a brief report every year (1 page) to the IGGP steering committee, which will facilitate a broader communication within the scientific community. These reports should emphasize the current priorities of the WG, activities, outcomes, and challenges faced by the WG. The steering committee will provide support, aligned with its capacity, to coordinate interactions between WGs through a virtual space for common topics and outputs. Renewal of the WG by the IGGP will be given with recommendations, if necessary. Failure of the WG to demonstrate progress in their research objectives may lead to discontinuation of the WG by the IGGP, subject to a majority vote. A WG can also decide on its discontinuation by itself, and should notify the IGGP in an appropriate time period.

**Timeline for proposal submission and final decision:** The deadline for submission of proposals is August 1st, 2018. The proposals must be submitted to laurent.deluc@oregonstate.edu and anne-francoise.adam-blondon@inra.fr. The IGGP steering committee will inform the coordinator of each WG for final recommendation by September 1st, 2018.



**The COST CA 17111 « Integrape »**  
**Data integration to maximise the power of omics for grapevine improvement**

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Grapevine (*Vitis vinifera* L.) is grown worldwide and is one of the most important and valuable horticultural crops. The berries are used as fresh, dried or canned fruit, and for juice or jam, but their main use is wine production. The major challenge for viticulture and oenology is to control berry composition and maintain yields while limiting the use of pesticides, water and other inputs, thus adapting to climate change and achieving environmental and economic sustainability. It is therefore necessary to understand and precisely modulate the complex mechanisms controlling adaptive, yield, quality and sensory traits, based on research to determine relationships between genotype, phenotype and environment (including management). To address these challenges, the grapevine research community must generate and integrate heterogeneous datasets (genomics, epigenomics, transcriptomics, proteomics, metabolomics and ampelography) describing genotypes, phenotypes and the environment. Integrating these multi-omic datasets will reduce the gap between data generation and the ability to analyse and understand the biological mechanisms underlying grapevine responses to the environment. This is achieved by applying high-throughput experimental techniques that generate large datasets (“omics” technologies and multiplex sampling/imaging). The resulting data are currently dispersed and difficult to access, hindering exploitation beyond their initial purpose. International gene data repositories do not store functional data (e.g. regulatory and metabolic networks), detailed plant materials or non-molecular phenotypes. Such data may be stored in regional or local databases but are often inaccessible to the wider research community.

The value of data from individual experiments is much enhanced when considered in a wider context through meta-analysis. In the Arabidopsis community, rich datasets supported by the TAIR portal are used to develop broader hypotheses (<http://www.arabidopsis.org>). The meta-analysis of these integrated datasets helps to identify mechanisms underlying the interactions between plants, their environment and plant management techniques. However, the interpretation and re-processing of data requires additional metadata to provide appropriate context. Ideally, data should be formatted in a standardised manner for automated processing to avoid errors caused by manual manipulation, especially in very large datasets (Stephens et al. 2015; Wilkinson et al. 2016). Such automated data re-processing is supported by recent advances in informatics that combine different computational environments (e.g. cloud computing). International consortia are also promoting the use of FAIR principles that ensure data are findable, accessible, interoperable and reusable (Wilkinson et al. 2016). Model system ontologies cannot always be applied directly to grapevine because its unique traits differ from model organisms, which is why current standardisation efforts have been unsuccessful when applied to this species, so the grapevine community must adapt current conventions to build a standardised system for data collection, processing, storage, access and analysis, and develop strategies to integrate them for a full description of the phenotypes.

**Keywords:** big data, data base, genomics, metabolomics, phenomics, transcriptomics

**Workshop**  
**Advances and applications of plant phenotyping in viticulture**

Reinhard Töpfer, Ulrich Schurr, Katja Herzog, Mario Pezzotti, Serge Delrot

Quantitative assessment of the plant phenotypes provides the vital link between genetic information and biological structure and function which is needed to improve plant performance, tolerance to biotic or abiotic stress or quality related traits. Due to technical advances within the last decade genomic data became easily accessible, but the generation of phenotypic information is not keeping pace with the explosion in available genomic information. The lack of reliable and available phenotypic data limits the possibilities to identify associations between phenotypic and genotypic data. This phenotypic gap is a major challenge in biological understanding of plant processes and their translation into practical application. Specifically phenotyping of perennial plants such as grapevine under field conditions represents challenge for quantitative non-invasive assessment of a variety of traits.

In this workshop, we will focus on discussing the requirements and challenges in viticulture where relevant high-throughput field phenotyping platforms may support quantitative assessment relevant traits over vast areas. Specifically, addressing these challenges requires interaction within the community. The EU funded project EPPN2020 provides access to some plant phenotyping facilities in Europe (<https://EPPN2020.plant-phenotyping.eu/>), while the ESFRI listed project EMPHASIS aims at a synergistic development and long-term operation of phenotyping infrastructure in Europe (<https://emphasis.plant-phenotyping.eu/>) by developing infrastructures and providing access for multi-scale phenotyping to analyze genotype performance in diverse environments and quantify the diversity of traits. One of the key elements of EMPHASIS is the development of well-instrumented field sites with high-resolution recording of the environmental conditions (including abiotic and biotic) and detailed imaging carried by proximal or remote sensing systems on airborne or ground based systems linked to relevant information systems.

To make progress within the grapevine community structures and common goals need to be defined. Uli Schurr from Forschungszentrum Jülich (Germany) will point out the possibilities in Europe to establish infrastructures to intensify phenotyping. Katja Herzog from JKI Institute for Grapevine Breeding Geilweilerhof will bring up some examples and summarize some possible needs. Very important in all this is the data organisation and data integration which will be addressed by Mario Pezzotti, from University Verona and representative of COST CA17111 INTEGRAPPE: Data integration to maximise the power of omics for grapevine improvement ([http://www.cost.eu/COST\\_Actions/ca/CA17111](http://www.cost.eu/COST_Actions/ca/CA17111))

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**Acknowledgments :** we are grateful to the below mentioned organizations which supported the organization of the congress :

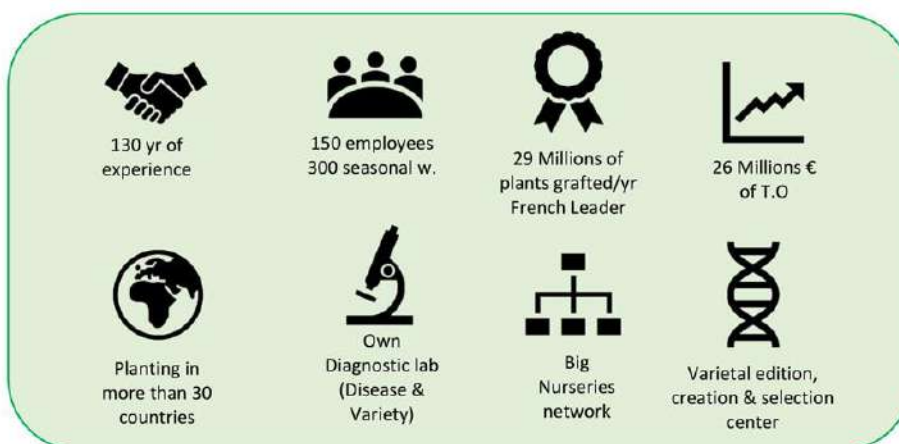
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Mercier is now in all the value channel of material production, controlling the traceability and the sanitary quality of its material.

Mercier start a strong financial program in R&D for varietal edition and creation and invite all the breeders, researchers and creators of new genetics in grapevine, but also the grapegrowers to be part of this project.



Contact: [olivier.zekri@mercier-groupe.Com](mailto:olivier.zekri@mercier-groupe.Com)

**Qualité - Traçabilité - Conseil - Service - Recherche - Innovation - Garantie - Suivi**

SARL MERCIER FRÈRES. Etablissement de pré-multiplication  
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**Beijing Key Laboratory of Grape Science and Enology (BKGSE)  
Institute of Botany, Chinese Academy of Sciences (IBCAS)**

Starting with germplasm collection and breeding at 1954, IBCAS makes systematical studies on grape germplasm collection and evaluation, genetics and regulation mechanisms of berry quality, stress tolerance and genetic control mechanisms. Moreover, to meet national demand of grape and wine industry, IBCAS specially makes great breakthroughs in breeding early-mature table grapes, and wine-making grapes with high resistance to cold.

A total of 24 grape cultivars have been released by IBCAS, including 14 table grapes of 'Jing' series and 7 wine-making grapes of 'Bei' series. All those cultivars have been widely extended in China, and makes significant economic benefits.

'Jing' cultivars fill the blank of early-mature grape market, planting area of 'Jingya' is more than 80,000 ha and the only Chinese cultivar of the three main table grape cultivars cultivated in China. 'Bei' series of wine-making grape cultivars do not need to be buried in winter in the main wine-making grape regions in China due to their high resistance to cold. Moreover, red and rose wines made from 'Bei' grapes have high quality and unique pleasant flavor. In the past few years, 'Beihong' and 'Beimei' have been planted for about 1,000 ha.



**Director  
Prof. Dr. Shaohua Li**



**The research group**



**Jingxiu**



**Jingxiangyu**



**Jingyan**



**Jingya**



**Beihong**



**Beimei**



**Wines made from 'Beihong' or 'Beimei'**

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### **The French Wine and Vine Institute**

The French Wine and Vine Institute (IFV) is the French research center specialized in vine and wine. Its missions are to conduct studies of general interest for the French wine and Nursery industries.

#### **IFV's activities**

French Wine and Vine Institute's activities are focused in 3 main sectors:

1. selection of grape plant material,
2. vineyard management including biocontrol, pest management,...
3. wine making issues.

#### **IFV's means**

The French Vine and Wine Institute offers a wide range of professional competences covering all the skills needed for wine production. 140 scientists and engineers work on multidisciplinary subjects in relation to vine production and wine quality: ampelography, agronomic studies, vine diseases, oenology, microbiology, processes, circular economy, mechanization etc. Laboratories are officially (Cofrac, ISO or others...) recognized for agronomy and oenology trials or studies.

IFV conducts its research on its experimental sites established in all different the French wine regions.

#### **IFV's missions**

IFV is the national viticultural Research and Development Organization. Officially recognized by the Ministry of Agriculture as the agricultural technical Institute for the French wine industry, IFV has a role of interface between professionals and research teams, and transfers research results to the wine industry.

Main qualities of the French Wine and Vine Institute are:

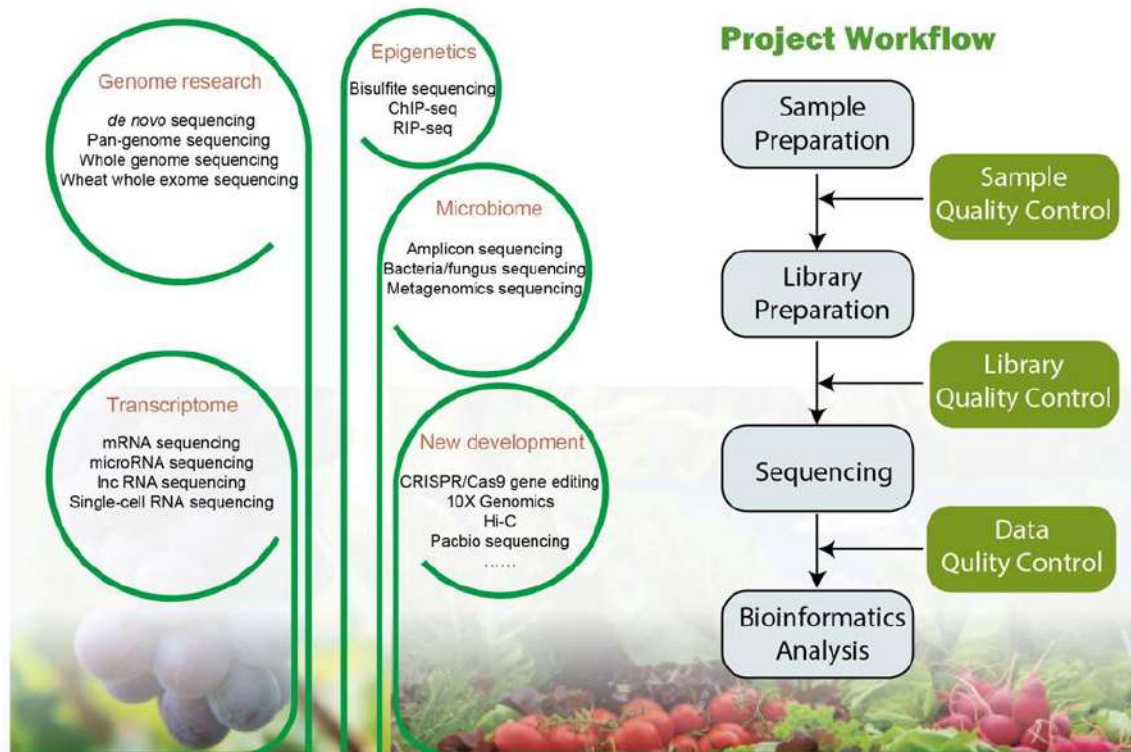
- a range of professional competences covering all the skills needed for the production of wines,
- a network established in the main French wine regions,
- multidisciplinary teams with means of experimentation,
- board members representing all professionals of the wine industry,
- an interface between professionals and research teams,
- a capacity to innovate, to transpose and to highlight research results.



### **ENTAV-INRA Trademark**

Since more than half a century, IFV and INRA have been leaders in Clonal Selection and Breeding programs backed by their unique know-how represented by over 400 varieties officially registered, over 1200 approved clones (wine grapes, table grapes and rootstocks) along with a new range of cultivars resistant to downy mildew and powdery mildew. Created in 1995 by IFV and INRA, the ENTAV-INRA® trademark represents a substantial Vine Selection in health and agronomy terms in view of spreading approved and authentic plant material throughout the world, directly from the repository of the IFV National Plant Material Pole located in the Grau du Roi in Southern France. Today, ENTAV International, a subsidiary of IFV and INRA created in 1999, which is in charge of developing the ENTAV-INRA® trademark in France, in Europe and on the other continents where today, there are 18 licensees in Canada, USA, Chile, Argentina, South Africa, Australia and New Zealand.





Novogene is the largest sequencing capacity in the world providing cutting edge NGS services. With around 2,000 employees mastering highly professional scientific expertise, Novogene commits itself to unsurpassed customer service and data quality to help researchers achieve pioneering academic goals in the rapidly evolving world of genomics. With intercontinental branches, 49 NGS-related patents, and >300 publications in top-tier journals, Novogene has rapidly arisen as a world leader in NGS services.

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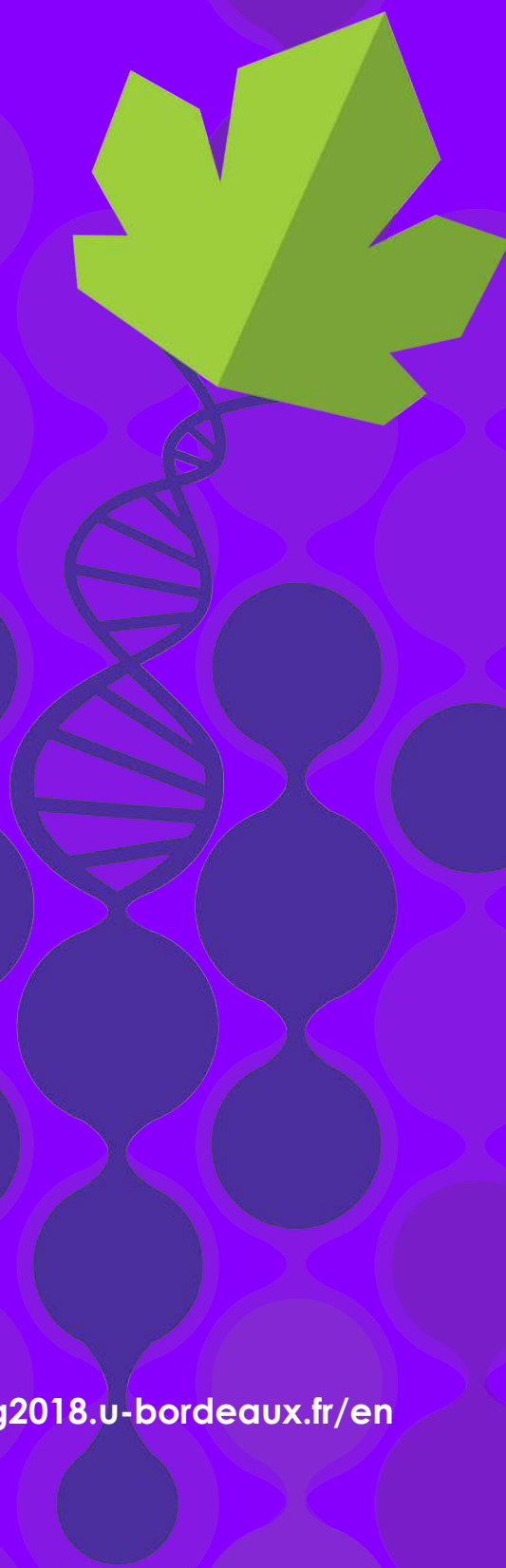
Situated in the heart of a 300 hectares domain, the vineyard of Château Giscours, Grand Cru classé 1855, includes 100 hectares in the Margaux appellation.

Château Giscours has a rich history dating back to the 14<sup>th</sup> century. Primarily a defensive tower overlooking “a wild and hostile” landscape. In 1552, Pierre de Lhomme, a rich Bordeaux draper bought the noble house of "Guyscoutz“, formed around a vast field. He planted the very first vines. The Wine adventure was running and the successors of this rich merchant made their contribution to a rich history through the centuries.

In the 19<sup>th</sup> century, under the leadership of the Promis, Pescatore and Cruse families Giscours dressed up in all its finery: transformation of the castle into a neoclassical palace, construction of a park with rare species by the landscape designer Eugene Bühler, modernization of production tools with the construction of huge farm buildings, including the "Ferme Suzanne".

In 1995, Eric Albada Jelgersma became the president of the estate. He immediately began a meticulous reorganization of the vineyards and the renovation of farm buildings. He knew how to surround himself with a renewed team who is today managing the estate. The ambition of this new generation perfectly matches with the heritage of winemakers who turned this domain into a worldwide renowned classified growth.





<http://gbg2018.u-bordeaux.fr/en>

