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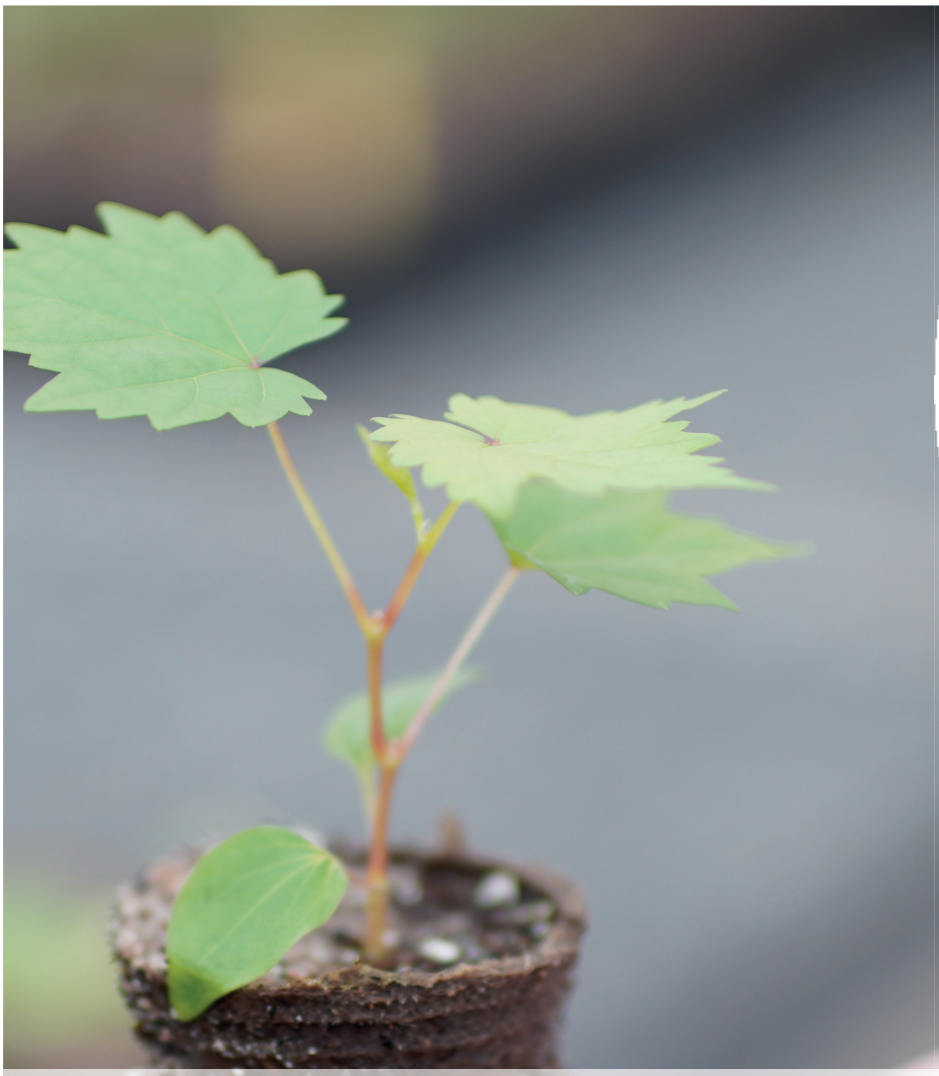

XIII. International Symposium on Grapevine Breeding and Genetics

Pioneering Wines (PIWIs) – Innovation and Tradition

Abstract Book

10th – 15th July 2022

Institute for Grapevine Breeding Geilweilerhof | Siebeldingen/Germany

Julius Kühn-Institut
Bundesforschungsinstitut für Kulturpflanzen

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Foreword

After decades of peaceful and prosperous development in many parts of the world, the coronavirus pandemic and other current crises have shown how fragile our daily lives can be. Of particular note is the ongoing climate change and with it the question of the sustainability of our actions. Today's grapevine breeders and geneticists are committed to these questions and are working on solutions building on the achievements of their predecessors. It is about nothing less than the preservation of our wine-growing cultural landscape, which is only possible through change. This change also includes adapted grapevine varieties. The symposium is therefore held under the motto "Pioneering Wines (PIWIs) - Innovation and Tradition". It is intended to be a forum for discussion, scientific exchange and getting to know PIWI wines as well as top wines from traditional cultivars.

Since COVID-19, digital communication has brought the world together again and has found its way into our everyday lives as a new tool. However, the experience of digital communication also shows its limits, as people also like to experience personal contact and exchange. We are therefore very pleased that the “XIII. Symposium for Grapevine Breeding and Genetics” under the auspices of the *Federal Ministry of Food and Agriculture* and the *International Organisation of Vine and Wine* takes place as a conference in attendance. Exchange and networking is easily possible in addition to the participation in lectures and poster sessions or workshops! A special feature of viticulture is not only theoretical consideration but also the sensory experience with a discourse about the product wine. This dimension is only possible in presence and is a cornerstone on the way from science to viticulture practice. We therefore very much regret the absence of colleagues who unfortunately cannot take part in the conference due to existing travel restrictions in some countries. However, they will at least be able to see the theoretical side of the coin by accessing the abstracts and proceedings of the conference that will be published later in "*Vitis* Journal of Grapevine Research".

Prof. Dr. Reinhard Töpfer

Head of the Institute for Grapevine Breeding Geilweilerhof, Siebeldingen, Germany

Conference Program

Sunday, 10.7.

17:00 – 22:30	Registration and welcome reception at the festival hall Landau/Pfalz

Monday, 11.7.

08:00 – 10:00	Registration and Opening address
09:00	Prof. Dr. Reinhard Töpfer Institute for Grapevine Breeding Geilweilerhof
09:15	Dr. Bettina Hartwig Federal Ministry of Food and Agriculture (BMEL)
09:30	President Klaus Schneider German Winegrowers' Association (DWV)
09:45	President Prof. Dr. Frank Ordon Julius Kühn Institute (JKI) - Federal Research Centre for Cultivated Plants
10:00	**Session 1: Genetic Resources** Moderation: Erika Maul Julius Kühn Institute (JKI), Institute for Grapevine Breeding Geilweilerhof, Siebeldingen, Germany
10:00	**Keynote lecture: *Vitis* genetic resources: current challenges, achievements and perspectives** Thierry Lacombe, AGAP, University of Montpellier, CIRAD, INRAE, Institut Agro, Montpellier, France
10:30	**Grapevine genetic resources of Armenia: molecular fingerprinting and phylogenetic relationship among wild and cultivated grapevine** Kristine Margaryan *et al.*, Research Group of Plant Genomics, Institute of Molecular Biology, National Academy of Sciences RA, Yerevan, Armenia
10:50	Coffee break
11:20	**Genetic characterisation of the Greek grapevine collection: belated but catching up quickly** Georgios Merkouropoulos, Hellenic Agricultural Organisation DIMITRA, Institute of Olive tree, Subtropical plants and Viticulture, Department of Vitis, Athens, Greece
11:40	**Analysis of Croatian wild and cultivated grapevine diversity by genotyping by sequencing** Luka Marinov *et al.*, Institute for Adriatic Crops and Karst Reclamation, Split, Croatia
12:00	**Genetic diversity in 200 years old Serbian grapevine herbarium specimens** Carolina Royo *et al.*, Instituto de Ciencias de la Vid y del Vino (ICVV, CSIC-CAR-UR), Departamento de Viticultura, Logroño, Spain

12:20	**Two main distinct evolutionary stories describe the Italian grapevine assortment** Manna Crespan *et al*., CREA, Research Centre for Viticulture and Enology, Conegliano, Italy
12:40	**Could a postglacial recolonization route be proposed for German *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi, based on nuclear microsatellite markers?** Erika Maul *et al.*, Julius Kühn Institute (JKI), Institute for Grapevine Breeding Geilweilerhof, Siebeldingen, Germany
13:00	Lunch break
14:30	**Session 2: Phenotyping** Moderation: Javier Tello Instituto de Ciencias de la Vid y del Vino (ICVV), Universidad de la Rioja, Logroño, Spain
14:30	**Keynote lecture: New and emerging digital technologies for plant phenotyping using Artificial Intelligence (AI)** Sigfredo Fuentes, Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Australia
15:00	**Dissecting foliar physiology and chemical properties with integrated high-throughput phenotyping and molecular markers in grape improvement** Ugochukwu Ikeogu *et al*., Cornell University, Cornell-AgriTech, Horticulture Section, Geneva, NY, USA
15:20	**Sensor based screening of yield relevant characteristics within grapevine breeding research** Hannes Engler *et al*., Julius Kühn Institute (JKI), Institute for Grapevine Breeding Geilweilerhof, Siebeldingen, Germany
15:40	**Single berry development - a new phenotyping and transcriptomics paradigm** Stefania Savoi *et al*., UMR AGAP, Montpellier University, CIRAD, INRAE, Institut Agro-Montpellier, Montpellier, France
16:00	Coffee break
16:30	**Risk prediction of Botrytis bunch rot based on physical characteristics of grapes** Katja Herzog *et al.*, Julius Kühn Institute (JKI), Institute for Grapevine Breeding Geilweilerhof, Siebeldingen, Germany
16:50	**Metabolic snapshot of grapevine-pathogen interactions through mass spectrometry imaging** Marisa Maia *et al.*, Laboratoire de Chimie et Physique-Approche Multi Échelle des Milieux Complexes (LCP-A2MC), Institut Jean Barriol (FR 2843), Université de Lorraine, Metz, France
17:10	**A ResNet-CNN for accurate quantification of grapevine leaf hair** Nagarjun Malagol *et al*., Julius Kühn Institute (JKI), Institute for Grapevine Breeding Geilweilerhof, Siebeldingen, Germany
17:30	End of session

19:00 Dinner at the Castle Landeck

Tuesday, 12.7.

09:00	**Session 3: Abiotic Stress** Moderation: Melané Vivier Department of Viticulture and Oenology, Stellenbosch University, Stellenbosch, South Africa
09:00	**Keynote lecture: Moving towards grapevine genotypes better adapted to abiotic constraints** Nathalie Ollat *et al.*, EGFV, Univ. Bordeaux, Bordeaux Sciences Agro, ISVV, Villenave d'Ornon, France
09:30	**Water use efficiency as a novel target for grapevine breeding programs** José M. Escalona *et al*., Research Group on Plant Biology under Mediterranean Conditions, Department of Biology, University of Balearic Islands (UIB). Palma, Balearic Islands, Spain
09:50	**The rootstock control on the scion transpiration even at night** Davide Bianchi *et al.*, EGFV, Bordeaux Sciences Agro, INRAE, Univ. Bordeaux, ISVV, Villenave d'Ornon, France
10:10	**Genetic structure exploration and association mapping for root related traits in *V. berlandieri*** Louis Blois *et al*., EGFV, Bordeaux Sciences Agro, INRAE, Univ. Bordeaux, ISVV, Villenave d'Ornon, France
10:30	**Influence of vegetative propagation on epigenetic rejuvenation and its effect on vine response to stress: a multi-omic study** Carlos M. Rodriguez Lopez, University of Kentucky, Environmental Epigenetics and Genetics Group, Dept. of Horticulture, Lexington, USA
11:00	Coffee break
11:30	**Session 4 (1): Biotic Stress** Moderation: Anne Fennell South Dakota State University, Brookings, South Dakota, USA
11:30	**Keynote lecture: Improving biotic stress resistance in grapevines: What's the path forward?** Silvia Vezzulli, Fondazione Edmund Mach, Research and Innovation Centre, San Michele all'Adige, Italy
12:00	**Grapevine *Rpv3-*, *Rpv10-* and *Rpv12*-mediated defense responses against *Plasmopara viticola*** Chantal Wingerter *et al*., State Education and Research Center of Viticulture, Horticulture and Rural Development, Institute of Plant Protection, Neustadt/Weinstraße, Germany
12:20	**Characterization of the *Rpv12* locus in a haplotype-separated grapevine genome sequence** Sophia Müllner *et al*., Julius Kühn Institute (JKI), Institute for Grapevine Breeding Geilweilerhof, Siebeldingen, Germany

12:40	**Towards marker-assisted breeding for black rot resistance: high-density linkage mapping and identification of a major QTL in the cultivar 'Merzling' (*V. rupestris* × *V. aestivalis* var. *lincecumii*)** Paola Bettinelli *et al*., University of Trento, Centre of Agriculture Food Environment, Trento, Italy
13:00	Lunch break
14:00	**Session 4 (2): Biotic Stress** Moderation: Claudio Moser Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy
14:00	**Functional evaluation of defensins in grapevine provide evidence that these peptides could be exploited for their stress protective roles** Helmien Barkhuizen and Melané A. Vivier, Stellenbosch University; South African Grape and Wine Research Institute, Department of Viticulture and Oenology, Stellenbosch, South Africa
14:20	**Identification of metabolic markers of grape infection with Esca complex disease** Florent Weiller *et al*., University of Lisbon, Faculty of Sciences, BIOISI, Lisboa, Portugal
14:40	**Grape phylloxera leaf galling - traits or triggered?** Astrid Forneck *et al*., University of Natural Resources and Life Sciences, Vienna (BOKU), Institute of Viticulture and Pomology, Tulln an der Donau, Austria
15:00	**Grapevine fanleaf disease: simple solution for complex problem?** Samia Djennane *et al.*, INRAE, Université de Strasbourg, UMR SVQV, Colmar, France
15:30 parallel	Workshop Phylloxera: Astrid Forneck and Joachim Schmid
15:30 parallel	Workshop *Vitis sylvestris:* **Objective: broad genetic basis of *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi for deep studies** Erika Maul *et al*., Julius Kühn Institute (JKI), Institute for Grapevine Breeding Geilweilerhof, Siebeldingen, Germany
15:30 parallel	Poster session
16:00	Coffee break
16:30	Poster session
17:50	End of session

Wednesday, 13.7.

09:00 – 18:00	**Technical Tours** Visit of local wineries/DLR Rheinpfalz and Geilweilerhof
19:00	Barbeque at Geilweilerhof (outdoors)

Thursday, 14.7.

09:00	**Session 5: Grapevine Breeding** Moderation: Bruce Reisch School of Integrative Plant Science, Cornell AgriTech, Cornell University, Geneva, New York, USA
09:00	**Keynote lecture: Grapevine breeding: progress, innovation, and opportunity** Peter Cousins, E. & J. Gallo Winery, Modesto, CA, USA
09:30	**Agronomical behaviour of 21 new disease resistant winegrape varieties grown in northeast Italy** Luigi Bavaresco *et al*., Università Cattolica S. Cuore, Dept. Sustainable Crop Production, Pomology & Viticulture section, Piacenza, Italy
09:50	**The differences in resistance levels of different FRCs and the impact of their deployment on fungicide use in viticulture** Birgit Eisenmann *et al*., State Education and Research Centre of Viticulture, Horticulture and Rural Development, Institute of Plant Protection, Neustadt/Weinstraße, Germany
10:10	**Contributions of the VitisGen2 project to grapevine breeding and genetics** Bruce Reisch *et al.*, Cornell University, Cornell-AgriTech, Horticulture Section, Geneva, NY, USA
10:30	**Producing and (epi)genotyping a large collection of intravarietal diversity for grapevine improvement** Darrell Lizamore *et al*., Bragato Research Institute, Grapevine Improvement Team, Lincoln, New Zealand
10:50	Coffee break
11:20	**Exploring clonal variation in 'Riesling'** Kai P. Voss-Fels *et al*., Hochschule Geisenheim University, Department of Grapevine Breeding, Geisenheim, Germany
11:40	**Reduced bunch compactness in somatic variants of 'Tempranillo' relate to genome structural variation and the sex locus** Noelia Alañon *et al*., Instituto de Ciencias de la Vid y del Vino (ICVV, CSIC-CAR-UR), Departamento de Viticultura, Logroño, Spain
12:00	**Genetic analysis of berry, flower and seed traits in two 'Tempranillo Tinto' populations: Influence of the Sex locus** Cristina Manso-Martínez *et al*., Instituto de Ciencias de la Vid y del Vino (ICVV), Logroño, Spain

12:20	**Genome Wide Association Study using table grape breeding families provide new QTLs for berry, seed and cluster traits** Tiago Carvalho *et al*., Centro de Biotecnologia y Genómica de Plantas (CBGP, UPM-INIA) Universidad Politécnica de Madrid (UPM) - Instituto Nacional de Investigación y Tecnologia Agraria y Alimentaria, Madrid, Spain
12:40	Lunch break
14:00	**Session 6: Novel Technologies** Moderation: Patricio Hinrichsen Instituto de Investigaciones Agropecuarias, INIA-La Platina, Santa Rosa, Santiago, Chile
14:00	**Keynote lecture**: **Genome editing in grapevine: a great opportunity or only a dream?** Riccardo Velasco, Consiglio per la Ricerca in Agricoltura e L'Analisi dell'Economia, Agraria-Centro di Ricerca Viticoltura ed Enologia (CREA-VE), Turi, Italy
14:30	**Breeding for grapevine downy mildew resistance via gene editing** Lisa Giacomelli *et al*., Fondazione Edmund Mach, Research and Innovation Centre, San Michele all'Adige, Italy
14:50	**Removal of a 10-kb *Gret1* transposon from *VvMybA1* of *Vitis vinifera* cv. 'Chardonnay'** Yingzhen Yang *et al*., USDA-Agricultural Research Service Grape Genetics Research Unit, Geneva, NY, USA
15:10	**Genomic and phenomic predictions for accelerating grapevine breeding** Charlotte Brault *et al*., UMT Geno-Vigne®, IFV-INRAE-Institut Agro, Montpellier, France
15:30	**Analysis of genomic prediction across populations in grapevine (*Vitis vinifera* L.)** Komlan Avia *et al*., Université de Strasbourg, INRAE, SVQV UMR-A 1131, Colmar, France
16:00	Coffee break
16:30 parallel	Poster session
16:30 parallel	Workshop PIWI: **Is a systemic innovation approach possible with PIWI?** Reinhard Töpfer and Oliver Trapp Julius Kühn Institute (JKI), Institute for Grapevine Breeding Geilweilerhof, Siebeldingen, Germany
17:30	End of session
19:00	Conference Dinner (Hohenstaufensaal, Annweiler)

Friday, 15.7.

09:00	**Session 7: Big Data** Moderation: Mario Pezzotti Department of Biotechnology, University of Verona, Verona, Italy
09:00	**Keynote lecture: Genomes as research tools: from loci to candidate genes** Dario Cantu, Department of Viticulture and Enology, University of California, Davis, CA, USA
09:30	**A large chimeric deletion associates with impairment of cuticle development in a dark berry somatic variant of 'Tempranillo Tinto'** Pablo Carbonell-Bejerano *et al*., Instituto de Ciencias de la Vid y del Vino (ICVV, CSIC-CAR-UR), Logroño, Spain
09:50	**Do we need to consider grape phyllosphere microbiome in breeding schemes?** Patrice This *et al*., UMR AGAP Institut, Univ. Montpellier, CIRAD, INRAE, Institut Agro Montpellier, Montpellier, France
10:10	**High quality phased assembly of grape genome offer new opportunities in chimera detection** Victoria Sichel *et al*., UMR AGAP Institut, Univ. Montpellier, CIRAD, INRAE, Institut Agro, Montpellier, France
10:30	**Genomics and bioinformatics strategies to tackle diversity and domestication in grapevine** Sara Freitas *et al*., CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Universidade do Porto, Vairão, Portugal
10:50	**Gene functional characterization assisted by genome-wide TF-binding site interrogation: Grapevine as a case of study** José Tomás Matus, Institute for Integrative Systems Biology (I2SysBio), Universitat de Valencia-CSIC, Spain
11:10	Coffee break
11:40	**Genetic characterization of the phylloxera resistance QTL Rdv1 in both haplotypes of the grapevine roostock variety 'Börner'** Bianca Frommer *et al*., Bielefeld University, Chair of Genetics and Genomics of Plants, Faculty of Biology and Center for Biotechnology (CeBiTec), Bielefeld, Germany
12:00	**Virulence-related metabolism may be activated in *Botrytis cinerea* mostly in the interaction with tolerant green grapes that remain largely unaffected** Flávio Soares *et al*., BioISI - Biosystems and Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, Campo Grande, Lisboa, Portugal
12:20	INTEGRAPE Workshop: **Beyond COST Action INTEGRAPE: The Grapevine Genomics Encyclopedia Initiative** José Tomás Matus *et al.*, The GRAPEDIA Consortium
13:00	Lunch break

14:00	**Session 8: Grape and Wine Quality** Moderation: Christian Zörb University of Hohenheim, Institute for Crop Sciences, Quality of Plant Products and Viticulture, Stuttgart-Hohenheim, Germany
14:00	**Keynote lecture: Oenological traits as targets for grape breeding** Ulrich Fischer, DLR Rheinpfalz, Institute for Viticulture and Enology, Neustadt/Weinstraße, Germany
14:30	**Differences on the transcriptomic profiles explain clonal phenotypic variation in *Vitis vinifera* L. 'Malbec'** Luciano Calderón *et al*., Instituto de Biología Agrícola de Mendoza (CONICET-UNCuyo), GenoVid, Chacras de Coria, Argentina
14:50	**Does the introgression of disease resistance genes impacts agro-œnological traits in grapevine varieties?** Elsa Chedid *et al*., SVQV, University of Strasbourg, INRAE, Colmar, France
15:10	**An independent haplotype responsible for white berry phenotype in *Vitis vinifera* arose from a large deletion at the berry color locus** Jean-Sébastien Reynard, Agroscope, Viticulture, Pully, Switzerland
15:30	**The real sour grapes: genetic loci, genes, and metabolic changes associated with grape malate levels** Noam Reshef *et al.*, Department of Food Science, Cornell University, Ithaca, NY, USA
15:50	**Genetic mapping of organic acids in a F1 white wine population with high variation in acidity and maturity date** Florian Schwander *et al*., Julius Kühn-Institut (JKI), Institute for Grapevine Breeding Geilweilerhof, Siebeldingen, Germany
16:10	Coffee break
16:40	Closing remarks and farewell
17:40	End of symposium

Session 1: Genetic Resources

Keynote lecture

Vitis genetic resources: current challenges, achievements and perspectives

Lacombe, Thierry

AGAP, University of Montpellier, CIRAD, INRAE, Institut Agro, Montpellier, France
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Abstract

Management and knowledge of *Vitis* genetic resources intend to help address the multiple current and future challenges facing the professional sector: i) preservation of wild and cultivated grapevine heritage, ii) clonal selection of existing cultivars, iii) creation of new cultivars by hybridisation or other technologies, iv) progress in fundamental and applied sciences on all viticultural and oenological issues. It is therefore a vast field of study and action that deals with all taxonomic levels within the *Vitis* genus (subgenus, species and their hybrids, cultivars and their variants, clones), in natural and cultivated environments, on all continents, both for fruit production (wine and table grapes) and for rootstocks. To meet the many expectations, different actors should ideally coordinate the following actions: 1) research and identification of sources of genetic diversity, 2) access, collection and sampling of this diversity, 3) *ex situ* and/or *in situ* conservation, 4) characterisation, 5) dissemination of plant material and related information, 6) use of genetic resources by the different applicants. Following this outline, we will try to assess the challenges and achievements over the last 5-10 years, and discuss the perspectives of this disciplinary field taking into account elements of the wine-growing, scientific, institutional and regulatory context.

Keywords: grapevine, germplasm, collection, preservation, characterisation, dissemination

Oral presentations

Grapevine genetic resources of Armenia: molecular fingerprinting and phylogenetic relationship among wild and cultivated grapevine

Margaryan, Kristine[1, 2*]; Gasparyan, Boris[3]; Harutyunyan, Frunz[1]; Töpfer, Reinhard[4]; Maul, Erika[4]

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[2]Department of Genetics and Cytology, Yerevan State University, Yerevan, Armenia
[3]Institute of Archaeology and Ethnography, National Academy of Sciences RA, Yerevan, Armenia
[4]Julius Kühn Institute (JKI), Institute for Grapevine Breeding Geilweilerhof, Siebeldingen, Germany
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Abstract

Armenia is characterized by high diversity of cultivated (*Vitis vinifera* L. subsp. *Vinifera*) and wild (*Vitis vinifera* L. subsp. *sylvestris*) grapes. The country has played a leading role in the centuries-lasting history of grapevine cultivation in Southern Caucasus. Varying climatic conditions and the existence of wild grapes lead to the formation and promotion of viticulture and winemaking, as evidenced by nearly 450 autochthonous varieties. Hundreds of unique indigenous cultivars are still preserved in old vineyards and abandoned gardens, though most of them are threatened by extinction. Wild grapes, thriving along riverbanks, climbing the rocks and embracing the trees can be found in Vayots Dzor, Tavush, Syuniq regions and in Artsakh.

With the main goal to estimate the phylogenetic relationships among Armenian wild grapes and indigenous cultivars, and to estimate the possible contribution of wild grapes to the genetic makeup of indigenous cultivars, we analyzed 79 unique cultivars and 111 putative wild plants, collected from different viticultural regions, with 25 nSSR markers.

The genetic diversity analysis conducted for wild grapes and indigenous cultivars unfolded the allelic richness of wild and cultivated gene pools and surprisingly for us revealed the absence of significant differences for all genetic parameters between the two subspecies. Moreover, the results registered for the number of different alleles (Na), effective number of alleles (Ne), Shannon's information index (I) have shown comparatively high values for wild grapes, while the observed negative value of Fixation index (F) for indigenous cultivars mirrored an abundance of heterozygote genotypes presuming random mating. The neighbour-joining (NJ) cluster analysis indicated clear separation between the two subspecies *vinifera* and *sylvestris* and formed two main clusters. Applied non-hierarchical horizontal clustering using Structure software assigned the 190 genotypes into two clusters. The delta K criterion (ΔK) suggested K = 2 as the optimal uppermost hierarchical level of structure. Obtained results were absolutely comparable with the NJ cluster analysis and confirmed the divergence of *sylvestris* from *vinifera*, indicating a clear separation between two subspecies. Meanwhile, results highlighted the role of gene flow between wild grapes and cultivars through observed overlaps and admixed ancestry values. Grapevine genetic resources of Armenia can contribute overcoming biotic and abiotic stresses and better adaptation to climate change

Keywords: wild grape, indigenious cultivar, genetic diversity, phylogeny, Armenia

Genetic characterisation of the Greek grapevine collection: belated but catching up quickly

Merkouropoulos, Georgios

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Abstract

Grapevine cultivation and wine production in Greece commenced during the Neolithic Period. As an outcome of this long cultivation period, many autochthonous varieties exist in the country; the recently updated National Catalogue contains 210 wine varieties together with 36 autochthonous table varieties, not considering clonal material that is getting registered for first time in the history of the country; considering the modern Greek bibliography, however, it is believed that a relatively large number of unidentified varieties occur in the countryside. Nearly all the registered genetic material is conserved in the collection of grapevine varieties, which is located in Lykovrysi (Athens, Greece) and maintained by the Hellenic Agricultural Organization DIMITRA. Collection of this material was performed progressively from the 1950's to the 1980's; this is the oldest and largest collection (hereafter: Collection) of autochthonous grapevine varieties in the country. Ampelographic description for each variety of the collection had been performed in the 1990's and they all are available. Molecular profiling, however, had never been performed in the past. The current work aims to cover this gap of information and characterization, representing a minor step towards modernisation of Greek viticulture. Ten microsatellite markers, including the six molecular descriptors introduced in the relevant 2009 OIV Catalogue plus the three markers incorporated in 2012, have been applied in order to create the molecular profile of each variety of the Collection. Within the frame of the markers used, a number of issues have been revealed: i) cases of synonymies/homonymies (as expected) even within the Collection's vineyard, ii) verbal similarities in the names (including color distinction) are not always accompanied by relevant genetic closeness, therefore, genetic analysis should be applied in order to define the degree of genetic similarity, iii) there have been cases of discrepancies and irregularities that came up upon comparison of the genetic material from the Collection with the material of the same name from the cultivation centres, indicating cases of misidentification.

Greece was among the pioneering countries in molecular profiling of the autochthonous grapevine resources; after a period of low activity, agricultural research has been re-activated and is catching up quickly.

Keywords: microsatellites, SSRs, native varieties

Analysis of Croatian wild and cultivated grapevine diversity by genotyping by sequencing

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Abstract

The traditional Croatian grapevine varieties (*Vitis vinifera* L. subsp. *vinifera*) represent a significant part of the germplasm of the so-called Balkan grapes. Over time and the rich history of this area, numerous varieties have accumulated and are still grown commercially. There are also several populations of wild grapevine (*Vitis vinifera* subsp. *sylvestris* Hegi Gmel) in natural sites in this region. Both methods, morphometric and SSR markers, have previously been used to validate identities and investigate genetic variability of Croatian germplasm, but without a detailed characterization of their genomes. Genotyping–by–sequencing (GBS) is a recent approach to effectively characterize individuals at thousands of variant sites based on next generation sequencing technology. The aim of this study was to use GBS to: (i) define a panel of single nucleotide polymorphisms (SNPs) and determine the identity of Croatian cultivated and wild grapevine accessions, (ii) verify the presence of homonyms and synonyms, (iii) determine the genealogical relationships and the varieties that acted as the main progenitors of the Croatian germplasm, (iv) determine the distance between wild and cultivated accessions. We successfully genotyped 192 grapevine accessions, including 132 cultivated (*vinifera*) and 60 wild (*sylvestris*) individuals. Approximately 50,000 genomic intervals in the gene space were analyzed using a targeted sequencing system based on single primer enrichment technology. The filtered DNA reads were aligned to the PN40024 v0 reference genome and more than 500,000 high quality SNPs were identified using the Genome Analysis Toolkit (GATK) software. Twelve synonyms were found within Croatian varieties, while eleven varieties were matched to international varieties, mostly from neighboring countries, indicating historical migration of cultivars. Distance- and model-based cluster analysis showed a clear genetic separation between cultivated varieties and wild accessions but also spotted the presence of rare feral individuals in natural populations of wild grapevines. For the prediction of relatedness, an identity-by-descent (IBD) analysis identified 'Plavac Mali', 'Bombino bianco', 'Bljuzgavac', 'Heunisch weiss' and 'Tribidrag' as varieties with highest numbers of first-degree relationships among the genotypes studied.

Keywords: single nucleotide polymorphism, native grapevines, Croatian germplasm, first degree relationship**,** genetic diversity

Genetic diversity in 200 years old Serbian grapevine herbarium specimens

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Abstract

A grapevine herbarium dated from 1812-1824, prepared by botanist Andreas Wolny Slovak, has recently been found in Sremski Karlovci (wine region of Vojvodina, Serbia). This collection should represent local cultivated grapevine diversity before *Phylloxera* was introduced to these areas. The herbarium collection comprises more than 100 samples, organized in two subcollections: red-berried varieties and white-berried varieties, totalling 47 different grape varieties. The goal here was to study the history of cultivated grapevines in the Balkans and Pannonia wine-growing areas with a long viticulture tradition. The obtention of DNA from plant remains older than 100 years requires the use of procedures of ancient DNA (aDNA) extractions in specific clean rooms, with positive pressure to avoid external contamination with modern DNA. Though, internal contamination from other organism is expected, such as bacteria and fungi associated to the living plant or herbarized samples. To avoid any cross-contamination with exogenous grapevine DNA, this work was performed following a protocol for recovering ultra-short DNA molecules from 10 mg of herbarized leaves in specific facilities for aDNA extraction at the University of Tübingen. In 80 samples, DNA could be quantified. In part of the samples, *Vitis psaA* chloroplast gene amplification was checked and confirmed the presence of grapevine DNA in these extractions. Furthermore, genotyping using standard DNA markers was performed in a specific laboratory at the ICVV where grapevine DNA had never been amplified. Different degrees of success were achieved in the genotyping analyses, from samples that did not produce any positive result to other that worked fine, like modern DNA samples. The genetic profiles obtained from the herbarized samples were compared to those stored in international databases (ICVV and *V*IVC). This task allowed us to successfully identify some of the herbarized samples as known varieties from the Western Balkans and neighbouring regions, such as Kadarka Kek, indicating their uninterrupted cultivation for more than 200 years. The joint analysis of ancient and modern samples allows establishing possible relationships among them, elucidating the historic evolution of the crop in Serbia.

Acknowledgements: Ministry of Agriculture, Forestry, and Water Management, Republic of Serbia.
Keywords: ancient DNA, herbarium, genotyping, grapevine

Two main distinct evolutionary stories describe the Italian grapevine assortment

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Abstract

A dataset of high-quality 7k SNP profiles of 1,038 unique Eurasian grapevine varieties was used to infer the most likely grapevine migration events, a spacial ancestry estimation, and a model about the origin of Eurasian grapevine germplasm. The comparison of putative gene flow scenarios from Caucasus throughout Europe aided to fit the more reliable spreading routes around the Mediterranean Basin. The same dataset was also used to assess the population genetic diversity, structure, and relatedness of Italian varieties. This data suggested a different history between Northern and Southern Italian grapevines.

More interestingly, the Italian genotypes were shown to be distinguishable from all the other Eurasian populations for the first time.

The same SNP panel was used to determine parental relationships, to identify the main parents of traditional Italian and closely related cultivars. The parentage network suggested that Italian germplasm largely originated from a few main parents distributed into several geographical areas of genetic influence, with more or less large overlaps. These key cultivars are 'Bombino bianco', 'Garganega'/'Grecanico dorato', 'Mantonico bianco', 'Orsolina', 'Sangiovese', 'Termarina'/'Sciaccarello', 'Visparola' and 'Vulpea'. The pedigree reconstruction by full-sib and second-degree relationships highlighted the pivotal role of some cultivars, like the little known 'Visparola'. A hypothetical migration of this variety from South to North of the Italian Peninsula along the Eastern side, as well as 'Sangiovese' migration from South to Central Italy along the Western side might be supposed. Moreover, 'Moscato bianco', mainly through its offspring 'Malvasia aromatica di Parma', furnished a consistent contribution to the development of many aromatic grapes grown in the Norther-Western part of the Italian Peninsula.

These results represent the most accurate and complete study of population genetics that has been carried out until now on the Italian germplasm.

Keywords: *Vitis vinifera* L., SNP markers, migration events, cultivar geographic areas, Italian founder varieties, parent-offspring relationships, second-degree relationships, pedigree

Could a postglacial recolonization route be proposed for German *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi, based on nuclear microsatellite markers?

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Abstract

Until the 19th century *Vitis vinifera* L. subsp. *sylvestris* C.C. Gmel., the wild compartment of our cultivated grape, was ubiquitous in many European flood-prone areas between 43° and 49° northern latitude. For the German wild grape, Bronner wrote in 1857 that they grow in thousands in the forests on the banks of the river Rhine. Since then, populations have diminished across Europe. Oberlin criticized the eradication of wild vines by the forest administration in the Rhine Valley in 1881. This happened even before the appearance of Phylloxera in that region. An approximately 100 individuals counting *V. sylvestris* population survived in the hardwood floodplain forest on the Rhine island Ketsch. The question arose: would it be possible to decipher the postglacial recolonization route taken by the ancestors of the Ketsch-population? To answer that question three main refuge areas Iberia, Italy and the Balkans were considered as survival zones. Therefore previously published and own nuclear microsatellite (SSR) data of 599 *V. sylvestris* genotypes were gathered from eight European countries: Austria (81 genotypes), Bosnia-Herzegovina (19), Croatia (70), France (46), Germany (57), Italy (Sicily – 105; mainly from Central and Southern Italy – 109), Slovenia (13), and Spain (99). SSR-marker data for Austrian and German accessions are own data. Eighteen overlapping SSR-markers were selected. They were standardised according to reference varieties and allelic frequency distribution patterns. Statistical analysis revealed that within the wild grapevines considered in the present study a distinct substructure could not be detected. Nevertheless STRUCTURE analysis with 3, 5 and 7 groups led to results indicating increasing discrimination among the different subpopulations. Italian refuge was clearly ruled out as a contributing gene pool. Austrian *V. sylvestris* formed an independent group, only slightly linked to Balkan compartment. Ketsch-population was placed within Western European (France and Northern Spain) *V. sylvestris*.

Keywords: *Vitis sylvestris*, population, postglacial recolonization, microsatellites, diversity

Session 2: Phenotyping

Keynote lecture

New and emerging digital technologies for plant phenotyping using Artificial Intelligence (AI)

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Oral presentations

Dissecting foliar physiology and chemical properties with integrated high-throughput phenotyping and molecular markers in grape improvement

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Abstract

As advances in sequencing technology continue to reduce the time and cost of generating genome-wide genotypic data, the need to develop complementary efficient technologies to likewise increase phenotyping throughput non-destructively and cost-effectively has become imperative in plant breeding. Hyperspectral reflectance, acquired by either imaging or non-imaging spectrometers, measures the interaction of light with vegetation at single leaves or entire canopy. This technology can be used to detect structural, chemical, and functional traits useful in accelerating crop breeding. We deployed a portable field spectroradiometer (SVC-HR1024i) with a wavelength range of 350 nm to 2500 nm to acquire grapevine foliar spectral signatures to improve our phenotyping throughput of foliar chemical and physiological properties including pigments, phenolics, flavonoids, sugars, and to better understand the variations, genetic architecture, and correlations with yield, fruit quality, vine health, and nutrition. Data were collected in Cornell University Grape Breeding Program mapping populations and the diverse genetic collection of the USDA Plant Genetic Resources Unit in Geneva, NY. Preliminary results regarding the accuracy of using the hyperspectral device as an alternative analytical tool for leaf chemical attributes, developed using Partial Least Square regression, showed a correlation between actual and predicted values of 0.46 to 0.99 in the training population (n~79 samples) and 0.28 to 0.63 in the test set (n~32) for leaf pigments – Neoxanthin, Violaxanthin, Lutein, Zeaxanthin, Chlorophyll a, Chlorophyll b and Beta-Carotene. Combined with publicly available resources (foliar prediction models in several other plants from the Ecological Spectral Model Library, ecosml.org), we are exploring QTL associated with phenotypic variation in grape foliar traits and hope to further understand how they relate to fruit quality, vineyard health, and nutrition. As more data points become available and using advanced machine learning models, integrating high-throughput spectral phenomics will help to facilitate the simultaneous improvement of leaf physiological and chemical composition of grape, complementing ongoing efforts to improve disease resistance, other biotic as well as abiotic stress resistances, fruit quality traits and yield.

Keywords: grapes, breeding, high-throughput phenotyping, hyperspectral, QTL

Sensor based screening of yield relevant characteristics within grapevine breeding research

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Abstract

In recent years, great progress has been achieved in grapevine breeding by dissecting the genetic basis of important resistance traits. Through the development and use of molecular markers, young seedlings can be selected for the presence of powdery and downy mildew resistances at an early stage in the breeding process (marker assisted selection, MAS). The extension of MAS to quantitative traits like yield has one huge bottleneck: precise phenotyping of multiple traits. So far, the recording of phenotypic traits has mainly been done manually. This process is very labor and time intensive and therefore only possible with selected breeding material. In addition, the ongoing breeding process has shown that the classification according to descriptive factors can only be used to a limited extent for marker development, especially in the case of very complex traits such as yield, which is influenced by different individual parameters and environmental factors. Thus, our main goal is to increase breeding efficiency and the selection of suitable breeding lines by the development of new field phenotyping methods - sensor-based, automated, fast and with high precision.

In a first step, a yield potential monitoring concept was developed. This is based on the quantification of various yield-related parameters in the vineyard with high temporal and spatial resolution. Key features include the quantification of shoots, clusters, and dormant pruning wood. For this purpose, a new embedded vision system was developed. The sensor system is transferable to different moving platforms. The yield potential concept combines the data from the image analysis with artificial intelligence trained by long-term yield and climate data as well as different sensor data for the recording of vitality and soil parameters.

Using this system, we expect to increase the precision, target specificity and throughput of screening plant material without reducing its accuracy over time. In addition, a weighting of the yield-relevant parameters is possible, which also allows a higher throughput to be achieved in phenotyping for QTL analysis. This opens up new possibilities for efficient plant evaluation in the scope of grapevine breeding and opens up new application possibilities for precision viticulture.

Keywords: PhenoQuad, PHENOboxx, Phenoliner, phenotyping, precicion viticulture, sensor, data management

Single berry development- a new phenotyping and transcriptomics paradigm

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Abstract

Present knowledge on berry development mostly arises from sampling and averaging hundreds of berries representing their diversity in the plot. According to recent studies, such chimeric samples formed from non-synchronized berries are unsuitable for detecting physiological changes faster than two weeks. It is thus necessary to revisit in-depth the physiological and transcriptional bases of berry development.

Over a three-month period, berry expansion characterized through image analysis was adjusted to a noticeably invariant (from-berry-to-berry) double sigmoid model. From this analysis, the second growth period lasts only three weeks on single fruits instead of five on non-synchronized samples. Hundreds of berries were then individually analyzed for tartaric and malic acids, glucose, fructose, and K^+ concentrations to calculate their respective accumulation rates with unprecedented precision. These individual fluxes allowed us to distinguish eleven developmental stages, during which specific pathways were switched ON or OFF. In all investigated genotypes, the new fluxes of malate and sugar quantitatively argue for the activation of a sucrose/H^+ exchange, providing a considerable sink strength during ripening. Finally, an RNAseq study was conducted on single berries from each physiological stage. Triplicates were well resolved on PCA plots of gene expression, while single berries inside the stages remained almost indistinguishable.

Switch genes abruptly set ON or OFF were easily identified and expressed explicitly during:
(i) the successive synthesis of tannins, tartaric, and then malic acids during the green phase;

(ii) the sudden activation of the apoplasmic pathway of phloem unloading of water and sugar in the pericarp, and the breakdown of malic acid during the second growth phase, in line with the immediate expression of specific membrane transporters and primary pump genes, indicating strong compartmentation control on berry ripening;

(iii) these genes were switched off with phloem unloading as abruptly as they were induced.

Moreover, genes inside multigenic families, such as cell wall-related proteins or aquaporins, were differentially expressed between the two growth phases.

Single berry monitoring evidenced sharp developmental phases and enlighted the mechanism underlying the malate/sugar ratio evolution during ripening.

New, high-throughput single berry phenotyping methods are now required to compare unambiguous developmental stages in genetic studies.

Keywords: grapevine, fruit development, image analysis, RNA-Sequencing, gene regulation

Risk prediction of *Botrytis* bunch rot based on physical characteristics of grapes

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Abstract

Botrytis bunch rot is the third economically most important disease in cool climates as most of the domesticated wine grape varieties are highly susceptible. In order to avoid or delay spreading of *Botrytis* infections until the grapes reach physiological ripeness, different management strategies as early defoliation or fungicide applications were developed.

The major task within the scope of the German grapevine breeding program is the selection of fungus-resistant, climatic adapted vines with balanced, healthy yield and outstanding wine quality. Facing the long-term procedure of breeding, marker-assisted selection (MAS) is the most efficient method for an early selection of grapevine seedlings. MAS and marker development work effective for qualitative single locus traits as *R*-gene mediated downy and powdery mildew resistance. Corresponding resistance genes are not described for *Botrytis* for neither grapevines nor any other affected crop. However, new grapevine selections can be equipped with natural infection barriers protecting against *Botrytis* bunch rot represented by physical and mechanical characteristics: loose grape bunch architecture, and thick, impermeable berry cuticles. At the same time, such quantitative traits are difficult to trace for the development of molecular markers and require objective phenotyping.

The present study aims at a new type of risk prediction for *Botrytis* bunch rot based on physical fruit traits (bunch architecture, berry impedance and berry texture) assessed with objective, high-throughput sensors. The preliminary predictive model is trained and tested on one-year data determined in 2021 including a mixture of 14 *Botrytis*-susceptible and –resilient varieties as well as elite breeding lines. Berry impedance and berry texture were identified as most effective traits enabling the forecast of *Botrytis* bunch rot infection with high accuracy. In addition, new QTLs were detected facilitating the development of molecular markers selecting for an increased resilience to *Botrytis* bunch rot. Furthermore, the physical barriers showed to be additional indicators for sunburn resilience and thus, are probably protecting berries against abiotic damages, too.

Keywords: grapevine phenomics, Botrytis bunch rot, predictive model, grape berry cuticle, berry impedance, berry texture, 3D grape bunch architecture, QTL analysis, sensor-based phenotyping

Metabolic snapshot of grapevine-pathogen interactions through mass spectrometry imaging

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Abstract

Every year, viticulture is facing several outbreaks of established diseases, such as downy and powdery mildews and gray mold, which possess different life cycles and modes of infection. To cope with these different aggressors, grapevine must recognize them and arm itself with an arsenal of defence strategies, including the accumulation of antimicrobial metabolites.

Plant metabolites are the first to be affected by changing conditions. Their rapid reactions activate and induce a series of defense mechanisms allowing the plant to adapt, defend and survive.

Despite the scientific community's efforts to characterize grapevine defence responses to pathogens, the molecules involved in pathogen recognition and their specific location upon pathogen interaction are still unknown.

Hence, to fully understand grapevine-pathogen interactions and to clarify metabolite specific roles in plants resistance/susceptibility to pathogens, it is not only important to identify the compounds involved in the first moments of the infection process but also to localise them *in vivo* and correlate their localisation with pathogens' recognition and development.

In our work, we investigate the metabolic responses during grapevine-pathogen interactions. We have been studying the metabolic events occurring in leaves, from different *Vitis* cultivars, infected with *Botrytis cinerea*, the causal agent for gray mold*,* through a time course infection using Matrix Assisted Laser Desorption Ionization-Mass Spectrometry Imaging (MALDI-MSI).

MALDI-MSI is a powerful technique that has the unique ability to analyse the sample surface directly by combining raster-scans of the sample surface with laser shots with high-mass resolution. This technique can reveal the molecules involved in the first stages of pathogen contact with the host leaf surface as well as their localisation, bringing new information and allowing a better understanding of pathogen recognition by grapevine and defense mechanism induction. In this work, we focus on the accumulation and spatial distribution of different phytoalexins (small compounds with antimicrobial activity synthesized *de novo* by plants in response to stresses) such as resveratrol and viniferins. Our results show that these compounds accumulate in areas close to the pathogen infection sites. Different genotypes are being compared and other phytoalexins as well as other compounds (involved in grapevine defence) are being investigated.

Keywords: mass spectrometry imaging, metabolomics, plant-pathogen interaction, phytoalexins

A ResNet-CNN for accurate quantification of grapevine leaf hair

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Abstract

The leaf hair density on the abaxial surface of *Vitis* species varies greatly. Generally, a dense indumentum is an effective protective mechanism to prevent damage from pests and pathogens, provide shelter for predatory species and influences transpiration. Most importantly for grapevine, this physical defence mechanism based on hydrophobicity prevents the infection by pathogens that rely on the presence of water for their spore germination. In particular, the entry of the oomycete *Plasmopara viticola* into the stomata on the lower side of the leaf is hampered. Leaf hair density is a complex morphological trait and is difficult to phenotype due to the lack of reliable and accurate tools for quantification. However, precise quantitative data are necessary to elucidate the genetic factors of leaf hair formation and density by e.g. quantitative trait locus (QTL) mapping. Many advanced Machine Learning (ML) models have been successfully implemented in plant morphological trait classification. Therefore, we trained a Residual Networks based Convolutional Neural Network (ResNet-CNN) model for accurate and precise quantification of leaf hair with an overall model accuracy of 95.41%. Performance evaluation of the model in correlation with experts evaluation data yielded a significant correlation of R=0.98 and R=0.92, and root-mean-square error values of 8.20 and 14.18, respectively. Furthermore, subsequent evaluation of a set of six varieties segregating for leaf hair density by experts and non-experts revealed a significant level of bias in terms of absolute error for non-experts. Moreover, validation with a panel of novice evaluators resulted in considerable over- and underestimation of the trait. In conclusion, the developed ResNet-CNN has significant potential for enhancing objective phenotyping accuracy for the leaf hair density, allowing a more precise examination and ultimately making it accessible for grapevine breeding.

Keywords: *Vitis*, trichome, machine learning, ResNet CNN, phenotyping, quantification

Session 3: Abiotic Stress

Keynote lecture

Moving towards grapevine genotypes better adapted to abiotic constraints

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Abstract

Vitis species, both in their cultivated and wild forms, grow in a large diversity of environments since thousands of years. Consequently, they have developed many adaptive mechanisms controlled by a range of regulatory processes. The cultivated species *Vitis vinifera* is fairly well-adapted to semi-arid conditions and its cultivation can be used to produce crops on marginal lands. However, this is under threat due to climate change, which is associated with a rise in temperature and CO_2 atmospheric content, modifications of water availability and a higher probability of extreme events, such as heat waves and early spring frosts. Indirect effects of climate change in solar radiation and soil mineral contents are also expected. Altogether, it is likely that cultivated grapevines will have to face more abiotic constraints occurring concomitantly or successively over one or several growing cycles. In addition to climate change, viticulture worldwide has to reduce pesticide use. Adapting to climate change and reducing pesticide use are challenging and increase the need to select new grapevine varieties more resistant to disease and better adapted to abiotic constraints. In this sense, the adaptive mechanisms from wild and cultivated Vitis species have to be leveraged. While major advances have already been made for disease resistance, the polygenic nature of adaptation to abiotic factors has slowed down research progress. To tackle this limitation, ambitious integrative strategies should be designed, from collection and characterization of genetic resources, genetic architecture studies and identification of underlying genes (including those involved in epigenetic regulation), to new breeding technologies and the development of genomic selection. An up-date on the state-of-the-art regarding these aspects will be presented.

Keywords: phenotyping, polygenicity, climate change, Vitis, diversity

Oral presentations

Water use efficiency as a novel target for grapevine breeding programs

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Abstract

Water scarcity is one of the main limiting factors for grapevine cultivation in semiarid regions, which is increasing in a context of climate change. To face this challenge, it could be useful to consider plant water use efficiency (WUE) as new criteria in current and future breeding programs. Considering the relation photosynthesis/ stomatal conductance (A_N/g_s) and $\delta^{13}C$ as selection targets, several long-term studies of WUE variability among grapevine cultivars, clones within a cultivar and rootstocks was carried out, considering field and pots experiments at different environmental and water availability conditions. Results showed a significant variation of WUE among cultivars according to the different stomatal regulation capacity under drought when comparing ancient and commercial cultivars. Moreover, up to 30% of clonal variability in WUE was found within Tempranillo and Grenache cvs, mainly under moderate water stress conditions. Although seasonal and environmental conditions affected this inter and intracultivar variability, a multilevel methodology analysis made it possible to rank genotypes by their WUE. Regarding rootstocks, some field and pot experiment comparing behavior of commercial and new lines of genotypes, showed both a variability in plant water status regulation and WUE under drought. This variability was clearly related with plant hydraulics capacity. All these results support the interest of exploring genetic resources to cope with the effects of climate change on viticulture considering WUE as a selection criteria for breeding programs.

Keywords: *Vitis vinifera*, rootstocvk, cvlone, drought

The rootstock control on the scion transpiration even at night

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Abstract

Water is the main limiting factor for yield in viticulture. Improving drought adaptation in viticulture will be an increasingly important issue under climate change. Genetic variability of water deficit responses in grapevine partly results from the rootstocks, making them an attractive and relevant mean to achieve adaptation without changing the scion genotype. The objective of this work was to characterize the rootstock effect on the diurnal and nocturnal regulation of scion transpiration.

A large panel of 55 commercial genotypes were grafted onto Cabernet Sauvignon. Three biological repetitions per genotype were analyzed. Potted plants were phenotyped on a greenhouse balance platform capable of assessing real-time water use and maintaining a targeted water deficit intensity. After a 10 days well-watered baseline period, an increasing water deficit was applied for 10 days, followed by a stable water deficit stress for 7 days. Pruning weight, root and aerial dry weight and transpiration were recorded and the experiment was repeated during two years. Transpiration efficiency (ratio between aerial biomass and transpiration) was calculated and $\delta^{13}C$ was measured in leaves for the baseline and stable water deficit periods.

A large genetic variability was observed within the panel. The rootstock had a significant impact on nocturnal transpiration which was also strongly and positively correlated with maximum daytime transpiration. The correlations with growth and water use efficiency related traits will be discussed. Transpiration data were also related with VPD and soil water content demonstrating the influence of environmental conditions on transpiration. These results highlighted the role of the rootstock in modulating water deficit responses and give insights for rootstock breeding programs aimed at identifying drought tolerant rootstocks. It was also helpful to better define the mechanisms on which the drought tolerance in grapevine rootstocks is based on.

Keywords: nocturnal transpiration, vapour pressure deficit, water deficit, plasticity, grapevine

Genetic structure exploration and association mapping for root related traits in *V. berlandieri*

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Abstract

In grafted plants like grapevine, increasing the choice of rootstocks available to growers is an ideal strategy to adapt plants to climate change whilst maintaining the wine typicity. In grapevine, the scion is *Vitis vinifera* and the roostock is generally a hybrid between various American Vitis species. *Vitis vinifera* has been domesticated and bred for centuries, but the use of American species is more recent and these genotypes remain poorly characterised. A few traits such as root architecture, water deficit responses, and traits related to biotic and abiotic stresses have been studied on American rootstocks; these studies have described genetic variability, but have rarely characterised the genetic architecture of traits of interest. Among the three main species used for breeding rootstocks, *V. riparia*, *V. rupestris* and *V. berlandieri*, *V. berlandieri* hybrids generally show high performances for chlorosis and water deficit tolerance. One hundred years ago when most rootstocks used today were created only few individuals were available as parents. In this project, seeds of 78 wild *V. berlandieri* were collected in Texas after open fertilization. A total of 286 genotypes were genotyped to describe the population genetic structure, and phenotyped for root related traits in order to perform genome wide association analysis (GWAS). De novo *V. berlandieri* long read whole genome sequencing allowed us to identify and filter 104378 SNPs. STRUCTURE analysis identified two gene-pools that were associated with differences in altitude. Root phenoyping revealed diverse root system architectures and GWAS allowed detecting 8 QTLs associated with root traits with medium to high heritability (from 0.36 to 0.82) on chromosomes 1, 5, 9, 10, 13, 14 and 17. This original work is the first GWAS study done with a population of grapevines sampled in natural conditions and phenotyped as grafted plants. Our results offer new insights into rootstock genetics and could offer the potential to use marker assisted selection in grapevine rootstocks improvement programs.

Keywords: Grapevine, Population genetics, Genome-wide association, Genotyping by sequencing, Root architecture

Influence of vegetative propagation on epigenetic rejuvenation and its effect on vine response to stress: a multi-omic study

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Abstract

Plants have developed a suite of processes to endure stress conditions. Previous work has shown how the memory of stress primes plants to be more resilient to subsequent stresses, but how such priming effect is maintained in perennial plants after winter dormancy and during vegetative propagation is less studied. Here we used a multi-omic approach to determine if abiotic stress induces an epigenetic priming in grapevine, and how winter dormancy and vegetative propagation affects its maitainance. Our results showed that exposure to abiotic stress induces the expression of genes associated with epigenetic modifications during stress and after stress removal, suggesting the establishment of epigenetic memory of stress. This was further supported by primed plants showing a small number of differentially expressed genes associated with stress response even in the absence of a second stress, and presenting a stronger response than naïve plants when re-exposed to stress one year later. Similarly, plants propagated from primed mother plants using layering presented more differentially expressed genes than plants propagated using callused cuttings. Also, only primed layered propagules showed differentially expressed genes in the absence of a second stress event, suggesting that the established stress memory is, at least partially, lost during callused cutting propagation. Whole-genome bisulphite sequencing analysis showed that callused cutting propagation induces a reduction in DNA methylation similar to that observed during sexual propagation. Additionally, we observed the expression of small RNAs previously associated to plant juvenility in plants propagated using callused cuttings. Taken collectively, our results indicate that abiotic stress induces an epigenetic memory of stress, and that such memory is maintained in primed plants over a year, affecting their response to a subsequent stress. Interestingly, callused cutting propagation results in a rejuvenation of the propagule's methylome and in the loss of such memeory of stress. This work presents the first evidence of stress memory establishment in grapevine and laids the foundation to understand the importance of epigenetic mechanisms during the vegetative propagation of perennial plants like grapevine.

Keywords: Epigenetic priming, DNA methylation, small RNAs, gene expression, stress response, plant rejuvenation

Session 4 (1): Biotic Stress

Keynote lecture

Improving biotic stress resistance in grapevines: What's the path forward?

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Abstract

Grapevine breeding for biotic stress resistance is a valuable strategy to embrace the principles of the European Green Deal, which will be one of the strongest drivers of the Agrifood research sector in the next decades. Grapevines are challenged by a range of diseases and pests, causing economic losses, and requiring often costly approaches to mitigate damage. Public interest in reducing the use of chemicals is a related challenge, along with climate change. All these aspects converge upon the urgent need for sustainable viticulture. The *Vitis* gene pool provides vast resources for the development of genetic resistance in rootstock and scion cultivars, but the search is not yet exhausted. According to BrAPI, germplasm consists of wild/acquired accessions as well as breeding products (breeding selections and cultivars). The enhancement of the entire germplasm is a crucial step. This is expressed in fingerprinting and high-throughput genotyping as well as phenotyping for disease and pest resistance, even in retroactive mode on traditional breeding products and acquired accessions. In fact, classical breeding approaches have made great strides in the development of cultivars with adaptive traits. Recent access to 'omic technologies, coupled with advanced phenotyping tools, has further facilitated the identification of useful loci, along with rapid trait introgression from wild species. Moreover, marker technologies (mainly accessible microsatellite-based systems) are now used in Marker-Assisted Selection to stack multiple loci/genes for the same trait into a single superior genotype. It would be relevant to phenotypically test these "stacked" genotypes in controlled lab settings as well as in different challenging environments worldwide. This effort would represent a step forward in terms of understanding genotype (loci) - pathogen (races) interaction. Genomic technologies will finally impact germplasm characterization, enabling the identification of candidate resistance genes and thereby facilitating "Breeding by Design" approaches.

Keywords: disease and pest resistance, genotyping, germplasm, marker-assisted breeding, phenotyping, *Vitis* spp.

Oral presentations

Grapevine *Rpv3-, Rpv10-* and *Rpv12*-mediated defense responses against *Plasmopara viticola*

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Abstract

The high susceptibility of European grapevine varieties (*Vitis vinifera*) to downy mildew (*Plasmopara viticola*) leads to intensive use of fungicides in viticulture. To reduce the use of fungicides, resistance loci from wild *Vitis* species have been crossed into *Vitis vinifera* varieties. The resulting new fungus-resistant grapevine cultivars (FRCs) represent an important tool for reducing pesticide applications in viticulture. Due to variety-specific resistance differences, little is known about the type and timing of the plant defense responses mediated by different resistance loci. Since the long-term goal of resistance breeding is to pyramidize several resistance loci, these should be based on different resistance mechanisms to increase the durability of the resistance. Therefore, detailed knowledge of the different defense mechanisms and resistance genes conferred by the respective *Rpv*-loci is essential to ensure sustainable resistance management and durable as well as stable resistance breeding.

Therefore, the resistance mechanisms mediated by the *Rpv10-*, *Rpv3-* and/or *Rpv12*-loci on downy mildew development, sporulation ability, onset of programmed cell death (PCD), production of hydrogen peroxide and stilbene levels were evaluated and compared. Furthermore, resistance-breaking isolates were used as a tool to additionally evaluate whether the resistance loci are based on different mechanisms. In order to understand the mechanistic basis of the defense responses mediated by the *Rpv12*-locus, cultivars containing this locus were examined in more detail.

The experiments revealed an early and locally precise defense response in *Rpv10-*, *Rpv12-* and *Rpv12/Rpv3*-genotypes, whereas a delayed defense response in *Rpv3*-genotypes was observed. These temporal differences correlated with an increase in the *trans*-resveratrol level and the formation of hydrogen peroxide shortly before onset of PCD. The differences in timing of onset of *Rpv*-loci specific defense reactions following downy mildew infection could be responsible for the observed differences in hyphal growth, sporulation and cultivar-specific susceptibility to this pathogen in the vineyard. Regarding the *Rpv12*-locus described first by Venuti et al. 2013, we were able to further narrow down the region, which confers resistance by new resistance markers. In this reduced region 10 putative disease resistance *R*-genes were found and are analyzed bioinformatically.

Keywords: Disease resistance, Downy mildew, Grapevine, *Rpv12*, *Rpv10*, *Rpv3*, *Vitis vinifera*, Stilbenes, *Plasmopara viticola*

Characterization of the *Rpv12* locus in a haplotype-separated grapevine genome sequence

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Abstract

Grapevine downy mildew, caused by *Plasmopara viticola*, imposes a major challenge on viticulture since the 19th century. Attempts to counteract the disease by crossing the noble European *Vitis vinifera* with resistant American *Vitis* wild species in the early 20th century remained discouraging due to transmission of undesirable characteristics. The recent development of genetic marker analyses allowed to identify resistance mediating genomic regions from extra-European *Vitis* species and to follow them in cascaded back crosses to *V. vinifera*. More than 31 genetic loci are currently known to contribute to resistance to *P. viticola*. One of these is *Rpv12* (resistance to *P. viticola* 12), initially identified in the Asian species *V. amurensis*. This locus was identified in 2013 (Venuti *et al.*) and it was compared to the American locus *Rpv3* in transcriptomic and metabolomic studies (Chitarrini *et al.*, 2020). However, these investigations were not yet able to identify candidate resistance genes. Thus, to delimit the locus and to reveal its possible resistance genes, the *Rpv12* carrying genotype Gf.99-03 (Geilweilerhof 2014-099-0003, *V*IVC: 27131) was sequenced in combination with its parental genotypes 65-153-18 (*V*IVC: 41129) and Gf.43-21 (Geilweilerhof 2904-043-0021, *V*IVC: 27130). Long read data were computed into a high quality haplotype separated genome assembly of Gf.99-03. Gene annotation of the newly assembled genome sequence was supported by RNA-Seq analyses from various tissues such as leaves, stems, tendrils and roots. Also, comprehensive differential gene expression analysis of experimentally inoculated leaf discs at different time points after inoculation with *P. viticola* was performed. Approximately 600 differentially expressed genes were identified.

The *Rpv12* locus is delimited by the simple sequence repeat (SSR)-markers UDV-014 and UDV-370. Differentially expressed genes in the resistance carrying haplotype of Gf.99-03 were identified and checked for uniqueness. In addition, the locus was searched for typical resistance gene analogs like *NLR*s (nucleotide binding site leucine rich repeats). A cluster of putative resistance mediating genes including *CNL*s was identified in this region.

Keywords: downy mildew**,** fully-phased genome assembly, infection experiment, leaf disc assay, *Plasmopara viticola*, resistance, *Rpv12,* TrioBinning, *Vitis spec*.

Towards marker-assisted breeding for black rot resistance: high-density linkage mapping and identification of a major QTL in the cultivar 'Merzling' (*V. rupestris* × *V. aestivalis* var. *lincecumii*)

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Abstract

Nowadays the value of human economic activities is no longer measured only in terms of efficiency and profit, but also in terms of sustainability. When it comes to agriculture, one of the biggest areas of improvement involves the decrease of chemicals. Although vineyards cover less than 5% of agricultural land in Europe, viticulture is responsible for the use of more than 60% of all fungicides. The exploitation of grapevine varieties resistant to mildews is an efficient strategy already implemented in integrated/organic farming to reduce treatments. However, from the beginning of this century, European viticulture has been threatened by severe outbreaks of black rot (BR), an emergent and destructive disease caused by the ascomycete *Phyllosticta ampelicida* (sexual morph *Guignardia bidwellii*). These events introduced the urgent need for the introgression of BR resistance in mildew-tolerant genotypes. For this purpose, a set of parental lines and breeding selections of the Fondazione Edmund Mach has been screened for BR resistance in a growing chamber with *in vivo* produced spores using an optimized artificial infection protocol. Given the good performance of 'Merzling' (a complex genotype derived from *V. vinifera* and *V. rupestris* × *V. aestivalis* var. *lincecumii*), this cultivar was used for a cross with the susceptible variety 'Teroldego' (*V. vinifera*) and the segregating offspring was genetically characterized by means of the GrapeReSeq 18K Vitis SNP chip. Five phenotypic experiments were carried out under controlled conditions on leaves of potted plants, and three on bunches in the field. A dense genetic map was constructed combining 7,175 SNP with 194 SSR markers of a previous map. All QTL analyses revealed the presence of a strong major BR resistance locus on chromosome 14. It explains up to 45% of the trait variability (LOD 10.5) and spans a genomic region of 1.36 Mb. A specific SNP marker was found robustly associated with the resistance trait. No minor QTLs were detected. The genes underlying this region are currently under investigation via bioinformatic analysis, and microscopic inspections of disease progression are in place to understand the biological causes of the resistance trait. Finally, new molecular markers will be developed and validated on segregating populations with different genetic backgrounds, to be implemented in marker-assisted selection for BR resistance in grapevine.

Keywords: disease resistance, *Guignardia bidwellii*, molecular breeding, plant phenotyping, SNP genotyping, QTL mapping, *Vitis* spp.

Session 4 (2): Biotic Stress

Functional evaluation of defensins in grapevine provide evidence that these peptides could be exploited for their stress protective roles

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Abstract

In contrast to plant defensins' *in vitro* antifungal activity, little is known about their *in vivo* roles, especially in grapevine. The goal of this study was to evaluate the *in vivo* functions of plant defensins in grapevine in terms of growth, biotic stress and protective potential. The impact of the peptides on grapevine growth parameters, as well as fungal pathogens and a major insect pest was studied by functionally characterising four transgenic populations of two *Vitis vinifera* cultivars overexpressing two different plant defensin peptides. Follow-up experiments with a chemically synthesised version of one of the peptides were used to evaluate and confirm peptide-specific protective effects. The defensins had little effect on plant growth when evaluating their *in vivo* functions in the transgenic populations, but significant protection against specifically *Erysiphe necator* and *Planococcus* (mealybug) infestation was observed. This protection was confirmed as a peptide-specifc response in experiments where plants treated with peptides displayed the same resistance responses against the same pathogen/pest, as well as against *Botrytis cinerea* infection. The defensin gene families of grapevine should be studied more comprehensively, particularly in context of the additional genomic resources available for grapevine. Defensin peptides displayd protective *in vivo* roles in grapevine towards biotic stress and when applied exogenously and hold great potential to be developed into a natural control agent.

Keywords: Plant defensin peptides, *Vitis vinifera* (Grapevine), Rs-AFP2, Hc-AFP1, Vvi-AMP1, *Planucoccus ficus* (mealybug), *Erysiphe necator* (powdery mildew fungus), exogenous application.

Identification of metabolic markers of grape infection with Esca complex disease

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Abstract

Grapevine trunk diseases (GTD) are caused by phytopathogenic fungi that degrade the woody part of the vine, leading to loss of vigor with decrease of grape production and quality, and eventually plant death. Widely spread, they are responsible for important economic loss for the viticulture industry worldwide. The Esca complex, caused by *Phaeoacremonium* spp., *Phaeomoniella chlamydospora* and *Fomitiporia* spp., has been shown to be one of the major GTD but information is still very scarce. Early identification of GTD is crucial, however due to their cryptic nature, visible symptoms in wood and foliage may take years to manifest. The identification of molecular and metabolic markers in grape and leaves may allow early detection and a better understanding of the factors responsible for disease progression.

In this work, grape berries and leaves were collected in 2016, at harvest stage, from control and symptomatic grapevines (five biological replicates each) from a 17 years old vineyard of the Portuguese cultivar Aragonez (= Tempranillo) that had been monitored for five years. Collected samples were subjected to gene expression and metabolomic analyses using qPCR and GC-MS. Expression differences of the fatty acid metabolism and wax and tocopherol biosynthesis pathways were targeted while a wide range of soluble and volatile metabolites were quantified. Total phenolic content was also measured.

Principal component analysis of GC-MS data revealed that infected samples were clearly discriminated from control ones. The metabolic reprogramming due to infection was interestingly more evident in berries than in leaves. Several volatiles, fatty acids, triterpenoids and phenylpropanoids putatively involved in defense were present in significantly higher amounts in infected berries, making them strong candidates as Esca disease biomarkers of infection. Additionally, these results will contribute to predict the impact of infection on wine quality.

Keywords: Trunk disease, Esca complex, Metabolomic, Infection biomarkers

Grape phylloxera leaf galling - traits or triggered?

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Abstract

Grape phylloxera, *Daktulosphaira vitifoliae* FITCH (Hemiptera, Phylloxeridae) is a notorious pest in viticulture. The insect feeds on roots and leaves of grapevines where it induces nodosities and tuberosities on roots and pocketlike galls on roots and leaves of grapevines. As an effective plant protection against phylloxera, resistant rootstocks are used worldwide in viticulture. In contrast to successful research efforts targeting root resistance, knowledge on the resistance in leaves is still limited. From the eight genetic loci published (*Rdv1-8*), one (*Rdv3*) could be characterized as relevant for leaf resistance. Moreover, phylloxera resistance in grapevine leaves seems to rely on several interacting QTLs. Foliar infestations in commercial vineyards on *V. vinifera* occur rarely, although leaf galls on cultivated cold-hardy or fungi-tolerant hybrids may frequently occur in commercial viticulture. As to date, studies of the damage of phylloxerated foliage on the yield and quality have provided no unambiguous results, and management options remain limited.

A knowledge gap remains with regard to the genetic background and environmental constraints on the leaf resistance (or susceptibility) towards grape phylloxera which include the insects' genotype(s) and feeding performance (biotypes).

In this article, we present a short review of the most urgent questions from our research on controlled studies of the phylloxera–leaf interaction.

Keywords: Grapevine, Grape Phylloxera, Foliar infection

Grapevine fanleaf disease: simple solution for complex problem?

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Abstract

Grapevine fanleaf disease, caused by grapevine fanleaf virus (GFLV) and transmitted by the soil-borne nematode *Xiphinema index*, is the most detrimental of the grapevine viral diseases. It provokes severe symptoms and economic losses, threatening vineyards worldwide. Many strategies of control based on prophylaxis, biotechnology, biocontrol have been explored. So far, no effective and environmentally friendly solution has been reached. Natural resistance to GFLV has equally been examined but, in the most comprehensive screening study, none of the tested accessions were found to be resistant. As recessive genetic determinisms have often been described for plant virus resistance, the challenges of finding a source of natural resistance to GFLV may be explained by the high rate of heterozygosity in grapevine.

We investigated the presence of recessive resistance to GFLV in grapevine genetic resources through the screening of the progenies from self-fertilization of various varieties and species. We discovered that the Riesling variety displays resistance to GFLV, although it is susceptible to *X. index*. This resistance is determined by a single recessive factor located on grapevine chromosome 1, which we have named *resistance to grapevine fanleaf virus 1* (*rgflv1*). *rgflv1* is located in a 5.7 cM interval which represents a physical distance of ~1.1 Mb. Analysis of recombinant allowed us to narrow the interval to a region of 600 kb encompassing 57 genes.

To our knowledge, this is the first and only instance of resistance to grapevine fanleaf virus identified in grapevine so far. This finding represents strong basement to go further in the identification and the characterization of the gene underlying the *rgflv1* resistance locus. In terms of innovation, it paves the way for the design of new ideotypes of multi-resistant grape varieties that combine resistance to root and aerial major diseases.

Keywords: grapevine fanleaf virus, resistance, recessive gene, Riesling

Session 5: Grapevine Breeding

Keynote lecture

Grapevine breeding: progress, innovation and opportunity

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Abstract

Thanks to the thoughtful, enthusiastic, informed, assiduous, and dedicated work of grapevine breeders, our grape and wine industry enjoys sustained benefits in improved plant material—new fruiting varieties, clones or selections, and rootstocks. We recognize the important substantial and meaningful improvements that make large, noticeable changes for the better in the way that grapevines are grown and in the products that consumers worldwide value wholeheartedly. For example, the Sunpreme grape variety dries into raisins without cane cutting and the raisins themselves may be harvested by machine directly from the vine, vastly reducing the labor requirement for raisin production. This variety is now in commercial production and shows how new grapevine varieties can radically change the traditional approach to cultivation, since typical practices for raisin production have very high requirements for hand labor, even when partly mechanized. Presently there is exciting and impactful progress being made in the breeding, validation, and commercialization of fruiting varieties that are meaningfully resistant to important diseases, especially downy mildew, powdery mildew, and Pierce's diseases. In some cases, the genetic sources of resistance are newly discovered and in other cases the resistance is derived from well-recognized sources. The important progress now being made is greater in magnitude and scope from the past for several reasons, including international cooperation among researchers, discovery, development, and implementation of molecular markers which help predict resistance phenotypes among the members of segregating populations, vastly improved grape and wine chemical analysis, and important technical advances in small scale research winemaking and grape quality assessment. Recently several seedless table grapes varieties have been commercialized with exceptional flavors and the origin of these flavors is *Vitis labrusca*. Through horticulture, post-harvest physiology and handling, and marketing and advertising, these special grapes can be targeted to appreciative consumers, meeting the diverse expectations and desires of grape consumers thanks to an expanding palette of grape flavors. Discoveries from the germplasm and from genetics populations provides exciting opportunities for future grape breeding. The discovery of genetic resistance against grapevine fanleaf virus, combined with our understanding of phylloxera and nematode resistance in germplasm, enables the breeding of new rootstock varieties with complete resistance against phylloxera and functional resistance against fanleaf degeneration, one of the leading virus disease of grapevine and a serious and persistent threat to viticulture worldwide. Important, useful resistance against other diseases and pests and meaningful improvements in quality and horticultural attributes will continue to distinguish newly-bred grapevine varieties, ensuring both consumer delight and the vitality of our grape and wine industry.

Oral presentations

Agronomical behaviour of 21 new disease resistant winegrape varieties grown in northeast Italy

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Abstract

The goal of the field trial was to evaluate the agronomical characteristics of 21 (10 red and 11 white) winegrape varieties obtained from recent breeding programmes for disease resistance developed in Hungary, Germany and Italy. The tested red varieties were as follows: Cabernet Carbon, Cabernet Eidos, Cabernet Volos, Julius, Merlot Kanthus, Monarch, Prior, UD. 31.103, Vinera. The tested white varieties were as follows: Aromera, Bronner, Fleurtai, Johanniter, Muscaris, Souvignier gris, Sauvignon Kretos, Sauvignon Nepis, Sauvignon Rytos, Solaris, Soreli. Cvs. Merlot (red) and Glera (white) were included as control. The experimental vineyard was established in Castelfranco Veneto (Treviso province – northeast Italy, 45° 40’ lat N; 11° 55’ Long E, temperate-warm climate) on the plain, in 2014. Spray treatments were applied against downy and powdery mildew, by using only copper and sulphur. Grape production, grape quality, and phenology were recorded over a six-year-period, while disease resistance (downy and powdery mildew, black rot and anthracnose) were detected only during a few years. The most significant findings were: a) red grape varieties had a earlier bud burst but a later veraison compared to Merlot; as concerning ripening, some varieties were earlier than Merlot, other ones were later; b) white varieties had a later bud burst but an earlier veraison and ripening time as compared to Glera; c) grape production and quality changed significantly depending on the varieties, being titratable acidity higher than 6.4 g tartaric acid/L and pH lower than 3.5; d) the following varieties (tested in unsprayed plots) resulted very downy mildew resistant: Cabernet Carbon, Monarch, Prior, UD 31.103, Muscaris, Solaris, Souvignier gris, Bronner, Fleurtai; e) Monarch, Muscaris, Solaris, Sauvignier gris also showed a high level of resistance toward black rot and anthracnose.

Keywords: grapevine, production, quality, diseases, phenology

The differences in resistance levels of different FRCs and the impact of their deployment on fungicide use in viticulture

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Abstract

European grapevine cultivars (Vitis vinifera spp.) are highly susceptible to the downy mildew pathogen Plasmopara viticola. To reduce the dependence of viticulture on chemical inputs, and thereby reduce the ecological and economic burden of wine production, a number of breeding programs have introgressed resistance loci from wild North American and Asian Vitis species into V. vinifera resulting in new fungus-resistant grapevine cultivars (FRCs). These FRCs are a promising strategy to reduce the impact of disease management. Due to variety-specific resistance differences, little is known about the required amount and the potential reduction of fungicide applications.

The aim of the project was to investigate the degree of resistance of new FRCs and thus the capability of fungicide reduction in the vineyard over a 6 year time period. For this purpose, the infection and sporulation ability as well as the development of P. viticola in different FRCs and susceptible cultivars were investigated and compared. Additionally, FRCs with reduced plant protection treatments were compared to traditional cultivars. On the basis of the results obtained, adjusted plant protection recommendations for FRCs were developed.

The on-farm experiments showed that the use of FRCs in combination with reduced plant protection management strategies offers the possibilities to significantly reduce the number of fungicide treatments required for grape production. Results obtained from this study demonstrated that the deployment of FRCs can save 50–85 % of fungicide applications in viticulture depending on the degree of the vairiety´s resistance level. The omission of all plant protection applications can ultimately lead to negative effects on yield, quality and even resistance durability. The latter was demonstrated by the identification of new P. viticola isolates capable of overcoming *Rpv3*- and *Rpv12*-mediated resistance. Therefore, this study demonstrates the importance of sustainable breeding and crop protection strategies.

Keywords: Disease resistance, Downy mildew, Fungus-resistant grapevine cultivars, Grapevine, *Vitis vinifera*, *Plasmopara viticola*

Contributions of the *Vitis*Gen2 project to grapevine breeding and genetics

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Abstract

The *Vitis*Gen projects (2011-2022) have improved the tools available for breeding new cultivars with regional adaptation, high quality and disease resistance. *Vitis*Gen2 (the second project in the series) is a multi-state collaboration (USDA-Geneva, New York; University of California, Davis; USDA-Parlier, California; Cornell University; Missouri State University; University of Minnesota; South Dakota State University; Washington State University; North Dakota State University; and E&J Gallo, California) to develop improved genetic mapping technology; to identify useful DNA marker-trait associations; and to incorporate marker-assisted selection (MAS) into breeding programs. A novel genetic mapping platform (rhAmpSeq) now provides 2000+ markers that are transferable across the *Vitis* genus. rhAmpSeq has been used in California, New York, Missouri, and South Dakota to identify new QTL for powdery and downy mildew resistance. In addition, fruit/flower traits that would normally take years to phenotype have been associated with predictive markers accessible from seedling DNA (e.g. malate metabolism, anthocyanin acylation, mono:diglucoside ratio, bloom phenology and flower sex). Over the past ten years, the project has used MAS to screen thousands of grape seedlings from public breeding programs in the United States, and and has produced "RenStack" public breeding lines to enable simultaneous access to 4 or 6 powdery mildew resistance loci from single source genotypes. High-throughput phenotyping for powdery and downy mildew resistance has been revolutionized with the Blackbird automated-imaging system powered by artificial intelligence for image analysis. Affordable DNA sequencing along with phenotyping innovations are transforming grapevine breeding.

Keywords: *Vitis*, breeding, marker-assisted selection, QTL, disease resistance, phenotyping, molecular markers

Producing and (epi)genotyping a large collection of intravarietal diversity for grapevine improvement

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Abstract

In New Zealand, wine production depends on a very limited amount of genetic diversity, with approximately 75% of all export revenue coming from the sale of Sauvignon Blanc. Only a small number of clones are used, and strict national biosecurity regulations make importing collections of new genetics impractical. However, the industry's largest research programme aims to generate new intravarietal diversity from which to select and propagate material for future plantings. This involves two approaches. The first is a grower-led identification and tagging of atypical vines using a mobile web app, supported by industry members working in vineyards across the country. The second is the production of somaclonal variation by exposing grapevine somatic embryos to calibrated stress treatments. Vines recovered from these cultures display diverse novel phenotypes. Using a target-enriched sequencing approach, combined with a multi-dimensional pooling strategy, we have identified genetic variation caused by transposable element activity. This same method can also distinguish among existing clones of the same variety. More recently, we have also characterised stable epigenetic changes in mature vines regenerated from somatic embryos. Some of these epigenetic changes persist after 3-4 years in the field. To date, we have trialled the use of tissue culture to trigger transposition events in three varieties. Based on these experiments, we are now working to produce a population of 12,000 – 20,000 Sauvignon Blanc vines displaying the maximum scope of intravarietal diversity possible. These will be used to select clones showing improved characteristics related to productivity, disease resistance and climate change. By cataloguing the genetic and epigenetic diversity of each vine, we hope to provide a repository of information that can be cross-referenced with phenotypic data, thereby creating a resource for functional genomics research.

Keywords: genotyping, epigenetics, transposable elements, somaclones, intravarietal diversity, sequencing

Exploring clonal variation in 'Riesling'

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Abstract

Clonal selection has a long history in grapevine breeding and was initially started as a means to improve reduced yields due to virus infections. The concept of single vine selection, introduced by Froelich in 1886, and its huge success laid the foundation for clonal selection activities in many public and commercial programs. It has been shown for several traditional varieties that substantial trait variation exists. For example, there is a broad range of different shoot growth types, cluster architectures, Botrytis tolerances, titratable acidity, anthocyanins, tannins and flavour between clones of several varieties. The variability between commercially available clones of most varieties gives growers an opportunity to individually select most optimal planting material.

Increasingly variable environmental conditions as a consequence of climate change make it difficult to determine which trait configuration(s) to prioritise in breeding. In fact, climate change and changing markets necessitate the systematic conservation and characterisation of clonal variation within traditional varieties as a basis sustainable and competitive viticulture in the future.

Therefore, the Department of Grapevine Breeding at Geisenheim University has established a large collection of almost 1,200 clones of the variety Riesling over the last decades. Clones were selected from single vines grown in old vineyards in Germany and neighbouring countries and planted at the Department trial site with three vines per clone. Over more than ten years, key traits including yield, soluble solids, acid composition and concentration have been measured to assess the level of clonal variation and identify clones with interesting characteristics.

Mixed model-based variance decomposition reveals substantial between-clone variation for important traits. While it is established that mutations and epigenetics are the most likely underlying drivers the relative importance of genetic vs. epigenetic variation in Riesling remains unclear. To shed light on this and uncover (epi-)genomic factors underyling clonal variation in Riesling we are applying new genomics and epigenetics approaches. This will help to improve our understanding of the genetic architecture of important traits.

Keywords: Clonal selection, Riesling, clonal variation, mutations, epigenetics

Reduced bunch compactness in somatic variants of 'Tempranillo' relate to genome structural variation and the sex locus

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Abstract

Grapevine cultivars are vegetatively propagated to keep their varietal attributes. Still, spontaneous somatic variation that emerges during long cycles of vegetative growth provides the opportunity for natural improvement of traditional grape cultivars. A lower bunch compactness is an advantageous trait for winegrowing as it decreases susceptibility to pests and fungal diseases and enables better adaptation to climate change by enabling a more homogeneous berry ripening. To understand the genetic and molecular mechanisms generating variation in bunch compactness, we studied here two somatic variants of Tempranillo Tinto cultivar that produce looser bunches. One of the somatic variants exhibits a male-like flower phenotype, with an underdeveloped but functional gynoecium (flower sex note 2 according to OIV 151 descriptor), instead of the hermaphroditic flowers regularly developed in this cultivar (note 3). Histological analyses revealed a reduced development of the style and stigma, as well as a remarkable thinning of the gynoecium septum. Genetic analyses of its self-progeny revealed the co-segregation of the male-like phenotype with the hermaphrodite allele of the grape sex locus (*SDR*). A treatment of flower clusters in stages prior to flowering with cytokinins, hormones involved in the gynoecium development, reversed the phenotype in the somatic variant. These findings suggest that some somatic mutation of the hermaphrodite *SDR* allele into male-like could hinder the correct fertilization of the ovules, leading to lower fruit set and looser bunches.

The other somatic variant of Tempranilllo Tinto with looser bunches presents a notable reduction in pollen viability, around 50%, compared to the >90% regularly observed in Tempranillo clones. Pollen sterility also segregated in the self-progeny of the somatic variant, suggesting the inheritance of this trait in the progeny. Whole genome DNAseq identifed structural variation specific of this clone, concretely a translocation breakpoint between chromosomes 1 and 3 that was confirmed by PCR and Sanger sequencing and was observed also in individuals of the self-progeny with low pollen viability. These findings together with previous reports in other somatic variants such as Tempranillo Blanco suggest that genome structural variation is a recurrent source of low bunch compactness phenotypes by causing deleterious effects on haploid gametes and, ultimately, limiting fruit set.

Keywords: bunch compactness, somatic variation, Tempranillo Tinto, flower development, pollen viability.

Genetic analysis of berry, flower and seed traits in two 'Tempranillo Tinto' populations: Influence of the *Sex* locus

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Abstract

Berry weight, berry shape, seed content and ripening date are relevant traits to viticulturists in order to establish the quality and consumer acceptance of wine grapes. We performed a genetic analysis of 12 berry (berry weight, berry diameter, berry length and berry shape), flower (ovary and pistil length, flower diameter and ovary and pistil shape), flowering date and seed traits (seed number and seed weight) relevant to breeding in order to elucidate their genetic control and the association with flower sex. QTL analyses were conducted in two segregant populations obtained from crossing Tempranillo tinto as the pollen parent, with Graciano (Gra x Te, 151 genotypes) and Grenache (Gre x Te, 133 genotypes), respectively. Populations were phenotyped during at least two vintages in different locations. Significant QTL were detected for berry diameter (25% variance explained) and berry weight (26%) in LG3, LG5 and LG18, and for berry shape in LG1 (18%) and LG9 in both genetic backgrounds.

Sex locus was mapped close to markers VVIB23 and VMD34 in LG2, in a region where QTL for flower morphology, seed traits and flowering date were mapped. *Sex* locus strongly influenced flower morphology traits such as ovary shape in both progenies as expected. In one population (Gre × Te), co-localization of QTL for flower morphology-related traits, and flowering date was observed in LG7 and LG13. Besides a QTL region in LG17 was found significantly associated to berry morphology and seed parameters, suggesting close linkage or pleiotropic effects. In Gra × Te progeny, co-localizations of QTL for flower morphology, seed traits and phenology events were detected in LG3 and LG11 explainig up to 30% of the variance. For that progeny QTL for seed traits in LG18 resulted associated with locus *SDI*, and significant effects for berry seed and flower traits on LG5 co-localized with the FERONIA locus. A candidate gene with a function in pollen morphology is proposed associated to the highly significant QTL detected in LG11 for flower traits in both progenies.

The present study provides new evidence that *Sex* locus has a broader influence on phenology and berry traits than just flower sex determination. This result may have significant implications for future breeding programs.

Keywords: QTL, flowering date, grape quality, grape breeding, berry shape, seed traits

Genome Wide Association Study using table grape breeding families provide new QTLs for berry, seed and cluster traits

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Abstract

Breeding programs use several sources of genetic diversity for the improvement of their objective traits, such as seedlessness, berry size, shape, firmness and good quality and condition after cold storage, generating several families derived from a good x good crossing strategy. In contrast, many QTL have been obtained using bi-parental mapping, which gains resolution power by using parents with contrasting phenotypes in one single large family. As result, these QTL must to be validated before their use in the complex genetic background of a breeding program.

In this work, we take advantage of low-coverage genotyping and phenotypic data produced for breeding materials at INIA's table grape breeding program during three consecutive seasons. We use a large set of 536 table grape progenies generated from from 7 related F_1 families, as well as a diverse germplasm panel of 68 seedless and seeded vines. Using genotyping-by-sequencing we produced a set of 20,013 reliable markers distributed across the 12X.2 grapevine reference genome. To perform genome wide association analysis, we used BLINK and a robust Bonberroni adjunted p-values to determine 78 statistically significant associations between these genetic variants and measurements of eight berry and seeds traits at harvest, as well as cluster weight both at harvest and after cold storage. About half of these markers co-localize with previously described QTL, but many others are novel and overlap, or are located nearby, genes of biological significance in grapevines' seed and berry development. Due to the suggestive functional impact of these genes on final trait outcomes of interest, these variants may represent potential useful loci for table grape breeding.

This work was fund by FONDECYT Grants 11161044, and CORFO 09PMG-7229

Keywords: GWAS, table grape, berry size, berry shape, cluster weight loss, QTL

Session 6: Novel Technologies

Keynote lecture

Genome editing in grapevine: a great opportunity or only a dream?

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Abstract

Genetic improvement has been a limited experience in grapevine due to the well know difficulties to improve by traditional approaches international successful or traditional varieties. The biggest disadvantage of traditional crossbreeding is that entire sets of chromosomes are recombined, resulting in numerous undesired traits in the offspring. Through lengthy selection, the best plants are selected from these offspring and registered and marketed as a variety. Thanks to the so-called new breeding techniques (NBTs), possibilities have opened up in recent years to achieve genetic improvement of individual traits in plants. The NBTs are genetic techniques that leave point mutations in selected genes in a mutagenic approach that leverages the capabilities of the CRISPR/CAS system. Genome editing leads to the introduction of mutations via double-strand breaks in the DNA upon endogenous DNA repair. In vine breeding, the main advantage of NBTs lies primarily in the possibility of introducing desired properties into well-known and valued traditional grape varieties. Several bottlenecks must be overcome in the next years to turn this possibility into reality. *In vitro* recalcitrant varieties, specific transformation protocols, protoplast regenerations are only some of the major difficulties that must be improved to realize a dream. Other social acceptance of NBTs as “non-GMOs” does the rest.

Oral presentations

Breeding for grapevine downy mildew resistance *via* gene editing

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Abstract

Downy mildew (DM) caused by the oomycete *Plasmopara viticola* ranks in the top diseases affecting grapevine (*Vitis vinifera* L.) cultivation and its control requires every year a large use of fungicides. The Farm to Fork strategy newly promoted by the EU aims to accelerate the transition to a sustainable food system and has set very ambitious targets including the reduction by 50% of the use and risk of pesticides by 2030. The introduction of disease-tolerant grapevine varieties or clones clearly represents a step forward to reach this goal.

The recent advent of new breeding tools such as genome editing and *cis*-genesis offers a great opportunity to obtain resistant plants with higher precision and speed than by conventional breeding, either by knocking down susceptibility genes or by introducing known resistance-genes in commercial cultivars. Based on reports in other crops, the family of *Downy Mildew Resistant 6* (DMR6) and DMR6-like oxygenases (DLOs) are candidate susceptibility genes for the control of DM resistance in *V. vinifera*.

Deep-sequencing the putative susceptibility genes in 190 genetically diverse grapevine genotypes identified several Single Nucleotide Polymorphisms then screened for their impact on protein structure/function and association with DM resistant genotypes. Gene expression and gene network analysis suggested that grapevine *DMR6* and *DLO* genes have distinct functions, and that *VviDMR6-1* is co-regulated with several Pathogenesis-related genes. Based on this evidence, we generated a large collection of *DMR6-1* and *DMR6-2* single and double knock-out mutants in multiple grapevine cultivars and evaluated their resistance to DM. Phenotypic resistance data upon artificial infection are being collected and will be presented here. In parallel, we also developed a new DNA-free gene editing methodology and obtained non-transgenic and non-chimeric edited grapevine plants regenerated from a single cell.

Keywords: *Vitis*, *Plasmopara viticola* resistance, new breeding technologies, susceptibility genes, DMR6, gene network analysis

Removal of a 10-kb *Gret1* transposon from *VvMybA1* of *Vitis vinifera* cv. Chardonnay

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Abstract

Many white grape cultivars have a nonfunctional *VvMybA1* gene due to the presence of a 10-kb *Gret1* transposon in its promoter. In this study, we successfully demonstrated removal of the 10-kb *Gret1* transposon from a *VvMybA1* allele in *Vitis vinifera* cv. Chardonnay through transgenic expression of Cas9 and two gRNAs simultaneously targeting two junction sequences between *Gret1* LTRs and *VvMybA1*. We generated 80 and 106 *Agrobacterium*-and bombardment-transformed transgenic vines, respectively, and conducted molecular analyses of editing efficiencies in these vines and their progenitor calli. While the editing efficiencies were as high as 17% for the 5' target site and 65% for the 3' target site, simultaneous editing of both 5' and 3' target sites resulting in the removal of *Gret1* transposon from the *VvMybA1* promoter was 0.5% or less in most transgenic calli and vines, suggesting that these calli and vines had very few cells with their *VvMybA1* alleles simultaneously edited at both target sites. Nevertheless, two bombardment-transformed vines were found to have the *Gret1* successfully edited out from one of their two *VvMybA1* alleles. Precisely removing more than a 10-kb DNA fragment from a grape gene broadens the possibilities of using gene editing technologies in modifying various trait genes in grapes and other plants. Detailed molecular and sequencing analyses of the edited events in transgenic calli and vines revealed many interesting features of gene-editing, including large structural changes likely resulting from illegitimate recombination of highly homologous *VvMybA* genes in the *VvMybA* complex loci.

Keywords: CRSPR-Cas9, berry color, grapes, *Gret1,* large DNA fragment deletion, *MybA1*

Genomic and phenomic predictions for accelerating grapevine breeding

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Abstract

The length of the breeding cycle in grapevine is a major obstacle to genetic progress. Several years are needed before phenotypic data are available. The required time between crossing and final variety registration is about 15 years in France. Genomic selection could accelerate breeding. It consists of using a training population, genotyped and phenotyped, in order to train a predictive model to be applied to a selection population, which is only genotyped. This way, phenotypes could be predicted at the seedling stage. Genomic selection is already used routinely in many species, but its application to grapevine remains rare in a breeding context. Recently, phenomic selection, based on near-infrared spectra, has also been proposed as a low-cost alternative to genomic selection, and so far, it has never been tested in grapevine. In this work, we implemented both genomic and phenomic selection within and between two contrasted grapevine populations: a diversity panel of 277 genotypes, chosen to represent the genetic diversity of *Vitis vinifera* L., and a half-diallel of 622 genotypes, which includes ten interconnected full-sibs families with five parents. These populations were genotyped by GBS (32 894 common SNPs) and phenotyped for 15 traits related to berry composition, yield, morphological, phenological and vigour traits, displaying various heritability and phenotypic structure. Genomic predictive ability was medium to high within populations and it tended to decrease in across populations. To study the determinants of genomic predictive ability, we decomposed it into between (cross mean) and within (Mendelian sampling) half-diallel crosses components. We found that distance between parents was the major determinant for cross mean, while heritability impacted Mendelian sampling component. For phenomic prediction, we used spectra collected on wood and leaf tissues, for two years. After having calculated the part of genetic variance in spectra, we used them for predicting phenotypes measured several years ago. Phenomic predictive ability was lower than genomic predictive ability but still encouraging for the application in breeding. Finally, we found that genomic and phenomic predictive abilities were correlated, underlining that phenomic prediction relies on genetics. These results showed that the delay for selecting new grapevine cultivars could be shortened by using genomic or phenomic prediction.

Keywords: genomic prediction, phenomic prediction, breeding, NIRS, BLUP

Analysis of genomic prediction across populations in grapevine (*Vitis vinifera* L.)

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Abstract

Genomic prediction (GP) of breeding values has developed widely both in animal and plant breeding programs for its ability to accelerate genetic gains, saving cost and time while handling complex traits. However, its routine deployment in most of the major plant species is still not effective, mainly because of the many factors that are likely to influence prediction accuracies. In grapevine, very few studies have evaluated potential usefulness of GP in breeding contexts. Although the French grapevine breeding program for durable resistance to downy and powdery mildew (INRAE-ResDur) has now proved to be effective with about ten new varieties already officially registered, the duration of each breeding cycle remains long (about 15 years). GP can potentially help us to accelerate and diversify our breeding schemes by targeting complex traits involved in the determination of the optimal productivity and quality of the berries and wines. The main objective is to reduce as much as possible, the long intermediate selection phase of the breeding scheme where every genotype is evaluated in the yard during six years.

Here we evaluated GP across four different breeding populations for main phenological and agronomic traits to identify main factor affecting accuracies. This study brings additional information to the grapevine community regarding the implementation of GP to accelerate breeding programs.

Keywords: grapevine, genomic prediction, INRAE-ResDur, disease resistance, genetic architecture

Session 7: Big Data

Keynote lecture

Genomes as research tools: from loci to candidate genes

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Abstract

Advances in sequencing technologies and assembly algorithms have led to astounding improvements in the quality of grape genome assemblies. Further development of scaffolding tools, like HaploSync, allow generating fully phased diploid and chromosome-anchored genomes. HaploSync (https://github.com/andreaminio/HaploSync) scaffolds sequences from a draft diploid assembly into phased pseudomolecules guided by a genetic map and/or the genome of a closely related species; it leverages the relationship between haplotypes to increase the assembly contiguity, improve completeness by filling gaps, correct scaffolding, and phase highly heterozygous, complex regions. Phased assemblies of grape genomes have revealed genomic complexities that were inaccessible in previous haploid representations, such as haplotype-specific structural variation events, trait-associated alleles, and allele-specific gene expression and methylation. The availability of wild and cultivated grape diploid genome references containing the genes and alleles underlying traits of interest has been instrumental in dissecting the genetic basis of disease resistance, flower sex determination, aroma, and flavor. Online services that provide public and timely access to these new genomic resources have also been developed. User-friendly web platforms, like www.grapegenomics.com and www.grapedia.org, aim to rapidly and broadly share genomic datasets and tools, and foster multidisciplinary collaborations and progress in grape research.

Oral presentations

A large chimeric deletion associates with impairement of cuticle development in a dark berry somatic variant of 'Tempranillo Tinto'

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Abstract

Color intensity is a relevant feature for red wine quality, which depends on the accumulation of athocyanins during berry ripening and their interaction with other compunds present in the wine. While grape color is a varietal feature, emerging spontaneous somatic variation for this trait can be selected for cultivar improvement. Here we studied the genomic origin of a darker berry clone (VN21) selected in 'Tempranillo Tinto' (TT) cultivar, which enables the production of wines with higher color intensity. Using a diploid genome assembly of TT produced following a trio binning approach from PacBio and Nanopore sequencing, and genome re-sequencing data of VN21 compared to other TT clones, we identified a 10 Mb deletion in chromosome 11 that likely affected only the L1 meristem cell layer of VN21. An RNA-seq analysis identified a general down-regulation of genes within the chimeric hemizygous segment in the berry skin of VN21. Down-regulated genes where also enriched in wax biosynthesis functional category genes, including one *CER1* and one *ABCG32* homologs located in the hemizygous segment. SEM images showed that wax accumulation is impaired in the berry cuticle of VN21, which likely leads to the shiny color of VN21 berries. Candidate loss of function polymorphisms remaing hemizygous in VN21 after the chimeric deletion were also detected from TT haplotype comparison. Our findings show that very large hemizygous deletions can stabilize as periclinal chimeras in grapevine clones, giving rise to pleiotropic mutant phenotypes that can be exploited for cultivar innovation.

Keywords: berry color, berry wax cuticule, genome structural variation, somatic variation, 'Tempranillo Tinto'

Do we need to consider grape phyllosphere microbiome in breeding schemes?

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Abstract

The aerial surface of the plant (phyllosphere) is the habitat of complex microbial communities. These communities may have profound effects on host plant health and its performance traits.

When breeding new cultivars, i.e. the aerial component of a grape plant, one can simply ignore the phyllosphere in breeding schemes if it's composition is mainly dependent on the environment or consider it as an important component if the genotype is the main driver of the phyllosphere composition. In order to answer this question, we have analysed several factors influencing the phyllosphere microbial community structuring. Using amplicon sequencing of the 16S rRNA gene and of the internal transcribed spacer (ITS), we explored the microbial diversity at genus level for both bacteria and fungi present in the phyllosphere of leaves and grape berries. We analysed it on different grape taxonomic level (between 5 *Vitis* species ora set of *Vitis vinifera* cultivars chosen to represent the 3 genetic pool of the species), for different years and on five commercially important varieties of Vitis vinifera that were sampled from three different French terroirs. Our results indicated the presence of complex microbial diversity and assemblages in the phyllosphere and the presentation will describe the observed diversity. A significant effect of several factors (organ, grape species, growing year and terroir) on taxa abundance was observed with varying degrees of effect. At a given location, genotypes have an impact on microbial assemblage in the phyllosphere of leaf and berries, most pronounced on fruits but the effect of terroir was much stronger than the cultivar identity when the leaf phyllosphere of five grapevine varieties grown in different agro-climatic zones was compared. Limitations of the study as well as implied consequences of this work will be discussed.

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

High quality phased assembly of grape genome offer new opportunities in chimera detection

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Abstract

In perennial plants and especially those propagated through cuttings, several genotypes can coexist in a single individual, thus leading to chimeras. When the variant induces a noticeable phenotype modification, it can lead to a new cultivar. Viticulture already took economic advantage of this natural phenomenon: for instance, the berry skin of cv. 'Pinot Gris' derived from cv. 'Pinot Noir' by the selection of a chimera. Chimeras could also impact other crucial traits without being visually identified. Periclinal chimera where the variant has entirely colonised a cell layer is the most stable and can be propagated through cuttings. In grapevine, two functional cell layers are present in leaves, L1 and L2. However, lateral roots are formed from the L2 cell layer only. Thus, comparing DNA sequences of roots and leaves could allow chimera detection. In this study we used new generation Hifi long reads sequencing and recent bioinformatics tools applied to 'Merlot' grape cultivar to detect periclinal chimeras. Sequencing of cv. 'Magdeleine Noire des Charentes' and 'Cabernet Franc', the parents of cv. 'Merlot', allowed haplotype resolved assembly. Pseudomolecules were built with few contigs, in some occasions only one per chromosome. This high resolution allowed haplotype comparison. Annotation from PN40024 was transferred to all pseudomolecules. Through variant detection, periclinal chimeras were found on both haplotypes. These results open new perspectives on chimera detection which is an important resource to improve cultivars through clonal selection or breed new ones. Detailed results will be presented and the validation of these positions will be discussed.

Keywords: Chimera, Hifi Sequencing, trio-binning, phased assembly, Whole genome

Genomics and bioinformatics strategies to tackle diversity and domestication in grapevine

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Abstract

Grapevine (*Vitis vinifera* L.) diversity richness results from a complex domestication history over multiple historical periods. Expansion of human activity led to the creation of thousands of varieties with extensive phenotypic diversity. Unfortunately, the recent favoring of specific varieties/clones, and the globalization-driven exposure to pathogens, has led to extensive genetic erosion in this widely cultivated and economically significant crop. Fighting this genetic erosion whilst addressing issues of resilience to climate change, yield and other traits, requires a crucial understanding of the genetic basis of grapevine variation. This scientific field has witnessed significant advances due to the use of genomics approaches, enabled by Next Generation Sequencing. Here, NGS-driven whole genome resequencing strategies have been used to tackle multiple aspects associated with the extant genetic diversity present in grapevine germplasm, including a clarification of different features of its recent evolutionary history that suggest a meaningful role of the Iberian Peninsula in grapevine domestication. These different aspects of grapevine biology, which require genome-level analysis, employ multiple genomics and bioinformatics strategies that help bridge the gap between population history, genomic variation and gene function. Funding: Fundação para a Ciência e Tecnologia (FCT/MCTES) for project GrapeVision (PTDC/BIA-FBT/2389/2020) and support to H.A. (CEECIND/00399/2017/CP1423/CT0004); FCT/MCTES and POCH/NORTE2020/FSE for support to S.F. (SFRH/BD/120020/2016); FCT/MCTES and POPH-QREN/FSE for support to M.C. (CEECINST/00014/2018/CP1512/CT0002).

Keywords: bioinformatics, domestication, genetic diversity, whole genome resequencing

Gene functional characterization assisted by genome-wide TF-binding site interrogation: Grapevine as a case of study

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Abstract

The study of transcriptional regulation through protein-DNA binding exploring methods can provide an outline of the roles a transcription factor (TF) may carry out based on what coding genes are bound in their promoter regions. In non-model plant species, TFs can be now examined in their genome-wide DNA-binding profiles (cistromes) by the in vitro technique DAP-seq, which involves expressing a tagged TF and then immobilizing it for subsequent exposure to genomic DNA fragments. The technique offers important advantages with respect to ChIP-seq (no need to develop a TF-specific antibody or generate transgenic organisms expressing a tagged TF). This is of particular benefit when working on slow-growing non-model organisms, where genetic transformation is time-consuming. Our group has implemented the DAP-seq method in grapevine to identify all the sites in the genome where transcription factors bind. Depending on each case we have been able to obtain thousands of binding events assigned to genes, with important TF family-specific differences of peak location distributions. The possibility of performing stable or transient over-expression experiments constitutes a second step in gene target discovery, as shown in the validation of MYB15/MYB14, HY5, MYBA7/A1 and MYBPA1 high confidence targets, or in the characterization of the main regulator of the onset of fruit ripening CARPO. In addition, to TF function discovery, DAP-seq also helped us to hypothesize and explore TF-TF interactions and to identify novel pathway genes that can later be characterized. To visualize the cistromes of different TFs we have created 'DAPBrowse', currently displayed in the Vitis Visualization platform, a publicly available resource with a range of analytical and visualization tools for grapevine. For the initial inspection of TF targets we demonstrate how bound genes can be overlapped with co-expression networks to look for potential targets. Finally, applying DAP-seq in grape calls for species-specific experimental considerations which are being gathered in a set of guidelines currently under preparation in the frame of the Integrape COST Action. The main aim of the guidelines is to provide a standardised framework for DAP-seq experiments in grapevine as well as to help the community in the preparation and analysis of experiments.

Keywords: DNA affinity purification sequencing, transcription factor, binding landscape, gene characterization

Genetic characterization of the phylloxera resistance QTL *Rdv1* in both haplotypes of the grapevine roostock variety 'Börner'

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Abstract

In the 19th century, phylloxera (*Daktulosphaira vitifoliae*) caused a crisis of unprecedented dimension for viticulture and questioned the survival of viticulture in general, as *Vitis vinifera* proved to be susceptible at the rootsystem. Only grafting of susceptible *V. vinifera* cultivars onto phylloxera-tolerant rootstocks derived from American wild *Vitis* species or their interspecific hybrids rescued viticulture. The phylloxera plague remains a serious burden even if it is controlled at the moment. One newly developed rootstock variety is the cultivar 'Börner', an interspecific hybrid derived from a cross of the American *Vitis* genotypes *V. riparia* GM183 and *V. cinerea* Arnold. 'Börner' inherited resistance to phylloxera from *V. cinerea* and plants afflicted by phylloxera do not develop nodosities or tuberosities. At the genetic level, the quantitative trait locus (QTL) *Rdv1* for resistance to phylloxera was identified on chromosome 13 in the 'Börner' genome.

We generated a fully haplotype-separated, high-quality 'Börner' genome sequence assembly in two phases using PacBio long reads. A comprehensive gene annotation was performed, genes were functionally annotated and resistance gene analogs were predicted. True haplotype phasing was verified and the *Rdv1* locus was selected for detailed characterization. This genetically mapped resistance locus was previously described with a size of approximately 1.5 – 3 Mbp, but could be reduced to about 300 kbp using a local mapping approach in which selected recombinant F1 individuals were analyzed with additional markers. Comparing the *Rdv1*-carrying haplotype BoeCin with other haplotypes from *Vitis* varieties conferring susceptibility or only tolerance, *Rdv1* was further delimited based on striking deviation of the sequence synteny. This region of the highly size-reduced *Rdv1* locus was characterized regarding its unique gene content. A set of putative disease resistance gene analogs was identified that represent likely candidates for conferring resistance to phylloxera.

Keywords: Assembly, PacBio sequencing, phylloxera, 'Börner', *Rdv1*, rootstock, genome, annotation, resistance, resistance gene analogs

Virulence-related metabolism may be activated in *Botrytis cinerea* mostly in the interaction with tolerant green grapes that remain largely unaffected

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Abstract

Botrytis cinerea is responsible for the gray mold disease, severely affecting *Vitis vinifera* grapevine and hundreds of other economically important crops. However, many mechanisms of this fruit-pathogen interaction remain unknown. The combined analysis of the transcriptome and metabolome of green fruits infected with *B. cinerea* from susceptible and tolerant genotypes was never performed in any fleshy fruit, mostly because green fruits are widely accepted to be resistant to this fungus.

In this work, peppercorn-sized fruits were infected in the field or mock-treated, and infected berries were collected at green (EL32) stage from a susceptible (Trincadeira) and a tolerant (Syrah) variety. RNAseq and GC-MS data suggested that Syrah exhibited a pre-activated/basal defense relying on specific signaling pathways (enrichment in protein kinases, transcription factors, Ca2+ signaling), hormonal regulation (jasmonates, and ethylene metabolism), and phenylpropanoid metabolism. In addition, putative defensive metabolites such as ursolic acid, trans-4-hydroxy cinnamic acid, and epigallocatechin were more present in Syrah than Trincadeira before infection. On the other hand, Trincadeira underwent a broad metabolism reprogramming upon infection but was unable to contain disease progression. RNA-seq analysis of the fungus in planta revealed an opposite scenario with higher gene expression activity in *B. cinerea* during infection of the tolerant cultivar and less activity in Trincadeira infected berries. The results suggested an active virulence state on the tolerant cultivar even without visible disease symptoms. Together, this study brings novel insights related to *B. cinerea* early infection strategies and the green berry defense involved in tolerance/susceptibility against necrotrophic fungi.

Keywords: *Botrytis cinerea*, fungus-plant interaction, transcriptome, metabolome, *Vitis vinifera*

Session 8: Grape an Wine Quality

Keynote lecture

Oenological traits as targets for grape breeding

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Abstract

With the exception of table grapes for direct consumption, most grapes undergo a sophisticated winemaking process to produce typical wines regarding the grape variety itself, geographic origin as well as the use of traditional oenological measures.

Achievements in molecular biology led to the identification and successful implementation of molecular markers for physiological traits such as yield, sugar graduation and improved resistance against fungal diseases in grapevine breeding. However, oenological traits for Quantative Traite Loci (QTL) analysis representing quality aspects appreciated by consumers and wine experts are still a big challenge.

Only 15 to 20 volatiles are classified as true impact aroma compounds in wine. Among these, only isobutyl-3-methoxypyrazine and ß-damascenon exist in their free form in grapes. They are already monitored by large producers to control grape quality. Exotic thiols, floral monoterpenes or volatile phenols however occur in grapes predominantly as odorless precursors. This poses the necessity to study a wide range of complex glycosides, glutathionyl and cysteinyl precursors to assess the aroma potential as well as carotenoids and fatty acids, from which C_{13}-norisoprenoids or green C_6-carbonyls are liberated.

Early grape breeding already addressed the mitigation of grape derived off-flavors such as hybrid related 2-APP or methylanthranilate contributing to foxy scents or the petrol note of TDN in Riesling. Anthocyanins and skin tannins are targets for improved red wine quality as well as early lignification of seeds to limit extraction of bitter and harsh polyphenols.

Defining wine quality however, is not as simple as the more the better. In fact, quality requires a large pool of sensory active compounds to enhance complexity, but they have to be in an appropriate balance. For that purpose, the analysis of free and bound aroma compounds, pigments, tannins and acids are complemented by sensory analysis, using trained panels to define distinct sensory profiles and consumers, expressing their preference for the tested wines. Combining both sources allows to determine sensory drivers of preferences or rejection. Comprehensive statistical modelling relates these sensory traits to analytical figures and in a further step to genetic information which in turn will allow to identify QTLs for wine quality aspects auch as varietal expression or even specific wine styles. Such advancements will add a new dimension of precision into grape vine breeding.

Keywords: aroma compounds, precursor, polyphenols, grape derived off-flavor, sensory analysis, drivers of preference.

Oral presentations

Differences on the transcriptomic profiles explain clonal phenotypic variation in *Vitis vinifera* L. 'Malbec'

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Abstract

Cultivated grapevines are clonally propagated, mainly to maintain phenotypic traits of productive interest; this practice turns particularly relevant in the wine industry to preserve the varietal typicity. Nonetheless, a wide clonal phenotypic diversity has been reported for several cultivars. Malbec is appreciated for producing high-quality red wines and recognized world-wide as the flag cultivar of the Argentine viticulture. Previous analyses demonstrated a notorious clonal phenotypic diversity for Malbec, in technologically relevant traits. On the other hand, clonal genetic diversity was shown to be scarce, affecting mostly the intergenic regions. Aiming to dissect the molecular bases of the reported phenotypic diversity, we studied 27 clonal accessions grown under the same environmental and cultural conditions at *Vivero Mercier Argentina* experimental vineyard. Phenotypic analyses were performed on berries at technological maturity (~23º Brix), during two consecutive seasons (2017-2019). More precisely, we meassured: i) phenolic composition, ii) analytical profile and iii) skin weight. Afterwards, we chose the six accessions exhibiting extreme contrasting values for the evaluated features. Whole RNA extractions were performed from veraison berries (75% colored), from the six selected clones. Illumina stranded paired-end reads (150 bp in length) were obtained, totaling ~122 Gb of transcriptomic data for 18 samples (6 clones x 3 biological replicates). In order to perform differential gene expression (DEG) and gene ontology (GO) enrichment analyses, the obtained transcriptomic data was aligned to a Malbec reference genome, assembled *de novo* in a truly-phased fashion and annotated by our group. After performing a discriminant analysis including all RNA-seq data, clone Cot-595 exhibited a highly differentiated transcriptomic profile. Moreover, this clone showed the highest total polyphenols and anthocyanins concentration, while clones Mb-506 and Cot-596 showed the lowest concentrations. Therefore, we focused the fore coming DEG and GO analyses to pairwise comparisons among the three mentioned accessions. Consistently, Cot-595 exhibited GO enrichment for genes involved in the anthocyanins biosynthesis pathways, while Mb-506 and Cot-596 showed enrichment for genes involved in metabolic pathways that regulate vegetative growth. These results suggest that phenotypic diversity observed among Malbec clones, might have solid ground on the described differences at the transcriptomic level.

Keywords: Intra-cultivar diversity, RNA-seq, Phenotyping, Malbec

Does the introgression of disease resistance genes impacts agro-œnological traits in grapevine varieties?

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Abstract

A major aim in modern grapevine (*Vitis vinifera* L.) breeding programs is the introgression of resistance genes along with desired cultural and œnological traits. Understanding the genetic links between resistance genes and agro-œnological traits is an important issue for grapevine breeders. In this work, we studied the genetic determinism of yield components and berry quality in the progeny of a cross between two grapevine hybrids carrying each several known quantitative trait loci (QTL) of resistance to fungal diseases. Several yield descriptors like number of inflorescences per shoot, berry weight and berries sugar content were recorded during three successive seasons for 209 genotypes in the vineyard. High density parental and consensus genetic maps were built with 'Genotyping by sequencing' technology using 239 individuals. Many QTL were detected for all studied traits. We found co-localisations between resistance genes and two of the studied traits: berry weight and berries sugar content. Detailed will be presented.

Keywords: *Vitis vinifera*, sugar content of the berries, berry weight, resistance, QTL, plant breeding.

An independant haplotype responsible for white berry phenotype in Vitis vinifera arose from a large deletion at the berry color locus

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Abstract

Since the seminal work of Gregor Mendel in the middle of the XIX century, the inheritance and genetic bases of color variation in plants have attracted significant scientific interest. For Eurasian grapevine (*Vitis vinifera*), the thousand different cultivars can be grouped into two main categories, based on the presence/absence of anthocyanins in the berry skin. One locus on chromosome 2 has been shown to be the major genetic determinant of berry skin coloration. This berry color locus contains a cluster of Myb transcriptional factors (*e.g.,* MybA1 and MybA2). In this study, we investigated a particular allele of MybA1 gene, MybA1_SUB (from haplotype F), originally described in the Sultanine cultivar. We analysed 528 cultivars originating from diverse countries and found 78 cultivars possessing the MybA1_SUB allele, but displaying both phenotypes: colored and non-colored berry skins. This allele is rare in western European cultivars. However, we identified this allele in 9 black-skinned autochthonous cultivars from the Alpine region. These cultivars are cultivated on both sides of the border in the Valais canton (Switzerland) and Aosta Valley (Italy). To gain a better understanding of the genetic mechanisms underlying the presence/absence of anthocyanin in berry skins, we characterized the berry locus region from several black and white-skinned berries cultivars possessing the MybA1_SUB allele. To facilitate genetic analyses, homozygous genotypes were created using selfing and investigated using long-read genome sequencing to reconstruct a large portion of chromosome 2. Our finding showed that the structural organization of the berry color locus for black-skinned variety with SUB allele is different from what has been described for the canonical haplotype of Pinot noir (PN40024). Examining the berry color locus for white-skinned berries SUB cultivars, we identified a large deletion (ca. 77kb). Furthermore, using RNAseq on berry skins tissues during ripening, we examined gene expression profiles for four cultivars carrying the MybA1_SUB allele. Our findings indicate that haplotype F can be divided into two subhaplotypes: one functional that can trigger anthocyanin production and one complete or partial loss-of-function allele due to the deletion. The non-functional haplotype F was identified only in table grape cultivars. In conclusion, contrary to what it has been previously proposed, our results suggest that not all white cultivars share a common origin, but rather that during the domestication process, at least two independent haplotypes were selected for white-skinned berries phenotype.

Keywords: *Vitis vinifera*, berry color, Myb genes, structural variation

The real sour grapes: genetic loci, genes, and metabolic changes associated with grape malate levels

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Abstract

Insufficient levels of malate and lack of sourness in commercial grape cultivars (*V.vinifera*) hinders the quality of fruit grown in warm climates. Conversely, excessive levels of malate and sourness in wild *Vitis* grape, leads to unpalatable fruit and complicates the introgression of valuable disease resistant alleles through breeding. Nonetheless, albeit decades of research, knowledge regarding the molecular regulation of malate levels in grape remains limited.

While malate dissimilation is a hallmark of grape ripening, it was found to be lacking or highly limited in wild *Vitis* fruit (*riparia*, *cinerea*). Hence, these genotypes serve as unique resources to deepen our understanding of malate regulation, with the overarching goal of controlling fruit acidity through breeding.

Our research aimed to (i) Identify genetic loci tightly associated with fruit malate levels in interspecific families, and (ii) highlight differences in metabolism and gene expression, associated with contrasting malate behavior between wild and commercial genotypes. For that, QTL mapping was performed using a novel set of amplicon-based markers (rhAmpSeq) and six years of phenotyping of a complex interspecific F1 family with strong and stable variation in malate at ripeness. In addition, a comparative RNAseq and primary metabolite profiling was performed during fruit development in *riparia* and *cinerea* accessions, and commercial *vinifera* cultivars.

Three significant QTL for ripe fruit malate on chromosomes 1, 7, and 17, accounted for over two-fold and 6.9 g/L differences, and explained 40.6% of the phenotypic variation. QTL on chromosomes 7 and 17 were stable in all and in three out of five years, respectively. Lack of malate degradation in wild genotypes was associated with higher fruit respiration rates, higher levels of amino acids, TCA and fermentation metabolites, and higher expression of their corresponding genes, some of which positioned within the identified QTL in the interspecific family. Compared to *vinifera* cultivars, wild genotypes had lower expression of a cytosolic malate dehydrogenase, and higher expression of malate/dicarboxylate transporters (ALMT/TDT). These results advance current knowledge regarding the regulation of malate at the mechanistic and metabolic levels, and highlight genetic markers and candidate regulatory genes for the control of grape sourness.

Keywords: Fruit sourness, Wild *Vitis*, marker-assisted breeding, rhAmpSeq, ALMT

Genetic mapping of organic acids in a F1 white wine population with high variation in acidity and maturity date

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Abstract

A balanced level of organic acids is of high relevance for wine production. New grapevine cultivars need acidity levels adjusted for the different growing regions and within a proper range to cushion the annual variations, especially under cool climate viticultural conditions as well as in the context of global warming. Organic acids protect berries from spoilage in the vineyard, stabilize the vinification process and play an important role in sensory perception of wines. The development of reliable molecular markers for organic acids to be used in marker-assisted selection (MAS) would increase breeding efficiency by early negative selection of poorly perfoming genotypes. Marker development needs a detailed long-term data acquisition to estimate the environmental factors. A white wine F1 population ‘Calardis Musqué’ x ‘Villard Blanc’ differing in acidity levels and ripening was investigated over ten years. The acidity profile was recorded by FTIR analysis between veraison and harvest under cool climate field conditions at Geilweilerhof (Palatinate, Germany). Genetic mapping of haplotype-based markers (HBMs) extracted from a genotyping-by-sequencing (GBS) approach resulted in a high-density genetic map with 2,260 mainly full-informative markers for QTL analysis. Investigated traits include total acidity, tartaric and malic acid levels as well as pH. Tartaric and malic acid are the two most important organic acids in grapes with major impact on acidity perception in wines. Best linear unbiased predictors (BLUP) were calculated over ten-year-data for maturity date. Major QTLs for total acidity were identified on chromosomes 4 and 14 explaining each 22 % of the variance when veraison was taken into account as covariant. Both loci are co-located with major malic acid QTLs having comparable values. The most pronounced tartaric acid QTLs were identified on chromosomes 7 and 13 (19 % and 18% of explained variance). They are co-located with QTLs for pH value. Thanks to a long examination period and a high-density genetic map, it is now possible to reliably identify functional genomic regions influencing acidity. This provides new insights and opens up new possibilites to increase efficiency in grapevine breeding by early selection for stable acidity level. This will lead to new cultivars well adapted to future climate conditions and combining resistance to pathogens with high quality potential.

Keywords: wine quality, climate change, tartaric acid, malic acid, QTL mapping, metabolic quality potential, genetic quality potential, cool climate

Workshops

Workshop Phylloxera

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Abstract

Colleagues are invited to brainstorm and discuss updates on the major topics and research problems of their work environments and communities, with focus on urgency of matters as well as the scientific perspectives. An important goal of the workshop is to detail the upcoming 8. International Phylloxera Symposium which was postponed due to the pandemic circumstances

Workshop *Vitis sylvestris*

Objective: broad genetic basis of *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi for deep studies

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Abstract

Vitis vinifera ssp. *sylvestris* is the wild relative of the cultivated grapevine *Vitis vinifera* ssp. *vinifera*. In order to better understand the differences between wild and cultivated compartment of *Vitis vinifera* molecular tools provide excellent diagnostic possibilities. The comparability of different studies would benefit greatly from a methodologically coordinated approach. The aim of the workshop is therefore to discuss a methodological toolkit that allows a broad genetic analysis of *Vitis sylvestris* in its entire distribution area and allows in-depth studies. The workshop will adress the

Identification, preservation, and population genetics of true *Vitis sylvestris*

- analysis of individuals, which were not genotyped yet
- exclusion of feral types and hybrids
- overall genetic diversity, differentiation etc.
- putative dispersal routes

Marker tools

- Microsatellite-markers
  - agreement and utilization of common reference varieties from cultivated gene pool
  - agreement on a common set of SSR markers
  - gathering of additional non published SSR-data
- SNP-markers
  - discussion on a common genotyping method (WGS, SNP chips, GBS etc.)
  - common data structure with alignment to most recent PN40024 version (at the moment v2; soon v4)
  - plastid genome analysis

Keywords: *Vitis sylvestris*, genotyping, microsatellites, SNPs

Workshop PIWI

Is a systemic innovation approach possible with PIWI?

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Abstract

Grapevine breeding looks back on more than 200 years of tradition. A time-lapse view reveals significant breeding progress with many new varieties. The dilemma of fighting harmful pathogens, losing plant protection agents, and at the same time facing increasing demands for sustainability poses a challenge for viticulture, as does the need of adaptation to climate change. The EU has set an ambitious goal by halving the use of pesticides in agriculture by 2030, which is particularly challenging in a crop protection-intensive sector such as viticulture.

Ambitious goals can only be achieved by implementing innovations. In view of the transformation goals towards sustainability, however, an individual entrepreneurial innovation is not enough to achieve the ambitious goals - a systemic innovation approach is required. This includes a wide use of fungus-resistant varieties (PIWI). However, in a system spoiled by success such as viticulture, there are considerable tendencies to persist instead of proactively tackling innovations. However, the way has been mapped out: climate change is forcing us to change varieties. This creates an opportunity for new, robust varieties. However, we must avoid frustration in the viticultural sector and discuss the existing problems:

- Slow market introduction.
- Lack of enological experience.
- Remaining plant protection.
- How durable can resistance be?
- Are NBT a supplement for breeding or an alternative?
- What can breeders do to implement better resistance concepts?

The discussion of these and other aspects within the workshop should take us a step further and open up possible options.

Keywords: PIWI, durable resistance, market introduction, systems innovation

Workshop INTEGRAPE

Beyond COST Action INTEGRAPE: The Grapevine Genomics Encyclopedia Initiative

Matus, José Tomás, Rustenholz, Camille; Grimplet, Jérôme; Cantu, Dario; Holtgräwe, Daniela; Bombarely, Aureliano ; Carbonell, Pablo ; Alaux, Michael; Fasoli, Marianna; Moretto, Marco; Mietton, Camille ; Sanseverino, Walter ; Gruden, Kristina; Ware, Doreen ; Adam-Blondon, Anne-Françoise; Pezzotti, Mario

The GRAPEDIA Consortium

Abstract

The COST Action CA17111 INTEGRAPE, ending in September 2022, has greatly contributed in providing the basis for integrating grape resources. The Integrape community has been active and productive, generating guidelines for FAIR treatment and generation of standardized data, allowing them to be findable, accessible, interoperable and reusable. We have contributed to the release of the latest and most updated reference genome assembly of grape, together with the world's largest gene catalog of functional data. This COST Action has also served as a hub of all tools generated by the community, allowing data exploration and analysis. Despite these advances, there is still much room for improvement. These *in house* resources do not full interoperate, neither are they linked to other important, highly-accessed resources generated by the community, such as Grapegenomics.com, Vespucci, Vitviz, etc. With the aim to integrate and intercommunicate these resources, offering them to the community in an innovative, dynamic and centralized web portal, we have established the Grapevine Genomics Encyclopedia (GRAPEDIA) Initiative. GRAPEDIA will be structured in a federated database system that will converge different sources of open access data, integrating biological knowledge, genetic and genomic resources and will also offer customized services for the research community and industry that will allow the database to be commercially exploitable. GRAPEDIA aims to develop grapevine-tailored application programming interfaces (APIs) and implement innovative technologies such as deep learning and artificial intelligence methods to provide the community the best tools to face near-future challenges: improve or design new cultivars and agricultural systems to cope with environmental transition and pests, whilst ensuring vineyard sustainability. There are companies who have been involved in INTEGRAPE activities and have explicitly manifested their interest in contributing to GRAPEDIA, but first we need to fully understand and unite the needs of the whole community. For this reason, in this workshop we expect to interact with members of the community and discuss possible outcomes of the portal. We welcome all public and private entities to join this effort. Visit http://grapedia.org/ for more details.

Poster presentations

P1 – Assessment of grapevine diversity in old vineyards from Northeast Portugal

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Abstract

The high diversity of grapevine varieties in Portugal is well known. In several municipalities in the northeast of Portugal at the beginning of the last century, more than one hundred different varieties were recognized. Currently are authorized for wine production with Protected Designation of Origin "Trás-os-Montes" 33 varieties and "Douro"/"Porto" 110, comprising 115 different varieties. Nevertheless, despite this huge diversity, only 22 (19.1%) have representativity in these wine regions higher than 1%, corresponding to 84,2% ("Trás-os-Montes") and 89.0% ("Douro"/"Porto") of the total vineyard area. Prospection and identification of grapevine material in ancient vineyards in these wine regions is of utmost importance to prevent its disappearance. Thirteen old vineyards, aged between 50 and over 100 years, were studied, comprising a total of 456 plants. Genotyping by SSR and SNP markers allowed the identification of 88 different molecular profiles, including 15 unknown genotypes. In fact, 18 genotypes were detected in only one plant which emphasizes the urgency in their preservation. Moreover, chlorotype diversity was also analyzed. Four chlorotypes (A, B, C and D) were detected: chlorotype A was the most frequent, followed by chlorotype D. Chlorotypes B and C were only present in four foreign grapevine varieties.

Keywords: *Vitis vinifera* L., grapevine germplasm, autochthonous varieties, halt genetic erosion

P2 – New genotypes for an old business: 'Criolla' and European grapevine varieties found in old vineyards from Chile

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Abstract

Chilean vineyards began with the arrival of the Spanish conquerors, by XVI century. Vines prospered very well in the Mediterranean climate of the southern countries of South America. Particularly in Chile, grapevines were planted all along the valleys of the central part of the country. A few genotypes were used, prevailing the red variety 'Listán Prieto' (LP), known in Chile as 'País'. Two centuries later supposedly arrived 'Muscat of Alexandria' (MA), and in a manner and timing not determined, these two founder varieties cross-polinated, originating the larger group or criolla varieties, up to now scarcely studied. Subsequently, since mid XIX century, a second wave of European varieties arrived, establishing also novel viticultural practices and wine styles. In this work we are presenting the identification and partial distribution of a number of new criolla genotypes, additional to the ones previously described in the region, as well as a few European varieties not previously registered in Chile. For their differentiation and characterization, the set of nine SSR markers proposed by the Vitis International Variety Catalogue was used, suplemented with 20 additional polymorphic SSRs to facilitate their paternity analysis. Up to now, we have found ca. 30 new criolla genotypes (according to their allelic patterns matching with LP and/or MA as the most probable parents), not included in the *V*IVC catalogue nor described before in any database. Their prevalence is quite variable, some corresponding to a single or a few vines identified in particular places, meanwhile others are repeatedly present in different valleys, suggesting they were "selected" and then propagated and shared by local growers. These criolla-type varieties co-exist with European (mostly French) old varieties; how and when each of these minor varities arrived to Chile is unknown, but most probably they traveled together with the importation of French varieties occurred during the "re-colonization" of the Chilean vineyards by mid XIX century. The discovery and documentation of these "new" genotypes is the first step of a long-term work that must be followed by evaluations of their enological characteristics, productivity and management systems, in the search for a diversification in the Chilean wine industry.

Keywords: *Vitis vinifera* L., criolla, hidden European varieties, South America, fingerprinting, microsatellites, genetic resources

P3 – *VviMybA1* and *VviMybA2* independent mutations trigger white to red berry skin color reversion in *Vitis vinifera* cv 'Albariño' and 'Verdejo'

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Abstract

Spontaneous somatic mutations within vegetative meristems may be related to changes in berry color of *Vitis vinifera*. Anthocyanin accumulation in the berry skin is triggered *VviMybA1* and *VviMybA2*, transcription factors, regulating the expression of genes encoding different enzymatic steps in the anthocyanin biosynthetic pathway. Null mutations in both *VviMybA1* and *VviMybA2* can lead to a white-skinned phenotype. The lack of berry color in white berry cultivars has been commonly associated with a recessive null allele harboring an insertion of *Gret1* retro-transposable element in the *VviMybA1* promoter region together with a two nucleotide deletion in *VviMybA2* coding sequence producing a null frameshift. On rare occasions, berry anthocyanin pigmentation is partially recovered in white-skinned cultivars giving rise to red-skinned variants. Here, we studied the origin of two spontaneous red somatic variants derived from white-skinned Verdejo and Albariño cultivars, through transcriptomic and targeted UPLC-QqQ-MS/MS metabolomic approaches. At veraison, RNA-seq analysis identified 386 and 84 differentially expressed genes in berry skin of Verdejo and Albariño variants, respectively, compared to their white-skinned ancestors (FDR < 0.05). In both red-skinned variants, flavonoid biosynthesis activation was detected, including the upregulation of *chalcone synthase*, *flavonone-3-hydroxylase*, *leucoanthocyanidin dioxigenase* or *UFGT*. Anthocyanin accumulation in berry skin was observed from veraison stage in both red-berried somatic variants, which was dominated by cyanidins. In addition, colored Albariño berries presented detectable amounts of delphinidins and peonidins. Anthocyanin accumulation was more pronounced at maturation stage. Besides, the Albariño red somatic variant showed greater anthocyanin concentration than the Verdejo variant in both stages. Additionally, targeted Nanopore sequencing and PCR validation identified molecular genetic alterations responsible of white to red skin color reversion. Colored Albariño recovered *VviMybA1* expression as consequence of the partial *Gret1* retrotransposon excision, leaving behind a solo LTR region. Colored Verdejo displayed a mitotic gene conversion between the two CR domains within *VviMybA2* exon 3, which restored the reading frame and protein function. This is the first time that a berry color recovery is related to a *VviMybA2* function regain, enabling to determine the specific role of the two major transcription factors regulating grape anthocyanin accumulations.

Keywords: Grapevine, Berry color, Anthocyanin, *VviMybA* genes, Somatic variation

P4 – A very strange ‘Riesling’

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Abstract

‘White Riesling’ is one of the most important grapevine varieties in Germany and the development of new clones is dependent on the genetic diversity within this traditional variety. For this purpose, the Department of Grapevine Breeding in Geisenheim houses the world’s largest genetic resources of ‘White Riesling’ with almost 1,200 clones.

Within this collection, some accessions originating from the single vineyard site “Saarburger Rausch” in the Moselle wine region were examined in detail as their ampelographic characteristics are deviating from those of ‘White Riesling’. To verify whether the accessions “Saarburger Rausch” are ‘White Riesling’, nine standard Simple Sequence Repeat markers (SSRs) for identification of varieties were analyzed. One accession (SB 17-117) has a profoundly unusual SSR-profile. That “very strange Riesling” shows all alleles of ‘White Riesling’ but a third allele is present at four out of the nine SSR-loci analyzed. White Riesling is diploid and usually has a maximum of two distinct alleles at one locus.

The most obvious explanation for the occurrence of more than two alleles are differences in the genotypes of the two distinct cell layers of grapevine, the outer tunica layer(L1) and the inner cell layers (L2). Grapevine varieties composed of genetically different cell layers are called periclinal chimeras and are very common with ‘Pinot meunier’ or ‘Pinot gris’ being prominent examples. To test the hypothesis of SB 17-117 being a periclinal chimera, the L2 cell layer was examined independently by planting dormant cuttings in order to produce adventitious roots which are composed of L2 only. Surprisingly the three alleles at four loci were also detected from DNA of adventitious roots and hence the three alleles of SB 17-117 cannot be explained by chimerism.

A second hypothesis assumes that SB 17-117 is polyploid. Polyploid grapevine varieties are carrying more than two alleles per marker which could theoretically be different and lead to the presence of three or more different alleles. Flow cytometry was used to analyze ploidy levels and unravel the genetic background of SB 17-117.

Keywords: genetic resources, White Riesling, SSR marker, cell layers, polyploidy, tri-allelic, flow cytometry

P5 – Screening for susceptibility to *Erysiphe necator* infection in Spanish minority grapevine varieties

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Abstract

Powdery mildew of grapevine (*Vitis vinifera*) is caused by the fungus *Erysiphe necator*. It is one of the diseases that has caused the greatest economic and quality losses in European vineyards since its introduction two centuries ago. Almost all *V. vinifera* varieties are susceptibles to this fungus, a differential response to infection has been observed. Understanding the diversity in the response of grapevine germplasm to the disease, as well as the molecular and genetic basis for this differential response, can provide tools for sustainable disease management. The aim of this work was to evaluate the susceptibility to powdery mildew of 32 Spanish minority varieties from different wine-growing areas. Young detached leaves sterilized were inoculated with a vacuum tower. Fungal development was evaluated on a scale from 1 to 8, from 1 to 6, the development stage of the fungus and from 6 to 8, the percentage of leaf occupied with the naked eye was recorded. Data was obtained at 7 and 14 days after inoculation. Differences in the susceptibility among varieties was found. The correlation between the disease incidence between 7 and 14 days was highly significant with a value of 0.5. The cluster analysis yielded 5 groups, the group containing the largest number of varieties was characterised as the most susceptible, giving the highest scale at both 7 and 14 days. On the other hand, *Kishmish vatkana* was left out of any grouping, this variety which contains the *Ren 1* gene was included as a control. The other 2 groups contained the least susceptible varieties, in one of them lower fungal development was observed on the 2 measurement dates, which could suggest the importance of the structural defence mechanisms of these varieties. In the other group, the differences were recorded at 14 days, suggesting a late recognition of the pathogen. Results reported differences in grapevine response to powdery mildew attack. This information can be useful for breeding programs, promoting the use of varieties that are well adapted to the environment and that require fewer treatments for their control. It would also allow the diversification of the wine market, which is becoming more competitive and demanding new products.

Keywords: Plant defence, Tolerant, Germplasm, Powdery mildew, *Vitis vinifera.*

P6 – *Rpv32* – A new downy mildew resistance locus from the unexploited wild species *Vitis coignetiae*

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Abstract

Downy mildew, caused by the obligate biotrophic oomycete *Plasmopara viticola*, is one of the most damaging threats to grapevines growing in a warm and humid climate. Extensive application of fungicides is necessary to avoid serious yield losses, but this leads to a severe environmental impact and decreasing acceptance in society. In contrast to the conventional downy mildew management strategies, cultivars showing durable resistance are in high demand due to their contribution towards a sustainable and environmentally friendly viticulture. Therefore, genetic resistances with different defense mechanisms are necessary to prevent pathogen adaptation and to breed durable resistant varieties. This study aims to identify and genetically map a resistance for downy mildew from the unexploited East Asian wild species *V. coignetiae*. The individuals of a bi-parental F1 population (N=496) derived from the cross of 'Morio Muskat' x COxGT2 (*V. coignetiae* x 'Gewürztraminer') were phenotyped for resistance to *P. viticola* in an artificial leaf disc infection assay in the laboratory. A first framework map was generated based on 109 SSR markers. Using 647 transferable rhAmpSeq haplotype markers, a high-resolution map was obtained with a total map length of 1147.36 cM on 19 linkage groups, accounting for 96% of the physical coverage and an average distance between loci of 3.2 cM. Quantitative trait locus (QTL) analysis with each genetic map detected a single and highly significant stable QTL on chromosome 14 in four independent experiments that explains up to 36.4% of the phenotypic variation. This QTL maps to a different position as *Rpv8* and *Rpv12* from *V. amurensis* and shares no SSR marker alleles with them. It was therefore named *Rpv32* (Resistance *Plasmopara viticola* 32). A rhAmpSeq haplotype allele and a SSR marker were identified to be strongly associated with the novel resistance locus and have the potential to be exploited as selection markers for introgression of *Rpv32* into breeding lines.

Keywords: Downy mildew, QTL, mapping, resistance locus, *Rpv32*, *Vitis coignetiae*

P7 – Optimization of preculture medium for *in vitro* microcuttings in the procedure of cryopreservation of the grapevine cultivar 'Graševina'

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Abstract

Grapevine (*Vitis vinifera* L.) is one of the oldest agricultural species. Wide application of this culture in the economy makes it one of the most important agricultural fruit culture in the world. Republic of Croatia is important gene center for native, as well as for introduced cultivars of grapevine, so the viticulture's aim is to conserve and revitalize its cultivation. Crypreservation is the most efficient procedure for the conservation of the plant material. In the cryopreservation procedure different preculture of microcuttings, cryoprotectants and steps during freezing can be toxic and make the stress within cultivars of grapevine. The aim of this study is the optimization of preculture medium for preculture of microcuttings with addition of antioxidants (salicylic acid) with the purpose of the successful growth of shoot tips of the cultivar 'Graševina'. The study was made on microcuttings of cultivar 'Graševina', planted on half-strength MS medium, with or without cytokinins benzylaminopurine and different concentrations of salicylic acids (0, 0.1, 0.5 and 1 mMol). The highest percentage of shooted microcuttings was achieved on the medium without the salicylic acid and BAP (68, 38%), and the lowest on the medium with addition of salicylic acid in concentration 0.1 mMol and supplement of 1µmol BAP (35, 00%). Microcuttings cryopreservation of cultivar 'Graševina' reached the highest results of regeneration in controled explants (15%) in comparison on the freezing ones (10%). Given results are implying that some additional studies should be done for successful cryopreservation of this cultivar.

Keywords: *Vitis vinifera* L., cultivar 'Graševina', preculture of microcuttings, antioxidants, salicylic acid, regrowth

P8 – Genetic diversity and population structure in Brazilian grapevine hybrids

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Abstract

The grapevine breeding program has been developed at the Agronomic Institute (IAC) since the 1940s to develop new cultivars adapted to the climatic conditions of Brazil. More than 2,000 crosses were carried out over 50 years, using 850 genotypes as parents. However, among the thousands of hybrids developed by the program, only 130 are still maintained in the IAC grapevine germplasm collection. Little is known about its genetic makeup and usefulness for the current breeding program. The present study evaluates the genetic diversity and population structure of these 130 Brazilian grapevine hybrids using 17 highly polymorphic microsatellite markers. A total of 202 alleles were obtained, and high expected heterozygosity was identified (0.81). The STRUCTURE analysis indicated that the hybrids represent three distinct genetic clusters. Based on a membership probability threshold of 0.70, 28 hybrids were assigned to cluster 1, 45 hybrids were assigned to cluster 2, and 44 hybrids were assigned to cluster 3. Thirteen hybrids did not sort to defined clusters and were assigned to the admixed group. The discriminant analysis of principal components (DAPC) showed several similarities to those achieved by STRUCTURE, and both analyses showed the same pattern of clustering. The genetic groups were based mainly on the use and combination of parental groups. In cluster 1 were allocated wine hybrids obtained by crossing Seibel hybrids with *V. vinifera* wine cultivars. Cluster 2 was formed by table grape hybrids obtained through crosses with fine muscat grapes. In cluster 3 there was no clear discrimination based on human usage, hybrids for wine, table, and rootstock are found in this group. However, all hybrids of this group have in common the presence of wild *Vitis* in their genealogy. The molecular characterization of this breeding hybrids bank collection contributes to understanding the genetic basis of the genotypes, guiding the efficient exploitation of available genetic diversity. These results could be applied to other breeding programs and assist in the selection of parents, management of the breeding collection, and conservation of the grapevine genetic resources.

Keywords: SSR markers, genetic resources, plant breeding, *Vitis* spp., grape

P9 – Phenotypes and genetic background of seedless grapevine varieties in Armenia

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Abstract

Seedlessness is a highly valuable trait in the EU and global grape markets. Genotyping and phenotyping of seedless varieties are an efficient tool for assessing the genetic diversity, determining the type of seedlessness, and optimizing their use in table breeding programs. In this work, a total of 42 Armenian *Vitis vinifera* L. seedless grapevine accessions were sampled from the Armavir region, mainly from the grape collections at Etchmiadzin (ARM006) and Nalbandyan (ARM011). True-to-type genotyping was performed with seven nuclear microsatellite loci and the molecular profiles obtained were compared to those stored in the *Vitis* International Variety Catalogue (*V*IVC, www.vivc.de), and the Armenian *Vitis* database (www.vitis.am). Phenotypic analysis of berries and seeds was carried out according to OIV descriptors. Molecular analysis of seedlessness was performed by the targeted sequencing of *VviAGL11* MADS-box gene, responsible for embryo and endosperm development and seed coat lignification.

Genetic profiling identified 11 unique genotypes, namely 'Anush', 'Eskeri', 'Hrushaki', 'Karmir Kishmish', 'Karmir Yerevani', 'Kishmish Chernyi', 'Kishmish Khishrau', 'Kishmish Moldavskii', 'Parvana', 'Sultanina' and 'Ushahas Nazeli'. According to their pedigrees in the databases the majority of investigated varieties are genetically Sultanina derived: 'Karmir Yerevani', 'Eskeri', 'Hrushaki' and 'Kishmish Moldavskii' are offspring of Sultanina, while 'Anush' and 'Ushahas Nazeli' have a second-degree relationship. Phenotypic analysis revealed a wide variation in berry size, as well as in the formation of seeds (from very small, to large and noticeable). Mean berry size ranged between 10.5 mm and 20.1 mm in length, and 9.5 mm to 16.9 mm in width. Berries with ovule residues and small rudimental seeds were detected in 'Karmir Yerevani', 'Karmir Kishmish', 'Sultanina' and 'Hrushaki'. Plants of the rest of cultivars produced large, but empty floating seeds, and rarely up to 0.4 well-developed seeds per berry. By contrast, berries of 'Ushahas Nazeli' showed large size 1.5 well-developed potentially viable sinker seeds per berry. Targeted sequencing of VviAGL11 gene revealed heterozygosity for the seedless dominant point mutation Arg-197Leu (A:C) in ten investigated seedless genotypes, which causes the stenospermocarpy in Sultanina-type seedless cultivars. The only exception was found for 'Ushahas Nazeli' which was homozygous for the seeded allele (C:C). We are currently investigating gametes viability, as well as endosperm and embryo development features, in order to integrate- phenotypic and genotypic data.

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Keywords: *Vitis vinifera* L, seedlessness, stenospermocarpy, 'Sultanina', *VviAGL11*

P10 – First description of wild grapevine (*Vitis vinifera spp. sylvestris*) locations in Slovenia

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Abstract

Vitis vinifera subsp*. sylvestris* is the only native wild grapevine in Eurasia (Europe and Western Asia) and the present ancestor of the grapevine varieties belonging to subsp*. vinifera*. The wild subspecies survived the Ice Age in small refugia (sites with isolated or relict populations) and spread from there through alluvial forests. A few natural populations of European wild grapevine still exist in small, disconnected populations in remnant habitats such as Szigetköz (Fertő-Hanság National Park) in Hungary and Germany in the Upper Rhine Valley. In Slovenia, the prevailing opinion was that there were no habitats of subsp. *sylvestris*. This study describes for the first time *Vitis vinifera* subsp*. sylvestris* in Slovenia and the aim was to get an overview of wild grapevine locations in the country.

In this project, a sample set of 83 accessions was studied using 24 SRR markers and 3 markers for flower sex determination. The accessions were found in forests on the left side along the Sava River in Slovenia at the border between alluvial soils and limestone and dolomite at 4 different sites, some of which were described for the first time. The proportion of female and male accessions differed at the different sites. At two sites female plants dominated, at others the ratio was balanced. Their genetic diversity and structure were compared with autochthonous and unique varieties of subsp. *vinifera* from old vineyards in Slovenia and with rootstocks escaped to nature from abandoned vineyards. Sylvestris was clearly separated from vinifera and the rootstocks.

The conservation of biodiversity has a practical value for mankind, as some of the subsp. *sylvestris* accessions showed relatively high tolerance to grapevine pathogens and represent a valuable genetic resource for resistance breeding.

Meanwhile, a complete genetic copy of the wild grapevine has been established at the University Centre Meranovo University of Maribor, Faculty of Agriculture and Life Sciences.

Keywords: Slovenia, Wild grapevine, SSR markers, V. V. Subsp. Sylvestris, conservation, genetic fingerprinting

P11 – Comparative genomics and phylogenetic analysis of *Vitis vinifera*: insides from the complete plastid genome sequences

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Abstract

Vitis L. belongs to one of the oldest Vitaceae family of flowering plants. The origins of the grapevine remain uncertain, this family possibly were originated on the boarder of Jurassic and Cretaceous periods and was widely distributed in the Old and New World. Its great age is testified by fossilised grape leaves and seeds in Palaeocene and Eocene deposits. Glacial period destroyed most of *Vitis* habitats and only in a certain area (refuges) they were survived. Such areas in Europe were located around Mediterranean basin and Southern part of Black and Caspian Seas. The current distribution of genus *Vitis* habitat includes three centres of diversity: East Asia, Northern south America, Central America and North America, Europe and Central Asia. Resent 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.

We present the analyses of plastid genome diversity of wild (*V. vinifera* ssp. sylvestris) and cultivated grapevines (*V. vinifera* ssp. sylvestris) from Georgia, Europe, Mediterranean basin and Asian and American spices by the Next-generation Sequencing and Comparative Genomics. In particular, in the frame of our research: 1). Next-generation Illumina Sequencing of more than 40 genomes of *Vitis vinifera* from different geographic origins were conducted; 2). SNPs and indel regions were detected in each sequenced genome; 3). By using of comparative genomic approaches, the phylogenetic linkage study of the analyzed plastid genomes was performed. According to our results, it is shown that GTA haplotype dominates in wild grapevines of Europe (i.e., Germany and France), ATA and ATT haplotypes were found in the Mediterranean basin and Anatolia (i.e., Corsica, Greece, Turkey). Genetically unique AAA haplotype was found in Georgian wild and cultivated samples and ATA haplotype was detected in Asian and American species. The obtained results will help to understand the genetic relationships between wild and cultivated grapes from different geographical locations and explain the molecular bases of grape origin and evolution.

Keywords: *Vitis vinifera*, Plastid DNA, Next-generation sequencing, Comparative genomics,

P12 – A virus-resistant *Vitis* germplasm

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Abstract

More than 80 viruses infect grapevines and spread among vineyards and nurseries, singly or collectively. The most prevalent are grapevine leaf roll-associated viruses (GLRaV). Grape breeders and researchers are seeking virus-resistant *Vitis* species and cultivars, a holy grail for them. Although 734 *Vitis* accessions were assessed for resistance against Grapevine fanleaf virus, GLRaV-1 and GLRaV-3 in the 1990s, no virus-resistant germplasms were found. After multiple years of evaluation, we found that a North American grapevine, *Vitis aestivalis* 'Norton', is resistant to Grapevine vein clearing virus (GVCV), a DNA virus spreading in cultivated and native grapevines in the Midwest of the USA. In another project, we infected 'Norton' by graft-transmission with seven grapevine viruses, Grapevine fleck virus (GFkV), GRLaV-1, -2, and -3, Grapevine virus A (GVA), Grapevine Pinot gris virus (GPGV), Grapevine rupestris stem pitting-associated virus (GRSPaV), all of which were infecting 'Kishmish Vatkana' (*Vitis vinifera*) grapevine. Five months later, we applied RNA-seq to compare viral small RNA (vsRNA) composition and genome coverage of the seven viruses in 'Norton' and 'Kishmish Vatkana'. Total vsRNAs of GLRaV-1, GLRaV-2, GLRaV-3, GVA, and GPGV were significantly less abundant in 'Norton' than in 'Kishmish Vatkana', but total vsRNAs of GFkV were more abundant in Norton than in 'Kishmish Vatkana'. vsRNAs of GLRaV-1, GLRaV-2, GLRaV-3, and GVA vsRNAs did not cover their specific viral genome in 'Norton' as fully as in 'Kishmish Vatkana'. vsRNAs of the seven viruses were composed of mainly 21- and 22-nt classes. Quantitative PCR assays showed that GLRaV-1 was not detected in 'Norton'; GLRaV-2, GLRaV-3, and GVA were less abundant in 'Norton'; and GFkV was more abundant in 'Norton' than in 'Kishmish Vatkana'. These results demonstrated that Norton grapevine resists GLRaV-1 and suppresses GLRaV-2, GLRaV-3, and GVA, but supports GFkV in comparison with 'Kishmish Vatkana'. This study revealed complex molecular interactions between grapevines and multiple viruses. We are currently conducting genetic analysis of a selfed 'Norton' population to discover genetic elements conferring resistance to viruses. Norton's broad-spectrum resistance to biotrophic and necrotrophic fungi as well as to multiple viruses merits its use as valuable germplasm in breeding and inspires further study on genetic and molecular mechanisms underlying grapevine virus resistance.

Keywords: resistance, virus, grapevine, *Vitis*

P13 – Investigations into shortened internodes degeneration of the variety 'Neuburger' for better selection

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Abstract

The cultivar Neuburger is an autochtonous variety for wine production in Austria. Since a couple of years the interest of growers for this cultivar is decreasing due to the short internode phenomenon. Vines concerned by the degeneration loose their normal growing behavior by stunting and shortening of internodes. This decline occurs surprisingly and changes growth behavior also from former healthy looking vines. Maintenance of the variety would require to find genetic indications if a vine will be stable or carries the potential to easily switch to stunting.

We pooled several genotypes of the cultivar with and without symptoms and looked for the obviously differences. Several gibberellines were quantified by mass spectrometry. Only one of the GA substances showed a significant difference between healthy and stunted vines. Finally several genes from the GA metabolic pathway were analysed. We could find differences and mutations but none of them explains the phenomenon for the whole. Involving qPCR we tried to find relationship of RNA level of selected genes to short-internodal vines. Genes from different parts of gibberellin synthesis and the signaling pathway were selected. Two cytochrome P450 monooxygenase genes showed a tendency towards upregulation in one of two sample sets. In the case of gibberellin oxidases, one locus showed a significantly lower level of expression in the short-internodal variant. In the case of the genes involved in the signaling pathway, ambiguous results are found between the sample sets. Furthermore full transcriptome analysis by sequencing was done. Genes that showed significant differences between short-internodal and normal-growing vines as a result of the transcriptome analysis, were selected for further analysis. In some cases, significant differences in expression levels of RNA could be discovered.

For the possible contribution of epigenetic effects we sequenced the genome by nanopore technology. Some genes showed in symptom carrying vines a high degree of methylation. One of them is a Della protein. These changes could also be responsible for differences in RNA metabolism. Furthermore defined markers to get access to the genes with relevant mutations were applied. The findings allow the conclusion that the shorten internodes in Neuburger is based on several changes in the genome and not a single mutation.

As a practical approach for deliberating the stunting of selected vines gibberelline applications were performed to them. In some trials the intensity of stunting could be alleviated.

Keywords: grapevine, gibberellines, qPCR, RNA, sequencing, genetic marker

P14 – Polyclonal selection for *Vitis vinifera* cv. 'Petite Arvine' in Switzerland

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Abstract

Petite Arvine is an emblematic white grape variety of the Valais vineyards (Switzerland). This autochthonous variety presents a high intravarietal diversity. Works has been done to characterise this clonal biodiversity. As results, the agronomic and partly oenological potential of around one hundred clones were analysed. Polyclonal selection consists in the selection of top-ranked set of clones concerning specific target traits. The massal or polyclonal approach is often opposed to the clonal approach in grapevine selection. Some advantages of polyclonal selections compared to the clonal approach that are usually mentioned are a greater potential for resilience to certain stress factors caused by changes in climate and cultivation techniques (e.g. cover crops) as well as a possible positive influence on wine complexity. The aim of this project is to compare the advantage of polyclonal selections focused on specific topics compared to the behaviour of homologated clones, from the agronomic level to the quality of the wines.

Especially in the context of climate change, we are exploring different polyclonal selections in order to mitigate the effect of abiotic stresses (higher temperature and more frequent drought) caused by the evolution of climate. Five polyclonal selections were constituted targeting specific traits, each composed of 10 clones. The following traits were selected to create these five populations: berry acidity (high level); yeast assimilable nitrogen (high level); yield at harvest (moderate/high level) and aromatic precursor P3MH (high level).

The aim of this study will be to evaluate the interest of polyclonal selection and clonal selection to mitigate the effects of climate change in viticulture and to valorize the work done to safeguard Petite Arvine genetic diversity.

Keywords: *Vitis vinifera* cv. Petite Arvine, clonal diversity, climate change, polyclonal selection

P15 – Elucidating the genetic determination of ampelographic traits by QTL analysis in a segregating F1 population

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Abstract

Ampelography (Greek: “ampelos” = vine) is the field of botany dealing with the identification and classification of grapevine cultivars. Starting with the birth of Mediterranean viticulture documented by ancient Greeks and Romans, such as Theophrastus (375 – 297 BC), Vergilius (70 BC – AD 19), Columella (4 - 70 AD), or Pliny (23/24 – 79 AD), grapevine varieties have been described based on their morphology. In the last centuries, detailed descriptions summarized in large compilations were published (e.g. Babo 1844, Hedrick 1908, Viala and Vermorel 1901-1910, Galet 1975) representing the foundation stone for modern ampelography as a scientific discipline. Historically this has been done by subjective visual description of morphological traits mainly related to vegetative growth characteristics, but more recently objective ampelometry and molecular DNA fingerprinting were introduced. The main focus in traditional ampelography lays on leaf description, due to the confirmed stability of leaf characteristics within the same variety grown in different environments, their abundance and availability over the long vegetative period and as herbarium material. However, less is known about the genetic determination of morphogenetic factors.

To identify quantitative trait loci (QTLs) and subsequent putative candidate genes influencing ampelographic descritpors, a segregating white wine population (150 F1 plants; ‘Calardis Musqué’ x ‘Villard Blanc’) with an established high-density genetic map was investigated in four consecutive years. The analysed traits can mainly be grouped in three classes related to: (1) hair formation (e.g. OIV004 or OIV053), (2) anthocyanin coloration (e.g. OIV010 or OIV070) and (3) leaf shape (e.g. OIV068 or OIV079). For all three groups, several promising QTLs could be detected and will be discussed.

Keywords: grapevine, morphology, OIV, phenotyping, *Vitis vinifera* L.

P16 – Epigenetic analysis reveals differentially methylated regionsbetween cultivated and wild grapevines

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Abstract

Grapevine domestication has traditionally been based in clonal propagation, with the aim of enhancing and selecting grapes traits (like fruit size or berry sugar content) and reducing heterogeneity in the vineyard. Due to the domestication process, *Vitis vinifera* L. was separed in two subspecies based on morphological differences: ***Vitis vinifera* ssp sylvestris** and ***Vitis vinifera* ssp vinifera**, which differences concentrate in reproductive phenotype and environmental adaptations. These phenotypic differences could be explained both by the selection of genetic and **epigenetic variation** occured during species evolution. The contribution of genetic variability towards the observed phenotypic diversity and plasticity of cultivated and wild *V. vinifera*, has been extensively studied. However, very little effort has been directed at the selection of epialleles during grapevine's domestication. To explore the **epigenomic differences between the two subspecies**, we characterized the methylome across 8 wild accessions (WT) of *Vitis vinifera* ssp sylvestris and 10 cultivated varieties (CV) of *Vitis vinifera* L. ssp vinifera using a reduced-representation genome sequening approach (**epiGBS**). Genome-wide analysis of differentially methylated regions (DMRs) identified a total of 9955 DMRs, of which, 7793 were hypermethylated and 2162 hypomethylated in cultivated varieties in comparison to wild accessions. Additionally, study of the location of DMRs in relation to genomic features, showed **higher DNA methylation levels in intergenic regions in WT than in CV in all methylation contexts (*i.e.*, CG, CHG, and CHH)**. Conversely, we found a **higher percentage of methylated regions (in all contexts) in gene promoters of the CV group** (and different methylation rates in **intron** and **exon** between CV and WT groups). The results suggested that, although the methylome of CV and WT groups were modelled under the same environmental conditions, we showed that regions harboring polymorphic methylation could contribute to functionally relevant phenotypic variation across them.

Keywords: Epigenetics, methylome, domestication, wild, cultivar, grapevine, DMR

P17 – Metabolomic discrimination of genetic and geographical groups of grapevine varieties (*Vitis vinifera* L.)

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Abstract

An important aspect of wine geographic origin is related to grapevine varieties used for wine productionin specific winegrowing regions or countries. Grapevine germplasm is highly variable and classified into geographical groups. These classifications were recently confirmed by genetic studies, and further classified into genetic-geographic (GEN-GEO) groups. Secondary metabolites, namely polyphenolic and volatile organic compounds (VOCs), have crucial role in winemaking industry due to their influence on quality, colour, and sensory properties of wine. The aim of the research was to investigate the polyphenolic and volatile profiles of 50 grapevine varieties from different GEN-GEO groups. The groups are C2 (varieites from Italy and France), C7 (varieites from Croatia), and C8 (varieites from Spain and Portugal). Polyphenolic compounds analysed belonged to the classes of anthocyanins, flavan-3-ols, flavonols, phenolic acids, and stilbenes. Classes of VOCs analysed were carbonyls, alcohols, acids, esters, and terpenoids. The most abundant class of polyphenols were anthocyanins, followed by flavan-3-ols and flavonols, while carbonyls were the most abundant class of VOCs, followed by alcohols and sesquiterpenes. Using discriminant analysis, the GEN-GEO groups were clearly separated by their polyphenolic and volatile profiles. In the case of polyphenolic profiles, compounds contributing the most to the discrimination of groups belong to classes of hydroxycinnamic acids, flavan-3-ols, and flavonols. Furthermore, some of the compounds contributing to discrimination are found in relatively small amounts. Regarding the discrimination based on volatile profiles, GEO groups were discriminated by their overall volatile profile. C2 group contains higher amounts of carbonyl compounds and alcohols, while C8 group contains higher amounts of sesquiterpenes and acids. Group C7 is characterized by low content of VOCs. This data demonstrates that geographical origin, combined with genotype, also influences the overall polyphenolic and volatile profiles.

Keywords: secondary metabolites, grapevine varieties, GEN-GEO groups, discriminant analysis

P18 – Chloroplasts and targeted nuclear DNA based genetic diversity among grapevine (*Vitis* sp.)

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Abstract

As for grapevine, there are many genetic resources in the Balkan region that have not yet been evaluated at the molecular level. Conventional molecular markers used for genotyping of grapevines have been largely replaced in recent years by HTS techniques that have dramatically expanded the possibilities for identifying allelic variations used in the application for genotyping by multiplexing. Multigene panels can be easily performed with gene-specific target enrichment probes. In this project we developed probes to capture information on key sites in the grapevine nuclear genome: 2271 highly variable SNP loci, 943 sites of the sex locus, 96 GAI1 loci associated with berry traits and phenology, 51 loci associated with resistance, 59 random loci, 312 MYB loci associated with color, and 47 TFL1 loci associated with flowering and phenology. For each polymorphic site, a 120-mer probe was designed with the expected variant in the central position. The panel was designed to genotype most of the diversity in grapevine from Balkan region (370 *Vitis vinifera* genotypes; 124 from Slovenia, 28 from Serbia, 76 from Croatia, 16 from Montenegro, 55 from BIH, 6 from Macedonia, 26 from Greece, 39 from Albania) and 23 *V. vinifera* references from France and 27 *Vitis* sp. species. The obtained data will allow to determine the exact calling of the variants for the evaluation of Balkan grapevines: their true-to-typness, important traits and kinships in the grapevine gene-pool.

For the same group of samples, we performed whole-genome shotgun sequencing to detect DNA variation in chloroplasts and performed sequence alignment and phylogenetic analyses. Analyses were performed at inter- and intra-specific levels to improve previous phylogenetic work that was limited in taxonomic scope or marker choice (Peros *et al.* 2011, Wan *et al.* 2013, Trondle *et al.* 2010,

Lozsa *et al.* 2015) and to improve parental analysis, particularly of grapevine varieties from the Balkans (Stajner *et al.* 2015), using maternally inherited chloroplast variation. Using low coverage DNA-seq, we were able to sequence a grapevine genome with an average coverage of 0.17, while the chloroplast genome reached up to 60-fold coverage, which was high enough to determine reliable SNPs.

Keywords: variability, chloroplast, targeted nuclear DNA, high-troughput sequencing

P19 – 'Roesleria' and the need to breed

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Abstract

The root rotting fungus *Roesleria subterranea* causes serious damage to viticulture in several wine-growing regions in Germany. The ascomycete affects roots and obstructs the water-transport vessels leading to the dieback of vines. No treatment or cure are available and *R. subterranea* is persistant in soil for years even after removing affected vines, leading to infection of newly planted material.

Although there have been various reports of this disease, there are still no reliable estimates of the geographic distribution and the severity in Germany and worldwide. To close this gap of knowledge the spread of *R. subterranea* in three German wine-growing regions (Palatinate, Rheingau and Rheinhessen) was analyzed using aerial photographs. The suspected areas were checked on site for infestation with *R. subterranea*. With this newly developed method, 10 % of the respective regions were examined. All three wine-growing regions are demonstrably affected, but to very different degrees.

All rootstock varieties commonly used in viticulture are affected by the disease and no resistance or defense mechanisms are known to date. Investigation is needed wether less susceptible or even resistant genotypes exist and consequently could be used for breeding.

Therefore, a project was started to test a broad genetic base of wild *Vitis* species from Asia, Europe and America for their response to an artificial inoculation with *R. subterranea*. This wide range of genotypes was selected because the origin of the fungus is unknown and accordingly it is unknown where potential resistances could have co-evolved with the pathogen.

This project lays a foundation for future breeding projects, both, by identifying candidate genotypes for breeding and by providing reliable estimates of infestation to call attention to the "need to breed" to tackle *R. subterranea*.

Keywords: *Roesleria subterranea*, root rot, rootstock breeding, wild *Vitis* species

P20 – Prospection and genetic identification of grape cultivars from old Serbian vineyards

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Abstract

Serbia has a long-standing viticulture tradition. As in other countries of the Western Balkans, current Serbian wine production relies in the cultivation of well-recognized international grape varieties together with some local varieties, such as 'Prokupac', 'Smederevka' (syn. 'Dimyat'), 'Plovdina' (syn. 'Pamid'), or 'Tamjanika Crna'. Nevertheless, many other cultivars can be found across the country, sometimes grown by small wineries. Here, we have performed the largest prospection of local grape cultivars performed in Serbia so far, collecting 163 samples in old vineyards from different viticulture regions across the country for their genetic identification. SSR and SNP marker analyses identified up to 60 different genetic profiles, which were compared to those stored in the *V*IVC and the ICVV databases. This work allowed the genetic identification of 49 grapevine cultivars, most of them autochthonous cultivars from Serbia and other Balkan countries, like 'Braghina Rosie', 'Pamid', 'Prokupac', 'Ruza Bijela', 'Tamjanika Crna', or 'Zacinak'. In addition, we found a considerable number of cultivars from other Eastern regions (like 'Agadai', 'Chaouch Blanc', or 'Parmak Cerven'), and other cultivars of Western origin (like 'Pinot Noir', 'Semillon', or 'Villard Blanc'). Thus, the current Serbian grapevine genetic pool includes a series of indigenous cultivars of local origin, and some exogenous cultivars from different regions that were introduced into Serbia for different purposes. In many cases, these cultivars were found to be grown under local synonymies, like 'Muscat Krokan' to refer to the French cultivar 'Muscat Fleur D'Oranger', or 'Tamjanika Bela' and 'Tamjanika Crvena' to refer to the Italian cultivar 'Moscato Giallo'. Interestingly, we revealed the genetic identity of the variety known in Serbia as 'Jagoda' ('Ferdinand de Lesseps'), locally appreciated for white wine production. In addition, we discovered 11 non-identified genetic profiles, some of them twice in vineyards of different viticulture regions, suggesting they could be old autochthonous varieties on the edge of extinction. Parentage analyses indicated that some of the identified and non-identified genetic profiles have a first-degree relationship with other local cultivars, suggesting that local genetic resources represented an important source of diversity for generating current Serbian grapevine varietal diversity.

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Keywords: Genetic resources, Genotyping, Grape diversity, SNP, SSR, *Vitis vinifera* L.

P21 – Development of cultivars with fast fruit development suited for growing in Northern Europe

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Abstract

For the first time breeding of grapes for a growing region as cold as in Northern Europe including Scandinavia has been initiated. The breeding is established in a cooperation between a small private breeding company 'FastGrapes' established by Toldam-Andersen, T. B. and the Julius Kühn Insitute. The cultivar 'Solaris' was introduced in 2003 and has quickly established as the dominant cultivars in especially Denmark and Sweden, but is even grown in Norway. In Denmark it uses in average 95 days from flowering to harvest maturity (based on contry average of the last 15 years). Harvest maturity, is in average reached by 1st October (+/- 15 days). This is an ideal time of maturity, as high quality grapes can be harvested every year. The successful growing of Solaris means it now represents approx. 40% of the commercial viticultural area in Denmark. In total 200 ha is now grown, and the area show exponential growth. Thus, in the new breeding programe, we use Solaris as reference cultivar. The work was initiated in 2020 where about 5000 seedlings at JKI Geilweilerhof was screened 15-18 August. 40 potential genotypes were identified from which 25 was selected for propagation and further testing. In 2021 an additional 2850 seedlings were screened adding 4 new selections to the list. Selection in further seedling fields will be performed in the coming years. In addition to time of maturity an array of parameters are evaluated (yield components, growth habit and disease tolerance). Depending on the crossing partners used it appears between 0,5 and 2 % secregates out as fast maturing genotypes. The genetic background of the seedling fields is complex including several *Vitis* species used in almost 200 years of breeding. Resently also a breeding line from cooperation between INRA and JKI is utilized. It bring in new resistance genes from Muscadinia. As a result, multiple resistance loci is found in the selected FastGrapes breeding lines against downy and powdery mildew. From the mother plants as many plants as possible are propagated (in average 40) in order to plant test plots in 6 Danish locations, 2 Swedish and 1 in Northern Germany in addition to JKI in Siebeldingen. A 'fast track' strategy has been developed, in which cultivar candidates are selected after two years of cropping (year 4 after planting). The candidates are then multiplied and planted in 5 locations with 200 of each in each place in order to allow for larger scale enology test. Final selection for variety nomination is made after 2 years of harvest. The fast track procedure allows new cultivars to be developed in 10 years after selection in the seedling fields.

Keywords: Robust cultivars, short development, resistance

P22 – FEMVitisDB: a FAIR data management system for data integration in grapevine

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Abstract

A grapevine germplasm collection including more than 2,000 unique genetic profiles, distinguished by their origin and/or distinctive phenotypic characteristics, is maintained and preserved at the Fondazione Edmund Mach (FEM, Italy). Each genotype is represented by 1-20 accessions. New acquisitions from repositories around the world and wild collections consist of released or naturalized *Vitis* hybrids as well as *Vitis* species, useful to scout new genetic resources for diversity studies and breeding purposes (wine/table grapes and rootstocks). Most accessions were genotyped with the reference set of 9 microsatellite markers to verify their trueness-to-type against international and national databases. Moreover, the entire germplasm collection has been phenotyped during three successive years for several traits related to ampelography, vine development (e.g. phenological stages, fertility), biotic stress response, berry and wine composition (e.g. chemical parameters). In parallel, crossbreeding programs have been carried out to obtain new genotypes coupling winemaking quality and resistance to different biotic stresses.

We developed the FEMVitisDB database to host the above information and that produced by the current and future grape breeding programs. This infrastructure is focused on the "FAIR" paradigm which emphasizes the importance of organizing data and metadata. The infrastructure core is a PostgreSQL database compliant with the MIAPPE (Minimum Information About a Plant Phenotyping Experiment) standard. The database can host both genotyping and phenotyping data allowing the storage of heterogeneous datasets including dense time series and high throughput genotyping data. The architecture is ontology driven (i.e. the semantics of all the terms can be specified by a suitable ontology) to provide a powerful system to integrate data originating from different experiments and platforms. Where feasible, the OIV code - as described in the Vitis ontology - has been applied for the standardization of the phenotype description; alternatively, the grape or plant ontologies have been used. The database is integrated with standardized RESTful Web Service API based on BrAPI (brapi.org) which provides access to the data. The data management system is completed by the BrAPIViewer, a web frontend returning all the necessary information in a user-friendly way.

Keywords: digitalization, data standardization, germplasm valorisation, genotyping, phenotyping

P23 – *Vitis vinifera* phenotyping by NIR spectroscopy and chemometrics

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Abstract

The phenotype of any plant is an expression of its genotype that is influenced by interactions with the environmental context. Plant phenotyping is conventionally performed with sampling methods that are costly, labor-intensive, time-consuming, and destructive. In modern plant phenotyping, several non-destructive techniques have been developed with numerous advantages over the traditional ones. In this preliminary study, a multivariate analysis combined with near-infrared (NIR) spectroscopy was employed to classify grapevine leaves. We analyzed two commercially important varieties of table grape, namely Red Globe and Sugraone collected from vines of equal age. Both varieties were grown in the same vineyard (Southern Italy) and were subjected to the same field treatments, ensuring identical pedoclimatic conditions. After collection, the samples were left with the stem immersed in water for 1 hour before the NIR spectrometric analysis (wavenumber range of 4000–10,000 cm^{-1}). The acclimatization step was performed to compensate for different solar exposure since samples were collected over a large time frame. To achieve a full characterization of the whole leaf, six points on each leaf face (three on each side) were measured. Leaves of various ages (young, intermediate, and mature) from different grapevines were collected for each variety. The analysis showed a difference in the NIR spectra of the two leaves' faces. The spectra of the lower faces were selected to perform a discriminant analysis between the varieties since showed a better discrimination capacity. Several pre-treatment techniques including Standard Normal Variate (SNV) and smoothing were compared, aiming to eliminate unnecessary information in the spectra and amplify relevant variations. The following classification techniques were compared: Linear Discriminant Analysis (LDA), Classification and Regression Trees, k-Nearest Neighbors (K-NN), Support Vector Machines (SNV), and Random Forest. The best fit model on the test data set was obtained with an LDA based on a principal component analysis (PCA) selection of SNV pretreated NIR spectral data. The discrimination ability of the NIR technique could thus be used as a tool for fast variety recognition. In the ongoing analysis of different grape varieties growing in our experimental vineyards, we plan to further verify and improve the discriminating capacity of the technique. In the context of precision agriculture or digital farming, this work shows an example of how the application of chemometric methods could be effectively used tosupport agronomic decision making.

Keywords: leaf, *Vitis vinifera*, NIR.

P24 – Assessing the impact of organic soil amendments on grapevine vigor using sensor-based phenotyping

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Abstract

Soil organic amendments are known to have severel beneficial effects on soil fertility by improving, for instance, water holding capacity or nutrient availability. Furthermore, the incorporation of organic amendments into soils might also contribute to climate protection goals by reducing carbon from the atmosphere. A slow degradation of such amendments can be expected especially in the subsoil through physical isolation and the binding of organic carbon to minerals. Vineyard soils are especially suitable for long-term carbon storage, since deep tilling is performed once before planting a new vineyard followed by a long resting periode of the subsoil. This is why two organic amendments (greenwaste compost and biochar compost substrate) were incorporated in 30 – 60 cm depth in a vineyard prior to planting with the fungus-resistant cultivar 'Calardis Musqué'. The impact of this approach on grapevine vitality and grape quality was assessed around veraison and harvest during three consecutive years using several sensor-based applications. For the analysis of different leaf parameters, two non-imaging sensor (spectroradiometer (VIS-NIR-SWIR) and chlorophyllmeter) were applied focusing especially on chlorophyll content as an important plant stress indicator. Thereby, a high correlation between sensor and ground-truth data could be confirmed making sensor-based analyses reliable for practical application. In this study, chlorophyll content did not vary significantly between plants grown on the two organic amendments. However, significant differences between plants grown on organic amendments and control plants (no amendment) could be observed with control plants having lower chlorophyll contents. This indicates that the incorporation of the two organic amendments does not affect this parameter negatively. Furthermore, vines' position within a vineyard can have an important influence and should therefore not be neglected in analyses. Preliminary results further imply differences in resilience to *Botrytis* bunch rot and grape quality parameters depending on the respective substrate. However, grapevines are typically grown for several decades and soil conditions change rather slowly making long-term assessment of this vineyard necessary for a holistic evaluation.

Keywords: plant phenotyping, grapevine vitality, grape quality, precision viticulture, chlorophyll, *Botrytis*, *Vitis vinifera*

P25 – Phenotype *versus* genotype

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Abstract

The classic resistant genes were evaluated by visual phenotyping and marker assisted selection. For downy mildew the genes *Rpv1*, *Rpv3.1* and *Rpv3.2* as well as *Rpv10* and *Rpv12* were compared. For powdery *Run1*, *Ren3* and *Ren9* and *Ren4* have been analyzed. Big differences occurred in the situation of the vineyards and the climatic conditions. The choice of the *Vinifera* partner plays a significant role. Different fungi isolates altered the results. Resistant genes interact together and change their expression.

Keywords: phenotype, genotype, selektion, downy mildew, powdery mildew, fungi isolates

P26 – Genetic variability of grapevine vegetative development parameters as described with LiDAR data and associated quantitative trait loci

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Abstract

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

Keywords: grapevine, pruning wood, leaf area, LiDAR, QTL

P27 – High-throughput phenotyping of water functioning and carbon metabolism in grapevine

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Abstract

Grapevine production and wine quality are affected by climate change, and identifying varieties adapted to climate change becomes an urgent necessity. Searching for genotypes that are more water-efficient and able to maintain high photosynthesis in drought condition seems to be the key to maintain production while preserving vineyards on the long term. In order to evaluate the genetic variability for these traits, it is a key requirement to phenotype hundreds of genotypes in the vineyard. However, this is often hampered because conventional methods for measuring water and carbon related traits are typically expensive, destructive, and not usable at high-throughput. In this project, we aimed at developing and testing new high-throughput phenotyping methods, based on the use of NIRS (Near InfraRed Spectroscopy) and leaf chlorophyll fluorescence, for studying the genetic variability and determinism of functional traits in grapevine. We used two populations, one resulting from a semi-diallel cross (600 genotypes), the other corresponding to a diversity panel of *279 Vitis vinifera* varieties. In this "calibration" phase, we combined, on a subset of genotypes, conventional low-throughput measurements of photosynthesis, stomatal conductance, leaf water potential, nitrogen and carbohydrates content, with fast leaf measurements using porometry/fluorimetry and NIRS. We used these datasets to calibrate models to predict the traits of interest from NIRS and porometry/fluorimetry, based on PLS (Partial Least Squares) regressions. In the next phase of the project, we are deploying the high-throughput methods alone on the whole populations, in order to predict the traits of interest using models established in the calibration phase, and to further analyse the genetic determinants of these traits with Genome Wide Association Study (GWAS).

Keywords: Phenotyping, high-throughput, near infrared spectroscopy, wineyard, drought, photosynthesis, transpiration

P28 – Using near-infrared spectroscopy to quantify quality-determining sugars and acids in grape must

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Abstract

The most useful quality-determining factors for grape must are sugars and acids due to their importance for the production of high quality wine. Moreover, German wine law divides different categories according to the sugar content of the must depending on the EU defined wine-growing zones. Therefore, in most cooperatives the sugar content in particular is one basic factor for payment.

Winegrowers have the possibility to manually measure the sugar and acid contents in their vineyards prior to harvest. However, these measurements are individual observations and do not represent the whole plot. Cooperatives check the quality of berries upon delivery for payment and further cellar logistics. An early detection of must characteristics prior to delivery on a grape harvester for example would improve the harvest and cellar logistics in big cooperatives.

While there are already many applications for near-infrared spectroscopy to detect ingredients in biological samples, there is still no applicable detector for determining ripeness in viticulture. To close this gap, an apparatus was built and tested in the laboratory. It consists of a near-infrared sensor and a flow cell, recording spectra in the range of 1100 nm to 1350 nm of grape must. This must was collected from 100 berries from each of four different red and white *Vitis vinifera* (L.) varieties from different vineyards. The spectra were acquired throughout the growing season in 2021 in Rhineland-Palatinate (Germany). Reference values for the main sugars (glucose, fructose), as well as acids (malic-, tartaric acid) were determined with high-performance liquid chromatography.

Using chemometric methods, i.e. partial least square regression and leave-one-out cross-validation, it was possible to estimate root mean square prediction errors (RMSEP) of 4.96 g/l and 0.60 g/l and a determination coefficient (R^2) of 83% for sugars and acids, respectively. Coefficient of determination and RMSEPs were higher for red varieties compared to white varieties for both substance classes. This high prediction accuracy could enable automatic ripeness measurements during the harvesting process on the harvester, thus reducing the workload during harvest and offering more planning security.

Keywords: grapevine, spectroscopy, NIRS, ripening, quality, harvest

P29 – Image-based phenotyping pipeline enabling an environment-independent screening for sunburn resilience of grapes based on a controlled heat stress-induction in the lab

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Abstract

Sunburn of grapes is an abiotic stress reaction triggered by high temperatures and solar radiation during berry ripening phase. Climate change with increasing risks for such high temperature events (> 40°C) may lead to sunburn with corresponding damages and yield loss. In 2019 and 2020, heat records were recorded in Germany and depending on the genotype, massive sunburn damages were observed in the experimental vineyards of JKI Geilweilerhof. Therefore, the present study aims at developing an environmental-independent, image-based tool with the capacity to objectively screen breeding material and mapping population regarding sunburn resilience and its application in QTL analysis. Berries from field grown plants were collected with intact pedicels (BBCH 75) and were placed on black grid boards providing space for 80 berries. RGB images were taken from each plate after a heat stress (HS)-treatment. From previous studies, it was known that the degree of brownish sectors on the skin of treated berries can be used as indicator for sunburn susceptibility. The proportion of healthy green tissue versus symptomatic tissue was extracted per berry image applying a semi-automated Matlab tool. The investigated genotypes showed significant differences in the appearance of browning, thus different degrees of sunburn susceptibility are expectable. For validation, results were compared with corresponding field scorings of sunburn damages (2019 and 2020). These data confirmed that genotypes with a susceptible phenotype in the field (high degree of grape sunburn) show high degree of browning due to HS. The other way around, genotypes with resilient phenotypes in the field showed no or very low degree of browning after the treatment. The screening of the mapping population Calardis Blanc x Villard Blanc, segregating for sunburn resilience, revealed one preliminary QTL (HS resilience) and thus is the starting point to identify genetic regions and regulators with influence on sunburn resilience. This opens the perspective to include an early selection by marker-assisted selection (MAS) for resilience of grapes towards abiotic damages in breeding programs. However, at present we do not exclude additional factors (e.g. water deficit, solar radiation) contributing to sunburn in the vineyard, but surly HS is one major contributor.

Keywords: abiotic stress resilience, climate change, objective screening, High-throughput phenotyping, QTL analysis, sunburn

P30 – Characterization of grapevine resistance to downy mildew using hyperspectral imaging in SWIR spectral range

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Abstract

Downy mildew (DM) caused by the oomycete Plasmopara viticola is a serious disease in viticulture, which causes high economical losses. Currently repeated applications of fungicides and copper are used to avoid massive damages or total losses. Beside the environmental pollution, fungicide resistances are frequently found in P. viticola populations. Therefore, the introgression of resistances is a major task for grapevine breeding programs.

While molecular genotyping methods are well established, the methods for the characterization of phenotypic expression are still labour- and time - consuming. *Rpv3* and *Rpv10* carrying varieties are known to differentiate in the accumulation of stilbenes. These differences contribute to the described differing level of resistance, while stilbenes like resveratrol, pterostilbene and viniferins play a crucial role in the defense reaction of grapevine to downy mildew. While proceeded DM infections can be detected, using a simple RGB-imaging workflow, hyperspectral imaging in the short-wave infrared (SWIR) range is a promising tool to detect and possibly quantifiy biochemical components, like e.g. stilbenes. These non-destructive and fast insights in plant's changed metabolism due to an infection with P. viticola allows on one hand a high throughput and can help on the other hand, gaining a better understanding of resistance mechanisms by enabling the measurement of resistance kinetics in time series. Both aspects are highly demanded in grapevine breeding and research.

The objective of the present study was to develop a SWIR-imaging based method for the differentiation of varieties with (I) no resistance locus, (II) *Rpv3*, (III) *Rpv10* and (IV) *Rpv3* and *Rpv10* loci. Our assumption is a stilbene-associated resistance reaction due to *Rpv10*. To test the performance of the hyperspectral sensor, resistance response of leaf discs was induced by UV-C radition on one hand and artificial inoculations on the other hand. HPLC-MS based reference data were used to model stilbene concentrations in leaf discs, using artificial intelligence based algorithms. These neural networks allow to group the tested varieties into different resistance levels and due to their intelligent and learning ability nature are promising tools for studies on an extended set of varieties in further research.

Keywords: Hyperspectral imaging, resistance, downy mildew, grapevine

P31 – Real-time high-throughput monitoring of grapevine berry ripening and development with near-infrared spectroscopy

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Abstract

It becomes particularly urgent to decipher the physiological mechanisms underlying the impact of climate change on berry ripening and to select new genotypes keeping an adequate balance between sugars and acids despite the increase in summer temperature. Classical genetic studies consist in studying the diversity of traits of interest, possibly in response to constraints, in order to identify alleles of interest. This type of study is particularly complex to implement regarding grape composition, which displays marked developmental changes during berry ripening, this phenological variation being itself genetically controlled. It is therefore essential to analyze berries from various varieties at the same developmental stage, so that the differences observed are not due only to differences in maturity level. This is further complicated by the noticeably asynchronous development of berries. In this context, it is crucial to develop non-destructive tools for monitoring ripening and berry development at high-throughput. Here, we report the use of near-infrared spectroscopy (NIRS), using a portable device in the field, to study the accumulation of sugar and acids in berries of four grapevine varieties. We sequentially acquired spectra on single berries from 50 clusters all along ripening, from the green stage to over-maturity, collecting a subset of these berries weekly, for quantifying sugar and acid concentrations with HPLC. We used the resulting data to train calibration models between spectra and sugar or acid concentrations, which proved to be quite accurate within cross-validation settings. These models were further applied to predict sugar and acid concentration on the berries that were followed with NIRS but not collected for HPLC measurements. This enabled the reconstruction of developmental trajectories of individual berries during the whole ripening period. These results pave avenues for genetic and physiological studies of berry ripening which are critical for selecting and developing new varieties in the context of climate change.

Keywords: Phenotyping, near infrared spectroscopy, single berry, ripening, sugar, acids

P32 – Improving high throughput sensor-based data acquisition of breeding material

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Abstract

Grapevine is a perennial woody plant whose characteristics, in addition to marker-assisted selection (MAS) on resistances, are mainly recorded in the field during the breeding steps. For material selection in breeding long-term observation and recording of the overall vitality, yield, phenology, growth architecture, resistance against diseases, and quality is done. However, the time window for recording characteristics during the growing season is narrow and the evaluation capacity is limited due to manual assessment. Thus, phenotypic characteristics cannot be documented in detail for early breeding stages for the large number of breeding material, which leads to a lack of information about the selected genotypes and later no retrospective data analysis of individual genotypes is possible. Therefore, a novel high throughput determination system is needed to increase the efficiency of phenotyping and to optimize data management within the breeding program. In the present study, the expanded phenotyping platform “Phenoliner 2.0” and the steps to develop such a phenotypic pipeline for breeding are shown. The platform, which is equipped with a prism-based simultaneous multispectral camera system consisting of a visible color channel and two near-infrared channels, was developed and tested in the vineyard to acquire images and spectra of grapevines. Compared to the previous version, the speed of data acquisition has been improved from one kmh to four kmh. The assignment of the sensor data is based on GPS information of the vine locations, which are recorded by a real-time kinematic GPS receiver mounted on the phenoliner. With the help of a plant detection algorithm, the absence of vines can be recognized and documented automatically. In order to develop the image analysis pipeline for the main phenotypic breeding traits: (1) vitality, (2) yield and (3) wood maturity, the system needs to be trained to distinguish among different structures of the grapevine (leaf, grape bunch, and stem) and between healthy and diseased structures. For this purpose, the recorded images were segmented and manually annotated. So far, the annotation software developed in this study is able to automatically recoginze leaf and grape bunch structures in the images and videos. To improve data management, the images and their results could be stored and linked to individual genotype metadata, such as breeding number, planting years, and crossing parents. The system can be applied to map phenotypic parameters of an entire vineyard. The information on vitality, yield, ripening, and resistances is important for further breeding steps and viticulture.

Keywords: grapevine, phenotyping, sensor, data management, annotation, healthy, diseased

P33 – Hormonal changes in tolerant and susceptible grapevine leaves under powdery mildew infection

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Abstract

The biotrophic fungus *Erysiphe necator* causes Powdery Mildew (PM) in grapevine. Phytohormones are major modulators of defensive responses in plants but the analysis of the hormonome associated with grapevine tolerance and susceptibility against this pathogen has not been elucidated. In this study, changes in hormonal profiling were compared between a tolerant (*Vitis rupestris × riparia* cv. 101-14 Millardet et de Grasset) and a susceptible (*Vitis vinifera* cv. Aragonêz) species upon *E. necator* infection. Control and PM-infected leaves were collected at 0, 6, 24, 96 hours post-infection (hpi), and analysed through LC-MS/MS. The results showed a distinct constitutive hormonome between tolerant and susceptible species. Constitutive high levels of salicylic acid (SA) and indole-3- acetic acid together with additional fast induction of SA within the first 6 hpi as well as constitutive low levels of jasmonates and abscisic acid may enable a faster and more efficient response towards PM. The balance among the different phytohormones seems to be species-specific and fundamental in providing tolerance or susceptibility. These insights may be used to develop strategies for conventional breeding and/or editing of genes involved in hormonal metabolism aiming at providing a durable resistance in grapevine against *E. necator*.

Keywords: *Erysiphe necator,* grapevine, hormones, powdery mildew, tolerance, susceptibility

P34 – Early remote detection of downy mildew on grape vine by fluorescence methods

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Abstract

Pathogenic fungi severely threatens the annual yield of grapes in quantity and quality. Therefore, viticulture requires intensive fungicide applications, compared to other crops. Aimed at a reduction of fungicide input, different techniques and combined strategies are applied in viticulture. To successfully reduce losses in yields, early detection techniques are required. In this work we present premeasurements to remotely detect a pathogenic fungus on the leaves of *Vitis vinifera* (Müller-Thurgau). We were able to detect a leaf infection with *Plasmopara viticola* (causal agent of downy mildew) on potted vines within the first week after inoculation. Our results could be reproduced over multiple samples and different methods. Fluorescence mapping was used to monitor the blue-green fluorescence and chlorophyll fluorescence behaviour over a wide area of excitation wavelengths. Based on this, spectral data of fluorescence emission was recorded via fluorometric measurements with excitation at different wavelengths in the visible and UV range. Furthermore, we used laser-induced fluorescence (LIF) and hyperspectral imaging to verify our results. Additionally, we combined hyperspectral imaging with LIF. The evaluation was based on the ratio of blue fluorescence to far-red fluorescence (BFRR_UV) and normalized difference vegetation index (NDVI), which represent an established method in many agricultural applications. Eventually, we evaluated the scalability of our methods for long term measurements. Our results form a fundamental approach for the design of a laser-based stand-off detection system for the practical application in vineyards. Such a system can serve as a model technology for early detection of pathogenic diseases and opens a window for early countermeasures. As mildew infection is considered the most damaging disease in European viticulture, these early countermeasures could greatly reduce the environmental and economic cost associated with fungicide application.

Keywords: remote detection, fluorescence, LIF, hyperspectral imaging, grapevine, pathogenic fungi, downy mildew

P35 – Multi-site QTL analysis of rootstock-scion interactions across varied climates

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Abstract

What is the genetic basis of rootstock modulation of scion phenotypes? Rootstock and environment contribute to scion phenotypic variability. Understanding and improving the genetics of rootstock scion interactions is essential to maintain sustainable grape production, particularly in the face of climate change. To determine the genetic underpinnings of rootstock affects on scion phenotype, a multi-site QTL mapping study was established. An F1 rootstock mapping population (200 individuals) derived from *V. rupestris* ('B38', seed parent) and *V. riparia* ('HP-1', pollen parent) was studied as ungrafted F1 vines and grafted with a common scion 'Marquette'. Contrasting phenotypes between these species include differences in leaf senescence, cold acclimation, dormancy length, budbreak date, sprawling versus climbing habitat, lateral versus primary meristem dominance, deep versus shallow rooting, fruit ripening, leaf shape, leaf ion concentration, and gene expression. To understand genotype-by-environment effects and rootstock-mediated scion plasticity, the segregating rootstock population was clonally replicated and the ungrafted and grafted populations were planted in a common garden arrangement in three climatically diverse regions of the US (New York, South Dakota, Missouri). Morphological, physiological, berry metabolite, leaf ionome and carbon isotope, and transcriptomic traits are explored to understand genetic and genotype by environment effects modulating scion phenotypes. An integrated GBS and rhampSeq map was used to identify the genetic basis of rootstock-scion interaction and amplitude of scion phenotypic modulation.

Keywords: QTL, rootstock, *V. rupestris*, *V. riparia*, phenotype, GxE

P36 – *VviNAC61* promotes molecular processes associated with late grapevine organ development

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Abstract

NACs (NAM/ATAF/CUC) are plant-specific transcription factors (TFs) and represent an interesting family due to their key role in plant development processes and stress responses. Among the 74 *Vitis vinifera* family members, *VviNAC61* was selected for functional characterization as one of the key candidates of the major transcriptome reprogramming occurring during grapevine development and for its role as a molecular marker of the grape postharvest withering process. *VviNAC61* is particularly expressed at veraison and post-veraison berry stages and shows a high expression also in lignified tissues such as roots, tendrils, seeds and rachis. In leaves, *VviNAC61* achieves its higher level of expression in the senescence stage; in line with this observation, a NAC family multispecies phylogenetic tree revealed that VviNAC61 clusters with VviNAC33 and AtANAC046, both involved in the senescence processes. The effect of *VviNAC61* over-expression was investigated through the agroinfiltration of *N. benthamiana* leaves, showing clear wilting symptoms and altered stomatal conductance. A transcriptomic analysis was performed on cv. 'Sultana' leaves transiently over-expressing *VviNAC61* and a search of TF-bound genes by DNA Affinity Purification and sequencing (DAP-seq) was performed to narrow down the list of VviNAC61-regulated candidate genes. The "secondary metabolism", "transcription facor regulation" and "oxidative stress" functional categories were found as mainly enriched among candidate target genes. More in detail, several laccases and genes encoding for the phenylpropanoid pathway-related enzymes, senescence-related genes and several *MYB* and *NAC* genes (including *VviNAC61*) were bound in their promoter region by this TF. The control of the stilbene synthase regulator *VviMYB14*, the chlorophyll degradation regulator *VviSGR1*, as well as the *VviNAC61* self regulation were validated through the luciferase assay. As last, the upstream regulation mechanism of VviNAC61 was further investigated demonstrating that VviNAC61 is controlled and interacts with CARPO (VviNAC60), a previously characterized master regulator of grapevine organ maturation.

Keywords: NACs, VviNAC61, cistrome, late organ development

P37 – Basal defense - variations in the EDS1 promoters of *Vitis vinifera* cv. 'Cabernet Sauvignon' and *V. aestivalis* cv. 'Norton' may define specific EDS1 expression pattern and influence associated salicylic acid signaling

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Abstract

In grapevine, the defense regulator *ENHANCED DISEASE SUSCEPTIBILITY* (*EDS*) is present as a multi-member gene family and plays a role in basal defense in plants. *EDS1* is highly conserved and functional in both, *Vitis vinifera* cv. 'Cabernet Sauvignon' and *Vitis aestivalis* cv. 'Norton'; hence, the regulation of *EDS1* - regarding tissue specificity and transcript level - may be a key for powdery mildew (PM) resistance in cv. 'Norton', while cv. 'Cabernet Sauvignon' is susceptible. Besides PM resistance, also salicylic acid (SA) signaling and the degree of accumulation in leaves is thought to be associated with *EDS1* expression. By preliminary analysis the SA content was determined in leaves of several resistant and susceptible accessions from both species, *V. vinifera* and *V. aestivalis*, which corroborates a tendency that SA accumulation and PM resistance may be linked. Further, the putative promoter sequences of the *EDS1* genes (pEDS1) from cv. 'Cabernet Sauvignon' and cv. 'Norton' were bioinformatically analyzed with the Genomatix software suite to predict transcription factor binding sites (TFBS) and higher order regulatory models. The analysis revealed that the promoter sequences contain some conserved but also highly variable regions as well as a different set of TFBS and regulatory models. In pEDS1 from cv. 'Cabernet Sauvignon' higher order models containing AHBP, DOFF, L1BX, MYBL, OPAQ and WRKY motifs are predicted. In contrast, in pEDS1 from cv. 'Norton' other putative models are present containing OPAQ and DOFF motifs only. The variations within the regulatory regions of pEDS1 are presumably responsible for specific EDS1 expression and associated SA signaling.

Keywords: powdery mildew resistance, EDS1 gene regulation, transcription factor binding sites, salicylic acid

P38 – Reaction of PIWI cultivars and selections to Ripe rot during grape physiological maturation in south of Brazil

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Abstract

The southern regions of Brazil conditions favor the occurrence of diseases, due to the presence of temperature and rain-induced diseases, which hamper the quality of the grapevine harvest and the productivity. Although genetic breeding developed cultivars that carries out resistance alleles to downy and powdery mildew, called PIWI varieties, but not yet to ripe rot of grape. Ripe rot is caused by several species of *Colletotrichum* and exhibit rotting on ripe fruits, which directly degrades the quality of the wine or require early harvest. Therefore, the objective of this work was to evaluate the incidence and severity of ripe rot of grape in different PIWI cultivars and selections. The work was carried out in the experimental stations of Epagri, Videira, and UFSC, Curitibanos, both in Santa Catarina; under periodic monitoring of average, maximum and minimum air temperatures, rainfall and relative humidity. All cultivars and selections used come from modified backcrossing, with 90% of *V. vinifera* genome. The incidence (I) and severity (S) of the ripe rot were performed at 2018/2019 and 2019/2020 harvests on five clusters of two randomly chosen plants, according to a diagrammatic scale of rot in vine clusters. A randomized block design was applied, with data normality using the Shapiro-Wilk test and Analysis of Variance (ANOVA), in the R 4.1.2 software. In Videira, the average temperature was 2°C and precipitation was 20-30% higher than in Curitibanos. In both years and locations, the relative humidity always was above 75%. Although distinct behavior of the PIWI cultivars and selections to the incidence and severity, all genotypes showed symptoms of ripe rot in both vintages and locations. While cultivars Sauvignon Blanc and Bronner showed the highest susceptibility, Gf.2004-043-0015 and Gf.2004-043-0024 showed the lowest susceptibility to ripe rot. Cultivars Regent, Baron, Calardis Blanc, Felicia, Bronner and Prior showed intermediate susceptibility.

Keywords: *Coletotrichum* spp., disease resistance, Ripe rot of grape, grapevine, *Vitis vinifera.*

P39 – How strong is the influence of different nitrogen sources on iron uptake of grapevine rootstocks?

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Abstract

Grapevine (*Vitis vinifera L.*) is the most economically important deciduous fruit crop in the world. Iron (Fe) is an essential micronutrient for plant development since its active form is involved in several biochemical processes, e.g., chlorophyll synthesis. The uptake of the elements depends on individual elements' interactions. Iron (Fe) nutrition of plants can be significantly affected by different nitrogen (N) forms through altering the uptake ratio of cations and anions, and changing rhizosphere pH. It is known, that most crop species grow best with access to nitrate (NO_3^-) as well as ammonium (NH_4^+) nitrogen, but little attention has been paid to changes in iron uptake under supply of different nitrogen (N) forms. The presented study aimed to decipher the specific interaction of different nitrogen (N) forms on Iron uptake of different grapevine rootstocks on the physiological as well as on the biochemical and molecular level. Four NH_4^+: NO_3^- ratios (0:1,1:5, 1:3, 1:1) were tested with a modified half-strength Hoagland solution with and without FeNa(III)-EDTA, summing up to eight treatments within a hydroponic system (Kick-Brauckmann, 7.5 L) under semi-controlled climatic glasshouse conditions in 2021. Rooted woody cuttings of the rootstocks Fercal (*V.berlandieri* x *V. vinifera*) and Couderc 3309 (*V. riparia* x *V. rupestris*) were used for the experiment. The results could differentiate iron deficiency effects, nitrogen form effects and rootstock effects. Biomass of leaves was negatively influenced by iron absent in both rootstocks with nitrate as only N source. This effect was observed for 3309C for all NH_4^+: NO_3^-ratios, while with Fercal the biomass of leaves increased with increasing amount of NH_4^+. A similar trend was overserved for root biomass, promoting in Fercal with increased NH_4^+ amount and not response in 3309C. The efficiency of the photsystem II (Y(II)) decreased under iron deficient conditions in both rootstocks showing the strongest effect with only nitrate as N source especially with rootstock 3309C. Increasing ammonium levels in the nutrient solution lead to a higher pH reduction by the rootstocks probably enhancing the Fe^{2+} availability and uptake. The ferric chelate reductase (FCR) activity was specifically increased in Fercal under iron-deficient conditions, while no effect was observed for 3309C in comparison to control plants. These first results of the study indicate that rootstocks differ in their preference on both the physiological and molecular level depending on the nitrogen form and in interaction with iron deficiency stress. In a next step the molecular mechanism will be analysed.

Keywords: grapevine, rootstock, nitrate, ammonium, iron, ferric chelate reductase

P40 – The grapevine Pectin Methylesterases gene family and its involvement in *Botrytis* bunch rot control

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Abstract

Plant Pectin Methylesterases (PMEs) represent a group of tissue-specific and developmentally regulated proteins. The gene family is involved in the plant cell wall (CW) remodelling process, by the control of the degree of cell wall pectin methylesterification. Pectin methylesterification also influence the susceptibility to pathogens as *Botrytis cinerea* (*Bc*), a necrotrophic fungus responsible of the *Botrytis* bunch rot in grapevine. In *Botrytis,* PME as well as other CW degrading enzymes have been identified as virulence factors. To further characterize the *PME* gene family and its role in the *Botrytis* bunch rot, the latest genome assembly and annotation were revised and through sequence homology search, a total of 63 PME domain containing proteins were identified, 16 more than a previous identification in grapevine. The *in-silico* analyses of the family by means of the *Vitis* gene expression database VESPUCCI as well as Aggregated Gene Co-expression Network approach (AggGCNs) allowed us to identify and enrich gene co-expression modules and build gene co-expression networks. Interestingly, one of the co-expression modules showed a high modulation in presence of *Botrytis cinerea* infection and particular attention was paid to it. To investigate the contribution of the genes of that module, their expression level in different organs and developmental stages from two grapevine cultivars with divergent *Bc* susceptibility was investigated. Furthermore, berries were artificially infected with *Bc* at mature stage to evaluate *PME* gene expression level and their possible role in the grapevine bunch rot susceptibility. The results obtained contribute to characterize the grapevine *PME* gene family and the role of specific members in the grapevine-*Bc* interaction and to select *PME* genes candidate to the control of *Botrytis* bunch rot in grapevine.

Keywords: grapevine, pectin methylesterase, cell wall, *Botrytis cinerea*, bunch rot, gene expression, co-expression, network analysis

P41 – DAP-Seq analysis on MYB108A/B transcription factors identified candidate target genes involved in anther development and biotic stress response in grapevine

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Abstract

Since the usability of a crop pass throught the full comprehension of the genetic mechanisms at the base of flower and fruit tissues development, which in grapevine represent the economically important part, we think it is pivotal make clear the molecular occurrence defining gene expression. For this purpose, we produced a floral expression atlas using an RNA-Seq approach isolating the absolutely and highly specific genes for each tissue using a τ and WGCN analysis. Amongst all the results, we focused attention on those transcription factors specifically expressed in each floral whorl. Of particular interest was *VvMYB108A,* a gene expressed exclusively in anther tissues before anthesis. This gene, which is paralogous of *VvMYB108B* and orthologous of the *Arabisopsis* gene *MYB108*, seems to be involved in male fertility and stamens development by controlling pollen viability, filament elongation and anther dehiscence. Moreover, *MYB108* was shown to be involved in plant-pathogen relationship during *Botrytis cinerea* infection. In order to identify the gene targets of *VvMYB108A/B,* we took advantage of a novel NGS tecnique, namely DAP-Seq (DNA-Affinity Purifcation Sequencing), able to identify all the genomic regions bound by a given transcription factor. Results were crossed with gene coexpression networks already available on public repositories. *MYB108A* and *MYB108B* overexpression in tomato and *Arabidopsis* plants together with dual reporter luciferase assays are now in progress aiming to functionally characterize these genes and to validate results obtain by DAP-Seq.

Keywords: grapevine, NGS, flower, *Botrytis cinerea*, MYB

P42 – Resistance variation to necrotrophic and biotrophic foliar pathogens in American hybrid grape cultivars

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Abstract

Most Northeastern US viticulture cannot rely on the European grape species (*Vitis vinifera*) due to its lack of cold and disease resistance. The Northeastern US is a center for grape biodiversity. In parallel with this host diversity, a myriad of foliar diseases infect grapes in the Northeast. Crosses of native American grape species (e.g. *Vitis riparia*) with *V. vinifera* are cultivars with a range of cold and disease resistance that have potential for Northeastern viticulture. While we expect the American hybrids to have acquired some disease resistance from the American lineages, reports of disease resistance are inconsistent. More specifically, whether there is a relationship between resistances to necrotrophs and biotrophs is unknown. Quantifying resistances and understanding resistance interactions are essential to breeding effort and integrated pest management. We quantified how disease resistances vary across cultivars and the relationship between resistances to different pathogen types (necrotrophs and biotrophs).

In 2021, we created a collection of grape leaf necrotrophs from cultivated and wild grapes from Massachusetts. Based on sequencing, most necrotrophs belonged to the genera *Botryosphaeria*, *Colletotrichum*, *Diaporthe*, and *Didymella*.

In the laboratory, we quantified host susceptibility to necrotrophs using leaf disc assays. Pathogenicity of a subset of three necrotrophs in the genera *Colletotrichum* and *Didymella* was measured on four hybrid grape cultivars. Regardless of cultivars, *Colletotrichum* caused larger necroses than *Didymella*. Resistance to necrotrophs also varied according to cultivar, ‘Vidal’ was the most resistant followed by ‘Frontenac’, ‘Riesling’ and ‘Noiret’.

In the field, we quantified host susceptibility to the biotroph downy mildew using the percentage of the leaf surface infected by the disease. We quantified the disease susceptibility of nine cultivars to downy mildew in our cultivar trial in Belchertown, Massachussetts in September 2021. Resistance to downy midlew varied according to cultivar, from most resistant to least resistant being as follows: ‘Marquette’, ‘Frontenac’, ‘Noiret’, ‘Chambourcin’, ‘St. Croix’, ‘La Crescent’, ‘Corot Noir’, ‘Riesling’, and ‘Vidal’. Based on the four cultivars tested both in the laboratory and in the field, we did not see a correlation between resistance to necrotrophs and resistance to biotrophs.

Keywords: American hybrid, biotroph, Botryosphaeria, ‘Chambourcin’, Colletotrichum, ‘Corot Noir’, *Diaporthe, Didymella*, downy mildew, ‘Frontenac’, ‘La Crescent’, ‘Marquette’, necrotroph, ‘Noiret’, ‘Riesling’, ‘St. Croix’, ‘Vidal’

P43 –Can nitrogen nutrition affect the grapevine resistance to downy mildew?

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Abstract

Downy mildew (caused by *Plasmopara viticola*) is a disease that strongly affect grapevine. To date, the use of fungicide is still the most effective way to prevent the spreading of the pathogen. However, concerns about the safety and the environmental impact of the fungicides make the search of new solutions an urgent problem. Nitrogen nutrition can be one of this. Indeed, *P. viticola*, that lack of some nitrogen related pathways, can take advantage of plant nitrogen. In this work, we hypothesized that *P. viticola* could suffer the absence of nitrogen, making the susceptible Pinot Noir cultivar less susceptible to the pathogen. Pinot noir plants were grown in hydroponic solutions with five different nitrogen concentrations (0, 0.05, 1, 2 and 5 mM) and leaf nitrogen content, photosynthetic performance, *P. viticola* sporulation and gene expression of four nitrogen-related genes (VvLBDIc3, VvLBDIf5, VvLBDIIa3 and VvLBDIIb2) were estimated at three different time points. Leaf nitrogen content, photosynthetic performance and disease severity were reduced at the 0 mM treatment. Nitrogen treatments modulated the expression of genes analysed. VvLBDIc3, VvLBDIf5 and VvLBDIIa3 were overexpressed at low nitrogen concentrations, suggesting a role during nitrogen starvation. While, VvLBDIIb2 was underexpressed at low nitrogen concentrations. A correlation between disease severity and gene espression has been highlighted. These data demonstrated that decrease in nitrogen content can have a positive impact on the incidence of downy mildew, stressing the importance to manage the vineyard nitrogen nutritional status and suggesting an appropriate use of fertilizers, with a beneficial impact for environment and human health.

Keywords: *Plasmopara viticola*, leaf nitrogen content, photosynthetic performance, disease severity, LBD genes.

P44 – Phenotyping of Croatian native grapevine (*Vitis vinifera* L.) varieties in susceptibility to the causal agent of downy mildew (*Plasmopara viticola*)

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Abstract

A long history of grapevine cultivation in diversified geographical regions in Croatia gave rise to a high number of native varieties. In the era of sustainable production, there is a growing demand to define their differences in susceptibility to downy mildew. By applying leaf disc bioassay in controlled laboratory conditions, it has been found that native varieties react differently to the infection of *Plasmopara viticola*. Therefore, they were ascribed to classes of resistance according to the OIV descriptor 452-1 [Leaf: degree of resistance to *Plasmopara* (leaf disc test)]. Chlorophyll fluorescence and multispectral imaging traits have been measured in time points before and upon inoculation to define which of them could be used as an early detector of infection and are these methods suitable for distinguishing genotypes of different susceptibility to downy mildew. Moreover, the leaves were analysed using high-performance liquid chromatography (HPLC) to define if their chemical background, i.e., polyphenolic composition, is responsible for native varieties' different levels of resistance. It has been found that the leaf disc test is a simple method to perform, and it brings about trustworthy results when genotypes with a known level of resistance are comparatively evaluated. Chlorophyll fluorescence and multispectral imaging are promising tools for precise monitoring of the photosynthesis transmission inside a leaf tissue upon *P. viticola* inoculation. This utility could be used as a phenotyping method in the absence of the pathogen to define the level of genotype's resistance to *P. viticola*. As far as secondary metabolites are concerned, polyphenolic compounds proved to be responsible for the discrimination of varieties among the OIV classes of resistance. It has been found that the constitutive polyphenolic profile contributes to the separation of susceptible OIV classes (1, 3, and 5) into three groups. The content of resveratrol-3-*O*-glucoside and total stilbenes discriminated non-infected and infected samples, whereas the content of piceatannol and total stilbenes discriminated completely resistant OIV class 9 (*V. riparia*) and the remaining OIV classes. Less susceptible grapevine varieties that belong to OIV class 5 (Malvazija istarska, Ranfol, Teran) could be interesting to use in breeding programs aiming to produce high-quality genotypes resistant to main fungal diseases.

Keywords: *Vitis vinifera* L., downy mildew, biotic stress, chlorophyll fluorescence, polyphenols

P45 – Metagenomic differences between 'Callet-R110' and 'Merlot-R110' under drought stress

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Abstract

The search of solutions to promote sustainable viticulture involves not only a rational and optimized use of water using cultivars with higher WUE (water-use efficiency), but use of innovative farming techniques. Plant-associated microorganisms that contribute to traits such as drought tolerance may help in adapting to the undesirable effects of climate change. Our hypothesis is based on numerous studies that have demonstrated that climate change may affect all types of beneficial plant-microorganism interactions which are an important factor modulating plant responses to climate change. Hence, we want to study the soil microbiome in two grapevine genotypes under different field drought conditions. We will use two grapevine cultivars with different response to drought: Merlot, a wide word cultivar with a near-anisohydric response, and 'Callet' a local cultivar from Mallorca, with near-isohydric response. 'Callet' is reputed to be a drought-resistant cultivar, it is used to produce high quality red wines, and it grows well in hot, dry and bright areas.

The sequencing dataset (16S and ITS) obtained was representative for 16 soil samples from two grapevine genotypes with different water availability conditions (deficit irrigation and irrigated). Biodiversity indexes and principle statistics analyses on taxonomic profiles were analyzed using QIIME2-2011.11, alpha diversity was measured as Pielou´s Evenness distance and beta diversity was determined as UniFrac unweighted. The result showed that the alpha and beta diversity in bacterial and fungal communities are different. For 16S sequences alfa and beta diversity shown similar trends, Merlot soils presents higher values for Evenness values and UniFrac distance than 'Callet' soils ($p = 0.0027$, $P=0.024$), but no differences were found between treatment or combination treatment-genotype. Meanwhile, ITS sequences alfa values do not presented differences in any case, but UniFrac values presented differences at genotype ($p = 0.02$) and at genotype-treatment combination ($p = 0.001$) When we analysed the differential abundance for 16S and ITS libraries it was observed than a higher value of fungal than bacterial species were differentially affected by both genotype and water stress conditions.

Keywords: *Vitis vinifera* L., metagenomics, ITS, 16S, drought, 'Callet', 'Merlot'

P46 – Genetic analysis of grapevine (*Vitis vinifera*) wood composition

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Abstract

Grapevine woody stems represent a valuable source of polyphenols with a broad spectrum of health benefits. In contrast to the large number of studies about genetic determinants of grape berry compostion, few works have investigated the genetic bases of grapevine wood composition.

In this work, we have used both targeted and non-targeted metabolomic approaches to perform a global metabolic quantitative trait loci (mQTL) analysis with a progeny from a cross between 'Riesling' (Ri) and 'Gewürztraminer' (Gw).

Analysis of woody canes extracts using high performance liquid chromatography coupled to mass spectrometry has revealed genetic variability in the Ri x Gw progeny for metabolites from different families such as stilbenoids and flavonoids. High-density genetic maps based on single nucleotide polymorphism (SNPs) allowed the detection of significant mQTLs for a large number of metabolites, including major polyphenols.

This poster presents information about the detected QTLs impacting the accumulation of major grapevine wood polyphenols. Furthermore, characterization of major determinants of grapevine wood composition may provide bases for a better understanding of sensitivity to wood diseases such grapevine trunk diseases.

Keywords: Grapevine, wood, metabolomics, genetics, QTLs

P47 – Adaptation of *Plasmopora viticola* isolates against defense mechanisms conferred by different resistance genes in grapevine

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Abstract

The grapevine breeding for disease resistance is a strategy to promote the viticulture sustainability worldwide. Due the perennial nature of grapevine, it is essential that the genetic resistance should be durable. However, biotrophic pathogens with a mixed reproduction systems associated with heterotalism, such as *Plasmopara viticola*, the causal agent of grapevine downy mildew, have a high evolutionary potential that confers a strong capacity to overcome resistance mechanisms. Thus, the main aim of this work was to evaluate the level of adaptation of *P. viticola* populations to resistant grape varieties/genotypes carrying different combinations of R-Gene alleles in Santa Catarina state, Brazil. For this purpose, *P. viticola* inocula were collected from two sites of the State (Curitibanos and Urussanga) in the year of 2021. The inoculum was composed of a mixture of sporangia sampled at the end of the grapevine cycles, from hosts containing the R-alleles *Rpv3*, *Rpv10*, *Rpv1+3*, *Rpv3+10*. The sporangia suspension was prepared from each population and used in cross inoculations using leaf discs of the susceptible variety Cabernet Sauvignon and a panel of ten cultivars/genotypes containing different R-alleles combinations (*Rpv3.1*, *Rpv3.1+3.2, Rpv3.1+3.3*, *Rpv1+3.1, Rpv10*, *Rpv10+3.3*, *Rpv10+3.1+3.3*, *Rpv12* and *Rpv12+3.1*). At six days after cross-inoculation (dai), disease severity, resistance level (OIV 452-1 descriptor) and sporulation incidence were evaluated; at 7 dai, sporangia production was measured. The data obtained were subjected to analysis of normality and variance, followed by the Freedman, Dunn's and Tukey tests. The *Rpv12* allele was the most effective source of resistance, limiting completely the sporulation of the pathogen. *Rpv10* allele was also highly efficient, and no additive effect was observed when combined with the *Rpv3* allele. In addition, an erosion of the resistance conferred by *Rpv3* allele was observed, manifested as a dense sporulation as Cabernet Sauvignon. However, when *Rpv3* was combined with *Rpv1*, a high level of resistance was generally observed. Isolates collected from Curitibanos and Urussanga were able to sporulate in host carrying *Rpv1+3*, and *Rpv3+10*, bringing concerns about the durability. This knowledge is fundamental for the definition of breeding strategies for the release of grapevine varieties with durable resistance.

Keywords: disease resistance, plant breeding, PIWI, durability

P48 – Abscisic acid and proline are not equivalent markers for heat, drought and combined stress in grapevines

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Abstract

Background and Aims: Viticulture will be particularly affected by increasing drought and heat waves in the future. It is of interest to find traits that indicate stress before symptoms become apparent. We investigated whether the commonly used traits, proline and abscisic acid (ABA) biosynthesis, are suitable markers for heat, drought or combined stress and whether gene expression of key enzymes of ABA biosynthesis is regulated in grapevine leaves under these stress conditions.

Methods and Results: Plant growth and gas exchange were measured to evaluate plant reactions to increased temperature and water deficit. Proline and ABA concentration in leaf material was measured, respectively, photometrically and with GC/MS. Gene expression analysis of NCED1, NCED2 and P5CS was done by real-time quantitative reverse transcriptionpolymerase chain reaction. Drought stress had a stronger effect on growth, gas exchange, proline, and ABA biosynthesis than heat stress. An interaction between heat and drought stress was observed for gas exchange and for proline biosynthesis.

Conclusions: Proline concentration and gene expression of P5CS are good markers for combined stress. The concentration of ABA is a suitable marker for drought stress and might be a suitable marker for combined stress. Gene expression of NCED1 in leaves was a good marker for drought stress and might be a suitable marker for combined stress, whereas NCED2 was not suitable.

Significance of the Study: These results provide insight into the response of grapevines to heat, drought and combined stress and show the suitability of ABA and proline as stress markers.

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

P49 – PIWIs meet drought: ecophysiology responses to soil dehydration

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Abstract

Grapevine interspecific hybrids (PIWIs) represent an interesting alternative to classic cultivars in several wine regions, especially under sustainable and organic production systems. While such gentopyes are well characterised for some agronomic aspects and mainly because their response to biotic stressors such as powdery and downey mildews, little is known about their response to abiotic stressors such as drought. Here we studied the response of three different commercial PIWI genotypes (Donauriesling, Muscaris, and Souvignier gris, all grafted on rootstock Kober 5BB) to drought by monitoring their leaf gas exchange and water potential during dehydration in two seasons (2020 and 2021). We quantified the leaf area development and some leaf hydraulic traits to understand how they relate to the drought response in a pot experiment under controlled conditions. The results showed slight differences between genotypes only during the first days of dehydration, where Souvignier gris exhibited a less tight stomatal control as compared with the other PIWIs and in coordination with a lower (more negative) osmotic potential and turgor loss point. However, after five days under drought, all genotypes exhibited similar stomatal behaviour (g_s<0.05 mol m^{-2} s^{-1}). Our study shows that PIWIs behave and operate within similar ranges of stomatal conductance and water potentials as compared with other *V. vinifera* cultivars used in this study (Grüner Veltliner and Riesling). Acclimation to long-term water deficit and impacts on fruit composition is needed to better understand PIWIs ability to grow under limited water scenarios.

Keywords: water stress, grapevine, grapevine hybrid, drought tolerance

P50 – Grapevine leaf size influences vine canopy temperature

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Abstract

Grapevine leaves have diverse shapes and sizes. Their shape and size is known to be influenced by genetics, vine phytosanitary status, environment, leaf and vine age, node position on the shoot, shoot order, and other factors. In order to determine the importance of grapevine leaf shape and size to canopy temperature, we examined the relationship in five seedling populations grown in a vineyard in the San Joaquin Valley, California, USA. The seedlings were individual genotypes and unreplicated. All of the populations had one parent or grandparent with compound leaves of the *Vitis piasezkii* type and each population had a different second parent with non-compound leaves. As expected based on published reports and our own observations in related populations, the *V. piasezkii* compound leaf phenotype segregated as a dominant or semi-dominant trait and the populations all showed noticeable segregation for leaf shape. We measured leaf shape using 21 homologous landmarks. We used an infrared thermometer to measure the temperature of the canopy in July and August 2018 and 2019. By recording time of sampling and temperatures, we were able to determine which vines were cooler or hotter than expected, using a linear model. We did not measure canopy architecture, photosynthesis, or water use efficiency, as the heterogeneity of individual seedling canopies is very high, and this substantially complicates these observations on unreplicated seedlings. We established a relationship between leaf size and leaf temperature: vines with larger leaves were cooler than expected. In contrast, leaf shape was not strongly correlated with variation in vine temperature. Ultimately, these findings indicate that vines with larger leaves may contribute to the reduction of overall vine canopy temperature, but further work is needed to determine if this is due to variation in leaf size, differences in the openness of the canopy, or other related traits.

Keywords: compound, shape, heat, tolerance, adaptation

P51 – Virome of Slovenian grapevine candidate clones and production of healthy plants by thermotherapy and meristem tip micrografting

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Abstract

The presented research was focused on virus screening using small RNA sequencing (sRNA-seq) technology, to get an overview of viruses and virus-like organisms that are present in preclonal candidates of six autochthonous and local grapevine varieties (*Vitis vinifera* L.) in Primorska wine-growing region in Slovenia. During the process of viral infection, the virus- and viroid-derived small RNAs (sRNAs) accumulate abundantly in plants and can be detected by deep sequencing of infected plants. sRNAs were isolated, twelve libraries were constructed, and sequenced on IonTorrent System. The sRNA-seq data were analyzed using the open-source bioinformatics pipeline VirusDetect. The used method revealed the presence of: grapevine fanleaf virus (GFLV), grapevine leafroll-associated virus 3 (GLRaV-3), grapevine rupestris stem pitting-associated virus (GRSPaV), grapevine fleck virus (GFkV), grapevine red globe virus (GRGV), grapevine rupestris vein feathering virus (GRVFV), grapevine Syrah virus-1 (GSyV-1), grapevine Pinot gris virus (GPGV), raspberry bushy dwarf virus (RBDV), hop stunt viroid (HSVd), and grapevine yellow speckle viroid 1 (GYSVd-1). In silico results were validated by RT-PCR and Sanger sequencing. Biotechnological approach in vivo thermotherapy combined with in vitro meristem tip (0.1-0.2 mm) micrografting onto in vitro growing seedling rootstocks of Vialla (*Vitis labrusca* x *Vitis riparia*) was used to study the elimination efficiency from selected samples infected with abovelisted viruses and viroids (except GRGV). The medium used for the growth and root development of micrografts and micropropagated plants (1/2 MS with vitamins, 30 g/L sucrose, and 8 g/L agar) proved to be highly efficient. The overall regeneration rate was very low (8.5%), but it is sufficient to obtain one virus-free regenerated plant per candidate that further can be micropropagated. The regenerated in vitro plants were tested with RT-PCR. Elimination success was 100% for all viruses, while for the viroids, HSVd and GYSVd-1, the elimination rate was significantly lower, 39.2% and 42.6%, respectively.

Keywords: *Vitis vinifera* L., viruses and viroids, *in vivo* thermotherapy, *in vitro* micrografting

P52 – Susceptible and tolerant *Vitis vinifera* cultivars present distinct cell wall metabolism during ripening and under infection by *Botrytis cinerea*

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Abstract

Vitis vinifera grape berries undergo complex molecular and metabolic changes during ripening and under infection by pathogens. The 'Trincadeira' and 'Syrah' cultivars are known for they respective susceptibility and tolerance to *Botrytis cinerea.*

This work explores how the cell wall components vary upon ripening of the two cultivars, and how this may reflect their susceptibility degree to the fungal pathogens.

Grape clusters were infected with *B. cinerea* at peppercorn size stage (EL29); and then sampled at green (EL32), veraison (EL35) and ripe (EL38) stages. Visual inspection showed infection of Trincadeira grapes at three ripening stages and Syrah at ripe; however, molecular analysis detected the presence of the fungus in 'Syrah' at EL32 and EL35, despite the absence of symptoms.

Analyses of cell wall components with CoMPP and GC-MS were then conducted to explore the mono/polysaccharide composition and seek metabolic markers of ripening and of infection with *B. cinerea.* Data showed that both cultivars show differences in their cell wall composition prior to infection. Increase in extensins and decrease in hemicelluloses were noticed during ripening in both cultivars. Ripe 'Syrah' berries presented higher levels of crystalline cellulose, but lower levels of AGPs and rhamnogalacturonan-I than 'Trincadeira'. Regarding changes upon infection, specific structural components that maintain cell wall integrity, such as cellulose, suffer major turnover, which can impact fruit quality. At green stage, 'Syrah' does not show changes between control and infected samples, unlike 'Trincadeira', where the sugars arabinose, fucose, mannose, rhamnose and xylose decrease. At veraison, infection leads to a faster decrease of cellulose in both cultivars, and, at ripe, infection causes decrease of xyloglucan in Syrah and increase in cellulose in 'Trincadeira', which may affect fruit quality.

Taken together, this data brings knowledge on how berry quality parameters strongly affected by cell wall metabolism (e. g. texture) change during ripening and under infection with pathogens and which changes are cultivar specific.

Keywords: *Vitis vinifera*, grapes development, *Botrytis cinerea*, cell wall, CoMPP

P53 – Geisenheim genetic resources against *Plasmopara viticola*

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Abstract

In the framework of the national German research project "*VITIFIT*" (*https://vitifit.de/*), genetic resources of resistance against the fungus, *Plasmopara viticola* (Peronospora) present within the collection of the department of Grapevine Breeding at the Hochschule Geisenheim University have been evaluated.

Leaf discs assays have been conducted during summers 2020 and 2021 on eighty different accessions, including 26 different American and Asian wild *Vitis* species, two PIWIs and five German grapevine varieties. During summer 2020, a total of 15.629 leaf discs were inoculated using a single fungus-solution with one fixed concentration, incubated in a growth chamber with specific light cycles and pictures of each leaf disc were taken eleven days after inoculation. From these pictures, several information were extracted: scores of sporulation and necrosis (following Possamai *et al.* 2020) were attributed, as well as the fungus-incidence and the percentage of leaf area covered by sporulation were measured. A total of 4.247 on 7.021 leaf discs presenting sporulation were used to measured the percentage of leaf area contaminated by the fungus. During summer 2021, the same protocol was used on similar sampling representing a total of 15.208 tested leaf discs.

Moreover, a screening survey of known *Rpv*-markers (genetic markers linked to a Resistance against *Plasmopara viticola*) present within these accessions was investigated. A total of twenty-seven pairs of primers relative to twelve already known *Rpv*-markers were used to genotype hundred two different accessions (including the eighty accessions tested for resistance plus twenty two more corresponding to additional grapevine varieties, PIWIs and *V. riparia* genotypes).

The comparison of results obtained from both datasets - 2-year leaf discs assays and *Rpv*-screen - allowed us to determine which wild *Vitis* species from Geisenheim collection could possess putative new resistance markers and should be used for future breeding programs in Germany or in the world.

Using these information, several crossings (between resistant wild *Vitis* species without already known *Rpv*-markers and non-resistant grapevine varieties) were conducted to generate F1-populations and to identify/localize new unknown *Rpv*-markers. Five different treatments (control, cold, GA_3, H_2O_2, $H_2O_2 + GA_3$) were tested on two different accessions (*V. berlandieri* and PIWI - 150 seeds per treatment per accession) in order to increase the germination rate of these „resistant x non-resistant" hybridizations and, then, the production of F1-offsprings.

Results of each experiment (leaf disc assay, *Rpv*-screen, crossings, seed germination) will be discussed at the light of previous results found in the literature.

Keywords: genetic resources, wild *Vitis* species, *Plasmopara viticola*, resistance, *Rpv* markers

P54 – Investigations on hot water treatment for the production of high-quality grapevine propagation material

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Abstract

Hot water treatment (HWT) applied on dormant woody plant material to control Grapevine Trunk Diseases (GTD) showed variable results in previous studies. In this project, a field experiment was carried out to investigate the effect of HWT on GTD pathogens (*Phaeomoniella chlamydospore* (Pch), *Phaeoacremonium minimum* (Pmi), *Botryosphaeriaceae* (BOT) species). Inoculated wood cuttings were treated in a hot water tank under practical conditions, grafted onto healthy rootstocks and planted in nurseries. Pathogen development was analysed in wood samples at distinct time points after treatment. Pch and BOT could be successfully eliminated through HWT. However, only a reduced infection by Pmi was observed in the treated samples. To determine the influence of HWT on the growth of *Trichoderma* and its long-term antagonistic effect following HWT on GTD pathogens, a commercially available biocontrol agent product, Vintec®, [*Trichoderma atroviride* strain SC1 (TASC1), Belchim Crop Protection Deutschland GmbH] was tested. No negative impact of HWT could be detected on the growth of TASC1, and thus its antagonistic ability against the GTD pathogens was also not affected. Heat tolerance of the distinct developmental stages of each GTD pathogens was assessed at different temperature-time combinations under laboratory conditions. In general, Pch and BOT were more sensitive to HWT than Pmi at both tested developmental stages. Pmi showed reduced sensitivity to HWT at the ungerminated spore stage. Conidial germination was not inhibited following an incubation of the spore suspension for 45 minutes at 50˚C, whereas no survival of treated mycelia of Pmi could be detected. These results suggest that HWT can be a useful tool to reduce infections with GTD pathogens in propagation material without affecting the antagonistic effects of TASC1.

Keywords: Grapevine trunk diseases, hot water treatment, *Trichoderma atroviride*

P55 – Molecular responses to sunburn in grapevine and preventive measures for viticulture

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Abstract

Climate change has a significant impact on viticulture. Particular phenological growth stages such as flowering and berry development, the challenge of certain grape varieties to adapt to higher temperatures and drought stress, as well as the occurrence of undesirable off-flavours in wine represent a wide range of impairments. The growing conditions of many autochthonous varieties become poorer as they are adapted to cooler climate due to the longstanding selection process. In addition, sunburn damages to grapes have frequently occurred in Germany since the 1990s, which is associated with an increase of global radiation exposures as well.

In ‘Riesling’ wines, sensory impairments known as "petrol off-flavour" have also occurred in this context in recent years. According to previous studies, the causes of this off-flavour are attributed to rising temperatures and an overall increase in radiation exposure whereby carotenoids in grape berries are degraded into C_{13} norisoprenoids. Further chemical reactions ultimately result in the compound 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) which gives a dull and petrol-like odour as well as a bitter and astringent taste to the wines. Therefore, sunburn damages significantly reduce both, yield quantity and wine quality.

One research objective of the project is to develop a prevention strategy in which optimized canopy management in combination with application of various compounds or shading reduces sunburn susceptibility of ‘Riesling’ grapes. First results suggest an early defoliation of the bunch zone and application of china clay or lime to significantly decrease the risk of sunburn. Alternatively, different nets could improve grape health. Sunburn avoidance needs to be as effective, economically affordable and sustainable as possible, but also without impairing wine quality.

Moreover, grape varietal differences in sunburn susceptibility could be observed. The fungus-resistant grape variety ‘Calardis Blanc’ visually showed a significantly higher tolerance compared to the genetically distantly related ‘Riesling’. To get further insights regarding sunburn tolerance, experiments in the field as well as in the climate chamber are conducted. Berry skins are investigated on a transcriptional and metabolic level to identify the underlying molecular mechanisms and evaluate their temporal regulation. For this purpose, a deeper understanding of the sunburn-inducing factors is essential as well.

Keywords: ‘Calardis Blanc’, climate change, drought stress, global radiation, grapevine, heat, prevention strategy, ‘Riesling’, sunburn, *Vitis vinifera*

P56 – Hydraulic safety and xylem morphological traits in a panel of *Vitis vinifera* varieties

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Abstract

Water deficit can significantly impact grapevine physiology, growth, yield, and berry quality. Recent studies provided evidence of interactive and complex relationships between leaf hydraulic and stomatal control of transpiration under drought, suggesting the need to integrate multiple traits in defining the so-called grapevine drought tolerance. However, to date, genotypic variation for xylem morpholigcal traits and hydrauylic safety truough the assessment of percent loss of conductivity (PLC) has never been explored in a large panel of grapevine varieties. In addition, significant discrepancy exists in the literature for vulnerability to water stress-induced cavitation in grapevine, potentially due to the different methods used (e.g., centrifuge, dehydration, air-injection methods). In our work, twelve own-rooted grapevine varieties adapted to different European wine regions and known to hold dissimilar hydraulic behavior were grown under greenhouse conditions and assessed for xylem morphological traits and PLC. Significant genotypic variation was observed for xylem vessel diameter (XVD), number and total area, with cultivars known to behave as anysohydric (Sémillon, Syrah, Sangiovese) showing larger XVD than so-called isohydric varieties (Cabernet Sauvignon, Grenache, Macabeo). Regarding xylem vulnerability, there also were distinctions between cultivars of iso- and anisohydric tendencies. To our knowledge, this is the first study providing evidence of a broad genotypic variation in grapevine for hydraulic traits.

Keywords: drought, embolism, grapevine, *Vitis vinifera*

P57 – VRIAACC PROJECT - CATALUNYA

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Abstract

VRIAACC (Varietats Resistents I Autoctones Adaptades al Canvi Climatic) it means resistant's autochthon varieties adapted to climate change.

The project starts on Albet i Noya property vineyards in Penedès on 1999 from the hand of Dr. Pierre Basler and Josep M. Albet i Noya, and after 13nt. years testing same existing resistant's varieties, we know we need our own ones, because we did not want to lose the profile, character and personality of our traditional wines.

On 2012 I contact with the Suis Ing. Mr. Valentin Blattner and we initiate VRIAACC project with two more cellars, and the ambitious objective to obtain downy and powdery mildew resistant varieties sons of the mainly Catalan and Penedès varieties Xarel.lo, Macabeu, Parellada, Ull de Llebre and Red Garnatxa, who represent more than 70 % of the total Catalan vineyards. (55.118 hectares)

Thanks to the evolved genetic basis of the plant material of Ing. Mr. Valentin Blattner, and after the hard and meticulous work during the last 10 years and more than 350.000 crossings, actually we have more than 400 polygenic varieties, same of them with 6 and 7 gens, and a lot of them are already producing incredible quality wines.

We expect put on the market 10-12 varieties on the next 4 years, praying for the bureaucrats of the Catalan and Spanish Agriculture Departments open his minds and permit to the winegrowers produce really organic grapes and offer to the consumers clean wines without heavy metal residues, and leaving the clayey earth of the Penedès Appellation without the compaction wo is actually destroying the life and the structure of our soils.

P58 – *VviAGL11* molecular mechanism in seed development

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Abstract

VviAGL11, the Arabidopsis SEEDSTICK homolog, has been proposed to have a causative role in grapevine stenospermocarpy. An association between a mutation in the coding sequence (CDS) and the seedless phenotype was reported, however, no working mechanisms have been demonstrated yet. Previous studies have demonstrated that *VviAGL11* has a causative role in stenospermocarpy in grape. Indeed, a full correlation was shown of a missense mutation Arg197Leu in exon 7 of *VviAGL11* with seedlessness in cultivated grapevine. The mutated seedless version has a partial dominance over seeded alleles, allowing breeding for seedlessness based in heterozygous seedlings. Moreover, sequence data on the regulative region of *VviAGL11* showed low recombination rates between the promoter and the CDS regions, thus suggesting the existence of a Linkage disequilibrium in that region. The existence of incomplete dominance of some alleles in the promoter region has been also observed. Besides this genetic and transcriptional data confirming a major role of VviAGL11 in grape stenospermocarpy, no functional evidence nor a working mechanism has yet been defined.

To this aim, we sequenced and analyzed the complete *VviAGL11* gene in grapevine varieties representing different seedlessness classes. We demonstrated the existence of specific promoter-coding sequence (CDS) combinations self-activation that directly affect the expression level demonstrating a dominant-negative effect of the mutated CDS (Arg197Leu). Transcriptomic analyses on ovule and developing seeds in seeded and seedless varieties highlighted the role of *VviAGL11* in hormone signaling and phenylpropanoid metabolism. In this regard, we identified and further functionally validated through luciferase assay and in situ hybridization a Methyl jasmonate esterase, an Indole-3-acetate beta-glucosyltransferase, and an Isoflavone reductase, as direct targets of *VviAGL11*. The dominant-negative effect of the mutated CDS was also functionally validated in target induction. All our findings allowed us to define a regulatory mechanism correlating the haplotype assortment, *the VviAGL11* expression level, and seedlessness class in grapevine.

Keywords: grapevine, hormone signaling, secondary metabolism, seed coat, self-activation, stenospermocarpy*, VviAGL11*, SEEDSTICK

P59 – In-depth genotyping-by-sequencing of a grapevine F1 mapping population towards the prediction of wine quality potential

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Abstract

The molecular and genetic characterization of grapevine is a basic requirement to increase grapevine breeding efficiency. The introduction of marker-assisted selection (MAS) increased the efficiency of grapevine breeding programs and paved the way from empirical to predictive design of crosses. Resource optimization is achieved by early seedling removal as well as the selection of parental plants with favourable alleles. MAS is particularly important for perennial crop breeding, where solely the period from planting to first fruit set lasts years, in grapevine usually three years. For the subsequent in-depth evaluation during the breeding process, in particular to assess the quality potential of breeding lines, at least two more decades have currently to be scheduled.

An improved high-density genetic map of the cross population 'Calardis Musqué' and 'Villard Blanc' (150 genotypes) was developed based on genotyping by sequencing (GBS) data. Our GBS and computational pipelines are designed to maximize the gain of information from DNA sequencing data. To improve the amount of initial loci, our computational pipeline is based on de novo clustering and alignment on the read length level. By choosing this procedure we were able to identify not only SNPs, but also InDels which might represent valuable candidates for marker development followed by application using selective PCR. All in all, our modular de novo approach is taxon independent, does not necessarily rely on a reference genome, and allows for the incorporation of different marker types (SNPs, SSRs, InDels). Consequently it is highly versatile and expandable to future application platforms. Therefore, we minimized early filtering steps at different levels of the approach, resulting in a large dataset of ~500,000 loci. Adjacent biallelic SNPs from individual reads were translated into 20.410 haplotype-based markers with informative segregation patterns. The map was validated and used in QTL analyses on quality related traits like véraison (onset of berry ripening) and linalool content. Thus, the genetic map derived from our GBS data was proven to be suitable for the identification of trait-linked markers to give MAS access to wine quality potential. Additionally, our highly efficient GBS strategy was applied to selected genotypes of the extended crossing population (850 genotypes). (style Standard)

Keywords: GBS, haplotype-based markers, high-density genetic map, wine quality potential (style Standard)

P60 – Variability of phenolic compound content of 'Tempranillo' varieties to *Erysiphe necator* infection

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Abstract

Powdery mildew is one of the most important disease of grapevine (*Vitis vinifera*) caused by the fungus *Erysiphe* necator. It is an obligate ectoparasite of species belonging to the genus *Vitis*, and particularly *V. vinifera* varieties, most of which are highly susceptible. This pathogen provokes important economic losses. Its control required numerous chemical treatments that cause environmental and health problems and lead to the appearance of resistance to the most commonly products. One of the alternatives to reach a sustainable management is based on the design of strategies that trigger a defence response in plants that allows them to defend against the attack of the fungus. The aim of this work was to analyse the changes in the phenolic biosynthesis pathway in diseased leaves by *E. necator* in 'Tempranillo' and 'Tempranillo Blanco' varieties. To compare susceptibility to the disease, *in vitro* tests were carried out using whole leaves. Phenolic compounds from hydroalcoholic extracts of infected and control leaves were analysed by UPLC-MS at 1, 5, and 14 days after-inoculation. Differences in susceptibility to powdery mildew between the varieties in the early stages of infection were found, being the fungal growth in 'Tempranillo' lower than 'Tempranillo Blanco'. A higher content of phenolic compounds was observed in the leaves of 'Tempranillo Blanco', mainly due to hydroxyphenolic acids. In this variety, no differences were found between the diseased leaves and the control in terms of phenolic content. In Tempranillo, the content of total phenolic compounds, hydroxycinnamic acids and stilbenes were higher in infected leaves. Thus, in Tempranillo the upregulation of this pathway was not directed to the flavonoid pathway, although a rearrangement of these compounds was found. When comparing the treated leaves of both varieties, differences in anthocyanidin content were found. The genetic background of both varieties is similar, as 'Tempranillo Blanco' comes from a natural mutation of 'Tempranillo' which could lead to the loss of some genetic information related to the *Myb* genes that are involved in the regulation of the anthocyanidin pathway.

Keywords: plant defence, phenylpropanoids, grapevine, powdery mildew, *Vitis vinifera*

P61 – Identification and functional annotation of candidate genes for the control of phenotypic differences between early and late ripening 'Pinot' cultivars

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Abstract

Grapevine cultivars of the Pinot family represent in the broader sense clonally propagated mutants with clear-cut phenotypes, such as different colour or shifted ripening time, that result in major phenotypic and physiological differences as well as changes in important viticultural traits. Specifically, the cultivars 'Pinot Noir' (PN) and 'Pinot Noir Precoce' (PNP, early ripening) flower at the same time, but vary for the beginning of berry ripening (veraison) and consequently for the harvest time. Apart from the genotype, seasonal climatic conditions also affect ripening times. To reveal possible regulatory genes affecting the timing of the start of ripening, we investigated differences in gene expression profiles between PN and PNP throughout berry development with a closely meshed time series and during two separate years.

The difference in the duration of berry formation was quantified to be about two weeks under the growth conditions applied, using plant material with a proven clonal relationship. Clusters of co-expressed genes and differentially expressed genes (DEGs) were detected which reflect the shift in the beginning of ripening at the level of gene expression profiles. Functional annotation of these DEGs fits to phenotypic and physiological changes during berry development. In total, we observed between PN and PNP 3,342 DEGs in 2014 and 2,745 DEGs in 2017. The intersection of both years comprises 1,923 DEGs. Among these, 388 DEGs were identified as veraison-specific and 12 were considered as candidates for a regulatory effect on berry ripening time. The expression profiles revealed two candidate genes for Ripening Time Control, designated *VviRTIC1* and *VviRTIC2* , that may contribute to controlling the phenotypic difference between PN and PNP.

Many of the 1,923 DEGs identified show highly similar expression profiles in both cultivars as far as accelerated berry formation of PNP is concerned. Putative ripening time controlling genes differentially expressed between PNP and PN as well as veraison-specific genes were identified. We point out potential connections of these genes to molecular events during berry development and discuss potential ripening time controlling candidate genes, two of which are already differentially expressed in the early berry development phase. Several down-regulated genes are annotated to encode auxin response factors / ARFs. Conceivably, changes in auxin signaling may implement the earlier ripening phenotype of PNP.

Keywords: *Vitis vinifera*, 'Pinot Noir', 'Pinot Noir Precoce', grapevine, berry ripening, fruit development, differential gene expression, transcriptome profiling, ripening time control, veraison

P62 – *Vitis vinifera* L. new crossings tolerance to downy mildew

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Abstract

Although the high susceptibility of *Vitis vinifera* to the fungus *Plasmopara viticola*, several elements in this field suggest a variety-specific response to this oomycete infection. To date, not much is known about the degree of susceptibility to downy mildew segregates in populations derived from breeding of *V. vinifera* varieties with different degrees of susceptibility. Thus, CREA-Viticulture and Enology of Turi (BA) breeding program, among its goals, focuses on the identification of new *Vitis* genotypes showing more tolerance to this biotic stress. Starting from 2021, different genotypes belonging to two segregating populations, Inzolia imperiale x Autumn royal seedless and N22/050 ('Red globe' x 'Regal seedless') x 'Melissa', have been phenotypically evaluated for their differential response to *P. viticola* infection. In detail, a leaf disc assay was performed, and each leaf disc has been inoculated with 50 µL of a suspension of *P. viticola* zoosporangia. Five days after the inoculation the incidence of the disease was calculated as a percentage ratio between the number of leaf discs showing symptoms and the total number of tested discs. Furthermore, the severity of the infection was evaluated by using an empirical 0-to-4 rating scale and for each genotype the Mckinney's index was calculated. Noteworthy, data show that at least 10% of the tested genotypes, for each segregating population, are highly tolerant to the fungus infection. Among the Inzolia imperiale x Autumn royal seedless tested individuals, 18 out of 113 genotypes showing different responses to the infection were selected and the leaf disc assay was repeated on them to confirm their degree of tolerance/susceptibility to the disease. Moreover, to further investigate the genotypes response, some infected leaf discs from each genotype were collected and frozen in liquid nitrogen at different time points after inoculation and stored for transcriptomics studies aiming to evaluate differences in genes expression with respect to the degree of tolerance registered for each sample. The tolerance to the *P. viticola* infection of these genotypes will be tested also in the field in the next future and other commercial characteristic will be evaluated. We believe this work will lay the ground for providing in the next future new cultivars of *V. vinifera,* whose management will allow a lower environmental impact.

Keywords: *P. viticola*, *V. vinifera*, tolerant genotypes

P63 – Setting up new tools to reduce the duration of the grapevine breeding process in the first French private breeding company

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Abstract

Since some years, the French wine sector faces strategical challenges, all linked to climate changes. Multiple issues have been observed like diseases development, early frost, hailstorms, drought, change in the precocity and maturity of grapes, each one resulting in loss of productivity and yield. In France, the varieties proposed today by nurseries are historical varieties that are not well adapted to those changes. Therefore, Mercier Frères, the first French and second world leader grapevine nursery, have decided to start its own research programs, with the help of its laboratory Novatech, to answer the growing demand for new grapevine varieties. One approach will be presented, the NATHY program, consisting in creating new varieties by traditional breeding, with the help of molecular tools and new production techniques. In partnership with breeders around the world, the aim is to develop and propose resistant varieties first to the most harmful fungi: downy and powdery mildew, and black rot. Traditional breeding of perennial species as grapevine can take 25 to 30 years. The challenge for the company is to reach a breeding cycle of 10 years from the seed to the registration of the variety. To achieve this goal, the combination of multiple tools is required. Marker assisted selection allows us to detect resistance genes in the early life of the plant, and to discard rapidly genotypes that don't meet our expectations. Another major improvement is to reduce the time to product scion. A tomato-like production system has been settled, enabling the plant to produce scions in only one year after the planting, instead of 3 years in a classic field process. Multiples other tools are tested to study all way to reduce the breeding cycle. With this rupture innovation program, we hope to create new genetic resources meeting growers' expectations about climate change challenges.

Keywords: viticulture, climate change, resistant varieties, greenhouse production, marker assisted selection

P64 – Genetic mapping of phenology-relevant, berry quality and aromatic potential traits from two graopevine bi-parental mapping populations

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Abstract

Grapevine breeding requires renewed efforts to meet the challenge of the ongoing climate change and the societal demand for low input viticulture. A breeding program supported by Bordeaux wine professional committee (CIVB) aims at generating new cultivars that should meet these two objectives by improving traits such as disease resistance (downy and powdery mildew, black rot), while retaining the typicality of Bordeaux wines.

As part of this program, two *V. vinifera* bi-parental mapping populations were generated by crossing, on the one hand, 'Petit Verdot' (PV) and 'Cabernet Franc' (CF) (n = 202) and, on the other hand, 'Ugni blanc' (UB) and 'Sauvignon blanc' (SB) (n = 214). These two mapping populations were investigated for quantitative trait loci (QTLs) detection of relevant segregating traits such as phenology or aromatic potential with GC-MS and LC-MS analyses.

Genotyping by sequencing (GBS) by Illumina HiSeq 3000 of both mapping populations was performed on GET-plage platform (Toulouse, France). After bioinformatics treatments, the construction of the related genetic maps was achieved using JoinMap 4.1.

High-density genetic maps, based on single nucleotide polymorphism (SNPs), allowed the detection of 25 QTLs related with phenology, berry quality, aroma composition and aroma precursors, as well as the identification of some relevant candidate genes. The QTLs identified typically accounted for 7-27% of the trait variation.

The molecular markers identified in this project will be used for marker-assisted selection in current and future grapevine breeding programs. The project will provide the basis of the genetic architecture of useful traits for the production of red and white wines with emblematic Bordeaux typicality and will thus contribute to the design of new ideotypes.

Acknowledgments: We thank the CIVB for the financial support to the NEWVINE project N° 901 and to the GENOGRAPE project n°1801053/54

Keywords: berry quality, genotyping by sequencing, phenology, QTL detection

P65 – Effect of salinity and water regime, mediated by rootstock, on *cv* 'Syrah' must metabolite profile and vine physiology

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Abstract

Diminishing availability of fresh water in the Mediterranean basin and increasingly polluted underground water prompts the use of reclaimed water for agriculture. The years' efforts are being put to develop tolerant grafts in wine grapevine. understanding the response of grapevine metabolism to altered water balance and salinity is of pivotal importance. We used Syrah grafted on rootstocks 1103Paulsen and SO4 for this study, under a set of combinations of salinity (0.5 and 2.5 dS/m) and irrigation levels (66%, 100% and 133%) in an experimental vineyard located at Sde Boqer campus (30°51'22.37"N and 34°46'52.98"E). Generally, grafts of SO4 had higher total yield and single berry weight as compare to 1103 Paulsen. Measured physiological parameters like photosynthetic activity was considerably reduced by salinity and deficit irrigation in both rootstocks. Stem water potential (SWP) steadily dropped throughout the first two months of the experiment and decreased sharply after sixty days. grafts with SO4 had a slightly higher SWP. Whereas grafts of SO4 showed greater accumulation of the Cl^- ion as compared to grafts of 1103 Paulsen although not statistically significant yet suggesting that the two rootstocks may have different salt exclusion capacity. In the must of Syrah berries harvested separately from the two graft combinations, a total of 44 primary metabolites were uniquely identified and annotated using a GC-MS based profiling. Among them, the accumulation of several major sugars and amino acids are affected by the interaction of salt, water regimes, and rootstocks. Relative content of amino acids, like proline and alanine, increased under salinity stress while lysine, valine, and leucine decreased. Grafts of 1103 Paulsen showed increased levels in amino acids (pyroglutamate, leucine, valine), sugars (lyxose, xylose, and trehalose), and some other metabolites (ethanolamine, cinammate, lactate, and galactarate) than SO4 graft. Taken together, the results showed that the two rootstocks possess different mediating effects on the metabolism and physiology of Syrah in response to salinity and water regime. The different response of agronomical, and physiological traits as well as metabolite changes to stress between SO4 and 1103 Paulsen may suggest the reason for the different tolerant abilities between SO4 and 1103 Paulsen.

Keywords: grapevine, salt stress, central metabolism, water deficit, rootstock, stress response

P66 – The diversity of condensed tannins in domesticated grapevine associates with an unsettled multicopy F3'5'H region on Chr6 that expanded before the allopatric speciation of the *Vitis* genus

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Abstract

Condensed tannins or proantocyandins (PA) are key players in oenology as they determine the colloidal stability, oxydo-reduction activity and astringency in the wine. It can therefore be hypothesized that their composition underwent selection during both domestication and modern breeding. An international network recently presented a vast phenotyping campaign on condensed tannins in the family of Vitaceae (ca 600 accessions) with emphasis on the *Vitis* genus. We therefore confirmed that the respective abundances of di, and trihydroxylated catechins as constitutive monomers in PA may vary strongly not only in this plant family but also within the *Vitis* genus (submitted).

In the present work, to investigate the impact of domestication on PA features, the diversity of PA composition was characterized throughout a representative set of more than 500 wild or domestic cultivated *V. vinifera* genotypes. This was made possible by the collaboration of a number of international partners and the contribution of the Vassal-Montpellier Grapevine Biological Resource Center (INRAE, France).

We found more EGC+EGCG in *V. sylvestris* as compared to *V. vinifera*, and globally more diversity in the East as compared to the West. For this trait, some old traditional cultivars could not be distinguished from *V. v.* subsp. *sylvestris*, suggesting the trihydroxylation ratio could have been counter-selected for oenological and taste reasons.

A GWAS approach was then conducted in cultivated grapevine, between these phenotypic data and 10k SNPs scattered along its genome. SNP flanking the multicopy Flavonoid 3'5' hydroxylase (F35H) region of Chr6 previously documented by Falginella et al. (2010) appeared significantly associated with the relative enrichment of condensed tannins in epigallocatechin subunits. Public DNA sequences based on long-range techniques allowed to compare the F35H copy number in this region among genotypes, together with its content in transposable elements. As it is notoriously difficult to place SNPs in such regions, present results highlight the interest in long-range sequencing to explore the impact of structural variants on the phenotype.

Keywords: Vitaceae, *Vitis vinifera*, tannin, proanthocyanidin subunits composition, domestication, genetics, diversity

P67 – Analysis of phenology and ripening quality traits in segregating populations derived by crossing 'Corvina' with divergent varieties

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Abstract

Adaptation of varieties to changing climatic conditions is a major breeding target, which includes the selection of late ripening varieties/clones, whose bunches may escape the warmer summer condition by postponing the ripening period. However, assessment of the genetic basis of phenology and quality related traits is a prerequisite to develop breeding programs for grapevine varieties adapted for the cultivation in specific viticultural areas and/or to identify the candidate genes for the new breeding technology approaches.

The present work reports a two-season evaluation of traits segregation in populations derived from crosses of the red skinned cv. 'Corvina', the principal local variety of the Valpolicella wine area (Verona, Italy), with other two cultivars: the white skinned cv. 'Solaris', highly divergent from 'Corvina' for the phenology and fruit ripening traits, and the red skinned cv. 'Cabernet Sauvignon', whose bunches shows distinctive ripening and post-ripening traits from 'Corvina'. One hundred and fourty-two and one hundred and 29 seedlings were developed respectively for each population, propagated and grown under field conditions. During each season the main phenological stages from budbreak to berry maturation were determined for each genotype in the populations. At full ripening, several clusters from each plant were harvested for measuring main morphological and technological parameters. The data collected was then used to evaluate the distribution of each trait across the individuals in each population. Correlation analyses have been performed between traits collected in the two seasons or across traits. The comprehensive information obtained will be used for QTL mapping. The identification of genetic markers associated with the studied traits will help accelerating the selection of new cultivars more adapted to the changing climatic conditions. Altogether, the described approaches will finally allow to improve our current understanding of the genetic control of phenology and berry quality traits in grape, thus helping and assisting breeding.

Keywords: cross population, phenotyping, 'Corvina', 'Solaris', 'Cabernet Sauvignon'

P68 – Dissecting the effect of soil on berry transcriptional plasticity in two Italian grapevine varieties (*V. vinifera* L.)

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Abstract

Grapevine (*Vitis vinifera* L.) is one the oldest and most widely cultivated perennial fruit crops in the world. Its global socioeconomic importance is due to the high-quality attributes of berries, principally employed for wine production and fresh consumption. Grapevine is characterized by a pronounced phenotypic plasticity, as different wines can be produced from the same genotype when cultivated in different environmental conditions. The growing interest of the scientific community and wine producers on genotype *per* environment (GxE) interactions and the strictly related concept of *terroir*, which itself embodies one of the most evident examples of phenotypic plasticity, led to a boost in studies on this issue. Dissecting the weight of each single terroir component in the grapevine plastic response is a notable effort, given the difficulty of comparing the behavior of different plants grown in different areas blocking all variables except one. This was the challenge of the present study: trying to underlie the genetic and molecular mechanisms at the base of phenotypic plasticity in response to a single terroir factor and not to a combination of variables. To do this we conducted a field experiment where all the terroir variables, except for the soil, were kept as constant as possible and taking advantage of the high-throughput expression profiling technologies, we analyzed gene expression on a global scale, trying to investigate the berry skin and flesh transcriptional plasticity throughout maturation in two varieties of great interest at the national and international level: 'Glera' and 'Corvina'. Molecular results, together with phenological and physiological parameters measured during the whole seasonal cycle over 72 plants, suggest a specific effect of soil factor on grapevine plasticity and indicated specific gene networks related to terroir that could be object of further studies with the ultimate aim of implementing agricultural practices in order to i) obtain the desired fruit characteristics for every climate/cultivar combination, ii) lead to more efficient use of resources and better management of vineyards, iii) maximize the terroir effect on the grapevine to highlight the uniqueness of their vineyards ultimately increasing the industrial competitiveness.

Keywords: Phenotypic plasticity, GxE interactions, terroir, transcriptomics, gene expression, 'Glera', 'Corvina'

P69 – Genome wide association mapping of flowering-veraison interval in *Vitis vinifera* L.

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Abstract

Grapevine cultivation is being afflicted, as for many other crops, by environmental changes. Indeed, high temperatures occurring during growth may advance the date of phenological stages changing climate conditions over ripening and thus negatively affecting grape and wine quality. In particular, starting of veraison, that is the onset of the berry ripening process, and its laps of time from flowering are variable among the different varieties. Therefore, dissection of genetic determinants driving the phenological stages of flowering, veraison, as well as the interval among these, represents an interesting target in the contest of adapting grape varieties to changing environmental conditions. This has been previously enquired by QTL studies conducted in bi-parental populations. By using large diversity panels, Genome Wide Association Study (GWAS) provides a further promising approach for mapping of these traits and associated variants.

To this aim, starting from a panel of more than 600 cultivars grown in the large germplasm collection of CREA-VE in Susegana (Treviso, Italy) a core of 132 genotypes representative of the genetic diversity of the whole panel was pulled out, based on genotypic data at 45 SSR markers. Using this core we have conducted a GWAS to identify loci linked to the extension of the interval between flowering and veraison times. Phenotypic data, collected over a period of 11 years, were managed together with GrapeReSeq *Vitis* 18K SNPChip genotyping data and bioinformatic analysis was conducted, with 3 different softwares. We identified a list of SNPs significantly associated to the phenotype by at least one of these softwares among which some were confirmed by more softwares. Alternative strategies for SNP validation were implemented, either based on further available SNP data in public repositories or on direct Sanger sequencing of some of the associated SNPs in genotypes with extreme behaviors (48 selected samples with long and 48 selected samples with short flowering-veraison interval). Potential candidate genes in the associated regions were also identified.

All togheter these results provide useful clues for the flowering-veraison interval genetic regulation and for the molecular breeding in *Vitis vinifera*.

Keywords: grapevine, climate change, GWAS, QTL

P70 – NAC family's cis-regulatory elements atlas in grapevine

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Abstract

The grapevine (*Vitis vinifera*) is one of the oldest known plants and at the same time represents an important fruit crop that is used in various productions such as wine, jams, juices and jellies, grape seed extracts, raisins, vinegar, and oil of grape seeds. In consideration of the huge importance that the vine represents, much genomic research has been conducted in the last decades. These studies were also conducted thanks to the sequencing of the entire genome and the accurate knowledge that is possessed about it. Today, based on published sequence data, it is possible to perform a complete analysis of a special gene family to discover their functions, evolution, and expression profiles as accurately as possible.

On this trend, we place our work which aims to develop the knowledge related on one of the largest family of the transcription factor and most important ones for grapevine: NAC (NAM, no apical meristem, ATAF and CUC). Moreover, it is believed that the NAC family of transcription factors (TFs) is among the plant TFs, in general but especially for the grapevine, which play significant roles in the growth, development, stress and defence responses of plants and therefore, offers the critical regulatory functions of the plant in the plant at various stresses. In studies conducted on grapevine it has been observed that NAC genes can represent important signalling components especially in the control of grape ripening processes such as late development of the berries and senescence of the leaves.

With a view to identify transcription factor binding site (TFBSs) and describe the grapevine cistrome, we used a novel high-throughput TF-DNA-binding assay called DAPseq (DNA Affinity Purification and sequencing) that combines next-generation sequencing of a genomic library with in vitro expression of affinity-purified TFs to generate cistrome and epicistrome maps. DNAseq libraries have been constructed using native genomic DNA from Cabernet franc young leaves by preserving tissue-specific cytosine methylations that are known to impact TF binding.

The data collected from DAPseq analysis will be combined with other new analysis methodologies based on next-generation sequencing (NGS) such as RNASeq for gene expression, ATACSeq for chromatin accessibility, BSSeq for DNA methylation, ChIPSeq for histone modifications in order to obtain the most exhaustive knowledge possible about gene regulation in grapevine.

Our study provides new useful information on NAC with the aim of obtaining a better understanding of the mechanisms of genetic regulation and more information on the signalling mechanisms of transcription factors in the different physiological processes.

Keywords: DAPseq, grapevine, NAC, gene regulation, cistrome

P71 – The genetic architecture of berry size in seedless grapevine: From QTLs discovery to the validation of a candidate panel of markers for assisted selection

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Abstract

Although the table grape breeding industry is achieving a maturity and programs are close to regime and release tens of new varieties every year, it is still a low performance innovation sector. The development of molecular techniques, combined with classic and newly genetic-genomic resources, allowed the discovery and description of genomic regions involved in qualitative, semi-quantitative and quantitative traits in more than 50 studies in the last 20 years, very few tools for assisted selection or the prediction of the phenotype for breeding purposes are being used.

In this work we have characterized the genetic architecture of the berry size in a framework of genetic improvement of seedless table grapes. To reduce the outcome of underpowered or biased results we used a large biparental cross (n~530), between a table seedless vine (Crimson Seedless) and a multipurpose vine (Muscat of Alexandria). The progeny was genotyped using a GrapeReSeq Illumina 20K SNP chip and phenotyped for five seasons to perform a fine QTL mapping experiment. To reduce the effects of environmental variance, Best Linear Unbiased Predictors were used to discover QTLs and individual seasonal data was used to evaluate the reproducibility across seasons. Up to 15 QTLs for berry weight describe a complex nature of this trait. For the five most stable QTLs we defined several candidate genes and developed SSR markers that were individually tested for its association in a larger panel of the biparental population (n~770). Considering an additive model built on the favorable alleles of the most associated five genes (SSR), up to 42% of the phenotypic variation of berry weight was explained. Finally, in a panel consisting in n~663 genotypes, including 100 common varieties and 563 seedlings derived from 13 crosses held by CREA-Turi and INIA breeding programs, in which 60% are seedless, we validated the associations and development of SSR markers for assisted selection for Berry Weight in a context of seedlessness. With five markers we are able to apply negative selection to close to 90% of breeding material, encouraging for an efficient breeding.

This work was fund by FONDECYT Grants 1170586, 11161044, and FONDEF G09i1007.

Keywords: berry size, table grape breeding, marker assisted selection

P72 – DAP-seq and GCN analyses to infer the role of the grapevine R_2R_3-MYB C2 repressors clade

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Abstract

In grapevine, the involvement of the *VviMYB* family of Transcription Factors (TFs) in the key physiological processes that determine phenotypic variability is well known. Especially for members of the *R_2R_3-MYB* subfamily, which are mainly involved in fine-tuning the biosynthetic pathways of secondary metabolism. Transcription activation is orchestrated by the close interaction of several TFs such as basic Helix-Loop-Helix (bHLH) and WD40 with MYB proteins, which act by specifically targeting the promoter region. Among these MYB, a handful belonging to subgroup 4 are characterized by the presence of the C2 repressor motif, involved in the repression of transcription. The integration of data belonging to different *in silico* and wet-lab approaches is becoming increasingly important for the inspection and scouting of relevant genomic regions involved in the regulation of gene transcription. In this regard, DAP-seq (DNA Affinity Purification-sequencing) is a molecular technique capable of providing a collection of CREs (Cis-Regulatory Elements) on a whole genomic scale by combining the *in vitro* expression of TFs and NGS (Next Generation Sequencing) analysis. On the other hand, the information provided by transcriptomic data stored on public databases can be exploited using tools for GCN (Gene Co-expression Network) analysis. Taking advantage of the abovementioned approaches, the present study aims to characterize the role of *VviMYB4b, VviMYBC2-L1, VvMYBC2-L2* and *VviMYBC2-L3* genes belonging to C2 repressor motif clade, drawing up a list of candidate target genes, some of which are involved in biosynthetic pathways linked to secondary metabolism. The preliminary results shown here pave the way for the use of innovative investigation techniques which, once the target regulatory regions of TFs have been identified, allow the isolation of the protein complexes that regulate gene expression. This will entail an expansion of knowledge concerning the interaction TF-promoter region to serve as a basis for future genome editing experiments on CREs.

Keywords: *Vitis*, TFs, CREs, NGS, genome editing

P73 – Identification of genetic determinants associated to cluster architecture and plant fitness in table grapes using genome-wide association studies

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Abstract

Grapevine (*Vitis vinifera* L.) is one of the most economically relevant fruit crops in the world. Grapevine phenology and physiology are highly dependent of environment fluctuations, which alter the progression of phenological states and interfere with plant physiology, being fruit development one of the most severely affected traits. Since the fresh consumption is one of the most profitable markets, the characterization of the genetic architecture of traits related to cluster structure and berry development, and the further determination of associated genetic markers, is highly relevant for the development of breeding selection tools. To elaborate on this, a diverse panel of 116 grapevine varieties containing table, wine and mixed-use varieties as well as segregant lines from INIA's breeding program, were used to perform a genotyping-by-sequencing (GBS) experiment. Subsequently, biallelic polymorphisms were filtered by MAF (>5%) and missing data (<5%) following a VCF pipeline, resulting in the identification of ca. 210,000 high-quality single nucleotide polymorphisms (SNPs), later annotated using SnpEff 12X.2 assembly and VCost3 annotation. In parallel, this panel was phenotyped over three seasons at harvest, including traits such as cluster weight (CW), rachis weight, berry fresh weight (BFW), leaf area index (LAIe), photosynthetic efficiency, the latter two as vigour and fitness indicators, among others. Genome-wide association analysis (GWAS) were performed using TASSEL software and GLM /MLM models. Our preliminary results point to the identification of chromosomic regions associated with CW, BFW, LAIe and photosynthetic efficiency, among others. Further studies, including experimental validation with transcript and metabolite characterization, are currently under development. Our ultimate goal is to identify markers with association to cluster architecture and plant fitness. These will be validated using amplicon sequencing to evaluate their relevance and potential application in MAS breeding programs.

Financed by FONDECYT/ANID Chile grants 11190936 and 1221410.

Keywords: SNPs, GWAS, molecular markers, cluster architecture, plant fitness, grapes, plant breeding.

P74 – Breeding of new phylloxera resistant rootstocks in Geisenheim

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Abstract

Phylloxera risk makes viticulture virtually impossible without grafted vines. Most rootstock varieties are sufficiently phylloxera tolerant but not resistant, allowing the formation of leaf galls and root nodosities. Genetic diversity of rootstocks is small worldwide. Rootstock cultivars of the *Vitis cinerea* genotype are highly resistant to phylloxera (e.g. Börner, Rici, Cina). The introduction of completely phylloxera resistant rootstocks is the chief goal of our breeding program at Geisenheim. New candidate varieties are evaluated for rooting and grafting capability comparing their performance in vineyard trials to commonly used rootstocks. The aim of this study is to gain information on some alternative rootstocks and new completely phylloxera resistant candidate varieties, which could help to enlarge the range of commercially used rootstocks.

Plants were bench-grafted with virus tested rootstock and Pinot Noir, Pinot Gris, Trollinger (Black Hamburg) and White Riesling as scion material, callused in a glasshouse and rooted in a field nursery. Rootstock trials were located in different wine growing regions in Germany representing a range of different soil types.

Rootstocks have a huge impact on the scion partner, its physiology and performance. Different yield levels are corresponding to the relative water holding capacities of the trial sites. While most rootstocks show variable results, SO4 is the most stable high-level performer at all sites. A number of new Geisenheim crosses show comparable performance characteristics on a medium to high level according to site specific soil conditions. Vigour, yield, berry size, concentration of minerals within berry juice, content of organic acids, pH and sugar concentration are affected by rootstocks considerably.

The introduction of new completely phylloxera resistant rootstocks will contribute to a larger biodiversity, which is a good protection measure against phylloxera and possible new root diseases. A number of new Geisenheim rootstock crosses show a good potential for commercial cultivation. In any case, an increase in rootstock biodiversity is crucial for the future development of viticulture. Two new rootstock varieties are already in the registration process.

Keywords: adaptation, phylloxera, rootstock

P75 – Towards resistance to downy mildew and powdery mildew in Portuguese vines: evaluation of a F1 progeny

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Abstract

Grapevine (*Vitis vinifera* L.) is the most important fruit crop in Portugal. Associated with a long history of wine production, there is also a significant diversity and richness in the Portuguese germplasm, both in the cultivated subspecies vinifera as well as in wild vine populations, (subspecies sylvestris). In the second half of the nineteenth century pest management became one of the main tasks in European viticulture after the introduction of the fungal pathogens causing powdery and downy mildew. Atlantic climate areas in Portugal may need up to 10 treatments per season to control these diseases. One of the most promising approaches to control these diseases is to introgress resistance traits relying on the breeding of new grapevine varieties.

A number of crosses between Portuguese varieties well adapted to the local terroir and with high enologic importance (Arinto, Fernão Pires and a Touriga Nacional) and varieties with known resistance (Chambourcin, Regent and two lines A and B of resistant grapevine varieties) were carried out in 2018 and 2019. More than 3000 seeds were obtained, these were germinated in 2020 at control condicions (temperature, humidity and light) in the greenhouse. During the 2021 growing season theses progenies were challenged with downy mildium (near 30000 sporangia ml^{-1}concentration, spread on lower suface of leafs) and with powdery mildium (direct brusch strocks on upper suface of leafs). Both challenges were done in the absence of Phytosanitary treatments at two growing stages (Baggiolini scale E and I). Only tolerante plants will be further studied, so far we have 239 progenies. As future perspective these new varieties will be screened with identified locus that identify the genetic profile of these grapevine varieties and check the correct progenitors. Additionaly resistant locus identified in the used progenitors, hermaphroditism flower and berry skin color will to be used to select the new grapevine varieties to be used on wine production or in new backcrosses in order to obtain F2 varieties.

Keywords: *Vitis vinifera*, Portuguese germplasm richness, new varieties, powdery and downy mildew resistance

P76 – Resistance breeding in Serbia: SK 13-7/5 is a promising red wine genotype

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Abstract

Grapevine breeding in Serbia was initiated in the middle of XX century. However, the work on the new cultivars tolerant to main fungal diseases started in 1979. Several white fungus tolerant cultivars were released almost twenty years ago but only two have found their place in the vineyards: 'Backa' (*V*IVC number 21272) in Serbia and South Hungary, and 'Morava' (*V*IVC number 23777) in Central Serbia, while the first colored fungus tolerant cultivar 'Dionis' (*V*IVC number 24064) was released in 2017. High resistance levels from these cultivars, that are the result of different back crossings, now could be combined with high-quality cultivars. 'Tamjanika crna' is an important high-quality cultivar, particularly in East Serbia, but sensitive to downy mildew (*Plasmopara viticola*). The aim of the crossing, performed in 2013, was to improve the tolerance of 'Tamjanika crna' to downy mildew. Tamjanjika crna was chosen as a mother due to its female flowers while the other parent was 'Dionis', which carries *Rpv3* and *Rpv12* genes. Initially, 56 seedlings were obtained but only four reached the final stage. In the paper the results of productive characteristics of the four candidates SK 13-7/1, SK 13-7/2, SK 13-7/5 and SK 13-7/6 are present. The results at harvest were obtained during two seasons in an organic vineyard at the Experimental field for Viticulture, University of Novi Sad. All genotypes were pruned to one cane with 12 buds and one spur with 2 buds and were harvested the same day (13 September on average). The genotype SK 13-7/5 had upward shoot orientation as Tamjanika crna and high tolerance to main fungal diseases as the other parent Dionis. SK 13-7/5 also had two clusters per shoot with 22.8% sugar in the must. This candidate had higher titratable acidity of the must compared to other candidates and Tamjanika crna, which in most of the seasons lose acids quickly just prior the harvest. It seems that SK 13-7/5 genotype has some better characteristics compared to its both parents and could be promoted as a new cultivar.

Keywords: *Plasmopara viticola*, 'Tamjanika crna', 'Dionis', 13-7/5, grape quality

P77 – Identification of a genetic locus associated with resistance to grapevine anthracnose (*Elsinoë ampelina*)

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Abstract

Grapevine anthracnose (*Elsinoë ampelina*) is a major disease in tropical and subtropical regions. This hemibiotrophic ascomycete attacks preferentially young green tissues. Typical symptoms on leaves and berries are brownish lesions with black margins that resemble a bird's eye. Most traditional European grapevine varieties are highly susceptible and regular plant protection measurements are necessary to control this disease. However, resistance was observed in some American tropical and East Asian *Vitis* species as well as in new bred varieties. Therefore, our study aimed at mapping QTLs associated with resistance to *E. ampelina*. The F1 half-sib individuals from crossings between the resistant genotype MGM4 ('Moscato Giallo' x 'Sibera') and two susceptible genotypes, A190 and A271, were phenotyped in six experiments for anthracnose symptoms on leaves of potted plants after artificial inoculation with spore suspensions from *E. ampelina*. In addition, the F1 plants were genotyped using SSR markers available for the *Vitis* genome. The data were used to create a genetic map for the resistant parent (MGM4) and to perform QTL analysis. In total, 182 SSR markers were mapped, spanning the 19 chromosomes, with an average distance of 9.3 cM between markers. A QTL designated *Rea1*, located on chromosome 18 and associated with anthracnose resistance on leaves, was detected in a region with genes coding for proteins linked with ROS activities. The F1 plants carrying this QTL showed less anthracnose spots on leaves compared to plants without the locus. This indicates that the locus, presumably derived from *Vitis amurensis*, attenuates anthracnose symptom formation and is the first resistance QTL linked to grapevine anthracnose. It is a starting point, which allows the development of genetic markers for marker-assisted selection (MAS) for this locus, saving time in grapevine resistance breeding.

Keywords: anthracnose, *Elsinoë ampelina*, black spot, QTL, mapping, *Vitis amurensis*

P78 – Uncovering genetic and epigenetic factors as a source for trait variation in ‘Riesling’

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Abstract

The Department of Grapevine Breeding has a collection of almost 1200 ‘Riesling’ clones that encompass a broad range of trait characteristics, e.g. growth types or cluster architecture. While the phenotypic variation in this collection is well described the underlying genetic and epigenetic mechanisms remain largely unknown. Therefore, we are applying state-of-the-art sequencing technologies like Oxford Nanopore Sequencing to generate long DNA sequences that enable simultaneous scoring of genome-wide methylation patterns. This type of data is especially suited to detect differences between the two haplotypes of the diploid ‘Riesling’ genome so that a reference assembly can be generated that contains both haplotypes of all 19 chromosomes. The new ‘Riesling’ reference genome assembly will enable to study the variation between the two haplotypes within clones while also being able to assess the degree of differential mutation and methylation between clones. This information is then analysed with comprehensive phenotypic data collected from over more than a decade to unravel underlying causal polymorphisms and determine the relative importance of genetics vs. epigenetic for trait variation in ‘Riesling’. This information can then be used to facilitate the identification and selection of clones that are better adapted to certain vineyards, which is especially important in the light of rapidly changing environments due to climate change.

Keywords: genomics, epigenetics, ‘Riesling’, clonal variation, genome sequencing, genome assembly

P79 – Identification of nematode-resistant grapevines for rootstock breeding by screening *via* glass vial assay

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Abstract

Rootstock breeding focuses on nematode resistance to fight indirectly the fanleaf degeneration disease caused by Grapevine fanleaf virus (GFLV), which is transmitted by the vector nematode *Xiphinema index*. Screening of candidate *Vitis* genotypes was performed based on nematode reproduction and fertility rates and virus diagnosis of test plants after inoculation with viruliferous nematodes and incubation for 35 days in glass vials. 11 *Vitis* candidates out of 68 genotypes were identified as nematode resistant and 3 of them remained GFLV-negative after exposure to viruliferous nematodes in the assay. The time and space saving setup of the assay enabled the efficient identification of promising nematode resistant genotypes with a genetic background from *Vitis aestivalis* or *Vitis labrusca*. The selected candidate genotypes may be considered for future rootstock resistance breeding

Keywords: *Xiphinema index*, resistance screening, nematode reproduction rate, *Vitis aestivalis*, *Vitis labrusca*,

P80 – Ribbon trichomes as a physical barrier for *Plasmopara viticola* infection

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Abstract

Plant hair or trichomes can be found on surfaces of different grapevine organs and are of diverse morphology, ontogeny and function. Trichome types in *Vitis* are described as either non-glandular, including ribbon and simple trichomes, or glandular. Ribbon trichomes are elongated and twisted and their occurrence can vary in extent from absent to sporadic up to a dense, highly hydrophobic indumentum. Ribbon trichomes can provide a first line of defense against infection with *P. viticola*, since the causal agent of downy mildew requires the presence of liquid water to successfully infect grapevine tissues. A dense layer of ribbon trichomes on the abaxial leaf surface holds back the sporangia and the hydrophobic environment prevents wetting after rainfall thereby impairing zoospores from hatching, approaching the stomata, and finally penetrating the leaf. In the present study, it was shown that this inhibitory effect on artificial infection in a leaf disc assay with pubescent varieties could be reduced by the use of detergents. In this way, genetic resistance can be studied independently of this physical barrier. Moreover, a F1 population of a cross between 'Lemberger' (*V. vinifera*) with glabrous leaves and 'Catawba' (*V. labrusca* x *V. vinifera*) with a dense indumentum was examined for the trait ribbon trichome density. QTL mapping led to the identification of a major locus on chromosome 5 of 'Lemberger' for hairlessness. Using a second biparental population, this locus was also detected in glabrous 'Morio Muskat' and is known as *Leaf Hair 1* (*LH1*) locus from 'Muscat of Alexandria'. Additional QTL are described e.g. on chromosomes 1, 8, 10 and 15 associated with either absence or presence of ribbon trichomes, indicating the complexity of this trait. Knowledge about such underlying genetic loci can be used in breeding programs to consider ribbon trichomes as a preformed physical barrier in addition to genetic resistances in form of *Rpv* loci. Thus, the resilience of new cultivars to downy mildew can be reinforced, green leaf tissue preserved and the infection pressure in vineyards kept low.

Keywords: leaf hair, ribbon trichomes, physical barrier, QTL, downy mildew, *P. viticola*

P81 – A high-density integrated map for grapevine based on three mapping populations genotyped by the *Vitis*18K SNP chip

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Abstract

The improvement of grapevine through biotechnology requires identification of the molecular bases of target traits by studying marker-trait associations. The *Vitis*18K SNP chip provides a useful genotyping tool for genome-wide marker analysis. The majority of linkage maps are based on single mapping populations, but integrated maps can support QTL studies increasing marker density and providing a reference for marker genetic order. Here we present the integration of three different maps genotyped using the *Vitis*18K SNP chip. The parents consist of the well-known wine cultivars 'Cabernet Sauvignon', 'Corvina' and 'Rhine Riesling', the lesser-known wine variety 'Deckrot', and a table grape selection, G1-7720.

Three high-density population maps with an average inter-locus gap ranging from 0.74 to 0.99 cM were developed. These maps show high correlations (0.9965 – 0.9971) with the reference assembly and validate, by genetic mapping, chromosomal location of 9340 of the SNPs from the *Vitis*18K. Only 93 markers with large order discrepancies compared to expected physical positions were found, of which a third consistent across multiple populations. More recently an additional mapping study, based on a similar approach, further increased the number of the SNPs belonging to *Vitis*18K chip with a validated genetic position. Moreover, these genetic data aid the further refinement of the grapevine genome assembly, by anchoring 104 yet unanchored scaffolds.

From the three population maps, an integrated map was constructed which includes 6 697 molecular markers and reduces the inter-locus gap distance to 0.60 cM, resulting in the densest integrated map for grapevine thus far. A small number of discrepancies, mainly of short distance, involve 88 markers that remain conflictual across maps. The integrated map shows similar collinearity to the reference assembly (0.9974) as the single maps. This high-density map increases our understanding of the grapevine genome and provides useful tools for its further characterization and the dissection of complex traits.

Keywords: *Vitis vinifera*, genetic linkage maps, single nucleotide polymorphism, Consensus map building

P82 – Characterization of the black rot resistance loci (*Rgb1* and *Rgb2*) of 'Börner' and development of associated markers suitable for marker-assisted selection in grapevine breeding

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Abstract

Since the beginning of the 21st century, an increased incidence of grapevine black rot (*Guignardia bidwellii*, anamorph: *Phyllosticta ampelicida*) disease has been reported from regions all over Europe. The hemibiotrophic ascomycete favours a warm and humid climate. Due to climate change, potential distribution areas for black rot will shift to northwestern winegrowing regions of Europe. With the intended reduction of fungicide use and increase of acreage of new mildew-resistant grapevine cultivars in the European Union, there is concern that diseases previously considered secondary, such as black rot, may emerge and become relevant. Therefore, the identification of resistances to black rot and their introgression in new varieties is an important task. In an initial study on a F1 population of a cross of V3125 ('Schiava Grossa' x 'Riesling') x 'Börner' (*Vitis riparia* x *Vitis cinerea*), two QTL (quantitative trait loci) were described conferring resistance to black rot. They were localized on chromosome 14 (*Rgb1*) and 16 (*Rgb2*) of 'Börner'. In the course of haplophase-specific analysis to reveal the origin of the black rot resistance, *Vitis riparia* was clearly identified as the resistance donor of both resistance loci. The use of an improved version of the integrated genetic map based on SSR markers led to a further reduction of the size of the *Rgb2* locus. Using SSR markers linked with the *Rgb1* locus, we could demonstrate the stable inheritance of the black rot resistance into the next generation (pseudo-backcross with 'Pinot blanc'). These SSR markers are highly suitable for marker-assisted selection (MAS) and valuable tools to extend the focus of grapevine breeding towards the introgression of black rot resistance into new varieties. To further reduce the size of the *Rgb* loci and define candidate genes for black rot resistance, a local mapping approach will be followed analyzing recombinant individuals of the enlarged V3125 x 'Börner' and derived pseudo-backcross populations.

Keywords: black rot, *Guignardia bidwellii*, resistance, QTL mapping, 'Börner', MAS, *Vitis riparia*

P83 – Technological implementations toward successfull application of marker-free genome editing in *Vitis vinifera*

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Abstract

New Plant Breeding Techniques (*NPBT*s) protocols have been developed to produce new grape varieties with improved quantitative and qualitative characteristics. Unlike traditional cross-breeding, the NPBTs allow modifying a chosen character, keeping intact the other typical characteristics of the variety by adding or modifying only target genes.

In order to perform marker free editing, the most promising route is the one that passes by embryogenic competent callus formation, isolation of viable protoplasts, transformation with CAS ribonucleoproteins, regeneration of the plants through embryos formation.

Here, we present the recent advancement of our research team in develop genomic tools for the application of next generation molecular breeding in *Vitis vinifera* L.

The applicability of these *NPBT*s is strictly dependent to some main factors: to identify the genes involved in interesting traits, the availability of reliable transformation protocols, the possibility of avoiding unwanted integrations of exogenous DNA in the genome of the plant that would transform it into a transgenic organism, the availability of reliable regeration protocols. By combinig genomic and trascriptomics we were able to in deep study some candidate genes important in grape breeding and use these informations to precise design of gRNAs/CAS modules to target specific sites of mutation. To fine-tune the whole procedure, we chose to target *VviAGL11*, the gene that induces seedlessness in table grapes.Notewothy, we optimized methods to improve the somatic embryogenesis ability of recalcitrant genotypes, such as the Italia cultivar. Indeed, in grapes a wide application of NPBTs in *Vitis* is hindered by the null or very low aptitude to generate embryogenic calluses of many important varieties. By testing different protocols, we observed that besides the strong genotype influence, in our system the formation of total and embryogenic calluses was influenced by the type of sterilization and the cultivation substrate.

Our modified protocol increases the production of embryogenic calluses, which was a fundamental aspect for the applicability of *NPBT*s such as cisgenesis and genome editing.

We optimized a protocol for *CAS12* production and purification suitable for laboratories not specialized in the production of recombinant proteins. We also produced *CAS9* and dCAS9-VN64, a protein lacking the nuclease activity but suitable for activatation of target genes. All produced proteins were *in-vitro* tested to be fully active. Another step forward was made by improving the protocols to isolate protoplasts and to induce their transformation and regeration. We tested several protocol and strategies in order to obtain the highest possible yeld of viable protoplasts. So far, some embryo regerenants were obtained.

Keywords: Genome editing, protoplasts*, NPBT, CAS9, CAS12, VviAGL11*

P84 – Diversity and distribution of viroids in German grape vines and possible future implications for product quality under global warming conditions

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Abstract

Grapevine is a perennial crop, which is cultivated intensively over many decades. This provides optimal conditions for viroids, which need several months or years to be fully established in their host. After the viroids are initially introduced through infected planting material, they are easily transmitted through crop management practices. Since most viroids do not lead to strong regular symptoms in grapes, they are considered to be latent, thus unimportant for the grape producer. This might be the reason, why viroids can be found in most, if not all, grape production areas worldwide. Currently, there are six viroids reported to infect grapevines, of which the Australien grapevine viroid is thought to have evolved through recombination of different viroids present in a single host. Studies in the 1980s showed that the hop stunt viroid (HSVd) is present in Germany. However, other viroids are also present and known to induce yellow spots especially under warm weather conditions like the grapevine yellow speckle viroid 1 (GYSVd-1). Because grapevine is a reservoir for hop-pathogenic viorids like the HSVd and also global warming is likely to increase symptom severity of GYSVd-1 in the future a preliminary survey and subsequent comparative sequence analysis was conducted to get an up-to-date overview of the infection status of German grapes.

The analysis of leaf samples of commercial grape cultivars with reverse transcription and subsequent PCRs show that HSVd and GYSVd-1 are widely distributed in Germany. Furthermore, Sanger sequencing revealed that the dominat HSVd-variant (similar to X06873) is different from variants typically found in other grapes, citrus or hops. The effect of this HSVd-variant as pathogene for hop has not yet been studied. The effect of the “latent” infection of HSVd and GYSVd for grape yield and subsequent wine quality has not yet been determined. These topics need to be addressed in further studies.

Keywords: global warming, risk assessment, reverse transcription, phylogenetic analysis

P85 – Predictive breeding for wine quality: sensory and chemical phenotyping of wines from a F1 grapevine population

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Abstract

New pathogen resistant grapevine varieties greatly reduce the use of fungicides and thus contribute to a more sustainable viticulture. However, the evaluation of the resulting wine quality of new varieties is a bottleneck in the selection process slowing down the breeding efficacy. Therefore, our major aim is to develop robust models to predict the genetic quality potential of grapevine varities. The implementation of metabolic markers for wine quality traits in marker-assisted selection (MAS) during grapevine breeding will result in an early and more efficient selection of promising genotypes.

A segregating white wine F1 population of ‘Calardis Musqué’ and ‘Villard Blanc’ consisting of 150 genotypes with 13 plants per genotype at two locations provides the basis for a broad set of genomic, metabolomic, and sensory data. A ‘Genotyping by Sequencing’ approach with a novel bioinformatics pipeline delivered a high-density genetic map of the breeding population. Authentic wines from standardized micro-fermentations were used for comprehensive sensory evaluation and chemical analysis of major and minor metabolites, including aroma compounds such as terpenoids, by SPE-GC-MS. Moreover, five annual repetitions at two locations allow refinement, evaluation, and validation of predictive models and an estimation of environmental impact on the phenotypic data.

The descriptive and quality score card for sensory evaulation was adapted to the large number of wine samples and the unusual broad range of wine qualities resulting from an unselected set of grapevine genotypes. With the annual repetition of the sensory evaluation of all wines from the 150 genotypes, a trained panel reproducibly differentiated a set of best and worst genotypes over five vintages. The intensity of the descriptive wine attribute “floral” correlates with the concentrations of the two aroma-active compounds linalool and cis-rose oxide in each vintage. Finally, linking sensory and analytical data from multiple vintages with genetic information gives new insights in genomic regions related to the quality potential. Thus, predictive models will provide descriptors for wine quality traits leading to a more efficient grapevine breeding.

Keywords: wine quality, phenotyping, metabolic quality potential, monoterpenes, genetic quality potential

P86 – Adequate nitrogen management of vine, effects on leaf and berry physiology and wine quality. A study from lab scale to field scale

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Abstract

Nitrogen (N) can be taken up by the roots in various forms such as nitrate, ammonium, urea or even amino acids which had to be proven. These N-forms and their possible differential assimilation directly and indirectly influence grapevine vegetative and generative growth. The application of different amounts and different forms of nitrogen may affect not only yield but also the metabolite patterns in grapevine leaves and may therefore also influence berries metabolite composition and finally affect the sensory profile of wine. The quality of grapevine berries, must and wine is of course influenced by environmental and viticultural inputs and their complex interactions. It was tested weather nitrogen forms such as nitrate, ammonium, urea or amino acid application has influence on the composition of phenolic compounds in leaves, berries and wine. The study was conducted in hydroponics, soil culture and in a vineyard to include all scales from greenhouse experiments which result in adequate physiological results and up to vineyard trials for obtaining results in terms of quality aspects. Aroma and flavour are decisive for quality and are mainly determined by primary and secondary metabolites. In particular, phenolic compounds contribute to berry and wine quality. Must and wine quality was evaluated by chemical analysis and sensory testing. Metabolomic profiling was also performed. Aroma and sensory profile were significantly changed by the application of nitrogen in contrast to cero nitrogen fertilisation. The levels of 33 metabolites in leaves and 55 metabolites in wine were significantly changed altered by fertilisation with the various nitrogen forms. In leaves, more metabolites were increased by the use of nitrate or ammonium but were decreased by the use of urea. In terms of wine, the used nitrogen forms decreased more metabolites compared with no fertilisation. Moreover, expression of key enzymes for nitrogen assimilation in grapevine rootstock in response to N-form and timing were analysed. Roots and rootstocks play an essential role in grapevine water and nutrient uptake and affect biomass allocation of the scion and the grape berry composition connected with wine quality. N assimilation is driven by N acquiring enzymes such as nitrate reductase (NR), nitrite reductase (NiR) and glutamine synthetase (GS). This assimilation physiology can be influenced by factors such as light conditions or substrate availability. Hydroponically grown grapevine rootstocks were fertilized with various N-forms, namely calcium nitrate, ammonium, urea, and glutamine. The transcript expression of the enzymes NR, NiR and GS and the enzymatic nitrate reductase activity (eNRA) were examined at various short time points (0 – 6 h) after N application. Resulting data suggest that the grapevine rootstock SO4 has the ability to assimilate the amino acid Gln. Furthermore, AM, UR, and in some organs Gln, can regulate the co-enzymes NR and NiR, both of which function as activators of the nitrate assimilation process. The eNRA is clearly defined by the plant organ. Roots option of a direct uptake of amino acids is new and is especially important for organic fertilization. Moreover, besides effects of fertilized N amounts, the N-form may directly effect on the leaf physiology the berry composition and the wine quality.

Keywords: nitrogen, ammonium, urea, amino acids, quality, nitrate reductase


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