2nd Annual Meeting

INTEGRAPE 2020

Multi-omics data integration for genotype-phenotype association

Ljubljana, 3 – 5 March, 2020

Book of Abstracts

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Table of Contents

Program overview

MONDAY March 2, 2020	
17:30	Registration
19:00	Welcome Reception at Hotel Slon (club 2+3 Hall)

TUESDAY March 3, 2020	
08:30-16:30	Registration
09:00-09:10	Welcome addresses (Kavarna Hall)
09:10-09:30	Introduction to 2019-2020 COST INTEGRAPE activities (Kavarna Hall)
09:30-10:40	Session 1: Genomes, genome variation and gene functional annotation (Kavarna Hall)

09:30-10:00 ***Network based potato gene function prediction from temporal gene expression and knowledge***

Blaž Škrlj (Slovenia)

10:00-10:20 ***The wild side of grape genomics***

Dario Cantu (USA)

10:20-10:40 ***Transposable elements, structural variation and epigenetic variation in grapevine***

Michele Morgante (Italy)

10:40– 11:10	Coffee Break
11:00-12:30	Session 2: Grapevine transcriptomics (Kavarna Hall)

11:00-11:30 ***Development and application of Spatial Transcriptomics from mammalian species to plants: progress and prospects*** (via remote presentation)

Stefania Giacomello (Sweden)

11:30-11:50 *GREAT (Grape Expression Atlas): all in one, a curated database, an analysis workflow and a web application to analyze Vitis vinifera public RNA-seq data*

Camille Rustenholz (France)

11:50-12:10 *Defining a model of molecular phenology for grape berry development*

Giovanni Battista Tornielli (Italy)

12:10-12:30 *Finding functional interactions among grapevine genes using transcriptomic data and NES²RA algorithm*

Stefania Pilati (Italy)

12:30-14:00 Lunch (Crystal Hall) and poster session (Kavarna Hall)

14:00-14:20 Session 2:
Grapevine transcriptomics - continued (Kavarna Hall)

14:00-14:20 *Report on the METHADA 2020 Training School*

Jérôme Grimplet and José Tomás Matus (Spain)

14:20-15:10 Session 3:
Grapevine metabolomics (Kavarna Hall)

14:20-14:50 *Introduction to FAIR principles about data, metadata and protocols in metabolomics*

Panagiotis Arapitsas and Pietro Franceschi (Italy)

14:50-15:10 *Lipid characterization of Ribolla Gialla grapes for the production of monovarietal sparkling wines*

Domen Škrab (Italy)

15:10-16:00 Session 4:
Genetics and genomics of traits of interest (Kavarna Hall)

15:10-15:40 *The search for SNP, InDel and SSR markers associated to berry size in table grape, or the dream of a MAS platform for a complex trait*

Patricio Hinrichsen (Chile)

15:40-16:00 *An optimization of the marker-assisted breeding process for downy and powdery mildew resistance in grapevine*

Paola Bettinelli (Italy)

16:00– 16:20 Coffee Break

16:20-17:20 Session 4:
Genetics and genomics of traits of interest - continued (Kavarna Hall)

16:20-16:40 *Berry shrivel a matter of "switch" gene manipulation – is there a bioinformatic solution?*

Michaela Griesser (Austria)

16:40-17:00 *Study of the diversity of Moroccan local vine varieties "Vitis vinifera ssp. Vinifera" basing on OIV ampelographic descriptors*

Salama El Fatehi (Marocco)

17:00-17:20 *Haplotyping the germplasm collections of the USDA National clonal germplasm repositories with rhAmpSeq and development of markers for sex locus and other breeding QTLs*

Qi Sun (USA)

19:00 Unformal networking at Union Pub (own expense)

WEDNESDAY March 3, 2020

08:00-16:30 Registration

09:00-10:30 Session 5:
Data integration, knowledge development (Kavarna Hall)

09:00-09:30 *Network analysis of multilevel integrated plant datasets*

Živa Ramšak (Slovenia)

09:30-09:50 *Phenotypic variation of ampelographic and technological traits of newly created grapevine genotypes*

Glišić Milica (Serbia)

09:50-10:10 *Omics approaches to investigate different susceptibility of grapevine varieties to Flavescence dorée*

Nadia Bertazzon (Italy)

10:10-10:30 *Volatile organic compunds from essential oils as a sustainable alternative to pesticides – deciphering the molecular basis underlying their mode of action and their role as plant immunity primers*

Markus Rienth, Sana Ghaffari, Marylin Cléroux, Arnaud Pernet, Julien Crovadore, Eric Remolif, Jean-Philipp Burdet, Francois Lefort (Switzerland)

10:30– 10:50 Coffee Break

10:50-12:10 Session 6:
Presentation of the work done through the STSM Program (Kavarna Hall)

10:50-11:10 *Case study: Impact of INTEGRAPE on documentation and molecular characterization of grape genetic resources in Armenia*

Kristine Margaryan (Armenia)

11:10-11:30	*The correlation of secondary metabolites contents with oxidoreductase enzyme activities in autochthonous grapevine varieties from Bosnia and Herzegovina*
	Dino Hasanagić (Bosnia and Herzegovina)
11:30-11:50	*Monitoring of a vineyard soil moisture and ground-zone temperature by automatic sensors*
	Barnabás Kovács (Hungary)
11:50-12:10	*Introduction to the pilot project sessions*
12:10-13:30	Lunch (Crystal Hall) and poster session (Kavarna Hall)
13:30-16:00	Break out sessions of the pilot projects

- *pilot project 1(Kavarna Hall 1) and 3 (Kavarna Hall 2): metadata for genome submission to ENA + alignment with requirements for transcriptome submissions, gene nomenclature*
- *pilot project 2 (Club Hall 1) : standardisation of the organs naming, development stages, Vitis ontology*

16:00 – 16:30	Coffee Break
16:30-17:00	*Break out sessions of the pilot projects (continued)*
17:00-17:30	*Report of the break out session to all: planning towards deliverables (Kavarna Hall)*
19:00-22:30	Networking dinner at Ljubljana Castle

THURSDAY March 5, 2020	
08:00-09:30	Registration
09:00-10:30	Annual meeting wrap-up (Kavarna Hall)
10:30– 10:50	Coffee Break
10:30-12:00	Core group meeting (Kavarna Hall)
12:00-13:00	Management committee meeting (Kavarna Hall)

Abstracts of Selected Talks

Network based potato gene function prediction from temporal gene expression and knowledge graphs

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Gene function prediction is one of the most commonly addressed tasks in systems biology. In the recent years, gene expression information is often recorded in time, and additionally annotated with concepts from existing background knowledge graphs – data structures containing typed nodes and links that are understandable and easily parsable. In this work, we addressed the task of gene function prediction based on temporal gene expression profiling, as well as existing background knowledge sources. We considered transcription factor and binding events, miRNA regulation, binary protein-protein interactions, literature associations and temporal expression networks. We fused the mentioned layers of information into a single multiplex network which we embedded into a low-dimensional real-valued representation, allowing us to associate individual genes with their corresponding molecular function spaces. We performed the task of multilabel classification (more possible functions per single gene) with deep neural networks, supporte vector machines and gradient boosting machines.. We achieved state-of-the-art predictive performance with deep neural networks and produced multiple novel predictions that were evaluated based on existing empirical evidence.

The wild side of grape genomics

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The cultivation of grapevines (*Vitis vinifera*) rely on wild *Vitis* species as sources of resistance to biotic and abiotic stresses. Despite the importance of non-vinifera *Vitis* species, very few genomic resources are available. We have been generating reference genomes for wild *Vitis* species that either have been used, or have demonstrated promise, for breeding. These include multiple accessions of *V. vinfera* ssp. *sylvestris,* as well as North American species such as *V. arizonica, V. girdiana, V. berlandieri, V. acerifolia, V. riparia, V. aestivalis, V. monticola, V. mustangensis,* and Central Asian species, such as *V. piazeskii* and *V. romanetii.* All genomes were sequenced using single molecule real-time sequencing (SMRT; Pacific Biosciences) and optical maps (Bionano), and are being assembled into completely phased pseudochromosomes. SMRT sequencing was also used to sequence full-length cDNA (Iso-Seq) and, together with high-depth short-read libraries, reconstruct the transcriptomes of all species. These annotated reference genomes will be shared with dedicated genome browsers as a community resource and have been used as a foundation for own studies. For example, living collections of thousands of North American genotypes have been genotyped and phenotyped to study wild grape evolution in the American Southwest as well as to identify novel sources of genetic resistance to Pierce's Disease resistance and tolerance to salinity. These resources have been valuable also to study the genetic basis of other important domestication and agronomic traits, such as resistance to fungal and bacterial diseases, and flower sex determination.

Transposable elements, structural variation and epigenetic variation in grapevine

Gabriele Magris1,2, Paloma Perez-Bello Gil3, Gabriele Di Gaspero2, Mirko Celii1,2, Emanuele De Paoli1, Rachel Schwope1,2, Elenonora Paparelli3, Michele Morgante1,2

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A large fraction of phenotypic variation appears to be determined by regulatory rather than coding variation and understanding how gene expression is controlled becomes a prerequisite for the exploitation of the full potential of genome editing techniques. Biochemical data collected in the Encyclopedia of DNA Elements (ENCODE) project, coupled with genetic and comparative genomics information, has led to considerable progress in the understanding of the regulatory elements in the human genome. Relatively little is known about the transcriptional regulatory structure of plant genomes in comparison to animals in terms of number, location and evolutionary conservation of cis-regulatory elements. The recent hyperactivity of transposable elements (TEs) observed in most Angiosperm species analysed so far is a specific characteristic of plant genomes. The impact of such movement has been described in terms of effects on structural variation at the DNA sequence level but much less is known on the global impact on regulatory variation and its effects on epigenetic variation and chromatin structure.

Vitis vinifera is both economically important and scientifically intriguing. In contrast to the small and relatively simple genome of Arabidopsis, grapevine contains a complex genome of 487 Mb that exhibits extensive colonization by transposon elements, making it a useful model in which to study how gene expression is regulated. We have exploited a high quality reference genome sequence, additional haplotype-specific sequences obtained through a combination of de novo and resequencing efforts, whole genome sequences for more than 120 genotypes (providing a full description of genome-wide single nucleotide and structural variation), transcriptome analysis using RNASeq and information on epigenetic features such as DNA methylation obtained through bisulfite sequencing to link the presence of structural variation due to transposable element movement to local changes in DNA methylation and in gene expression using a haplotype-specific approach in heterozygous individuals.

Development and application of spatial transcriptomics from mammalian species to plants: progress and prospects

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In the past few years we have seen a growth in the understanding of the transcriptional complexity led by technologies like single-cell RNA-sequencing, which have allowed to discover the heterogeneity of gene expression among cells of the same tissue. Currently, the scientific field is taking a step further to localize such transcriptional information within the spatial context of the tissue itself.

Here, we show an innovative, high-throughput technology originally developed for mammalian tissues, Spatial Transcriptomics, which we extended to plant tissues. The method enables the simultaneous quantification and visualization of transcriptional profiles in thin tissues at 100- and 55-µm resolution. We are applying Spatial Transcriptomics to young wheat spikes and *Pseudomonas* droplet infected *Arabidopsis thaliana* leaves. To this end, we developed several advancements to the original method in order to study the concerted bacterial infection process and plant response.

Our results demonstrate that Spatial Transcriptomics allows not only to study unexplored spatial gene expression patterns in young wheat spikes but also to detect the spatial gene expression profiles of the plant response to bacterial infection. This opens up the possibility of extending our approach to different plant systems to elucidate complex infection processes where the spatial component is key for their understanding.

GREAT (Grape Expression Atlas): all in one, a curated database, an analysis workflow and a web application to analyze *Vitis vinifera* public RNA-seq data

Amandine Velt, Lauriane Renault, Gautier Arista, Thuy-Thanh Truong, Philippe Hugueney, Éric Duchêne, Camille Rustenholz

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Since several years, RNA-seq is the leading technology to assess global gene expression profiling. RNA-seq was massively used to study transcriptomes of a large amount of samples to answer large diversity of questions. With the creation of public databases to store high-throughput sequencing data, it is possible to retrieve public RNA-seq data and reuse them for new discoveries. However, it can be difficult for biologists, seeking for candidate genes for a given trait, for example, to make the most out of these databases. First, it is very time-consuming to go through the metadata to find suitable RNA-seq data for a given biological question. Second, the raw RNA-seq data need to be re-analyzed so the results may be comparable across various experiments. Third, the obtained results are difficult to visualize and to investigate in a user-friendly manner.

To tackle all these problems and help biologists to explore RNA-seq public databases, we developed a project for the grapevine scientific community, called GREAT (GRape Expression ATlas, http://great.colmar.inra.fr/*). GREAT is composed of three parts that can be adapted to any RNA-seq data of any given organism:

1. A curated and quality-checked database for *Vitis vinifera* species integrating metadata from main RNA-seq public databases (SRA, EBI and GEO). It currently contains more than 900 and more than 1,000 more samples will soon be added.
2. A RNA-seq analysis workflow developed with Snakemake. It starts with raw data (.sra or .fastq files) as input and generates gene count tables for all publicly available data for *Vitis vinifera* as output.
3. A web interface developed with R Shiny to explore gene expression in all these samples. It allows visualizing heatmaps, clustering graphs, analyses of differentially expressed genes and downloading the chosen results for further investigation.

In conclusion, it is important for biologists to have tools, such as GREAT, to speed-up the exploration of public RNA-seq data to easily access the genes expression of all samples of its species of interest. Thus, GREAT makes it possible to quickly explore gene expression in various varieties of *Vitis vinifera* and/or for a wide range of biotic and abiotic treatments, in order to have a global vision of the expression of one or more genes. Moreover, GREAT was developed to be used broader than the grapevine community as it is easily transferrable to any RNA-seq data of any given organism.

* For early access, please request an account by email.

Defining a model of molecular phenology for grape berry development

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The Modified E-L and the Extended BBCH are the phenology scale systems most adopted by viticulturists. These systems describe the annual phenology of the plant, including grape berry development from fruit set to maturity, and number the main developmental stages by increasing order. However, although some stages can be easily described (e.g. fruit set, veraison), defining a comparable developmental stage for grapes of the same cultivar when grown in different conditions or for grapes of different cultivars can very likely generate mistakes, in particular after the onset of ripening. In the last years the application of genomic tools to the analysis of gene expression during grape berry development have generated a huge amount of transcriptomic data from different varieties and growing conditions. It has been shown that the variations of a portion of the transcriptome (the core transcriptome) along berry development seem to be conserved across cultivars and growing condition of grapevines, and thus may be used to describe the developmental stage of berry development. In this work we explore the possibility of using the transcriptomic data generated from grape berries weekly sampled from Cabernet Sauvignon and Pinot noir vines grown in the same location over three consecutive vintages to map the development of the grape berry. We used the most variable portion of the transcriptome to build a preliminary transcriptomic model of berry development, which allowed to precisely define the progression of development during berry formation and ripening phases. The Pinot noir and Cabernet Sauvignon samples mostly aligned in a 3D transcriptomic map (~80% of the variance described by Principal Component Analysis), allowing to define a general model of berry development based on gene expression. The performance of the model in describing the development of other grape varieties was accessed projecting RNA-seq samples of fruit development of ten Italian cultivars onto the model. Both red and white-skin berry samples mapped on the transcriptomic map. Moreover, we validated that berry maturation of the same cultivar cultivated in different international growing regions can be well represented and aligned by means of our transcriptomic map. These results showed that the transcriptomic information can be accessed to precisely define a model of molecular phenology that can be used to map the ontogenetic development of the fruit with high precision and to align the stage of berry development of different grapes.

Finding functional interactions among grapevine genes using transcriptomic data and NES2RA algorithm

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More than two hundred transcriptomic studies are currently publicly available for grapevine. They have been collected, normalized and annotated into the Vitis Expression Studies Platform Using COLOMBOS Compendia Instances (VESPUCCI updated version, Moretto et al., in preparation). Mining all this information to extract novel findings, such as gene networks that control agronomically relevant traits, remains a challenge. In particular, climatic changes and the shift to more sustainable practices affect diseases and yield behaviors in grape production, thus urging the scientific community to propose new strategies to cope with them. System biology approaches can represent an opportunity to boost our knowledge of the grapevine physiology. Gene networks are a convenient way of representing as graphs the functional interactions (edges) among the genes (nodes) of an organism. Gene networks can be co-expression networks, based on Pearson's correlation, or association and regulatory networks, in which direct and possibly causal relationships are represented. We would like to present the tool NES2RA (Network Expansion by Sub-Setting and Ranking Aggregation) - based on the PC-algorithm (Spirtes and Glymour, 1991)- that finds causal relationships from observational data. It performs a systematic test for conditional independence to retain significant relations between pairs of genes. It starts from a fully connected network and removes interactions between genes, whenever it finds a set of genes that supports that interaction. Due to the computational power requirements of the NES2RA algorithm, it has been implemented on a distributed computation platform, as part of the gene@home project, which relies on thousands of volunteers' computers by means of TN-Grid, an infrastructure based on the BOINC system (Asnicar et al., 2015). In order to accomplish to the FAIR (Findable, Accessible, Interoperable and Reusable) requirements for the information produced by NES2RA, the expansion gene list of each single gene has been pre-computed and annotated and can be downloaded from our website (http://ibdm.disi.unitn.it/, in preparation). The user can consider the lists can as such or analyze them further for example by aggregating them to reconstruct a gene network. A case study example concerning the regulatory network and biosynthetic pathway of the grapevine leaf cuticle will be presented to show how this information can help the biologist in gene function discovery, candidate gene prioritization and planning functional studies in grapevine.

Introduction to FAIR principles about data, metadata and protocols in metabolomics

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In 2016, a consortium of scientists (cheminformaticians, bioinformaticians, biologists, data scientists, computer scientists and representatives from data archives and publishers congregated) with the intention to provide guidelines to improve the findability , accessibility , interoperability and reusability of the digital assets, published the 'FAIR Guiding Principles for scientific data management and stewardship'(Wilkinson et al., 2016). FAIR Guidelines are based on 15 Principles divided in 4 categories and cover Data, Metadata and Protocols.

Fondazione Edmund Mach (FEM) has a long experience in food, grape and wine metabolomics, while over the last years it's Metabolomic platform shared several data using public repositories. This presentation will discuss FEM experiences, by reporting the workflow and the tools used from the experimental design to the data sharing, in order to respect the FAIR guidelines. In detail, will be discussed: a) the meta-data collection and organization; b) the instrumental parameters and set ups; c) the various protocol needed; d) the ontologies; e) the metabolites ID; f) the informatic tools; and g) the repositories.

Reference:
Wilkinson et al. The FAIR Guiding Principles for scientific data management and stewardship. Scientific Data, 3, 160018 (2016).

Lipid characterization of Ribolla Gialla grapes for the production of monovarietal sparkling wines

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Due to their biological functions, lipids are essential biomolecules in all plant cells. The knowledge of grape lipid composition results limited to date. A few investigators have shown that lipid profile depends on grape maturity, the variety and their location in the berry. However, none of the previous studies focuses on the analysis of lipids, as one of the principal chemical constituents, in grapes of Ribolla Gialla variety from different vineyard sites. Moreover, the scope of the present work was to study the physiological and technological characteristics of grape lipid profile in correlation with cluster thinning, as a commonly adopted viticultural technique for increased accumulation of metabolites in the fruit, and increased sparkling wine quality at the end of winemaking process. The grape samples were collected during three consecutive growing seasons in two commercial vineyards in controlled designation of origin DOC (Corno di Rosazzo and Casarsa della Delizia) in the Friuli Venezia Giulia region in North-East Italy. A completely randomized design with two treatments (UNT, no thinning; and CT, 20% cluster thinning) and three replicates was imposed in each vineyard site. Furthermore, the grapes were sampled at several different maturity levels in all three vintages, in order to inspect the influence of harvest date on the quality properties of sparkling wines. By using liquid chromatographic electrospray ionization tandem mass spectrometry, twenty-nine lipid compounds were determined, among which the saturated long-chain fatty acids (LCFAs) were the predominant ones. Ribolla Gialla showed a higher total average concentration of saturated FAs in CT samples from Corno di Rosazzo (3.34 mg kg-1), compared to the unsaturated FAs (1.66 mg kg-1), which may lead to higher foam height in the later production of sparkling wines, and represents one of the key quality features of sparkling wines in general. Advancing the dates of the harvest does not change the ratio of saturated to unsaturated FAs, which may be profitable for the foaming properties of wine. Nevertheless, the availability of unsaturated FAs can affect yeast metabolism by maintaining integrity and function of the *Saccharomyces cerevisiae* membrane, as well as adapting to fermentation stresses.

The search for SNP, InDel and SSR markers associated to berry size in table grape, or the dream of a MAS platform for a complex trait

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The domestication of the grapevine occurred in different sites, from the Caucasus to Central-South Europe, and derived in very different phenotypes, depending on their final intended use as table or wine grape. These two main types of grapevines differ in a number of characteristics, with berry size as one of the most striking. In the case of table grapes, growers tended to select larger berries and clusters, in contrast to wine varieties, harboring small seeded berries. In modern times, berry size has been one of the most important selection criteria for table grape breeding programs, among other berry quality traits. Therefore, we decided to study its genetic determinants, in search of markers that could be used to implement a molecular-based selection scheme, as it has been successfully done for other traits in grapevine. In a first step, we used contrasting segregants for berry size in two developmental stages (pea-sized berries and pre-veraison) in order to compare their transcriptomes, identifying hundreds of differentially expressed genes for each combination. After the application of a series of filters, 350 genes potentially associated to berry size were detected. From them, we selected a group of 30 SNP and eight InDel markers unevenly distributed on eight chromosomes. However, even when this set of markers could explain up to ca. 30% of phenotypic variance, their implementation became technically complex, especially in the framework of a breeding program, when thousands of samples must be analyzed per season in a brief period of time. Then, we decided to move to an analytically simpler platform, such as microsatellites (SSRs). Also, we included at this stage 10 candidate genes located on nine chromosomes reported as linked to berry size, such as transcription factors of the bHLH family, VvCEB1, VvNAC26, VvGDSL, VvSTBsynt, among others. An in-silico search for the presence of mono- to penta-nucleotide SSR repeats in the periphery (1 Mb) of each marker and gene yielded a list of over 500 SSRs. Subsequently, these SSRs were evaluated for amplification quality and polymorphism level in a testing set of four genotypes, and the selected ones were further tested on 12 cultivars and segregants, always contrasting large vs. small berry size genotypes. In this way, 24 SSRs were selected and evaluated on three populations: two crossings (‘Ruby seedless’ x ‘Sultanina’, and ‘Muscat of Alexandria’ x ‘Crimson seedless’) and a set of 94 cultivars, in an effort to cover the species genetic diversity. The association analyses, currently in progress, is showing only partial association of specific alleles with smaller or larger berries for a subset of the 24 SSRs, which in turn were related to a subset of the SNPs/InDels and genes previously associated to berry size. The applicability of these markers as selection tools for breeding will be discussed.

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An optimization of the marker-assisted breeding process for downy and powdery mildew resistance in grapevine

Paola Bettinelli, Tiago Camponogara Tomazetti, Daniela Nicolini, Benedetta Tanzi, Chiara Dolzani, Alessandra Zatelli, Monica Dallaserra, Monica Visentin, Giulia Betta, Silvano Clementi, Cinzia Dorigatti, Luca Zulini, Marco Stefanini, Silvia Vezzulli

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Today the reduction in pesticide use is imperative and the implementation of sustainable viticulture is an urgent necessity. With this awareness and vision, the genetic improvement program for biotic stress resistances began at the Fondazione Edmund Mach (FEM) in 2010. Initially a genotypic characterization of grapevine materials acquired from other European and extra-European breeding programs, as well as wild material collected in 2011 in northeastern America, was conducted.

In parallel, investments were addressed to the development and optimization of phenotyping protocols for the evaluation of the symptoms of downy (DM) and powdery (PM) mildew both in laboratory conditions on detached organs and in greenhouses on seedlings and potted plants. A series of parents has therefore been identified suitable for different objectives and used over the years in the process of introgression and pyramiding of resistance (R) loci (genomic regions).

In 2017, through a Marker-Assisted Parental Selection (MAPS) procedure, a high number of stacked (or pyramided) genotypes was reached in the open field, 48.4% against DM and 59.5% against PM; in particular, 30.3% of genotypes resulted pyramided for R-loci against both mildews. Having achieved this result, it is timely to make the process of Marker-Assisted Seedling Selection (MASS) more efficient: investment in low-cost genotyping and in a phenotyping workflow capable of guaranteeing the scouting of PM as well as the maintenance of DM resistance. After the phenotyping tests, approximately 1,200 progeny individuals were sampled in the 2018 season, which were then characterized at nine R-loci. Updated and detailed results will be presented regarding the level of pyramiding now reached and the correlation between genetic makeup and levels of resistance to downy and powdery mildews in greenhouse conditions.

Thanks to this application study, starting from the 2019 season we have been able to adopt a pure MAS process −that is without upstream phenotyping screening− especially for those parental lines by now well established and with a known behavior in the breeding program.

Berry shrivel a matter of “switch” gene manipulation – is there a bioinformatic solution?

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Grape berry ripening follows distinct metabolic processes and complex regulations via phyothormones. The physiological ripening disorder berry shrivel (BS) is characterized by reduced sugar accumulation, low anthocyanin contents, and high acidity in affected berries. The processes leading to BS induction are unknown, but recent transcriptional data on reduced expression of switch genes hint towards a disturbed ripening onset with a lack or delay in “switch” gene induction at veraison. To decipher the regulation of this set of gene would bring us a major step forward to identify the causes leading to BS induction in grape berries. In a first step, we investigated the phytohormone composition throughout grape berry ripening in healthy and BS berries in Vitis vinifera L. cultivar Blauer Zweigelt. Thereby we hypothesize that phytohormones are key players for BS induction and suppress the expression of switch genes at veraison. We observed the induction of many phytohormonal biosynthesis pathways (ABA, auxin, and cytokinin) in BS berries after veraison on the transcriptional level, while ethylene and brassinosteroids are suppressed. One may question which process(es) keeps BS berries metabolically active during the ripening phase, as shedding or abscission of such berries would be resourceful. Two distinct phytohormone profiles in BS berry phenotypes were determined: pre- and post-veraison. Firstly, an ACC peak about 2 weeks before veraison was determined in BS berries and the reciprocal ethylene-auxin crosstalk needs to be taken into consideration in a next step. The application of ACC pre-veraison led to BS symptoms while ethephon induced berry abscission. Temporal and spatial sensitivity towards phytohormone changes in grape berries throughout the ripening process and its consequences both in healthy and induced BS phenotypes are unclear. Secondly, we propose that the induction of several phytohormone pathways prevent fruit abscission as e.g. observed with bunch stem necrosis or sunburn, post-veraison. The similarities and differences in transcriptional patterns of ripening disorders and withering processes need to be determined as well as the role of iP (and possibly also of ABA-GE and IAA-Asp) in berry ripening as well as the consequences of its decreased accumulation for sink activity in berries. Sophisticated approaches and defined experiments are needed to decipher in detail the pivotal role of phytohormones in BS induction pre-veraison and in the development of BS symptoms after veraison. In a first step we aim to analyse available transcriptomics data from grape berry ripening and relate their expression profiles to our data with a focus on the processes at veraison and switch genes. We aim to look for the profile/networks of already determined marker genes for ripening start and include stress related datasets. This future bioinformatic project perspective will be in accordance with the objectives of INTEGRAPE and will greatly benefit from the accumulated knowledge.

Study of the diversity of Moroccan local vine varieties "*Vitis vinifera ssp. Vinifera*" basing on OIV ampelographic descriptors

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Morocco, with its Mediterranean climate and its various potentialities, contains an important space for the extension of viticulture especially that of traditional grape varieties, which has undergone profound upheavals linked to economic, social and environmental constraints, which has had a negative impact on genetic diversity. We seek to highlight in order to recognize the local phylogenetic heritage, taking into account the phylogenetic richness that the region of the North West has in this matter by a study of characterization of the traditional vine leaves, which was discussed an ampelographic study of a collection of 1617 leaves, 162 feet and 27 different traditional varieties. This collection was processed using an ampelometric and ampelographic approach with SUPER AMPELO software. The study was conducted with a statistical approach in order to highlight the most discriminating parameters by the ANOVA test, namely the angles, the depth of the lateral sinuses with respect to the lengths of the ribs and the relations between all the parameters. In this sense, the qualitative parameters (OIV Codes) confirmed the presence of a morphological diversity within the grape varieties studied, the study of the general averages made it possible to specify the varieties with the large values of distance / angles and ratio and a showed the presence of a large intra-varietal diversity in addition to that which is inter-varietal. The analysis in principal components allowed to grouping the grape varieties in 5 groups according to their expressions vis-à-vis the quantitative parameters and it confirmed the hypothesis of the influence of the external environment in addition to the gene pool on the grape varieties. This leads us to provide more efforts to maintain inter and intra-varietal variability and to fight against genetic erosion and the threat of changes environmental.

Haplotyping the germplasm collections of the USDA National clonal germplasm repositories with rhAmpSeq and development of markers for sex locus and other breeding QTLs

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VitisGen2 is an USDA funded project to develop novel genotyping and phenotyping technologies for grape breeding. The genetics team of the project has developed a DNA marker system from the core genomic regions of major *Vitis* species, using the rhAmpSeq technology. Genetic linkage mapping of hybrid breeding families showed that these markers have great transferability among populations across the *Vitis* genus. In collaboration with USDA Breeding Insight Project, we sequenced 2057 targeted loci from 21,500 grape vines, including 6,000 accessions from the USDA germplasm repositories, and genotyped the diverse population for both haplotype alleles and SNP alleles. The results show that the rhAmpSeq markers can be used for both euvitis and muscadine subgenera. Even though the markers were developed to work across different *Vitis* species, majority of the haplotype markers are polymorphic within *vinifera*. Work are in progress to resolve haplotype diversity in *Vitis*, especially in the major QTL regions with breeding values. One region of interest is the grape sex locus, as accurate prediction of sex is important for hybrid grape breeding. *De novo* assemblies were built for male, female and hermaphroditic haplotypes. By integrating genetics and genomic resource of sex locus of multiple *Vitis* species, we were able to narrow down the genes for sex determination, and identify the origin of the hermaphroditic allele in the domesticated cultivars. A set of SNP markers and a machine learning model was developed to accurately identify the male, female and hermaphroditic haplotypes in all grape species. The sex locus markers and markers for other major disease resistance QTLs have been incorporated into the rhAmpSeq core marker set.

Network analyses of multilevel integrated plant datasets

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The need to better understand stress-mitigating mechanisms in crop plants is increasing rapidly. Most often discussed biological systems are networks of genes or proteins. Infection of a plant by a pathogen initiates a complex interaction between both players involved, leading to changes in the complex signalling network, which result in gene activity changes and reprogramming of the cell metabolism. A systems biology approach was adopted for the purpose of modelling complex biological processes in order to understand the mechanisms involved in potato plant defense following the infection with potato virus Y (PVY).

A mechanistic model of plant immune signalling was constructed from literature, describing the biosynthesis and signal transduction pathways for three crucial phytohormones involved in plant defence: salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). We additionally built a comprehensive knowledge network, combining the information from publically available databases and high-throughput experiment datasets, namely protein-protein interactions, transcription factor regulation and non-coding RNAs. The resulting knowledge network was translated from Arabidopsis to potato using PLAZA as a converter reference. Lastly, the Arabidopsis network was superimposed with an ensemble network from own produced experimental data – a short transcriptomics time-series (five time points) measuring potato response to viral infection.

These three data sources (PIS model, Arabidopsis comprehensive knowledge network and networks inferred from experimental data) were combined and analysed for presence of intriguing clusters. We aimed a particular subset of network analyses towards examining targeted identification of novel cross-talk connections between receptors and transmitters of seven plant hormonal pathways. One of the most interesting findings was the shortest path from the ET pathway transmitter EIN3 to the SA receptor NPR1. It showed several potential transcription regulation paths, including a cascade of transcription factors (e.g. ERF, WRKY). The connection was confirmed experimentally, in Arabidopsis and potato, thus confirming that, in addition to protein level regulation of NPR1, transcriptional regulation of the NPR1 gene also plays a role in plant immune signalling.

Phenotypic variation of ampelographic and technological traits of newly created grapevine genotypes

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The identification of grapevine varieties can be done by several complementary methods. Ampelographic methods are based on morphological, i.e. phenotypic characteristics and rely on descriptors to express the results uniformly. Production-technological characteristics describe the yield characteristics, the elements of the structure of the bunch and berry, as well as the characteristics of the wine. The aim of this study was to investigate some of the ampelographic and most important technological characteristics of perspective grapevine genotypes: H2 (Merlot x Župski Bojadiser), H12 (Prokupac x Župski Bojadiser) and OCB (Alicante Henri Bouschet x Vranac) intended for the production of red wines. The listed genotypes with these codes are on the *Vitis* International Variety Catalog (*V*IVC). It was necessary to determine similarities and differences with their parental partners in the studied genotypes. The experimental vineyard where the plant material was tested and collected, during the three years (2016–2018), belongs to the Faculty of Agriculture, University of Belgrade. The ampelographic description included 21 characteristics in the tested genotypes and parental partners, recommended by the International Plant Genetic Resources Institute (IPGRI, 1997) for the Gene Bank. The examined genotypes showed many similarities among themselves, as well as in comparison with their parental partners, but for some traits, differences were found, and as such, they represent unique genotypes. The H12 genotype differed by 5 characters, while the H2 and OCB genotypes differed by 6 characters from both of their parental partners. The technological characteristics of the examined genotypes were at the same level or better than their parental partners. The OCB genotype showed the highest yield, the highest bunch weight and the largest berry size, while the H2 genotype showed the highest average sugar and total acid content in the must. The wine of the tested genotypes was drinkable, harmonious with specific aroma and taste. Alcohol content varied from 13.5% v/v (OCB genotype) to 14.2% v/v (H2 genotype). The tested genotypes also differed from each other in total phenol content ranging from 0.95 g/l (OCB genotype) to 1.2 g/l (H12 genotype). Since the ampelographic description has determined that each of the examined genotypes represents a unique genotype, their application has been submitted to the Commission for the recognition of new grapevine varieties in Serbia.

Omics approaches to investigate different susceptibility of grapevine varieties to Flavescence dorée

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Flavescence dorée (FD) is the most serious grapevine yellows disease in Europe. It is caused by phytoplasmas which are transmitted from grapevine to grapevine by the leafhopper *Scaphoideus titanus* (St). Grapevine varieties show differences in susceptibility to FD, suggesting the existence of specific genetic traits associated with resistance to the disease. In the present study, different omics approaches were used to investigate the mechanisms responsible for the intraspecific variability in the susceptibility to FD. Firstly, the transcriptomic response induced by the FD phytoplasma and by its vector was investigated on two cultivars, Chardonnay and Tocai friulano, which display very different susceptibility to FD. Results showed that constitutive differences related to defense strategies between the two varieties were amplified after the challenging with the insect or with the FD phytoplasma. Those molecular mechanisms caused defense responses different in the type, amplitude and kinetics of gene induction, thus defining the diverse susceptibility to FD of the two grapevine cultivars. The search for gene traits upstream of these molecular mechanisms is underway through two different genomic approaches. The first consists in phenotyping and genotyping of a segregating population deriving from the cross between Chardonnay and T. friulano, to find out Quantitative Trait Loci (QTL) related to FD resistance and susceptibility. The second strategy involves the deep whole genome sequencing of two clones of Chardonnay displaying very different susceptibility to FD coupled with the study of transcriptomic response during the three-trophic FD-St-grapevine interaction. Based on the obtained results, further resequencing of genes putatively associated to resistance/susceptibility will be performed in a panel of other resistant/susceptible grapevine varieties, in order to confirm that specific genetic traits are present in the varieties with the same phenotype. Preliminary data, that suggested an involvement of jasmonic acid in grapevine defense against FD, are also used to investigate the ability of some microbial volatile organic compounds (mVOCs) in activating this specific defense pathway. Experimental trials, with controlled FD infections with St, together with transcriptomic and metabolomic analyses, will be performed to evaluate the efficacy of mVOCs treatments.

Volatile organic compunds from essential oils as a sustainable alternative to pesticides – deciphering the molecular basis underlying their mode of action and their role as plant immunity primers

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The amount of synthetic pesticides applied in viticulture is relatively high compared to other agricultural crops, due to the high sensitivity of the grapevine (*Vitis vinifera L.*) to fungal diseases such as downy mildew (*Plasmopara viticola).* Alternatives to reduce fungicides are utterly needed to ensure a sustainable vineyard-ecosystems and consumer acceptance.

Essential oils (EOs) are amongst the most promising natural plant protection products due to their antibacterial, antiviral and antifungal properties. However, the efficiency of EOs depends highly on timing and method of application and the molecular interactions of host, pathogen and EO, which underlie the efficiency of EOs, are not well understood. To circumvent the drawbacks of a direct application, the presented study aimed a) to evaluate whether a continuous fumigation of EO can control downy mildew and b) to decipher molecular mechanisms that are triggered in host and pathogen by EO application.

Therefore, we customized a climatic chamber, which permitted a continuous fumigation of potted vines with different EOs. Several experiments with vines, infected with *Plasmopara viticola* and subsequently exposed to continuous fumigation of different EOs with different concentrations and application times were conducted. Experiments were stopped when signs of infections were clearly visible on the control after sporulation was induced. Strikingly oregano oil vapor treatment reduced downy mildew development to 95%. RNA. Analysis of differentially expressed genes yielded in a total of 4800 EO modulated transcripts in vines. Strikingly many genes linked to the plant immune system were triggered by EO vapour (ethylene synthesis, phenylpropanoids and flavonoid synthesis), which indicates for the first time, that the antifungal efficiency of EO is mainly due to the priming of resistance pathways inside the host plants. These results are of major importance for the production and research on biopesticides, plant stimulation products as well as for resistance breeding strategies.

Case study: Impact of INTEGRAPE on documentation and molecular characterization of grape genetic resources in Armenia

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Grapevine (*Vitis vinifera* L.) is one of the earliest domesticated crops and consists of two forms cultivated *V. vinifera* L. subsp. *sativa*, Beck and wild *V. vinifera* L. subsp. *sylvestris* Beck. Armenia is considered to be one of the primary centres of origin of viticulture and winemaking confirmed by archaeological, palaeobotanical and cultural findings. Confirmations for long-lasting cultivation of grapevine in Armenia stem also the huge genetic and morphological diversity of both wild and cultivated grapes in the country.

During the last years, the documentation and comprehensive characterization of the *Vitis* biodiversity becomes one of the main pillars of national strategy towards conservation of grape genetic resources. The ultimate goal of our group is to enrich the number of old, less known Armenian grape varieties which can be achieved by exploring and evaluating grape genetic resources and to strengthen the *in situ* conservation strategy of wild grape genetic resources in Armenia, which could represent a valuable genetic resource for future breeding programmes, as well as for conservation of biological diversity in natural environments.

In 2019 by the support of INTEGRAPE, CA17111 and Institute for Grapevine Breeding, JKI two hundred forty-five (245) grapevine accessions were characterized using a set of 25 simple sequence repeat (SSR) markers encompassing the nine SSR markers recommended by the European project GrapeGen06. Grapevine samples were collected during recent prospections carried out in the main wine-growing regions throughout the country. Thus the analyzed material encompasses grape varieties grown since ancient times. Besides major wine and table varieties, minor varieties of local importance, grown especially in private and very old vineyards, as well as neglected local varieties, at risk of extinction were collected and conserved in the National Grapevine Collection of Armenia. Wild grapes included in the analysis were selected in the spontaneous flora and were checked for typical morphological characteristics. The distances between wild accessions and cultivated grapevines were taken into consideration in accordance with the principles defined in the frame of the European GrapeGen06 project.

The determination of 245 grapevine accessions identity requires a combination of molecular data and morphological characteristics. Molecular analysis of Armenian grape samples revealed the three main cases: synonyms, homonyms and questionable cases. The SSR profiles comparison based on *Vitis* International Variety Catalogue (*V*IVC) (http://www.vivc.de/) database assisted to determine accessions identities. Unique profiles, additional synonyms, homonyms and duplicates also were identified.

The obtained results reveal the uniqueness of the great part of analyzed grape samples and unlock a substantial level of genetic variation within the Armenian *Vitis* resources. Based on the realized

large scale investigation a true-to-type inventory of Armenian grape varieties already documented in the Armenian *Vitis* database (www.vitis.am) and in the *Vitis* International Variety Catalogue. The realized activities during 2019 promoted also to increase the quantity and quality of data of Armenian grape varieties documented in *V*IVC, where the encyclopedic information for 334 Armenian grape varieties already is available. There is a strong need to continue to explore the poorly known *Vitis* biodiversity still preserved in Armenia, which can provide new understanding for the future genetic improvement of grapevine.

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The correlation of secondary metabolites contents with oxidoreductase enzyme activities in autochthonous grapevine varieties from Bosnia and Herzegovina

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Phenolic compounds are secondary metabolites highly responsible for sensory characteristics and quality of wines, but their stability and antioxidant properties are associated with the activities of enzymes from oxidoreductase group, primarily with peroxidase (PD, EC 1.11.1.7) and polyphenol oxidase (PPO, EC 1.10.3.2). This study included the identification and quantification of the anthocyanins and phenols in the peel and pulp of berries of two autochthonous grapevine varieties (Blatina and Trnjak) cultivated in the wine region of Bosnia and Herzegovina. In addition, this research included the antioxidant activities of berry extracts and biochemical characterization of peroxidase and polyphenol oxidase. The samples were collected in the experimental fields of company Agroherc d.o.o, Čapljina-Višići, which area was 67 ha. Standard agricultural and viticultural practices were used, which along with growing conditions (modified Mediterranean climate) ensured steady yields. The most abundant phenolic compounds in the peel of both varieties were quercetin and trans-resveratrol, while in the pulps prevail catechin derivates and gallic acid. The quantification of anthocyanins was done by HPLC-MS analysis and confirmed that malvidin-3 glucoside and malvidin-3- (*p*-coumaroyl glucoside) in the peel of both varieties have the highest concentration in comparison with contents of other anthocyanins. At both varieties POD reactions with caffeic and chlogenic acid were more expressed in pulps in comparison to the peels. The pulp extracts of both grape varieties had higher PPO activity in comparison to the activities of their peel extracts. Very high positive correlation between catechin content and PPO activity was observed. On the other side very high negative correlation between content of individual anthocyanin compounds and PPO as well as POD activity was noticed. The role of PPO and POD activity in the antioxidant activities of phenolic compounds and their distribution in analyzed tissues was discussed.

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Monitoring of a vineyard soil moisture and ground-zone temperature by automatic sensors

GROW Observatory: a European database building by citizens

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GROW Observatory is a project funded under the European Union's Horizon 2020 research and innovation program. Its aim is to establish a large scale (>20,000 participants), resilient and integrated 'Citizen Observatory' and community for environmental monitoring that is self-sustaining beyond the life of the project. The scientific objectives within GROW, creating a soil moisture database using low cost soil moisture sensors to provide an extensive dataset of in-situ soil moisture observations. This database can serve as a reference to validate satellite-based soil moisture products, whilst there is an overarching vision to address land use and management issues.
We set up our monitoring along this last idea, in a long-term experimental vineyard that exposed to soil erosion (46°78'83,1" N; 17°48'92,4" E) in all the eight set variable. In each treatment, we installed three devices in the middle row (five rows per treatment). Sensors (Total: 24) measured every 15 minutes four parameters (soil moisture, air temperature, light: photosynthetically active radiation (PAR) and soil conductivity) and stored in their memory until the monthly manual uploading to the cloud by the researcher's phone connected to the sensors one by one.
After an eight month long monitoring we collected the data in .csv format and analyzed by R and ArcGIS 10.2 software packages. We also compared our data to measurements made by the nearest certified weather station.
It has been concluded, that these low cost sensors have a relatively good performance. In the case of a few devices we noticed significant bias in terms of soil moisture measurement, but as this bias was systematic, it could be easily corrected by calibration.
Building of a database from a cheap, fast and precise analyses, support correct decision-making and allows farmers to choose sustainable and more precision soil management in response to climate phenomena and trends.

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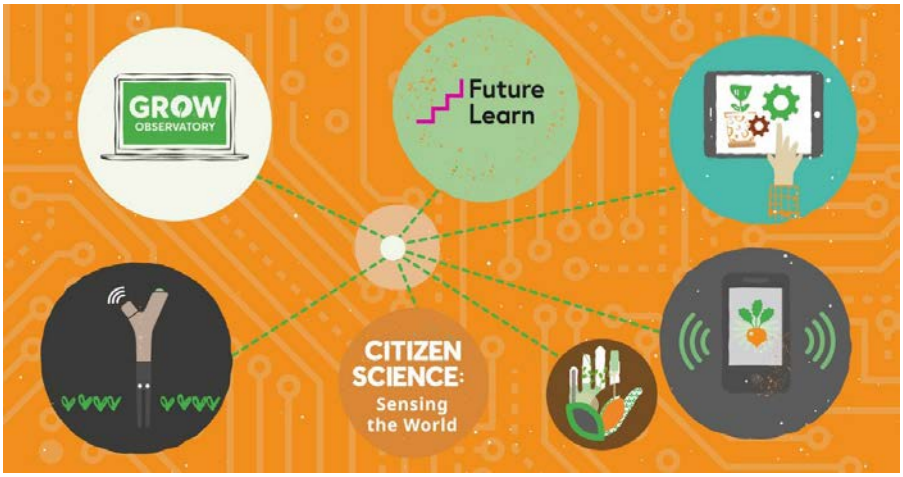

Abstracts of Posters

Towards understanding the aroma biosynthesis in wine grape Greek varieties

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The aromatic substances of the grape berries contributing to the aromas of the wine have been extensively studied in different cultivars worldwide. However, work on the functional characterization of the corresponding genes and enzymes in these cultivars is still behind. Most of the known aromatic substances are terpenoids (linalool, geraniol, nerol, terpineol, limonene) originated from the MVA/MEP pathway, phenylpropanoids (2-phenylethanol, eugenol, isoeugenol) from the phenylpropanoid pathway, methoxypyrazines (IBMP, IPMP) originated from leucine, volatile thiols (3-sulfanylhexan-1-ol (3SH) and 4-methyl-4-sulfanylpentan-2-one (4MSP)) from cysteine or glutathione, whereas aliphatic alcohols ((Z)-3--hexen-1-ol) from the fatty acid degradation pathway. Since the genome sequence of *Vitis vinifera* has been completed, the uncovering of the involved genes became easier. In order to understand and reveal the aromatic complexity of the Greek wine cultivars at the gene level, we initially studied the gene expression of 27 genes participating in the above mentioned biochemical pathways in four stages of development, ripening, green, véraison, mid-ripe and ripe, in four Greek wine-grape cultivars namely Assyrtiko, Moschofilero, Rhoditis and Xinomavro to select the appropriate sampling time point for RNA-sequencing. The Real-Time PCR analysis revealed that most of the monoterpene synthases had higher levels of expression during véraison, whereas most of the genes involved in the production of phenylpropanoids, volatile thiol precursors and fatty acid degradation pathways were increased towards maturation, i.e. red stage. As previously shown and because methoxypyrazines have a greenery odor, the expression levels of the genes involved in methoxypyrazines' synthesis were higher during green stage and decreased thereafter proportionally. Genes of thiol precursor's synthesis exhibited higher expression in the white wine cultivars Assyrtiko and Moschofilero, compared to reddish Rhoditis and Xinomavro, especially at the mid-ripe and ripe stages. It seems that the mid-ripe stage will be selected for RNA-sequencing in cultivars with high and low aromatic profiles. Finally, based on the expression pattern and the aromatic profile of each cultivar, the following first group of genes was cloned into yeast vectors and the functional characterization is in progress: from Assyrtiko, pUTDH3myc-terpineol synthase, pUTDH3myc-eugenol synthase, pUTDH3myc-carotenoid cleavage dioxygenase 1, pGem-Furaneol UGT, pUTDH3myc-zingerone synthase, and pGem-4-hydroxybenzalacetone synthase; from Moschofilero, pUTDH3myc-wine lactone synthase, and pUTDH3myc-aromatic decarboxylase; from Xinomavro pUTDH3myc-4-hydroxybenzaldehyde synthase). Uncovering the transcriptome differences between these four cultivars and functionally characterizing the genes participating in the aroma formation of grape berries will lead to better understanding of the mechanism of the formation of their characteristic aromas and possibly will help in identifying molecular markers link to aroma trait.

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The traditional agroecosystem of mountain as conservatories of the agrodiversity of vine «*Vitis vinifera* ssp. *vinifera*” in the north of Morrocco

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The vine (*Vitis vinifera* ssp. *vinifera*) is an ancient culture steeped in history and symbols. in fact, the history of viticulture and the vineyard is intimately linked to the history of the regions where it is practiced. The legacy of local knowledge which corresponds to traditional agricultural practices, local varieties, traditional processing techniques and methods of grape consumption is the result of this history. In Morocco, the culture of the vine is very old and goes back to the Phoenician period. Currently, modern vineyards are mainly composed of varieties originating in Europe, spread over an area of approximately 45,000 ha including 39,000 ha for table grapes, 12,000 ha for wine vines, 1,000 ha for raisins and 3,000 ha of young plantations. However, at the level of traditional mountain agroecosystems in northern Morocco, the vineyards are indigenous and composed of local grape varieties whose inventory and description are partial, as well as that they are exploited for the production of local products. In this sense, this contribution aims to inventory the local varieties of vines and to draw attention to the importance of the agrodiversity of these rare local grape varieties whose characterization has not yet been fully achieved.

PyrrouAmpelos: Phenotypic characterization, molecular fingerprinting and oenological evaluation of indigenous *Vitis* cultivars from the Epirus Region of Greece

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The project “PyrrouAmpelos” comprises a cooperation among two companies operating in the region of Epirus, Zoinos Winery and IPER, and three research organizations: the Hellenic Agricultural Organization-Demeter, the University of Ioannina and the Agricultural University of Athens. It aims at preserving and promoting the uniqueness of indigenous grapevine genetic resources of the Epirus region of Greece, through their proper identification, characterization and valorization, in the context of the ongoing climate change. The ultimate goal of the project is to strengthen the regional viti-vinicultural sector and place differentiated branded products in the market.

The main objectives of the project are:

- to localize autochthonous grapevine varieties (either registered or not registered in the National Catalogue), perform ampelographic descriptions and phenotypic evaluation
- to assess the phytosanitary status of the grapevine germplasm
- to perform genotyping and epigenetic analyses towards developing diagnostic molecular tools which pertain to the identification, traceability and stability of genetic resources
- to investigate genes that are associated with desired agronomical traits related to yield, stress residence and wine quality
- development of molecular biomarkers of genetic or epigenetic base towards selection of appropriate genotypes in breeding programs
- to identify and characterize of main secondary metabolites which determine the aromatic profile of wine
- to evaluate the oenological potential of different grapevine varieties through vinification, chemical analysis, metabolic analysis and organoleptic assessments
- to reveal associations among phenotypic, molecular and oenological characteristics
- to generate a grapevine database with the phenotypic, genetic and epigenetic information complemented by geo-referenced data.

Morphological characterization and phenotypic variability of autochthonous Greek grapevine varieties of the Epirus region

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As part of a larger effort towards characterization and valorization of the Greek grapevine germplasm, and within the framework of the Greek National project '*PyrrouAmpelos*' (Phenotypic characterization, molecular fingerprinting and oenological evaluation of indigenous *Vitis* cultivars from the Epirus Region of Greece) the present study investigated a series of 8 autochthonous wine grapevine varieties (among others included in the project) from the district of Epirus, in Northwestern Greece.

In particular, the current work focused on the phenotypic characterization of 5 white (B-Blanche) and 3 red (N-Noir) varieties from the prefecture of Ioannina and specifically from the regions of Zitsa, Metsovo and Pogoni. The name and respective origin of the varieties were as follows: a) Debina (B) from the area of Zitsa; b) Piknoassa (B), Proimo Metsovou (N), Goudaba (N) and Blachavona (B) from Metsovo; c) Voska (B), Votsiki (B) and Mavroudi (N) from the area of Pogoni. Debina is the most commonly used variety with PDO certification and has been used extensively by the local viticulturalists and wine industry for the production of high-quality wines. The rest are as yet under-explored and under-exploited varieties, nevertheless, they are very well adapted to the particular agro-climatic conditions of the Epirus regions.

Full and credible records of morphological and genetic analysis are necessary for providing proper variety identification and protecting the rich grapevine diversity in the Epirus region. The current study focused on morphological evaluation of different grapevine varieties by use of ampelographic characterization. Field trips were undertaken in order to localize and mark varieties and full photographic records were obtained. The Ampelographic description was based on 27 ampelographic descriptors of mature leaves, as specified by the OIV Descriptor List (OIV 2009). An exploratory statistical analysis of ampelographic measurements was performed in order to acquire an initial understanding of the structure of phenotypic diversity among the grapevine varieties examined. A dendrogram was constructed utilizing the Manhattan dissimilarity index and the UPGMA clustering algorithm employing NTSYSpc software program, displaying inter-cultivar variability.

This study will provide a significant contribution to i) proper identification of indigenous varieties, ii) a proposal on the phylogenetic relations among the examined varieties and iii) characterization of the regional grapevine genetic resources aiming at exploiting the full potential of the rich grapevine germplasm of the Epirus district and promoting the economy of the local communities, in view of the changes in the regional climatic conditions.

Virus testing for woodland grape (*Vitis sylvestris* C.C. GMEL.) genotypes from Hungary

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Based on theoretical and practical researches it is supposed, that *Vitis sylvestris* C.C. GMEL (woodland grape) itself, or crossing with other species could be the progenitor of the European grapevine (*Vitis vinifera* L.).

During our research we collected propagation material (young shoots) from woodland grapes originated from Szigetköz and Fertő-Hanság National Park. Then, we grafted the collected materials with green grafting to rootstocks in the NARIC Research Institute for Viticulture and Oenology, Badacsony for an *ex-situ* conservation.

International studies have shown that woodland grapes catch diseases from cultivated grapes, which resulted in a loss of vitality and tolerance. GLRaV1 (Grapevine leafroll associated virus 1) and SLRV (Strawberry Latent Ringspot Virus) viruses were detected from the tested samples.

In our research we used the so-called Double Antibody Sandwich ELISA (DAS ELISA) for ELISA testing. During the sample collection, 32 woodland grape genotypes were collected and studied from Badacsony and 21 individuals from Szigetköz from their original habitat. We tested the samples for the following viruses: GFLV (Grapevine fanleaf virus), ArMV (Arabis mosaic virus), GCMV (Grapevine chrome mosaic virus), TBRV (Tomato black ring virus), GFkV (Grapevine fleck virus), GLRaV 1, 2, 3 (Grapevine leafroll associated virus), GVA (Grapevine virus A) and GVB (Grapevine virus B).

Based on the obtained results, the presence of TBRV virus was detected from four woodland grape genotypes originated from Badacsony. The GVA virus was detected from three genotypes originated from Badacsony and from two individuals originated from Szigetköz. GLRaV-1 virus was detected in three Badacsony and two Szigetköz individuals, while GLRaV-2 and 3 viruses were detected in one Badacsony sample.

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Carbon quantum dots conjugated with proline confer tolerance to grape plants against salt stress

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Salinity represents one of the main abiotic stress factors leading to major yield losses in crop plants worldwide. Nanotechnology and nano-materials in particular have evolved as a novel, promising approach towards protection of plants against climate change-related phenomena. Carbon quantum dots (CQDs) have unique properties (e.g. very small dimension, high water solubility, biocompatibility, biodegradability with no or low toxicity) and recorded beneficial influences on plant growth and physiological parameters. Proline (Pro), an essential amino acid, is well known to confer tolerance to osmotic stress conditions. The present study attempted to examine the potential additive or synergistic effect of CQDs-Pro conjugates in a dose-dependent manner. An experiment was therefore conducted to evaluate the impact of this advanced nanomaterial as a novel priming agent on grape plants cv. 'Rasha'. For this purpose, Pro, CQDs and CQDs-Pro were applied four times on grape plants with 12 h intervals at three concentrations (0, 50 and 100 mg L^{-1}), and salinity stress (0 and 100 mM NaCl) was imposed 48 h after the last priming agent application. Three days after NaCl treatment, biochemical measurements were recorded while other parameters were measured after one month. Results revealed that Pro treatments at both concentrations and CQDs and CQDs-Pro at 50 mg L^{-1} positively affected grape plants under both control and stress conditions. Optimal protection was achieved with 100 mg L^{-1} Pro and 50 mg L^{-1} CQDs-Pro. Proline treatment at 100 mg L^{-1} increased chl a, b and Pro content, SOD activity and Y (II) at both non-stress and stress conditions, as well as protein content, carotenoids and CAT activity under control conditions. Furthermore, this treatment significantly lowered electrolyte leakage under both growth conditions and Y (NO) under control conditions. CQDs-Pro treatment at 50 mg L^{-1} enhanced total phenol, anthocyanin and carotenoid contents, APX and GP activities and $^{Fv}/_{Fo}$ at both conditions, as well as protein content and CAT activity under salinity conditions. In addition, both treatments significantly decreased MDA and H_2O_2 content at both conditions and Y (NO) under salinity conditions. Overall, although Pro demonstrated positive impacts at both concentrations applied, CQDs-Pro treatment at low concentration displayed optimal results, suggesting that the conjugation of CQDs enhanced Pro efficiency. This opens new horizons in the development of promising plant priming technologies in plants for improved growth under stress conditions through the combined application of advanced nanomaterials with chemical compounds.

Optimization of the protocol for capture and sequencing of targeted DNA libraries of *Vitis* samples

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NGS has dramatically expanded the capabilities of laboratories by multiplexing and streamlining DNA sequencing workflows. Replacing traditional target amplification techniques with in-solution enrichment technologies has simplified sequencing template preparation, greatly increasing the productivity of individual laboratories. Multigene panels can be easily performed with gene-specific target enrichment probes.

In the present project 2271 probes were designed to target grapevine SNPs based on polymorphism information content, position, annotation, etc. corresponding to the GrapeReSeq diversity panel (GrapeReSeq GENOT 783; Laucou et al.); 943 probes were designed to capture information of sex locus on chr2, 24 probes for capturing variable regions of the chloroplasts, 96 GAl1 loci linked to berry traits and phenology, 51 loci linked to resistance, 59 random loci, 312 MYB loci linked to colour in grape and 47 TFL1 loci linked to flowering and phenology. For each polymorphic site, a 120-mer probe was designed with the expected variant in the central position. The panel was developed to genotype most of the diversity in *Vitis* sp. species from Balkan region, covering sites known to be informative for identification of grape cultivars, their important traits and analyzing of parentships.

For sequencing of 393 *Vitis vinifera* genotypes and 27 *Vitis* species fro different Western Balkan countries we used in-house sequencing facility based on the Ion Proton sequencer, which can generate up to 80 million reads of modal length up to 200 bp. DNA NGS libraries were constructed using laboratory developed protocol, which greatly reduces the price of library construction compared to commercial kits (>10 times). Briefly, HMW DNA was sonicated using water bath sonicator and resulting DNA fragments were end-repaired using T4 polynucleotide kinase and T4 DNA polymerase. The reaction was cleaned using 1.8 vol of magnetic beads (e.g. MagSi-NGSPREP Plus) and two Proton adapters ligated to each end of the DNA fragments (P1 and barcoded adapter A) and afterwards cleaned by magnetic beads. Libraries were quantified using Agilent DNA chip and by means of qPCR. Up to 24 NGS libraries representing 24 grapevine samples were pooled together and enriched following capture procedure of the designed probes panel including hybridization and washing steps (Arbor Biosciences). Up to 96 NGS libraries were sequenced (e.g. from 4 capturing experiments) together on one Proton PII chip following manufacturer protocol.

The sequencing revealed the equal distribution of reads between samples reaching almost 1 M reads per sample on average with the modal length of 188 bp. Mapping of the sequencing data confirmed grapevine origin with more than 98% of the reads aligned to the reference *Vitis vinifera* cultivar PN40024. Looking at the targeted loci, more than 80% of the reads originated from captured loci confirming the efficiency of capturing protocol. Analyzing the data distribution across single SNP loci resulted in high average coverage (up to 95X). The data obtained will enable us to determine precise calling of variants for evaluation of Balkan grapevines: their true-to-typness, important traits and kinships in the grapevine genepool.

State and prospects for the preservation, evaluation and improvement of Bulgarian grapevine genetic resources

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The germplasm potential in grapevines remains undiscovered with the big number of officially registered varieties (about 10,000) from which only 35 account for approximately 70% of the worldwide vineyards. This negative trend is fully valid for Bulgaria, as a country whose viticulture is one on the main economically important agricultural sectors. Many local varieties (both autochthonous and newly bred) and promising wild grape forms are lost as their commercial potential is ignored. The results from the long-term monoclonal propagation are suppression of inter and intra-varietal variability, decreasing of the genetic flexibility and finally- irreversible erosion of the grapevine biodiversity. That is critical problem compromising Bulgarian, European and global viticulture. Mentioned above deviations suggest an urgent need for new updating of national and international grapevine genetic resources programs, networks and other initiatives leading to improve the existing germplasm preservation and evaluation efforts. Progress in state and prospects for the preservation, evaluation and improvement of Bulgarian grapevine genetic resources are discussed. "Omics" technologies as a successful approach for improvement of the existing Bulgarian grapevine genotypes are also considered. Following the best practices of the European Cooperative Programme for Plant Genetic Resources (ECPGR) and the European Genebank Integrated System (AEGIS), a total of 172 accessions were preserved, developed and evaluated (since 1995) in the Agrobioinstitute grapevine genebank, including varieties, rootstocks, wild grapes, local autochthonous varieties and new selected forms. An updated, long-term and strategically targeted programme for the preservation and sustainable development of Bulgarian grapevine genetic resources is absolutely necessary. That will contributing directly to increasing the quality of the planting material and profitability of vineyards as well as will improving significantly the development of the Bulgarian grape and wine industry.

Genotyping 8 newly bred Bulgarian wine and table grapevine varieties using SSR markers

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Eight newly bred Bulgarian table and wine grapevine varieties including cv. Kristalen, cv. Garant, cv. Troya, cv. Katya, cv. Misket viking, cv. Elitsa, cv. Nikopolski mavrud and candidate cv. Gigant were genotyped at 9 microsatellite loci adopted by the GrapeGen06 project (https://www1.montpellier.inra.fr/grapegen06/accueil.php) for genetic identification of grapevines. A total of 54 alleles were scored. The most informative markers were VVS2 and VVMD7 with PIC values of 0.82, while the least informative was VVMD32 (PIC = 0.48). Both expected heterozygocity (He) and observed heterozigocity (Ho) showed high values with an average of 0.76 ± 0.09 and 0.83 ± 0.19 respectively thus demonstrating high genetic diversity for the studied varieties. Clustering analysis was performed based on the SSR allele data from the newly genotyped varieties as well as on previously genotyped newly bred Bulgarian varieties, varieties developed during the late 20-eth century, old local varieties and the French varieties cv. Merlot and cv. Cabernet Sauvignon. The performed clustering showed that all mavrud based varieties including the newly bred cv. Nikopolski mavrud, which also has cv Mavrud in its pedigree (cv. Buket was obtained as a cross between cv. Mavrud and cv. Pinot noir) formed a separate cluster. Cultivar Kristalen showed to be closely related to cv. Misket Viking and was clustered together with the previously genotyped cv. Rubin. Five of the genotyped varieties including cv. Garant, cv. Troya, cv. Katya, cv. Gigant, cv. Elitsa formed a separate cluster and grouped together with the old variety with Eastern origin cv. Tamyanka as well as with cv. Velika, which was developed during the 1980s. The obtained SSR fingerprints were included in the Bulgarian Vitis Database (http://bulvitis-db.com).

IN VITRO propagation of *Phylloxera* resistant rootstock cultivar 'Teleki 5C'

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Grapevine is one of the important fruit crops in Armenia. At present, phylloxera is a serious hazard to viticulture in Armenia, which has caused considerable problems for grape growers in recent years and the successful means of controlling phylloxera will be by planting vines grafted onto phylloxera-resistant rootstock. Conventional method of grapevine propagation allows disease transmission. Therefore, *in vitro* propagation as an alternative method for propagating grapevines is very important. The objective of this study was to investigate micropropagation protocol for phylloxera resistant rootstock 'Teleki 5C' *(V. berlandieri x V. riparia).* Explant surface sterilization is the most important stage for micropropagation, because controlling fungal and bacterial contamination of field plant sources is very difficult. Contamination rate, survival and development of shoots from excised shoot were analyzed after sterilization. Sterilization of explants using 1.0 % Calcium hypochlorite for 10 min followed by 70 % ethanol for 30 second duration was optimum. Determination of the most optimal types and concentrations of plant growth regulators as medium components is one of the important aspects of successful plant regeneration. Murasige Skoog (MS) medium supplemented with different concentrations of 6-bezylaminopurine (BAP) and kinetin (KIN) were used for shoot regeneration and proliferation. The maximum shoot regeneration (95.0%) was obtained on MS medium supplemented with BAP (0.6 mgl-1) + KIN (0.2 mgl-1). Successful rooting of microshoots is a prerequisite to facilitate their establishments in soil. To optimize root induction, different concentrations of Indole-3-acetic acid (IAA) and Indol-3-butyric acid (IBA) were used. Microshoots induced root for all the treatments used, but good roots were found on MS medium supplemented with 0.5 mgl-1 IAA +0.2 mg/L of IBA. Rooted plantlets of about 10 cm in length were transplanted into the plastic pots filled with mixture of sand: soil (1:1) under artificial diffuse light conditions, and then covered with polyethylene bags and survival percentage was about 85 %. Thus, the achievements of this study will play a big role in the grape vine culture program.

MedVitis: Diversity of rare Slovenian Vitis genotypes

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Viticulture in Europe and in particular in the Mediterranean basin is an old agricultural activity, dating to antiquity, and intimately associated with the history and culture of the grapevine cultivation regions. The project, MedVitis (ARIMNET2 programme), proposes an integrated effort by four Mediterranean partners originating from Greece, Slovenia, and Morocco, aiming to protect the diversity of grapevine germplasm across the three countries, in order to better manage issues of grapevine identification, genetic erosion, climate change and *Vitis* pathogenicity. In Slovenia, some old grapevine varieties are preserved in national collections; but there are still many old varieties which are typical for specific agro-climatic areas and some of them have become important for the production of regional vines. The identification of local, unknown varieties is often difficult by morphological descriptors alone because there are many synonyms/homonyms of particular local varieties. A set of 36 *Vitis* grapevine genotypes/varieties have been identified and selected for further phenotypic (ampelometric) characterization and genetic studies. This set consists of rare, unknown, resistant and standard grapevine cultivars from all three wine-growing regions in Slovenia, i.e. Podravje, Posavje in Primorska. Ampelographic characterization will consists of 85 different O.I.V (International Organization of Vine and Wine) descriptors related to young shoot, shoot, woody shoot, young leaf, mature leaf, flower, bunch and berry. During the growth period several samplings were performed and photo documented on each individual vine. Leaf samples from the selected Slovenian genotypes were collected for subsequent genotyping procedures. A set of different microsatellite markers (Simple Sequence Repeats-SSR) will be utilized, including the SSR markers recommended as grapevine-specific molecular descriptors by the O.I.V. Diversity parameters, genetic structure and linkages of analyzed Slovenian genotypes will be assessed and evaluated through different algorithms implemented into selected bioinformatics programs and software packages. Ampelographic description and molecular characterization of selected Slovenian vine genotypes will enable the identification, characterization and preservation of valuable autochthonous *Vitis* germplasm. Collected data will be used to upgrade and improve the national collection inventories and databases.

The chloroplast DNA sequence polymorphism (SNP) of grape cultivars

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Grapevine is an important fruit crop, as the source of table grapes and wine. In our study, we employed whole-genome shotgun sequence data to target DNA variation in the chloroplasts and performed their sequence alignment and phylogenetic analyses. The chloroplast sequence analyses were performed on inter- and intra-specific levels with aims to improve earlier phylogeny works that were 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 the parentship analysis particularly of Balkans grape cultivars (Stajner et al 2015) using maternally inherited chloroplast variation. The low coverage DNA-Seq was performed on Ion Torrent sequencer, a sequencing platform allowing cheap, fast and precise sequencing. Genotypes of *Vitis vinifera* linné subsp. *vinifera* were obtained from different regions: Slovenia (124), Serbia (28), Croatia (76), Montenegro (16), BIH (55), Macedonia (6), Greece (26), Albania (39) and France (23). To get insights of chloroplast variation on the interspecific level of genus *Vitis* 27 different Vitis species were additionally sequenced.

In the poster we will represent results on a subset of 21 sequenced samples representing grapevine cultivars of *Vitis vinifera* L. originating from Slovenia (1), Greece (4), Albania (4), BIH (4), Serbia (4), Croatia (4). Low coverage whole-genome DNA-seq of samples was performed and the data were used for reference-guided assembly using 'Map Reads to Reference' Tool implemented in the CLC Genomics Workbench with default settings. The chloroplast sequence of the grapevine 'Maxxa' (NC_007957) was used as the reference plastid genome. For variant detection 'Fixed Ploidy Variant Detection' Tool was used in CLC Genomics Workbench with haploid settings and ignoring non-specific matches. To obtain the reliable set polymorphism the defined SNPs were further filtered out using the criteria of zygosity, frequency and of removing indels. Phylogentic tree was constructed based on multiple whole genome chloroplasts alignments using Neighbour Joining algorithm implemented in CLC Genomics Workebench.

Using low coverage DNA-seq we were able to sequence a grapevine genome at average 0.17 coverage while chloroplast genome reached up to 60x coverage which was high enough to call reliable SNPs positions. Eighty-four SNPs in 21 grape cultivars were identified in comparison to the reference 'Maxxa' chloroplast genome. The highest number of cultivars having individual SNP was seventeen and it appeared for 27 SNPs. Ten SNPs were assigned to only one cultivar, and nine out of ten were specific for 'Drenjak Crni' from Serbia. The number of noncoding substitutions

was 45 and coding substitutions 40. In 9 cases, nonsynonymous substitutions were observed, which altered the amino acid sequence. In 75 cases, synonymous substitutions were detected. In gene ycf1, 6 SNPs (4 nonsynonymous and 2 synonymous) were observed.

In the dendrogram the clustering resulted in four separated groups. Based on these data, a new methodology of simultaneous resequencing of a large number of high coverage chloroplast DNA was achieved without preliminary chloroplast isolation or chloroplast enrichment. This method has great potential for expanding both phylogenetic and population genetic information on the evolution of domesticated crops.

NGS approach for SSR fingerprinting in grapevine

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The conservation of plant genetic resources that are threatened with extinction is the main task of many international projects. In some countries, particularly in south-eastern Europe, the level of information about the indigenous germplasm and its genetic diversity is still low. Obtaining this information is necessary both for the proper management of plant resources and for conservation. The ambiguities can be successfully overcome by molecular markers typing. They are a very suitable tool which, in contrast to phenotypic characterization, is independent of environmental conditions and has proven to be a powerful tool for identity and kinship analysis in a variety of species.

For more than two decades, genotyping projects were dominated by multiplex PCR and capillary electrophoresis (CE), which enabled the identification of amplified length polymorphisms. The main challenge associated with this approach is the cross-laboratory standardization of allele sizes. This step requires the inclusion of standards and in particular manual sizing and processing to avoid false results. Furthermore, the CE data approaches limit the information to the length polymorphism and do not allow the determination of a complete marker sequence, which also complicates a simple comparison of the data sets and masks alternative alleles of equal size. As an alternative, Next Generation Sequencing (NGS) methods offer information about DNA sequences including the identification of their flanking regions. The advanced approach provides deeper insight and more accurate assessment of allele variants. In this work we investigated the suitability of a semi-quantitative sequencing approach for microsatellite genotyping and validate the methodology by comparing the data generated by NGS with CE-based data. Twelve microsatellite loci, that are well established for grapevine CE typing, were analysed on 96 grapevine samples from 6 different countries. Primers were redesigned to the length of the amplicon for short sequencing (~100 bp) and amplified using the established protocol. The primer pair was flanked with an 10 bp overhang for introduction of barcodes on both sides of the amplicon in the secondary PCR which enable the sequence to be assigned to the sample. The concentrations of amplicons were measured and all samples were mixed equimolarly and sequenced using IonTorrent single-end and Illumina paired-end technology. The raw data were demultiplexed by barcodes to assign the sequence to cultivar and in a second step by the SSR primer sequence to assign the sequences to the locus using fastq-multx tool. For further analysis, only full-length sequences were considered. The sequences were analyzed in two ways, namely by full length and by the number of microsatellite repeats and the data were presented as histograms. The highest data peaks were detected as SSR alleles and compared with CE dataset on the basis of 12 reference samples. The comparison showed that NGS SSR genotyping can replace the CE system in new experiments. The NGS sequencing approach facilitates high multiplexing of high number of loci and/or high number of samples and allows accurate identification of variations. We believe that with NGS it is still possible to improve genotyping in terms of speed, accuracy and price.

Virome status of old Slovenian grapevine varieties as determined by NGS of virus-derived small RNAs

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The presented research was focused on virus screening using next-generation sequencing (NGS) technology, to get an overview of all viruses and virus-like organisms that are present in old plants of autochthonous Slovenian varieties. As a method of choice, small RNA sequencing was chosen. Virus discovery by NGS and subsequent assembly of small RNAs has proven to be highly efficient in plant virus detection (Kreuze et al., 2009). These small RNAs, which frequently cover the whole genome of the infectious agent, are 21–24 nt long and are known as virus- or viroid-specific RNAs. During the process of viral infection, the virus-derived small RNAs can be detected by deep sequencing of infected host plants (Wu et al., 2010). The isolation of small RNAs was performed by enrichment procedure using the "mirVana™ miRNA Isolation Kit" (Ambion, Life Technolgies), which enables that RNA molecules of <200 nt can be efficiently purified from the larger RNA species. Using the Ion Total RNA-Seq kit, miRNA libraries were constructed according to the manufacturer's protocol. Thus, constructed and barcode-labeled miRNA libraries (cDNA libraries) were sequenced using the Proton ™ system (Ion Torrent ™; Life Technologies). After deep sequencing (average 11 880 007 reads per sample), free, open-source bioinformatics pipeline VirusDetect (Zheng et al., 2017) was employed, which can efficiently analyse small RNA datasets to identify both known and novel viruses. Together, 33 grapevine plants of 4 different cultivars were analyzed. By employing bioinformatics automatic pipeline VirusDetect, 6 viruses and 2 viroids were identified: *Grapevine pinot gris virus* (GPGV), *Grapevine fleck virus* (GFkV), *Grapevine rupestris stem pitting-associated virus* (GRSPaV), *Raspberry bushy dwarf virus* (RBDV), *Grapevine leafroll-associated virus 3* (GLRaV-3), *Grapevine rupestris vein feathering virus* (GRVFV), *Hop stunt viroid* (HSVd) and *Grapevine yellow speckle viroid* (GYSVd). *Grapevine pinot gris virus* (GPGV), *Hop stunt viroid* (HSVd) and *Grapevine yellow speckle viroid* (GYSVd) were common for all analyzed samples. The results of NGS analysis will be further confirmed by viruses specific RT-PCR and Sanger sequencing to allow an efficient validation of identified virus and viroid genome sequences. In the next future elimination of viruses will be utilized using thermotherapy, meristem tissue culture and cryotherapy. The elimination of the viruses is especially important because some old grapevine varieties are infected with many different viruses and viroids and there is no virus-free material that can be used for plantation.

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How to express grape quality? Which unit is correct?

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Secondary metabolites are very important and abundant groups compounds present in grape and in the other parts of grapevine. Among them, polyphenolic compounds and volatile compounds are very well studied. These compounds have a great impact on the quality of grape and wine and can be markers for phenotyping. The analysis of them is often used in scientific purposes but also in routine analysis for the prediction of wine quality. According to the Scopus and WoS in the last 30 years, there have been published more than 500 scientific papers related to the content and composition of polyphenolic and volatile compounds in different parts of grapes. In most cases, the data published in different papers cannot be directly compared because of the different quantification approaches. For example content of anthocyanins can be expressed using 11 different units such as μg/g; μg/g DW; mg/kg; mg/g DM; mg/kg berry, dry basis; mg/kg grape; mg/kg DW; mg/kg FW berry; mg/g berry; mg/100g DM; mg/g skin. Most of these units are interchangeable. In most of published papers method for the analysis of the same group of compounds which include extraction and quantifications are quite different thus from the quantitative point of view the obtained results cannot be comparable. Due to the great importance of the secondary metabolites and the content and composition of these compounds between different researchers, it is necessary to standardize the expression of the obtained content, and the analytical procedure, as well. This approach would allow improvement and higher integration of data repositories and interoperability between datasets as well as re-use of grapevine metabolomics data beyond the original experiments.

The application of modern molecular data for grapevine breeding and governance of resistance

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Within the researches and works during in the previous years in the Republic of Moldova were obtained a number of grapevine varieties, resistant to the adverse winter conditions, characteristic for this region, located on the northern limit of industrial viticulture, which allowed the considerable reduction of losses of resources and investments in the process of production of grapes and increased the money income and efficiency of the local producers. At the same time, it is strictly necessary during the nearest time to obtain new varieties with multiple resistance to pests and diseases in conditions of high heat and humidity, that will also keep already the obtained properties of the high quality of production and resistance to adverse winter conditions. At the same time, under the current and future climate and socio-economic challenges with their multiple and unpredictable effects on the viticulture, the spectrum of necessary features required by the producers become more wide, as well as the limits of the expression of these features.

The genetic traits highlighted and confirmed in the ampelographic collections can be used as criteria in evaluating, selecting and introducing the necessary genetic resources as sources of useful characters. This process involves searching, associating of the necessary ones, first of all, in the frame a very wide and diverse informative material, that becomes at the same time more and more voluminous. Rapid and efficient evaluation can significantly accelerate their mobilization and use in the process of improving of the assortment.

The accumulation of a large volume of information in international Data Bases, including free access, offers new opportunities in the evaluation of the existing grapevine genetic resources, therefore, the faster identification of the useful characters for the breeding process, the eventually geographical location of the sources of biological material for introduction.

For the purpose of the initial processing, under various aspects, of the available genetic-molecular information, the functionality of the Information System for the management of genetic resources from the Institute's Genofond was extended. The ampelographic description was completed, according to the OIV Descriptor, with the descriptors OIV 801 - OIV 806 (SSR-markers), and the information carried out in the previous projects (INTAS, SeedNet , COST FA1003 et al.), concerning some autochthonous genotypes, were introduced in database. The modules of Information System, which allow reading, storing, fragmenting or aggregating information, according to the formulated criteria have been developed.

An attempt was made to test the hypothesis of subtraction of genetic information, which would possibly show the difference in resistance to pathogens between *V. rotundifolia* Michx. and *V. Vinifera* L., based on the *V. rotundifolia* Michx. limited DNA sequences available in the NCBI database.

The presence in the same Informational System of the heterogeneous data (phenological, phenotypic, ampelographic, agrobiological, genetic-molecular), which include a broad spectrum of genotypes, will ensure easier association of traits useful for the genetic improvement process. The development of the functionalities is envisaged in the direction of completing, ensuring the junction with the bioinformatic methods.

"*MedVitis*": Protecting the diversity of Mediterranean *Vitis* in a changing environment

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Viticulture and wine production constitute important sectors of agriculture in Mediterranean countries, such as Greece, Slovenia and Morocco. Diverse geographical terrains and regional climate variations within each country have favored wide *Vitis* diversity and led to local varieties that are well adapted to specific agro-climatic conditions supporting sustainable agricultural systems of low inputs. However, introduction of foreign commercial varieties over the years have resulted in genetic erosion and loss of genetic diversity, necessitating collaborative actions aiming to preserve the diversity of *Vitis* genetic resources in this area. Moreover, environmental changes across the Mediterranean region, triggered by the global climate change, as well as the threat of diseases predict negative impacts for Mediterranean viticulture. '*MedVitis*', a project within the framework of the ARIMNET2 programme, is an integrated effort by Greek, Slovenian and Moroccan partners, aiming to protect Mediterranean grapevine biodiversity and enrich national grapevine collections. '*MedVitis*' entails phenotypic and molecular assessment of commonly used as well as rare grapevine varieties in order to enable proper variety identification, resolve issues of ambiguous identity (synonyms, homonyms) impacting the authenticity of final products, and allow for the updating of existing, or development of new national grapevine databases. Furthermore, exploring rare grapevine germplasm may provide novel information about varieties with tolerance to environmental changes such as drought, elevated temperature and increased rainfalls, or resistance to common pathogens. Exchange of knowledge and expertise among partners with respect to phenotypic and genetic characterization of grapevine varieties and information about potential resilience of different varieties to adverse climatic conditions would provide valuable tools for promoting sustainable viticulture in each country. Overall, the project aims to protect the diversity of grapevine germplasm across Greece, Slovenia and Morocco, and set the ground for addressing more efficiently issues of grapevine identification, genetic erosion, climate change and *Vitis* pathogenicity in the Mediterranean basin. Hence, the research proposed by '*MedVitis*' is expected to contribute to the conservation of Mediterranean grapevine genetic resources, promote sustainable viticulture, and enhance rural development affecting the economy and growth of local communities in the Mediterranean region.

Initial characterization of indigenous grapevine varieties from the Preveza region of Greece

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The global climate change predicts negative impacts for the sustainability of Mediterranean viticulture. In this context, the project "*MedVitis*-Protecting the diversity of Mediterranean *Vitis* in a changing environment" within the framework of the Arimnet2 programme, proposes the implementation of collaborative actions among three Mediterranean countries, Greece, Slovenia and Morocco, aiming at protecting Mediterranean *Vitis* biodiversity through detailed phenotypic and genetic analysis of grapevine genetic resources.

The current work describes initial studies on phenotypic evaluation of a series of five indigenous wine grapevine varieties from the region of Preveza, Epirus, in the Northwestern part of Greece. In particular, morphological characterization was performed for five red wine grapevine varieties from the prefecture of Preveza. The variety names and respective cultivation sites were as follows: Dichali/Managiatiko, Korithi Erithro, Tourkopoula, Alpoura and Koutsoupia from the location of Oropos (average altitude 30 m) as well as Dichali and Korithi Erithro from the historical site of Zalongo/Kryopigi (average altitude 500 m).

Ethno-botanical surveys with local farmers and viticulturalists were conducted in order to exchange information and knowledge with the local community with respect to the activities and significance of the project. The aforementioned varieties are very well adapted to the agroclimatic conditions of the Epirus region requiring low input systems. For a number of them, local wine production at small-scale have indicated, thus far, promising vinification potential for quality wine production. Addressing issues such as proper variety identification constitutes an important factor for protecting the rich grapevine diversity of the Epirus region as a whole and of Preveza, in particular. The current study focused on morphological evaluation of different grapevine varieties by use of ampelographic characterization. Field trips were undertaken in order to localize and mark the different varieties (total of 27 markings) and full photographic records were obtained. The Ampelographic description was based on 52 ampelographic descriptors of young shoots, young leaves and mature leaves, as specified by the OIV Descriptor List (OIV 2009). An exploratory statistical analysis of ampelographic measurements was performed in order to acquire a preliminary understanding of the structure of phenotypic diversity among the grapevine varieties examined. An initial dendrogram was constructed utilizing the Manhattan dissimilarity index and the UPGMA clustering algorithm employing NTSYSpc software program, displaying inter-cultivar variability.

This study provides an initial morphological characterization of the autochthonous grapevine varieties of the area of Preveza which will be followed by complete morphological and molecular evaluation in future work and ultimately contribute to the overall endeavor of phenotypic and genetic assessment of Mediterranean grapevine germplasm.

Analysis of the grapevine genetic diversity existing in Montenegro using ICVV-SNP and *VI*VC databases

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Montenegro has a long winemaking tradition dated back to Greek and Illyrian period times. Current Montenegrin wine-growing regions have evolved following globalization rules to fit international market needs. Nevertheless, this modern system co-exists with another Montenegrin viticulture that still maintains most of its traditional practices, including the cultivation of autochthonous cultivars. This traditional way is particularly important in Montenegro's viticulture because of the significant number of small grape growers that keep it alive, converting this region in an invaluable opportunity to study traditional ways to produce new varieties and genetic diversity which are currently extinct in Western European countries. Here, we performed the widest prospection of grapevine genetic resources carried out in Montenegro so far, which involved the collection of 419 grapevine leaf samples from old vines found in traditional vineyards across the different viticulture regions of the country. These samples were analyzed together to 57 accessions from the *ex situ Vitis* collection of the Biotechnical Faculty of the University of Montenegro (BTF *Vitis* collection), which was created in 1960 to preserve local grapevine genetic resources. Samples were genotyped by a combination of single nucleotide polymorphisms (SNP) and simple sequence repeats (SSR), and unique genetic profiles were compared with international databases (ICVV-SNP and *VI*VC databases, respectively) for proper grapevine varietal identification and for the detection of synonymies (different names for the same genotype) and homonymies (different genotypes with the same name). SNP genotyping at 48 loci revealed 144 different genetic profiles, of which 68 corresponded to prospected cultivated plants in ancient vineyards and 43 to plants prospected as wild vines. In addition, 33 genetic profiles were exclusively found in the *ex situ* BTF *Vitis* collection. The comparison of the SNP and SSR profiles obtained from old vineyards with those stored in the ICVV-SNP and *VI*VC databases allowed the full identification of 32 grapevine cultivars, including autochthonous cultivars from the Western Balkans (such as cvs. Kratošija (found 106 times), Vranac (76), Lisica (35) or Krstac (22)), others from Eastern countries (such as cvs. Razaklija (27), Kadarun (5) or Chaouch blanc (4)) and others from Western countries (such as cvs. Vulpea (3), Muscat Hamburg (2) or Merlot (1)), cultivated in many cases under synonym names. We did not find any match for up to 33 unique profiles, six of which were found at least twice across Montenegrin vineyards, indicating they are likely true, old autochthonous varieties on the edge of extinction. The high genetic diversity found in one of smallest European countries (13.812 km^2) reflects historic reports that indicate multiple introductions of plant material in the country from diverse European viticultural regions in different times and with different purposes. In addition, we found a complex parentage network linking multiple autochthonous cultivars, in which two varieties (Razaklija and Kratošija) played a leading role on the generation of local genetic resources. Our findings demonstrate that isolated, local niche-selection can represent an important mechanism contributing to the generation of current grapevine varietal diversity.

Antioxidant properties of phenolic compounds as residues in fermented grape pomace of cv. Cabernet Sauvignon

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In recent 20 years, it was discovered that there are many compounds in wine that have positive healthy effects. The most powerful compounds for humans health are phenolic compounds. Their extraction depends on winemaking technique, and it was investigated how much phenolic compounds stay in its by-product (pomace) as residue. These compounds possess various biological effects such as prevention of cardiovascular diseases and anti-inflammatory and anti-cancerogenic properties. The polyphenolic molecules have a functional role, in that they behave as antioxidants against the free radical species and show a physiologic role as well. In fact, they increase the antioxidant capacity and beneficial health effect wine consumption. Grape variety Cabernet Sauvignon was harvested in the state of technological maturity. Phytosanitary state was: 100% health, sugar in the must 23% and total acid in the must 6.8 g/l. Alcohol fermentation with maceration was carried out by microvinification method at temperature of 25°C using the "pigeage" system. Free sulfur dioxide 5 g/hl was added to the grape pomace. Yeast *Saccharomyces cerevisiae* (BDX, Lallemand, Canada) in the amount of 20 g/hl and Enzyme EXV (pectolytic) (Lallemand, Canada) in the amount of 2 g/hl were used. Liquid parts were separated from the start of fermentation (3, 5, 7, 14, 21 day, respectively), and fermented without contact with the solid phase (seeds and skin). Control sample was pomace separated immediately after crashing. Samples of pomace for each day, were frozen (-80°C) and after that lyophilized. Their extracts (extraction-methanol: water) were used for determination of total phenol compounds and anti-DPPH radical activity. Extraction of phenolic compounds depends on increasing of alcohol content, temperature and other yeasts nutritients (NH_4^+, pH, etc.). It was evaluated increasement until 10^{th} day from start of maceration (236.7 g/kg fresh pomace), and after that their content decreased until 21^{st} day (155.5 g/kg fresh pomace). Also, it was found that anti-DPPH radical activity of pomace extracts descrease with prolonged maceration time and at 21^{st} day was 1.8 %. Except for the amount of phenolic compounds in grapes (seeds and skin), their extractability during the vinification process is also important.

Two-omics data revealed commonalities and differences between Rpv12– and Rpv3–mediated resistance in grapevine

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Plasmopara viticola is the causal agent of grapevine downy mildew (DM). DM resistant varieties deploy effector–triggered immunity (ETI) to inhibit pathogen growth, which is activated by major resistance loci, the most common of which are *Rpv3* and *Rpv12*. We previously showed that a quick metabolome response lies behind the ETI conferred by *Rpv3* TIR–NB–LRR genes. Here we used a grape variety operating *Rpv12*–mediated ETI, which is conferred by an independent locus containing CC–NB–LRR genes, to investigate the defence response using GC/MS, LC/MS and RNA–Seq analyses. Eighty–eight metabolites showed significantly different concentration between inoculated resistant leaves and controls. RNA–Seq analysis showed 432 differentially expressed genes. Most metabolite changes in sugars, fatty acids and phenols were similar in timing and direction to those observed in *Rpv3*–mediated ETI but some of them were stronger or more persistent. Activators, elicitors and signal transducers for the formation of reactive oxygen species were early observed in samples undergoing *Rpv12*–mediated ETI and were paralleled and followed by the upregulation of genes ontology categories associated with salicylic acid signalling, signal transduction, WRKY transcription factors and synthesis of PR–1, PR–2, PR–5 pathogenesis–related proteins.

The study of cell wall metabolism in Trincadeira and Syrah cultivars indicates potential mechanisms involved in basal tolerance against *Botrytis cinerea* infection

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Grapes (*Vitis vinifera* L.) are fruit of major economic importance but are strongly affected by fungal diseases such as grey mould caused by the necrotrophic fungus *Botrytis cinerea*. The Portuguese cultivar Trincadeira is highly susceptible to *B. cinerea*, and presents severe infection symptoms even in green berries

In this work, changes in cell wall metabolism in grapevine were compared between a susceptible (Trincadeira) and a tolerant (Syrah) cultivar upon infection with *B. cinerea*. Peppercorn-sized fruits were infected in the field and mock-treated and infected berries were collected at green stage (EL32) for RNAseq analysis (Illummina) and cell wall profiling (GC-MS, CoMPP and FT-IR).

Regarding basal responses Syrah presented an enrichment in the categories for Cellulose synthases, Pectinesterases and Xyloglucan hydrolase families. On the other hand, Trincadeira showed an enrichment in Pectin methylesterase inhibitors and Polygalacturonase inhibiting proteins. Once infected Trincadeira grapes underwent an intense reprogramming of cell wall metabolism which was not observed in Syrah grapes. Classes such as Cellulose synthases, Xyloglucan endotransglycosylases and hydrolases, Pectinesterase, were enriched and up-regulated whereas classes endo-*beta*-glucanases, polygalacturonases and Fasciclin Arabinogalactan-protein were enriched as down-regulated.

Regarding cell wall profiling PCA analysis confirmed the lack of significant differences between control and infected Syrah samples. However, Trincadeira grapes responded to infection by decreasing the levels of the sugars arabinose, fucose, glucose, mannose, rhamnose and xylose. Additionally, Trincadeira grapes tend to present once infected the cell wall composition characteristic of Syrah grapes. This specific composition may be involved in Syrah's basal tolerance against *Botrytis cinerea*.

Mapping out the *Plasmopara viticola*-related metabolites of artificially infected grapevine

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One of the most economically important diseases of grapevine is Downy mildew (DM) caused by the oomycete *Plasmopara viticola*. The majority of cultivated grapevines originate from *Vitis vinifera*, a Eurasian species known for its remarkable flavor. However, this species is highly susceptible to the *P. viticola*, which implies a dependency of the grape production on the frequent use of fungicides. One of the most promising strategies to diminish the use of fungicides is to focus on the selection of grapevine varieties showing pathogen-specific resistance. Several genetic factors derived from *Vitis* species have been identified with resistance to DM, but occasionally the protection offered by these resistance genes can be overcome by virulent strains of the pathogens. Interspecific hybrids of *V. vinifera* and North American species, that showed a better resistance, have yielded cultivars with good wine-grape qualities and greater resistance to the pathogens.

The most frequent type of resistance is based on a gene for gene interaction with the pathogen followed by a more promising strategy, the pyramiding resistance where several resistance genes are associated in the same variety.

We hypothesize that grapevine plants having one single resistant locus have a different response to the development of *P. viticola* as to the genotypes carrying pyramided loci.

A metabolomics approach can help in exploring the interaction between grapevine and *P. viticola* and in extending the current knowledge about the perturbations occurring in the plant system after biotic stresses.

In our study, we evaluated the metabolic changes in four resistant or tolerant genotypes containing different sources of resistance (Bianca, Jasmine, BC4 and Solaris) and one susceptible genotype (Pinot noir). Additionally, work is being carried on to study also two genotypes carrying pyramided loci (F12P127, F12P60). With the aim to elucidate if different sources of resistance are associated with different degrees of resistance and implicitly with different responses to the pathogen, we considered the most important classes of plant metabolites. Thus, we identified and quantified primary compounds (GC-MS), lipids (LC-MS/MS), phenols (LC-MS/MS), and semi-quantified volatile compounds (GC-MS) at 0, 12, 48 and 96 hours post artificial infection.

The objective of this project is the evaluation of grapevine hybrids with one or more than one different sources of resistance against the pathogen considering a metabolome approach. The expected results are a different characterization of the resistant mechanisms underlying the hybrids-pathogen interaction and their fight against the diseases. This project is directed towards a better understanding of plant defense mechanisms and characterization of the plant pathogen interactions affecting the *Vitis* species.

Getting the best of ancient DNA data using new bioinformatics tools specifically designed to deal with short query sequence and mismatches

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Evolutionary history of viticulture practices and wine making may be studied through ancient biological samples such as waterlogged seeds found in different archaeological excavations or herbarium specimens kept in botanical museums. More specifically, paleogenomics is one of the most promissing approaches to trace the grapevine migration whose origins have been suggested to surround the South Caucasus and spread along the Mediterranean coast (McGovern et al. 2017).

The recovery of this genomic information also informs about genetic shift during domestication and links ancient population to modern varieties. So, we know that Romans made wines with varieties closely related to modern ones two thousands years ago (Ramos-Madrigal et al. 2019).

However, ancient samples presents several problems due to degradation process. The small quantity of endogenous DNA in these fossil and damaged samples hampers their extraction. On the other hand, genome fragmentation and specific alterations arise from cytosine deamination, depurination or crosslinkng make sequencing and analysis more difficult.

Ancient DNA extraction and amplification methods have been optimized in this field of biomolecular archeology, for instance, with the Illumina T/A ligation when library preparation, or the solid-phase target enrichment based on modern DNA oligos in order to retrieve genomic regions with informative single nucleotide polymorphisms. But data treatment and analysis of these short, incorrect or incomplete query sequences still poses important computational challenges.

Mapping of such raw reads to a modern reference genome is one of the key steps for genotyping analysis. In the last years, many algorithms have been developed to address short query sequences and high error rate in the tail, which is typical to Illumina sequencing data. However, conservative pipelines are still recommended in ancient DNA literature and extensively used.

We evaluated mapping efficiency and phylogenetic results comparing a classic pipeline based on adapter trimming and Burrows-Wheeler Aligner (BWA) seed disabling (Schubert et al. 2014) and a new version that employs adapter masking and BWA-mem alignment. BWA-mem uses a new algorithm that improves productivity and is strongly recommended by the authors to handle similar features with ancient DNA (Heng and Durbin, 2010)

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Aspects of reproductive biology of Armenian grapevine genetic resources for novel breeding strategies

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Armenia is rich by numerous aboriginal and bred Vitis vinifera L. cultivars and V.vinifera subsp. silvestris wild genotypes. Nowadays the major challenge for Armenian viticulture is the productivity and berry quality under climate change condition. The yield and qualitative and quantitative characteristic of berries is based on the grapevine reproductive biology of the different genotypes. The determination of flower sex and development relate to complex mechanism in grapes. Vitis vinifera cultivars are generally characterized by hermaphroditic flowers and only small quantity of cultivars with female flowers which are usually less productive with smaller berries than hermaphroditic. Wild Vitis species are dioecious with separated plants with male or female flowers. There are several candidate genes involved in flower formation in grapevine.

In our research the complex analysis of thirty Armenian grapevine cultivars and wild genotypes was carry out. Data of genetic and phenotypic study of flowers and their sex were obtained. Genetic characterization of Armenian grapevine genotypes using three SSR molecular markers GF02-31, GF02-49 and APT3 Indel detected genetic diversity for flower sex loci. The majority of the cultivars characterized by hermaphroditic flowers production with presence of both male and any female allels of all three genes. Cultivars with female flowers were segregated in two groups with different allels of APT3 including female flowers named Fc cultivated and Fw wild. Homozygotes of Fc were identified for Arevik and Shaheni. Heterozygotes with FcFw were found in Qrdi khaghogh and Spitak Areni. By genetic analysis of flower sex loci two cases of misnamed genotypes with hermaphroditic flowers were identified which were characterized in our research by female flowers production.

In the frame of COST INTEGRAPE new approaches including genomics, transcriptomics and proteomics will be applied for the detailed characterization of the different stages of flower formation in Armenian grapevine genetic resources. Future applications of our data for genotype-phenotype association will be useful for increasing the yield and improvement of the grape quality in novel breeding strategies.