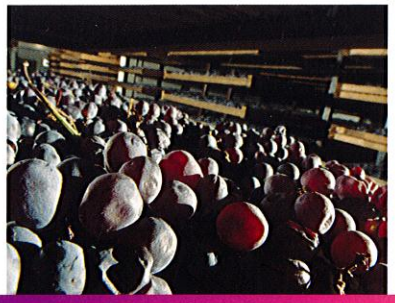
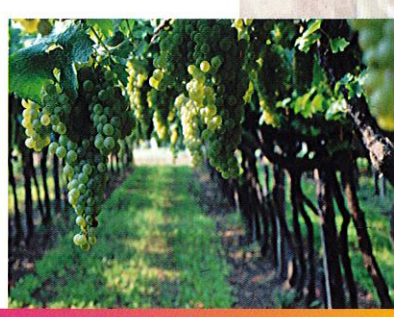
UNIVERSITÀ di VERONA
Dipartimento di BIOTECNOLOGIE

ISHS

X INTERNATIONAL SYMPOSIUM ON GRAPEVINE PHYSIOLOGY AND BIOTECHNOLOGY



BOOK OF ABSTRACTS

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Protocol and tissues employed in grapevine RNA extraction

*Tiago Tomazetti [1] - Marcia Rossarolla [1] - **Isadora Malohlava** [1] - Raissa Podesta [1] - José Afonso Voltolini [1] - Rubens Nodari [1] - Miguel Guerra [1] - Leocir Welter [1] - Aparecido da Silva [1]*

Centro de Ciencias Agrárias, Universidade Federal de Santa Catarina, Florianopolis, Brazil [1]

The aims with this work was test RNA extraction protocols from *Vitis labrusca* tissues for use in RT-qPCR technique. Three samples of different parts of the vine were used. For RNA extraction the organic extraction protocol with modified Trizol®, the extraction with CTAB detergent and the commercial kit RNeasyPlant(Qiagen) were used. The tissues used were kept at low temperature, transported the laboratory and stored at -20°C until RNA extraction, using 100mg of tissue for each extraction. The concentration of the extracted RNA was measured with a spectrophotometer NanoDrop ND-1000, the proportion of protein (A260/A280) and polyphenols (A_{260}/A_{230}), and subsequently the reaction of RT-qPCR was carried out with 20ng of sample RNA using mastermix QuantiTectProbe (OneStep) with labeled probe (Zen™) for the 18SrRNA gene. For C_t comparison the data were analyzed using the statistical test SNK($\alpha = 0.05$). The RNA concentration was higher with the commercial kit, except in the branches. The A_{260}/A_{280} and $A_{260}/_{A230}$ relations showed no significant interactions, the kit provided greater purity and Trizol the lower purity. Due to the unsatisfactory results of the extraction with Trizol, the RT-qPCR reaction was carried out only with samples from the other protocols, except for the sample obtained from the twigs. The kit provided smaller amounts and variation of Ct compared to CTAB, indicating greater integrity of the extracted RNA. This kit provided higher RNA concentration, purity and quality compared to other protocols, except for the samples obtained from the twigs. Trizol does not present satisfactory results for RNA extraction in the tested tissues.

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Genomic basis of differentiation between wild and cultivated grapevines

Annarita Marrano *[1] - Giovanni Birolo [2] - Diego Micheletti [1] - Silvia Lorenzi [1] - Maria Lucia Prazzoli [1] - Giorgio Valle [2] - Maria Stella Grando [1]*

Research and Innovation Centre Genomics and Biology of Fruit Crops, Fondazione Edmund Mach, San Michele all'adige, Italy [1] - CRIBI-department, University of Padua, Padova, Italy [2]

The selective pressure applied by humans to domesticate plants is thought to have reduced the genetic diversity of genes contributing to elected traits. This selection process left genomic signs known as "signatures of selection". While domestication produced crops with high yield and rapid growth, it arguably led to a decrease of plants resilience. Today wild ancestors are considered valuable sources of resilience factors, whose re-discovery can be fundamental for future sustainable agriculture. During domestication, changes in berry size and a transition from dioecious to hermaphrodite plants occured in cultivated grapevines (*Vitis vinifera* subsp. *sativa*) from its wild form (*V. vinifera* subsp. *sylvestris*). Population genetic analysis can help to clarify how these changes happened and map genes contributing to adaptive traits in grapevine. In this regard, we investigated the genetic diversity of a grapevine germplasm collection composed by 42 *V. sylvestris* and 46 *V. sativa* accessions. We genotyped the whole population using the commercial GrapeReSeq Illumina 20K SNP chip and through a novel RAD-seq procedure, obtaining a high density panel of 30K solid polymorphisms. Population genetic structure highlighted a clear separation among wild and cultivated accessions with a low level of admixure. Therefore, we evaluated the FST metric

between cultivated and wild accessions along the whole genome. Over one thousand of SNPs showed a significant high value of FST, validated empirically with permutation test. These loci fall within putative "signatures of selection" that contain genes presumably involved in adaptation during domestication in grapevine. Moreover genome wide association mapping is also being performed for nine traits, including berry size and composition, as an alternative approach for the identification of domestication-related loci. Finally, the application of both population genetic methods and association mapping enabled the discovery of numerous signals of selection which will be extremely useful to better understand the plant response to environmental stimuli.

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A new and easy way to purify nucleic acid from grape leaves, grapes and other relevant plants

Céline Ménager *[1] - Elisa Ruiz [2] - Laurence Delaurière [1] - Samantha Lewis [3] - Becky Carroll [3] - Trista Schagat [3]*

PETAL, Promega France, Charbonnières les Bains, France [1] - PETAL, Promega France, Charbonnières les bains, France [2] - promega corp, promega corp, Madison, United States [3]

Genotyping, epigenetic modifications, gene expression, authentication, GMO detection, and viral contamination detection are important areas for the study of plants at a molecular level. All these applications require starting with nucleic acids of high quality.
In plants, purification of nucleic acids provides a variety of unique challenges.
The diversity of the species, the different tissue types (e.g. leaves, seeds, fruits, and roots), the maturity stage of the sample and the variety of extracellular structures make lysis and extraction difficult and lead to decreased nucleic acid yield. In addition, the different endogenous compounds (e.g. polysaccharides, phenolics) can co-purify with nucleic acids and inhibit downstream enzymatic assays. These difficulties highlight the need for robust, reliable chemistries for molecular biology studies in plants.
We present several solutions to purify and amplify nucleic acids from plant tissues. We developed a manual approach using spin columns and an automated approach using novel cellulose-based paramagnetic particles. These particles are easily automated for low to medium throughput isolation using the small bench-top Maxwell® RSC Instrument or for high throughput 96well purification on large automated platforms such as the KingFisher Flex.
Data are presented for DNA and RNA extraction from grape leaves, grapes and other relevant plants. Extracted DNA and RNA are evaluated for yield, purity, amplification in qPCR or RT-qPCR and for presence of inhibitors. Additional data show the capacity of our extraction systems to extract viral RNA from other plant leaves and seeds.

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Identification of QTL for acidity in wine grape based on a dense SNP genetic map

S. Song [1, 2, 3] - P. J. Martínez-García [4] - M.M. Hernández [2, 3] - ***Cristina M. Menéndez*** *[2, 3]*

Department of Plant Science, School of Agriculture and Biology, Shanghai JiaoTong University, Shanghai, 200240, P.R. China [1] -Instituto de Ciencias de la Vid y el Vino (ICVV, CSIC-Universidad de la Rioja-Gobierno de La Rioja), Logroño, 26006, Spain (2) - Departamento de Agricultura y Alimentación. Universidad de La Rioja, Logroño, 26006, Spain (3) - Department of Plant Sciences, University of California, Davis, CA 95616, USA [4]