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X INTERNATIONAL SYMPOSIUM ON GRAPEVINE PHYSIOLOGY AND BIOTECHNOLOGY









BOOK OF ABSTRACTS

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parenchyma cells in the vascular tissues.

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Based on these results, we propose a two-step model for retrieval of leaked sugar in the pedicel: first, sugar transporters may provide the molecular machinery to actively return sugar from xylem vessels (part of the apoplast) to the symplast; next, the presence of symplastic connections supports the possibility of symplastic sugar movement after its retrieval from the apoplast.

P6

Comparative analysis of the expression of candidate genes in male, female and hermaphrodite flowers of wild grapevine (Vitis vinifera L ssp sylvestris)

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We have searched the expression analysis of functional candidate genes to sex determination in the new defined sex locus described by Picq et al (2014). We have found significant differential RNA expression in male and female flowers of wild grapevine (*Vitis vinifera* L ssp *sylvestris*) at the different flower development stages (15-18) for the enzyme 1 aminocyclopropane-1-carboxylic acid synthase (ACS) and ETO1 protein that specifically inhibits the enzyme activity of ACS. The ETO1 copy is localized in the LG2 in the sex determination region. In the other hand, the ACS a copy is localized in the LG2 but outside of the sex locus. However, association between ACS sequence polymorphisms and the sex trait was found. These elements converge to include this ACS copy as a functional candidate gene for sex determinism in grapevine. Although this gene it is outside of the flower sex locus, could be possible that some protein from the sex locus region it is affected the ACS gene expression. Our data support that ACS and ETO1 genes could be implicated in grapevine flower development.

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Characterization of grapevine somatic variants differing in seed content

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Seedlessness is an important objective in grape breeding programs being highly appreciated by consumers. Substantial advances in the comprehension of the molecular underlying mechanisms have been achieved. However, most studies inspected the stenospermocarpic variety Sultanine, whereas different sources of seedlessness have been much less investigated. With the aim of providing additional insights into the regulation of seed formation and develop testable candidate gene hypotheses, we explored our grapevine germplasm collections searching for clones with contrasting seed content. In total, we identified nine



variant pairs that differ only in those characteristics related to the presence/absence of seeds while showing identical microsatellite marker profile. Here we report their characterization, with special emphasis on Sangiovese and its seedless variant, for which we previously performed a comprehensive transcriptomic comparison. A subset of the paired accessions and some additional genotypes were characterized during 4 years for fruit and seed set in control, self-pollinated and emasculated conditions. Our observations suggest that when pollination is not prevented stenospermocarpy may be responsible for the seedless phenotype and is potentially driven by pollen and/or ovule defects. Two genotypes were unexpectedly found to develop fruits also without pollen contribution, which occasionally showed normal-like seeds. The possible origin of this phenomenon is currently under investigation. In the seek for structural variation each seedless mutant was compared to its seeded reference variety by using the GrapeReSeq_Illumina_20K_SNP_chip. None of the putative SNPs was validated by resequencing, proving they are near-isogenic lines. Conversely, RNA-Seq-based variant calling allowed the identification of 5 nonsynonymous SNPs that were experimentally confirmed in the original and additional Sangiovese accessions. These polymorphisms are suitable to be tested as diagnostic markers in clone identification and as functional candidates for the seedless phenotype.

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Functional evaluation of terpenoid biosynthesis during fruit development and stress conditions in metabolic engineered microvines

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Terpenoids, the largest family of natural compounds, derive from the plastidial MEP- or from the cytosolic MVA-pathway and are involved in important biological functions in plants. Volatile and non-volatile terpenoids are implicated in the attraction of pollinators and predators of herbivores, in protecting against photo-oxidative stress, in mediating thermo-tolerance and in plant defense against pathogens. They have also a huge commercial value *in primis* for their use as flavors and fragrances. In grapevine, monoterpenes are the main responsible for Muscat aroma, a highly appreciated quality trait in wine and table grape. The first enzyme of the MEP pathway, VvDXS1, was suggested as a candidate gene for this trait, having been colocalized with a major QTL for monoterpenoids in berries. In addition, a non-synonymous SNP responsible for an amino acid substitution in the VvDXS protein was found to be significantly associated with muscat flavored varieties. In this study a functional analysis of VvDXS1 has been carried out in 'microvine', a grapevine model system suitable for rapid reverse genetics studies.

The two allelic forms of VvDXS1 have been overexpressed in 'microvine' by Agrobacterium tumefaciens-mediated gene transfer. The expression pattern of several genes involved in terpenoid biosynthesis was assessed at various stages of berry development and significant differences were found between transgenic and wild-type plants. Moreover, the transgenic plants are currently under evaluation in abiotic stress conditions to disclose possible advantages in plant stress response resulting from an increased MEP pathway flux.