Model-assisted comparative analysis of sugar accumulation in fleshy fruits: grape, tomato, and peach

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COST Action FA1106 "QualityFruit"

2nd Annual Conference

Fleshy Fruit Development & Ripening

BOOK OF ABSTRACTS

Chania 22-25 September 2013

Mediterranean Agronomic Institute of Chania (MAICh)

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19:30-21:30	***Welcome reception***

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End of general meeting	

ABSTRACTS
OF
ORAL PRESENTATIONS

SESSION 1: “Fruit Development and Ripening”

GENETIC REGULATION OF TOMATO FRUIT RIPENING: FROM GENES TO GENOMES TO THE EPIGENOME.

Giovannoni James

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Tomato is a primary model for fruit development and shelf-life in addition to a vegetable crop of increasing production and nutritional importance the world over. Annual per capita tomato consumption in the US alone is near 70 pounds making tomato primary sources of vitamins A and C in US diets. Pioneering work in tomato biology has elucidated mechanisms of pathogen response, ethylene hormone synthesis and perception, carotenoid metabolism and transcriptional control of fruit ripening. Translational biology of tomato discoveries has, for example, demonstrated conservation of nutrient metabolism and ripening mechanisms in a range of species and suggests that additional discoveries will have wide ranging effects on food security and nutrient content. Novel and well characterized germplasm resources, efficient transformation and a high quality genome sequence have accelerated the pace of tomato biology facilitating genome, expression and metabolite/trait analyses and the ability to exploit systems approaches toward biological discovery with practical implications to crop improvement. In highlighting these resources and their applications, the roles of multiple transcriptional regulators of ripening and nutrient content will be described along with preliminary data suggesting a role for epigenome modification in control and manifestation of the ripening process.

LONG-TERM *IN VITRO* CULTURE OF GRAPE BERRIES AND ITS APPLICATION TO ASSESSING THE EFFECTS OF SUGAR SUPPLY ON ANTHOCYANIN ACCUMULATION

Zhan Wu Dai[1], Messaoud Meddar[1], Christel Renaud[1], Isabelle Merlin[1], Ghislaine Hilbert[1], Serge Delrot[2], Eric Gomès[2]

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Grape berry development and metabolism are controlled by the import of nutrients and hormones, and by the environment sensed by the berry. The study of nutritive, hormonal and environmental effects on berries still attached to intact plants is very difficult because of the large size of the plants, because of interplant, intercluster and interberry variability, and because it is not possible to control strictly the nutrient and hormonal import, and the environment. It would be interesting to be able to grow isolated berries for several weeks on artificial media where the nutritive and hormonal composition, and the environment, can be strictly controlled. To this end, a long term *in vitro* culture system of intact detached grape berries was developed by coupling the production of greenhouse fruiting-cuttings and *in vitro* organ culture techniques. ^{13}C and ^{15}N labeling experiments showed that this system enables intact and detached berries to actively absorb and utilize carbon and nitrogen from the culture medium. It was further used to study the effects of sugars on anthocyanin accumulation. Sucrose concentration higher than 2% could induce anthocyanin synthesis in the absence of additional exogenous ABA. The higher was the sucrose concentration, the earlier was the induction of anthocyanin accumulation. Increased anthocyanin accumulation was induced by glucose, fructose, and sucrose, with glucose and fructose being more effective than sucrose. The increase in anthocyanin accumulation under high sugar supply was not due to an increase in its precursor level, since the precursor phenylalanine was decreased by high sugar supply. Instead, genome-wide transcriptome analysis suggests that the sugar-induced enhancement of anthocyanin accumulation results from a modification of regulatory and structural gene expression levels (especially UFGT) by high sugar concentration, together with massive reprogramming in signaling transduction pathways. Overall, this *in vitro* system may serve to study the response of berry quality to other nutrient factors (e.g. nitrogen and sulfur) and their interaction effects with environmental factors (e.g. light and temperature), which can be precisely controlled.

ENDOREDUPLICATION AND FRUIT GROWTH IN TOMATO: EVIDENCES IN FAVOR OF THE KARYOPLASMIC RATIO THEORY.

Chevalier Christian[1], Julien Pirrello[1], Matthieu Bourdon[1], Catherine Cheniclet[1], Mickaël Bourge[2], Spencer Brown[2], Jean-Pierre Renaudin[1] and Nathalie Frangne[1]

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Endopolyploidy occurs in many plant species and supports the process of differentiation of cells and organs. The functional role of endopolyploidy in plant cells remains poorly understood, mainly because the analysis is hampered by the fact that complex polyploid tissues usually include cells with different ploidy levels. During the development of tomato fruit, cells from the (fleshy) pericarp tissue become highly polyploid reaching DNA content barely encountered in other plant species (between 2C and 512C). To investigate the spatial and temporal distribution of endopolyploidy, it is necessary to address the DNA content of individual nuclei *in situ*. Populations of nuclei with different ploidy levels were isolated to characterize at the cytological level the consequences of endopolyploidy on the ultrastructure of nuclear and nucleolar chromatin, the nuclear shape and the relationship with other cellular organelles such as mitochondria. We were able to develop a new method based on BAC-FISH to determine *in situ* the ploidy level of different nuclei and consequently establish a ploidy map of tomato fruit pericarp. Based on this map, we demonstrated a link between the ploidy level, the complexity of nuclear shape and the number of mitochondria in the vicinity of polyploid nuclei. We were able to provide the first direct evidence that endoreduplication plays a role in the increased transcription of rRNA and mRNA in plant cells. We thus provided quantitative data in favor of the 'karyoplasmic ratio' theory and showed that endoreduplication is associated with a complex cellular re-organization during development of tomato fruit.

NEW ANNOTATION OF THE GRAPE GENOME: A FIRST STEP TOWARDS UNIFYING TOMATO AND GRAPE RESEARCH

Mohamed Zouine[1], Stéphane Rombauts[2], Maria Luisa Chiusano[3], Mathieu Lauvernier[1], Mario Pezzotti[4] and Bouzayen Mondher[1]

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[2]Department of Plant Systems Biology, Vlaams Instituut voor Biotechnologie, B-9052 Ghent, Belgium & Department of Plant Biotechnology and Bioinformatics, Ghent University, B-9052 Ghent, Belgium
[3]Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, 80055 Portici, Naples, Italy.
[4]Department of Biotechnology,Università degli Studi di Verona, 37134 Verona, Italy

One of the main objectives of the COST Action "*QualityFruit*" is to provide tools, methodologies and biological resources for high throughput data processing and visualization as well as means for comparative genomics between tomato and grape. One of the milestones set by the project is to deliver a high quality annotation, both structural and functional, for grapevine and tomato. These predictions will be made broadly accessible to both communities. The tomato genome published in 2012, provided the scientific community with a high quality whole genome assembly and annotation. This structural and functional annotation was produced by the international tomato annotation group (iTAG), a multinational consortium. The strength of the pipeline used by iTAG resides in its high level of integration, taking full advantage of the available ESTs and RNA-seq data. On the other hand, A first version of the grape genome (8x base coverage) sequence has been published in 2007 and subsequently in 2010 additional sequencing brought the coverage to 12x, leading to the release of an improved assembly. Together with this new assembly, a corresponding structural and functional annotation was generated (Grimplet et al. 2012). Since the main focus of the COST Action "QualityFruit" is to set up suitable comparative analysis between the grapevine and tomato, it is therefore important to have for both genomes gene models of comparable quality produced by highly similar pipelines. It was thus decided to adapt and apply the iTAG pipeline to the grapevine genome. In *vitis,* available RNA-Seq and EST sequences generated from different grape tissues have been aligned with the latest version of the 12x reference genome. Protein sequences from *Arabidopsis thaliana* (TAIR10), tomato (SOL-iTAG2.3) and SwissProt were also mapped. The existing repeat library has been enriched with sequences obtained using RepeatModeler and REPET which improved the masking of transposable elements. These data were combined within the iTAG pipeline to produce the structural gene predictions. In addition, to further improve the annotation, the GUI/ORCAE, has been setup to receive support for structural and functional annotation by experts, from both, tomato and grapevine communities. This online platform will work as a centralized repository for both species gene annotations. The quality of the resulting gene prediction is currently under evaluation before being released to the scientific community. Moreover, comparative analyses are being organized to fix a preliminary set of computationally defined orthologs from grape and tomato reference annotations.

DISTANT COUSINS? THE HORMONAL CONTROL OF FRUIT DEVELOPMENT IN GRAPES HAS POINTS OF SIMILARITY AND DIFFERENCE WITH TOMATOES.

Davies C.[1], C. Böttcher[1], P. Boss[1], T. Peat[2], J. Newman[2]

[1]CSIRO Plant Industry, Adelaide, Australia, [2]CSIRO Materials, Science and Engineering, Melbourne, Australia.

There are obvious differences in the morphology and metabolism of grapes (non-climacteric) and tomatoes (climacteric) and how they develop. Similarly, there are numerous examples of differences in the roles of various hormones during fruit development and in particular ripening. Close examination however, reveals some common elements underlying the way various hormones participate in the ripening of these two important fruit. Interactions between the biosynthesis and perception pathways of the different hormones are important in both fruit and the role of different hormones is strongly dependent upon developmental stage. In grapes, levels of abscisic acid (ABA) and the brassinosteroid castasterone increase rapidly at veraison. ABA and castasterone application before veraison can advance ripening and an inhibitor of brassinosteroid biosynthesis delayed ripening. ABA may also act as a promoter of tomato ripening and appears to be involved in cell wall changes. As in grapes, brassinosteroids can advance tomato disc ripening. The role of ethylene in grape berry ripening is less clear than in tomatoes. An increase in ethylene evolution at veraison is so small as to be difficult to measure. However, much of the 'machinery' of synthesis and perception are similar in both fruit species. Increasing evidence demonstrates that ethylene can play a role in grape berry development albeit a less dominant role than that played in climacteric fruit. Grapes appear to lack autocatalytic ethylene production and exhibit a biphasic response to ethylene during the first half of development. Auxins delay ripening in tomato and other climacteric fruit if applied early enough in development to avoid the induction of a climacteric response. In all cases the timing of hormone application is crucial to effect. Grape ripening is also delayed by auxin application pre-veraison. The pattern of auxin accumulation is similar in both being higher in young fruit decreasing to low levels prior to the onset of ripening. The family of indole-3-acetic acid (IAA)-amido synthetases (GH3 enzymes) important for auxin homeostasis appears to be a critical factor in the control of grape berry ripening and there is an accumulation of the IAA-Asp conjugate after veraison. A similar situation occurs in tomatoes and two GH3 genes are upregulated at the onset of tomato ripening. We have recently elucidated the 3-D structure for one of the grape IAA-amido synthetases that provides insights at the molecular level into an important mechanism involved in auxin homeostasis.

ETHYLENE AND AUXIN-DEPENDENT TRANSCRIPTIONAL CONTROL UNDERLYING THE RIPENING PROCESS IN TOMATO FRUIT.

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The making of a fruit is a developmental process unique to plants involving a complex network of interacting genes and signalling pathways. In fleshy fruits, this involves three main stages known as fruit set, fruit growth and fruit ripening each corresponding to a transition step associated to major metabolic reorientations and structural changes. The co-ordinated changes in the levels of several plant hormones associated with the triggering of the ripening process strongly suggest their dynamic involvement in these processes. To address the general role of hormone cross-talk involved in fruit ripening combined genome-wide transcriptomic profiling and reverse genetics approaches were adopted to gain further insight on the molecular events underlying tomato fruit ripening. Overall, the data emphasize the role of auxin and ethylene-dependent transcriptional control of gene expression as part of the mechanism by which the fruit developmental program switches into a ripening process. Transcription factors belonging to the *ERF* (Ethylene Response Factors) family display an auxin-dependent regulation and altering their expression results in modified ripening behaviour. some ERF genes are shown to mediate ethylene responses during ripening and the exploring the mechanisms by which ERFs select their target genes shed new light on the molecular basis underlying the specificity of ethylene responses. For instance, over-expression of a chimeric repressor construct of *Sl-ERF.B3* results in altered fruit shape and size, inhibition of lycopene accumulation and accelerated fruit senescence. Consistently genes involved in ethylene biosynthesis, perception and in cell wall degradation are up-regulated whereas those involved in carotenoid biosynthesis pathway are down-regulated. Moreover, the expression of key regulators of ripening such as *RIN, CNR* and *HB-1* is stimulated in *ERF.B3-SRDX* dominant repressor fruits. Noteworthy, a number of *ERF* genes show altered expression patterns in *ERF.B3-SRDX* ripening fruits suggests the existence of a complex network enabling interconnection between different *ERF* genes accounting for the pleiotropic alterations in fruit maturation and ripening. Altogether, the data uncover a mean for uncoupling some of the main features of fruit ripening such as fruit softening and pigment accumulation, and bring new insight on the role of *ERFs* in regulating fruit maturation and ripening in a pleiotropic way. Likewise, the study shows that down-regulation of some members of the *ARF* (Auxin Response Factors) gene family results in ripening phenotypes with major metabolic reorientation and structural changes. This uncovers new roles for ARFs during fleshy fruit development and adds auxin to the list of the important cues controlling fruit ripening and overall fruit quality.

SUPPRESSION OF A TOMATO PROLYL 4 HYDROXYLASE AFFECTS FRUIT AND SEED DEVELOPMENT.

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Proline hydroxylation is a major post-translation modification of hydroxyproline-rich glycoproteins (HRGPs) that is catalyzed by prolyl 4-hydroxylases (P4Hs). Their involvement in plant growth and development has been recently investigated in Arabidopsis, tobacco and carnation while little is known about their role in tomato. The tomato genome comprises 10 putative P4Hs with most of them being expressed during fruit development. Preliminary experiments to partially suppress their expression using Virus Induced Gene Silencing resulted in alterations on cell division and expansion of tomato leaves. Therefore, transgenic tomato plants expressing an RNAi construct were produced in order to suppress a tomato P4H highly expressed during fruit development and ripening. Several independent lines down-regulating the target P4H were identified and nine of them were further characterized. The expression of the target P4H was completely suppressed during fruit ripening and the total hydroxyproline content in fruits was lower in most of the lines. All of the lines exhibited a reduction in fruit diameter while the number of viable seeds reduced by 80%. Collectively, these results indicate that the target P4H play a significant role in tomato fruit and seed development.

DAILY OSCILLATORY TRANSCRIPTIONAL PROGRAMS IN GRAPEVINE RIPENING BERRIES.

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Temperature and solar radiation are factors influencing grapevine berry ripening. High temperatures decrease anthocyanin accumulation and hasten organic acid metabolization, while light promotes proanthocynidin and terpenes accumulation. Temperature and radiation conditions cyclically fluctuate in a daily period under field environment. Indeed, the strength of this daily variation affects berry ripening and quality. The intensity of daily temperatures fluctuation modifies flavonoid partitioning, while cold and light during night time enhance anthocyanin accumulation. Transcriptome alterations in grapes under different set temperature and light conditions has been documented. Additionally, a circadian clock signaling that modulate the expression of a high proportion of the genome is present in plants. This circadian clock in connection with other factors controls multiple developmental processes like flowering or dormancy. Nevertheless, the effect of these daily cycling elements over the ripening transcriptional program has not been characterized in detail. In order to determine whether different ripening transcriptional programs are activated during a day in different grape tissues, we followed the berry skin and flesh transcriptome in six time points throughout a 24-hours cycle using the NimbleGen Vitis HX12 microarray. Tempranillo berries with the same density (~19º brix) were selected in all time points from plants grown under controlled greenhouse conditions. In that manner, four different profiles of differentially expressed genes (5% FDR in Limma and ≥2-fold change) were identified in both tissues. Two different oscillatory cycles correlated with them, one involving temperature variation along the 24-hours cycle, while the other presented two opposite peaks at the end of the light and the dark period, respectively. Functional enrichment analysis showed that a thermotolerance response including the activation of HSP chaperones took place at noon time in both tissues. More genes with expression changes following light oscillation were identified in the skin. They were enriched in regulatory genes involved in circadian clock and ethylene signaling pathways as well as in WRKY and AP2 transcription factors and protein kinases. Analogous thermotolerance and signaling responses were found in Verdejo whole pericarp from field grown conditions, while other specific changes could be related with the experimental settings. Genes involved in metabolism were not greatly altered, suggesting that daily variations of conditions could affect berry ripening by mechanisms other than transcriptional control. These results indicate daily oscillatory changes in the grapevine berry transcriptome to occur and they could help to a better understanding of the progress of berry ripening in short term time scales.

SESSION 2: “Fleshy Fruit Metabolism”

RIPENING, ROTTING AND REGULATION: TOMATO FRUIT METABOLISM AND SUSCEPTIBILITY TO *BOTRYTIS CINEREA*

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Developmental processes contribute to the outcomes of interactions between plants and pathogens. Regulated in part by transcription factors, early development of fruit in tomato (*Solanum lycopersicum*), establishes structural and metabolic environments within the fruit flesh that are reconfigured later as other transcription factors and hormones activate and regulate maturation and ripening programs. Ripening leads to significant losses of mature and harvested products from the maceration or rotting of flesh of the fruit caused by microbial infections and growth. As much as 80% of the ripened fruit from over 200 species are destroyed due to their susceptibility, in particular to necrotrophic fungal pathogens, such as the filamentous ascomycete *Botrytis cinerea.* No robust genetic source of resistance to this pathogen has been identified. The limited growth of *B. cinerea* on unripe fruit is altered by developmental and ripening processes in the fruit and by the differences in the infection strategies deployed by the pathogen on ripe and unripe fruit.. Although unripe tomato fruit are typically resistant to infections by *B. cinerea*, the pathogen expresses virulence functions that eventually enable aggressive hyphal growth and the precocious activation of selected aspects of fruit ripening. As tomato fruit ripen around mature seeds, the changing physiology and cell wall polysaccharide architecture of the epidermis and pericarp flesh contribute to the increasing susceptibility of fruit. The susceptibility of ripe fruit could simply be a default outcome of the entire syndrome of ripening. However, tomato lines with mutations that affect fruit development and ripening provide evidence of the key roles of selected aspects of early development and subsequent ripening in the acquisition of susceptibility in ripened fruit. Mutations in ripening regulators reveal that specific ripening processes and changes in the polysaccharides within the cell walls of the softening flesh are required to render ripe fruit supportive of active *B. cinerea* growth. Some non-ripening mutants are susceptible to *B. cinerea*, leading to the conclusion that not all ripening processes are required for fruit to become susceptible to infections. By identifying the regulators of fruit ripening and fungal virulence processes which are crucial for the susceptibility of ripened fruit, acceptably mature fruit with reduced rotting may eventually be developed and yield improved fresh and stored products.

THE ACTION OF POLYPHENOLIC ANTIOXIDANTS IN DETERMINING SHELF-LIFE OF TOMATO: LESSONS FOR IMPROVING THE POST-HARVEST QUALITY OF FLESHY FRUIT?

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We have engineered tomatoes that produce very high levels of anthocyanins (delphinidin 3-o (coumaroyl) rutinoside 5-o glucoside and petunidin 3-o (coumaroyl) rutinoside 5-o glucoside) and tomatoes that accumulate high levels of flavonols (quercetin and kaempferol rutinosides). The purple, high anthocyanin tomatoes show extended shelf life due to both slower over-ripening and reduced susceptibility to grey mould, *Botrytis cinerea*. The orange, high flavonol tomatoes also display extended shelf life, although their shelf life is not as long as that of purple tomatoes. The longer shelf life of orange, high flavonol tomatoes is due to slower over ripening, but there is no decrease in their susceptibility to *B.cinerea* compared to controls. The slower over-ripening of tomatoes with high levels of polyphenols is probably due to the general antioxidant activity of anthocyanins and flavonols, which reduce the generation of reactive oxygen species late in fruit ripening and thereby may slow over-ripening directly (by reducing membrane damage and cell leakage) and indirectly (by reducing ROS-signalling. The reduced susceptibility to *B.cinerea*, appears specific to anthocyanins, but by combining mutants in anthocyanin biosynthesis with high levels of flavonoid production, we have shown that susceptibility is dependent on the antioxidant capacity of individual flavonoids, and so susceptibility is low in purple tomatoes because they accumulate high levels of delphinidin 3-o (coumaroyl) rutinoside 5-o glucoside and petunidin 3-o (coumaroyl) rutinoside 5-o glucoside, which are excellent antioxidants due to the presence of two pairs of adjacent hydroxyl groups on the B-ring of these anthocyanins. Together,the total antioxidant capacity and the specific antioxidant capacity of individual flavonoids determine the extension of shelf life in tomato, through delaying over-ripening and reducing susceptibility to *B.cinerea* respectively. Engineering antioxidant levels (both general and specific antioxidants) may offer new strategies to improve the shelf life of fleshy fruit, either by breeding or by metabolic engineering) and contribute significantly to reducing waste and improving the quality of fruit.

A MYB FACTOR, IDENTIFIED THROUGH EXPRESSION QUANTITATIVE LOCUS MAPPING, NEGATIVELY REGULATES THE PROANTHOCYANIDIN PATHWAY IN GRAPE BERRY.

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Flavonoids are plant secondary metabolites with multiple functions. In grape, the most abundant flavonoids are proanthocyanidins (PA), major quality determinants for fruit and wine. Complex PA composition in grapevine suggests a fine regulation of PA synthesis, about which little is known. The goal of this work was to identify novel regulators of this pathway using integrative approaches. Expression quantitative trait locus (eQTL) mapping was performed on transcript abundance of five downstream PA synthetic genes (VvDFR, VvLDOX, VvLAR1, VvLAR2 and VvANR) measured by Real-Time quantitative PCR on a 191-individual pseudo F1 population in two growing seasons. Twenty-one eQTL were detected. Both cis-eQTLs of large effect, corresponding probably to the gene promoters, and trans-eQTL of smaller effect, corresponding probably to regulation genes, were identified. Seventeen of them didn't overlap with known candidate transcription factors norcis-regulatory sequences. In a genomic region co-locating eQTL for VvDFR, VvLDOX and VvLAR1, a candidate gene, VvMYBC2-L1, belonging to the R2R3 MYB protein family was selected. This Myb factor was previously found to be induced by the overexpression of VvMybPA1 and VvMybPA2, two transcription factors activating the PA pathway (Terrier et al., 2009). Phylogenetic analysis showed its high similarity to characterized negative MYB factors. Its spatio-temporal expression profile in grape is correlated to PA synthesis. Association genetics using a core-collection of 141 individuals as sample (Barnaud et al., 2006; Fournier-Level et al., 2009) revealed a significant link between a molecular polymorphism found into this gene and the variation of skin PA concentration (Carrier et al., 2013). Its functional characterisation via overexpression in grapevine hairy-root demonstrated its ability to reduce PA amount and to down-regulate expression of PA genes.

COPPER TRANSPORTERS AND GRAPE BERRY METABOLISM IN RESPONSE TO BORDEAUX MIXTURE

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Fungal diseases present a powerful threat to the productivity of grapevine and fruit and wine quality. Fungicides based on copper salts have been extensively used since the late 1800s upon the development of the 'Bordeaux mixture', a broad spectrum fungicide that consists of copper sulfate and lime. Despite being an essential element for plant health, excess copper may be a cause of toxicity, and several mechanisms such as chelation and compartmentation of metal ions have evolved to avoid negative effects at cell level while insuring their appropriate delivery within the cell compartments. Previous studies have shown that the viability of grape cells decreases with the increase in copper concentration in a dose-dependent manner and that the sequestration of the metal ion in the vacuole may constitute an effective mechanism to avoid its inherent toxicity (Martins et al., 2012). Copper transporters (COPT/Ctr) operate at the plasma membrane level and in internal membranes, such as the tonoplast. These high-affinity transporters have been mostly characterized in yeast, humans and in some plant species. In the present study, eight putative *Vitis vinifera* Copper Transporters (*VvCTrs*) were identified. *In silico* analysis of VvCTrs revealed typical features of Ctr transporters, including the presence of three transmembrane domains and several key amino acid residues reported to be essential in copper transport. The expression of each *VvCTr* was studied by qPCR throughout the season in grape berries and leaves from grapevines of a commercial vineyard treated with Bordeaux mixture or with a conventional triazol-based fungicide following regular vineyard management practices. In plants not treated with copper, transcript levels of *VvCTr1* and *VvCTr8* were the most abundant in leaves and berries, respectively, suggesting a preponderant role in copper homeostasis. *VvCTrs 4*, *5* and *7* were the least expressed genes in both organs. The application of copper resulted in differential regulation of the *VvCTrs* in grapes and leaves, depending on the plant developmental stage. Atomic spectrometry showed that copper content in the berries from grapevines treated with Bordeaux mixture was 7 to 14-fold higher than in control fruits and decreased from the green phase onwards. The highly complex expression pattern of *VvCTr* genes and their specific regulation by copper highlight the intricate metabolic pathways involved in copper homeostasis of the grapevine. Furthermore, the localization of VvCTr1 was assessed in plant cells after transient transformation with fusion proteins of VvCtr1 with GFP and RFP. *VvCTr1* successfully restored the growth-defect of yeast mutants lacking Ctr transporters, validating its function as a copper transporter and giving insights on its contribution for copper mobilization within the cell. A metabolomics approach showed that copper application in the vineyard impacts in particular N metabolism in the grape berry and leaves.

THE COMPLEXITY OF ETHYLENE RESPONSE DURING FRUIT RIPENING IN APPLES

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In fleshy fruit species that have a strong requirement for ethylene to ripen, ethylene is synthesised auto-catalytically, producing increasing concentrations as the fruit ripen. Suppression of the ethylene biosynthesis gene, ACC OXIDASE 1 (*ACO1*) in apples, stops autocatalytic ethylene production at fruit maturation. Using these apples, applied with different concentrations of ethylene, we have shown ripening traits are controlled in an ethylene sensitivity-dependency manner. Some early traits such as starch breakdown have a high sensitivity to ethylene, but low dependency, while others such as flesh softening have a lower sensitivity, yet high dependency on ethylene. Further investigation of this model suggests that apple fruit responds to ethylene in a dose-by-time mechanism, with sustained lower ethylene concentrations giving the same response as shorter periods of high ethylene. Focussing on fruit softening, at the molecular level, high levels of ethylene (>100 $uL.L^{-1}$) causes multiple waves of cell wall gene expression. This contrasts to expression patterns observed in receptor genes, which are rapidly and consistently up regulated by these concentrations. At lower ethylene concentrations (>1 $uL.L^{-1}$) the expression patterns become more variable, especially with early response waves. This data suggests multiple levels of transcriptional control during fruit ripening that are carefully orchestrated to a set program of ripening steps.

REGULATION OF CUTICULAR LIPIDS BIOSYNTHESIS AND ITS INTEGRATION WITH EPIDERMAL CELL DIFFERENTIATION AND ORGAN FORMATION IN TOMATO FRUIT

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The biosynthesis of cuticular waxes, particularly in the model species Arabidopsis, has been explored for many years by this time. Pathways associated with formation of cutin and waxes, the major cuticular constituents, were identified to a certain extent and involvement of specific genes and enzymes was established. Yet, many open questions remain with respect to the regulatory network controlling these pathways under diverse environmental conditions. Moreover, formation of the cuticle is expected to be an integral part of the wider process of aerial organ formation and this was not investigated to date. Indeed, many cuticular mutants examined till now displayed clear epidermis cell differentiation phenotypes. In the past years we have investigated the involvement of MIXTA, an R2-R3 MYB-type transcription factor known to play a key role in epidermis cell differentiation, in formation of the cuticle. We first showed that in tomato, *MIXTA* (*SlMIXTA*) displays a fruit skin/peel enriched expression profile. Analysis of *SlMIXTA* RNAi and over-expressing tomato lines showed clear phenotypic changes, particularly those related to epidermal cell shape in developing tomato fruit. Additionally, histological, chemical and transcriptomic analysis showed dramatic changes to cuticle biosynthesis suggesting that *SlMIXTA* might be a major transcriptional regulator of cutin biosynthesis. Putative target genes of SlMIXTA (SlCYP86A68, SlCYP77A1 and SlCYP77A2) were functionally characterised and demonstrated to perform crucial roles in cutin monomer biosynthesis. The results suggest that SlMIXTA is a positive regulator of conical epidermal cell shape and cuticle development in tomato fruit. This work highlights and an interesting and significant association between cuticle biosynthesis and epidermal cell differentiation mediated by the MIXTA transcription factor.

ARGININE BIOSYNTHESIS DURING DEVELOPMENT AND RIPENING OF TOMATO (*Solanum lycopersicum*) FRUIT

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Arginine is an important amino acid for human nutrition. Arginine is an essential aminoacid for the fetus and the neonate, as well as for adults under conditions. In addition, there is increasing evidence for a role of arginine in wound healing, in the response of the immune system and other pathophysiological phenomena including cancer. In plants, arginine and arginine metabolism are of great importance. Apart from participating in the biosynthesis of proteins, arginine serves as an important nitrogen source in seed storage proteins as well as a free aminoacid in germinating seeds. Furthermore, an intermediate metabolite in the arginine anabolic pathway, citrulline,, has been proven to be an important form of transported nitrogen.

In Kalamaki et al. 2009, the tomato *SlNAGS* gene, the first step in the arginine biosynthesis pathway, was cloned and introduced into *Arabidopsis* plants under a constitutive *35S* promoter, resulting in plants with higher levels of ornithine. These transgenic *Arabidopsis* plants exhibited increased drought and salt tolerance, revealing a role for arginine biosynthesis in stress responses.

Here, we report the identification and cloning of genes involved in the arginine biosynthesis pathway in tomato (*Solanum lycopersicum*). We used the tomato annotated genome to identify genes with high sequence homology to the *Arabidopsis* genes and to genes from other species. The expression levels of these genes were investigated in several developmental stages of the ripening tomato fruit. In addition, the tomato *SlNAGS* gene was cloned and introduced into the tomato genome with stable transformation under the control of the fruit specific polygalacturonase (PG) promoter. The data show that PG promoter regulates an increase of the *SiNAGS* expression levels as the tomato fruit matures. A combination of transcriptomic and metabolomic approach will be used to compare the *PG::SlNAGS* transgenic lines with the wild type, in order to identify possible bottlenecks in the arginine biosynthesis pathway. Elucidating the arginine biosynthesis pathway, as well as its possible bottlenecks, will allow us to identify possible candidate genes in our efforts to achieve higher arginine levels in the tomato fruit and eventually producing molecular markers that will be used in our tomato breeding project.

Kalamaki, et al., 2009. Over-expression of a tomato N-acetyl-glutamate synthase gene in *Arabidopsis thaliana* results in increased tolerance in salt and drought stresses. J. Exp. Bot. 60: 1859-1871.

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MICRO-TOM MUTANTS FOR IDENTIFICATION OF TARGET GENES CONTROLLING FRUIT GROWTH IN TOMATO.

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Mechanisms involved in fruit size/growth are difficult to unravel because of complex interactions between cell specification, polarity, asymmetric division, rearrangement and growth. In the recent years, genomic approaches including transcriptome, proteome and metabolome analyses have produced a wealth of candidate genes possibly involved in the control of biological processes or traits of interest, such as fruit size. Following their discovery, the relationships between the candidate genes identified and the processes or traits of interest must be confirmed, and gene function needs to be assed. Forward genetics appears as the most powerful approach for the identification of new gene functions and mutant collections offer invaluable resources for discovering new phenotypes and new allelic variants. Thanks to the recent availability of tomato genomic sequence and the current availability of deep sequencing tools, linking genotypic variations to associated phenotypic changes is now more accessible. We generated highly-mutagenized tomato EMS mutant resources using the miniature tomato cultivar Micro-Tom for genetics approaches in tomato. This collection has been used, in part, for identifying novel fruit size mutants in tomato and, last, give some hints on how current deep sequencing technologies may be used for identifying unknown mutations responsible for phenotypic changes in Micro-Tom mutants. Towards this end, a Micro-Tom EMS mutant collection of 3500 phenotyped lines available at INRA Bordeaux was screened for fruit size (small or large fruit) and pericarp thickness (thick or thin) mutants. Among these, 36 mutant lines were selected (21 fruit size mutants, 15 pericarp thickness mutants). Following confirmation of the phenotype, 20 mutant lines were submitted to detailed fruit developmental analysis in view of identifying fruit growth processes altered in these mutants. The results illustrate how screening mutant collections, which is relatively straightforward once the initial characterization of the mutant collection and the construction of the database have been done, can successfully contribute to the isolation of a large number of new fruit size/growth mutants and thus provide new genetic material for deciphering the mechanisms involved in the control of fruit growth and size.

SESSION 3: “Molecular breeding for fruit quality traits”

CAROTENOID METABOLISM IN DEVELOPING AND RIPENING BERRIES: BUILDING UP AND BREAKING DOWN.

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Terpenes are a large group of organic compounds that are involved in diverse functions in plants that range from defense responses to phytohormone production. Carotenoids are C_{40}-tetraterpenes and their metabolism in plants and fruits has received much attention. From a human perspective carotenoids are an important source of antioxidants and serve as vitamin A (retinol) precursors in our diet. From a plant perspective, they fulfill crucial roles in photosynthesis (i.e. light harvesting and photo-protection). Similarly, carotenoid catabolism (via carotenoid cleavage) has received much attention due to the production of the phytohormones ABA and strigolactone; as well as the formation of norisoprenoids, impact flavour and aroma compounds in a number of commercially important fruits and flowers. The carotenoid metabolic pathway is well characterised in plants and the orthologues of the pathway members have been identified in the grapevine genome and selected enzymes characterised[1]. The availability of the grapevine genome and associated tools such as transcriptional and metabolite profiling, facilitated a carotenoid pathway analysis, using berries from a model (highly characterised) Sauvignon blanc vineyard. Methods have been optimised for profiling the carotenoids (carotenes and xanthophylls) and chlorophylls[2]; as well as the flavor and aroma related volatile apocarotenoids[3]. During berry development in grapevine, the major carotenoids (i.e. β-carotene and lutein) decrease in a developmental pattern that closely mirrors chlorophyll decline. Specific carotenoids (especially the xanthophylls) were shown to increase during development depending on the environmental conditions. Carotenoid degradation via enzymatic cleavage is catalysed by the carotenoid cleavage dioxygenases (CCDs). Grapevine possesses orthogoues for VvCCD1 and VvCCD4 (involved in norisoprenoid production), VvCCD7 and VvCCD8 (involved in strigolactone formation) and NCEDs (involved in ABA production). A transcriptomic analysis of Sauvignon blanc berries harvested in three stages of development showed that VvNCED3 expression corresponded with the sudden increase in ABA levels in ripening berries[1]. The expression profile, functional analysis and substrate specificity of VvCCD1, VvCCD4a and b are providing important clues to their potential *in planta* roles in berries. The transcriptomic profiling, as well as the pigment and apocarotenoid volatile profiling from the same samples during berry development and ripening allow the possibility to evaluate carotenoid metabolism in a holistic manner. The results obtained for grapevine fruits will be discussed with reference to what is known in tomato and other fruits.

THE USE OF METABOLOMICS IN PATHWAY ELUCIDATION, METABOLIC ENGINEERING AND BREEDING.

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The maturation and development of tomato fruit has received much attention due both to the complexity and intricacy of the changes which occur during this process and due to the importance of these fruits as a component of the human diet. Whilst great advances have been made in understanding molecular genetic aspects of fruit development our knowledge concerning the metabolic shifts underpinning this process remains largely confined to primary metabolism. Conversely, the majority of the metabolites considered to have health benefits are secondary or specialised metabolites. Here, I will review advances in tomato primary and secondary metabolism focussing on the use of metabolomics strategies and where applicable the enabling of these strategies by their coupling to information resident in the tomato genome sequence in order to define metabolite pathway structure and its regulation. The use of wide breeding populations will be discussed with regard to the use of breeding in metabolic engineering strategies.

GENE REGULATORY NETWORKS AND QTL MAPPING IN TOMATO

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Fruit ripening is a complex developmental process. There is a major drive to encourage people to eat more fruit and vegetables because of the health benefits. Fruits and vegetables are known to promote healthy ageing by protecting against heart disease, osteoporosis and certain cancers. The Government estimates that the number of elderly people in the UK will be around 19 million by 2050. Healthy ageing is vital unless health services are to be completely overwhelmed by increasing costs of care. A major challenge is to produce better quality fruit products, but manage costs and reduce postharvest waste. To achieve these goals requires significant control over the ripening process. Two approaches we are using at Nottingham to identify key genes controlling ripening will be described, (1) FruitNet is a novel gene regulatory network utilizing an intuitive point-and-click interface comprising the expression profiles of several 1000 genes associated with different stages of the ripening tomato. It provides a novel tool for the identification of new and important ripening-related genes and a foundation for the analysis of the 'molecular circuits' that control this complex biological process. (2) The tomato genome sequence has allowed us to identify candidate genes under QTL for fruit texture and colour. Progress is described on the identification of texture related genes and their utility in commercial lines. A major goal now is to use our gene regulatory networks to allow rapid identification of QTL candidate genes.

THE HIGH POLYPHENOL CONTENT OF VITIS VINIFERA CV. TANNAT BERRIES IS CONFERRED MOSTLY BY THE PRIVATE AND DISPENSABLE PORTION OF THE GENOME.

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The grapevine (Vitis vinifera) variety Tannat is cultivated mainly in Uruguay for the production of high-quality red wines. Tannat berries have unusually high levels of polyphenolic compounds, producing wines with an intense purple color and remarkable antioxidant properties. We investigated the genetic basis of these important characteristics by sequencing the genome of the Uruguayan Tannat clone UY11 to 134x coverage using Illumina technology, followed by a mixture of de novo assembly and iterative mapping onto the PN40024 reference genome. RNA-Seq data were processed using a combination of reference-guided annotation and de novo transcript assembly, allowing 3673 previously uncharacterized genes to be defined and resulting in the discovery of 2228 genes that were not shared with the reference genome and could therefore be considered as private to the Tannat variety. Expression analysis showed that the private genes contributed substantially (up to 50%) to the overall expression of enzymes involved in the synthesis of phenolic and polyphenolic compounds. The dispensable portion of the grapevine genome therefore contains many private genes that contribute to the unique characteristics of the Tannat berries and the health-promoting properties of the resulting wines.

THE PLASTICITY OF GRAPEVINE BERRY TRANSCRIPTOME

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Phenotypic plasticity refers to the range of phenotypes a single genotype can express as a function of its environment. We investigated phenotypic plasticity in grapevine by comparing the berry transcriptome in a single clone of the vegetatively-propagated common grapevine species *Vitis vinifera* (cv Corvina) through three consecutive vintages cultivated in 11 different vineyards in the Verona area of Italy. Most of the berry transcriptome clustered by vintage rather than common environmental conditions or viticulture practices, and transcripts related to secondary metabolism showed high sensitivity towards different climates, as confirmed also by metabolomic data obtained from the same samples. When compared 11 vineyards during one harvesting season, finding that the environmentally-sensitive berry transcriptome comprised 5% of all protein-coding genes and 18% of transcripts modulated during berry development. Specific plastic transcripts were associated mainly with transcription factors, translation, transport, secondary metabolism and stress boxes, and plastic transcriptome reprograming was more intense in the vintage characterized by extreme weather conditions. We also identified a set of genes that lacked plasticity, showing either constitutive expression or similar modulation in all berries. Our data provide the first step towards the characterization of grapevine transcriptome plasticity under different agricultural systems.

GENETICAL GENOMIC APPROACHES TO UNRAVEL AND RECONSTITUTE VOLATILE PRODUCTION IN FRUIT.

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Volatile compounds are produced by fruits as an strategy to atract seed dispersors and therefore contribute to fruit organoleptic quality. Variability in the volatile chemical composition is responsible for the wide variety of aroma and flavours exhibited by fruits. Despite of that modern breeding has resulted in fruit with low volatile profile and therefore low organolepetic quality. The reasons for that are many but the complexity of the traits, and our lack of understanding the molecular mechanisms underlying volatile production in fruit has contributed in part to the loss of quality. Fortunately a combination of different strategies including QTL mapping, candidate gene and biochemical approaches has changed this situation in the last 10 years and we are now better positioned to use the available genetic variability and to include volatile composition in the fruit quality breeding programs. We have been using recombinant inbred and introgression lines representing the whole genome of selected wild relatives in cultivated tomatoes and segregating populations of strawberry and peach to identify volatile QTLs. Our results indicated novel vol QTLs across species and some of the QTLs could be confirmed in derived ILs for specific volatiles. We have also been using a combination of other genomic approaches such as differential gene expression of lines with extreme levels for specific volatiles to identify candidate genes in volatile production or metabolism. Although some of the volatile QTLs colocalize in genes involved in volatile production this is not always the case and some may be involved in volatile metabolism by conjugation to produce compounds that either non volatile or with different odor-hreshold or description. I will exemplify some of the progress obtained for the cases of strawberry, peach and tomato.

REGULATION OF TOMATO ASCORBATE LEVELS: A CANDIDATE GENE INVOLVED IN FRUIT NUTRITIONAL VALUE AND STRESS TOLERANCE.

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Ascorbate is a powerful antioxidant in plants, and its levels are an important quality criterion in commercial species. Factors influencing these levels include environmental variations, particularly light, and the genetic control of its biosynthesis, recycling and degradation. Our previous work has identified QTL for fruit vitamin C content and a candidate gene, encoding a monodehydroascorbate reductase (MDHAR) isoform, an enzyme catalysing reduction of the oxidized radical of ascorbate, monodehydroascorbate, to ascorbate. The activity of this enzyme is also correlated with fruit tolerance to post-harvest chilling stress in introgression lines containing the vitamin C QTL. In order to understand the role of this gene in fruit physiology and metabolism we produced transgenic lines in different genetic backgrounds. We show that the isoform encodes a cytosolic and peroxisomal MDHAR, and that this enzyme negatively regulates ascorbate levels in certain genotypes of *Solanum lycopersicum* (tomato). Transgenic lines overexpressing MDHAR show a decrease in ascorbate levels in leaves, whereas lines where MDHAR is silenced show an increase in these levels in both fruit and leaves. Furthermore, the intensity of these differences is light-dependent. The unexpected effect of this MDHAR on ascorbate levels cannot be explained by changes in the expression of Smirnoff-Wheeler pathway genes, or the activity of enzymes involved in degradation (ascorbate peroxidase) or recycling of ascorbate (dehydroascorbate reductase and glutathione reductase) suggesting a previously unidentified mechanism regulating ascorbate levels. The impact of under-expression of this gene in processing tomato on fruit post-harvest physiology has also been examined.

AVAILABILITY OF MAS FOR SEEDLESSNESS IS LIMITED IN MULTIRESISTANT TABLE GRAPE BREEDING

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Seedlessness is basic requirement in table grape breeding. In a breeding program with multiple resistance genes combined with seedlessness, the number of appropriate combinations is very low. As such, the availability of early selection is crucial. Marker assisted selection (MAS) offers an efficient tool for genotyping stenospermocarpic seedlessness in grapevines. During the last 15 years, several markers were developed for the selection of seedlessness of grapes, from which the cleaved amplified polymorphic sequence SCC8 and the simple sequence repeat p3_VvAGL11 are the two best characterized. Although these markers have been tested on a wide range of seeded and seedless genotypes, the involvement of different disease resistance sources initiated the review of the general applicability of these markers. Several seeded and seedless varieties with diverse geographical origin, further families with different genetic background for fungal disease resistance (complex hybrids of *Vitis vinifera* and North-American *Vitis* species, *Muscadinia rotundifolia* back cross, *V. vinifera* 'Kishmish vatkana') were tested with SCC8 and p3_VvAGL11 markers. The investigated genotypes are involved in a breeding program of the Institute of Viticulture and Oenology at the University of Pécs, where seedless grapes with immunity grade resistance against powdery and downy mildew, suitable for organic vine growing are bred. In the case of SCC8, the high frequency of the SCC8 null allele (which is linked to seededness) and the relatively high number recombinants among seeded resistance sources makes the applicability of the marker limited. p3_VvAGL11 is proposed to be an in-promoter marker of seedlessness located in the very close vicinity of VvAGL11, which is in turn supposed to be the best candidate for the seed development inhibitor gene (*SdI*), the main genetic determinant of seedlessness. This marker does not suffer from the drawback of null alleles. Seeded varieties however, which are recombinant between SCC8 and *SdI* (*V. vinifera* 'Katta kurgan', 'Dshandshal kara'; *Muscadinia rotundifolia* hybrids), were also recombinant between p3_VvAGL11 and *SdI*. Stenospermocarpic seedlessness is a complex trait, where seed development also depends on several minor effect genes. As a consequence, MAS based on *SdI* is prone to be less reliable by nature. Based on our results, the applicability of available markers for MAS of grapevine seedlessness is even more limited, if modern fungal resistance sources are applied.

CHARACTERIZATION OF B-CAROTENE ACCUMULATING TOMATO FRUITS REVEALS MULTI-LEVEL CONTROL OF CAROTENOIDS ON TOMATO FRUIT RIPENING.

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Tomato fruits accumulate, during ripening, the linear carotene lycopene, partially due to the transcriptional repression of the pathway leading to β-carotene and β-xanthophylls. Increasing evidence is accumulating for a role of ABA in controlling ripening of both climacteric and non-climacteric fruits. We generated transgenic tomato fruits overexpressing the *LYCOPENE BETA CYCLASE* (*LCY*) gene and accumulating β-carotene. Unexpectedly, these fruits also show enhanced shelf-life and delayed softening. Cell wall composition in ripe fruits was changed, with an increase of sugars present in the pectic backbone. LCY fruits also showed moderate changes in the levels of several metabolites, many of which can be explained in terms of delayed fruit ripening. Affymetrix microarray analyses showed changes in the expression of cell wall-remodeling enzymes, induction of ABA-regulated genes and repression of ethylene-regulated ones. In keeping with these observations, ABA and ethylene levels were, respectively, increased and decreased in LCY fruits. The transcript levels of several master regulators of fruit ripening (Non-ripening or NOR, and Ripening Inhibitor or RIN) showed strong co-regulation with ABA levels (Fig. 1). The above phenotypes were observed in several independent transgenic lines. We propose that the increased β-carotene levels affect the profile of tomato fruits at several different levels, and that one of the effectors of this action is the β-carotene metabolite, ABA.

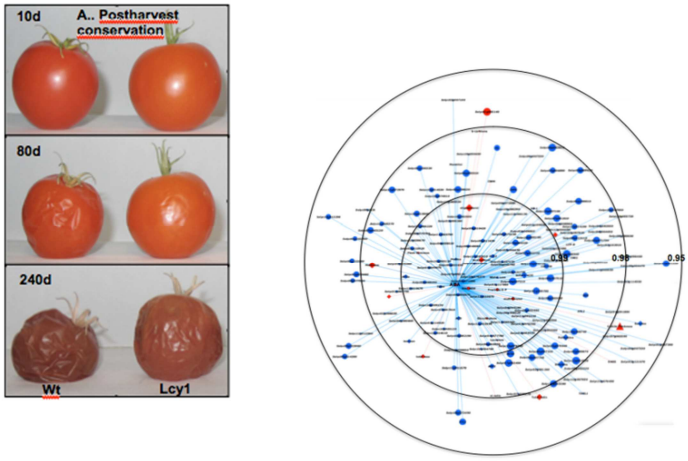

Figure1. Elongated shelf-life phenotype (left) and correlation network using ABA as central hub (right)

KINETIC MODELING OF THE ETHYLENE BIOSYNTHESIS PATHWAY DURING TOMATO FRUIT DEVELOPMENT AND RIPENING.

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To better understand the regulation of ethylene biosynthesis, a targeted systems biology study of tomato (*Solanum lycopersicum* L.) fruit ripening was conducted. A kinetic model was developed describing the dynamics of the entire ethylene biosynthesis pathway and the first step of the Yang cycle. The kinetic model consists of ordinary differential equations describing the transfer of genetic information into protein abundance and their corresponding metabolic activity. This approach requires only gene expression data, to describe the behavior of all downstream components. Model parameters were estimated from measured data. The model was able to describe the rate of change of all important enzymes and related metabolites during tomato fruit development, climacteric ripening and postharvest storage with high accuracy. The model explained 85 % of the variability and it was further analyzed using a Monte Carlo analysis and validated against various ethylene biosynthesis mutants. The model was used to evaluate in silico the importance of the biological inactive derivate 1-(malonylamino)cyclopropane-1-carboxylic acid (MACC). Altering the rate constant of MACC formation and the possible reverse reaction (MACC hydrolysis), showed that MACC is an essential metabolite that regulates the pool of 1-aminocyclopropane-1-carboxylic acid (ACC) and consequently ethylene production.

EXPLOITING WILD RELATIVES OF *S. lycopersicum* FOR TOMATO QUALITY IMPROVEMENT.

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Commercial tomato, *Solanum lycopersicum*, is the most domesticated and studied species from the *Lycopersicon* group within the *Solanum* genus (Grandillo et al., 2011). Still, *S. lycopersicum* only represents ~4.5% of the total genetic diversity within *Solanum* species (Miller & Tanskley, 1990). Knowledge about flavour and nutritional value in wild germplasm can result in variation that could lead to an expansion of quality traits to be further incorporated into breeding programs (Jenks and Bebeli, 2009; Causse et al., 2010). In the application of molecular breeding, genotyping has been a major bottle neck for a long time. Although several marker systems have been developed and applied, most of them fall short in the genomics era, either by lack of reproducibility or by being too low throughput (Agarwal et al., 2008). This is radically changing as a result of the genome (re)sequencing projects that are carried out and delivering large numbers of informative markers as single nucleotide polymorphisms (SNP). We developed a 5528 SNP array platform and used it to genotype various cultivated tomatoes, landraces, wild relatives and several mapping populations (Víquez-Zamora et al, 2013). This large-scale genotyping platform allowed the visualization of polymorphisms, recombination patterns and the identification of trait loci among different populations. We are currently applying high throughput genotyping and metabolomics platforms to characterize the genetics of fruit quality traits in a Recombinant Inbred Line (RIL) population between *Solanum lycopersicum* cv Moneymaker and *Solanum pimpinellifolium*. Three different metabolomic platforms were used to analyse the RIL population: Liquid chromatography (LC) mass spectrometry (MS) to detect semi-polar compounds such as flavonoids, alkaloids, phenylpropanoids, saponins, phenolic acids, polyamines and products thereof (De Vos et al., 2007), gas chromatography (GC) coupled with to electron impact time of flight (TOF)-MS for detection of primary metabolites (Lisec et al., 2006) and solid phase microextraction (SPME)-GC-MS for the analysis of volatiles (Tikunov et al., 2005). Using this combination of genomics and metabolomics approaches, we obtained an overview of metabolite quantitative trait loci (mQTLs) in this population. Although most mQTLs were dispersed over the genome, we identified metabolic hot-spots particularly on chromosomes 1 and 10. These results provide valuable insight in the genetics of fruit quality traits and may aid to breeding strategies aimed at improving fruit quality traits.

MODEL-ASSISTED COMPARATIVE ANALYSIS OF SUGAR ACCUMULATION IN FLESHY FRUITS: GRAPE, TOMATO, AND PEACH

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Sugars are one of the key determinants for fruit quality by determining sweetness in fresh fruits and alcohol content in processed products (e.g. wine). Despite considerable divergences in sugar concentration among fruit species, sugar accumulation in a fruit is a result of the complex interplay among sugar import, sugar metabolism, and water dilution. Therefore, multispecies comparison would help to identify common and/or species-specific modes of regulation in sugar accumulation. A mechanistic-based model that can simulate the dynamic sugar accumulation over berry development was used to compare the sugar accumulation in three fruits: grape, tomato and peach. We collected representative datasets of the temporal sugar accumulation over fruit development, composing of 104 combinations of species (3), genotypes (32), and growing conditions (19 years and 16 trophic and environmental treatments). At maturity, grape showed the highest sugar concentrations (16.5-26.3 g /100 g FW), followed by peach (2.2 to 20 g /100 g FW) and tomato (1.4 to 5 g /100 g FW). The models previously developed for one species were not able to satisfactorily simulate sugar accumulation in the other species. Therefore, the existing models were generalized by identifying a novel and generic function of sugar metabolism, which rebuilt a single model to accurately simulate all the 104 sugar accumulation profiles. In the novel generic function, there are two parameters, *k1* and *k2*, describing the utilization of imported carbon for synthesizing non-sugar compounds. A global sensitivity analysis on the two parameters revealed that *k1* is more influential than *k2* on sugar concentration. Model selection analysis showed that *k1* and *k2* are stable for a given genotype under various growth conditions, suggesting *k1* and *k2* are genotype-dependent and environment independent. Analyses on the three main processes determining sugar accumulation in the model, namely the sugar import, sugar metabolism and water dilution, uncovered that the difference of sugar concentration mainly results from the distinct sugar metabolism in the three fruit species, while sugar import and water dilution are comparable among the three fruits.

SESSION 4: “COST FA1106 STSM highlights”

RIPENING INCEPTION AND QUALITY PARAMETERS OF CABERNET SAUVIGNON GRAPE BERRIES ARE DIFFERENTIALLY AFFECTED BY GRAPEVINE ROOTSTOCKS.

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Berry ripening evolution is affected by grapevine rootstocks, but mechanisms relying on this effect are poorly understood. The onset of ripening (véraison) is regulated by a complex network of mobile signals among which hormones (auxin, ethylene, Abscissic acid and brassinosteroids) as well as by microRNAs (miRNA) play a pivotal role. In this study, the effect of 1103P and M4 rootstocks on Cabernet Sauvignon (CS) berry development was monitored at different phenological stages, from pre-véraison to harvest. Physical (volume, diameter), biochemical (brix°) and colorimetricparameters, showed that M4 induce an acceleration of ripening evolution of CS berries in comparison to thatobserved in CS/1103P berries. This behavior is paralleled by significant differences in transcripts and miRNas profiles, detected at pre-véraison, véraison and harvest stagesin CS/1103P and CS/M4 berries. In order to cluster together genes and miRNA with the same behavior, a time course cluster analysis using the Mfuzz R package was performed on Differentially Expressed Genes (DEGs) and miRNA. This analysis allowed us to identify six and seven clusters for the DEGs with different kinetic in skin and pulp, respectively, and six clusters for differentially expressed miRNA in both skin and pulp. In order to shed light about differences in CS/M4 and CS/1103P ripening regulation mediated by miRNA, differentially expressed miRNA and the respective target genes were identified in both pulp and skin clusters. In skin tissues, genes that showed an opposite behavior in comparison to the respective miRNA were related to metabolic categories belonging to plant growth, transport and detoxification. On the other hand, in pulp tissue genes which seem to show a control mediated by miRNA belonged to growth regulation, auxin transcription factors, Homeobox-leucine zipper transcription factors and detoxification metabolic categories. Considering the large numbers of DEG related to auxin transcription factors identified in the grape berries, a characterization of grape ARF and AUX/IAA gene families and the consequent association with the gene expression data was carried out. Among the genes belonging to these families, were identified thosewith a statistically significanttranscripts expression. Expressionanalysis showed that, except in pré-veraison stage, auxin metabolisms were generally repressed in CS/M4, in comparison to CS/1103P berries. In addition, miRNA-seq analysis highlighted a role in the regulation of ripening of miR160 and miR167,which respectively have ARF10 and ARF8 as target, suggesting the impact of rootstocks on ripening is due to the combination of different transcriptional regulators.

CHARACTERIZATION OF TWO NEW "*Y-LIKE*" MUTANTS IN TOMATO FLESHY FRUIT.

Fernandez-Moreno Josefina-Patricia[1,2]; Hen Avivi Shelly[2]; Lashbrooke Justin[2]; Tzfadia Oren[2]; Rogachev Ilana[2]; Meir Sagit[2]; Ariizumi Tohru[3]; Granell Antonio[1]; Aharoni Asaph[2].

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The phenylpropanoid represents a diverse group of secondary metabolites with health-promoting properties desirable in genetic improvement programs. The phenylpropanoid pathway in tomato fleshy fruit is well known at structural gene level, however not much is known at regulation level. The transcription factor *SlMYB12* is accepted as the key regulator of the phenylpropanoid pathway, but how operate and if it's doing it alone or together with other transcription factor still is unclear in tomato fleshy fruit. Two different mutant lines with a characteristic pink fruit phenotype were recently identified in an EMS mutant population generated in the cultivar MicroTom (see TOMATOMA DB). As described previously for the "*y*" mutant, the pink phenotype in both "*y-like*" mutants was due to the lack of the yellow pigment in the fruit skin. Metabolic analysis by the use of high resolution mass spectrometry technology (UPLC-QTOF-MS) revealed that both "*y-like*" mutants exhibited a similar metabolic profile, largely resembling the one observed in "*y*". Naringenin and Naringenin chalcone were downregulated in both "*y-like*" mutants as expected from a pink fruit phenotype. Furthermore, the metabolic differences found in both "*y-like*" mutants included changes to dihydroflavonols, flavonols and hydroxycinnamic acids. Gene expression analysis by new generation sequencing technology (RNAseq) revealed a high similarity in expression changes between both "*y-like*" mutants. Structural genes of the flavonoid pathway as well as regulatory genes were altered in the mutants. Surprisingly, *SlMYB12* displayed a normal expression level in both "*y-like*" mutants. Furthermore, the *SlMYB12* gene sequence in both mutants was truncated downstream to exon-3, missing the last exon of the gene. Taken together, these results suggest that: (i) both "*y-like*" mutants could be only new one allele of the same gene, *SlMYB12*; (ii) the lack of yellow skin pigmentation is not due to reduced expression of *SlMYB12* gene expression; and (iii) the missing region in *SlMYB12* of both "*y-like*" mutants could be responsible of the pink phenotype by producing a truncated version of the protein.

ABSTRACTS

OF

POSTER PRESENTATIONS

POSTER SESSION 1

P1.

SIMULTANEOUS TRANSCRIPTOME ANALYSIS OF THE FUNGAL PATHOGEN *COLLETOTRICHUM GLOEOSPORIOIDES* AND TOMATO FRUITS RESPONSE AT DIFFERENT STAGES OF PATHOGENICITY.

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Colletotrichum gloeosporioides is widely distributed fungus that causes the economically significant anthracnose fruit disease. After *C. gloeosporioides* breach fruit cuticle by appressoria formation it remains quiescent until fruit ripening, at which time a necrotrophic infection will ensue. Here we simultaneously characterized the fungal transcriptome and the tomato fruit response to each of the fungal colonization stages, ie, appressoria formation, quiescent stage and necrotrophic colonization. Transcriptome analysis of the appressoria stage prior to fungal penetration through the fruit cuticle showed activation of pathways involving glycerol accumulation, melanin biosynthesis, cAMP, MAPK kinase, TCA cycle, and glycerolipids. In the initial stages of appressoria formation the fruit tissue responds in a manner similar to that found for the latter quiescent stage; including activation of the hormone JA, ethylene and ABA pathways and phenylalanine pathway that lead to phytoalexins together with enhanced lipid-turnover. After *C. gloeosporioides* penetration of the cuticle two distinct quiescent structures become evident; dendritic-like structure which develop in the fruit cuticle and swollen hyphae that appear within epidermal cells. The quiescent stage possesses a large degree of active cuticle degrading enzymes and appears to undergo chromatin remodeling, possibly to facilitate dormancy until fruits ripen. Upon fruit ripening the fungus activates an arsenal of pathogenicity factors including proteases and cell wall degrading enzymes. The fruit in turn appears to activate chiefly salicylic acid mediated responses including respiratory burst, cell wall thickening, carbohydrate metabolism and regulation of plant cell death. Thus, using RNA-seq we were able to simultaneously characterize *C. gloeosporioides* and tomato fruit transcriptome regulation during different stages of colonization and thus gain insight into novel host and pathogen interactions.

P2.

LON3, A KEY ORGANELLE PROTEASE PLAYER DURING POLLINATION.

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Protein quality control and turnover is essential for cellular homeostasis. In plant organelles this biological process is predominantly performed by ATP-dependent proteases. Arabidopsis has four genes encoding for Lon proteases. They contain a Serine (S)-Lysine (K) catalytic dyad and, like their bacterial and eukaryotic homologs, may have combinatorial proteolytic and chaperone-like activities. Lon1, Lon2 and Lon4 genes have been thoroughly studied. Lon3 (At3g05780) protein function is unknown. Lon3 was considered a pseudogene solely based on the absence of transcripts in RNA samples extracted from different tissues or environmental conditions. Nevertheless, bioinformatics analysis through public available microarrays showed that Lon3 gene is predominantly expressed in sperm cells. Indeed, transcript analysis of Lon3 gene revealed its exclusive expression in mature pollen grain. Lon3 preserves an open reading frame with an in silico deduced cDNA smaller by one exon compared with Lon4. The deduced Lon3 polypeptide is predicted to have an ambiguous presequence as Lon4. Bioinformatics analysis showed that Lon3 and Lon4 are duplicated genes in a *head*-to-*tail* orientation and their evolutional status in reference to Lon1 gene remains elusive. This protease is the only member of the Lon gene family with a potential nuclear localization signal. This amino acid signature motif has been deleted from the other three mebers. Three-dimensional reconstruction modeling demonstrates that the highly ordered α-helical structures of Lon3 SSD (**S**ensor and **S**ubstrate **D**iscrimination) domain are highly contrasted to that of Lon1 and Lon4. Further analysis using mutant characterization and a combinatorial approach is required to decipher the molecular role of Lon3 protease in mitochondria/nuclei function during pollination.

P3.

CHANGES IN TABLE GRAPES TRANSCRIPTOME AT TWO RIPENING STAGES IN RESPONSE TO LOW TEMPERATURE AND HIGH CO_2 LEVELS.

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Table grapes (*Vitis vinifera* Cv. Cardinal) are highly perishable and their quality deteriorates during postharvest storage at low temperature due mainly to sensitivity to fungal decay and desiccation of stems and pedicels. The application of a 3-day CO_2 treatment (20% CO_2+20% O_2+60% N_2) at 0ºC reduced total decay and retained fruit quality in early and late-harvested table grapes during postharvest. To gain a better understanding of the molecular mechanisms involved in the responses of grapes to low temperature and high CO_2 levels, we performed a genome-wide transcriptional profiling analysis of RNA isolated from the skin of CO_2-treated and non-treated 'Cardinal' berries at two ripening stages using a custom-made Affymetrix GeneChip®. The Principal Components Analysis (PCA) showed that most of the samples were separated based on the first principal component (PC1), which accounted for 50.17 % of the variance in the dataset. Moreover, with the exception of the 3 days air sample, separation seems to be related with the stage of ripening (early vs. late-harvested). Significance Analysis of Microarrays (SAM) revealed that the transcriptome of 3-day CO_2-samples was affected to a lesser extent than non-treated samples however the ripening stage. Functional enrichment analysis indicated ERF transcription factors to take part in the regulation of CO_2-induced processes that participate in the maintenance of fruit quality. We have focused on five ERFs from the gene family of plant-specific DNA-binding (GCC box) factors, analyzing their changes in gene expression in fruit and non-fruit tissues of grapes.

P4.

STORAGE POTENTIAL OF LONG-SHELF LIFE TOMATO CULTIVARS AS AFFECTED BY TEMPERATURE AND 1-MCP TREATMENT

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Research Institute of Horticulture, Skierniewice, Poland

The aim of experiments conducted was the comparison of storage potential of three cv. Strada F_1, Zafiro F_1 and Habana F_1 of tomatoes with long shelf life (LSL) gene grown in field conditions. Fruits were harvested at mature-green stage and stored at temperature 12.5°C and 20°C and relative humidity 85-90% in normal air. Tomato fruits of Habana F_1 were treated with 1.0 $\mu l \cdot L^{-1}$ or 2.0 $\mu l \cdot L^{-1}$ of 1-MCP for 21 h at 20ºC. Firmness, weight loss, postharvest maturation , ripening, market value and storage potential of tomato fruit were determined after 4 weeks. The results of the experiments showed some marked differences in ripening rate, fruit firmness and storage potential depending on cultivar, 1-MCP treatment and storage temperature. Tested cultivars were characterized with high firmness and compression strength due to presence of gene that delays maturation time (days to breaker). Treatment of Habana F_1 cultivar with 1-MCP was found to be beneficial for firmness and compression strength during storage in comparison with untreated fruits of other cultivars. The highest firmness and the greatest compression strength was recorded for fruits of Habana F_1 treated with 1-MCP at 2.0 $\mu l \cdot L^{-1}$ and stored at temperature of 12.5 ºC, while the lowest firmness and the greatest compression strength was found for untreated cultivars, specially stored at 20 ºC. Maturation time (days to breaker) and subsequent ripening (days from breaker to table ripe) was affected by 1-MCP treatment, cultivar and storage temperature. Treatment with 1-MCP and storage at 12.5 ºC extended the maturation time near double as compared to the rate of maturation of other cultivars. It was found that 1-MCP treatment inhibited tomato ripening as they reached table ripe stage after 40 days, while untreated after 21-33 days at 12.5°C. Habana F_1 (LSL) fruits treated with 1-MCP at 1.0 $\mu l \cdot L^{-}1$ had the best storage potential (still marketable after 52 days), good market value and the lowest weight losses as held at12.5^{o}C.

P5.

REGULATION OF CUTICULAR LIPIDS BIOSYNTHESIS AND ITS INTEGRATION WITH EPIDERMAL CELL DIFFERENTIATION AND ORGAN FORMATION IN TOMATO FRUIT.

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The biosynthesis of cuticular waxes, particularly in the model species Arabidopsis, has been explored for many years by this time. Pathways associated with formation of cutin and waxes, the major cuticular constituents, were identified to a certain extent and involvement of specific genes and enzymes was established. Yet, many open questions remain with respect to the regulatory network controlling these pathways under diverse environmental conditions. Moreover, formation of the cuticle is expected to be an integral part of the wider process of aerial organ formation and this was not investigated to date. Indeed, many cuticular mutants examined till now displayed clear epidermis cell differentiation phenotypes. In the past years we have investigated the involvement of MIXTA, an R2-R3 MYB-type transcription factor known to play a key role in epidermis cell differentiation, in formation of the cuticle. We first showed that in tomato, *MIXTA* (*SlMIXTA*) displays a fruit skin/peel enriched expression profile. Analysis of *SlMIXTA* RNAi and over-expressing tomato lines showed clear phenotypic changes, particularly those related to epidermal cell shape in developing tomato fruit. Additionally, histological, chemical and transcriptomic analysis showed dramatic changes to cuticle biosynthesis suggesting that *SlMIXTA* might be a major transcriptional regulator of cutin biosynthesis. Putative target genes of SlMIXTA (SlCYP86A68, SlCYP77A1 and SlCYP77A2) were functionally characterised and demonstrated to perform crucial roles in cutin monomer biosynthesis. The results suggest that SlMIXTA is a positive regulator of conical epidermal cell shape and cuticle development in tomato fruit. This work highlights and an interesting and significant association between cuticle biosynthesis and epidermal cell differentiation mediated by the MIXTA transcription factor.

P6.

GROWTH AND PROTEOMIC ANALYSIS OF TOMATO FRUITS IN WILD TYPE AND *FLACCA* MUTANT

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To better understand the metabolic regulation of tomato fruit growth and the role of plant hormones ABA, the proteomic analyses in the phase of intensive growth of fruits of wild type and *flacca* mutant (ABA-deficient) was done. The phase of fruit development was determined by following fruit growth rate in the experiment conducted in controlled conditions. These results showed that lower fruit growth rate in *flacca* fruits compared to the fruits of wild type plants, resulted in significantly smaller final fruit size of mutant plants with the smaller ABA content in a pericarp. For proteomics analyses, pericarps of investigated fruits were collected in the phase of intensive cell expansion (30 days post-anthesis). Phenol method was used for protein extraction, while proteins were separated by two-dimensional gel electrophoresis (2-DE) and analyzed by LC-MS/MS mass spectrometry. Identification was done using the SGN tomato unigene database. Proteins were classified according to their function in several categories: related to carbon metabolism, amino acid metabolism, protein translation, processing and degradation, energy metabolism, cell wall related, oxidative stress, stress defense, and other metabolic process. In total 50 proteins showed variation between two genotypes. Most proteins related to carbon, amino acid metabolism and protein translation, processing and degradation showed increased expression in wild type compared to *flacca*. These results indicate that faster metabolic flux in wild type compared to *flacca*, might be responsible for higher fruit growth rate and final bigger fruit size in wild type than in *flacca*.

P7.

BERRY TRANSCRIPTOME COMPARISON OF TEN ITALIAN GRAPEVINE VARIETIES.

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Grape berry development and maturation have been intensively studied and can be described as a succession of several physiological and biochemical changes reflecting the transcriptional modulation of many genes; nonetheless little is known about these transcriptional changes and their regulation. The aim of this research project is to understand the physiological and biochemical differences among ten Italian cultivars by analyzing and comparing the evolution of all the transcriptomes during berry development, as well as to find common transcriptomic traits. Ten varieties, five red and five white, were chosen among several hundreds of Italian varieties for their diversity in agronomical and oenological traits, and for adaptation to different growing locations. The ten varieties were all grown in the same experimental vineyard (Conegliano, Veneto region, Italy) and grape berry samples were collected during the same growing season (2011) at four different phenological stages, two pre- and two post-véraison, in triplicate. In total, 120 RNA samples were sequenced using the Illumina HighSeq technology. An average of 33.4 million single-ended reads for each sample was obtained, with a read length of 100 bases. The raw reads were aligned onto the 12X sequence of the Pinot Noir 40024 reference genome. An average of 28.5 million reads per sample was mapped, representing a mapping quality of 85.3%. At present, we are analyzing all data in order to obtain an overview of gene expression profiles during grape berry development for each variety. By comparing red or white varieties transcriptomes among themselves, and likewise red varieties transcriptomes versus the white ones at each berry growth stage, it will be possible to gain insights into the specific gene expression profiles of each variety and each colour-group. By this approach, it will be also possible to uncover commonly expressed genes in all the cultivars which will be considered as putative molecular biomarkers involved in berry development.

P8.

THE INTERACTION BETWEEN *parthenocarpic fruit* AND *Curl* INDICATES A ROLE FOR CLASS I KNOX GENES IN TOMATO FRUIT SET

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Knotted1-like homeobox (KNOX) transcription factors, which have been described as negative regulators of gibberellin (GA) biosynthesis genes, are highly expressed at anthesis in the ovary of wild-type (WT) tomato plants and transcript levels decrease after pollination. In the *parthenocarpic fruit* (*pat*) mutant, where the ovary develops into seedless fruits independently of pollination and fertilization, the expression levels of *Tomato Knotted 2* (*TKn2*/*LeT6*), the ortholog of the Arabidopsis *SHOOTMERISTEMLESS* gene, decrease prior to anthesis. These findings indicate that members of the KNOX gene family might act as negative regulators of fruit growth repressing GA biosynthesis in unpollinated WT ovaries. Following these observations, a phenotypic and molecular characterization of the interaction between the mutations *pat* and *Curl* (*Cu*, overexpressing *TKn2* and showing abnormal leaf development) was carried out by studying segregating progenies. All phenotypes typical of the *pat* syndrome were evaluated in F_3 and F_4 individuals representing all the genotypic combinations (*Pat cu*, *pat cu*, *Pat Cu* and *pat Cu*). For all the reproductive traits examined, including the frequency of stamen and ovule aberrations and yield traits such as fruit and seed set, a reduction of the *pat* expressivity was observed in the *pat Cu* double mutant background. When specifically testing for parthenocarpy, emasculated not-pollinated flowers of *pat Cu* plants did not set fruit or developed fruitlets significantly smaller than those formed by the *pat* single mutant. Thus, the GA-overdose phenotypes showed by the *pat* mutation are likely mediated by a deregulation of *Tkn2* in the mutant ovary at anthesis. Expression analysis of *Tkn2* in parthenocarpic systems other than *pat* indicated that the developmental regulation of the gene in the ovary is conserved in different WT genetic backgrounds, but it is not paralleled in all the parthenocarpic mutants. In conclusion, the phenotypic and molecular characterization of the *pat Cu* double mutant strongly suggests that deregulation of *Tkn2* mediates the parthenocarpic phenotype induced by the *pat* mutation through changes in GA biosynthesis gene expression.

P9.

A TRANSCRIPTOMIC APPROACH TO IDENTIFY REGULATORY GENES INVOLVED IN FRUIT SET OF WILD TYPE AND PARTHENOCARPIC TOMATO GENOTYPES

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The *parthenocarpic fruit* (*pat*) mutation in tomato (*Solanum lycopersicum* L.) associates a strong competence for parthenocarpy with homeotic transformation of anthers and aberrancy of ovules. To dissect this complex floral phenotype and to detect genes involved in the pollination-independent fruit set of the *pat* mutant, a transcriptomic approach was used. Two pre- and one post-anthesis stages were selected to monitor and compare the expression profile of genes in the wild type (WT) and mutant ovary during flower-to-fruit transition. A customized microarray platform was used in this study. Normalized microarray expression data were subjected to one-way ANOVA and 3,627 transcripts showed significant expression differences ($P \leq 0.01$). Among them, 1,714 displayed a greater than 3-fold change in at least one of the pair-wise comparisons analyzed between genotypes and stages. By clustering analysis, co-expressed genes were grouped into 20 clusters and clusters showing a similar expression pattern in the WT and/or in mutant ovary were grouped according to their centroid expression graph and based on their putative biological function. Using this approach, five biological trends were identified and clusters classified as Controlling Complex (CC), Pollination-Dependent (PD), Fruit Growth-related (FG), Always Different (AD) and Always Similar (AS). CC clusters contained putative negative or positive regulators of fruit set (genes up- or down-regulated at pre-anthesis in the WT ovary and deregulated in the *pat* mutant). Interesting genes belonging to this group encoded orthologs of Arabidopsis transcription factors (TFs) regulating the meristem differentiation and development of floral organs, such as SHOOTMERISTEMLESS, BIG PETALp, AINTEGUMENTA and CRABS CLAW. These findings represented new insights, because so far these genes belonging respectively to the KNOX, bHLH, AP2/ERF and YABBY families of TFs had never been so directly associated to fruit set and parthenocarpy. Their expression profile was confirmed by real-time PCR, which was also extended to other TFs already known to be involved in controlling tomato fruit set, such as AUXIN RESPONSE FACTOR7 (ARF7), ARF8 and INDOLE-3-ACETIC ACID9. Finally, selected genes showing a deregulated expression pattern in *pat* were also studied in other parthenocarpic genotypes either genetically anonymous (*pat-2* and *pat-3*/*pat-4*) or carrying lesions in known gene sequences (*EMS-iaa9* and *RNAi-ARF7*). This comparative approach raised interesting cues for improving the present molecular understanding of fruit set and parthenocarpy in tomato.

P10.

EVOLUTION OF ANTIOXIDANT ACTIVITY AND BIOACTIVE COMPOUNDS IN TOMATO (*Lycopersicon esculentum* Mill.) FRUITS DURING DEVELOPMENT AND RIPENING

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The interest in the consumption of tomato (*Lycopersicon esculentum* Mill.) is, to a large extent, due to its content of bioactive compounds and their importance as dietary antioxidants. During the development and ripening process, there are quantitative and qualitative changes in the fruit composition which will determine the nutritional quality and antioxidant potential at each stage. Two half determinate, early hybrids cultivars (Prekos and Balkan) and one indeterminate mid-early hybrid cultivar (Reyana) were considered for this study. Fruits from plants grown on sandy soil in an unheated greenhouse were collected at three development and six maturity stages (mature green, breaker, turning, pink, light red and red). Antioxidant activity, total and soluble solids, total organic acids, ascorbic acid, lycopene, β-carotene, chlorophylls and total phenolic contents were monitored. During fruit development, total and soluble solids and also total organic acids recorded a slight decrease, polyphenols and β-carotene contents remained almost the same while ascorbic acid content and antioxidant activity increased continuously. The stage of ripening significantly influenced the content of all bioactive compounds as well as the antioxidant activity of tomato fruits. The first stages of ripening were characterized by a slight decrease of the total solids content and by an increase of the total organic acids content, while in the last two stages of ripening these variations reversed. Ascorbic acid and total phenolics content increased as maturity progressed from mature green to pink or light red stage and decreased afterward. Lycopene started to accumulate since turning and significant increases occurred in the last three stages, the highest increase being recorded in the last stage of ripening. From the antioxidant point of view, depending on the cultivar, the pink or light red stages were the ones with the greatest potential, since tomato fruits had the highest levels of ascorbic acid (198.5-327.4 mg/kg) and polyphenols (293.9-385.6 mg GAE/kg) and the highest antioxidant activity (0.73-1.20 mmol Trolox/kg). Although there were significant differences among the contents of bioactive compounds and antioxidant activity of the three cultivars studied, their patterns of variation during the nine stages were quite similar.

P11.

THE ONSET OF GRAPEVINE BERRY RIPENING IS CHARACTERIZED BY ROS ACCUMULATION AND LIPOXYGENASE-DERIVED GALACTOLIPID PEROXIDES.

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The ripening of fleshy fruits is a complex developmental program characterized by extensive transcriptomic and metabolic remodeling in the pericarp tissues (pulp and skin). The onset of ripening is modulated by a network of external and endogenous signals. Previous studies reported the accumulation of hydrogen peroxide (H_2O_2) and extensive modulation of reactive oxygen species (ROS) scavenging enzymes at véraison in grape berries, suggesting ROS may participate to fruit development control. In order to better characterize the oxidative events occurring at ripening onset, *Vitis vinifera* cv. Pinot Noir berries sampled during seven weeks centered on véraison were analyzed for ROS identification and quantification. We show that H_2O_2 and singlet oxygen ($^1O_2$) accumulate in berry skin cells at softening, in the cytosol and plastids, respectively. H_2O_2 peak at véraison is followed immediately by a peak of catalase activity. Also a specific class of membrane lipids, i.e. galactolipids, shows the accumulation of oxidized species at véraison. Monogalactosyl diacylglycerols (MGDGs) and digalactosyl diacylglycerol (DGDGs) are peroxidized on one or both □-linolenic fatty acid chains, with a 13(S) absolute configuration implying the action of a specific enzyme. We identified a lipoxygenase (PnLOXA) which is expressed at véraison and localizes at the plastid thylakoid membranes. This enzyme is indeed able to catalyze membrane galactolipid peroxidation in tobacco leaves overexpressing *PnLOXA*, strongly supporting its role in grapevine berry lipid peroxidation. This reaction could represent the first step of oxylipins biosynthesis or a mechanism to regulate membrane proteins redox state, possibly involved in ripening control.

P12.

IN THE MICROVINE PERICARP, CRITICAL TRANSCRIPTOME CHANGES OCCUR DURING THE NIGHT

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Many transcriptomic studies have been conducted on different *vitis vinifera* L. cultivars to better understand physiological changes occurring during berry development (Lijavetzky et al., 2012, Terrier et al., 2005, Pilati et al., 2007). To our knowledge very few time course studies, assessing both day and night transcriptome, have been performed on any fleshy fruits until now. Still, it was demonstrated on model plants such as Arabidopsis thaliana (Schaffer et al., 2001, Chow and Kayb, 2013) that important changes in gene expression following diurnal patterns occur in all living organisms and might influence organoleptic quality of the grapes. With the macrovine, which presents an annual reproductive cycle, studying day and night fruit development under controlled conditions is almost impossible. In our study, we useed the microvine (DRCF-mutant; Boss and Thomas, 2002) a recently proposed grapevine model (Chaib et al., 2010), which is a dwarf and present a rapid cycling and a continuous production of fruits. Gene expression profile was studied on two pre - and two post-véraison stages of berry development using a whole genome microarray assay (Nimblegen® vitis 12x). Equivalent developmental stages were sampled at day and night time. A total number of 9273 probesets were developmentally modulated between all stages day and night with a high number of genes being modulated specifically at night (1755). Eight very similar clusters of gene expression profiles could be obtained independently for day and night development. Our study allowed highlighting development stage specifics mechanisms of diurnal modulation of genes in the fruit that have been overlooked in other transcriptomic studies on fruit development so far. In the green berry functional enrichment analysis indicated major changes in cellular organization and photosynthesis whereas in the ripe berry mainly secondary metabolism was affected during the night.

P13.

EVOLUTION OF POLYPHENOLIC CONTENT DURING MATURATION OF RED GRAPES

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In Romania consumption of red wines has a special place. Red grapes are considered to be the most important source of phenolic compounds in terms of flavonoids, phenolic acids and stilbenes. Phenolic compounds accumulation in the red grape is depending of many factors including maturity degree of the grape, temperature, light and vine water status during grape ripening.It is already known that red grapes are rich especially in anthocyanins which are responsible for the final color of red wines. It is generally accepted that phenolic compounds are beneficial for human health. Red grapes have been shown to have antioxidant activity due to their richness in phenolic compounds. Fetească neagră and Băbească neagră are two Romanian autochthonous red variety of *Vitis vinifera*. Fetească neagră variety (Black maiden) is an old pre-phylloxeric variety grape cultivated in several romanian areas. These grapes produce red wines, with an alcohol content of 12-12.5% (v/v), a deep red colour with ruby shades, and a black currant flavour, which becomes richer and smoother with aging. Băbească neagră variety (Black grandmother) is an old native Romanian wine grape variety. It is cultivated in the south eastern part of Romania. The wines produced from Băbească neagră are light and fruity red wines. Both of them acquire its superior quality in the Dealu Bujorului vineyard. These grape varieties are very important in production of high-quality red wines in Romania. Phenolic composition of Fetească neagră and Băbească neagră grapes from Dealu Bujorului vineyard (south eastern of Romania) was studied by using spectophotometric and High Performance Liquid Chromatographic (HPLC) methods. The results revealed significant differences between these cultivars. The total anthocyanins ranged from 251 mg/kg berries in Băbească neagră to 422 mg/ kg berries in Fetească neagră. Both cultivars were characterized by an interesting anthocyanin profile for winemaking with a prevalence of malvidin-3-glucoside. The skins and seeds both had small amounts of flavonoids. However, Fetească neagră berries had more flavonoids in the seeds (55%) than in the skins. The most important parameters for differentiating between the cultivars were the peonidin and malvidin derivative forms, and the total amounts of acetyl-glucosides.

P14.

ANALYSIS OF THE ORIGIN OF PARTHENOCARPY IN GRAPEVINE CULTIVAR CORINTO BIANCO

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Seedless fruits appear spontaneously in grapevine (*Vitis vinifera* L.) as a result of somatic variation. Stenospermocarpic and parthenocarpic seedlesness are known. The first type is widely used in table grapes production because seed development aborts after fertilization giving rise to seed traces and almost normal size berries. In contrast, small berries without seed traces develop in absence of fertilization in parthenocarpic cultivars that are appreciated for raisin production. The cultivar Corinto Bianco is a parthenocarpic somatic variant of the Spanish seeded cultivar Pedro Ximénez. Morphological and molecular comparison of flower development and gametogenesis between both genotypes were directed to understand the genetic and molecular basis of this parthenocarpic phenotype. Histological analyses showed that ovules developed similarly in both genotypes. However, macrogametogenesis was altered in Corinto Bianco showing disorders during mitosis after mother cell meiotic reduction. Microgametogenesis was also altered in Corinto Bianco, which pollen was 100% sterile in 2012 and 2013, compared with Pedro Ximénez pollen that showed only 8.47 and 21.64% of sterility in those years. The average number of seeds per berry was 1.35 in Pedro Ximénez berries, which weight was six times higher than in Corinto Bianco parthenocarpic berries with zero seeds. In addition, we observed partial phenotypic reversion in 2.6% of Corinto Bianco berries that carried one seed and displayed a comparable size to that of Pedro Ximénez berries. These seeds were unable to germinate in soil under normal conditions. Fortunately, following an *in vitro* germination protocol we were able to rescue 41 seedlings derived from revertant berries for further analyses. Gene expression alterations between Pedro Ximénez and Corinto Bianco, that could lead to the identification of candidate genes responsible for the phenotypic change, were analyzed by comparing closed flowers of both genotypes at 50% bloom time using the NimbleGen Vitis HX12 microarray. We identified 441 genes upregulated and 949 downregulated in the parthenocarpic mutant (≥2-fold change and 5% FDR). Interestingly, genes related with cell cycle and gametogenesis were downregulated in Corinto Bianco, including a cyclin (CYB1;2-like) and a MADS-box gene (AGL66-like), whose function in Arabidopsis is related with mitosis and pollen development, respectively. The expression of these genes could be related with defects in gametogenesis in Corinto Bianco. These preliminary results suggest the presence of defects in the meiotic mechanisms central to the process of gametogenesis in Corinto Bianco, providing clues for further characterization of the origin of parthenocarpy in this cultivar.

P15.

SAGE ESSENTIAL OIL ENHANCES TOMATO FRUIT QUALITY IN DIFFERENT RIPENING STAGES.

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Tomato fruits (*Solanum lycopersicum* L.) at breaker and red ripening stage were exposed to sage volatile oils (50ppm or 500ppm) for 7 days, at 11^{o}C and 95% RH. Quality-related attributes were examined during vapour treatment. Fruit treated with volatiles (500ppm) increased respiration rate (up to 63% for breaker and up to 42% for red tomatoes) while ethylene production increased in both 50ppm and 500ppm volatile concentration, compared to control (un-treated) fruits. Essential oil (50ppm and 500ppm) application decreased weight losses up to 46% for breaker ripening stage tomatoes and this effect consists following ripening only for 50ppm application for the red tomatoes. Indeed, 500ppm volatiles application for red tomatoes, increased (up to 25%) the weight losses comparing with the control fruit. Tomatoes-enriched with 50ppm sage oils maintained fruit firmness comparing with higher concentration (500ppm) in red fruits while no differences observed with controlled fruits. Sage oil-treated red fruit at 50ppm maintained lycopene content while fruits exposed to 500ppm revealed a 28% reduction in lycopene content comparing with the control fruits. No differences in lycopene content marked for the breaker fruits. In breaker fruits, increased sage oil concentration marked a steady increase in b-carotene content while no differences observed in red tomatoes. Vapour-treatment did not affect the content of total phenolics and citric acid as well as total soluble solids in both breaker and red tomatoes. Natural volatiles may maintain fruit quality in addition to the well documented antimicrobial protection during fresh produce storage and transit.

P16.

APPLICATION OF NEW RESEARCH APPROACHES IN THE EVALUATION OF VITICULTURAL POTENTIAL OF DIFFERENT TERROIRS (PRELIMINARY RESULTS)

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A combined study has been started in 2010 in 4 different growing sites of the Eger wine district of Hungary. The aim of the investigation was to follow the grape ripening processes and to describe viticultural potential of different terroirs using new research tools: i.e. Multiplex Research®, berry texture analyser and hyperspectral camera. Multiplex Research® Nr. 3 a hand-held battery operated instrument, having four excitation light emitting diode sources in the UV-A (370 nm), blue (460 nm), green (516 nm) and red (637 nm) and three detection channels in the blue-green, red and far red spectral regions (Cerovic et al., 1999 ; Tuccio et al., 2011) was used to estimate phenolic maturity of the grapevine clusters in the field. Berry texture parameters were analyzed by SMS Texture Analyzer during the ripening period. Skin break force (F_{sk}), skin thickness (Sp_{sk}), seed break force (F_s), and berry hardness (BH) were investigated according to Letaief *et al*. 2008. In parallel, Cell Maturity Index (CMI) and Seed Maturity Index (SMI) were also measured according to Saint-Criq *et al* 1998. Digital spectral images were taken by a push-broom typed Aisa Eagle II hyperspectral camera (www.specim.fi) in the visible and near-infrared range (VNIR). With the help of the devices, we followed differences in ripening phases of grape berries grown in different growing sites, and we also analysed the vineyard's canopy growth. Our first results show that the new tools are promising for detection of phenolic maturity (i.e. anthocyanin content of berries, berry texture parameters). Detailed mathematical analysis is needed to smooth the heterogeneity of bunches. Hyperspectral images can support description of vineyard's growing uniformity. Its further and wider use can ease detection of stress symptoms of growing sites.

P17.

THE ETHYLENE-AUXIN BALANCE CHANGES THE INTENSITY OF ORANGE/RED COLOR IN ONE FLESHY FRUIT MODEL, TOMATO (*SOLANUM LYCOPERSICUM*), BY MODULATING THE EXPRESSION OF PHYTOENE SYNTHASES AND LYCOPENE CYCLASES.

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The application of auxin and/or ethylene precursor was shown to impact the tomato ripening and in particular the color change. Auxin (indole-3- acetic acid, IAA) delayed the transition from green to orange/red, and the ethylene precursor (1-aminocyclopropane-1-carboxylic acid, ACC) accelerated it, as measured by chromametry. The combination of IAA+ACC led to tomatoes less orange/red than controls, suggesting that the response to IAA is dominant over the response to ACC. The application of an auxin antagonist, the *p*-chlorophenoxy isobutyric acid (PCIB), led to tomatoes nearly as ripe as with ACC. Four days after the treatments, the lycopene and the carotene contents were increased in ACC and PCIB trials, when they were still low in controls, IAA and IAA+ACC. Studying the RNA accumulation of several key genes of the carotenoid pathway showed that the transcript profile matching the best the lycopene accumulation was the decrease of a lycopene cyclase following the ACC and PCIB treatments, this cyclase being a key step of the lycopene conversion to carotenes. This confirms with previous reverse genetics experiments. It was also shown that IAA also inhibited one phytoene synthase and a associated MADS-box RIN. Our study also suggests that IAA blocks lycopene accumulation by down regulating the accumulation of most transcripts of the pathway between the geranyl-geranyl pyrophosphate and the lycopene, giving also information on accumulation levels of the following transcripts: geranyl-geranyl pyrophosphate synthase, the phytoene desaturase, the carotene desaturase, the carotenoid isomerase, and also carotene *β* -hydroxylase.

P18.

SEARCH FOR TRANSCRIPTIONAL AND METABOLIC MARKERS OF GRAPE PRE-RIPENING AND RIPENING AND INSIGHTS INTO SPECIFIC AROMA DEVELOPMENT IN THREE PORTUGUESE CULTIVARS

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Grapes (*Vitis* species) are economically the most important fruit crop worldwide. However, the complexity of molecular and biochemical events that lead to ripening of berries as well as how aroma is developed are not fully understood. In an attempt to identify the common mechanisms associated with the onset of ripening independently of the cultivar, grapes of Portuguese elite cultivars, Trincadeira, Aragonês, and Touriga Nacional, were studied (Agudelo-Romero et al. 2013 Plos One). The mRNA expression profiles corresponding to *veraison* (EL35) and mature berries (EL36) were compared. Across the three varieties, 9,8% (2255) probesets corresponding to 1915 unigenes were robustly differentially expressed at EL 36 compared to EL 35. Eleven functional categories were represented in this differential gene set. Information on gene expression related to primary and secondary metabolism was verified by RT-qPCR analysis of selected candidate genes at four developmental stages (EL32, EL35, EL36 and EL 38). Gene expression data were integrated with metabolic profiling data from GC-EI-TOF/ MS and headspace GC-EI-MS platforms. Putative molecular and metabolic markers of grape pre-ripening and ripening related to primary and secondary metabolism were established and revealed a substantial developmental reprogramming of cellular metabolism. Altogether the results provide valuable new information on the main metabolic events leading to grape ripening. Furthermore, we provide first hints about how the development of a cultivar specific aroma is controlled at transcriptional level.

P19.

DEVELOPMENTAL REGULATION OF SPECIALIZED METABOLISM IN GRAPE BERRIES OF CABERNET SAUVIGNON AND SHIRAZ

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Grape berry development is a process regulated by genetic and environmental cues. The extent of metabolite accumulation in the berry is dependent on factors such as temperature, light, nutrient, soil water status, and genetics. The combined effect of these factors on the chemical composition of the berry will eventually affect the wine quality. As part of the berry biochemistry, phenolic compounds - found mainly in the berry skin- are known for their health-promoting activity. In the present study, developmental changes in metabolite and transcript profiles were assessed in two wine grape cultivars, Shiraz (SH) and Cabernet Sauvignon (CS). Apparently, distinct metabolite patterns of change were shown in the two cultivars during berry development. The relative abundance of non-methylated anthocyanins (Cyanidin-3-glucoside and delphinidin 3-O-glucoside) was comparable in the two cultivars. However, all the acylated forms (p-coumaroyl derivatives) of anthocyanins were significantly higher ($P<0.01$) in Shiraz compared to CS. Among identified flavonols, kaempferol-3-glucoside was significantly higher throughout development in CS compared to Shiraz. Intermediates of the upstream section of the flavonoid pathways including naringenin chalcone hexose, catechin and hydroxybenzoic acid hexoside were increased in Cs compared to Shiraz at mid-ripening and harvest. RNAseq analysis showed stilbene synthase genes to be highly induced particularly in Shiraz as berry developed from veraison to mid-ripening stage. Increase in transcript level was coupled to the accumulation of resveratrol and piceid suggesting a direct link between transcription and metabolite level. Flavonoid 3', 5'-Hydroxylase (F3'5'H) gene showed significant induction from veraison to mid-ripening stage in both cultivars, which associated with increased levels of delphinidin 3-O-glucoside and other downstream intermediates. When taken together the results suggest a differential partitioning across polyphenol metabolism between the cultivars.

P20.

PHYSIOLOGICAL DISORDERS AS A TOOL TO INVESTIGATE GRAPE BERRY RIPENING AND SINK SOURCE BALANCES IN VINES

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Grape berry ripening is a process following a double sigmoid curve with distinct growth phases determined by different physiological processes. Several aspects of this complex ripening process of a non-climateric fruit have been investigated and the understanding concerning phloem and xylem unloading, metabolism of organic acids, biosynthesis of secondary metabolites and the influence of abiotic and several biotic stresses on the ripening process is quite high. Nevertheless there are some processes, especially concerning the interplay of the vine with each individual grape cluster, the phytohormon biosynthesis and signaling and the details of the phloem unloading process that are not fully elucidated. Our group is investigating these aspects of grape berry ripening, linked to the source sink balance of the plant, by analyzing the physiological ripening disorder Berry Shrivel (BS). Grapes affected by BS are of low quality characterized by low sugar contents, high acidity, reduced anthocyanin content and strong off flavors. Thereby we are following two strategies: the transport of assimilates and nutrients towards ripening berries and the strong changes in the metabolic activity of berries during the ripening process. Applying a set of methods comprising of chemical analyses, transcriptional profiles, enzymatic assays and microscopic methods, we gain important knowledge about the BS induction process as well as the development in healthy grape clusters. In a first effort we focused on transcriptional changes in the rachis and in berries of BS clusters and we will extent the analytical part towards phytohormon and sugar analyses.

P**21.**

THE HEAT STRESS TRANSCRIPTION FACTOR HSFA2 IS IMPLICATED IN HEAT STRESS RESPONSE AND DEVELOPMENTAL PROCESSES IN TOMATO (*SOLANUM LYCOPERSICUM*).

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Heat stress transcription factors (HSFs) trigger the transcriptional activation and rapid accumulation of heat shock proteins to compensate for the disturbed proteostasis under adverse stress conditions. Apart from heat stress, Hsfs are also involved in response mechanisms against other abiotic stresses as well as in different plant developmental processes. Tomato genome comprises 24 HSF genes but major aspects of heat stress response and recovery are regulated by the interaction of HsfA1a with HsfA2 and HsfB1. Using a bioinformatics co-expression approach we identified a putative regulatory network for HsfA2 with potential role in abiotic stress responses. HsfA2 is expressed at basal levels in vegetative tissues under non-stress conditions but is strongly induced in early stages of pollen development indicating possible developmental function. To investigate the role of HsfA2 in plant development and heat stress response, we used tomato lines (*S. lycopersicum* cv Moneymaker) transformed with expression cassettes encoding HsfA2 in sense and antisense orientation either causing its constitutive expression or RNAi-mediated knock-down, respectively. Phenotypic analyses showed that HsfA2 is involved in different developmental aspects including pollen quality and fruit development. Plants overexpressing HsfA2 produced lower number of germinating and viable pollen grains. Fruits from both sense and antisense lines require longer time to reach the stage of red ripe fruit, while fruits from sense lines produce significantly lower number of seeds which also exhibit germination defects. To get more insights into the role of HsfA2 in both vegetative and reproductive tissues, we performed a transcriptome analysis using Next Generation Sequencing of control and heat stressed leaves and anthers. Our results show major differences between the heat stress response in vegetative and male reproductive tissues and point to the direction of HsfA2 as an important transcriptional regulator of many genes related with thermotolerance. In addition, gene expression analysis showed that HsfA2 regulates the expression of several heat shock proteins that are highly expressed during early stages of pollen development suggesting that components of protein homeostasis are involved in the development of male gametophyte. These observations are of particular biological and agricultural interest, considering that the early stages of pollen development are the most sensitive to high temperatures and exposure of plants to heat stress conditions at this stage can cause significant crop losses.

P22.

GRAPEVINE GENE NOMENCLATURE SYSTEM

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A major breakthrough in grapevine genomics was achieved in 2007 with the sequencing of the *Vitis vinifera* cv. PN40024 genome. Subsequently, invaluable data related to the structural and functional characterization of the genes accumulated exponentially. Unfortunately, data that were acquired prior to the genome release are largely under-exploited. Furthermore, because of heterogeneous sources these data remain often incompatible but also decentralized storage is making retrieval more difficult. Classically, a large amount of useful data describing gene functions only appeared in printed articles describing experiments carried out to answer specific questions. These data remain inaccessible for automatic text mining. High throughput "omics" data are typically stored in public repositories, but generally are confined to their initial aim. With the objective of providing a high quality and highly accessible annotation of grapevine genes, the International Grapevine Genome Project (IGGP) commissioned an international Super-nomenclature Committee for Grape Gene Annotation to coordinate the expert-annotation effort of the grapevine genes. The goal of the committee is to provide a standard nomenclature for locus identifiers and to define conventions for a gene naming system. Using previous experience acquired on similar initiatives for other plant species such as *Arabidopsis*, rice and tomato, a versatile system was implemented anticipating on future developments and issues. Specific emphasis has focused on directions for the expert annotators by: (i) providing a clearly-identified common annotation platform that also enables community-based gene curation (ii) emphasizing a gene naming scheme reflecting the biological features of gene products, (iii) and using a genetic ontology consistent with other organisms to facilitate evolutionary comparison of genes.

P23.

THE BZIP TRANSCRIPTION FACTOR VVABF2 MEDIATES ABA SIGNALLING IN GRAPE BERRIES

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In grape (*Vitis vinifera* L.), abscisic acid (ABA) accumulates during fruit ripening and is thought to play a pivotal role in this process, but the molecular basis of this control is poorly understood. The present work characterizes VvABF2, a grape bZIP transcription factor belonging to a phylogenetic sub-group previously shown to be involved in ABA and abiotic stress signalling in other species. *VvABF2* transcripts mainly accumulated in the berry, from the onset of ripening to the harvesting stage, and were up-regulated by ABA. Microarray analysis on transgenic grape cells overexpressing *VvABF2* showed that this transcription factor upregulates and/or modifies existing networks related to ABA responses. In addition, grape cells overexpressing *VvABF2* exhibited enhanced responses to ABA treatment compared to control cells. Among the VvABF2-mediated responses highlighted in this study, the synthesis of phenolic compounds and cell wall softening were the most strongly affected. VvABF2 overexpression strongly increased the accumulation of stilbenes that play a role in plant defense and human health. The function of VvABF2 was also checked by overexpression in tomato. The firmness of fruits from tomato plants overexpressing *VvABF2* was strongly reduced. These data indicate that VvABF2 is an important transcriptional regulator of ABA-dependent grape berry ripening.

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P24.

METABOLITE FLUXES PATTERN IN GRAPE BERRY CELL SUSPENSION CULTURE (*Vitis vinifera* L. Var Gamay Red) INFLUENCED BY LIGHT, TEMPERATURE AND JASMONIC ACID

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Grape, *Vitis vinifera,* is an important crop for its economical and health benefits. However, abiotic and biotic stresses significantly limit its yield, quality and distribution. However the complex interaction of these environmental factors hampers our understanding of the commonal and stress-specific responses at the molecular level. Accordingly, we set an *in vitro* cell suspension culture experiment from berry skin explants. Treatments were provided with different light exposure (50 and 100 µmol $m^{-2}s^{-2}$), temperatures (20 and 30 ^{0}C) and 0.1 mM Jasmonic acid. The metabolic flux pattern in phenylpropanoid pathway were analysed by measuring the relative metabolite abundance during 4, 8 and 12 hours of the experiment. Further, a stable isotope-labeled 0.1mM L-Phenylalanine-1-^{13}C was used in jasmonic acid treatment to trace isotopic enrichment in the phenylpropanoid pathway. An established UPLC-QTOF-MS method was used for metabolite profiling. The result indicated that jasmonic acid treatment enhanced stilbene synthesis and glycosylated quercetin, but more unexpectedly it also increased anthocyanin accumulation. In addition, light and temperature significantly induced the accumulation of anthocyanins in the form of acetylated and $_{P}$-coumaroyl. Further analysis is underway to associate it with detached berry experiments at pre-veraison and veraison stage of berry development.

P25.

CATABOLIC PATHWAYS OF L-METHIONINE IN THE FORMATION OF SULFUR AND NON-SULFUR AROMA VOLATILES IN RIPE MELON FRUIT

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Melon cultivars and accessions differ in the content of sulfur-containing and other aroma volatiles. Sulfur containing aroma volatiles are important contributors to the distinctive aroma of melon and other fruits. Some of these volatiles have classic melon aromas, while others have off-flavor and sulfuric aromas. L-Methionine has been postulated to serve as a precursor of these volatiles. Incubation of melon fruit cubes with ^{13}C- and ^{2}H- labeled L-methionine revealed two distinct catabolic routes into volatiles: one apparently involving the action of an L-methionine aminotransferase and preserving the main carbon skeleton of L-methionine. The majority of these volatiles depict classic cantaloupe aromas. The second route apparently involves the action of an L-methionine-γ-lyase activity, releasing methanethiol, a backbone for the formation of thiol-derived aroma volatiles. The majority of these volatiles have off-flavor aromas. Exogenous L-methionine also generated non-sulfur volatiles by further metabolism of α-ketobutyrate, a product of L-methionine-γ-lyase activity. α-Ketobutyrate was further metabolized into L-isoleucine and to other important melon volatiles including non-sulfur branched and straight-chain esters. Cell-free extracts derived from ripe melon fruit exhibited L-methionine-γ-lyase enzymatic activity. A novel gene (*CmMGL*) was ectopically expressed in *E. coli* and shown to possess L-methionine-γ-lyase enzymatic activity. The expression of *CmMGL* was relatively low in early stages of melon fruit development, but increased in the flesh of ripe fruits depending on the cultivar tested. Moreover, the expression levels of *CmMGL* in recombinant inbred lines population co-segregated with the levels of the S-volatiles enriched with +1 m/z unit and postulated to be produced via this route. This suggests that *CmMGL* expression has an important role in determine melon off-flavor scent. These results indicate that L-methionine is a precursor of both sulfur and non-sulfur aroma volatiles in melon fruit.

P26.

APPLICATION OF HIGH-THROUGHPUT GENOTYPING TECHNOLOGIES FOR THE RAPID DEVELOPMENT OF A GENOMIC LIBRARY OF INTROGRESSION LINES OF *SOLANUM PIMPINELLIFOLIUM*

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Genomic libraries of introgression lines (IL), i. e., collections of genetic stocks, each IL carrying a different single defined chromosome segment from a donor species in a uniform genetic background, have been extensively used to genetically dissect complex traits and to facilitate the use of unadapted germplasm in modern plant breeding. The number of IL libraries available in tomato is currently low due to the high cost in time and resources necessary for their development. Fortunately high-throughput genotyping methods are now increasingly applied in tomato breeding to facilitate the development of molecular breeding designed genetic stocks. We selected a *Solanum pimpinellifolium* accession TO-937 with interesting quality and stress adaptation traits to develop a genomic library of ILs in the "Moneymaker" (MM) genetic background. We first developed a large collection of Single Nucleotide Polymorphic (SNPs) markers based on the comparison of the reference tomato genome and RNA-seq reads of TO-937 and designed a 756 SNP genotyping array to characterize a BC2 population for the TOxMM cross, from which we selected a minimum numbers of plants to fund the BC3 population. A large population of BC3 seedlings were genotyped with a new 96 SNP genotyping array to result in a collection of pre-ILs containing 1-4 introgressions per IL. A collection of single introgression lines were finally developed by performing within-family selection by "High Resolution Melting" SNP genoytping. The genotypes of the selected lines were verified with the 8K SOLCAP Infinium array. A total number of 54 ILs have been developed, containing on average 3.7% (range 0.5 -7.7%) of TO-937 genome, and 4.6 IL/chromosome (range 4-6), defining 68 bins and covering 97.3 % of donor genome. This collection of ILs is currently evaluated for fruit quality traits in three experimental trials in Valencia (IBMCP), Orihuela (UHM) and Málaga(EELM).

P27.

THE USE OF A MULTI-LOCUS MIXED MODEL FOR GWAS REVEALS ASSOCIATIONS FOR METABOLIC TRAITS IN THE TOMATO, *SOLANUM LYCOPERSICUM*

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Genome-wide association studies have been successful in identifying genes involved in polygenic traits notably in crops, and can be a useful way towards crop improvement. We have applied in a major crop, tomato, a recently developed multi-locus mixed model as a general method for mapping complex traits in structured populations (Segura et al., 2012). Among major crops worldwide, tomato (*Solanum lycopersicum*) is a highly valuable fruit with excellent nutritional value (Causse et al., 2010). SNP beadchips (Hamilton *et al.*, 2012) are available and enable GWAS for traits of interest. Recently, a pilot study defined the optimal conditions for GWAS by using cherry tomato accessions (Ranc et al., 2012). In our study, we examined a core collection of 180 tomato varieties composed of 20 wild accessions (*S. pimpinellifollium*), 130 admixed accessions (*S. cerasiforme*) and 30 domesticated accessions (*S. lycopersicum*). Multi-locus GWAS analysis was conducted using the MLMM package (Segura et al., 2012) with 7700 SNP markers and a set of sugar-related, vitamin C-related and morphological traits as well as a broad range of metabolites involved in central carbon metabolism. The present study is the first one in tomato reporting associations for a large set of traits at the genome scale. We found significant associations for 89 loci with a total of 19 traits including fresh weight, sucrose, ascorbate, malate or citrate notably. Identified loci were also concordant with published quantitative trait loci (eg malate), while new loci were identified (for tocopherol). Moreover, several related metabolites, such as citrate and malate (both involved in the Krebs cycle) displayed two identical associations. These results (1) provide a list of candidate loci to be functionally validated and (2) provide a powerful analytical approach for finding genetic variants that can be directly used for crop improvement and deciphering the genetic architecture of complex traits.

P28.

ROLE OF *SEPALLATA1/2*-LIKE GENES AND AUXIN DURING APPLE FRUIT DEVELOPMENT: TOWARDS A BETTER UNDERSTANDING OF FLESHY FRUIT MORPHOGENESIS

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Auxin is an important phytohormone for fleshy fruit development, having been shown to be involved in the initial signal for fertilisation, fruit size through the control of cell division and cell expansion, and ripening related events. There is considerable knowledge of auxin-related genes, mostly from work in model species. With the apple genome now available, it is possible to carry out genomics studies on auxin-related genes to identify genes that may have roles in specific stages of apple fruit development. An expression analysis screen of auxin-related genes involved in auxin reception, homeostasis, and transcriptional regulation showed complex patterns of expression in each class of gene. In particular, the expression analysis of an *ARF* gene was linked to a strong QTL for fruit weight suggesting that the auxin signal regulating fruit size could partially be modulated through the function of this gene. In parallel, we are investigating the role of an apple *MADS-box* gene, *MdMADS8* (a *SEPALLATA1/2-like* gene) during apple fruit development. Transgenic apple lines, down-regulated for *MdMADS8,* lack the fleshy part of the fruit, presenting the characteristics of a dry-like fruit and do not ripen at maturity. To date the best example of gene affecting flesh development has been identified and characterised in tomato. Transgenic tomato down-regulated for the MADS-box gene *TAGL1* (*TOMATO AGAMOUS-LIKE 1*) show a reduced pericarp tissue, but the remaining tissue maintained its fleshy character, as well as full or partial inhibition of some ripening characters. Our results suggest a plasticity of function both within the *SEP* clade as well as within the MADS box family, making it important to look widely within the MADS box family when trying to identify functionally related genes in different species.

P29.

IDENTIFICATION AND CHARACTERIZATION OF TWO NEW TRANSCRIPTION FACTORS IMPLICATED IN TOMATO FRUIT DEVELOPMENT

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In the last years, our understanding of the mechanisms involved in fruit ripening has been considerably furthered, thanks to the increased availability of genetic and genomic tools. In tomato and in other species, recent studies highlighted the crucial role played by regulatory networks involving several transcription factors in the onset of fruit ripening. Several evidences further indicate that in tomato the ripening process can be initiated well before the mature green stage of the fruit. In a recent study (Lemaire-Chamley et al., 2009) we identified through correlation network analysis of combined transcriptome/metabolome data from early developing fruit tissues, several regulatory hubs possibly involved in the transition between the cell expansion stage and the mature stage of the fruit. Among these were two new regulators, a bZip and a HD-Zip transcription factors (TFs). Fruit-specific silencing of both TFs with CRES-T technology using the *SlPPC2* promoter targeting the cell expansion phase till the onset of ripening (Fernandez et al. 2009) leads to profound and opposite fruit phenotypic changes. Fruits from HD-Zip silenced lines (MicroTom cultivar) are greener, display increased starch content in immature-green (IG) fruit and strong increase in sugar content (glucose levels X 2) in the ripe fruit. Fruits from b-Zip silenced lines are pale, with decreased starch in IG fruit and reduced sugar content in the ripe fruit. In addition, b-Zip silencing has profound effects on fruit ripening including delayed and uneven carotenoid accumulation, increased malate content and enhanced firmness. Detailed results from the developmental and metabolic characterization of available lines (OE, RNAi, CRES-T and TILLING lines) will be presented and discussed in relation to the known regulations involved in the control of fruit ripening.

P30.

UPLC-QTOF-MS BASED PROFILING AND SNP GENOTYPING INDICATE A PRINCIPAL ROLE FOR QUERCITIN 3 GALACTOSIDE, A BIOLOGICALLY ACTIVE FLAVONOID O-GLYCOSIDE, IN THE VARIETAL METABOLIC DIVERSIFICATION OF MUSCAT GRAPE.

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The chemical composition of grape berry is mainly varietal dependent, as well as influenced by climate, soil and viticulture practices. The ratio of different terpene compounds is responsible for the characteristic flavor of muscat grapes. The composition of phenolic compound also plays a significant role on the organoleptic property of the wine. In the present study, we investigated the natural diversity in specialized metabolites including flavonoids and nonflavonoids of grape origin in a Muscat collection. Metabolite profiling was performed on 18 Moscato bianco clones and 43 natural grape varieties of muscat using ultra perfomance liquid chromatography–quadrupole time of flight–mass specrometry (UPLC-QTOF-MS/MS). The metabolite analysis was coupled with SNP genotyping, in order to generate a genetic relationship among the accessions. Metabolite profiling based principle component analysis and hierarchical clustering showed a separation of the genotypes into six main groups, three red and three white. The level of quercitin galactoside contributed mostly to the separation between white groups, while the red groups were separated largely according to the relative amount and form of anthocyanins. The overall distribution pattern of metabolic variation among the different groups and the genetic analysis suggests a strong post-transcriptional regulation accountable for the varietal differences in specialized metabolites across the Muscat collection. Nevertheless, leaf based SNP (20K) genotyping identified a genetic marker which explained the variance among white varieties shown at the metabolite level. The marker, located on gene sequence kegg 2.1.1.76 and encoding for quercetin 3-O-methyltransferase and involved in flavone and flavonols biosynthesis.

P31.

THE PLASTICITY OF GRAPE BERRY METABOLOME

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An untargeted LC-ESI-MS-based metabolomics approach was used to investigate on plasticity of non volatile metabolites of Corvina grape berries, i.e. the ability of plant to adapt the levels of various metabolites within the berry in response to different environmental conditions. According to the experimental design, samples from 7 commercial vineyards located in three different macroarea around Verona (North of Italy) during three growing season and considering three ripening stages (early veraison, mid-ripening, full-ripening) were included. Moreover, we use only one cultivar (Corvina) and only one clone (clone 48). The LC-ESI-MS analysis allowed detecting 551 features in negative ionization mode and 422 features in positive mode. About 100 of these features corresponded to individual putatively identified metabolites, while other 173 feature corresponded to isotopes, fragments and adducts of the identified metabolites. The identified metabolites included hdroxycinnamic and hydroxybenzoic acids, anthocyanins, flavan 3-ols and procyanidins, other flavonoids, stilbenes and viniferins. The dataset was analyzed both by unsupervised (PCA) and supervised (PLS-DA, O2PLS-DA) multivariate methods. The ripening-dependent metabolites (i.e. metabolites that increased or decreased from veraison to full ripening) and the metabolites less influenced by ripening (i.e. metabolites whose level did not change or change less from veraison to full ripening) were identified and analyzed separately. These analyses showed that the two groups of metabolites have differential plasticity, since the ripening-dependent metabolites were less influenced by the environmental and climatic conditions. Moreover, focusing on the various classes of metabolites, the stilbenes and the quercetin-based flavonoids proved to be the more plastic group of berry chemicals while, on the other side, the procyanidins levels were quite constant and stable over the different environmental conditions. The results suggest that the accumulation of metabolites within the berry is controlled by various and partially overlapped programs, depending on ripening program and/or climatic and environmental stimuli.

P32.

INTEGRATED BIOINFORMATICS: A KEY STEP TOWARDS the ANNOTATION OF METABOLIC PATHWAYS. AN EXAMPLE FOR ASCORBIC ACID IN TOMATO.

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The completion of genome sequencing, the availability of further related -omics collections and suitable bioinformatics are key tools for expanding knowledge on gene components involved in metabolic pathways. We present here a bioinformatics strategy for improving the gene annotation taking advantage of immediate integrated resources. To this aim we mainly exploited the bioinformatics platform ISOL@ (Chiusano et al. 2008) which was set-up initially to support the tomato genome annotation. Thanks to the expansion of the available data collections and to the integration of several data sources, ISOL@ is now oriented to support deeper analyses on gene structure organization and function with the aim to still provide a contribution to understand the information content embedded in the sequenced solanaceae genomes. Our strategy is here proposed as a contribution to the annotation of the pathway of the ascorbic acid in tomato. Despite the relevance of the ascorbic acid as a nutrient for human health, the full description of the tomato genes associated to this pathway is still ambiguous, even at a year of distance from the release of the draft genome. Moreover, several evidence indicate that multiple ascorbic acid biosynthetic routes are functioning in plants, although some of the steps haven't been characterized yet. For this reasons we investigated the genes expected to contribute to the entire pathway, their genome distribution, their possible redundancy in terms of multi copy genes or possible transcript variants, for a suitable targeting and assignment of their specificity, which are key steps for further functional validation, breeding practice and evolutionary analyses. Our preliminary results reveal multiple gene copies for the majority of the genes involved in the pathway. Often the analysis highlighted a still incomplete annotation in terms of gene structure or functional description when considering the current official one. Moreover, several alternative transcript sequences have been detected from most of the genes, suggesting possible adaptive routes in this biosynthetic way. Our approach represents a model that highlights the complexity of these studies, that can be expanded to other pathways and other species, further contributing to the enrichment from "omics" to human knowledge and practice.

P33.

ANTHOCYANIN ACYLATION IN GRAPEVINE BERRIES

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Anthocyanin acylation is very common in grape and contributes to pigment stabilization during winemaking and to colour diversity among grape genotypes. The molecular mechanisms underlying anthocyanin acylation in grape, however, have not been elucidated. In plants, anthocyanin acyltransferases (AATs) catalyse the transfer of aliphatic or aromatic acids to the glycosyl group of anthocyanins. Based on homology to a *Petunia hybrida* gene, we isolated two putative AATs from grape (*AAT1* and *AAT2*). While *AAT1* is mostly expressed in leaves and is not affected by ripening stage in berries, *AAT2* transcripts are absent in leaves and peaks at véraison in berries, as shown qRT-PCR assays. Microarray transcriptomic analysis also includes *AAT2* among genes overexpressed in *VvMYBA1*-overexpressing, and acylated anthocyanin-accumulating, root cultures, and this result is confirmed using qRT-PCR. The expression of *AAT2* is induced, together with expression of *VvMYBA1*, by ABA treatment in suspension cell cultures, although the level of *AAT2* transcripts remains overall low . Berries of two grape genotypes were sampled before véraison, were incubated *in vitro*, and were treated with ABA: qRT-PCR analysis showed also in these tissues an accumulation of *AAT2* together with *VvMYBA1*. The functional characterization of *AAT2* was attempted by overexpressing it into grapevine leaves using a DNA infiltration technique with *VvMYBA1* and *AAT2* constructs: however no increase in anthocyanin acylation was observed. This can be explained by activity of endogenous *AAT2* and thus a silencing experiment is currently underway. Expression of *AAT2* moreover is observed also in Pinot noir, which notably does not accumulate acylated anthocyanins, suggesting that additional protein(s) may be required for enzymatic action of this *AAT*.

P34.

MULTIPLE ROLES FOR GRAPEVINE FLAVONOID REGULATORS

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The R2R3-MYB family is one of the largest groups of transcriptional regulators in plants, with diverse roles in development, physiology and metabolism. Phylogenetic analysis has shown that in grapevine the R2R3-MYB proteins clustering in functionally-conserved clades associated with flavonoid and anthocyanin metabolism, have expanded compared to other species, but only a few genes have been functionally characterized and unequivocally assigned to the control of particular branches of the pathway. VvMYB5a and VvMYB5b were previously proposed to control the spatiotemporal expression of flavonoid structural genes during berry development. On the basis of the comparative analysis with other plant systems, we hypothesized that these two regulators may play additional roles, not strictly related to the flavonoid biosynthesis. To gain information about the function of the two MYBs we used functional complementation analyses of well characterized petunia pH/anthocyanin regulatory mutants. Moreover, we produced grapevine transgenic plants with an altered expression of VvMYB5a and VvMYB5b, and transgenic plants silenced for the TTG2-like transcription factor putatively acting downstream these two MYBs in the regulatory cascade. All transgenic lines displayed severe phenotypic alterations affecting leaves and sometimes the whole plant. Transcriptomic analysis revealed that a small set of modulated genes was shared among different transgenic lines. These genes seem not directly involved in the flavonoid pathway and, more likely, are related to lipid metabolism and ion transport across membranes. Altogether these results indicate that VvMYB5a and VvMYB5b may act in a transcriptional regulatory network conserved among plant species.

P35.

WATER LIMITATION AND ROOTSTOCK GENOTYPE AFFECT GRAPE BERRY METABOLISM AND GENE EXPRESSION.

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The biochemical composition of grapevine berry is a major determinant of wine quality. This composition depends on complex interactions between the genotypes of the rootstock and the scion, vineyard management and environmental factors, which affect both fruit development and metabolism. Climate change affectsthe physiologyof the vine, yield andfruit composition, and ultimatelythe qualityand typicity ofwines. In this context, a better understanding of the physiological mechanisms and the genetic background underlying the interactions between the grapevine plants and the environment is needed, and this will be helpful for the development of sustainable viticulture under changing environmental conditions. Using field experiments, we studied the effects of two water regimes (natural rainfall or moderate water stress obtained by covering the soil with a plastic sheet) on berry composition of *Vitis vinifera* L. cv. Pinot noir grafted on two different rootstocks (110R, drought-tolerant and 125AA, drought-sensitive). We integrated the analysis of primary (sugars, organic acids, amino acids) and secondary (anthocyanins, flavonols) metabolisms, as well as transcriptomic analysis with the 30 K Nimblegene microarrays. Biochemical analysis were made at 4 developmental stages, over 3 years (2009, 2010 and 2011), with 3 biological replicates. Transcriptome analysis were made with berries collected at 50 and 100 % veraison, for 2 years (2009 and 2010) and with 3 biological replicates. Water stress did not significantly affect berry weight and sugar content. However, water limitation, especially at véraison, caused a substantial increase of anthocyanins, among which malvidin-3-0-glucoside was the most affected, whatever the rootstock. The berry amino acid content at harvest depends on interactions between water supply and rootstocks. Transcriptomic analysis indicate that water stress affects the expression of stress-related gene families, as well as particular genes encoding for enzymes of the amino acid, organic acid and flavonoid pathways.

P36.

GENETIC MAPPING OF RIPENING TIME TRAITS IN GRAPEVINE

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Grapevine plants (*Vitis* spec.) provide the basis of viticulture, a particular branch of agriculture with long history and tradition. Current breeding efforts aim to improve quality and pathogen resistance of European *Vitis vinifera* cultivars for more sustainable viticulture. Nowadays marker-assisted selection is employed to follow the introgression of relevant genomic regions carrying genetic factors affecting traits of interest. Besides resistance characteristics, phenological traits recently catch elevated attention. This is due to climatic changes that make the breeders wish to be able to select grapes with a better defined "window" of ripening time during the yearly growing season. Ripening behavior also affects pathogen susceptibility and therefore influences quality. In grapevine the maturation phase of fruit ripening is accompanied by cell wall softening. The transition point when small, hard green berries start to turn soft is defined as "Véraison". The time of this physiological change can be tactually screened and serve as an indicator of fruit maturation. To identify genomic regions carrying genes affecting ripening time we constructed a genetic map using 151 individuals from the cross of grapevine breeding line Gf.Ga-47-42 (maternal genotype, `Bacchus´x `Seyval´) with `Villard blanc´ (paternal genotype, Seibel 6468 x Seibel 6905). Both parental lines differ considerably in ripening time. While Gf.Ga-47-42 is rather early, `Villard blanc´ exhibits middle to late ripening. The trait segregates considerably in the cross population and was repeatedly scored over six growing seasons. Recombination analysis used newly developed SNP markers analyzed with two different technologies. An overlapping SNP marker set was employed to verify the reliability of both techniques. Furthermore a comprehensive set of microsatellite polymorphisms was used, many of these loci newly developed based on the *Vitis* reference genome sequence of PN40024 (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/). The resulting map was then scanned for QTL influencing the time of "Véraison" and other traits. Results will be presented.