Genetic dissection of grape berry ripening and composition

Sara Zenoni
Fruit ripening

Fruit is the organ specialized for *seed dispersal* and the transition from unripe to ripe fruit represents a *crucial survival strategy*

- irreversible phenomenon
- tightly coordinated with seed development
- genetically and epigenetically programmed system
  - phytohormone signalling pathways
  - transcription factor networks

**TOMATO**

*model for flesh fruit ripening*  
*climateric fruit*
Grape berry ripening

- non climateric fruit
- very long ripening, almost 3 months
- strongly affected by environment
- ripening in the grape berry originates in pulp near the stylar end
- the onset of ripening is characterized by an accumulation of specific reactive oxygen species (ROS)
Chemical and physiological changes during berry development

- Cell division
- Cell expansion
- Water accumulation
- Dry mass accumulation
- Structural C accumulation
- Slow K accumulation
- Rapid sugar accumulation
- Rapid K accumulation
- Cell dehydration
  - Water loss
  - Dry mass loss
  - Concentration of existing sugars
  - Concentration of existing K

**Stage 1**
Growth phase

**Stage 2**
Lag phase

**Stage 3**
Ripening & growth phase

- Sugar
- Potassium
- Water

**Stage 4**
Senescence phase

- Berry Mass
- Organic acid/anion accumulation

- Growth changes
- Colour changes
- Flavour & aroma changes
- Softening
- Malic acid degradation

- Loss in cell vitality
- Volume loss

**Days after flowering**

Xylem flow
Phloem flow
Symplastic unloading
Apoplastic Unloading
Respiration

Rogiers et al., 2017
Large-scale transcriptional changes during berry development

DAVIES AND ROBINSON

PILATI et al., DELUC et al.,

GRIMPLET et al.,

TERRIER et al., WATERS et al.,

BURGER et al.,

LUND et al.,

ZAMBONI et al., ZENONI et al.,

FORTES et al.,

GUILLAUME et al.,

SWEETMAN et al., LIJAVETZKY et al.,

AGUDELO-ROMERO et al.,

DAL SANTO et al.,

RINALDO et al.,

PALUMBO et al., CRAMER et al.,

MASSONNET et al.,

GHAN et al.,

DAL SANTO et al.,

FASOLI et al.,

JAILLON et al.,

GRAPEVINE GENOME SEQUENCING


CONSTANT IMPROVEMENT OF TRANSCRIPTOMIC PLATFORMS

cDNA-AFLP macroarrays microarrays RNA-seq

Genome-wide transcriptome profiling Bioinformatic tools System biology approaches

GRAPEVINE GENOME SEQUENCING

JAILLON et al.,
Identification and definition of "GRIP"
grape ripening-induced protein
Large-scale transcriptional changes during berry development

Expression profile of the principal molecular events during berry development
THE SHIFT FROM THE GROWTH TO RIPENING PHASE IN BERRY INVOLVES A PROFOUND TRANSCRIPTOMIC REARRANGEMENT
Large-scale transcriptional changes during berry development

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DAL SANTO et al.,
FASOLI et al.,

Transcriptomic changes during berry development in **pulp and skin separately**
Ripening program is anticipated in pulp

Lijavetzky et al., 2012
Large-scale transcriptional changes during berry development

RNA-seq approach to dissect the transcriptional complexity during berry development
Ripening transcriptomic program in red and white grapevine varieties

5 white varieties:
- Vermentino
- Garganega
- Moscato bianco
- Glera
- Passerina

5 red varieties:
- Sangiovese
- Barbera
- Negro amaro
- Refosco
- Primitivo

Massonnet et al., 2017
Number of expressed genes and biomarkers

THE NUMBER OF EXPRESSED GENES DECREASES DURING BERRY DEVELOPMENT

BIOMARKERS OF BERRY DEVELOPMENT STAGES AND PHASES WERE DEFINED
Core transcriptomic traits during berry development

Massonnet et al., 2017

CORE TRANSCRIPTOMIC TRAITS WERE PROFLED
Relation with anthocyanin accumulation and ripening progress at transcriptional level.

TRANSCRIPTOMIC PROGRAM OF FRUIT RIPENING SEEMS MORE DIRECTLY RELATED TO ANTHOCYANIN ACCUMULATION RATHER THAN SUGAR CONTENT

Massonnet et al., 2017
Relation with anthocyanin accumulation and ripening progress at transcriptional level

About **6000** genes are responsible for transcriptional differences among red varieties at harvest.

**ANTHOXYANIN LEVELS MAY INFLUENCE MANY OTHER PROCESSES**
- **INCREASE OF SKIN OPACITY TO SUNLIGHT**
- **INVOLVEMENT IN SIGNALLING PATHWAYS NOT YET DESCRIBED**

**Secondary metabolic process**
- Transport
- Carbohydrate metabolism
- Transcription factor activity

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**Syrah**
Rinaldo et al., 2015

Massonnet et al., 2017
Correlation of gene expression between genotypes increases as haplotype distance decreases

- Sequencing of ten genomes
- Pairwise comparison to define the haplotype distance
- Identification of local IBD (segment identical by descent)

Shared chromosome segments between ‘Garganega’ and ‘Passerina’

Two shared haplotypes IBD2
One shared haplotype IBD1
No shared haplotypes IBD0

Shared chromosome segments between ‘Passerina’ and ‘Vermentino’

Magris et al., unpublished
Correlation of gene expression between genotypes increases as haplotype distance decreases

- Genes for which the pair of individuals are IBD0 (Sharing 0)
- Genes for which the pair of individuals share one haplotype (IDB1-Sharing 1)
- Genes for which the pair of individuals share two haplotypes (IDB2-Sharing 2)

Correlation of transcript expression level

Fraction of non differentially expressed genes

Developmental stages

HAPLOTYPE SHARING ACCOUNTS FOR CORRELATION OF GENE EXPRESSION

Magris et al., unpublished
Large-scale transcriptional changes during berry development

DAVIES AND ROBINSON

PILATI et al.,

DELUC et al.,

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TERRIER et al.,

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GHAN et al.,

TERRIER et al.,

WATERS et al.,

BURGER et al.,

Large-scale transcriptional changes during berry development.

Highly Variable Plasticity, strong Genotype-by-Environment Interaction.
Molecular dissection of the grapevine GXE interaction

Changes in performance of genotypes in different environments are defined as genotype X environment (GXE) interaction
Molecular dissection of the grapevine GXE interaction

Data Mining Pipeline

Screening
- Whole Transcriptome → 18122 genes

Profiles definition
- K-means clustering → 300 clusters

Profiles characterization
- Variable Importance Measure (VIM)
  measure of how each variable affects the expression
  STAGE
  GENOTYPE
  YEAR
  AREA

ANOVA 2-way (p<0.01)

Year
- Cabernet Sauvignon: 5324, Sangiovese: 5449

Area
- Cabernet Sauvignon: 230, Sangiovese: 834

Interaction
- Cabernet Sauvignon: 445, Sangiovese: 418

Genes

SANGIOVESE RESULTED MORE RESPONSIVE THAN CABERNET SAUVIGNON

Dal Santo et al., 2018
Variable-specific clusters

Median VIM of each Variable

Photosynthesis

R-proteins

Dal Santo et al., 2018
GxE clusters are enriched in secondary metabolism, signal transduction and abiotic stress response.

IDENTIFICATION OF SEVERAL CANDIDATE GENES THAT COULD BE USED AS MARKERS OF BERRY QUALITY TRAITS IN GxE INTERACTIONS

Dal Santo et al., 2018
Large-scale transcriptional changes during berry development

Expression profile of the principal molecular events during postripening phase
Transcriptional changes during berry post-harvest

Days after harvest

% of initial berry weight

~30% weight loss

Zenoni et al., 2016
Transcriptional changes during berry post-harvest

DURING THE POST-HARVEST PHASE THERE IS AN ACTIVE METABOLIC REARRANGEMENT AND NOT ONLY A PASSIVE CONCENTRATION

Zenoni et al., 2016
What triggers the ripening transition?
Berry softening is one of the earliest ripening events.
Identification of putative regulators of ripening transition

Grapevine expression atlas as a starting point

**THE PROFOUND TRANSCRIPTOMIC REARRANGEMENT IN BERRY FROM IMMATURE TO MATURE PHASE WAS OBSERVED FOR ALL GRAPEVINE PLANT ORGANS**

54 samples representing different plant organs during development

Fasoli et al., 2012
Identification of putative regulators of ripening transition

Differentially expressed genes between vegetative/green and mature/woody samples

During the transition to mature phase many processes are inhibited rather than activated.

Topological properties and roles were analysed.

Palumbo et al., 2014
Identification of switch genes

Heat cartography map

SWITCH GENES

• interact highly with genes outside their own module
• interact poorly with genes inside their own module
• mainly anti-correlated with their partners

Palumbo et al., 2014
Switch genes model of action

- All switch genes are down regulated during growth phase and up during mature phase.
- Switch genes could act as an electric switch able to switch-off the expression of vegetative-related genes and to switch-on the expression of mature-related genes.

Palumbo et al., 2014
Switch genes in red and white berries

Massonnet et al., 2017
# Transcription factors among switch genes

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Massonnet et al., 2017
Two transcriptional transition mark the onset of ripening

Weekly sampled
Three vintages

VERAISON

PC2 (12.0%)

PC1 (34.0%)

FIRST TRANSITION
SECOND TRANSITION

Cabernet sauvignon
Pinot noir

Fasoli et al., 2018 in press
Identification of markers of the onset of ripening

Positive and negative markers of the two transitions define important transcriptional changes during the two weeks before verasion.

POSITIVE MARKERS OF THE FIRST TRANSITION SEEM TO PLAY A MAJOR ROLE AS TRIGGERS
Switch genes are positive markers of the two transitions

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Functional analysis of VvNAC33 and VvNAC60

- **35S VvNAC33 TER Prom eGFP TER**
- **35S VvNAC60 TER Prom eGFP TER**

> Embryogenic calli

- **Transient expression after 48 h**

- **Stable GFP expression after 2-3 months**

Greenhouse and phenotypic analysis after 1-2 years

In vitro plantlets

Transgenic plantlets
Overexpression of VvNAC33 in transgenic Syrah

CTRL	A4	A5	A8

2,4	2,8	3,2	3,6

µg Chl/cm²

CTRL	A4	A5	A8

Chl/Car

D’Incà, unpublished
Overexpression of VvNAC60 in transgenic Syrah

CTRL  35S::VvNAC60

Clear senescence symptoms

Poster P119 of Chiara Foresti

Poster P148 of Edoardo Bertini

“IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF MASTER REGULATORS OF THE ONSET OF BERRY RIPENING IN GRAPEVINE”

ChIP-Seq analysis of VvNAC60

“REGULATORY NETWORK BEHIND THE BERRY RIPENING: THE ROLE OF VITIS VINIFERA NAC60 TRANSCRIPTION FACTOR”
Transcriptomic analysis of VvNAC33 and VvNAC60 overexpressed plants

VvMYB15
4 ERF/AP2 genes
3 Methyl jasmonate esterases

VvMYBF1
VvMYBPA1
16 Auxin responsive proteins
3 Auxin induced proteins
17 Ankyrins
4 Calmodulin binding proteins

VvMYBA1p:LUC
Alessandra Amato

VvMYBA1
VvMYBA2
Anthocyanin permease (VvAnthoMATE1)

VvMYB14
2 STs
Trans-resveratrol di-O-methyltransferase - VvROM
6 ERF/AP2 genes (VvERF075)
Lateral organ boundaries proteins (LOB1,LOB39)
3 Nitrate transporters

VvNCED1
5 XHTs
MADS-box Agamous 1 (VvAG1)
MADS-box delta 2b (VvMADSD2b)
5 NACs (VvNAC11 and VvNAC26)

VvMYBC2-L2
6 Gibberellin-regulated proteins (GASA4)
Auxin responsive/induced proteins
Vegetative storage protein

5 MADS-boxes Short Vegetal Phase (SVPs)
5 Photosystem reaction centers
4 Light-harvesting chlorophyll binding (LHCB) proteins
Accessible germplasm resources
Simple diploid genetics
Efficient greenhouse propagation
Short life cycle
Ease of transformation
Recombinant inbred lines
High-quality genome sequence
Natural mutants of ripening

- **developmental window** in which fruit responds to ethylene that corresponds to seed maturation;
- fruit tissues do not mature uniformly;
- a core set of **ripening regulators** has been defined, including **RIN-MADS**, **NOR-NAC**, **CNR-SPL** and **TAGL1**;
- **hypomethylation** contributes towards the start of ripening regulatory cascade

Tomato NOR mutant

Growth phase
- cell division + cell expansion

Ripening phase

Pollination
0
7
17
27
Immature fruit

39
42
47
52
57
dap

MG
BK
BK+10

Wild type

nor/nor

Ethylene production impaired
Ripening strongly inhibited
VvNAC33 and VvNAC60 partially complement the NOR function
VvNAC60 induces ethylene biosynthesis

Wild type

nor/nor

35S::VvNAC33  #1

35S::VvNAC60  #1

Ethylene (nL/gfw/h)

WT

nor/nor

VvNAC33

VvNAC60

“FUNCTIONAL COMPLEMENTATION OF non-ripening (nor) TOMATO MUTANT WITH FOUR NAC TRANSCRIPTION FACTORS, PUTATIVE MASTER REGULATORS OF THE VEGETATIVE-TO-MATURE ORGAN TRANSITION IN GRAPEVINE”
### VvbHLH075 and VvWRKY19

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<td>VIT_08s0007g07670</td>
<td>NAC domain-containing protein</td>
<td>VvNAC60</td>
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<td>VIT_07s0005g01710</td>
<td>WRKY Transcription Factor</td>
<td>VvWRKY19</td>
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<td>VIT_05s0020g04730</td>
<td>Zinc finger (C3HC4-type ring finger)</td>
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<td>VIT_08s0040g01950</td>
<td>Zinc finger (C3HC4-type ring finger)</td>
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<td>VIT_18s0001g01060</td>
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<td>VIT_03s0091g00260</td>
<td>Zinc finger protein 4</td>
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Functional analysis of VvbHLH075 and VvWRKY19

The YFP fluorescence is mainly localized in the first and second leaves.

Transcriptomic analysis

Thompson seedless plants grown in vitro

First leaf
Second leaf

First leaf
Second leaf

35S YFP Term 35S WRKY19 Term
35S YFP Term 35S BHLH75 Term

d.p.i. 7

Edoardo Bertini

Fasoli et al., 2018 in press
Putative targets of VvbHLH075 and VvWRKY19

Fasoli et al., 2018 in press
Putative hierarchy of transcription factors in the onset of berry ripening

- bHLH075
- NAC60
- NAC11
- NAC17
- NAC26
- NAC33
- WRKY19
- MYBA2
- MYBA1
- MYB14
- MYBF1
- MYBPA1

Genes:
- NAC17: Marker of the first transition
- NAC11: Marker of the second transition
- MYB14: Stilbenoid synthesis
- MYBA1: Anthocyanin metabolism

Pathways:
- Berry size and shape
- Flavonol synthesis
- Proanthocyanin synthesis
- Switch gene
- Berry ripening
Conclusions

- New technological advancements in gene expression analysis have generated a huge amount of transcriptomic data that needs to be deeply interpreted.
- The intricate transcriptional network of the onset of ripening has been partially disentangled through co-expression and statistical pipelines.
- Biomarkers and putative regulators have been identified.
- Functional studies are needed to understand the role of these candidates in triggering ripening transition.

Next steps

- Identify direct targets
- Investigate role of methylation in berry ripening control
- Characterize potential microRNAs that could control expression of identified regulators
The berries are ripe and my time is finished....

THANKS FOR YOUR ATTENTION