

# Genetic dissection of grape berry ripening and composition



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# 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*

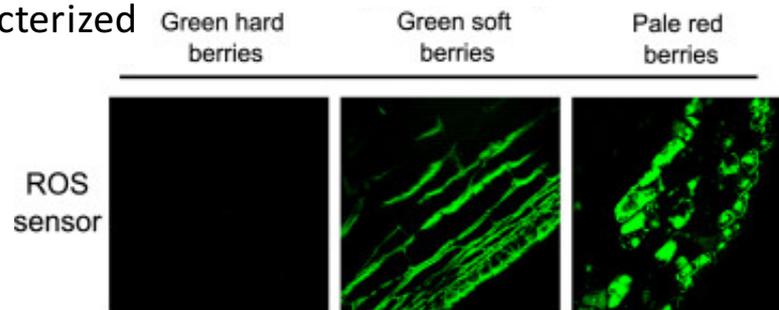
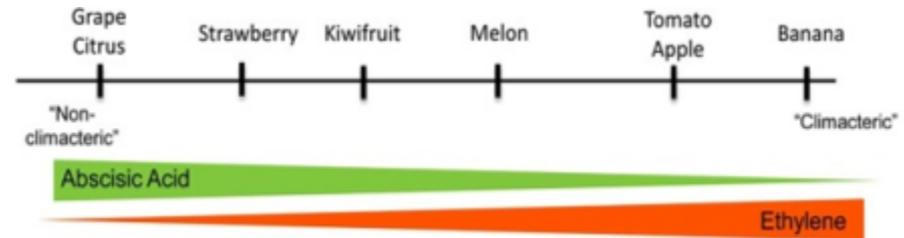
*climateric fruit*



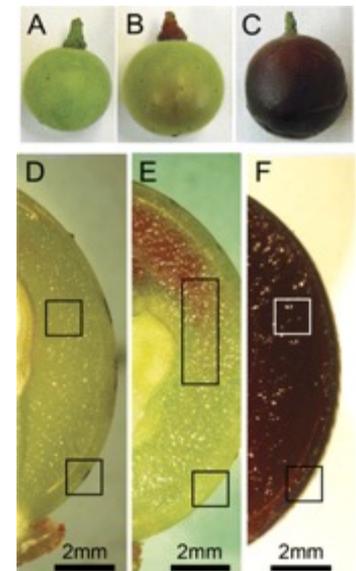
# Grape berry ripening



- non climacteric 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)



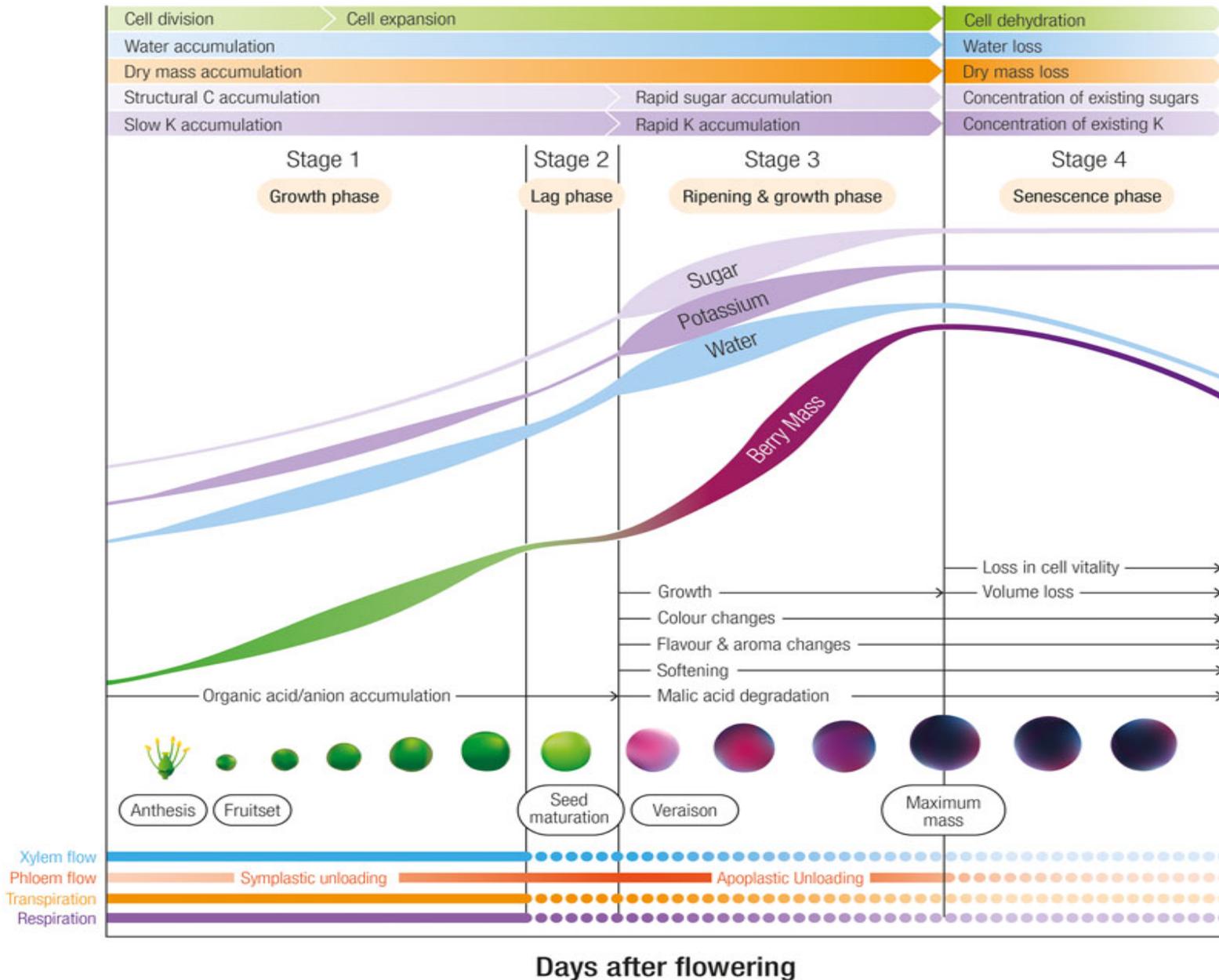
*Pilati et al., 2014*



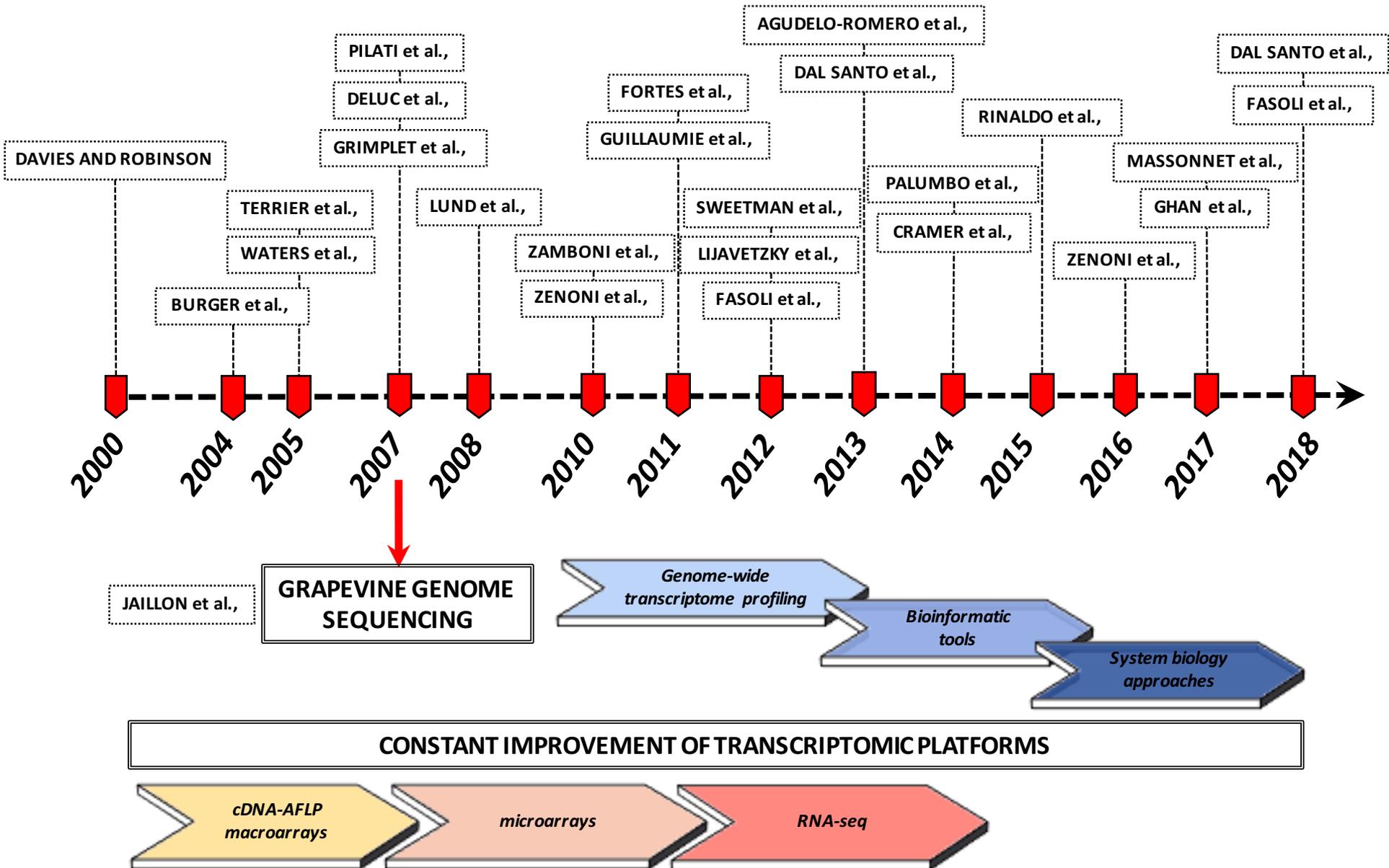
(G)reen (T)ransition (R)ed

*Castellarin et al., 2011*

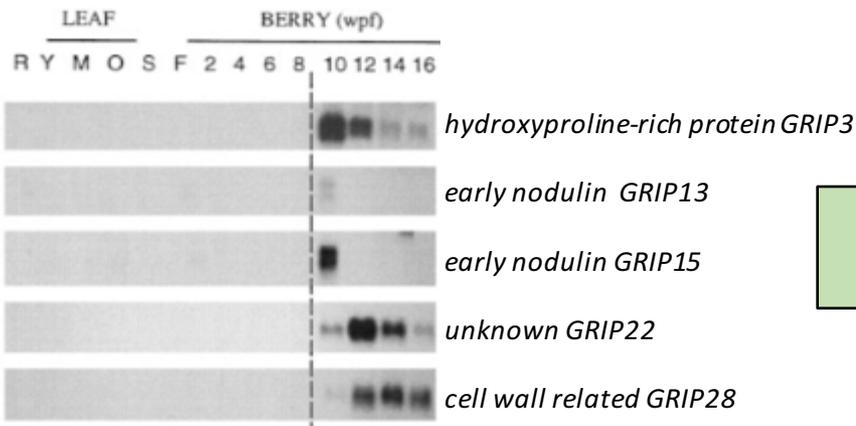
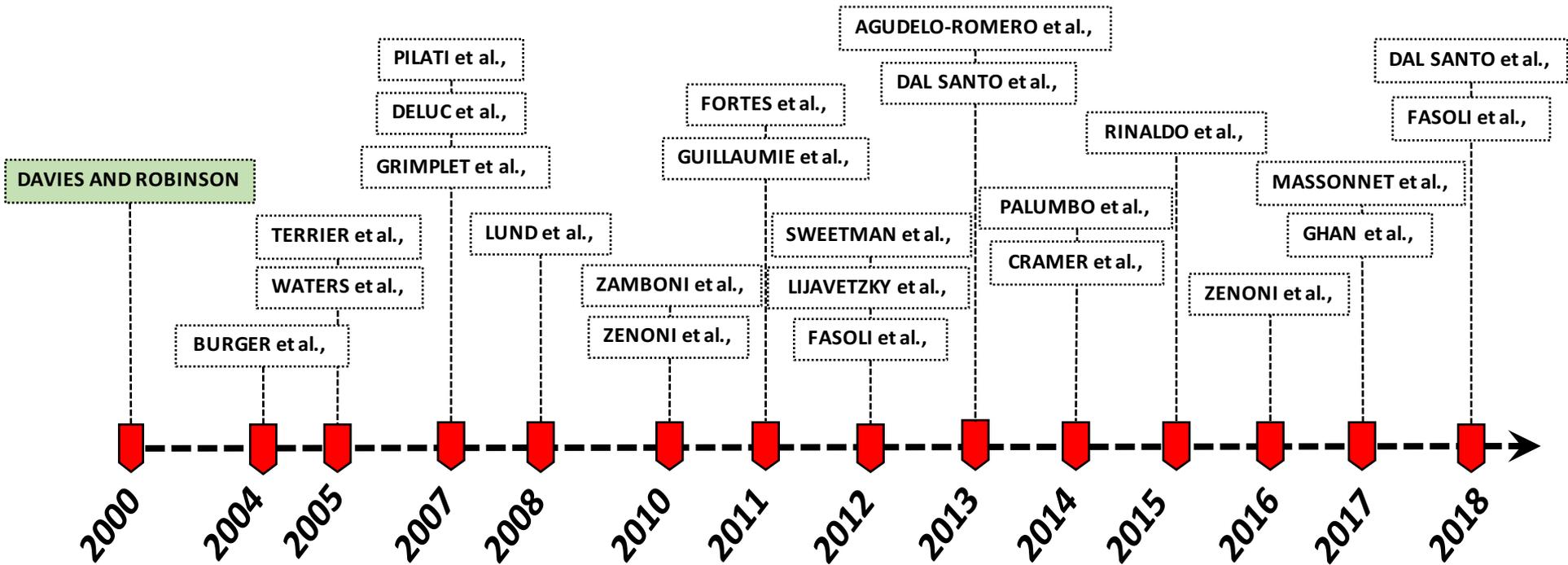
# Chemical and physiological changes during berry development



# Large-scale transcriptional changes during berry development

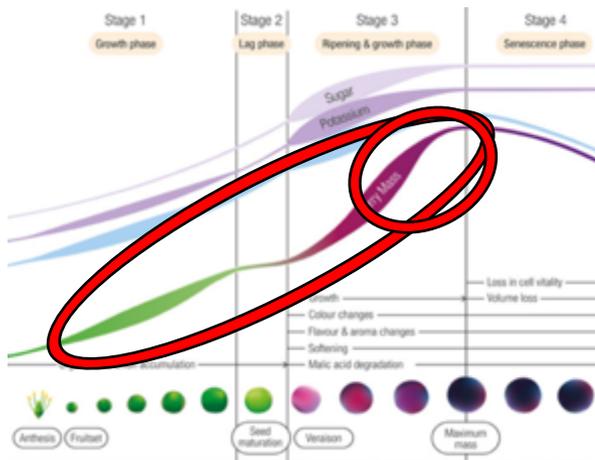
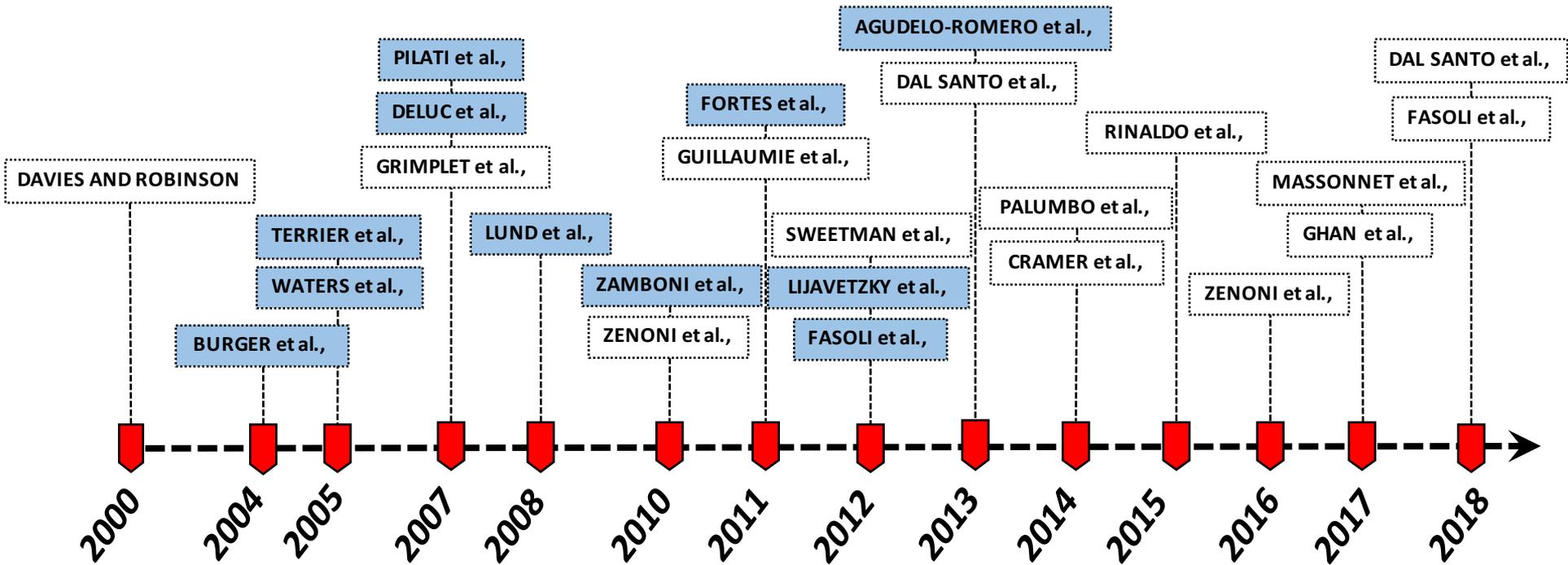


# Large-scale transcriptional changes during berry development



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*

# Transcriptomic rearrangement during the ripening transition

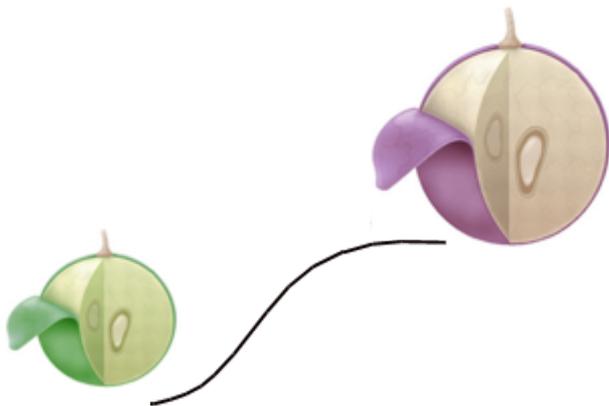
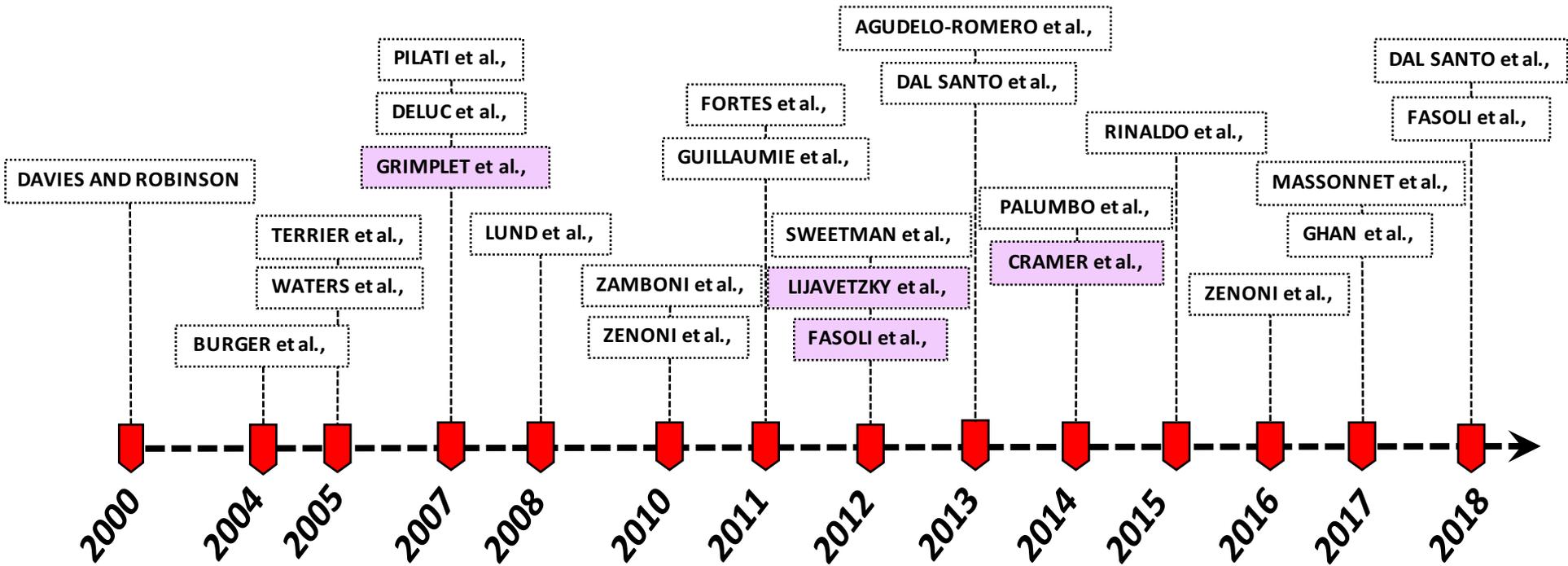
- Photosynthesis
- Cell cycle
- Cellular component organization
- Hormone (auxin) signalling responsive transcripts



- Secondary metabolism
- Sugar metabolism
- Starch degradation
- Regulation of gene expression
- Biotic stress response
- Cell wall metabolism

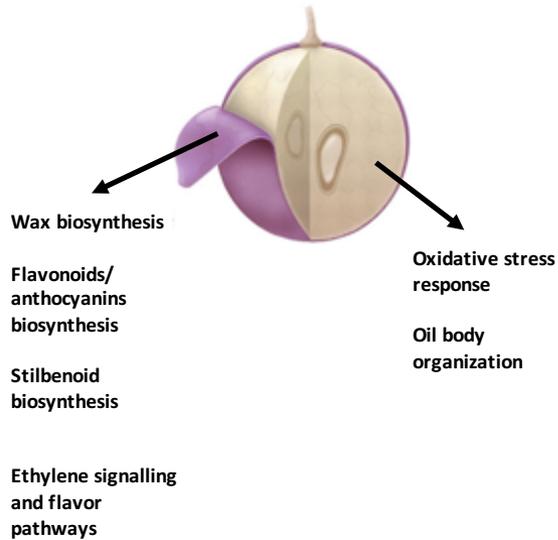
**THE SHIFT FROM THE GROWTH TO RIPENING PHASE IN BERRY INVOLVES A PROFOUND TRANSCRIPTOMIC REARRANGEMENT**

# Large-scale transcriptional changes during berry development

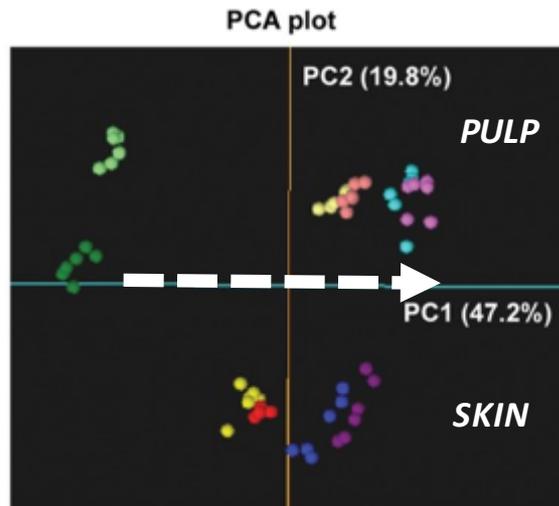


*Transcriptomic changes during berry development in pulp and skin separately*

# Ripening program is anticipated in pulp

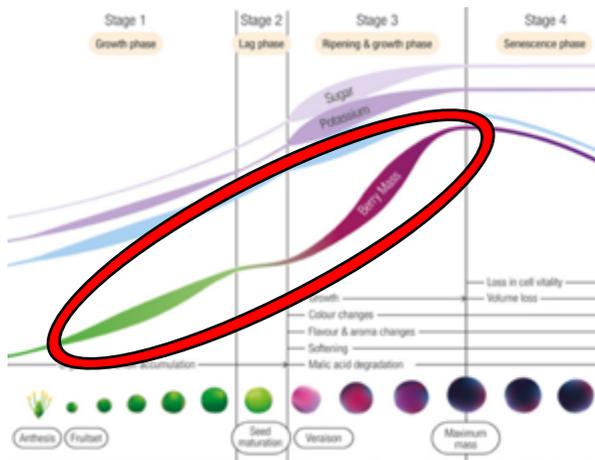
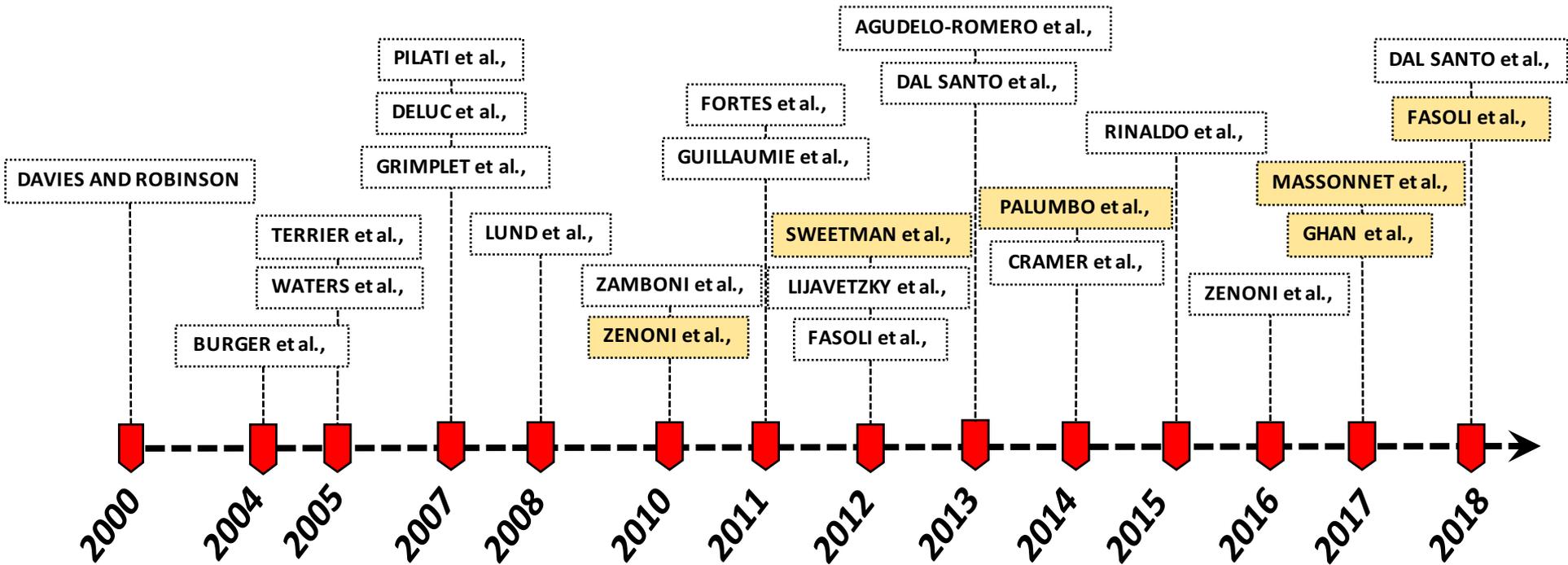


| Functional terms  |   | P      | V1-V2 | R1    | R2    |
|---|---|--------|-------|-------|-------|
| Auxin signalling, bHLH TF, Homeobox TF, GA biosynthesis           | S | Black  | Grey  | Grey  | Grey  |
|   | F | Black  | Grey  | Grey  | Grey  |
| Wax biosynthesis  | S | Black  | Grey  | Grey  | Grey  |
|   | F | Black  | Grey  | Grey  | Grey  |
| ABC transporters, Cellulose biosynthesis, Glycerolipid metabolism | S | Black  | Black | Black | Black |
|   | F | Black  | Black | Black | Black |
| Photosynthesis  | S | Black  | Black | Black | Black |
|   | F | Black  | Black | Black | Black |
| Microtubule organization and biogenesis                           | S | Black  | Black | Black | Black |
|   | F | Black  | Black | Black | Black |
| Flavonoid and anthocyanin biosynthesis, ABC transporters          | S | Black  | Black | Black | Black |
|   | F | Black  | Black | Black | Black |
| C2C2-DOF TF, Macromolecule transport                              | S | Black  | Black | Black | Black |
|   | F | Black  | Black | Black | Black |
| Oxidative stress response   | S | Black  | Black | Black | Black |
|   | F | Yellow | Black | Black | Black |
| Oil body organization and biogenesis, C2H2 TF                     | S | Black  | Black | Black | Black |
|   | F | Yellow | Black | Black | Black |
| Biotic stress response, Xyloglucan modification                   | S | Black  | Black | Black | Black |
|   | F | Black  | Black | Black | Black |
| Stilbenoid biosynthesis, WRKY TF, Macromolecule transport         | S | Black  | Black | Black | Black |
|   | F | Black  | Black | Black | Black |



**TRANSCRIPTOMIC PROGRAM IS ANTICIPATED IN PULP IN COMPARISON TO THE SKIN**

# 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

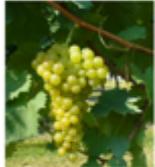
## 5 white varieties



Vermentino



Garganega



Moscato bianco



Glera



Passerina

## 5 red varieties



Sangiovese



Barbera



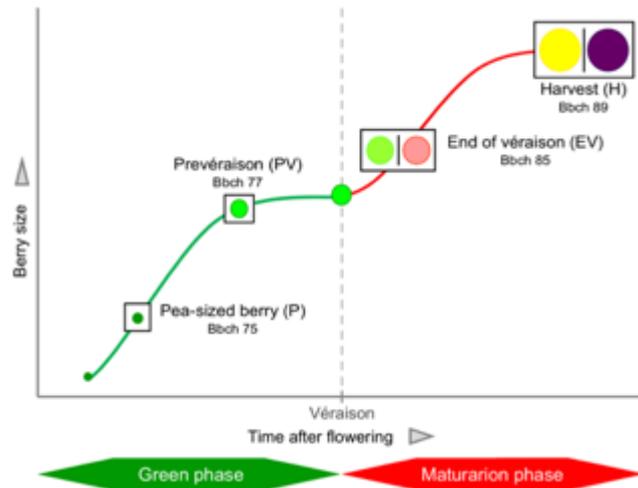
Negro amaro



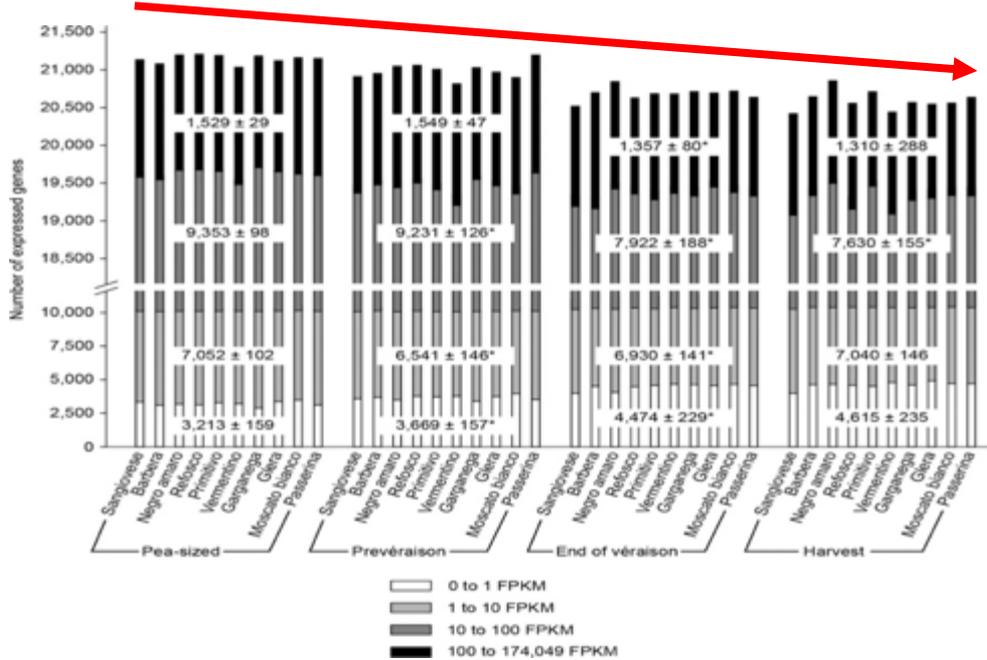
Refosco



Primitivo

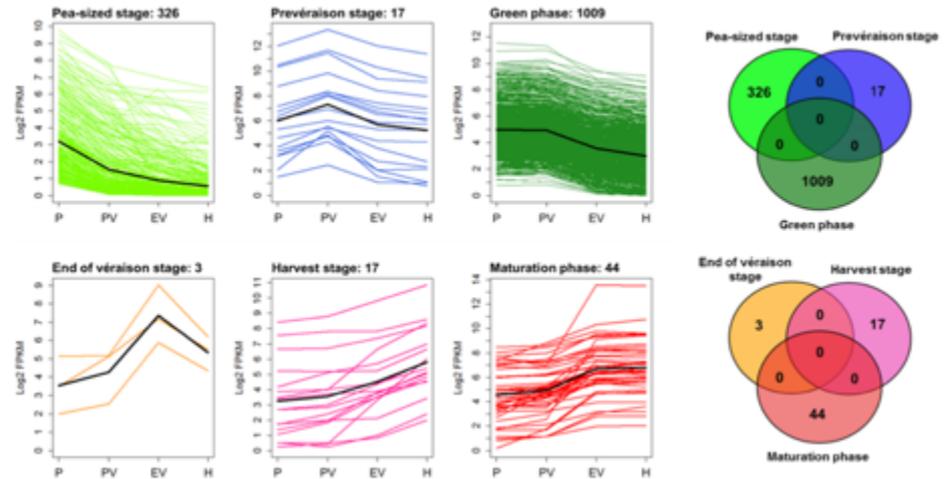


# Number of expressed genes and biomarkers

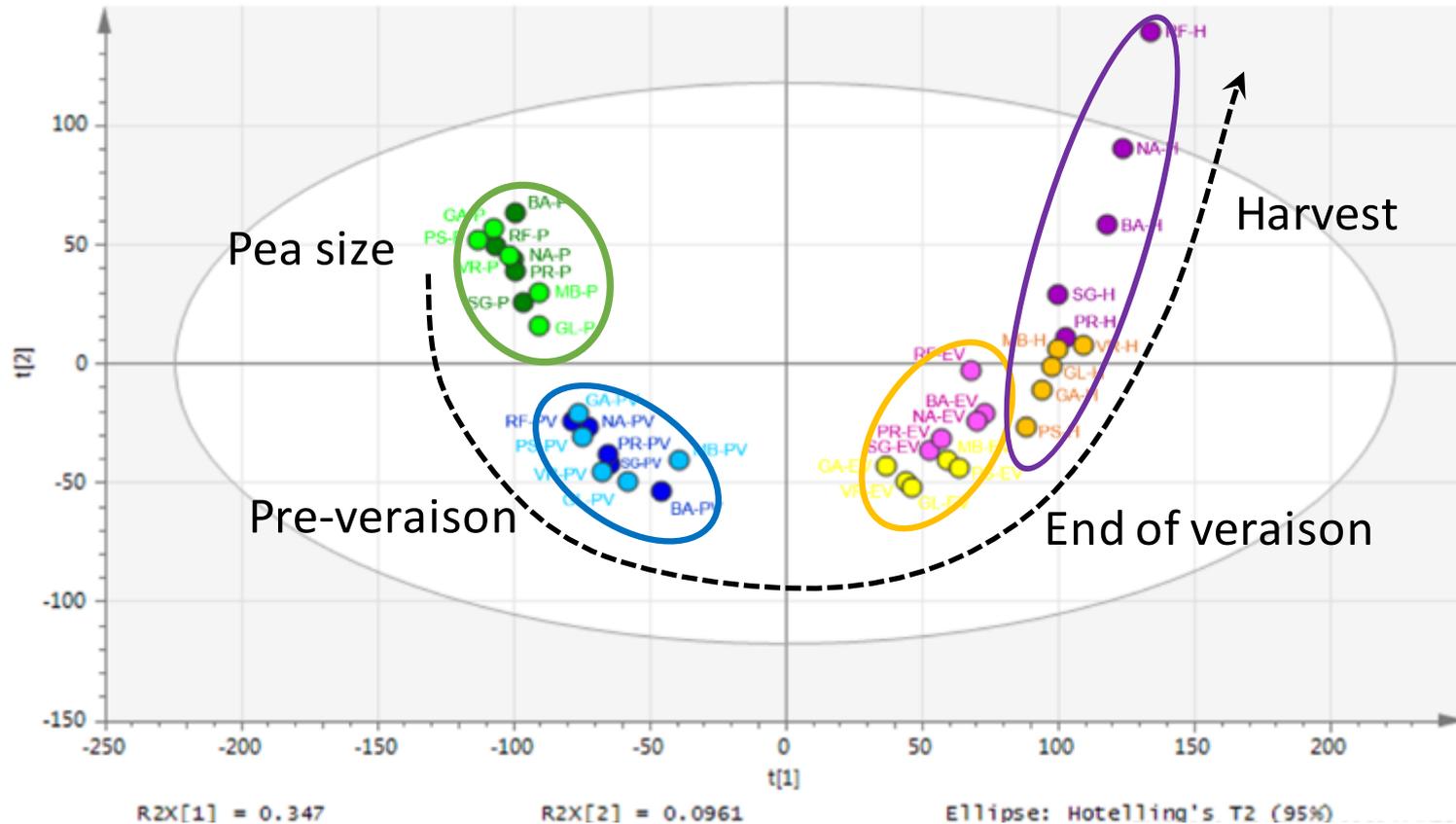


THE NUMBER OF EXPRESSED GENES DECREASES DURING BERRY DEVELOPMENT

BIOMARKERS OF BERRY DEVELOPMENT STAGES AND PHASES WERE DEFINED



# Core transcriptomic traits during berry development



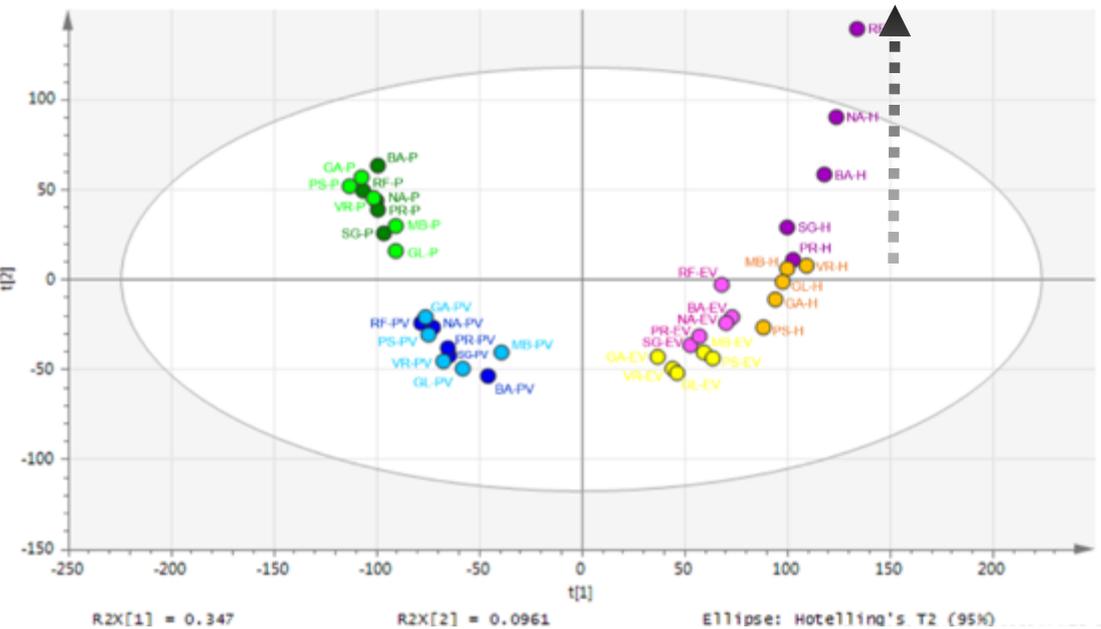
Red varieties      White varieties

- Pea-sized
- Prevéraison
- End of véraison
- Harvest

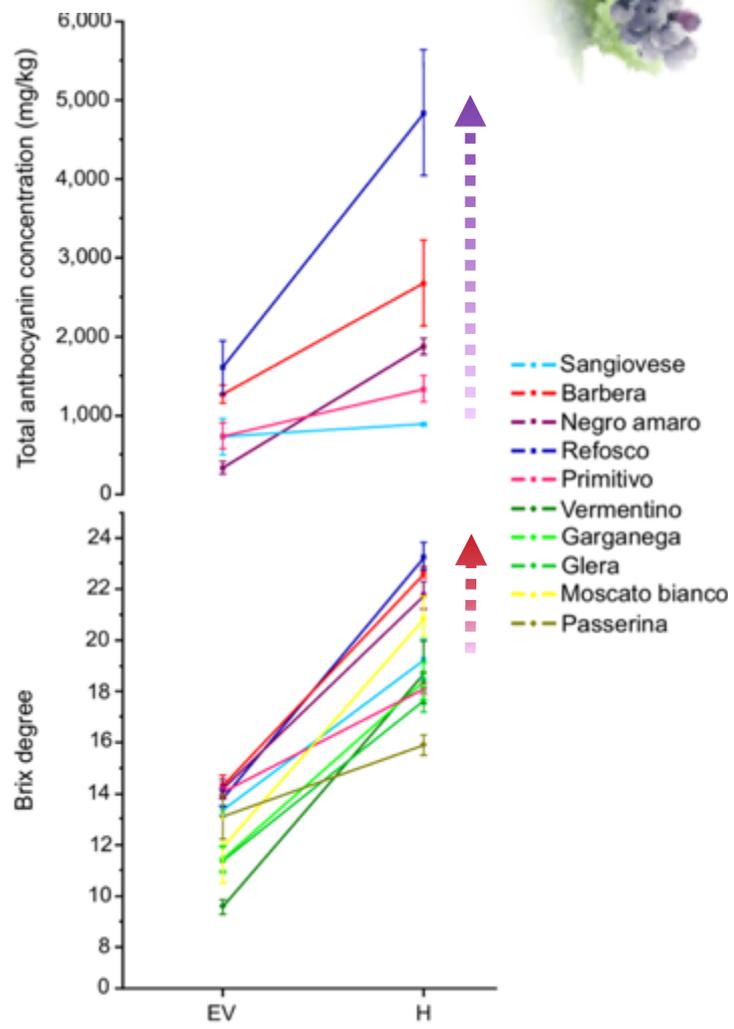
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**CORE TRANSCRIPTOMIC TRAITS WERE PROFILED**

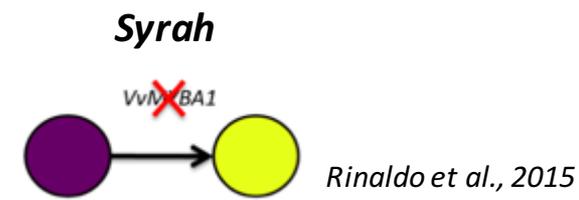
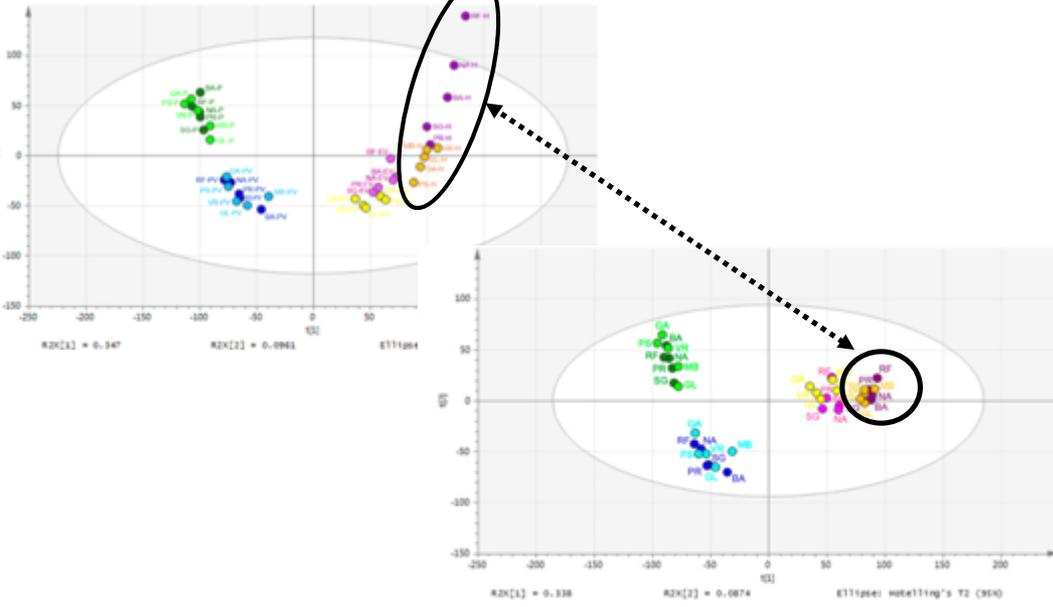
# Relation with anthocyanin accumulation and ripening progress at transcriptional level



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



# Relation with anthocyanin accumulation and ripening progress at transcriptional level



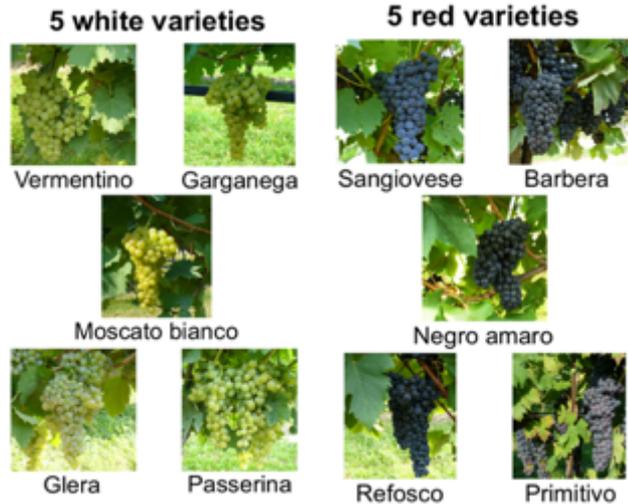
About **6000** genes are responsible for transcriptional differences among red varieties at harvest

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

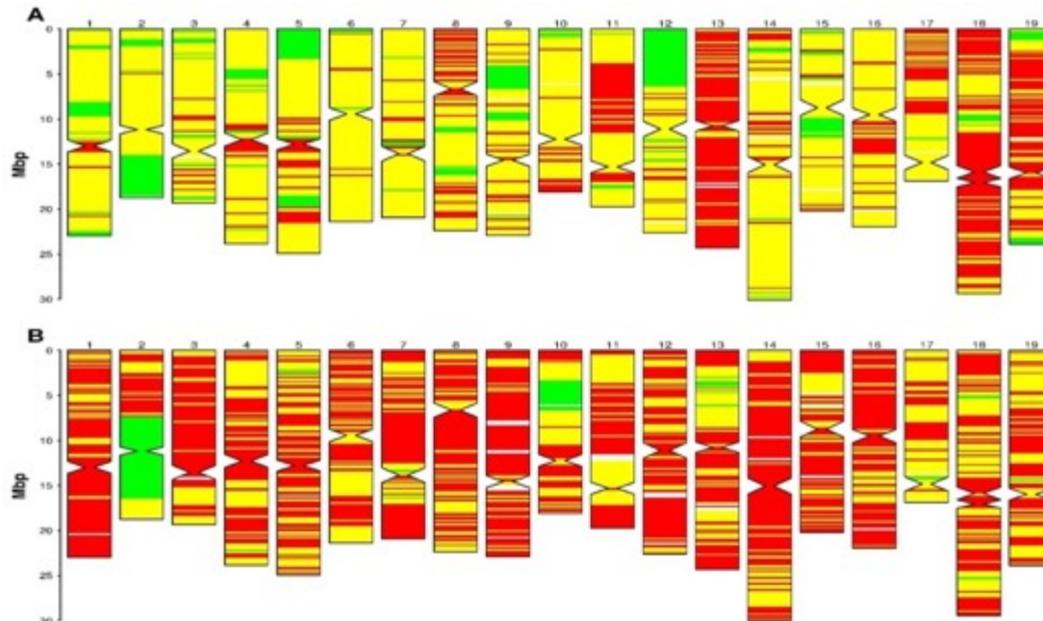
**ANTHOCYANIN LEVELS MAY INFLUENCE MANY OTHER PROCESSES**

- INCREASE OF SKIN OPACITY TO SUNLIGHT
- INVOLVEMENT IN SIGNALLING PATHWAYS NOT YET DESCRIBED

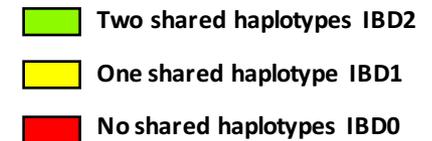
# Correlation of gene expression between genotypes increases as haplotype distance decreases



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



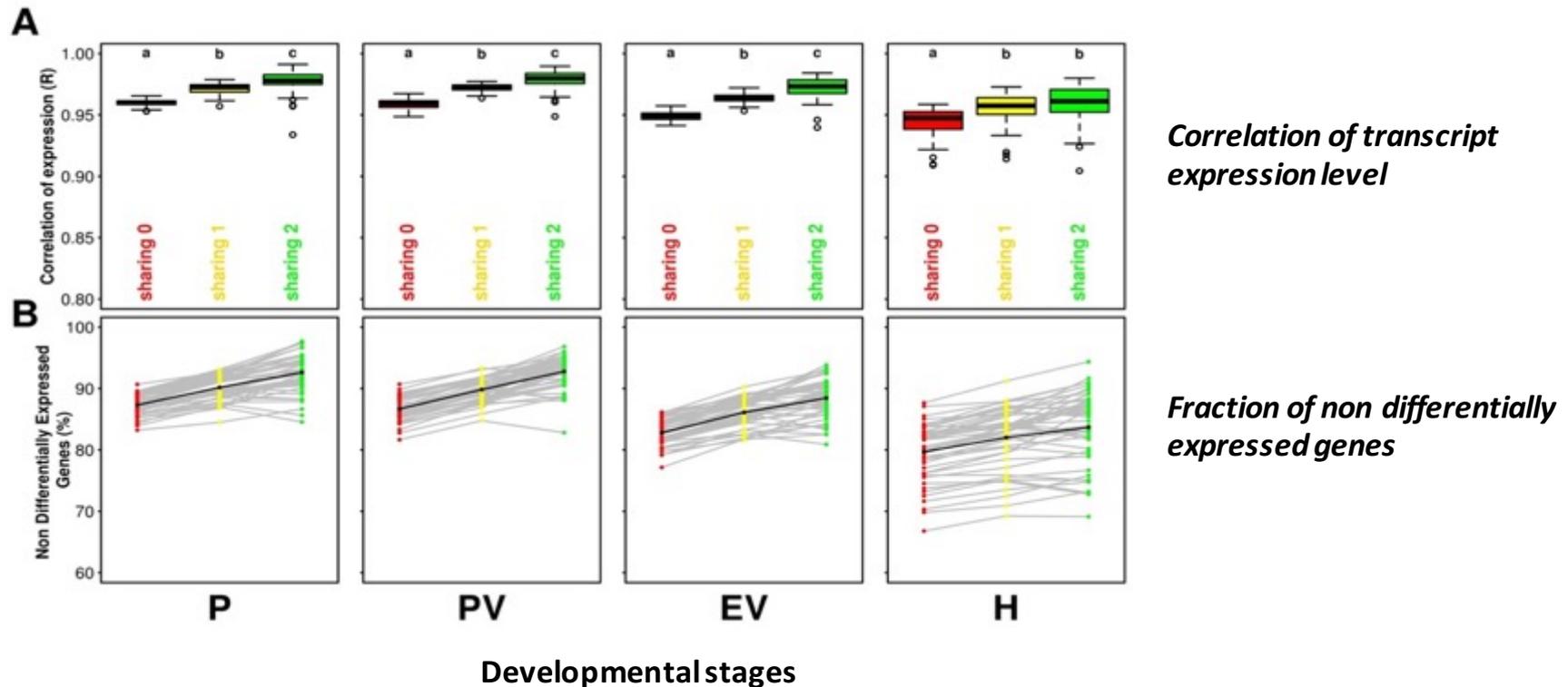
Shared chromosome segments between 'Garganega' and 'Passerina'



Shared chromosome segments between 'Passerina' and 'Vermentino'

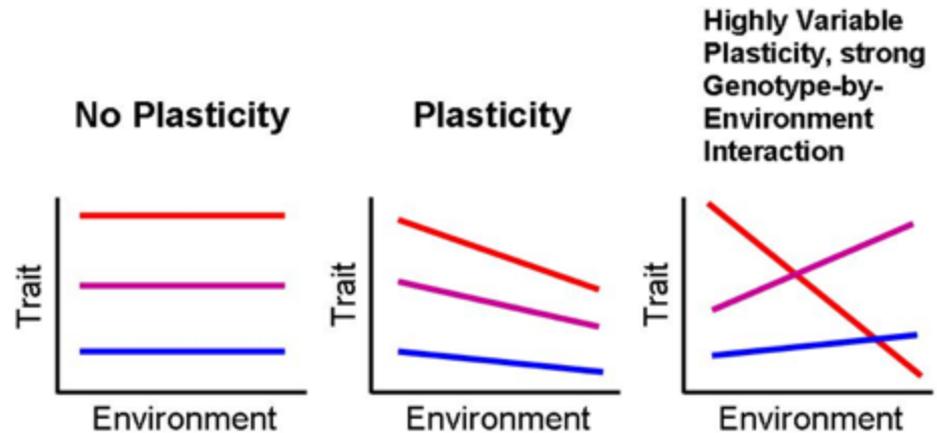
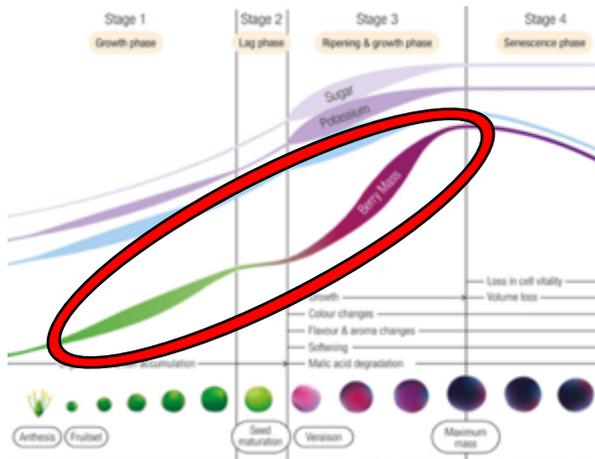
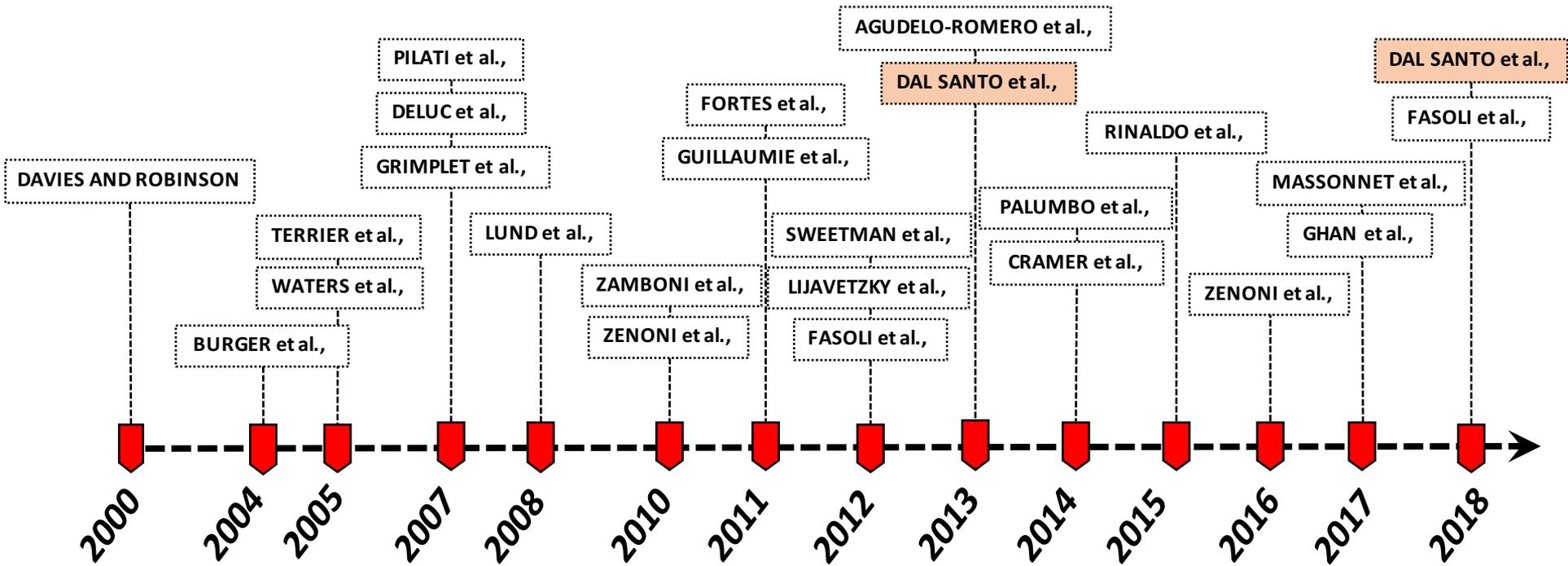
# Correlation of gene expression between genotypes increases as haplotype distance decreases

- 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**)



**HAPLOTYPE SHARING ACCOUNTS FOR CORRELATION OF GENE EXPRESSION**

# Large-scale transcriptional changes during berry development



# Molecular dissection of the grapevine GXE interaction

Changes in performance of genotypes in different environments are defined as genotype X environment (**GXE**) interaction



## 2 Genotypes

Sangiovese and Cabernet sauvignon

## 4 Developmental stages

## 2 Years

## 3 Areas

Adjacent Vineyards per each Area

Pea Size



Pre-Veraison



Mid-Ripening



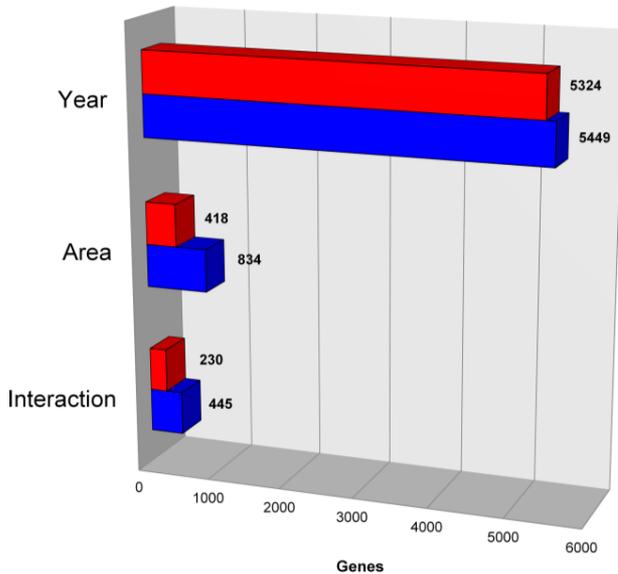
Ripe



*Dal Santo et al., 2018*

# Molecular dissection of the grapevine GXE interaction

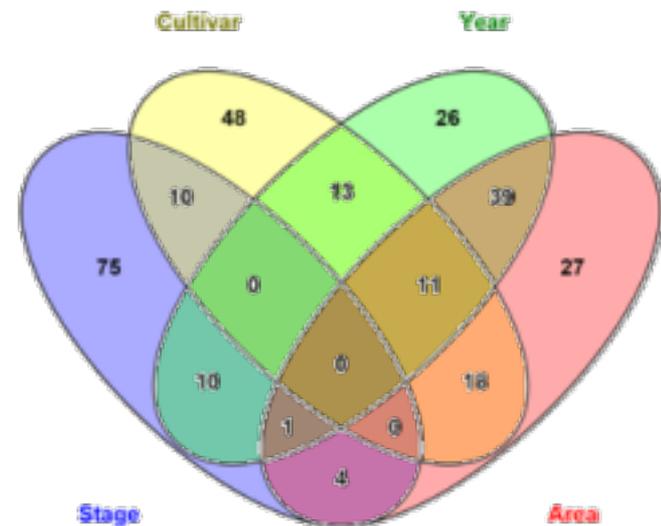
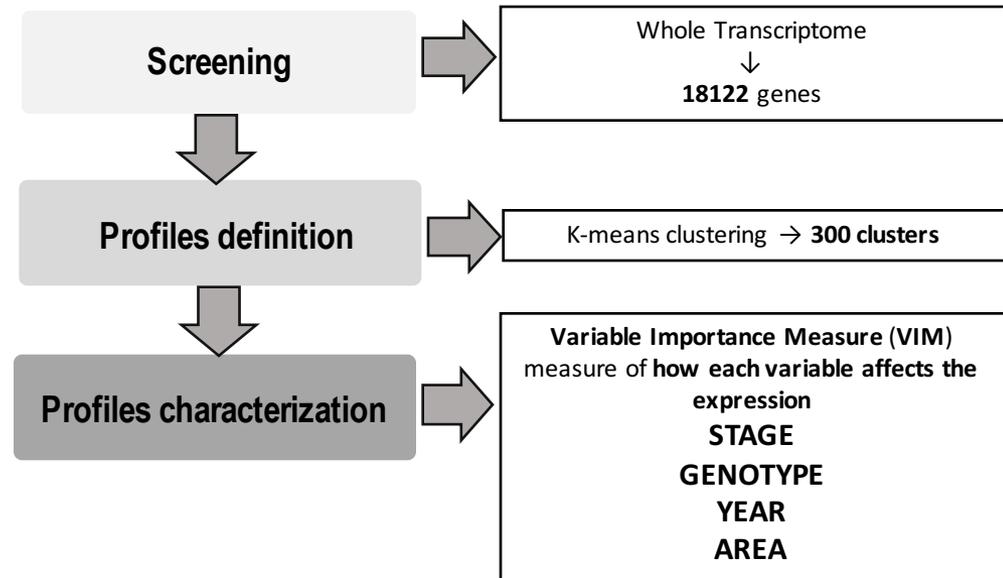
ANOVA 2-way (p<0.01)



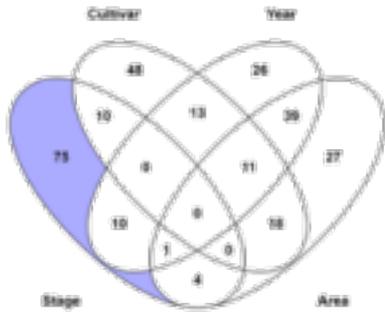
■ Cabernet Sauvignon  
■ Sangiovese

**SANGIOVESE RESULTED MORE RESPONSIVE THAN CABERNET SAUVIGNON**

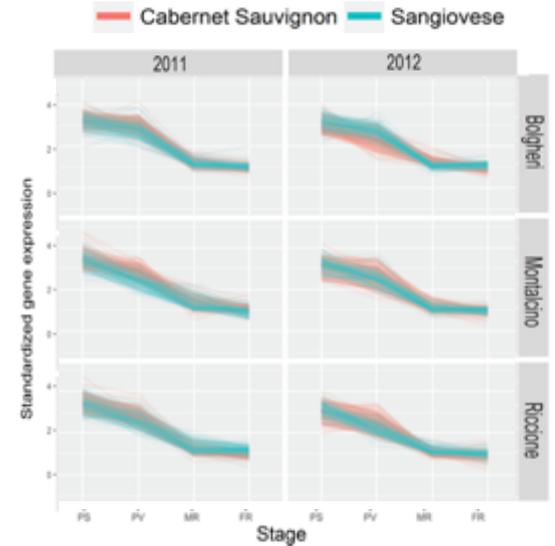
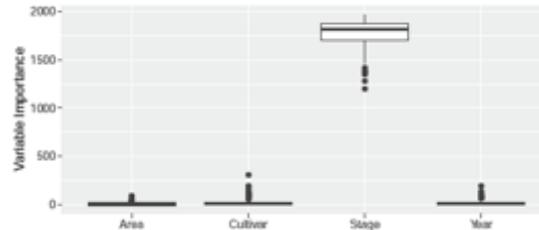
## Data Mining Pipeline



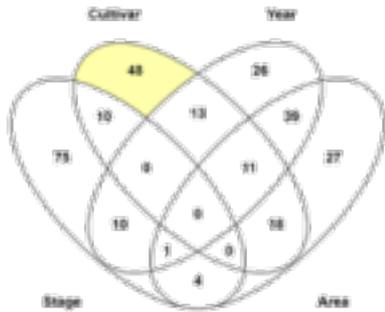
# Variable-specific clusters



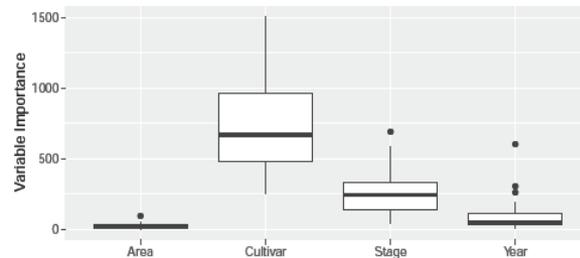
Median VIM of each Variable



Photosynthesis

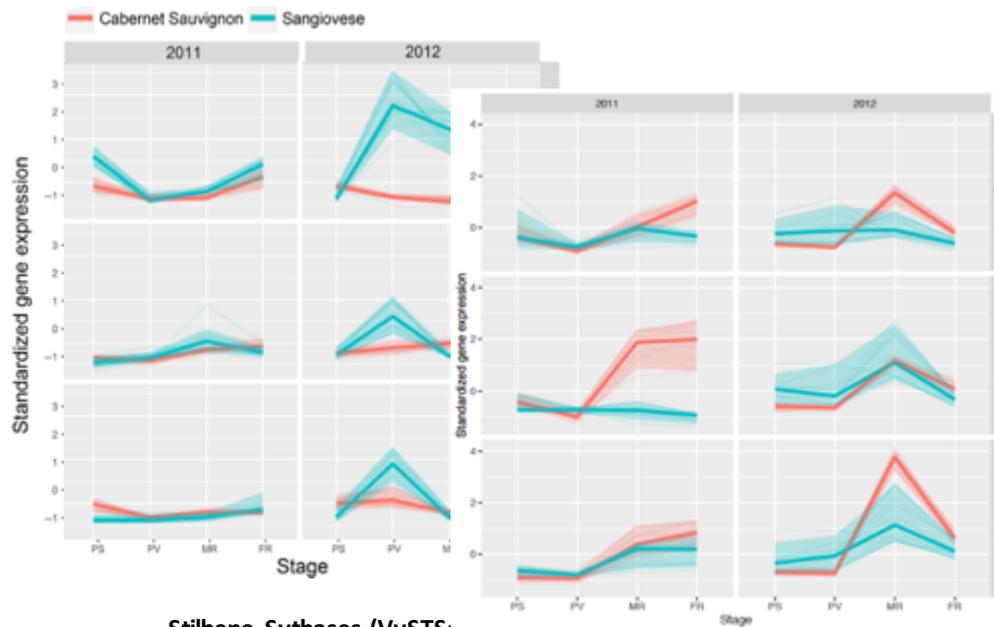
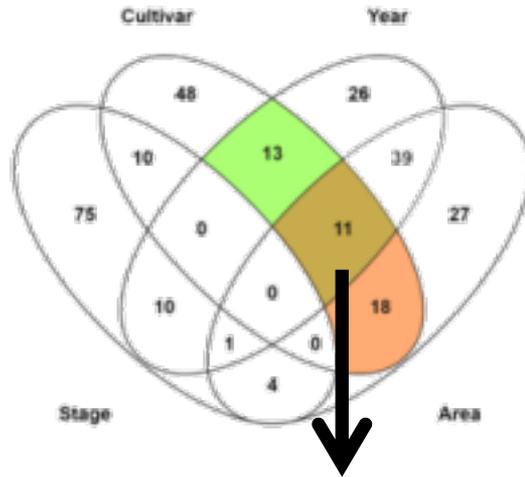


Median VIM of each Variable



R-proteins

# GxE clusters are enriched in secondary metabolism, signal transduction and abiotic stress response

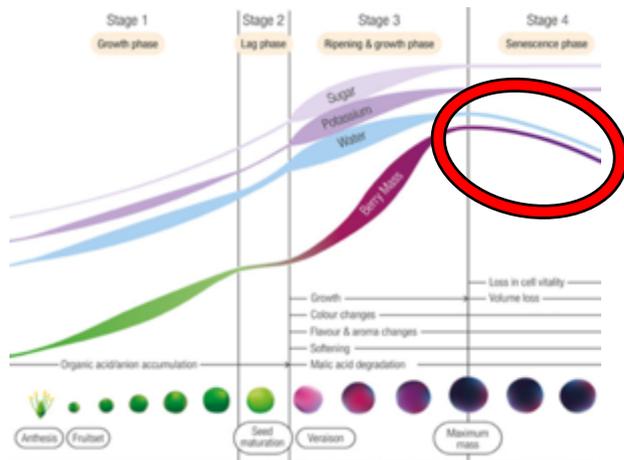
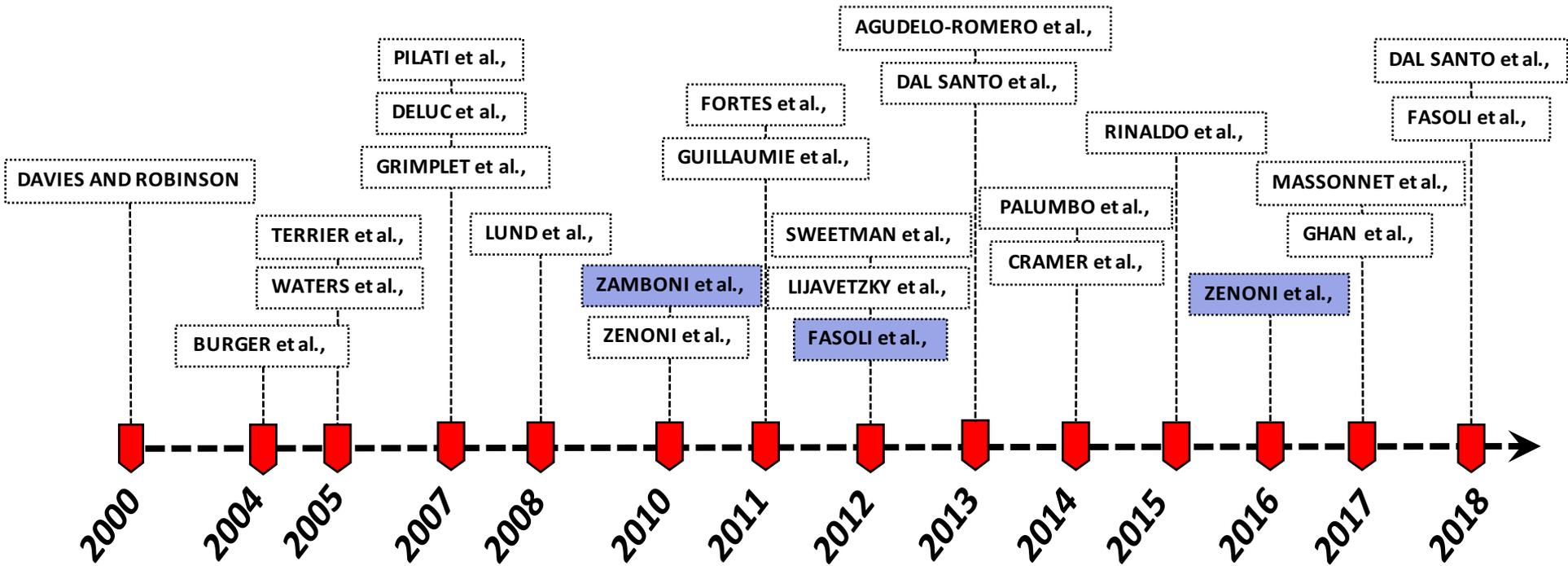


Stilbene Synthases (VvSTSs),

Linalool Synthases (VvSPSs)

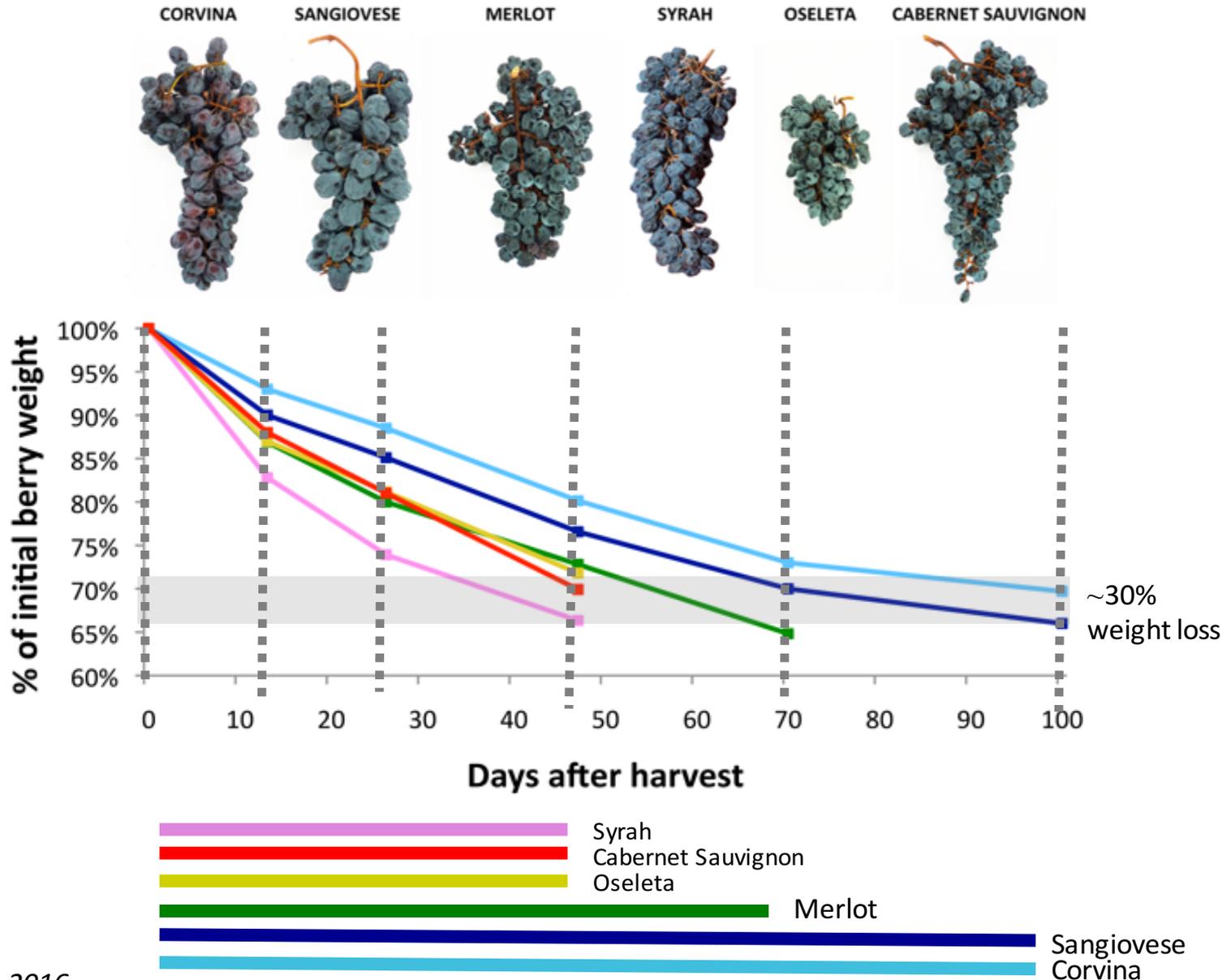
**IDENTIFICATION OF SEVERAL CANDIDATE GENES THAT COULD BE USED AS MARKERS OF BERRY QUALITY TRAITS IN GXE INTERACTIONS**

# Large-scale transcriptional changes during berry development

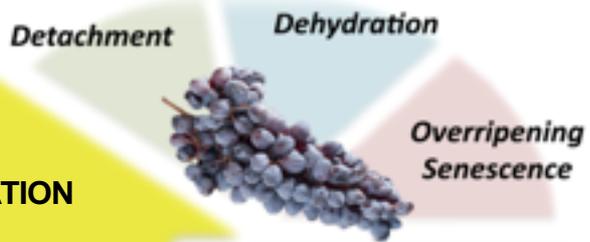


*Expression profile of the principal molecular events during postripening phase*

# Transcriptional changes during berry post-harvest



# Transcriptional changes during berry post-harvest

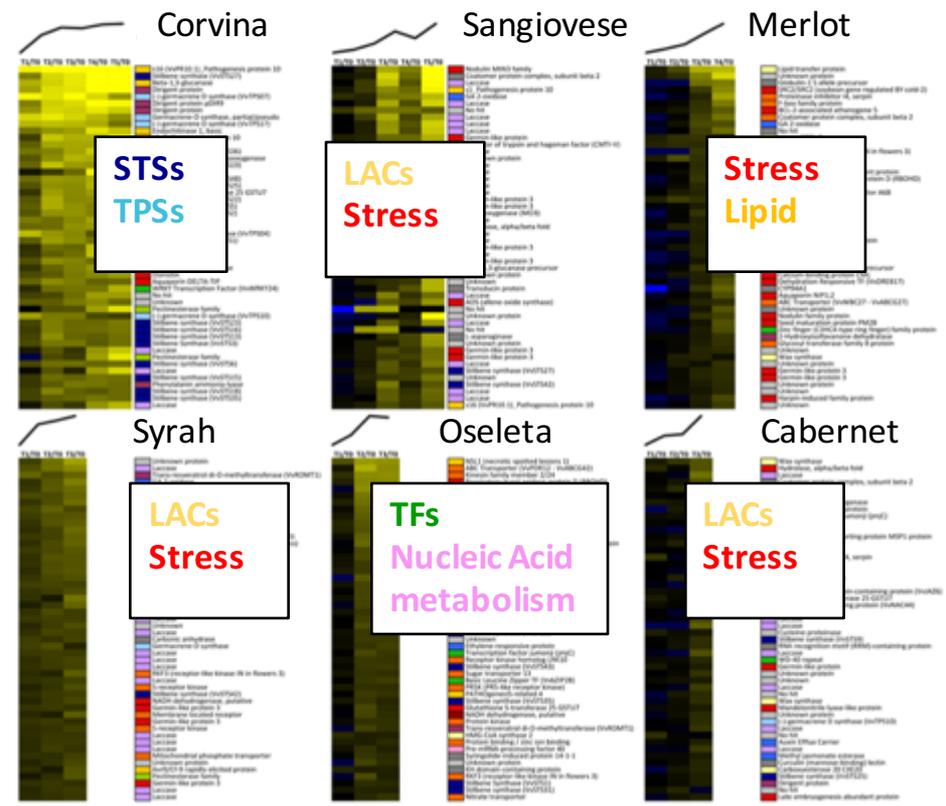


UP REGULATION

- Hormone metabolism**  
ACC1  
Ethylene-responsive protein (2)  
Auxin efflux carrier  
Nitrilase (4)  
VvJAZ29
- Secondary metabolism**  
PAL (11) GENERAL PHENYLPROPANOID  
C4H (2)  
4CL  
STB (19) STILBENES  
ROMT  
COMT  
CCR  
Lignin protein (3)  
Laccase (8) LIGNIN
- Oxidative stress**  
GST (2)  
Peroxidase  
Respiratory burst oxidase
- Aerobic/Anaerobic respiration**  
Pyruvate kinase  
Lactate dehydrogenase
- Defense**  
Pathogenesis protein (2)  
Beta-1,3-glucanase
- Osmotic stress**  
Osmotin (2)
- Transcription factors**  
VvNAC60 VvNAC61  
WD40 (2)  
WRKY16 WRKY29  
Zinc finger C2HC4-type  
Myb domain protein

- Hormone metabolism**  
VVERF045  
IAA (3)  
Auxin-independent growth promoter  
Brassinosteroid-6-oxidase
- Secondary metabolism**  
CCR  
LDOX  
Cytb5  
AnthoMATE1 ANTHOCYANINS  
GST4  
VvMYBA1 similar  
VvMYBA2
- Oxidative stress**  
Catalase (2)  
Lipoxygenase LOX2
- Aerobic/Anaerobic respiration**  
Alcohol dehydrogenase
- Carbohydrate metabolism**  
Malic enzyme  
Sucrose synthase (2)  
Ribulose-1,5-bisphosphate carboxylase  
Tonoplast monosaccharide transporter
- Cell wall metabolism**  
1,4-beta mannan endohydrolase  
Cellulase  
Endo-1,4-beta glucanase  
Expansin (4)  
Extensin-like  
Pectate lyase  
Pectinacetylesterase (2)  
Polygalacturonase  
XTH
- Transcription factors**  
bHLH (2)  
VvZIP07  
Zinc Finger  
MYB Divalent  
Zinc finger C2HC4-type

DOWN REGULATION

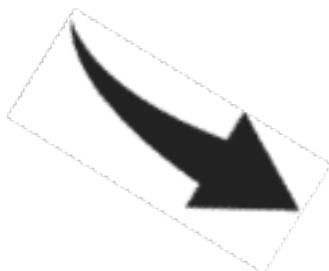
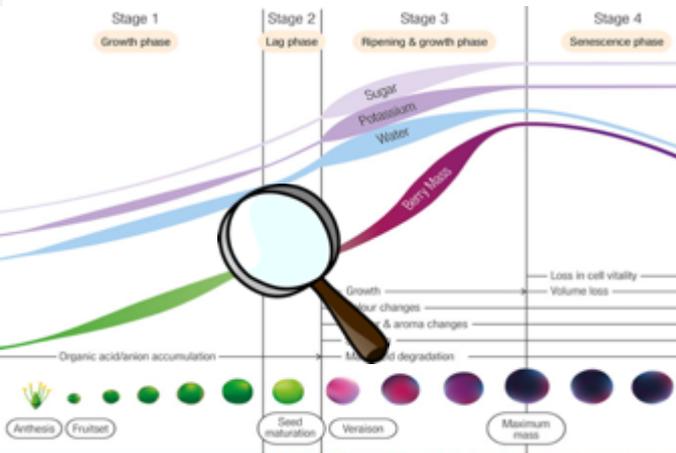


DURING THE POST-HARVEST PHASE THERE IS AN ACTIVE METABOLIC REARRANGEMENT AND NOT ONLY A PASSIVE CONCENTRATION

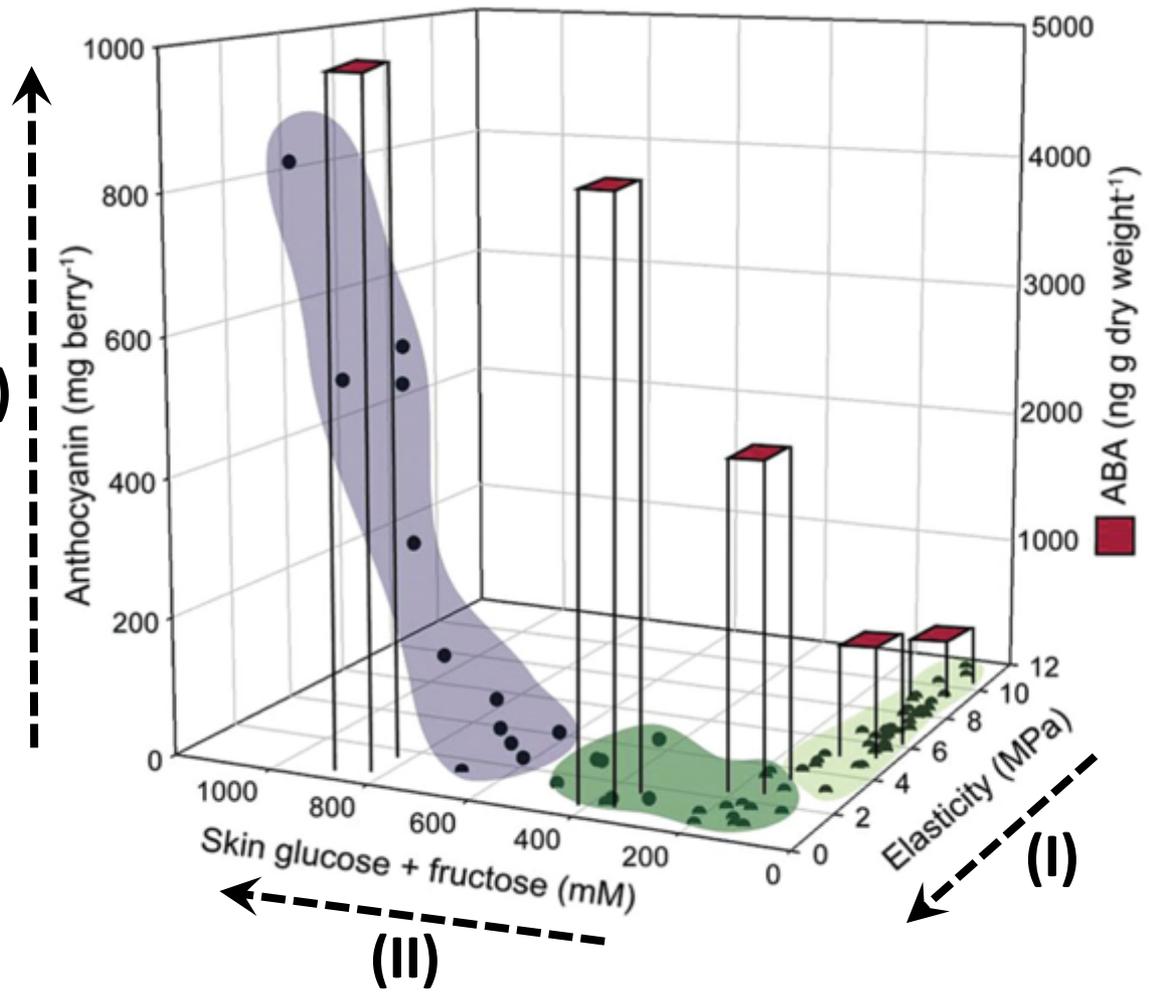


*What triggers the ripening transition?*

# Berry softening is one of the earliest ripening events



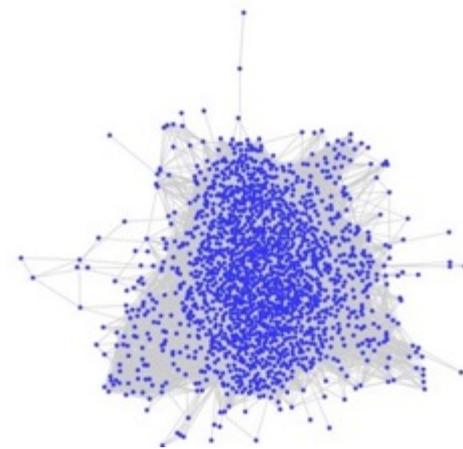
(III)



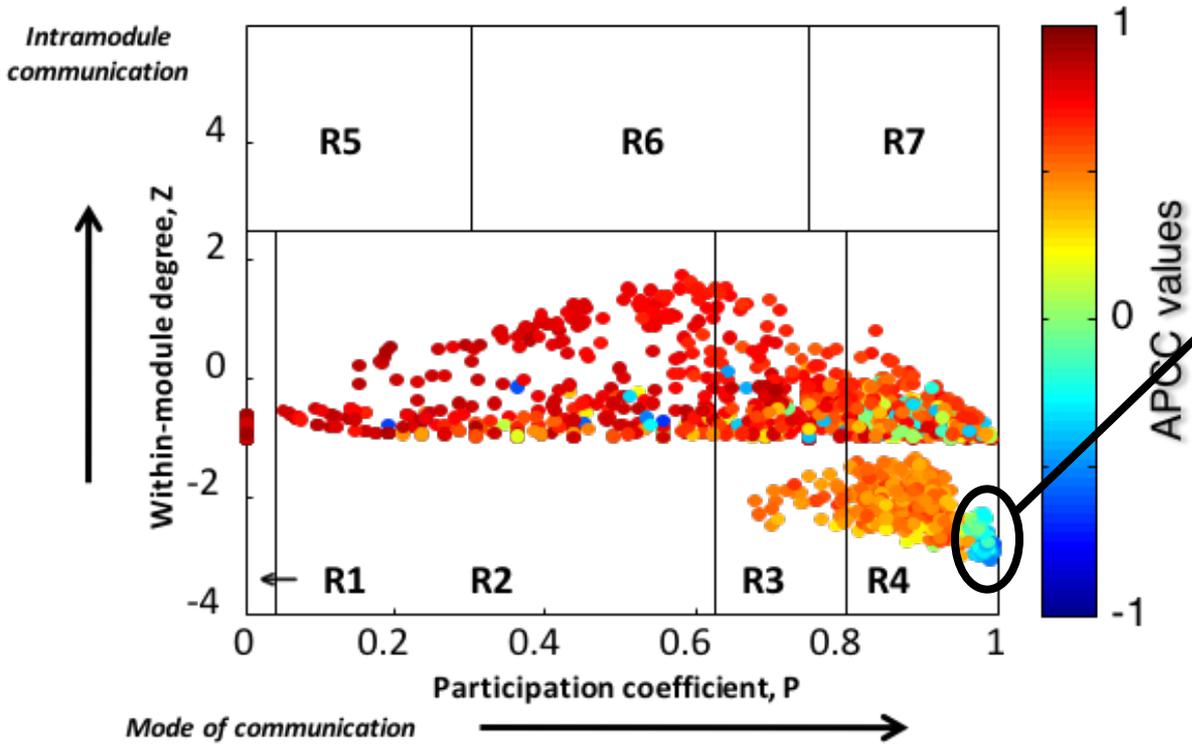




# Identification of switch genes



## Heat cartography map

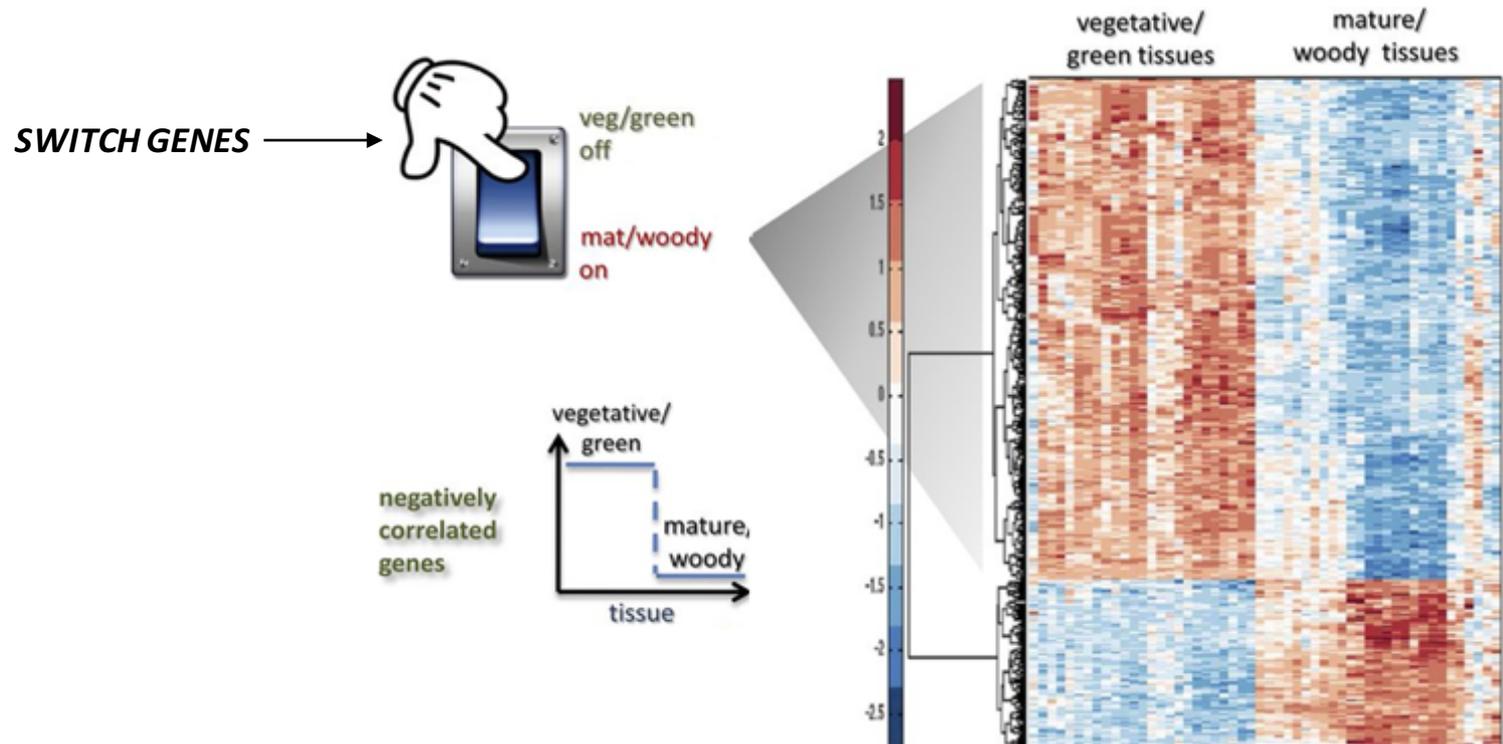


## SWITCH GENES

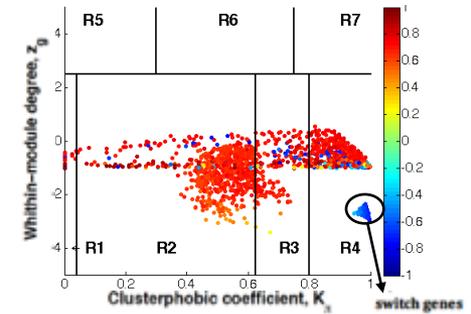
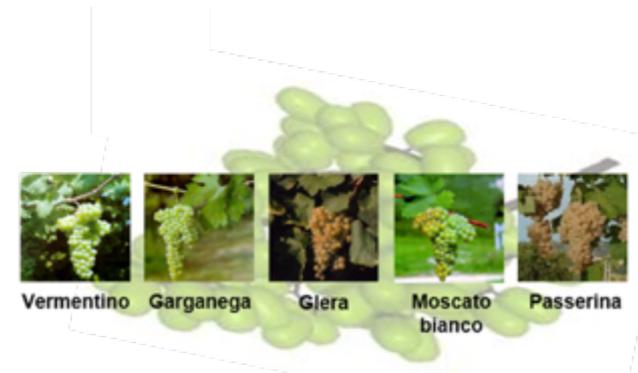
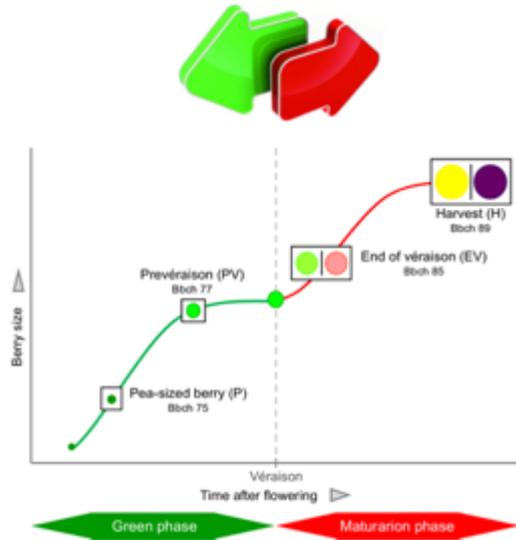
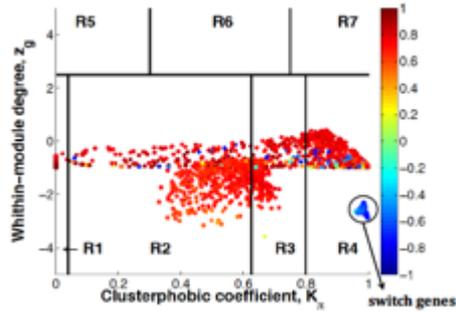
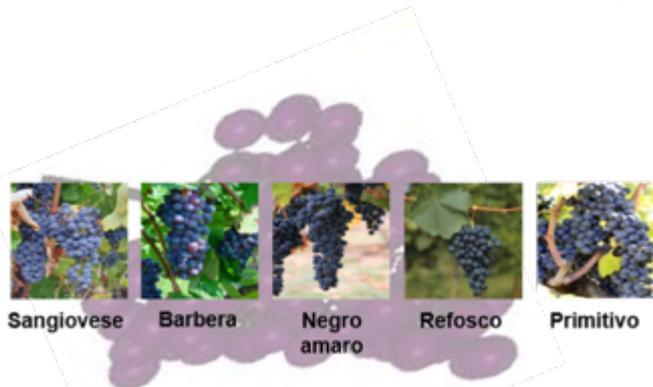
- *interact highly with genes outside their own module*
- *interact poorly with genes inside their own module*
- *mainly anti-correlated with their partners*

# Switch genes model of action

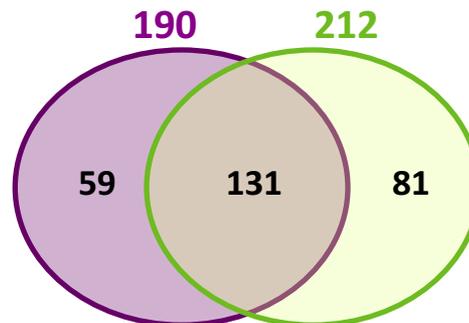
- ✓ All switch genes are down regulated during growth phase and up during mature phase
- ✓ Switch genes could act as an electric switch able to switch-off the expression of vegetative-related genes and to switch-on the expression of mature-related genes



# Switch genes in red and white berries



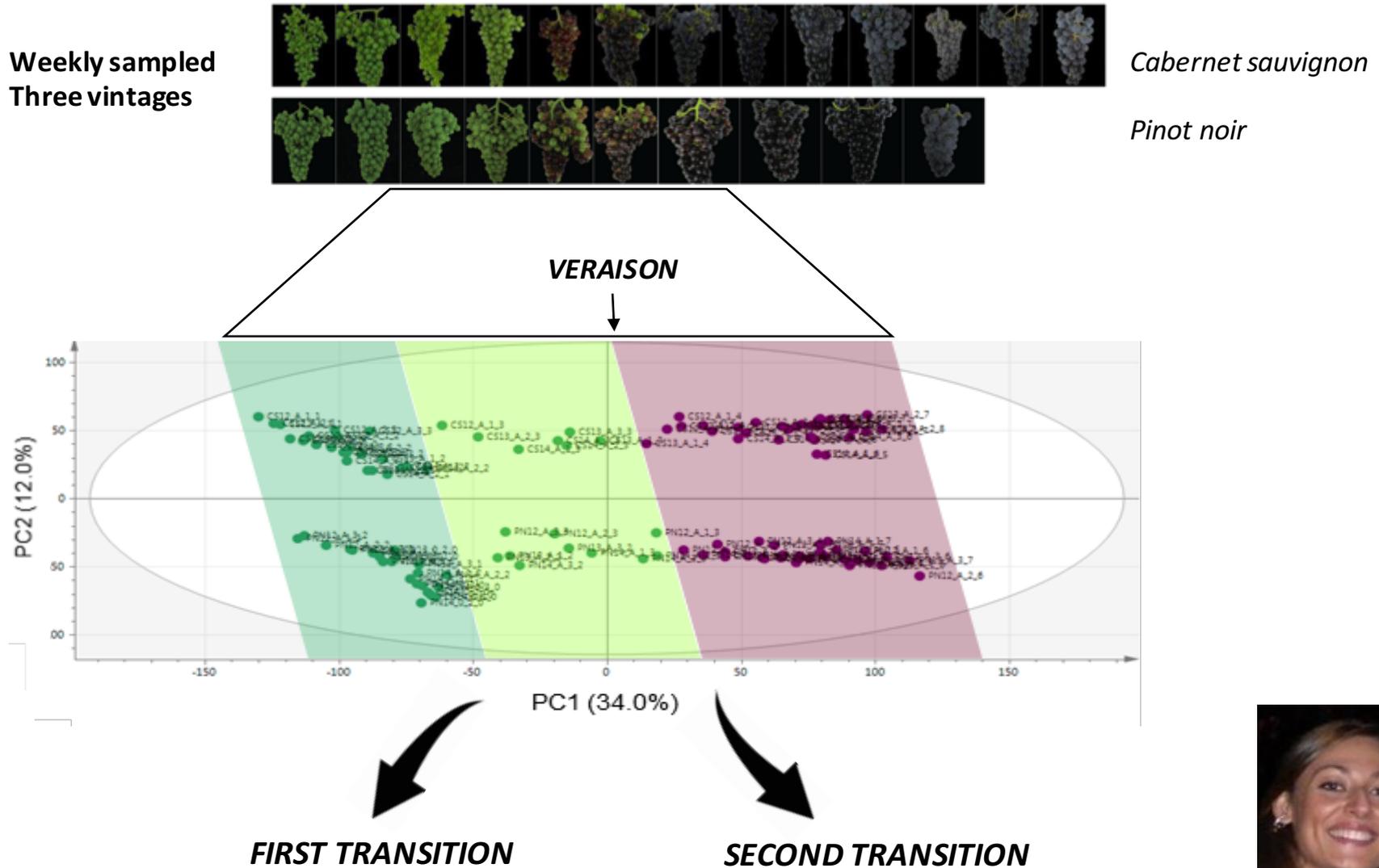
## BERRY SWITCH GENES



# Transcription factors among switch genes

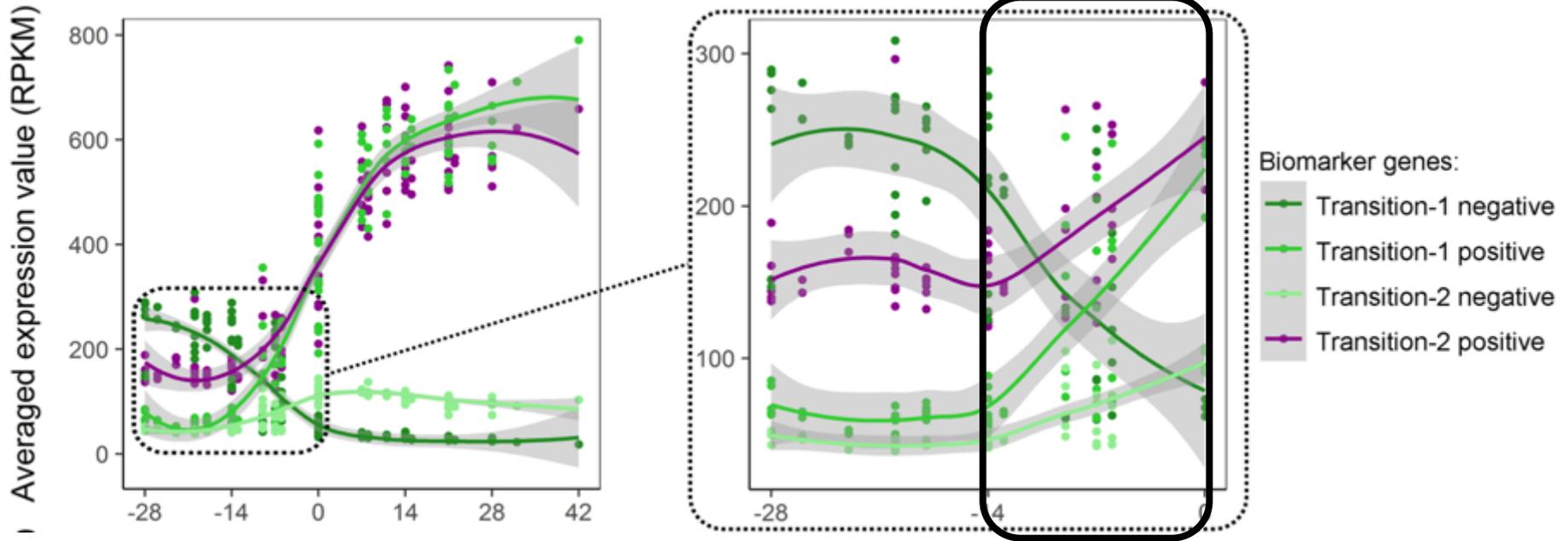
| <i>GENE_ID</i>           | <i>GENE_description</i>                     | <i>GENE_Name</i>        | <i>Atlas</i> |
|--------------------------|---|-------------------------|--------------|
| <i>VIT_17s0000g00430</i> | basic helix-loop-helix (bHLH) family        | <i>bHLH075</i>          |              |
| <i>VIT_15s0046g00150</i> | DOF affecting germination 1                 | <i>DAG1</i>             |              |
| <i>VIT_06s0004g07790</i> | Lateral organ boundaries domain 15          |                         | *            |
| <i>VIT_03s0091g00670</i> | Lateral organ boundaries protein 38         |                         |              |
| <i>VIT_13s0158g00100</i> | putative MADS-box Agamous-like 15a          | <i>VviAGL15a</i>        |              |
| <i>VIT_07s0031g01930</i> | myb TK11 (TSL-KINASE INTERACTING PROTEIN 1) |                         |              |
| <i>VIT_02s0033g00380</i> | R2R3MYB transcription factor                | <i>VvMybA2 (C-term)</i> |              |
| <i>VIT_02s0033g00390</i> | R2R3MYB transcription factor                | <i>VvMybA2</i>          |              |
| <i>VIT_02s0033g00450</i> | R2R3MYB transcription factor                | <i>VvMybA3</i>          |              |
| <i>VIT_14s0108g01070</i> | NAC domain-containing protein               | <i>VvNAC11</i>          |              |
| <i>VIT_02s0012g01040</i> | NAC domain-containing protein               | <i>VvNAC13</i>          |              |
| <i>VIT_19s0027g00230</i> | NAC domain-containing protein               | <i>VvNAC33</i>          | *            |
| <i>VIT_08s0007g07670</i> | NAC domain-containing protein               | <i>VvNAC60</i>          | *            |
| <i>VIT_07s0005g01710</i> | WRKY Transcription Factor                   | <i>VvWRKY19</i>         |              |
| <i>VIT_05s0020g04730</i> | Zinc finger (C3HC4-type ring finger)        |                         |              |
| <i>VIT_08s0040g01950</i> | Zinc finger (C3HC4-type ring finger)        |                         | *            |
| <i>VIT_18s0001g01060</i> | Zinc finger (C3HC4-type ring finger)        |                         | *            |
| <i>VIT_03s0091g00260</i> | Zinc finger protein 4                       |                         |              |

# Two transcriptional transition mark the onset of ripening



# 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

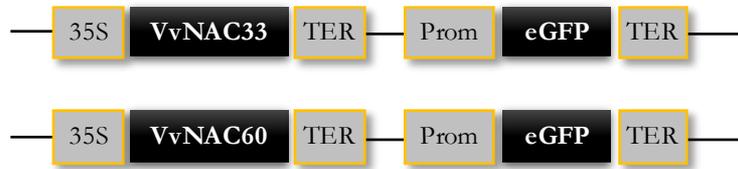


**POSITIVE MARKERS OF THE FIRST TRANSITION  
SEEM TO PLAY A MAJOR ROLE AS TRIGGERS**

# Switch genes are positive markers of the two transitions

| <i>GENE_ID</i>           | <i>GENE_description</i>                     | <i>GENE_Name</i>        | <i>Atlas</i> | <i>Marker of the first transition</i> | <i>Marker of the second transition</i> |
|--------------------------|---|-------------------------|--------------|---------------------------------------|--|
| <i>VIT_17s0000g00430</i> | basic helix-loop-helix (bHLH) family        | <i>bHLH075</i>          |              | *                                     |  |
| <i>VIT_15s0046g00150</i> | DOF affecting germination 1                 | <i>DAG1</i>             |              |                                       |  |
| <i>VIT_06s0004g07790</i> | Lateral organ boundaries domain 15          |                         | *            | *                                     |  |
| <i>VIT_03s0091g00670</i> | Lateral organ boundaries protein 38         |                         |              |                                       |  |
| <i>VIT_13s0158g00100</i> | putative MADS-box Agamous-like 15a          | <i>VviAGL15a</i>        |              |                                       |  |
| <i>VIT_07s0031g01930</i> | myb TK11 (TSL-KINASE INTERACTING PROTEIN 1) |                         |              | *                                     |  |
| <i>VIT_02s0033g00380</i> | R2R3MYB transcription factor                | <i>VvMybA2 (C-term)</i> |              |                                       | *                                      |
| <i>VIT_02s0033g00410</i> | R2R3MYB transcription factor                | <i>VvMybA1</i>          |              |                                       |  |
| <i>VIT_02s0033g00390</i> | R2R3MYB transcription factor                | <i>VvMybA2</i>          |              |                                       | *                                      |
| <i>VIT_02s0033g00450</i> | R2R3MYB transcription factor                | <i>VvMybA3</i>          |              |                                       |  |
| <i>VIT_14s0108g01070</i> | NAC domain-containing protein               | <i>VvNAC11</i>          |              |                                       |  |
| <i>VIT_02s0012g01040</i> | NAC domain-containing protein               | <i>VvNAC13</i>          |              |                                       |  |
| <i>VIT_19s0027g00230</i> | NAC domain-containing protein               | <i>VvNAC33</i>          | *            | *                                     |  |
| <i>VIT_08s0007g07670</i> | NAC domain-containing protein               | <i>VvNAC60</i>          | *            | *                                     |  |
| <i>VIT_07s0005g01710</i> | WRKY Transcription Factor                   | <i>VvWRKY19</i>         |              | *                                     | *                                      |
| <i>VIT_05s0020g04730</i> | Zinc finger (C3HC4-type ring finger)        |                         |              |                                       | *                                      |
| <i>VIT_08s0040g01950</i> | Zinc finger (C3HC4-type ring finger)        |                         | *            | *                                     |  |
| <i>VIT_18s0001g01060</i> | Zinc finger (C3HC4-type ring finger)        |                         | *            |                                       |  |
| <i>VIT_03s0091g00260</i> | Zinc finger protein 4                       |                         |              | *                                     |  |

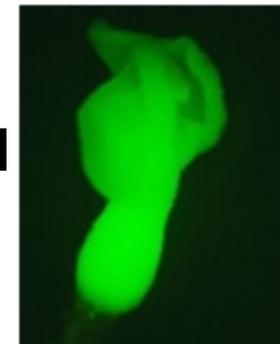
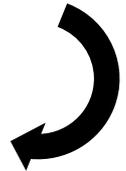
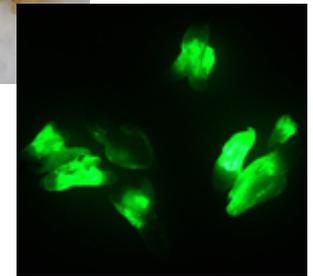
# Functional analysis of VvNAC33 and VvNAC60



*Embryogenic calli*



*Transient expression after 48 h*



*Stable GFP expression after 2-3 months*

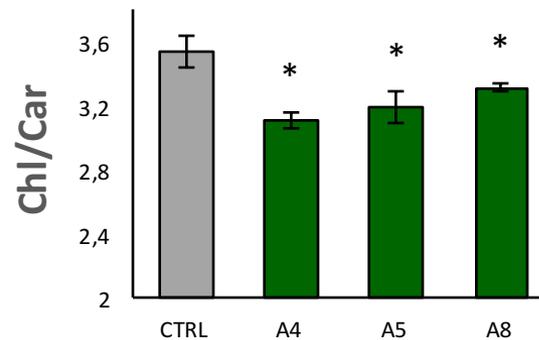
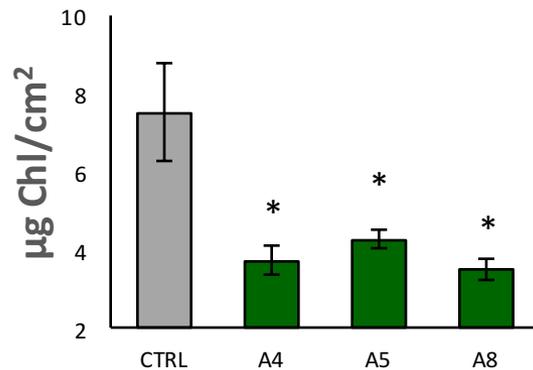
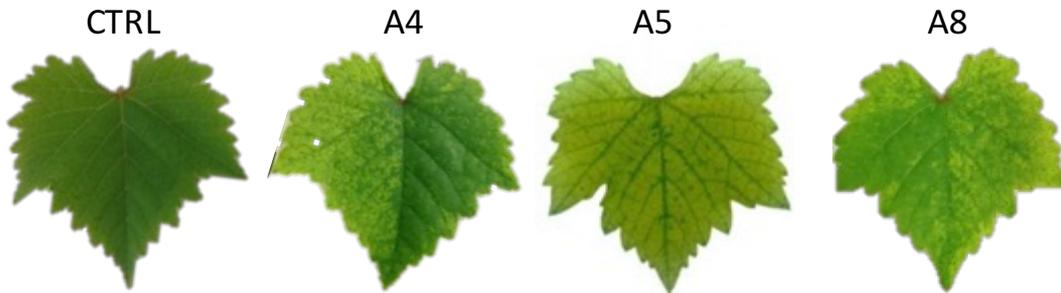
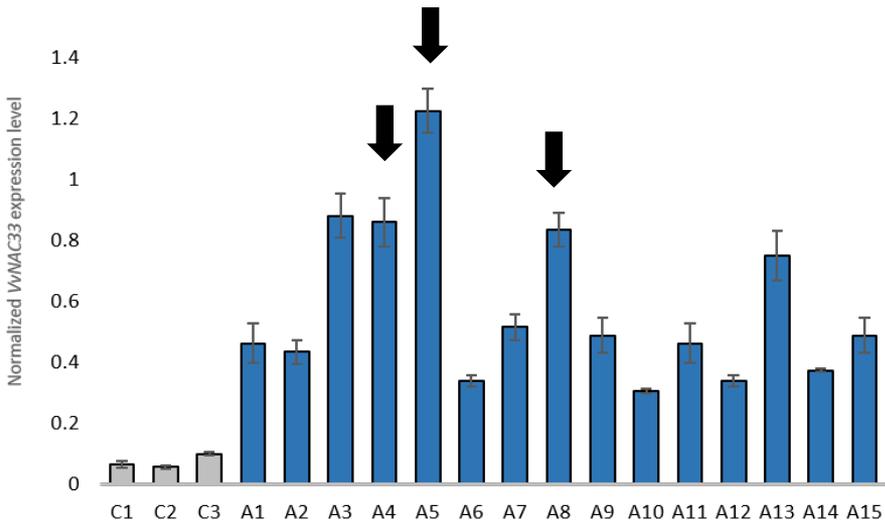


*In vitro plantlets*



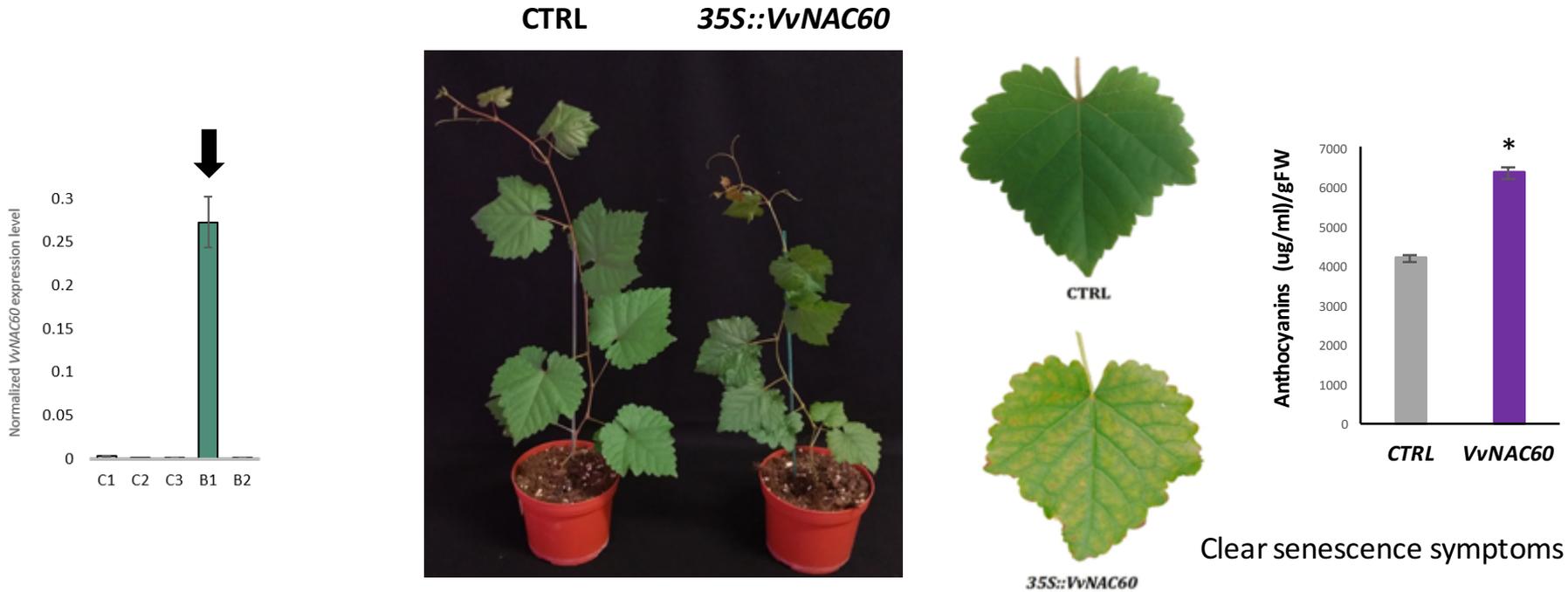
*Greenhouse and phenotypic analysis after 1-2 years*

# Overexpression of VvNAC33 in transgenic Syrah

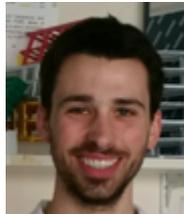


*D'Incà, unpublished*

# Overexpression of VvNAC60 in transgenic Syrah



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**

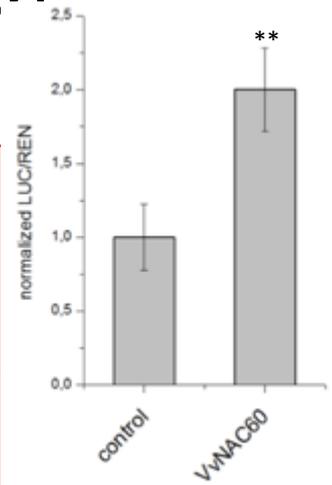
Poster P119 of  
Chiara Foresti



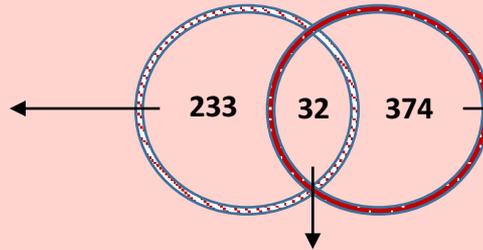
“REGULATORY NETWORK BEHIND THE BERRY RIPENING: THE ROLE OF VITIS VINIFERA NAC60 TRANSCRIPTION FACTOR”

# Transcriptomic analysis of VvNAC33 and VvNAC60 overexpressed plants

VvMYBA1p:LUC



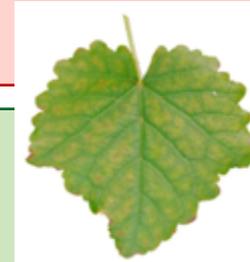
35S:NAC33 35S:NAC60



**VvMYB15**  
4 ERF/AP2 genes  
3 Methyl jasmonate esterases

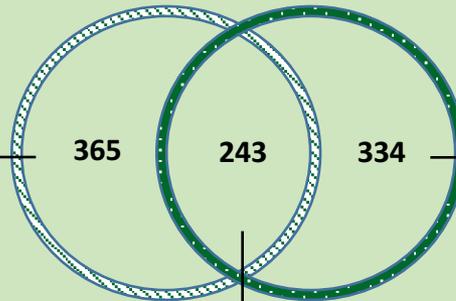
**VvMYBA1**  
**VvMYBA2**  
Anthocyanin permease (VvAnthoMATE1)  
**VvMYB14**  
2 STSs  
Trans-resveratrol di-O-methyltransferase - VvROM  
6 ERF/AP2 genes (VvERF075)  
Lateral organ boundaries proteins (**LOB38**)  
5 XHTs  
MADS-box Agamous 1 (VvAG1)  
MADS-box delta 2b (VvMADSD2b)  
5 NACs (**VvNAC11 and VvNAC26**)  
VvNCED1

2 Galactinol synthases  
2 Lateral organ boundaries proteins (**LOB1,LOB39**)  
3 Nitrate transporters



UP R

35S:NAC33 35S:NAC60



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

5 MADS-boxes Short Vegetal Phase (SVPs)  
5 Photosystem reaction centers  
4 Light-harvesting chlorophyll binding (LHCB) proteins

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



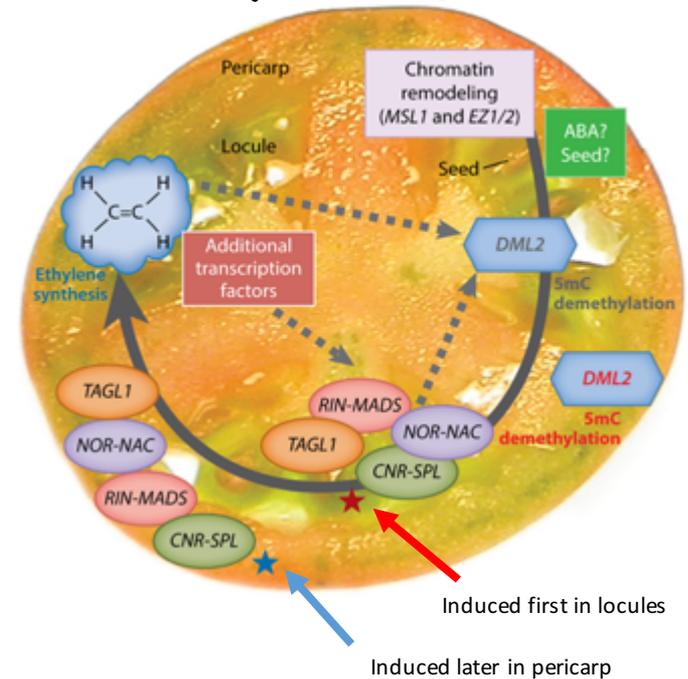
Alessandra Amato

DOWN REGULATED

# Tomato as model to study flesh fruit ripening

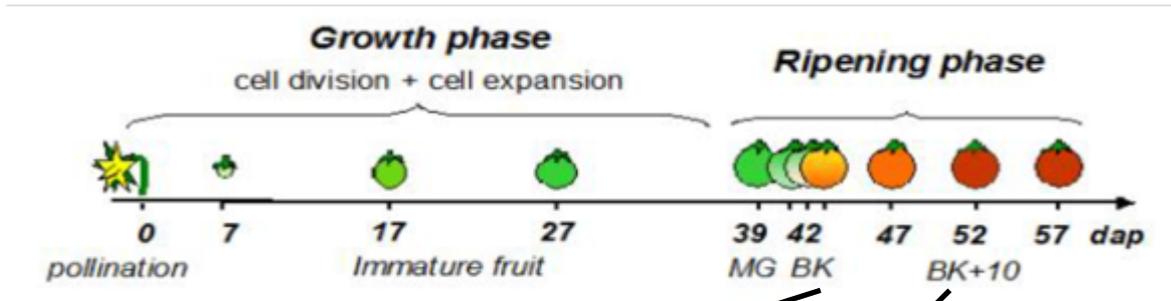


- Accessible germoplasm resources
- Simple diploid genetics
- Efficient greenhouse propagation
- Short life cycle
- Ease of transformation
- Recombinant inbred lines
- High-quality genome sequence
- Natural mutants of ripening



- **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

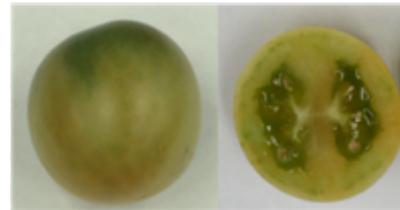
# Tomato NOR mutant



*Wild type*



*nor/nor*



***Ethylene production impaired  
Ripening strongly inhibited***

# VvNAC33 and VvNAC60 partially complement the NOR function

Wild type



*nor/nor*



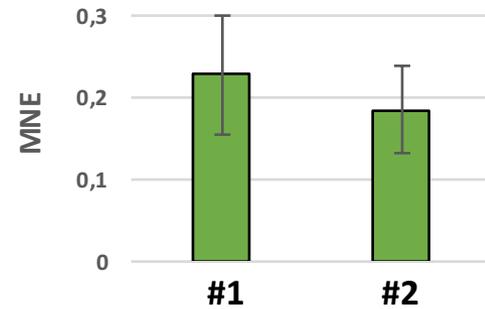
35S::VvNAC33



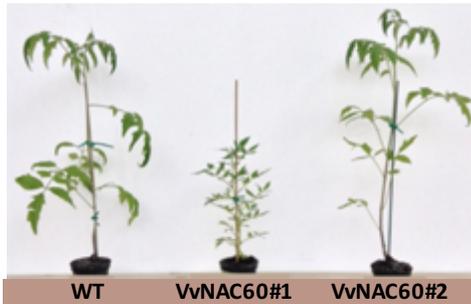
#1



#2



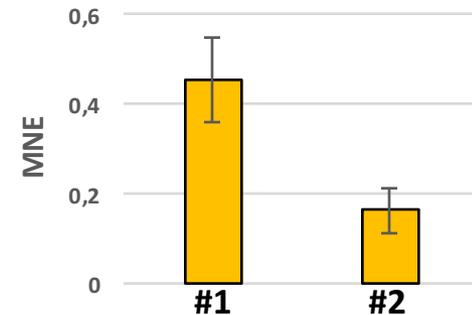
35S::VvNAC60



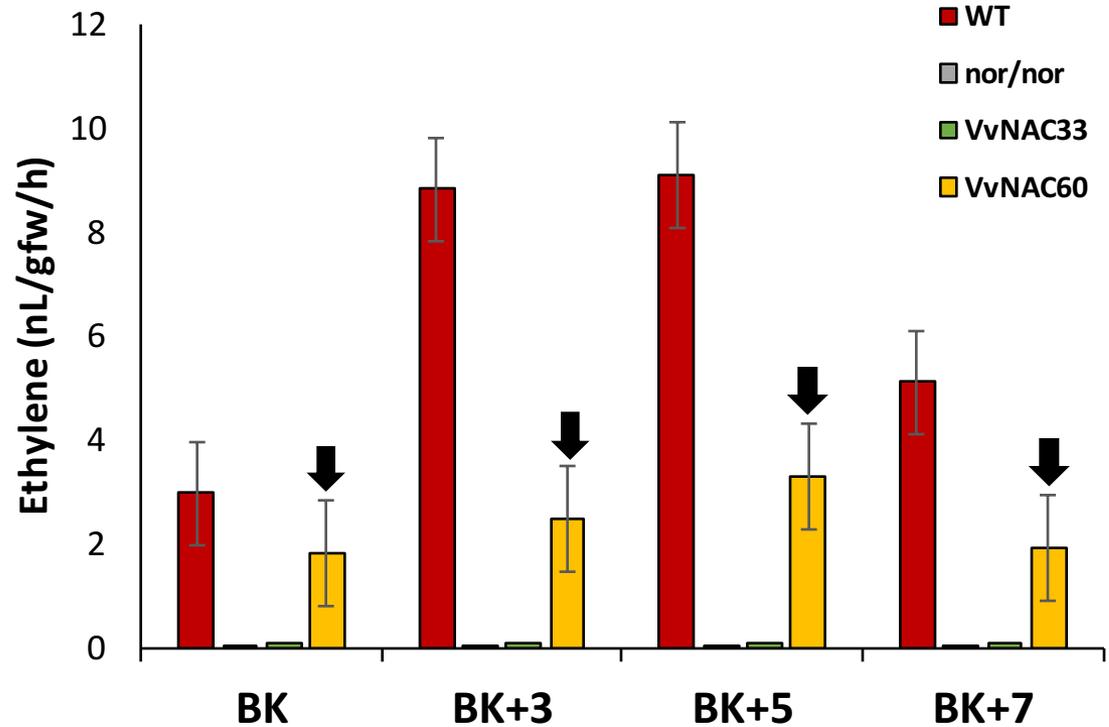
#1



#2



# VvNAC60 induces ethylene biosynthesis



Poster P145 of  
Erica D'Inca



“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”

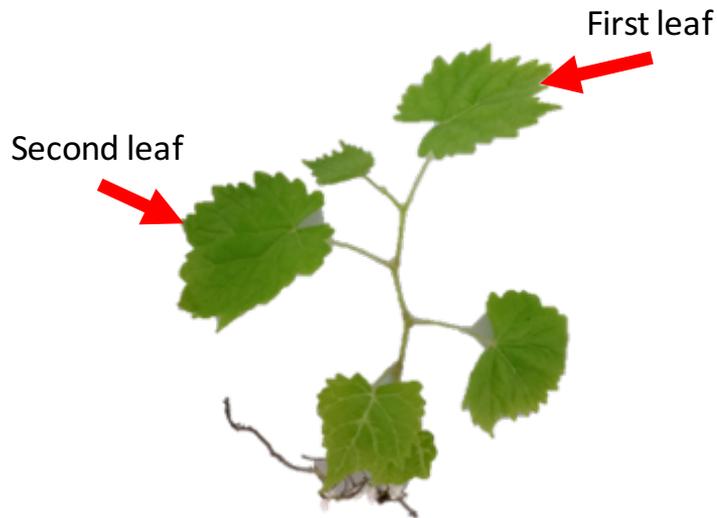
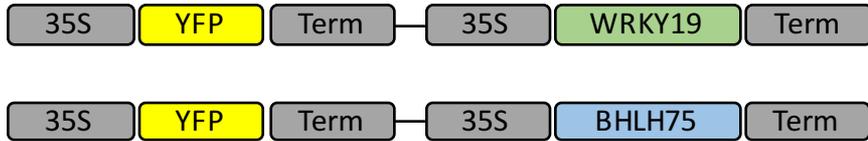
# VvbHLH075 and VvWRKY19

| <i>GENE_ID</i>           | <i>GENE_description</i>                     | <i>GENE_Name</i>        | <i>Atlas</i> | <i>Marker of the first transition</i> | <i>Marker of the second transition</i> |
|--------------------------|---|-------------------------|--------------|---------------------------------------|--|
| <i>VIT_17s0000g00430</i> | basic helix-loop-helix (bHLH) family        | <i>bHLH075</i>          |              | *                                     |  |
| <i>VIT_15s0046g00150</i> | DOF affecting germination 1                 | <i>DAG1</i>             |              |                                       |  |
| <i>VIT_06s0004g07790</i> | Lateral organ boundaries domain 15          |                         | *            | *                                     |  |
| <i>VIT_03s0091g00670</i> | Lateral organ boundaries protein 38         |                         |              |                                       |  |
| <i>VIT_13s0158g00100</i> | putative MADS-box Agamous-like 15a          | <i>VviAGL15a</i>        |              |                                       |  |
| <i>VIT_07s0031g01930</i> | myb TK11 (TSL-KINASE INTERACTING PROTEIN 1) |                         |              | *                                     |  |
| <i>VIT_02s0033g00380</i> | R2R3MYB transcription factor                | <i>VvMybA2 (C-term)</i> |              |                                       | *                                      |
| <i>VIT_02s0033g00410</i> | R2R3MYB transcription factor                | <i>VvMybA1</i>          |              |                                       |  |
| <i>VIT_02s0033g00390</i> | R2R3MYB transcription factor                | <i>VvMybA2</i>          |              |                                       | *                                      |
| <i>VIT_02s0033g00450</i> | R2R3MYB transcription factor                | <i>VvMybA3</i>          |              |                                       |  |
| <i>VIT_14s0108g01070</i> | NAC domain-containing protein               | <i>VvNAC11</i>          |              |                                       |  |
| <i>VIT_02s0012g01040</i> | NAC domain-containing protein               | <i>VvNAC13</i>          |              |                                       |  |
| <i>VIT_19s0027g00230</i> | NAC domain-containing protein               | <i>VvNAC33</i>          | *            | *                                     |  |
| <i>VIT_08s0007g07670</i> | NAC domain-containing protein               | <i>VvNAC60</i>          | *            | *                                     |  |
| <i>VIT_07s0005g01710</i> | WRKY Transcription Factor                   | <i>VvWRKY19</i>         |              | *                                     | *                                      |
| <i>VIT_05s0020g04730</i> | Zinc finger (C3HC4-type ring finger)        |                         |              |                                       | *                                      |
| <i>VIT_08s0040g01950</i> | Zinc finger (C3HC4-type ring finger)        |                         | *            | *                                     |  |
| <i>VIT_18s0001g01060</i> | Zinc finger (C3HC4-type ring finger)        |                         | *            |                                       |  |
| <i>VIT_03s0091g00260</i> | Zinc finger protein 4                       |                         |              | *                                     |  |

# Functional analysis of VvbHLH075 and VvWRKY19

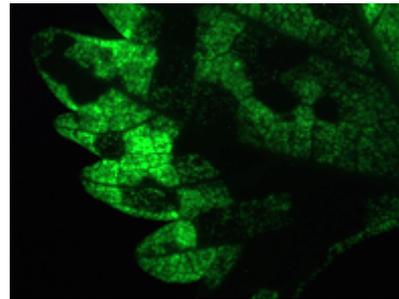


Edoardo Bertini

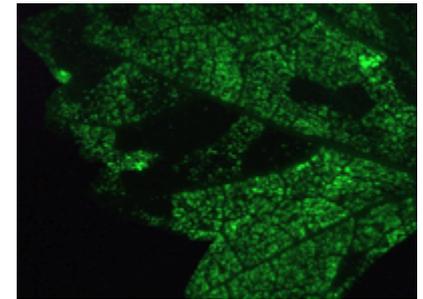


Thompson seedless plants  
grown *in vitro*

d.p.i. 7



First leaf



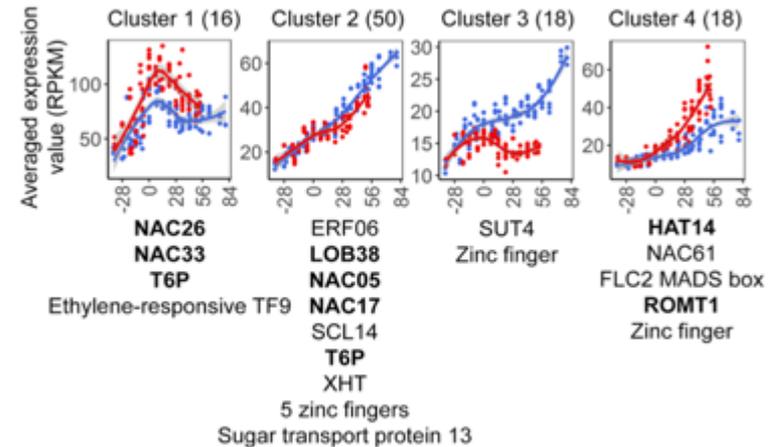
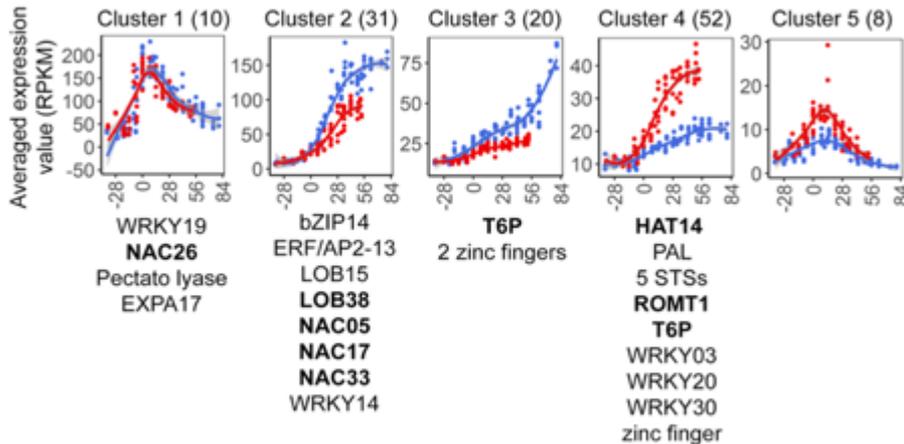
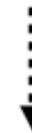
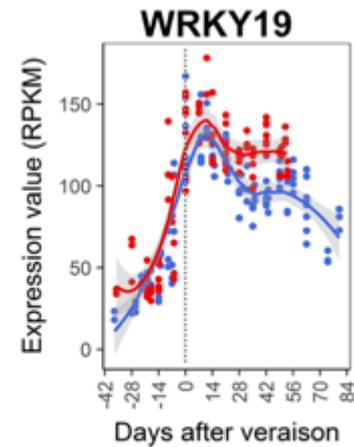
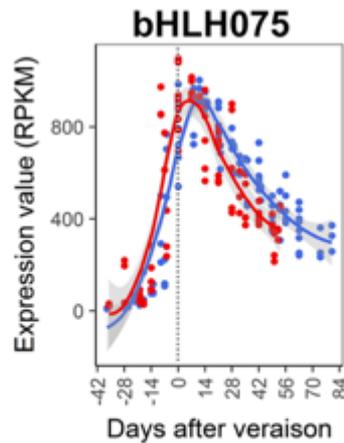
Second leaf

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



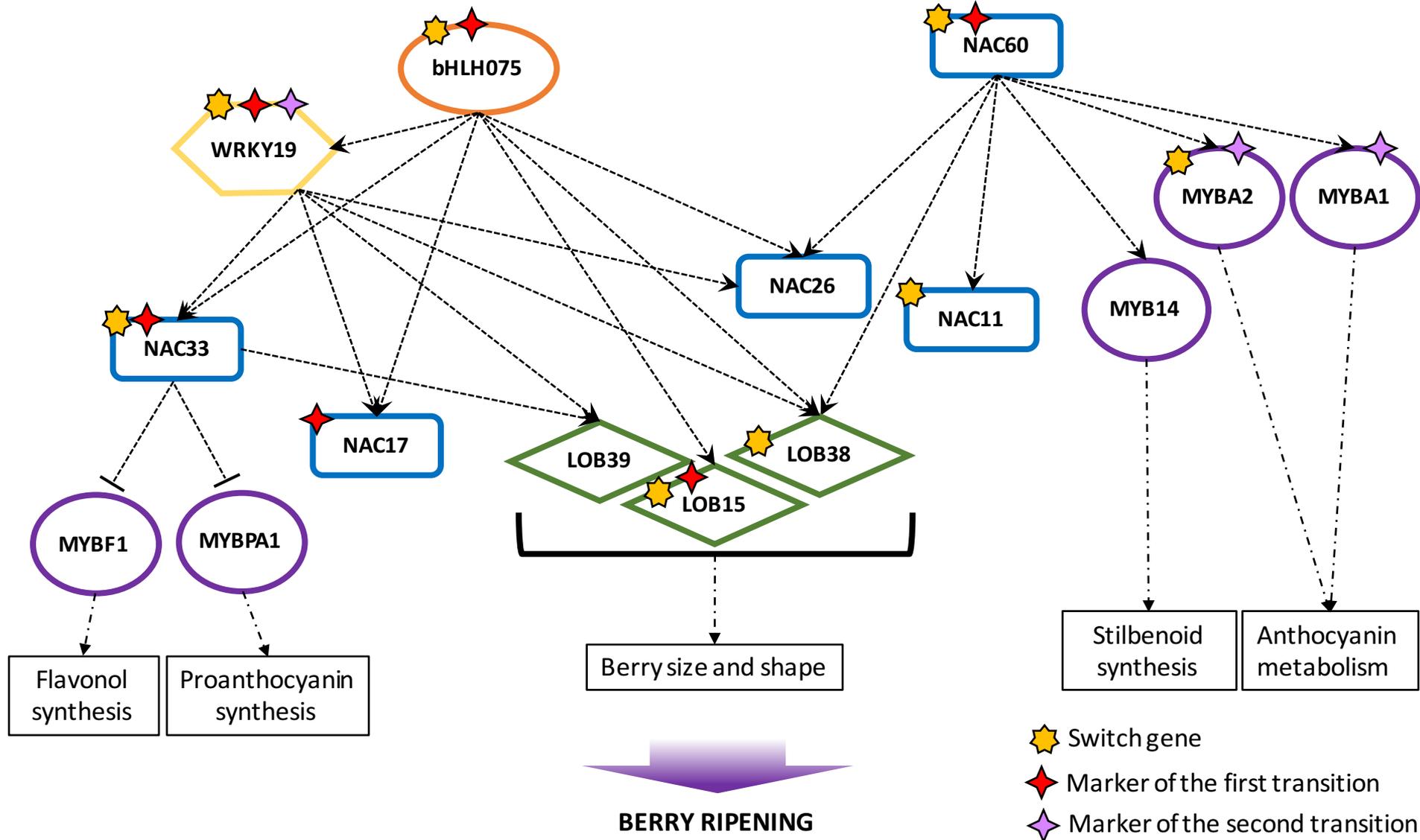
Transcriptomic analysis

# Putative targets of VvbHLH075 and VvWRKY19



— Cabernet Sauvignon — Pinot noir

# Putative hierarchy of transcription factors in the onset of 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



# Thanks to

**Mario Pezzotti**  
**Giambattista Tornielli**  
**Silvia Dal Santo**  
**Melanie Massonnet**  
**Erica D'Inca**  
**Alessandra Amato**  
**Edoardo Bertini**  
**Chiara Foresti**



**Lucio Brancadoro**  
**Gabriella De Lorenzis**



**Michele Morgante**  
**Gabriele Di Gaspero**  
**Gabriele Magris**  
**Emanuele De Paoli**



**Paola Paci**  
**Lorenzo Farina**



**Jim Giovannoni**  
**Julia Vrebalov**



**Paola Zuccolotto**  
**Marco Sandri**



**Massimo Gardiman**



**Nick Dokoozlian**  
**Marianna Fasoli**  
**Chandra Richter**



**E&J. Gallo Winery**



*The berries are ripe  
and my time is finished....*



# THANKS FOR YOUR ATTENTION