



UNIVERSITÀ
di **VERONA**

Dipartimento
di **BIOTECNOLOGIE**

Il contributo della trascrittomica nella valutazione della vocazionalità dei *terroir* del Sangiovese.



Mario Pezzotti

Montalcino

Sanguis Jovis 26 Settembre 2017

From Genotype to Phenotype

Genotype



Phenotype



OUTLINES

Phenotypic Plasticity in Plants: definition, role in evolution and in plant breeding

Grapevine Plasticity: identification and previous studies

Grapevine Plasticity in the genomic era

Grapevine Genotype X Environments in the genomic era

Phenotypic plasticity

Phenotypic plasticity—the ability of a single genotype to produce multiple phenotypes in response to the environment— and responses often quantified and depicted as **reaction norms**

PLANTS

- Plants are remarkable because of their **ability to thrive** despite their limited capacity to alter their surroundings.
- Plants are exquisite in their **ability to sense, respond** to, and **survive** a variety of abiotic stresses.
- This is largely the result of **acclimation to the environment** through changes in physiology, metabolism, growth, and indeterminate development.

Phenotypic Plasticity and Genotype X Environment Interaction

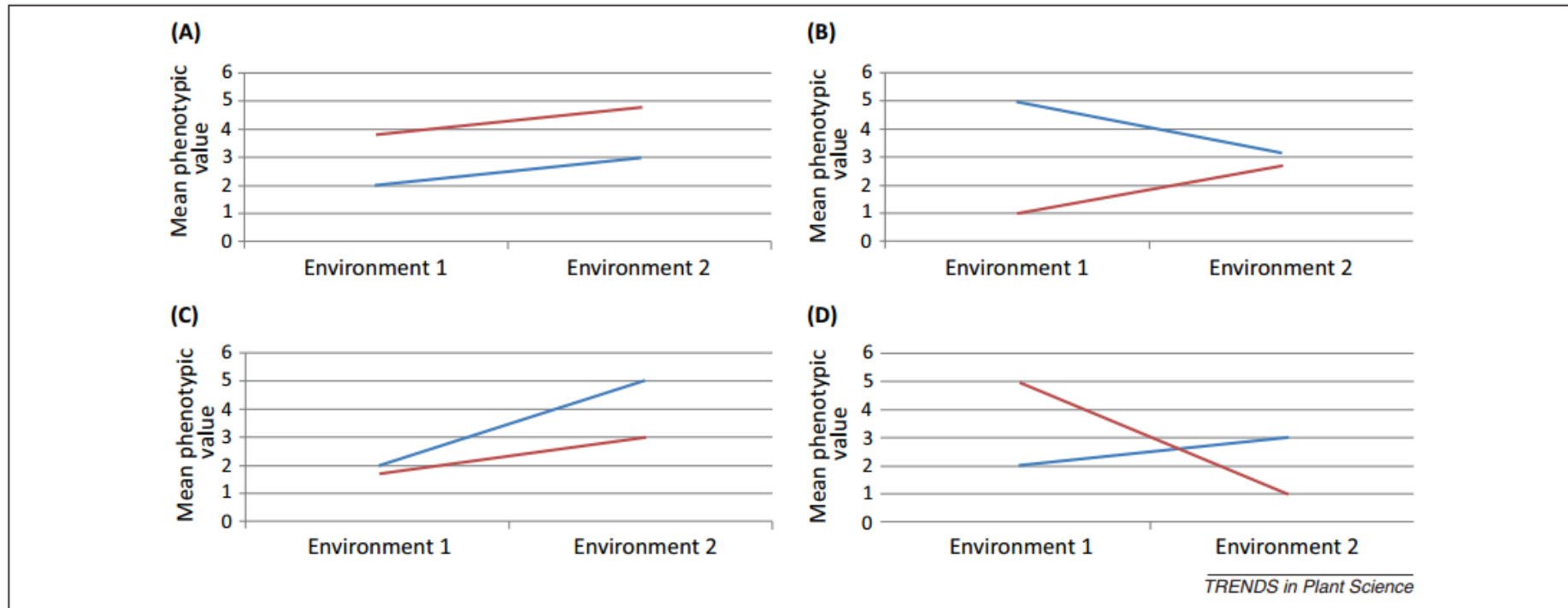


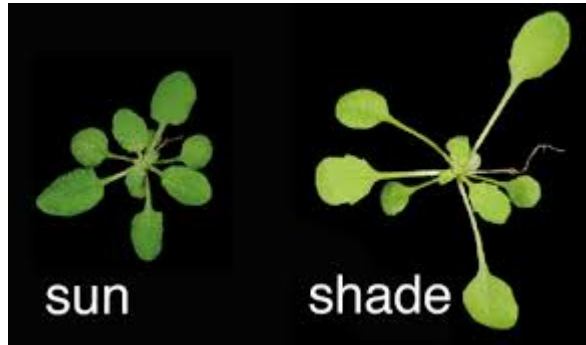
Figure 1. Phenotypic plasticity and genotype \times environment interaction ($G \times E$). Four examples of reaction norms illustrating **(A)** the phenotypic plasticity of genotypes between environments, and **(B–D)** a genetic difference between genotypes for plasticity indicating $G \times E$. Genotype 1 is the red line and genotype 2 the blue line in each graph. In **(A)**, the trait values change across environments but the reaction norms run parallel because the response to the environmental conditions is similar for both genotypes. In **(B,C)**, there is a scale-effect interaction indicating $G \times E$, because each genotype has a different response to each of the environments, but without the reaction norms to cross, whereas in **(D)**, there is a stronger genetic effect on the phenotype in response to the different environments, also indicating $G \times E$, causing reaction norms to cross.

From El-Soda et al.2014

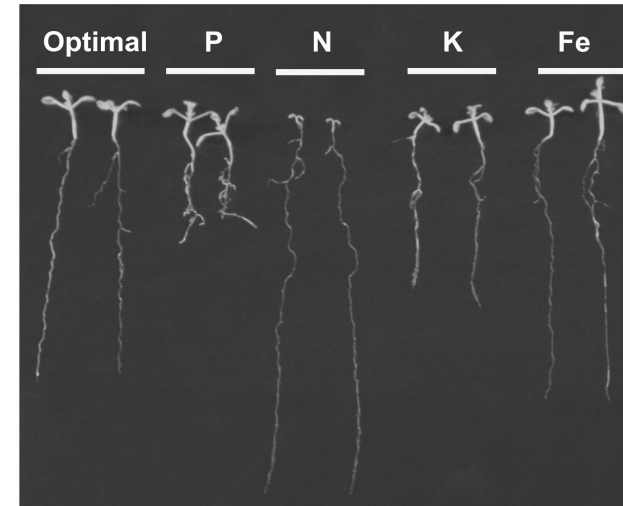
$$P = G + E + GXE$$

Examples of phenotypic plasticity in plants

shade avoidance



root architecture changes



Many examples are clearly **adaptive** (e.g., shade avoidance) and may result in local adaptation. However, plastic responses can also be passive consequences of the resources available for growth and development. As such, **neutral** or **maladaptive** plasticity occurs and even adaptive plasticity involves costs and limits that may constrain evolution

OUTLINES

Phenotypic Plasticity in Plants: definition, role in evolution and in plant breeding

Grapevine Plasticity: identification and previous studies

Grapevine Plasticity in the genomic era

Genotype X Environment in plants : Cloned Genes, Gene Expression Studies

Grapevine Genotype X Environments in the genomic era

Phenotypic Plasticity in *Vitis vinifera*

Vitis vinifera spp : a single genotype is able to produce berries with different quality, thus different wine qualities, depending on the micro-environment where it is cultivated.

Berry plasticity is very high

Environmental factors (e.g., temperature, light, and soil moisture) and viticulture practices (e.g., pruning, irrigation, and cluster thinning) are known to cause variability:

within berries,

among berries within a cluster,

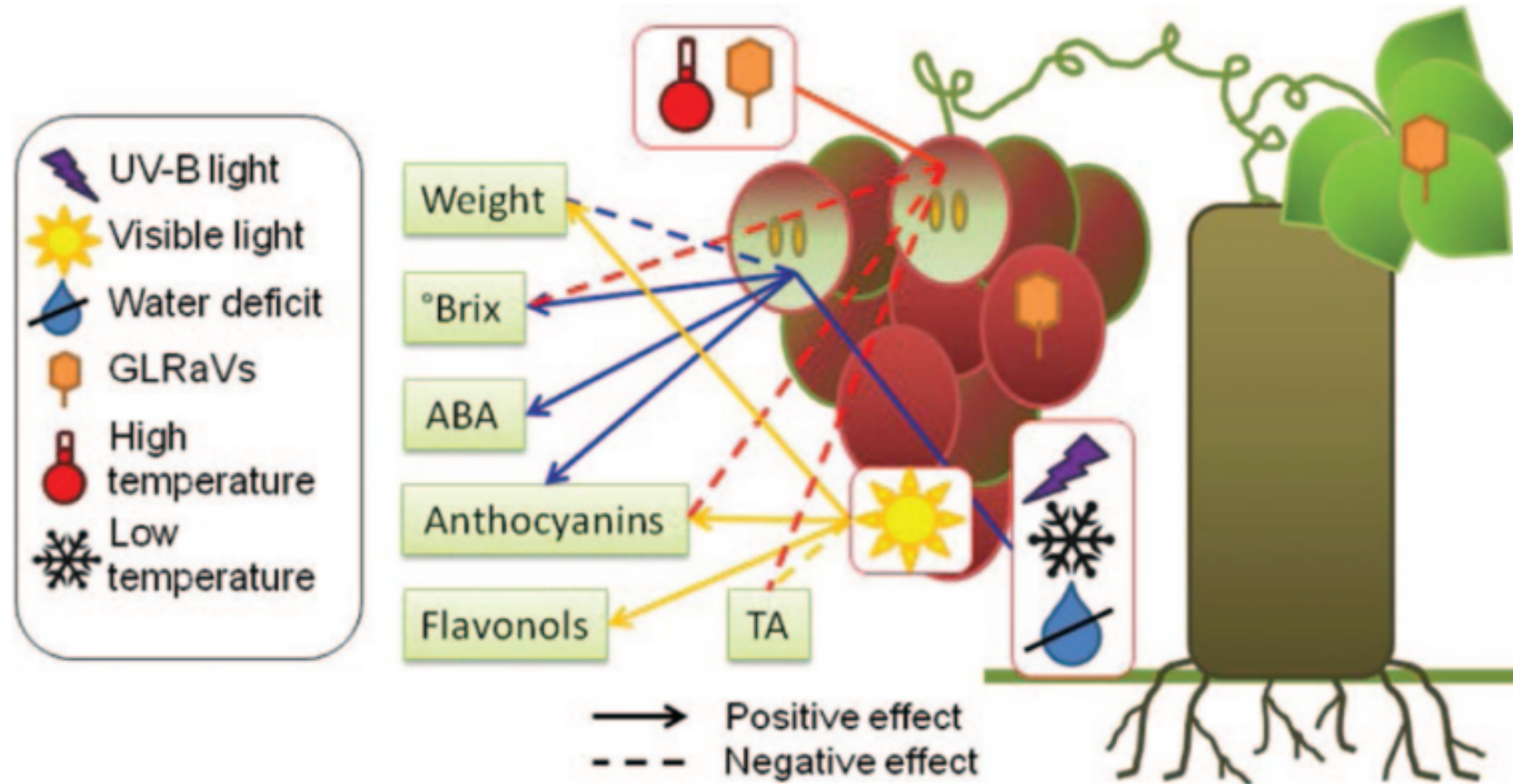
among clusters on a vine,

and among vines within a vineyard

(Gray 2002, Keller 2010).



Environmental cues affecting typical parameters associated with berry ripening in coloured grapes



From Kuhn et al. 2013

Use of phenotypic plasticity in grapevine

PROS

- Adaptation existing cultivars to a specific growing region
- Production a wide range of different wines from the same cultivar
- Coping with environmental changes

CONS

- Uneven maturity
- Interseasonal fluctuation

OUTLINES

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Grapevine Plasticity: identification and previous studies

Grapevine Plasticity in the genomic era

Grapevine Genotype X Environments in the genomic era

The French-Italian Public Consortium for the Sequencing of the Grapevine Nuclear Genome



VIGNE

Università degli Studi di Verona

Vigna Vigne

L'iniziativa bilaterale Italia - Francia per il sequenziamento e la caratterizzazione funzionale del genoma della vite (*Vitis vinifera* L.)

Lunedì, 13 marzo 2006

Università di Verona
Palazzo Giuliani - Sala "G. Barbieri"
Via dell'Artigliere, 8



The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla

The French–Italian Public Consortium for Grapevine Genome Characterization*

The analysis of the first plant genomes provided unexpected evidence for genome duplication events in species that had previously been considered as true diploids on the basis of their genetics^{1,2}. These polyploidization events may have had important consequences in plant evolution, in particular for species radiation and adaptation and for the modulation of functional capacities^{3,4}. Here we report a high-quality draft of the genome sequence of grapevine (*Vitis vinifera*) obtained from a highly homozygous genotype. The draft sequence of the grapevine genome is the fourth one produced so far for flowering plants, the second for a woody species and the first for a fruit crop (cultivated for both fruit and beverage). Grapevine was selected because of its important place in the cultural heritage of humanity beginning during the Neolithic period⁵. Several large expansions of gene families with roles in aromatic features are observed. The grapevine genome has not undergone recent genome duplication, thus enabling the discovery of ancestral traits and features of the genetic organization of flowering plants. This analysis reveals the contribution of three ancestral genomes to the grapevine haploid content. This ancestral arrangement is common to many dicotyledonous plants but is absent from the genome of rice, which is a monocotyledon. Furthermore, we explain the chronology of previously described whole-genome duplication events in the evolution of flowering plants.

All grapevine varieties are highly heterozygous; preliminary data showed that there was as much as 1.3% sequence divergence between alleles, which would hinder reliable contig assembly when a whole-genome shotgun strategy was used for sequencing. Our consortium therefore selected the grapevine PN40024 genotype for sequencing. This line, originally derived from Pinot Noir, has been bred close to full homozygosity (estimated at about 93%) by successive selfings, permitting a high-quality whole-genome shotgun assembly.

A total of 6.2 million end-seeds were produced by our consortium, representing an 8.4-fold coverage of the genome. Within the assembly, performed with Aschene⁶, 316 supercontigs represent putative allelic haplotypes that constitute 11.6 million bases (Mb). These values are in good fit with the 7% residual heterozygosity of PN40024 assessed by using genetic markers. When considering only one of the haplotypes in each heterozygous region, the assembly (Table 1a) consists of 19,577 contigs (N_{50} = 65.9 kilobases (kb), where N_{50} corresponds to the size of the shorter supercontig or contig in a subset representing half of the assembly size) and 3,514 supercontigs (N_{50} = 2.07 Mb) totalling 487 Mb. This value is close to the 475 Mb previously reported for the grapevine genome size⁷.

Using a set of 409 molecular markers from the reference grapevine map⁸, 69% of the assembled 487 Mb, arranged into 45 ultracontigs

Table 1 | Global statistics on the genome of *Vitis vinifera*

(a) Assembly						
	Status	Number	Size (kb)	Longest (kb)	Size (Mb)	Percentage of the assembly
Contigs	All	19,577	65.9	357	46.75	–
Supercontigs	All	3,514	2,065	12,675	40.71	100
	Anchored on chromosomes	191	3,199	12,675	33.56	68.9
	Anchored on chromosomes and oriented	143	3,827	12,675	29.69	61.9
(b) Annotation						
	Number	Median size (bp)	Total length (Mb)	Percentage of the genome	NGC	
Gene	30,434	3,399	225.6	4.63	36.2	
Exon CDS	149,351	130	33.6	6.9	44.5	
Intron CDS	118,917	213	17.86	3.67	34.7	
Intergenic	30,453	3,544	26.15	34.7	33.0	
tRNA*	600	73	0.04	NS	43.0	
mRNA†	164	103.5	0.002	NS	35.9	
(c) Orthology						
	Number of orthologous proteins	Mean identity (%)				
<i>P. trichocarpa</i>	1,299.6	72.7				
<i>A. thaliana</i>	1,140.4	65.5				
<i>O. sativa</i>	973.1	59.8				
Common to eudicotyledons‡	1,054.7					
Common to Magnoliophytals§	812.1					

* Transfer RNA (tRNA) values were computed on rice.

† MicroRNA (miRNA) are members of known conserved miRNA families.

‡ Eudicotyledons are represented by *P. trichocarpa* and *A. thaliana*.

§ Magnoliophytals (or flowering plants) are represented by *P. trichocarpa*, *A. thaliana* and *O. sativa*.

*A list of participants and their affiliations appears at the end of the paper.



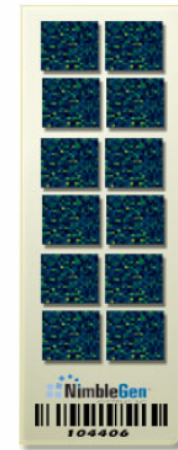
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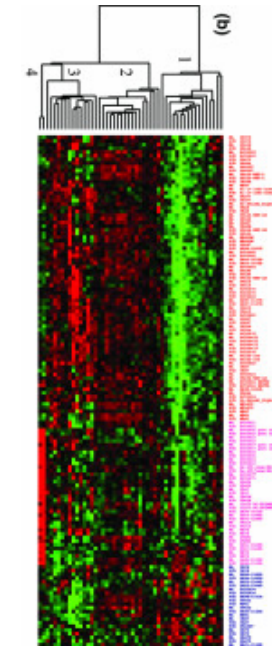
FONDAZIONE
Cariverona

Centro di Genomica Funzionale

Con il supporto di Fondazione Cariverona



12-plex
12 x 135 K



A dynamic gene expression atlas of cv. Corvina

Sample/organ	N. of developmental stages
Bud	5
Inflorescence	4
Tendrils	3
Leaf	3
Berry pericarp	8
Berry skin	7
Berry flesh	7
Seed	4
Rachis	5
Stem	2
Root ("in vitro")	pool
Seedling	pool
Stamen	pool
Pollen	pool
Carpel	pool
Petal	pool
Total	54



Bud

Inflorescence



Tendrils



Leaf



Senescent Leaf

Berry development



Post Harvest Withering



Seed

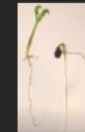


Rachis (axis of the cluster)



Stem (from the cane)

In vitro root

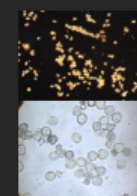


Seedling (seed germination)



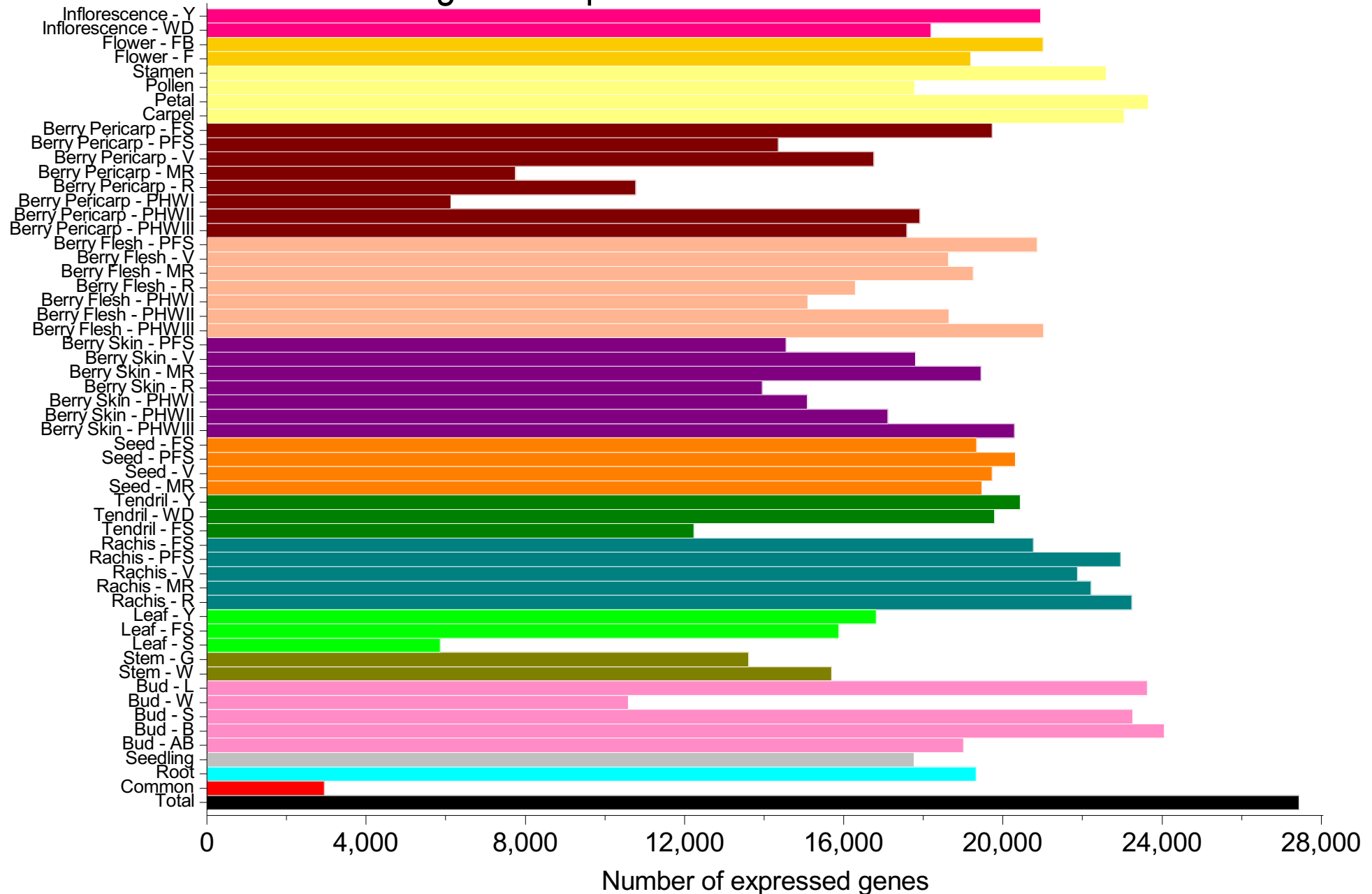
Flower:
- Stamen
- Petal
- Carpel

Pollen



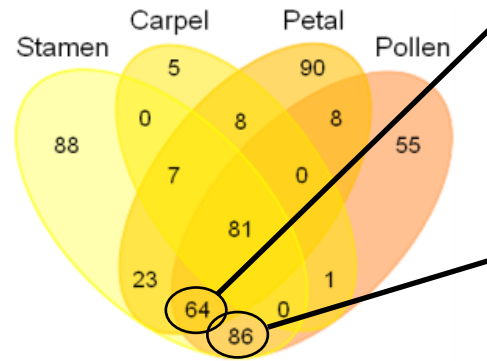
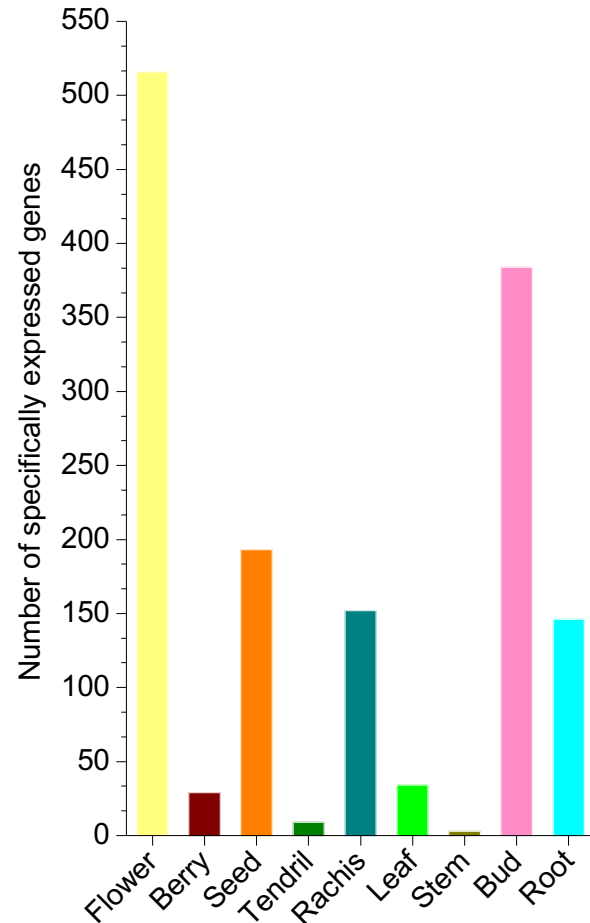
Global patterns of tissue gene expression

Number of genes expressed in each of the 54 tissues



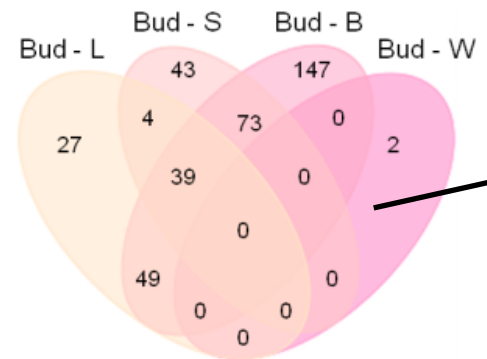
Global patterns of tissue gene expression

Tissue specific genes



- among genes stamen, petal and pollen specific: MYB33-like transcription factor (JGVV59.65), involved in the onset of flowering (Miller and Gluber, 2005)

- among genes pollen and stamen specific: embryogenic pollen-abundant phosphoprotein NtEPc (JGVV0.545), which appeared in the cells undergoing a dedifferentiation process from immature pollen grains to embryogenic cells (Kyo et al., 2000)



Bud specific genes distribution showed shared transcripts in active growth stages, but not during dormancy (Bud - W), undergoing into a rest period during winter.

Shared expression profiles were more common among different organs than at different developmental stages in the same organ

Grapevine Plasticity in the Genomic era

The mechanisms determining plastic changes are still largely unknown especially for **plants cultivated in open fields**, where the simultaneous challenge of different environmental signals leads to complex responses.

Questions need to be answered in the genomic era

- How should plasticity be described and quantified?
- Do genes for plasticity really exist?
- How do the genes affecting the plastic response operate?

Micro-Environmental and Agricultural Features



Altitude
100-450 m. a.s.l.



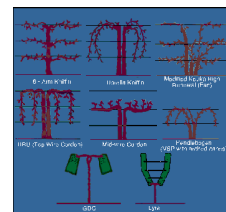
Vineyard Planting Layout



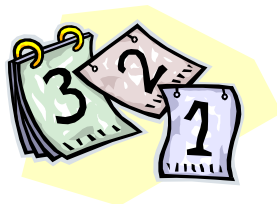
Type of Soil
Sandy-Clay



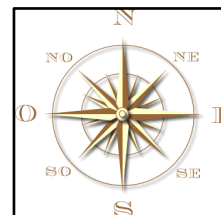
Type of Rootstock
K5BB/41B/SO4/420A



Vineyard Training System
Replacement Cane Guyot
Parral System



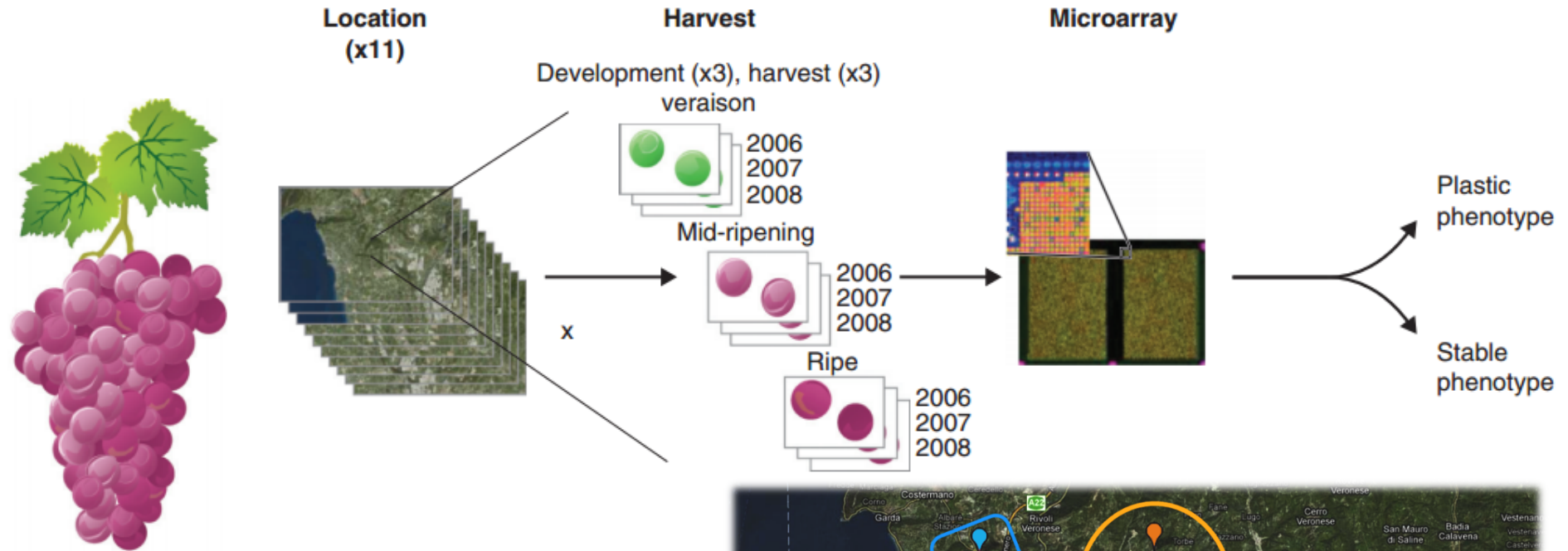
Vineyard Age
6-18 years



Rows Facing Direction
North-South
East-West

Grapevine Plasticity in the Genomic era

The experiment



Vitis vinifera CV *Corvina* – 48 Clone

- Red berry variety
- Major component of “Amarone” wine
- Most important clone cultivated in the Verona area



Phenotypic Plasticity I : the Vintages and Vineyards

The impact of season climate on the berry transcriptome

4 Vineyards sampled at **3** berry developmental stages:

Veraison

3 Vintages studied: 2006

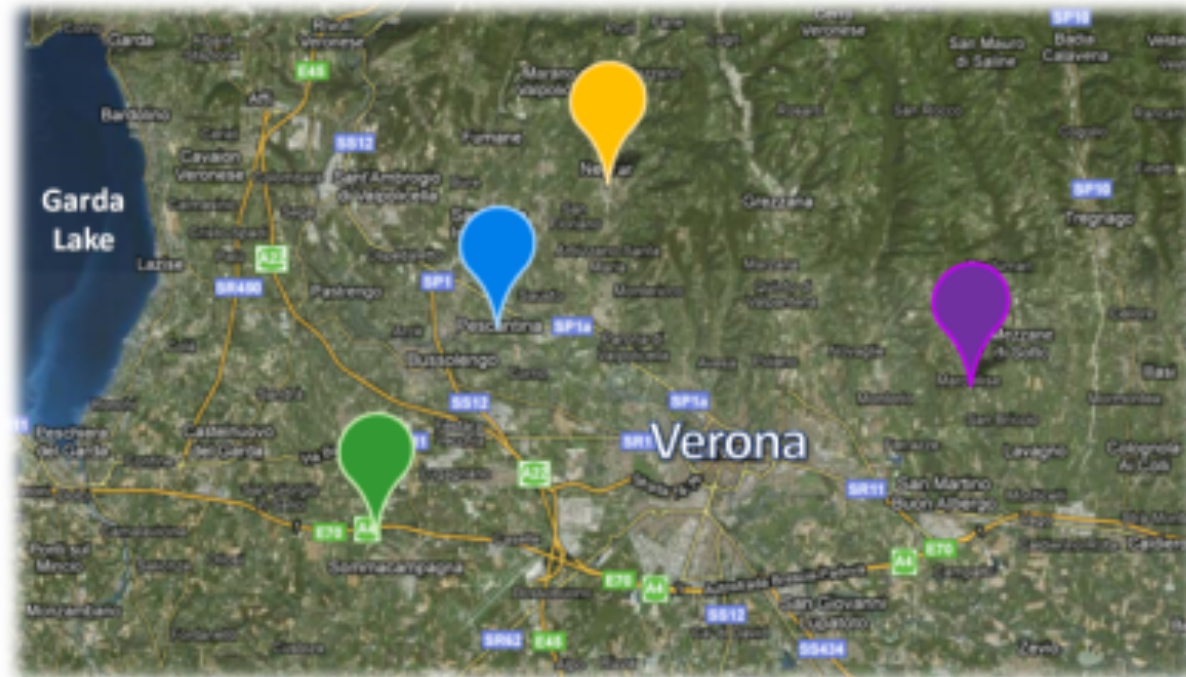
Pre-Ripening

2007

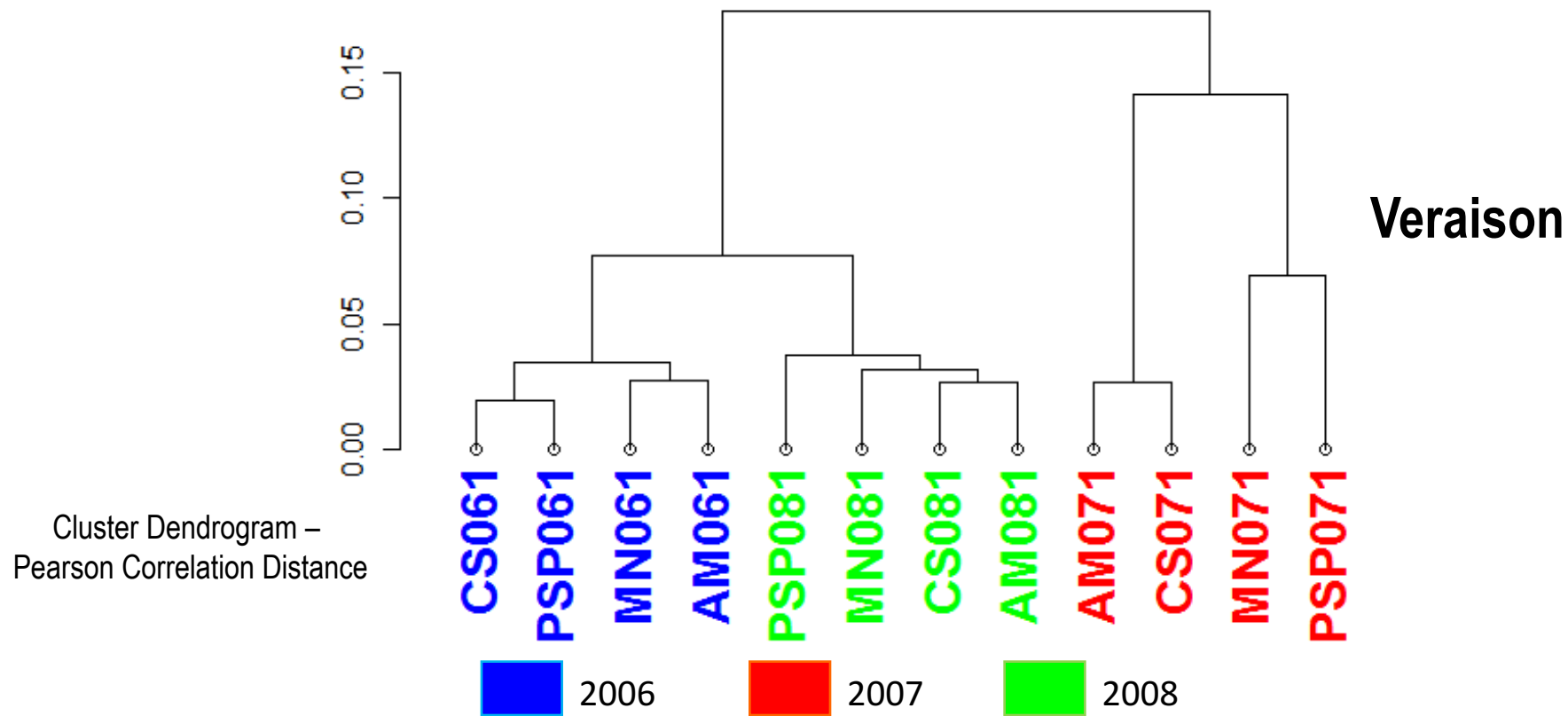
Ripening

2008

4X3x3x3=108 hybridizations



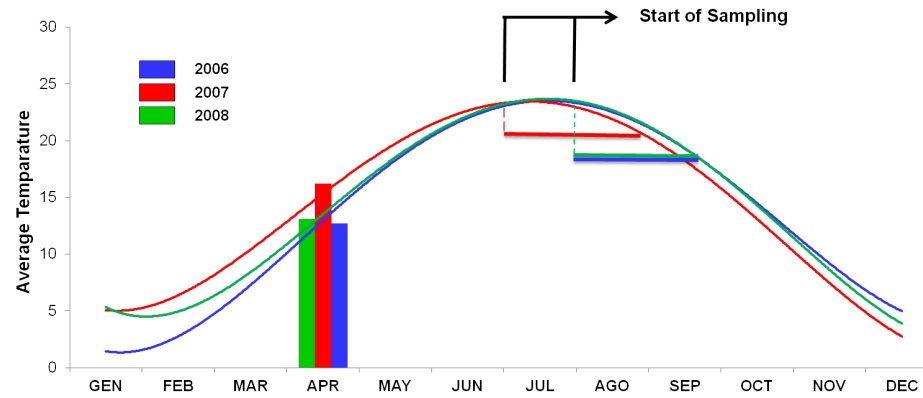
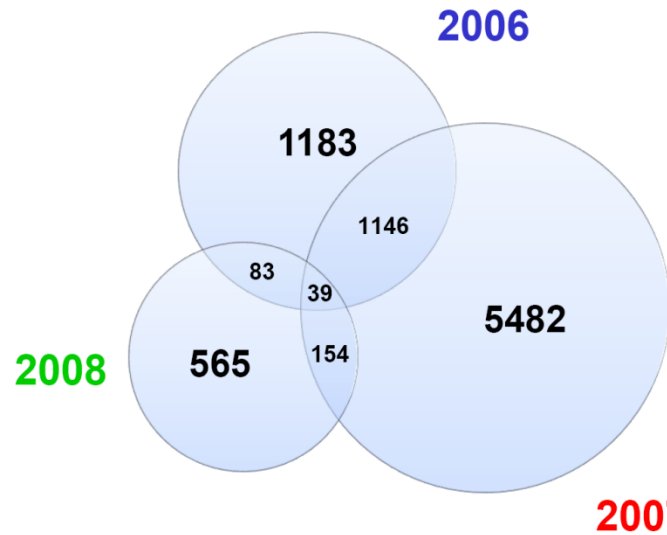
Phenotypic Plasticity I: Vintages and Vineyards



Vintage effect impacts more than vineyard on total variability

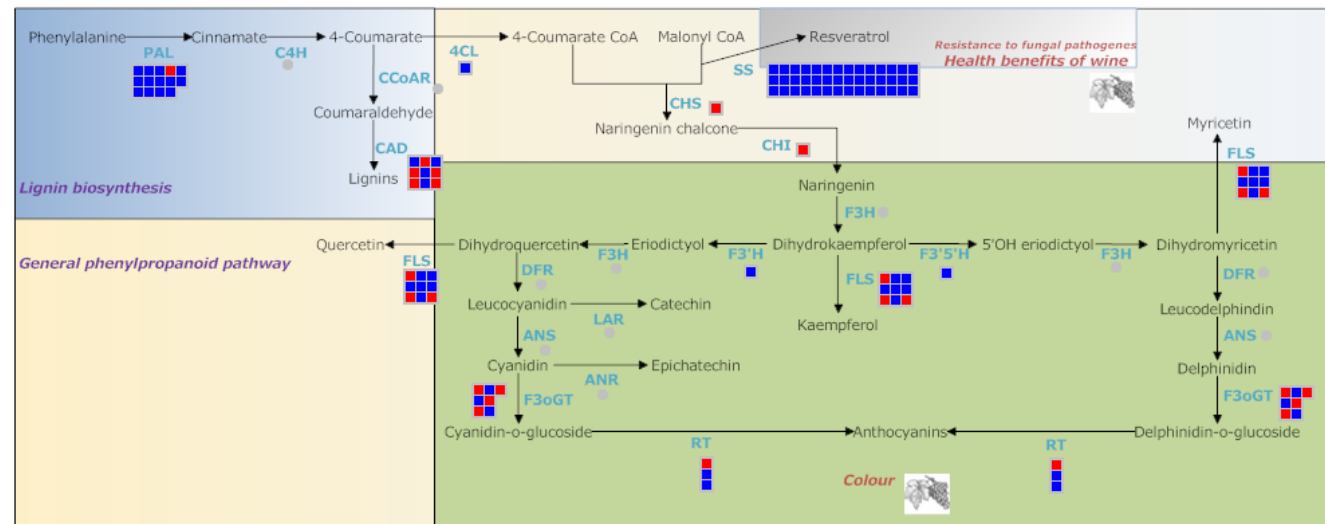
Vintage 2007 is significantly different from 2006/2008 in all vineyards

Differential Gene Expression



2007 scored the highest average temperature over a 45-year period

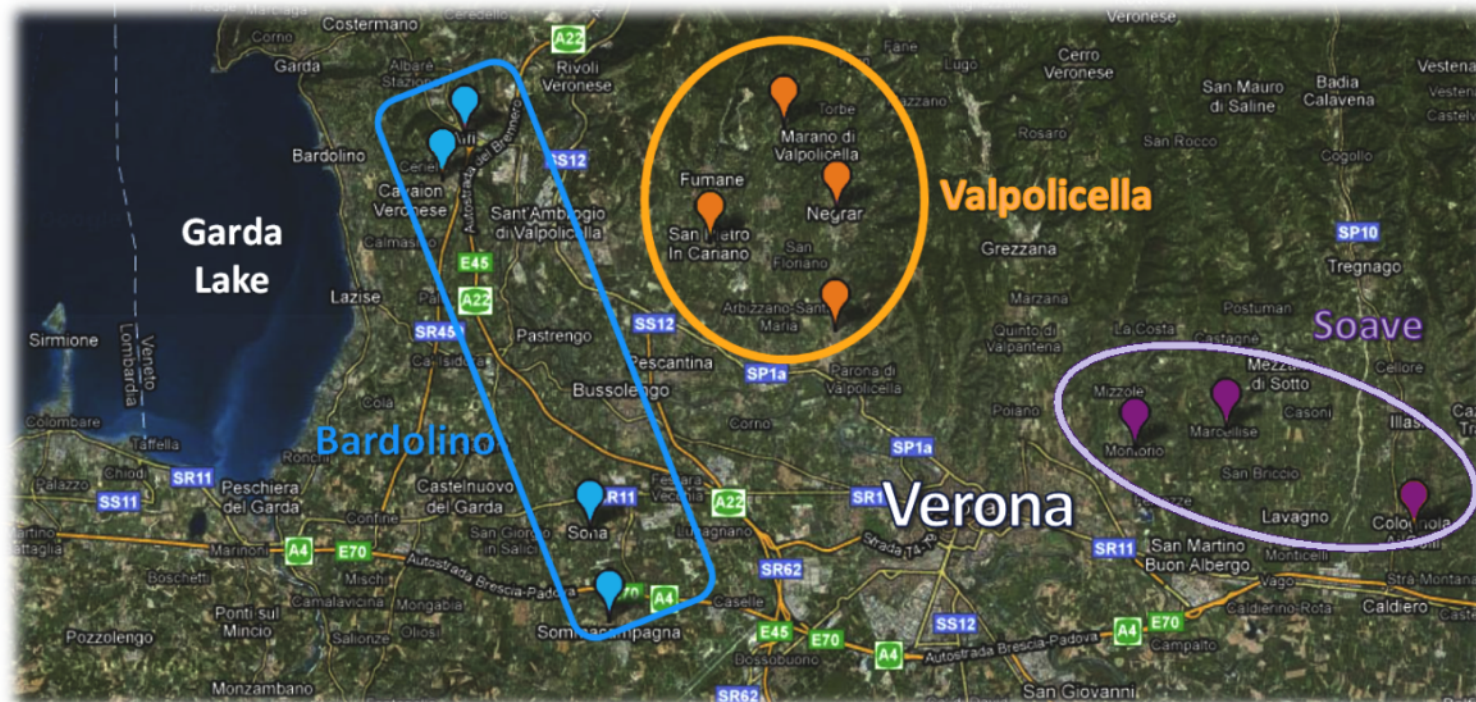
Differentially-modulated genes among the four vineyards studied in each year



Phenotypic Plasticity II: Berry Development

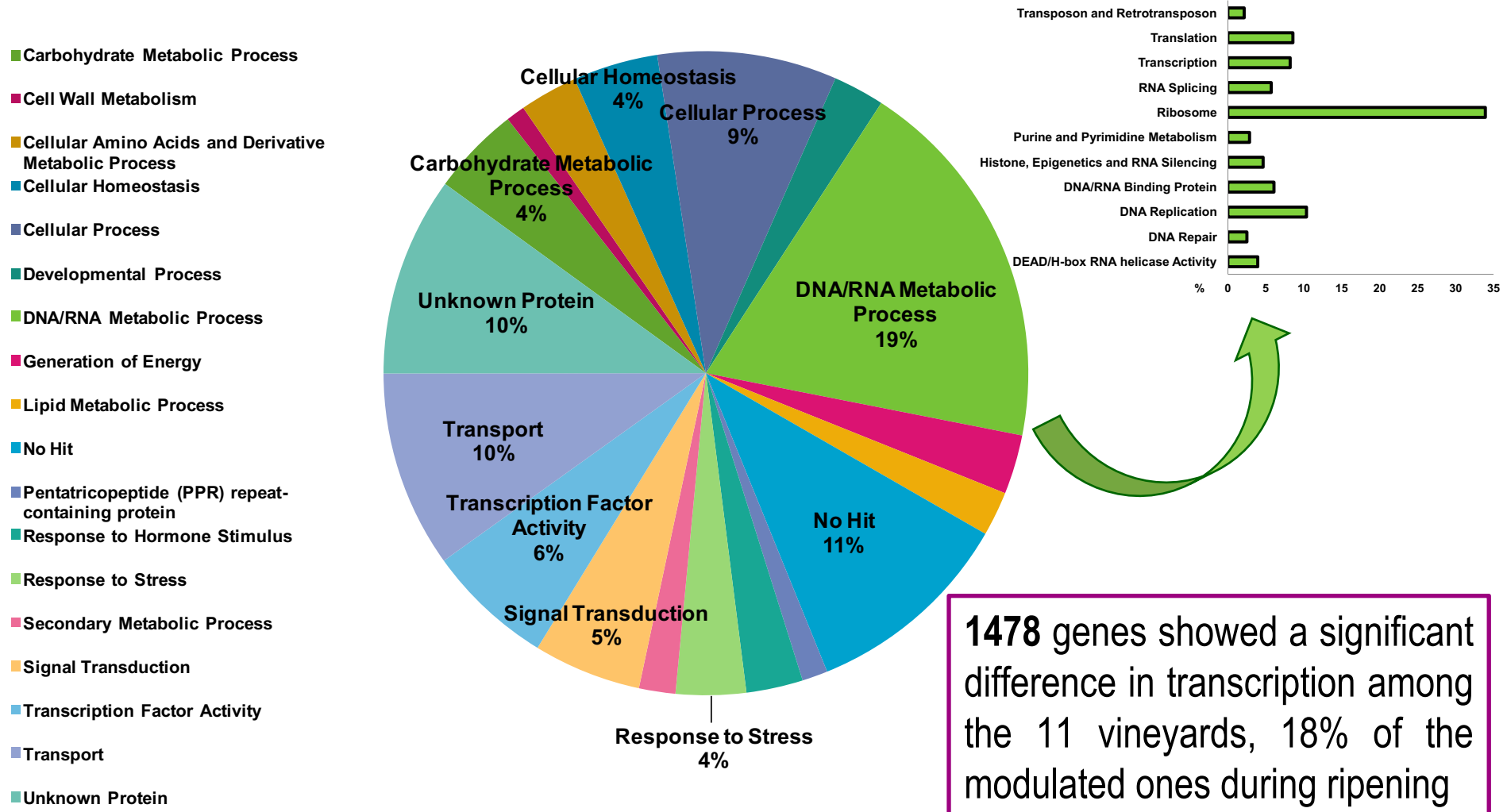
- 11 Vineyards sampled at 3 berry developmental stages: **Veraison**
1 Vintage studied: **2008**
Pre-Ripening
Ripening

11x3x3=99 hybridizations

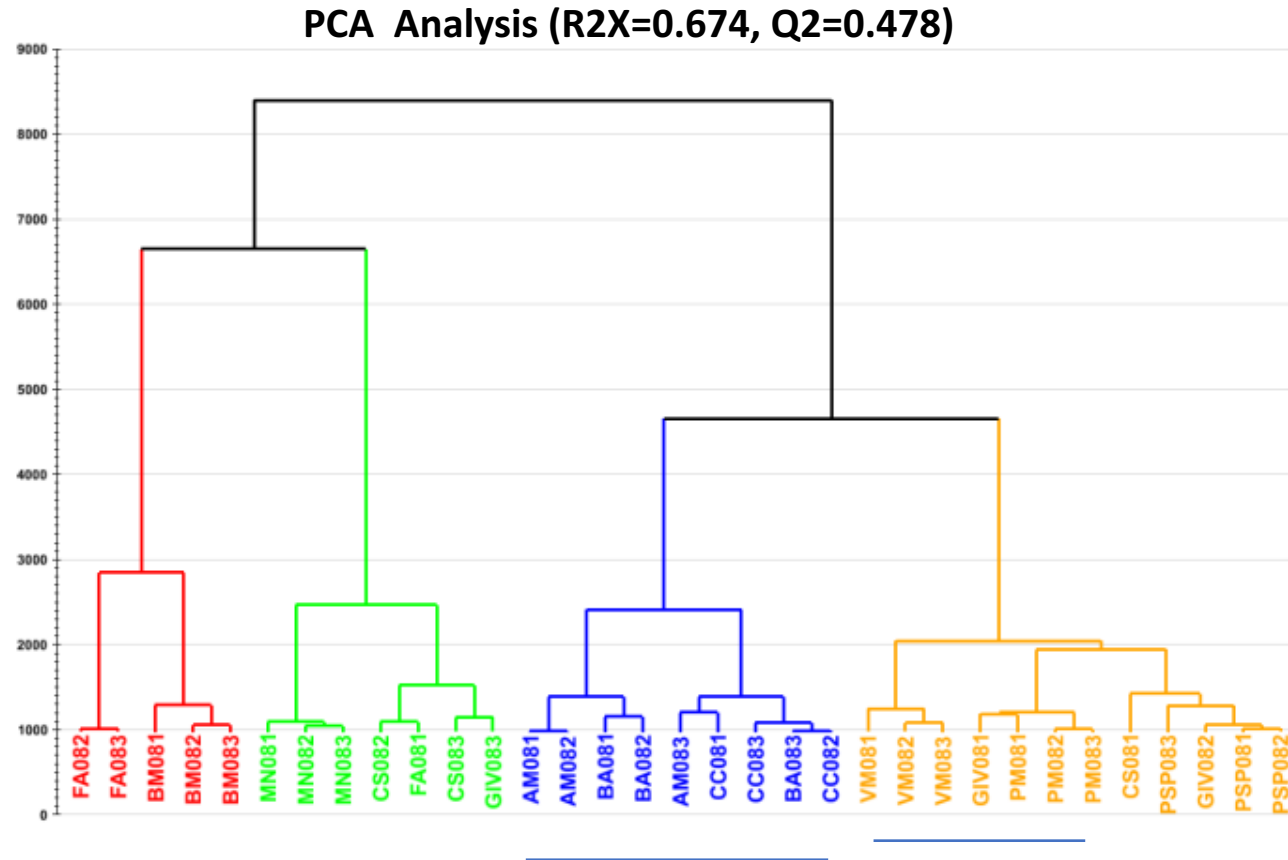


Transcriptome Plasticity during Berry Development

Samples from **2008** harvested in **11** Vineyards in **3** developmental stages



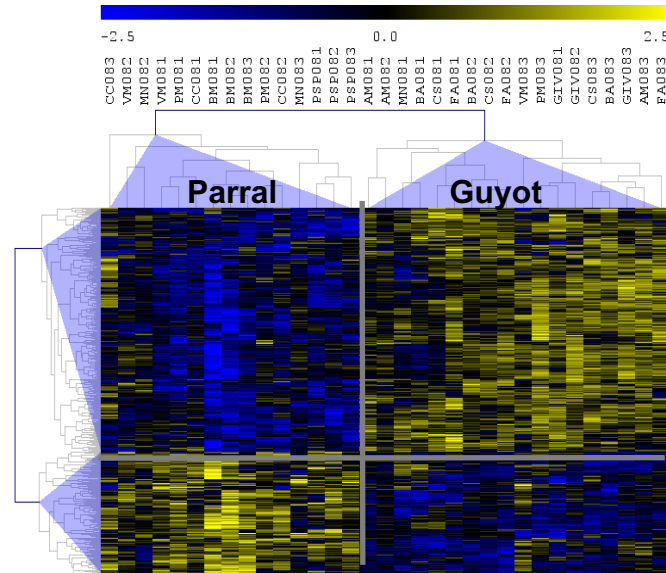
Plasticity In Clusters of Vineyards



Vineyards with a shared “transcriptome signature” during berry development cluster together

Plasticity and Farming/Environmental Conditions

Kruskal-Wallis Test (FDR=0.25%) - Bi-clustering (Pearson Correlation)



Plastic genes preferentially transcribed in vineyards using **specific farming practices**

Vineyard Training System

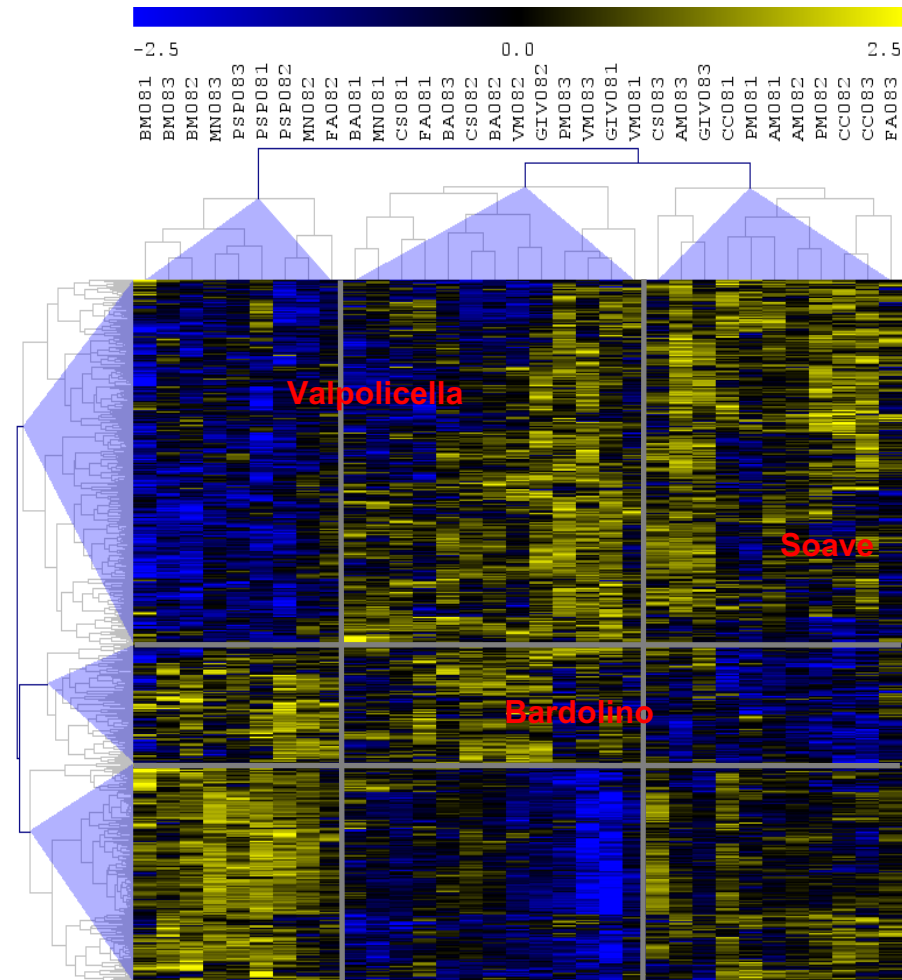


Plasticity and Farming/Environmental Conditions

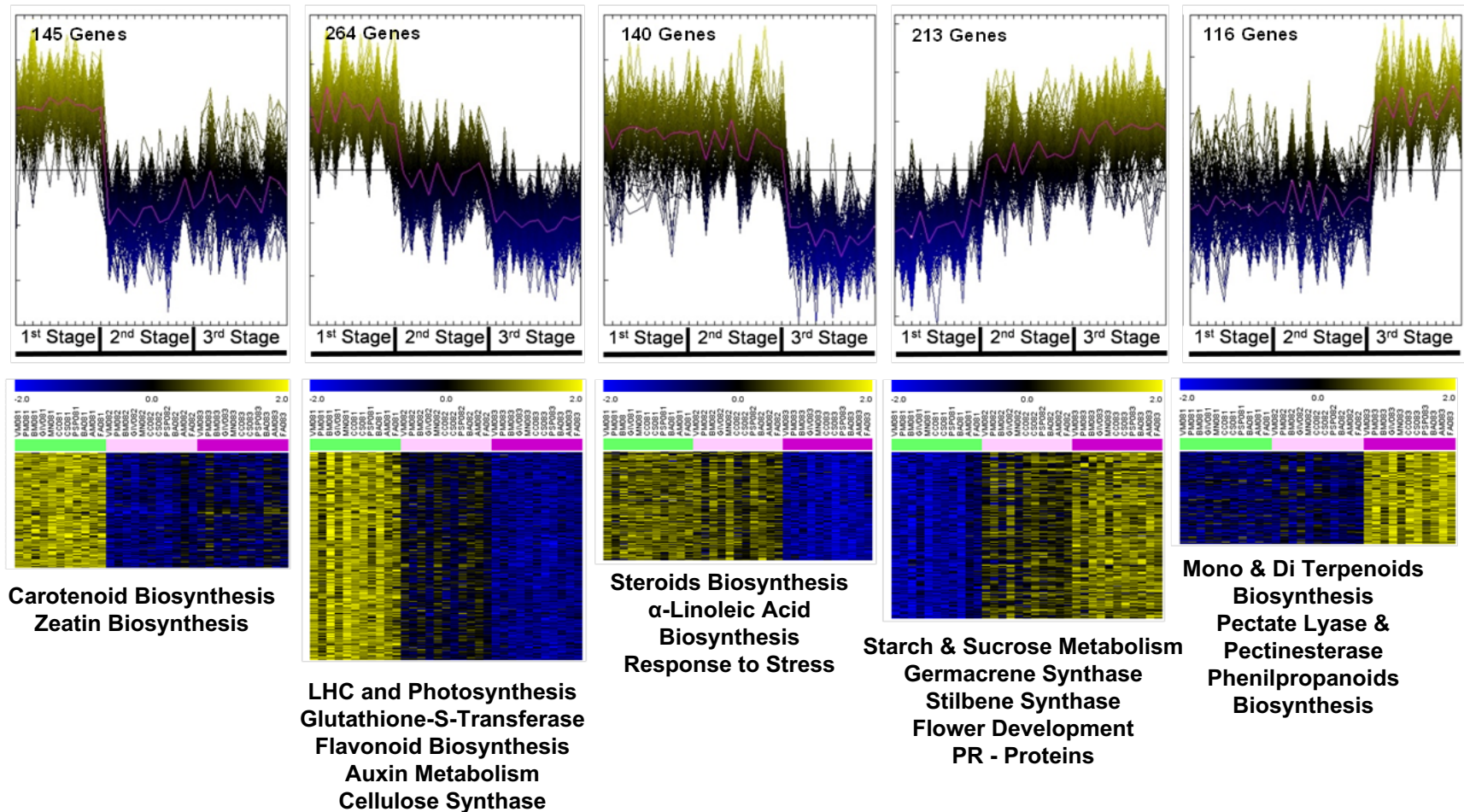
Kruskal-Wallis Test (FDR=0.25%) - Bi-clustering (Pearson Correlation)

Vineyard Geographical Area

Plastic genes preferentially transcribed in vineyards having specific environmental conditions



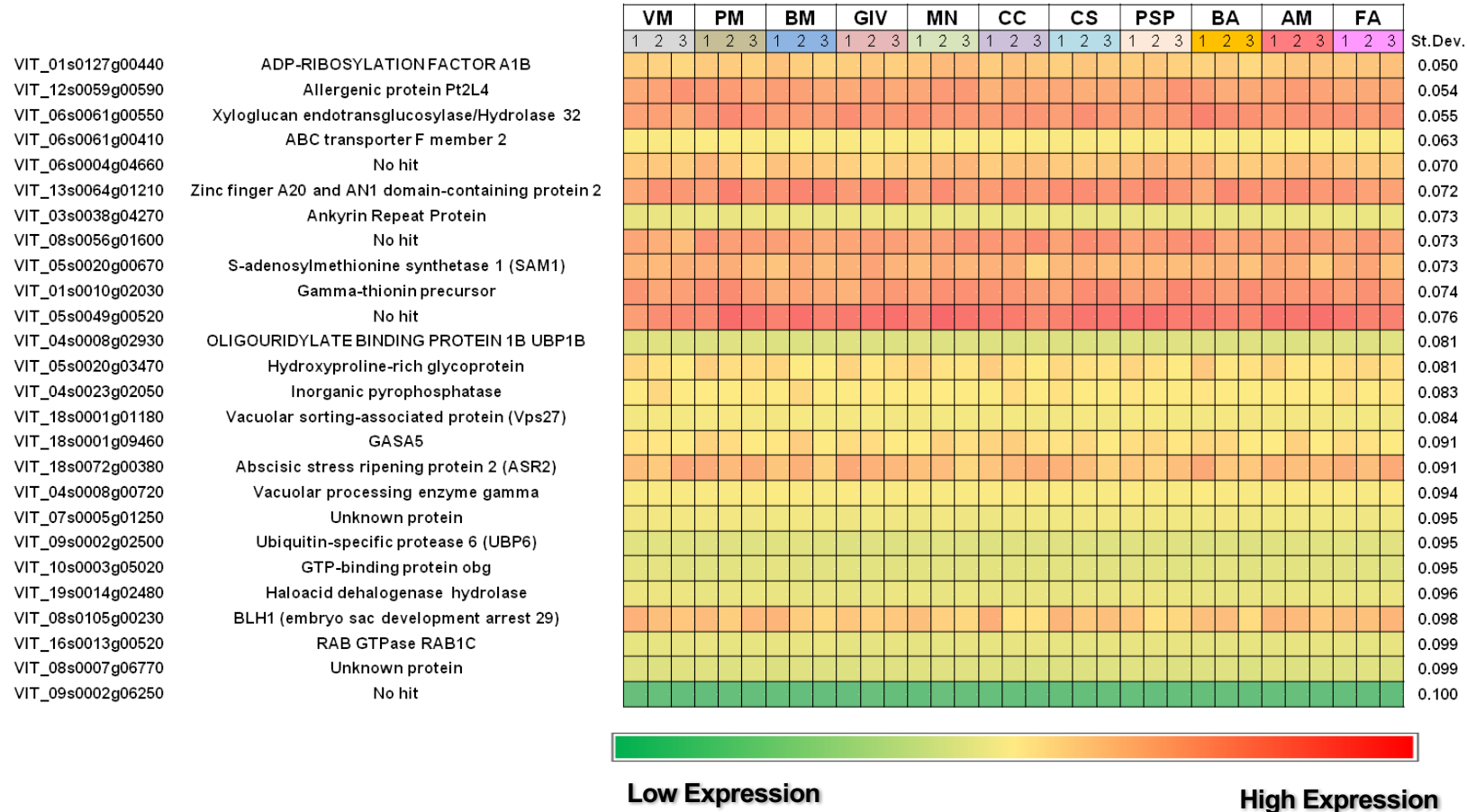
Non plastic transcripts



These transcripts could be developed into **universal markers** suitable for the monitoring of grape ripening in the field, regardless of cultivar and environment.

Non-Plastic Transcripts - Constitutive

Transcripts neither plastic (no variation among the 11 vineyards) nor developmentally modulated (no variation among the three developmental stages)



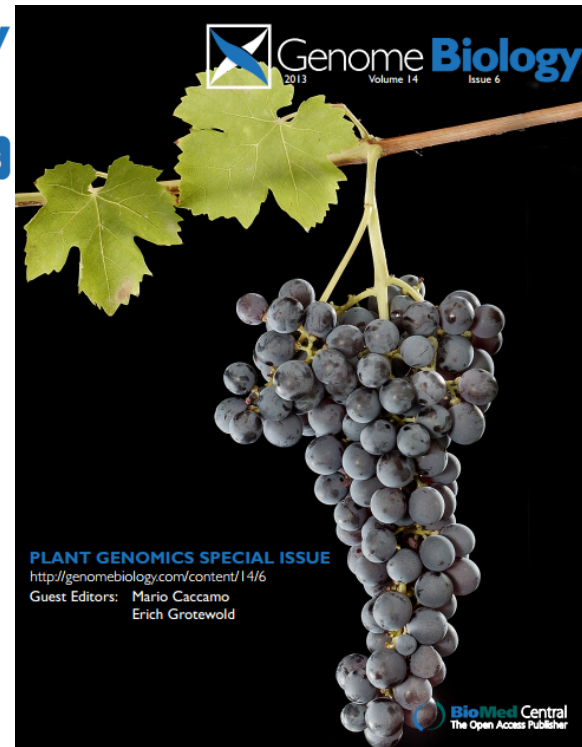
These non-plastic constitutive genes are candidate reference genes for quantitative gene expression analysis.

RESEARCH PAPER

Open Access

The plasticity of the grapevine berry transcriptome

Silvia Dal Santo¹, Giovanni Battista Tornielli¹, Sara Zenoni¹, Marianna Fasoli¹, Lorenzo Farina², Andrea Anesi¹, Flavia Guzzo¹, Massimo Delledonne¹ and Mario Pezzotti^{1*}



- Phenotypic plasticity can be investigated at transcriptomic/genomic level.
- Plastic genes do operate for adapting the plants to environmental changes.
- Non-plastic genes are also very crucial for adaptation.

OUTLINE

Phenotypic Plasticity in Plants: definition, role in evolution and plant breeding

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Grapevine Plasticity in the genomic era

Grapevine Genotype X Environments in grape in the genomic era

Phenotypic plasticity and Genotype X Environment Interaction

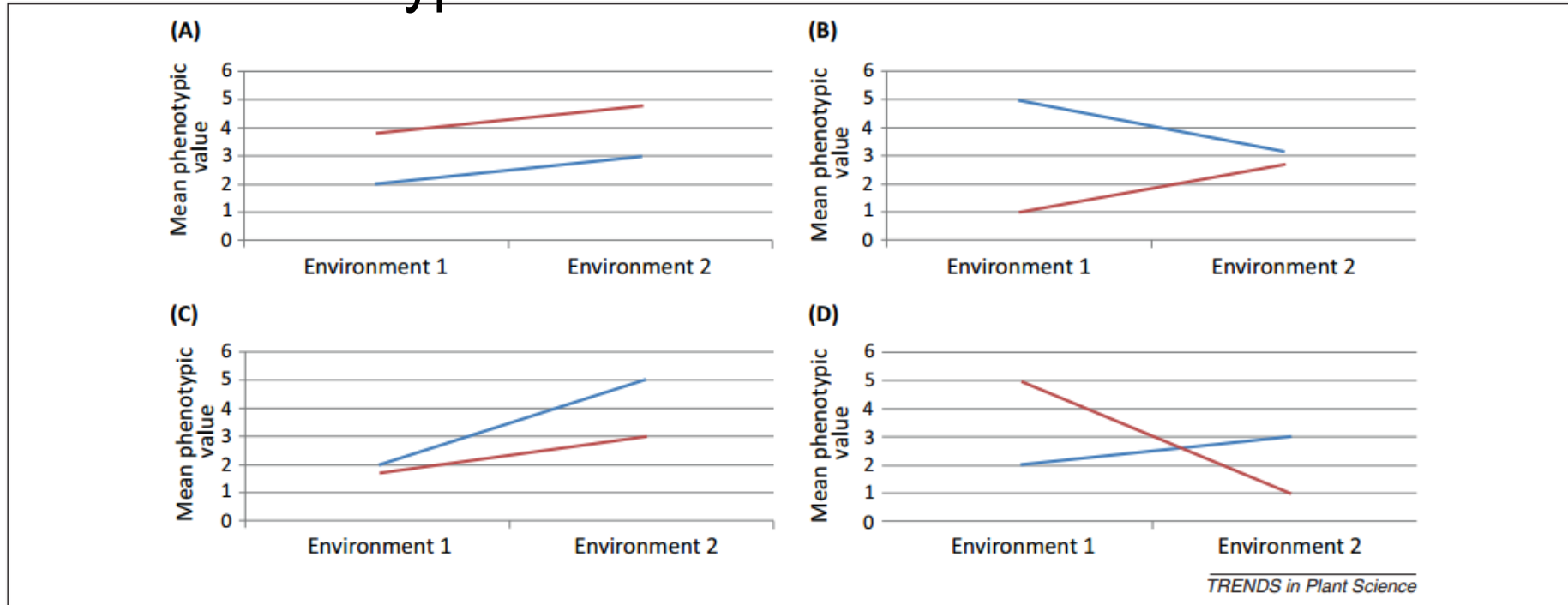
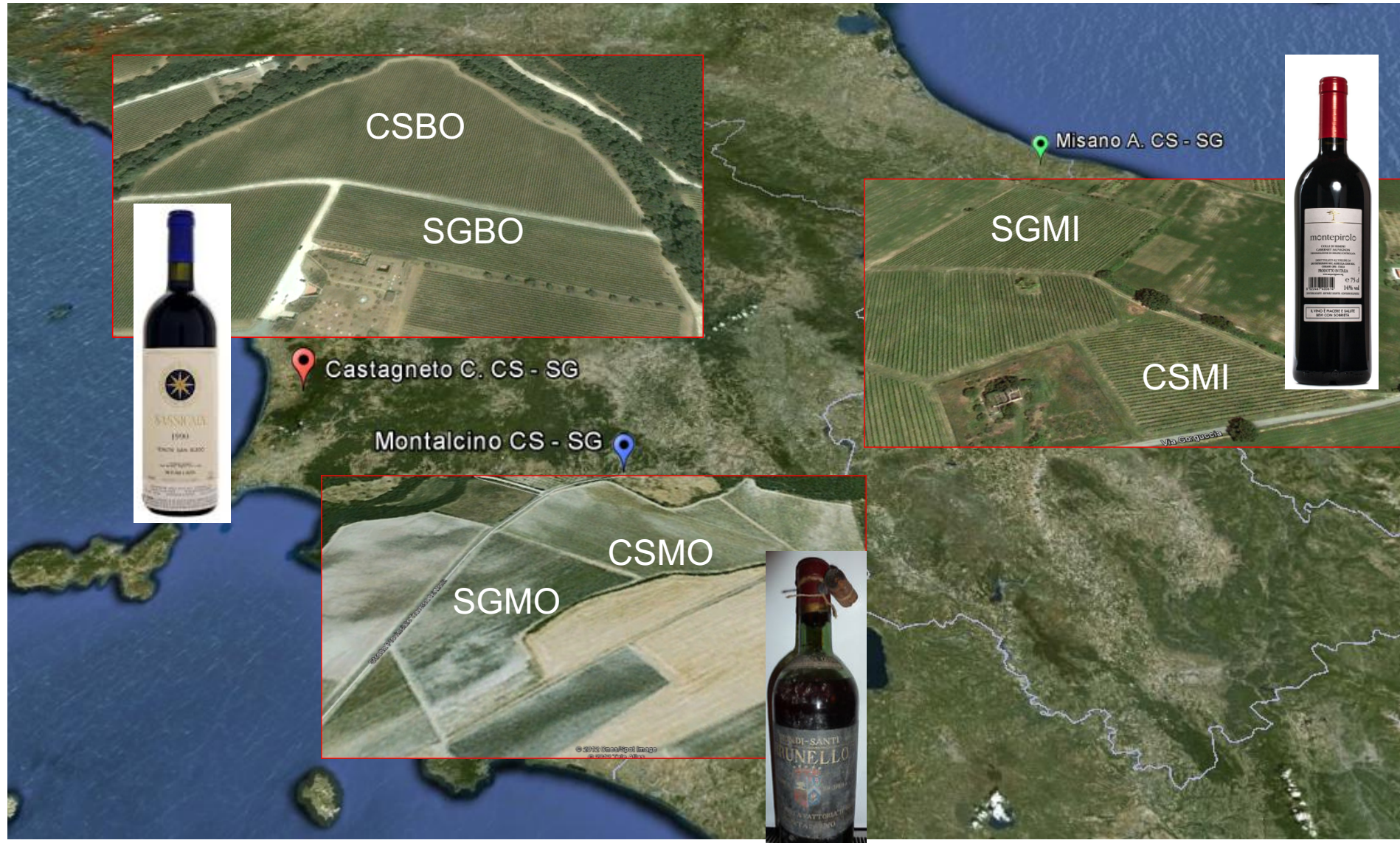


Figure 1. Phenotypic plasticity and genotype × environment interaction (G × E). Four examples of reaction norms illustrating (A) the phenotypic plasticity of genotypes between environments, and (B–D) a genetic difference between genotypes for plasticity indicating G × E. Genotype 1 is the red line and genotype 2 the blue line in each graph. In (A), the trait values change across environments but the reaction norms run parallel because the response to the environmental conditions is similar for both genotypes. In (B,C), there is a scale-effect interaction indicating G × E, because each genotype has a different response to each of the environments, but without the reaction norms to cross, whereas in (D), there is a stronger genetic effect on the phenotype in response to the different environments, also indicating G × E, causing reaction norms to cross.

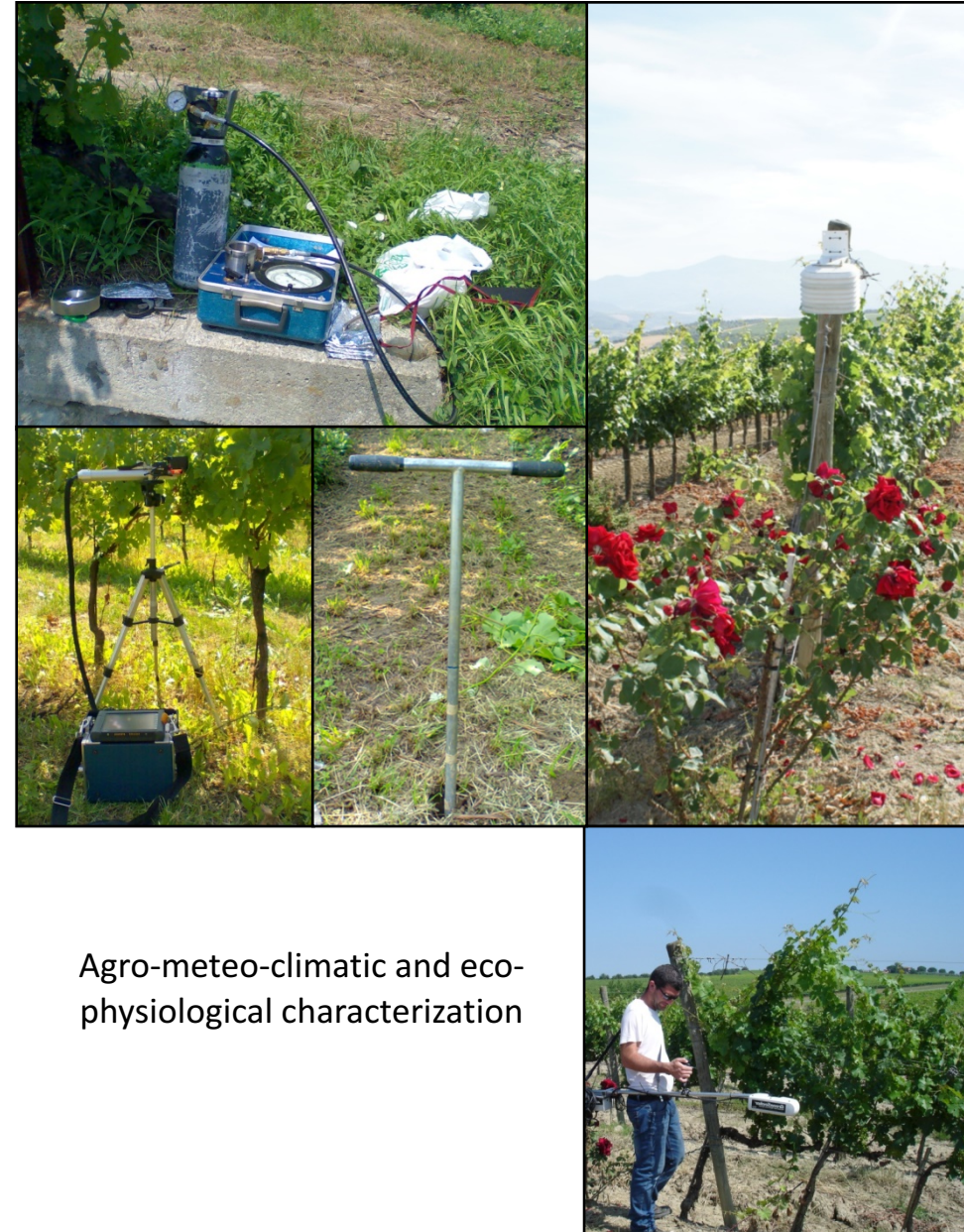
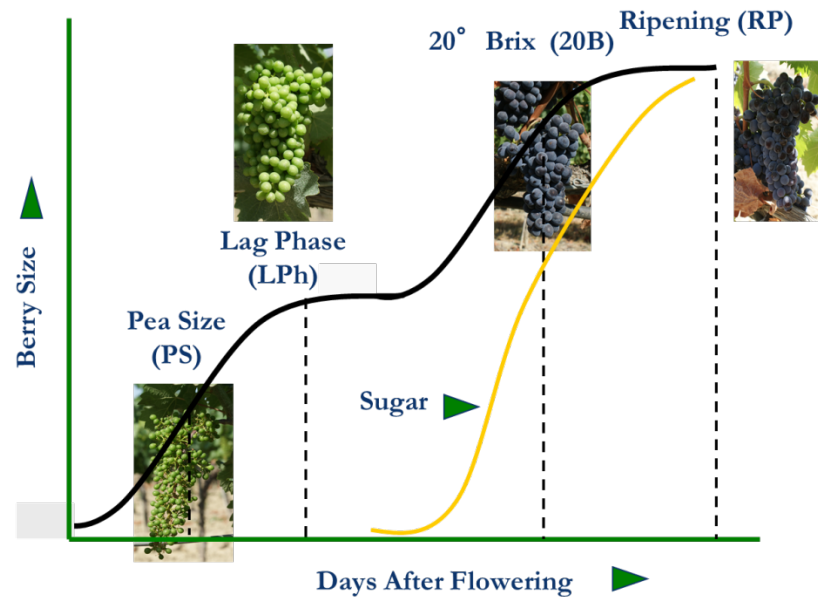
From El-Soda et al.2014

$$P = G + E + GXE$$

G X E Interaction in Grape: The Vineyards



The Sampling Procedure



Agro-meteo-climatic and eco-physiological characterization

The Experimental Plan

Bolgheri
Littoral Tuscany

Montalcino
Central Tuscany

Romagna
Foothill Area

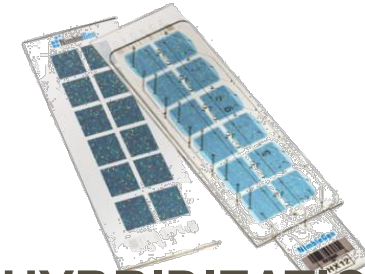
Sangiovese



Cabernet
Sauvignon



- 2011 and 2012
- 3 Biological Replicates



144 HYBRIDIZATIONS

Pea Size



Pre-Veraison



Mid-Ripening

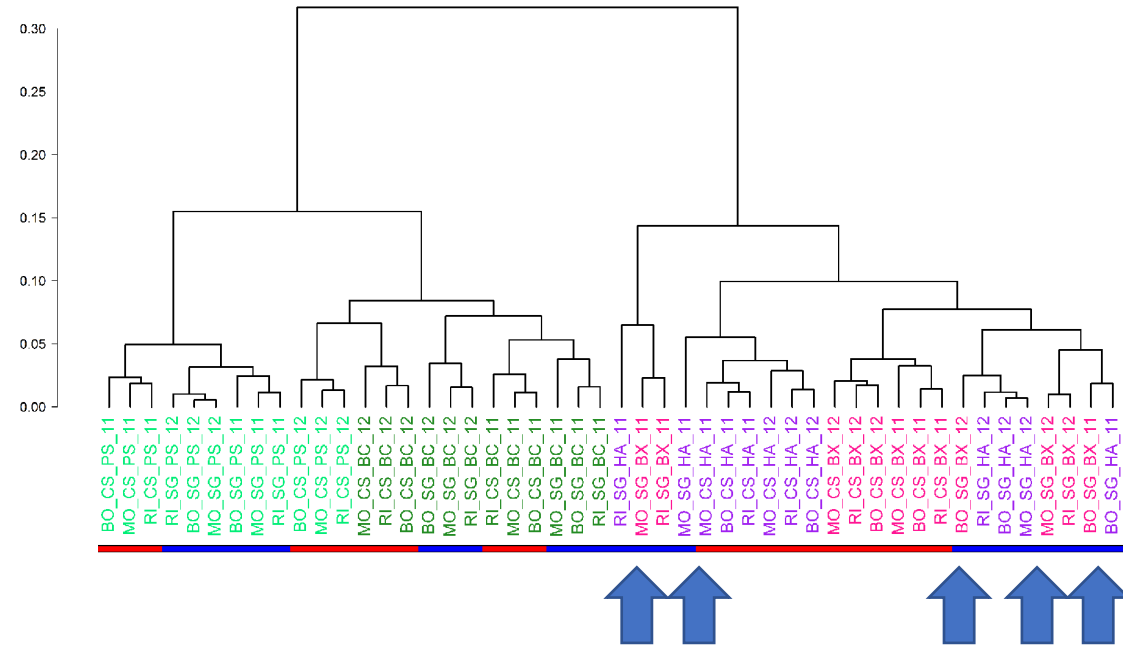
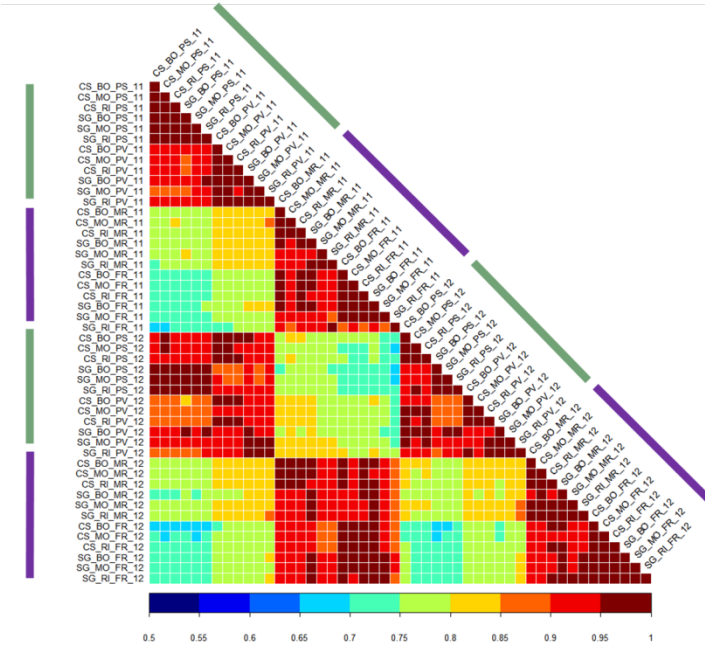


Ripe



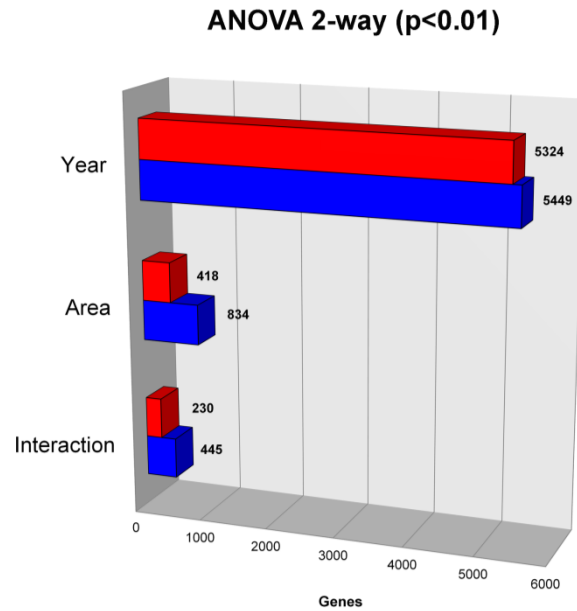
Dataset Exploration

Pearson's Correlation Analysis



- In a multi-year experimental design the Area variable *per se* has a slight impact on the grapevine transcriptome plasticity.
- The Cultivar Sangiovese samples (MR and FR) clustered less uniformly

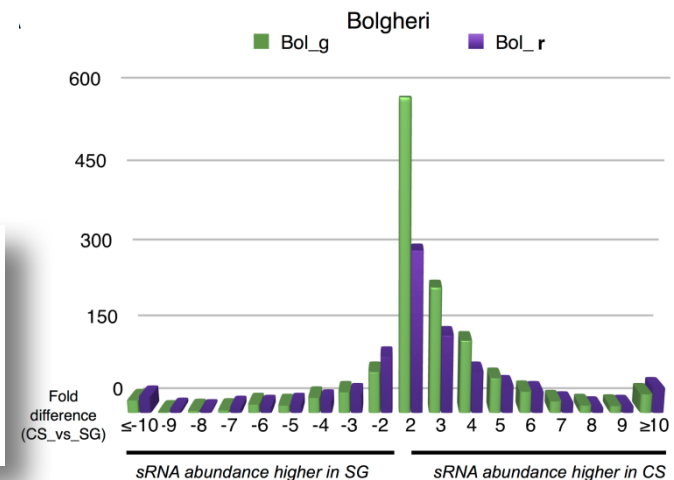
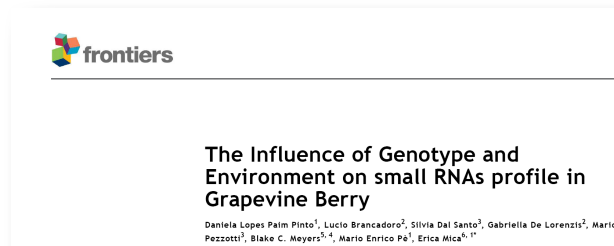
Dataset Exploration



Sangiovese modulates more gene among the 3 Areas and in Year x Area Interaction

■ Cabernet Sauvignon
■ Sangiovese

Cabernet Sauvignon berries have a higher number of expressed sRNA-generating loci than Sangiovese berries, collected in the same conditions.

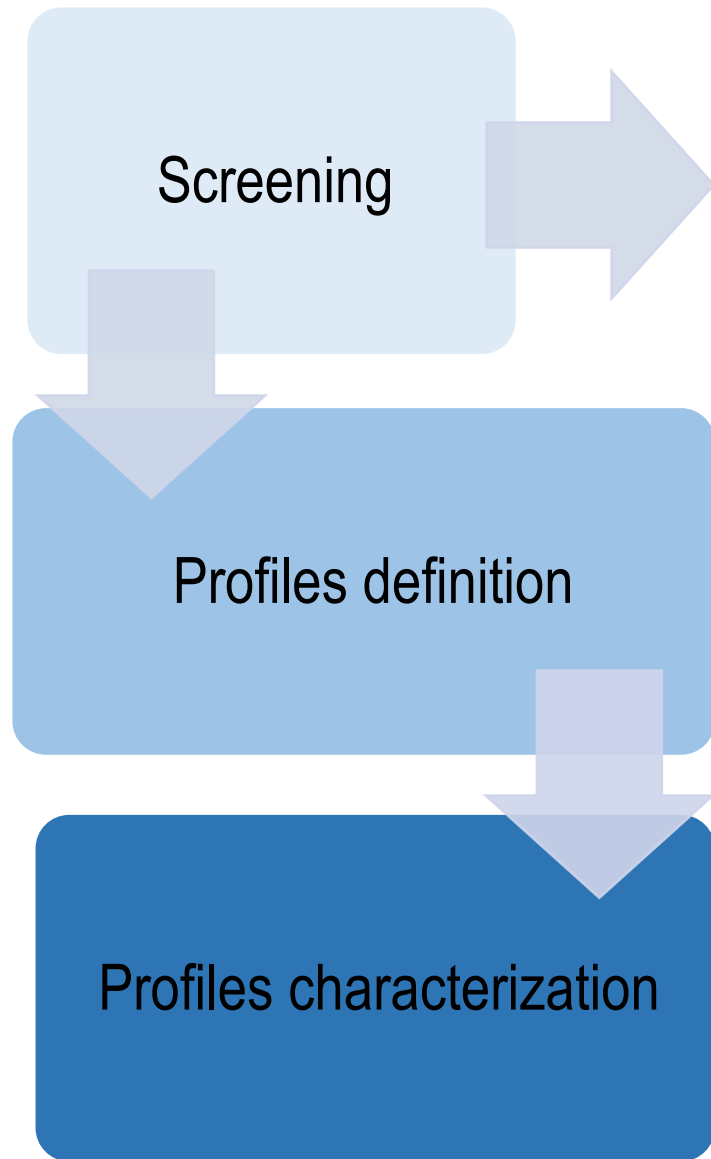


Mica et al., 2016, in press

G X E Interaction in Grape: Dissection of P value

2011	Genotype/Site	Montalcino	Bolgheri	Riccione
	Sangiovese			
	Cabernet			
2012	Genotype/Site	Montalcino	Bolgheri	Riccione
	Sangiovese			
	Cabernet			

$$P = G + E + G \times E$$

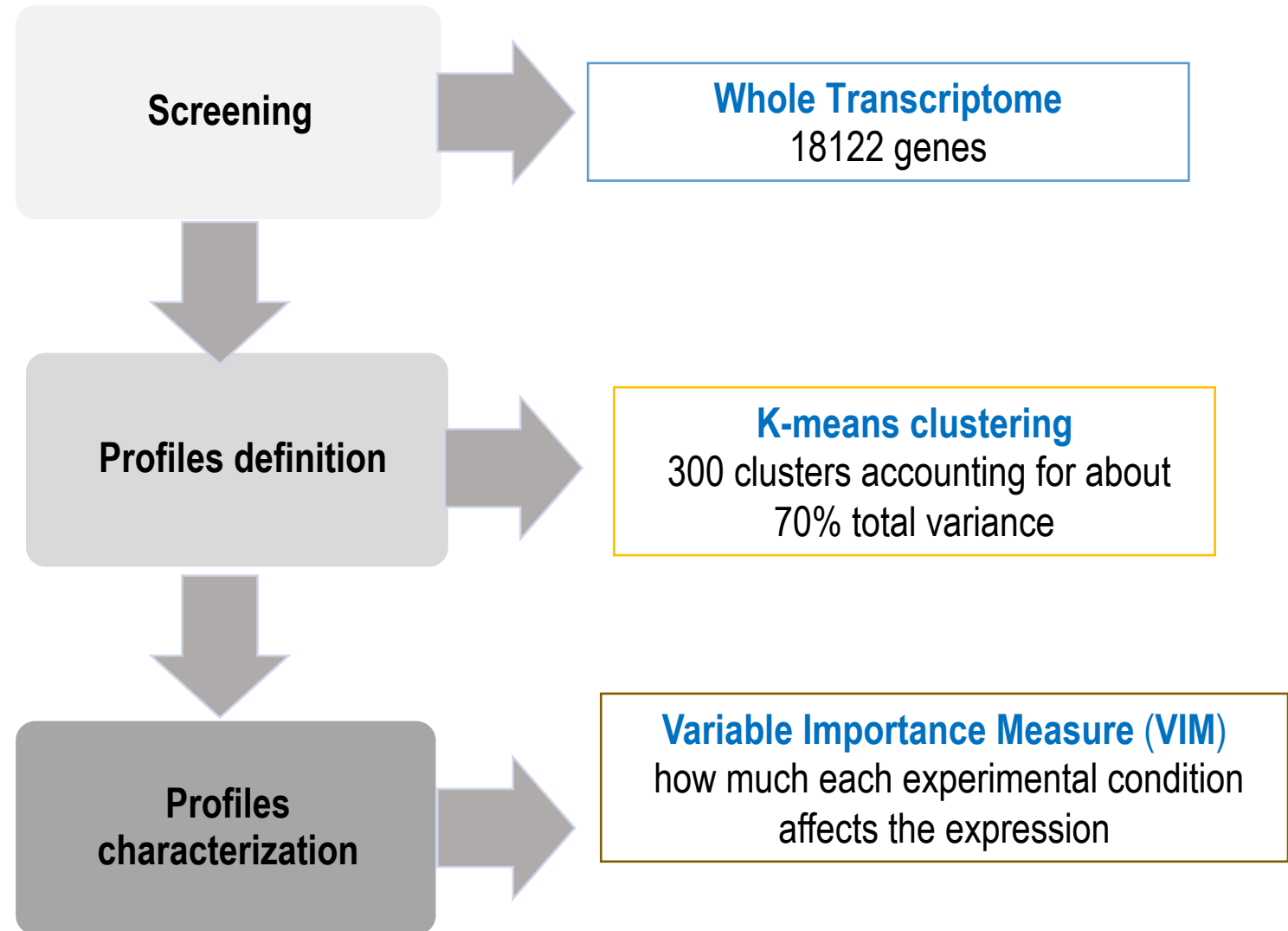


We slimmed down the list:

- Unexpressed genes (threshold) – **5507 genes**
- Genes with low variability among the experimental conditions (low ratio between over total variance) – **4209 genes**
- Genes with outliers expressions – **238 genes**
- Genes with pattern not linked to any experimental conditions (i.e, stage; cultivar; year; area) – **1473 genes**

18122 variables (genes) remaining

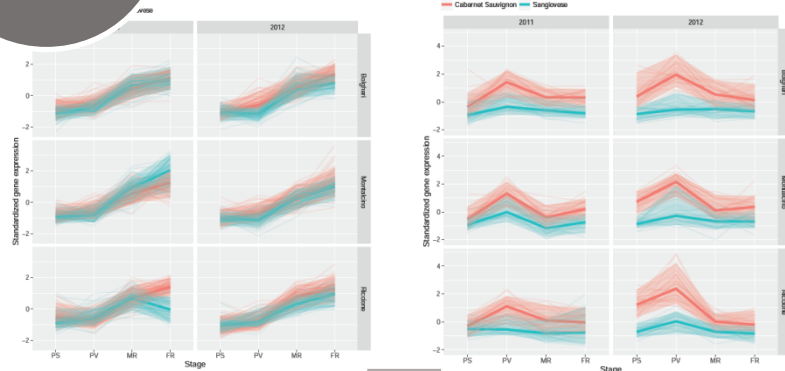
A new Statistical Pipeline



Gene clusters characterization

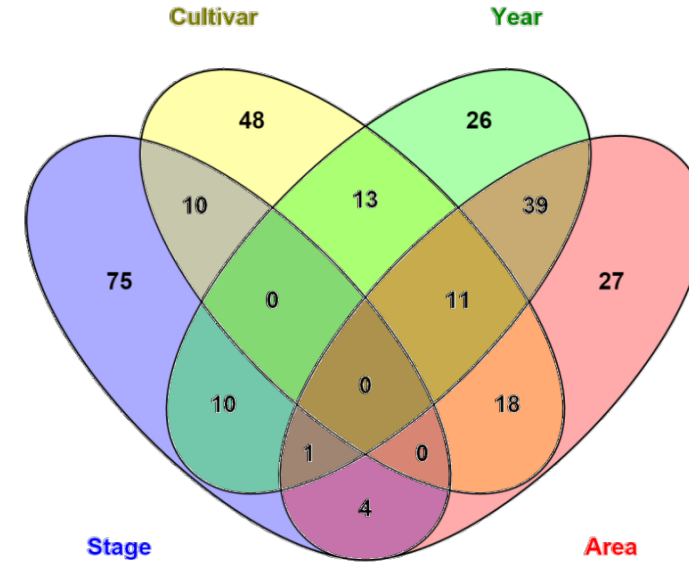
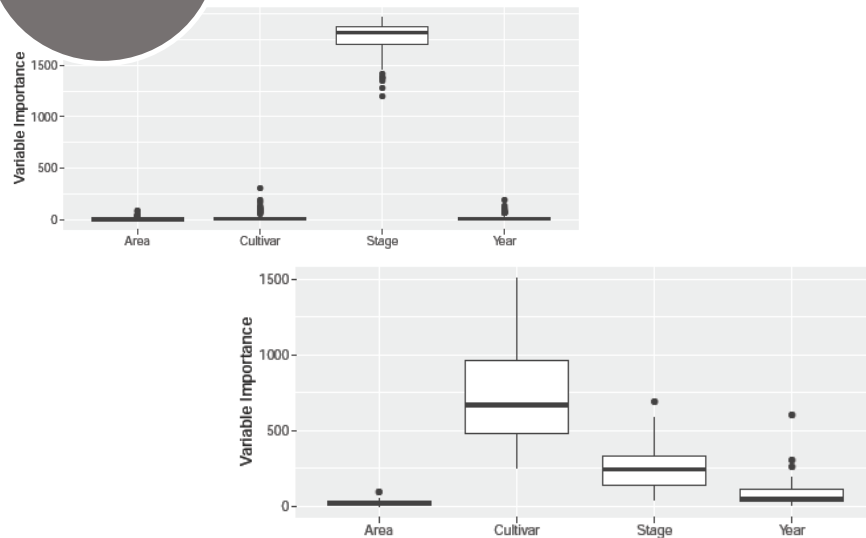
300x

Clusters



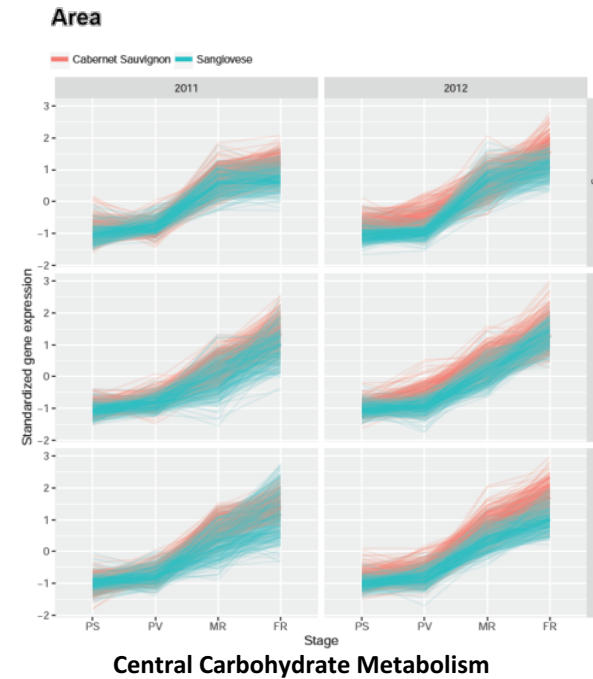
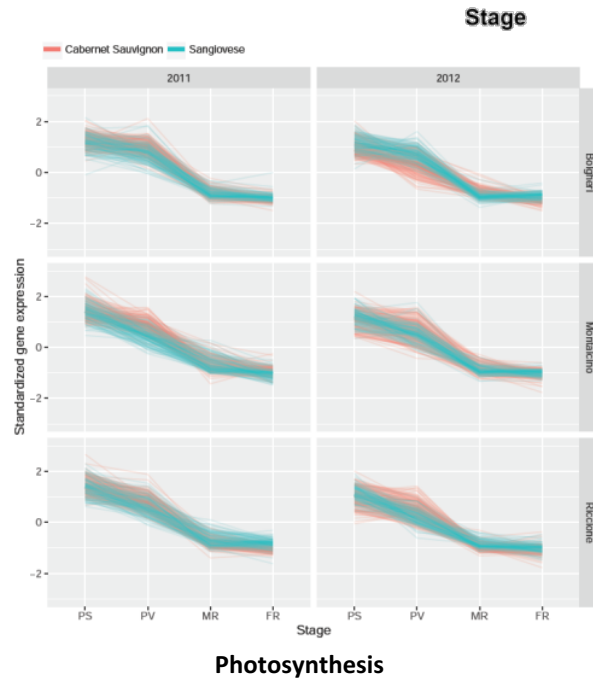
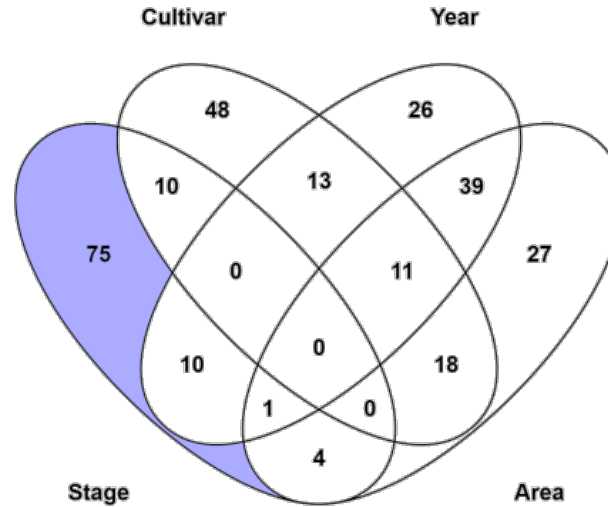
300x

Median VIM of each Variable



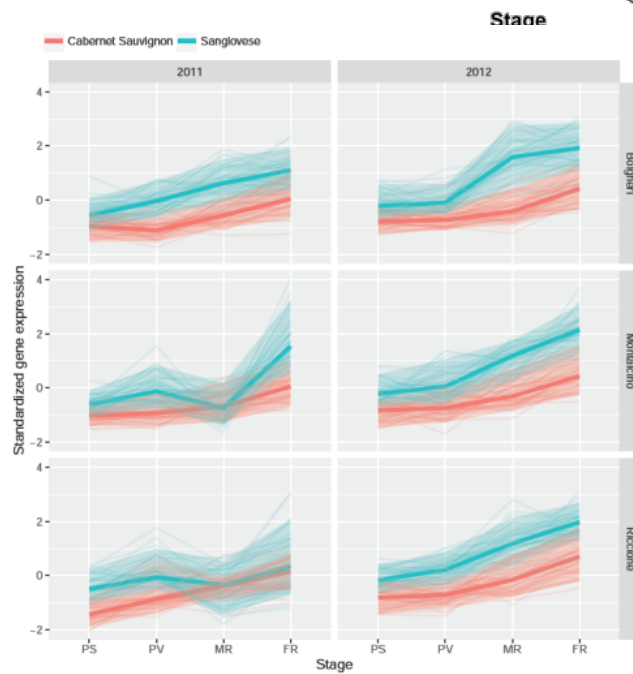
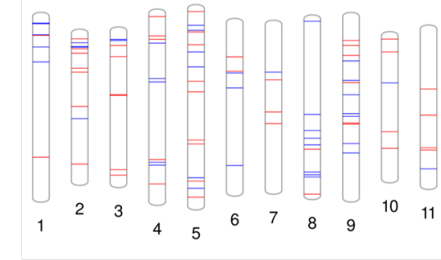
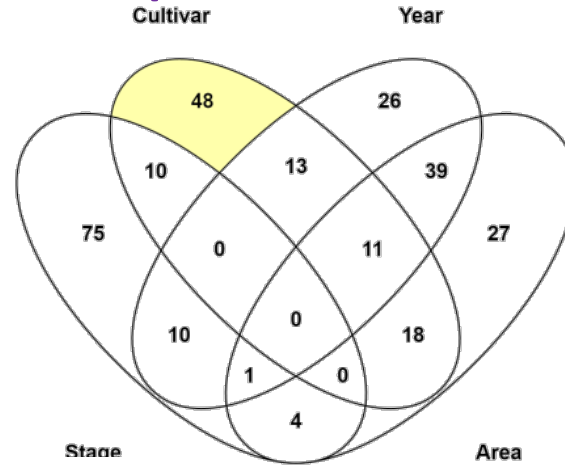
Multiple Variable Dependent Clusters
Single Variable Dependent Clusters

Stage Dependent Gene Clusters

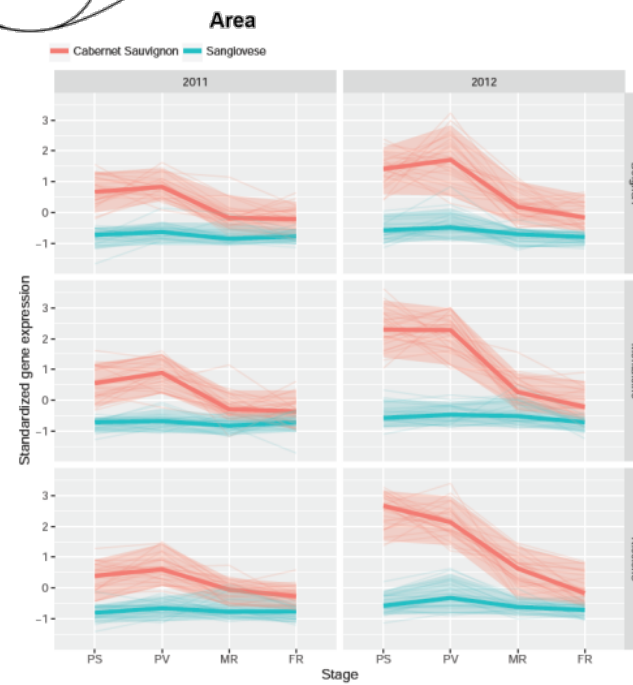


Stage-dependent clusters are enriched in Primary Metabolism GOs

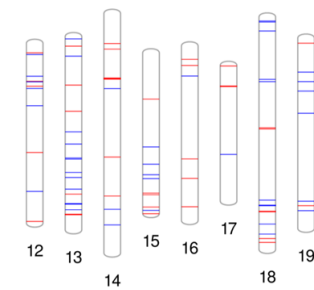
Cultivar Dependent Gene Clusters



TIR-NBS-LRR disease resistance

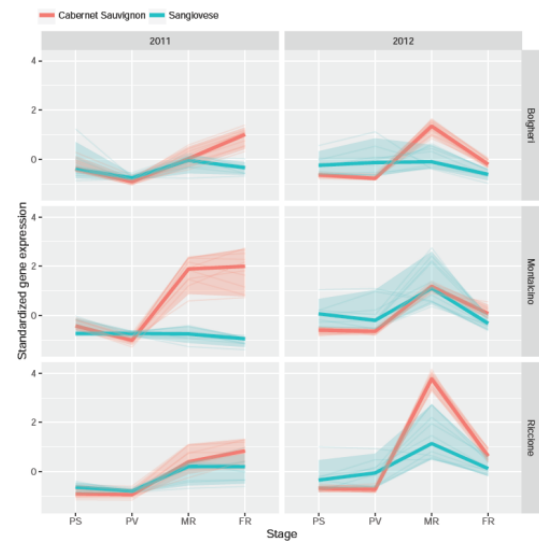
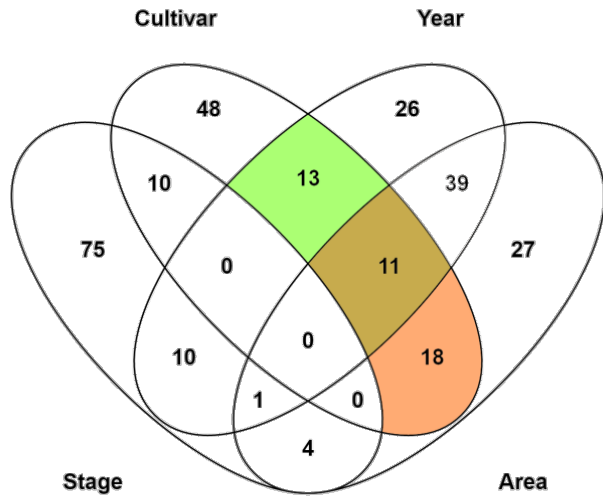


R-proteins

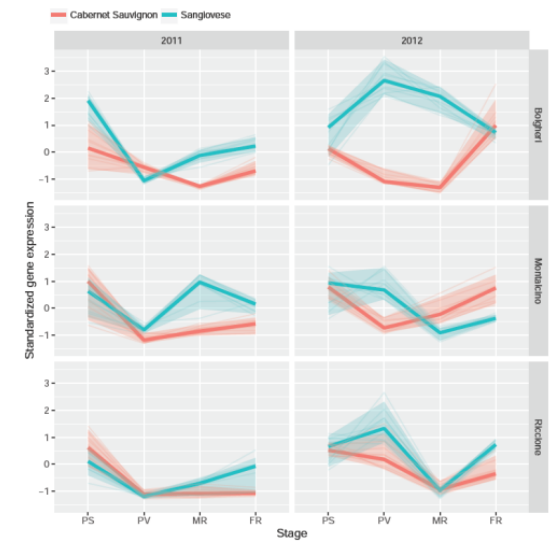


Cultivar-dependent clusters are enriched in **Response to Biotic Stimuli** GOs

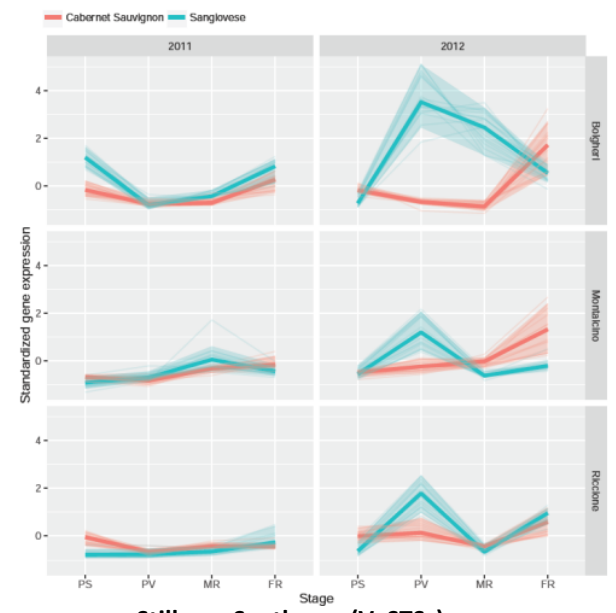
GxE Gene Clusters



Linalool synthases (VvTPSs)



Phenylalanine ammonium lyases (VvPALs)

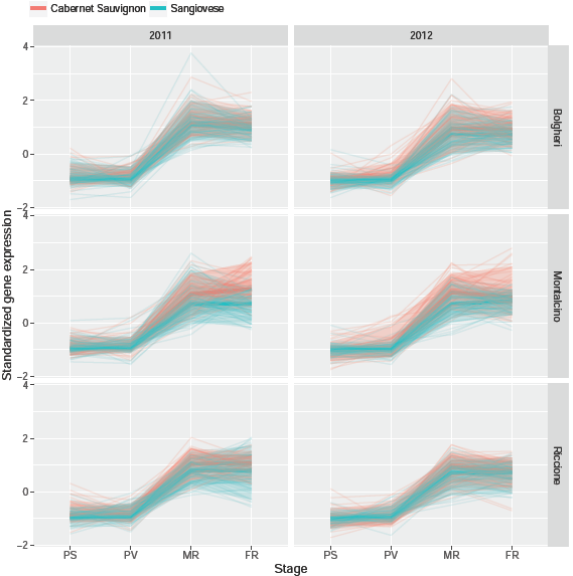


Stilbene Synthases (VvSTs)

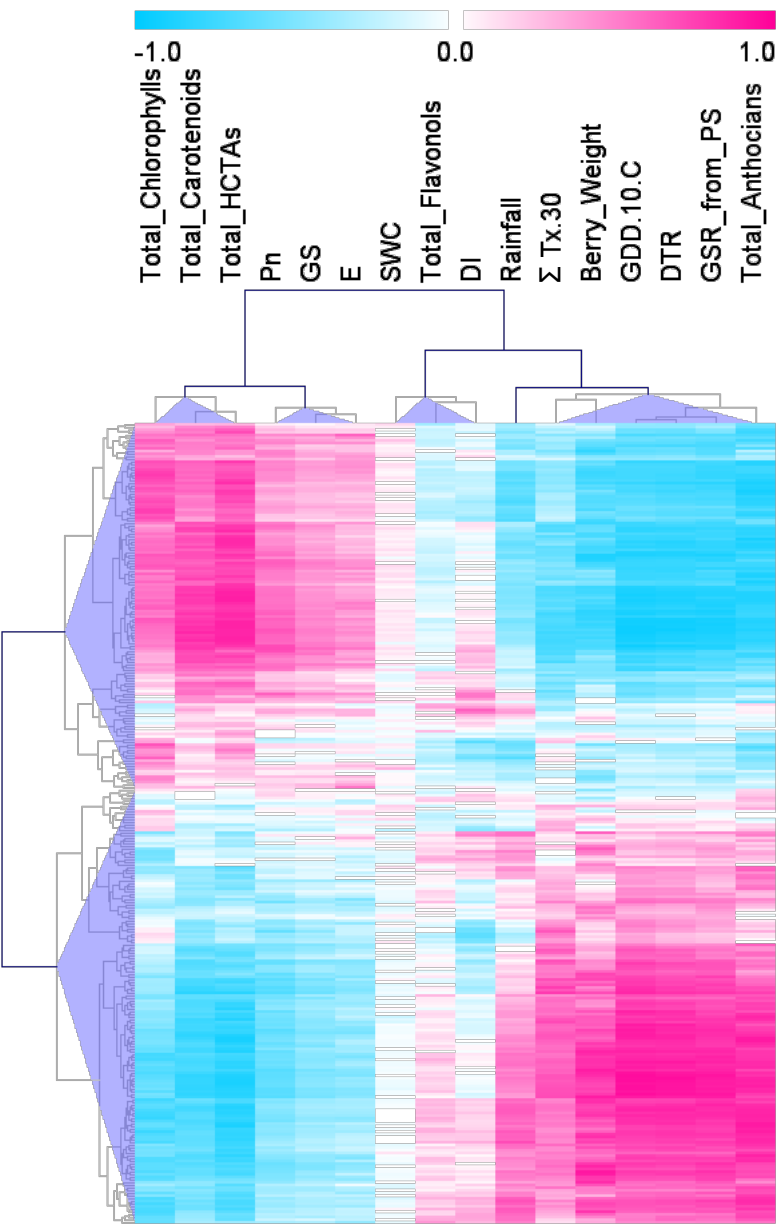
GxE clusters are enriched in Secondary Metabolism GOs

Environmental Parameters Correlation

Cluster JD = 186
 Spearman's Correlation
 vs Total Anthocyanins
 Pedoclimatic/Viticultural Parameters
 Correlation = 0.90
 vs Clusters Expression Metaprofile



- VIT_15s0021g02700 - Expansin (VvEXPB4) ←
- VIT_02s0033g00390 - VvMybA2 ←
- VIT_06s0061g00550 - Xyloglucan endotransglucosylase/hydrolase 32 ←
- VIT_04s0079g00690 - Glutathione S-transferase (VvGST4) ←
- VIT_11s0118g00200 - Sucrose-phosphate synthase
- VIT_07s0005g01710 - WRKY Transcription Factor (VvWRKY19)
- VIT_02s0012g02220 - Xyloglucan endotransglucosylase/hydrolase 30
- VIT_08s0056g01240 - No hit
- VIT_02s0025g01450 - Unknown protein
- VIT_16s0022g00960 - Invertase/pectin methylesterase inhibitor
- VIT_19s0090g01370 - No hit
- VIT_02s0012g01040 - NAC domain-containing protein (VvNAC13)
- VIT_11s0016g04920 - Early nodulin 93 ←
- VIT_02s0033g00410 - VvMybA1 ←



CONCLUSIONS

- We have used Data Mining Techniques to obtain a grapevine transcript mapping highlighting the **relations between genes expression and environmental variables**.
- We have defined 300 clusters with high intra-cluster homogeneity and characterized them with respect to the analyzed variables, thanks to the combined application of a machine learning algorithm, a Principal Component Analysis and a flexible graphical tool useful to identify **hidden relationships**.
- Our **multi-year experimental design** allowed to show that Area variable *per se* has a slight impact on the grapevine transcriptome plasticity.
- The **GxE influence on phenotype** in open field grown plants should rather be explored by considering the interactions among **Genotype, Area and Year**.
- Our statistical pipeline could be applied to other GxE studies in different crops.

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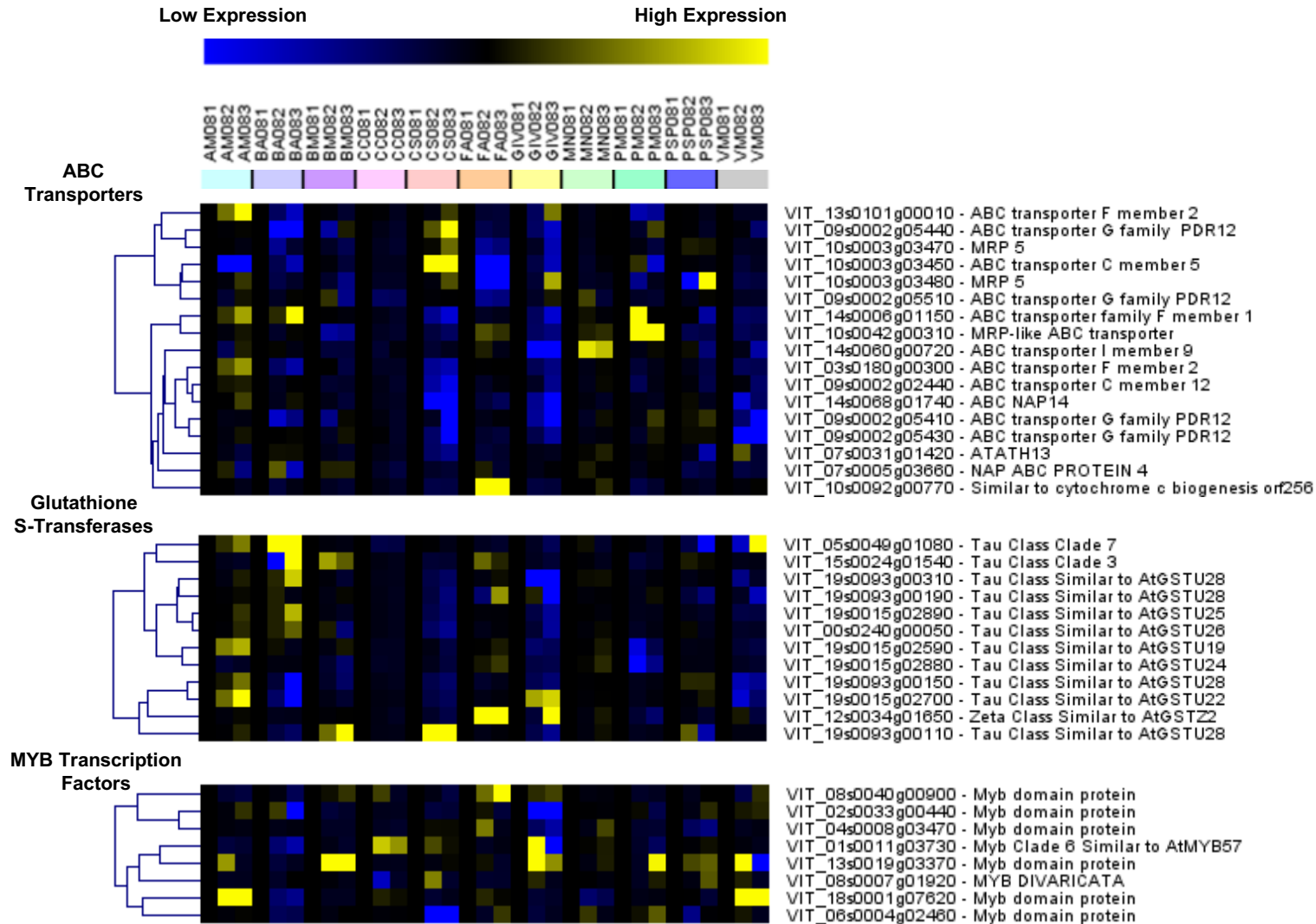
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Gabriele Di Gaspero
Gabriele Magris
Michele Morgante



Gruppo Italiano Vini



Plasticity in Gene Families during Berry Development



Concluding remarks:

We have used Data Mining Techniques in order to obtain a grapevine genes mapping able to put in evidence the relations between genes expressions and environmental variables.

We have defined 300 cluster with high intra-cluster homogeneity and we have been able to designate them according to their behavior with respect to the analysed variables, thanks to the joint application of a machine learning algorithm, a Principal Component Analysis and a flexible graphical tool useful to identify hidden relationships.

G X E Interaction in Grape: Global gene expression

