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ASSEMBLEE GENERALE 2017
2017 General Assembly

NOUVEAUX OUTILS POUR LE SUIVI DE LA QUALITE DES RAISINS :

Capteurs, analyse des données, outils d’aide à la décision

New tools for monitoring grapes quality : sensors, data analysis, decision

High throughput transcriptomic and metabolomic, grape quality, climate and grape cultivars

Sara Zenoni

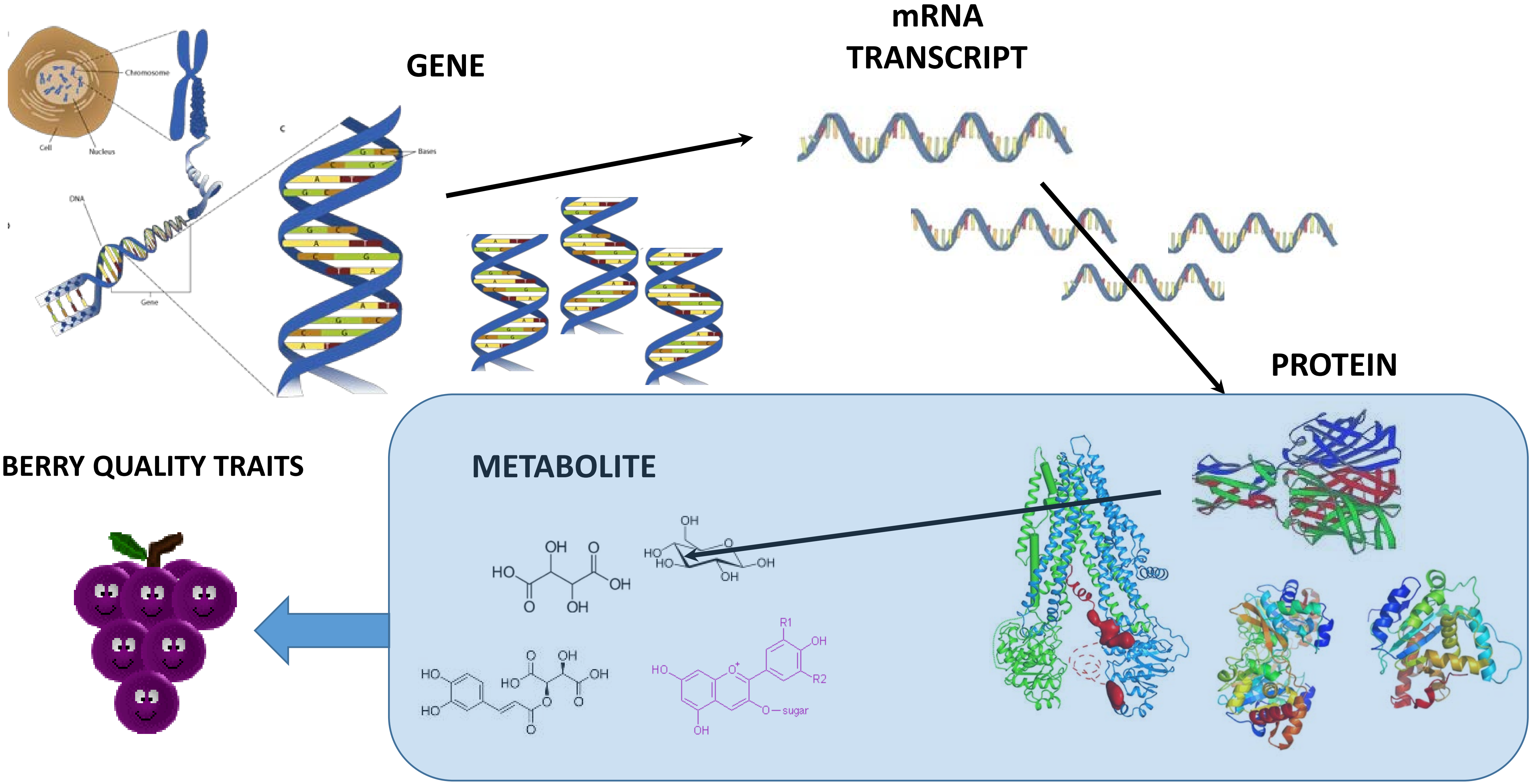
UNIVERSITÀ
di VERONA
Dipartimento
di BIOTECNOLOGIE

OUTLINE OF PRESENTATION

- **Brief introduction of the principal "Omic" (high throughput) approaches**
- **Trancriptomic and metabolomic in grape: platforms and technological advances**
- **Some of the most recent results obtained by transcriptomic and metabolomic for berry ripening and plant responses to biotic and abiotic stresses**

THE FLOW OF BIOLOGICAL INFORMATION

GENOME is the total DNA of a cell



GENE

mRNA TRANSCRIPT

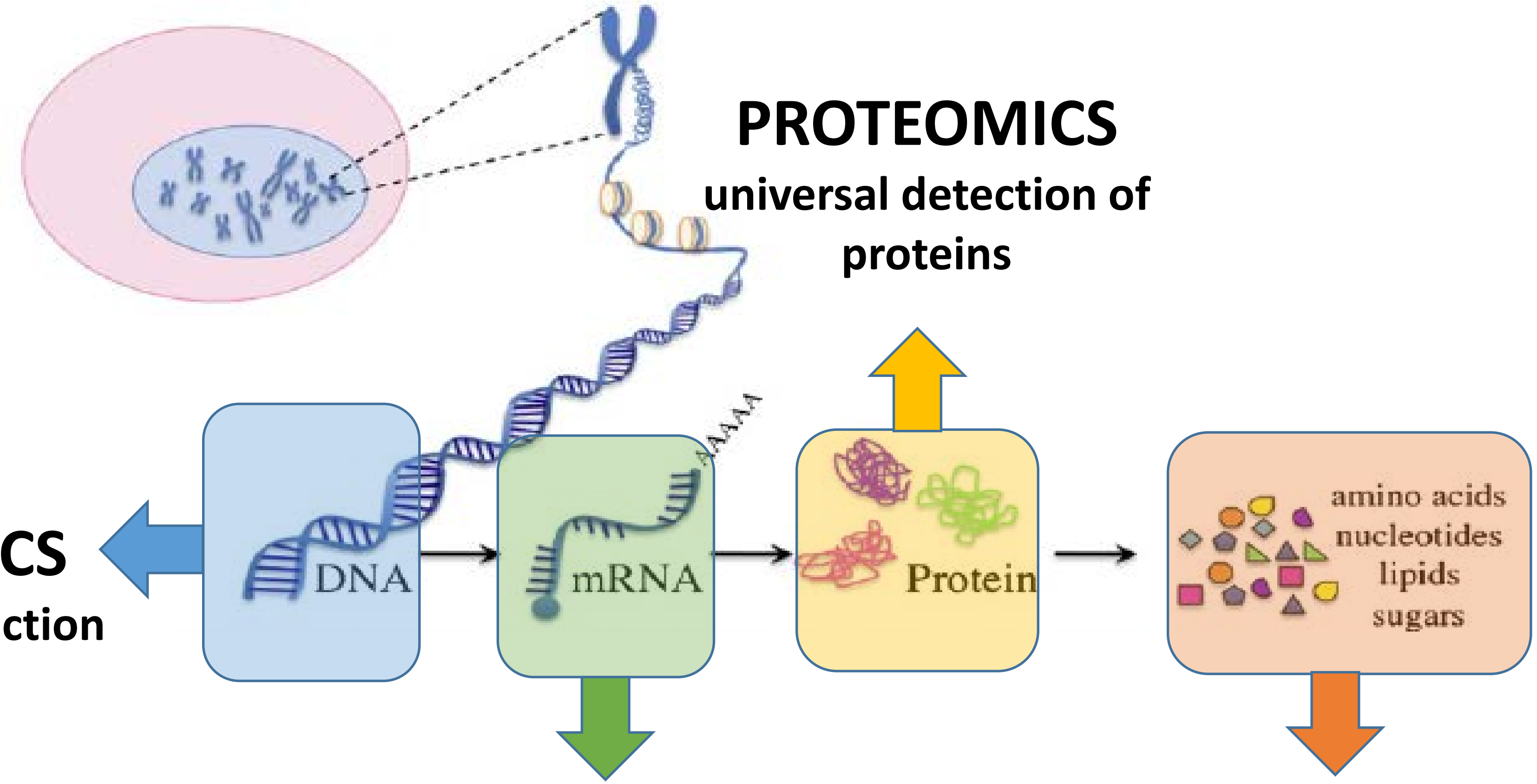
PROTEIN

METABOLITE

BERRY QUALITY TRAITS

“OMIC” APPROACHES

‘Omic’ approaches adopt a holistic view of the molecules that make up a cell, tissue or organism



PROTEOMICS
universal detection of proteins

GENOMICS
universal detection of genes

TRANSCRIPTOMICS
universal detection of transcripts

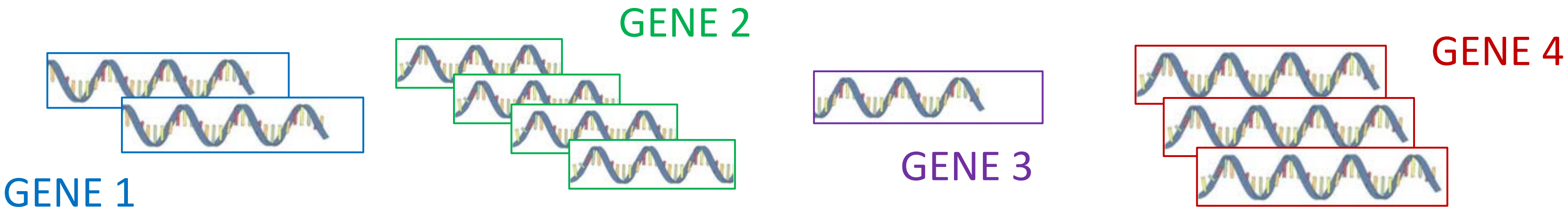
METABOLOMICS
universal detection of metabolites

TRANSCRIPTOMICS

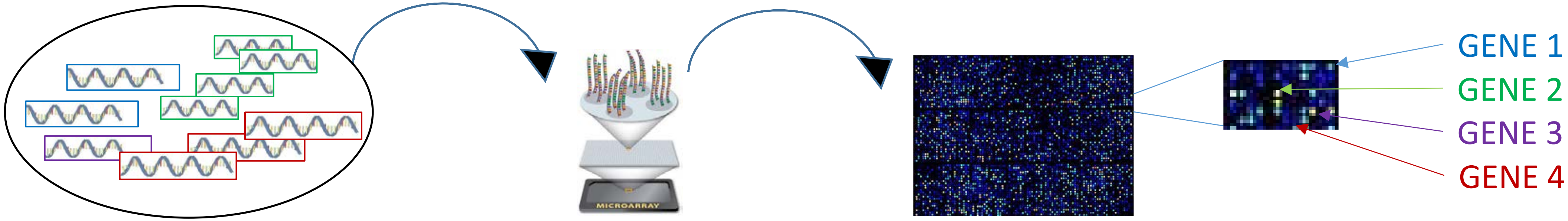
- **The transcriptome depends on gene expression and therefore changes qualitatively and quantitatively according to cell type, developmental stage and in response to external conditions or physiological states**

- **Transcriptomic methods are based on one of two principles, both of which allow for a highly parallel analysis of thousands of transcripts:**

1) the digital analysis of sequence data by counting the number of times a particular sequence is represented in a sample;



2) the quantitative analysis of hybridisation data (array hybridization).

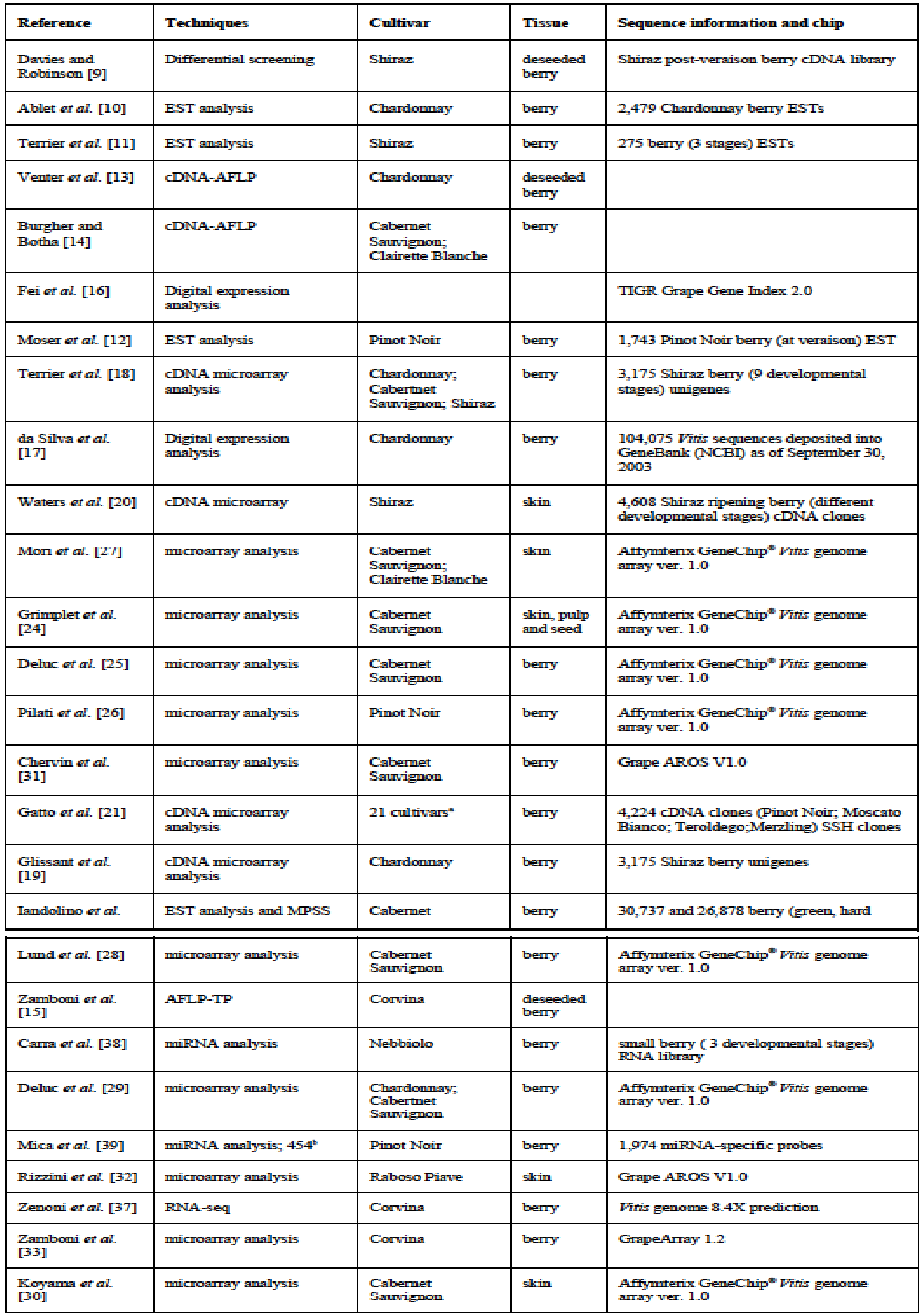


TRANSCRIPTOMICS

2000 the first study aimed to identity genes expressed during ripening using low-throughput Sanger sequencing approach

In **2004**, the first commercial high-density oligonucleotide arrays became available for the grapevine (about 14.000 genes)

In **2007** the complete genome was published and became possible to monitor gene expression at whole-genome level.



Reference	**Techniques**	**Cultivar**	**Tissue**	**Sequence information and chip**
Davies and Robinson [9]	Differential screening	Shiraz	deseeded berry	Shiraz post-veraison berry cDNA library
Ablet *et al.* [10]	EST analysis	Chardonnay	berry	2,479 Chardonnay berry ESTs
Terrier *et al.* [11]	EST analysis	Shiraz	berry	275 berry (3 stages) ESTs
Venter *et al.* [13]	cDNA-AFLP	Chardonnay	deseeded berry	
Burgher and Botha [14]	cDNA-AFLP	Cabernet Sauvignon; Clairette Blanche	berry	
Fei *et al.* [16]	Digital expression analysis			TIGR Grape Gene Index 2.0
Moser *et al.* [12]	EST analysis	Pinot Noir	berry	1,743 Pinot Noir berry (at veraison) EST
Terrier *et al.* [18]	cDNA microarray analysis	Chardonnay; Cabertnet Sauvignon; Shiraz	berry	3,175 Shiraz berry (9 developmental stages) unigenes
da Silva *et al.* [17]	Digital expression analysis	Chardonnay	berry	104,075 *Vitis* sequences deposited into GeneBank (NCBI) as of September 30, 2003
Waters *et al.* [20]	cDNA microarray	Shiraz	skin	4,608 Shiraz ripening berry (different developmental stages) cDNA clones
Mori *et al.* [27]	microarray analysis	Cabernet Sauvignon; Clairette Blanche	skin	Affymterix GeneChip® *Vitis* genome array ver. 1.0
Grimplet *et al.* [24]	microarray analysis	Cabernet Sauvignon	skin, pulp and seed	Affymterix GeneChip® *Vitis* genome array ver. 1.0
Deluc *et al.* [25]	microarray analysis	Cabernet Sauvignon	berry	Affymterix GeneChip® *Vitis* genome array ver. 1.0
Pilati *et al.* [26]	microarray analysis	Pinot Noir	berry	Affymterix GeneChip® *Vitis* genome array ver. 1.0
Chervin *et al.* [31]	microarray analysis	Cabernet Sauvignon	berry	Grape AROS V1.0
Gatto *et al.* [21]	cDNA microarray analysis	21 cultivars^a	berry	4,224 cDNA clones (Pinot Noir; Moscato Bianco; Teroldego;Merzling) SSH clones
Glissant *et al.* [19]	cDNA microarray analysis	Chardonnay	berry	3,175 Shiraz berry unigenes
Iandolino *et al.*	EST analysis and MPSS	Cabernet	berry	30,737 and 26,878 berry (green, hard
Lund *et al.* [28]	microarray analysis	Cabernet Sauvignon	berry	Affymterix GeneChip® *Vitis* genome array ver. 1.0
Zamboni *et al.* [15]	AFLP-TP	Corvina	deseeded berry	
Carra *et al.* [38]	miRNA analysis	Nebbiolo	berry	small berry (3 developmental stages) RNA library
Deluc *et al.* [29]	microarray analysis	Chardonnay; Cabertnet Sauvignon	berry	Affymterix GeneChip® *Vitis* genome array ver. 1.0
Mica *et al.* [39]	miRNA analysis; 454^b	Pinot Noir	berry	1,974 miRNA-specific probes
Rizzini *et al.* [32]	microarray analysis	Raboso Piave	skin	Grape AROS V1.0
Zenoni *et al.* [37]	RNA-seq	Corvina	berry	*Vitis* genome 8.4X prediction
Zamboni *et al.* [33]	microarray analysis	Corvina	berry	GrapeArray 1.2
Koyama *et al.* [30]	microarray analysis	Cabernet Sauvignon	skin	Affymterix GeneChip® *Vitis* genome array ver. 1.0

THE GRAPEVINE GENOME

The Grapevine genome was sequenced by the Italian –French consortium

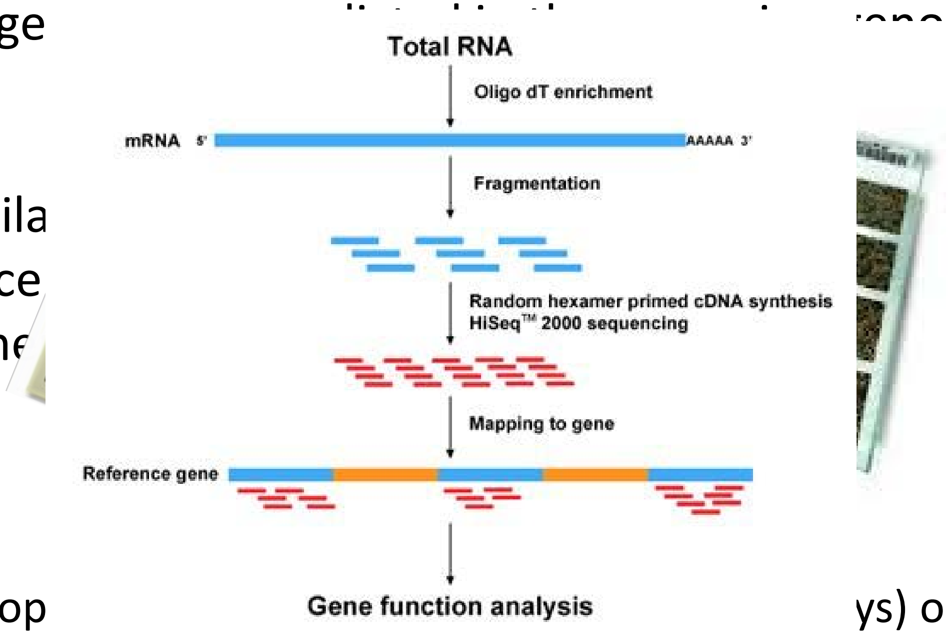
HYBRIDIZATION approach

SEQUENCING approach

29,971 ge

enome

The availa
sequence
experime



Develop ys) on which almost all grapevine genes, more than 29,000, are represented

The recent technological advances in next-generation sequencing methods have been applied to transcriptomics. This approach, known as RNA-seq, gives a more complete information about gene expression

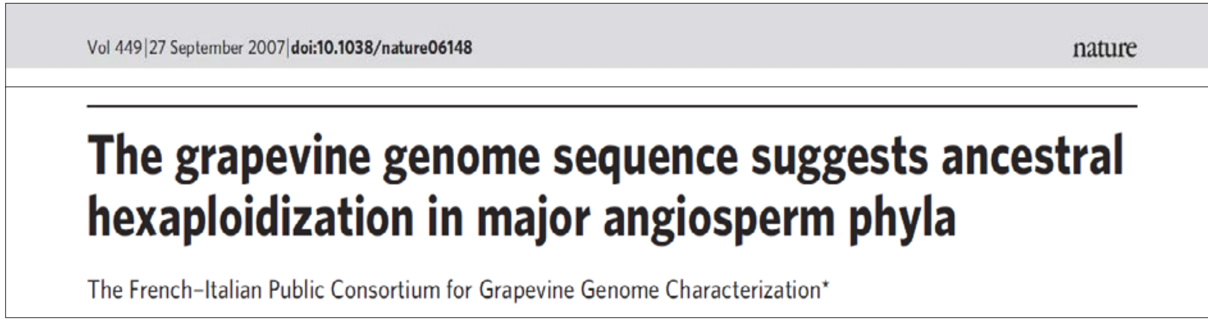
Università degli Studi di Verona

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



Vol 449|27 September 2007|**doi:10.1038/nature06148** nature

The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla

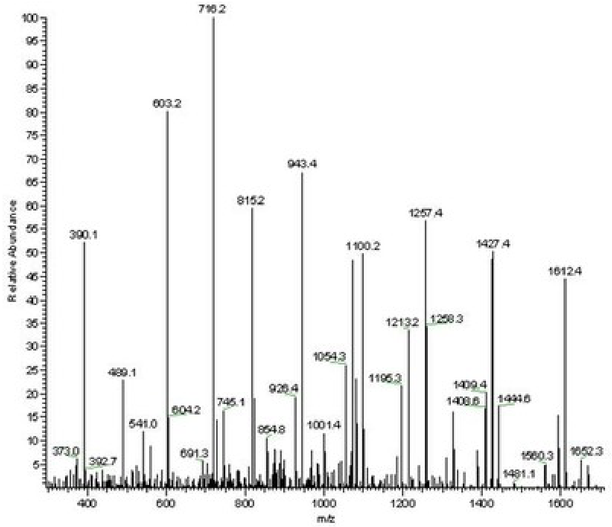
The French–Italian Public Consortium for Grapevine Genome Characterization*

METABOLOMICS

- **Metabolomics can generally be defined as the study of a complete set of metabolites (small molecules Mr< 1,000D) produced in a single cell, tissue or organ.**
- **Like the transcriptome, the metabolome is dynamic and varies by cell type, developmental stage and in response to different stimulus.**
- **Unlike the transcriptome, the metabolome can only be achieved using multiple analytical platforms to cover the chemical diversity of metabolites**

Mass spectrometry is the most commonly-used method to investigate/identify metabolites.

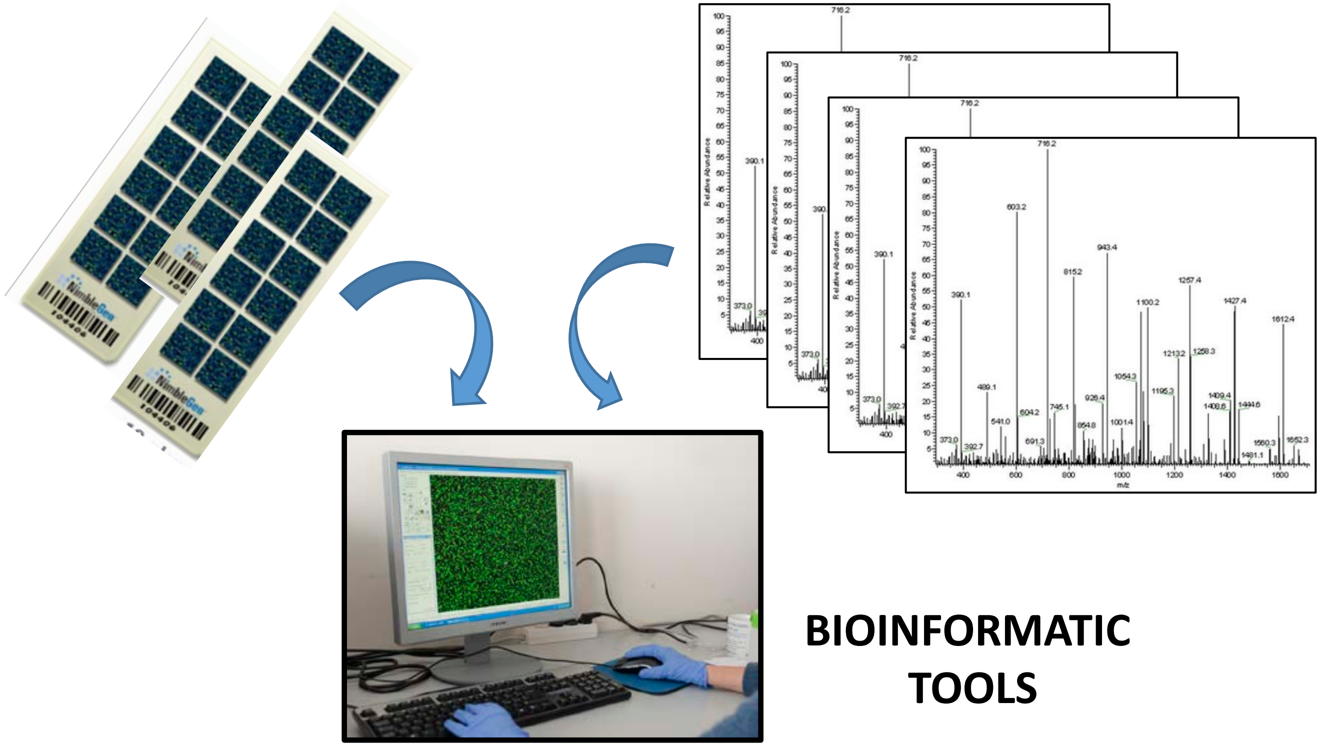
Ions are created from neutral metabolites, which are then separated according to their mass-to-charge ratio (*m*/*z*) and detected to create a mass spectrum, which is characteristic of the molecular mass and/or structure.



MS associated with Gas chromatography (**GC-MS**) is used for the analysis of volatile metabolites
MS associated with Liquid chromatography (**LG-MS**) is used for the analysis of non volatile metabolites

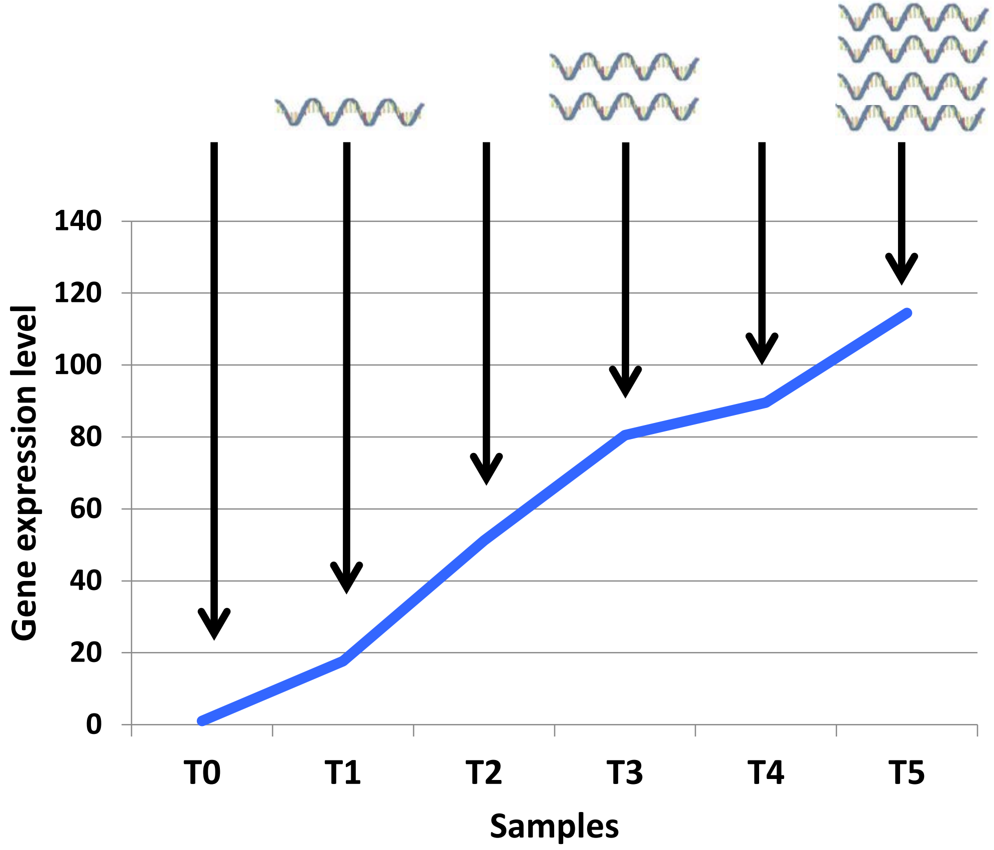
TRANSCRIPTOMIC AND METABOLOMIC DATA ANALYSIS

Both transcriptomic and metabolomic analysis produce a huge amount of data



BIOINFORMATIC TOOLS

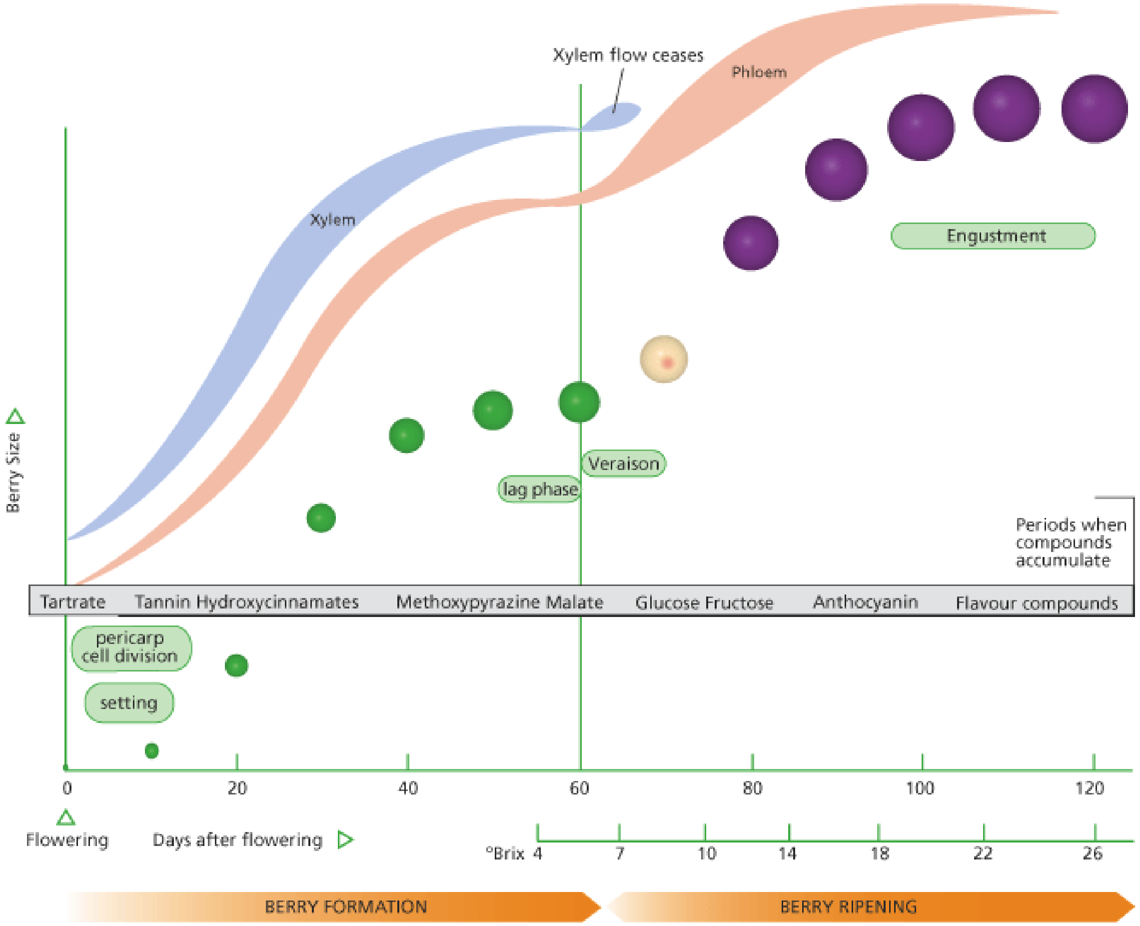
EXPRESSION PROFILE



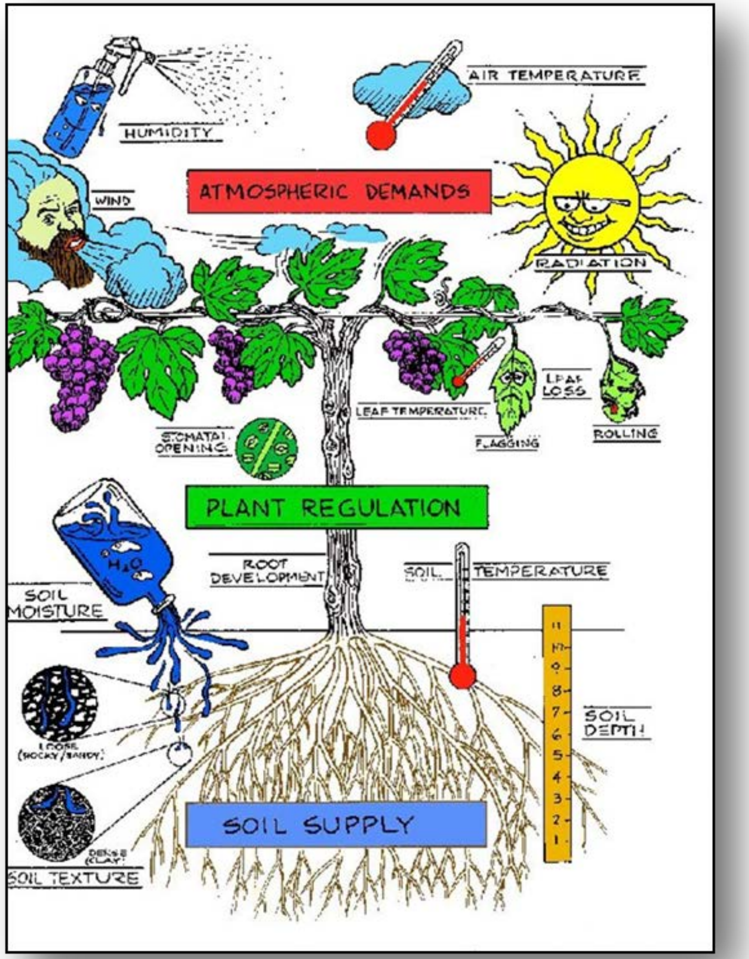
Genes and metabolites that significantly modify their expression or accumulation levels

PRINCIPAL DEVELOPMENTAL AND PHYSIOLOGICAL PROCESSES INVESTIGATED BY TRANSCRIPTOMIC AND METABOLOMIC IN GRAPE

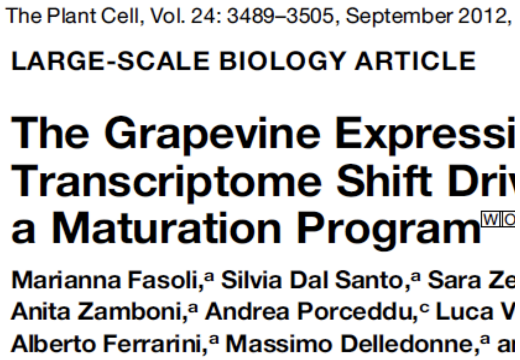
BERRY FORMATION AND RIPENING



RESPONSES TO BIOTIC AND ABIOTIC STRESSES



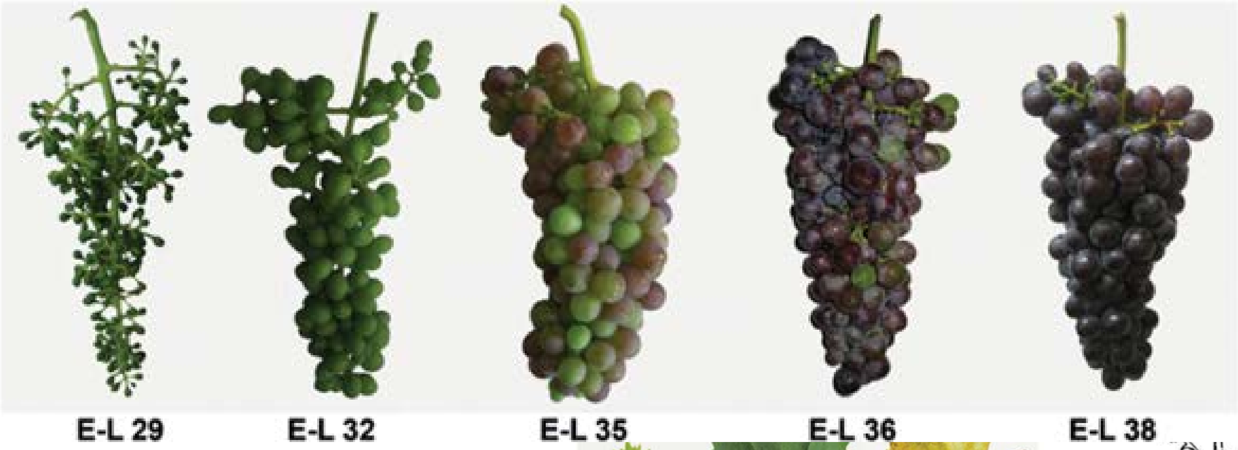
BERRY FORMATION AND RIPENING


The Plant Cell, Vol. 24: 3489–3505, September 2012,

LARGE-SCALE BIOLOGY ARTICLE

The Grapevine Expressi
Transcriptome Shift Dri
a Maturation Program

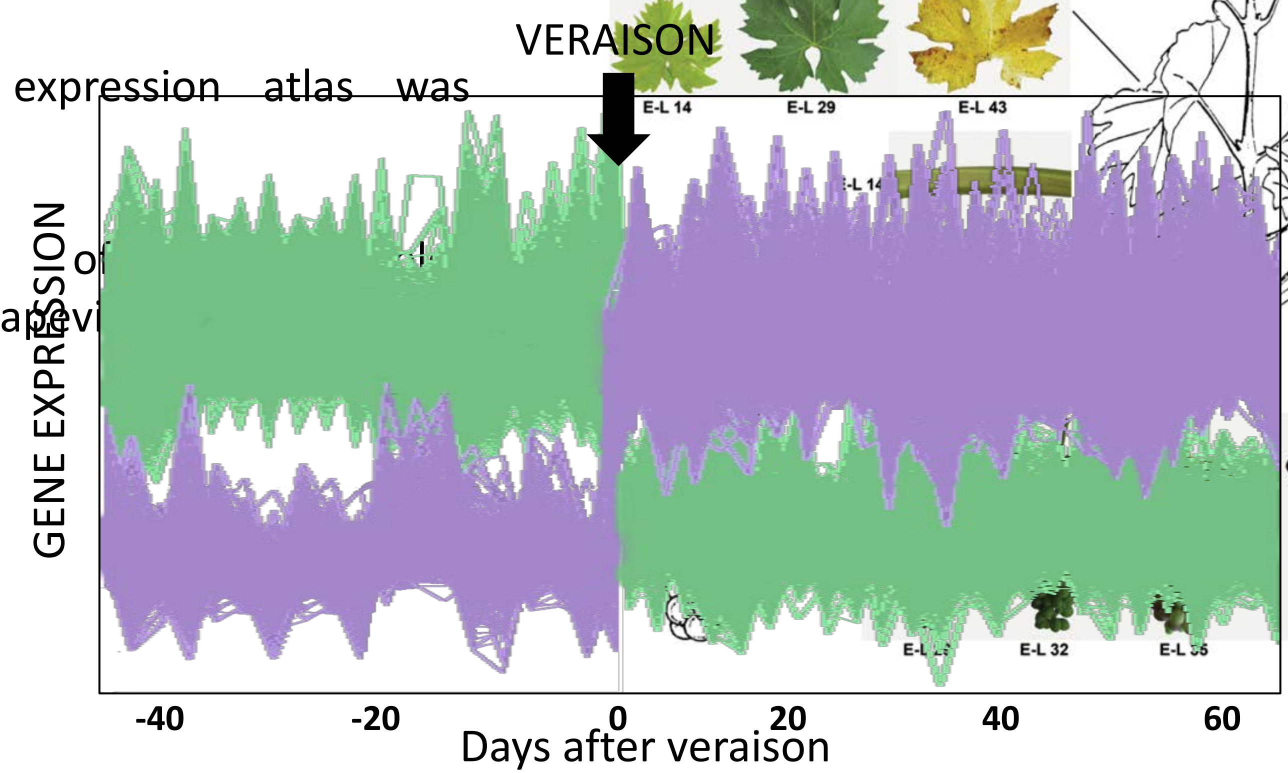
Marianna Fasoli,[a] Silvia Dal Santo,[a] Sara Ze
Anita Zamboni,[a] Andrea Porceddu,[c] Luca V
Alberto Ferrarini,[a] Massimo Delledonne,[a] a

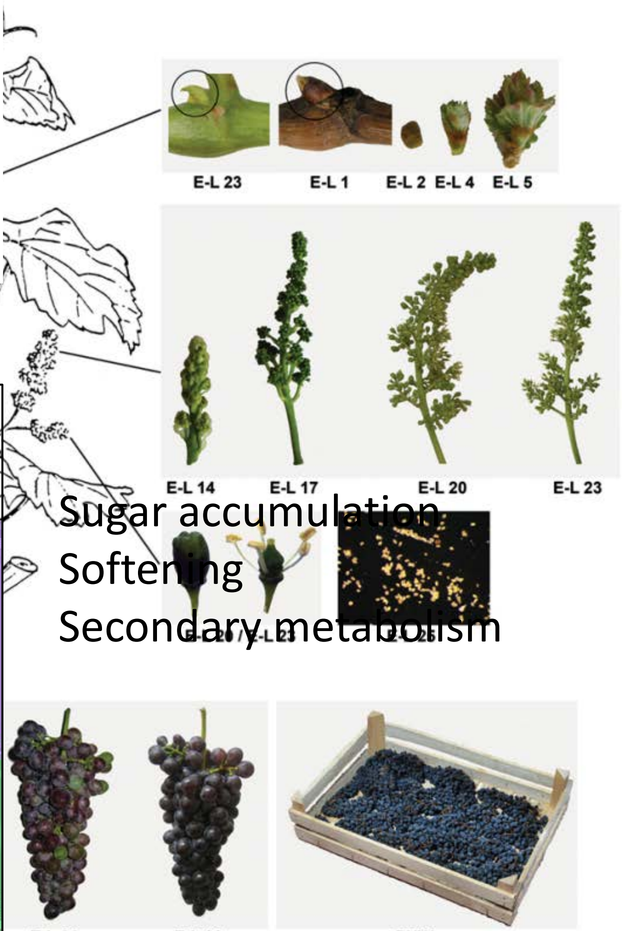


2012_ The first gene expression atlas was established for grapevine

Transcriptomic analysis of
representing different grapevi
development

Photosynthesis
Cell cycle
Primary metabolism





Sugar accumulation
Softening
Secondary metabolism

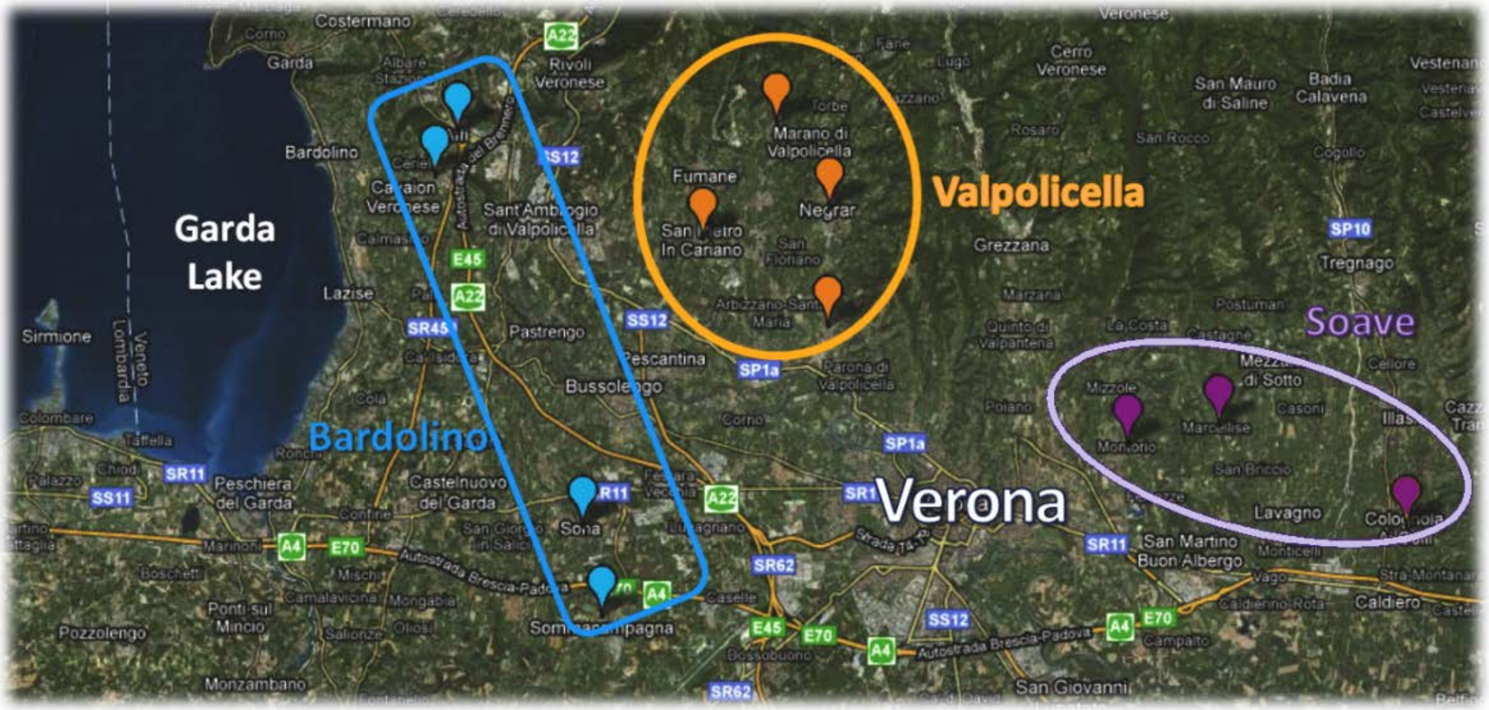
BERRY FORMATION AND RIPENING

RESEARCH PAPER **Open Access**

The plasticity of the grapevine berry transcriptome

Genome **Biology**

Silvia Dal Santo[1], Giovanni Battista Tornielli[1], Sara Zenoni[1], Marianna Fasoli[1], Lorenzo Farina[2], Andrea Anesi[1], Flavia Guzzo[1], Massimo Delledonne[1] and Mario Pezzotti[1*]



2013

~~Samples from 11 vineyards sampled at 3 developmental stages:~~
Vèraison, Mid-Ripening and Fully Ripe

Vitis vinifera Cv Corvina – Clone 48

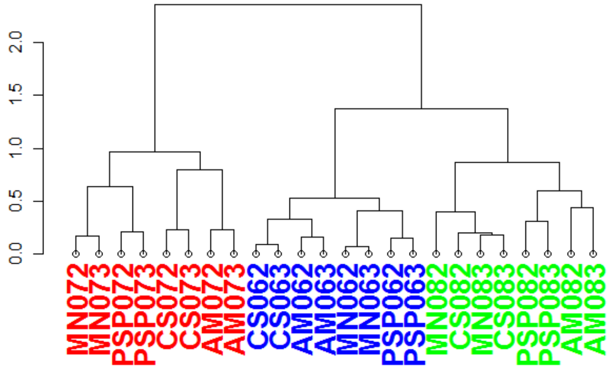
- ➢ Red berry variety
- ➢ Major component of "Amarone" wine
- ➢ Most important clone cultivated in the Verona area

3 Vintages studied:

2006

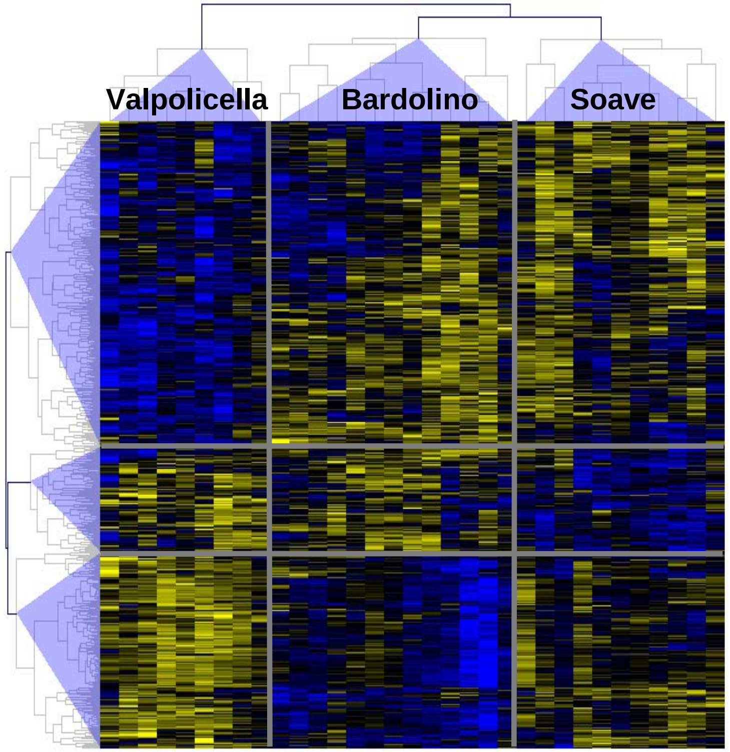
2007

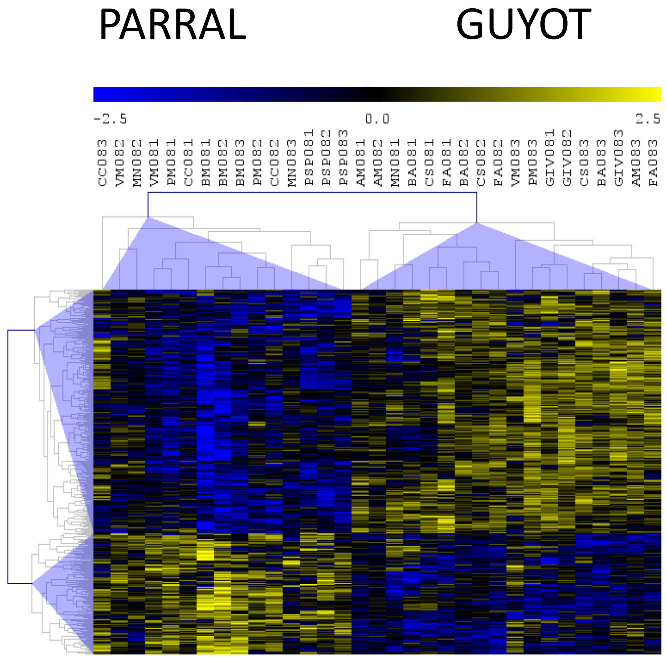
2008



Vintage impacts more than vineyard on total variability

BERRY FORMATION AND RIPENING





Genes expressed in a particular growing condition or associated to a particular growing system have been identified

BERRY FORMATION AND RIPENING

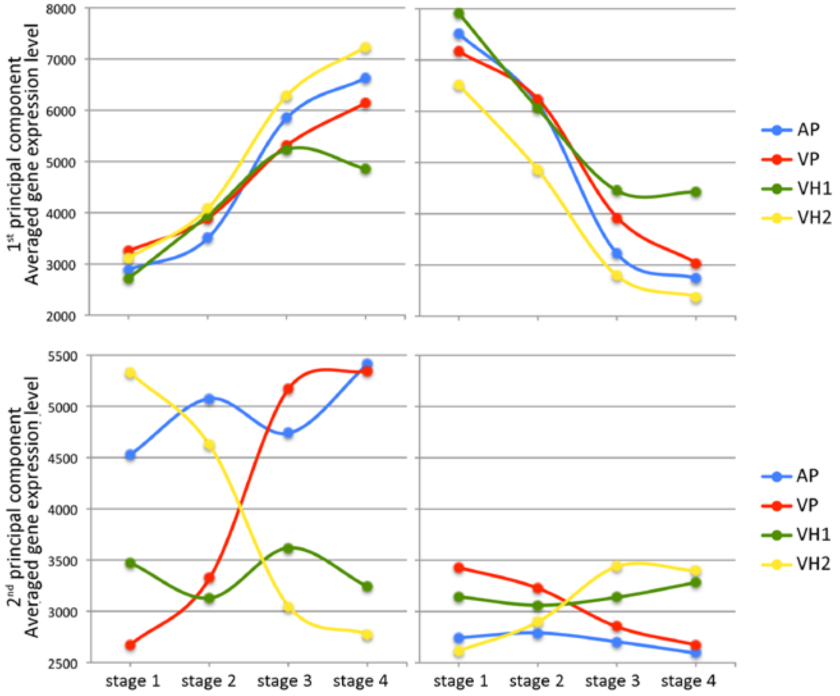
2016

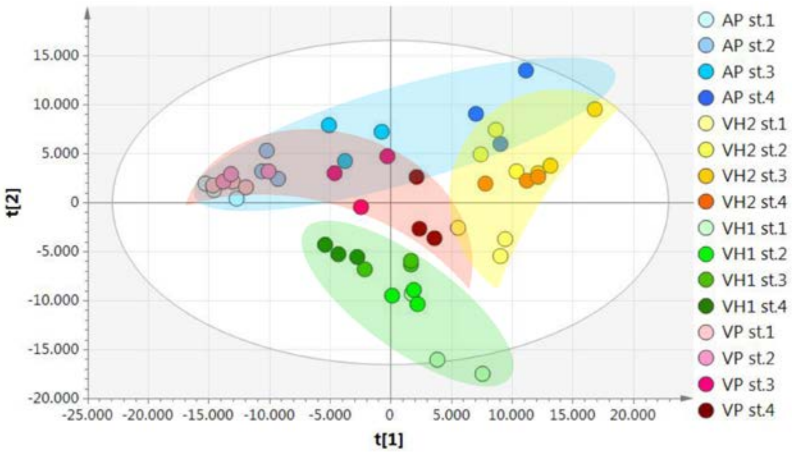
frontiers in Plant Science

ORIGINAL RESEARCH
published: 12 July 2016
doi: 10.3389/fpls.2016.00970

Plasticity of the Berry Ripening Program in a White Grape Variety

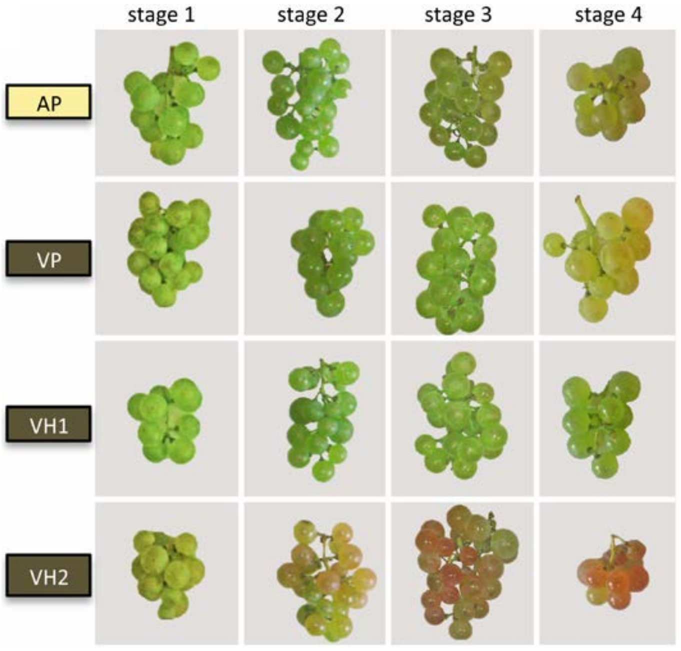
Silvia Dal Santo[1†], Marianna Fasoli[1,2†], Stefano Negri[1†], Erica D'Incà[1], Nazareno Vicenzi[3], Flavia Guzzo[1], Giovanni Battista Tornielli[1], Mario Pezzotti[1] and Sara Zenoni[1]*





Alluvional soil

Volcanic soil

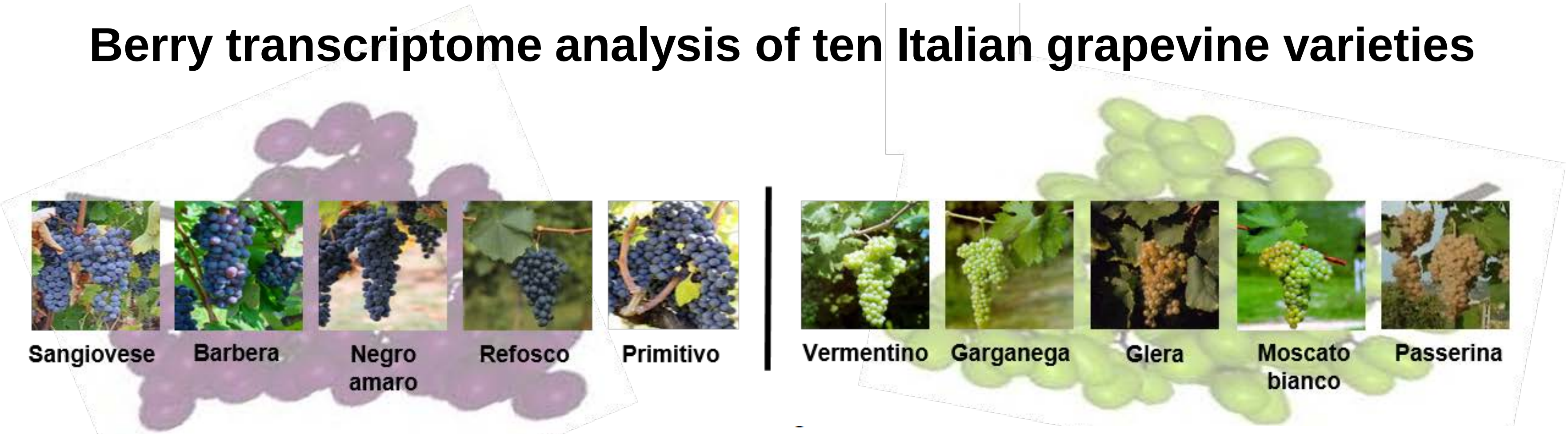


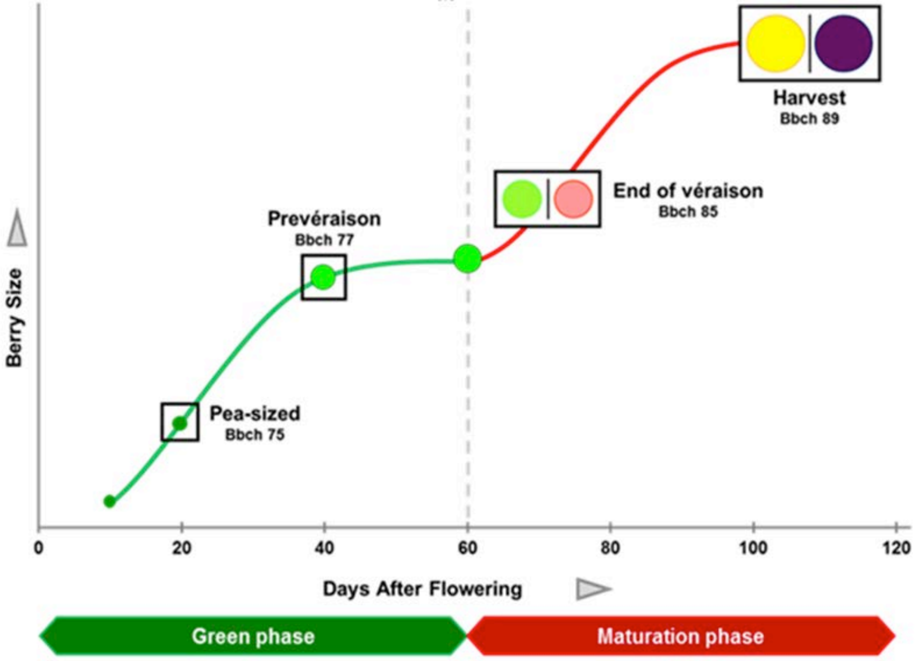
Transcriptomic and metabolomic responses to the different growing sites of Garganega were revealed

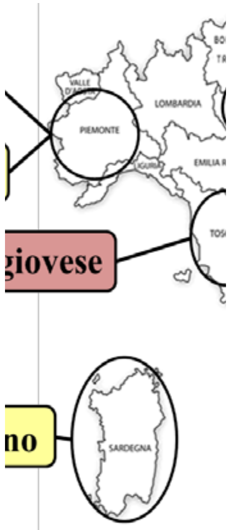
Genes and metabolites belonging to the phenylpropanoid metabolism showed strongest responses

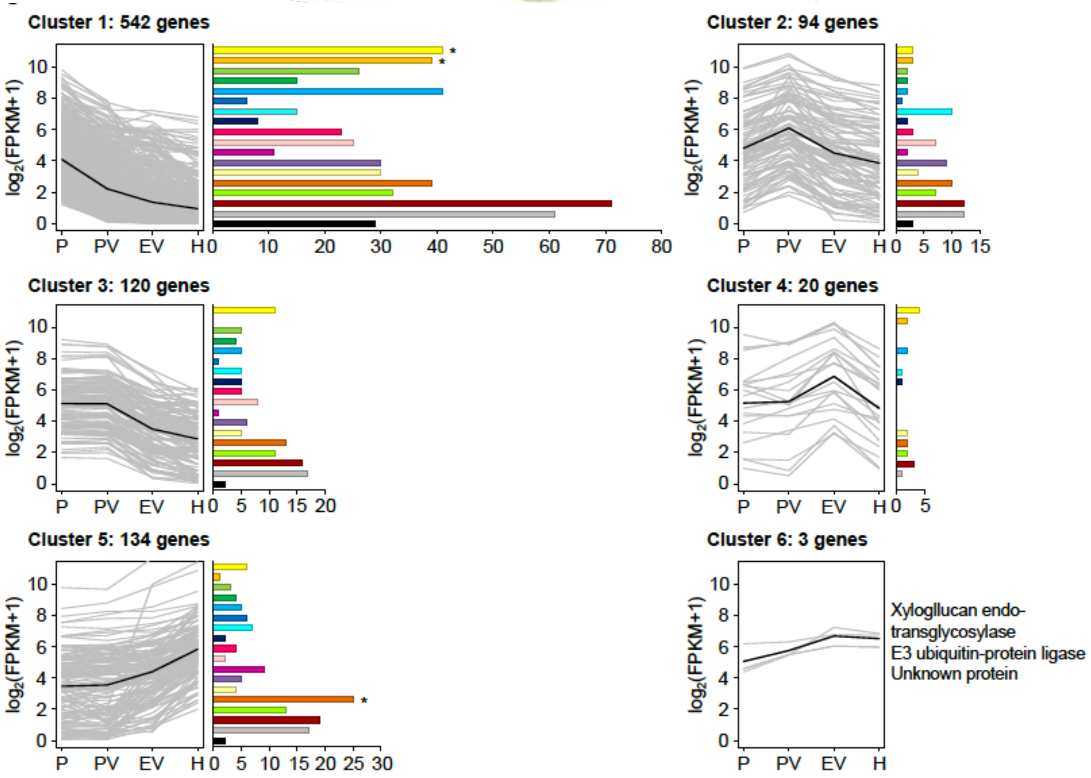
BERRY FORMATION AND RIPENING

Berry transcriptome analysis of ten Italian grapevine varieties





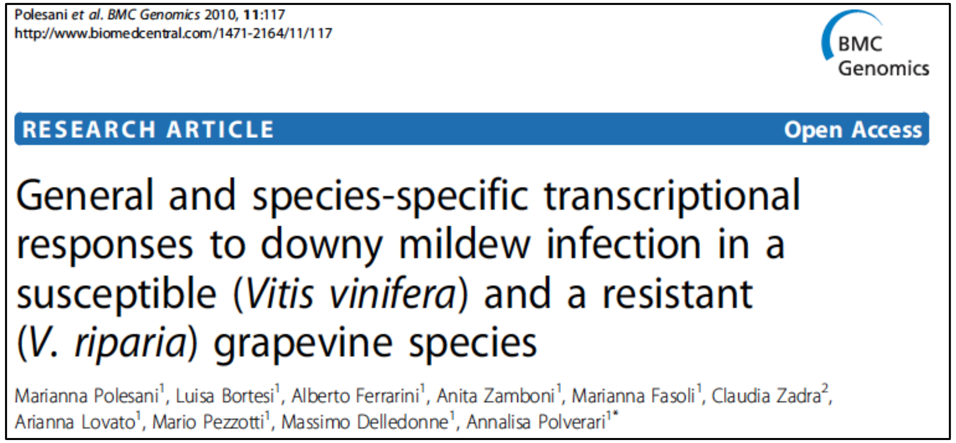




RESPONSES TO BIOTIC AND ABIOTIC STRESSES

BIOTIC STRESSES

- Plasmopara viticola - downy mildew
- Erysiphe necator - powdery mildew
- Leafroll-associated virus 3 (GLRaV-3)



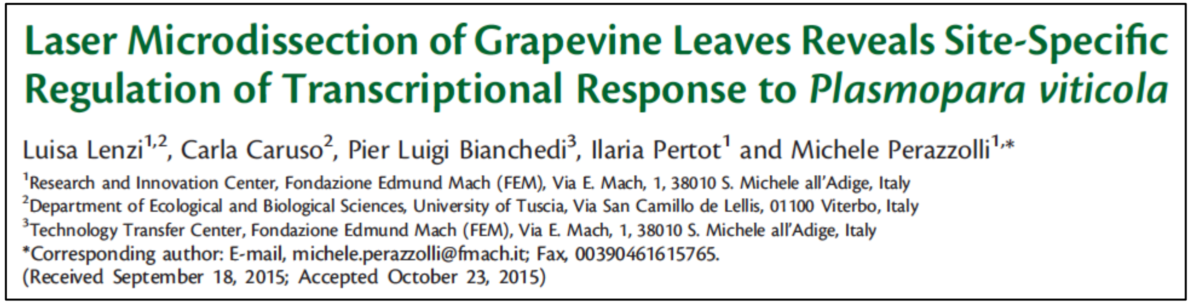
Polesani *et al. BMC Genomics* 2010, **11**:117
http://www.biomedcentral.com/1471-2164/11/117

BMC Genomics

RESEARCH ARTICLE **Open Access**

General and species-specific transcriptional responses to downy mildew infection in a susceptible (*Vitis vinifera*) and a resistant (*V. riparia*) grapevine species

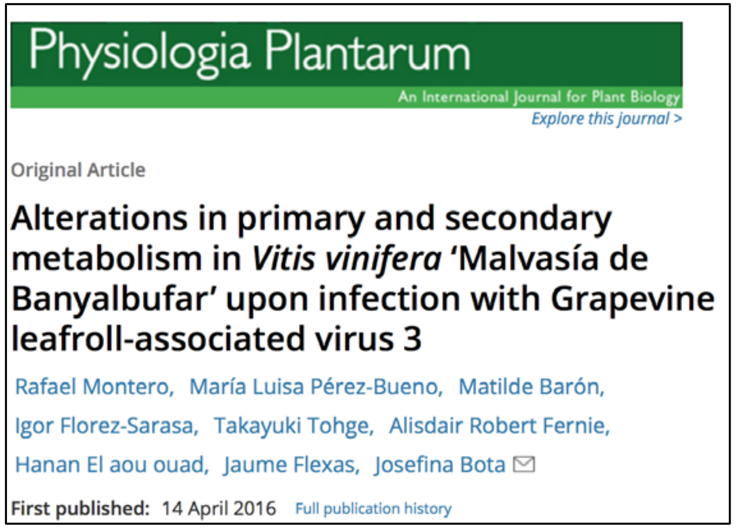
Marianna Polesani[1], Luisa Bortesi[1], Alberto Ferrarini[1], Anita Zamboni[1], Marianna Fasoli[1], Claudia Zadra[2], Arianna Lovato[1], Mario Pezzotti[1], Massimo Delledonne[1], Annalisa Polverari[1*]



Laser Microdissection of Grapevine Leaves Reveals Site-Specific Regulation of Transcriptional Response to *Plasmopara viticola*

Luisa Lenzi[1,2], Carla Caruso[2], Pier Luigi Bianchedi[3], Ilaria Pertot[1] and Michele Perazzolli[1,*]

[1]Research and Innovation Center, Fondazione Edmund Mach (FEM), Via E. Mach, 1, 38010 S. Michele all'Adige, Italy
[2]Department of Ecological and Biological Sciences, University of Tuscia, Via San Camillo de Lellis, 01100 Viterbo, Italy
[3]Technology Transfer Center, Fondazione Edmund Mach (FEM), Via E. Mach, 1, 38010 S. Michele all'Adige, Italy
*Corresponding author: E-mail, michele.perazzolli@fmach.it; Fax, 00390461615765.
(Received September 18, 2015; Accepted October 23, 2015)



Physiologia Plantarum
An International Journal for Plant Biology
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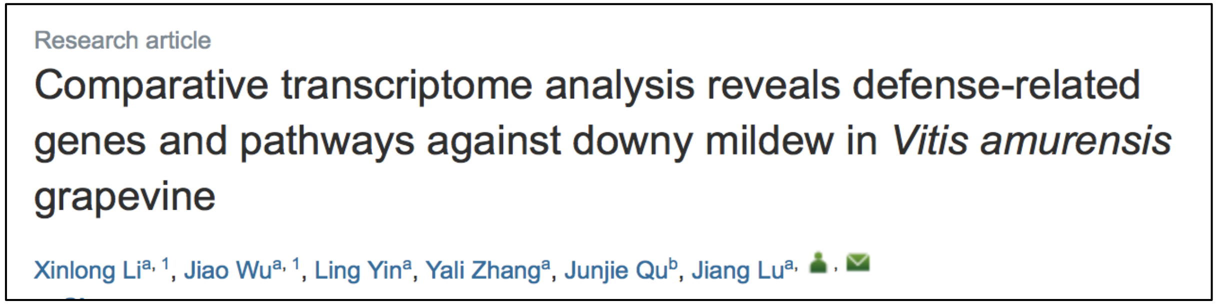
Original Article

Alterations in primary and secondary metabolism in *Vitis vinifera* 'Malvasía de Banyalbufar' upon infection with Grapevine leafroll-associated virus 3

Rafael Montero, María Luisa Pérez-Bueno, Matilde Barón, Igor Florez-Sarasa, Takayuki Tohge, Alisdair Robert Fernie, Hanan El aou ouad, Jaume Flexas, Josefina Bota ✉

First published: 14 April 2016 Full publication history



Research article

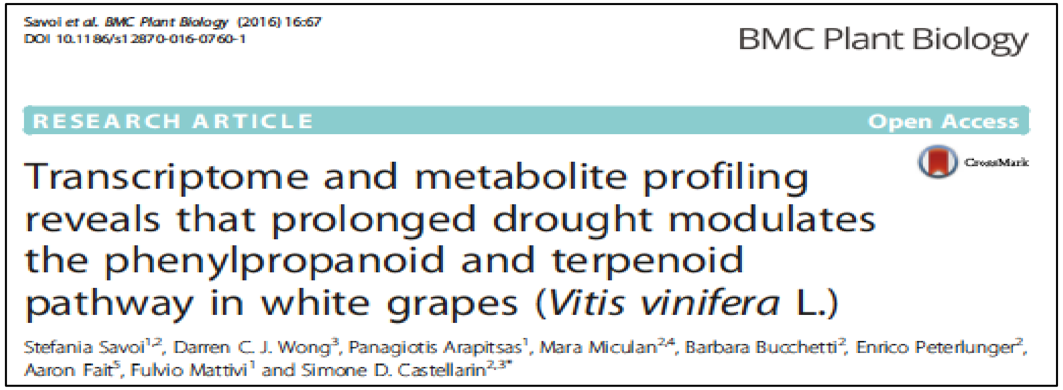
Comparative transcriptome analysis reveals defense-related genes and pathways against downy mildew in *Vitis amurensis* grapevine

Xinlong Li[a, 1], Jiao Wu[a, 1], Ling Yin[a], Yali Zhang[a], Junjie Qu[b], Jiang Lu[a,] 👤, ✉

RESPONSES TO BIOTIC AND ABIOTIC STRESSES

ABIOTIC STRESSES

WATER LIMITATION and DROUGHT



Savoi *et al. BMC Plant Biology* (2016) 16:67
DOI 10.1186/s12870-016-0760-1

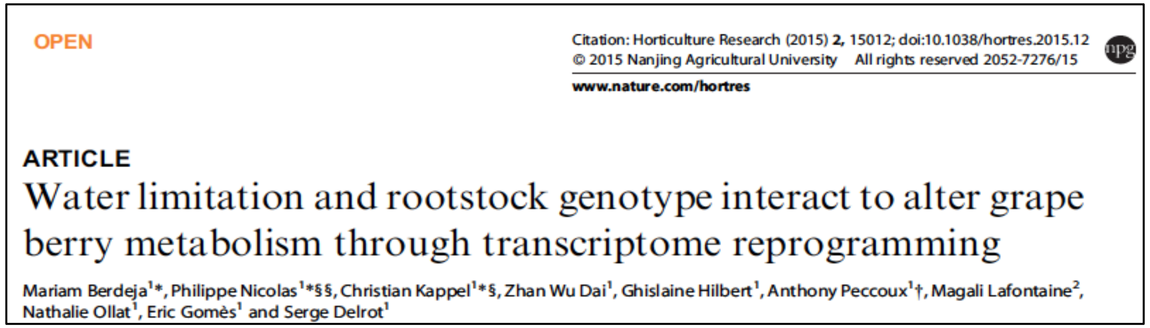
BMC Plant Biology

RESEARCH ARTICLE Open Access

Transcriptome and metabolite profiling reveals that prolonged drought modulates the phenylpropanoid and terpenoid pathway in white grapes (*Vitis vinifera* L.)

Stefania Savoi[1,2], Darren C. J. Wong[3], Panagiotis Arapitsas[1], Mara Miculan[2,4], Barbara Bucchetti[2], Enrico Peterlunger[2], Aaron Fait[5], Fulvio Mattivi[1] and Simone D. Castellarin[2,3*]

Grapevine berries respond to drought by modulating several secondary metabolic pathways, with potential effects on grape and wine antioxidant potential, composition, and sensory features



OPEN

Citation: Horticulture Research (2015) 2, 15012; doi:10.1038/hortres.2015.12
© 2015 Nanjing Agricultural University All rights reserved 2052-7276/15
www.nature.com/hortres

ARTICLE

Water limitation and rootstock genotype interact to alter grape berry metabolism through transcriptome reprogramming

Mariam Berdeja[1]*, Philippe Nicolas[1]*§§, Christian Kappel[1]*§, Zhan Wu Dai[1], Ghislaine Hilbert[1], Anthony Peccoux[1]†, Magali Lafontaine[2], Nathalie Ollat[1], Eric Gomès[1] and Serge Delrot[1]

Involvement of genes related to secondary metabolism and JA during drought stress and the effect of different rootstocks

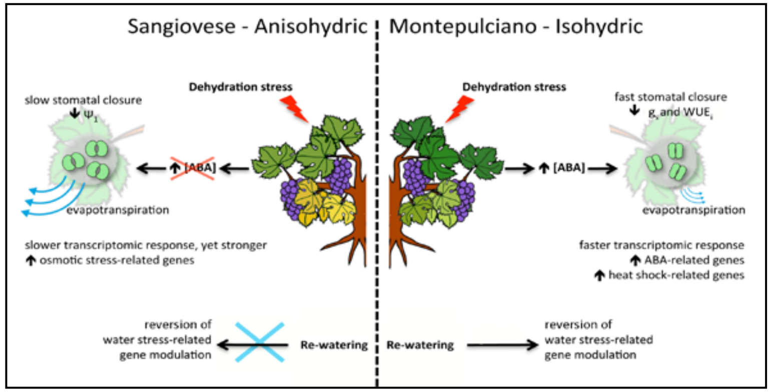
Dal Santo *et al. BMC Genomics* (2016) 17:815
DOI 10.1186/s12864-016-3136-x

BMC Genomics

RESEARCH ARTICLE Open Access

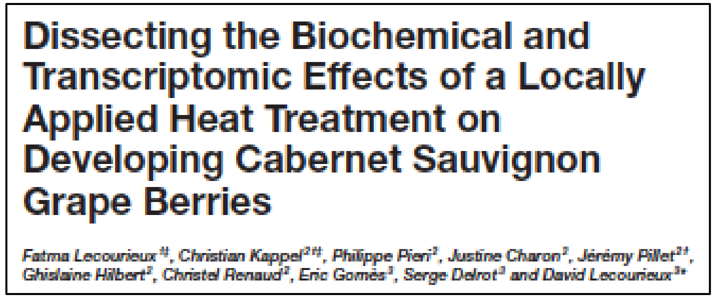
Distinct transcriptome responses to water limitation in isohydric and anisohydric grapevine cultivars

Silvia Dal Santo[1†], Alberto Palliotti[2†], Sara Zenoni[1], Giovanni Battista Tornielli[1], Marianna Fasoli[1,3], Paola Paci[4], Sergio Tombesi[2], Tommaso Frioni[2], Oriana Silvestroni[5], Andrea Bellincontro[6], Claudio d'Onofrio[7], Fabiola Matarese[7], Matteo Gatti[8], Stefano Poni[8] and Mario Pezzotti[1*]



RESPONSES TO BIOTIC AND ABIOTIC STRESSES

TEMPERATURE


Dissecting the Biochemical and Transcriptomic Effects of a Locally Applied Heat Treatment on Developing Cabernet Sauvignon Grape Berries

Fatma Lecourieux[‡], Christian Kappel[2‡], Philippe Pieri[2], Justine Charon[2], Jérémy Pillet[2†], Ghislaine Hilbert[2], Christel Renaud[2], Eric Gomès[3], Serge Delrot[3] and David Lecourieux[3]*

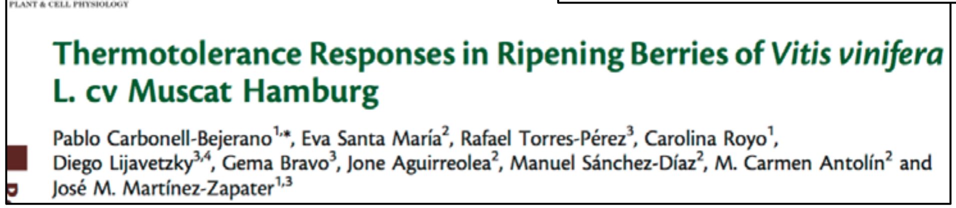

Rienth *et al. BMC Plant Biology* (2016) 16:164
DOI 10.1186/s12870-016-0850-0

BMC Plant Biology

RESEARCH ARTICLE Open Access

Temperature desynchronizes sugar and organic acid metabolism in ripening grapevine fruits and remodels their transcriptome

Markus Rienth[1,2,3], Laurent Torregrosa[1], Gautier Sarah[1], Morgane Ardisson[1], Jean-Marc Brillouet[4] and Charles Romieu[1*]


PCP
PLANT & CELL PHYSIOLOGY

Thermotolerance Responses in Ripening Berries of *Vitis vinifera* L. cv Muscat Hamburg

Pablo Carbonell-Bejerano[1,*], Eva Santa María[2], Rafael Torres-Pérez[3], Carolina Royo[1], Diego Lijavetzky[3,4], Gema Bravo[3], Jone Aguirreolea[2], Manuel Sánchez-Díaz[2], M. Carmen Antolín[2] and José M. Martínez-Zapater[1,3]

VITICULTURAL PRACTICES

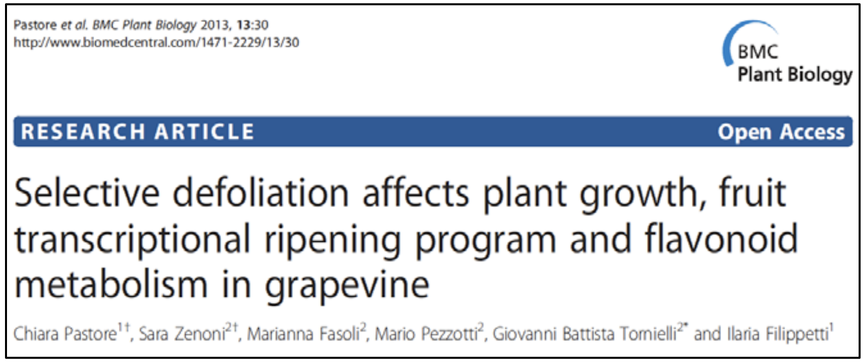

Pastore *et al. BMC Genomics* 2011, 12:631
http://www.biomedcentral.com/1471-2164/12/631

BMC Genomics

RESEARCH ARTICLE Open Access

Increasing the source/sink ratio in *Vitis vinifera* (cv Sangiovese) induces extensive transcriptome reprogramming and modifies berry ripening

Chiara Pastore[1†], Sara Zenoni[2†], Giovanni Battista Tornielli[2], Gianluca Allegro[1], Silvia Dal Santo[2], Gabriele Valentini[1], Cesare Intrieri[1], Mario Pezzotti[2*] and Ilaria Filippetti[1]


Pastore *et al. BMC Plant Biology* 2013, 13:30
http://www.biomedcentral.com/1471-2229/13/30

BMC Plant Biology

RESEARCH ARTICLE Open Access

Selective defoliation affects plant growth, fruit transcriptional ripening program and flavonoid metabolism in grapevine

Chiara Pastore[1†], Sara Zenoni[2†], Marianna Fasoli[2], Mario Pezzotti[2], Giovanni Battista Tornielli[2*] and Ilaria Filippetti[1]


DPF

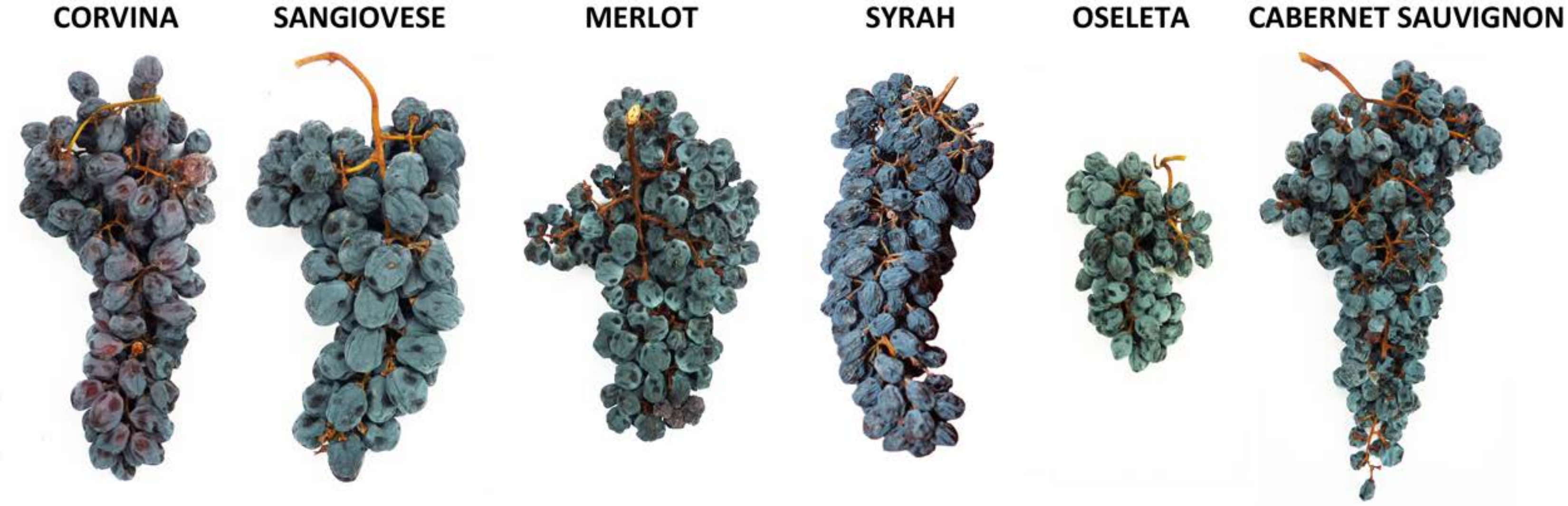

DI

RESPONSES TO ABIOTIC STRESSES, DEVELOPMENT AND CULTIVARS

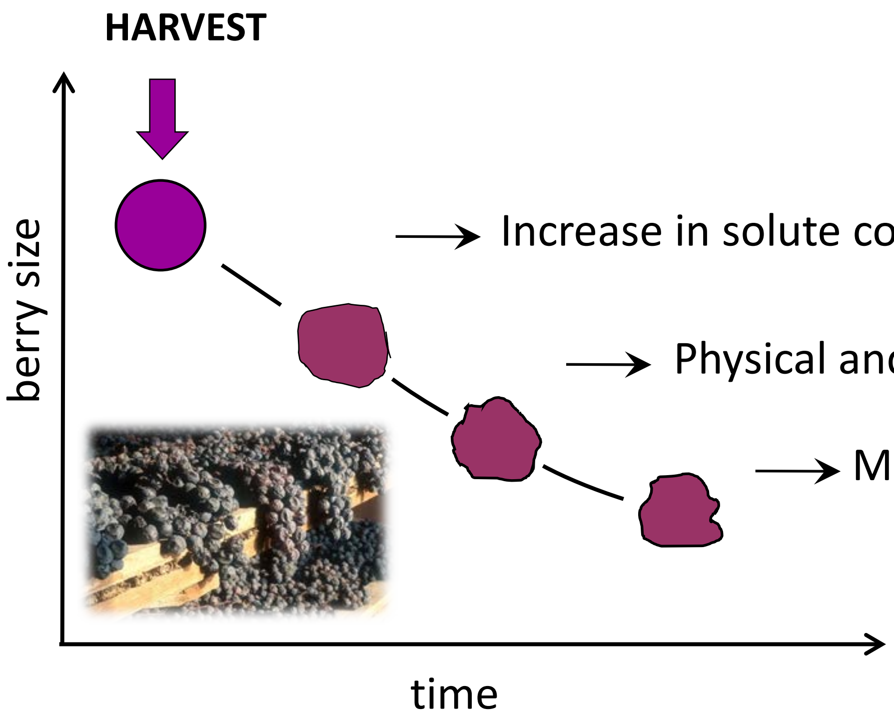
Disclosing the Molecular Basis of the Postharvest Life of Berry in Different Grapevine Genotypes[1]

November 2016

Sara Zenoni[2], Marianna Fasoli[2,3], Flavia Guzzo, Silvia Dal Santo, Alessandra Amato, Andrea Anesi[4],
Mauro Commisso, Markus Herderich, Stefania Ceoldo, Linda Avesani, Mario Pezzotti, and
Giovanni Battista Tornielli*



GRAPE POSTHARVEST DEHYDRATION



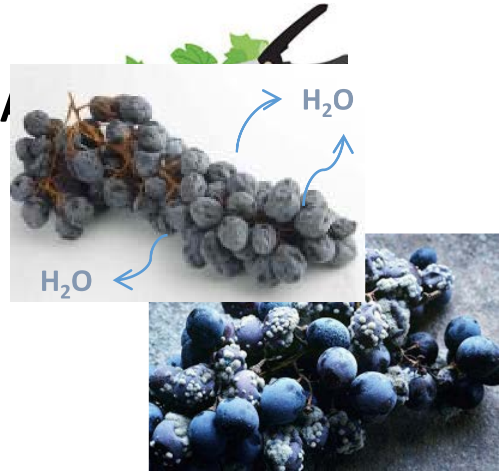
→ Increase in solute concentration

→ Physical and chemical changes

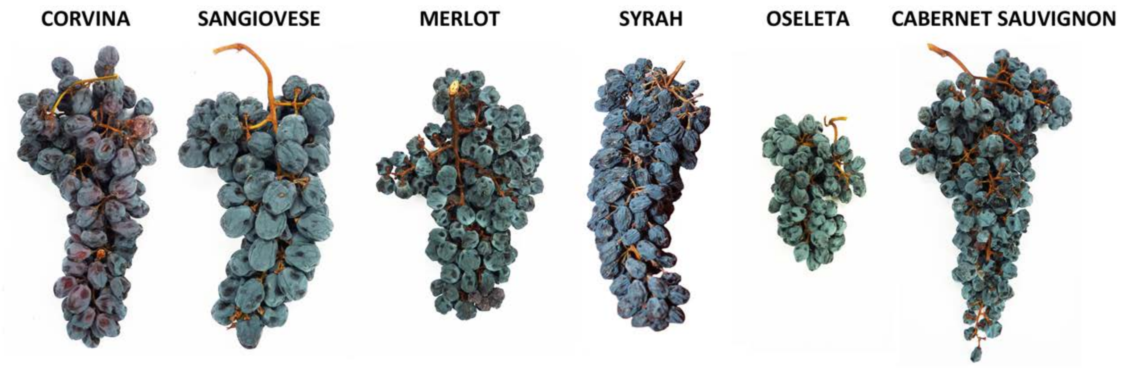
→ Modulation of primary and secondary metabolism

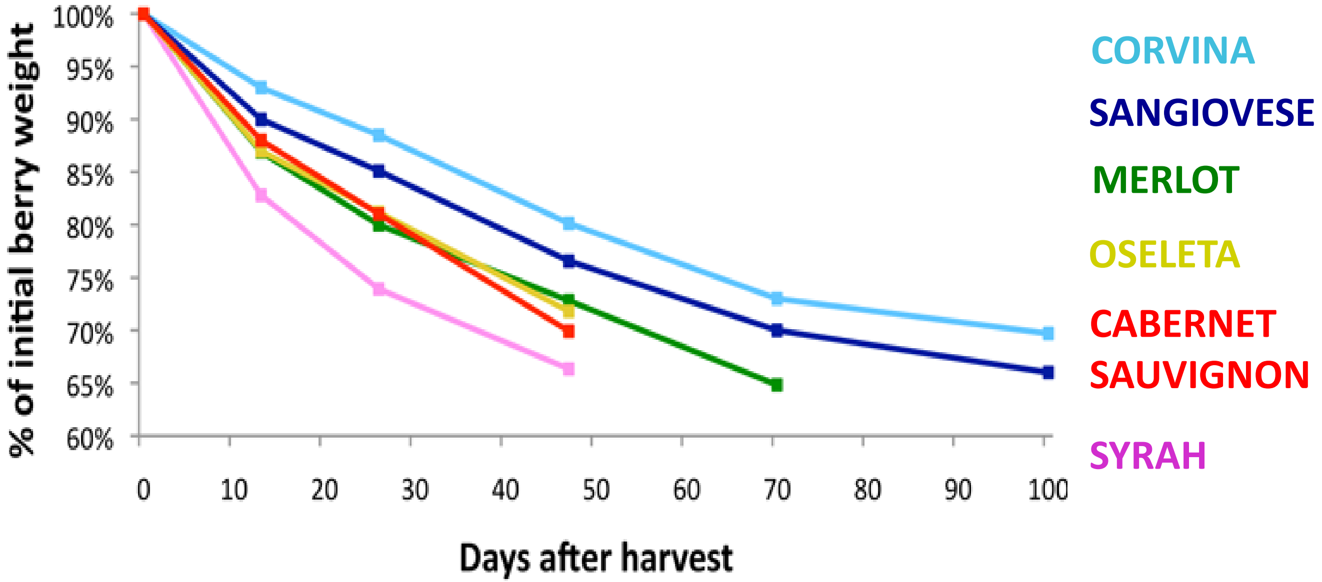
- **OVERRIPENING / SENESCENCE**

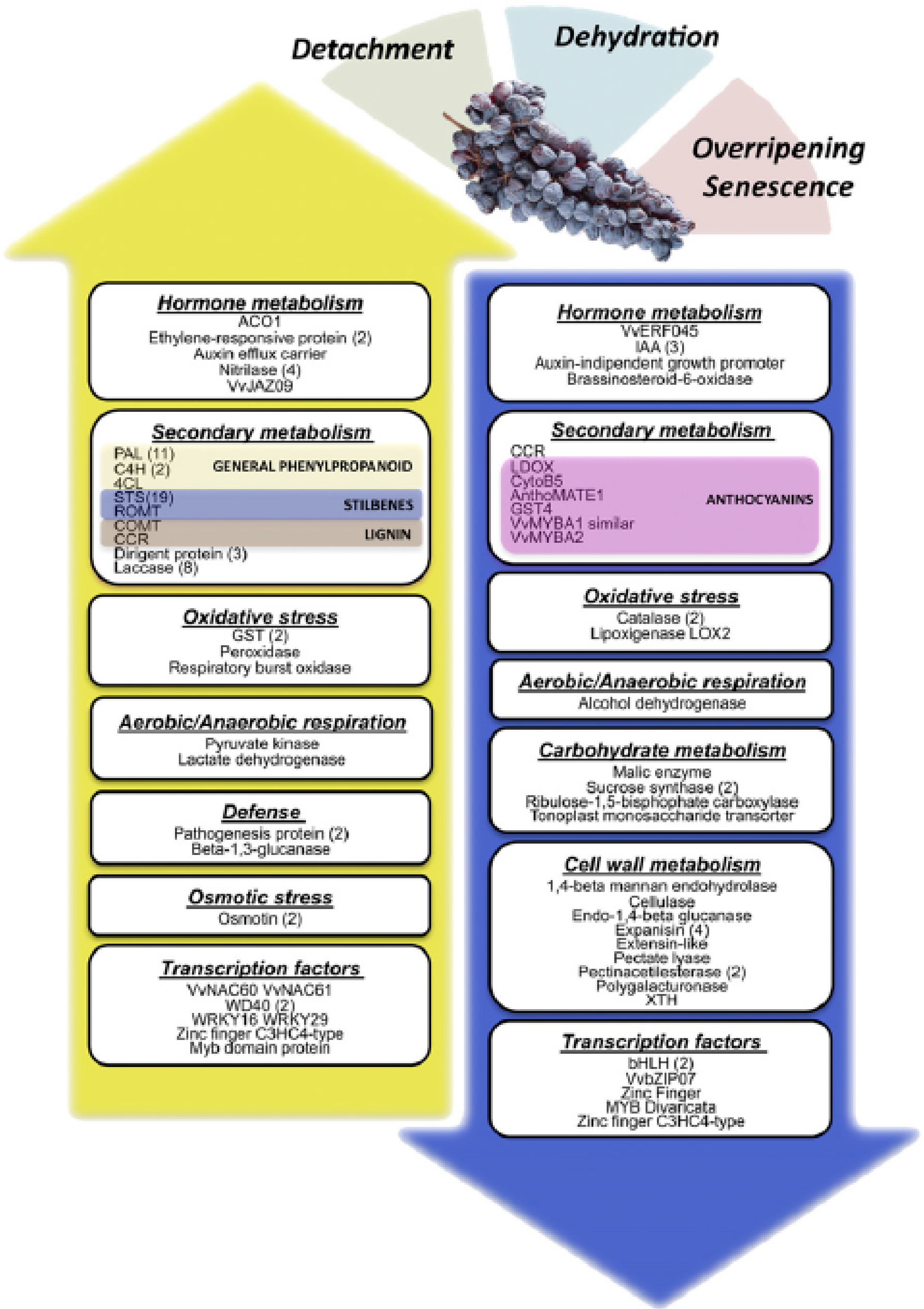
- **STRESS RESPONSE**
  - **DETACHMENT FROM THE PL**
  - **DEHYDRATION STRESS**
  - **ABIOTIC FACTORS**
  - **BIOTIC FACTORS (Botrytis)**



POSTHARVEST DEHYDRATION IN SIX VARIETIES







Hormone metabolism
ACO1
Ethylene-responsive protein (2)
Auxin efflux carrier
Nitrilase (4)
VvJAZ09

Secondary metabolism
PAL (11)
C4H (2)
4CL
GENERAL PHENYLPROPANOID
STS(19)
ROMT
STILBENES
COMT
CCR
LIGNIN
Dirigent protein (3)
Laccase (8)

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 C3HC4-type
Myb domain protein

Hormone metabolism
VvERF045
IAA (3)
Auxin-indipendent growth promoter
Brassinosteroid-6-oxidase

Secondary metabolism
CCR
LDOX
CytoB5
AnthoMATE1
GST4
VvMYBA1 similar
VvMYBA2
ANTHOCYANINS

Oxidative stress
Catalase (2)
Lipoxigenase LOX2

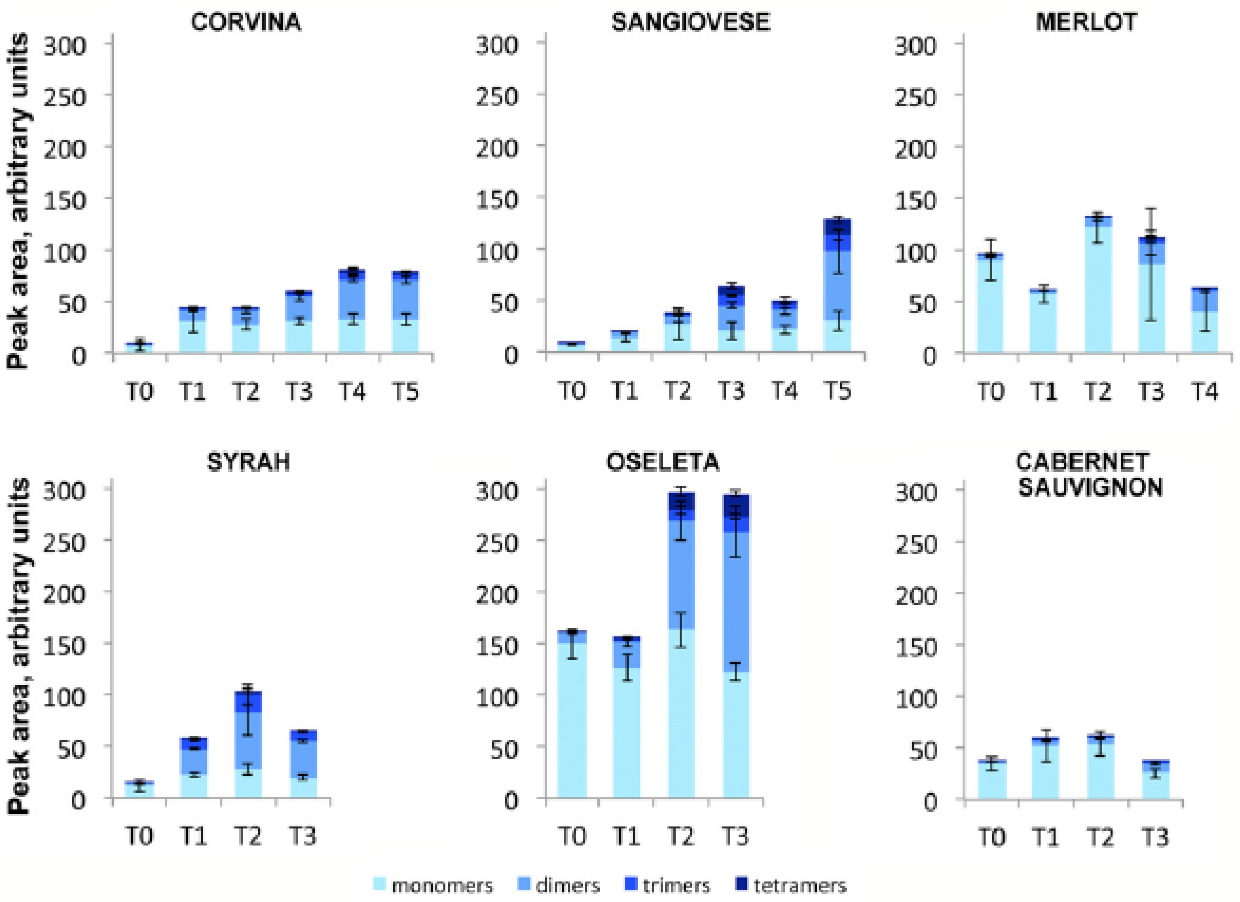
Aerobic/Anaerobic respiration
Alcohol dehydrogenase

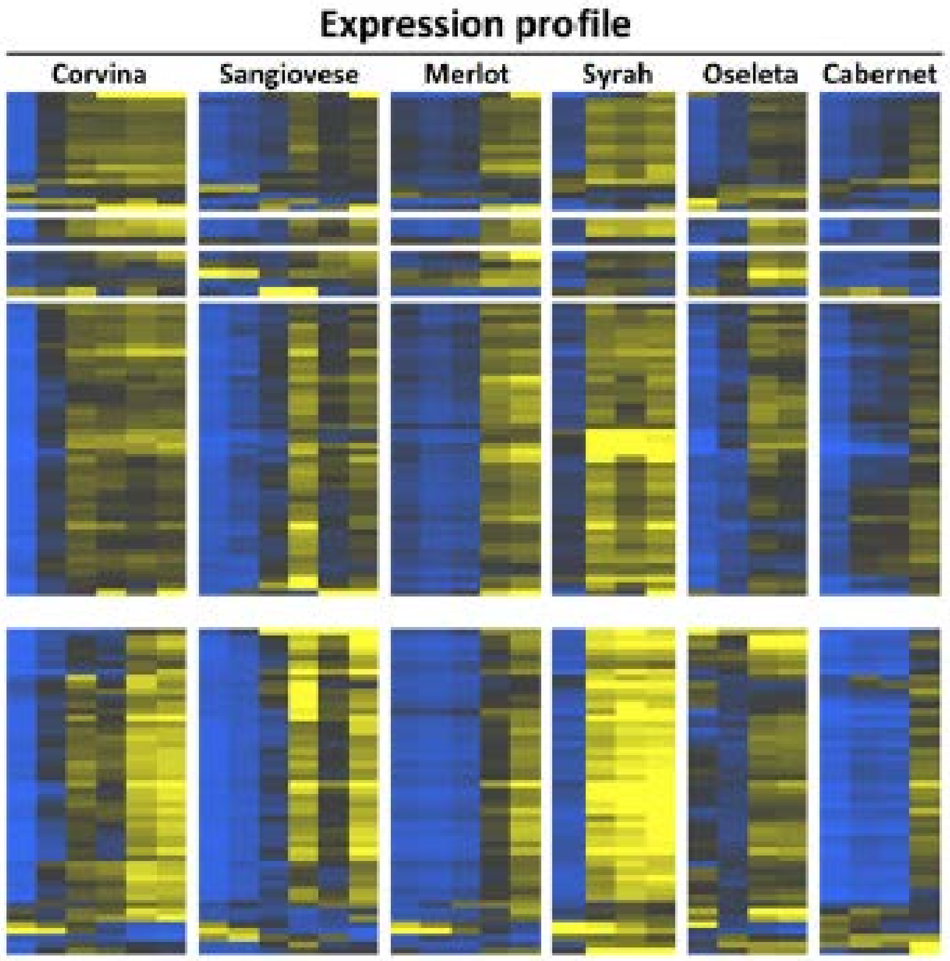
Carbohydrate metabolism
Malic enzyme
Sucrose synthase (2)
Ribulose-1,5-bisphophate carboxylase
Tonoplast monosaccharide transorter

Cell wall metabolism
1,4-beta mannan endohydrolase
Cellulase
Endo-1,4-beta glucanase
Expanisin (4)
Extensin-like
Pectate lyase
Pectinacetilesterase (2)
Polygalacturonase
XTH

Transcription factors
bHLH (2)
VvbZIP07
Zinc Finger
MYB Divaricata
Zinc finger C3HC4-type

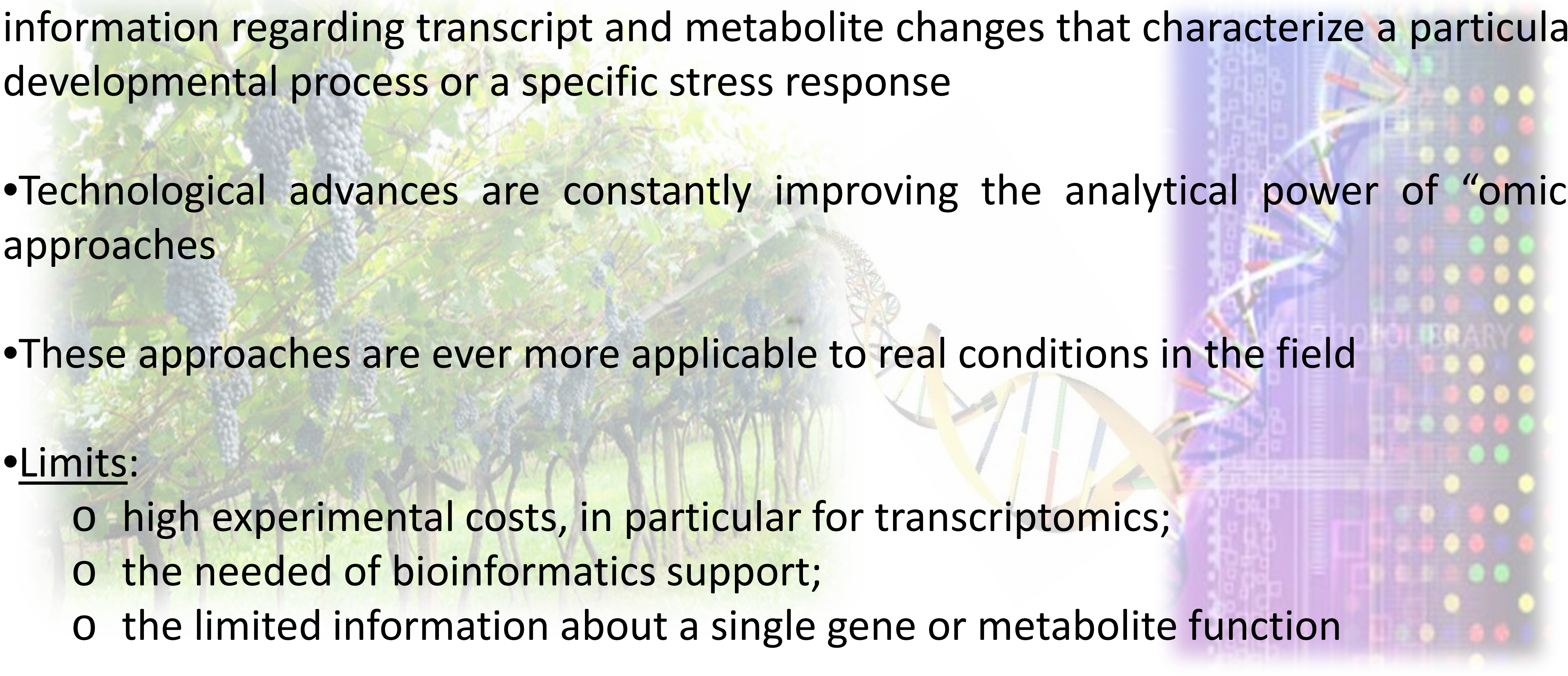
POSTHARVEST DEHYDRATION IN SIX VARIETIES





The activation of stilbene metabolism seems to represent a general molecular event that occurs in dehydrating berries after harvest, although the modulation intensity of stilbene accumulation clearly distinguished the behavior of genotypes.

CONCLUSIONS



- Transcriptomic and metabolomic analyses allow us to obtain a large amount of information regarding transcript and metabolite changes that characterize a particular developmental process or a specific stress response

- Technological advances are constantly improving the analytical power of “omic” approaches

- These approaches are ever more applicable to real conditions in the field

- Limits:
  - high experimental costs, in particular for transcriptomics;
  - the needed of bioinformatics support;
  - the limited information about a single gene or metabolite function

acknowledgements

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