A review of marker development to monitor the appasimento process in grapes

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Appasimento Process

- Can be performed under controlled or uncontrolled conditions.
- This will affect grape metabolism and the production of
  - volatile aroma compounds and other organic molecules including polyphenols (caftaric acids, flavonoids, resveratrols, anthocyanins and tannins)
- During appasimento process grape dehydration monitored
  - by weight to determine berry water loss
  - and use destructive measures to determine the relative sugar concentration.
  - Costly, labor intensive and time consuming
  - Not always effective
- How to develop an effective and reproducible process
  - Rapid, accurate and broadly applicable.

Appassimento leads to:

- Sugar concentration (elimination of water)
- Concentration of other substances (elimination of water)
- Increased aromatic, colouring and phenolic substances in the must (activation of enzymes)
- Transformation of aromatic substances from simple to complex
- Development of phenolic substances with consequent disappearance of “rustic grassiness”, and bitterness and increase of “smooth and rounded” tannins
- Possible effects “desirable Botrytis” or undesirable “grey mould”:

Appassimento leads to:

- appearance of glycerine
- breakdown of malic and tartaric acid
- Degradation of varietal aromas
- Production of various acids and various colloidal substances.
- Expression of catalysts that break down or modify phenolics (flavonoids, catechins, resveratrols, anthocyanins) or aroma compounds.

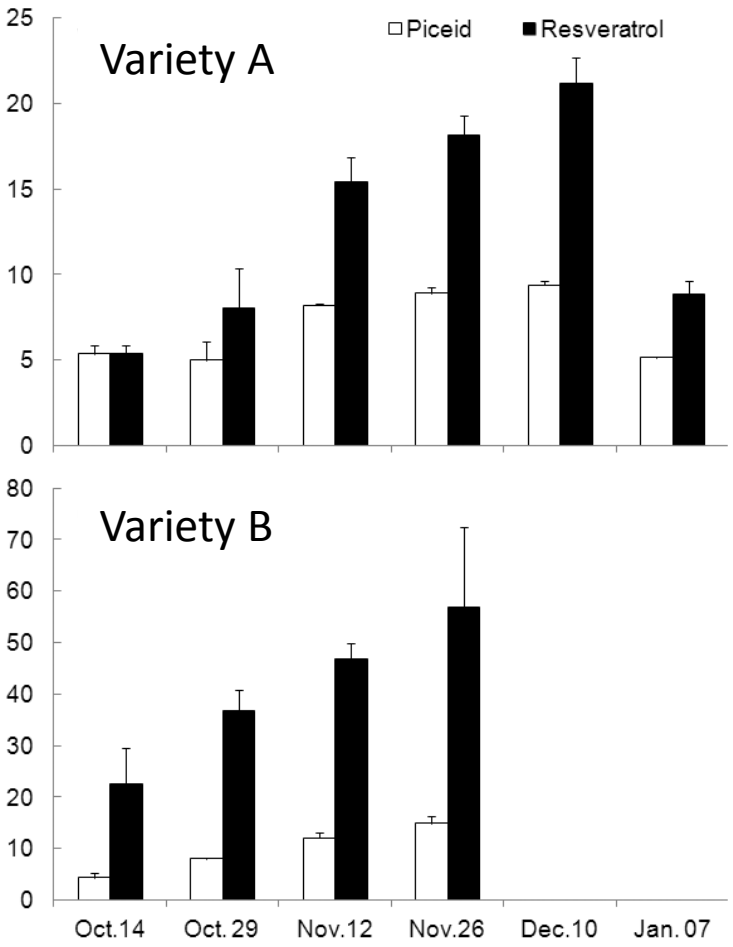
Outline

- Appasimento Project Metabolite Analyses (2011-2013) of grapes dried by different processes.
  - Vine
  - Barn
  - Greenhouse
  - Drying Chamber
  - Kiln
- Transcriptomic, Proteomic and metabolomic approaches to find markers in Italy

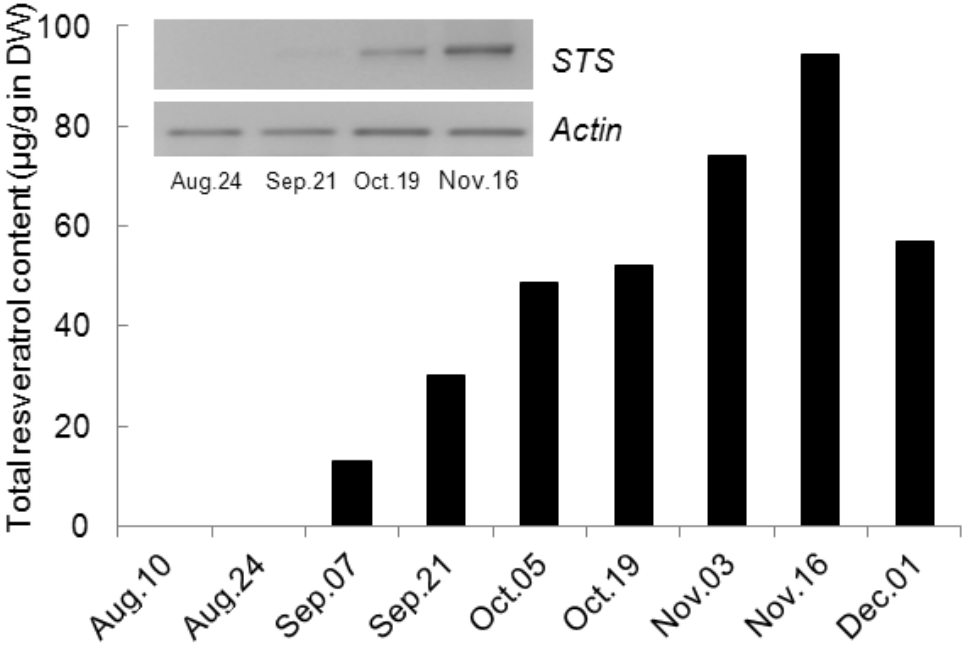
- What happens in Ice wine grapes?
- Extraction and Identification of **25-30** different polyphenols
  - Simple phenols (Gallic acid, Galloyl glucoside, Caftaric Acid)
  - Resveratrols (transresveratrol, cis and trans-piceid)
  - Procyanidins (Procyanidin, Procyanidin Dimers, Catechin, Epicatechin
  - Flavonoids (Kaempferol, Kaempferol Glucosides, Quercetin, Quercetin glucoside, Quercetin glucuronide, Isorhamnetin glucoside, Myricetin, Myricetin glucoside, Myricetin galactoside, Myricetin rhamnoside
  - Anthocyanins (Delphinidin-3-0-glucoside, Petunidin 3-O-glucoside, Malvidin-3-O-glucoside, Malvidin 3-O-acetylglucoside, Malvidin 3-O-coumaroylglucoside

Contents of Resveratrol and Resveratrol glucoside (Piceid) during the formation of ice wine grapes

- Accumulation of resveratrol and piceid increased throughout the fall and early winter period before the harvest of Ice wine grapes for making ice wine.
- Does this involved de novo synthesis of resveratrol?

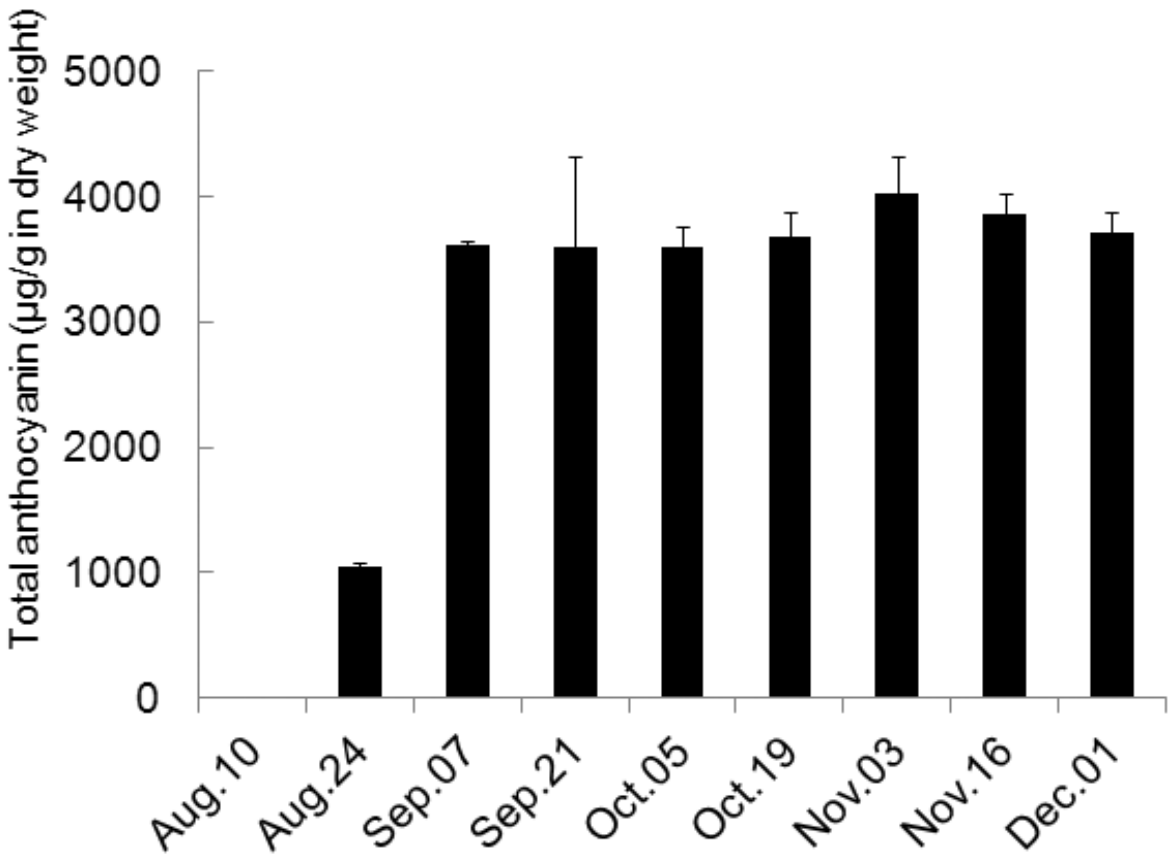


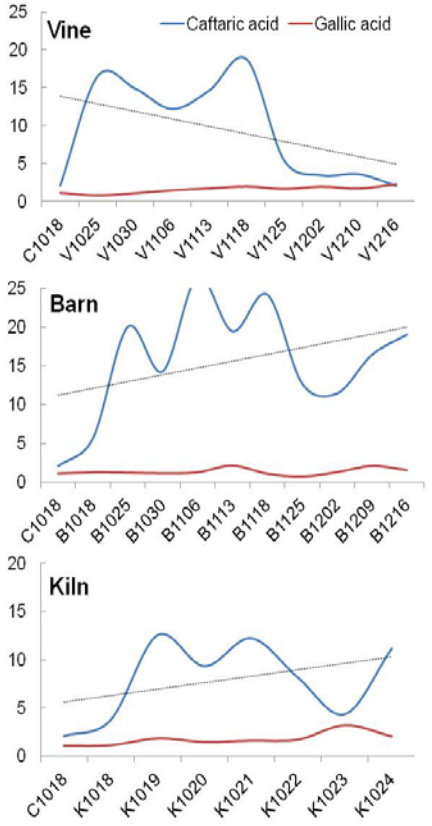
Transcript levels of Stilbene Synthase rise in ice wine grapes



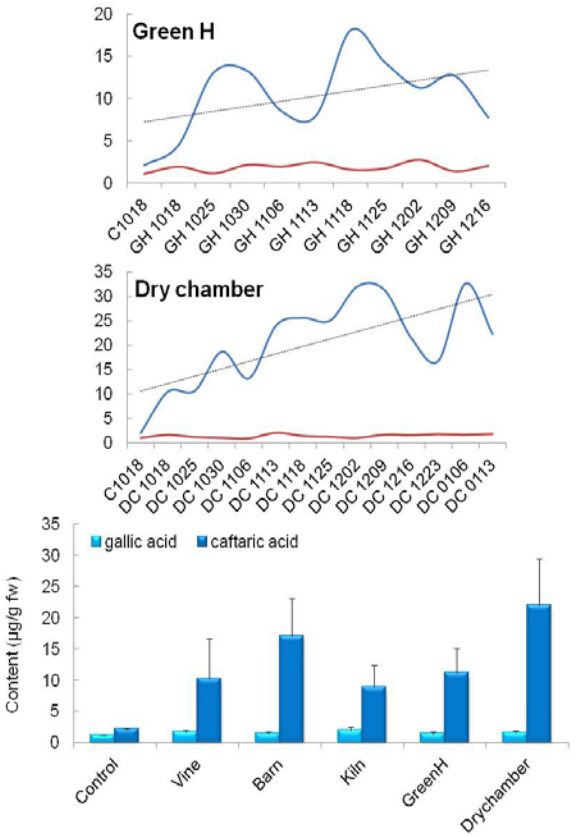
- The data suggests that stilbenes are made de novo during the fall and early winter.
  - Grapes are therefore still biologically active
- Are grapes grown under appasimento conditions also active in Stilbene production and is this a good marker for the appasimento process?

Anthocyanin levels do not change during the development of Ice Wine Grapes

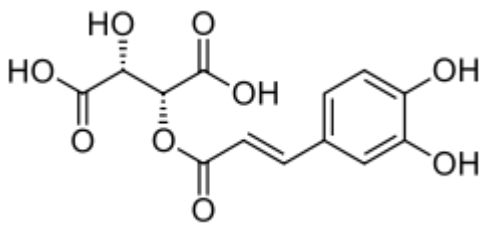


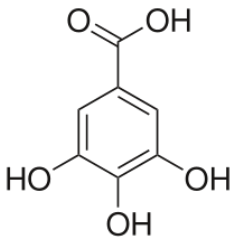


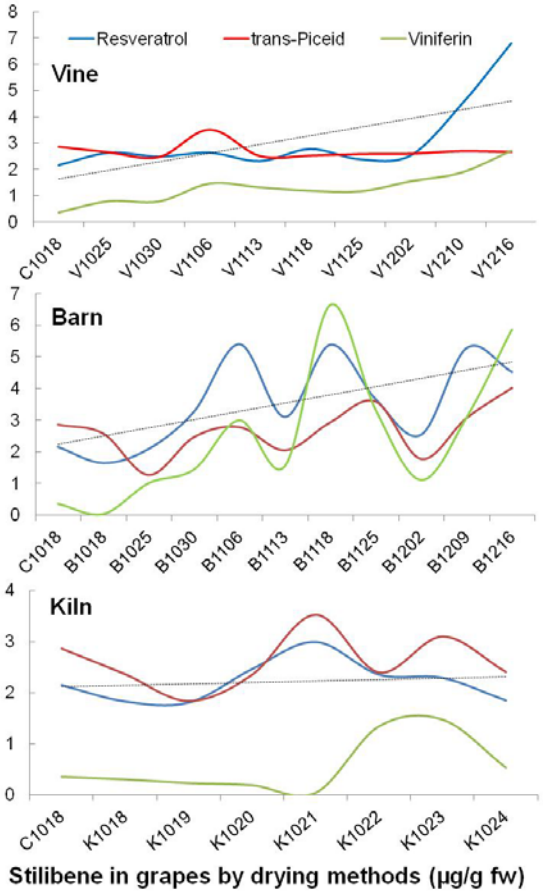
Caftaric and gallic acids in grapes by drying methods (μg/gfw)



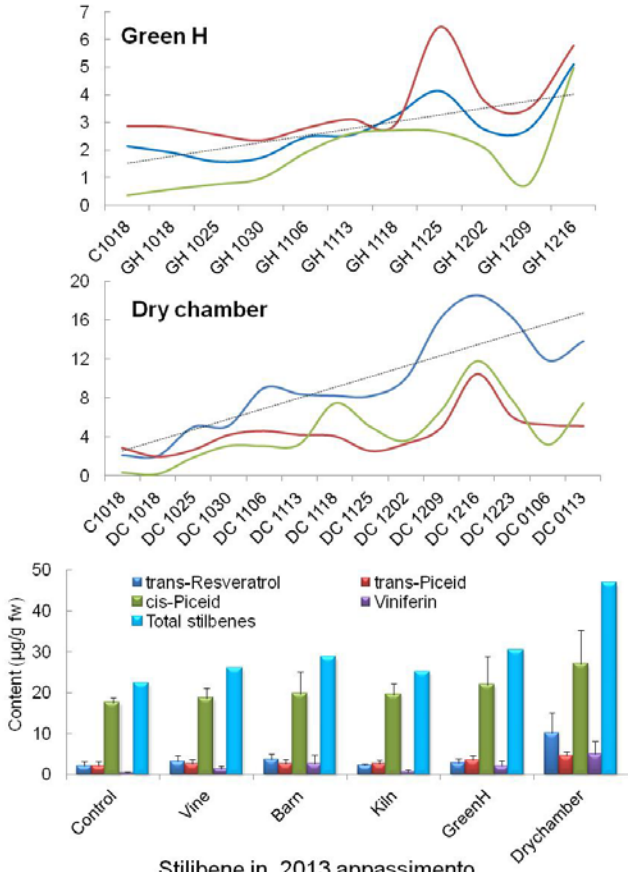
Caftaric and gallic acids in 2013 appassimento



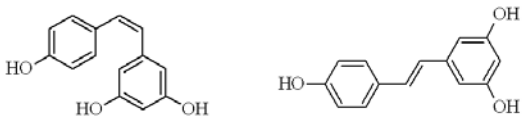




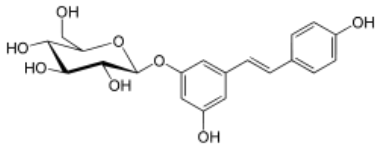
Stilibene in grapes by drying methods (µg/g fw)



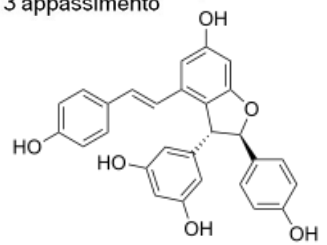
Stilibene in 2013 appassimento



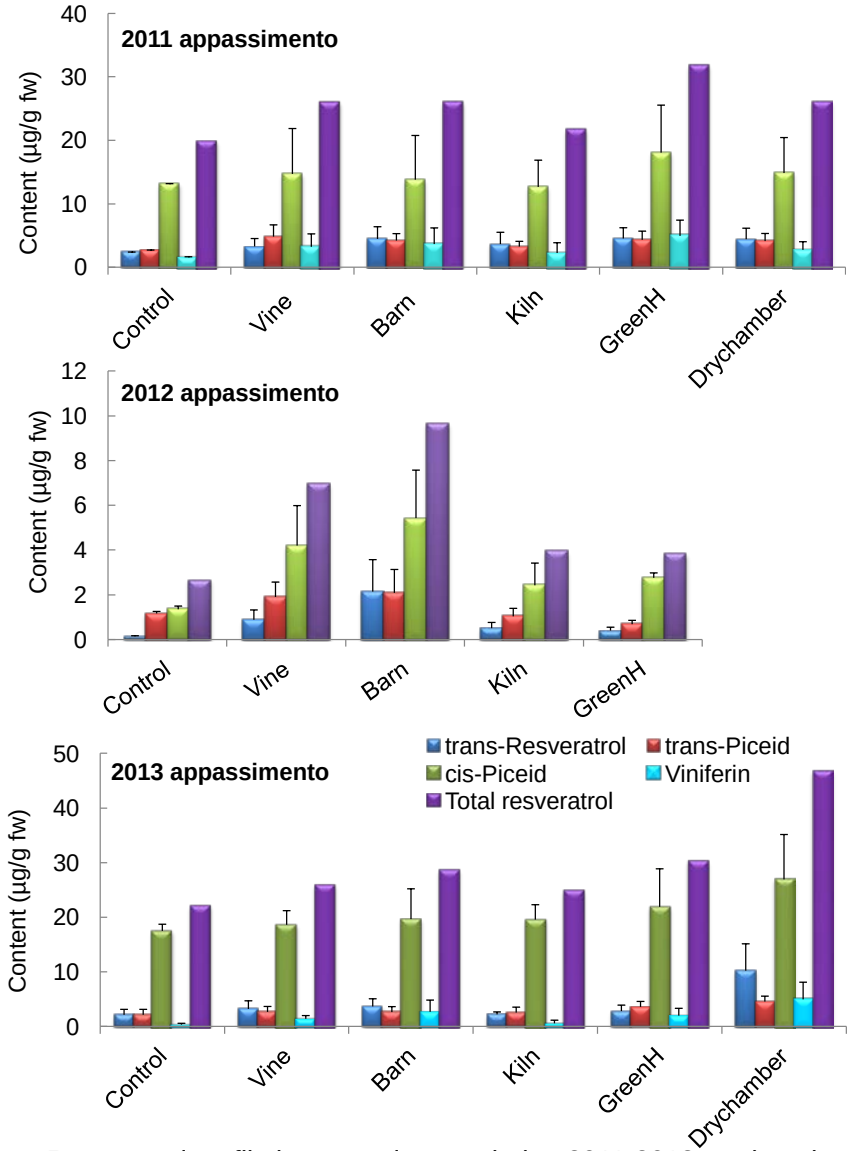
Cis and trans resveratrol



Trans Piceid

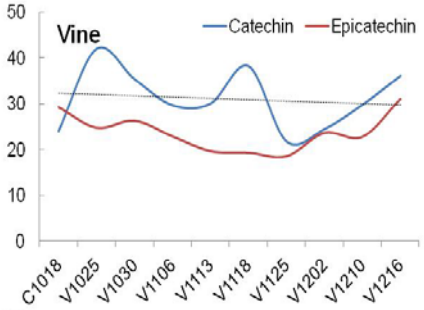


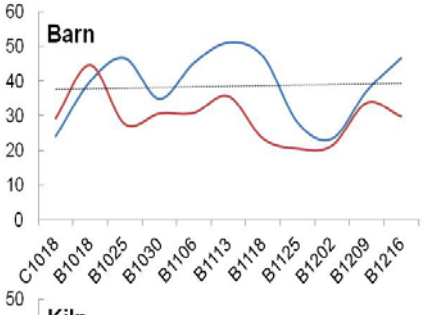
Viniferin dimer

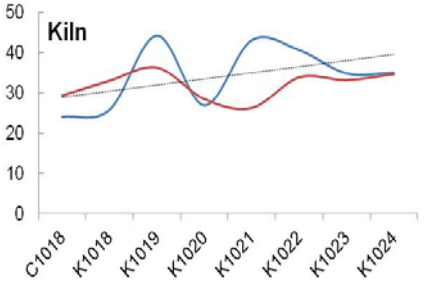


Resveratrol profile in appassimento during 2011-2013 produced by different drying methods

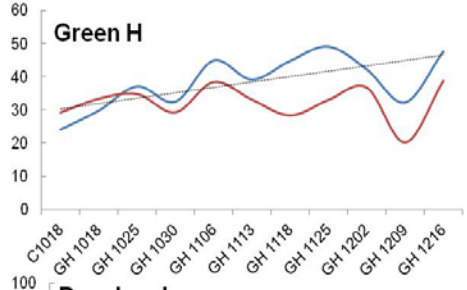
1. Resveratrol normally found associated to sugar (glucoside) named piceid.
2. Total Resveratrol includes the addition of free and bound forms.
3. Note that levels were 4 to 8 times larger in 2011 and 2013 than in 2012.
4. Excellent marker for level of fungal pressure during growing season.
5. When total resveratrol is compared to control levels of harvested grapes, their levels do not rise significantly

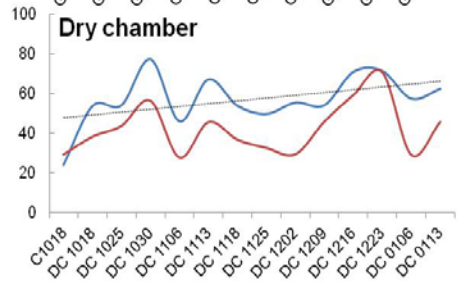


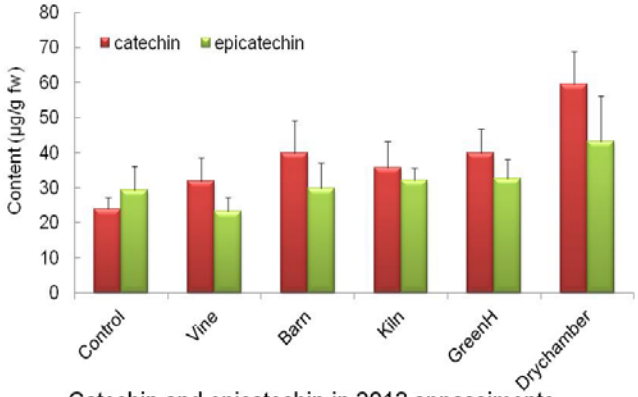




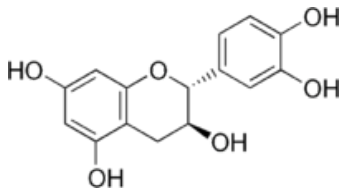
Catechin and epicatechin in grapes by drying methods (μg/gfw)

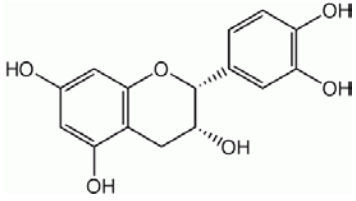


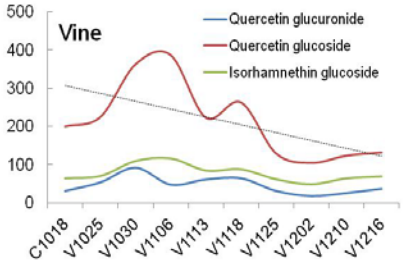


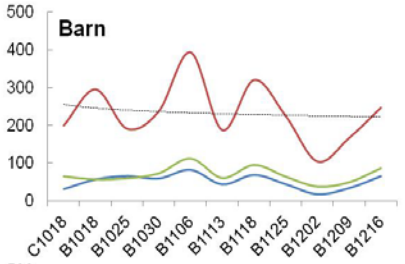


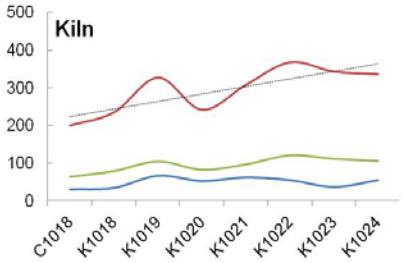
Catechin and epicatechin in 2013 appassimento

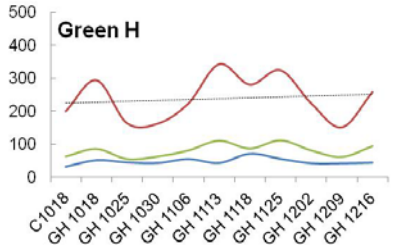


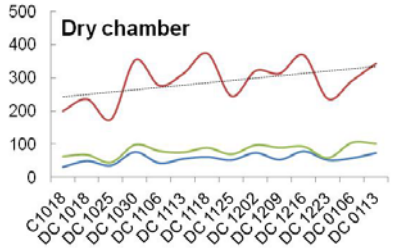


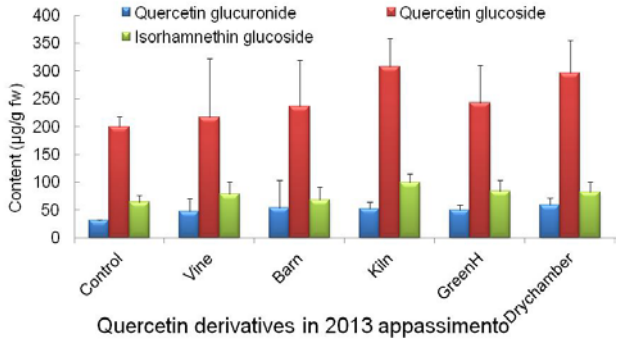






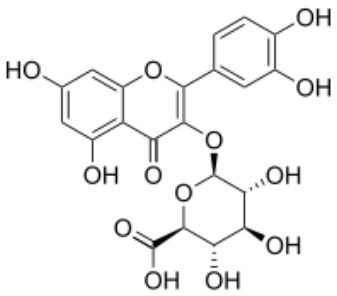


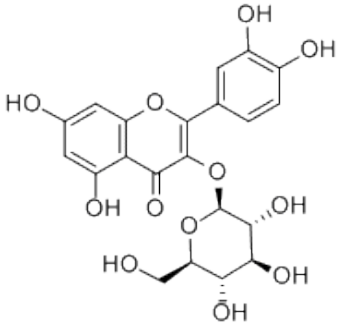


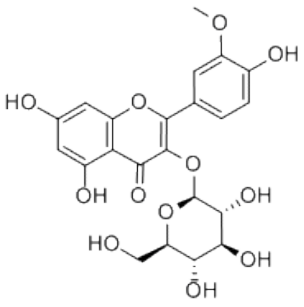


Quercetin derivatives in grapes by drying methods (µg/gfw)

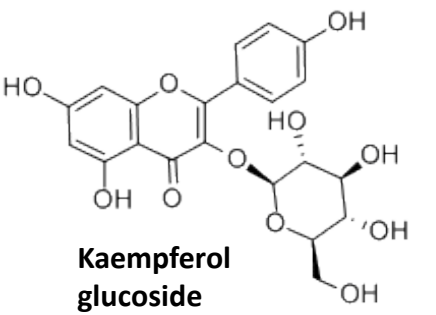
Quercetin derivatives in 2013 appassimento



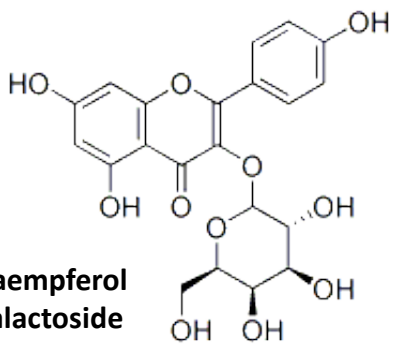




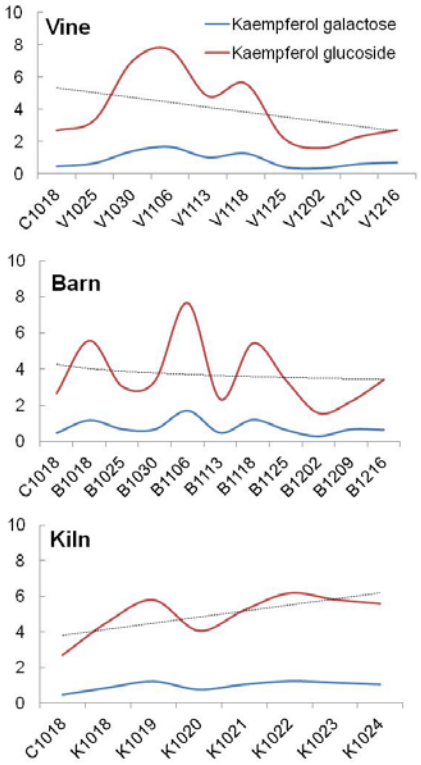
Quercetin glucuronide **Quercetin glucoside** **Isohramnetin glucoside**



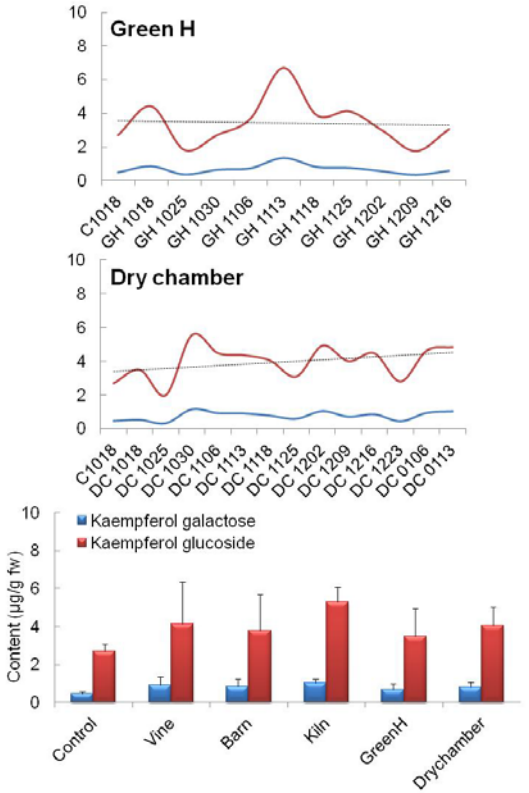
Kaempferol glucoside



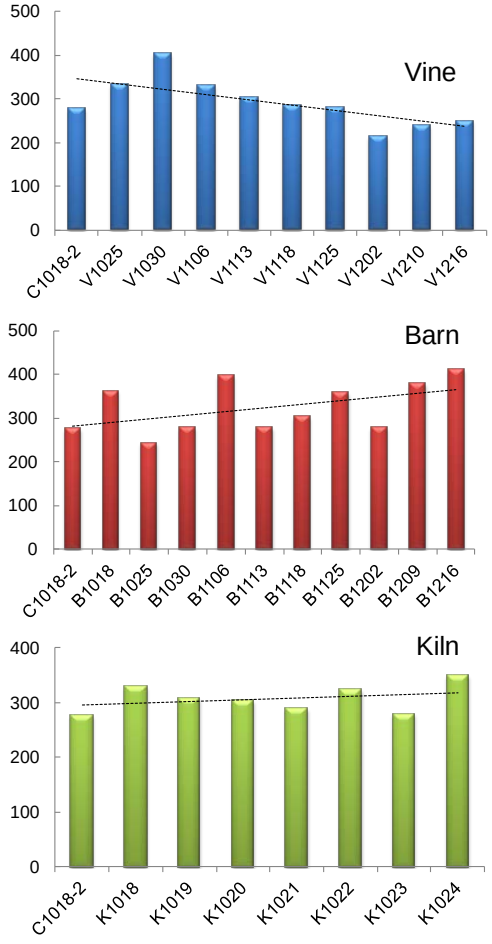
Kaempferol galactoside



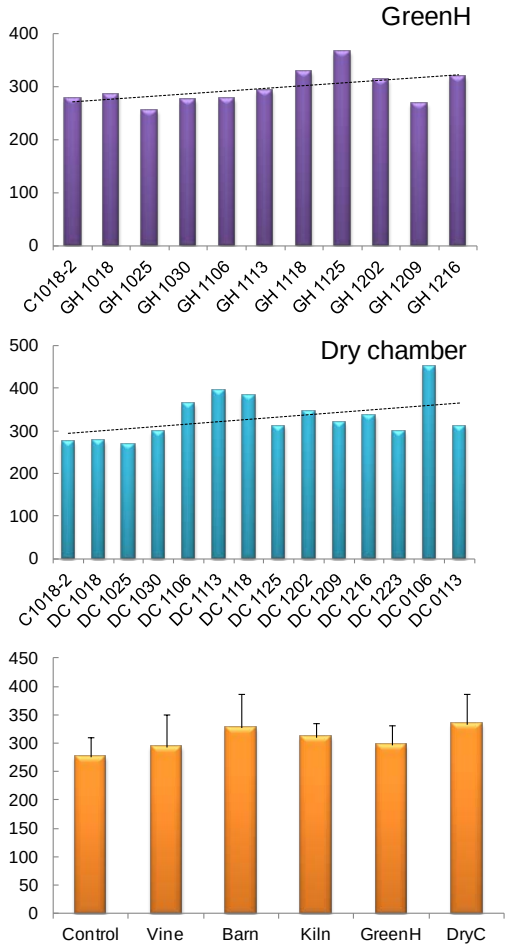
Kaempferol glycosides in grapes by drying methods (μg/gfw)



Kaempferol glycosides in 2013 appassimento (μg/gfw)

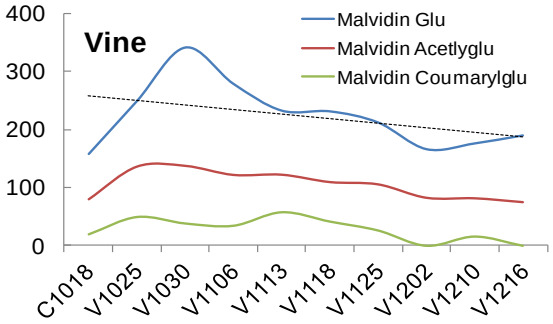


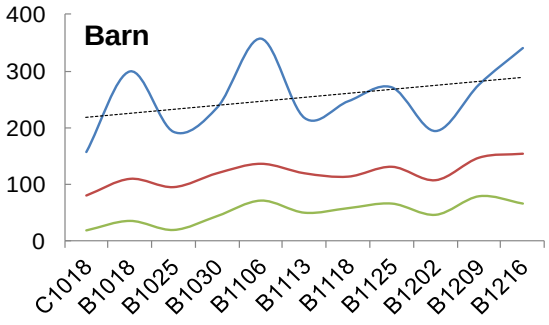
Total anthocyanin in appassimento (µg/g fw)

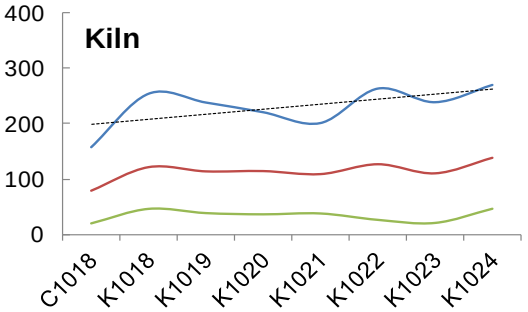


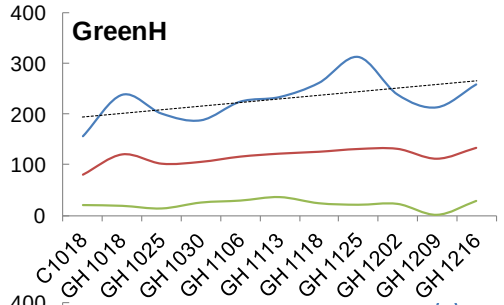
Total anthocyanin in appassimento (µg/gfw)

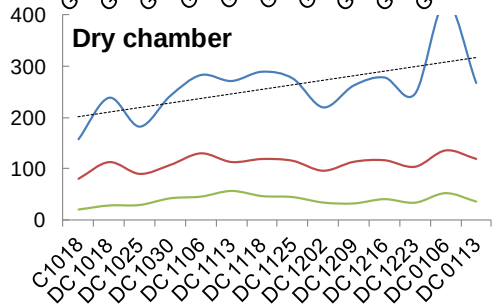
2013

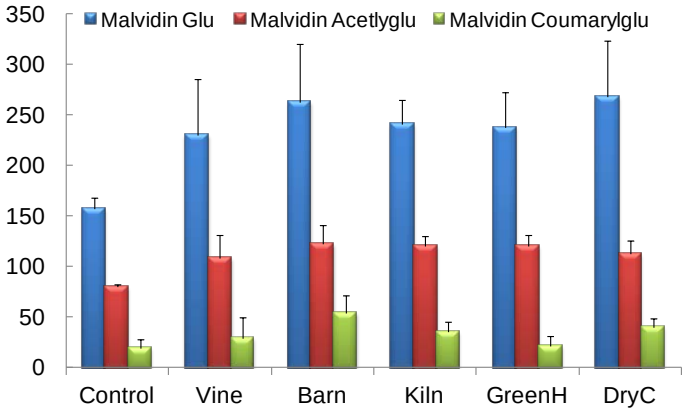




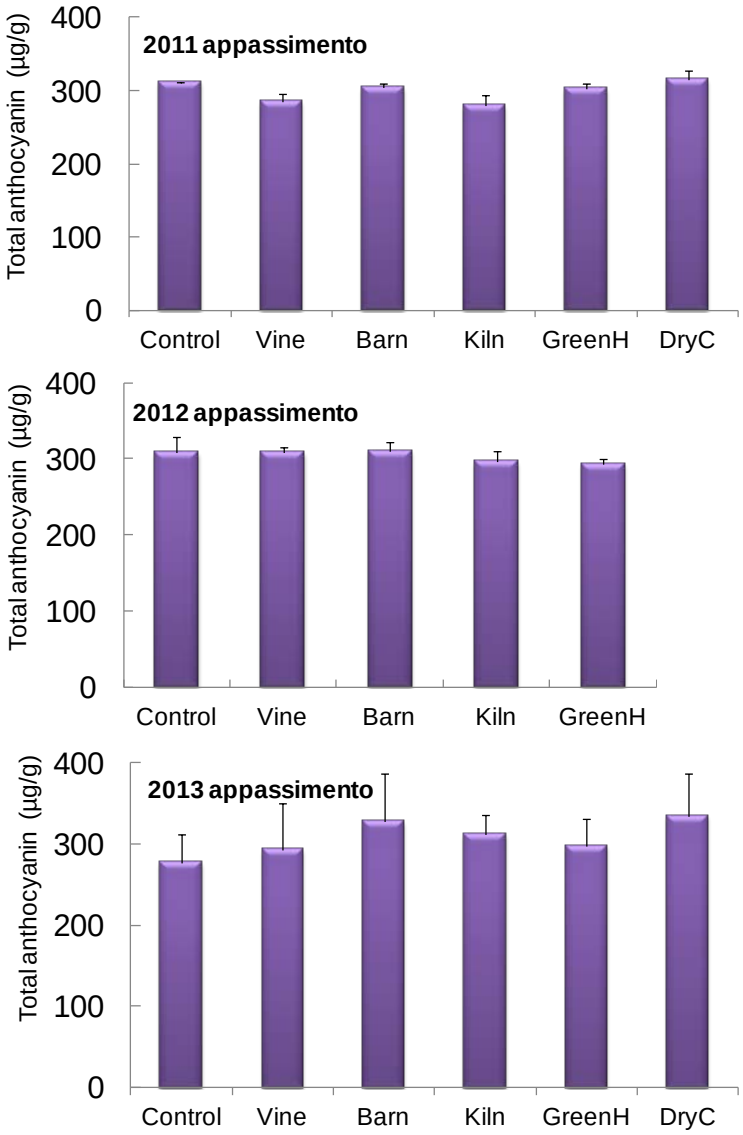








Anthocyanin in appassimento (µg/gfw) **2013**



Total anthocyanin in appassimento during 2011-2013 produced by different drying methods (µg/g)

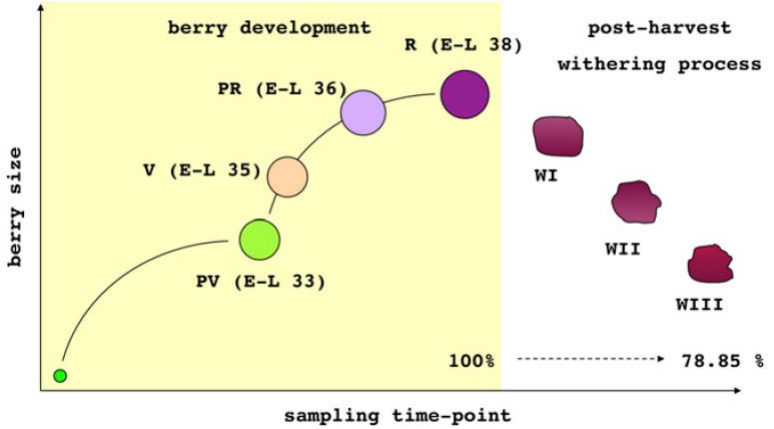
Zamboni 13 others from Italy and UK **Plant Physiol. 2010;154:1439-1459**

- Identification of Putative Stage-Specific Grapevine Berry Biomarkers and Omics Data Integrationinto Networks

- Transcriptome
  - set of all RNA molecules, including mRNA, eRNA, tRNA and other non-coding RNA transcribed in one cell or a population of cells.

- Proteome
  - entire set proteins expressed by a genome, cell, tissue or organism at a certain time.

- Metabolome
  - refers to the complete set of small molecule chemicals found wirthin a biological sample (a cell, tissue, organism)



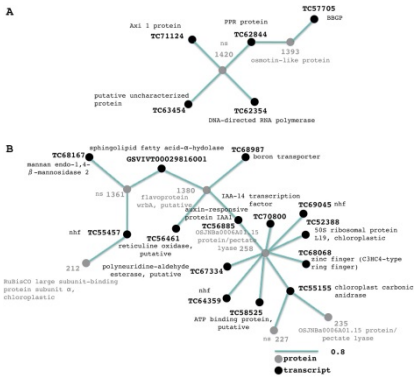
- **PV, Preveraison**
- **V, veraison**
- **PR, preripening**
- **R, ripening**
- **WI, withering I**
- **WII, withering II**
- **WIII, withering III**

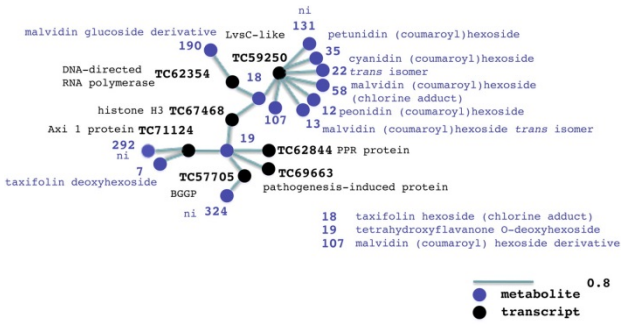
- Microarray
  - Older technology
- RNA seq
  - More recent

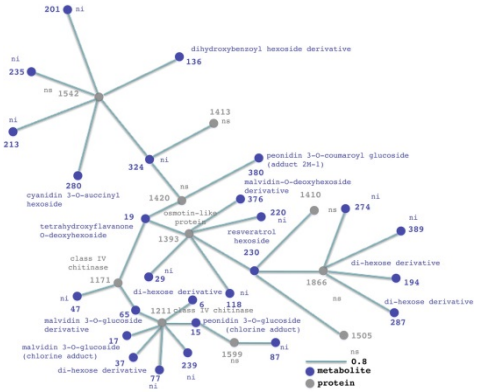
- Isoelectrofocussing
- & SDS polyaccrylamide gel elctrophoresis
- Digestion and MS/MS analysis

- HPLC-MS

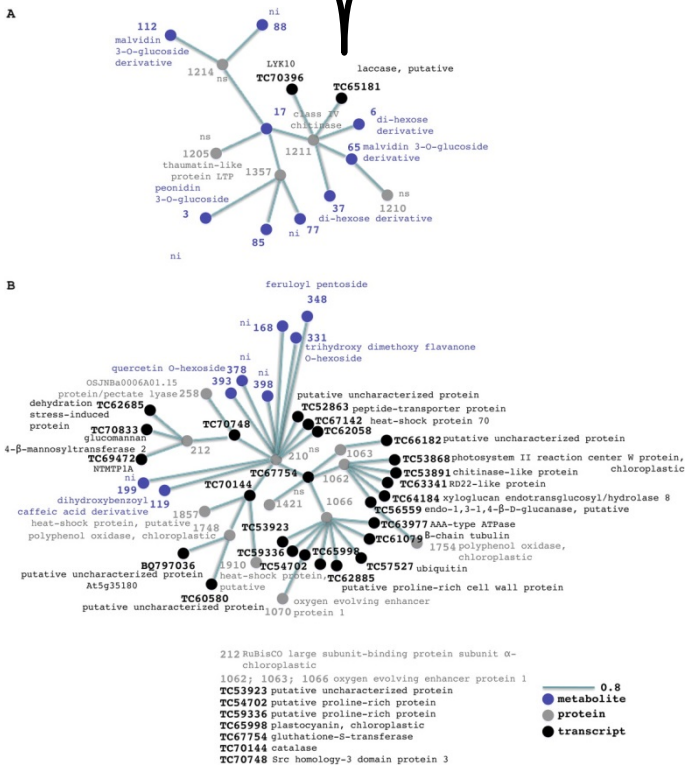
NETWORKS



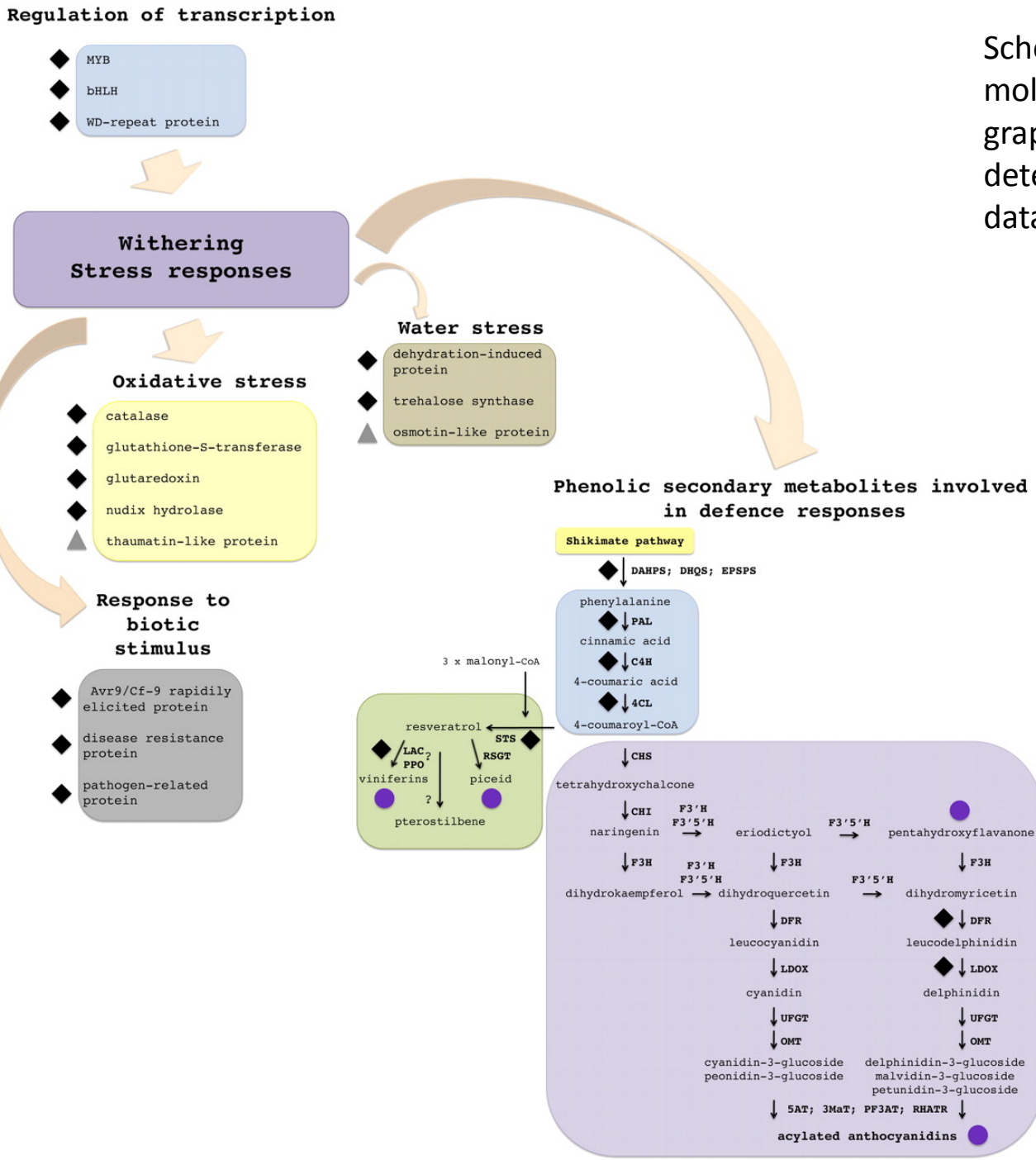




Transcript-Protein | **Transcript metabolite** | **Protein Metabolite**



Transcript-Protein-Metabolite Network



Schematic representation of the molecular events characterizing grapevine berry withering determined by hypothesis-driven data integration

transcript

protein

metabolite

Conclusions

- Potential markers for Quality
  - Transcript/Protein Markers
    - Oxidative stress
    - Osmotic Stress
    - Biotic Stress
  - Small Molecules
    - Sugars
    - Acids
    - Phenolics

- Simple and inexpensive
  - Single entity
  - Dip stick
- More complicated
  - Multiple entities
  - More costly and time consuming