Sustainability in grape production and commerce

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  - Supported by OMAFRA, NSERC and Industry
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    - Dawn Hall, PhD
    - Dr. Kyung Hee Kim

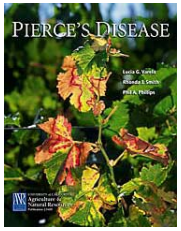
Introduction

1. Research Interests: cell specialization and biochemical production
2. Acyltransferases involved in grape volatile production.
   - Acyl esters of phenols and terpenes.
   - Relationship between anthocyanin 5 glucosides and methyl anthranilate.
3. Glucosyltransferases and grape natural products.
   - Water solubility, stability, transport and compartmentation
   - Bifunctional GT and its role in resveratrol and phenol tartrates production
4. Identification of Grape Pomace secondary metabolites.
   - Value as food additives

Brock University

Sustainability

- Wine grapes
  - Are clonally propagated
  - Large numbers of identical individuals growing together
  - Susceptible to similar diseases
  - Unique to wine grapes
  - Lack of large scale breeding efforts
- Unique to wine grapes
  - Lack of large scale breeding efforts
  - Heavy dependence on management and chemical pesticide use
- Regulatory issues
  - Demands by governments to decrease the pesticide footprint for wine grape production.
    - Contamination of land, water and air.
    - Population risks
  - Needs for biocontrol measures over pesticide use
  - Need for genetically superior disease resistant genotypes
- Risks
  - Appearance of new diseases?
    - Pierce's Disease
    - Powdery Mildew
    - New forms of Phylloxera

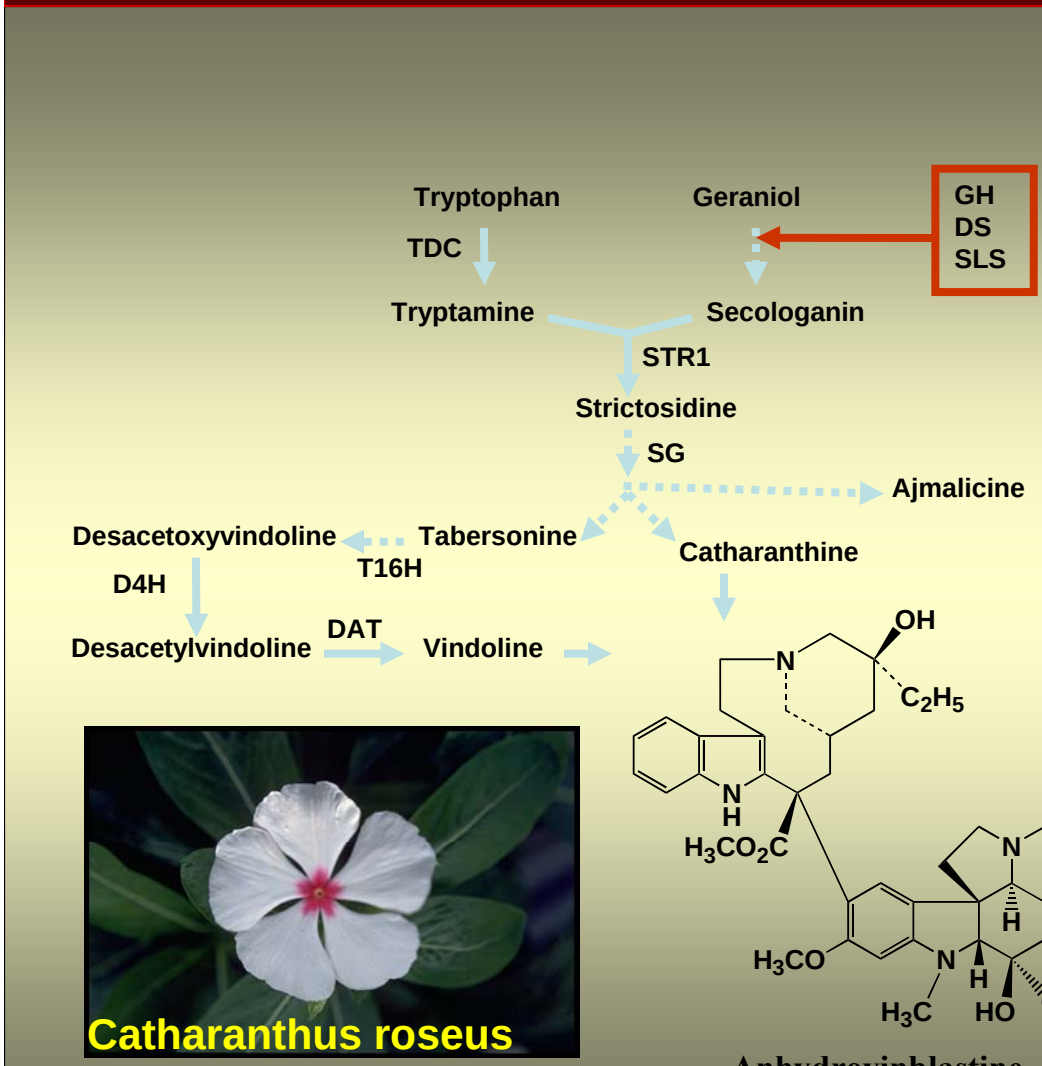


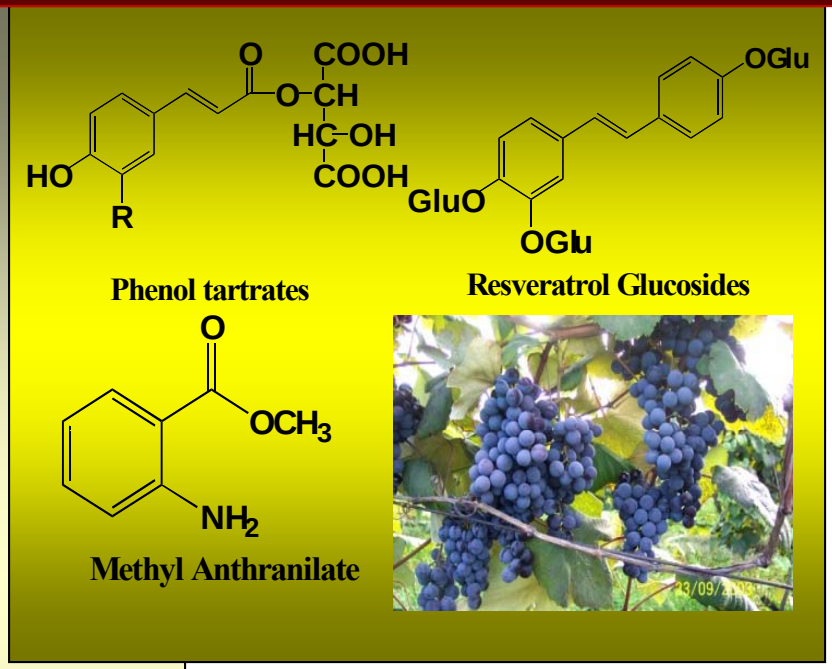

Approaches to grape improvement

- Tools available
  - Genomics
    - Grape genome sequence (Pinot Noir)
    - Synteny
    - Marker assisted breeding
  - Metabolic profiles
  - Pathways
    - Genes
    - Proteins
    - Mutations

- Examples of important wine related pathways
  - Anthocyanins
    - Very well known in plants
  - Resveratrol
    - Very well known in grape
  - Acylated aroma and flavor cpds
    - Methyl anthranilate
  - C-13 Norisoprenoids
    - β-damascenone

Cell specific specialization for secondary metabolism





Cell factories & biosynthesis of plant natural products

Scanning electron microscopy of aromatic Sage: The Scientist Nov 17 2003 V17: p12

Brock, April 19, 2010

Research Interests of our Laboratory

- Biosynthesis of Novel and commercially important metabolites
- Evolution of biosynthetic pathways
- Cell and biochemical specialization involved

Mint gland on the surface of a mint leaf

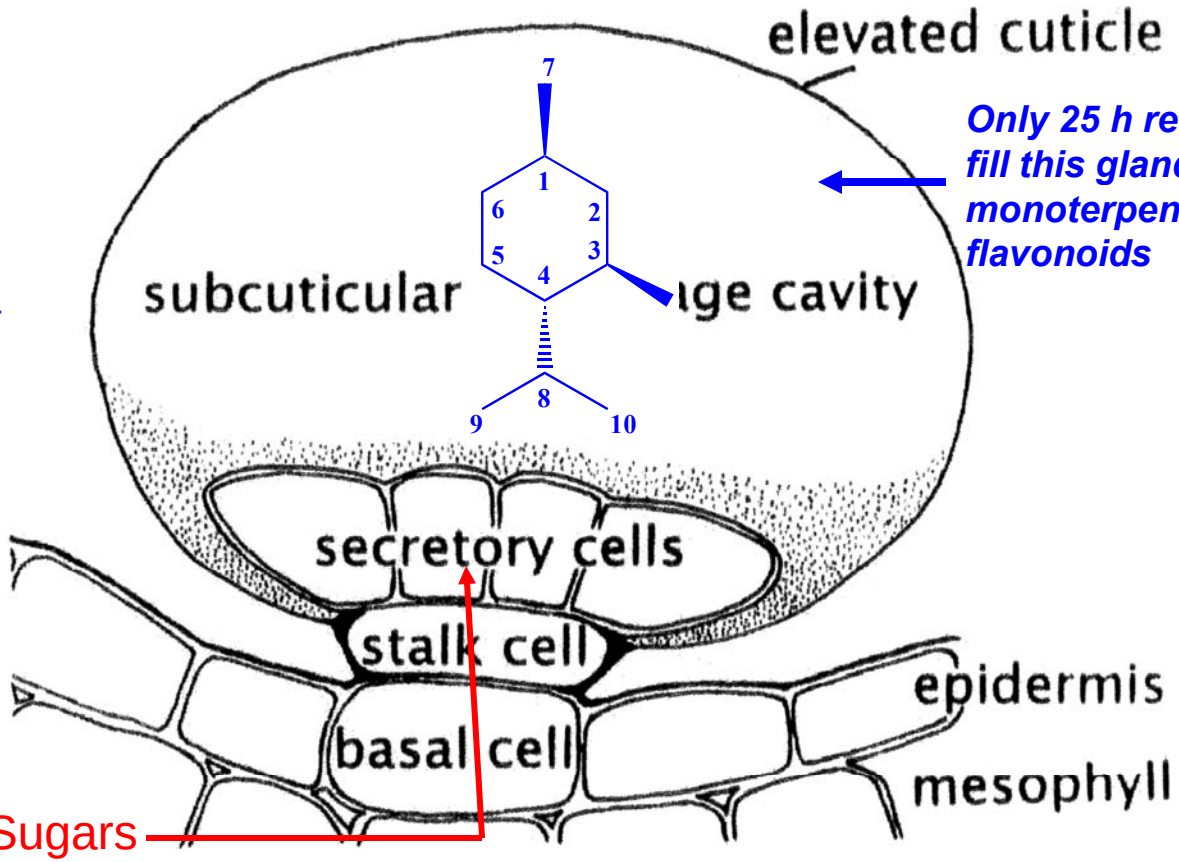
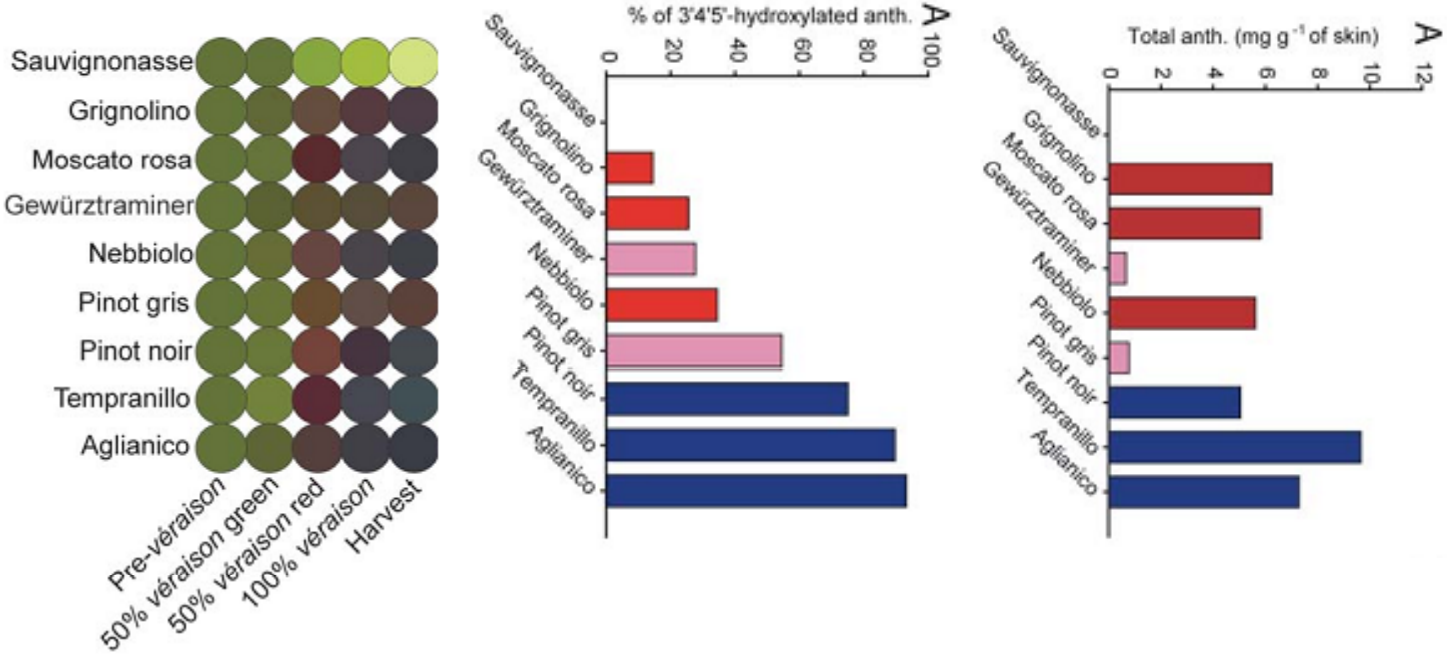


Diagram from: Plant Physiology 136:4215-4227 (2004)

Anthocyanin Biosynthesis
is the best know pathway of Secondary metabolism in plants

The pattern of grape pigmentation in nine cultivars is regulated by differential gene expression of anthocyanin biosynthesis and their transcription factors

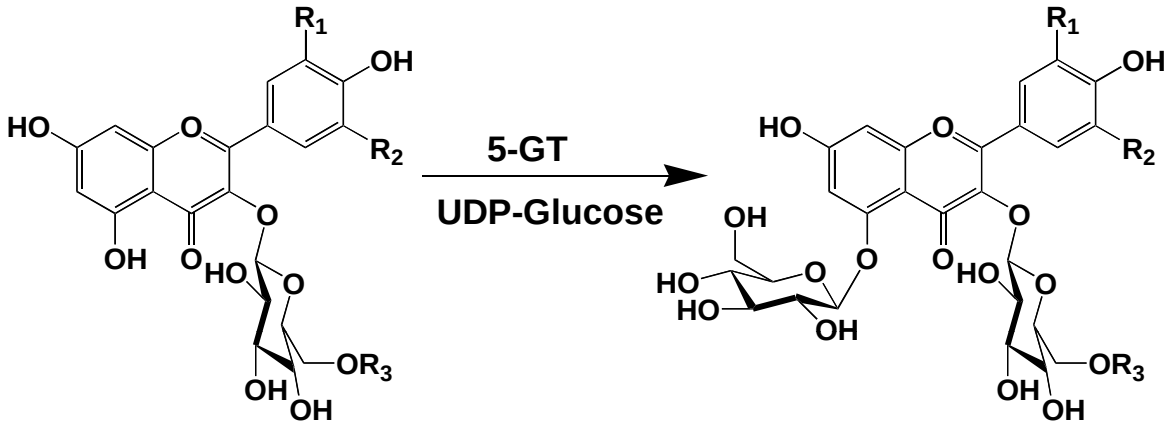


Castellarin and Di Gaspero BMC Plant Biology (2007) 7: 46

Anthocyanin Biosynthesis
is the best know pathway of Secondary metabolism in plants

- **The sugar pattern is used to define wines contaminated with North American grapes.**
- **However anthocyanins with sugars on the 5 position are more stable and their presence could be valuable for wine color stability?**

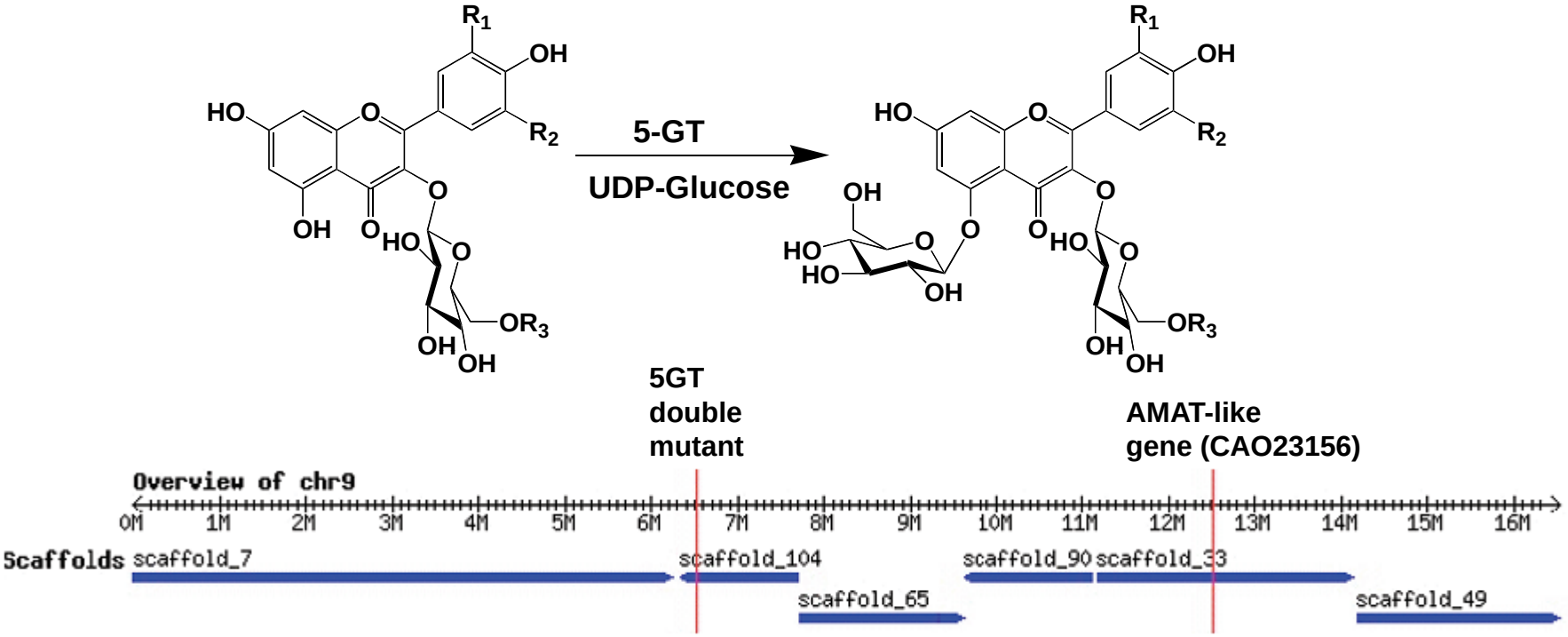
Identification of the 5GT responsible for the formation of anthocyanin diglucosides



- **The inability of most European grapevines to produce 3,5-di-O-glucosides has long been used to classify wines according to their varietal origin.**
  - **This study showed that *V. vinifera* has a 5GT gene with 2 mutations that render the protein inactive.**
  - **Correction of the 2 mutations reactivated the *V. vinifera* 5GT gene**
  - **This explains why revertants have not been observed**

Janvary et al, Agric Food Chemistry (2009) 57: 3512-3518

Colocalization of *5GT* and *AMAT* to the same area of chromosome 9 explains why FOXY & diglucosides are linked!

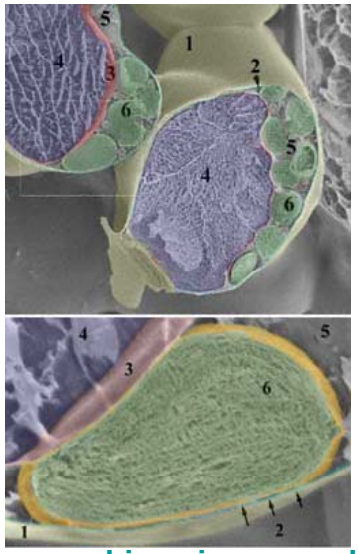


- ***V. vinifera* (Pinot Noir) genome shows that :**
  - CAO23156 is 95% identical on the amino acid level with V. labrusca anthraniloyl-CoA:methanol anthraniloyl transferase (AMAT).
  - Colocalization of the two genes would explain genetic linkage between these 2 traits in hybrid cultivars.

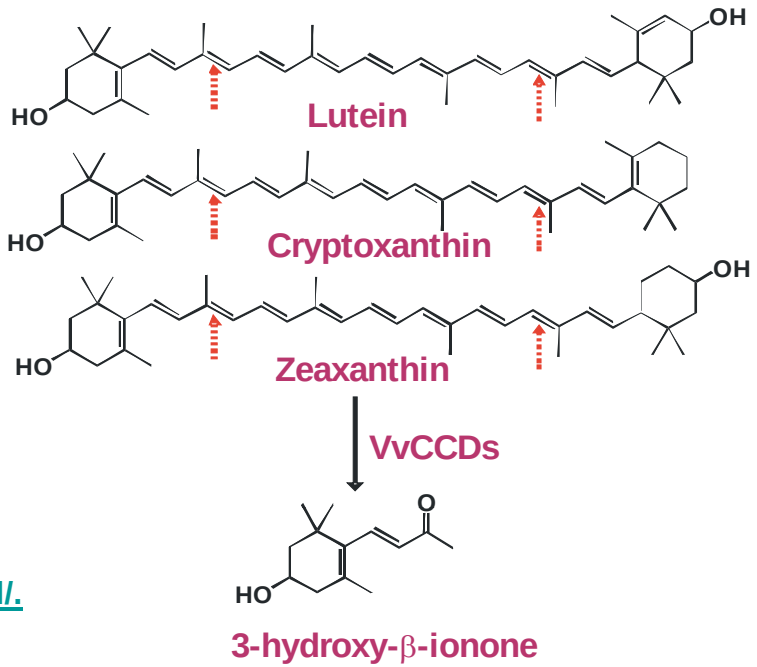
Janvary et al, Agric Food Chemistry (2009) 57: 3512-3518

How are floral norisoprenoids made?

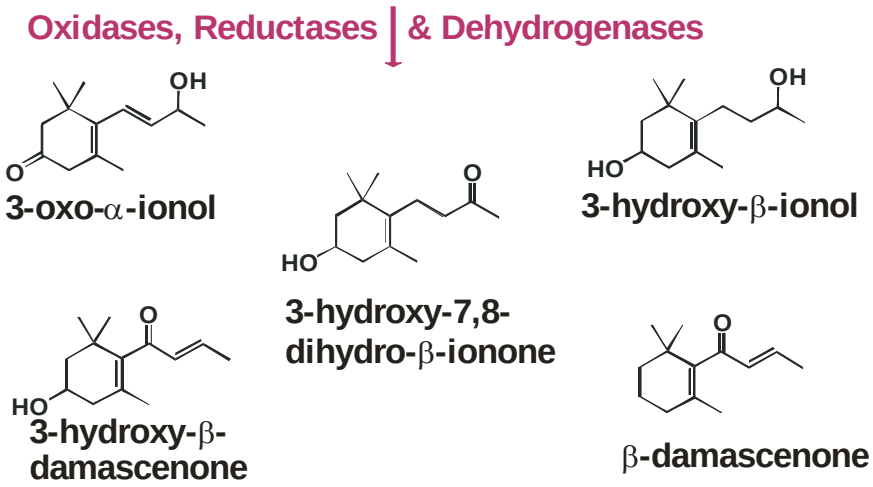
Journal of Experimental Botany, Vol. 56, No. 420, pp. 2721–2731, October 2005



www.vcbio.science.ru.nl/../applets/chloroplast/

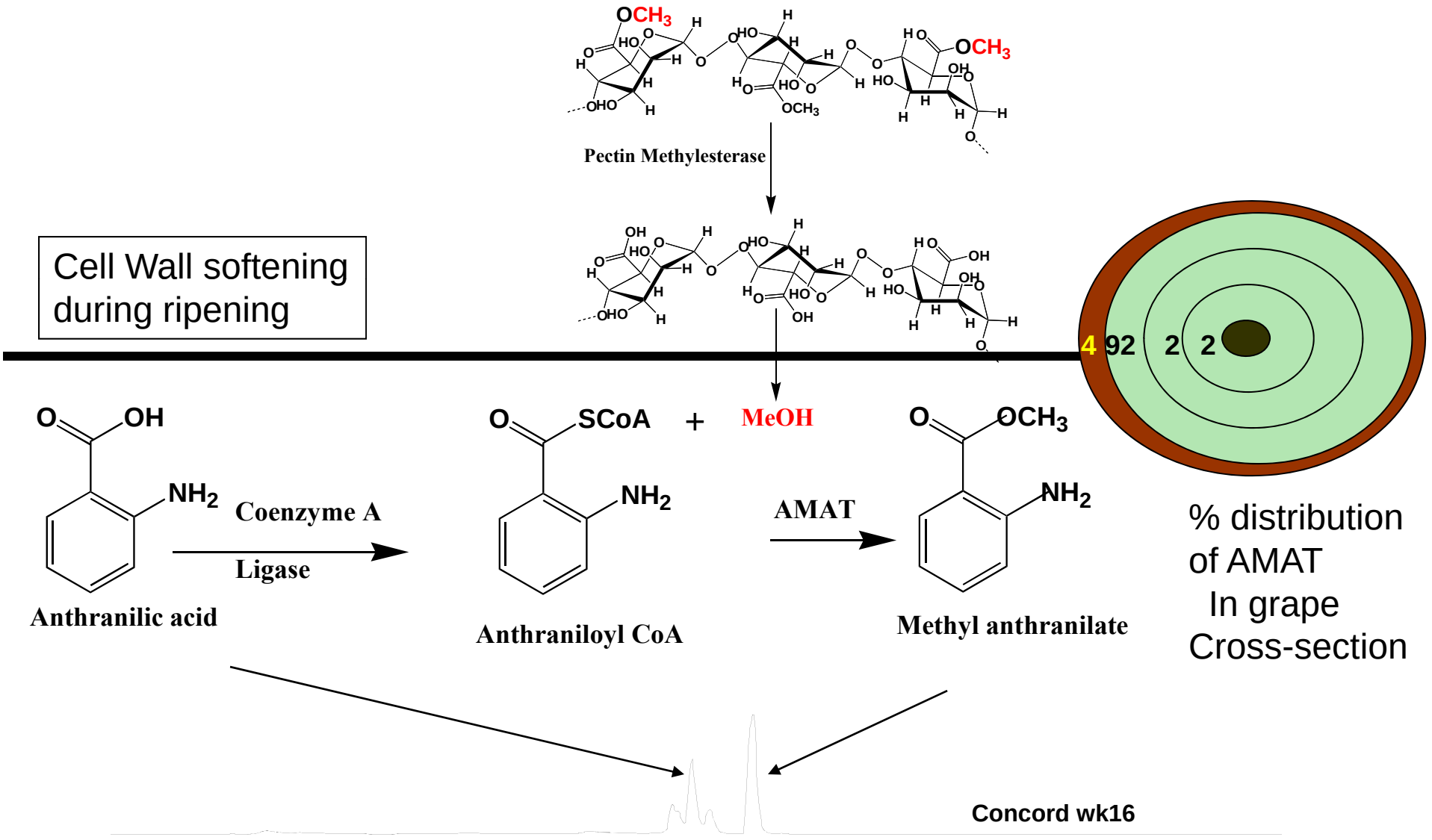


- **Grapes**
  - **Muscat grapes make more norisoprenoids than in Muscat of Alexandria grapes than in those of Shiraz**
  - **Single VvCCD gene appears to be involved**
  - **VvCCD molecular marker can be used for selection purposes in breeding program to enhance the norisoprenoid profiles of wine grapes**

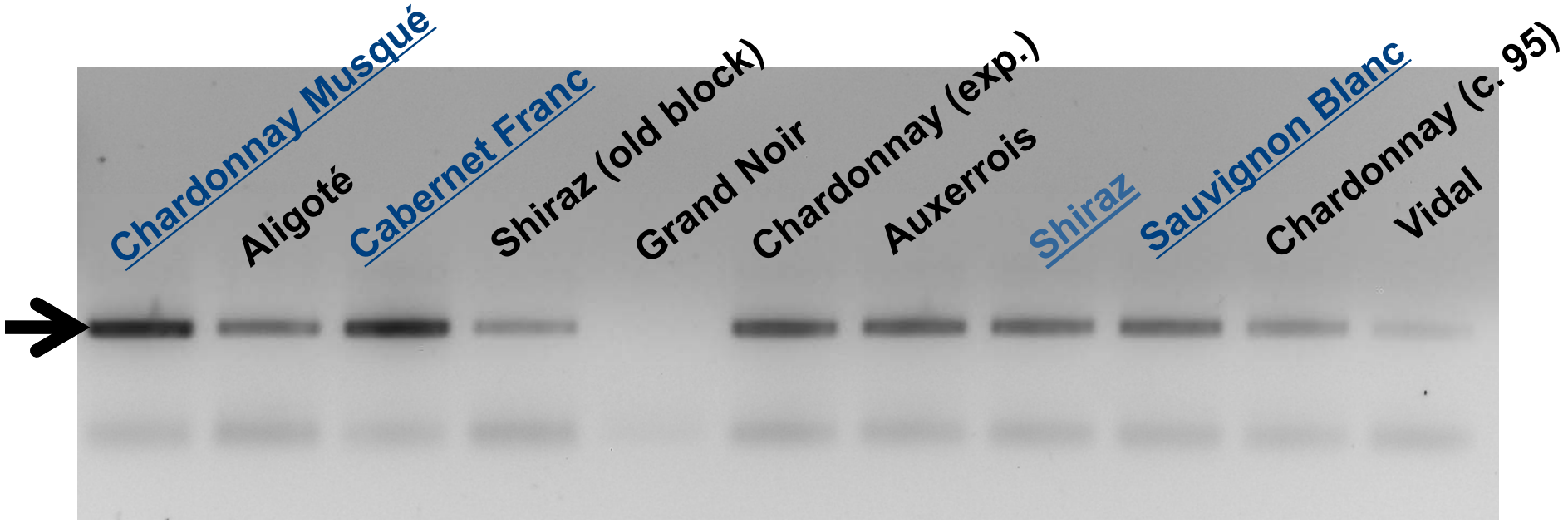


How are floral volatile acylated flavor compounds made made?

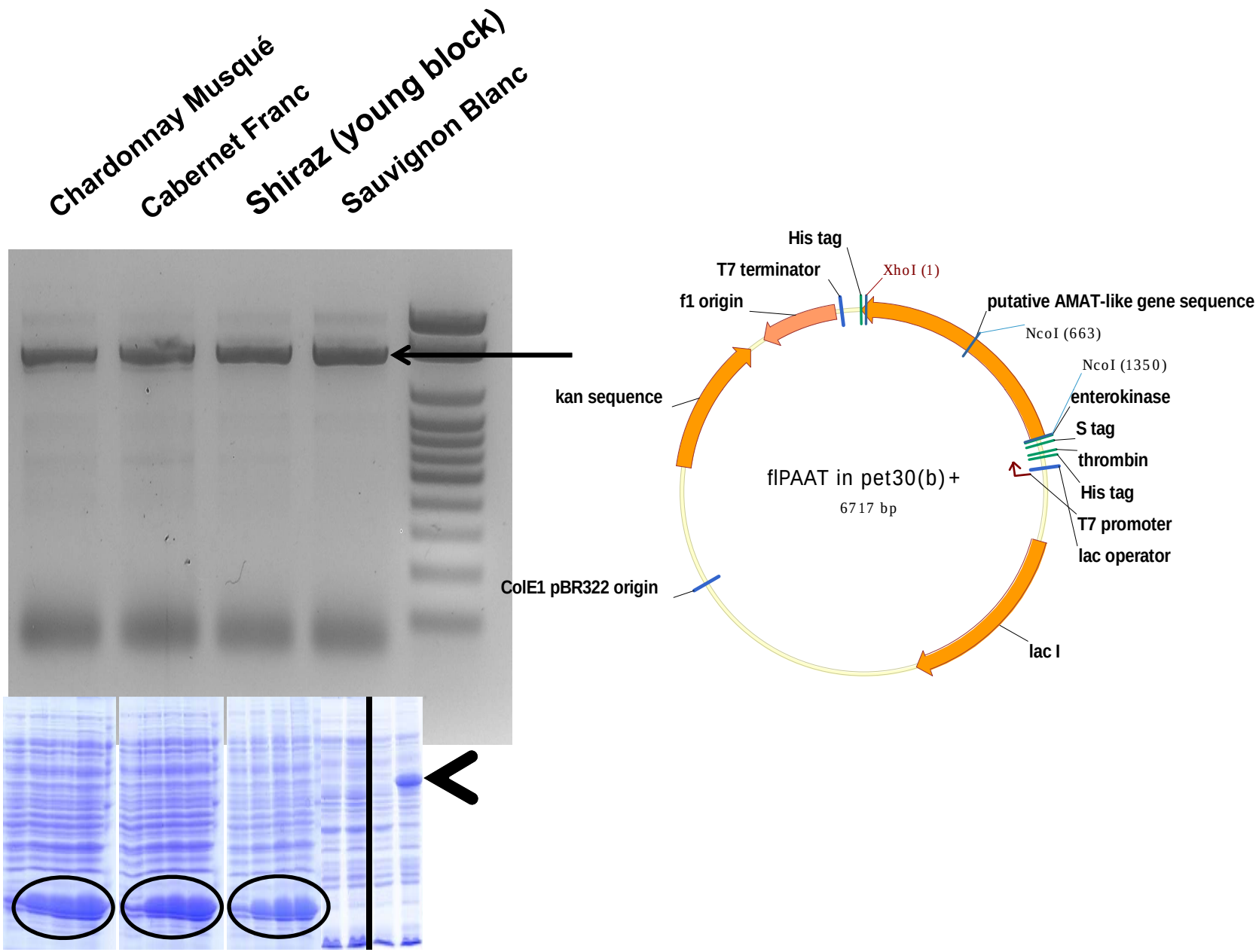
Example: Grapes make methyl anthranilate from pectin derived methanol and the CoA ester of anthranilate Plant J (2005) 44, 606–619



European Grape cultivars have almost identical transcripts to those of AMAT of Concord Grape?



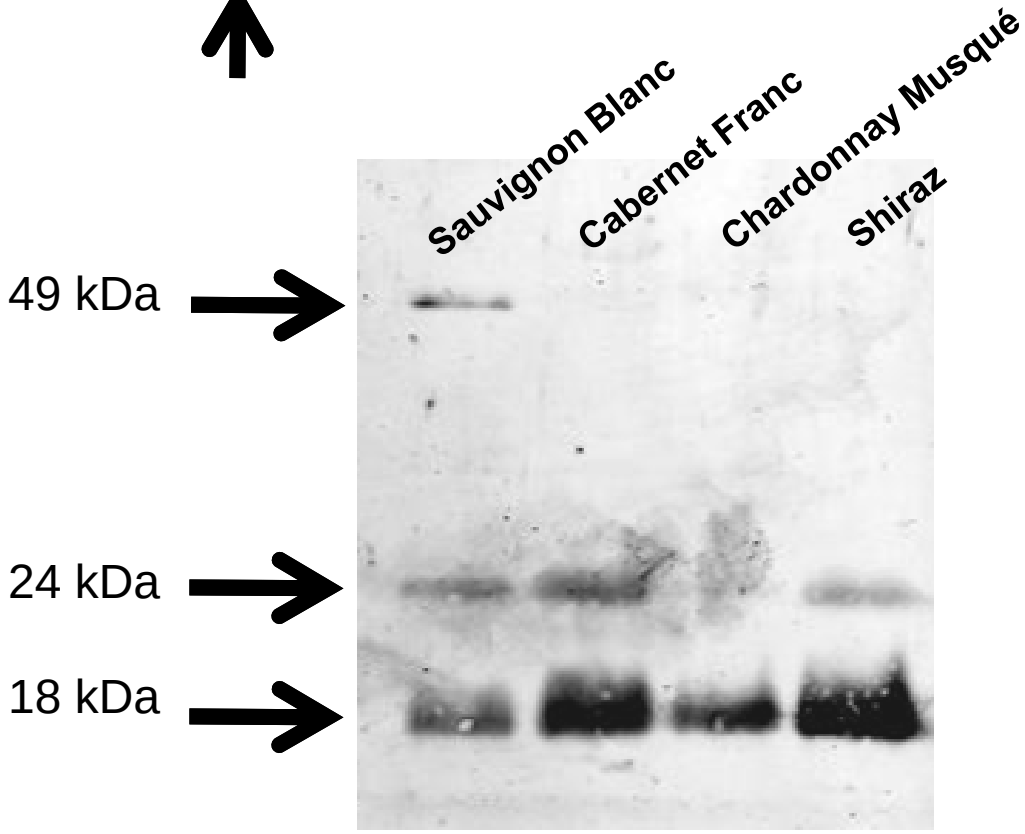
Different varieties of mature *Vitis vinifera* grapes contain AMAT-like acyltransferase (pAAT) transcripts. The arrow denotes positives: a 331 base pair product amplified with primers designed against the C-terminal region of the pAAT. Varieties in **blue** font were selected for cloning of a full-length cDNA gene.


Chardonnay Musqué
Cabernet Franc
Shiraz (young block)
Sauvignon Blanc
His tag
T7 terminator
XhoI (1)
f1 origin
putative AMAT-like gene sequence
NcoI (663)
NcoI (1350)
kan sequence
enterokinase
S tag
thrombin
flPAAT in pet30(b)+
6717 bp
His tag
T7 promoter
lac operator
ColE1 pBR322 origin
lac I

Grape varieties produce slightly different transcripts responsible for the short truncated proteins produced in Cabernet franc, Chardonnay Musque and Shiraz cultivars.

```
                          (410) 410        420        430        440        450        460        470
                AMAT      (410) CCACGACAATTCTCGGTTCCCCTCTTATCTTAATTCAGGTGACCCGATTGAGGTGCGGAGGATT
Sauvignon Blanc pAAT      (410) CCACGAGAATTCTTGGTTCCCCTCTTATCTTAATTCAGGTGACCCGATTGAGGTGCGGGGGATT
Cabernet Franc pAAT       (410) CCACGAGAATTCTTGGTTCCCCTCT-ATCTTAATTCAGGTGACCCGATTGAGGCGCGGGGGTT
Shiraz pAAT               (410) CCACGAGAATTCTTGGTTCCCCTCT-ATCTTAATTCAGGTGACCCGATTGAGGCGCGGGGGTT
Chardonnay Musque pAAT    (410) CCACGAGAATTCTTGGTTCCCCTCT-ATCTTAATTCAGGTGACCCGATTGAGGCGCGGGGGTT
```

- Sauvignon blanc grapes make a protein of the appropriate size & a truncated protein as well?
- Other cultivars only accumulate truncated protein forms.
- Do truncated proteins have other biological activities?
- What is the biochemical role of Sauvignon blanc acyltransferase?

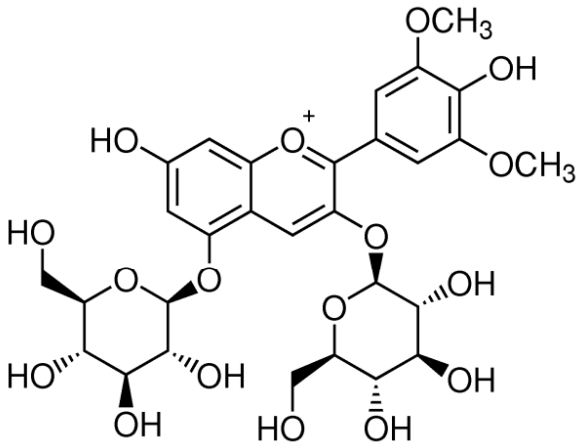


Summary of Vitis vinifera AMAT-Like protein

- One full-length functionally active form of acyltransferase exists
- One mutated form that produces a truncated protein exists
- Scan all commercial grape varieties for presence of the active form during grape ripening.
  - Documentation of genes expressed vs useful wine metabolites produced
  - Fingerprint of the genetic make up of the cultivar
- Use as a marker for introduction of the trait in other vinifera cultivars
  - Breeding vs genetic engineering

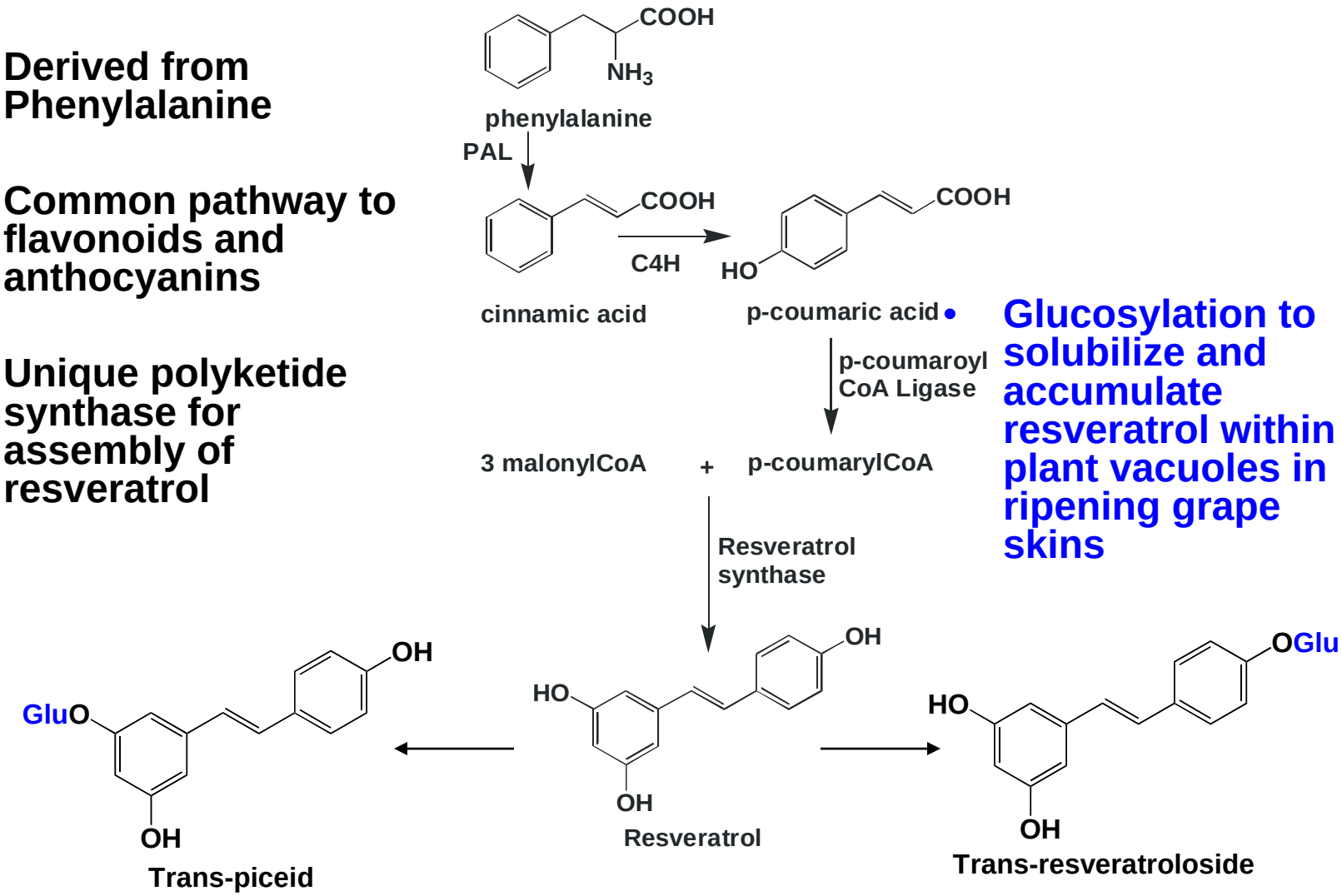
Grape Glucosyltransferases

- Grapes have hundreds of GTs
- Their roles are to increase the solubility and stability of secondary metabolites such as anthocyanins
- Glycosides may be transported or stored in vacuoles
- Glycosides may also ‘activate’ the molecule for further reactions and biosynthesis



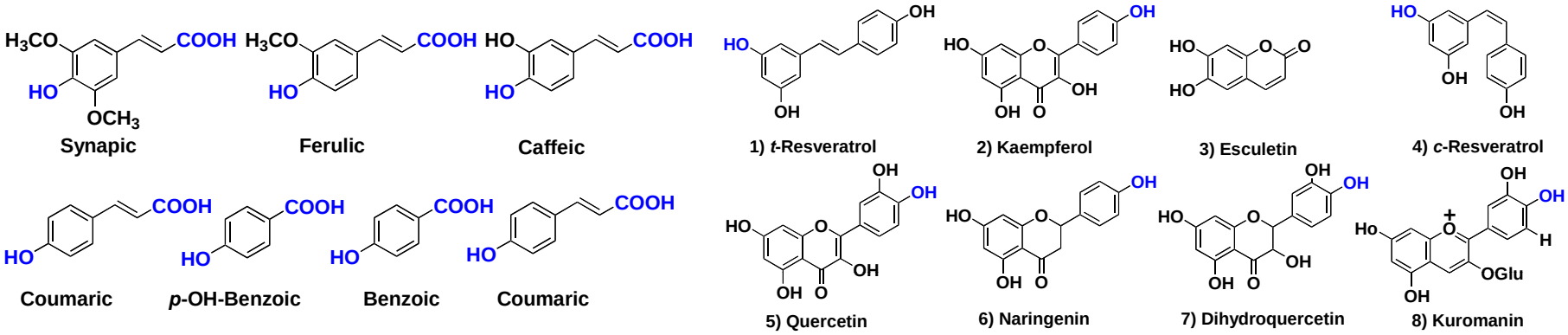
Grapes accumulate resveratrol in the form of Glucosides

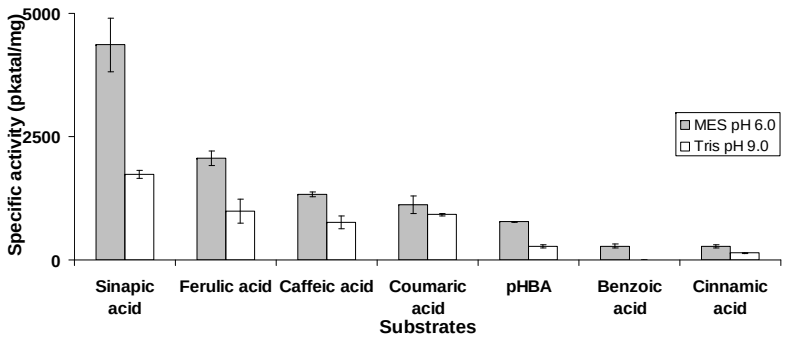
- **Derived from Phenylalanine**
- **Common pathway to flavonoids and anthocyanins**
- **Unique polyketide synthase for assembly of resveratrol**

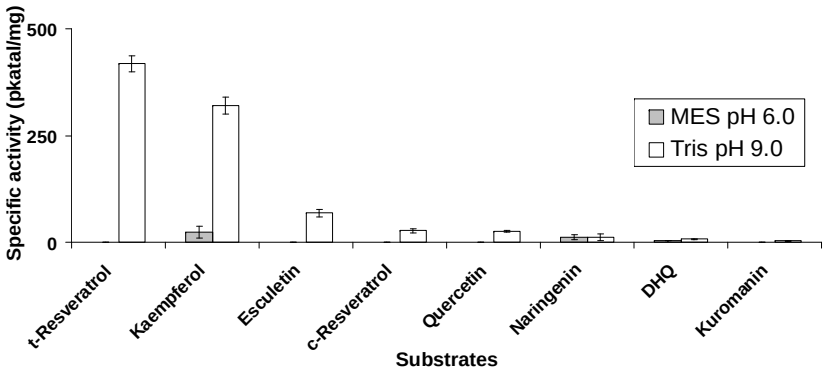


Glucosylation to solubilize and accumulate resveratrol within plant vacuoles in ripening grape skins

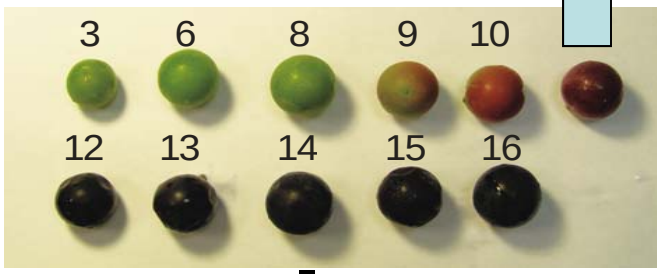
Bifunctional rVLRSgt of Concord grape



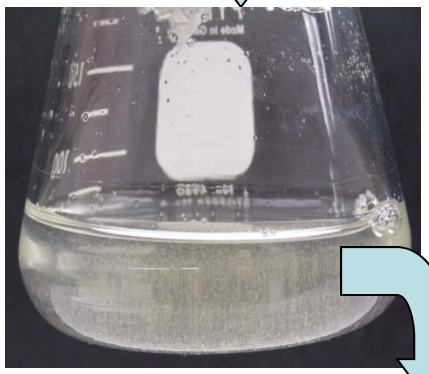




Biotechnological production of substituted resveratrols

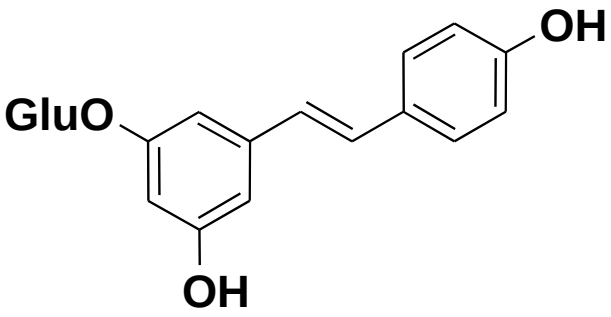


Grape callus & cell suspension cultures

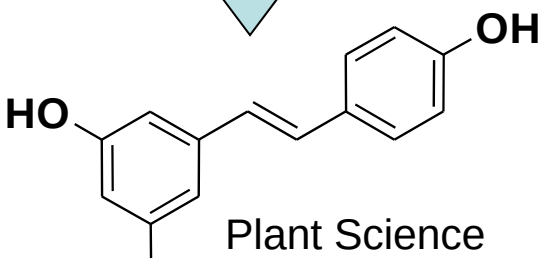


Alanine triggers resveratrol biosynthesis & accumulation

Cloning of functional Resveratrol Glucosyltransferase & transfer of functional gene to bacteria. Plant J (2006) **49:** 579–591



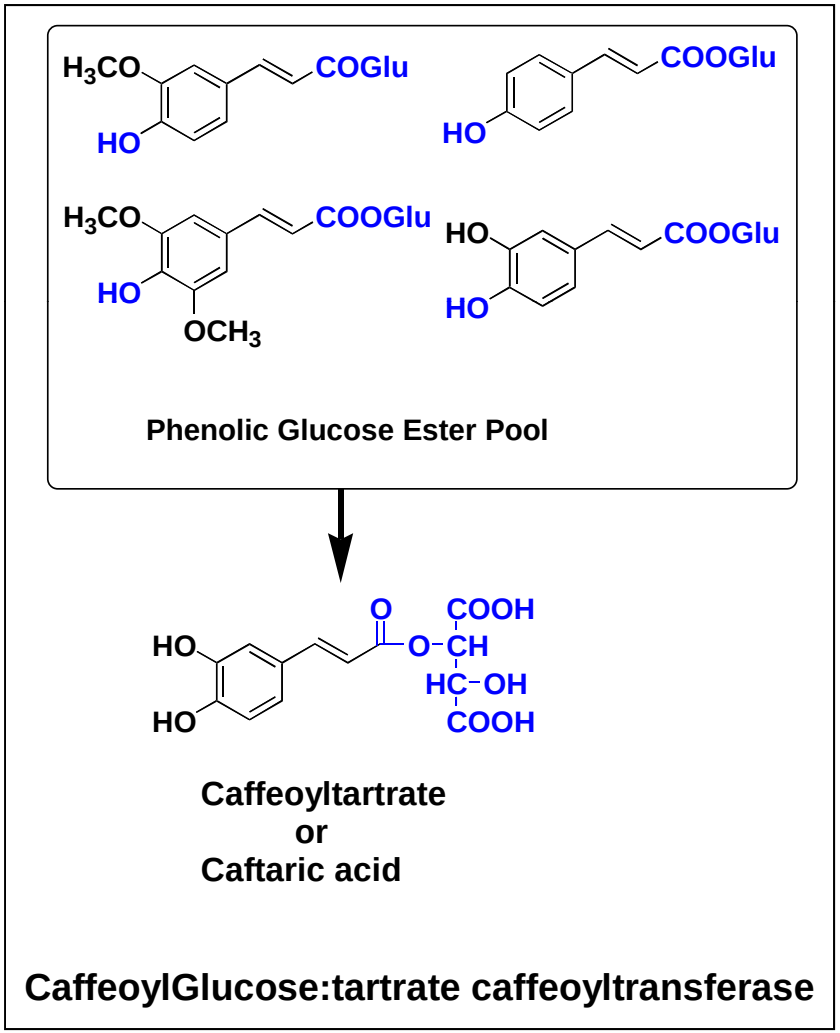
Bacteria expressing Resveratrol GT



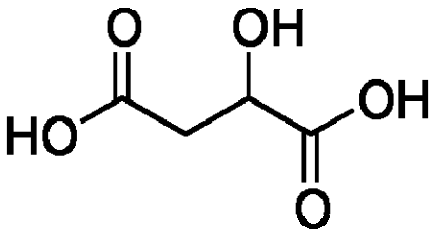
Plant Science (2006) **171:** 734–744

Collaboration with Christophe Clement, Eric Courot, David Donnez, Philipe Jeandet

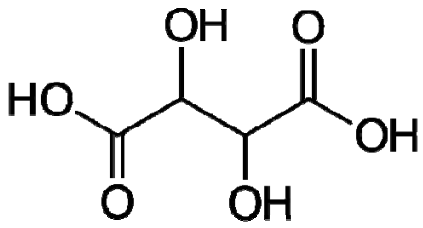
Involvement of VLRSgt in biosynthesis glucose esters and biological role in biosynthesis of caffeoyl tartrate



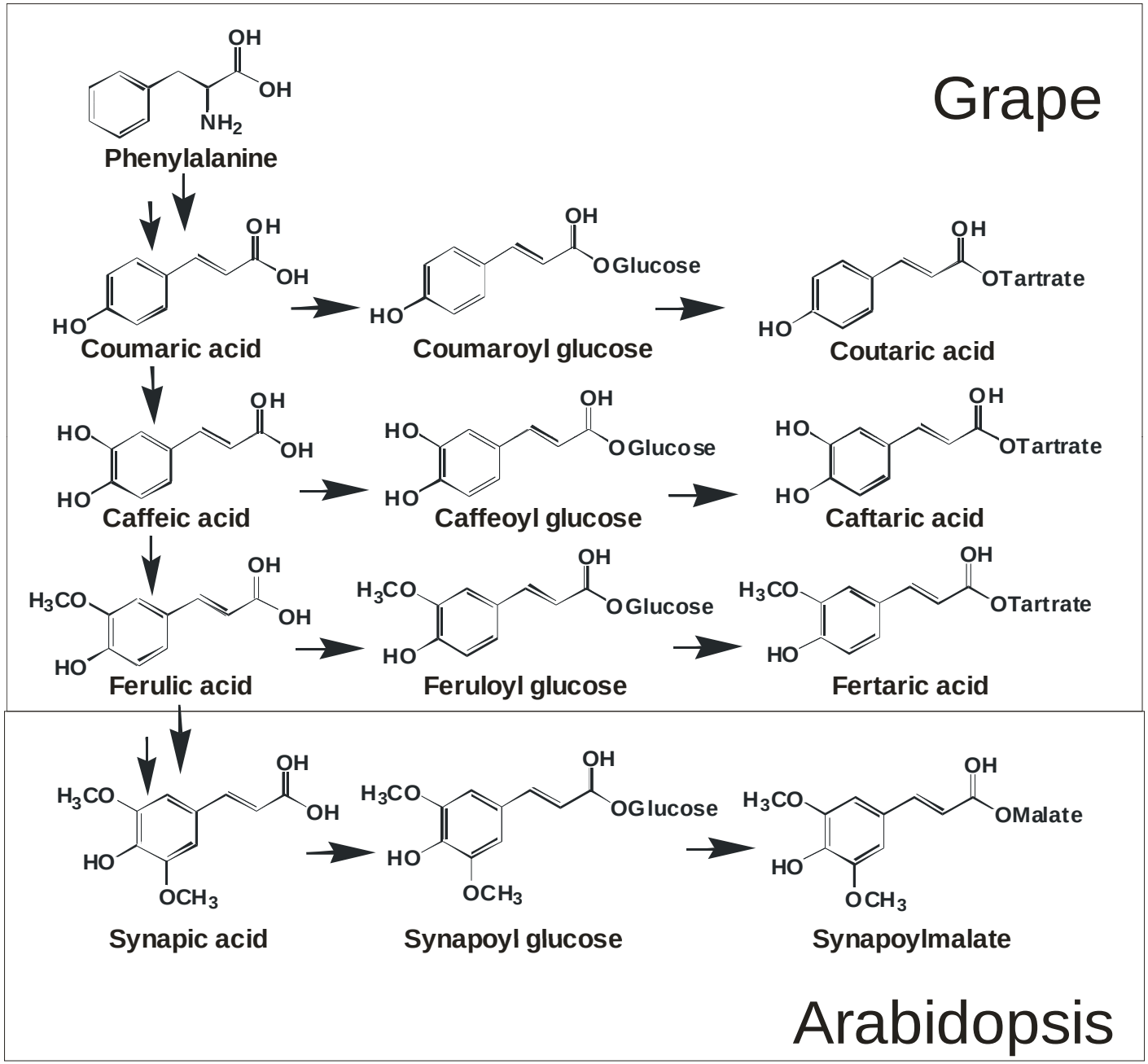
- **Caftaric acid**
  - **Major phenol in wine**
  - **Involved in browning reaction**
  - **Reacts with glutathione to produce S-glutathionyl caftaric acid**
  - **Quantities in wine >30mg/l**



Malic



Tartaric acid


Grape
Phenylalanine
Coumaric acid
Coumaroyl glucose
Coutaric acid
Caffeic acid
Caffeoyl glucose
Caftaric acid
Ferulic acid
Feruloyl glucose
Fertaric acid
Synapic acid
Synapoyl glucose
Synapoylmalate
Arabidopsis

Characterization of the Nutraceutical Value of (VFL) Grape pommace sourced powder

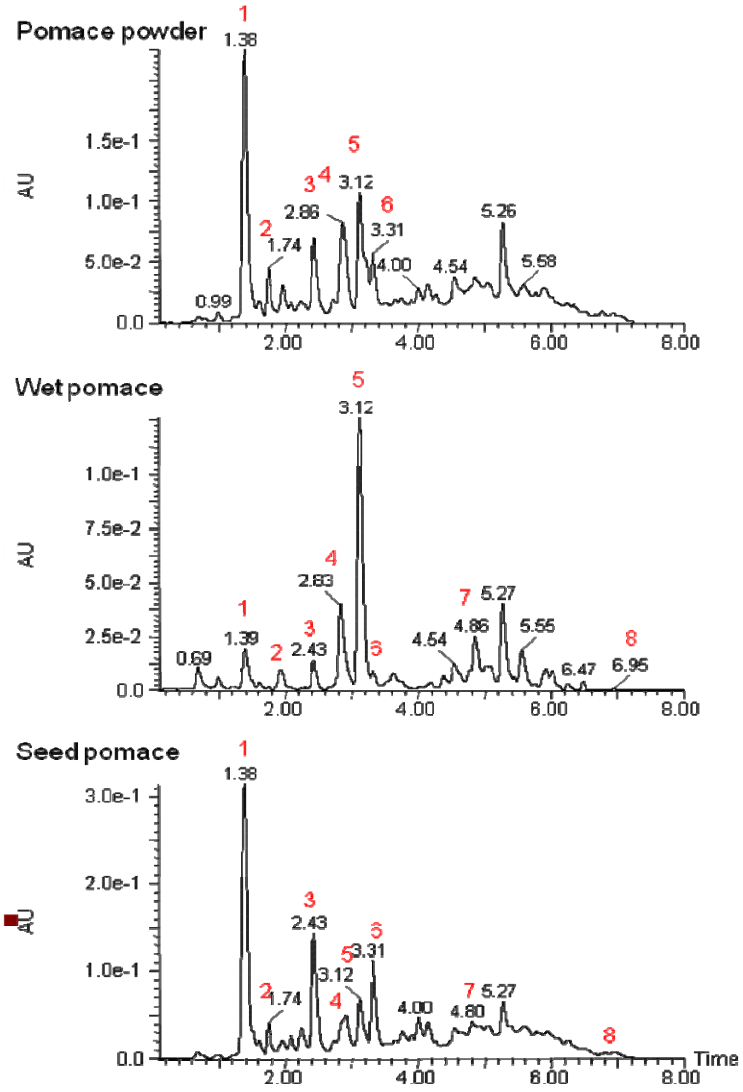
- Identifying antioxidant activity and other health benefits –Polyphenols in this powder
  - Flavonoids
  - Anthocyanins
  - Proanthocyanidins
  - Catechins
  - Triterpenes (Saponins)

Free and Bound secondary metabolites.

UPLC MS facility for metabolite analysis

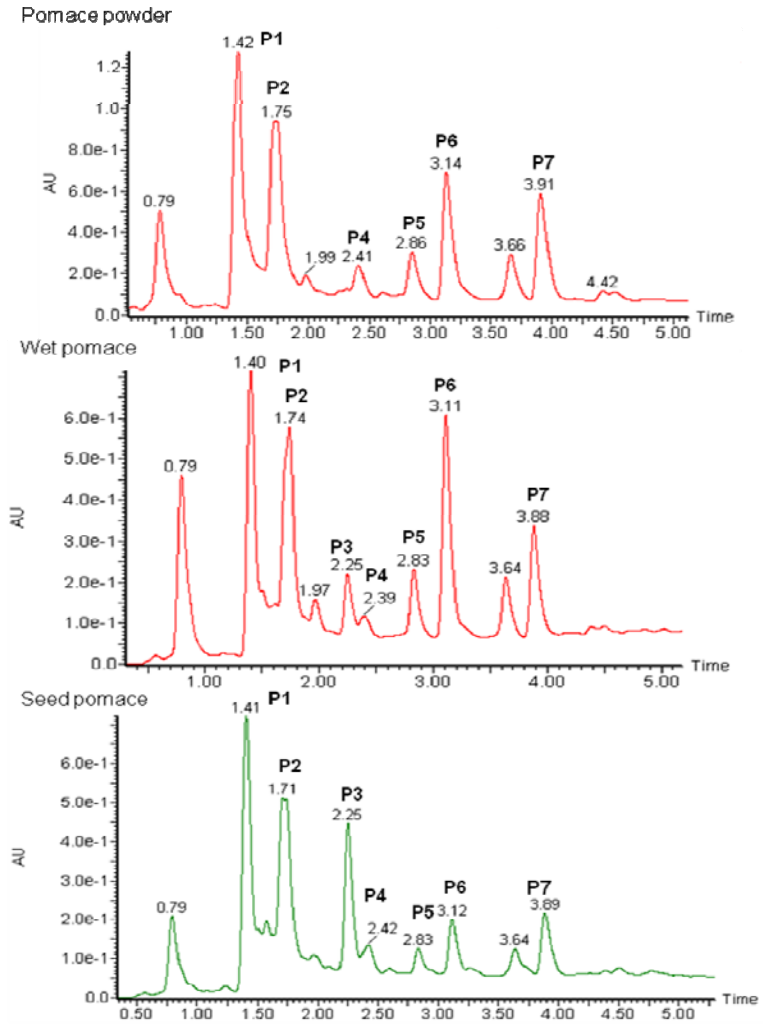
- Screening capabilities based on UPLC system from Waters.
  - Traditional HPLC Systems normally take 30 to 45 min to run a sample **(32 to 48/24 hr)**
  - UPLC has been standardized to run 7 min per sample **(205/24hr)**
  - If a particular peak is desired, the run can be decreased to 1min **(1440/24 hr)**
  - Very short run times suggest system can be used for mutant screens
  - Program can be produced to automatically select promising candidates

UPLC chromatogram of SOLUBLE phenolic acids in pomace powder, wet pomace and seed pomace at 280 nm



1) gallic acid
2) protocatechuic acid
3) Catechin
4) procyanidin B
5) procyanidin B
6) Epicatechin
7) quercetin glucuronide
8) quercetin.

UPLC chromatogram of BOUND phenolic acids in pomace powder, wet pomace and seed pomace at 280 nm

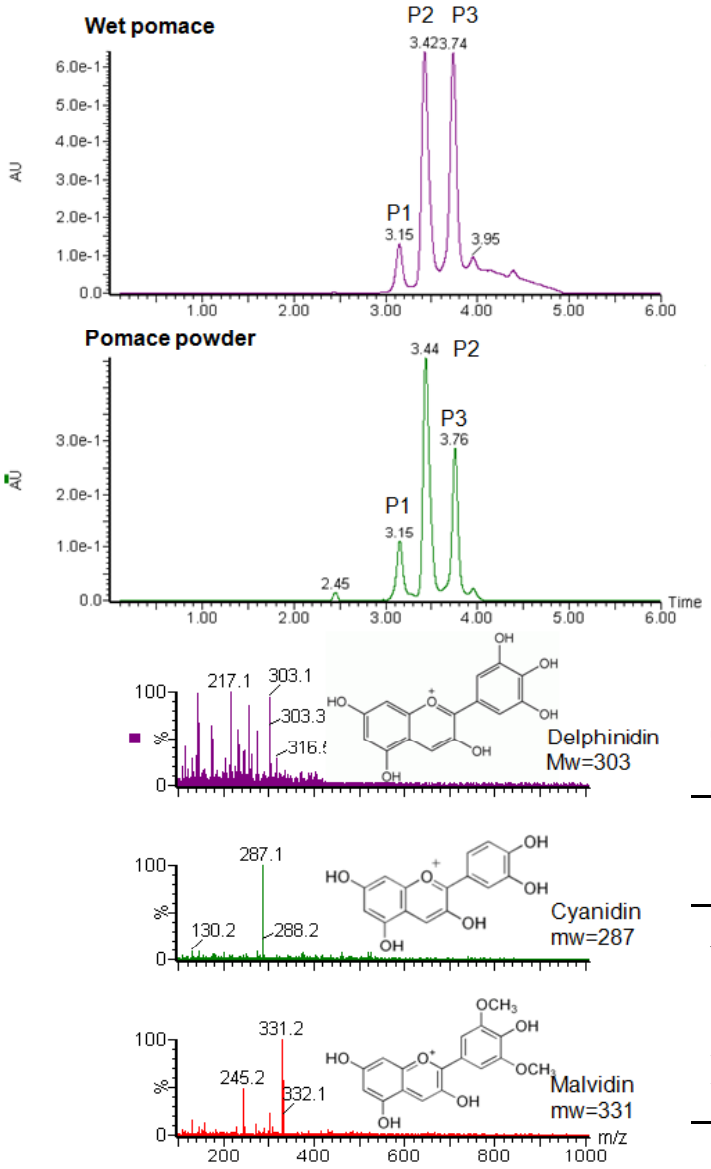


P1 gallic acid
P2 protocatechuic acid
P3 *ρ*-hydroxybenzoic acid
P4 gentisic acid
P5 caffeic acid
P6 (-)-epicatechin
P7 *ρ*-coumaric acid

Table 1. Concentration of major phenolic acids in grape pomaces (µg/g)

Phenolic acids	Methanol extract			Residue		
	Powder	Wet pomace	Seed	Powder	Wet pomace	Seed
Gallic acid	260	12	511	3398	990	874
Catechin	111	23	237	-	-	-
Epicatechin	280	412	166	1132	856	232
Caffeic acid	-	-	-	111	80	31

*** ( ) has been calculated as a percentage, %.**



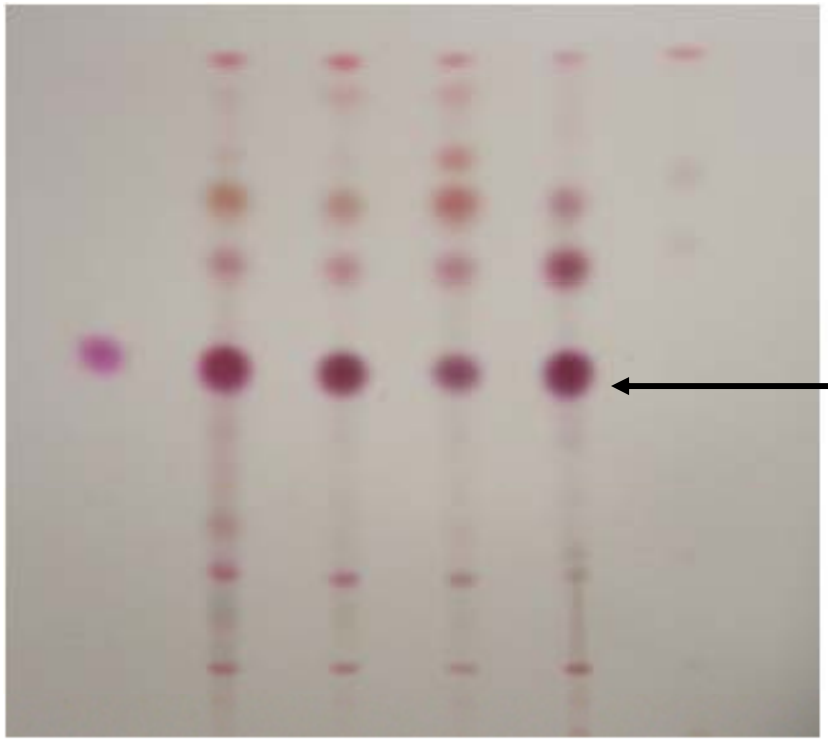
- **Anthocyanins in wet pomace and pomace powder**
  - **P1) dephinidin**
  - **P2) cyanidin**
  - **P3) malvidin**

Table: [major anthocyanins] in grape pomaces (µg/g)

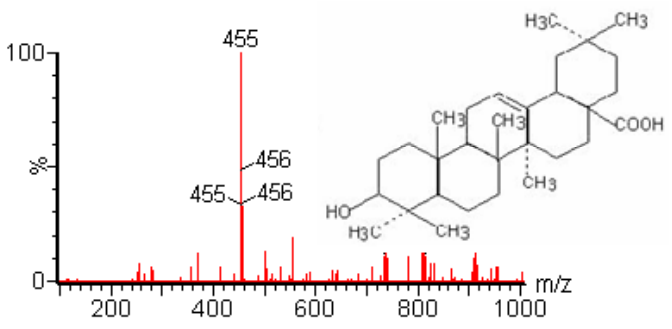
	Delphinidin	Cyanidin	Malvidin
Wet pomace	**35**	**172**	**388**
Pomace powder	**32**	**117**	**165**

* ( ) has been calculated as a percentage, %.

Triterpenes in grape pomace power



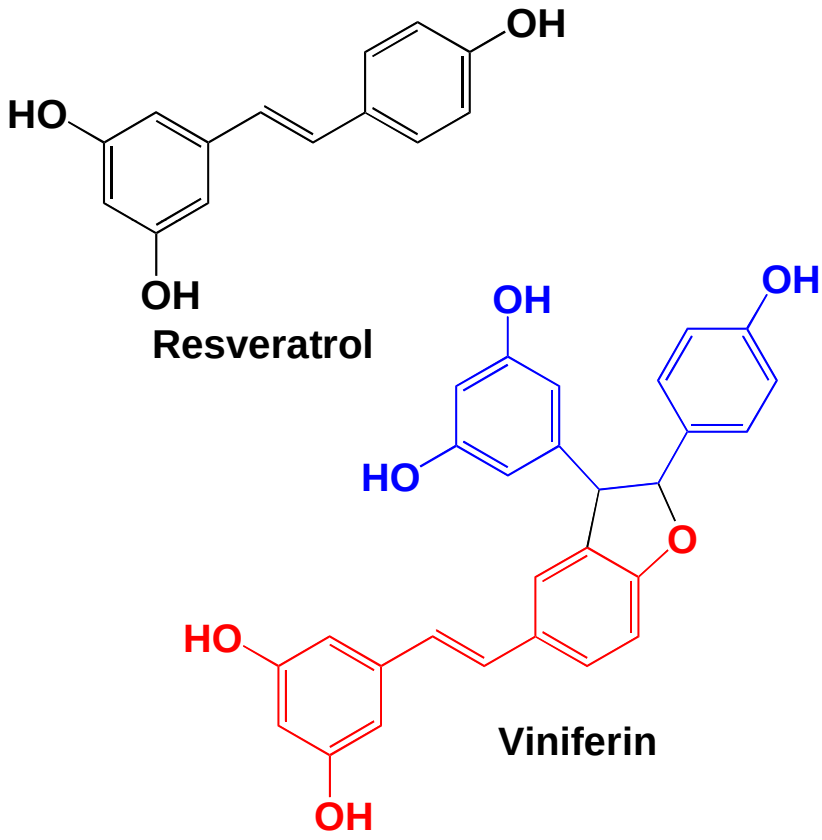
(D) Dry pomace
(W) Wet pomace
(S) grape seed
(SK) fresh grape skin
(SC) seed coat



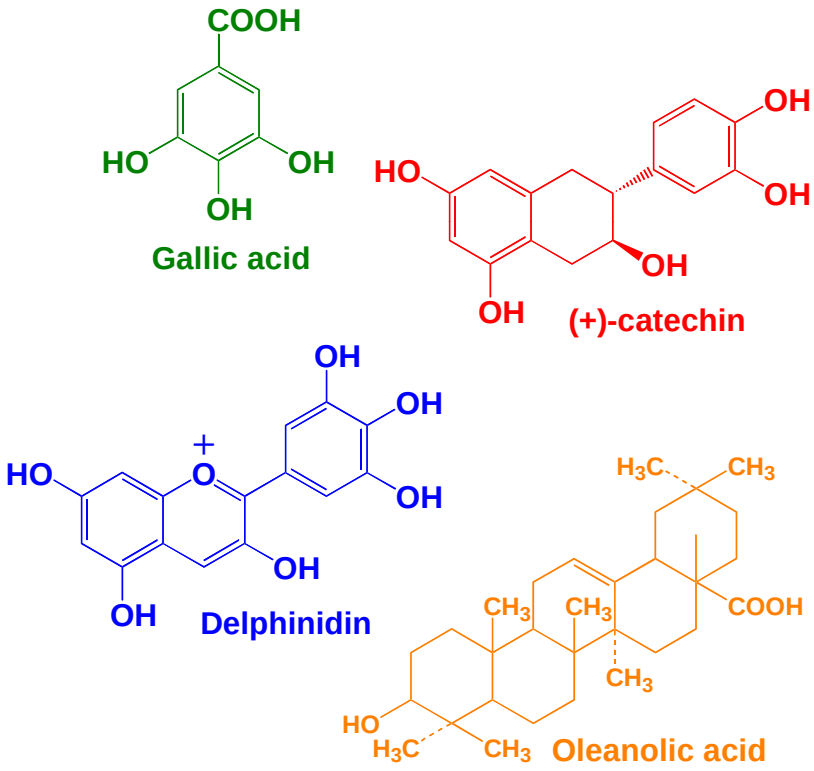
Oleanolic acid mw=456

Summary of grape pomace metabolites identified by UPLC-ESI-MS.

- Analyses of different grape pomaces:
  - Little or No resveratrol found in wet or dry pomace.
  - We have identified pomace source with significant levels of resveratrol & viniferin can be found



Summary of grape pomace metabolites identified by UPLC-ESI-MS.



- Main Phenolic acid: Gallic acid
- Procyanidin (+-catechin)
- dephinidin, cyanidin and malvidin as major anthocyanins.
- Wet pomace contained higher concentration of anthocyanins than powder and seed pomace
- High content of oleanolic acid

Summary: Future Prospects

- The biochemistry and molecular biology of wine chemistry is rapidly being characterized
  - Anthocyanins
  - Resveratrol
  - AMAT-like aroma compounds
  - C-13 norisoprenoids
  - Phenolics
  - Terpenes
- Tools will be used to:
  - Fingerprint differences between cultivated wine grapes
    - Marketing and commecial protection of cultivars
    - Identity preservation
  - So far documented differences appear to be small involving single or double gene mutations
  - Breeding programs (Classical genetics/Genetic Engineering)to improve
    - Color, Flavor and Aroma
    - Stress tolerance of grapes (Cold, Drought, Disease, Herbivore)
  - Grape Pomace analysis and potential uses as value added sources of anti-oxidants
    - Improved marketing of byproducts for incorporation into foods
    - New health claims
    - Identity preservation of certain pomace sources