

#### Introduction of research activities



#### Laboratory focus:

- Medicinal plants and their biosynthetic pathways
  - Madagascar periwinkle (anticancer agents, vinblastine and vincristine)
  - Genome Canada funded project
- Grape funded Projects
  - Vinifera For Life
     Phytochemical analysis of grape pomace powders
  - Appasimento
    - Phytochemical analyses of appasimento grapes when submitted to different drying techniques
  - Sweet and Sticky
    - Phytochemical analyses of icewine grapes from late summer to winter harvest
  - Acyltransferases and Aroma production

### Genome Canada Funded October 2009-September 2013



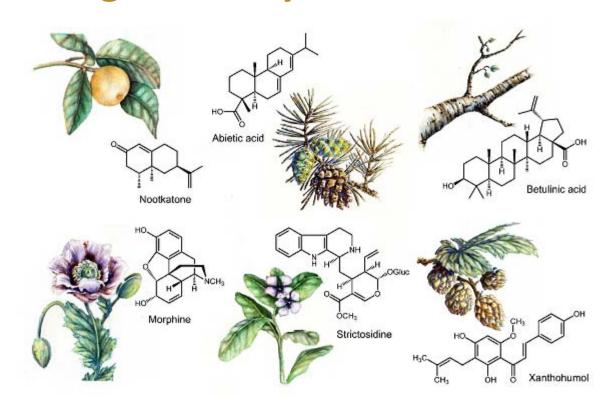
Large scale metabolomic and sequencing analysis of 75 target medicinal plant species

- University of British Columbia
  - University of Calgary
    - NRC, Saskatoon
  - University of Toronto
  - Concordia University
    - Brock University
- PDF Dr. Sayaka Atsumi Masasda
- PhD Candidates: Dylan Levac, Vonny Salim

### Genome Canada Funded October 2009-September 2013

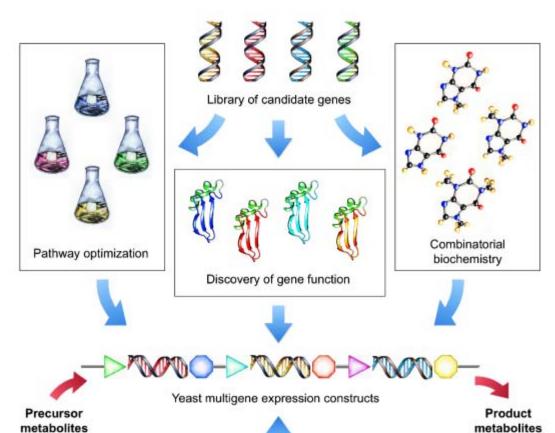


#### Saving humanity with small molecules



**Trends in Biotechnology (2012)** 

#### High throughput sequencing



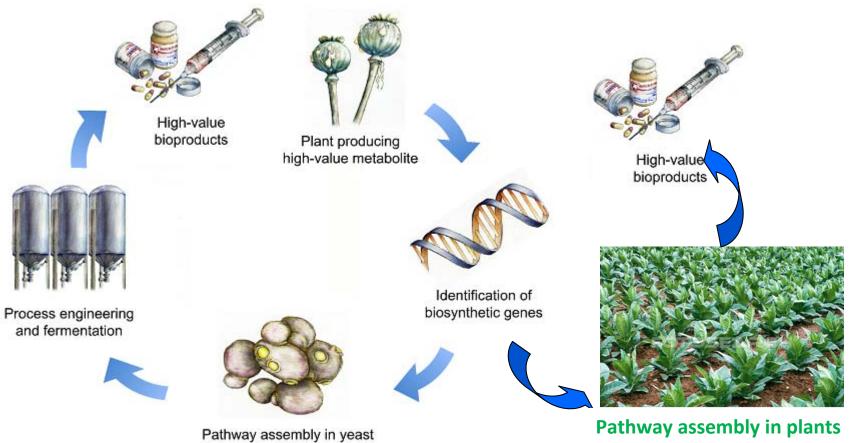
Library of gene expression control elements



**Trends in Biotechnology (2012)** 

#### A sustainable future





**Trends in Biotechnology (2012)** 

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     Phytochemical analysis of grape pomace powders
  - Appasimento (Dr Kyung Hee Kim)
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  - Acyltransferases and Aroma production (MSc Brent Wiens)

## Grape pomace contains numerous phenolics



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

| Methanol<br>extract |        |            | Residue |        |            |      |
|---------------------|--------|------------|---------|--------|------------|------|
| Phenolic acids      | 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   |

<sup>\* ( )</sup> has been calculated as a percentage, %.

## Wet and dry grape pomace contain anthocyanins



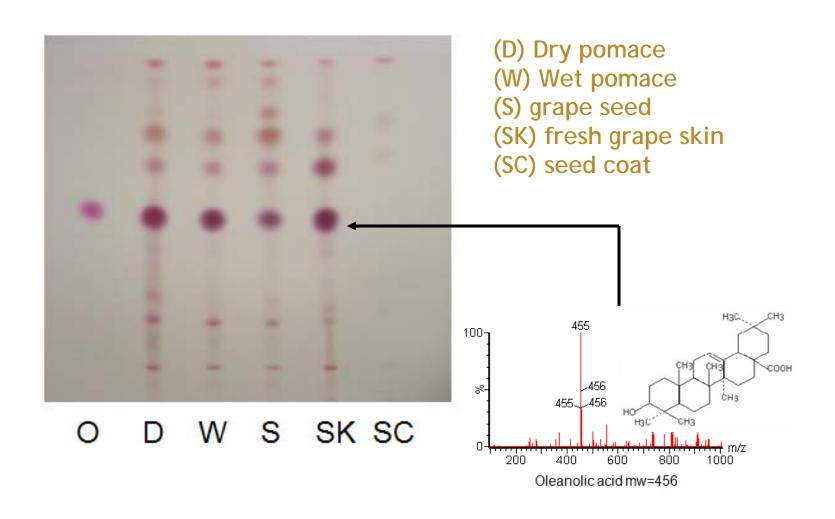
Table: [major anthocyanins] in grape pomaces (µg/g)

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

 $<sup>\</sup>ensuremath{^*}$  ( ) has been calculated as a percentage, % .

# Wet and dry grape pomace contains high levels of triterpenes like oleanolic acid





### Wet and dry grape pomace contains no resveratrol or viniferin



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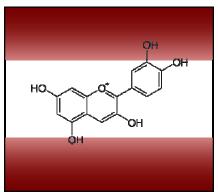
### Living tissues are involved in biosynthesis as they mature.

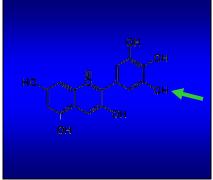


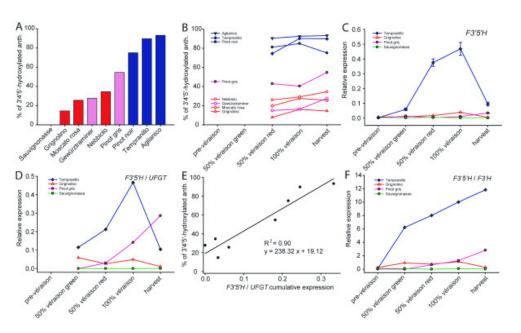


Castellarin & DeGasparo BMC Plant Biol (2007) 7: 46.

- 1. Genotype-specific anthocyanin biosynthesis.
- 2. Variation based on differential expression of anthocyanin pathways.
- 3. Color shifts from red to blue with increased hydroxylation of anthocyanins



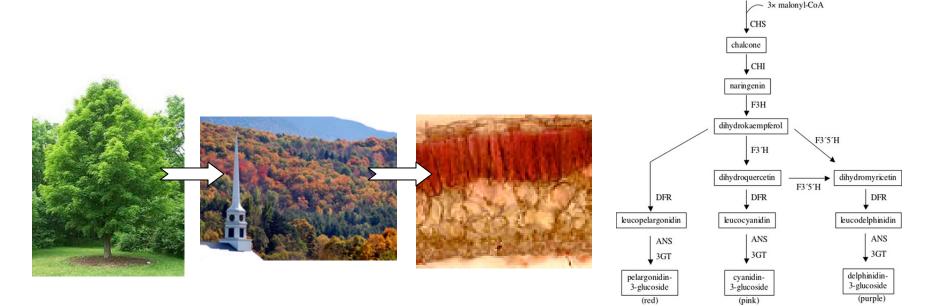




### Are living tissues involved in biosynthesis as they die? Yes



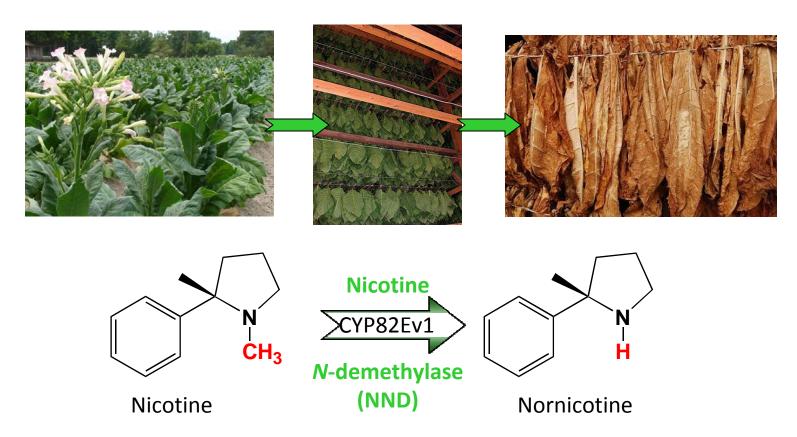
p-coumaroyl-CoA



- The arrival of autumn triggers activation of anthocyanin pigmentation in Maple
- Colors vary from yellow to orange to red
- Due to activation of flavonoid and anthocyanin biosynthesis
- Accumulation of pigments in palisade mesophyll cells produce colors.
- Ecological value (light screen to prevent stress from photoxidative dammage)?

### Are living tissues involved in biosynthesis as they die? Yes

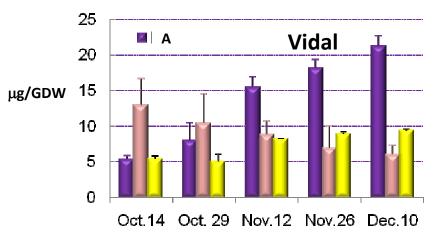


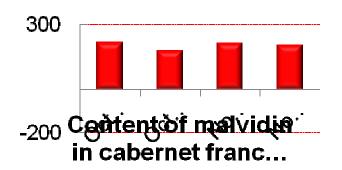


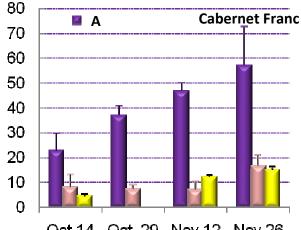
- NND activated during tobacco drying
- Ethylene mediated Induction of CYP82Ev1
- Nornicotine converted to nitrosamines and glycation byproducts

#### Identification of an key chemical marker for real vs 'fake' ice wine grapes (2010).







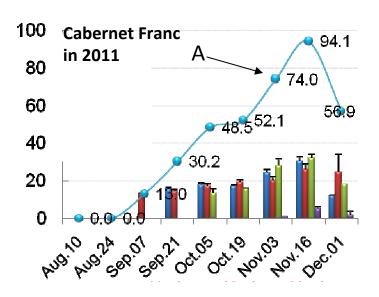


Oct.14 Oct. 29 Nov.12 Nov.26

- Metabolite A increases about 4-fold by December in Vidal grapes
- Metabolite A increases about 3-fold by the end of **November in Cab franc grapes**
- 3. Other metabolites do not change substantially (B, C and Malvidin).
- **Conclusion:** 
  - Mature grapes are biochemically active to make this metabolite in spite of the fact that they are dying (dead?)
  - Metabolite A is an important physiological marker for real vs 'fake' ice wine grapes.

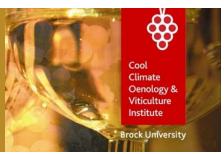
### Identification of an key chemical marker for real vs 'fake' ice wine grapes (2011).

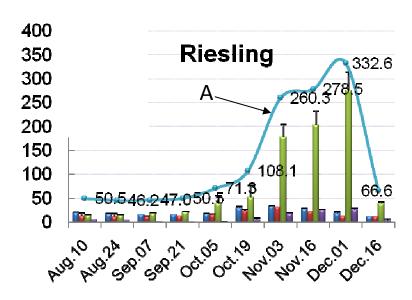




- 1. Experiments of 2010 were repeated in 2011 with Cabernet franc grapes.
- 2. Metabolite A increases to 94 μg/GDW by December in Cabernet franc grapes.
- 3. Conclusion:
  - Reproducibility of the results from 2010 confirms that Metabolite A can be used as a physiological marker for real vs 'fake' ice wine grapes.

### Identification of an key chemical marker for real vs 'fake' ice wine grapes (2011).





#### Present studies:

- •Expression analyses of pathway-specific genes during ice grape formation.
- Demonstration of functional pathway enzymes during ice grape formation.

- 1. Experiments of 2010 were repeated in 2011 with Reisling grapes.
- 2. Metabolite A increases about 10-fold by December in Reisling grapes!
- 3. Conclusion:
  - Mature grapes of <u>different</u>
     <u>varieties may all be</u> biochemically active to make this metabolite in spite of the fact that they are dying (dead?)
  - There appear to be varietyspecific differences in the amounts of Metabolite A that are made during the production of ice wine grapes.
  - Reproducibility of the results in another variety suggests that Metabolite A can be used as a physiological marker for real vs 'fake' ice wine grapes.

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#### Vitis labrusca vs. Vitis vinifera



| "Foxy", wines distasteful  | Non-"foxy", wine sales \$120B/yr  |
|--|---|
| Native to North America  | Native to Europe  |
| Used for juices, jams, jellies   | Used in winemaking  |
| Welch's SMUCKERS COUNTY OF THE PROPERTY OF THE | SHANA |

### Vitis labrusca (Concord) AMAT (VIAMAT) is responsible for "foxy" methyl anthranilate

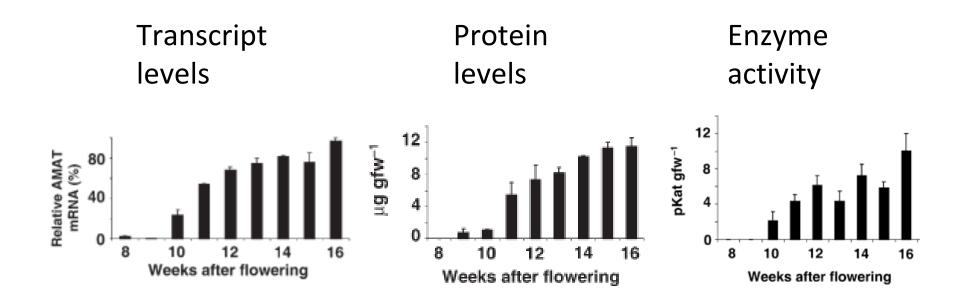


VIAMAT is a member of the BAHD family of plant acyltransferases

Wang & De Luca, 2004.

### VIAMAT transcript, protein levels, and activity increase throughout ripening



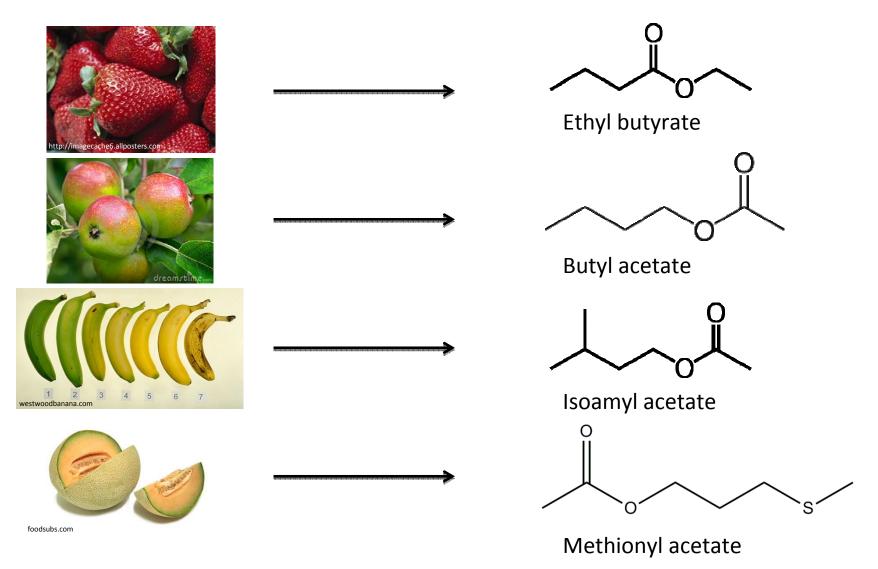


At the onset of ripening, methyl anthranilate starts to accumulate as well.



# Ripening-induced pattern is common with other fruit scent-related acyltransferases





#### Phylogeny of 88 biochemically characterized BAHD acyltransferases Climate Oenology & Viticulture **Brock University** ÔН Aromatic, polyamine, Alkaloid..., acylation Methyl anthranilate **Anthocyanin** HQ\_CO<sub>2</sub>H acylation HO" ŌΗ Phenylpropanoid ОН acylation Salutaridinol-7-0-

Alkaloid and benzoic acid acetylation

#### What about *V. vinifera*?



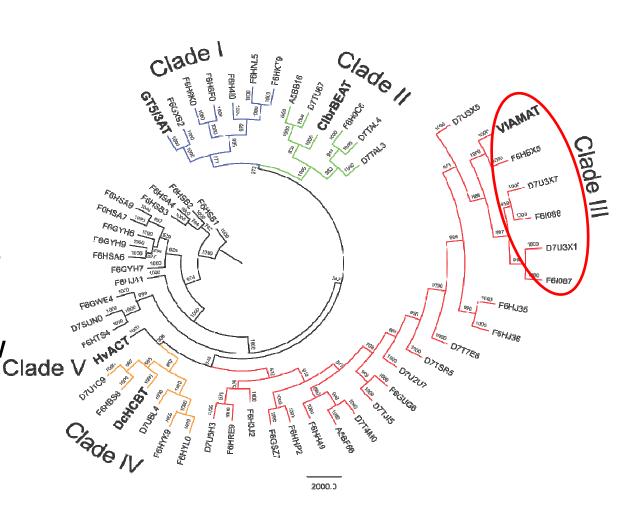
- BAHD acyltransferases in wine grape?
- Biological roles in determining characteristic sensory qualities?
- Practical uses in grape cultivation/breeding programs

### V. vinifera genome contains 5 highly similar VIAMAT-like genes



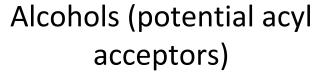
 Bioinformatic analysis shows at least 51 putative BAHD acyltransferases in *V. vinifera* genome (none yet characterized)

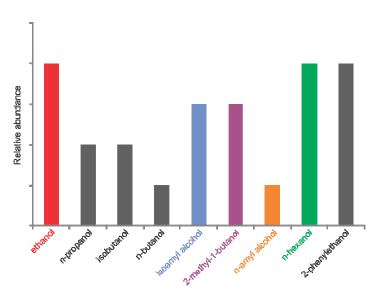
 5 candidates show 86 to 95% sequence identity to VIAMAT



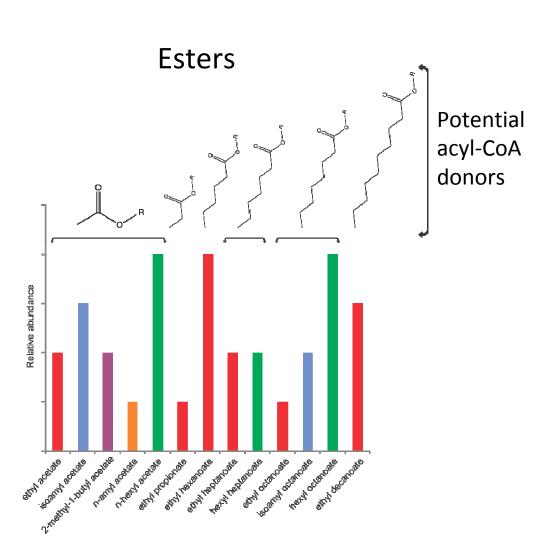
### The volatile alcohols & esters of mature Sauvignon Blanc berries?





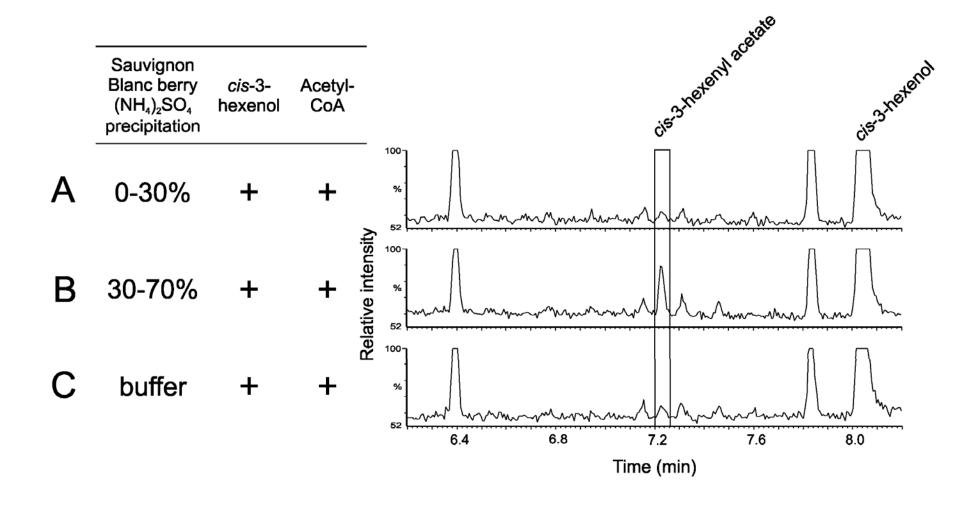


Data from Chaudhary et al., 1964



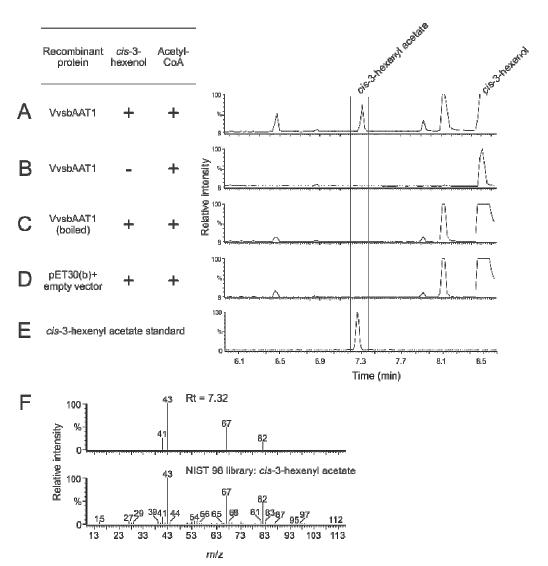
## Acetyl CoA: *cis*-3-hexenol acetyltransferase (CHAT) of Sauvignon blanc berries





## VvsbAAT1 is a CHAT rather than an AMAT!





#### Conclusions



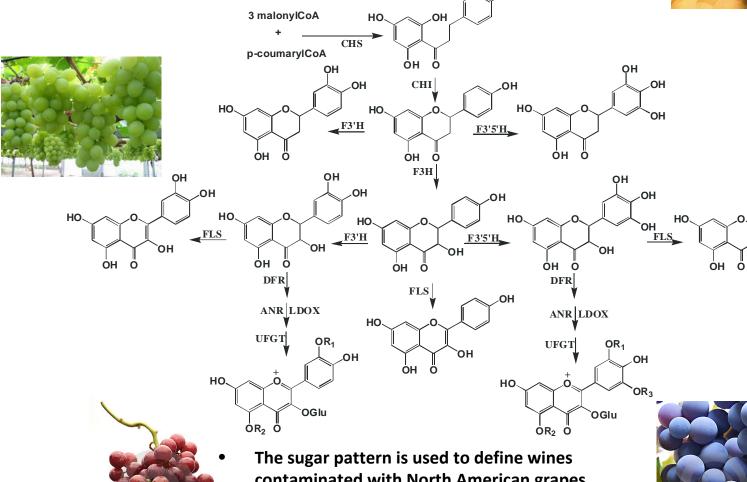
- Recombinant VvsbAAT1 catalyzes the formation of cis-3-hexenyl acetate.
- Compared with VIAMAT, VvsbAAT1 displays a completely different expression profile.
- These results suggest VvsbAAT1 may have a different biological role in V. vinifera compared with VIAMAT in V. labrusca.

#### Anthocyanin Biosynthesis

is the best known pathway of Secondary metabolism in plants



.OH



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

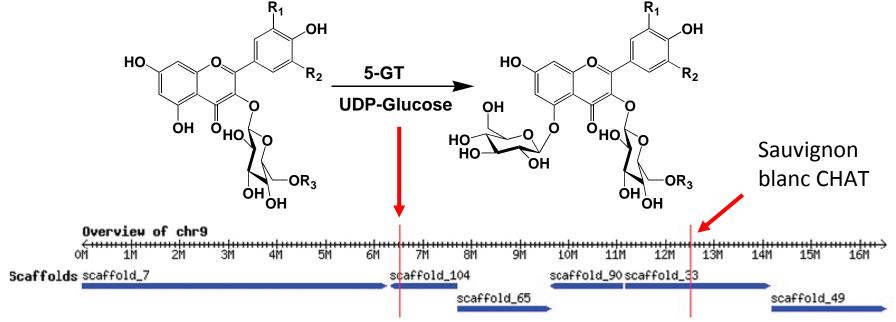


- The inability of most European grapevines to produce 3,5-di-O-glucosides has long been used to classify wines according to their varietal origin.
  - This study showed that V. vinifera has a 5GT gene with 2 mutations that render the protein inactive.
  - Correction of the 2 mutations reactivated the 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

#### Summary



#### Phytochemical analysis of grape pomace powders

### Phytochemical analyses of icewine grapes from late summer to winter harvest

Metabolite A can be used as a physiological marker for real vs 'fake' ice wine grapes.

### Acyltransferases and Aroma production (MSc Brent Wiens)

VvsbAAT1 catalyzes the formation of cis-3-hexenyl acetate



#### Acknowledgements

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Researchers:
Dr Kyung Hee Kim
Brent Wiens