

# Grape Breeding Program at Florida A&M University

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# RESEARCH ACTIVITIES

## Muscadine beneficial characteristics:

- Anticancer activity (African American Breast Cancer and African American Prostate Cancer);
- **Berry color and its relation to antioxidant activity;**
- Antimicrobial activity of muscadine grape against ripe rot fungus (*Colletotrichum* sp.);
- **Produce large berry seedless muscadine grapes for fresh consumption using CRISPR – technology;**
- Biochemical and molecular comparison of aroma profile spectrum in ripe muscadine and bunch grape berries;
- **Breeding new high quality Southern grape cultivars for meeting industry demands in Florida.**
- Identify molecular mechanisms underlying drought, salinity, and hypoxia tolerance in grapes.

# RESEARCH ACTIVITIES

## Berry Color & Antioxidant Activity



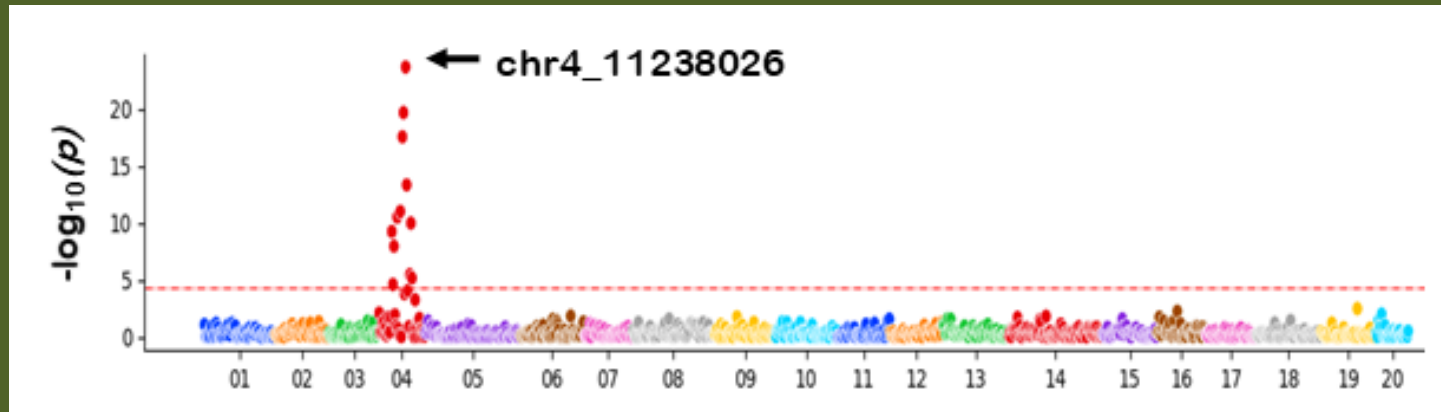
Muscadine berry color:  
Purple, Dark-red, Red, Bronze, and Green.

Bunch grape berry color:  
Purple, Dark-red, Red, Bronze,  
Yellow, Gray, and Green.

**Reason????**

**Reason: MYB**

# Genome-Wide Association Studies (GWAS) – Color Trait



Loss of color in muscadine is associated with a SNP in GST4b encodes glutathione S-transferase gene, resulting in change P<sub>171</sub> (c<sub>cg</sub>) to Leu (c<sub>tg</sub>).

**a**

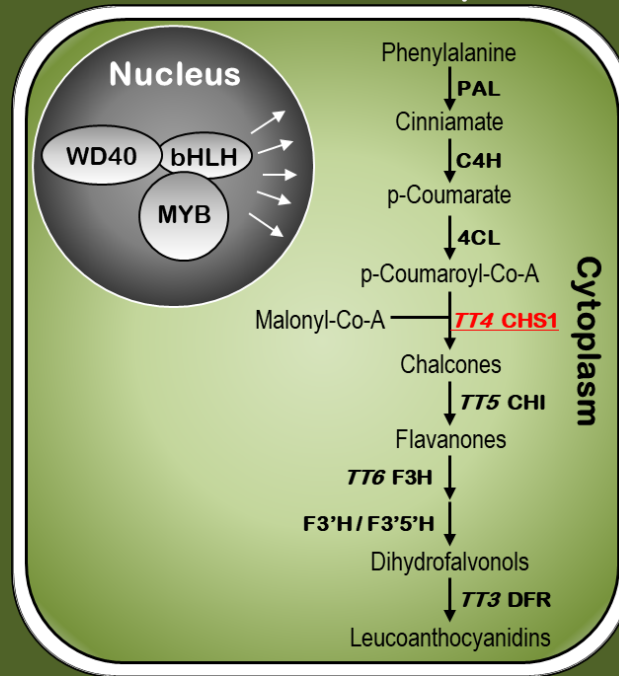
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GST4b1 : MWMKVYGFVRAACPQRVLACLVEKGVFEVWHVDLDSGEQKRPDFLLRQPPFGQVPVVEDGDFRLFESRAIIRYIAAKYAEQGPDLLGKSLEEKAVVDQWLEVEAHNFNEL : 110
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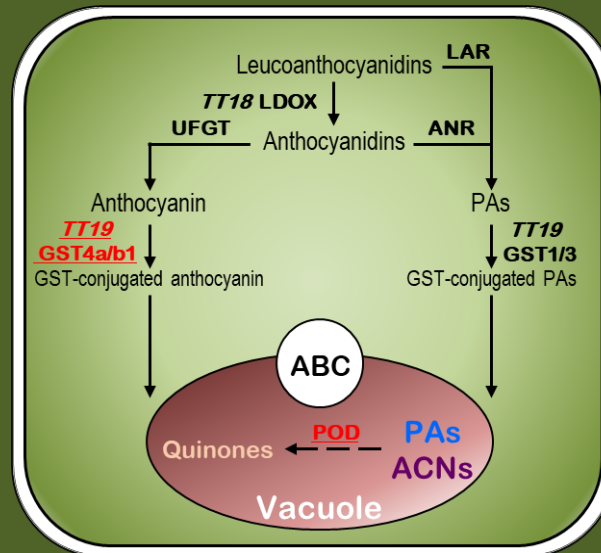
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GST4b1 : VYTLVLQLLILPRMGERGDLALAHTCEQKLEKVFVDVYEQRLSKSRYLAGDSFTLADLSHLEA*IRNLVKEAGMAHLVTERKSVSAWWEDISRAAWKKVMELAS : 213
GST4b2 : VYTLVLQLLILPRMGERGDLALAHTCEQKLEKVFVDVYEQRLSKSRYLAGDSFTLADLSHL*EAIRNLVKEAGMAHLVTERKSVSAWWEDISRAAWKKVMELAS : 213
    
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- CHS and GST4 enzymes control pigment intensity among colored muscadines;
- Homozygote muscadine genotypes for GST4 have more anthocyanin levels than heterozygote genotypes;
- Non-colored muscadines are due to a mutation that made the mGST4 not able to transport anthocyanins into vacuole.
- The cytoplasmic anthocyanins are converted into proanthocyanidins that can be transported to the vacuole by GST1 and GST3.

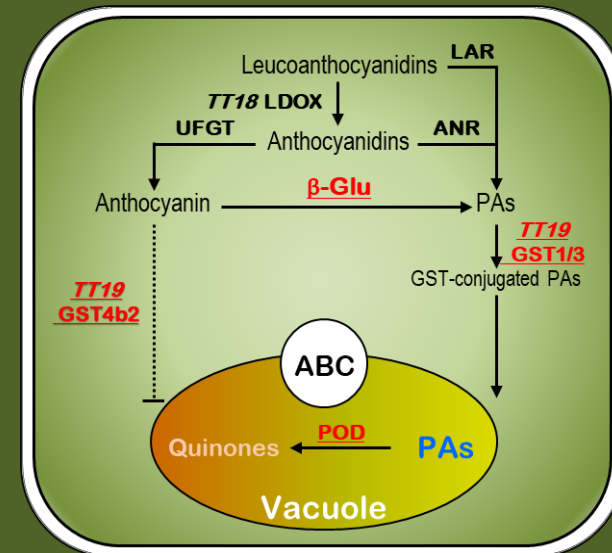
## Muscadine Grapes



## Colored Berries



## Colorless Berries



# Questions

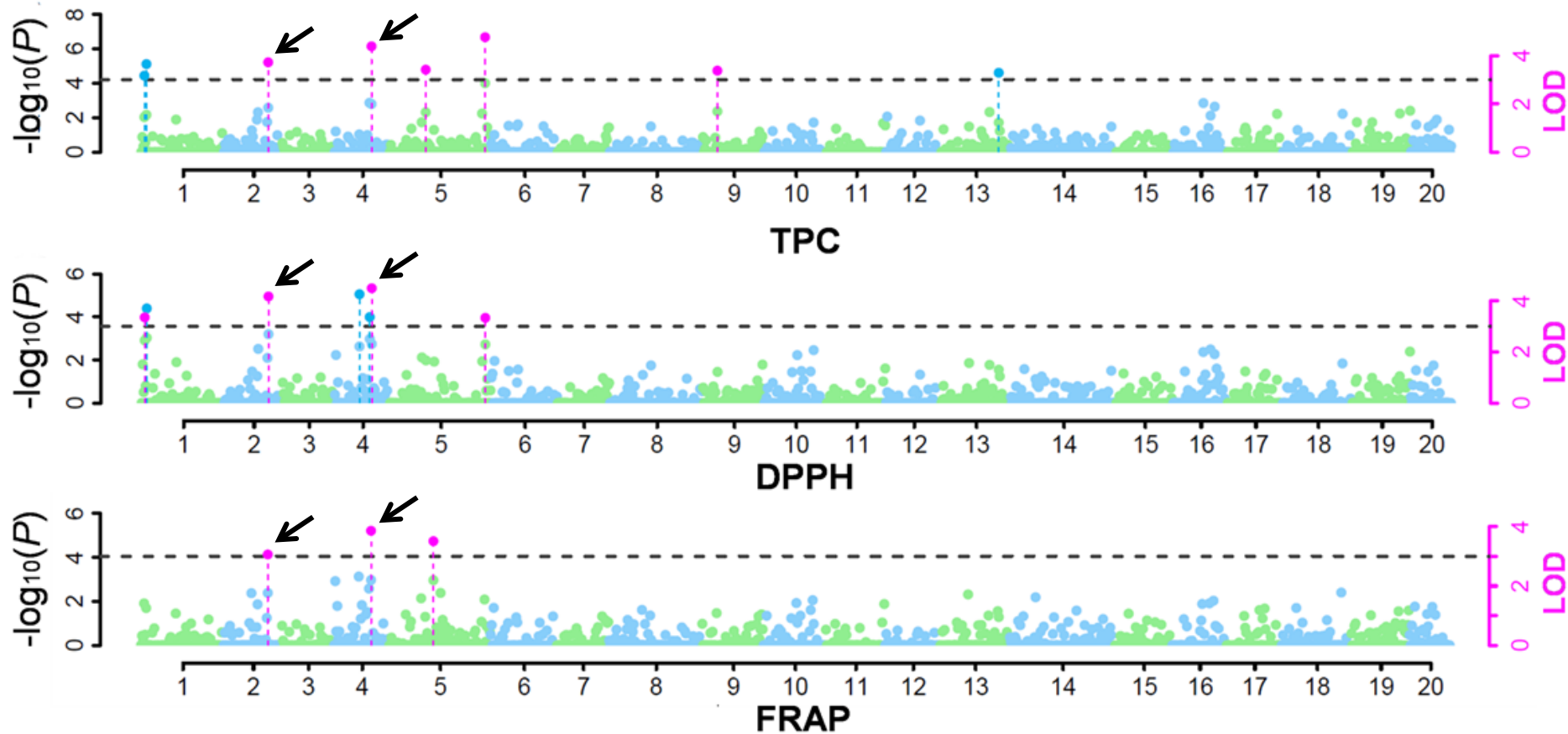
Is anthocyanin is positively involved in antioxidant activity? **Yes**

Red muscadines has more antioxidant activity the bronze muscadines? **Not necessarily**

Why wine generated based on colored muscadine berries is healthier than wine generated based on unpigmented berries?

Why muscadines exhibit diversity in antioxidant activity?

# Genome-Wide Association Studies (GWAS) – Antioxidant Trait



- [chr2\\_1446718](#): UDP-glycosyltransferase enzyme positively associated with high antioxidant activity.
- [chr4\\_16491374](#): 4-hydroxy-4-methyl-2-oxoglutarate aldolase involved in gallic acid degradation. Gallic acid is a key metabolite player involved in antioxidant activity in muscadine grapes.

# Produce large berry seedless muscadine grapes for fresh consumption using CRISPR –technology

## Fruit set programs in bunch grapes



Seeded/Female

Seeded/Perfect

Parthenocarpy  
seedless



# Fruit set programs in bunch grapes



Seeded

Stenospermocarpy  
seedless

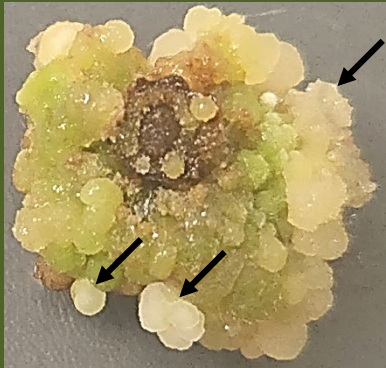
Parthenocarpy  
seedless

- Our target is to generate muscadine grapes exhibiting stenospermocarpy fruit set.
- Introducing the trait by conventional breeding with bunch grapes is not possible due to differences in chromosomes number between the two species.
- Several research groups were able to identify a potential gene candidate underlying the trait.
- We decided to use the gene-editing technology to generate a non-GMO large seedless muscadine berry suitable for fresh consumption.

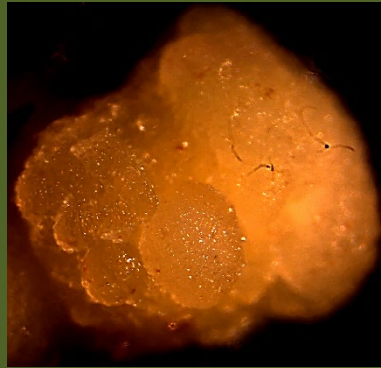
# Problems

- Grapes are transformed by using somatic embryos as a gene delivery system. Highly efficient regeneration protocol has been developed at the CVSFR for the purpose of this project.
- The CRISPR technology can interrupt the gene either by insertion or deletion. However, we wanted to investigate the option of nucleotide substitution.

# CRISPR technology for improving muscadine grapes



Pre-embryonic callus



Somatic embryos



Somatic embryos propagation



Somatic embryos transformation



Plants development in a selection media



Adaptation

# Breeding new high quality Southern grape cultivars for meeting industry demands in Florida

## Blanc Du Soleil

- Wine aroma of 'Blanc Du Soleil' is comparable to 'Pinot Gris' with pear, apple, peach, and pineapple notes.
- Wine exhibit medium acidity compared to 'Stover' (low) and 'Blanc Du Bois' (high), which makes it versatile for sparkling, still, and dessert style wines.
- Wine has a superior color characteristics compared to Blanc Du Bois and Stover. It is lighter in color than Blanc Du Bois and less brown/pink than Stover, which gives it better quality for white wine production.
- Plants will be available by the end of 2023 from Double A Vineyard, Fredonia, NY.

