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FLORIDA AGRICULTURAL AND MECHANICAL UNIVERSITY

FAMU FLORIDA A&M UNIVERSITY VITICULTURE & SMALL FRUIT RESEARCH
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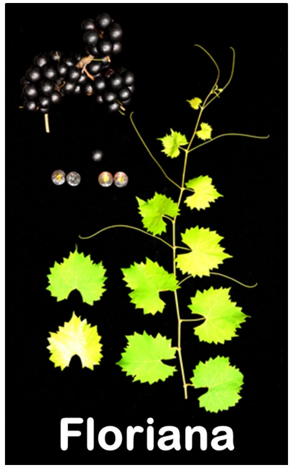
Grape Breeding Program at Florida A&M University



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Research Activities

Muscadine beneficial characteristics:

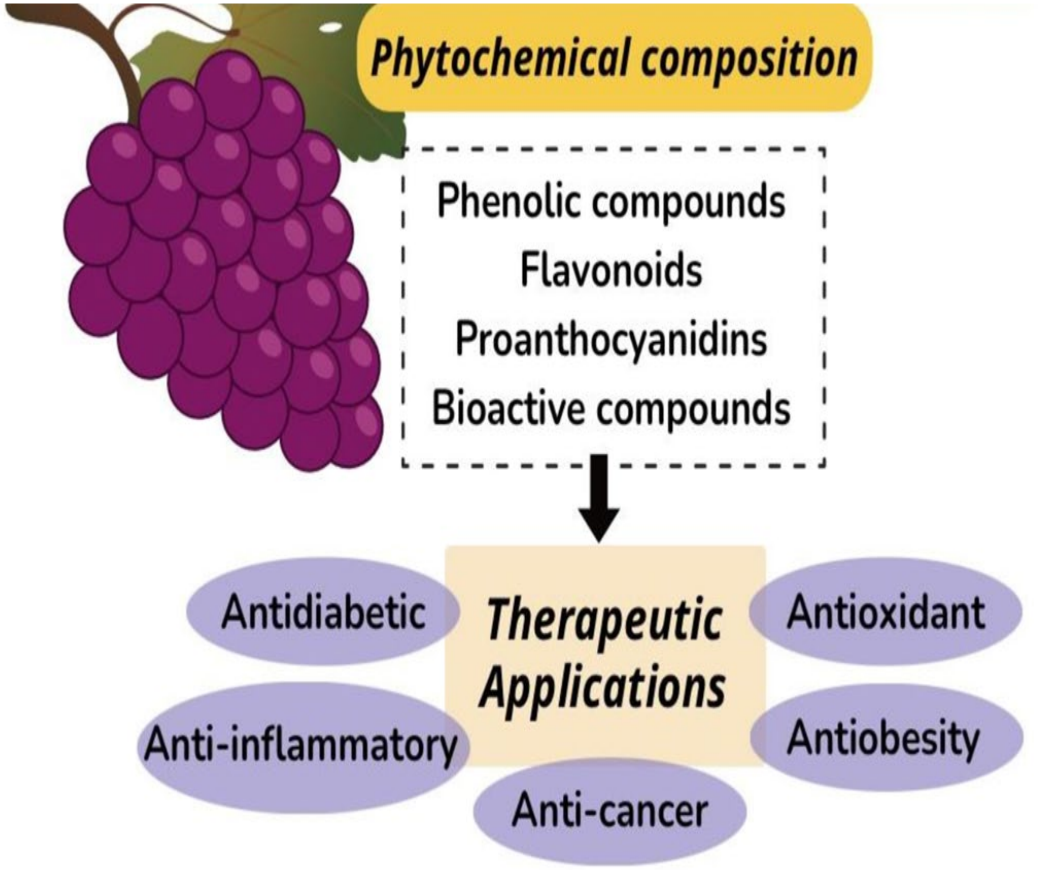
- Biochemical and molecular comparison of aroma profile spectrum in flowers and ripe muscadine and bunch grape berries;
- Breeding new high quality Southern grape cultivars for meeting industry demands in Florida;
- Identify molecular and biochemical markers associated with abiotic stress resistance (i.e., drought, salinity, and hypoxia) in grapes;
- Berry color and its relation to antioxidant activity;
- Produce large berry seedless muscadine grapes for fresh consumption using gene editing CRISPR–Cas technology;
- Anticancer activity (African American Breast Cancer and African American Prostate Cancer);
- Identify molecular and biochemical markers associated with biotic stress resistance (i.e., ripe rot and gray mold) in grapes.

Phytochemical Properties of Muscadine Grapes

- Muscadine grapes attract significant attention from the food, winemakers, pharmaceutical, and nutraceutical sectors, due to their chemical compositions and nutritional benefits.

- Muscadine grape contains unique sets of primary and secondary metabolites, including fruit acids, carbohydrates, and phenolics (i.e., gallic acid, ellagic acid, proanthocyanidins, anthocyanins, catechins, quercetin, resveratrol, and myricetin).

- These metabolites play important roles in plant growth and defense, but also benefit human health and contribute to the taste, color, and mouthfeel of grapes and wine.



Schematic Representation of the Muscadine Grape Metabolites Extraction

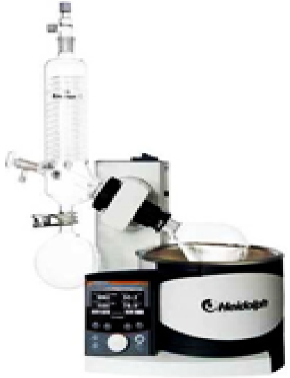
Muscadine berry collected & frozen

Samples ground to fine powder under freezing

2010 Spex Grinder

Samples subjected to methanol extract for 24 h

Methanol separated from extracts



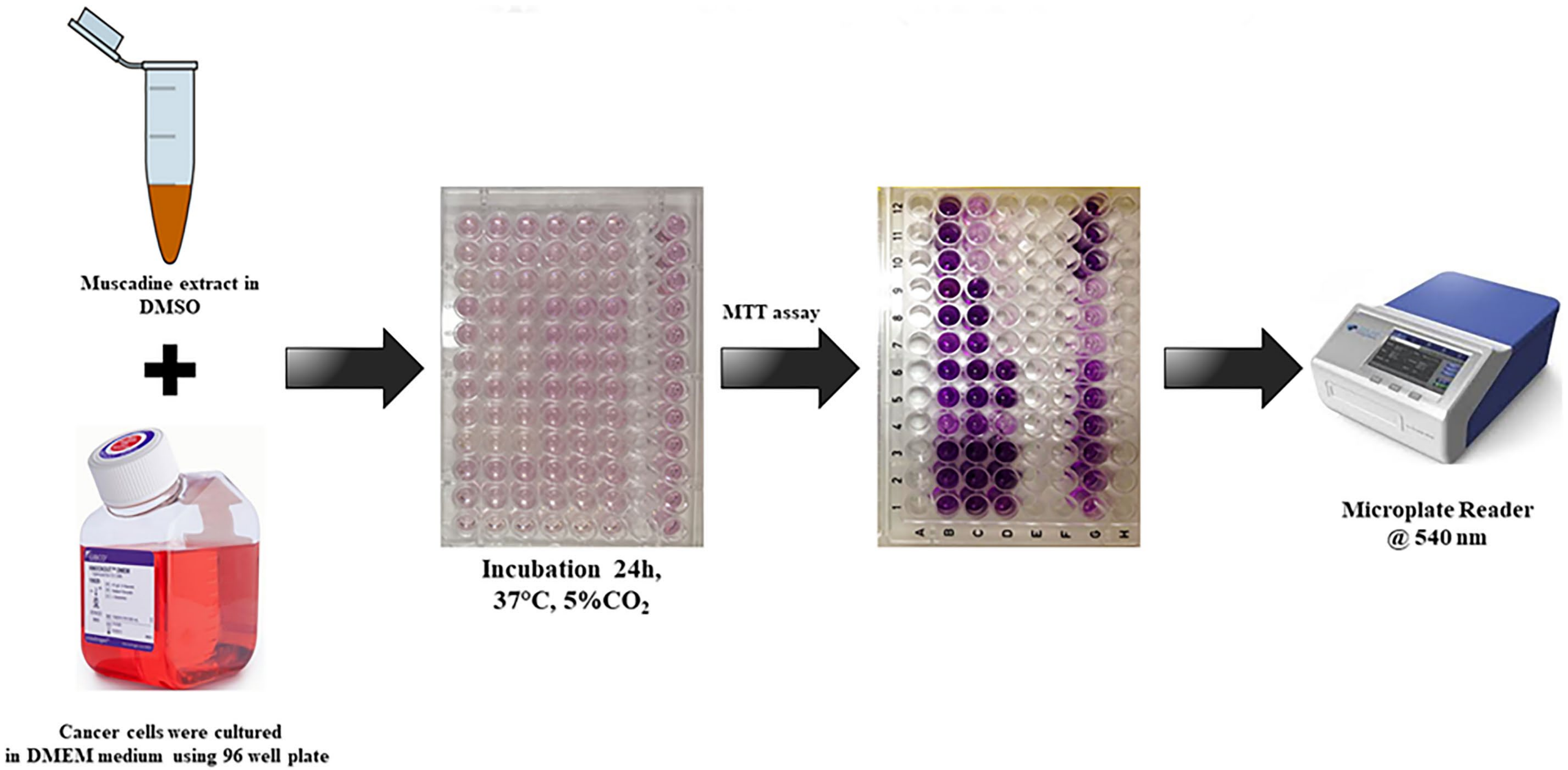
Digital Rotary Evaporator Heidolph

Samples totally dried using SpeedVac

All samples stored under dry dark conditions for analysis

Prostate Anticancer Activity (MTT assay)

- Seed and skin extract of 360 individual muscadine genotype from ripe berries.
- Prostate cancer cell lines tested are C42B (Caucasian) and MDA PCa 2b (African American).
- The extracts were used at concentrations of 100 ng/µl and 250 ng/µl for seed and skin tissues, respectively.



Prostate Anticancer Activity

Factor	Caucasian (C42B)		African American (MDA)	
	Seed	Skin	Seed	Skin
Cytotoxicity range	0 – 100%	0 – 100%	15.2 – 61.4%	0 – 29.7%
Average cytotoxicity	78% ±13.2	22.1% ±22.6	47.1% ±9.4	8.6% ±7.5
Median cytotoxicity	80.2%	16.6%	48.5%	6.3%

Position of Noble and Carlos cultivars among muscadine population (354 individual):

- Noble seed x C42B cytotoxicity: ranked at the position 328 with cytotoxicity level of 62.3% ±5.4.
- Noble skin x C42B cytotoxicity: ranked at the position 40 with cytotoxicity level of 51.3% ±7.
- Noble seed x MDA cytotoxicity: ranked at the position 327 with cytotoxicity level of 62.3% ±6.1.
- Noble skin x MDA cytotoxicity: cytotoxicity level of 0%.

- Carlos seed x C42B cytotoxicity: ranked at the position 344 with cytotoxicity level of 55.3% ±6.3.
- Carlos skin x C42B cytotoxicity: ranked at the position 198 with cytotoxicity level of 10.4% ±1.8.
- Carlos seed x MDA cytotoxicity: ranked at the position 343 with cytotoxicity level of 55.2% ±4.6.
- Carlos skin x MDA cytotoxicity: ranked at the position 189 with cytotoxicity level of 5.1% ±0.9.

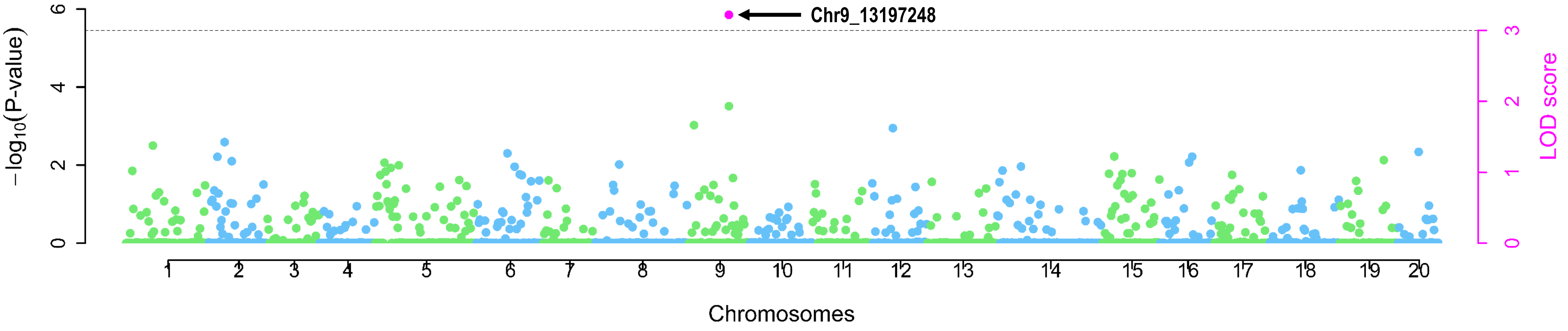
Correlation Coefficient and Calculated Probability with Nutraceutical Traits

		TPC		TFC		TAC		DPPH		FRAP	
		r^2	*P*	r^2	*P*	r^2	*P*	r^2	*P*	r^2	*P*
C42B	Seed	NS	NS	NS	NS	nd	nd	NS	NS	NS	NS
	Skin	**0.33**	**1.8×10^{-10}**	**0.22**	**3.3×10^{-5}**	**0.19**	**1.4×10^{-3}**	**0.31**	**2.5×10^{-9}**	**0.27**	**4.7×10^{-7}**
MDA	Seed	NS	NS	0.11	**3.9×10^{-2}**	nd	nd	NS	NS	NS	NS
	Skin	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

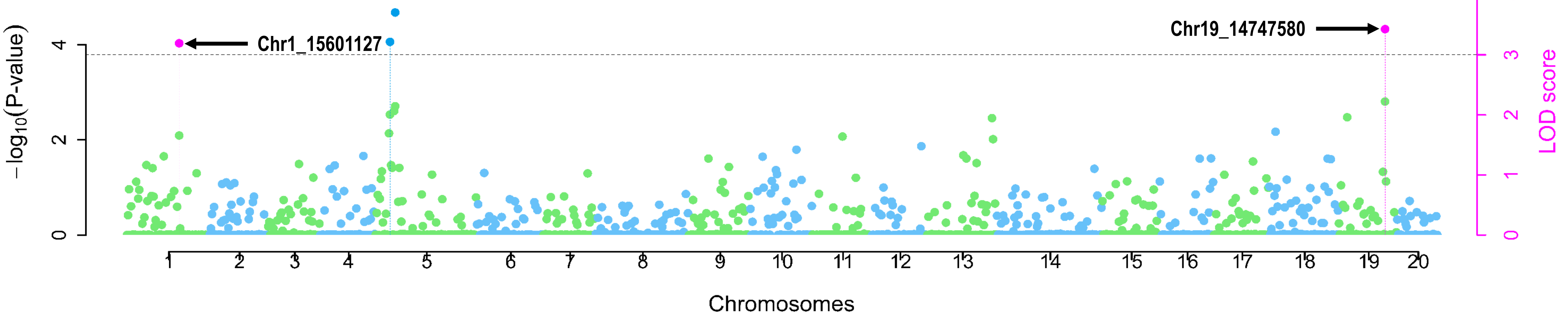
Total phenolic content (TPC), total flavonoid content (TFC), total anthocyanin content (TAC), 1,1-diphenyl-2-picrylhydrazyl antioxidant activity (DPPH), and Ferric Reducing Antioxidant Power (FRAP). Statistically significant differences are represented by probability levels [*n*=360].

Genome-Wide Association Studies (GWAS)

C42B x Seed



C42B x Skin



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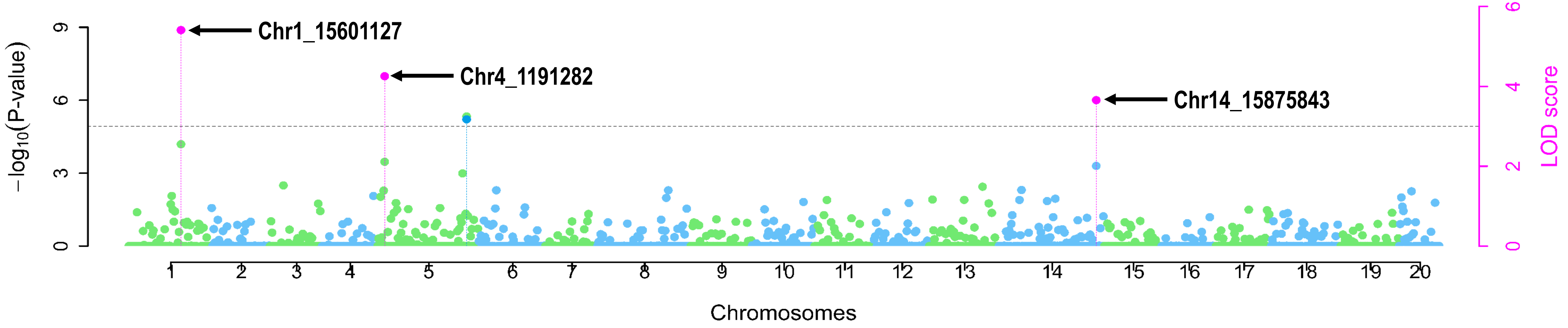
Genome-Wide Association Studies (GWAS)

- **MDA x Seed**



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Prostate cancer cytotoxicity during berry development

- Muscadine genotypes exhibiting the highest and lowest prostate anticancer activity with respective cell line and tissue used were identified.
- Seed or skin tissue from each genotype were collected at the berry developmental stages, fruit-set (FS), green (G), veraison (V), and ripening (R).
- All samples were subjected to methanolic extraction and assessed for prostate anticancer activity.

Fruit-Set (FS) | Green (G) | Veraison (V) | Ripening (RIP) | Ripening-Skin/Flesh (RIP-SF) | Ripening-Seeds (RIP-S)

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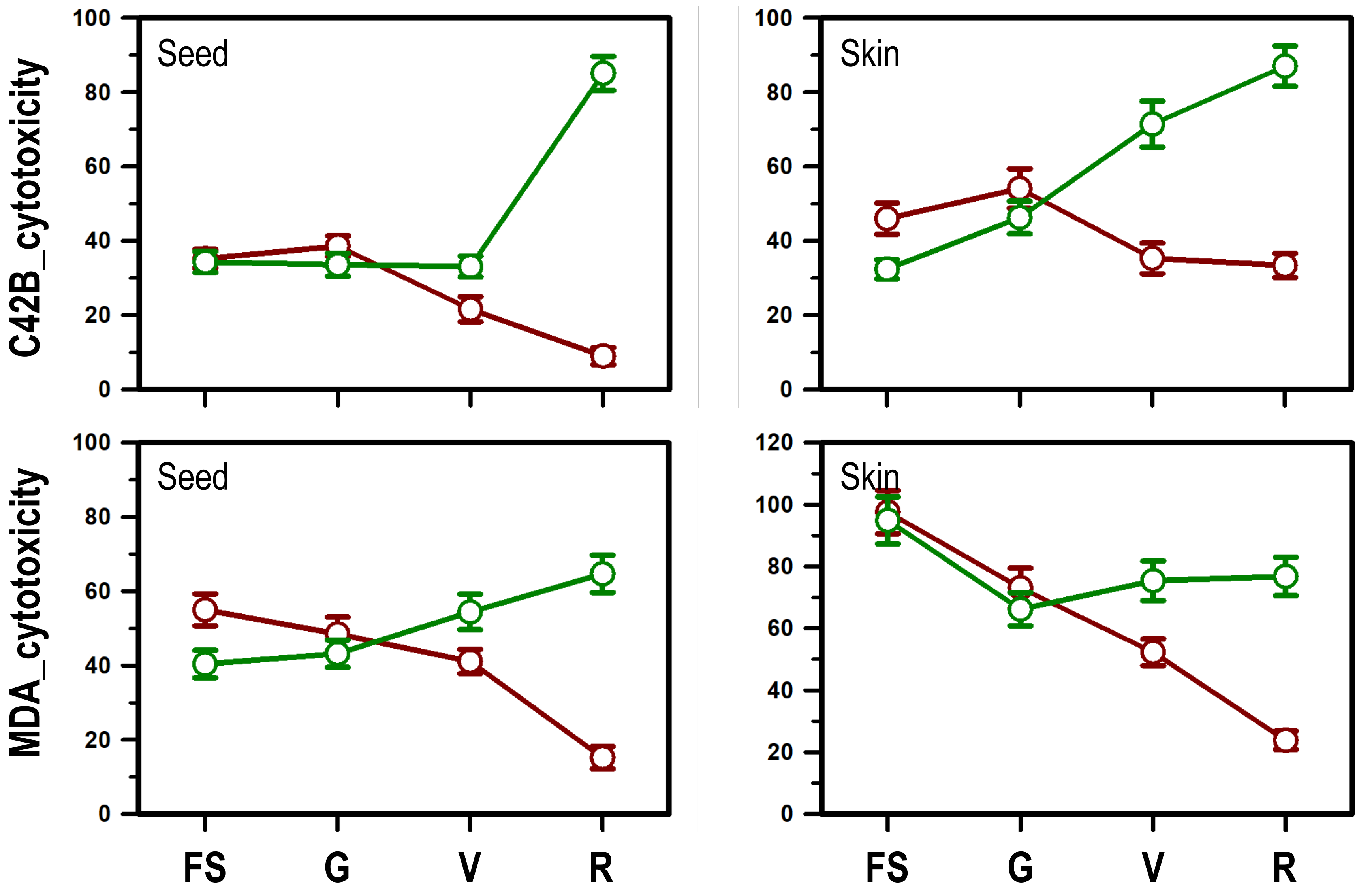
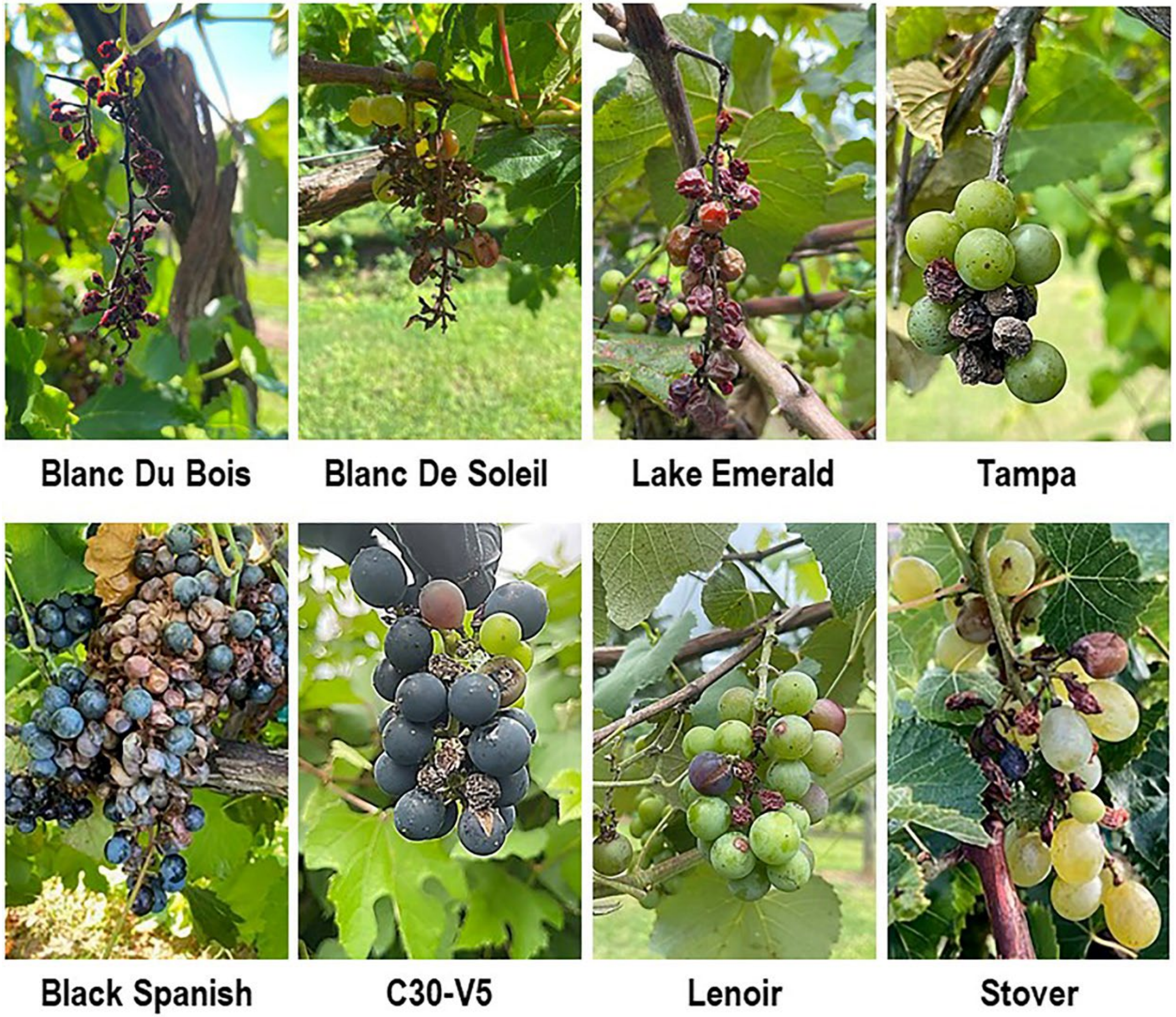
Prostate cancer cytotoxicity during berry development

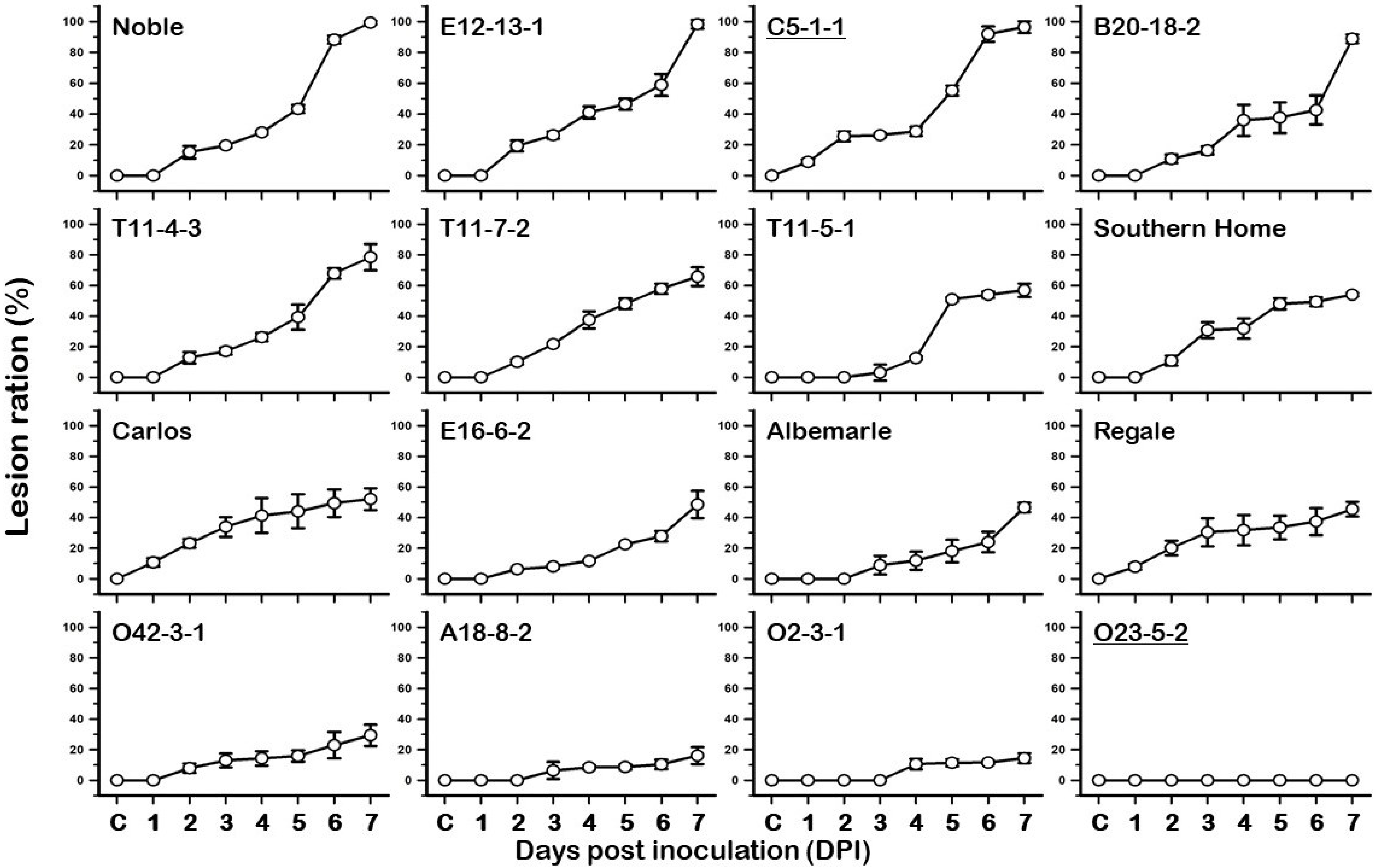


Characterization of muscadine grape for ripe rot (*Colletotrichum* sp.) resistance

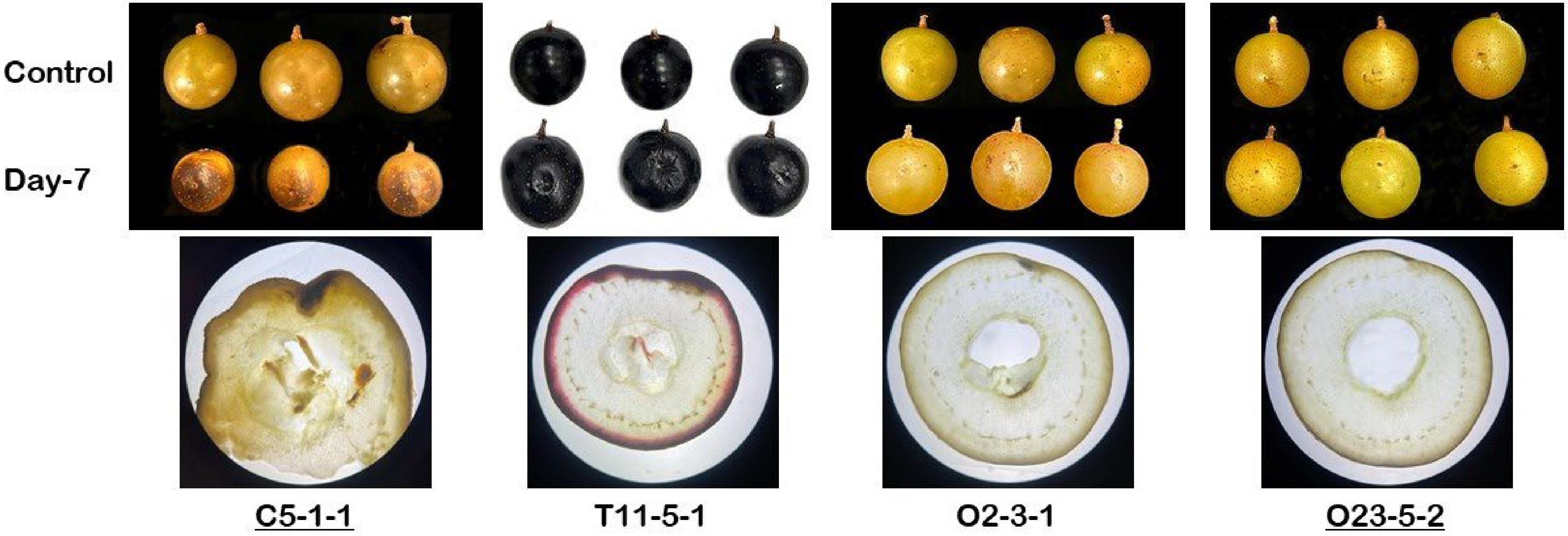
Close-up view of several southern bunch grape cultivars showing naturally occurring ripe rot (*Colletotrichum* spp) incidents in the field.



Characterization of muscadine grape for ripe rot (*Colletotrichum* sp.) resistance



Inoculation Assay of Ripe Rot Fungal Spores



Muscadine grapes exhibit a wide range of responses to ripe rot infection ranged from:

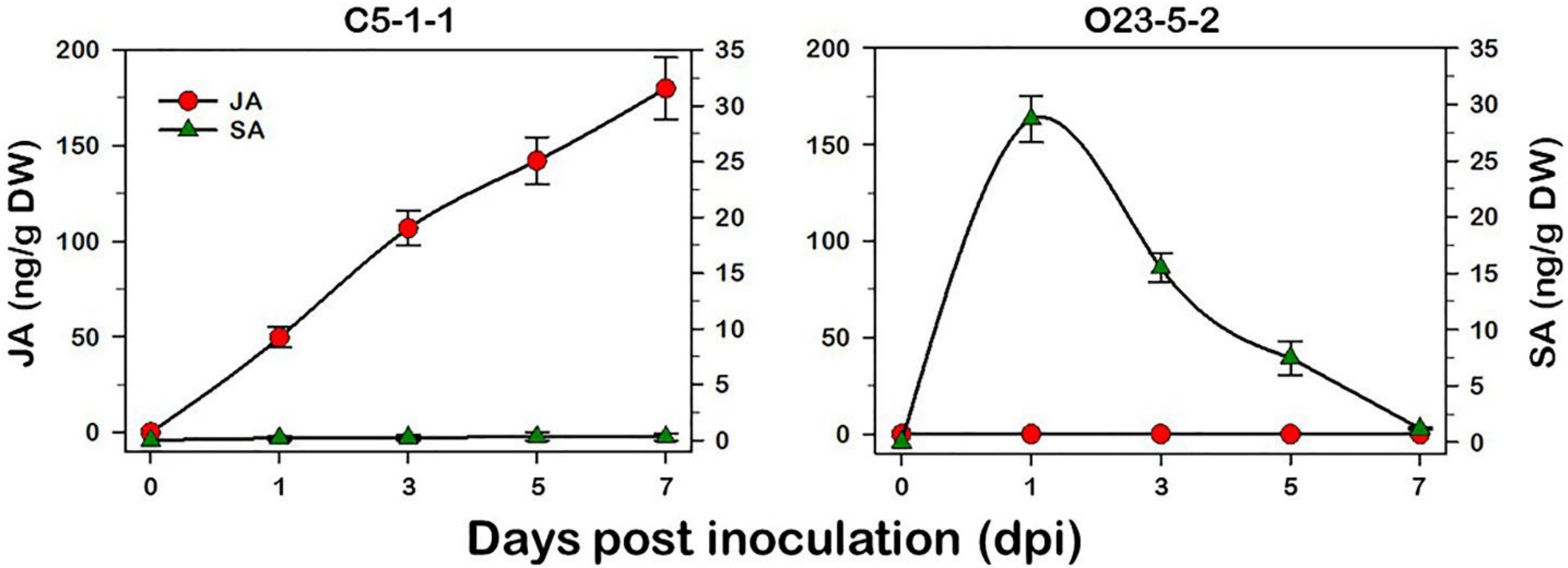
1- Susceptible (78.5 - 100% lesion zone).

2- Tolerant (29.4 - 65.8% lesion zone).

3- Resistant (12.4 – 16.6% lesion zone).

4- Immune (0% lesion zone).

Changes in defense hormones during infection of sensitive and resistant muscadine genotypes



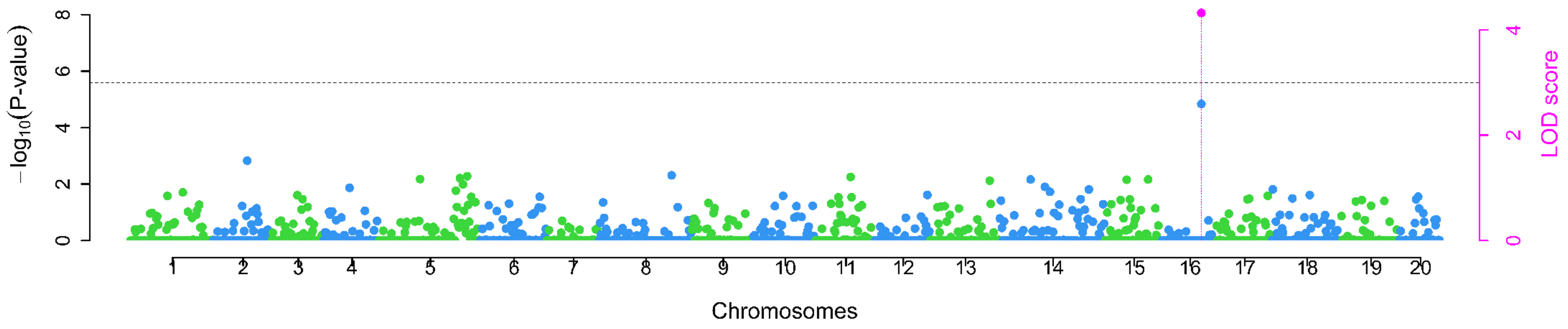
Genome-Wide Association Studies (GWAS) – Antifungal Trait

Determine the antifungal property of muscadine seed extract to inhibit ripe rot spores growth:

1- A population of 354 individual muscadine extracts were used in the assay.

2- Muscadine seed extracts exhibited wide fungal inhibition rate (22.3 – 90.4%).

3- Inhibition of fungus growth was not associated with TPC, TFC, or TAC.



Preliminary genomic and molecular analysis:

1- We have an indication of the master gene responsible for resistance.

2- We were able to identify of muscadine antifungal proteins responsible for killing fungal spores.

3- Resistance is due hyper-sensitive alert signal that was able to early detect the disease presence and react in a SA-dependent mechanism.

4- Susceptibility is due to defected disease detection signal associated with delayed response to the disease presence that occurred in JA/ET dependent mechanism.

Acknowledgement

FLORIDA SPECIALTY CROP FOUNDATION

Florida Wine & Grape Growers Association
Established 1923

USDA
United States Department of Agriculture
National Institute of Food and Agriculture

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