The First Version of the Whole-Genome Sequencing of the Muscadine Grape

(Muscadinia rotundifolia cv. Noble)

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Our Muscadine Breeding Program

- Currently muscadine grapes is commercially grown in 12 states in the US.

- There is a demand for new muscadine cultivars with enhanced fruit/vinification qualities.

- Over the years, conventional breeding strategy has resulted in several muscadine cultivars suitable for table and wine markets with improved quality traits.

- However, the procedure is lengthy, laborious and expensive, which does not match with the rapid, ever-increasing industry needs.

- Our goal is capitalizing on the latest achievements of genomic technologies to develop breeding platform that meets the growing industry demands.
Grapevine Cultivars & Germplasm at CVSFR

<table>
<thead>
<tr>
<th>Background</th>
<th>Total No. of Vines</th>
<th>No. of Individual Genotypes</th>
<th>No. of Cultivars</th>
<th>Adult (^a)</th>
<th>Juvenile (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Muscadinia</em> hybrids</td>
<td>3925</td>
<td>2537</td>
<td>51</td>
<td>664</td>
<td>1877</td>
</tr>
<tr>
<td><em>Vitis</em> hybrids</td>
<td>3380</td>
<td>2432</td>
<td>172</td>
<td>589</td>
<td>1843</td>
</tr>
<tr>
<td><em>Muscadinia</em> x <em>Vitis</em> (MV)</td>
<td>106</td>
<td>106</td>
<td>-</td>
<td>103</td>
<td>3</td>
</tr>
</tbody>
</table>

\(^a\) 3-year old and /or elder; Reproductive Phase.

\(^b\) 2-year old and or younger; Growth Phase.
Grape

**Muscadinia Genus (3)**
- *M. rotundifolia*
- *M. munsoniana*
- *M. popenoei*

40 chromosomes (n=20)

**Euvitis Genus (14)**
- *V. vinifera*
- *V. labrusca*
- *V. riparia*
- *V. aestivalis*
- *V. rupestris*

38 chromosomes (n=19)

**Morphological Differences:**
Leaves, flower type, tendrils, cluster size, berry size, and age of cutting for propagation.
Muscadinia sp. hold particular agronomical and reproductive traits:

- Resistance to most of diseases that limit the worldwide production of V. vinifera.
- Adapted to poor soil.
- Grow well under warm and humid conditions.
- Exotic fruit quality traits (berry size, unique flavor spectrum, therapeutic phytochemicals content).
- Unique vinification qualities.
Muscadines have obviously different challenges from those of traditional ‘*vinifera*’ grapes

- Cold hardiness.
- Extended ripening season.
- Perfect flower with large berry size.
- Fruit quality traits (diversity in berry color & shape, seedlessness, large cluster, thinner skin, dry stem scar, uniform ripening, extended shelf-life).

**Male**  
**Female**  
**Perfect**  

*Wet stem scar*

*Photos by UGA CAES - University of Georgia*
Whole – Genome Sequencing of Muscadine Grape Noble cv.

Thomas (♀) x Tarheel (♂)

- High vigor growth.
- Disease resistant.
- Self-fertile (perfect flower).
- Midseason.
- Uniform ripening.
- Black small berries (3.8 – 4.3 g).
- High yield (9.6 – 10.7 tone/acre).
- Stable purple pigments.
- Suitable for wine and juice production.

Muscadine Genome Sequencing & Assembly

- **Sequencing**
  - Illumina HiSeq 2500 (80 M reads)
  - Chicago (181 M reads)
  - Hi-C (223 M reads)

- **Genome HiRise Assembly**
  - Contigs
  - Scaffold

- **Annotation**
  - Related Genome
  - Annotated Draft Genome
### Muscadine Genome Assembly Statistics

<table>
<thead>
<tr>
<th>Metric</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated genome size</td>
<td>414 Mb (82%)</td>
</tr>
<tr>
<td>Assembly Size</td>
<td>400.26 Mb (96.6%)</td>
</tr>
<tr>
<td>Contig N50</td>
<td>107 kb</td>
</tr>
<tr>
<td>Contig L50</td>
<td>1,011 contigs</td>
</tr>
<tr>
<td>Scaffold N50</td>
<td>20.045 Mb</td>
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<tr>
<td>Scaffold L50</td>
<td>9</td>
</tr>
<tr>
<td>Scaffold N90</td>
<td>16.486 Mb</td>
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<tr>
<td>Scaffold L90</td>
<td>18</td>
</tr>
<tr>
<td>Number of scaffolds</td>
<td>1,629</td>
</tr>
<tr>
<td>Heterozygosity</td>
<td>1.47%</td>
</tr>
<tr>
<td>Unplaced sequence</td>
<td>2.47%</td>
</tr>
<tr>
<td>Contig gaps</td>
<td>8,168</td>
</tr>
</tbody>
</table>

**Diagram:**

- **X-axis:** Muscadine Genome (V. vinifera) chromosome name.
- **Y-axis:** Chromosome size (Mbp).
- **Legend:**
  - *V. vinifera*
  - *M. rotundifolia*
Benchmarking Universal Single-Copy Ortholog (BUSCO)

Assessment of muscadine genome assembly and gene set using BUSCOs indicates highly-complete representation of protein-coding genes.

Statistics searching Embryophyta BUSCOs:

1382 Complete BUSCOs (96%).
  - 1339 Complete and single-copy BUSCOs (93%).
  - 43 Complete and duplicated BUSCOs (3%).

17 Fragmented BUSCOs (1.2%).

41 Missing BUSCOs (2.8%).

1440 Total BUSCO groups searched.
M. rotundifolia and V. vinifera display high colinearity

The extra Muscadinia chromosome is composed of two large segments homologous to Vitis chromosome 7.
Characterization of muscadine population

A population of 400 muscadine genotypes (50 standard cultivars and 350 lines) has been carefully selected to ensure diversity and subjected to:

1. **Phenomic Characterization**
   - Fertility traits (bud fertility, bud fertility coefficient, position of first fruiting bud);
   - Cluster physical traits (intensity, size, weight, number and weight of berries/cluster);
   - Berry physical traits (scar pattern, color, size, weight, number and weight of seeds/berry, firmness);
   - 50 berries traits (berries weight, pomace weight, juice volume);
   - Yield traits (No. of clusters/vine, yield/vine); and
   - Berry quality traits (TSS, acidity, pH).
Characterization of muscadine population

2. Phenology traits

- Bud break.
- Green shoot visible.
- Visible inflorescences.
- Partial bloom.
- Full bloom.
- Fruit-set.
- Cluster closure.
- Veraison.
- Post-Veraison.
- Ripening.

3. Biotic stress traits

- Leaf roll virus (several strains); and
- Fungal diseases (powdery and downy mildew).
Characterization of muscadine population

4. Metabolomic and sensorial traits

- Aroma volatile esters production.
- Total phenolic.
- Total flavonoid.
- Antioxidant activity.
- Antimicrobial activity (*E. coli*).
- Anticancer activity (Breast cancer cell lines MDA-MB-231 and MDA-MB-468).
Anticancer activity of muscadine extracts using African American breast cancer cell lines
Classification of muscadine genotypes based on their anticancer activity
Undergoing

- Generating Pacbio Iso-seq and Illumina RNA-seq data on multiple tissues to generate accurate and comprehensive gene predictions.

- Performing ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing) to determine the chromatin accessibility of muscadine genome.

- Analyzing of *M. rotundifolia* genes’ PAV (presence-absence variation) and their predicted functions compared to *V. vinifera*.

- Complete QC analysis and submission to Phytozome.
Acknowledgement
Team Members

D. Vera  
D. Kambiranda  
V. Tsolova  
I. El-Sharkawy