

SERA IEG-14 State Report

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1. Developing Seedless Muscadine Grapes

During the 2003- 2004 season, we continued field-evaluation of the putative seedless hybrids produced from previous years for disease resistance, fruit quality, yield etc. Several putative muscadine-bunch hybrids resulted from 2003 pollination and embryo rescue were planted into the field early this spring. For moving the seedless trait into muscadine grapes from *Vitis vinifera* grapes, pollination and embryo rescue was focused on the 'JT hybrid', a seedless muscadine x *V. vinifera* hybrid (Table 1). Back-cross to muscadine proved to be difficult, as evidence that only 5 viable embryos were obtained from over 5,000 berries, and no seedlings were regenerated from this backcross effort. Majority of the seed traces were found empty (without an embryo and endosperm). We also dissected many seed traces from open pollinated berries of JT hybrids, which yielded 10 plantlets. We concluded that reinstating the fertility of the infertile 39-chromosome hybrid is the key for further moving the seedless trait into the muscadine grapes.

Four crosses of bunch x muscadine grapes were made by using 'Orlando Seedless' as female parent and several muscadine grapes as pollen sources. Three hundred and thirty seven seed traces were extracted from 1,385 berries, which resulted in 20 embryos and 14 plantlets. These plants are in the process of being transferred into the greenhouse.

Several seeded muscadine x bunch grape hybrids were also used for back-cross into seedless *V. vinifera* and muscadine grapes. Twenty-three crosses, with flower cluster ranging from 2 -12 in each of the crosses, were pollinated. Eight out of the twenty-three crosses produced fruits and seeds (Table 2). Thirteen crosses of muscadine x muscadine grapes were also made, which resulted in 1,160 hybrid seeds. In addition, the 'seedless' trait was genetically engineered into muscadine cv. 'Fry' and the putative transgenic muscadine grapes were established in the greenhouse.

Table 1. Developing Seedless Grapes Via Embryo Rescue

Cross combinations	Seed trace	Embryo	Plantlets
11	1,205	25	14

2. Improvement of disease resistance and fruit quality of muscadine grapes

The objective of this project is to improve quality and productivity of commercial muscadine grape cultivars. Three major breeding goals are: 1) breed perfect-flower cultivars with large berries and fruit quality similar to or better than 'Fry'; 2) select new cultivars with better fruit qualities for fresh fruit (e.g. higher soluble solids content, edible skin and better texture) and that are disease resistant; and 3) develop new cultivars with high disease resistance for processing into quality wine, juice and jelly. Hybrid seeds obtained in 2003 were summarized in Table 2.

During the 2003 – 2004 seasons, we continued evaluating the advanced breeding lines of muscadine grapes. About a dozen of breeding lines that showed better viticultural characteristics and fruit quality are being propagated. A collaborative evaluation /trial with Florida grape growers will be started this fall.

Table 2. Seeds obtained from 2003 pollination

Bunch x Bunch Grapes

Twelve cross combinations, 13,918 seeds

Muscadine x Muscadine Grapes

Eleven cross combinations, 1,160 seeds

VM hybrids* x Seedless *V. vinifera*

Twenty-three cross combinations, 131 seeds

Bunch Grapes x Muscadine Grapes

Four cross combinations, 84 seeds

*VM hybrids = *vinifera* x muscadine hybrids

3. Bunch Grape Breeding and Product (wine) Evaluation

Evaluations of hybrid bunch grapes produced from previous years were conducted throughout the 03-04 season. Important viticultural characteristics (disease resistance, vine vigor), flower type, productivity and fruit quality were recorded for about thirty advanced selections. Among them, a half dozen advanced lines received special attention for their good viticultural characteristics, productivity, and disease resistance. Experimental wines were made from seven selections (Table 3). In comparison with other Florida hybrid bunch grapes used, six out of the seven selections have higher sugar. Wine-quality evaluation was delayed due to the renovation of the Viticulture Center Building. These seven advanced selections have been propagated. They are ready to be distributed to other locations for further evaluation.

Table 3. Bunch grape selections were used for making wine in 2003

Crosses	Color	SSC	Acid
A21-4-5	Red	16.3	0.6
A21-3-1	White	17.0	0.5
A22-2-4	Red	14.0	1.0
A22-5-10	Red	17.5	0.7
A23-7-8	White	18.0	0.7
A24-1-4	Red	17.5	0.7
A24-5-6	White	17.5	0.6
Blanc du Bois	White	15.2	0.8
Conquistador	Red	12.0	0.8
Stover	White	15.1	0.5
Suwannee	White	14.0	0.8

4. Develop New Grape Cultivars by Interspecific Somatic Hybridization

Protoplasts from 'Autumn Royal Seedless' and 'Tara' somatic embryos were fused with protoplasts from 'Orlando Seedless' leaves. About 30% and 70% fusions were achieved depending on the quality of the protoplasts. Cell division and callusing

are observed among the fused cells but no plantlets have been produced. Studies are being focused on culture media and conditions.

5. Developing Seedless Grape Cultivars by Genetic Engineering

A 'seedlessness' gene was transferred into muscadine grape cv. 'Fry'. Twenty putative transgenic lines of muscadine cv. 'Fry' have been established in a greenhouse. The transgenic 'Fry' grapes are being evaluated for viticultural characteristics. Seedlessness and overall fruit quality will be evaluated upon fruiting.

6. Develop Disease Resistant Grape Cultivars by Genetic Engineering

Vectors carrying Chitinase and several antifungal peptides genes were constructed. Transformation with these genes has been conducted. In the mean time, the EGFP reporter gene has been sub-cloned and transformed into *Agrobacterium* EHA 105. The putative transgenic 'Merlot' and 'Chardonnay' carrying the universal 35S promoter and "Osmotin" (wound inducible) promoters and the reporter gene were subjected to DNA analysis, with a negative result. Additional testing is necessary to confirm the putative transgenic plants.

7. Grape Genomics Research

Vitis shuttleworthii is one of the most disease and pest resistant grape species originated and distributed in the southeast United States. It is highly resistant to: Pierce's Disease, Anthracnose, Black rot and Downy mildew diseases, which are the limiting factors to grow *Vitis vinifera* grapes in this region. For these reasons, *V. shuttleworthii* cDNA libraries were constructed from mRNA isolated from leaves and flowers harvested during anthesis. We have sequenced 15,000 cDNA, from which 12,008 clean ESTs (Expressed Sequence Tag) were obtained. After assembling, 5776 unigenes (2106 contigs and 3670 singletons) were generated. ESTs distribution based on protein function using a modified MIPS MATDB *Arabidopsis* Scheme revealed that 7% of the *V. shuttleworthii* ESTs were related to disease/pest defense or stress tolerance genes. Over 300 contigs containing complete or 90% open coding region of known functional genes were obtained. The ESTs that were annotated as

pathogenesis-related proteins, enzymes in Salicylic Acid, Jasmonic Acid and Ethylene Signaling were selected for further study in order to elucidate the role and interaction of them in the signal transduction cascade that leads to grape defense gene activation upon treatment of a bacterial pathogen. We plan to conduct global profiling of the pathogen-induced transcriptome between disease resistance and susceptible grapevines to identify novel disease resistant genes based on preliminary pathogenesis-relative pathways network analysis. In addition, over 800 putative SSR and SNP markers were isolated.