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The Grape Genetics and Breeding Program
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Bunch Grape Breeding and Product (wine) Evaluation

During the 2005 pollination season, twenty-four cross combinations were made between the Florida hybrid bunch grapes, advanced breeding lines, selective American hybrids, and *Vitis vinifera* premier wine grape cultivars (Table below)

Cross combinations and seeds obtained in 2005

Crosses	# Clusters	# Seeds
Bunch grape		
N18-6 x Blanc Du Bois	48	3402
N18-6 x Cabernet Sauvignon	20	652
N18-6 x Chardonnay	29	1195
N18-6 x Chenin Blanc	3	258
N18-6 x Merlot	18	974
N18-6 x Suwannee	31	1036
N18-6 x Zinfandel	3	47
O46-10-10 x O47-16-8	1	100
O46-10-10 x O47-15-5	1	221
O46-10-10 x O46-12-3	1	262
O46-10-10 x O47-16-10	4	733
O46-13-10 x Cabernet Sauvignon	11	1170
O46-13-10 x Cynthiana	9	765
O46-19-10 x O46-20-10	1	217
O46-19-10 x O46-18-8	4	106
O46-19-10 x O46-19-8	1	83
O46-19-10 x O46-23-10	1	211
O47-20-7 x O46-20-10	2	46
O47-20-10x O46-23-10	2	297
O47-23-7 x O47-22-4	1	257
O47-23-9 x V_2-66	5	962

O47-23-9 x O47-23-2	1	0
O47-23-7 x Cabernet Sauvignon	4	439
QB21-17 x Merlot	5	53

Evaluation of the advanced breeding lines was focused on viticultural characteristics /vine vigor, disease resistance, fruit quality for processing. Ten to twelve of the hybrid seedlings showed some good characteristics, high yield and disease resistance. These seedlings were multiplied for evaluation in further detail.

Experimental wines were made from some of these breeding selections at FAMU since 2003. Wine-qualities were evaluated by taste panel and a couple of them received favorable comments. Specific attention has been given to one of these selections, with FAMU trial number of C30-5-1. The vine is highly disease resistant and vigorous. Fruit qualities of C30-5-1 were comparable and better than other commercial bunch grape varieties. The berries were held very well until fully ripened. The berry has dark purple color, and color of the wine is deep red and stable.

Rootstock evaluation and selection

To understand the adaptation of grape rootstocks commonly used in major grape production areas worldwide to Florida, where Pierce's Disease (PD) and black rot are prevailing, ten major grape rootstocks were planted at the FAMU experimental vineyard. Their performance was evaluated based on the severities of diseases, vine vigor, adaptability to Florida soil and climate conditions. Data showed that none of these grape rootstocks was completely resistant to PD, black rot, anthracnose, and downy mildew. The overall growth performance suggested that 'St George' and 'Ramsey' are the most suitable rootstocks in northern Florida environment.

A few muscadine x bunch hybrids continue to show superior vigor and disease resistance. Experiments on graft compatibility, rooting ability were set up and it will take a few more years to know if these muscadine x bunch hybrids could be used as rootstocks.

Breeding New and Improved Muscadine Grape Varieties

The muscadine-breeding project is an important part of the overall grape breeding at FAMU. During the 05 pollination season, we made a total of 28 muscadine cross combinatios and produced a total of 2,766 seeds including:

1). Eight seeded muscadine cross combinations - 1,021 seeds obtained
2). Eleven muscadine /*V. vinifera* hybrids back crossing into seedless *V. vinifera* grapes – 1,148 seeds produced
3). Six cross combinations of tetraploids x diploids – 313 seeds obtained
4). Three combinations of wine grape varieties – 284 seeds produced

For continuing moving the seedless trait into muscadine grapes, pollination and embryo rescue was focused on the 'JT hybrid', a seedless muscadine x *V.*

vinifera hybrid. A total of 15 cross combinations with 187 flower clusters were backcrossed to either the seedless bunch grapes or the muscadine grapes. About 1,225 seed traces were extracted from at least 2,392 berries, which resulted in 102 embryos and 31 plantlets.

During the 05 growing season, we continued field-evaluation of the seeded and putative seedless hybrids produced from previous years for disease resistance, fruit quality, yield etc. About 10 more muscadine selections were added into the advanced selection group. Among them, two were 'seedless' muscadine hybrids. We found that the two seedless muscadine hybrids selected in 04 season were not true (genetically stabled) seedless. However, we can still use these 'seedless' lines as breeding parents for further breeding effort. We continued the on-going research of integrating the 'seedless' trait into seeded muscadine cultivars via genetic engineering technology.

Establishment of an Efficient Somatic Embryogenesis and Regeneration System in Muscadine Grape (*Vitis rotundifolia*)

Successful genetic transformation for grape improvement requires a reliable and efficient in vitro regeneration system. Somatic embryogenesis provides a unique capability for consistent plant regeneration and long-term supplies of materials for genetic improvement of muscadine grape cultivars. Somatic embryos were successfully induced from various tissues including ovules, anthers, petioles and leaves, with different medium and supplement combinations. The somatic embryogenic cell lines were well preserved and repeatedly multiplied in both solid and liquid media without losing its embryogenesis competence. A high productivity of synchronized somatic embryos was achieved in suspension culture. More than 95% of these somatic embryos could germinate and develop into normal plantlets within 30 days after transferring to regeneration media with appropriate supplements.

Isolation, Culture and Fusion of Grape Protoplasts from Somatic Embryos and Leaves

Somatic hybridization offers the possibility of manipulating plant genomes between species. Successful application of this technique in plant cultivar improvement requires a high efficient plant regeneration system. A method for preparing viable protoplasts from suspension culture of somatic embryogenic cells was established in *Vitis vinifera* grape 'Autumn Royal Seedless' (ARS) and *V. rotundifolia* 'Tara' (TR). The protoplasts were cultured on 0.6M BH3 medium (Grosser et al., 2000) containing 125 g/l sucrose and 0.5 g/l malt extract and MS (Murashige and Skoog, 1962) basal medium containing 20 g/l maltose and 0.5 g/l glutamine supplemented with 1 mg/l β-naphthoxyacetic acid (NOA). The highest plating efficiency was obtained on MS medium. Somatic hybridization between the muscadine and the bunch grapes were attempted for overcoming the inter-specific incompatibility. Protoplast fusions were

achieved between somatic embryogenic protoplasts from ARS and leaf protoplasts from 'Alachua' (*V. rotundifolia*), and between the somatic embryogenic cell line of TR and leaf protoplasts of 'Orlando Seedless' (*Vitis* hybrid) by using polyethylene glycol (PEG). Cell division and cell wall formation were observed three weeks after cultivation on MS basal medium with supplements described above.

Grape Functional Genomics / Bioinformatics Research

Diseases such as Pierce's disease, anthracnose, and downy mildew are the limited factors of growing the European grapes (*Vitis vinifera*) in the southeast United States. On the other hand, many Native North American grape species are tolerant / resistant to these diseases. However, little has been know about the mechanism and genetic basis of the host resistance, which has been greatly hampering our ability to genetically manipulate the host resistant genes for cultivar improvement. To improve disease resistance of the European grape varieties by using the host resistance from the native American species, a functional genomic project with emphasis on identifying, isolating, and characterizing disease resistant genes was initiated at the Center for Viticulture and Small Fruit Research, Florida A&M University, in collaboration with the USDA-ARS Horticultural Research Laboratory at Fort Pierce, Florida. *Vitis shuttleworthii* and *V. rotundifolia* (muscadine grape), the most disease / pest resistant grape species originated from the southeast United States, were chosen for the EST sequencing. We have sequenced 22,500 ESTs, from which 8,700 unigenes were generated. Functional annotation revealed that about 8% of them were correlated to disease resistance. Comparative genomic analysis indicated that about 20% of the genes found in the native grapes did not have homology in the European grapes (screening approximately 170,000 ESTs and 24,000 unigenes). Among the homologous gene sequences, a high frequency of SNPs and SSRs were detected between the *V. shuttlwworthii* and the *V. vinifera* genomes. Functional analysis revealed that high percentage of these SNP variations maybe correlated to disease resistance. With the combination of gene expression analysis and assistance of molecular markers, isolation and characterization of the host resistant genes will have great impact for the grape breeding process and genetic transformation.

Influences of Pollinators on Fruit Setting and Quality of Muscadine cv. 'Pam'

'Pam' is a popular muscadine grape characterized with large berry, nice appearance, good flavor, and high disease resistance. This cultivar, however, requires pollinators since it is pistillate. To search for better pollinators of this pistillate cultivar, a 2-year study was conducted at Florida A&M University in 2003 and 2005. Fresh pollen of muscadine grape 'Alachua', 'Nesbitt', and 'Noble' was used for pollination. 'Nesbitt' pollen resulted in 100% of the cluster pollinated setting fruits in both years, while 'Alachua' pollination yielded 70% (2003) to 87% (2005) of fruiting clusters, and 'Noble'

pollination yielded 72% (2003) and 97% (2005), respectively. Fruit numbers per fruiting cluster also varied among pollen sources. 'Alachua' pollen resulted in 7.2 (2003) and 8.1 (2005) fruits per cluster, while ‘Nesbitt’ produced 10.1 (2003) to 10.5 (2005) fruits per cluster, and ‘Noble’ produced 8.3 (2003) and 9.0 (2005) berries per cluster. Open pollinated ‘Pam’ had 100% clusters set fruits, with about 11 fruits per cluster in both seasons. Both sugar contents and acids levels were a little bit higher in 2005 than that in 2003. However, no differences of sugar content and acid level were found among the fruits derived from different controlled pollen sources, except the open pollinated fruits showed slightly higher sugar content. These data suggested that 'Nesbitt' is a better pollinator than 'Alachua' and 'Noble' for 'Pam' muscadine grape.

Publications

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