

The Grape Genetics and Breeding Program at FAMU

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Development of new grape cultivars is critical for the continuous growth of Florida wine industry. Since breeding perennial fruit crop like grape is a long-term process, this report is a continuation of on-going breeding effort for producing new grape cultivars for Florida Viticulture industry. During last few years, we have made good progress in the wine grape (hybrid bunch grapes) and Table (muscadine grapes) breeding project. Several breeding selections have demonstrated good potential in terms of disease resistance, productivity and general viticultural characteristics.

1) Advanced selections

Around 50 hybrid selections that showed some promising characteristics were established during last few years. These seedlings/advanced selections are the products of hybridization between Florida hybrids, American species originated from the southeast, and *Vitis vinifera* wine grapes. These hybrids were derived from advanced selections crossed with major *vinifera* wine grapes, including 'Chardonnay', 'Merlot', and 'Cabernet-Sauvignon'. Some of these hybrids are highly vigorous and disease resistant. During last few seasons, we have been evaluating all these hybrids with data of disease resistance, vine vigor, productivity, brix, acid, pH. We have set up 6 to 8 vines trial for most of these selections in FAMU vineyard. Some of the selections were planted in several other locations throughout the state.

Among the wine grape selections, the red wine grape breeding line #C30-5-1 exhibited excellent vine vigor and productivity, high disease resistance, and good fruit quality. This hybrid fully ripens in mid- August with uniformity. The vines hold the berries extremely well until harvesting, and no sign of fruit rotting was observed. Brix reaches about 16.0%, similar or better than the Florida hybrid bunch grapes that are being used by Florida wineries. PH and acidity are also favorable for processing. We have made wines from the C30-5-1 fruits during last three seasons and the red wine turned out to be good quality. The wine had stable deep red color. For almost three years, the color of the first batch made in 2003 has still been held pretty much the same. The wine was evaluated during the growers' Field Day in May, 2005 at FAMU, and again during the FGGA Annual Meeting in Ocala 2006, and received very good comments from researchers, wine makers and general publics in both events. Cuttings of this selections has been distributed to several growers and wineries for further test and evaluation. At this point, we are very optimistic about this red wine hybrid selection.

2). Interspecific hybridization with wine grape cvs. Of *V. vinifer*, American hybrids, and advanced selection

A total of 6,173 seeds were obtained in 2006, including 26 bunch-grape cross combinations and 39 muscadine grape crosses. These are the typical number of seeds produced annually. Seedlings will be planted in the field in the late fall /early winter.

3) Making wine from the advanced selection

We also conducted fermentation and other necessary procedures for making wines from the wine grape selections. During the 06 -07 season, a total of 24 selections were used for making wines. Evaluation of wine qualities among the selected hybrid lines is continuing.

4) Rootstock trial 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 in 2001. PD and fungal diseases were scored during each of the growing seasons for last five years. Based on the severities of leaf symptoms. Data showed that none of these grape rootstocks was completely resistant to PD and the fungal disease, and the severity of diseases varied. For example, 'Ramsey' and 'St George' showed least PD symptoms, while 'Freedom' and '3309C' had the highest PD scores. Vine vigor was also evaluated during last few growing seasons, and varied among the rootstocks as evidenced by trunk diameter, annual shoot length, annual shoot node number, internode length and shoot diameter. Based on the evaluation data, the overall growth performance suggested that 'St George' and 'Ramsey' are the most suitable rootstocks in northern Florida environment.

In the mean time, we have selected several muscadine x bunch hybrids that are showing superior vigor and disease resistance, for the rootstock trial. These muscadine x bunch grape hybrids may have a potential for new rootstock selection. During the 06 -07 season, we have been testing these hybrid selections for rooting ability and graft compatibility. The research will be continued in the following season.

5) Somatic Hybridization

Six somatic embryogenic cell lines were successfully established in muscadine grapes 'Fry', 'Tara', 'Triumph', and seedless *V. vinifera* grape cv. 'Autumn Royal Seedless', and wine grape cvs. 'Chardonnay', and 'Merlot'. We continued to propagate these cell lines in both solid and liquid medium. In addition, A new embryogenic lines were established of 'Orlando Seedless', 'Thomson Seedless', 'Alachua', which ensures constant supplies of protoplasts all year long.

Protoplasts were successfully isolated from somatic embryogenic suspension cultures of *V. vinifera* 'Autumn Royal Seedless' ($2n=2x=38$), and *V. rotundifolia* 'Tara' ($2n=2x=40$). Protoplasts were also isolated from in vitro

propagated plant leaves of Florida hybrid 'Orlando Seedless', *V. vinifera* 'Autumn Royal Seedless', 'Cabernet Sauvignon', and *V. rotundifolia* 'Alachua'. These cultures were established from shoot tip cultures and maintained on Lloyd and McCown Woody Plant (LMWP) medium supplemented with 1 μ M 6-benzyladenine (BA) by subculture every six weeks.

Purified protoplasts were used directly for culture experiment and fusion. The leaf protoplasts filled with green chloroplasts were easily distinguished from somatic embryogenic suspension culture protoplasts. They also provide an excellent marker for identification of fused products/somatic hybrids with the green chloroplasts of leaves integrated in somatic embryonic protoplasts. The preliminary result indicated that leaf protoplasts didn't show any sign of cell wall formation, cell division, or callus formation. Cell wall formation from embryonic protoplasts began 3-4 days after liquid cultivation. Cell division was observed in 5 days after cultivation, and calli were gradually formed. However, the efficiency of callus formation from the grape protoplast was generally low.

6) FAMU Grape Genomics Project

To identify the genes associated with disease / insect resistance, we use Expressed Sequence Tagged, or EST strategy, which are small genetic sequences of the genes being expressed inside the growing grapevines. The EST technique is the most effective technique for gene identification and isolation, which are widely used today to identify genes being expressed by a plant in response to a disease, environmental condition, or insect pest.

The grape ESTs are thoroughly investigated in the European grape (*V. vinifera*). Studies on grape transcriptional expression analysis for stress and defense relative gene identification are being conducted among the International Grape Genomics Community for years. More than 450,000 pieces of gene sequence (ESTs), which could be assembled up to 30,000+ functional genes, have been sequenced by many laboratories around the world. While most of the EST projects are focusing on the European grapes (*V. vinifera*), the FAMU (Florida A&M University) genomics project emphasizes on the native North American grapes. We chose a *V. shuttleworthii* and a *V. rotundifolia* (muscadine) grape, the most disease / pest resistant grape species originated from the southeast United States, for the EST sequencing. About 32,000 pieces of ESTs have been sequenced, from which about 10,000 genes were assembled.

These sequences have been analyzed for their corresponding proteins by comparing to the GenBank database. By isolating these sequences from the plants, we have been able to identify genes associated to disease resistance, fruit quality, drought tolerance, insect resistance and more. The sequence analysis also leads to the discovery of genes uniquely found in the native North American grape species. By comparison of gene sequences between resistant and susceptible grape species, we were able to identify genes only from the native *V. shuttleworthii* and muscadine grapes, but not present in the genetic dataset from other *Vitis* species. This unique set of genes was included on the

commercially available, 'The GeneChip® *Vitis vinifera* Genome Array', produced by Affymetrix®. Microarrays are a method wherein thousands of genes can be screened at once for their expression levels in a plant, under set conditions. The conditions may be infection with a plant disease, fed on by insect pests, or specific to a development stage such as fruit set, or fruit ripening. Expression analysis advances the understanding of which genes are functioning in specific plant pathways. We can then determine if disease resistant grape plants also express this pattern and use these genes to screen (test) our seedling populations to select those seedlings which have these genes. The ability to examine grape seedlings for multiple disease resistance in this rapid manner expedites the selection of seedlings to be saved and planted into the vineyard for further evaluations. Similarly, the 'gene chips' technology is being used for identification of anthracnose and Pierce's disease resistance genes from the native grape species. A dozen of putative disease /pest resistant genes have so far been identified.

The FAMU/ARS marker development project focuses on developing SSR and SNP molecular markers based on the grape EST sequences. This approach takes advantage of the gene sequences (ESTs) available in the public databases to identify and validate the potential markers correlated with disease /pest resistance. These EST-derived markers are within transcribed regions of the DNA, and are expected to be more transferable than anonymous markers. Furthermore, the SNPs found in the transcribed regions could be the direct causes for observed phenotypic variation.

Of the 30,000 *V. shuttleworthii* and *V. rotundifolia* gene (EST) sequences, around 600 putative SSR markers have been identified. Together with the *V. vinifera* EST sequences in the public database, over 1,100 genes containing unique SSR sequences have been mined. The potential usefulness of each SSR is being validated by PCR amplification on selective grape DNA templates, and some of which are found to be useful for marker development .

The gene to gene comparison between the native North American grapes and *V. vinifera* genomes is being used to identify single nucleotide substitution, which could be used for the development of Single Nucleotide Polymorphisms (SNPs) markers. For example, when we aligned about 10,000 ESTs between *V. shuttleworthii* and *V. vinifera* 'Cabernet Sauvignon' ESTs, we found about 400 SNP sites between these two genomes. These single nucleotide replacements, or SNPs, are being used for marker development in our laboratory.

