

SERA014 Progress Report 2005

The Genetics and Breeding Program Florida A&M University

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Research Projects

1. Breeding New and Improved Muscadine Grape Varieties

The muscadine-breeding project is an important part of the overall grape breeding at FAMU. The funding for the 2004-2005 year is a continuation of our long-term muscadine breeding, including seedless muscadine grape breeding effort. Three major breeding goals for the muscadine grapes are: 1) breed perfect-flower cultivars with large berries and fruit quality similar or better than 'Fry'; 2) select new cultivars with better fruit qualities for fresh fruits (e.g. higher soluble solid 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.

For continuing the moving of the seedless trait into muscadine 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. Several crosses of bunch x muscadine grapes were also made by using embryo rescue (Table 1). More than three hundred seed traces were extracted from over 1,000 berries, which resulted in 15 embryos and 6 plantlets. These plants are in the process of transferring into the greenhouse.

Several seeded muscadine x bunch grape hybrids were also used for back-cross into seedless *V. vinifera* and muscadine grapes. Twenty-five crosses, with flower cluster ranging from 5 -15 in each cross, were pollinated. However, many of these crosses produced no fruits and seeds (Table 2).

During the 2004-2005 season, we continued field-evaluation of the putative seedless hybrids produced from previous years for disease resistance, fruit quality, yield etc. About 12 more muscadine selections were added into the advanced selection group. Among them, two were seedless muscadines in the 2004 season.

However, seeds were found in the berries of these two muscadines in the 2005 season. In addition, the 'seedless' trait that was engineered into muscadine cv. 'Fry' was further evaluated in the greenhouse in the 2005 season.

Table 1. Breeding Seedless Muscadine Grape by Embryo Rescue (2004)

Cross	# Bags	# Clusters	# Berries	# Seed	Plants
JT x Orlando Seedless (JTO)	42	9	130	13	0
JT x Burger Seedless (JTB)	12	7	15	1	0
x Thompson Seedless	44	2	2	3	0
x Cowart	26	0	0	0	0
x S21	10	1	13	5	1
x Tara	10	0	0	0	0
x Triumph	10	0	0	0	0
x A2-14-7	5	0	0	0	0
x A12-4-8	3	3	0	0	0
x B27-22-2 (A)	10	10	1	3	0
x B27-23-2 (B)	12	10	241	40	5
JT Open pollinated	16 Plates	0			

2. Bunch Grape Breeding and Product (wine) Evaluation

2.1. Advanced selections

Around 70 hybrid selections that showed some promising characteristics were established during last five years. Evaluations of hybrids produced from previous years were conducted throughout the 04-05 season. Viticulturally important characteristics, flower type, productivity and fruit quality were recorded for individual selections. Half a dozen advanced lines among the best were evaluated in further detail, including vine vigor and disease resistance, viticulturally important characteristics, productivity and fruit quality. Experimental wines were made from these selections in 2003 and 2004 season (Table 3). Wine-qualities were evaluated by taste panel and a couple of them received favorable comments. One of them, C30-5-1, has very good color that has been held very well for over two years. It also contains some unique flavor and aroma. These advanced selections were propagated (dormant cutting) and planted up to eight

vines each in FAMU Experimental Vineyard. A State-wide trial for these selections has been arranged. Some of the selections have been planted at several wineries for further evaluation.

Table 2. Summary of Muscadine Grape Pollination (2004)

Crosses	# Cluster	# Harvest	# Berries	#Seeds
JT Hybrid				
x Cowart	26	0	0	0
x Tara	10	0	0	0
x Triumph	10	0	0	0
x A2-14-7	5	0	0	0
x A12-4-8	3	3	0	0
x B27-22-2 (A)	10	10	1	3
x B27-23-2 (B)	12	10	241	40
MV hybrids x Seedless				
A12-3-6 x Alachua	6	3	0	0
MV hybrids x muscadine				
A11-4-4 x Alachua	9	9	58	142
x Cowart	10	9	65	77
x Tara	10	8	2	4
A12-3-6 x Alachua	10	8	0	0
A12-4-8 x Alachua	5	5	0	0
4x x 2x muscadine				
O25-14-8 x Alachua	10	8	3	75
x Tara	5	5	0	0
B20-1-2 x Alachua	3	1	0	0
Muscadine hybrids (MH) x MH				
A2-13-4 x A2-15-7	4		76	0
A5-16-3 x A5-15-5	6		69	0
A5-16-3 x A5-14-3	3		0	0

Table 3. Bunch grape selections used for making wine in 2003 and evaluated in 2004

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
A30-5-1	Red	17.0	0.7
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

2.2. New selections

Seedlings produced in the last few years were established in the D, E and O Blocks of the FAMU vineyard. Evaluations/selections were conducted for those setting fruits during the 04 and 05 seasons. Five wine grape hybrids that showed some good characteristics, high yield and disease resistance were selected. In addition, we found two seedless grapes that have potential for table grape development. All these selections will be further evaluated during the next few years, and selections are expected as more hybrids set fruit.

2.3. Crosses of bunch grapes

Crosses were made between the *Vinifera* wine and Table grapes, Florida hybrid bunch grapes, advanced breeding lines, and selective American hybrids, and hybrid of muscadine/bunch grapes (Table 4). Due to the early season diseases or other unexplained reasons, many flower clusters of the bunch grapes dried right before anthesis or after bagging or after pollination. Many of the flowers pollinated (listed in Table 4) were from the secondary flowers, which were forced out in May by cutting the short back. As the result, the number of seeds obtained was less than planned.

2.4. Testing existing wine grape selections and cultivars from other breeding program

(1). Q21B#17, Dr. Olmo's selection derived from a cross between a tropical grape in South America and *vinifera*. It has been under field trial at Hammond Research Station, Louisiana, for a few years. It is reported to be PD resistant and used for making a good red wine in Mississippi and Louisiana (personal communication). The hybrid QB21-17 showed large clusters, good productivity and moderate disease resistance. Wine was made from the fruits of this grape. Our conclusion is that even if it can't be used as a wine grape cultivar right away, it would be a very good breeding parent for wine grape development.

(2). Cynthiana/Norton grape The Cynthiana (Norton) is a popular red wine grape in Arkansas and Missouri. This grape has been tested at FAMU for about 7 years. The vines deteriorated in about 4-5 years and we have now lost 75% of the original planting, and the rest of them do not look good and are expected to die within 1-2 years. We therefore concluded that Norton grape is not hardy enough for the Florida environment and may be PD susceptible, and it is unnecessary to conduct a further testing.

3. Develop New Grape Cultivars by Interspecific Somatic Hybridization

Somatic hybridization offers the possibility of manipulating plant genomes between species. Successful application of this technique in plant cultivar improvement requires a highly 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 the MS medium. Somatic hybridization between the muscadine and the bunch grapes was 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.

Table 4. Summary of bunch grape pollination in 2004

Cross	# Cluster	# Harvest	# Berries	#Seeds
N18-6 x Orlando Seedless	5	5	5	104
N18-6 x P48	10	0		
QB21-17 x Blanc du Bois	10	10	10	1137
x S21	10	10	9	403
x V2-66	10	10	10	288
JT Hybrid				
x Burger Seedless	12	12	15	13
x Orlando Seedless	42	40	130	1
x Thompson Seedless	44	2	2	3
x S21	10	1	13	5
x B27-22-2 (A)	10	10	1	3
x B27-23-2 (B)	12	10	241	40
Hybrid Selections x				
O45-17-7 x O45-12-10	3	2	0	0
O45-12-10 x O45-17-7	5	3	8	29
O47-23-9 x O47-23-2	5	3	20	10
MV hybrids x Seedless Bunch Grapes				
A11-4-4 x Burger Seedless	20	19	89	251
x Orlando Seedless	10	3	46	24
x Princess Seedless	10	9	?	176
x Thompson Seedless	10	0	0	0
A12-4-7 x Orlando Seedless	3	2	0	0
A12-4-8 x Orlando Seedless	10	9	0	0
MV hybrids x wine grapes				
A11-4-4 x Blanc du Bois	10	10	0	0
x Cabernet Sauvignon	10	10	0	0

4. Grape Somatic Embryogenesis, Suspension Culture and Genetic Engineering

Somatic embryogenesis is an efficient tool for rapid propagation, genetic transformation, somatic hybridization, and somaclonal variation. Pre-embryogenic calli were obtained from the culture of fertilized immature ovules of muscadine (*Vitis rotundifolia* Michx.) and seedless bunch grapes (*Vitis vinifera* L.). Somatic embryogenesis was achieved in seedless bunch grape cultivars 'Autumn Royal Seedless', 'Crimson Seedless', and muscadine grape cultivars 'Alachua', 'Summit', and 'Tara'. The frequency of somatic embryogenesis was greater in callus derived from white crystal callus than others and was highly genotype-dependent and thus far was confined to these cultivars. Embryogenic calli maintained their regeneration capacity for 24 months. Somatic embryos proliferated rapidly and germinated and developed into normal plantlets after transfer to Lloyd and McCown Woody Plant (WP) medium supplemented with 1 μ M BA and 1.5% (w/v) sucrose.

Grape biotechnology has great potential for grape genetic improvement. However, successful implementations of grape biotechnology, e.g. transformation and *in vitro* selection, is based on a high yield productivity of synchronized somatic embryos as well as an efficient single cell regeneration system. Proembryonic mass (PEM) suspension culture of 'Autumn Royal' seedless was established from primary somatic embryos in liquid medium. The PEMs, yellowish in color, grew rapidly in the liquid medium. Browning was not observed in the liquid medium nor the PEMs. More than 1,000 mature somatic embryos could be produced from approximately 50 mg of SPEMs within 14-16 weeks on solid MSA medium. The somatic embryos were easily germinated (100%) in MSA medium and up to 95% of them were converted into normal plantlets.

Several antifungal peptides genes are being transferred into disease susceptible *V. vinifera* seedless grape cv. "Autumn Royal Seedless". In the mean time, the EGFP reporter gene is being used for improving the transformation system, for both Biolistic and *Agrobacterium* system. The regeneration system (section 5) has been very well developed for the grape transformation work.

The twenty putative seedless transgenic lines of muscadine cv. 'Fry' were maintained and evaluated in the greenhouse, and subjected to lab evaluation /confirmation.

5. Grape Genomic / Bioinformatics Research

We have sequenced 25,000 ESTs from a clone of *Vitis shuttleworthii* grape that has been using extensively in several grape breeding programs in the southeast United States. Blasting analysis revealed that 13% of the *V. shuttleworthii* ESTs are unique when compared to the existing *Vitis vinifera* NCBI databases, and 3% of the ESTs did not find any homologous sequences to all plant ESTs reported in NCBI. Overall, approximately 7% of the ESTs were related to disease / pest defense or stress tolerance genes. To develop molecular markers for marker-assisted grape breeding, SSRs from our *V. shuttlewothtii* sequences and *V. vinifera* ESTs in the public domain were screened. Of the 12,056 *V. shuttleworthii* ESTs, 401 putative SSR loci were identified. Among them, tri-SSR appeared to be the most abundant repeats, and CAA/GTT and ACC/TGG were the most abundant tri-SSR. GA/CT was the most abundant repeat type among the di-SSR. The potential usefulness of each of the SSRs is being verified by PCR amplification on selective grape DNA templates

6. Field Evaluation of Grape Rootstock Response To Natural Infection By Pierce's Disease

Ten major grape rootstocks commonly used in other viticulture areas were evaluated in FAMU experimental vineyard for last four years. Evaluation data showed that none of these grape rootstocks was completely resistant to PD and black rot, 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 three 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 accumulated evaluation data (three years total), the overall growth performance suggested that 'St George' and 'Ramsey' are the most suitable rootstocks in northern Florida environment.

Publications:

- Jiang Lu, Hong Huang, Wayne Hunter, Phat Dang. Towards to Identifying Pierce's Disease Resistant Genes from A Native American Grape Species *Vitis Shuttleworthii* – a Genomics Approach. Proceedings of Pierce's Disease Research Symposium
- Cousins, P., J. Lu and Z. Ren. Rootstock variety influence on Pierce's disease symptoms in grafted Chardonnay (*Vitis vinifera* L.) grapevines. Proceedings of Pierce's Disease Research Symposium
- Paraiso O., Lu J., Bloem K., Sheick M., Hopkins D. L., and H. Yun. Correlation between Resistance to Pierce's Disease and the *Xylella* Strain Virulence Using Partially Purified Culture Filtrate. Proceedings of Pierce's Disease Research Symposium. .
- Xia Xu, Jiang Lu*^T, Zhongbo Ren, and Stephen Leong. Callus Induction and Somatic Embryogenesis in Muscadine and Seedless Bunch Grapes (*Vitis*) from Immature Ovule Culture. Proceedings of Florida State Horticulture Meeting.
- Z. Ren, J. Lu, X. Xu, and S. Leong. Influences of pollinators on fruiting and fruit qualities of muscadine cv 'Pam'. Proceedings of Florida State Horticulture Meeting.

Presentations in professional meetings:

- Hong Huang, Jiang Lu, Wayne Hunter, Phat Dang. Microsatellite (SSR) Frequency and Distribution among the Grape ESTs and Identification of Disease Related EST-SSRs. International Conference of Plant and Animal Genome. San Diego, California
- Lu, J., H. Huang, W. Hunter, P. Dang, S. Leong. Comparative analysis of *Vitis* species ESTs (Expressed Sequence Tags) and *Arabidopsis* genome sequences. American Society for Plant Biologist Annual Meeting, Orlando Florida.
- Huang, H., J. Lu, W. Hunter, P. Dang. Identification of pathogenesis related genes of *Xylella fastidiosa* by comparative analysis of transcriptional profiling using microarrays. American for Plant Biologist Annual Meeting, Orlando Florida.
- Lu, J., H. Huang, W. Hunter, P. Dang. Pathogenesis-Relative Pathway and Relative Putative Gene Annotation in *Vitis shuttleworthii* Grape through EST Analysis. Amer. Soc. For Hort. Sci. Annual Meeting. Austin, Texas.
- Lu, J., H. Huang, W. Hunter, P. Dang, S. Leong. Identification of Disease Defense- and Stress –Related Genes in the Grape *Vitis shuttleworthii* Grape though EST Analysis. International Conference of Plant and Animal Genome. San Diego, California
- Xia Xu, Jiang Lu, And Zhongbo Ren. Somatic Embryonic Line Establishment from Ovules of Muscadine and Seedless Bunch Grapes. FSHS Annual Meeting. Orlando, Florida.