PROJECTS FUNDED BY THE SOUTHERN REGION SMALL FRUIT

CONSORTIUM FOR 2011

Title: **Determination of Flower Type and Other Traits in Muscadine Grape Using Molecular Markers**

Final or Progress Report(Indicate which): **Progress Report**

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Objectives:
1. To determine phenotypically the flower type (perfect, pistallate, staminate) of muscadine grape cultivars and selections along with appropriate seedling populations segregating for flower type.
2. To genetically map the flower sex locus in muscadine grapes and to develop molecular markers suitable for screening seedlings for flower type prior to planting in the field. This technology would then be available to all muscadine grape breeders.

Justification:
Muscadine grapes (subgenus *Muscadinia, Vitis rotundifolia)* have three flower types, perfect (self-fruitful with male and female flower parts on the same flower), pistillate (female flower parts only and require a pollen source to set fruit), and staminate (male flower parts only, no fruit produced). Commercially there are both female and perfect-flowered cultivars used. However, the most desirable type is perfect flowered, as no concerns for pollenizer source must be considered by the grower in designing vine placement in the vineyard. A primary objective of the University of Arkansas muscadine breeding program, along with all other muscadine improvement programs, is the development of perfect-flowered cultivars. Parents in the program are both pistallate and perfect-flowered.

Conversely, *V. vinifera*, the primary grape in commerce in the world, is most often perfect-flowered, and breeding programs using only this species, or only selections of *Euvitis* (bunch grape subgenus) species that are perfect flowered, do not have as great a concern with flower type segregation.

Until 1946 all fruiting muscadine cultivars were pistillate and required interplanting of pollinizers (Goldy, 1992). Loomis (1948) and Loomis et al. (1954) investigated the genetics of flower type inheritance and concluded that inheritance in muscadines is a qualitative trait similar to *Euvitis*. Additionally, Loomis found that when self pollinated, 21 perfect-flowered parents were placed in to two groups: those producing perfect flowered and pistillate progeny, and those producing perfect, pistillate, and staminate progeny. The first group was found to have the same parent, Dearing's selection H1, and the second group was found to have Dearing's selection H2. H1 and H2 were two of the first observed hermaphroditic muscadine vines developed (Dearing,1917). Based on this work it was clear that the perfect-flowered parents were heterozygous for perfect flowers. More recently, Zhongbo and Lu (1999) found that among 65 seedlings of 'Summit' (pistillate) X 'Noble' (perfect), the ratio of pistillate:perfect flowers was 35:30 and was a close fit to a 1:1 Mendelian ratio. The genotype of 'Noble' was therefore interpreted as heterozygous Hh, and 'Summit' as homozygous hh.

However, flowering type cannot be determined until the vines are mature enough to flower, which is the third growing season in Arkansas. Therefore, pistillate and a limited number of staminate vines are grown out along with perfect-flowered genotypes in these populations, and substantial management costs (planting, training, weed control, pruning, fertilization, seedling removal, etc.) along with vineyard space are expended to grow the vines to maturity. Additionally, at the time of fruit evaluation, a seedling cannot be identified as pistillate or perfect at the time of fruiting and both types bear fruit. Identification of flower type can only be determined by examining the genotype at the time of flowering in late May to early June. Therefore, selection efficiency is not as high as the fruiting vines could be pistillate or perfect-flowered and identifying superior perfect-flowered vines will not be done until the following year. A breeding program could then carry more pistillate selections than desired.

Recent work by Dr. Chris Owens with the USDA-ARS Grapes Genetics Research Unit at Geneva, NY has focused on *Euvitis* (bunch grape) flower type determination. A key reason to research this topic is that there are bunch grape breeding programs that are not exclusively *V. vinifera* in the U.S. and have flower type segregation in their populations. The best example is the University of Minnesota program, where *V. riparia* genotypes (that vary in flower type) are used in breeding due to their superior cold hardiness. In working with Dr. Jim Luby at the University of Minnesota, Dr. Owens began work on molecular determination of flower type in 2009 . The flower sex locus has previously been genetically mapped to linkage group #2 in several interspecific populations (Dalbo et al. 2000; Lowe and Walker, 2006; Riaz et al 2006; Marguerit et al. 2009). Dr. Owens has recently conducted fine-mapping of the flower sex locus in 2 segregating populations and localized the locus to a narrow region of 91 kb. The DNA sequence of the genes within this region has been determined in selected progeny of these crosses and several individuals of *V. vinifera* and *V. riparia* that vary in flower type. Strong genetic evidence suggests that one of these genes in this region is a strong candidate for controlling flower sex in *Euvitis* species. SNPs identified within this candidate gene are now

being used to screen previously phenotyped selections from the University of Minnesota breeding program to confirm the suitability of these markers for screening seedlings. There is value in expanding work to include muscadine grapes in this effort. It's entirely possible there is some other genetic mechanism occurring in muscadines. However, the first step is to genotype/sequence muscadine cultivars or selections of known flower type, to compare these results to those found in *Euvitis*. Subsequent to this is to collect DNA from a segregating population of muscadines (that have flowers examined at bloom for flower type identification) to determine the genomic location of the muscadine flower sex locus and identify tightly linked molecular markers that would be useful in marker-assisted breeding. An additional gain from this work would be to learn more about the muscadine genome, and muscadine flower type locus in the genome.

Methodologies:
The proposed work includes the following procedures:

Step 1. Leaves will be collected from known flower type muscadine genotypes (selections and/or varieties) at an early growth stage (late April to May). Also, a limited number of seedlings in selected populations segregating for flower type will be sampled. These will be shipped to Dr. Owens for DNA extraction.

Step 2. Molecular analysis will be conducted on the DNA, using the methods and molecular markers used for the *Euvitis* research.

Step 3. Flower type of seedlings sampled will be determined at flowering. Seedling vines will be characterized as perfect, pistillate, or staminate. Flower type will also be re-confirmed on parental genotypes. Genetic map will be constructed and flower sex located in the genome.

Step 4. Evaluation of molecular success will be undertaken, and if a successful marker is identified, a second year of work will be conducted on additional seedling populations to confirm repeatability of the methods.

Vines for all work will be grown at the University of Arkansas Fruit Research Station, Clarksville. All vines are trained to a single-wire trellis, cordon trained, and spur pruned. Selection and variety vines are routinely spaced 20 ft between vines, with two, 10-ft. cordons. Seedling vines are spaced approximately 2.5 ft., with a 2 ft. cordon established. Vines will be irrigated, and have annual routine vineyard management practices conducting including annual dormant pruning, fertilization, weed control, and irrigation.

DNA will be extracted using established protocols for grape leaf tissue. Candidate genes will be PCR amplified, cloned, and resulting clones will be Sanger sequenced to identify polymormpisms between flower sex types. SNP markers for genetic mapping will be simulatenously identified and genotyped by a modified Genotyping-by-Sequencing (GBS) protocol (Gore et al. 2009) that will genotype several 1000 genome-wide SNP markers. Standard protocols for double pseudo-testcross mapping will be employed using JoinMap.

Results/Progress:

Two populations and their parents were identified for study at the University of Arkansas Fruit Research, Clarksville and flower type phenotyping and results are as follows:

Black Beauty (pistillate) X Nesbitt (perfect) with 172 plants. Flower type was identified for 149. First-year results show 85 are perfect-flowered (57%) and 64 are pistillate (43%.)

Supreme (pistillate) X Nesbitt (perfect) with 174 plants. Flower type was identified for 155 plants. First-year results show 109 are perfect-flowered (70%) and 46 are pistillate (30%.)

Additionally, the study was expanded from what was proposed to include additional traits to phenotype including berry weight, berry juice and pH, berry color, percent dry picking scar, texture, stem and petiole color, and dates of bloom and ripening. Additional data collection is complete for this year except for berry weight, soluble solids and pH measurements which are still being taken from frozen fruit samples.

Leave samples were collected from all seedlings in the above populations and their parents in April. Leaves were sent to USDA-ARS, Geneva, NY for molecular work. Leaf tissue was received in good condition, and DNA was extracted from all samples. Genotyping-by-sequencing (GBS) libraries have been prepared from the two populations, as well as the parents. The GBS protocol allows for 96-fold multiplexing of DNA samples. DNA sequence data has been collected for the two segregating populations and approximately 60,000 SNPs have been identified in each cross that are suitable for genetic mapping. Data from the molecular work will be analyzed and combined with the phenotypic data for marker associations. This portion of the project is still underway.

The two populations and their parents have been maintained in the field using routine cultural inputs and these cultural inputs will be continued in 2012.

Request for funding to continue this study has been made in 2011-2012 proposal submission for Consortium review.

Conclusions:
No conclusions determined at this time.

Impact Statement:
This study has not been completed thus no impact can be stated as of this progress report.

Citation(s) for any publications arising from the project:
No publications have resulted from this project.

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