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

Final or Progress Report (Indicate which): **Progress Report for 2012 Activities**

Grant Code: **SRSFC Project # 2012-02**

Research or Extension Proposal: **Research**

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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 (abbreviated from proposal):
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). 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.

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. 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. 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. 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 for 2012:

Step 1. Molecular analysis will be continued on on the DNA, using the methods and molecular markers used for the Euvitis research. 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.

Step 2. Flower type of seedlings sampled will be determined at flowering for a second year. Additionally, characterization of traits berry weight, berry juice and pH, berry color, percent dry picking scar, texture, stem and petiole color, and dates of bloom and ripening will be done for the second year (was not in the 2011 proposal).

Step 3. A Genetic map will be constructed and flower sex and other traits as possible located in the genome.

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.The proposed work includes the following procedures:

Results/Progress:

A progress report for 2011-funded research up thru activities of November 2011 was made last year. Progress from that date forward until November 2012 is reported here.

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

Black Beauty (pistillate, black) X Nesbitt (perfect, black) had the following phenotyping results

Flower type had 85 perfect, 64 pistillate, 23 no data (57% perfect, 43% female) (only two vines conflict with 2011 data for flower type, indicating very consistent flower type phenotyping and those conflicting were usually due to very limited flower numbers to examine). The ratio of flower types is very near 1:1, which agrees with work of L.R. Detjen reported in 1917 and later by Harold Loomis reported in 1957.

Berry color had 119 black, 42 bronze (74% black, 26% bronze) for 2012 fruits and in 2011 fruits 102 were black and 41 bronze (71 and 29%, respectively) with the difference being more vines fruited in 2012 than 2011, not that any errors in berry color were made in phenotyping. The ratio of black to bronze is very near 3:1 as more black fruit would be expected and the two parents are heterozygous for fruit color (both have the bronze Fry cultivar as a parent).

Avg. weight per berry of the population was 4.8 g from frozen samples from 2011.

Avg. soluble solids was 15.7% measured on frozen samples from 2011 and measurements completed in May, 2012.

Avg. pH was 3.2; same vines as SS.

Avg. titratable acidity 0.66%; same vines as SS and pH.

Avg. flavor (using a 1-10 scale with 10 best) 8.4 from 161 fruiting vines in 2012.

Avg. number of dry scars 40% (out of 10 berries per vine) from the same fruiting vines.

Fruit texture was 53 crisp,102 not crisp (34% crisp, 66% not crisp) from 155 vines (2012). In general Black Beauty is considered “crisp’ to some degree, so the recovery of crisp from this cross with the non-crisp Nesbitt is encouraging although large populations might be needed to get the combination of crisp with other characters since texture is likely a quantitative character.

Skin texture was 60 thick skin, 95 not thick skin (39% thick, 61% not thick) with 172 plants (2012). Again, Black Beauty is the thinner skinned of the two parents, and recovering the not-thick skin is encouraging to a degree, much like fruit texture.

Supreme (pistillate, black) X Nesbitt (perfect, black)

Flower type was 109 perfect (70%) and 46 pistillate (30%.) (only two vines conflict with 2011 data). Again this is very similar to the Black Beauty x Nesbitt population in inheritance.

Berry color was 129 black, 34 bronze (79% black, 21% bronze) for 2012 fruits and in 2011 fruits 139 were black and 34 bronze (80% black, 20% bronze). Again this is very similar to the Black Beauty x Nesbitt population.

Avg. weight per berry of the population was 4.5 g from frozen samples from 2011.

Avg. soluble solids 16.4% measured on frozen samples from 2011 and measurements completed in May, 2012.

Avg. pH was 3.2 same vines as SS.

Avg. titratable acidity 0.63%; same vines as SS.

Avg. flavor (using a 1-10 scale with 10 best) 7.0 using 163 vines in 2012.

Avg. number of dry scars 40% (out of 10 berries per vine) from the same fruiting vines.

Fruit texture was 77 crisp and 78 not crisp (50 % crisp, 50% not crisp) from 163 vines (2012). In general Supreme is considered "crisp" to some degree, so the recovery of crisp from this cross with the non-crisp Nesbitt is encouraging and Supreme might transmit this trait more effectively than Black Beauty although this is a very subjective trait and more measurements are needed to verify this

Skin texture was 109 thick skin and 44 not thick skin (71% thick, 29% not thick), markedly different from the Black Beauty x Nesbitt cross. Possibly Supreme transmits better skin texture than Black Beauty but more observations are needed to verify this preliminary finding.

Genomic DNA was isolated from leaf tissue collected in 2011 from 172 progeny of the Black Beauty x Nesbitt population and 173 progeny from the Supreme x Nesbitt population as well as the parents. Genotyping of the individual progeny and parents was conducted by Genotyping-by-Sequencing (GBS) (Elshire et al. 2011). 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.

The typical GBS analysis pipeline utilizes a reference genome for aligned sequence reads and calling SNPs. Utilizing the *V. vinifera* reference genome to call SNPs from *V. rotundifolia* has proven to be a challenge, and many low-quality SNPs were produced. An alternative method was chosen to identify SNP markers that does not require alignment to a reference genome (Buckler, unpublished). Dense genetic maps have now been generated for these two crosses. Testing of a genetic marker that has been shown to correlate with flower sex in Euvitis (Fechter

et al.) has shown no correlation with flower sex in these two *V. rotundifolia* populations. Work is now underway to genetically map flower sex and the other fruit quality traits onto the genetic maps.

Conclusions:
Genetic ratios for flower type and berry color were generated and agree with prior reports of the inheritance of these traits. Until further molecular analysis of the data is conducted, no further conclusions can be reported 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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