

**Title: Development of Simple Sequence Repeat DNA markers for Muscadine Grape Cultivar Identification.**

**Progress Report**

**Grant Code: SRSFC Project # 2018 R-06**

**Research Proposal**

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**Objectives:** 1) To develop a set of simple sequence repeat (SSR) DNA markers suitable for identifying muscadine grape cultivars 2) to use the SSR markers to elucidate several discrepancies in muscadine cultivar identification.

**Justification and Description**

Muscadine grapes, *Vitis rotundifolia*, were first cultivated in North America in the 16th century by European settlers, but didn't become commercial until the middle of 18th century. Early cultivars were usually collected from the wild, but current cultivars all come from breeding programs. These breeding efforts have resulted in large collections of *V. rotundifolia* cultivars and germplasm. However, accessions within these collections are only documented by comparing breeding records and morphological traits, which can be both unauthentic and equivocal. We have been approached several times in the last couple years by nurserymen and growers alike asking about the possibility of DNA testing of vines to authenticate muscadine cultivars. Some of the issue that seem to exist are: 1) at least two different 'Triumph' cultivars, one of which that is relatively large with a firm flesh and a second that is smaller with a more pink coloration; 2) more than one possible 'Carlos' in North Carolina with differing fruit quality; 3) two different 'Tara' cultivars with one seeming to yield less than the other; 4) more than one 'Noble' cultivar with differences in fruit size; 5) two different 'Granny Val' cultivars located at UGA and Univ. of Ark. It is difficult to resolve these issues based on morphological characteristics as differences are often relatively small and could be explained by cultural or environmental differences. We also are approached with the normal questions growers often have about the identification of unknown cultivars and whether a different looking vine is a sport or perhaps an unknown cultivar.

In order to answer some of these questions, we propose to develop a database of simple sequence repeat (SSR) marker profiles using all widely planted and historically important muscadine cultivars. SSR markers, otherwise called microsatellites, are a tract of repetitive DNA in which certain DNA motifs (normally ranging in size from 2-5 bp) are repeated from 5-50 times. SSRs occur at thousands of locations in a genome and have a higher mutation rate than other areas of DNA leading to high genetic diversity.

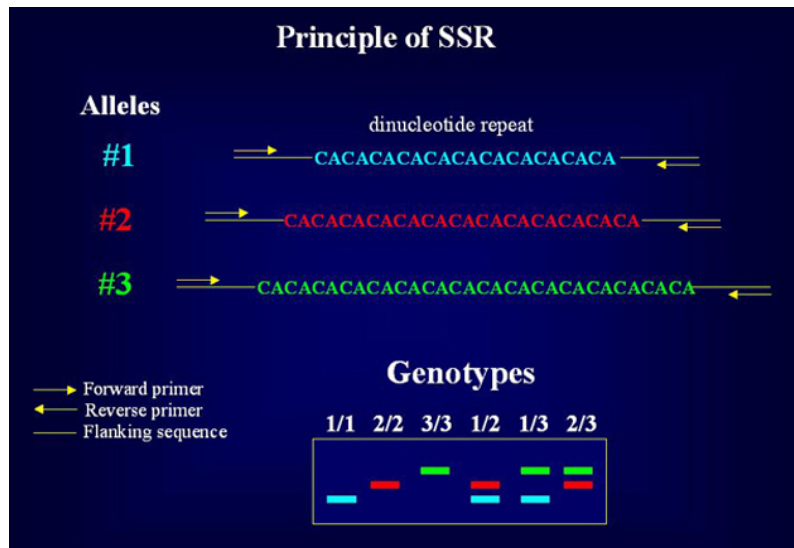


Fig. 1. Principle of SSRs. By looking at enough different SSR markers, cultivars can be unambiguously identified via their SSR genotypes.

Image from <http://www.cdfd.org.in/SILKSAT/>

There are a wide array of DNA-based markers that have been developed for cultivar identification, and each has their own advantages and disadvantages. We propose using SSR markers for several reasons: 1) a wide array of SSR primers have been developed in *V. vinifera* and previous research has shown that many of these are useful in *V. rotundifolia* (Riaz et al., 2012); 2) SSR marker protocols are relatively simple, allowing our lab to complete most steps “in house” which increases the speed of the analysis and lowers its cost; 3) SSR markers have good lab to lab transferability making an SSR database useful to the greater muscadine research community; 4) an SSR marker database would be useful for other studies including studying pollen flow within a vineyard and the effect of cross-pollination on berry size (metaxenia).

SSR markers have been used in muscadine in both genetic mapping (Blanc et al., 2012; Merdinoglu et al., 2003; Riaz et al., 2012) and in pedigree and diversity studies (Riaz et al., 2008). The study by Riaz et al. (2008) is an excellent starting point for this work as it developed an SSR profile for 39 muscadine cultivars using 14 SSR loci. Unfortunately, the cultivar collection utilized by Riaz et al. consisted of a large number of historical cultivars and lacked most of the cultivars released by Ison’s Nursery which form the basis of much of the fresh-fruit industry including: ‘Black Fry’, ‘Darlene’, ‘Early Fry’, ‘Granny Val’, ‘Ison’, ‘Late Fry’, ‘Pam’, and ‘Sweet Jenny’. Also lacking were more recent releases including ‘Delicious’, ‘Hall’, ‘Lane’, ‘Paulk’, ‘Scarlett’, and ‘Tara’. Another limitation of this work was that most of the cultivars were obtained from the University of California, Davis, breeding blocks and may not consist of verified genotypes. Thus we propose to expand on the Riaz et al. study by including a wider array of cultivars, increasing the number of markers used, and using a multiple accessions of each cultivar as a form of verification of cultivar identity.

### **Experimental Plan:**

*Plant Material:* This project will develop SSR profiles for 180 muscadine accessions and 10 *Vitis vinifera* and *Vitis labrusca* standard accessions. The muscadine accessions consist of 83 different current and historically important muscadine cultivars as well as several wild accessions related species. In the summer of 2017 leaf samples were obtained from several locations including the University of Georgia, University of North Carolina, University of

Arkansas, USDA Poplarville, USDA Davis, Paulk Vineyards, and two Georgia muscadine nurseries. Since most muscadine cultivars are not represented in a verified germplasm collection, at least two separate accessions from different locations for each cultivar were obtained wherever possible.

*DNA Isolation and SSR genotyping:* DNA will be isolated using a simple CTAB isolation procedure and bead grinding. Previous work in our lab shows that this protocol is relatively quick and inexpensive and the DNA is of suitable quality for PCR amplification. PCR amplification of SSR markers will be according to previous published methods for the given primer sets except that all markers will be labelled fluorescently and sizing will be done at the UGA Genomics Facility on an ABI 3730 DNA sequencer. SSR primers will consist of primers developed for use in *V. vinifera* that have been shown to be useful in *V. rotundifolia* in previous studies (Riaz et al., 2008) as well as primers that have not previously been tested in *V. rotundifolia* (Doligez et al., 2006; Troggio et al., 2007). Primers will be tested on a panel of 12 cultivars representing a diversity of muscadine pedigrees. Those that amplify well and show polymorphism will be used for this study. The goal is to use two markers for each of the 20 chromosomes, giving 40 total markers. We have previously tested a few of these markers in muscadine and have found a good proportion of the primers are usable in muscadine. The majority of the work involves finding those primers that amplify well in muscadine and are polymorphic, and then running them on the larger data set.

*Data Analysis:* Multiple accessions of each cultivar will be checked for differences in SSR profiles. In the event of differences, the correct cultivar will be identified via phenotyping flower sex and fruit morphology, pedigree analysis using SSR profiles, and SSR analysis of additional accessions of the cultivar. In this way we hope to develop an unambiguous SSR profile for each muscadine cultivar. At the end of the experiment the results will be published and made available to the muscadine research community.

## Results

This project developed SSR profiles for 180 muscadine accessions and 10 *V. vinifera* and *V. labrusca* standard accessions. The muscadine accessions consist of 33 current cultivars, 40 historical cultivars, 10 wild accessions and 6 unknown muscadine samples where identification was requested. In the summer of 2017, leaf samples were obtained from several locations including the University of Georgia, University of North Carolina, University of Arkansas, USDA Poplarville Station, USDA Davis Grape Germplasm Repository, Paulk Vineyards, and two Georgia muscadine nurseries. Since most muscadine cultivars are not represented in a verified germplasm collection, at least two separate accessions from different locations for each cultivar were obtained wherever possible.

Each accession was evaluated with 20 SSR loci. Of those 20 loci, currently 10 loci have been scored for all samples. Using those 11 loci, for Muscadinia accessions a total of 94 alleles were detected with an average of 8.5 alleles per locus. Polymorphic Information Content (PIC) scores ranged from 0.27 to 0.79 with an average of 0.68 (Table 1). The 11 SSR markers were able to uniquely identify 69 or 73 muscadine cultivars.

Specific information that we have found when examining the muscadine cultivars included the following:

1. Tested six different ‘Scuppernong’ clones and the original “mother plant” from Roanoke NC. Four ‘Scuppernong’ clones were identical to the mother plant. Two

- ‘Scuppernong’ clones were identical to the cultivar ‘Roanoke’, a 1962 release from North Carolina.
2. Fourteen muscadine accessions were shown to not be true-to-type. These were largely historical cultivars in germplasm blocks.
  3. ‘Triumph’ accessions from multiple locations were shown to be identical, however, this accession can not be a parent of ‘Scarlett’ and it is uncertain still if this is the originally released ‘Triumph’.
  4. The lower yielding ‘Tara’ was identified as ‘Scarlett’.
  5. All ‘Noble’ samples were identical despite differences in fruit size.
  6. An off-type ‘Granny Val’ was shown to be ‘Fry’.
  7. An off-type ‘Carlos’ was shown to be ‘Triumph’.
  8. An off-type ‘Magnolia’ was shown to be ‘Carlos’.
  9. An off-type ‘Southland’ was shown to be ‘Hunt’.

Table 1. SSR loci used to investigate muscadine germplasm.

Locus	LG	Na	Ho	He	PIC
VMC6f1	2	7	0.87	0.75	0.75
VVIB23	2	11	0.88	0.79	0.79
VMC7h3	4	8	0.13	0.27	0.27
VVMD27	5	11	0.86	0.79	0.78
VVMD7	7	6	0.78	0.74	0.74
VMC3d7	10	12	0.78	0.80	0.75
VMC2a5	14	7	0.55	0.56	0.56
VMC6e1	14	9	0.30	0.72	0.71
VMC5a1	16	7	0.76	0.71	0.71
VVMD5	16	11	0.69	0.70	0.70
VMC7c3	17	5	0.47	0.68	0.68
<b>Total</b>	<b>8</b>	94	7.07	7.51	7.44
<b>Mean</b>		<b>8.5</b>	<b>0.65</b>	<b>0.67</b>	<b>0.68</b>

Na: No. of detected alleles

Ho: Observed heterozygosity (%)

He: Expected heterozygosity (%)

PIC: Polymorphism Information Content

### Conclusions

SSR markers are an ideal tool for investigating identity in muscadine grape. With this information we now have the ability to confirm identity and investigate pedigrees in muscadine grape. This project is still in the process of being completed by a MS graduate student in my program. I expect to have a final report with additional information on relatedness of muscadine germplasm in the summer of 2019, at which time this information will be updated with a final report.

### Literature Cited

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