Genetic Diversity in Muscadine and American Bunch Grapes Based on Randomly Amplified Polymorphic DNA (RAPD) Analysis

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Abstract. Two morphologically distinct types of grapes belonging to the subgenera *Euvitis* and *Muscadinia* in the genus *Vitis* are cultivated in the United States. The former is commonly called bunch grapes while the latter is usually called muscadine. Genetic diversity among these grapes was investigated using RAPD markers. Sixteen grape cultivars, with parentage including *V. rotundifolia* Michx., *V. vinifera* L., and several American *Vitis* species, were used for the RAPD analysis. A total of 156 RAPD markers was produced from 19 random primers, over 90% of which was polymorphic among the muscadine and the bunch grapes. Polymorphisms were lower within each subgenus. Relationships between these two subgenera were estimated based on band-sharing and cluster analysis. The average genetic distance between the bunch and the muscadine grape cultivars was 0.45. The results based on DNA analysis agree with isozyme data obtained from a separate study, which demonstrated that muscadine grapes share very few common alleles with American bunch grapes and European grapes.

Grapes botanically belong to the family Vitaceae, which is divided into 12 genera. *Vitis* is the only genus with economic importance (Goldy et al., 1988). The genus *Vitis* is divided into two subgenera, *Euvitis* Planch. and *Muscadinia* Planch. The former has 38 chromosomes and many berries borne in each cluster so that a general term bunch grape is given to all species of *Euvitis*. *Muscadinia* has 40 chromosomes and smaller clusters, with a common name of muscadine grape.

The *Euvitis* species, with hundreds of known cultivars, can be divided into three geographical groups: the American group, Asiatic group, and European and middle-Asian group (Mullins et al., 1992; Olien, 1990). *Vitis vinifera* L., a predominant commercial species grown all over the world, is the only member in the third group and has given rise to thousands of cultivars. The American group accounts for about 30 species (Alleweldt et al., 1991), and a similar figure was estimated for the Asiatic group (Zhang et al., 1990). *Muscadinia* is native to the southeastern United States where the climate is warm, rainy, and humid during most of a year. Two genuine species, *Vitis rotundifolia* Michx. and *V. munsoniana* Simpson ex Munson, and one questionable species, *V. popenoei* Fennell, are found in this subgenus (Alleweldt et al., 1991). *Vitis rotundifolia*, normally referred to as the muscadine grape, is the only species within *Muscadinia* with commercial value.

Muscadine grapes, and hybrid bunch grapes selected from crosses between American species and *V. vinifera*, are the predominant grape cultivars in the southeastern United States. The hybrid bunch grapes cultivated in this region can be traced in recent ancestry to one or more species native to Florida and the southeastern United States, such as *V. shuttleworthii* House, *V. aestivalis* Michx. ssp. *smalliana* Bailey, and *V. aestivalis* Michx. ssp. *simpsoni* Munson (Table 1). They are distinct from American hybrids, which largely contain *V. labrusca* L. (Hedrick, 1908), French-

American hybrids, which are traced to *V. rupestris* Scheele and *V. riparia* Michx., and other American species originating from areas other than the southeastern United States (Snyder, 1937). Morphological differences among *V. vinifera* and the American species have been described in various reports. Until recently, little work was done to identify genetic diversity of grapes at the molecular level. Thomas et al. (1993) used repetitive DNA sequences as probes to detect polymorphisms in grapevines and found that a moderate to high level of heterozygosity existed in grapevine cultivars belonging to *V. vinifera*. They concluded that DNA markers could be used as alternative means for grapevine identification and could also be used to investigate taxonomic relation-

Table 1. Grape (*Vitis*) cultivars used in this study and species ancestry involved in the cultivar development.

Cultivar		Species ancestry ²
<i>Bunch grapes</i>		
1	Blanc du Bois	1, 2, 5, 6, 10
2	Blue Lake	2, 5, 10
3	Lake Emerald	1, 5, 10
4	Miss Blanc	4, 5, 6, 10
5	Orlando Seedless	1, 2, 5, 6, 10
6	Stover	3, 6, 7, 8, 9, 10
7	Suwannee	1, 2, 3, 5, 6, 7, 8, 10
8	Tampa	2, 5
<i>Muscadine grapes</i>		
9	Alachua	<i>V. rotundifolia</i>
10	Carlos	<i>V. rotundifolia</i>
11	Dixie	<i>V. rotundifolia</i>
12	Fry	<i>V. rotundifolia</i>
13	Higgins	<i>V. rotundifolia</i>
14	Magnolia	<i>V. rotundifolia</i>
15	Noble	<i>V. rotundifolia</i>
16	Welder	<i>V. rotundifolia</i>

²1 = *Vitis aestivalis* Michx. ssp. *simpsoni* Munson; 2 = *V. aestivalis* Michx. ssp. *smalliana* Bailey; 3 = *V. berlandieri* Planch.; 4 = *V. bourquiniana* Munson; 5 = *V. labrusca* L.; 6 = *V. lincecumii* Buckl.; 7 = *V. riparia* Michx.; 8 = *V. rupestris* Scheele; 9 = *V. shuttleworthii* House; 10 = *V. vinifera* L.

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ships among *Vitis* species. Bourquin et al. (1993) compared restriction-length polymorphic DNA (RFLP) markers among 46 accessions of *V. vinifera* and generated more than 100 unique restriction fragments. However, most of the DNA-based studies were limited to *V. vinifera*. This study investigates the feasibility of using RAPD technology to test the genetic relationship among the muscadine and bunch grape cultivars and estimate the taxonomic values of RAPD markers.

Materials and Methods

DNA isolation. Plant materials used for DNA extraction were obtained from the experimental vineyard at the Center for Viticultural Science, Florida A & M Univ., Tallahassee (Table 1). Two plants of each cultivar were used for DNA isolation, except for three cultivars, 'Miss Blanc', 'Magnolia' and 'Higgins', each of which had only one plant available. After quickly freezing dry in liquid nitrogen, young leaves were stored at -70°C until DNA isolation. DNA was extracted using a CTAB method modified by Lodhi et al. (1994) from an original method of Doyle and Doyle (1987).

DNA amplification and electrophoresis. Nineteen random 10-mer primers obtained from Biotechnology Laboratory, Univ. of British Columbia, Canada, were used for RAPD amplification (Table 2). The reaction mixture in a 0.5-mL tube included 4 μL of 1:10 diluted template DNA, 2.5 μL of $10\times$ reaction buffer, 0.5 unit of Taq DNA polymerase, 1 μL of 25 mM MgCl_2 , 0.25 μL of 1 mg $\cdot\mu\text{L}^{-1}$ BSA, 0.5 μL of 15 μM primer, and 1 μL of 10 mM dNTPs. dH_2O was used to bring the final volume to 25 μL . Thirty microliters of mineral oil was overlaid on top of the reaction mixture. DNA amplification was carried out in a thermal controller (MJ Research, Watertown, Mass.) programmed at 94°C for 1 min, 35°C for 2 min, 72°C for 2 min, with a total of 40 cycles. Eight minutes at 72°C was added for a final extension.

The amplified products were separated in 2% agarose gel [a mixture of 1% of high melting point agarose and 1% of NuSieve GTG (FMC Bioproducts, Rockland, Maine) low-melting-point agarose] in $1\times$ TBE. The gel was run at 100 v for 3.5 to 4 h. The gel was stained with 500 ppm ethidium bromide and examined and photographed on a UV light box (Fotodyne, Inc., Hartland, Wis.).

Table 2. Sequences of the primers used for RAPD analysis.

Primer	Sequence
UBC 219	5'-TGC ACT GGA G
UBC 230	5'-CGT CGC CCA T
UBC 237	5'-CGA CCA GAG C
UBC 238	5'-CTC TCC AGC A
UBC 247	5'-TAC CGA CGG A
UBC 249	5'-GCA TCT ACC G
UBC 250	5'-CGA CAG TCC C
UBC 264	5'-TCC ACC GAG C
UBC 265	5'-CAG CTG TTC A
UBC 266	5'-CCA CTC ACC G
UBC 268	5'-AGG CCG CTT A
UBC 272	5'-AGC GGG CCA A
UBC 273	5'-AAT GTC GCC A
UBC 280	5'-CTG AGT GTG G
UBC 281	5'-GAG ACT GGA A
UBC 282	5'-GGG AAA GCA G
UBC 283	5'-GGG CCA CCG T
UBC 284	5'-CAG GCG CAC A
UBC 292	5'-AAA CAG CCC G

Analysis of relationships among the cultivars. The amplified DNA fragments normally ranged from 100 to 1500 bp. They were scored by presence (+) or absence (-). These markers were identified by primer and fragment size in base pairs. Genetic variation among the cultivars was estimated using Nei's coefficient of genetic distance (Nei and Li, 1979):

$$S = (2N_{xy}) / (N_x + N_y)$$

$$D = -\text{Log}_{10} S$$

where S is the pairwise similarity coefficient, N_x and N_y are the total number of bands in cultivar X and Y, N_{xy} is the number of bands shared by X and Y, and D is the genetic distance between X and Y. Relationships among the 16 cultivars were represented in dendrographic form based on the matrix of distance coefficients (D) by unweighted pair-group method, arithmetic average (UPGMA) analysis. A program called Multivariate Statistical Package (Kovach Computing Service, Pentraeth, U.K.) was used for the computer analysis.

Results and Discussion

The 156 RAPD bands generated from the 19 primers were consistently reproduced in both plants of the same cultivar. The number of bands for each primer varied from 2 to 12, with an average of 8.2 bands/primer. The size of the amplified fragments ranged from 100 to 1600 bp. Among the 156 scored bands, 11 (7.1%) were monomorphic among all cultivars. Of the remaining 145 variable bands, 11 (7.6%) were constant in all bunch grape cultivars, while 22 bands (15.2%) appeared uniformly in all 8 muscadine cultivars. For example, a 490-bp fragment generated by primer UBC-237 appeared in all the muscadine cultivars but not in any bunch grape cultivar (Fig. 1A, arrow), while the 1250- and 810-bp fragments appeared uniformly in muscadine and bunch grapes. The remaining RAPD fragments generated by UBC-237 were polymorphic among the cultivars. Another primer, UBC-238, produced two unique bands to distinguish the bunch and the muscadine grapes. The 1600-bp fragment appeared in all the bunch grape cultivars but not in any muscadine cultivars, while the 460-bp fragment was present in all the muscadine cultivars but was absent in the bunch grapes (Fig. 1B, arrows). Other products amplified by UBC-238 were polymorphic bands.

Genetic distances derived from pairwise similarity coefficients among the 16 grape cultivars are summarized in Table 3. The smallest genetic distance was found in a pair of muscadine cultivars, 'Fry' and 'Carlos', which had a distance coefficient of 0.06. The largest distance was found between the muscadine cultivar 'Higgins' and the bunch grape 'Stover', which had the distance coefficient of 0.55. Moderate genetic distances were exhibited among the eight bunch grape cultivars, with an average of 0.21. Small genetic variation, an average of 0.12, was found in the muscadine grape cultivars.

Small genetic variation among the muscadine cultivars is predictable since all the muscadine cultivars were intraspecific hybrids of *V. rotundifolia*. More genetic variability observed in the bunch grapes can be attributed to the fact that they are interspecific hybrids derived from at least 10 *Vitis* species (Table 1), including the American species and *V. vinifera*. This finding is consistent with the results from an isozyme analysis in a separate study (Lu et al., 1994). The level of polymorphisms among the bunch grape cultivars identified by the RAPD analysis were also similar to previous reports of other American hybrids based on RFLP analysis (Weeden et al., 1988).

Based on the RAPD analysis, high genetic variation was found

Fig. 1. Amplification products obtained from primer 237 (A), and 238 (B). Lanes were read from left to right. Lanes 1 to 15 are bunch grape cultivars; Lanes 16 to 29 are muscadine cultivars. Lane 30 (M) is a marker of 100-bp ladder.

Lanes 1, 2 = Lake Emerald
Lanes 3, 4 = Suwannee
Lanes 5, 6 = Blanc du Bois
Lanes 7, 8 = Stover
Lanes 9, 10 = Orlando Seedless
Lanes 11, 12 = Tampa
Lanes 13, 14 = Blue Lake
Lanes 15 = Miss Blanc
Lane 16 = Magnolia
Lanes 17, 18 = Dixie
Lanes 19, 20 = Fry
Lanes 21, 22 = Carlos
Lanes 23, 24 = Welder
Lanes 25, 26 = Alachua
Lanes 27, 28 = Noble
Lane 29 = Higgins

able in the bunch grapes. One subcluster contained 'Miss Blanc', 'Blue Lake', and 'Tampa', where the last two cultivars formed one group. The other subcluster contained 'Lake Emerald', 'Suwannee', 'Blanc du Bois', 'Orlando Seedless', and 'Stover', with the first two in one group. The muscadine grapes were further divided into two subclusters, with one subcluster consisting of 'Higgins' only.

between the muscadine and the bunch grapes. The average genetic distance between the bunch and the muscadine grape cultivars was 0.45. Thomas et al. (1993), using the repetitive DNA technique, also demonstrated that *V. rotundifolia* is very different from *Euvitis* species. Results from this study indicate that the RAPD technique is useful for germplasm analysis and taxonomic study of American *Vitis* species. The level of polymorphisms revealed from this study also suggest that RAPD could be a useful tool for identifying North American grape cultivars and an alternative to ampelography.

Relationships among the sixteen cultivars, based on their genetic distances, were clustered in a dendrogram (Fig. 2). Two clusters, muscadine grapes versus bunch grapes, were clearly resolved on the dendrogram. The cluster consisting of the bunch grapes appeared less uniform because of relatively low levels of similarity among its members. Two subclusters were distinguish-

Relationships represented in the dendrogram are consistent with the available pedigree information. For instance, 'Blue Lake' and 'Tampa' are clustered together, a result that agrees with the genetic background of these cultivars. They share the same female parent 'Fla. 43-47', an open pollinated seedling of *V. aestivalis* ssp. *smalliana* Bailey (Mortensen and Stover, 1982; Stover, 1960), and have an ancestor of *V. labrusca* in common. 'Blanc du Bois', 'Suwannee', and 'Lake Emerald' are also clustered in a group. This result again agrees with their pedigree background. 'Lake Emerald' is one ancestor of 'Suwannee' (Mortensen, 1983), while 'Suwannee' and 'Blanc du Bois' share another common ancestor, 'Fla. 449'. In addition, all three cultivars share some genes of *V. vinifera* (Halbrooks and Mortensen, 1989). 'Miss Blanc' may be distinct from other groups due to the parentage of *V. bourquiniana* Munson, which is not shared with any of the other cultivars that were screened (Table 1).

Table 3. Genetic distances among sixteen grape cultivars.

Cultivar	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 Blanc du Bois	0.00															
2 Blue lake	0.19	0.00														
3 Lake Emerald	0.15	0.17	0.00													
4 Miss Blanc	0.26	0.18	0.24	0.00												
5 Orlando Seedless	0.18	0.23	0.17	0.29	0.00											
6 Stover	0.23	0.24	0.20	0.29	0.19	0.00										
7 Suwannee	0.16	0.23	0.15	0.25	0.20	0.16	0.00									
8 Tampa	0.17	0.11	0.20	0.22	0.21	0.22	0.25	0.00								
9 Alachua	0.44	0.46	0.45	0.45	0.47	0.47	0.50	0.50	0.00							
10 Carlos	0.39	0.37	0.37	0.36	0.38	0.44	0.43	0.44	0.12	0.00						
11 Dixie	0.47	0.44	0.46	0.43	0.50	0.51	0.53	0.51	0.11	0.12	0.00					
12 Fry	0.41	0.36	0.35	0.38	0.40	0.43	0.42	0.45	0.09	0.06	0.12	0.00				
13 Higgins	0.49	0.46	0.46	0.41	0.49	0.55	0.50	0.49	0.15	0.17	0.15	0.16	0.00			
14 Magnolia	0.47	0.45	0.45	0.39	0.46	0.47	0.54	0.48	0.09	0.12	0.11	0.13	0.16	0.00		
15 Noble	0.47	0.46	0.46	0.44	0.47	0.48	0.52	0.51	0.10	0.15	0.10	0.15	0.13	0.09	0.00	
16 Welder	0.42	0.42	0.41	0.38	0.45	0.48	0.47	0.48	0.10	0.10	0.07	0.10	0.12	0.09	0.07	0.00

*Blanc du Bois, **Lake Emerald, ***Orlando Seedless

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Fig. 2. Dendrogram among the 16 grape cultivars resulting from UPGMA cluster analysis based on Nei's genetic distances obtained from the 19 RAPD primers.