Use of SSR Markers to Assess Identity, Pedigree, and Diversity of Cultivated Muscadine Grapes

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ABSTRACT. The North American muscadine grape (*Muscadinia rotundifolia* Small) is a valuable source of resistance to powdery mildew [*Uncinula necator* (Schw.) Burr], root-knot nematode (*Meloidogyne Goeldi*), dagger nematode (*Xiphinema index* Thorne and Allen), grape phylloxera (*Daktulosphaira vitifoliae* Fitch), and Pierce's disease (*Xylella fastidiosa* Wells et al.). Efforts to breed muscadine grapes commenced in the early 1900s and have generated a large number of cultivars and a limited number of hybrids with *Vitis vinifera* L. and other *Vitis* L. species. Collections of this germplasm are currently maintained with accession identity based on declared identity when collected, breeding records, and comparisons of morphological traits. This study reports on the first use of DNA-based simple sequence repeat (SSR) marker profiles to authenticate *M. rotundifolia* cultivars and hybrids. A total of 57 accessions [39 *M. rotundifolia* cultivars, 3 *V. vinifera* cultivars, 3 *Vitis* spp. hybrids, and 12 *V. vinifera* × *M. rotundifolia* (VR) hybrids] from collections at the U.S. Department of Agriculture National Clonal Germplasm Repository and the University of California (Davis) Department of Viticulture and Enology were analyzed with 14 SSR markers. The fingerprint profiles were used to verify published breeding records of 31 *M. rotundifolia* cultivars and hybrids by comparing the shared alleles of parents and progeny. Marker data indicated that four cultivars were incorrectly identified; their alleles did not match respective parent/progeny relationships at more than five loci. Two *M. rotundifolia* accessions had the same fingerprint profile as a third accession at all 14 markers, implicating a likely planting error. The *M. rotundifolia* cultivars exhibited 88 unique alleles that were not present in a database of more than 600 *V. vinifera* cultivars.

Muscadine grape is genetically and morphologically distinct from species within the genus *Vitis*. The most obvious genetic difference between these two taxa is the number of somatic chromosomes: *Muscadinia* Small species have 40, and *Vitis* species have 38. *Muscadinia* species also differ from *Vitis* species in their seed, bark, tendrils, and cluster morphology. There is disagreement as to whether the differences between *Muscadinia* and *Vitis* warrant generic status (Olmo, 1995) or whether these taxa are best considered subgenera or sections (Liberty Hyde Bailey Hortorium, 1976). *Muscadinia* species possess very strong resistance to grape pests and diseases (Olmo, 1986), which prompted efforts to cross *Muscadinia rotundifolia* with *Vitis vinifera* cultivars. Although *Vitis* species hybridize freely, *Vitis* × *Muscadinia* crosses are difficult, and hybrids are rare and normally sterile, with 39 chromosomes.

Crop improvement within *M. rotundifolia* began with the selection of high-quality pistillate vines from within the native range (southeastern and south-central United States) of this dioecious species. Muscadine grapes were cultivated in the 16th century by European settlers, and they likely used high-quality forms selected by Native Americans before this date. 'Eden', 'Flowers', 'James', 'Memory', 'Mish', 'Scuppernong', and 'Thomas' are among the oldest cultivars and were all collected from the wild (Husmann and Dearing, 1913; Reimer and Detjen, 1914). Since then, there have been three primary objectives in muscadine breeding programs: 1) the development of hermaphroditic, self-fertile cultivars with enhanced

fruit quality and yields; 2) the introgression of *M. rotundifolia* into *V. vinifera* to develop disease-resistant fruiting cultivars with high-quality fruit; and 3) the utilization of *M. rotundifolia* in rootstock breeding. Dearing led the first large-scale muscadine breeding program in a cooperative effort between the U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS) and North Carolina State University. He reported on the first self-fertile muscadine genotypes (Dearing, 1917), leading to a number of cultivar releases (Dearing, 1948). Muscadine breeding programs were also conducted in Mississippi, Georgia (Lane, 1980), and Florida (Bates et al., 1980; Mortensen, 1971).

Efforts to introgress disease and pest resistance from *M. rotundifolia* into *V. vinifera* or other *Vitis* species and hybrids were confounded by the differences in chromosome numbers and the sterility of the resulting hybrids. However, fertile *V. vinifera* × *M. rotundifolia* (VR) hybrids have been produced (Detjen, 1919; Olmo, 1986). One such hybrid, NC6-15, produced by Detjen, is the backbone of the powdery mildew disease resistance breeding program in France (Pauquet et al., 2001). Olmo (1986) was also able to create a few fertile VR hybrids, which continue to be used in the current grape breeding program at the University of California, Davis. Although VR hybrid fruiting cultivars have not been released, a VR hybrid, 'O39-16', was released as a rootstock for use in controlling fanleaf degeneration (Walker et al., 1991).

These breeding efforts have resulted in large collections of *M. rotundifolia* cultivars and VR hybrids. At present, accessions within these collections are documented by comparing breeding records and morphological traits. A molecular marker-based database to aid in establishing a reference collection or aid in authenticating accession identification does not exist. DNA-based molecular markers, particularly simple

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Table 1. Parentage and origin of the studied plant material. The flower type and fruit color was observed on plants in the National Clonal Germplasm Repository at Davis, CA (NCGR-Davis) or University of California, Davis, Department of Viticulture and Enology (UCD-VE). Genotypes are grouped in sets: A to F are linked to the parentage analysis in Fig. 1; G contains reference *Vitis vinifera* cultivars, hybrids known to be in the parentage of other tested accessions, and *V. vinifera* × *Muscadinia rotundifolia* (VR) hybrids that are being used in breeding programs at UCD and elsewhere; H contains *M. rotundifolia* accessions without parentage information; I consists of accessions whose simple sequence repeat fingerprints did not match known parentages.

Accession (DVIT ^z no.)	Sex ^y	Fruit color ^x	Species or parentage	Geographical origin	Reference ^w
A					
‘Cowart’ (2189)	SF	B	‘Higgins’ × GA28	Georgia	3, 7
‘Dixieland’	SF	Br	‘Fry’ × GA29-49	Georgia	3, 7
‘Fry’ (1764)	F	Br	GA19-13 × USDA19-11	Georgia	3, 7
‘Higgins’ (2191)	F	Br	‘Yuga’ × White Male	Georgia	3, 7
P9-15 (2395)	SF	B	Z67-7-5 selfed	South Carolina	8
‘Nesbitt’ (2195)	SF	B	‘Fry’ × ‘Cowart’	North Carolina	3, 7
‘Sanmonta’ (1726)			(‘Scuppernong’ OP ^v seedling × <i>Vitis</i> sp.) × ‘Brilliant’ (‘Lindley’ × ‘Delaware’)	Texas	7, 9
‘Southern Home’	SF	B	‘Summit’ × P9-15	Florida	7, 8
‘Sugargate’ (1769)	F	B	‘Fry’ × GA29-49	Georgia	3, 7
‘Summit’ (2185)	F	Br	‘Fry’ × GA29-49	Georgia	3, 7
‘Supreme’	F	B	‘Black Fry’ × ‘Dixieland’	Georgia	3, 7
‘Yuga’ (1729)	F	Br	‘Sanmonta’ × White Male #1	Georgia	3, 7
B					
‘Albemarle’ (1738)	SF	B	‘Topsail’ × ‘Burgaw’	North Carolina	3, 7
‘Latham’	F	B	<i>M. rotundifolia</i>	North Carolina	6, 7
‘Topsail’ (2629)	F	Br	‘Latham’ × ‘Burgaw’	North Carolina	3, 7
C					
‘Dearing’ (2622)	SF	Br	‘Luola’ × ‘Burgaw’	North Carolina	3, 7
‘Welder’ (1771)	SF	Br	‘Dearing’ × unknown	Florida	3, 7
D					
‘Magnolia’ (2197)	SF	Br	(‘Thomas’ × ‘Scuppernong’) × (‘Topsail’ × ‘Tarheel’)	North Carolina	3, 7
‘Regale’ (2096)	SF	B	‘Hunt’ × ‘Magnolia’	North Carolina	4, 12
‘Sterling’ (2194)	SF	Br	(‘Scuppernong’ × NC9-199) × ‘Magnolia’	North Carolina	3, 7
E					
‘Onslow’ (1759)	F	B	(‘Scuppernong’ × ‘Male’) × ‘Burgaw’	North Carolina	3, 7
‘Scuppernong’ (2196)	F	Br	<i>M. rotundifolia</i>	North Carolina	6, 7
White Male	M		Male seedling from ‘Scuppernong’	Florida	1
F					
b55-41			‘Thomas’ OP	UCD	10
‘Magoon’ (2623)	SF		‘Thomas’ × ‘Burgaw’	Mississippi	3, 7
NC6-15	SF	B	Malaga seedling #1 × G52 (‘Thomas’ × ‘Hope’)	North Carolina	4
‘Southland’ (1768)	SF	B	‘Thomas’ × seedling of ‘Topsail’	Mississippi	3, 7
‘Thomas’ (1772)	F	B	<i>M. rotundifolia</i>	North Carolina	6, 7
Trayshed	M		<i>M. rotundifolia</i>	UCD	10
U62-56 (1689)			‘Thomas’ × ‘Trayshed’	UCD	10
U62-61 (1690)			‘Thomas’ × ‘Trayshed’	UCD	10
G					
‘Thompson Seedless’	SF	W	<i>V. vinifera</i>	Middle East	5
‘Riesling’	SF	W	<i>V. vinifera</i>	Germany	5
‘Grenache’	SF	B	<i>V. vinifera</i>	Spain	5
‘Verdelet’	SF	W	S.5455 × S.4938	Hybrid direct producer, France	
‘Villard Blanc’	SF	W	S6468 × S6905	Hybrid direct producer, France	5
‘Seneca’	SF	W	‘Lignan blanc’ × ‘Ontario’	American hybrid, New York	3
Z93-6-2	SF		VR hybrid	Zehnder	11

continued next page

Table 1. Continued.

Accession (DVIT ^z no.)	Sex ^y	Fruit color ^x	Species or parentage	Geographical origin	Reference ^w
Z97-60-3	SF		VR hybrid	Zehnder	11
Z01-20-4			VR hybrid	Zehnder	11
DRX73-026 (2711)	SF	B	Fla W1521 × DRX69-99 (has NC6-15 in its parentage)	Florida	2
NC194-1	SF	B	‘Seneca’ × M15 [‘Thomas’ × NCB6-19 (‘Latham’ × ‘Burgaw’)]	North Carolina	2
NC 74CO49-10	SF	B	UCD e4-12 (UCD Y14-14 × ‘Grenache’) × <i>M. rotundifolia</i> ‘Magnolia’	North Carolina	2
JB81-107-11	SF	B	NC74C049-10 (UCD e4-12 (UCD Y14-14 × ‘Grenache’) × <i>M. rotundifolia</i> ‘Magnolia’) × ‘Verdelet’	North Carolina	2
H					
Barrett Mn-1 (2435)			<i>Muscadinia munsoniana</i>	UCD	10
‘Chowan’ (1745)	SF	Br	‘Creswell’ × ‘Burgaw’	North Carolina	3, 7
‘Flowers’ (1749)	F	B	<i>M. rotundifolia</i>	North Carolina	6, 7
‘James’ (1752)	F	B	<i>M. rotundifolia</i>	North Carolina	6, 7
‘Male’ (Georgia)	M		<i>M. rotundifolia</i>	Georgia	10
‘Pride’ (1766)	F	B	GA19-13 × USDA19-11	Georgia	3, 7
‘Tarheel’ (1728)	SF	B	‘Luola’ × (‘Eden’ × V23R4B2)	North Carolina	3, 7
Thornhill			<i>M. munsoniana</i>	Florida	1
I					
‘Creswell’ (1746)	M		<i>M. rotundifolia</i>	North Carolina	3, 7
Farrer F2 #5 (1730)	F	B	NC6-15 OP	Georgia	10
‘Irene’ (1751)	M		‘Thomas’ × Black male	Georgia	3, 7
‘Jumbo’ (2188)	F	B	‘Higgins’ × USDA19-11	Georgia	3, 7
‘Triumph’ (2183)	SF	Br	‘Fry’ × GA29-49	Georgia	3, 7

^zDVIT numbers are accession numbers for *Vitis* in NCGR-Davis.

^ySF = self-fertile, F = pistillate, M = staminate.

^xB = black to dark purple, W = green to white, Br = bronze.

^w1 = H.C. Barrett breeding records; 2 = J.P. Bloodworth breeding records; 3 = Brooks and Olmo, 1997; 4 = Detjen, 1919; 5 = Galet, 1998; 6 = Husmann and Dearing, 1913; 7 = Mortensen, 2001; 8 = Mortensen et al., 1994; 9 = Munson, 1909; 10 = H.P. Olmo breeding records; 11 = D.W. Ramming breeding records.

^vOpen pollinated.

sequence repeat (SSR) markers, are excellent fingerprinting tools and have been used to identify grape cultivars and to assist in the maintenance of grape germplasm collections (Dangl et al., 2001; Thomas et al., 1994). The study presented here reports on the first use of SSR markers to manage *M. rotundifolia* germplasm. The resulting fingerprint profiles from 14 SSR markers were used to verify reported pedigrees and to assess the genetic diversity of muscadine cultivars.

Materials and Methods

PLANT MATERIAL. Table 1 presents the parentage, geographic origin of the *M. rotundifolia* cultivars, breeder selections, and hybrids studied. A few *V. vinifera* cultivars were also included to act as allele size references for comparison with other fingerprint databases. All plant material with a DVIT (Davis-*Vitis*) number is maintained at the USDA National Clonal Germplasm Repository, Davis, CA (NCGR-D). The remaining accessions are maintained in breeding blocks at the Department of Viticulture and Enology, University of California, Davis (UCD-VE).

DNA ISOLATION AND MICROSATELLITE GENOTYPING. DNA was extracted from young leaves and shoot tips using a modified hexadecyltrimethylammonium bromide procedure as described

by Lodhi et al. (1994). In cases where actively growing shoot tips and young leaves were not available, DNA was extracted from shoot cambium tissue. A total of 20 SSR markers selected from 11 different linkage groups from the *Vitis rupestris* Scheele × *Vitis arizonica* Engelm. genetic map (Riaz et al., 2006) was used to establish DNA profiles for the each of the tested accessions (Table 2). The sequences of primers were obtained from previous published studies: VVS2 (Thomas et al., 1994); VVMD07 and VVMD27 (Bowers et al., 1996, 1999); VrZAG62 (Sefc et al., 1999); VMC4f3.1 (Di Gaspero et al., 2000); VMC8b5 (Adam-Blondon et al., 2004); and VVIN16 (Merdinoglu et al., 2005). The *Vitis* Microsatellite Consortium (VMC) primer sequences are available on National Center for Biotechnology Information (NCBI) uni-STS (unified sequence tagged sites) database for *Vitis* (NCBI, 2005) and from publicly available expressed sequence tagged (EST)-derived microsatellites developed by D.R. Cook (Department of Plant Pathology, University of California, Davis), which are available at the Core Genome Facility (CGF; University of California, 2003). The conditions for marker amplification were previously described in Riaz et al. (2004), except that the amount of reaction mixture was reduced from 20 to 10 µL. The amplified products were separated on denaturing 5% polyacrylamide sequencing gels and were visualized by silver

Table 2. Marker name and linkage group location as determined from the *Vitis vinifera* reference map (Doligez et al., 2006), amplification status in *Muscadinia*, and references for the markers used in this study.

Marker name	Linkage group		Reference ^c
	no.	Amplified	
VVMD27	5	Yes	Bowers et al., 1999
VVMD7	7	Yes	Bowers et al., 1996
VrZAG62	7	Yes	Sefc et al., 1999
VMC5h2	8	Yes	NCBI, 2005
VMC5c1	9	Yes	NCBI, 2005
VMC3d7	10	Yes	NCBI, 2005
VVS2	11	Yes	Thomas and Scott, 1994
VMC8g9	12	Yes	NCBI, 2005
VMC4f3.1	12	Yes	Di Gaspero et al., 2000
VMC1g3.2	12	No	Di Gaspero et al., 2000
VMCNg2h7	12	Multiple loci	Di Gaspero et al., 2000
ctg1010863	12	No	University of California, 2003
VMC2b1.1	18	No	University of California, 2003
VMC2a3	18	No	University of California, 2003
VMC8b5	18	No	Adam-Blondon et al., 2004
VVIN16	18	Yes	Meridinoglu et al., 2005
VMC6e1	14	Yes	NCBI 2005
VMC2a5	14	Yes	NCBI, 2005
VMC5a1	16	Yes	NCBI, 2005
VMCNg3a10	19	Yes	Unpublished

^cNCBI = National Center for Biotechnology Information.

staining with a commercial kit (Promega, Madison, WI). Useful markers were amplified, run on polyacrylamide gels, and scored twice. Allele sizes in base pairs were determined by direct comparison with a sequencing reaction (Promega) run on each gel, as well as by comparisons to three common *V. vinifera* cultivars included in the study set. All gels were visually examined and scored on a light box and were then digitally scanned to preserve the images.

DATA ANALYSIS. To verify the published parentage and identity of the *M. rotundifolia* cultivars, allele size data were subjected to several analyses for polymorphic SSR markers with the assumption that there were no mistakes in the identity of genotypes in the collections. Expected heterozygosity (H_e) was calculated according to Nei (1987), using the microsatellite tool kit software (Park, 2001), which was also used to calculate allele frequencies. Observed heterozygosity was calculated as the ratio between heterozygous genotypes and the total number of genotypes analyzed for each marker. Pairwise similarity between the multilocus genotypes was estimated by using the “proportion of shared alleles” (ps) as described by Bowcock et al. (1994). The $-\ln$ (ps) option of MICROSAT, version 2.0 (Minch, 1997), was used to calculate the genetic distance between all pairwise combinations of the 57 genotypes with 14 SSR markers. The ps statistic is a measure of the dissimilarity between two samples. Thus, two individuals that are identical at all tested loci would have zero distance between them. A dendrogram was constructed with the unweighted pair-group method with arithmetic means (UPGMA) algorithm (Sneath and Sokal, 1973) using PHYLIP software, version 3.6 (Felsenstein, 2006), for the estimation. TreeView (Page, 1996) was used to construct the dendrogram.

Results

SSR MARKER AMPLIFICATION. Nineteen SSR markers used in this study were developed from repeat-rich genomic libraries made from nuclear DNA of *V. vinifera* and *Vitis riparia* L. cultivars. The 20th marker, ctg1010863, is an EST-derived SSR marker from *V. vinifera* (Table 2). No *M. rotundifolia*-based SSR or EST-SSR markers are available in public databases. Table 2 also presents the linkage group (LG) location of the 20 SSR markers tested. Five markers from LG12 and four markers from LG18 were selected because these two LGs are reported to harbor disease resistance genes for fungal diseases. Markers VMC8g9, VMC1g3.2, and VMC4f3.1 from LG12 have been linked to powdery mildew resistance and have been used to screen VR hybrid mapping populations segregating for powdery mildew resistance (Barker et al., 2005). Three of the nine markers from LG12 and 18 amplified successfully (two from LG12 and one from LG18; Table 2). The remaining six markers (VMC1g3.2, VMCNg2h7, ctg1010863, VMC8b5, VMC2a3, and VMC2b1.1) failed to amplify *M. rotundifolia* genomic DNA or amplified multiple loci, however, these markers amplified *V. vinifera*-based DNA successfully. Eleven markers from nine other linkage groups amplified successfully and resulted in a high level of polymorphism across the *M. rotundifolia* cultivars (Tables 2, 3, and 4). Table 4 presents the genotyping data for the entire study set.

AFFIRMATION OF PARENT-PROGENY RELATIONSHIPS TO VERIFY *M. ROTUNDIFOLIA* CULTIVAR IDENTITY. First order parentage analysis (one common allele between putative parent-progeny pairs for all markers) was carried out with data from the 14 polymorphic markers. An error in accession identification or reported pedigree was assumed when a purported parent-progeny pair did not share a common allele at every marker. Figure 1 presents the parent-progeny relationships that were consistent across all 14 markers (i.e., progeny had shared alleles from specified parents). In this way, allele comparisons were

Table 3. Genetic parameters of SSR markers, with number of alleles detected across all studied accessions (those unique to *Muscadinia* are listed in Table 5), and the observed and expected heterozygosity for 35 verified *Muscadinia* accessions.

Locus name	Alleles detected	Observed heterozygosity	Expected heterozygosity
	(no.)	(%)	(%)
VVMD27	16	0.81	0.78
VVMD07	11	0.77	0.75
VrZAG62	12	0.62	0.63
VMC5h2	15	0.77	0.77
VMC5c1	15	0.82	0.74
VMC3d7	13	0.94	0.74
VVS02	14	0.91	0.80
VMC4f3.1	19	0.88	0.74
VMC8g9	15	0.94	0.68
VMC2a5	11	0.77	0.72
VMC6e1	12	0.66	0.74
VMC5a1	10	0.77	0.73
VVIN16	5	0.05	0.05
VMCNg3a10	16	0.94	0.85
Total	184	10.65	9.72
Avg no./locus	13	0.76	0.69

Table 4. Allele size of studied plant material across 14 SSR markers. Dashes denote where DNA amplification failed.

Accession	Allele sizes (bp) of select SSR markers													
	VVM27	VVMD07	VrZAG62	VMC5h2	VMC5c1	VMC3d7	VVS02	VMC8g9	VMC4f3.1	VVIN16	VMC6e1	VMC2a5	VMC5a1	VMCNg3a10
'Thompson Seedless'	181-194	239-253	189-189	192-192	145-151	162-164	146-152	164-171	186-190	156-160	144-144	169-169	170-170	110-116
'Riesling'	181-189	249-257	195-205	192-206	145-173	160-164	144-152	168-173	172-188	154-156	144-152	154-187	170-170	118-120
'Grenache'	193-193	239-243	189-189	191-192	145-151	160-164	138-146	158-174	186-205	156-162	138-152	177-183	170-170	110-112
'Verdelet'	185-185	249-253	195-205	188-199	145-145	160-172	134-140	164-174	178-188	154-160	138-152	177-183	170-174	104-122
'Villard Blanc'	181-189	237-253	181-195	190-192	149-149	158-160	134-144	173-190	174-182	152-154	134-144	177-187	160-166	110-120
'Seneca'	185-185	235-247	195-203	192-200	145-145	162-164	126-156	168-184	172-172	156-162	144-170	154-154	156-166	104-120
Z93-6-2	199-199	237-243	189-197	194-194	167-184	168-172	136-148	138-140	182-222	152-152	140-140	152-152	170-172	110-116
Z97-60-3	211-215	235-235	215-215	198-200	—	172-174	148-154	138-138	222-222	152-152	140-140	152-159	170-172	116-130
Z01-20-4	181-215	237-239	189-209	199-202	151-167	162-172	134-150	140-174	166-192	152-160	138-166	—	170-170	98-134
DRX73-026	181-185	235-245	195-203	192-201	145-171	160-164	136-152	150-174	174-178	152-152	144-162	154-169	160-170	90-104
NC194-1	185-215	237-247	195-215	198-200	145-163	158-164	126-154	137-168	172-200	152-162	126-170	152-154	156-170	104-130
NC 74CO49-10	185-185	239-245	189-215	191-212	163-163	164-178	146-148	140-164	186-208	152-156	124-124	154-173	160-170	112-130
JB81-107-11	185-185	239-253	189-205	191-199	163-163	172-178	134-146	140-174	178-208	152-160	124-152	152-177	170-170	104-112
'Coward'	211-211	237-245	215-223	198-212	163-181	174-178	154-156	138-140	188-192	152-152	140-140	152-154	170-172	124-130
'Dixieland'	197-211	243-245	199-215	194-198	163-163	172-178	150-156	138-140	192-202	152-152	—	152-152	170-174	110-116
'Fry'	197-215	235-243	199-215	198-212	163-163	172-178	150-156	138-140	192-222	152-152	124-124	152-152	170-174	116-124
'Higgins'	199-211	237-243	199-223	200-212	163-167	172-174	150-156	138-140	192-222	152-152	140-140	152-152	168-172	110-124
P9-15	185-215	237-237	209-209	200-202	163-184	168-172	148-166	138-138	222-222	152-152	138-148	152-165	163-170	116-134
'Nesbitt'	211-215	235-245	215-215	198-212	163-181	172-174	150-156	138-140	188-192	152-152	140-140	152-154	170-174	124-124
'Sannonta'	195-199	235-245	215-226	194-206	163-163	174-178	148-150	136-138	184-202	152-152	124-140	152-173	163-170	110-116
'Southern Home'	199-215	235-237	209-215	198-202	163-184	168-172	150-166	138-140	192-222	152-152	124-138	152-165	170-170	124-134
'Sugargate'	199-215	235-243	199-215	198-198	163-163	172-178	150-156	138-140	192-222	152-152	124-140	152-154	170-170	116-130
'Summit'	197-199	235-245	215-215	198-198	163-184	172-178	150-156	137-140	192-202	152-152	124-126	152-159	170-174	124-124
'Supreme'	211-215	245-245	199-215	198-212	163-163	168-178	150-156	138-140	192-222	152-152	—	152-152	170-172	116-124
'Yuga'	199-211	235-237	223-226	194-200	163-184	172-174	150-154	138-140	184-192	152-152	140-140	152-152	163-172	110-118
'Albemarle'	199-199	245-245	215-215	194-200	163-181	158-172	152-154	140-158	192-208	152-152	142-142	154-159	168-170	130-134
'Latham'	179-199	233-245	199-215	194-198	145-163	170-172	150-166	138-140	206-222	152-152	124-124	152-159	170-174	112-124
'Topsail'	199-211	245-245	215-215	198-200	163-181	172-178	150-154	138-140	208-222	152-152	124-124	159-159	170-170	124-130
'Dearing'	199-215	235-237	199-215	200-212	167-181	172-178	150-154	138-159	192-222	152-152	142-142	152-154	163-170	116-134
'Welder'	211-215	235-245	215-215	194-200	181-184	172-178	148-150	138-138	192-222	152-152	—	154-159	170-174	116-130
'Magnolia'	185-215	243-245	199-215	194-212	163-184	172-178	148-148	138-140	208-222	152-152	142-142	152-173	170-170	122-130
'Regale'	—	243-243	199-199	194-212	184-184	172-172	148-148	138-138	222-222	152-152	124-124	152-173	170-170	116-130
'Sterling'	211-215	245-245	215-215	194-199	181-184	170-172	148-150	138-140	202-222	152-152	—	152-159	168-170	102-130
'Onslow'	199-215	245-245	215-215	200-210	163-184	168-178	150-154	138-158	192-222	152-152	124-124	152-159	170-172	116-134
'Scuppernong'	199-215	243-245	199-215	194-194	163-184	172-178	148-150	138-140	222-222	152-152	124-124	159-173	163-170	116-124
White Male	199-215	243-243	199-199	194-210	145-163	172-178	150-156	137-138	206-222	152-152	124-126	154-173	163-163	116-116
b55-41	211-215	235-237	205-215	194-200	163-184	158-172	148-154	137-138	182-202	152-152	124-142	154-173	163-170	112-116
'Magoon'	199-215	235-245	215-215	200-200	163-171	172-178	146-166	138-159	192-222	152-152	142-142	154-159	170-172	112-124
NC6-15	183-211	243-243	205-215	192-198	145-163	160-178	134-150	158-176	188-192	152-152	126-144	152-169	160-172	110-130
'Southland'	199-215	235-245	215-215	198-200	181-184	158-178	—	137-138	202-202	152-152	126-126	154-159	170-170	126-130
'Thomas'	211-215	235-237	215-223	198-200	181-184	158-178	146-154	137-158	192-202	152-152	126-142	152-154	170-172	112-130
'Trayshed'	—	237-245	205-215	194-194	163-163	172-178	146-148	137-138	182-192	152-152	124-124	171-173	163-163	116-124

continued next page

Table 4. Continued.

Accession	Allele sizes (bp) of select SSR markers															
	VVMD27	VVMD07	VrZAG62	VMC5h2	VMC5c1	VMC3d7	VVS02	VMC8g9	VMC4f3.1	VVIN16	VMC6e1	VMC2a5	VMC5a1	VMCNg3a10		
U62-56	211-215	235-237	215-215	198-198	163-181	158-172	146-146	137-138	192-192	152-152	124-126	152-173	163-170	112-116		
U62-61	211-215	235-237	205-215	200-200	163-184	158-172	146-146	138-138	192-192	152-152	124-126	152-173	163-170	124-130		
Barrett Mn-1	187-187	—	209-209	198-198	143-175	220-220	150-154	136-136	194-206	—	124-152	152-152	160-168	—		
'Chowan'	197-211	241-245	199-215	194-198	163-169	158-174	150-154	138-140	222-222	152-152	124-142	154-173	170-170	116-134		
'Flowers'	177-177	237-241	199-199	204-212	161-161	172-172	150-156	138-140	192-192	152-152	142-142	152-159	168-174	108-116		
'James'	197-209	237-245	205-215	194-198	159-188	172-196	148-150	138-140	184-186	152-152	124-140	152-173	163-170	100-116		
'Male' (Georgia)	199-215	237-245	205-215	194-194	163-184	168-172	146-148	137-140	192-222	152-152	124-124	152-173	163-167	100-116		
'Pride'	211-211	237-245	215-223	198-212	163-181	172-178	154-156	138-140	192-192	152-156	138-140	152-154	170-172	124-130		
'Tarheel'	197-215	241-245	199-215	194-212	167-184	168-172	148-148	138-140	192-202	152-152	—	152-152	168-174	102-116		
Thornhill	177-215	235-235	203-225	204-208	161-163	174-180	146-154	138-138	182-204	—	148-148	152-152	167-167	110-110		
'Creswell'	199-215	237-245	205-215	194-194	163-184	168-172	146-148	137-140	192-222	152-152	124-124	152-173	163-167	100-116		
Farrer F2 #5	179-233	239-245	189-215	194-206	145-163	164-202	130-150	138-164	170-222	152-154	126-166	163-169	164-170	100-122		
'Irene'	199-215	237-245	205-215	194-194	163-184	168-172	146-148	137-140	192-222	152-152	124-124	152-173	163-167	100-116		
'Jumbo'	—	239-243	189-189	198-204	—	160-178	136-150	—	—	152-152	126-144	152-159	163-170	112-130		
'Triumph'	199-199	243-243	199-199	194-200	167-184	174-178	148-150	137-138	202-222	152-152	142-162	152-152	170-172	102-110		

shown to support the designated identities of 30 *M. rotundifolia* cultivars and hybrids (Fig. 1). The profiles of four *M. rotundifolia* accessions, White Male, 'Jumbo', 'Triumph', and Farrer F2 #5 were not consistent with published pedigree records (Table 1).

Breeding records report that White Male is a male seedling of 'Scuppernong', and SSR data indicate that it shares one allele with the NCGR-D 'Scuppernong' at all amplified markers (data not shown). White Male is also reported to be in the pedigree of 'Higgins' and 'Yuga', yet White Male did not share alleles with 'Higgins' or 'Yuga' at five and seven markers, respectively (allele size data not shown). This observation indicates that White Male in the UCD-VE collection is not the accession reportedly used to generate 'Higgins' and 'Yuga'. It is important to point out that the name White Male is purely descriptive and may have been used for different genotypes. It is also possible that multiple collections have accessions with the same name, but different genetic profiles. The accession 'Jumbo' was not closely related to its reported parent 'Higgins' (Brooks and Olmo, 1997), but it was closely related to the VR hybrid 'NC6-15' (Fig. 2). Similarly 'Triumph' did not share alleles with its reported parent 'Fry' (Brooks and Olmo, 1997). Patel and Olmo (1955) reported that Farrer F2 #5 originated from an open pollinated NC6-15 cluster and that it has 39 chromosomes. However, the NCGR-D Farrer F2 #5 has a very unique allelic profile that did not group closely with any other genotype, and it was more closely related to *M. rotundifolia* than to *V. vinifera* or the other VR hybrids (Fig. 2).

Two *M. rotundifolia* cultivars, 'Creswell' and 'Irene', matched each other and also matched 'Male' (Georgia) at all 14 markers. Breeding records indicate that 'Creswell' and 'Irene' have pistillate flowers. However, both of these genotypes have staminate flowers at the NCGR-D, indicating that a mistake was made during the original importation or subsequent replanting and the same genotype was planted with different names. These three cultivars also grouped together in the UPGMA-based dendrogram (Fig. 2). Comparisons could not be made for 12 other *M. rotundifolia* cultivars and hybrids because potential parents and progeny were not part of the study set.

The validation of 'Thomas' as a grandparent of the VR hybrid NC6-15 was an important result given its current use in breeding for powdery mildew resistance (Pauquet et al., 2001). Detjen (1919) made the *V. vinifera* Malaga seedling #1 × *M. rotundifolia* G52 ('Thomas' × 'Hope') cross to produce NC6-15 (Table 1). Reimer and Detjen (1914) described 'Hope' as the first recorded *M. rotundifolia* hermaphrodite, but noted that it was incapable of producing viable seed. 'Hope' does not exist in the NCGR-D or UCD-VE collections. The genotype G52 may have been lost when the North Carolina State breeding program was discontinued during the U.S. Prohibition era, and no other copy is listed in the national or international collection records. The cultivar Thomas is the only link available to the powdery mildew-resistant selection NC6-15. Therefore, the verifying the correct identity of 'Thomas' is critical. 'Thomas' has been used as a parent in breeding to develop hybrid *M. rotundifolia* cultivars (Fig. 1, section F). In four of the reported cases, 'Thomas' shares one allele at all loci examined to the respective progeny, suggesting that the NCGR-D holds the correct 'Thomas' accession. Moreover, 'Thomas' as a grandparent of NC6-15 should share one-quarter of its alleles with

Fig. 1. Diagram of six partial pedigrees (A through F) where published parental relationships of *Muscadinia rotundifolia* cultivars could be confirmed with SSR marker data. See Table 4 for complete marker data; OP = open pollinated.

NC6-15. They had one common allele at 11 of 14 studied markers, which is consistent with 'Thomas' being a grandparent of NC6-15.

GENETIC DIVERSITY OF CULTIVATED *M. ROTUNDIFOLIA*. The allelic distribution and allele frequency were analyzed with SSR markers. The distribution analysis was carried out within *M. rotundifolia* cultivars, VR hybrids, and with selected *V. vinifera* cultivars and selections that are in the background of VR hybrids to determine the specificity and frequency of alleles unique to *M. rotundifolia* cultivars. A total of 184 alleles was detected in the 57 genotypes with an average of 13 alleles per marker (Table 3). The average observed heterozygosity of SSR markers was slightly higher for the complete set of 57 genotypes (all *V. vinifera* cultivars, VR hybrids, and *M. rotundifolia* cultivars) when compared with the set composed only of 35 pure *M. rotundifolia* cultivars, but the difference was not significant (results not shown). The difference between the observed and expected heterozygosity values for the *M. rotundifolia* cultivars was also small (Table 3). For 10 of the studied markers, observed heterozygosity was higher than expected, indicating that the studied set of *M. rotundifolia* cultivars is diverse and there is no indication of inbreeding depression (Table 3). For four markers (VVM27, VVM7, VrZAG62, and VVS2), alleles were also compared with a database of more than 600 *V. vinifera*-based cultivars to identify *M. rotundifolia*-specific alleles (G. Dengl, personal communication). However, it is important to note that *Vitis* and *Muscadinia* can have alleles of the same size (base pairs) and still have a unique origin. Only six alleles from four of the compared markers were common to the *V. vinifera* cultivars (Table 5). The comparisons found important differences in *M. rotundifolia* allelic composition reflected by the existence of a unique set of alleles with different frequencies. There were 88 unique *M. rotundifolia* alleles (Table 5). Within the studied set, eight alleles from eight markers had frequencies higher than 30.0 (VrZAG62-215, VMC5c1-163, VMC3d7-172, VMC8g9-138, VMC2a5-152, VMC6e1-124, VMC5a1-170, and VVIN16-152), whereas 47 alleles were present at frequencies lower than 1.0. It is also worth noting that the alleles with higher frequency at six of eight markers were unique to *M. rotundi-*

folia, further emphasizing the differences in allelic composition of this species (Table 5).

The genetic similarity of *M. rotundifolia* cultivars and VR hybrids was also analyzed using genetic distance analysis (Fig. 2). Four VR hybrids (NC74-10, JB81-107-11, NC194-1, and DRX73-026) clustered with *V. vinifera* accessions and grouped separately from all other genotypes. The accessions Farrer F2 #5, Thornhill, and Barrett Mn-1 were outliers. Thornhill and BarrettMn-1 are *Muscadinia munsoniana* (Simpson) Small accessions that were collected from Florida. As noted above, Farrer F2 #5 did not match its published parentage. The *M. rotundifolia* cultivars were clustered in small groups with common genetic backgrounds (Fig. 2), further validating the breeding records.

Discussion

Confirming the identity of grape cultivars based on comparisons with their published morphological traits is difficult. The environment can alter the expression of morphological traits, and identification is further complicated by record-keeping mistakes and propagation errors. Molecular markers like SSRs are ideal tools to differentiate cultivars, to determine phylogeographic structure of wild grapevines for conservation biology, to determine parent-progeny relationships, to identify grapevine accessions, to decipher homonyms and synonyms in grapevine cultivars, and to develop genetic maps of *V. vinifera* and *Vitis* species (Aradhya et al., 2003; Bowers et al., 1999; Dengl et al., 2001; Doligez et al., 2006; Lowe and Walker, 2006; Riaz et al., 2004, 2006). Thus, the use of SSR markers is invaluable in managing germplasm collections.

Many *V. vinifera*- and *V. riparia*-based SSR markers have been developed and have been shown to amplify sequences for other *Vitis* species (Sefc et al., 1999). However, the use of *Vitis*-based SSR markers to manage collections of *M. rotundifolia* cultivars and hybrids has not been reported. This study used SSR markers to evaluate the identity of the *M. rotundifolia* cultivars and hybrids maintained at the NCGR-D and UCD-VE collections. It was also intended to stimulate the use of SSR markers in other muscadine collections so that reference standards for identification can be agreed upon and the genetic diversity of *M. rotundifolia* germplasm can be maintained.

Fourteen SSR markers from 11 different linkage groups amplified *M. rotundifolia* DNA successfully, indicating that *M. rotundifolia* has a high degree of homology to *Vitis* species even though the chromosome number of the two genera is different. However, six markers from LG12 and 18 that have been previously mapped in a *V. rupestris* × *V. arizonica* background (Riaz et al., 2006) did not amplify *M. rotundifolia* alleles or resulted in multiple loci, indicating that the genetic sequences of these LGs are different between *Vitis* and *Muscadinia*. The powdery mildew resistance gene *Run1*

Fig. 2. Dendrogram displaying relationships among 57 grape accessions (*Vitis vinifera*, *V. vinifera* × *Muscadinia rotundifolia* hybrids, and *M. rotundifolia* accessions) based on cluster analysis (UPGMA) of genetic dissimilarity estimated using the $[-\ln(ps)]$ transformation of the proportion of the shared alleles (ps). Accessions in bold print did not match expected relationships. Scale bar represents 0.1 nucleotide substitutions per site.

has been genetically mapped on LG12 in populations that have NC6-15 in their background (Pauquet et al., 2001). Barker et al. (2005) reported that the physical map of *Run1* locus spans 450 kb with major gaps (i.e., the BAC contigs could not be extended), has extremely low recombination in vicinity of the resistance locus, and contains two different families of resistance gene analogs (RGA). Interestingly, Di Gaspero et al. (2007) reported that more than 50% of RGA markers mapped to LG12 and LG18. Although powdery mildew resistance from NC6-15 seems to segregate as a single dominant gene (Pauquet et al., 2001), the nature of this resistance is likely more complex given that powdery mildew resistance is controlled by multiple genes and alleles in other crop species (Calenge and Durel, 2006; Tommasini et al., 2006). Regardless of how powdery mildew resistance from *M. rotundifolia* is inherited, more information on the genetics of this resistance and how it functions under different environmental conditions is essential for it to be efficiently used in breeding programs.

Eighty-eight SSR alleles were unique to *M. rotundifolia* cultivars, indicating that the genome of *M. rotundifolia* is different from that of *V. vinifera* and consists of different sets of frequent alleles (Table 5). Genetic divergence between *M. rotundifolia* and *V. vinifera* was also supported by the analysis of genetic distance based on the proportion of shared alleles. The proportion of shared allele analysis makes no assumptions about the population under study or the frequency of alleles within a population. It has been used to construct trees of human individuals that reflected their ancestral origin with remarkable accuracy (Bowcock et al., 1994). A dendrogram depicting relatedness in the tested germplasm placed the *M. rotundifolia* cultivars in one large group and placed the *V. vinifera* accessions and the VR hybrids in a separate group (Fig. 2). The subgroupings of *M. rotundifolia* cultivars reflected their reported pedigrees.

Table 5. Recorded allele sizes (AS) and allele frequencies (AF) for 14 SSR markers evaluated across 57 accessions. Alleles in bold font were unique to *Muscadinia rotundifolia*. Underlined alleles were present in other *Vitis vinifera* accessions that were not part of this studied set (G. Dangl, personal communication).

VVMD27		VVMD7		VrZAG62		VMC5h2		VMC5c1		VMC3d7		VVS2	
AS (bp)	AF (%)	AS (bp)	AF (%)	AS (BP)	AF (%)	AS (bp)	AF (%)	AS (bp)	AF (%)	AS (bp)	AF (%)	AS (bp)	AF (%)
177	2.78	<u>233</u>	0.89	181	0.88	188	0.88	143	0.91	158	7.89	126	1.79
<u>179</u>	1.85	235	18.75	189	8.77	190	0.88	145	12.04	160	6.14	130	0.89
181	4.63	237	19.64	195	5.26	191	2.63	149	1.85	162	2.63	134	4.46
183	0.93	239	6.25	<u>197</u>	0.88	192	7.02	151	2.78	164	7.02	<u>136</u>	2.68
185	11.11	241	2.68	199	17.54	194	24.56	159	0.91	168	7.89	138	0.89
187	1.85	243	14.29	203	2.63	198	21.93	161	2.78	170	1.75	140	0.89
189	1.85	245	28.57	205	9.65	199	3.51	163	38.89	172	33.33	144	1.79
193	1.85	247	1.79	209	5.26	200	16.67	167	5.56	174	7.89	146	11.61
194	0.93	249	1.79	215	42.11	201	0.88	169	0.93	178	21.05	<u>148</u>	20.54
195	0.93	253	3.57	223	4.39	202	2.63	171	1.85	180	0.88	<u>150</u>	24.11
197	5.56	257	0.89	225	0.88	204	2.63	173	0.91	196	0.88	152	3.57
199	21.30			226	1.75	206	2.63	175	0.91	202	0.88	154	12.50
209	0.93					208	0.88	181	10.19	220	1.75	156	10.71
211	17.59					210	1.75	184	19.09			166	3.57
215	25.00					212	10.53	188	0.91				
233	0.93												

VMC4f3.1		VMC8g9		VMC2a5		VMC6e1		VMC5a1		VVIN16		VMCNg3a10	
AS (bp)	AF (%)	AS (bp)	AF (%)	AS (BP)	AF (%)	AS (bp)	AF (%)	AS (bp)	AF (%)	AS (Bp)	AF (%)	AS (bp)	AF (%)
166	0.89	136	2.68	152	41.96	124	32.69	156	1.75	152	84.55	90	0.89
170	0.89	137	10.71	154	16.96	126	10.58	160	4.39	154	3.64	98	0.89
172	3.57	138	36.61	159	11.61	134	0.96	163	14.91	156	5.45	100	3.57
174	1.79	140	25.00	163	0.89	138	5.77	164	0.88	160	3.64	102	3.57
178	2.68	150	0.89	165	1.79	140	15.38	166	1.75	162	2.73	104	4.46
182	4.46	158	6.25	169	5.36	142	13.46	167	4.39			108	0.89
184	2.68	159	1.79	171	2.68	144	7.69	168	5.26			110	10.71
186	3.57	164	3.57	173	11.61	148	2.88	170	48.25			112	8.04
188	4.46	168	2.68	177	3.57	152	4.81	172	10.53			116	23.21
190	0.89	171	0.89	183	1.79	162	1.92	174	7.89			118	1.79
192	27.68	173	1.79	187	1.79	166	1.92					120	2.68
194	0.89	174	4.46			170	1.92					122	2.68
200	0.89	176	0.89									124	14.29
202	8.93	184	0.89									126	0.89
204	0.89	190	0.89									130	15.18
205	0.89											134	6.25
206	2.68												
208	4.46												
222	26.79												

There were a relatively high number of alleles that were unique to *M. rotundifolia* cultivars when compared with those found in *V. vinifera*. However, these alleles were common across the group of *M. rotundifolia* cultivars, suggesting that the genetic base of these cultivars is limited. The wild populations of *M. rotundifolia* in the south-central and southeastern United States are a valuable source of germplasm with high resistance to many pests and diseases. Further study of wild *M. rotundifolia* populations is needed to clarify the nature and extent of their genetic and phylogeographic diversity. Wild populations of *M. rotundifolia* are not facing extinction, but their native growing areas are under increased human population pressure that could eventually lead to the erosion of natural genetic divergence in wild *M. rotundifolia*.

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