TRACING THE PEDIGREE OF CYNTHIANA GRAPE
BY DNA MICROsatellITe MARKERS

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Additional index words. Vitis aestivalis, Michaux, SSR markers,
ampelography, grape genetics

Abstract. While there are good quality wines being made from
muscadine and white bunch grapes in Florida, there is no iden-
tified well-adapted Florida grape for quality red wine that is
color-stable. ‘Cynthiana’ (also known as ‘Norton’) is consid-
ered one of the best American grape varieties for fine wine
making and is suspected to have good tolerance to PD
(Pierce’s disease) and low susceptibility to foliar and fruit dis-
ease. It is being successfully grown for commercial wine pro-
duction in southern Louisiana, as well as Missouri, Arkansas,
and Virginia. ‘Cynthiana’ grape produces color stable wines. It
has strong potential in Florida but needs to be evaluated be-
fore it can be recommended. Most of the grape varieties in ex-
istence today are centuries old and are considered to have
arisen by various means: domestication of wild vines, sponta-
neous crosses between wild vines and cultivated varieties,
and crosses between two varieties. Knowledge of a variety’s
parentage can have a great impact in its culture. DNA analysis
of microsatellite (simple sequencing repeat—SSR) markers
can determine the pedigree of varieties if the DNA profile of the
parents has been analyzed and documented. It is assumed
that ‘Cynthiana’/Norton originated from Vitis aestivalis,
Michaux. We have investigated the parentage of ‘Cynthiana’
grape via data mining in the existing North American grape
germplasm collections, ampelographic analyses and specifi-
cally expressed in the variety microsatellite markers.

The pedigree of North American grape ‘Cynthiana’
needs adequate clarification while this variety is evaluated for
commercialization in Florida. Because this cultivar is known
to produce high-quality wines, its tolerance to Pierce’s Dis-
ease, low vulnerability to fruit and foliar disease, and its genet-
ic background needs to be verified under Florida en-
vironmental conditions. By determining the parentage of
‘Cynthiana’, a well-adapted grape yielding high quality red,
color-stable wine may be scientifically recommended for
commercial production in Florida. While molecular marker types
such as isoenzyme and RAPD techniques are of limited use
for parentage studies (Buscher et al., 1994; Ohmi et al.,
1995), microsatellites have proven to be the marker of choice
for this purpose since they are transmitted in a codominant
Mendelian manner. In a cross, each of the parents passes one
allele per locus to the offspring and in consequence, each al-
lele displayed by the offspring must also be present in at least
one of the two parents. By examining the SSR allele composi-
tion of an individual and its two presumptive parents, it is pos-
sible to confirm or reject the proposed parentage. Currently,
numerous research projects implement the use of microsatel-
lite markers to detect parentage in other grape cultivars and
also in forensic studies.

‘Cynthiana’ is reported to be of predominantly Vitis aesti-
valis, Michaux, ancestry, and thought to have been developed
during the mid part of the 19th century (Reisch et al., 1993).
Vitis aestivalis can be found almost anywhere in the eastern
and central USA, from New England to Florida and from Wis-
consin to Texas (Galet, 1998). Vitis aestivalis has several close-
ly related species and many variants creating numerous
confusions and contradictions between the taxonomists try-
ing to classify these native American grape species. ‘Cyn-
thiana’ has excellent wine characteristics and is particularly
well suited to humid regions with comparatively long growing
seasons (Reisch et al., 1993). The disease resistance that ‘Cyn-
thiana’ exhibits is attractive to wine growers, especially in this
area of environmental protection and pesticide avoidance.
Therefore, a clarification of the pedigree of the variety is of
important not only to the Florida grape and wine industry,
but also nationwide.

In our studies we aimed to reconstruct the parentage of
‘Cynthiana’ by combining the use of DNA fingerprinting via
microsatellite markers, Simple Sequence Repeats (SSR) and
Random Amplified Polymorphic DNA (RAPD) markers via
data mining of existing germplasm collections records, and
comparative morphological description and ampelographic
analysis of the variety itself and close wild grape relatives. This
paper presents some preliminary results accumulated in the
course of a study, and our findings for the variety via em-
ployed comparative morphological descriptors, ampelo-
graphic analysis and DNA fingerprinting results.

Materials and Methods

Plant material. A 0.7 acre experimental plot was planted
with ‘Cynthiana’ grape plants in 2003 at FAMU/Cesta Center
for Viticulture & Small Fruit Research located in Leon, Co.,
Fla. For proven authenticity the planting material was taken
from Post Winery & Vineyard, Altus, Ark., the major recog-
nized nursery-distributor for ‘Cynthiana’ in the U.S. Three
year old, well established vines at “anthesis” and “fruit set”
were marked in the vineyard to serve as a donor material for
morphological similarity and DNA isolation as a ‘Cynthiana’
accession. One accession of Southern V. aestivalis, vine grow-
ing at the site of “Carriage Factory Restaurant”, Quincy, Fla.
was identified and included in this study. From the National
Clonal Germplasm Repositories at Geneva, N.Y. and Davis,
Calif., seven more accessions (five V. aestivalis; two V. aestivalis
var. aestivalis) from wild aestivalis were identified for inclusion
in the study. Noble vines from the experimental vineyard
(Henscratch Nursery, Lake Placid Fl.), Chardonnay vines
(Vintage Nursery, Indio, Calif.) and Concord vines (Ision’s
Nursery, Brooks, Ga.) were used as individual accessions for
V. muscadinina, V. vinifera and V. labrusca grape species, respec-

*Ampelographic analysis. Morphological characterizations
were performed following the descriptor list for the distinc-

This research is funded by FDACS, VAC Grant # 009083.
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Photographic plates with fully expanded leaf samples, growing tips of the fruiting branch and grape clusters at fruit set were made of the 'Cynthiana' and Southern V. aestivalis for morphological assessment. Galet (1998) drawings and photograph of North American V. aestivalis served as the standard for this species.

DNA Isolation and Molecular Analysis. DNA was extracted using the Qiagen® Protocol for Isolation of DNA from Plant Tissue DNAeasy Plant Mini Kit (2003). DNA was extracted and kept in a freezer at -20°C. Isolated DNA from each of the seven samples was quantified using a Hoefer® DyNA Quant 200 Fluorometer.

Eight Intersimple Sequence Repeat (ISSR) primers were utilized to amplify the repeat regions in the grapevine samples. The ISSR primers were acquired from the University of British Columbia (UBC) SSR Primer (RAPD) Synthesis Project Oligonucleotide Set 100/9. The primers are coded as #810, #816, #820, #826, #873, #875, #878, and #900. Nine RAPD primers also obtained from the University of British Columbia (UBC) RAPD Primer Synthesis Project Oligonucleotide set 100/8 were used to amplify the regions within the samples. The primers are coded as #701, #702, #703, #704, #705, #706, #707, #709, and #750.

Polymerase Chain Reaction (PCR) were carried out in 100 µL volume containing four samples of 28-33 ng of genomic DNA, 0.5 µM of each primer, 0.2 mM each of 10 mM dNTP, 1x of 10× PCR buffer, 1.5 mM of 50 mM MgCl2, and 2.5 units of 5 U/µL of Taq DNA Polymerase. PCR reactions were carried out using a MJ Research® PTC-200 Peltier Thermal Cycler with the following profile: (i) 94°C for 3 min; (ii) 94°C for 45 s, 55°C for 30 s, 72°C for 1 min, and 30 seconds per 35 cycles; (iii) 72°C for 10 min. A 100bp DNA ladder was used. Amplification was confirmed after running the PCR product in 2% regular agarose gel, 1% regular agarose + 1% NuSieve® agarose and observed under UV light.

Twenty-five pairs of SSR primers previously described by Thomas and Scott (1993), Bowers et al. (1996), Lamboy and Alpha (1998), and Sefc et al. (1999) were synthesized by Genosys-Sigma and used to test in 'Cynthiana' and wild V. aestivalis accessions in later efforts to attempt to identify specific loci and DNA fingerprints for use in reconstruction of the pedigree of the variety.

Results and Discussion

The systematics of V. aestivalis are a particularly difficult area of taxonomy. The legitimacy of any classification is still difficult. The definition of a species may be based on comparative morphology with support from environmental characteristics and geographical location (Subden et al., 1987). Evidence for natural hybridization may be based on the phe-
the 'Cynthiana' (Table 3; Fig. 3). The use of ISSR primers #816 and #900 within *V. aestivalis* is recommended.

Random Amplified Polymorphic DNA (RAPD) fragments are frequently used as molecular markers in genetic diversity studies. The theory associated in producing RAPDs is that a single, short oligonucleotide primer is used to amplify random sequences from a complex DNA template, which binds to many different loci. RAPD markers have been used to study the genetic diversity of wild and cultivated *V. vinifera* grapevines (Grando et al., 1994). In our experiments for identifying specific RAPD markers for 'Cynthiana' a RAPD profile was generated from primer #750 (Table 3; Fig. 4). This specific RAPD primer demonstrated an unambiguous amplification within 'Cynthiana'.

SSRs primers have been used to distinguish genetically different grapevines. Genetic markers produced with SSR primers have several advantages over DNA-based markers. SSRs are detected at specific loci, they are highly reproducible, and because SSRs use the PCR process, the amount of sample tissue DNA is very low (Lin and Walker, 1998). SSR markers are ubiquitously distributed throughout genomes.

Table 1. Leaf Blade Measurements of Southern *Vitis aestivalis*.

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Σ area = 184721 mm² (7272.5 in).
Mean area = 9236 mm² (363.6 in).

Table 2. Leaf Blade Measurements of 'Cynthiana'.

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Σ area = 180477 mm² (7105.4 in).
Mean area = 9023 mm² (355.2 in).
making these markers particularly useful in parentage studies (Meredith et al., 1999), genetic mapping studies (Blondon-Adam et al., 2004), and “fingerprinting” plant varieties (Bowers et al., 1993). Our experiments with twenty-five pairs of primers isolated and previously used for variety identification in V. vinifera and V. riparia are under way.

**Conclusion**

Few other crops can claim as many varieties as grape (Bowers et al., 1993). Estimates place the number of cultivars in V. vinifera alone between 5,000 and 15,000 (Galet, 1979). When viticulture was a traditional pastime centered on native cultivars, differentiating the parentage of varieties was less important. Today, the demands of international wine trade, the need to protect patented cultivars, and improved communication among researchers in various countries have made accurate identification of grape pedigrees essential.

By discovering the pedigree of ‘Cynthiana’, a new and suitable cultivar for growth in Florida may be recommended for commercial production. The use of ampelographic description and DNA fingerprinting (ISSR, RAPD, and SSR molecular markers) can serve as a template that can confirm and clarify the genetic background of ‘Cynthiana’ when compared to numerous native V. aestivalis accessions.

**Table 3. Sequences of 3 DNA molecular primers with specific amplification in Cynthiana grape.**

<table>
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<th>Primer Code</th>
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<tr>
<td>ISSR #816</td>
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<td>ISSR #900</td>
<td>ACT TCC CCA CAG GTT AAC ACA</td>
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<td>RAPD #750</td>
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**Fig. 4. Specific amplification of RAPD primers #750 in muscadinia, vinifera and Cynthiana (DNA ladder 100 bp): Lane 1: Cynthiana, Lane 2: labrusca, Lane 3: muscadinia, Lane 4: vinifera, Lane 5: Southern aestivalis, Lane 6: + Control, Lane 7: - Control, Lane 11: DNA ladder 100 bp.**

**Literature Cited**


