Mapping and Detection of Downy Mildew and Botrytis bunch rot Resistance Loci in Norton-based Population

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Norton has been grown in Missouri for over 160 years, but little is known about the genetics of its disease resistance, cold hardiness and berry quality.

**Vitis aestivalis**-derived
‘Norton’
State Grape of Missouri
Genetics of Norton (Missouri State) Grapes

Norton has naturally evolved resistance to
1. Powdery mildew
2. Downy mildew
3. Berry rot complex including *Botrytis*
   Bunch rot, Bitter rot and Black rot
4. Insect Phylloxera
5. Cold hardiness
Norton vs Cabernet Sauvignon

*Vitis aestivalis*-derived ‘Norton’
Cold hardy and Resistant to most fungal pathogens
Good wine quality

*Vitis vinifera* ‘Cabernet Sauvignon’
Cold sensitive and Susceptible to most fungal pathogens
Great wine quality

A need exits to breed for grapevines that would combine the superior wine quality of *V. vinifera* with the disease resistance and cold hardiness of Norton.
Interspecific Hybrid Identification

Genetic profiles (allele sizes in bp) of grape varieties at various SSR loci

<table>
<thead>
<tr>
<th>Primers</th>
<th>VVMD5</th>
<th>VVMD7</th>
<th>VVMD27</th>
<th>VVS2</th>
<th>VrZAG62</th>
<th>VrZAG79</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norton</td>
<td>233/247</td>
<td>237/246</td>
<td>184/186</td>
<td>135/137</td>
<td>181/205</td>
<td>250/254</td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>231/240</td>
<td>239/239</td>
<td>173/187</td>
<td>141/154</td>
<td>189/195</td>
<td>246/246</td>
</tr>
</tbody>
</table>

Crosses tested for interspecific hybrid production

<table>
<thead>
<tr>
<th>Crosses</th>
<th># Plants evaluated</th>
<th># True hybrids</th>
<th>% True hybrids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norton (♀) x Cabernet Sauvignon (♂)</td>
<td>286</td>
<td>252</td>
<td>88.1</td>
</tr>
<tr>
<td>Norton (♂) x Cabernet Sauvignon (♀)</td>
<td>24</td>
<td>21</td>
<td>87.5</td>
</tr>
</tbody>
</table>

Scientia Horticulturae (2014) 179: 363
Genotyping
Norton Linkage Map Construction

1. 1,157 SSR markers were tested on the parents and 4 progenies for polymorphism.
2. 414 polymorphic markers were identified and screened through a-182 genotype population.
3. 411 markers clustered in 19 linkage groups.
There are 26 gaps larger than 10 cM.
VitisGen I Project (2011-2016)

• A 5-year project funded by the USDA-National Institute of Food and Agriculture (NIFA) Specialty Crops Research Initiative
• Combines the expertise of breeders, geneticists, pathologists, physiologists, chemists, enologists, computational biologists, sociologists, economists, and the grape industry
• 12 research institutions
VitisGen Project

Genotyping

Genotyping-by-Sequencing (GBS)

Single Nucleotide Polymorphism (SNP)

Ultimate Goal: 50,000 SNPs/Population

43,971 SNPs have been identified.

A consensus map of 3,825 SNPs has also been developed.
Genotyping:
Genotyping-by-Sequencing (GBS)
Single Nucleotide Polymorphism (SNP)
Simple Sequence Repeat (SSR)

43,971 SNPs have been identified
A consensus map of 3,825 SNPs
A consensus map of 411 SSRs

Integration of Genetic Maps to construct a high-resolution map with both SSR and SNP markers using JoinMap 4.1 software
R/QTL software using a 4-way cross format for composite interval mapping (CIM)

Consensus Map

407 SSRs  1,665 SNPs  Total: 2,072 markers
There are only 4 gaps larger than 10 cM.
Downy Mildew

Norton

Cabernet Sauvignon

Norton

Cabernet Sauvignon

Adaxial Side

Abaxial Side
Laboratory Assay

Visual rating

1 2 3 4 5

Norton  Cabernet Sauvignon

Field Assay

r = 0.94
Strong correlations were observed among data sets (Spearman correlation coefficient = 0.57 to 0.79)
Downy Mildew Resistance (\textit{Rpv} 25)

*LOD value of 16.4 explaining 33.8% of the total phenotypic variation flanked by markers VVCS1H077H166R1-1 (56.6 cM) and UDV737 (60.9 cM).

*Three SNP-trait association were detected between the two flanking SSR markers, further reducing the interval distance to 0.7-2.3 cM.
Botrytis Bunch Rot

Botrytis cinerea

Cabernet Sauvignon

Norton
**Botrytis Bunch Rot**  
*p. cinerea*

<table>
<thead>
<tr>
<th>Harvesting Stage</th>
<th>Disease Incidence</th>
<th>Disease Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2012</td>
<td>2013</td>
</tr>
<tr>
<td></td>
<td>Norton</td>
<td>CS</td>
</tr>
<tr>
<td>E-L Stage 36</td>
<td>4.2</td>
<td>100.0</td>
</tr>
<tr>
<td>E-L Stage 37</td>
<td>0.0</td>
<td>83.3</td>
</tr>
<tr>
<td>E-L Stage 38</td>
<td>0.0</td>
<td>91.7</td>
</tr>
<tr>
<td>E-L Stage 39</td>
<td>4.1</td>
<td>100.0</td>
</tr>
<tr>
<td>E-L Stage 40</td>
<td>0.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Values are mean for five replicates for each stage between two cultivars for both years. Significant differences (two-sampled t test) are designed at P>0.05.
Botrytis Bunch Rot

<table>
<thead>
<tr>
<th>Population Size</th>
<th>158</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repetitions</td>
<td>8</td>
</tr>
<tr>
<td>Duration</td>
<td>10 DAI</td>
</tr>
</tbody>
</table>

![Image of disease severity graphs for 2015 and 2016]
Botrytis Bunch Rot Resistance

*LOD value of 7.1 explaining 18.4% of the total phenotypic variation flanked by markers VMC6F1 (42.7 cm) and VMC3B10 (46.9 cm).

*Four SNP-trait association were detected between the two flanking SSR markers, , further reducing the interval distance to 0.3-1.9 cm.
Norton x Vignoles
Summary

1. GBS data can be used to saturate the grape genome with SNPs in a pseudo-testcross population.

2. SNPs and SSRs can have complementary roles: first, to identify genome regions associated with traits of interest using SNPs, and second, to perform marker-assisted selection using SSRs.

3. The overall goal of this program is to provide molecular genetic support to expedite a Norton grape breeding effort with the ultimate goal of developing improved cultivars well adapted to Missouri conditions.
North American Grape Breeders Conference
Arkansas, California, Florida, Georgia, Minnesota and New York
2015 Cornell University; 2017 UC Davis
2019 Missouri State University
August 15-16, 2019

Field Day – Thursday
Missouri State Fruit Experiment Station, Mtn. Grove. MO
MSU breeding vineyard (VitisGen II) & winery; Virus cleaning network
St. James Winery, St. James, MO

Research Presentation – Friday
Bond Learning Center, Springfield, MO
1. Di Gaspero et al. (2012) studied the selective sweep in *Rpv3* using its flanking SSR loci UDV305 and UDV737. Seven different haplotypes were generated; however, none of the haplotypes were present in ‘Norton’/’Cynthiana’.

2. The *Rpv3* related SSR markers including UDV305, UDV108, UDV112 and VMC7F2 didn’t show polymorphism in Norton.

3. Downy mildew resistance in ‘Norton’ is most likely due to the presence of a new locus within the shared region of *Rpv3* (UDV305 and UDV737), or in the unique region between markers of VVCS1H077H16R1-1 and UDV305.
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VitisGen I&II

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Missouri Department of Agriculture