



QTL Mapping of Botrytis bunch rot Resistance in a *Vitis aestivalis*-derived 'Norton'-based Population

Chin-Feng Hwang, Ph.D.
State Fruit Experiment Station
Darr College of Agriculture

Dean Volenberg, Ph.D.
Grape and Wine Institute
University of Missouri



***Vitis aestivalis*-derived
'Norton'
State Grape of Missouri**

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.



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 exists to breed for grapevines that would combine the superior wine quality of *V. vinifera* with the disease resistance and cold hardiness of Norton.

Marker Development (PCR & DNA Sequencing)

- 1. Restriction Fragment Length Polymorphism (RFLP)**
- 2. Random Amplified Polymorphic DNA (RAPD)**
- 3. Amplified Fragment Length Polymorphism (AFLP)**
- 4. Simple Sequence Repeats (SSRs)**
- 5. Single Nucleotide Polymorphism (SNP)**

- More than 1,000 SSR markers available in grape society**
- Grape with a high level of “Heterozygosity”; SSR marker “Transferability” between populations**



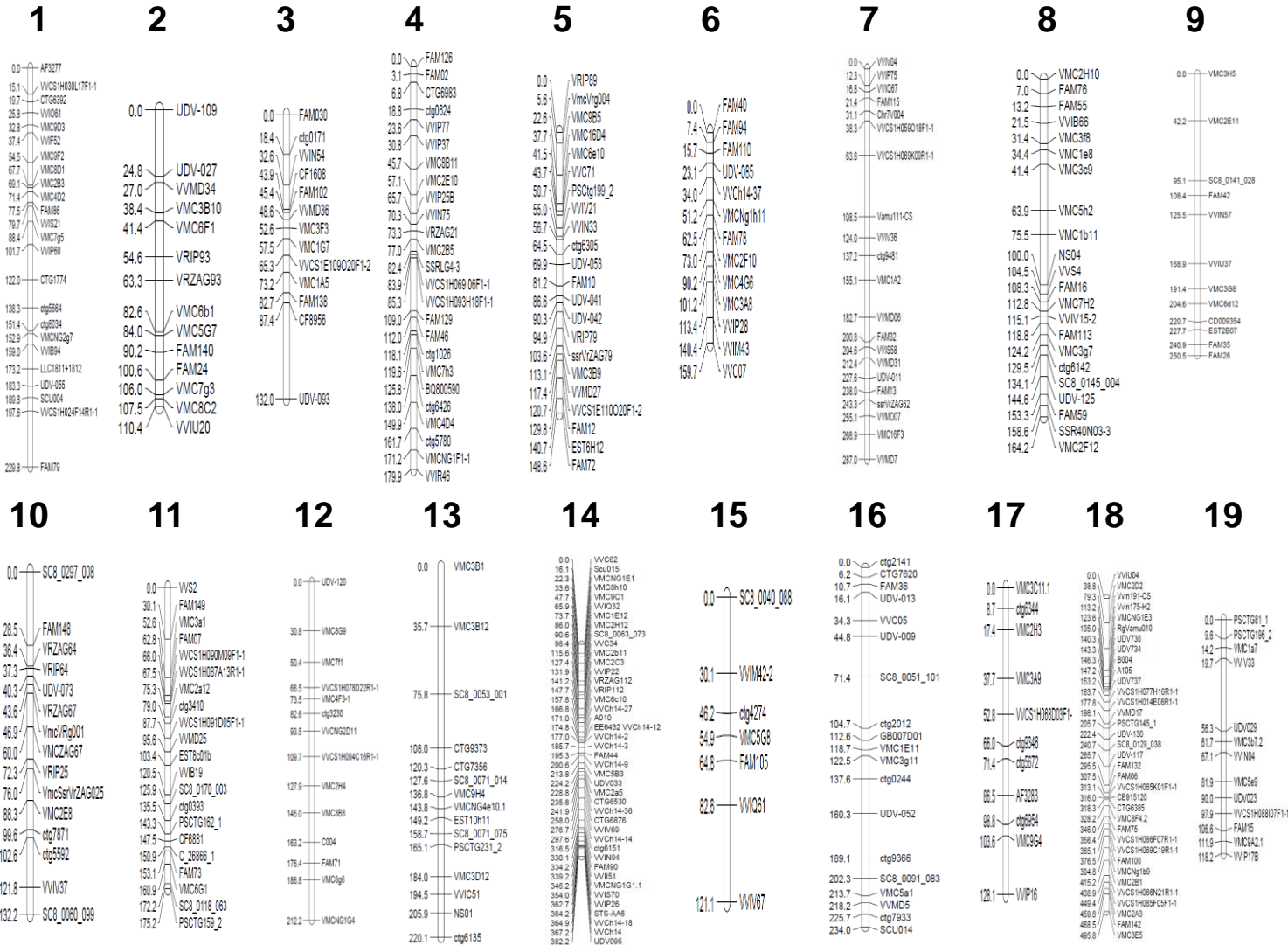
Genotyping



Norton Linkage Map Construction

1. 1,157 SSR markers were test on the parents (**Norton & Cabernet Sauvignon**) 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

Norton Haploid Map (411 SSRs)

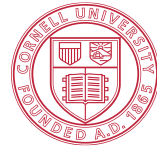


There are 26 gaps larger than 10 cM.

VitisGen I&II Project (2011-Present)



- A 10-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



Dr. Bruce Reisch
Dr. Lance Cadle-Davidson
Cornell University



OKLAHOMA CITY UNIVERSITY



Cornell University
Cooperative Extension



Missouri
State
UNIVERSITY





VitisGen I Project

Genotyping

Genotyping-by-Sequencing (GBS)

Single Nucleotide Polymorphism (SNP)

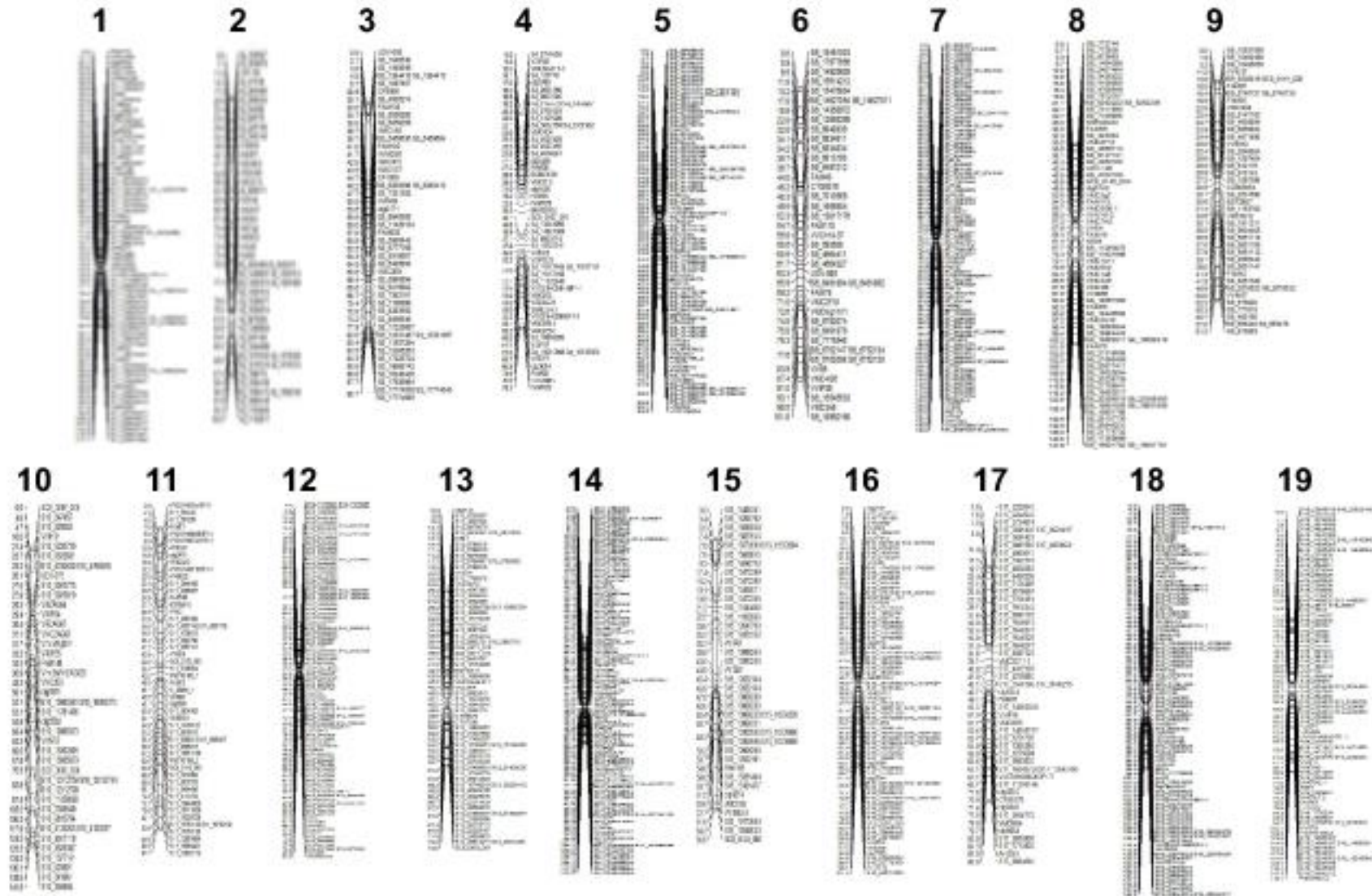
A consensus map of 3,825 SNPs

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**

JoinMap 4.1

Norton Haploid Map (407 SSRs + 1665 SNPs)



There are only 4 gaps larger than 10 cM.



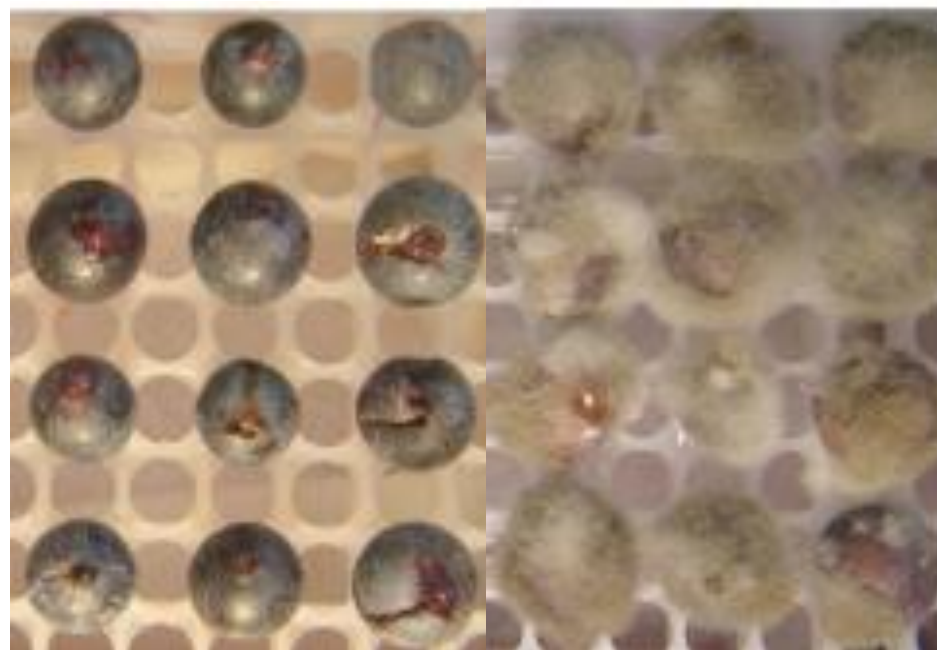
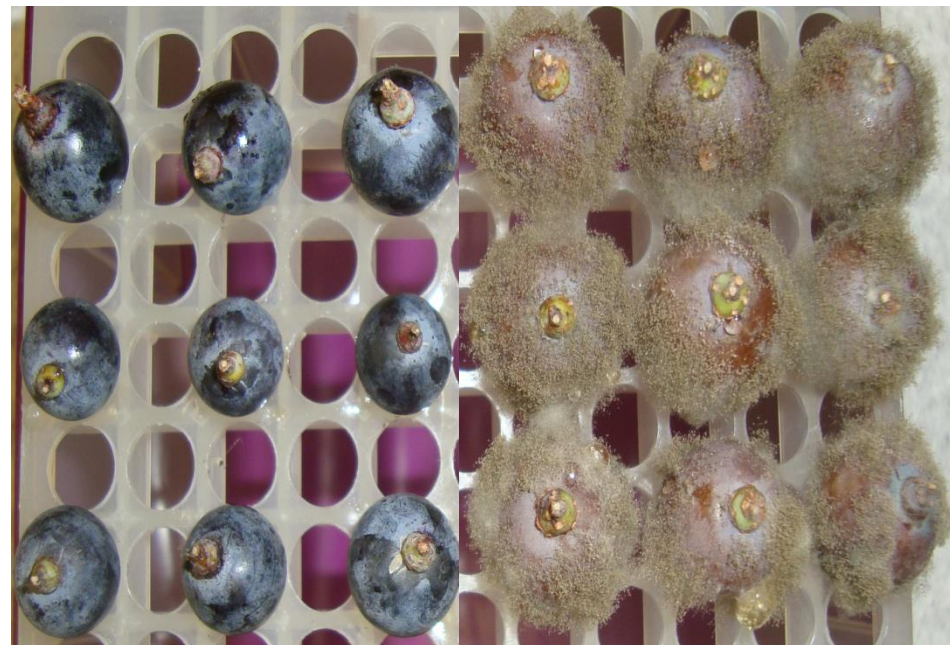
Phenotyping

Botrytis Bunch Rot

Botrytis cinerea

Cabernet
Sauvignon

Norton



Botrytis Bunch Rot

Botrytis cinerea

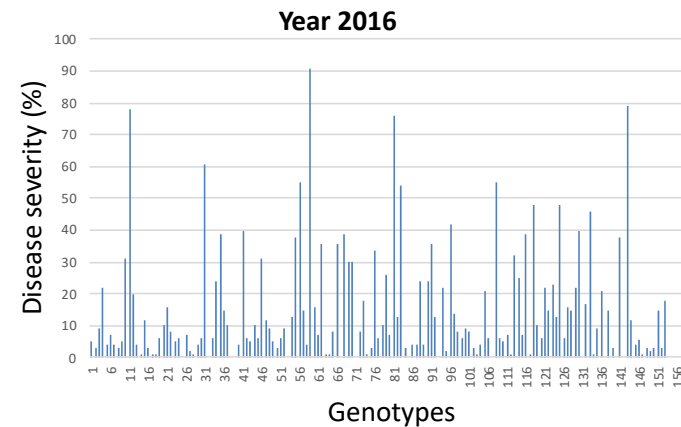
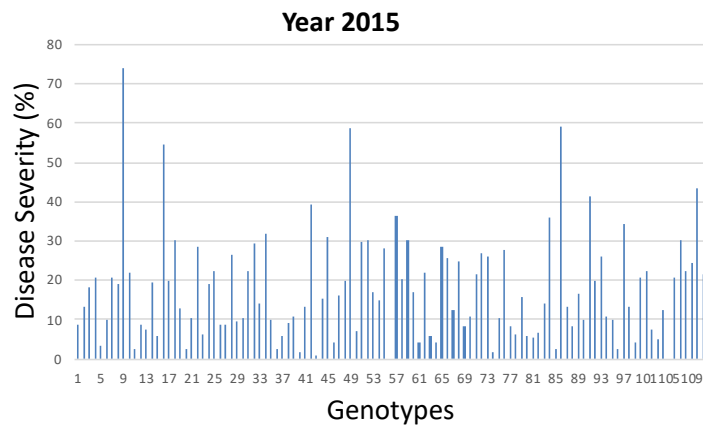
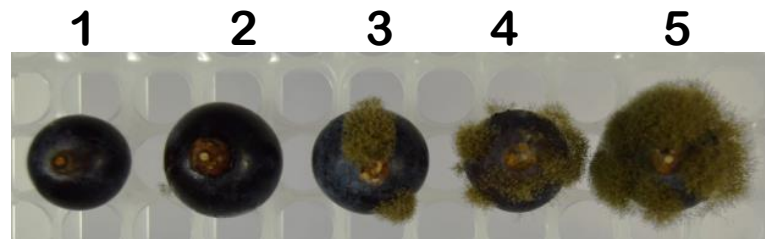
Table 1. Average disease incidence and severity between two grape cultivars; Norton and Cabernet Sauvignon (CS) under different stages.

Harvesting Stage	Disease Incidence				Disease Severity			
	2012		2013		2012		2013	
	Norton	CS	Norton	CS	Norton	CS	Norton	CS
E-L Stage 36	4.2	100.0	8.3	100.0	1.7	96.7	3.3	96.7
E-L Stage 37	0.0	83.3	12.5	95.8	0.0	80.8	5.8	86.7
E-L Stage 38	0.0	91.7	16.7	100.0	0.0	91.7	6.7	96.7
E-L Stage 39	4.1	100.0	16.7	100.0	1.6	96.7	10.8	100.0
E-L Stage 40	0.0	100.0	10.0	100.0	0.0	100.0	6.7	98.3

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

Population Size	158
Repetitions	8
Duration	10 DAI

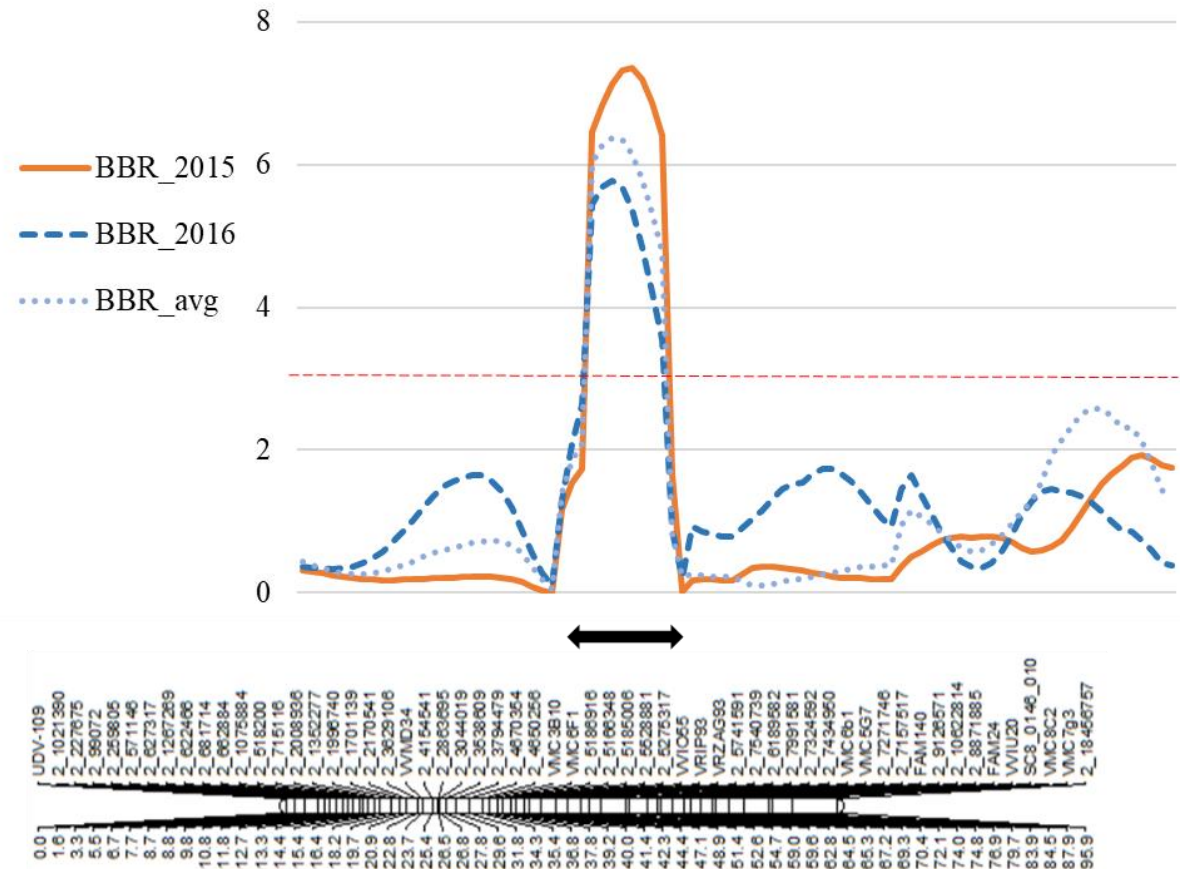


Botrytis Bunch Rot Resistance

*LOD value of 7.5 explaining 20.4% of the total phenotypic variation flanked by markers VMC6F1 (36.8 cM) and VMC3B10 (44.4 cM) which are 7.6 cM apart.

*Five SNP-trait associations were detected between the two flanking SSR markers, further reducing the interval distance to 0.8-2.1 cM.

Quantitative Trait Locus (QTL) Mapping



Linkage Group 2



Summary

- 1. GBS data can be used to saturate the grape genome with SNPs in a Norton x Cabernet Sauvignon mapping 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.**

Summary

The overall goal of this program is to provide molecular genetic support to expedite grape breeding effort with the ultimate goal of improving viticultural performance and enological quality with new grape cultivars well adapted to Missouri conditions.



Acknowledgements

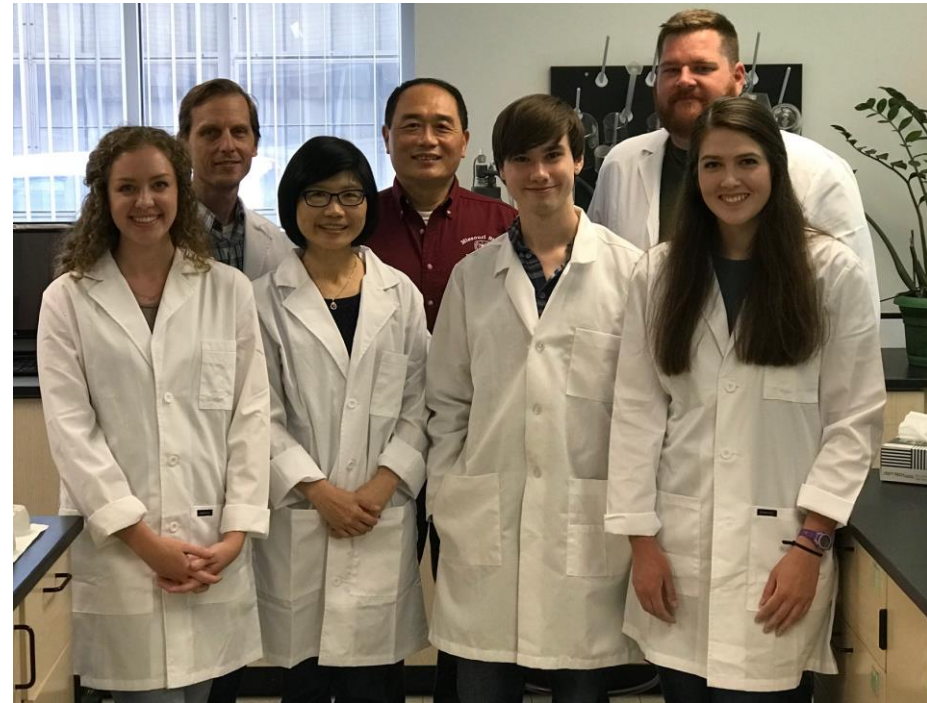
Surya Sapkota
Jacob Schneider
Will Knuckles

Li-Ling Chen
Karlene Negus
Kavya Tummala

UC Davis
Dr. Andy Walker

University of Missouri
Dr. Jim English
Dr. Dean Volenberg
Dr. Misha Kwasniewski

Cornell University
Dr. Bruce Reisch
Dr. Lance Cadle-Davison



E & J Gallo Winery, Modesto, CA

Jim Anderson, Missouri Grape & Wine Board

Funding Sources

USDA USDA-NIFA-NLGA Program
USDA-NIFA-AFRI Competitive Grant

Missouri State
UNIVERSITY

Missouri Grape & Wine Board



Missouri Department of
Agriculture