Summer 2021

Marker-Assisted Selection to Determine the Introgression of Rpv-3 Mediated Downy Mildew Resistance in 'Chambourcin' X 'Caberenet Sauvignon' Grapevine Population

Kavya Sri Tummala
Missouri State University, KavyaSri999@live.missouristate.edu

MARKER-ASSISTED SELECTION TO DETERMINE THE INTROGRESSION OF *Rpv-3* MEDIATED DOWNY MILDEW RESISTANCE IN 'CHAMBOURCIN' × 'CABERNET SAUVIGNON' GRAPEVINE POPULATION

A Master's Thesis

Presented to

The Graduate College of

Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science, Plant Science

By

Kavya Sri Tummala

July 2021

MARKER-ASSISTED SELECTION TO DETERMINE THE INTROGRESSION OF *Rpv-3* MEDIATED DOWNY MILDEW RESISTANCE IN 'CHAMBOURCIN' × 'CABERNET SAUVIGNON' GRAPEVINE POPULATION

Environmental Plant Science and Natural Resources

Missouri State University, July 2021

Master of Science

Kavya Sri Tummala

ABSTRACT

Downy mildew caused by the fungus, *Plasmopara viticola* in the grapes is one of the major diseases affecting crop yield throughout the world. Breeding grapes via conventional methods for the downy mildew resistance will take approximately 25-30 years to fully evaluate and release new cultivars. Because of the long breeding cycle in woody plant species like grape, developing DNA markers linked to genes or quantitative trait loci (QTLs) for horticultural traits can greatly accelerate the breeding process and allow a much more accurate selection of progeny. This study aims to determine the presence of resistance in the 267 F_1 genotypes developed by crossing the downy mildew resistant cultivar, *Vitis* interspecific hybrid 'Chambourcin', and the disease susceptible cultivar, *V. vinifera* 'Cabernet Sauvignon' using DNA markers. 'Chambourcin' has the *Rpv3* downy mildew resistance locus. Using 4 simple sequence repeats (SSR) markers linked to the *Rpv3* locus, UDV730, UDV734, UDV736, UDV737, the *Rpv3* resistance alleles were examined among the hybrid progenies via marker-assisted selection. The data was analyzed to confirm which resistance allele sizes were passed down from the pedigree of the 'Chambourcin' to the hybrid progeny. This study will help provide the industry stakeholders from the grape and wine community with new cultivars that are adapted to Missouri.

KEYWORDS: marker-assisted selection, downy mildew, susceptible, resistant, DNA markers.

MARKER-ASSISTED SELECTION TO DETERMINE THE INTROGRESSION OF *Rpv-3* MEDIATED DOWNY MILDEW RESISTANCE IN 'CHAMBOURCIN' × 'CABERNET SAUVIGNON' GRAPEVINE POPULATION

By

Kavya Sri Tummala

A Master's Thesis
Submitted to the Graduate College
Of Missouri State University
In Partial Fulfillment of the Requirements
For the Degree of Master of Science, Plant Science

July 2021

Approved:

Chin-Feng Hwang, Ph.D., Thesis Committee Chair

Li-Ling Chen, M.S., Committee Member

Steven Jacob Schneider, M.S., Committee Member

Julie Masterson, Ph.D., Dean of the Graduate College

ACKNOWLEDGEMENTS

I will always be grateful to my advisor Dr. Chin-Feng Hwang for accepting me into his lab and providing me opportunities and for guiding me in every step. I would also like to specially express my gratitude towards Li-Ling Chen for not only teaching me all the research techniques and lab protocols but also for constantly supporting me as a person. I will forever be grateful for my advisor Dr. Chin- Feng Hwang and Li-Ling Chen. I would also like to thank Jacob Schneider for being great support whenever needed. I would also thank my fellow lab member, Karlene Negus for helping me with the research and also in the field.

I dedicate this thesis to my parents, Nageswara Rao Tummala, Sandhya Tummala and to my lovely brother Hemanth Chowdary Tummala, for supporting me to do everything and anything that I ever wanted to do and for also being a keen part of the Graduate Journey along with me in every situation. Aside from my family, it would never have been possible to achieve a graduate degree without the blessings of Shri Shirdi Sai Baba.

|| Satchidananda Sadguru Shri Sai Nath Maharaj Ki Jai ||

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Grapes belong to the family Vitaceae and are mostly cultivated in the regions of Asia, North America, and Europe (Terral et al. 2010). There are 11 genera and about 600 different species of grapes. Cultivation of the grapes dates to 6500 B.C, which coincides with the Neolithic era, and was mainly for use as fresh table fruit, dried fruits (raisins), and for wine. Wine is one of the major products obtained from fermenting grapes and Egyptian records show that winemaking was happening since 2500 BC. However, the first wine is thought to be derived from the Middle East (UK Essays 2018). Wine was discovered out of luck, obtained by observing the damaged grapes fermenting in vessels and when farmers tasted the juice of the fermented grapes, they liked it (Chambers et al. 2010).

Grapes have several health benefits. They contain a compound called resveratrol which has anti-cancer properties and it also reduces the risk of paralysis. Resveratrol compound is also good for the large intestine (Fahad et al. 2020). By using the ointments made from polyphenols obtained from wine, skin problems and joint issues can also be treated (Feher et al. 2007). Grapes play an important role economically. In the year 2019, there were about 69.28 million hectares of grape area, 91.22 million hectograms per hectare of grape yield and 77.22 million tons of production quantity harvested (FAOSTAT 2019). There was an approximate total of 5.7 billion U.S dollars' worth of grape production during the year 2019 (Shahbandeh 2020).

Plant Breeding

Since 1750, naturalists like Linnaeus and Fairchild have been doing experiments to understand hybridization. But it was not until 1900 when plant breeding was introduced as a

discipline of study (Carlson 2004; Kingsbury 2009). The technique of developing cultivars with desirable traits that are heritable and permanent for the benefit of mankind is called Plant Breeding and it can also be referred to as "Plant Improvement". There are two different phases of plant breeding. First is conventional breeding based on selection of phenotypes and second is molecular breeding using molecular tools and DNA markers (Flavio et al. 2013; Yacoub 2010).

In the conventional breeding, plants with desirable qualities are identified first, and then crossing of the selected plants is carried out. After crossing, selection is done to separate the crosses with the desirable traits (Allard 2019). Before any breeding procedure is carried out, brief goals of the needs are listed as per the consumer preferences and producer needs. Usually, the main goals of breeding would be to develop plants that are resistant to lodging, are high yielding, adaptable to different environments, resistant to biotic and abiotic stress. Plants which are resistant to pests are being developed so that the amount of spraying required by the farmer to control the pests can be reduced thereby making farming economical while reducing the environmental pollution. Breeders can also develop new "cultivars" which are completely packed with the desirable qualities and will be released into the market so that the farmer who uses the cultivar can increase their income (Yacoub 2010). Crop yield losses caused by biotic stresses are quite high (Hasan et al. 2015). Also, the new kind of biotic stresses keeps on evolving so that the need for new and improved approaches is desirable so that plants can survive the attack. In conventional breeding, along with the desirable genes, few undesirable genes tend to inherit to the next generations, and it is difficult to get rid of those unwanted genes even with backcrossing.

Molecular Breeding

Plant Breeding has a long history of combining the latest innovations of biology and genetics to intensify crop improvement (Moose et al. 2008). Molecular breeding can be defined as using DNA markers to help in identification of plants that have the desirable characters (Jaradat 2015). Principles of hybridization and selection were outlined by Charles Darwin and the fundamental association between the phenotype and genotype was defined by Mendel (Shull 1909). In the previous years, plants showed desirable phenotypes were selected and cultivated to increase production. This can be considered the earliest example of biotechnology (Harlan 1992). Even though Mendelian genetics was thought important, it was only after quantitative genetics coming into light that united the Mendelian principles considering them important (Paul et al. 1988).

Molecular breeding of grapes is done to study the characters like disease resistance and berry quality. Chemical spraying needs to be done at a higher level to reduce the incidence level of diseases in grapes and to obtain good yield of high-quality berries. However, there is abundance of genetic diversity in *Vitis* species (Lijavetzky et al. 2007). This genetic variation is used to develop disease resistant cultivars in grapes using molecular breeding (Di Gaspero et al. 2010). There are different types of molecular markers developed since the 1970's, which are used to study the differences between the cultivars and to look for the presence of desired genes (Bautista et al. 2008).

Genetic Markers

Genetic markers are closely located to the gene of interest and act as flags. Classical markers and DNA markers are two types of genetic markers. Morphological, cytological, and biochemical are three different kinds of classical markers. Restriction Fragment Length

Polymorphism (RFLP), Randomly Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR) and Single-Nucleotide Polymorphism (SNP) are types of DNA markers (Nadeem et al. 2017) (Table 1).

Morphological markers are used to differentiate traits like flower color, seed structure, growth habit, and other agronomic traits (Nadeem et al. 2017). They are very easy to use, and they do not need any special molecular techniques. The disadvantage with the morphological markers is that they are very limited in number and can easily be influenced by the stage of plant growth (Nadeem et al. 2017). Cytological markers are used to study the variations present in the number, banding pattern, size, shape, order, and position of the chromosome. These markers are commonly used to differentiate between normal and mutated chromosomes. They can also be used to identify linkage groups and in physical mapping (Nadeem et al. 2017). Biochemical markers also known as isozymes are the forms of enzymes that are coded by different genes but have the same function. Biochemical markers are used to study genetic diversity, population structure, and gene flow. These markers are co-dominant markers and are economical to use. But the downside with the isozymes is that they are limited in number and are influenced by the different stages of plant growth and also by various extraction methodologies (Nadeem et al. 2017). The different types of molecular markers are discussed below.

RFLP : RFLP is the first and only hybridization based DNA marker that is used to determine the polymorphisms present in the species which could be due to insertions, deletions, point mutations, translocations, duplications, and inversions (Nadeem et al. 2017). DNA is isolated and mixed with the restriction enzymes isolated from bacteria to cut the DNA at a particular locus. Restriction enzymes will not perform their function if there are any changes in the

recognition sites. Later, agarose or polyacrylamide gel electrophoresis (PAGE) will be carried out to look for polymorphisms (Kundan 2014).

RAPD : RAPD markers are used to look for the polymorphisms present at primer sites or between the primer sites. A combination of short 10 nucleotide primer and a random primer are used for the amplification of genomic DNA. Amplification during PCR takes place when two hybridization sites are similar to each other and are in opposite direction (Jiang 2013). The primers selected for the amplification should have a minimum of 40% GC content as without which, it will not be able to sustain a temperature of 72^{o} required for the DNA polymerase for elongation (Williams et al. 1990). Gel electrophoresis can be used to study the fragment differences (Nadeem et al. 2017). However, the specificity of the primer to the DNA sequence is low thus making it difficult to study the results (Walker et al. 2010).

AFLP : AFLPs are used to see the variations in the fragment banding patterns which are caused by the mutation. For using AFLP markers, initially digestion of DNA will be performed followed by the PCR (Leal 2001). Frequent and rare cutters are the two restriction enzymes used for the digestion of DNA and the resulting ends are ligated using an oligonucleotide. PCR products are visualized by running either an agarose gel or polyacrylamide gel (Kundan 2014). Both AFLPs and RAPDs are dominant markers and it is very difficult to identify heterozygosity (Walker et al. 2010; Mueller and Wolfenbarger 1999).

SNP : SNP markers show the differences present at a single nucleotide location (Vignal et al. 2002). The frequency of SNPs varies between 1 SNP in every 100-300bp in plants and animals (Graham 2011). Thousands of SNPs in a mapping population can be generated by using next-generation sequencing (Cipriani et al. 2011; Barba et al. 2014). There are also recently developed marker platforms like genotyping-by-sequencing (GBS) and RNase H2 enzyme-dependent amplicon sequencing (rhAmpSeq) to study the grape genetics in depth. GBS is used to study the

genetic linkage and genomic diversity, however, there is some level of ascertainment and high cost involved (He et al. 2014). There was also undesirable target Polymerase Chain Reaction (PCR) amplification observed when amplicon sequencing (AmpSeq) was employed and the marker multiplexing is limited. The rhAmpSeq markers were developed to overcome the drawbacks of AmpSeq, and the rhAmpSeq markers have higher transferability compared to other SNP protocols in the entire *Vitis* genus (Zou et al. 2020).

Microsatellites/SSR : SSR markers are co-dominant markers which can have short tandem repeats with the size of 1-6 base pairs and can be referred to as di-, tri-, or tetranucleotide repeats (Tautz 1989; Vieira et al. 2016). PCR can be used to amplify the alleles located at the microsatellite loci (Saiki et al. 1988). Small sample of the genomic DNA can be used to amplify the alleles and the alleles can be separated using either polyacrylamide gel or an agarose gel. The bands visible can be either one or two and this difference can be used to study the genetic variations among the species in a population (Connell et al. 1997). SSR markers are very useful as they are efficient in differentiating heterozygotes and homozygotes, highly polymorphic, and have transferability (Abdul-Muneer 2014).

Several laboratories have developed *Vitis* microsatellite markers which have gained a lot of importance since then (Thomas and Scott 1993; Goto et al. 2006). Six *Vitis* species, twenty-six grapevine cultivars and *Muscadinia rotundifolia* L were identified by Thomas and Scott (1993) by using the microsatellites and they have established VVS microsatellite loci from the genomic library of *V. vinifera* L. cultivar Sultana. Bowers et al. (1996) developed VVMD microsatellites from the genomic library of *V. vinifera* L. cultivar Pinot Noir. Additionally, Bowers et al. (1999) developed VVMD microsatellite loci from the genomic library of Pinot Noir and Cabernet Sauvignon. ssrVrZAG markers were developed by an Austrian research group from *Vitis riparia*.

(Sefc et al. 1999). Microsatellite loci, ssrVvUCH were developed by Lefort et al. (2002) from the genomic DNA library of cultivar Syrah. UDV markers used in this study were developed at the University of Udine, Italy by Moroldo et al. (2008). SSR markers are mainly known to be helpful for identification of the grape genotypes and also for the parentage analysis (Tomic et al. 2013).

Marker-Assisted Selection

Marker-assisted selection is the use of DNA markers to select the plants that have desirable traits and is a combination of traditional genetics and molecular biology (Marker Assisted Selection 1914). There are several techniques such as recurrent selection, backcrossing, mutation breeding that are being used in conventional plant breeding. But these techniques need several generations to develop the plant and to know if the desired trait is fixed or not. However, using molecular markers that are tightly linked to the desired traits can facilitate the testing of plants at young age to know if the plant has the desirable traits or not thus reducing the number of generations of plants that need to be developed (Hasan et al. 2015).

Downy Mildew

Downy mildew is one of the major diseases affecting crop yield throughout the world. The disease is caused by the fungus *Plasmopara viticola* (Lazazzara et al. 2018) in grapes. It was thought to be originated in North America (Taylor 2021). The symptoms include circular yellow spots with an oily appearance on leaves. The spots can coalesce and can cover the entire leaf surface when they grow and can cause defoliation. White fungal growth can be observed under the surface of the leaf when the climate turns humid. Young berries are more susceptible to the disease when compared with the mature berries and under severe infection, effected berries will

drop. This disease is named Downy mildew as it causes downy white fungal growth (Ash 2000). Downy mildew causes 20-80% of the yield loss in grapes (Sawant 2010).

Rpv3 (Resistance to *Plasmopara viticola*)

There are 31 Mildew resistant loci identified so far using the DNA markers (Table 2). Out of the 31 loci, *Rpv3* is the major loci which is located on Chromosome 18 and was identified in the cultivar, Bianca (Bellin et al. 2009). The *Rpv3* locus is mainly found in the grape cultivars derived from North America. Six flanking SSR markers are useful in identifying the presence of the *Rpv3* locus; they are UDV 305, UDV 730, UDV 732, UDV 734, UDV 736, and UDV 737. UDV 305 and UDV 737 are the flanking markers on chromosome 18 (Moroldo et al. 2008, D. Copetti unpublished data). The *Rpv3* region contains nucleotide-binding site–leucine-rich repeat (NBS-LRR) genes that help the downy mildew affected plant to survive by triggering a hypersensitive response (HR) (Di Gaspero et al. 2007). There are seven different *Rpv3* conserved haplotypes which are *Rpv3* $^{299\text{-}279}$, *Rpv3* $^{\text{null-}297}$, *Rpv3* $^{361\text{-}299}$, *Rpv3* $^{299\text{-}314}$, *Rpv3* $^{\text{null-}287}$, *Rpv3* $^{321\text{-}312}$ and *Rpv3* $^{\text{null-}271}$ that confer to downy mildew resistance. 'Chambourcin' has a combination of *Rpv3* $^{299\text{-}279}$ and *Rpv3* $^{\text{null-}297}$ (Di Gaspero et al. 2012).

Characteristics and pedigree of the 'Chambourcin'

Vitis interspecific hybrid 'Chambourcin' was developed in France by Joannes Seyve and is gaining importance in the Missouri vineyards for its quality of wine (Galet 1979; Scheef 1991). It can withstand a freezing temperature of -20 ^{0} F and has greater disease resistance when compared to *V. vinifera* (Ferree et al. 1997; Dami et al. 2005; Homich et al. 2016).

The pedigree of the 'Chambourcin' is complex involving several Seibel hybrids (Thomas et al. 2020). *V. vinifera*, *V. rupestris*, *V. labrusca, V. riparia, V. labruscana, V. aestivalis,* and *V. cinerea* are involved in its pedigree (Maul and Eibach 2003). There are two different pedigrees available for the 'Chambourcin'. It was suspected since long time to be an offspring of 'Seyve Villard 12-417' and 'Seibel 7053' (Chancellor). 'Seyve Villard 12-417' was inferred as a female parent, with the allelic haplotype being compatible leaving a hypothesis that 'Seyve Villard 12-417' could have contributed to the downy mildew resistance in 'Chambourcin'. But the allelic haplotype of the 'Chancellor' does not seem to introgress to 'Chambourcin' confirming it not to be a male parent (Di Gaspero et al. 2012). The other pedigree says 'Joannes Seyve-11369' as the female parent and 'Planet' as the male parent which was previously confirmed through markers (Maul and Eibach 2003) (Figure 1 and 2). There are two different pedigrees available for 'Joannes Seyve-11369' where one of them was given by the breeder and the other pedigree confirmed through molecular markers. The pedigree of 'Joannes Seyve-11369' given by the breeder is 'Seibel 6468' and 'Seibel 7053' (Chancellor). The pedigree confirmed through molecular markers is 'Seibel 6468' and 'Seibel 6905' (Subereux) (Maul and Eibach 2003), which is the right one when the allele sizes are traced back (Di Gaspero et al. 2012) (Figure 3 and 4).

Study objectives

The main objectives of this study are to determine which allele at the *Rpv3* locus in 'Chambourcin' × 'Cabernet Sauvignon' population is responsible for the disease resistance, which resistant allele was passed down from the pedigree of the 'Chambourcin' to the F_1

progeny screened and the resistant allele sizes were traced back to the grand parents to see which pedigree fits to be the right one for the ‘Chambourcin’.

MATERIALS AND METHODS

Study Design

In this study, 267 hybrid progeny obtained by crossing *V.* interspecific hybrid 'Chambourcin' and *V. vinifera* 'Cabernet Sauvignon' were used to determine the introgression of *Rpv3*-mediated Downy Mildew resistance. Both the alleles of the 'Chambourcin' are resistant to downy mildew, and those of the 'Cabernet Sauvignon' are susceptible to downy mildew (Di Gaspero et al. 2012). DNA was isolated from the young leaf samples of the F_1 progeny. By using PCR, DNA was amplified using a set of four SSR (Simple Sequence Repeats) primers. The quality of the PCR product was checked by running agarose gel electrophoresis. Capillary electrophoresis was performed to determine the allele sizes.

Plant Materials

The mapping population obtained by crossing *Vitis* interspecific hybrid 'Chambourcin' and *V. vinifera* 'Cabernet Sauvignon' consisted of 306 progeny. Crosses were generated in three consecutive years, 100, 26 and 180 plants in the year of 2014, 2015 and 2016, respectively. This population has been maintained at the Missouri State Fruit Experiment Station (MSFES) in Mountain Grove, Missouri. Crossing was carried out between 'Chambourcin' as the female parent and 'Cabernet Sauvignon' as the male parent. Before anthesis, emasculation was performed in the female parent, 'Chambourcin' and the emasculated buds were covered with paper bags to prevent unwanted pollination. Pollen collected from the male parent, 'Cabernet Sauvignon' were placed under 60W lamp and were dried overnight and stored until further use at room temperature. The bagged emasculated buds were pollinated the next morning with a brush

and then were tagged and re-bagged until the berries have reached veraison for harvest. After harvest, the seeds were collected and washed neatly without any pulp and were stratified for 3 months by placing in sterilized sand at 4^0 C. The seeds were planted in the seedling trays and were allowed to germinate until 3-4 leaf stage. Then the leaf samples were collected for true hybrid identification. After finding the true hybrids, they were transferred into half-gallon pots and were grown in green house for 3-4 months. These seedlings were then transferred to an open shade house for 2 -3 weeks before being transplanted to the vineyard (Adhikari et al. 2014). The vines were planted with a spacing of 10-foot (3.05 m) between-row and 8-foot (2.44 m) within-row and were trained to a high bilateral cordon system.

DNA Extraction

Young leaf samples were collected from all the hybrid progeny and also from the parents, 'Chambourcin' and 'Cabernet Sauvignon'. DNA was isolated by using the Synergy™ 2.0 Plant DNA Extraction Kit (OPS Diagnostics LLC, Lebanon, NJ). The used protocol is with a slight modification with using 50 mg of leaf sample for the DNA isolation. Bead Beater was adjusted to run at the highest speed for 4 minutes such that the leaf sample gets homogenized and finally, elution of the DNA was done in 200 µL. The concentration of the isolated DNA was determined using a Nanodrop Spectrophotometer (Thermo Fisher Scientific, Waltham, MA). After determining the concentration of the DNA, the DNA samples were preserved at -20ºC until further use.

PCR Amplification and DNA Fragment Analysis

Four SSR markers, UDV 730, UDV 734, UDV 736 and UDV 737 were amplified by performing PCR reaction. Each reaction had a total volume of 8.4 µL comprising of 0.8 µL of H_2O, 0.8 µL of DNA template normalized to 15ng / µL, 0.8 µL of 0.1 µM of the forward primer, 0.8 µL of 2 µM of the reverse primer, 0.8 µL of M13 WellRed Primer at 2 µM, 0.2 µL of $MgCl_2$ at25 mM, 4.2 µL of AmpliTaq GoldR 360 Master Mix buffer (Thermo Fisher Scientific, Waltham, MA). M13 (TGTAAAACGACGGCCAGT) tailed WellRed primers were used to facilitate the economical pooling of PCR products for capillary electrophoresis and also to increase the specificity of PCR amplification (Boutin et al. 2001). D2, D3 and D4 are the three different WellRed primers used. The touchdown PCR protocol used for the amplification has one cycle of an initial denaturation stage at 95°C for 10 minutes, 10 cycles of touchdown with denaturation stage at 94°C for 30 seconds, annealing stage at 62°C for 30 seconds with temperature decreasing at 1°C for each cycle and an extension stage for 1 minute at 72°C. This was followed by 24 cycles which has denaturation stage at 94°C for 30 seconds, annealing stage at 56°C for 30 seconds and an elongation stage at 72°C for 1 minute. Finally, elongation stage at 72°C for 7 minutes was done to complete the reaction.

A 1.5% agarose gel was prepared to run the agarose gel electrophoresis. Three µL of the PCR product was loaded into the gel by mixing with 6X loading dye. After running the gel for 90 minutes, UV light visualization was carried out to decide on the amount of PCR product to be used for capillary electrophoresis based on the gel band intensity (Bio-Rad, Hercules, CA). GenomeLab GeXP Genetic Analysis System (Beckman Coulter, Brea, CA) was used to perform capillary electrophoresis (CE). Based on the agarose gel band intensity, the PCR product combined with sample loading solution and an internal size standard of 400bp (base pairs) was loaded into the machine for determining the allele sizes. WellRed primers were used during PCR

to facilitate the multiplexing of 4 different PCR products. GenomeLab GeXP Genetic Analysis software has the Fragment Analysis module which was used to determine the resistant allele sizes.

RESULTS

The *Rpv3* resistant allele sizes were traced back to the Grandparents from the hybrid progeny. The resistant allele sizes, 211 bp, 239 bp, 271 bp and 284 bp in the 'Chambourcin' match the 'Seyve Villard 12-417' which is recognized as one of the parents of 'Chambourcin'. However, allele sizes, 213 bp, 243 bp, NULL and 303 bp did not match 'Chancellor' and it is also inferred as one of the parents of the 'Chambourcin' (Di Gaspero et al. 2012). But we were able to successfully trace the allele sizes back to the 'Joannes Seyve-11369' and 'Plantet' pedigree as they were the ones previously confirmed through markers. The resistant allele sizes, 211 bp, 239 bp, 271 bp and 284 bp introgressed from 'Joannes Seyve – 11369' to the 'Chambourcin' to the hybrid progeny and the resistant allele sizes, 213 bp, 243 bp, NULL and 303 bp introgressed from 'Plantet' to the 'Chambourcin' to the hybrid progeny (Maul and Eibach 2003).

For the marker UDV 730, we have 'Chambourcin' with allele sizes 211 bp / 213 bp and 'Cabernet Sauvignon' with homozygous allele sizes of 198 bp / 198 bp. We observe two different allelic combinations in the progeny which are 211 bp / 198 bp and 213 bp / 198 bp having 152 progeny showing 211 bp / 198 bp and 115 progeny showing 213 bp / 198 bp. For the marker UDV 734, we have 'Chambourcin' with allele sizes 239 bp / 243 bp and 'Cabernet Sauvignon' having 247 bp / 255 bp. We have four different allelic combinations among the population with 84 progeny showing 239 bp / 247 bp, 69 progeny showing 239 bp / 255 bp, 38 progeny showing 243 bp / 247 bp and 76 progeny showing 243 bp / 255 bp. For the marker UDV 736, we have 'Chambourcin' showing 271 bp / NULL alleles and 'Cabernet Sauvignon' showing 281 bp / 303 bp allelic sizes. We have four different allelic combinations among the population

with 88 progeny showing 271 bp / 281 bp, 68 progeny showing 271 bp / 303 bp, 74 progeny showing 281 bp / NULL and 37 progeny showing 303 bp / NULL. Finally, for the marker UDV 737, we have 'Chambourcin' with 284 bp / 303 bp as the allele sizes and 'Cabernet Sauvignon' with 291 bp / 301 bp. We again have four different allelic combinations among population with 82 progeny showing 284 bp / 291 bp, 73 progeny showing 284 bp / 301 bp, 44 progeny showing 291 bp / 303 bp and 68 progeny showing 301 bp / 303bp. The allele sizes of both the parents for the four different markers and the number of progeny showing such allelic distribution as discussed are also given in (Table 3).

For the marker UDV 730, 211 bp is the resistant allele size that was passed down from 'Joannes Seyve-11369' and 213 bp is the resistant allele size from 'Plantet' that was passed down to the 'Chambourcin' and down to the hybrid progeny as per our lab data. As per the published data, 226 bp and 228 bp are the resistant allele sizes from 'Joannes Seyve-11369' and 'Plantet' that were passed down to the 'Chambourcin' for the marker UDV 730. For the marker UDV 734, 239 bp is the resistant allele size that was passed down from 'Joannes Seyve-11369' and 243 bp is the resistant allele size from 'Plantet' that was passed down to the 'Chambourcin' to the hybrid progeny as per our lab data. The published data have shown that 251 bp and 255 bp are the resistant allele sizes from 'Joannes Seyve-11369' and 'Plantet' that were passed down to the 'Chambourcin'. For the marker UDV 736, 271 bp is the resistant allele size that was passed down from 'Joannes Seyve-11369' and NULL is the resistant allele size from 'Plantet' that was passed down to the 'Chambourcin' down to the hybrid progeny as per our lab data but what was given by the published data was that 283 bp and NULL were the resistant allele sizes that passed down to the 'Chambourcin' from 'Joannes Seyve-11369' and 'Plantet'. For the marker UDV 737, 284 bp is the resistant allele size that was passed down from 'Joannes Seyve-11369' and

303 bp is the resistant allele size from ‘Plantet’ that was passed down to the ‘Chambourcin’ down to the hybrid progeny as per our lab data. The published data showed that 279 bp and 297 bp are the resistant allele sizes that were passed down from ‘Joannes Seyve-11369’ and ‘Plantet’ to the ‘Chambourcin’ for the marker UDV 737 (Table 4; Table 5). The distribution of different allelic combinations in 267 Ch X CS F_1 progeny for the four different markers were shown in figures 5,6,7 and 8.

DISCUSSION

According to our data, all the progeny are resistant to the downy mildew as they got the resistant alleles from the female parent, 'Chambourcin'. Progeny are heterozygous for downy mildew resistance with one resistant allele and one susceptible allele. There are two different pedigrees available for the 'Chambourcin'. One of them is 'Seyve Villard 12-417' and 'Chancellor' which was obtained from the breeder, and the other one is 'Joannes Seyve-11369' and 'Plantet' which was confirmed through molecular markers (Maul and Eibach 2003). The pedigree of the 'Chambourcin' with 'Joannes Seyve-11369' as a female parent and 'Plantet' as a male parent was successfully determined by tracing the resistant alleles back to the pedigree from the hybrid progeny. The grandparents of the hybrid progeny, 'Joannes Seyve-11369' and 'Plantet' contributed to the Downy Mildew resistance in 'Chambourcin' × 'Cabernet Sauvignon' hybrid progenies.

'Seibel 7053' (Chancellor) is a French interspecific hybrid which was involved in the controversial pedigree of the 'Chambourcin' and also in the pedigree of 'Joannes Seyve-11369' as the male parent. 'Seibel 7053' is obtained by crossing 'Seibel 5163' as the female parent and 'Seibel 880' as the male parent (Maul and Eibach 2003). However, we were not able to confirm 'Seibel 7053' to be a male parent in either of the pedigrees of 'Chambourcin' or 'Joannes Seyve-11369'.

All the hybrid progeny have the resistant alleles to the downy mildew and are believed to be resistant and show greater resistance when compared with the susceptible cultivars. The resistant allele sizes obtained from the previous research are used as a reference to move forward and trace back the pedigree of the 'Chambourcin' (Di Gasepero et al. 2012). The great

grandparents' pedigrees are also available but are not used in tracing resistant alleles as there is no resistant allele data available for all the great grandparents. Both the parents of the 'Chambourcin' are resistant to the downy mildew and 'Chambourcin' also has both the alleles resistant to the disease. The combination of phenotype data along with the genotype data obtained for 267 F1 'Chambourcin' × 'Cabernet Sauvignon' hybrid progeny can help in better understanding of the resistance capacity of the 'Chambourcin' in the future.

The resistant allele sizes obtained in our lab showed variation from the resistant allele sizes used as a reference from Di Gaspero et al. 2012. There was a variation that ranged in between 5 to 15 base pairs for each of the four markers used in this study. For the marker UDV 730, the variation was -15 base pairs. For the marker UDV 734, the variation was -12 base pairs. For the marker UDV 736, the variation was -12 base pairs. Finally, for the marker UDV 737, the variation was +5 base pairs.

We inferred the sizes obtained in our lab by the resistant allele sizes from the published paper based on how close they were. For the marker UDV 730, we obtained sizes 211 bp and 213 bp in our lab and the sizes given in the reference paper are 226 bp and 228 bp. We have inferred 211 bp to 226 bp and 213 bp to 228 bp, respectively. For the marker UDV 734, we have obtained sizes 239 bp and 243 bp and the sizes available in the reference paper are 251 bp and 255 bp. We have inferred 239 bp to 251 bp and 243 bp to 255 bp. For the marker UDV 736, we have obtained sizes 271 bp and NULL and the sizes available in the reference paper are 283 bp and NULL. We have inferred 271 bp to 283 bp and the NULL allele is the same as there would be no allele. For the marker UDV 737, we have obtained sizes 284 bp and 303 bp and the sizes available from the reference paper are 279 bp and 297 bp. We have inferred 284 bp to 279 bp and 303 bp to 297 bp. The allele sizes were inferred based on how close they are and also by

taking into account the difference between the two allele sizes for each of the markers. There was a difference in allele sizes between the reference paper and the genotype data obtained in our lab.

For the marker UDV 730, the difference between the alleles 226 bp and 228 bp is 2 bp and the difference is same for our sizes 211 bp and 213 bp. For the marker UDV 734, the difference between the alleles 251 bp and 255 bp is 4 bp and this difference is same for our sizes 239 bp and 243 bp. For the marker UDV 736, there is 283 bp and NULL from the reference paper and we have got 271 bp and NULL and we cannot infer the difference between the allele sizes with a NULL allele. For the marker UDV 737, the difference between the alleles 279 bp and 297 bp is 18 bp and the difference for the allele sizes 284 bp and 303 bp we have obtained in the lab is 19 bp giving us 1 bp extra difference (Table 6).

The resistant allele sizes used as reference from Di Gaspero et al. 2012 were carried out in a laboratory which might have a different lab setting from our research lab. The equipment and chemicals purchased by the lab is no standard and might differ between each and every lab (Pasqualotto et al. 2007). There are several factors listed below which could have conferred for the differences in the allele sizes.

The reasons for such change in the allele sizes obtained could be due to:

- Difference in the PCR conditions used
- Different PCR Machine
- Different CE Machine
- Different CE running conditions
- Different Internal Standard used for running CE
- Different chemicals used to run the PCR
- Temperature fluctuations in the lab can also cause the differences in the allele sizes

CONCLUSION

The two different pedigrees of the ‘Chambourcin’ were evaluated with the help of genotype data and were traced back to identify the correct pedigree. We were able to conclude that ‘Joannes Seyve-11369’ and ‘Plantet’ are the parents of the ‘Chambourcin’. The alleles responsible for the *Rpv3*- mediated resistance to downy Mildew in each of the progeny obtained by crossing the downy mildew resistant parent ‘Chambourcin’ and the susceptible parent ‘Cabernet Sauvignon’ were determined.

REFERENCES

Abdul-Muneer PM (2014) Application of microsatellite markers in conservation genetics and fisheries management: recent advances in population structure analysis and conservation strategies. Genet Res Int 2014:691759. https://doi.org/10.1155/2014/691759

Adhikari P, Chen L-L, Chen X, Sapkota SD, Hwang C-F (2014) Interspecific hybrid identification of *Vitis aestivalis*-derived 'Norton'-based populations using microsatellite markers. Sci Hort 179:363–366

Allard RW (2021) Plant breeding. Encyclopedia Britannica. https://www.britannica.com/science/plant-breeding. Accessed 18 March 2021

Alston JM, Sambucci O (2019) Grapes in the World Economy. In: Cantu D., Walker M. (eds) The Grape Genome. Compendium of Plant Genomes. Springer, Cham. https://doi.org/10.1007/978-3-030-18601-2_1

Ash G (2000) Downy mildew of grape. The Plant Health Instructor. https://doi.org/10.1094/phi-i-2000-1112-01

Barba P, Cadle-Davidson L, Harriman J, Glaubitz JC, Brooks S, Hyma K, Reisch B (2014) Grapevine powdery mildew resistance and susceptibility loci identified on a high-resolution SNP map. Theor Appl Genet 127:73-84

Barker CL, Donald T, Pauquet J, Ratnaparkhe MB, Bouquet A, Adam-Blondon AF, Thomas MR, Dry I (2005) Genetic and physical mapping of the grapevine powdery mildew resistance gene, Run1, using a bacterial artificial chromosome library. Theor Appl Genet. 2005 ;111(2):370-7. doi: 10.1007/s00122-005-2030-8. Epub 2005 May 18. PMID: 15902396

Bautista J, Dangl GS, Yang J, Reisch B, Stover E (2008) Use of Genetic Markers to Assess Pedigrees of Grape Cultivars and Breeding Program Selections. Am. J. Enol. Vitic. 59

Bellin D, Peressotti E, Merdinoglu D, Wiedemann-Merdinoglu S, Adam-Blondon A-F, Cipriani G, Morgante M, Testolin R, Di Gaspero G (2009) Resistance to *Plasmopara viticola* in grapevine 'Bianca' is controlled by a major dominant gene causing localized necrosis at the infection site. Theor Appl Genet 120:163–176

Blasi P, Blanc S, Wiedemann-Merdinoglu S, Prado E, Rühl EH, Mestre P, Merdinoglu D (2011) Construction of a reference linkage map of *Vitis amurensis* and genetic mapping of *Rpv8* , a locus conferring resistance to grapevine downy mildew. Theor Appl Genet 123:43–53

Bowers JE, Dangl GS, Vignani R, Meredith CP (1996) Isolation and characterization of new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). Genome, 39(4), 628–633. https://doi.org/10.1139/g96-080

Bowers J, Boursiquot JM, This P, Chu K, Johansson H, Meredith C (1999) Historical Genetics: The Parentage of Chardonnay, Gamay, and Other Wine Grapes of Northeastern France. Science (New York, N.Y.), 285(5433), 1562–1565. https://doi.org/10.1126/science.285.5433.1562

Carlson EA (2004) Mendel's legacy: the origin of classical genetics. Cold Spring Harbor Laboratory Press, New York

Chambers PJ, Pretorius IS (2010) Fermenting knowledge: the history of winemaking, science and yeast research. EMBO reports, 11(12), 914–920. https://doi.org/10.1038/embor.2010.179

Cipriani G, Di Gaspero G, Canaguier A, Jusseaume J, Tassin J, Lemainque A, Thareau V, Adam-Blondon AF, Testolin R (2011) Molecular linkage maps: Strategies, resources and achievements. In: Adam-Blondon AF, Martinez-Zapater JM, Kole C (eds.) Genetics, Genomics and Breeding of Grapes, CRC Press, New York pp. 111-136

Dami I, Bordelon B, Ferree DC, Brown M, Ellis MA, Williams RN, Doohan D (2005) Midwest grape production guide. Ohio State University Extension 919:19

David T (2013) Grapes: A Brief History. Missouri Environment and Garden News Article. Integrated Pest Management, University of Missouri. https://ipm.missouri.edu/meg/2013/8/Grapes-A-Brief-History/. Accessed 3 March 2021

Divilov K, Barba P, Cadle-Davidson L, Reisch RI (2018) Single and multiple phenotype QTL analyses of downy mildew resistance in interspecific grapevines. Theor Appl Genet 131:1133–1143

Di Gaspero G, Cipriani G, Adam-Blondon A-F, Testolin R (2007) Linkage maps of grapevine displaying the chromosomal locations of 420 microsatellite markers and 82 markers for R-gene candidates. Theor Appl Genet 114:1249–1263

Di Gaspero G, Cattonaro F (2010) Application of genomics to grapevine improvement. Aust J Grape Wine Res 16:122–130

Di Gaspero G, Copetti D, Coleman C, Castellarin SD, Eibach R, Kozma P, Kovács L (2012) Selective sweep at the *Rpv3* locus during grapevine breeding for downy mildew resistance. Theor Appl Genet 124:277–286

Fahad S, Faisal S, Naushad M (2020) Grape Production Critical Review in the World. SSRN Electronic Journal. 10.2139/ssrn.3595842

FAOSTAT [WWW Document], data, URL http://www.fao.org/faostat/en/#data/QC. Accessed 20 April 2021

Feher J, Lengyel G, Lugasi A (2007) The cultural history of wine - theoretical background to wine therapy. cent.eur.j.med 2, 379–391 https://doi.org/10.2478/s11536-007-0048-9

Ferree DC, Riesen R, Gallander J (1997) Cultivars desired by Ohio wineries. Proc. Ohio Grape-Wine Short Course. pp. 118–122

Fischer B, Salakhutdinov I, Akkurt M, Eibach R, & Edwards K, Toepfer R, Zyprian E (2004) Quantitative trait locus analysis of fungal disease resistance factors on a molecular map of grapevine. Theor Appl Genet. 108. 501-15. 10.1007/s00122-003-1445-3

Flavio B, Alexandre C (2013) Traditional and Modern Plant Breeding Methods with Examples in Rice (Oryza sativa L.). J. Agric.Food Chem. 61. 10.1021/jf305531j

Fu P, Wu W, Lai G, Li R, Peng Y, Yang B, Wang B, Yin L, Qu J, Song S, Lu J (2020) Identifying *Plasmopara viticola* resistance Loci in grapevine (*Vitis amurensis*) via genotyping-by-sequencing-based QTL mapping. Plant Physiol Biochem 154:75-84. doi: 10.1016

Galet P (1979) A practical ampelography: Grapevine identification. Cornell University Press, New York and London, pp. 166-167

Goto YN, Mouri H, Azumi M, Edwards KJ (2006) Development of grape microsatellite markers and microsatellite analysis including oriental cultivars. Am. J. Enol. Vitic 57 1 105 108

Graham J (2011) Molecular Plant Breeding by Y. Xu. Wallingford, UK: CABI (2010), pp. 734. ISBN 978-184593-392-0. Exp. Agric. 47(1), 173–173. http://doi.org/10.1017/S0014479710000669

Harlan JR (1992) Crops and Man. American Society of Agronomy and Crop Science Society of America, Madison, WI

Hasan MM, Rafii MY, Ismail MR, Mahmood M, Rahim HA, Alam MA, Ashkani S, Malek MA, Latif MA (2015) Marker-assisted backcrossing: a useful method for rice improvement. Biotechnol Biotechnol Equip. 29, 237–254. doi: 10.1080/13102818.2014.995920

He J, Zhao X, Laroche A, Lu Z-X, Liu H, Li Z (2014) Genotyping-by sequencing (GBS), an ultimate marker-assisted selection (MAS) tool to accelerate plant breeding. Front Plant Sci 5:484

Homich LJ, Scheinberg JA, Elias RJ, Gardner DM (2016) Effects of co-inoculation on wine quality attributes of the high-acid, red hybrid variety 'Chambourcin'. Am J Enol Vitic doi: 10.5344/ajev.2015.15084

Isabelle-BG, Marco R, Martine R, Christian D (2001) M13-Tailed Primers Improve the Readability and Usability of Microsatellite Analyses Performed with Two Different Allele- Sizing Methods. Biotechniques. 31. 24-6, 28. 10.2144/01311bm02

Jaradat, AA (2015) Breeding Oilseed Crops for Climate Change. Breeding Oilseed Crops for Sustainable Production. https://www.sciencedirect.com/science/article/pii/B9780128013090000185

Jiang GL (2013) Molecular Markers and Marker-Assisted Breeding in Plants, Plant Breeding from Laboratories to Fields, Sven Bode Andersen, IntechOpen, DOI: 10.5772/52583. Available from: https://www.intechopen.com/books/plant-breeding-from-laboratories-to-fields/molecular-markers-and-marker-assisted-breeding-in-plants. Accessed 26 March 2021

Kingsbury N (2009) Hybrid: the history & science of plant breeding. The University of Chicago Press, Chicago

Kundan MK, Ballani F, Vinita T, Yachana J, Madhumati B (2014) Potential and application of molecular markers techniques for plant genome analysis.

Lazazzara V, Bueschl C, Parich A, Pertot I, Schuhmacher R, Perazzolli M (2018) Downy mildew symptoms on grapevines can be reduced by volatile organic compounds of resistant genotypes. Sci. Rep. 8(1). https://doi.org/10.1038/s41598-018-19776-2

Leal S M (2001) Genetics and Analysis of Quantitative Traits. Am J Hum Genet 68(2):548-549

Lefort F, Kyvelos C, Zervou M, Edwards K, Roubelakis-Angelakis K (2002) Characterization of new microsatellite loci from *Vitis vinifera* and their conservation in some *Vitis* species and hybrids. e. https://epublications.marquette.edu/liana_articles/507/

Lijavetzky D, Cabezas JA, Ibáñez A, Rodríguez V, Martínez-Zapater JM (2007) High throughput SNP discovery and genotyping in grapevine (*Vitis vinifera* L.) by combining a re-sequencing approach and SNPlex technology. BMC Genomics 8:424–435

Lin H, Leng H, Guo Y, Kondo S, Zhao Y, Shi G, Guo X (2019) QTLs and candidate genes for downy mildew resistance conferred by interspecific grape (*V. vinifera L.* × *V. amurensis Rupr*.) crossing. Sci. Hortic., 244, 200-207. doi:10.1016/j.scienta.2018.09.045

Marguerit E, Boury C, Manicki A, Donnart M, Butterlin G, Némorin A, Wiedemann-Merdinoglu S, Merdinoglu D, Ollat N, Decroocq S (2009) Genetic dissection of sex determinism, inflorescence morphology and downy mildew resistance in grapevine. Theor Appl Genet 118(7), 1261–1278 https://doi.org/10.1007/s00122-009-0979-4

Marker assisted selection ii - iowa state university (n.d.) Accessed May 10, 2021, from https://www.biotech.iastate.edu/publications/mendel/ModuleIIP1.pdf

Maul E, Eibach R (2003) *Vitis* international variety catalogue. http://www.genres.de/idb/*vitis*/. Accessed February 10 2021

Merdinoglu D, Wiedeman-Merdinoglu S, Coste P, Dumas V, Haetty S, Butterlin G, Greif C (2003) Genetic analysis of downy mildew resistance derived from *Muscadinia rotundifolia*. Acta Hort 603:451–456

Moose SP, Mumm RH (2008) Molecular plant breeding as the foundation for 21st century crop improvement. Plant Physiol. 147(3), 969–977 https://doi.org/10.1104/pp.108.118232

Moreira FM, Madini A, Marino R, Zulini L, Stefanini M, Velasco R, Kozma P, Grando MS (2011) Genetic linkage maps of two interspecific grape crosses (*Vitis* spp.) used to localize quantitative trait loci for downy mildew resistance. Tree Genet Genomes 7:153–167

Moroldo M, Paillard S, Marconi R, Fabrice L, Canaguier A, Cruaud C, De Berardinis V, Guichard C, Brunaud V, Le Clainche I, Scalabrin S, Testolin R, Di Gaspero G, Morgante M, Adam-Blondon AF (2008) A physical map of the heterozygous grapevine 'Cabernet Sauvignon' allows mapping candidate genes for disease resistance. *BMC Plant Biol*. *8*(1), 66. https://doi.org/10.1186/1471-2229-8-66

Mueller UG, Wolfenbarger LL (1999) AFLP genotyping and fingerprinting. TREE 14:389-394

Nadeem MA, Nawaz MA, Shahid MQ, Doğan Y, Comertpay G, Yıldız M Hatipoğlu R, Ahmad F, Alsaleh A, Labhane N, Özkan H, Chung G, Baloch FS (2017) DNA molecular markers in plant breeding: Current status and recent advancements in genomic selection and genome editing. Biotechnol Biotechnol. Equip. 32(2), 261-285. doi:10.1080/13102818.2017.1400401

Ochssner I, Hausmann L, Töpfer R (2016) *Rpv14*, a new genetic source for *Plasmopara viticola* resistance conferred by *Vitis cinerea*. *Vitis* 55, 79–81. doi: 10.5073/*vitis*.2016.55.79-81

O'Connell M, Danzmann RG, Cornuet J, Wright JM, Ferguson MM (1997) Differentiation of rainbow trout (*Oncorhynchus mykiss*) populations in Lake Ontario and the evaluation of the STEPWISE mutation and Infinite allele Mutation models using microsatellite variability. Can. J. Fish. Aquat. Sci 54(6), 1391-1399 doi:10.1139/f97-043

Pasqualotto AC, Denning DW, Anderson MJ (2007) A cautionary tale: Lack of consistency in allele sizes between two laboratories for a published multilocus microsatellite typing system. J Clin Microbiol.;45(2):522-528. doi:10.1128/JCM.02136-06

Paul DB, Kimmelman BA (1988) Mendel in America: theory and practice. In R Rainger, KR Benson, J Maienschien, eds, The American Development of Biology. University of Pennsylvania Press, Philadelphia, pp 281–310

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239(4839):487-91. doi: 10.1126/science.2448875

Sapkota S, Chen L-L, Yang S, Hyma KE, Cadle-Davidson L, Hwang C-F (2019) Construction of a high-density linkage map and QTL detection of downy mildew resistance in *Vitis aestivalis*-derived 'Norton. Theor. Appl. Genet. 132, 137–147 doi: 10.1007/s00122-018-3203-6

Sargolzaei M, Maddalena G, Bitsadze N, Maghradze D, Bianco PA, Failla O, Toffolatti SL, De Lorenzis G (2020) *Rpv29, Rpv30 and Rpv31*: Three Novel Genomic Loci Associated with Resistance to *Plasmopara viticola* in *Vitis vinifera*. Front. Plant Sci 11:562432 doi: 10.3389/fpls.2020.562432

Sawant Indu S, Sawant SD, Upadhyay Anuradha, Sharma J, Upadhyay AK, Shetty D, Bhirangi R (2010) Crop loss in grapes due to downy mildew infection on clusters at pre- and post bloom stages under non-epiphytotic conditions. Indian J. Hort. 67: 425-432

Scheef RF (1991) Vintage Missouri. The Patrice Press, Missouri

Schwander F, Eibach R, Fechter I, Hausmann L, Zyprian E, Töpfer R (2012) Rpv10: a new locus from the Asian *Vitis* gene pool for pyramiding downy mildew resistance loci in grapevine. Theor Appl Genet. 124(1):163-76. doi: 10.1007/s00122-011-1695-4. Epub 2011 Sep 21. PMID: 21935694

Sefc KM, Regner F, Turetschek E, Glössl J, Steinkellner H (1999) Identification of microsatellite sequences in *Vitis riparia* and their applicability for genotyping of different *Vitis* species. Genome, 42(3), 367–373

Shahbandeh M (2020) Grape production worldwide https://www.statista.com/statistics/237600/world-grape-production-in-2007-by-region/ Accessed March 08 2021

Shtaya, Munqez Jamil Yacoub (2010) History and Role of Plant Breeding in Society | An-Najah Staff An-Najah Staff, staff-old.najah.edu/m /course-material/1-history-and-role-plant-breeding-society.

Shull GH (1909) A pure-line method in corn breeding. Am Breeders Assoc Rep 5 51–59

Tautz D (1989) Hypervariability of simple sequences as a general source for polymorphic DNA markers, Nucleic Acids Res. vol. 17, no. 16, pp. 6463–6471

Taylor A Downy mildew of grapevines. Agriculture and Food. https://www.agric.wa.gov.au/table-grapes/downy-mildew-grapevines?page=01. Accessed March 10 2021

Terral JF, Tabard E, Bouby L, Ivorra S, Pastor T, Figueiral I, Picq S, Chevance JB, Jung C, Fabre L, Tardy C, Compan M, Bacilieri R, Lacombe T (2010) Evolution and history of grapevine (*Vitis vinifera*) under domestication: new morphometric perspectives to understand seed domestication syndrome and reveal origins of ancient European cultivars. Ann Bot.105(3):443-55. doi: 10.1093/aob/mcp298

Thomas MR, Scott NS (1993) Microsatellite repeats in grapevine reveal DNA polymorphisms when analyzed as sequence-tagged sites (STSs). Theor. Appl. Genet 86 8 985 990

Thomas AL, Harris JL, Bergmeier EA, Striegler R (2020) Performance of 'Chambourcin' Winegrape on Nematode-resistant Rootstocks in Missouri.HortTechnology hortte, 30(5), 597-602

Tomic L, Stajner N, Javornik B (2013) Characterization of Grapevines by the Use of Genetic Markers. The Mediterranean Genetic Code - Grapevine and Olive. doi:10.5772/52833.

UK Essays (2018) History of Wine and History of Wine Making Processes.https://www.ukessays.com/essays/history/wine-history-grapes.php?vref=1. Accessed February 19 2021

Van Heerden CJ, Burger P, Vermeulen A, Prins R (2014) Detection of downy and powdery mildew resistance QTL in a 'Regent' × 'RedGlobe' population. Euphytica 200:281–295

Venuti S, Copetti D, Foria S, Falginella L, Hoffmann S, Bellin D, Cindrić P, Kozma P, Scalabrin S, Morgante M, Testolin R, Gaspero GD (2013) Historical Introgression of the Downy Mildew Resistance Gene *Rpv12* from the Asian Species *Vitis amurensis* into Grapevine Varieties. PLOS ONE. https://journals.plos.org/plosone/article?id=10.1371%2Fjournal.pone.0061228

Vieira ML, Santini L, Diniz AL, Munhoz Cde F Microsatellite markers: what they mean and why they are so useful. Genet Mol Biol. 2016;39(3):312-328. doi:10.1590/1678-4685-GMB-2016-0027

Vignal A, Milan D, SanCristobal M, Eggen A (2002) A review on SNP and other types of molecular markers and their use in animal genetics. Genet Sel Evol 34:275-305

Walker DR, Monteros MJ, Yates JL (2010) Molecular Breeding. In: Bilyeu K, Ratnaparkhe MB, Kole C (eds.), Genetics, Genomics and Breeding of Soybean, CRC Press, New York pp. 123-159

Welter LJ, Gokturk-Baydar N, Akkurt M, Maul E, Eibach R, Reinhard T, Zyprian EM (2007) Genetic mapping and localization of quantitative trait loci affecting fungal disease resistance and leaf morphology in grapevine (*Vitis vinifera* L.). Mol Breed 20:359–374

Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18(22):6531-6535. doi:10.1093/nar/18.22.6531

Zou C, Karn A, Reisch B, Nguyen A, Sun Y, Bao, Y, Campbell MS, Church D, Williams S, Xu X, Ledbetter CA, Patel S, Fennell A, Glaubitz JC, Clark M, Ware D, Londo JP, Sun Q, Cadle-Davidson L (2020) Haplotyping the *Vitis* collinear core genome with rhAmpSeq improves marker transferability in a diverse genus. Nat. Commun. 11(1), 413. https://doi.org/10.1038/s41467-019-14280-1

Zyprian E, Ochssner I, Schwander F, Šimon S, Hausmann L, Bonow- Rex M, Moreno-Sanz P, Grando MS, Wiedemann-Merdinoglu S, Merdinoglu D, Eibach R, Töpfer R (2016) Quantitative trait loci affecting pathogen resistance and ripening of grapevines. Mol Genet Genomics 291:1573–1594

Table 1. Characteristics of the Molecular Markers used commonly.

Characteristics	RFLP	RAPD	AFLP	SNP	SSR
DNA quality required	High	High	Moderate	Moderate	High
PCR based	No	Yes	Yes	Yes	Yes
Reproducibility	High	Not reliable	High	High	High
Dominant/Co-dominant	Co-dominant	Dominant	Dominant/Co-dominant	Co-dominant	Co-dominant
Effort level to develop and use	Difficult	Easy	Easy	Easy	Easy
Price per analysis	High	Low	Moderate	Low	Low
Cost involved to develop	Low	Low	Moderate	High	High
Accuracy	Very high	Very low	Medium	High	Very high
Abundance in genome	High	Very high	Very high	Medium	Medium
Developmental labor required	High	Low-medium	Low-medium	High	High

Table 2. Different *Rpv* loci discovered in the grapes with their location on the chromosome.

Attribute	Symbol	Chromosome	Reference Paper
Plasmopara viticola	*Rpv*1	12	Merdinoglu et al. 2003
Plasmopara viticola	*Rpv*2	18	Wiedemann-Merdinoglu et al. 2006
Plasmopara viticola	*Rpv*3	18	Welter et al. 2007; Bellin et al.2009; van Heerden et al. 2014; Di Gaspero et al. 2012; Zyprian et al. 2016
Plasmopara viticola	*Rpv*4	4	Welter et al. 2007
Plasmopara viticola	*Rpv*5	9	Marguerit et al. 2009
Plasmopara viticola	*Rpv*6	12	Marguerit et al. 2009
Plasmopara viticola	*Rpv*7	7	Bellin et al. 2009
Plasmopara viticola	*Rpv*8	14	Blasi et al. 2011
Plasmopara viticola	*Rpv*9	7	Moreira et al. 2011
Plasmopara viticola	*Rpv*10	9	Schwander et al. 2012
Plasmopara viticola	*Rpv*11	5	Fischer et al. 2004; Bellin et al. 2009; Schwander et al. 2012
Plasmopara viticola	*Rpv*12	14	Venuti et al. 2013
Plasmopara viticola	*Rpv*13	12	Moreira et al. 2011
Plasmopara viticola	*Rpv*14	5	Ochssner et al. 2016
Plasmopara viticola	*Rpv*15	18	Pap et al. in preparation
Plasmopara viticola	*Rpv*16	Unpublished	Pap et al. in preparation
Plasmopara viticola	*Rpv*17	8	Divilov et al. 2018
Plasmopara viticola	*Rpv*18	11	Divilov et al. 2018
Plasmopara viticola	*Rpv*19	14	Divilov et al. 2018

Table 2. continued

Attribute	Symbol	Chromosome	Reference Paper
Plasmopara viticola	*Rpv*20	6	Divilov et al. 2018
Plasmopara viticola	*Rpv*21	7	Divilov et al. 2018
Plasmopara viticola	*Rpv*22	2	Fu et al. 2020
Plasmopara viticola	*Rpv*23	15	Fu et al. 2020
Plasmopara viticola	*Rpv*24	18	Fu et al. 2020
Plasmopara viticola	*Rpv*25	15	Lin et al. 2019
Plasmopara viticola	*Rpv*26	15	Lin et al. 2019
Plasmopara viticola	*Rpv*27	18	Sapkota et al. 2018
Plasmopara viticola	*Rpv*28	In preparation	Bhattarai et al. in preparation
Plasmopara viticola	*Rpv* 29	14	Sargolzaei et al. 2020
Plasmopara viticola	*Rpv* 30	3	Sargolzaei et al. 2020
Plasmopara viticola	*Rpv* 31	16	Sargolzaei et al. 2020

Table 3. Allelic distribution of ‘Chambourcin’ × ‘Cabernet Sauvignon’ hybrid genotypes with 4 SSR markers.

Markers	Parents	Hybrid Genotypes	No. of progeny showing the combination
UDV 730	Ch- 211/213	211/198	152
	CS- 198/198	213/198	115
UDV 734	Ch- 239/243	239/247	84
		239/255	69
	CS- 247/255	243/247	38
		243/255	76
UDV 736	Ch- 271/NULL	271/281	88
		271/303	68
	CS- 281/303	281/NULL	74
		303/NULL	37
UDV 737	Ch- 284/303	284/291	82
		284/301	73
	CS- 291/301	291/303	44
		301/303	68

Table 4. Resistant allele sizes from the parents of the ‘Chambourcin’ passing down to the progeny.

Marker	Parents of Chambourcin	
	‘Joannes Seyve-11369’	‘Plantet’
UDV 730	211 bp	213 bp
UDV 734	239 bp	243 bp
UDV 736	271 bp	NULL
UDV 737	284 bp	303 bp

Table 5. Parent plants ('Chambourcin' and 'Cabernet Sauvignon') and 267 F_1 progeny screened using UDV 730, UDV 734, UDV 736, and UDV 737 SSR markers with associated allele sizes.

Primer number	UDV730	UDV734	UDV736	UDV737
Chambourcin	211/213	239/243	271/NULL	284/303
Cabernet Sauvignon	198	247/255	281/303	291/301
Ch X CS 001	198/211	239/247	271/303	284/291
Ch X CS 002	198/211	239/255	271/281	284/301
Ch X CS 003	198/211	239/247	271/303	284/291
Ch X CS 004	198/213	243/247	303/NULL	291/303
Ch X CS 005	198/213	243/247	281/NULL	301/303
Ch X CS 006	198/211	239/255	271/281	284/301
Ch X CS 007	198/211	239/255	271/281	284/301
Ch X CS 008	198/211	239/247	271/303	284/291
Ch X CS 009	198/213	243/255	281/NULL	284/301
Ch X CS 010	198/211	239/255	271/281	284/301
Ch X CS 011	198/211	239/247	271/303	284/291
Ch X CS 012	198/213	243/255	281/NULL	301/303
Ch X CS 013	198/213	243/247	303/NULL	291/303
Ch X CS 014	198/211	239/247	271/303	284/291
Ch X CS 015	198/211	239/247	271/281	284/301
Ch X CS 016	198/213	243/255	281/NULL	301/303
Ch X CS 017	198/211	239/247	271/281	284/301
Ch X CS 019	198/213	243/255	303/NULL	291/303
Ch X CS 020	198/213	243/247	303/NULL	291/303
Ch X CS 021	198/211	239/247	271/303	284/291
Ch X CS 022	198/211	239/247	271/303	284/291
Ch X CS 023	198/213	243/255	281/NULL	301/303
Ch X CS 024	198/213	243/247	303/NULL	301/303
Ch X CS 025	198/211	239/247	271/281	284/291
Ch X CS 026	198/211	239/247	271/281	284/301
Ch X CS 027	198/213	243/255	303/NULL	291/303
Ch X CS 028	198/213	243/247	281/NULL	291/303
Ch X CS 030	198/213	243/247	281/NULL	291/303
Ch X CS 031	198/213	243/255	303/NULL	291/303
Ch X CS 032	198/211	239/247	271/281	284/301
Ch X CS 033	198/213	243/255	303/NULL	291/303
Ch X CS 034	198/213	243/247	303/NULL	291/303
Ch X CS 035	198/213	243/255	281/NULL	301/303
Ch X CS 036	198/211	239/247	271/303	284/291

Table 5. continued

Primer number	UDV730	UDV734	UDV736	UDV737
Chambourcin	211/213	239/243	271/NULL	284/303
Cabernet Sauvignon	198	247/255	281/303	291/301
Ch X CS 037	198/211	239/247	271/303	284/291
Ch X CS 038	198/213	243/255	281/NULL	301/303
Ch X CS 039	198/211	239/255	271/281	284/301
Ch X CS 040	198/213	243/255	281/NULL	301/303
Ch X CS 041	198/211	239/255	271/281	284/301
Ch X CS 042	198/213	243/255	281/ NULL	301/303
Ch X CS 043	198/211	239/255	271/303	284/291
Ch X CS 044	198/211	239/255	271/281	284/301
Ch X CS 045	198/211	239/247	281/NULL	284/291
Ch X CS 046	198/213	243/255	271/281	301/303
Ch X CS 047	198/213	243/255	281/NULL	301/303
Ch X CS 048	198/211	239/255	271/303	284/291
Ch X CS 049	198/211	239/255	271/281	284/301
Ch X CS 050	198/213	243/255	281/NULL	301/303
Ch X CS 051	198/213	243/255	281/NULL	301/303
Ch X CS 052	198/211	239/255	271/281	284/301
Ch X CS 053	198/211	239/247	271/303	284/291
Ch X CS 054	198/213	243/255	281/NULL	301/303
Ch X CS 055	198/213	243/255	281/NULL	301/303
Ch X CS 056	198/213	243/255	303/NULL	291/303
Ch X CS 057	198/211	239/255	271/281	284/301
Ch X CS 058	198/211	239/247	271/303	284/291
Ch X CS 059	198/211	239/247	271/303	284/291
Ch X CS 060	198/211	239/255	271/281	284/301
Ch X CS 061	198/211	239/247	271/281	284/301
Ch X CS 062	198/213	243/255	281/NULL	301/303
Ch X CS 063	198/211	239/255	271/281	284/301
Ch X CS 064	198/213	243/255	303/NULL	291/303
Ch X CS 065	198/213	243/255	281/NULL	301/303
Ch X CS 066	198/211	239/247	271/281	284/301
Ch X CS 067	198/211	239/255	271/281	284/301
Ch X CS 069	198/211	239/255	271/303	284/291
Ch X CS 070	198/211	239/255	271/281	284/301
Ch X CS 071	198/211	239/255	271/281	284/301
Ch X CS 072	198/211	239/247	271/303	284/291
Ch X CS 073	198/211	239/247	271/281	284/301

Table 5. continued

Primer number	UDV730	UDV734	UDV736	UDV737
Chambourcin	211/213	239/243	271/NULL	284/303
Cabernet Sauvignon	198	247/255	281/303	291/301
Ch X CS 074	198/213	243/255	281/NULL	301/303
Ch X CS 075	198/211	239/255	271/303	284/291
Ch X CS 076	198/213	243/247	281/NULL	301/303
Ch X CS 077	198/211	239/255	271/303	284/291
Ch X CS 078	198/213	243/255	281/NULL	284/301
Ch X CS 080	198/213	243/255	281/NULL	301/303
Ch X CS 081	198/211	239/247	271/303	284/291
Ch X CS 082	198/211	243/247	271/281	284/301
Ch X CS 083	198/211	239/255	271/303	284/291
Ch X CS 084	198/211	239/255	271/303	284/291
Ch X CS 085	198/211	239/247	271/303	284/291
Ch X CS 086	198/211	239/255	271/303	284/291
Ch X CS 087	198/213	243/255	281/NULL	301/303
Ch X CS 088	198/211	239/247	271/303	284/291
Ch X CS 089	198/211	239/247	271/303	284/291
Ch X CS 090	198/211	239/255	271/303	284/291
Ch X CS 091	198/211	239/255	271/303	284/291
Ch X CS 092	198/213	243/255	281/NULL	301/303
Ch X CS 093	198/211	239/247	271/303	284/291
Ch X CS 094	198/213	239/255	271/303	291/303
Ch X CS 095	198/211	239/247	271/303	284/291
Ch X CS 096	198/213	243/247	303/NULL	291/303
Ch X CS 097	198/213	243/247	281/NULL	284/301
Ch X CS 098	198/213	243/255	281/NULL	291/303
Ch X CS 099	198/213	243/247	303/NULL	291/303
Ch X CS 101	198/211	239/247	271/303	284/291
Ch X CS 102	198/211	239/255	271/281	284/301
Ch X CS 103	198/211	239/255	271/281	284/301
Ch X CS 104	198/213	243/255	281/NULL	301/303
Ch X CS 105	198/211	239/255	271/281	284/301
Ch X CS 106	198/211	239/247	271/281	284/291
Ch X CS 107	198/211	239/255	271/281	284/301
Ch X CS 108	198/213	243/255	281/NULL	301/303
Ch X CS 109	198/213	243/255	281/NULL	291/303
Ch X CS 110	198/211	239/247	271/281	284/291
Ch X CS 111	198/213	243/255	281/NULL	301/303

Table 5. continued

Primer number	UDV730	UDV734	UDV736	UDV737
Chambourcin	211/213	239/243	271/NULL	284/303
Cabernet Sauvignon	198	247/255	281/303	291/301
Ch X CS 114	198/213	243/255	281/NULL	301/303
Ch X CS 115	198/211	239/255	271/281	284/301
Ch X CS 117	198/213	243/255	281/NULL	301/303
Ch X CS 118	198/211	239/255	271/281	284/301
Ch X CS 119	198/211	239/247	271/281	284/291
Ch X CS 120	198/211	239/247	271/303	284/291
Ch X CS 121	198/211	239/255	271/281	284/301
Ch X CS 122	198/211	239/247	271/281	284/301
Ch X CS 124	198/211	239/255	281/NULL	284/291
Ch X CS 125	198/213	243/255	271/281	291/303
Ch X CS 126	198/211	239/247	271/281	284/301
Ch X CS 127	198/213	243/255	281/NULL	284/301
Ch X CS 128	198/213	243/247	303/NULL	291/303
Ch X CS 129	198/211	239/247	271/303	284/291
Ch X CS 130	198/211	239/255	271/281	284/301
Ch X CS 131	198/213	243/247	303/NULL	291/303
Ch X CS 132	198/211	239/255	271/281	284/301
Ch X CS 133	198/213	243/255	281/NULL	301/303
Ch X CS 134	198/211	239/247	271/303	284/291
Ch X CS 135	198/211	239/247	271/303	284/291
Ch X CS 136	198/211	239/255	271/281	284/301
Ch X CS 137	198/211	239/247	271/303	284/291
Ch X CS 138	198/211	239/247	271/303	284/301
Ch X CS 139	198/211	239/255	271/281	284/301
Ch X CS 140	198/211	239/247	271/303	284/301
Ch X CS 141	198/213	243/255	281/NULL	301/303
Ch X CS 143	198/211	239/247	271/281	284/291
Ch X CS 144	198/211	239/255	271/281	284/301
Ch X CS 145	198/211	239/247	271/303	284/291
Ch X CS 146	198/211	239/247	271/303	284/291
Ch X CS 147	198/211	239/247	271/303	284/291
Ch X CS 148	198/213	243/255	281/NULL	301/303
Ch X CS 149	198/211	239/247	271/303	284/291
Ch X CS 150	198/211	239/247	271/303	284/291
Ch X CS 151	198/211	239/255	271/281	284/301
Ch X CS 152	198/213	239/255	303/NULL	291/303

Table 5. continued

Primer number	UDV730	UDV734	UDV736	UDV737
Chambourcin	211/213	239/243	271/NULL	284/303
Cabernet Sauvignon	198	247/255	281/303	291/301
Ch X CS 153	198/213	243/255	281/NULL	301/303
Ch X CS 154	198/213	243/247	303/NULL	291/303
Ch X CS 155	198/211	239/255	271/281	284/301
Ch X CS 156	198/211	239/255	271/281	284/301
Ch X CS 157	198/211	239/247	271/303	284/291
Ch X CS 160	198/211	239/255	271/281	284/301
Ch X CS 161	198/211	239/255	271/281	284/301
Ch X CS 163	198/211	239/255	271/281	284/301
Ch X CS 164	198/213	243/255	281/NULL	301/303
Ch X CS 165	198/211	239/247	271/303	284/291
Ch X CS 166	198/213	243/255	281/NULL	301/303
Ch X CS 167	198/213	243/255	281/NULL	301/303
Ch X CS 168	198/213	243/247	303/NULL	291/303
Ch X CS 169	198/211	239/247	271/303	284/291
Ch X CS 170	198/213	243/247	303/NULL	291/303
Ch X CS 171	198/211	239/247	271/303	284/291
Ch X CS 172	198/213	243/247	303/NULL	291/303
Ch X CS 173	198/213	243/247	303/NULL	291/303
Ch X CS 174	198/213	243/255	281/NULL	301/303
Ch X CS 176	198/211	239/247	271/303	284/291
Ch X CS 177	198/211	239/247	271/303	284/291
Ch X CS 178	198/211	239/247	271/303	284/291
Ch X CS 179	198/213	243/255	281/NULL	301/303
Ch X CS 180	198/211	239/255	271/281	284/301
Ch X CS 181	198/211	239/247	271/303	284/291
Ch X CS 182	198/211	239/247	271/303	284/291
Ch X CS 183	198/213	243/247	303/NULL	291/303
Ch X CS 184	198/211	239/247	271/303	284/291
Ch X CS 185	198/213	243/255	281/NULL	301/303
Ch X CS 186	198/213	243/247	303/NULL	291/303
Ch X CS 187	198/213	243/247	303/NULL	291/303
Ch X CS 189	198/211	239/247	271/303	284/291
Ch X CS 190	198/211	239/255	271/281	284/301
Ch X CS 191	198/211	239/255	271/281	284/301
Ch x CS 192	198/213	243/255	281/NULL	291/303
Ch x CS 193	198/211	239/247	271/281	284/291

Table 5. continued

Primer number	UDV730	UDV734	UDV736	UDV737
Chambourcin	211/213	239/243	271/NULL	284/303
Cabernet Sauvignon	198	247/255	281/303	291/301
Ch x CS 194	198/213	243/255	281/NULL	301/303
Ch x CS 195	198/211	239/255	271/281	301/303
Ch x CS 196	198/211	239/255	271/281	284/301
Ch x CS 197	198/213	243/255	271/281	284/301
Ch x CS 198	198/213	243/255	281/NULL	301/303
Ch x CS 199	198/211	239/255	271/281	284/301
Ch x CS 200	198/211	239/255	271/281	284/301
Ch x CS 201	198/211	239/255	271/281	284/301
Ch x CS 202	198/211	239/247	271/281	284/291
Ch x CS 204	198/213	243/247	303/NULL	291/303
Ch x CS 205	198/211	239/255	271/281	284/301
Ch x CS 206	198/213	243/247	303/NULL	301/303
Ch x CS 207	198/213	243/255	281/NULL	301/303
Ch x CS 208	198/211	239/247	271/303	284/291
Ch x CS 209	198/211	239/247	271/303	284/291
Ch x CS 210	198/213	243/255	281/NULL	301/303
Ch x CS 211	198/213	243/255	281/NULL	301/303
Ch x CS 212	198/213	243/255	281/NULL	301/303
Ch x CS 213	198/213	243/255	281/NULL	301/303
Ch x CS 214	198/211	239/247	271/303	284/291
Ch x CS 215	198/211	239/255	271/281	284/301
Ch x CS 216	198/213	243/255	281/NULL	301/303
Ch x CS 217	198/213	243/255	281/NULL	301/303
Ch x CS 218	198/211	239/247	271/303	284/291
Ch x CS 219	198/213	243/255	281/NULL	301/303
Ch x CS 220	198/213	243/255	281/NULL	301/303
Ch x CS 221	198/211	239/247	271/281	284/291
Ch x CS 222	198/211	239/247	271/303	301/303
Ch x CS 223	198/211	239/255	271/281	284/301
Ch x CS 224	198/213	243/255	281/NULL	301/303
Ch x CS 225	198/211	239/247	271/281	284/291
Ch x CS 226	198/211	239/247	271/303	284/291
Ch x CS 227	198/211	239/255	271/281	284/301
Ch x CS 228	198/211	239/247	271/281	284/291
Ch x CS 229	198/211	239/247	271/303	284/291
Ch x CS 230	198/213	243/247	303/NULL	291/303

Table 5. continued

Primer number	UDV730	UDV734	UDV736	UDV737
Chambourcin	211/213	239/243	271/NULL	284/303
Cabernet Sauvignon	198	247/255	281/303	291/301
Ch x CS 231	198/213	243/247	271/281	291/303
Ch x CS 232	198/213	243/255	281/NULL	301/303
Ch x CS 233	198/211	239/247	271/281	284/291
Ch x CS 234	198/213	243/247	271/281	291/303
Ch x CS 235	198/213	243/255	281/NULL	301/303
Ch x CS 236	198/213	243/255	281/NULL	301/303
Ch x CS 237	198/213	243/255	281/NULL	301/303
Ch x CS 238	198/211	239/247	271/281	284/291
Ch x CS 239	198/211	239/255	271/281	284/301
Ch x CS 240	198/211	239/255	281/NULL	284/301
Ch x CS 241	198/211	239/247	271/281	284/291
Ch x CS 242	198/213	243/247	303/NULL	291/303
Ch x CS 243	198/211	239/255	271/281	284/301
Ch x CS 244	198/213	243/247	271/281	291/303
Ch x CS 245	198/213	243/255	281/NULL	301/303
Ch x CS 246	198/211	239/247	271/303	284/291
Ch x CS 247	198/213	243/247	303/NULL	291/303
Ch x CS 248	198/211	239/247	271/281	284/291
Ch x CS 249	198/213	243/255	281/NULL	301/303
Ch x CS 250	198/213	243/255	281/NULL	301/303
Ch x CS 251	198/213	243/255	281/NULL	301/303
Ch x CS 252	198/213	243/255	281/NULL	301/303
Ch x CS 253	198/213	243/255	281/NULL	301/303
Ch x CS 254	198/211	239/255	271/303	284/301
Ch x CS 255	198/213	243/247	303/NULL	291/303
Ch x CS 256	198/211	239/247	271/281	284/291
Ch x CS 257	198/213	243/255	303/NULL	301/303
Ch x CS 258	198/213	243/247	303/NULL	291/303
Ch x CS 259	198/211	239/255	271/281	284/301
Ch x CS 260	198/211	239/255	271/281	284/301
Ch x CS 261	198/213	243/255	281/NULL	301/303
Ch x CS 262	198/211	239/247	271/303	284/291
Ch x CS 263	198/213	243/255	281/NULL	301/303
Ch x CS 264	198/213	243/247	303/NULL	291/303
Ch x CS 265	198/211	239/247	271/281	284/291
Ch x CS 266	198/213	243/255	281/NULL	301/303

Table 5. continued

Primer number	UDV730	UDV734	UDV736	UDV737
Chambourcin	211/213	239/243	271/NULL	284/303
Cabernet Sauvignon	198	247/255	281/303	291/301
Ch x CS 267	198/213	243/247	303/NULL	291/303
Ch x CS 268	198/213	243/255	281/NULL	301/303
Ch x CS 269	198/211	239/247	271/281	284/291
Ch x CS 270	198/213	243/247	303/NULL	291/303
Ch x CS 271	198/211	239/247	271/281	284/291
Ch x CS 272	198/211	239/255	271/281	284/301
Ch x CS 273	198/211	239/255	271/281	284/301
Ch x CS 274	198/211	239/247	271/303	284/291
Ch x CS 275	198/211	239/247	271/303	284/291
Ch x CS 276	198/213	243/247	303/NULL	291/303
Ch x CS 277	198/213	243/255	281/NULL	301/303
Ch x CS 279	198/213	243/247	303/NULL	291/303
Ch x CS 280	198/211	239/255	271/281	284/301
Ch x CS 281	198/211	239/247	271/303	284/291
Ch x CS 282	198/211	239/255	271/281	284/301
Ch x CS 283	198/211	239/247	271/303	284/301
Ch x CS 284	198/211	239/255	271/281	284/301

Table 6. Difference between the allele sizes used as a reference from Di Gaspero et al. 2012 and the allele sizes obtained in our lab for each of the four SSR markers.

Marker	'Joannes Seyve-11369'		'Plantet'		'Seyve Villard 12-417'	'Chancellor'	'Chambourcin'		Difference in allele sizes (bp)
	Reference sizes	Our sizes	Reference sizes	Our sizes	Reference sizes	Reference sizes	Reference sizes	Our sizes	
UDV 730	226	211	228	213	226/213	213/210	226/228	211/213	-15
UDV 734	251	239	255	243	251/255	239/243	251/255	239/243	-12
UDV 736	283	271	NULL	NULL	283/NULL	NULL/295	283/NULL	271/NULL	-12
UDV 737	279	284	297	303	279/299	287/312	279/297	284/303	5

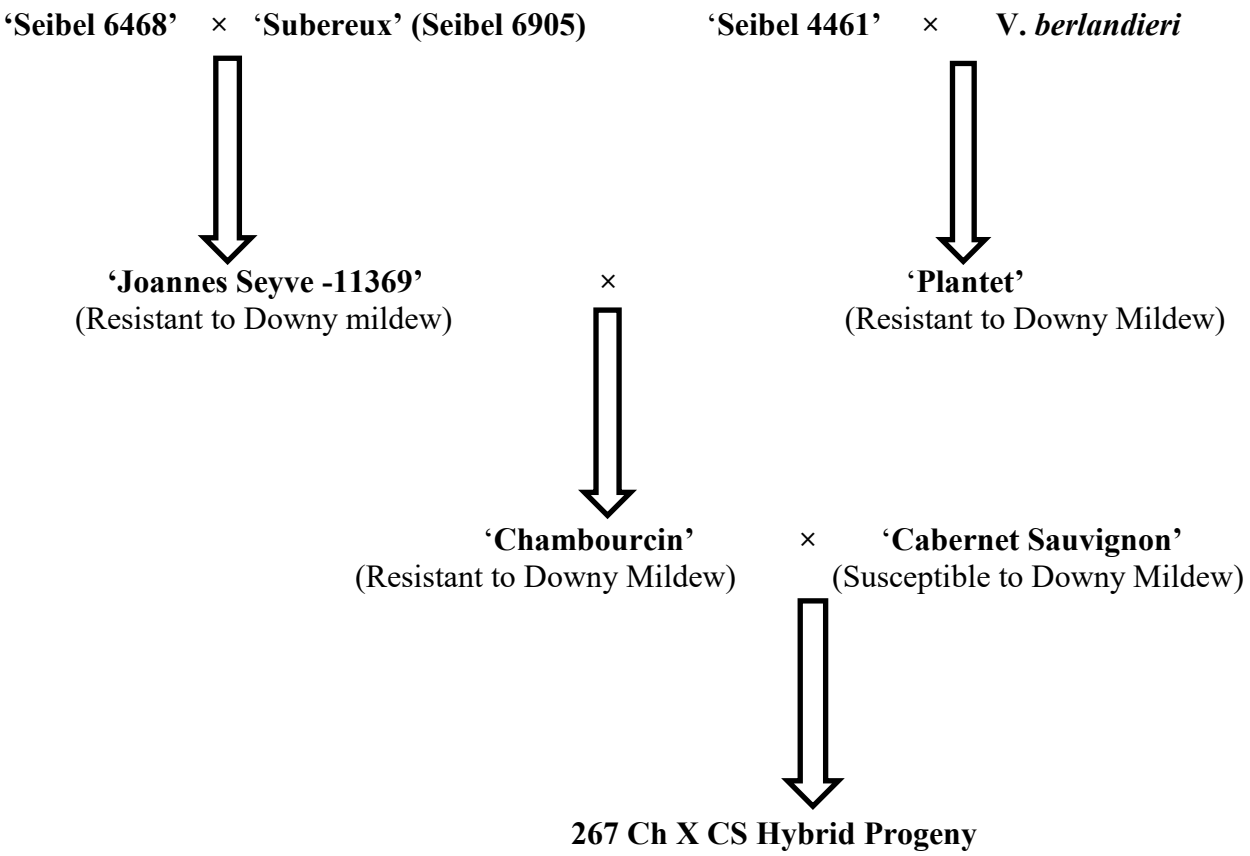


Figure 1. Pedigree of the ‘Chambourcin’ confirmed with DNA markers.

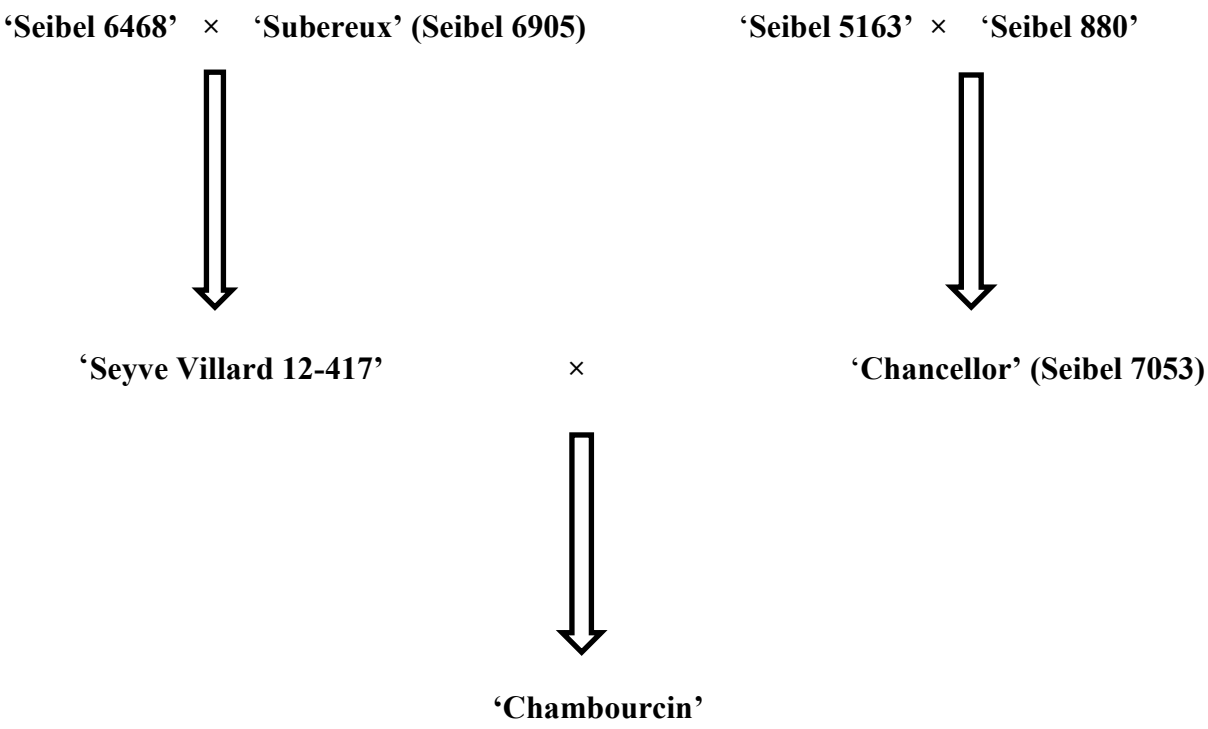


Figure 2. Pedigree of the ‘Chambourcin’ obtained through breeder/bibliography.

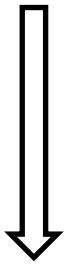
‘Seibel 6468’

UDV 730	226/213
UDV 734	251/251
UDV 736	283/318
UDV 737	279/293

×

‘Seibel 7053’ (Chancellor)

UDV 730	213/210
UDV 734	239/243
UDV 736	NULL/295
UDV 737	287/312



‘Joannes Seyve – 11369’

UDV 730	226/228
UDV 734	251/255
UDV 736	283/NULL
UDV 737	279/297

Figure 3. Pedigree of the ‘Joannes Seyve-11369’ obtained through breeder/bibliography along with the genotype data.

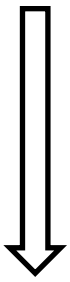
'Seibel 6468'

UDV 730	226/213
UDV 734	251/251
UDV 736	283/318
UDV 737	279/293

×

'Subereux' (Seibel 6905)

UDV 730	213/228
UDV 734	255/255
UDV 736	NULL/NULL
UDV 737	299/297



'Joannes Seyve – 11369'

UDV 730	226/228
UDV 734	251/255
UDV 736	283/NULL
UDV 737	279/297

Figure 4. Pedigree of the 'Joannes Seyve-11369' confirmed with DNA markers along with the genotype data.

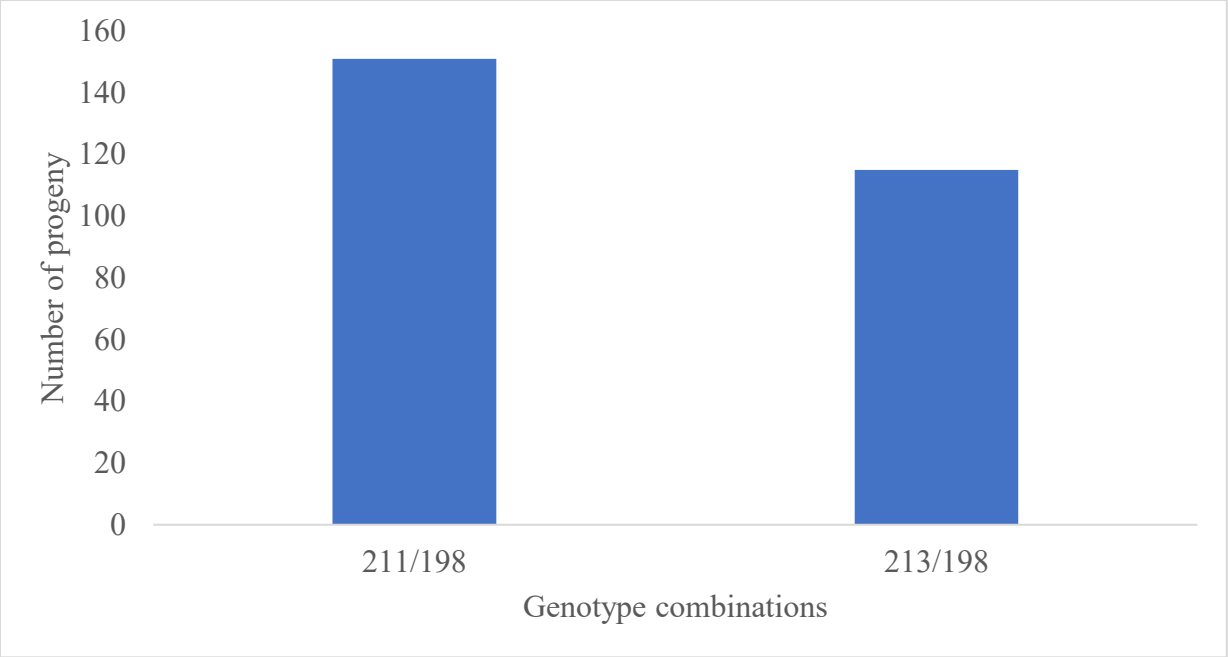


Figure 5. Clustered column chart showing the distribution of genotype combinations for the marker UDV 730 in 267 Ch × CS F_1 progeny.

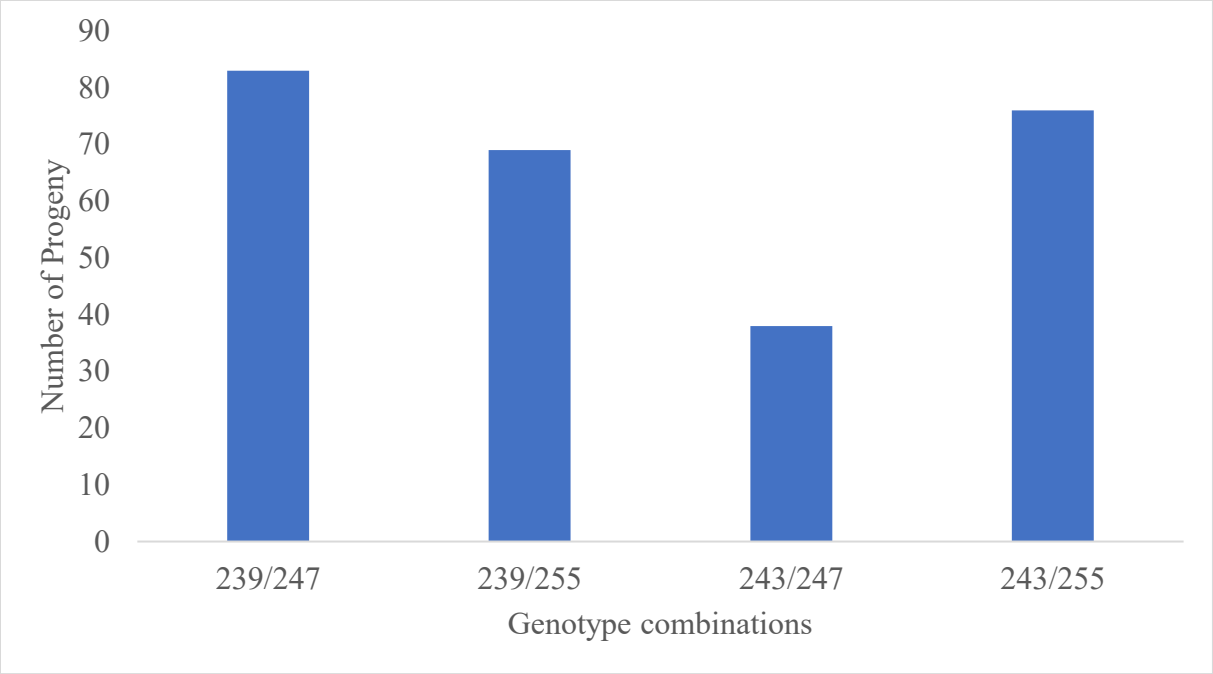


Figure 6. Clustered column chart showing the distribution of genotype combinations for the marker UDV 734 in 267 Ch × CS F_1 progeny.

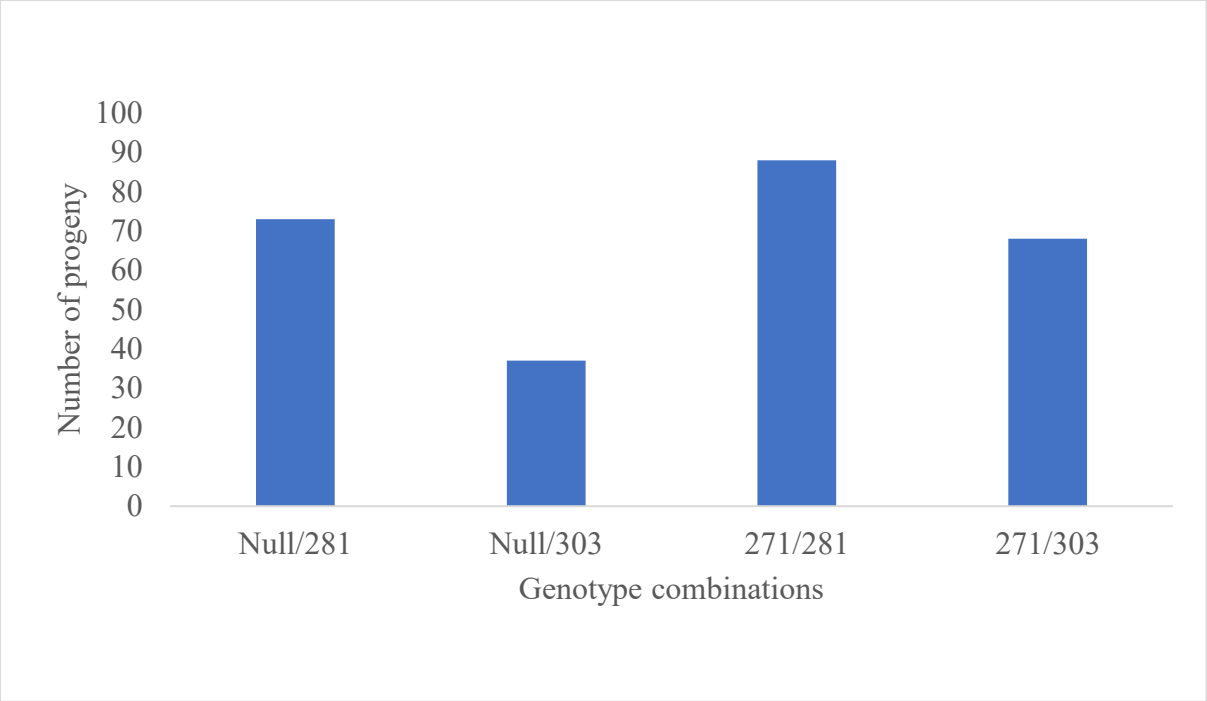


Figure 7. Clustered column chart showing the distribution of genotype combinations for the marker UDV 736 in 267 Ch × CS F_1 progeny.

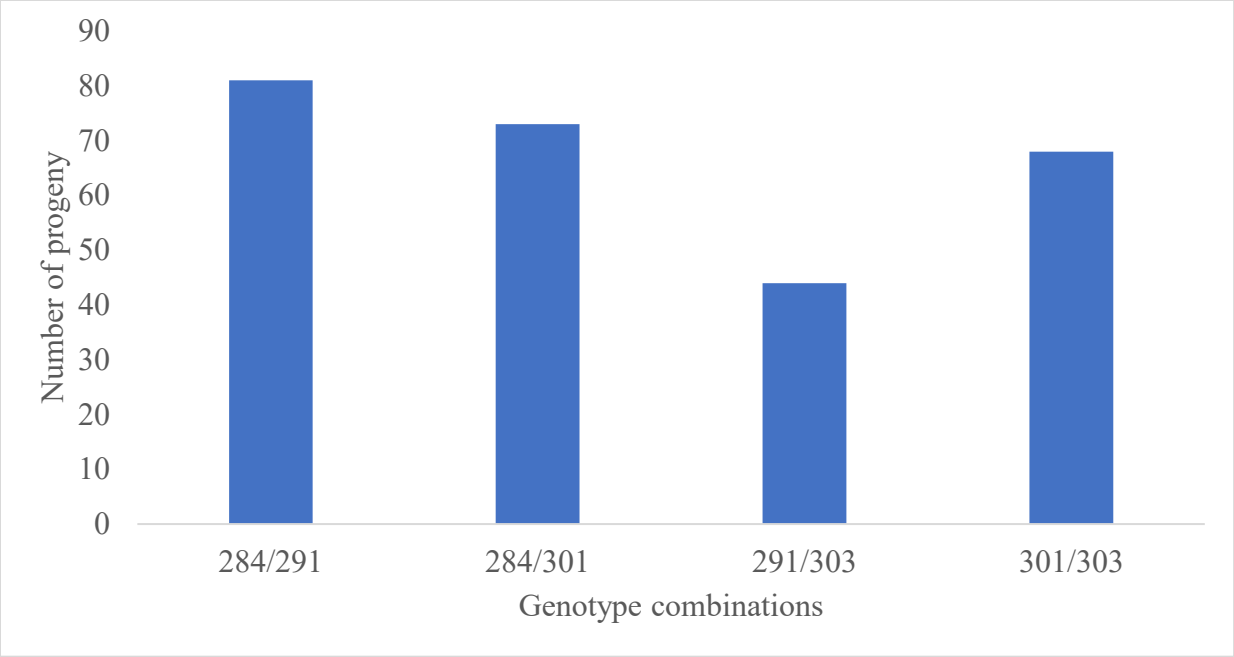


Figure 8. Clustered column chart showing the distribution of genotype combinations for the marker UDV 737 in 267 Ch × CS F_1 progeny.