Spring 2019

Mapping a New Disease Resistance Locus in an F1 Progeny Derived from Two Grape Wild Relatives

Gaurab Bhattarai
Missouri State University, Gaurab2017@live.missouristate.edu

MAPPING A NEW DISEASE RESISTANCE LOCUS IN AN F_1 PROGENY DERIVED FROM TWO GRAPE WILD RELATIVES

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, Biology

By

Gaurab Bhattarai

May 2019

MAPPING A NEW DISEASE RESISTANCE LOCUS IN AN F_1 PROGENY DERIVED FROM TWO GRAPE WILD RELATIVES

Biology

Missouri State University, May 2019

Master of Science

Gaurab Bhattarai

ABSTRACT

Linkage maps and quantitative trait loci (QTL) analysis have become essential tools for the positional cloning of agronomically important genes and for marker-assisted breeding. In this study, two North American grape species, *Vitis rupestris* and *Vitis riparia*, and their 294 F_1 progeny were used to construct parental linkage maps and to perform QTL analysis for downy mildew resistance. Single nucleotide polymorphism (SNP) discovery was accomplished using genotyping-by-sequencing (GBS) and resulted in 348,888 SNPs. Of these, 11,063 informative SNP markers (3.17% of the original SNP dataset) were derived after filtering for various quality parameters and missing data. A two-way pseudo-testcross strategy was followed for map construction using JOINMAP®5.0. The 1,115 and 1,177 significant markers (threshold LOD ≥ 14) for *V. riparia* and *V. rupestris* were grouped into 19 linkage groups covering 1657.4 and 1401.3 centimorgan (cM) of genetic distances with an average marker interval of 1.49 and 1.19 cM, respectively. Maps were validated by pinpointing a single significant QTL which determined maleness on chromosome 2 in the genetic background of the *V. riparia* male parent. Phenotype data for leaf downy mildew resistance were collected with both *in vitro* and naturally inoculated leaves of 86 and 136 F_1 progeny, respectively. With both methods, QTL analysis for reduced leaf area coverage by mildew lead to a significant peak on chromosome 10 in *V. rupestris* explaining 15-45% of the phenotypic variance. For *in vitro* inoculation, a significant QTL was detected for reduced sporangiophore density on chromosome 8 of *V. riparia*, explaining 15% of the variance. These are the first SNP-based linkage maps of these native North American grape species. The maps are expected to serve as a resource for breeding modern varieties for environment-friendly grape cultivation.

KEYWORDS: linkage map, genotyping-by-sequencing, single nucleotide polymorphism, marker-assisted selection, QTL, downy mildew, resistance

MAPPING A NEW DISEASE RESISTANCE LOCUS IN AN F_1 PROGENY DERIVED FROM TWO GRAPE WILD RELATIVES

By

Gaurab Bhattarai

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, Biology

May 2019

Approved:

Laszlo G Kovacs, Ph.D., Thesis Committee Chair

John S Heywood, Ph.D., Committee Member

Chin-Feng Hwang, Ph.D., Committee Member

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

ACKNOWLEDGEMENTS

I would like to acknowledge my major advisor, Dr. Laszlo G. Kovacs for his commendable guidance and continuous support throughout my study, course work, and research activities toward the completion of my graduate degree. I am also grateful to my thesis committee members, Drs. John S. Heywood, and Chin-Feng Hwang for the guidance they provided to my thesis research. I also greatly appreciate support from the Graduate College and the Biology Department at Missouri State University and the National Science Foundation Plant Genome Research Program (NSF-PGRP) which made this research project possible. Finally, I want to express my heartfelt gratitude to my colleagues, Drs. Zoë Migicovsky, Avinash Karna, Laura Klein, and Ms. Mani Awale for their help and support for various aspects of my research.

I dedicate this thesis to my beloved mother Indira Bhattarai and father Ram Chandra Bhattarai and family members.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

OVERVIEW

Linkage maps serve as important tools for the exploration of the genetic basis of traits in organisms. In perennial crops such as grapes, linkage maps also help accelerate the introgression of traits into cultivated varieties by facilitating positional cloning and marker-assisted selection (MAS). Naturally evolving plant species maintain high allelic diversity, but their domesticated relatives have lost much of this polymorphism during the domestication process. In the face of rapid climate change, the wild relatives of a crop can be utilized as valuable genetic resources to harness their potential for the improvement of environmental stress tolerance in the cultivated plants. With this as a long-term objective, I constructed a single nucleotide polymorphism (SNP)-based linkage map of two wild native North American grape species: *Vitis rupestris* and *Vitis riparia.* An F_1 mapping progeny of 353 plants was generated from a cross between these wild species and maintained in the field at the Grape Genetics Research Unit, United States Department of Agriculture at Geneva, New York, USA. The F_1 progeny and the its parents were analyzed employing the genotyping-by-sequencing (GBS) method for SNP discovery. I filtered the resulting SNPs for various quality parameters and kept only those informative SNP markers that had a heterozygous genotype in one parent and a homozygous genotype in the other. Significantly distorted and identical markers were removed from the analysis and parental linkage maps were constructed in JOINMAP®5.0 with threshold LOD ≥ 14, following a two-way pseudo-testcross strategy.

To verify the maps, the sex-determining region was mapped on chromosome 2 of *V. riparia* with quantitative trait loci (QTL) analysis near the locus where it had been previously reported in *Vitis vinifera.* The QTL study was further extended to map loci which confer

resistance to downy mildew (DM) disease. Grape DM is an important disease under temperate climatic conditions in regions where warm temperatures coincide with periods when liquid water covers leaf and young berry tissues. The *V. rupestris* x *V. riparia* F_1 progeny were challenged with a strain of the grape DM pathogen, *Plasmopara viticola*, which was isolated from a natural infection event. The strain was characterized by sequencing the internal transcribed spacer-1 (ITS-1) region of its rRNA gene and by evaluating its pathogenicity on three different grape species. Disease screening of the F_1 *V. rupestris* x *V. riparia* progeny was performed under two different conditions. The first screening was performed in a greenhouse where the pathogen naturally infected the F_1 individuals and disease severity was visually assessed based on the area of leaf surface covered by DM colonies. The second screening was performed under *in vitro* conditions where 8 leaf disks for each F_1 genotype were artificially inoculated with the sporangial suspension and incubated in the growth chamber for 7 days. Disease severity was measured for two different phenotypes: (1) area of the leaf disk covered by the pathogen and (2) sporangiophore density. QTL analysis for reduced leaf area coverage by mildew under both greenhouse and *in vitro* conditions lead to the discovery of a significant locus on chromosome 10 of *V. rupestris,* explaining 15-45% of the phenotypic variance. Under *in vitro* inoculation, a significant QTL was detected for reduced sporangiophore density on chromosome 8 of *V. riparia*, explaining 15% of the phenotypic variance. So far, these are the first SNP-based linkage maps of these wild grape species and the first QTL detected for DM resistance on chromosome 10 in *V. rupestris*.

CHAPTER 1: CONSTRUCTION OF AN SNP-BASED LINKAGE MAP OF TWO NATIVE NORTH AMERICAN GRAPEVINES: *VITIS RUPESTRIS* AND *VITIS RIPARIA*

Introduction

Grape (*Vitis vinifera* L.) is one of the oldest cultivated fruit crops. Since the dawn of civilization, wine, the fermented product of grapes, has been an important agricultural commodity (McGovern et al., 1995). Today, the wine industry is one of the largest food-producing sectors worldwide. The cultivated grape belongs to the family Vitaceae which includes woody and herbaceous perennial lianas (tree-climbing plants) (Bouquet, 2011). Being a perennial crop with an extended juvenile phase, grapevine requires several years to grow to maturity. The maintenance of grapevines also requires considerable amount of space and labor. Therefore, the evaluation of many important traits in the progeny from a cross between wild and commercial grape cultivars is a lengthy and expensive process. Repeated backcross of progeny to a recurrent parent, a common approach to restore desirable traits of elite varieties, is also impractical in grapevine because of its tendency for severe inbreeding depression (McClure et al., 2014). For these reasons, once growers identify a grape genotype with desirable horticultural characteristics and with good adaptation to local climatic conditions, they maintain that genotype through vegetative propagation for centuries.

Myles (2013) has described vegetative propagation as a double-edged sword. On one hand, it preserves the desirable traits of an elite cultivar, but on the other hand, it prevents these cultivars from evolving and adapting to new environmental conditions. Today, global climate change is forcing the grape and wine industry to deploy new hybrid cultivars, most of which have been developed through introgression of alleles from wild grape relatives to enable them to

better tolerate environmental stress and resist emerging pathogens (Firoozabady and Olmo, 1982; Walker et al., 1991; Reisch et al., 2014). Wild grape species also have many undesirable traits, particularly in aroma and flavor characteristics of the fruit. When such traits are introduced into the hybrid progeny through linkage drag, they reduce the market value of the new cultivars. Therefore, molecular tools and advanced genetic techniques, such as linkage-based mapping and marker-assisted selection (MAS), are promising tools to improve the precision of the breeding process and accelerate the exploration and utilization of alleles in wild grape relatives.

With the advent of high-throughput next-generation sequencing (NGS) platforms, whole-genome sequencing and the discovery of large number of single nucleotide polymorphisms (SNPs) have become readily achievable. The high frequency of SNPs in the heterozygous genome of grapevines makes the sequencing of the entire genome unnecessary (Liang et al., 2019), because a large enough number of informative SNPs can be identified in reduced genome representation sequencing (Luca et al., 2011). An approach based on this principle is genotyping-by-sequencing (GBS). GBS is a simple, highly multiplexed and economical technique of marker development which is based on high-throughput, next-generation sequencing at sites targeted by restriction enzymes (Elshire et al., 2011). Due to the high coverage and large number of SNP markers generated by this technique, dense linkage maps can be constructed which are superior to traditional low-marker-density linkage maps.

The high heterozygosity of grapes makes it possible to construct linkage maps in a two-way pseudo-testcross strategy (Grattapaglia and Sederoff, 1994). In virtually any two grapevines, a large number of SNPs will be heterozygous in one parent and homozygous in the other and will segregate in a 1:1 ratio in the F_1 progeny, following a testcross configuration. In this way, taking

heterozygous markers in one parent and homozygous markers in another parent, a separate linkage map can be constructed for each.

Linkage maps facilitate the localization of the allelic variants of genes for important horticultural traits and, therefore, are indispensable tools for the development of modern grape varieties. In grapevine genetics and breeding, linkage maps have become essential for identification of candidate genes, positional cloning experiments and marker assisted selection.

In this study, an F_1 mapping population from the cross between *V. rupestris* and *V. riparia* was analyzed with the GBS method and the resulting SNP markers were used to construct parental maps.

Materials and methods

Mapping Population and Genotyping. An F_1 mapping population was developed by crossing *V. riparia* Michx. 'PI583271' (2n=38, ♂) and *V. rupestris* 'PI588160' (2n=38, ♀). Both parents were collected in the United States and are maintained at the repository of the USDA Grape Genetics Research Unit in Geneva, New York. Crosses were made in the field by manually removing floral caps on the female *V. rupestris* parent and applying dried pollen collected from the male *V. riparia* parent. Clusters were bagged with paper bags to prevent contamination from other male vines in the germplasm. Seeds were collected from berries at the end of the season, vernalized for 4 weeks at 4°C and germinated under greenhouse conditions. In the spring of 2014, seedlings were planted into a grapevine nursery. Genomic DNA was extracted from leaves of 353 F_1 progeny vines and their parents. Genotyping-by-sequencing (GBS) was performed based on the protocol described by Hyma et al. (2015). Barcode adapters were ligated to DNA from each individual sample, and the resulting fragments

were paired-end sequenced on an HiSeq 2000 instrument (Illumina Inc., San Deigo, CA, USA). The raw reads were demultiplexed, processed and aligned against 12x reference genome sequence of *V. vinifera* 'PN40024' (Jaillon et al., 2007; accessible at http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/) for SNP discovery. GBS and SNP discovery was performed at Cornell University by Dr. Jason Londo, Research Geneticist at USDA, Agricultural Research Service (ARS) in Geneva, New York, USA.

Marker Generation, Filtering, Segregation Analysis and Map Construction. GBS reads that aligned to the *V. vinifera* reference genome sequence (RefSeq) were screened for single nucleotide polymorphisms (SNPs). SNPs identified were filtered for various quality parameters using VCFtools (Danecek et al., 2011). In the first filtering step, only biallelic SNPs at a sequencing depth of 6 or deeper were retained and all others were removed. SNPs with lower than 20% missing genotypes and of minor allele frequency (MAF) of 0.2 or higher were retained. SNP data in the VCF file were then converted into JOINMAP®5.0 (Van Ooijen, 2018) format using NGSEP software (Duitama et al., 2014). SNPs that were likely the result of sequencing error and F_1 genotypes with more than 10% missing SNP markers were discarded. A goodness-of-fit (χ^2) test was performed to filter out the markers which deviated from the 1:1 segregation ratio in the progeny. Because segregation distortion is a natural phenomenon in out-crossing species such as grape, markers showing a moderate degree of segregation distortion were retained for map construction. Significantly distorted markers ($p<0.0005$) were discarded for both parental maps. Maternal and paternal population nodes were created in JOINMAP®5.0 (Van Ooijen, 2018) with marker type "lm x ll" and "nn x np", respectively, and parental maps were constructed following a two-way pseudo-test cross approach (Grattapaglia and Sederoff, 1994). Markers of the "hk x hk" type were excluded for map construction. Identical markers

(markers with identical genotypes across all mapping progeny) were discarded from each pair, because they likely resulted from DNA contamination. Linkage groups were constructed with a threshold LOD value of 14, maximum recombination frequency of 0.4 and jump threshold of 5. Marker order was determined with a regression mapping algorithm and genetic distances were expressed in Kosambi distance with parameters at default settings. Only 2nd round maps were accepted. Map charts were constructed using the MAPCHART 2.32 software (Voorrips, 2002).

Results

Filtering 348,888 SNPs for various quality parameters (see Materials and Methods) yielded 11,063 SNPs across 294 F_1 individuals. The overall data summary, allele summary, minor allele frequency distribution, and average sequencing depth pre- and post-filtering SNP data are presented in appendices A, B, C, D, E, F, G, and H. Of the SNPs that satisfied the filtering criteria, 3,436 SNPs were discarded because both parents were homozygous for these sites. An additional 1,276 sites with unexpected genotypes (therefore, likely the result of sequencing error) were excluded from downstream analysis. Population nodes were created in JOINMAP®5.0 for each parent separately. An additional 360 and 331 markers were removed from paternal and maternal nodes, respectively, because their segregation was distorted from the expected 1:1 ratio as determined by Chi-squared test ($p<0.0005$). Upon the removal of identical markers from each parental node, 1,351 male and 1,462 female parent-informative markers following "nn x np" and "ll x lm" segregation types were used for linkage map construction. For the male and female parents, 1,115 and 1,177 significant markers (threshold LOD $\geq$ 14), respectively, were grouped into 19 different linkage groups covering 1657.4 and 1401.3 cM of genetic distance **(Table 1)**. Linkage groups were numbered according to assignment of *V.*

vinifera RefSeq chromosome map-anchored SNP markers (**Figure 1)**. Linkage map charts for *V. riparia* and *V. rupestris* are shown in appendices I and J, respectively. Correlation coefficients between marker genetic positions on linkage map and their corresponding physical coordinates in the reference genome sequence ranged from 0.24 to 0.98 and from 0.5 to 0.98 for the male and the female parent, respectively (Appendices K, L). Map quality was then further tested using the R script R/qtl. Pairwise recombination fractions and LOD scores are shown in **Figure 2**. An examination of recombination frequency between pairs of markers for each parent demonstrated tight linkage within, but not across, different linkage group.

Discussion

The availability of the *V. vinifera* reference genome sequence and the advent of efficient and cost-effective genotyping methods, such as restriction site-associated DNA sequencing (RAD-seq) (Baird et al., 2008), genotyping-by-sequencing (GBS) and Amp-seq (Yang et al., 2016), have facilitated the rapid mapping of quantitative trait loci in diverse grapevine genotypes.

We propose that the exploration of the vast genetic diversity of wild grape germplasm can be further accelerated by the creation and use of mapping populations derived from crosses between wild parents which have adapted to widely different environmental conditions.

In species of large and complex genomes, such as grapevine, GBS offers an alternative to more complex SNP discovery methods (Elshire et al., 2011). In this work, I utilized GBS-generated SNP markers, following the protocol described by Hyma et al. (2015). This protocol makes it possible to both reduce the sequenced portion of the genome and to readily scale up the library preparation procedure by multiplexing DNA digestion and adapter ligation in 96-well

plates. Even with a dramatic reduction of the represented genome, hundreds of thousands of SNPs can be identified with sufficiently high confidence for the construction of dense genetic maps. Some of the drawbacks of the GBS method are the large number of missing data and the shallow depth of sequence coverage. Use of markers generated from low sequence-depth coverage increases the probability of generating an erroneous linkage map. To reduce the probability of errors in my map, I applied stringent quality parameters for filtering the SNP data. Stringent filtering, on the other hand, inadvertently leads to the elimination of several correct markers and leads to a small number of final markers. There is a trade-off, therefore, between the reliability and the marker density of the generated linkage map. Filtering procedures reduced the number of SNPs to 11,063, which is only about 3.17% of the original number of SNPs obtained from variant calling. This number was further reduced when only markers heterozygous in one parent and homozygous in another parent (ll x lm or nn x np) were kept while those homozygous or heterozygous in both parents were discarded.

Grapes are highly heterozygous. Bacilieri et al. (2013) reported 77% expected heterozygosity (H_e) over 20 loci in 2,096 cultivated grapevine varieties. In *V. rupestris,* Pap et al. (2015) reported observed heterozygosity (H_o) of 0.30 to 0.77. genotyping in 100 wild accessions representing 5 different subpopulations at 14 microsatellite loci. Similarly, genotyping of 96 *Vitis* accessions at 19 different SSR loci covering all linkage groups, resulted in H_o values ranging from 0.18 to 0.90 (Jahnke et al., 2011). In this study, even after filtering SNP data with stringent quality parameters, 63% of the genotyped F_1 individuals proved heterozygous for all SNP markers. These values are in agreement with heterozygosity reported from several other obligate outcrossing perennial plants species (Pap et al., 2015 and references therein).

Despite stringent filtering, we obtained more than one thousand markers for each parent segregating in 1:1 ratio, which clearly indicates the effectiveness of the GBS technique for SNP discovery and marker generation in highly heterozygous species. Because of the high heterozygosity and the obligatory outcrossing nature of grapes, it is a common practice to follow a two-way pseudo-test cross strategy (explained by Grattapaglia and Sederoff, 1994) to construct parental maps separately using an F_1 mapping progeny. In this study, the resulting map coverages were consistent with the other SNP-based maps reported in *Vitis* species (Chen et al., 2015; Wang et al., 2017; Yang et al., 2016).

The maps were then further characterized using R/qtl (Broman et al., 2003). We obtained, in most cases, a good correlation between the genetic position of the markers in the linkage maps and their physical position in the *V. vinifera* RefSeq. It was observed, however, that 9.94% of the markers (117 out of 1,177) in *V. rupestris* and 8.87% of the markers in *V. riparia* (99 out of 1,115) were not assigned to the linkage group which corresponded to their physical position in the *V. vinifera* RefSeq. Gardner et al. (2014) and Antanaviciute et al. (2012) also reported similar discrepancies between genetic and physical position of 18.3% and 13.7% of SNP markers in apple, respectively. The conflicts between the genetic and RefSeq coordinates may stem from the presence of paralogous genomic regions or the incorrect sequence assembly of parts of the *V. vinifera* RefSeq.

References

Antanaviciute, L., Fernández-Fernández, F., Jansen, J., Banchi, E., Evans, K. M., Viola, R., et al. (2012). Development of a dense SNP-based linkage map of an apple rootstock progeny using the Malus Infinium whole genome genotyping array. *BMC Genomics* 13, 203. doi: 10.1186/1471-2164-13-203.

Bacilieri, R., Lacombe, T., Le Cunff, L., Di Vecchi-Staraz, M., Laucou, V., Genna, B., et al. (2013). Genetic structure in cultivated grapevines is linked to geography and human selection. *BMC plant biol. 13*, 25. doi:10.1186/1471-2229-13-25

Baird, N. A., Etter, P. D., Atwood, T. S., Currey, M. C., Shiver, A. L., Lewis, Z. A., et al. (2008). Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PloS One* 3(10), e3376. doi: 10.1371/journal.pone.0003376

Bouquet, A. (2011). "Grapevines and Viticulture" in *Genetics, Genomics and Breeding of Grapes*, eds. A., Adam-Blondon, J., Martinez-Zapater, and C., Kole (Florida: CRC Press), 1-5

Broman, K.W., Wu, H., Sen, Ś., and Churchill, G.A. (2003). R/qtl: QTL mapping in experimental crosses. *Bioinformatics* 19, 889-890

Chen, J., Wang, N., Fang, L., Liang, Z., Li, S., and Wu, B. (2015). Construction of a high-density genetic map and QTLs mapping for sugars and acids in grape berries. *BMC Plant Biol.* 15(1), 28. doi:10.1186/s12870-015-0428-2

Collard, B. C. Y., Jahufer, M. Z. Z., Brouwer, J. B., and Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica*. doi: 10.1007/s10681-005-1681-5

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., et al. (2011). The variant call format and VCFtools. *Bioinformatics* 27(15), 2156–2158. doi: 10.1093/bioinformatics/btr330

Duitama, J., Quintero, J. C., Cruz, D. F., Quintero, C., Hubmann, G., Foulquié-Moreno, M. R., et al. (2014). An integrated framework for discovery and genotyping of genomic variants from high-throughput sequencing experiments. *Nucleic Acids Res.* 42(6). doi: 10.1093/nar/gkt1381

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., and Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One*, 6(5). doi: 10.1371/journal.pone.0019379

Firoozabady, U. X., and Olmo, H. P. (1982). Resistance to grape phylloxera in *Vitis vinifera* x *V. rotundifolia* grape hybrids. *Vitis* 21, 1-4.

Gardner, K. M., Brown, P., Cooke, T. F., Cann, S., Costa, F., Bustamante, C., et al. (2014). Fast and Cost-Effective Genetic Mapping in Apple Using Next-Generation Sequencing. *G3 (Bethesda)* 4(9), 1681-1687. doi: 10.1534/g3.114.011023

Grattapaglia, D., and Sederoff, R. (1994). Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross: Mapping strategy and RAPD markers. *Genetics*. doi: 10.1007/s11033-010-0612-2

Hyma, K. E., Barba, P., Wang, M., Londo, J. P., Acharya, C. B., Mitchell, S. E., et al. (2015). Heterozygous mapping strategy (HetMappS) for high resolution genotyping-by-sequencing markers: A case study in grapevine. *PLoS One*, 10(8). doi: 10.1371/journal.pone.0134880

Jahnke, G., Kocsisné Molnár, G., Májer, J., Szöke, B., Tarczal, E., Varga, P., and Kocsis, L. (2011). Analysis of grape rootstocks by SSR markers. *J. Int. Sci. Vigne Vin* 45(4), 199–210.

Jaillon, O., Aury, J.-M., Noel, B., Policriti, A., Clepet, C., Casagrande, et al. (2007). The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature*, 449(7161), 463–467. doi: 10.1038/nature06148

Liang, Z., Duan, S., Sheng, J., Zhu, S., Ni, X., Shao, J., et al. (2019). Whole-genome resequencing of 472 Vitis accessions for grapevine diversity and demographic history analyses. *Nat. Commun.* 10(1). doi: 10.1038/s41467-019-09135-8

Luca, F., Hudson, R. R., Witonsky, D. B., and Rienzo, A. D. (2011). A reduced representation approach to population genetic analyses and applications to human evolution. *Genome Res.* 21, 1087–1098. doi: 10.1101/gr.119792.110.

Mammadov, J., Aggarwal, R., Buyyarapu, R., and Kumpatla, S. (2012). SNP Markers and Their Impact on Plant Breeding. *Int. J. Plant Genomics* 2012. doi: 10.1155/2012/728398
Mason, A.S. (2015). SSR Genotyping. In: Batley, J. (ed) Plant Genotyping. Springer, New York, NY, pp 77-89.

McClure, K. A., Sawler, J., Gardner, K. M., Money, D., and Myles, S. (2014). Genomics: A potential panacea for the perennial problem. *Am. J. Bot.* 101(10), 1780–1790. doi: 10.3732/ajb.1400143

McGovern, P. E., Fleming, S. J., and Katz, S. (Eds.). (1995). The origins and ancient history of wine. New York: Gordon and Breach.

Myles, S. (2013). Improving fruit and wine: What does genomics have to offer? *Trends Genet.* 29: 190 – 196

Nadeem, M. A., Nawaz, M. A., Shahid, M. Q., Doğan, Y., Comertpay, G., Yıldız, M, et al. (2018). 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

Pap, D., Miller, A. J., Londo, J. P., and Kovács, L. G. (2015). Population structure of *Vitis rupestris*, an important resource for viticulture. *Am. J. Enol. Viticult.* 66(4), 403–410. doi: 10.5344/ajev.2015.15012

Reisch, B. I., Stephen Luce, R., and Mansfield, A. K. (2014). “Arandell”-a disease-resistant Red wine grape. *HortScience* 49(4), 503–505. doi: 10.21273/HORTSCI.49.4.503

Van Ooijen, J. W. (2006). *JoinMap® 5, Software for the calculation of genetic linkage maps in experimental populations of diploid species*. Kyazma B.V., Wageningen, Netherlands.

Voorrips, R.E. (2002). MapChart: Software for the graphical presentation of linkage maps and QTLs. *J. Hered.* 93 (1): 77-78.

Walker, M.A., Lider, L. A., Goheen, A. C., and Olmo, H. P. (1991). VR 039-16 Grape Rootstock, *HortScience*, 26(9), 1224-1225. doi: 10.21273/HORTSCI.26.9.1224

Wang, J., Su, K., Guo, Y., Xing, H., Zhao, Y., Liu, Z., et al. (2017). Construction of a high-density genetic map for grape using specific length amplified fragment (SLAF) sequencing. *PLoS One* 12(7): e0181728. doi: 10.1371/journal.pone.0181728

Yang, S., Fresnedo-Ramírez, J., Sun, Q., Manns, D. C., Sacks, G. L., Mansfield, A. K., et al. (2016). Next Generation Mapping of Enological Traits in an F2 Interspecific Grapevine Hybrid Family. *PLoS One* 11(3). doi:10.1371/journal.pone.0149560

Yang, S., Fresnedo-Ramírez, J., Wang, M., Cote, L., Schweitzer, P., Barba, P., et al. (2016). A next-generation marker genotyping platform (AmpSeq) in heterozygous crops: A case study for marker-assisted selection in grapevine. *Hortic. Res.* 3. doi: 10.1038/hortres.2016.2

TABLE 1 | Distribution of SNP markers and the total genetic length in Kosambi distance across 19 linkage groups of *V. rupestris* 'PI588160' and *V. riparia* 'PI583271'.

LGs	***Vitis rupestris* (Female parent)**		***Vitis riparia* (Male parent)**	
	Number of SNP markers	Total Genetic Length (cM)	Number of SNP markers	Total Genetic Length (cM)
1	42	80.5	64	94.8
2	50	64.5	43	77
3	51	65.4	57	75.7
4	61	71	64	88.9
5	63	71.8	59	83.5
6	31	58.6	53	86.8
7	98	95.9	92	116.7
8	57	75.7	50	89.8
9	66	63.2	37	63
10	65	65.8	33	80.9
11	42	65.2	39	75.2
12	68	87.6	71	74.3
13	48	80	80	98.3
14	110	88.4	86	96.4
15	55	65.6	54	95.7
16	78	66.3	49	82.1

TABLE 1 | Continued. Distribution of SNP markers and the total genetic length across 19 linkage groups.

LGs	***Vitis rupestris* (Female parent)**		***Vitis riparia* (Male parent)**	
	Number of SNP markers	Total Genetic Length (cM)	Number of SNP markers	Total Genetic Length (cM)
17	41	64.4	53	72.7
18	78	108.7	74	125.1
19	73	62.7	57	80.5
Total	**1177**	**1401.3**	**1115**	**1657.4**

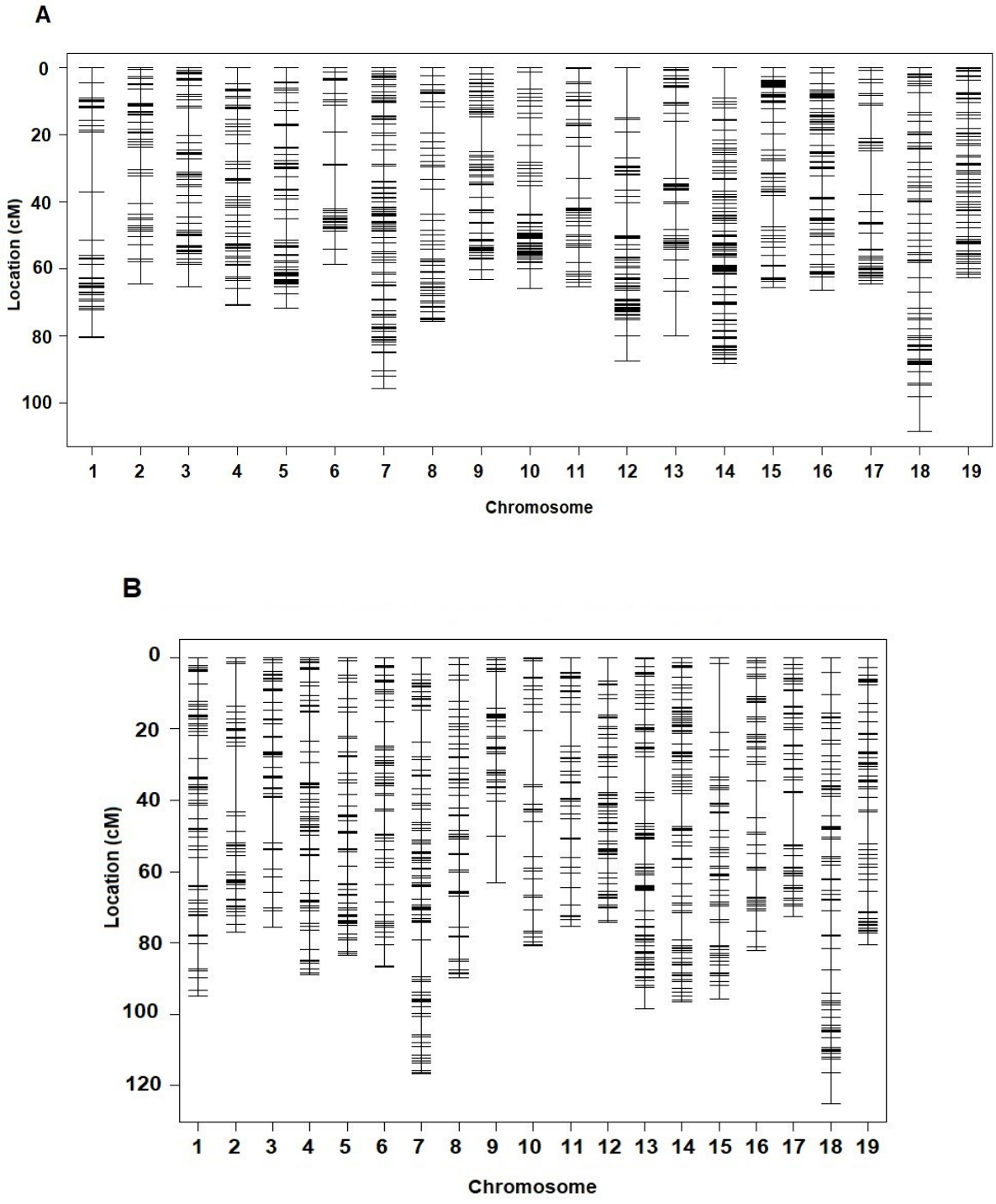


FIGURE 1 | Linkage map and marker distribution in linkage groups of (A) *V. rupestris* and (B) *V. riparia*

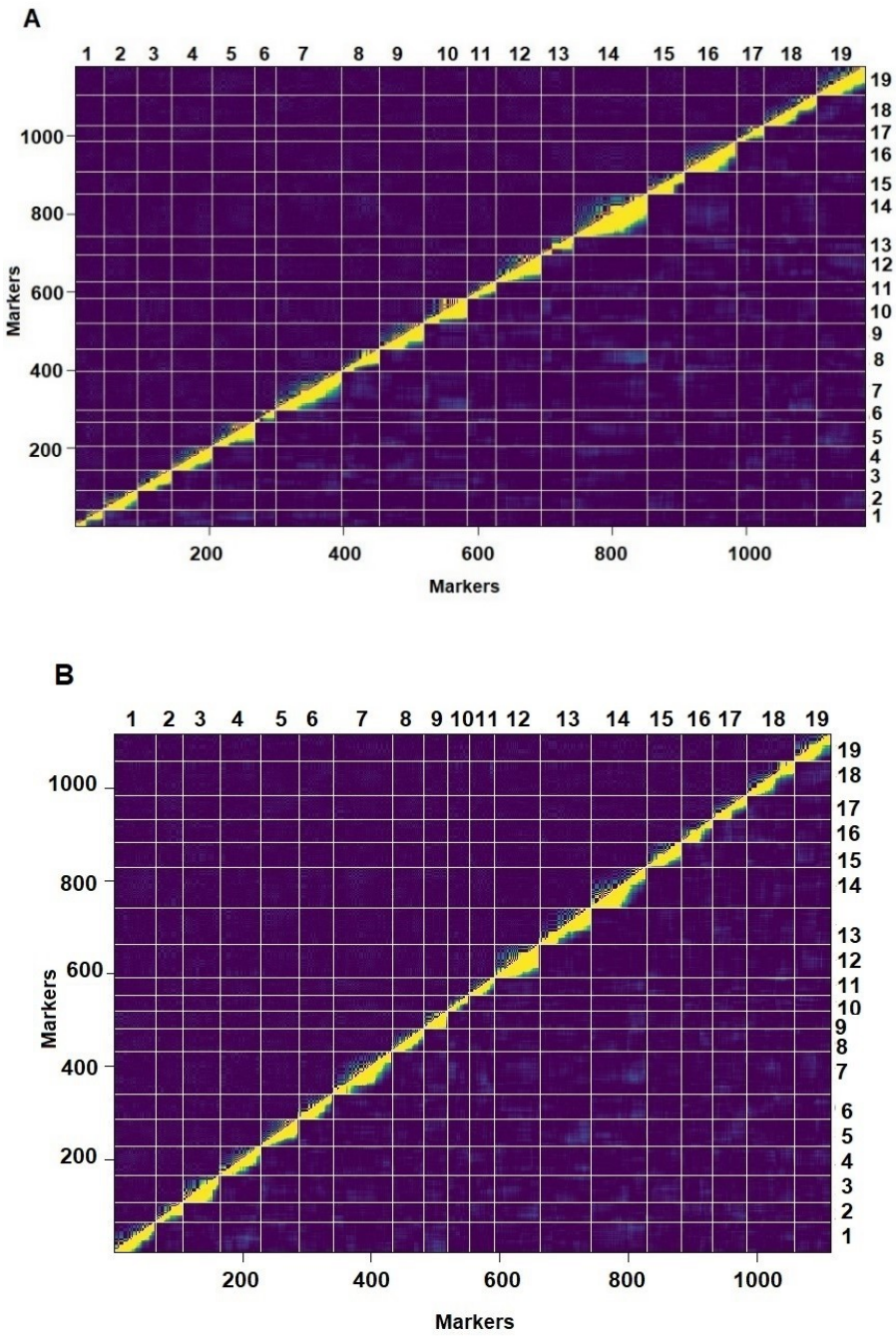


FIGURE 2 | Pairwise recombination fractions and LOD scores in (A) *V. rupestris* and (B) *V. riparia* linkage groups. Yellow and blue coloring below the identity line represent low and high cross-over frequency regions, respectively. Yellow and blue coloring above the identity line represent low and high LOD values, respectively. The top X-axis and the right Y-axis are numbered according to linkage groups; The bottom X-axis and the left Y-axis chart the position of markers in the 19 linkage groups.

CHAPTER 2: MAPPING QUANTITATIVE TRAIT LOCI (QTL) FOR DOWNY MILDEW DISEASE RESISTANCE

Introduction

Downy mildew (DM), caused by the obligate biotrophic oomycete, *Plasmopara viticola*, is one of the most destructive diseases of grapevines in regions where spring and summer rainfall are frequent and the average temperatures is above 10˚C (Ash, 2000). *Plasmopara viticola* zoospores enter the intercellular spaces of grape leaves through the stomata and germinate to form a germ tube which then develops into primary hyphae. Primary hyphae develop haustoria which enter the lumen of the host cell and obtain resources for additional hyphal growth. The hyphae branch to rapidly colonize the intercellular spaces of the host tissue and induce the characteristic "oil spot" symptoms, yellowish translucent areas of the leaf. Severely infected leaves become necrotic and die. Young berries, rachis and tendrils also are susceptible to infection in the spring (Unger et al., 2007). Finally, the mycelia fill the intercoastal field and develop sporangiophores which emerge through the stomata to produce sporangia. In the presence of liquid water, resulting from rain or dew, mature sporangia burst open and release the next generation of zoospores which will restart the infection process at different site. If conditions are favorable, several such asexual reproductive cycles take place in a single season, amplifying disease pressure and causing serious economic losses in unprotected plants.

Most of the commercially grown cultivars of the Eurasian grape species *Vitis vinifera* are highly susceptible to *P. viticola*. The pathogen was inadvertently introduced to Europe in the late 1870's while transporting grapevine propagation material from North America for rootstocks to manage the epidemic caused by phylloxera (*Daktulosphaira vitifoliae*). Phylloxera is an aphid-

like insect pest that had been introduced also from North America two decades earlier (Gessler et al., 2011). Large variation in the level of resistance against the downy mildew pathogen has been detected in various grape species. Yu et al. (2012) found a significant amount of callose deposition in downy mildew-immune *Vitis rotundifolia* and in the highly resistant Asian grapevine *Vitis pseudoreticulata* when infected with the DM pathogen. Callose depositions were also detected, albeit to a lesser extent, in the stomata and around the haustoria in the moderately resistant *Vitis amurensis* grapevines. Importantly, no callose deposition was observed in DM-susceptible *V. vinifera* grapevines. Callose and phenol depositions and a hypersensitive response are considered key features of disease resistance which halt pathogen growth in grapevines (Godard et al., 2009; Hamiduzzaman et al., 2005). Larger numbers of branched hyphae were detected around the stomata in susceptible *V. vinifera* than in resistant grapevines, indicating extensive proliferation of the pathogen in the infected tissue. The management of the DM disease is based almost exclusively on the recurrent use of chemical pesticides. Not only does this reliance on chemical control increase the cost of grape production, but it also negatively impacts human health and the ecosystem. Therefore, from the perspective of sustainable viticulture, the cultivation of newly developed DM-resistant grape varieties offers an alternative approach to control downy mildew disease.

Plant disease resistance is a complex trait governed by one or several genes. Quantitative trait loci (QTL) represent genomic regions that correlate with quantitative traits. Linkage maps are used to detect QTL by identifying markers tightly linked with a trait. So far, a number of major and minor quantitative trait loci (QTL) conferring varying degrees of resistance against downy mildew have been identified in muscadine and several North American and East Asian native grape species (Medrinoglu et al., 2003; Welter et al., 2007; Bellin et al., 2009; Van

Heerden et al., 2014; Di Gaspero et al., 2012; Zyprian et al., 2016; Marguerit et al., 2009; Blasi et al., 2011; Moreira et al., 2011; Ochssner et al., 2016; Divilov et al., 2018). In this study, an F_1 progeny generated from the cross between two native North American grapevines, *V. rupestris* and *V. riparia*, were screened and QTL analysis was performed for DM resistance. The GBS-based linkage maps, described in the first chapter of this thesis, were first validated by pinpointing the Mendelian factor determining the sex of F_1 progeny and later used for QTL analysis of DM resistance.

Materials and methods

Phenotyping for Downy Mildew Resistance in Naturally Infected Leaves. Phenotyping for downy mildew resistance was carried out by quantifying disease severity on the leaves of 136 F_1 individuals. To quantify disease severity, the growth of the pathogen was evaluated by visual scoring of the leaf area covered by sporangiophores and the density of sporangiophores within the mildew-covered areas. Disease severity was expressed on a scale ranging from 1 to 10 (1 denoting high susceptibility and 10 high resistance). One to five leaves were scored for each genotype. This method of phenotyping was carried out in the greenhouse located at South Dakota State University by Dr. Anne Fennel.

The DM disease in the greenhouse developed as a result of a natural infection. To characterize the *P. viticola* strain that was responsible for this infection, the pathogen was cultured on surface-sterilized grape leaves under *in vitro* conditions, its genomic DNA was extracted, and a 235-bp long internal transcribed spacer (ITS) sequence was PCR-amplified using the ITS-1 primer pair (Rouxel et al., 2014). The PCR product was then sequenced and aligned to the corresponding ITS nucleotide sequence of other *P. viticola* cryptic species. The

ITS-1 sequence alignment data were then used to construct a phylogenetic tree using the software MEGA7 (Kumar et al., 2016). The phylogeny was inferred using the Neighbor-Joining method. Evolutionary distances were computed using the Jukes-Cantor method and bootstrap values were determined using 1,000 bootstrap replicates.

To characterize the virulence of the *P. viticola* strain, leaf disks of three different grape genotypes, *V. vinifera* 'F2-35', *V. riparia* 'Gloire de Montpellier' and *V. rotundifolia* 'Thomas', were inoculated, and disease severity was evaluated as described below.

Quantifying Downy Mildew Resistance under *in vitro* Conditions. The resistance of 86 F_1 individuals to downy mildew was also quantified in an *in vitro* assay. These 86 individuals were part of the same segregating progeny as the plants phenotyped under natural infection under greenhouse conditions, but only 20 of the 86 individuals were shared between the two cohorts. Healthy leaves from the third and fourth nodes from the apical meristem were surface sterilized by submerging them in 1% NaOCl solution for two minutes and rinsing them for five minutes four times in sterile distilled water (dH_2O). Four circular leaf disks, 2 cm in diameter, were excised for each leaf and placed abaxial-side up on 0.8% water-agar plates in Petri dishes. A *P. viticola* sporangial suspension was prepared by suspending sporangia (stored at -20˚C) with sterile dH_2O and incubated for two hours at 4˚C. The density of the suspension was adjusted to 70,000 sporangia/ml and sprayed over the leaf disks uniformly. Inoculated leaf disks were incubated overnight in the dark under axenic conditions and transferred to a growth chamber with a temperature of 21˚C and a 5-hour/19-hour dark/light diurnal cycle. The leaf disks were visually scored for disease severity seven days after inoculation **(Figure 3)**. Leaf surface area coverage and sporangiophore density were estimated using the International Organization of Vine and Wine (OIV) standard disease severity chart which uses scores of 1 through 9 (Bellin et

al. 2009). A score of 1 for coverage represented more than 90% of mildew coverage of the leaf disk, and 9 indicated no growth at all. For intensity, a score of 1 represented very high sporangiophore density and 9 indicated the absence of sporangiophores.

Phenotyping the F_1 Progeny for Flower Sex. To determine the sex of an individual, 169 F_1 plants were phenotyped based on the morphological development of reproductive organs. Vines were recorded as male if they had long, well developed stamens with prominent anthers and an immature, relatively small pistil with an inconspicuous ovary (staminate flowers). Vines were recorded as female if they had small, rudimentary stamens and a large, well-developed pistil (pistillate flower). Male and female vines were then coded as 0 and 1 respectively for the QTL analysis.

QTL Analysis. Composite interval mapping (CIM) was used for the QTL analysis in R/qtl software (Broman et al., 2003). Genome-wide LOD thresholds were determined for each phenotype by performing 1000 permutation at 5% level of significance ($p<0.05$). The genetic positions for significant LOD peaks were identified and corresponding 95% Bayesian credible intervals (BI) and explained variances were calculated in R.

Results

Characterization of the Downy Mildew Strain. Nucleotide sequence alignment of the ITS-1 fragment (Appendix M) from the *P. viticola* strain used in phenotyping revealed 100% identity to the corresponding fragment of the riparia (clade-A) cryptic species of *P. viticola* **(Figure 4)**. To characterize the virulence of the strain (named GDM-MO-1), it was used to inoculate three different grapevines, *V. riparia* 'Gloire de Montepellier', *V. rotundifolia*

'Thomas' and *V. vinifera* 'F2-35'. *V. rotundifolia* 'Thomas' appeared immune to the strain, *V. riparia* 'Gloire de Montpellier' proved partially resistant, while *V. vinifera* 'F2-35' was highly susceptible **(Figure 5)**. This indicated that the strain represents an aggressive pathogen on cultivated grapes.

QTL Mapping of the Sex-Determining Locus. To verify the correctness of the linkage maps, flower sex data were used to map the maleness-determining locus in the parents (Appendix N). A single major QTL was detected on chromosome 2 (chr2) in the male parent with a peak LOD score of 42.4 at genetic position of 19.88 cM **(Figure 6)**. This position is close to the chromosomal location of the sex-determining locus in grapevine, as previously determined in *V. Vinifera* **(Figure 7)** (Dalbó et al., 2000; Riaz et al., 2006; Marguerit et al., 2009). This QTL explained 56.15 % of the phenotypic variance, which provides strong evidence that this genomic region determines maleness. No significant QTL was detected for maleness in the genome of the female parent (*V. rupestris*) **(Figure 6)**.

QTL Mapping of Downy Mildew Resistance. A significant QTL for leaf resistance to downy mildew was detected at genetic position of 8.6 cM on chr10 of *V. rupestris* based on leaf area covered by sporangiophores as phenotype (Appendix O) under natural infection with an LOD peak of 20.6. It explained 45.75% of the phenotypic variance in the progeny for disease resistance **(Figure 8)**. Similar, but not identical, results were obtained with data generated with the *in vitro* inoculation assay: a significant QTL for resistance was detected at a genetic position of 6.0 cM on chr10 of *V. rupestris* based on mildew coverage as phenotype (Appendix P), explaining 16.01% of phenotypic variance **(Figure 8)**. No significant QTL for leaf resistance to downy mildew was detected in the male parent for disease coverage. A significant QTL for

resistance was detected, however, at genetic position of 47 cM on chromosome 8 (chr8) of the male parent (*V. riparia)* parent based on sporangiophore density within mildewed areas as phenotype (Appendix Q) in response to *in vitro* inoculation **(Figure 9)**. This QTL had an LOD value of 4.8 and explained 15.42% of the phenotypic variance. A detailed summary of the QTL analysis for sex and disease resistance is presented in **Table 2**.

Discussion

Most cultivated grape varieties bear hermaphroditic flowers, but in nature, grapevines are obligate outcrossing dioecious plants. The maleness or femaleness of a plant is determined by the sex of the flower they bear. Male flowers have well-developed stamens with prominent anthers and an immature, relatively small pistil with inconspicuous ovary (staminate flowers). Female flowers have small, rudimentary stamens and a large, well-developed pistil (pistillate flower). Expression of flower sex in *Vitis* is thought to be controlled by a major locus with three alleles male (M), hermaphroditic (H), and female (F) with allelic dominance series of M>H>F (Oberle, 1938; Antcliff, 1980). The genetic locus for this trait has been previously mapped to chr2 in *V. vinifera* (Dalbó et al., 2000; Riaz et al., 2006; Marguerit et al., 2009). Fechter et al., 2012 have delimited this region to 143 kb, between coordinates 4,907,434 and 5,050,616 bp on chr2 in *V. vinifera* RefSeq 12x_v0.

Because the locus for this Mendelian trait has been well established, its localization to chr2 in this F_1 progeny offers strong support for the correctness of the *V. riparia* linkage map. For this study, the sex of 169 F_1 progeny was recorded and composite interval mapping was performed with R/qtl software. The male parent showed a significant QTL ($p<0.05$) on chr2 at 19.9 cM with a highest LOD peak of 42.4, whereas the female parent showed no significant

QTL. The linked marker in *V. riparia* corresponds to position 4,350,083-bp in the *V. vinifera* RefSeq 12x_v0, which is near, but not at the same, region where the sex-determining locus had been previously reported (4,907,434 - 5,050,616 bp). As femaleness is a recessive trait, the female parent is homozygous at this locus, which precludes its detection in a two-way pseudo-testcross approach.

Once the parental genetic maps were constructed and validated, I performed QTL analyses for DM resistance. If left uncontrolled, DM can cause as high as 75% crop loss (Buonassisi et al., 2017). The conventional method of controlling this disease is the recurrent application of copper-containing preventive pesticides, which requires considerable energy input and is harmful to the environment. In traditional grape growing regions of Europe, the regular application of copper-containing chemicals over the past century has led to copper accumulation in the soil to hazardous levels (Ruyters et al., 2013). An alternative control method is the application of curative organic pesticides which can be expensive and cut deeply into growers' profit. In addition, consumers are becoming increasingly concerned with pesticide residues in wine and grape products and have strong preference for those that were treated with the least amount of chemicals. This is coupled with growing consumer interest in supporting environment-friendly production systems. Therefore, there are strong economic incentives to develop DM-resistant grape varieties which will require lower or no pesticide input.

To assess the virulence of downy mildew pathogens isolated from the F_1 progeny, leaf disks of three grape cultivars were infected under *in vitro* conditions. *Vitis vinifera* F2-35, *V. riparia* 'Gloire de Montpellier', and *V. rotundifolia* 'Thomas' are grape cultivars of different geographic and phylogenetic background. *Vitis vinifera* F2-35 is derived from cultivated wine grapes of Eurasian origin, whereas *V. riparia* 'Gloire de Montpellier' is a rootstock cultivar of

North American origin. *Vitis rotundifolia* 'Thomas' is a muscadine grape cultivar widely cultivated in the southern states of the US. While the former two grapes belong to the Euvitis subgenus (2n = 38), *V. rotundifolia* 'Thomas' is member of the Muscadinia subgenus (2n= 40). The results of these inoculation experiments demonstrated that the cultivated grapevine *V. vinifera* is highly susceptible to the pathogen strain while pathogen growth was partially or completely halted by the other two cultivars. This indicated that GDM-MO-1 represents an aggressive cryptic species of the pathogen on *V. vinifera* grapes, and therefore biological resistance against it is a valuable resource. The data also corroborate the commonly held view that native North American grapevines, which coevolved with *P. viticola,* are a valuable source of biological resistance for the cultivated grape.

Identification of markers linked to disease resistance loci has proven useful for marker-assisted selection in grape breeding programs. Designing markers which are closely linked to novel DM resistance QTLs will provide opportunities for breeders to work with new disease resistance genes, which potentially represent novel disease resistance mechanisms. Pathogen strain-specific resistance loci are notoriously unstable in crops, as pathogens evolve to eventually evade the defense mechanism of the plant. This problem can be alleviated by combining several resistance loci into a single variety, an approach named "gene pyramiding". Combining various resistance loci will potentially lead to a combination of different resistance mechanisms, which is the most promising approach to develop crops with stable resistance. Furthermore, marker-assisted selection is a requirement for gene pyramiding, as the phenotypes of all disease resistance loci are manifested in the same trait: resistance. Thus, the only way breeders can select for plants that combine more than one such locus is by phenotyping the progeny using tightly linked molecular markers. Furthermore, working with markers greatly accelerates the progress of

breeding work even if the goal is to introduce a single locus. This is because seedlings from a new cross can be tested as soon as real leaves are formed on progeny seedlings and can yield DNA samples. A rapid screen to select out and discard those plants that do not contain the desired loci can be achieved in a matter of days, and the maintenance of only those plants which are of interest will be required. In this way, the use of molecular markers not only accelerates breeding progress but saves considerable resources and reduces breeding cost.

In this work, DM resistance phenotyping was performed under two different conditions: first, under greenhouse conditions by allowing the F_1 progeny to be naturally infected by the pathogen and, second, with *in vitro* inoculation in a growth chamber, providing optimal conditions for mildew growth. The former method included the scoring of disease severity of the 136 F_1 genotypes from the scale of 1 to 10 with increasing level of resistance. The progeny showed extensive variation against pathogen attack (mean ± SD disease severity of 6.183 ± 3.09). The 95% BI of significant QTL for disease resistance on chr10 corresponds to the physical position of 1,285,522 to 2,674,703 bp (~1.39 Mbp) in the *V. vinifera* RefSeq. QTL analyses for the disease coverage under *in vitro*-infection revealed significant loci on the same *V. rupestris* chromosome, albeit not at the identical position. It is important to note, however, that the natural infection-generated QTL was within the Bayesian 95% credible interval (BI) of the *in vitro*-generated QTL, which was as large as 2 to 11.3 cM. This interval spans the region between positions 1,285,522 and 3,167,847 bp (~ 1.9 Mbp) of chr10 in the *V. vinifera* RefSeq 12x_v0. Potential reasons for the discrepancy in the QTL positions are the different conditions under which the mildew developed, the relatively small sample size of the *in vitro*-inoculation experiment, and that only 20 of the F_1 individuals were shared between naturally infected and the

in vitro-infected cohorts. Thus far, this is the only downy mildew resistance QTL that has been detected on chr10 in any *Vitis* spp.

Additionally, based on the sporangiophore density phenotype, a significant QTL ($p<0.05$) for disease resistance was detected on chr8 in *V. riapria* at the genetic position of 47.0 cM, explaining 15.42% of the phenotypic variance. The 95% BI for this locus is 46.0-50.83 cM which spans the physical position of 13,468,856 – 14,133,706 bp (~665 Kbp) in the reference genome. Divilov et al. (2018) have identified a significant QTL of DM resistance, Rpv17, on chr8 of 'Horizon' grape cultivar which is derived from *V. vinifera* and North American *Vitis* spp. (Reisch et al., 1983). Rpv17 was obtained by QTL analysis using hypersensitive reaction as a disease resistance phenotype and corresponds to the 95% credible interval of 11.4-12.2 Mbp in the *V. vinifera* RefSeq.

Conventionally, QTL mapping studies in grapevine emphasize the use of experimental populations derived from a cross between a wild species with very few, sometimes only one, specific trait of interest, and an elite cultivar of *V. vinifera*. In this work, I took a different approach: I used an F_1 progeny from a cross between two wild *Vitis* species adapted to widely different environmental conditions. The American grape species *V. riparia* and *V. rupestris* have different geographical distributions, are adapted to disparate environmental conditions and evolved to have different growth habits. *V. riparia* forms large vines with leaves above the tree canopy and grows in the moist and nutrient-rich alluvial soil of floodplains. *Vitis rupestris*, on the other hand, forms a shrub which grows along the surface of nutrient-poor and occasionally drought-stricken gravel bars of intermittent rivers. I hypothesize that in this single F_1 population, it will be possible to map QTLs for several traits of horticultural importance.

North American grapevines have evolved to withstand various biotic and abiotic stresses which represent major challenges of the viticulture industry as it faces the impacts of global climate change. The past 150 years of grapevine breeding have provided ample evidence that these plants possess many desirable vegetative and reproductive traits which can be mapped and applied successfully in the breeding program through marker-assisted selection or transgenesis. Nonetheless, their vast genetic diversity has not been adequately explored. Recently, Riaz et al. (2019) have reported a very narrow genetic base of grape rootstocks that are being used in grape breeding programs. Previously, Di Gaspero et al. (2012) documented a similarly narrow genetic base of fruit-bearing hybrid grape varieties. These data lend strong support to the argument for extending the exploration and use of diverse grape genetic resources for the improvement of commercial cultivars.

In this study, I have demonstrated that disease resistance traits segregate in the F_1 progeny derived from a cross between two wild grape species. The novel QTLs identified in this study can be further tested and validated under different genetic backgrounds. The markers developed can be immediately applicable in marker-assisted breeding programs and for the further exploration of candidate genes through positional cloning.

References

Antcliff, A.J. (1980). Inheritance of sex in *Vitis*. *Ann. Amélior. Plant.* 30:113–122.

Ash, G. (2000). Downy mildew of grape. *The Plant Health Instructor.* doi: 10.1094/PHI-I-2000-1112-01

Bellin, D., Peressotti, E., Merdinoglu, D., Wiedemann-Merdinoglu, S., Adam-Blondon, A. F., Cipriani, G., et al. (2009). Resistance to Plasmopara viticola in grapevine "Bianca" is controlled by a major dominant gene causing localised necrosis at the infection site. *Theor. Appl. Genet.* 120(1), 163–176. doi: 10.1007/s00122-009-1167-2

Bioletti, F.T., Flossfeder, F.C.H., and Way, A.E. (1921). *Phylloxera-resistant stocks*. Agricultural Experiment Station, Berkeley

Blasi, P., Blanc, S., Wiedemann-Merdinoglu, S., Prado, E., Rühl, E. H., Mestre, P., and 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(1), 43–53. doi: 10.1007/s00122-011-1565-0

Buonassisi, D., Colombo, M., Migliaro, D., Dolzani, C., Peressotti, E., Mizzotti, C., et al. (2017). Breeding for grapevine downy mildew resistance: a review of "omics" approaches. *Euphytica* 213(5). doi: 10.1007/s10681-017-1882-8

Burruano, S. (2000). The life-cycle of *Plasmopara viticola*, cause of downy mildew of vine. *Mycologist* 14:179-182.

Canaguier, A., Grimplet, J., Di Gaspero, G., Scalabrin, S., Duchêne, E., Choisne, N., Mohellibi, N., Guichard, C., Rombauts, S., Le Clainche, I., Bérard, A., Chauveau, A., Bounon, R., Rustenholz, C., Morgante, M., Le Paslier, M. C., Brunel, D., et al. (2017). A new version of the grapevine reference genome assembly (12X.v2) and of its annotation (VCost.v3). *Genomics Data* 14, 56-62. doi:10.1016/j.gdata.2017.09.002

Dai, G. H., Andary, C., Mondolot-Cosson, L., and Boubals, D. (1995). Histochemical studies on the interaction between three species of grapevine, Vitis vinifera, V. rupestris and V. rotundifolia and the downy mildew fungus, Plasmopara viticola. *Physiol. Mol. Plant Pathol.* 46(3), 177–188. doi: 10.1006/pmpp.1995.1014

Dalbó, M.A., Ye, G.N., Weeden, N.F., Steinkellner, H., Sefc, K.M., and Reisch, B.I. (2000). A gene controlling sex in grapevines placed on a molecular marker-based genetic map. *Genome* 43(2):333-40.

Di Gaspero, G., Copetti, D., Coleman, C., Castellarin, S. D., Eibach, R., Kozma, P., et al. (2012). Selective sweep at the Rpv3 locus during grapevine breeding for downy mildew resistance. *Theor. Appl. Genet.* 124(2), 277–286. doi: 10.1007/s00122-011-1703-8

Divilov, K., Barba, P., Cadle-Davidson, L., and Reisch, B. I. (2018). Single and multiple phenotype QTL analyses of downy mildew resistance in interspecific grapevines. *Theor. Appl. Genet.* 131(5), 1133–1143. doi: 10.1007/s00122-018-3065-y

Eibach, R., Zyprian, E., and Töpfer, R. (2009). The use of molecular markers for pyramiding resistance genes in grapevine breeding. *Acta Hortic.* 827, 551-558. doi:10.17660/actahortic.2009.827.96

Fechter, I., Hausmann, L., Daum, M., Sörensen, T.R., Viehöver, P., Weisshaar, B., and Töpfer, R. (2012). Candidate genes within a 143 kb region of the flower sex locus in *Vitis*. *Mol. Genet. Genomics* 287(3):247-59.

Gessler, C., Pertot, I., and Perazzolli, M. (2011). *Plasmopara viticola*: a review of knowledge on downy mildew of grapevine and effective disease management. *Phytopathol. Mediterr.* 50(1), 3-44. doi: 10.14601/Phytopathol_Mediterr-9360

Godard, S., Slacanin, I., Viret, O., and Gindro, K. (2009). Induction of defence mechanisms in grapevine leaves by emodin- and anthraquinone rich plant extracts and their conferred resistance to downy mildew. *Plant Physiol. Biochem*. 47:827-837.

Hamiduzzaman, M. M., Jakab, G., Barnavon, L., Neuhaus, J. M., and Mauch-Mani, B. (2005). Beta-aminobutyric acid-induced resistance against downy mildew in grapevine acts through the potentiation of callose formation and jasmonic acid signaling. *Mol. Plant–Microbe Interact*. 18:819-829.

Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol. Evol.* 33, 1870-1874.

Marguerit, E., Boury, C., Manicki, A., Donnart, M., Butterlin, G., Némorin, A., et al. (2009). Genetic dissection of sex determinism, inflorescence morphology and downy mildew resistance in grapevine. *Theor. Appl. Genet.* 118(7), 1261–1278. doi: 10.1007/s00122-009-0979-4

Merdinoglu, D., Wiedemann-Merdinoglu, S., Coste, P., Dumas, V., Haetty, S., Butterlin, G., et al. (2003). Genetic analysis of downy mildew resistance derived from *Muscadinia rotundifolia*. *In Acta Hortic.* 603, 451–456. doi: 10.17660/ActaHortic.2003.603.57

Moreira, F. M., Madini, A., Marino, R., Zulini, L., Stefanini, M., Velasco, R., et al. (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(1), 153–167. doi: 10.1007/s11295-010-0322-x

Oberle, G.D. (1938). A genetic study of variations in floral morphology and function in cultivated forms of Vitis. N Y State Agric Exp Stn. 250:1–63.

Ochssner, I., Hausmann, L., and Töpfer, R. (2016). Rpvl4, a new genetic source for *Plasmopara viticola* resistance conferred by *Vitis cinerea*. *Vitis* 55(2), 79–81. doi: 10.5073/vitis.2016.55.79-81

Reisch, B., Robinson, W.B., Kimball, K., Pool, R., and Watson, J. (1983) 'Horizon' Grape. *HortScience* 18:108–109

Riaz, S., Krivanek, A.F., Xu, K., and Walker. M.A. (2006). Refined mapping of the Pierce's disease resistance locus, PdR1, and Sex on an extended genetic map of *Vitis rupestris* x *V. arizonica*. *Theor. Appl. Genet.*113(7):1317-29.

Riaz, S., Pap, D., Uretsky, J., Laucou, V., Boursiquot, J. M., Kocsis, L., and Andrew Walker, M. (2019). Genetic diversity and parentage analysis of grape rootstocks. *Theor. Appl. Genet*. doi: 10.1007/s00122-019-03320-5

Rouxel, M., Mestre, P., Baudoin, A., Carisse, O., Delière, L., Ellis, M. A., Gadoury, D., Lu, J., Nita, M., Richard-Cervera, S., Schilder, A., Wise, A., and Delmotte, F. (2014). Geographic distribution of cryptic species of *Plasmopara viticola* causing downy mildew on wild and cultivated grape in eastern North America. *Phytopathology* 104:692-701

Ruyters, S., Salaets, P., Oorts, K., and Smolders, E. (2013). Copper toxicity in soils under established vineyards in Europe: A survey. *Sci. Total Environ.* 443:470-477.

Saifert, L., Sánchez-Mora, F. D., Assumpção, W. T., Zanghelini, J. A., Giacometti, R., Novak, E. I., et al. (2018). Marker-assisted pyramiding of resistance loci to grape downy mildew. *Pesqui. Agropecu. Bras.* 53(5), 602-610. doi:10.1590/s0100-204x2018000500009

van Heerden, C. J., Burger, P., Vermeulen, A., and Prins, R. (2014). Detection of downy and powdery mildew resistance QTL in a "Regent" × "RedGlobe" population. *Euphytica* 200(2), 281–295. doi: 10.1007/s10681-014-1167-4

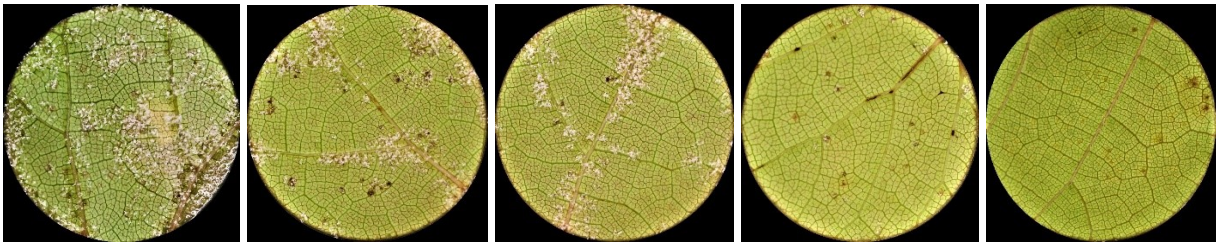
Viala, P., and Ravaz, L. (1903). American vines (resistant stock): their adaptation, culture, grafting and propagation. Press of Freygang-Leary Company, San Francisco

Welter, L. J., Göktürk-Baydar, N., Akkurt, M., Maul, E., Eibach, R., Töpfer, R., and Zyprian, E. M. (2007). Genetic mapping and localization of quantitative trait loci affecting fungal disease resistance and leaf morphology in grapevine (*Vitis vinifera* L). *Mol. Breeding* 20(4), 359–374. doi: 10.1007/s11032-007-9097-7

Yu, Y., Zhang, Y., Yin, L., and Lu, J. (2012). The mode of host resistance to *Plasmopara viticola* infection of grapevines. *Phytopathology* 102:1094- 1101.

Zyprian, E., Ochßner, I., Schwander, F., Šimon, S., Hausmann, L., Bonow-Rex, et al. (2016). Quantitative trait loci affecting pathogen resistance and ripening of grapevines. *Mol. Genet. Genomics* 291(4), 1573–1594. doi: 10.1007/s00438-016-1200-5

A



1 **3** **5** **7** **9**

B

1 **3** **5** **7** **9**

FIGURE 3 | *P viticola*-infected leaf disks. (A) Mildew coverage: 1- Full coverage, 3- At least half of the leaf disk, 5- Multiple sections on less than half of the leaf disk, 7- Few small spots, very little coverage, 9- No sporulation present (B) Mildew intensity: 1- Thick and full colonies, 3- Colonies not at full growth, 5- Thin visible colonies, 7- Very thin, hardly visible colonies, 9- No sporulation

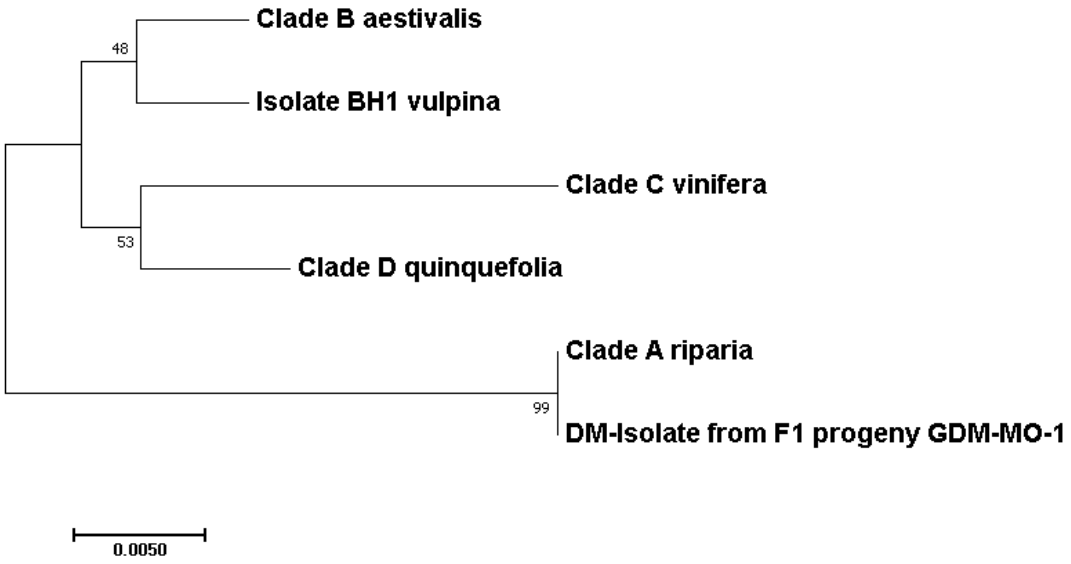


FIGURE 4 | ITS-1 sequence-based phylogeny of *P. viticola* cryptic species. The nucleotide sequence of *P. viticola* strain (GDM-MO-1) identical to that of Clade A (riparia) cryptic species.

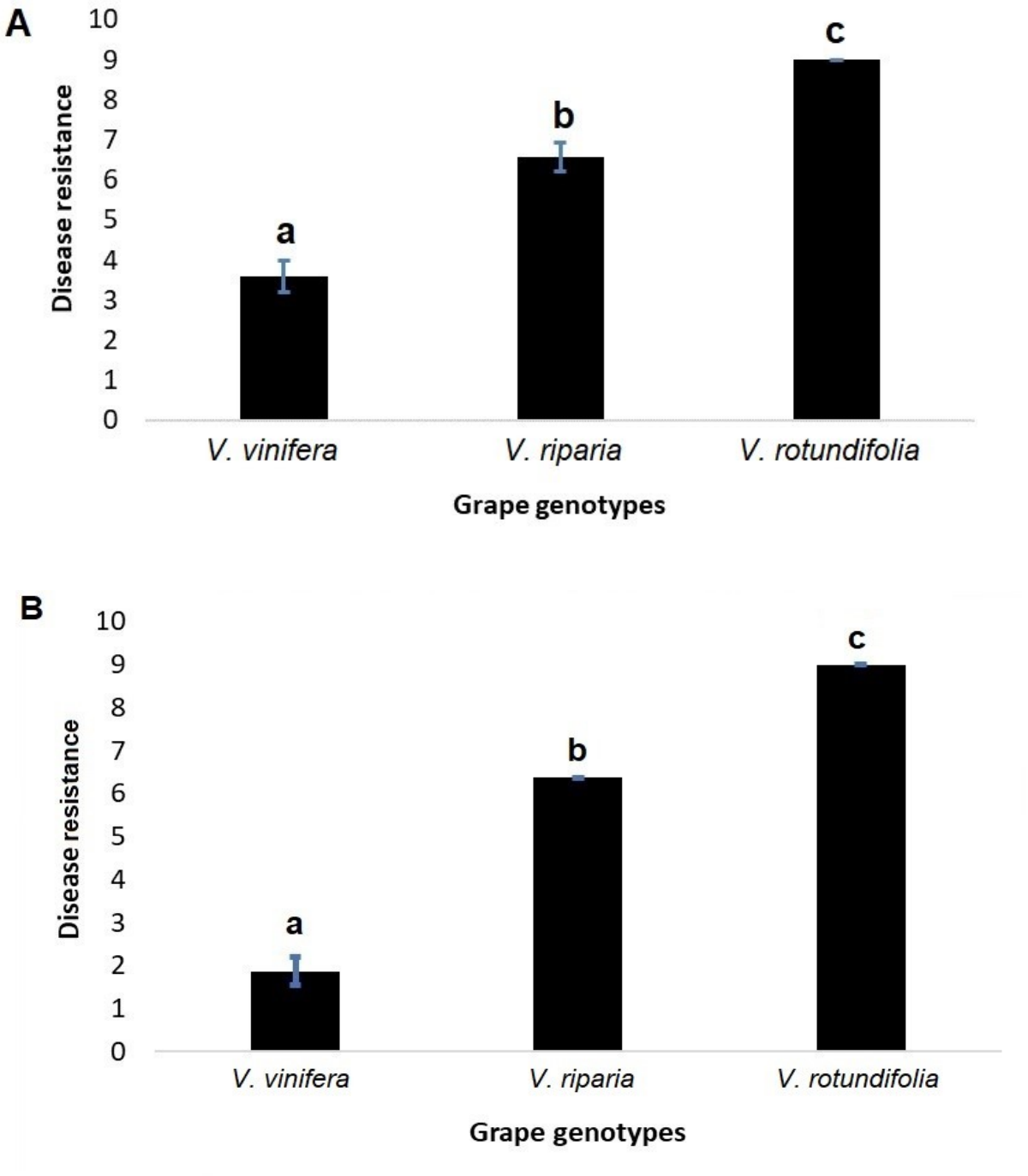


FIGURE 5 | Response of three grapevine species to inoculation with *P. viticola* GDM-MO-1. Disease severity was measured on leaf disks in terms of (A) coverage and (B) sporangial density of growth.

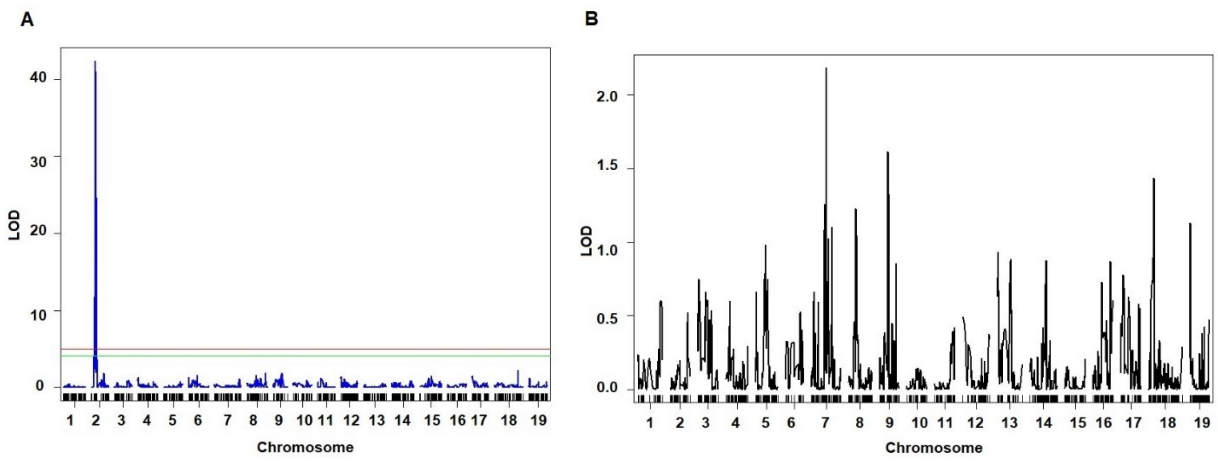


FIGURE 6 | QTL mapping of sex trait in the F_1 progeny. (A) Significant QTL ($p<0.05$) on chr2 of male the parent *V. riparia.* Genome-wide LOD thresholds after 1000 permutation were 4.0 and 4.9 at 5% (green line) and 1% (red line) level of significance respectively. (B) No significant QTL for flower sex was detected in the female parent *V. rupestris*.

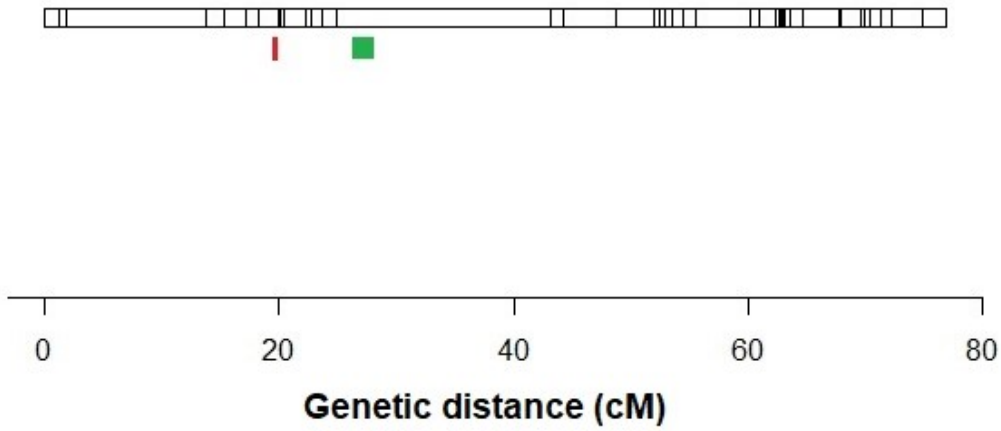


FIGURE 7 | Genetic map of chr2 in *V. riparia*. Vertical black lines represent the markers throughout the linkage group. Red box marks the QTL interval which determines flower sex in *V. riparia* from this experiment. Green box marks the genomic interval which corresponds to the position of the previously reported to determine flower sex in *Vitis vinifera* (Fechter et al., 2012).

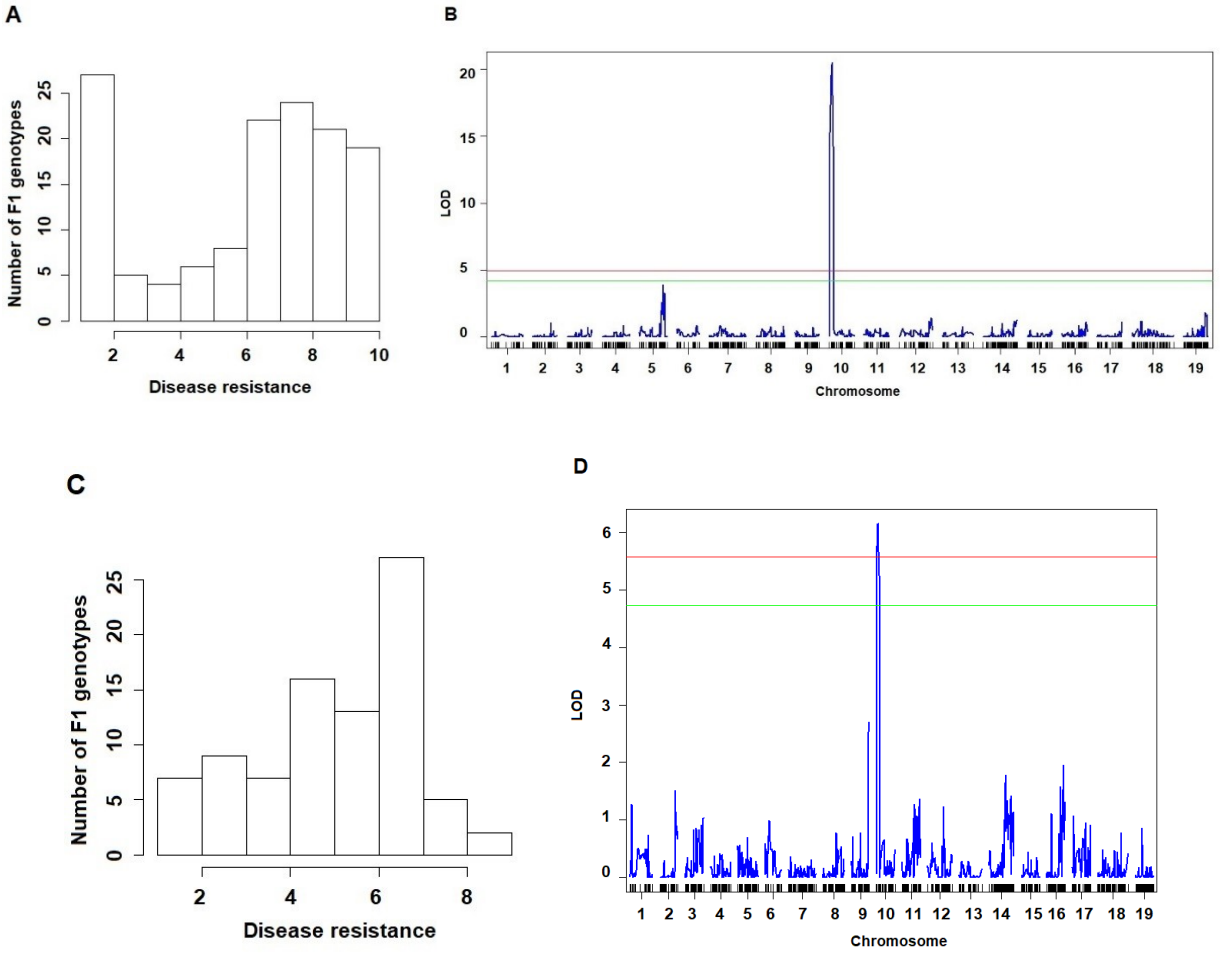


FIGURE 8 | QTL mapping of downy mildew resistance. Frequency distribution of F_1 progeny for the various categories of disease resistance measured (A) as coverage of leaf area by mildew and (C) as sporangiophore density within colonies. Significant QTL at $p \leq 0.05$ for (B) leaf area coverage on chr10 of the *V. rupestris* parent, and (D) sporangiophore density within colonies on chr8 of the *V. riparia* parent. In panels (B) and (D), green and red lines indicate genome-wide LOD thresholds at 5% and 1%, respectively after 1000 permutations. The 5% and 1% LOD thresholds were 4.7 and 5.6 in (B) and 4.2 and 4.9 in (D), respectively.

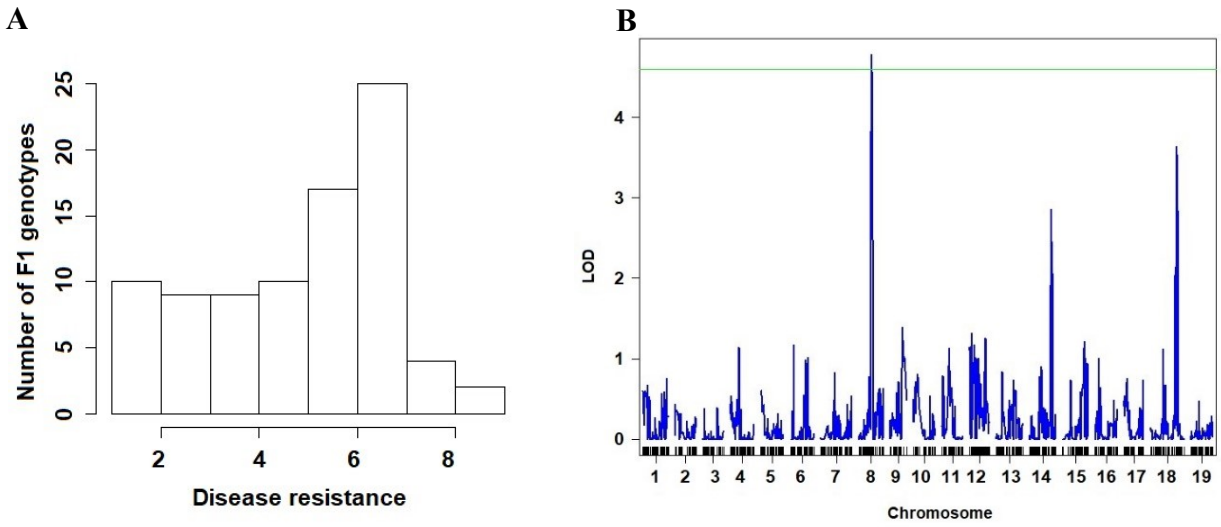


FIGURE 9 | QTL mapping of downy mildew resistance under *in vitro* conditions. (A) Frequency distribution of F_1 progeny for the various categories of disease resistance measured as hyphal density within colonies. (B) Significant QTL ($p<0.05$) on chr8 of *V. riparia.* Genome-wide LOD thresholds after 1000 permutation was 4.77 at 5% (green line) level of significance.

TABLE 2 | Summary of QTL analysis of flower sex and downy mildew resistance traits.

Phenotype	Inoculation method	Mean value/SD	Parent of origin	LOD threshold ($p<0.05$)	LOD $_{maximum}$	LG	Position (cM)	Explained variance (%)	Closest marker	95% BI (cM)
Coverage	Natural	6.183/3.09	*V. rupestris*	4.18	20.561	10	8.6	45.75	S17_17189484	6-9
Coverage	*In-vitro*	5.19/1.83	*V. rupestris*	4.73	6.153	10	6.0	16.01	S10_1285522	2-11.3
Intensity	*In-vitro*	5.0/1.99	*V. riparia*	4.59	4.777	8	47.0	15.42	S8_13784711	46-50.83
Sex	-	-	*V. riparia*	4.03	42.385	2	19.88	56.15	S2_4350083	19.88

SUMMARY

Large number of SNPs have been discovered using the GBS method in the North American grapevines *V. riparia* and *V. rupestris* and their F_1 progeny. The raw SNP data were filtered for various quality parameters and SNPs that qualified as markers were used to construct linkage maps for the parents following a two-way pseudo-testcross strategy. So far, the maps constructed in this study are the first SNP-based linkage maps in these species. Before using linkage maps to identify QTL for economically important traits, their quality was assessed by QTL mapping a known genomic region which determines flower sex in *V. vinifera*. Once the maps were verified by successfully localizing a Mendelian factor for male flower sex to the expected region of chr2 of the male parent, further QTL analysis was performed to test if loci influencing downy mildew disease resistance could be identified. Under two different conditions of disease phenotyping, the F_1 progeny showed a significant variation in response to attack by the grape downy mildew pathogen *P. viticola*. QTL analysis identified a novel locus on chr10 in *V. rupestris* for coverage of leaf area by the mildew. A significant QTL for sporangiophore density in mildew colonies was also detected on chr8 in *V. riparia*. These novel QTLs can be further explored to precisely identify the region conferring resistance against the downy mildew pathogen and used in breeding programs.

The maps constructed for these two wild grapevines will serve as important genetic tools to localize and introgress multiple important horticultural traits into the cultivated grape varieties. The ultimate goal of this study was to explore two wild grape relatives for traits which potentially can help the viticulture industry in facing the challenges of a changing climate.

APPENDICES

Appendix A | Overall summary of the raw SNP data

Number of Taxa	353.00
Number of Sites	348888.00
Sites x Taxa	123157464.00
Number Not Missing	90460909.00
Proportion Not Missing	0.73
Number Missing	32696555.00
Proportion Missing	0.27
Number Gametes	246314928.00
Gametes Not Missing	180921818.00
Proportion Gametes Not Missing	0.73
Gametes Missing	65393110.00
Proportion Gametes Missing	0.27
Number Heterozygous	14383229.00
Proportion Heterozygous	0.12
Average Minor Allele Frequency	0.17

Appendix B | Allele summary of the raw SNP data

Allele	Number	Proportion	Frequency
N	32696555	0.27	0.36
A	20449485	0.17	0.23
T	20403948	0.17	0.23
G	15960968	0.13	0.18
C	15888695	0.13	0.18
-	3374584	0.03	0.04
Y	3246414	0.03	0.04

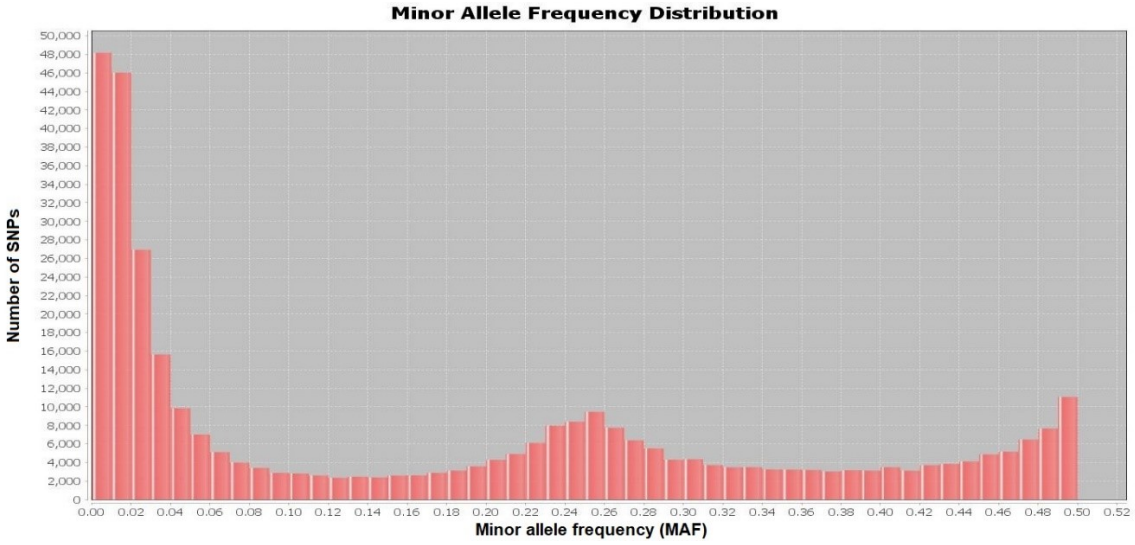
Appendix B | Continued. Allele summary of the raw SNP data

Allele	Number	Proportion	Frequency
R	3242613	0.03	0.04
0	3000073	0.02	0.03
W	1606419	0.01	0.02
M	1283701	0.01	0.01
K	1255057	0.01	0.01
S	748952	0.01	0.01
G:A	44727	0.13	NaN
C:T	44712	0.13	NaN
A:G	32527	0.09	NaN
T:C	31849	0.09	NaN
A:T	25239	0.07	NaN
T:A	25133	0.07	NaN
A:C	22830	0.07	NaN
T:G	22614	0.06	NaN
C:A	16950	0.05	NaN
G:T	16950	0.05	NaN
A:-	8466	0.02	NaN
T:-	8245	0.02	NaN
-:A	7343	0.02	NaN
-:T	7008	0.02	NaN
G:C	6993	0.02	NaN
C:G	6971	0.02	NaN
C:-	5346	0.02	NaN
G:-	5270	0.02	NaN
-:C	4422	0.01	NaN
-:G	4262	0.01	NaN
A:A	399	0.00	NaN
T:T	367	0.00	NaN

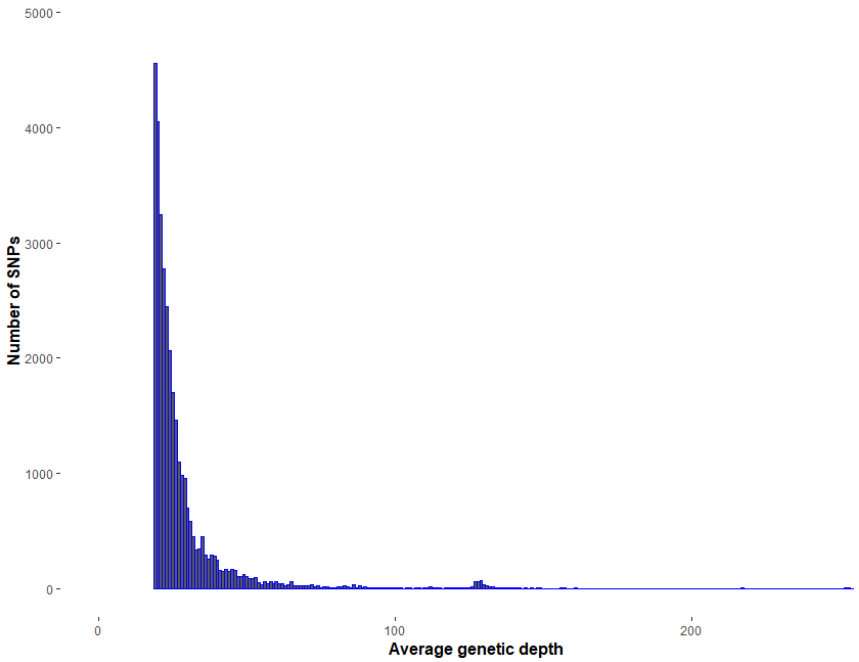
Appendix B | Continued. Allele summary of the raw SNP data

Allele	Number	Proportion	Frequency
C:C	131	0.00	NaN
G:G	130	0.00	NaN
-:-	4	0.00	NaN

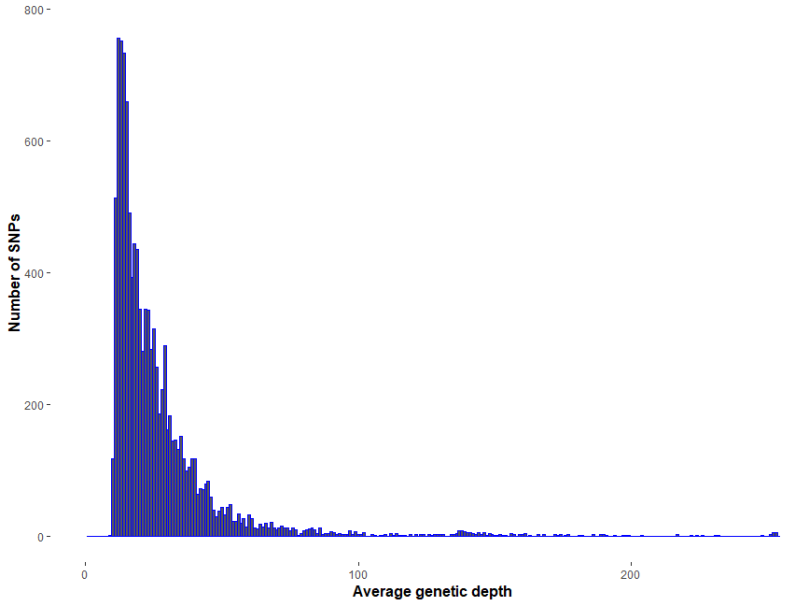
Appendix C | Minor allele frequency (MAF) distribution in raw SNP data



Appendix D | Distribution of average sequencing depth for SNP sites throughout 353 F_1 progeny before filtering for minimum genetic depth, missing percentage and MAF



Appendix E | Distribution of average sequencing depth for SNP sites throughout 353 F_1 progeny after filtering for minimum depth, missing percentage and MAF



Appendix F │ Overall summary of the filtered SNP data

Number of Taxa	353.00
Number of Sites	11063.00
Sites x Taxa	3905239.00
Number Not Missing	3629650.00
Proportion Not Missing	0.93
Number Missing	275589.00
Proportion Missing	0.07
Number Gametes	7810478.00
Gametes Not Missing	7259300.00
Proportion Gametes Not Missing	0.93
Gametes Missing	551178.00
Proportion Gametes Missing	0.07
Number Heterozygous	2460401.00
Proportion Heterozygous	0.63
Average Minor Allele Frequency	0.35

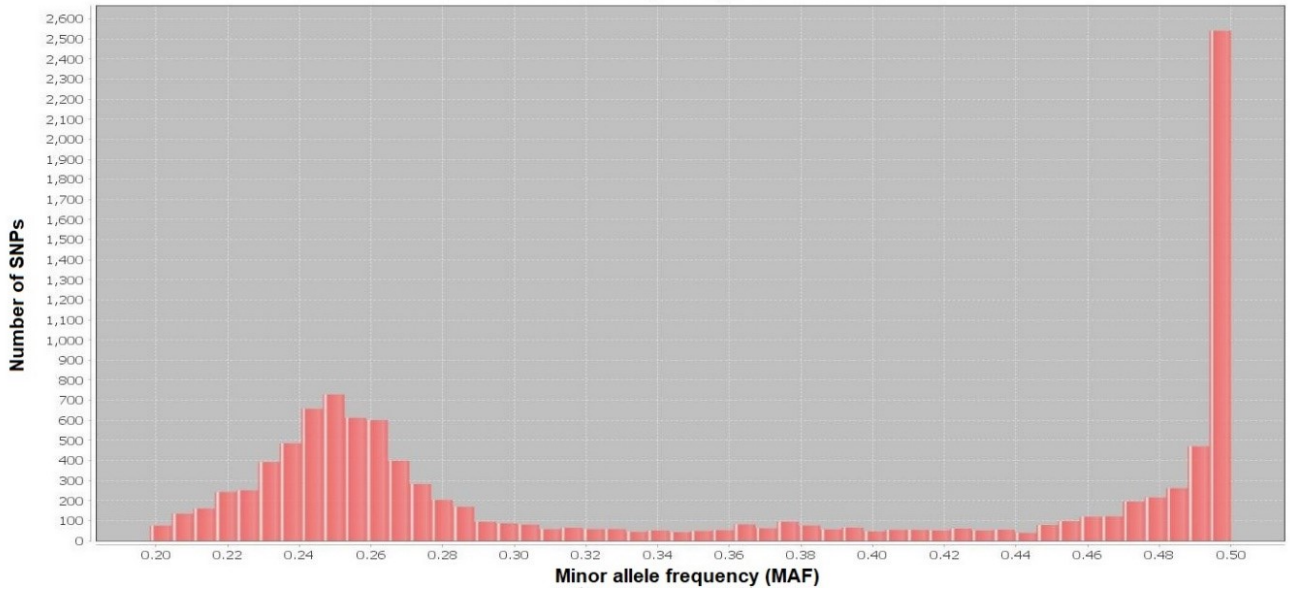
Appendix G │ Allele summary of the filtered SNP data

Allele	Number	Proportion	Frequency
Y	626507	0.16	0.17
R	620157	0.16	0.17
0	440919	0.11	0.12
C	304952	0.08	0.08
G	295753	0.08	0.08
N	275589	0.07	0.08
T	249490	0.06	0.07
A	243690	0.06	0.07
W	221851	0.06	0.06
K	192558	0.05	0.05
M	189336	0.05	0.05

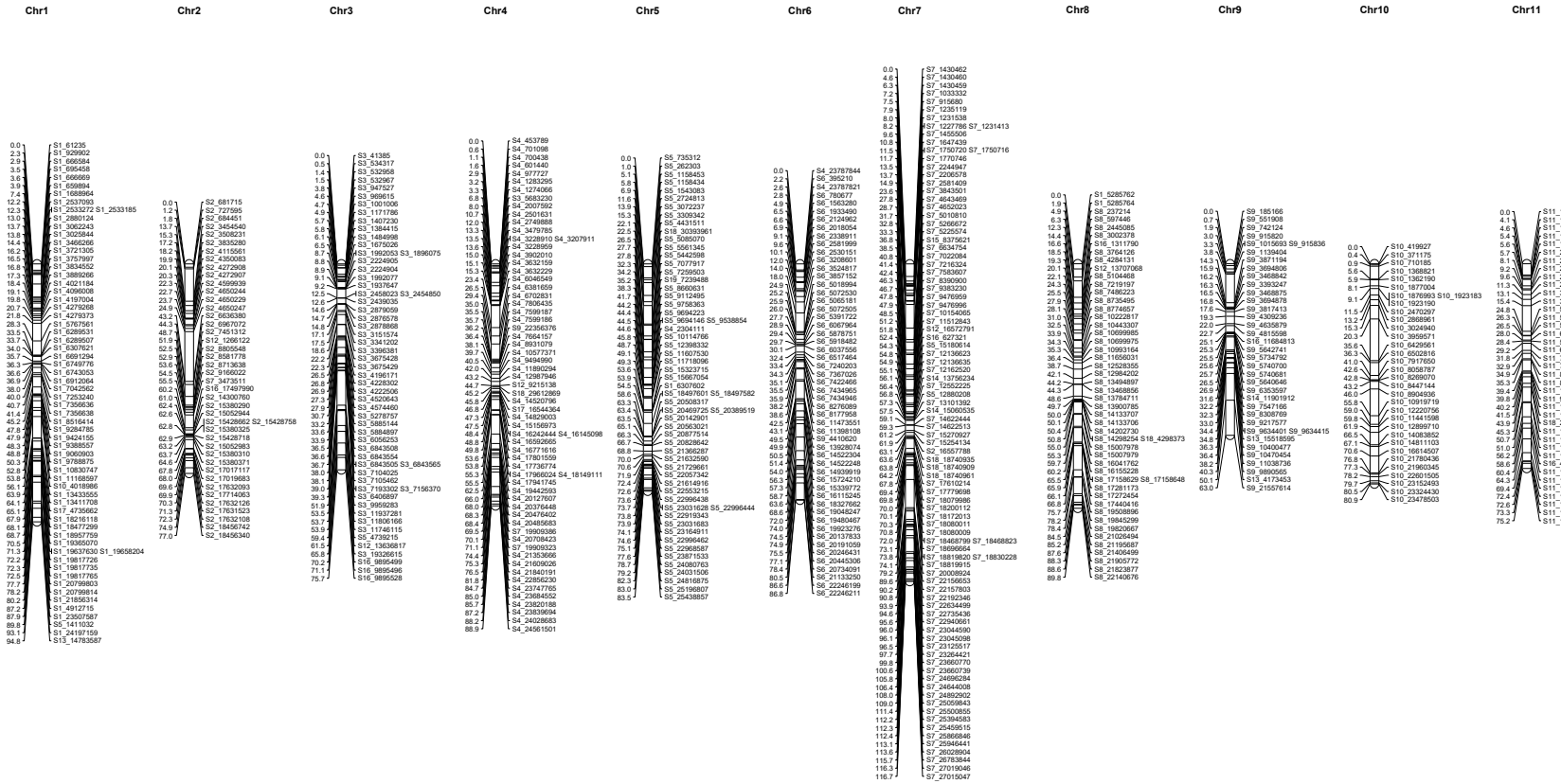
Appendix G | Continued. Allele summary of the filtered SNP data

Allele	Number	Proportion	Frequency
S	169073	0.04	0.05
-	75364	0.02	0.02
C:T	1757	0.16	NaN
G:A	1520	0.14	NaN
A:G	1292	0.12	NaN
T:C	1119	0.10	NaN
A:T	550	0.05	NaN
G:T	495	0.04	NaN
C:A	445	0.04	NaN
T:A	438	0.04	NaN
C:G	403	0.04	NaN
A:C	402	0.04	NaN
T:G	377	0.03	NaN
G:C	374	0.03	NaN
A:-	304	0.03	NaN
T:-	291	0.03	NaN
C:-	287	0.03	NaN
G:-	266	0.02	NaN
-:A	216	0.02	NaN
-:T	194	0.02	NaN
-:C	167	0.02	NaN
-:G	166	0.02	NaN

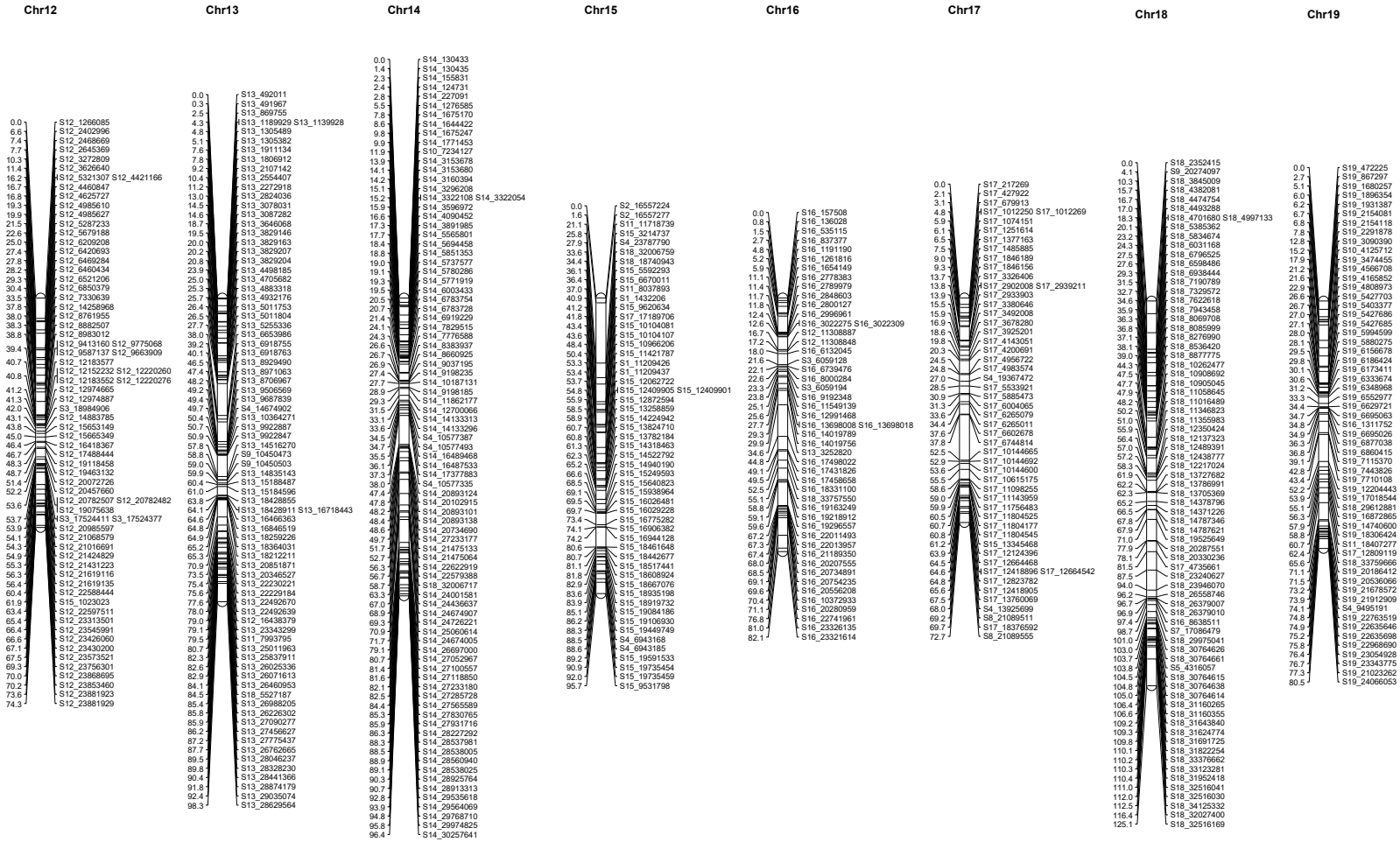
Appendix H │ Minor allele frequency (MAF) distribution in filtered SNP data



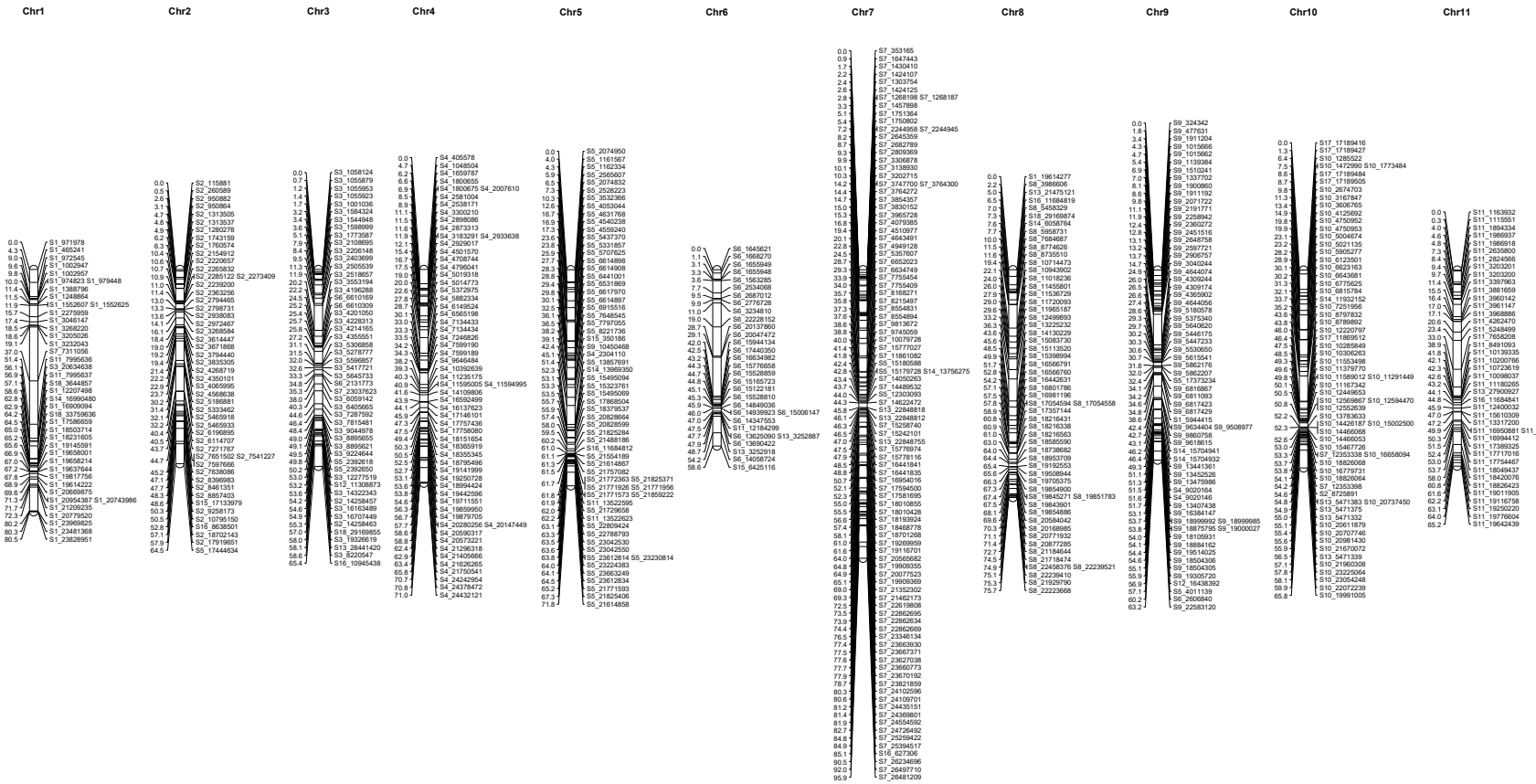
Appendix I | Map chart for 19 linkage groups for *Vitis riparia*. Chromosomes 1-11.



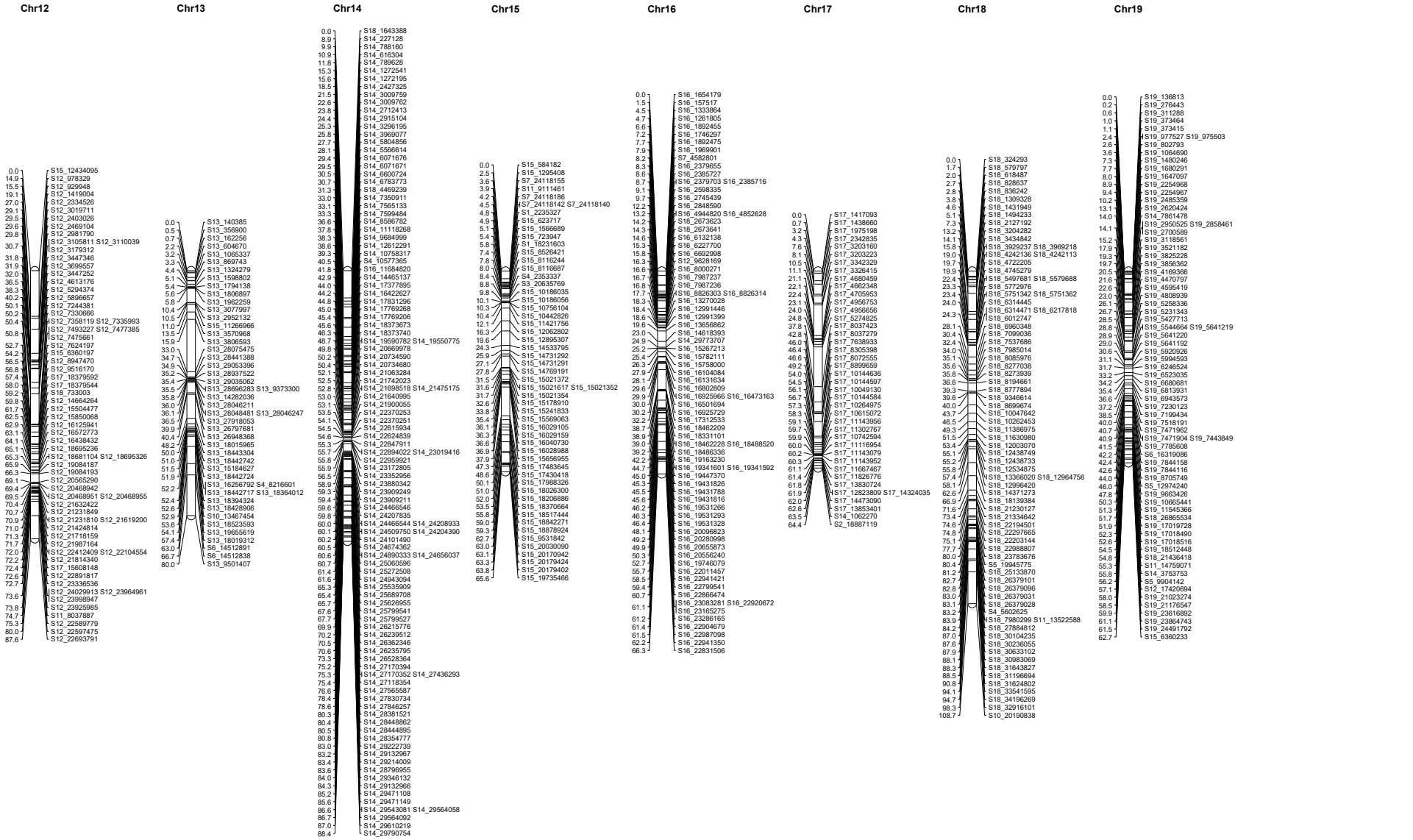
Appendix I | Continued. Chromosomes 12-19.



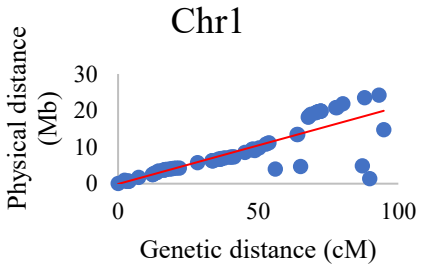
Appendix J | Map chart for 19 linkage groups for *Vitis rupestris*. Chromosomes 1-11.



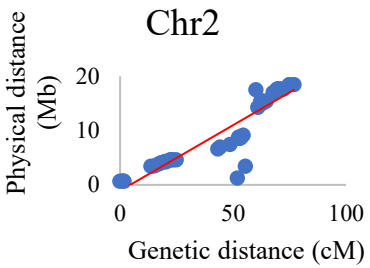
Appendix J | Continued. Chromosomes 12-19.



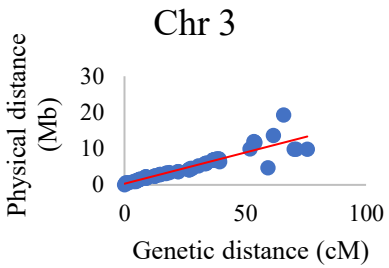
Appendix K | Correlation between genetic distance in *V. riparia* and physical distance in the *V. vinifera* reference genome sequence.



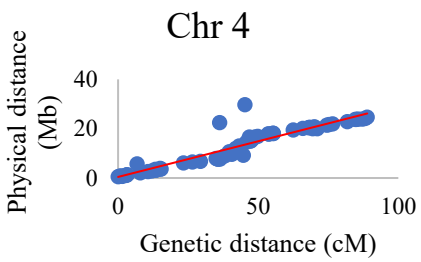
r= 0.828925



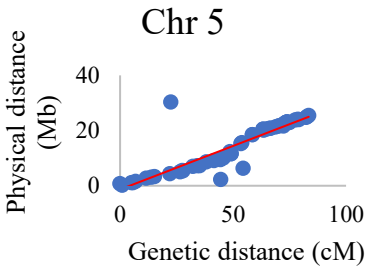
r= 0.901722



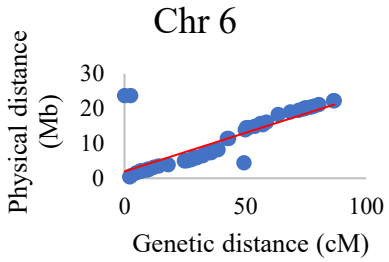
r= 0.910372



r= 0.934749

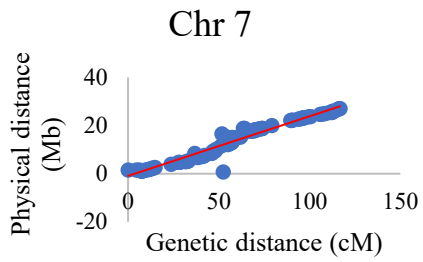


r= 0.882738

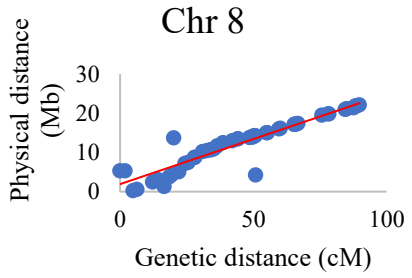


r= 766742

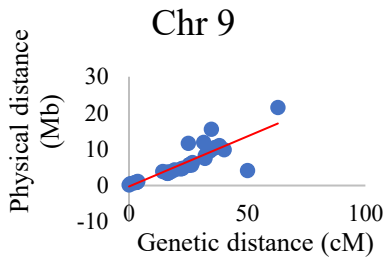
Appendix K | Continued**.** Correlation between physical and genetic distances.



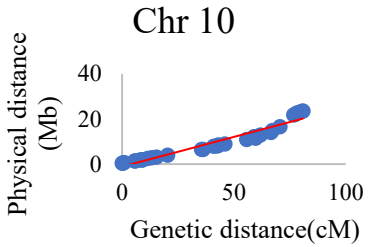
r= 0.976116



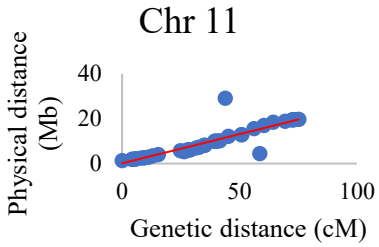
r= 0.935306



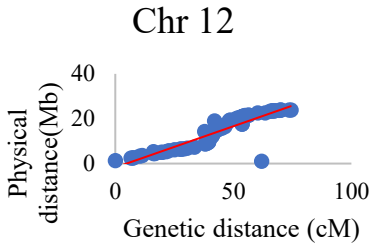
r= 0.857028



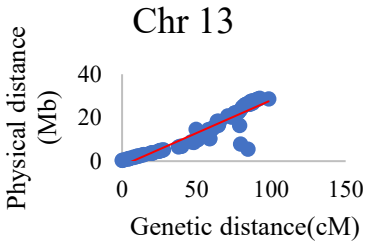
r= 0.966011



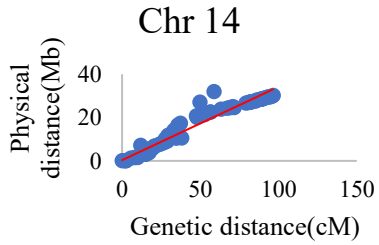
r= 0.863223



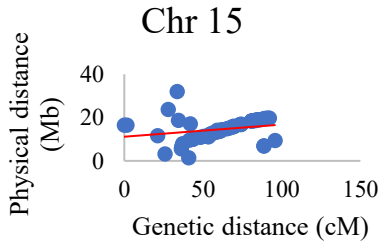
r= 0.9057



r= 0.929271

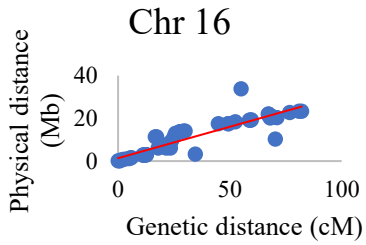


r= 0.968104

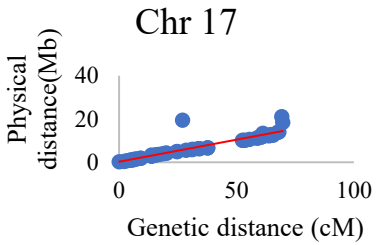


r= 0.244324

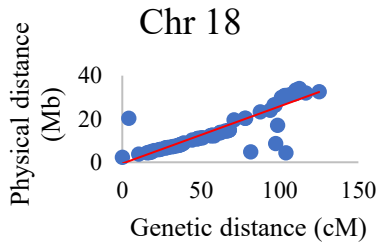
Appendix K | Continued**.** Correlation between physical and genetic distances.



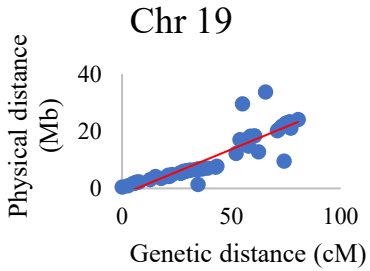
r= 0.894743



r= 0.904886

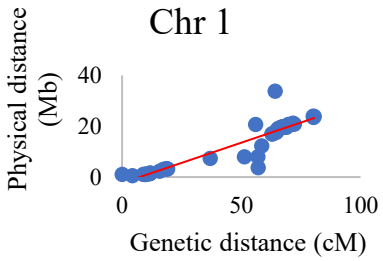


r= 0.877371

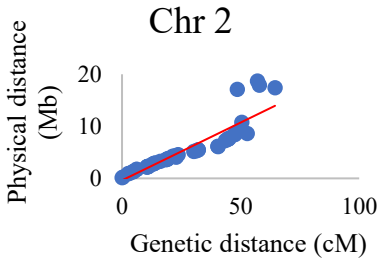


r= 0.896601

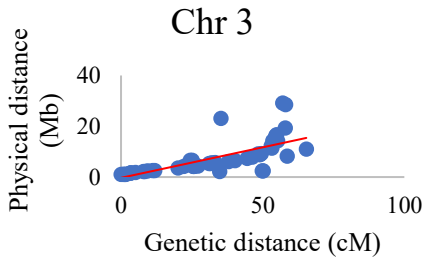
Appendix L | Correlation between genetic distance in *V. rupestris* and physical distance in the *V. vinifera* reference genome sequence



r= 0.921483

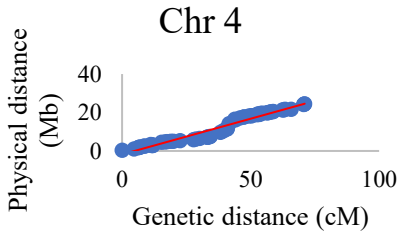


r= 0.901925

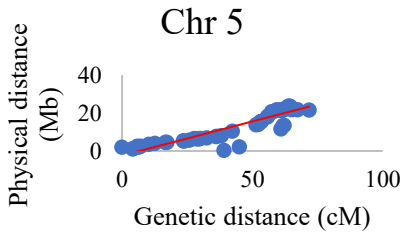


r= 0.716771

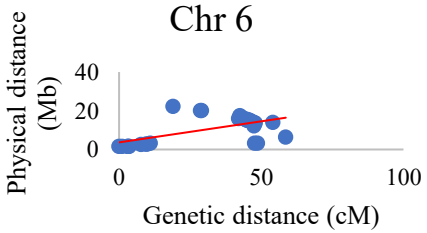
55



r= 0.978176

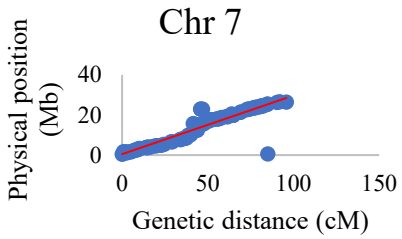


r= 0.917028

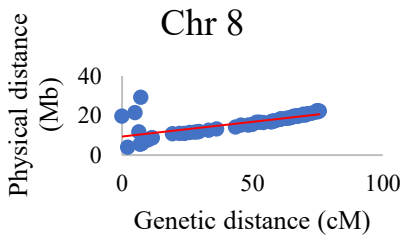


r= 0.608212

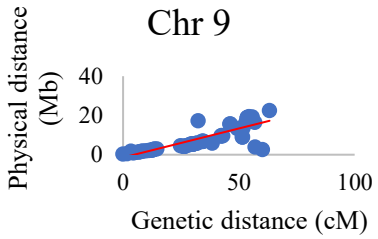
Appendix L | Continued. Correlation between physical and genetic distances.



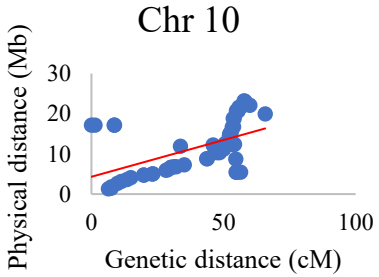
r= 0.928728



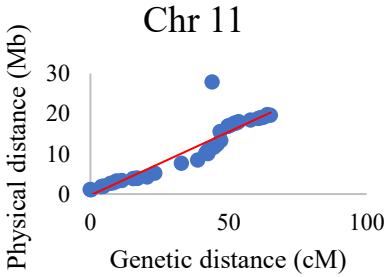
r= 0.705111



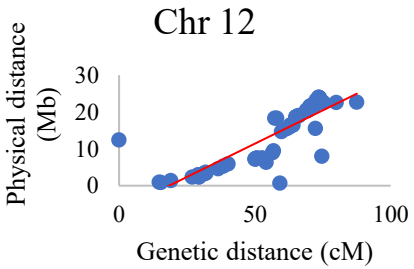
r= 0.842568



r= 0.535341

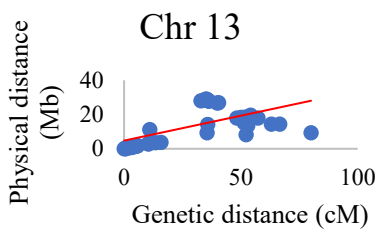


r= 0.926283

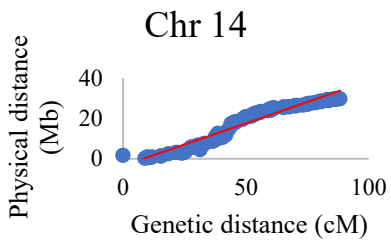


r= 0.849205

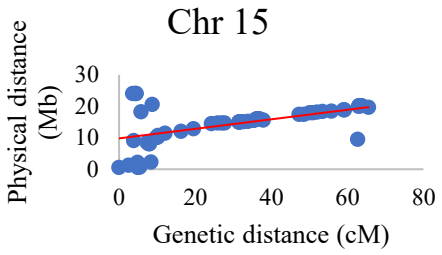
Appendix L | Continued. Correlation between physical and genetic distances.



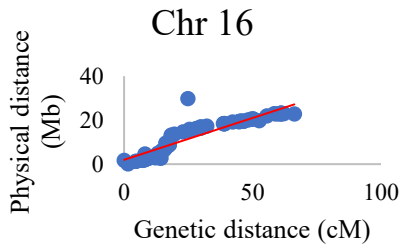
r= 0.60892



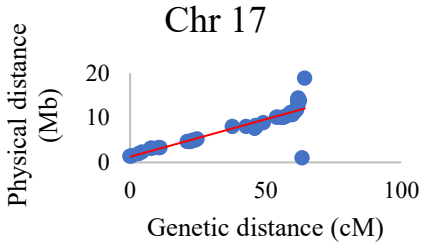
r= 0.960804



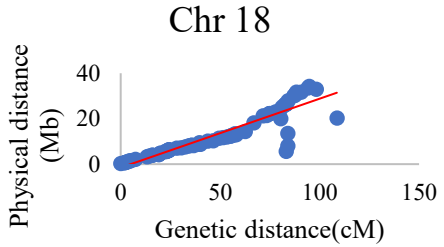
r= 0.508053



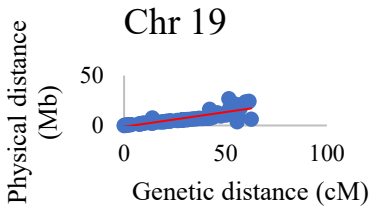
r= 0.912178



r= 0.85979



r= 0.913035



r= 0.836277

Appendix M │ ITS-1 sequence of the downy mildew isolate used in this study

5'TTTCAACCAAATAGTTGGGGATGAAATAGGCAGCGATAACTGACTTTATTGTCGGT

GGTTGCAGCTAATTGATTCCTATCATAGTGAAATAGCTTGGAATTTATTCCGAGCTA

GTAGCTATTTTTAAACCATTACTAAATACTGATTATACTGTGAGGACGAAAGTCTTT

GCTTTTACTAGATACAACTTTCAGCAGTGGATGTCTAGGCTCGCGCATCGATGAAGA

ACGCAGCA**3'**

Appendix N │Phenotype data for flower sex of F_1 mapping progeny. 0, 1, and NA represent male, female, and missing phenotype, respectively.

Genotype ID	Sex	Genotype ID	Sex	Genotype ID	Sex
160_271_001	0	160_271_119	1	160_271_317	NA
160_271_002	1	160_271_120	1	160_271_318	NA
160_271_003	NA	160_271_121	0	160_271_319	NA
160_271_004	NA	160_271_122	0	160_271_320	NA
160_271_005	1	160_271_123	1	160_271_321	NA
160_271_006	NA	160_271_125	0	160_271_322	NA
160_271_007	1	160_271_126	1	160_271_323	NA
160_271_009	0	160_271_127	0	160_271_324	NA
160_271_010	0	160_271_128	1	160_271_325	NA
160_271_011	1	160_271_129	1	160_271_326	NA
160_271_012	1	160_271_130	0	160_271_327	NA
160_271_013	1	160_271_131	1	160_271_328	NA
160_271_014	0	160_271_132	1	160_271_329	NA
160_271_015	1	160_271_134	0	160_271_330	NA
160_271_016	0	160_271_135	0	160_271_331	NA
160_271_017	1	160_271_136	0	160_271_332	NA
160_271_018	0	160_271_137	0	160_271_333	NA
160_271_019	0	160_271_138	0	160_271_334	NA

Appendix N | Continued. Phenotype data for flower sex.

Genotype ID	Sex	Genotype ID	Sex	Genotype ID	Sex
160_271_020	0	160_271_139	1	160_271_335	NA
160_271_021	NA	160_271_140	0	160_271_336	NA
160_271_022	1	160_271_141	0	160_271_337	NA
160_271_023	1	160_271_143	1	160_271_338	NA
160_271_024	1	160_271_144	1	160_271_339	NA
160_271_026	1	160_271_145	0	160_271_340	NA
160_271_027	0	160_271_146	1	160_271_341	NA
160_271_028	0	160_271_147	0	160_271_342	NA
160_271_029	0	160_271_148	0	160_271_343	NA
160_271_030	0	160_271_149	0	160_271_344	NA
160_271_031	0	160_271_150	0	160_271_345	NA
160_271_032	1	160_271_151	0	160_271_346	NA
160_271_033	1	160_271_152	1	160_271_347	NA
160_271_036	0	160_271_153	1	160_271_348	NA
160_271_037	1	160_271_154	1	160_271_349	NA
160_271_039	0	160_271_155	1	160_271_350	NA
160_271_040	NA	160_271_156	0	160_271_351	NA
160_271_041	1	160_271_157	1	160_271_352	NA
160_271_042	0	160_271_158	0	160_271_353	NA
160_271_044	0	160_271_159	0	160_271_355	NA
160_271_047	1	160_271_160	1	160_271_356	NA
160_271_048	0	160_271_161	0	160_271_357	NA
160_271_049	0	160_271_162	0	160_271_358	NA
160_271_050	1	160_271_163	1	160_271_360	NA
160_271_051	1	160_271_164	1	160_271_361	NA
160_271_052	0	160_271_165	1	160_271_362	NA
160_271_055	1	160_271_166	1	160_271_363	NA

Appendix N | Continued. Phenotype data for flower sex.

Genotype ID	Sex	Genotype ID	Sex	Genotype ID	Sex
160_271_056	0	160_271_167	0	160_271_364	NA
160_271_057	1	160_271_169	1	160_271_365	NA
160_271_058	1	160_271_171	NA	160_271_366	NA
160_271_059	1	160_271_172	1	160_271_367	NA
160_271_060	1	160_271_173	0	160_271_368	NA
160_271_061	0	160_271_174	0	160_271_370	NA
160_271_062	1	160_271_175	1	160_271_371	NA
160_271_063	1	160_271_176	1	160_271_372	NA
160_271_064	0	160_271_177	1	160_271_373	NA
160_271_065	0	160_271_178	0	160_271_374	NA
160_271_066	NA	160_271_179	1	160_271_375	NA
160_271_067	0	160_271_180	1	160_271_376	NA
160_271_068	0	160_271_181	0	160_271_377	NA
160_271_069	1	160_271_183	0	160_271_378	NA
160_271_071	1	160_271_184	1	160_271_379	NA
160_271_072	1	160_271_185	1	160_271_380	NA
160_271_073	0	160_271_187	0	160_271_381	NA
160_271_074	0	160_271_189	1	160_271_382	NA
160_271_075	0	160_271_193	1	160_271_383	NA
160_271_076	1	160_271_194	0	160_271_384	NA
160_271_080	0	160_271_196	1	160_271_385	NA
160_271_081	NA	160_271_197	0	160_271_386	NA
160_271_082	0	160_271_198	0	160_271_387	NA
160_271_083	0	160_271_200	0	160_271_388	NA
160_271_084	0	160_271_208	1	160_271_389	NA
160_271_085	1	160_271_209	0	160_271_390	NA
160_271_086	1	160_271_210	0	160_271_391	NA

Appendix N | Continued. Phenotype data for flower sex.

Genotype ID	Sex	Genotype ID	Sex	Genotype ID	Sex
160_271_089	1	160_271_213	0	160_271_392	NA
160_271_092	0	160_271_214	1	160_271_393	NA
160_271_094	0	160_271_215	0	160_271_394	NA
160_271_095	1	160_271_217	1	160_271_395	NA
160_271_096	0	160_271_218	NA	160_271_396	NA
160_271_097	1	160_271_219	0	160_271_397	NA
160_271_098	1	160_271_221	1	160_271_398	NA
160_271_099	0	160_271_222	0	160_271_399	NA
160_271_100	1	160_271_235	NA	160_271_400	NA
160_271_101	0	160_271_300	NA	160_271_401	NA
160_271_102	0	160_271_301	NA	160_271_402	NA
160_271_103	0	160_271_302	NA	160_271_403	NA
160_271_104	1	160_271_303	NA	160_271_404	NA
160_271_105	1	160_271_304	NA	160_271_405	NA
160_271_106	0	160_271_305	NA	160_271_406	NA
160_271_107	1	160_271_306	NA	160_271_407	NA
160_271_108	0	160_271_307	NA	160_271_408	NA
160_271_109	1	160_271_308	NA	160_271_409	NA
160_271_110	0	160_271_309	NA	160_271_410	NA
160_271_111	1	160_271_310	NA	160_271_411	NA
160_271_112	1	160_271_311	NA	160_271_412	NA
160_271_114	1	160_271_312	NA	160_271_413	NA
160_271_115	1	160_271_313	NA	160_271_414	NA
160_271_116	0	160_271_314	NA	160_271_415	NA
160_271_117	1	160_271_315	NA	160_271_416	NA
160_271_118	0	160_271_316	NA	160_271_417	NA

Appendix O | Phenotype data for downy mildew resistance (coverage) under greenhouse condition

Genotype ID	Average disease score	Genotype ID	Average disease score	Genotype ID	Average disease score
160_271_001	5	160_271_119	2.33333	160_271_317	NA
160_271_002	1	160_271_120	9.5	160_271_318	NA
160_271_003	NA	160_271_121	10	160_271_319	NA
160_271_004	9	160_271_122	7	160_271_320	NA
160_271_005	8	160_271_123	NA	160_271_321	NA
160_271_006	NA	160_271_125	1	160_271_322	NA
160_271_007	8.5	160_271_126	3	160_271_323	NA
160_271_009	6.5	160_271_127	1	160_271_324	NA
160_271_010	NA	160_271_128	6	160_271_325	NA
160_271_011	8.5	160_271_129	9	160_271_326	NA
160_271_012	6.66	160_271_130	NA	160_271_327	NA
160_271_013	9.5	160_271_131	1	160_271_328	NA
160_271_014	NA	160_271_132	NA	160_271_329	NA
160_271_015	6.5	160_271_134	NA	160_271_330	NA
160_271_016	7	160_271_135	7.5	160_271_331	NA
160_271_017	4.66	160_271_136	10	160_271_332	NA
160_271_018	NA	160_271_137	3.5	160_271_333	NA
160_271_019	9.33	160_271_138	NA	160_271_334	NA
160_271_020	4	160_271_139	9.5	160_271_335	NA
160_271_021	NA	160_271_140	8	160_271_336	NA
160_271_022	9	160_271_141	7.5	160_271_337	NA
160_271_023	1	160_271_143	NA	160_271_338	NA
160_271_024	NA	160_271_144	9	160_271_339	NA
160_271_026	8	160_271_145	6.5	160_271_340	NA
160_271_027	7.5	160_271_146	8.5	160_271_341	NA
160_271_028	5	160_271_147	6	160_271_342	NA

Appendix O | Continued. Phenotype data for downy mildew resistance (Coverage).

Genotype ID	Average disease score	Genotype ID	Average disease score	Genotype ID	Average disease score
160_271_029	9	160_271_148	7.5	160_271_343	NA
160_271_030	6	160_271_149	9	160_271_344	NA
160_271_031	8	160_271_150	10	160_271_345	NA
160_271_032	3	160_271_151	1	160_271_346	NA
160_271_033	1	160_271_152	10	160_271_347	NA
160_271_036	9	160_271_153	1	160_271_348	NA
160_271_037	7	160_271_154	9.5	160_271_349	NA
160_271_039	9	160_271_155	6	160_271_350	NA
160_271_040	NA	160_271_156	8	160_271_351	NA
160_271_041	6	160_271_157	6.5	160_271_352	NA
160_271_042	NA	160_271_158	NA	160_271_353	NA
160_271_044	6.5	160_271_159	NA	160_271_355	NA
160_271_047	NA	160_271_160	10	160_271_356	NA
160_271_048	6.5	160_271_161	9	160_271_357	NA
160_271_049	1	160_271_162	1	160_271_358	NA
160_271_050	7	160_271_163	7.5	160_271_360	NA
160_271_051	NA	160_271_164	8	160_271_361	NA
160_271_052	7.5	160_271_165	8.5	160_271_362	NA
160_271_055	4	160_271_166	1	160_271_363	NA
160_271_056	8	160_271_167	6.33333	160_271_364	NA
160_271_057	10	160_271_169	10	160_271_365	NA
160_271_058	NA	160_271_171	NA	160_271_366	NA
160_271_059	NA	160_271_172	1	160_271_367	NA
160_271_060	NA	160_271_173	7	160_271_368	NA
160_271_061	5	160_271_174	1	160_271_370	NA
160_271_062	7.5	160_271_175	8	160_271_371	NA
160_271_063	NA	160_271_176	1	160_271_372	NA

Appendix O | Continued. Phenotype data for downy mildew resistance (Coverage).

Genotype ID	Average disease score	Genotype ID	Average disease score	Genotype ID	Average disease score
160_271_064	7	160_271_177	6.5	160_271_373	NA
160_271_065	NA	160_271_178	NA	160_271_374	NA
160_271_066	NA	160_271_179	9	160_271_375	NA
160_271_067	1	160_271_180	4.5	160_271_376	NA
160_271_068	4	160_271_181	6.5	160_271_377	NA
160_271_069	1	160_271_183	1	160_271_378	NA
160_271_071	7	160_271_184	9	160_271_379	NA
160_271_072	10	160_271_185	6.5	160_271_380	NA
160_271_073	10	160_271_187	7.5	160_271_381	NA
160_271_074	1	160_271_189	NA	160_271_382	NA
160_271_075	1	160_271_193	7	160_271_383	NA
160_271_076	NA	160_271_194	8.5	160_271_384	NA
160_271_080	NA	160_271_196	10	160_271_385	NA
160_271_081	NA	160_271_197	10	160_271_386	NA
160_271_082	1	160_271_198	1	160_271_387	NA
160_271_083	6	160_271_200	NA	160_271_388	NA
160_271_084	NA	160_271_208	NA	160_271_389	NA
160_271_085	6.5	160_271_209	1	160_271_390	NA
160_271_086	9	160_271_210	8	160_271_391	NA
160_271_089	NA	160_271_213	9	160_271_392	NA
160_271_092	10	160_271_214	6.25	160_271_393	NA
160_271_094	NA	160_271_215	8	160_271_394	NA
160_271_095	1	160_271_217	7.5	160_271_395	NA
160_271_096	5	160_271_218	NA	160_271_396	NA
160_271_097	3	160_271_219	1	160_271_397	NA
160_271_098	1	160_271_221	9	160_271_398	NA
160_271_099	10	160_271_222	5.5	160_271_399	NA

Appendix O | Continued. Phenotype data for downy mildew resistance (Coverage).

Genotype ID	Average disease score	Genotype ID	Average disease score	Genotype ID	Average disease score
160_271_100	3	160_271_235	NA	160_271_400	NA
160_271_101	8	160_271_300	NA	160_271_401	NA
160_271_102	1	160_271_301	NA	160_271_402	NA
160_271_103	9	160_271_302	NA	160_271_403	NA
160_271_104	NA	160_271_303	NA	160_271_404	NA
160_271_105	8	160_271_304	NA	160_271_405	NA
160_271_106	NA	160_271_305	NA	160_271_406	NA
160_271_107	1	160_271_306	NA	160_271_407	NA
160_271_108	8	160_271_307	NA	160_271_408	NA
160_271_109	9.33	160_271_308	NA	160_271_409	NA
160_271_110	8	160_271_309	NA	160_271_410	NA
160_271_111	NA	160_271_310	NA	160_271_411	NA
160_271_112	6	160_271_311	NA	160_271_412	NA
160_271_114	NA	160_271_312	NA	160_271_413	NA
160_271_115	6.5	160_271_313	NA	160_271_414	NA
160_271_116	9	160_271_314	NA	160_271_415	NA
160_271_117	8	160_271_315	NA	160_271_416	NA
160_271_118	NA	160_271_316	NA	160_271_417	NA

Appendix P | Phenotype data for downy mildew resistance (coverage) under *in vitro* conditions

Genotype ID	Average disease score	Genotype ID	Average disease score	Genotype ID	Average disease score
160_271_001	NA	160_271_119	NA	160_271_317	NA
160_271_002	NA	160_271_120	NA	160_271_318	NA
160_271_003	NA	160_271_121	NA	160_271_319	NA
160_271_004	NA	160_271_122	NA	160_271_320	NA
160_271_005	NA	160_271_123	NA	160_271_321	NA
160_271_006	NA	160_271_125	NA	160_271_322	6.625
160_271_007	NA	160_271_126	NA	160_271_323	3.75
160_271_009	NA	160_271_127	NA	160_271_324	NA
160_271_010	NA	160_271_128	NA	160_271_325	NA
160_271_011	NA	160_271_129	NA	160_271_326	6.5
160_271_012	NA	160_271_130	NA	160_271_327	7
160_271_013	6.33	160_271_131	NA	160_271_328	NA
160_271_014	NA	160_271_132	NA	160_271_329	3
160_271_015	5	160_271_134	NA	160_271_330	7
160_271_016	NA	160_271_135	6.75	160_271_331	4.25
160_271_017	NA	160_271_136	NA	160_271_332	1
160_271_018	NA	160_271_137	5	160_271_333	NA
160_271_019	NA	160_271_138	NA	160_271_334	NA
160_271_020	NA	160_271_139	NA	160_271_335	5.75
160_271_021	NA	160_271_140	NA	160_271_336	6.75
160_271_022	NA	160_271_141	NA	160_271_337	NA
160_271_023	5.75	160_271_143	NA	160_271_338	NA
160_271_024	NA	160_271_144	NA	160_271_339	4
160_271_026	NA	160_271_145	NA	160_271_340	2.75
160_271_027	NA	160_271_146	NA	160_271_341	NA
160_271_028	NA	160_271_147	NA	160_271_342	NA
160_271_029	5.75	160_271_148	NA	160_271_343	6.5

Appendix P | Continued. Phenotype data for downy mildew under *in vitro* (Coverage).

Genotype ID	Average disease score	Genotype ID	Average disease score	Genotype ID	Average disease score
160_271_030	NA	160_271_149	NA	160_271_344	3.5
160_271_031	NA	160_271_150	NA	160_271_345	6.25
160_271_032	NA	160_271_151	NA	160_271_346	3.25
160_271_033	7.25	160_271_152	NA	160_271_347	9
160_271_036	6.75	160_271_153	NA	160_271_348	1.75
160_271_037	NA	160_271_154	7.25	160_271_349	NA
160_271_039	NA	160_271_155	NA	160_271_350	2.25
160_271_040	NA	160_271_156	NA	160_271_351	NA
160_271_041	6.25	160_271_157	NA	160_271_352	2
160_271_042	NA	160_271_158	NA	160_271_353	6
160_271_044	NA	160_271_159	NA	160_271_355	1.75
160_271_047	NA	160_271_160	NA	160_271_356	NA
160_271_048	5.75	160_271_161	NA	160_271_357	2.875
160_271_049	NA	160_271_162	NA	160_271_358	7
160_271_050	NA	160_271_163	NA	160_271_360	NA
160_271_051	NA	160_271_164	NA	160_271_361	1.5
160_271_052	NA	160_271_165	NA	160_271_362	6.75
160_271_055	NA	160_271_166	NA	160_271_363	NA
160_271_056	NA	160_271_167	7	160_271_364	NA
160_271_057	NA	160_271_169	NA	160_271_365	NA
160_271_058	NA	160_271_171	NA	160_271_366	3.75
160_271_059	NA	160_271_172	NA	160_271_367	NA
160_271_060	NA	160_271_173	7	160_271_368	2.75
160_271_061	NA	160_271_174	NA	160_271_370	NA
160_271_062	NA	160_271_175	NA	160_271_371	NA
160_271_063	NA	160_271_176	NA	160_271_372	6
160_271_064	NA	160_271_177	NA	160_271_373	4.25

Appendix P | Continued. Phenotype data for downy mildew under *in vitro* (Coverage).

Genotype ID	Average disease score	Genotype ID	Average disease score	Genotype ID	Average disease score
160_271_065	NA	160_271_178	NA	160_271_374	NA
160_271_066	NA	160_271_179	NA	160_271_375	NA
160_271_067	NA	160_271_180	NA	160_271_376	8.75
160_271_068	NA	160_271_181	NA	160_271_377	6.75
160_271_069	NA	160_271_183	NA	160_271_378	NA
160_271_071	NA	160_271_184	NA	160_271_379	7
160_271_072	NA	160_271_185	NA	160_271_380	4.25
160_271_073	NA	160_271_187	NA	160_271_381	NA
160_271_074	NA	160_271_189	NA	160_271_382	3
160_271_075	NA	160_271_193	NA	160_271_383	NA
160_271_076	NA	160_271_194	NA	160_271_384	4.5
160_271_080	NA	160_271_196	NA	160_271_385	5.375
160_271_081	NA	160_271_197	6.75	160_271_386	4
160_271_082	NA	160_271_198	NA	160_271_387	NA
160_271_083	NA	160_271_200	NA	160_271_388	4.5
160_271_084	NA	160_271_208	NA	160_271_389	NA
160_271_085	7.5	160_271_209	5.75	160_271_390	7
160_271_086	7.75	160_271_210	NA	160_271_391	5.75
160_271_089	NA	160_271_213	NA	160_271_392	2.5
160_271_092	NA	160_271_214	NA	160_271_393	NA
160_271_094	NA	160_271_215	NA	160_271_394	3
160_271_095	NA	160_271_217	5	160_271_395	NA
160_271_096	NA	160_271_218	NA	160_271_396	6.75
160_271_097	NA	160_271_219	NA	160_271_397	5.5
160_271_098	5.75	160_271_221	NA	160_271_398	NA
160_271_099	NA	160_271_222	NA	160_271_399	6.5
160_271_100	NA	160_271_235	NA	160_271_400	7.25

Appendix P | Continued. Phenotype data for downy mildew under *in vitro* (Coverage).

Genotype ID	Average disease score	Genotype ID	Average disease score	Genotype ID	Average disease score
160_271_101	NA	160_271_300	4.5	160_271_401	6
160_271_102	NA	160_271_301	5	160_271_402	NA
160_271_103	NA	160_271_302	4.75	160_271_403	NA
160_271_104	NA	160_271_303	5	160_271_404	5
160_271_105	NA	160_271_304	NA	160_271_405	4.25
160_271_106	NA	160_271_305	NA	160_271_406	NA
160_271_107	NA	160_271_306	5	160_271_407	NA
160_271_108	NA	160_271_307	6.75	160_271_408	6.25
160_271_109	NA	160_271_308	5	160_271_409	3.25
160_271_110	6.33	160_271_309	NA	160_271_410	1.25
160_271_111	NA	160_271_310	7	160_271_411	6.25
160_271_112	NA	160_271_311	NA	160_271_412	NA
160_271_114	NA	160_271_312	NA	160_271_413	6.5
160_271_115	NA	160_271_313	5.5	160_271_414	NA
160_271_116	NA	160_271_314	2.75	160_271_415	2
160_271_117	NA	160_271_315	NA	160_271_416	NA
160_271_118	NA	160_271_316	NA	160_271_417	NA

Appendix Q | Phenotype data for downy mildew resistance (sporangiophore density) under *in vitro* conditions

Genotype ID	Average disease score	Genotype ID	Average disease score	Genotype ID	Average disease score
160_271_001	NA	160_271_119	NA	160_271_317	NA
160_271_002	NA	160_271_120	NA	160_271_318	NA
160_271_003	NA	160_271_121	NA	160_271_319	NA
160_271_004	NA	160_271_122	NA	160_271_320	NA
160_271_005	NA	160_271_123	NA	160_271_321	NA

Appendix Q | Continued. Phenotype data for downy mildew resistance (sporangiophore density)

Genotype ID	Average disease score	Genotype ID	Average disease score	Genotype ID	Average disease score
160_271_006	NA	160_271_125	NA	160_271_322	6.75
160_271_007	NA	160_271_126	NA	160_271_323	3.75
160_271_009	NA	160_271_127	NA	160_271_324	NA
160_271_010	NA	160_271_128	NA	160_271_325	NA
160_271_011	NA	160_271_129	NA	160_271_326	5
160_271_012	NA	160_271_130	NA	160_271_327	7
160_271_013	6.75	160_271_131	NA	160_271_328	NA
160_271_014	NA	160_271_132	NA	160_271_329	2.25
160_271_015	5.75	160_271_134	NA	160_271_330	6.5
160_271_016	NA	160_271_135	5.875	160_271_331	3.75
160_271_017	NA	160_271_136	NA	160_271_332	1
160_271_018	NA	160_271_137	5.25	160_271_333	NA
160_271_019	NA	160_271_138	NA	160_271_334	NA
160_271_020	NA	160_271_139	NA	160_271_335	5.5
160_271_021	NA	160_271_140	NA	160_271_336	6.75
160_271_022	NA	160_271_141	NA	160_271_337	NA
160_271_023	7	160_271_143	NA	160_271_338	NA
160_271_024	NA	160_271_144	NA	160_271_339	3.25
160_271_026	NA	160_271_145	NA	160_271_340	2.25
160_271_027	NA	160_271_146	NA	160_271_341	NA
160_271_028	NA	160_271_147	NA	160_271_342	NA
160_271_029	6.25	160_271_148	NA	160_271_343	5.75
160_271_030	NA	160_271_149	NA	160_271_344	1.75
160_271_031	NA	160_271_150	NA	160_271_345	6.25
160_271_032	NA	160_271_151	NA	160_271_346	3.5
160_271_033	7.25	160_271_152	NA	160_271_347	9
160_271_036	6.75	160_271_153	NA	160_271_348	3.25
160_271_037	NA	160_271_154	7	160_271_349	NA

Appendix Q | Continued. Phenotype data for downy mildew resistance (sporangiophore density)

Genotype ID	Average disease score	Genotype ID	Average disease score	Genotype ID	Average disease score
160_271_039	NA	160_271_155	NA	160_271_350	2.25
160_271_040	NA	160_271_156	NA	160_271_351	NA
160_271_041	6.5	160_271_157	NA	160_271_352	1.25
160_271_042	NA	160_271_158	NA	160_271_353	5.75
160_271_044	NA	160_271_159	NA	160_271_355	1
160_271_047	NA	160_271_160	NA	160_271_356	NA
160_271_048	5.75	160_271_161	NA	160_271_357	3.375
160_271_049	NA	160_271_162	NA	160_271_358	7
160_271_050	NA	160_271_163	NA	160_271_360	NA
160_271_051	NA	160_271_164	NA	160_271_361	1.5
160_271_052	NA	160_271_165	NA	160_271_362	6.5
160_271_055	NA	160_271_166	NA	160_271_363	NA
160_271_056	NA	160_271_167	6.75	160_271_364	NA
160_271_057	NA	160_271_169	NA	160_271_365	NA
160_271_058	NA	160_271_171	NA	160_271_366	3.5
160_271_059	NA	160_271_172	NA	160_271_367	NA
160_271_060	NA	160_271_173	7	160_271_368	1.25
160_271_061	NA	160_271_174	NA	160_271_370	NA
160_271_062	NA	160_271_175	NA	160_271_371	NA
160_271_063	NA	160_271_176	NA	160_271_372	4.75
160_271_064	NA	160_271_177	NA	160_271_373	4.25
160_271_065	NA	160_271_178	NA	160_271_374	NA
160_271_066	NA	160_271_179	NA	160_271_375	NA
160_271_067	NA	160_271_180	NA	160_271_376	8.75
160_271_068	NA	160_271_181	NA	160_271_377	7
160_271_069	NA	160_271_183	NA	160_271_378	NA
160_271_071	NA	160_271_184	NA	160_271_379	7
160_271_072	NA	160_271_185	NA	160_271_380	2.25

Appendix Q | Continued. Phenotype data for downy mildew resistance (sporangiophore density)

Genotype ID	Average disease score	Genotype ID	Average disease score	Genotype ID	Average disease score
160_271_073	NA	160_271_187	NA	160_271_381	NA
160_271_074	NA	160_271_189	NA	160_271_382	2.75
160_271_075	NA	160_271_193	NA	160_271_383	NA
160_271_076	NA	160_271_194	NA	160_271_384	4.5
160_271_080	NA	160_271_196	NA	160_271_385	5.375
160_271_081	NA	160_271_197	7	160_271_386	3.25
160_271_082	NA	160_271_198	NA	160_271_387	NA
160_271_083	NA	160_271_200	NA	160_271_388	3
160_271_084	NA	160_271_208	NA	160_271_389	NA
160_271_085	7.25	160_271_209	4.5	160_271_390	7
160_271_086	7.75	160_271_210	NA	160_271_391	5.25
160_271_089	NA	160_271_213	NA	160_271_392	2
160_271_092	NA	160_271_214	NA	160_271_393	NA
160_271_094	NA	160_271_215	NA	160_271_394	2
160_271_095	NA	160_271_217	4.25	160_271_395	NA
160_271_096	NA	160_271_218	NA	160_271_396	6.25
160_271_097	NA	160_271_219	NA	160_271_397	5.75
160_271_098	4.5	160_271_221	NA	160_271_398	NA
160_271_099	NA	160_271_222	NA	160_271_399	6
160_271_100	NA	160_271_235	NA	160_271_400	7.25
160_271_101	NA	160_271_300	5.5	160_271_401	6.5
160_271_102	NA	160_271_301	5.75	160_271_402	NA
160_271_103	NA	160_271_302	5.25	160_271_403	NA
160_271_104	NA	160_271_303	5.25	160_271_404	4.75
160_271_105	NA	160_271_304	NA	160_271_405	3.25
160_271_106	NA	160_271_305	NA	160_271_406	NA
160_271_107	NA	160_271_306	3	160_271_407	NA

Appendix Q | Continued. Phenotype data for downy mildew resistance (sporangiophore density)

Genotype ID	Average disease score	Genotype ID	Average disease score	Genotype ID	Average disease score
160_271_108	NA	160_271_307	7	160_271_408	6.25
160_271_109	NA	160_271_308	5	160_271_409	2
160_271_110	6.66	160_271_309	NA	160_271_410	3
160_271_111	NA	160_271_310	6.75	160_271_411	5.5
160_271_112	NA	160_271_311	NA	160_271_412	NA
160_271_114	NA	160_271_312	NA	160_271_413	6
160_271_115	NA	160_271_313	5	160_271_414	NA
160_271_116	NA	160_271_314	2.5	160_271_415	1.5
160_271_117	NA	160_271_315	NA	160_271_416	NA
160_271_118	NA	160_271_316	NA	160_271_417	NA