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Research Article

A Novel Grape Downy Mildew Resistance Locus from *Vitis rupestris*

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Abstract: The viticulture industry needs advanced grape cultivars encoding genetic information that enhances disease resistance and environmental stress tolerance to meet the challenges of a changing climate. To discover beneficial allelic variants of grape genes, we established an F_1 mapping population from a cross between two North American grapevines, *Vitis rupestris* Scheele and *Vitis riparia* Michx. We generated genotyping-by-sequencing (GBS) markers and constructed parental linkage maps consisting of 1,177 and 1,115 GBS markers, respectively (LOD threshold ≥ 14), which were validated by mapping the sex-

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determining locus to chromosome 2. Taking advantage of loci heterozygous in both parents, we also constructed an integrated map containing 2,583 markers. We mapped a major QTL for downy mildew (*Plasmopara viticola*) resistance to chromosome 10 of *V. rupestris* using both greenhouse- and *in vitro*-generated leaf resistance data. This QTL explains 66.5% of the phenotypic variance under greenhouse conditions, and its 2-LOD confidence interval corresponds to region 2,470,297 to 3,024,940 bp on chromosome 10 in the *Vitis vinifera* L. PN40024 reference genome sequence (assembly 12X.v2). We provide PN40024-projected positions of the GBS markers, which can be used as anchors to develop additional markers for the introgression of this *V. rupestris* haplotype into cultivated grape varieties.

Key words: disease resistance, linkage map, QTL, *Plasmopara viticola,* QTL, *Vitis riparia, Vitis rupestris*

Introduction

Grape (*Vitis vinifera* L.) cultivation relies heavily on the recurrent application of fungicides, a disease control method that is costly, and potentially harmful to the environment and human health. Cultivation of disease-resistant grape varieties carrying genes that enhance defense against pathogens is an approach that helps reduce the amount of fungicides applied in viticulture. Traditionally, the source of such defense-related genes has been the North American wild relatives of *V. vinifera*, as they have coevolved with now pandemic pathogens and acquired allelic diversity that strengthens resistance against fungal and oomycete diseases (Alleweldt and Possingham 1988). Grapevine breeding has a long history of introgressing genetic information from wild grapevines (*Vitis* species) into *V. vinifera*. The first interspecific grapevine crosses were made in the United States during the

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54 early and mid-19th century, followed by a surge of breeding activity in Europe in the wake of
55 the phylloxera epidemic in the late 19th and early 20th centuries (Reisch et al. 2012). This
56 pioneering work focused on developing disease-resistant fruit-producing varieties through
57 crosses between *V. vinifera* and North American grape species, and breeding phylloxera- and
58 lime-tolerant rootstocks through interspecific crosses among various American grapevines.
59 These efforts met with great success, to the extent that hybrids from the late 19th century are
60 still popular fruit-producing varieties in the eastern and mid-western US, and that several of
61 the interspecific American hybrids are among the most widely used rootstocks worldwide
62 even today (Di Gaspero et al. 2012, Migicovsky et al. 2016, Riaz et al. 2019).

63 While the introgression of disease resistance trait s continued through the 20th
64 century, the exploration of wild grape relatives for new resistance sources has lagged. Most
65 of the breeding work during the past century focused on germplasm that had been introduced
66 to Europe from North America during the 1800s. This resulted in a narrow genetic base for
67 both fruit-producing and rootstock hybrids as documented by Di Gaspero et al. (2012) and
68 Riaz et al. (2019), respectively. The high degree of polymorphism reported in North
69 American wild grapevines (Liang et al. 2019) suggests that the genetic diversity of this
70 germplasm is vastly more extensive than what is represented in hybrid cultivars today.
71 Recent examples in which a broader exploration of this germplasm led to the discovery and
72 deployment of valuable haplotypes include the introgression of Pierce's disease resistance
73 from *Vitis arizonica* (Riaz et al. 2009) and powdery and downy mildew resistance from
74 *Muscadinia rotundifolia* (Feechan et al. 2013, Agurto et al. 2017).
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In this paper, we describe linkage map construction and QTL analysis in an F_1 family from a cross between *V. rupestris* and *V. riparia*, two species that vary in habitat and in adaptation to different environmental conditions. The seed parent used in this cross is *V. rupestris* B38, which was collected by Herbert C. Barrett in Texas in 1951 and donated to the National Germplasm Repository in Geneva, NY (PI588160) by Bruce Reisch in 1985. The pollen parent is *V. riparia* HP-1, which was collected by Neils E. Hansen in Bismarck, ND and donated to the National Germplasm Repository in Geneva, NY (PI588271) by Ronald Peterson in 1987. *V. rupestris* forms shrubs that sprawl along the surface of nutrient-poor sand or gravel bars in intermittent streams or stony outcroppings, and grows in small populations within a limited geographic area. *V. riparia*, a sister taxon of *V. rupestris*, forms high-climbing lianas in moist but well-drained alluvial soils along rivers, and thrives in large populations across great expenses of the continent. As these evolutionary closely related grapevine species (Klein et al. 2018), have adapted to contrasting environmental conditions in their native habitats, their alleles will likely influence horticultural traits in different ways. The parent plants were selected because they have large differences in fall photoperiod response, which is likely tied to cold tolerance, and because we wanted to pseudo-replicate the specific cross that had produced the commercial rootstock 3309C. We expected to find in the F_1 hybrid progeny of this cross abundant potential in segregation for other important viticultural traits, such as branching, angle of growth, leaf shape, root growth, periderm formation, and disease resistance. We hypothesized, therefore, that their F_1 hybrid progeny would allow for the mapping of economically relevant genomic loci.

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Materials and Methods

Mapping population.

An F_1 mapping population was developed by crossing *V. rupestris* accession PI588160 (female parent) with *V. riparia* accession PI588271 (male parent) (Germplasm Resources Information Network 2019) in 2014. Crosses were made in the field by manually removing floral caps on the *V. rupestris* parent and applying dried collected pollen from the *V. riparia* parent. Inflorescences were covered with paper bags for 3 weeks to prevent unintended pollination. Seeds were collected from berries (fully colored and soft), vernalized for 4 weeks at 4°C, and germinated under greenhouse conditions in a 1:1 mix of PRO-MIX and perlite. In the spring of 2015, seedlings were planted in the vineyard nursery and grown without irrigation or fertilizer, using pest management treatments as needed for powdery mildew, downy mildew, black rot, phomopsis, and anthracnose. In 2018, the surviving seedlings (n = 257) were transplanted into a permanent research vineyard at the USDA clonal germplasm repository in Geneva, NY (42.895208N, -77.009853W). An additional 100 seedlings were germinated and maintained as potted plants at South Dakota State University as part of a phenotyping project. The combined 357 vines were genotyped for the development of the genetic maps.

Genotyping.

A young leaf was collected from each vine into a well of a 96-well plate and promptly frozen and stored in a -80˚C freezer until processing. Two grinder beads were then placed into each tube and leaf tissue was ground using a Geno/Grinder 2000 (OPS Diagnostics LLC, Lebanon

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NJ, USA). Genomic DNA was extracted from each F_1 progeny plant and parents with DNeasy 96-well DNA extraction kits (Qiagen, Valencia CA, USA). Genotyping-by-sequencing (GBS) was performed following the protocol design described by Elshire et al. (2011) and modified by Hyma et al. (2015). Barcoded adapters were ligated for each individual sample and single-end sequencing of 100 bp was performed using HiSeq 2000 (Illumina Inc., San Diego, CA, USA) at the Institute of Biotechnology, Genomics Facility, Cornell University, Ithaca, NY. Illumina reads were submitted to the NCBI BioSample Database (SAMN13512746 through SAMN13513110). The raw reads were demultiplexed, parsed, trimmed for quality. Processed reads were aligned using BWA version 0.6.2-r126 (Li and Durbin 2009) against the 12X.v2 *V. vinifera* PN40024 reference genome sequence (RefSeq) (Jaillon et al. 2007, Canaguier et al. 2017). SNP genotypes were called using TASSEL-GBS pipeline version 3.0.139 (Glaubitz et al. 2014).

Marker generation and map construction.

GBS reads that aligned to the *V. vinifera* RefSeq were screened for single nucleotide polymorphisms (SNPs). The identified SNPs were filtered using VCFtools v0.1.13 (Danecek et al. 2011) to retain only biallelic SNPs at a sequencing depth of $\geq$ 6. Further, only SNPs with missing genotypes of $\leq$ 20% and with minor allele frequency of $\geq$ 0.2 were retained. The resulting SNP data in the VCF file were then converted to JOINMAP®5.0 (Van Ooijen 2006) format using NGSEP (Duitama et al. 2014). F_1 genotypes with more than 10% missing SNP markers were discarded, and a goodness-of-fit (χ^2) test was performed to filter out test-cross and intercross markers deviating from the 1:1 and 1:2:1 segregation ratio in the

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progeny, respectively. Because segregation distortion is a natural phenomenon in outcrossing species such as grape, markers showing a moderate degree of segregation distortion were retained for the map construction and only significantly distorted markers ($p < 0.0005$) were discarded. Identical markers were identified and removed from the analysis. Maternal and paternal population nodes were created in JOINMAP 5.0 with marker types "lm × ll" and "nn × np", respectively, and parental maps were constructed following the two-way pseudo-test cross approach (Grattapaglia and Sederoff 1994). Only 2nd round maps were accepted for each parent, using the jump threshold of 5 (default), to maximize the number of markers included in the maps while limiting the inclusion of markers with weak linkage. Markers of "hk × hk" type were then used to integrate parental linkage maps into a consensus map. Each linkage group was constructed with a threshold logarithm of odds (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 map units with parameters at default settings. Linkage maps were visualized using the software LinkageMap View (Ouellette et al. 2017).

Phenotyping the F_1 progeny for downy mildew resistance.

In the greenhouse, phenotyping was carried out by quantifying downy mildew (DM) resistance on naturally infected leaves of two replicate plants for each of 136 F_1 genotypes 5 days after the symptoms first appeared. Disease developed on naturally infected plants in the greenhouse, was monitored during development and evaluated at a single time point when shoots were at the 8-10 node stage. Scoring was performed using a disease resistance scale of

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1 to 10, where 1 represented the highest susceptibility (100% of the leaves had more than 50% of the leaf area on the abaxial side covered with sporangiophores) and 10 the highest resistance (all of the leaves had minimal or no sporangial growth). All leaves on a shoot were used to provide the score (coverage over the entire shoot). Plants were then stripped and pruned to 2 buds and sprayed with Dithane.

An *in vitro* disease assay was then performed to determine if the symptoms seen in the greenhouse were reproduceable under more tightly controlled conditions. The 86 individuals phenotyped *in vitro* were part of the same F_1 population as those phenotyped following natural infection, but only 20 individuals were shared between the two cohorts. Healthy leaves from the third and fourth nodes from the apical meristem were surface sterilized in 1% NaOCl solution for two minutes and then rinsed four times in sterile deionized water (dH_2O) for five minutes per rinse. Four circular leaf disks, 2 cm in diameter, were excised from each leaf and placed abaxial-side up on 0.8% water-agar plates in Petri dishes. Downy mildew was collected from infected leaves in the greenhouse and propagated on susceptible leaves to amplify the inoculum. A sporangial suspension of *P. viticola* was prepared by suspending sporangia in dH_2O at a density of 70,000 sporangia/ml, which was then sprayed over the leaf disks uniformly. Inoculated leaf disks were incubated overnight in darkness under axenic conditions and transferred to a growth chamber with a temperature of 21℃ and a 5-hour/19-hour dark/light diurnal cycle. The leaf disks were visually scored for disease resistance seven days after inoculation. Leaf surface area coverage was estimated using OIV standard disease resistance chart 452 (International Organization of Vine and Wine 2009), which uses a scale

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of 1 to 9, where 1 and 9 represent the highest susceptibility and the highest resistance, respectively (Supplemental Figure 1). Each leaf disk was evaluated by two observers independently and their scores were averaged. Though the of scales of the greenhouse and *in vitro* phenotyping had slightly different grading, they both had the same direction.

Characterization of the downy mildew strain.

To characterize the *P. viticola* strain responsible for this infection (named MO-1), the genomic DNA of the pathogen was extracted by boiling sporangiophores in the presence of 5% Chelex, and a 235 bp-long internal transcribed spacer-1 (ITS-1) sequence of the 5.8S ribosomal RNA gene was PCR-amplified using the ITS-1 primer pair specified by Rouxel et al. (2014). The PCR product was then sequenced and aligned to the corresponding ITS nucleotide sequence of other *P. viticola* cryptic species. To assess the virulence of MO-1 on different grapevine species, the *in vitro* disease assay was performed using leaf disks of three different grapevines, *V. riparia* Gloire de Montepellier, *M. rotundifolia* Thomas, and *V. vinifera* F2-35, plus the parents of the F1 population. Leaf surface area coverage was estimated using the 1-to-9 scale of the OIV standard disease resistance chart 452 (International Organization of Vine and Wine 2009),

QTL analysis.

QTL analysis was performed in MapQTL 6.0 (Van Ooijen 2009) using the integrated map. The interval mapping (IM) method was applied to detect significant associations between phenotypic traits and markers using a regression approach. Genome-wide LOD thresholds ($p < 0.05$) were determined for each phenotype by performing 1000 permutations. The genetic

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regions for significant LOD peaks were identified with corresponding 2-LOD intervals, the predicted gene content in this region was identified using the most recent annotation of the RefSeq (Grimplet et al. 2012, Canaguier et al. 2017), and the percentage of phenotypic variance explained by each QTL was calculated. QTL graphs were generated in MapChart version 2.32 (Voorrips 2002).

Results

Linkage map construction.

The removal of F_1 individuals with more than 10% missing data reduced the number of individuals in the mapping population to 294. Filtering 348,888 SNPs across this population for various quality parameters yielded 11,063 SNPs. 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 were excluded from downstream analysis. First, "lm × ll"- and "nn × np"-type SNP markers were used to construct parental maps. Population nodes were created in JOINMAP®5.0 for each parent separately. An additional 331 and 360 markers were removed from the maternal and paternal nodes, respectively, because their segregation was distorted from the expected 1:1 ratio as determined by χ^2 test ($p < 0.0005$). Upon the removal of identical markers from each parental node, 1,462 and 1,351 female and male parent-informative markers following "nn × np" and "ll × lm" segregation types were used for linkage map construction. For the female and male parents, 1,177 and 1,115 significant markers (LOD threshold ≥ 14) were grouped into 19 different linkage groups covering 1,401.3 cM and 1,657.4 cM of genetic distance (Table 1), respectively. Linkage groups were numbered according to the assignment of *V. vinifera*

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RefSeq chromosome map-anchored SNP markers. For *V. rupestris,* the number of SNP markers on each linkage group varied with a maximum of 114 on LG14 to a minimum of 31 on LG6. The longest and shortest linkage groups for *V. rupestris* were LG18 (108.7 cM) and LG6 (58.6 cM), respectively. In *V. riparia*, LG7 and LG10 included maximum (92) and minimum (33) number of SNP markers, respectively, and LG18 (125.1 cM) and LG9 (63 cM) were the longest and shortest linkage groups, respectively. Although the female map contains a greater number of markers than the male map, it spans a shorter genetic length. Furthermore, 291 "hk × hk"- type markers were combined with the male and female maps to construct an integrated map. The integrated linkage map consists of 2,583 markers distributed on 19 linkage groups and spans a genetic distance of 1,634.1 cM with an average marker interval of 0.63 cM (Supplemental Figure 2). Synteny between marker genetic positions on the linkage maps and their corresponding physical coordinates in the RefSeq are shown in Supplemental Figure 3. The detailed genotype information for each marker across 294 F_1 progeny are shown in Supplemental Tables 1, 2, and 3 for the *V. rupestris,* the *V. riparia*, and the integrated map, respectively. Parental map quality was then further tested using R/qtl (Broman et al. 2003, script provided in Supplemental File 1). Pairwise recombination fractions demonstrated tight linkage within, but not across, different linkage groups (Supplemental Figure 4).

QTL mapping of the sex-determining locus.

To verify the correctness of the linkage maps, pistillate/staminate flower data were used to perform interval mapping to map the sex-determining locus. Of 203 flower-bearing F_1

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252 individuals, 101 had pistillate, 102 had staminate, and none had hermaphroditic flowers,
253 indicating that the female parent was homozygous for the recessive female allele and the
254 male parent was heterozygous for the dominant male allele. A single major QTL was
255 detected at a genetic position of 21.99 cM on chromosome 2 (chr2) in the integrated map
256 with a peak LOD score of 60.32 (Supplemental Figure 5). This QTL (QTL.Sex) explained
257 80.7% of the phenotypic variance, and its localization to chr2 is in agreement with earlier
258 reports (Dalbó et al. 2000, Riaz et al. 2006, Marguerit et al. 2009).

259
260 *Characterization of the DM pathogen.*

261 The nucleotide sequence of the ITS-1 fragment amplified from the DM strain was identical to
262 the corresponding fragment of Clade-A of the *P. viticola* species complex (Supplemental
263 Figure 6), which established it as a member of the riparia cryptic species of *P. viticola*.
264 (Rouxel et al. 2014). To characterize the virulence of the MO-1 strain, it was used to
265 inoculate three different grapevines, *V. riparia* Gloire de Montepellier, *M. rotundifolia*
266 Thomas, and *V. vinifera* F2-35. *M. rotundifolia* Thomas appeared immune to the strain, *V.*
267 *riparia* Gloire de Montpellier proved partially resistant, while *V. vinifera* F2-35 was highly
268 susceptible (Fig. 1), indicating that the strain represents an aggressive pathogen of cultivated
269 grapes. Both parents of the F_1 progeny had higher levels of resistance to MO-1 than *V.*
270 *vinifera* (Fig. 1). While, based on its phenotypic appearance, we consider the downy mildew
271 population used in this study to be a single strain, we have not propagated it from a single
272 sporangium to ensure that inoculations were done with a pure culture.

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QTL mapping of DM resistance.

Segregation of the DM resistance phenotype in the F_1 progeny suggested that this trait was quantitative and determined by multiple loci (Supplemental Figure 7). Of the 20 individuals that were present in both the naturally and *in vitro*-infected cohorts, 16 had similarly moderate resistance ratings under both conditions. The four that substantially differed in DM coverage were all rated as highly susceptible (1 on a 1-to-10 point scale) in response to natural infection, but moderately resistant under *in vitro* conditions. Analysis of resistance levels in naturally infected vines led to the detection of a major QTL at a genetic position of 12.46 cM on chr10 in the integrated map (Rpv28.1, Fig. 2). This QTL, which independently mapped to the female parent but not to the male parent, had an LOD value of 32.32, and explained 66.5% of the phenotypic variance for disease resistance. QTL analysis of resistance levels in *in vitro*-inoculated leaf disks led to similar results: a significant QTL for resistance was detected at a genetic position of 15.09 cM on chr10 on the integrated map explaining 24.3% of the phenotypic variance (Rpv28.2, Fig. 2; mean and standard deviation scores for each genotype are reported in Supplemental Table 4). The *in vitro*-mapped QTL encompassed the entire Rpv28.1 interval. GBS markers that fall within the 2-LOD interval of Rpv28.1 and Rpv28.2, their LOD scores and their projected position in the 12X.v3 assembly of *V. vinifera* (Canaguier et al. 2017) are listed in in Supplemental Table 5. The 2-LOD interval surrounding Rpv28.2 is delimited by the GBS markers S10_419927 and S10_3959571, which correspond to the physical interval of chr10:419,927..3,959,571. Predicted genes within Rpv28.1 as projected to the *V. vinifera* 12X.v3 reference genome sequence are listed in Supplemental Table 6. No significant QTL for resistance was detected

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in the *V. riparia* parent under natural or *in vitro* conditions. Results of QTL analyses are summarized in Table 2; effect plots are shown in Fig. 3.

Discussion

The American grape species *V. rupestris* and *V. riparia* have adapted to disparate environmental conditions and occupy different but overlapping geographic ranges (Callen et al. 2016). They also evolved to have contrasting characteristics in dormancy, morphology, and growth habits (Munson 1909). Exploration of the genetic basis of their environmental adaptation is warranted because the viticulture industry is in need of genetic resources to mitigate the environmental impact of global climate change. The economic value of these species is evidenced by their status as cornerstone resources for the development of disease-resistant, phylloxera- and stress-tolerant, fruit-bearing and rootstock cultivars during the past one and a half centuries (Reisch et al. 2012). Despite their proven value, *V. rupestris* and *V. riparia* have yet to be explored for their vast genetic diversity across North America. *V. rupestris* has been under pressure due to habitat loss and is threatened by genetic erosion (Pap et al. 2015). These conditions add urgency to a broader examination of its native populations. In a recent study, Klein et al. (2018) used GBS markers to examine the genetic diversity of 27 *V. rupestris* and 80 *V. riparia* accessions housed at the USDA-ARS Grape Germplasm Collection. While their data are limited to the accessions maintained in the repository, their work has set the technological and phylogenetic foundations for a broader exploration of the natural populations of these and other wild grape relatives (Klein et al. 2018).

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We report here the construction of genetic linkage maps for *V. rupestris* and *V. riparia* based on an F_1 population produced from a cross between these two species. In terms of marker position, the genomes of both parents had a high degree of synteny with the genome of *V. vinifera*. (Supplemental Figure 3). We observed that *V. rupestris* and *V. riparia* had only 9.94% and 8.87% of their markers assigned to a linkage group that was different from the *V. vinifera* RefSeq linkage group assignment (Supplemental Figure 2). Gardner et al. (2014) and Antanaviciute et al. (2012) also reported similarly conflicting results between genetic and RefSeq positions for 18.3% and 13.7% SNP markers in apple. Such disagreements between linkage maps and RefSeqs do not necessarily indicate mapping errors, but may result from the presence of paralogous genomic regions or incorrect RefSeq sequence assembly. By performing a map validation step using the flower sex phenotype, we were able to verify the linkage maps we generated, since the genomic position of the sex locus was already known. This gave us confidence in the accuracy of our map and the ability to reproduce the mapping of a well-known locus with our set of markers, placed as they are on the genetic maps.

Defense-related genes tend to be in the heterozygous state in plants (McDowell and Simon 2006), and genes that confer resistance to the same pathogen are often located at different loci in various grape genotypes (Gadoury et al. 2012, Buonassisi et al. 2017). Consequently, two resistant grapevine parents will likely produce an F_1 population in which defense related traits will segregate, as recently demonstrated by Divilov et al. (2018). We followed a similar approach as described by Divilov et al. (2018) in that we established an F_1 hybrid population from a cross between two DM-resistant accessions. DM resistance

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segregated in the F_1 progeny, which enabled us to map a major QTL (LOD of 32.32), Rpv28.1, in the female parent accounting for 66.5% of the phenotypic variance in naturally infected plants under greenhouse conditions. Repeating this analysis using a leaf disk DM inoculation assay led to mapping of a resistance QTL, Rpv28.2 (also from the female parent), which overlaps with Rpv28.1 confirming the contribution of this locus to defense against DM. This QTL, however, explained only 24.3% of the phenotypic variance and had an LOD value of 5.2, indicating that the trait is strongly influenced by the environment. Other possible reasons for the lower LOD value with respect to the QTL detected in the greenhouse assay might include limited population size and/or phenotyping errors. Notably, of the 20 vines shared between the naturally infected and the *in vitro*-inoculated cohorts, 16 had similar and 4 had different phenotypes. All four of the latter were rated as highly susceptible in the greenhouse, but moderately resistant *in vitro.* Unfortunately, the number of plants is too low to address the question as to why these four had so much lower resistance in the greenhouse. No QTL were identified from the male parent, which is surprising given the results of our virulence assay on multiple species (Figure 1), in which the *V. riparia* parent showed an even higher level of resistance to this DM strain than the *V. rupestris* parent. One possible reason for this finding may be the presence of multiple components of resistance that contribute at low levels to the observed resistance phenotype in the male parent, which were below the threshold of detection in the offspring. Other possibilities include a major QTL that was present in a homozygous state or a in region of the genome with low marker coverage. We report here the identification of two overlapping DM resistance loci, Rpv28.1 and Rpv28.2. While these may in fact represent one single resistance locus, we identify them

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in separate assays that produced different LOD scores, different levels of variance explained, and different numbers of markers included under the peaks. Therefore, we think it more prudent to maintain separate nomenclature for these loci.

While the high LOD value for Rpv28.1 and its reproducibility lend strong support for the presence of a resistance QTL on chr10, our experiments have limitations. Importantly, resistance was likely assessed against a single strain of *P. viticola*. Furthermore, our results lack multi-season field data. At the time of writing, the entire F_1 population has been established in the field in both New York and Missouri. Both locations are known for high DM disease pressure, but represent different climates where the prevailing DM populations are likely dominated by different cryptic species of *P. viticola* (Rouxel et al. 2014). In the future, it will be important to test the resistance of this progeny under vineyard conditions. The New York and Missouri plantings will enable us to collect data on how various *P. viticola* strains and climatic conditions influence the performance of this QTL in the field.

Rpv28.1 is responsible for 66.5% of resistance against an aggressive DM pathogen and, therefore, it may be utilized for breeding grape cultivars with reduced requirement for fungicide input. The applicability of this locus is all the more relevant because, to our knowledge, it is the first defense-related QTL in this region of chr10. Previously, Kortekamp et al. (2008) hypothesized on the basis of gene expression measurements that DM resistance in the hybrid cultivar Regent was encoded by three CC-NBS-LRR-type resistance genes on chr10. These genes mapped, however, at a physical distance of at least 13 Mb away from Rpv28.1 and Rpv28.2 near the end of the opposite arm of chr10. Interestingly, the DM-

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384 resistant parent, *V. rupestris* B38, was identified by Divilov et al (2018) to harbor another
385 QTL for DM resistance on a different chromosome from the QTL identified in our study
386 (Rpv19 on chr14). We did not detect any QTL for DM resistance at this locus in neither the
387 greenhouse assay nor the *in vitro* assay performed in this study. The most likely reason that
388 we did not observe this QTL is that the DM isolate in this study was different from the isolate
389 that elicits disease resistance conveyed by Rpv19. Additionally, Rpv19 resistance is
390 characterized by a hypersensitive reaction that was not observed in the Rpv28 resistance
391 phenotype. Therefore, *V. rupestris* B38 is a promising source for multiple resistance loci and
392 may prove to be a useful component of "gene pyramiding" schemes. The premise of gene
393 pyramiding is that combining various defense mechanisms against the same class of
394 pathogen will result in more stable resistance than the introgression of a single resistance
395 gene, particularly when considering the ability of different resistance gene products to
396 recognize different pathogen isolates. Based on insight into the evolution of R gene in several
397 plant species (McDowell and Simon, 2006), it is not surprising that *V. rupestris* B38 several
398 resistance factors against the same pathogen. The combination of potentially multiple
399 resistance mechanisms represented by Rpv19 and Rpv28, afford protection against different
400 isolates of the pathogen and provide a survival advantage in nature. Future work will assess
401 the virulence of other DM isolates from different *P. viticola* clades on Rpv28 DM resistant
402 individuals to ascertain the breadth of recognition for this locus.

403 Introgression of several loci to provide resistance against the same pathogen is only
404 possible with marker-assisted selection (MAS). The GBS markers that define the new QTL
405 may prove valuable for the development of molecular markers for MAS (Table 2 and

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406 Supplemental Table 5). Myles et al. (2010) found that only 24.3% of the SNPs segregated
407 within both *V. vinifera* and wild *Vitis* germplasm, suggesting that a portion of the
408 heterozygous *V. rupestris* GBS markers can be readily selected when this resistance
409 haplotype is introgressed into a predominantly *V. vinifera* background. Although SNP-based
410 genotyping has been gaining popularity in grape breeding, many breeding programs still rely
411 on simple sequence repeat (SSR) markers. A significant number of SSR markers have been
412 shown to be transferable from *V. vinifera* to wild *Vitis* species and hybrid grapes (Pap et al.
413 2015, Hammer et al. 2017, Garris et al. 2009). We have identified six SSR markers that fall
414 within or closely flank the region spanning Rpv28.1 and Rpv28.2 (Supplemental Table 7).
415 These markers were originally developed for *V. vinifera*, and their applicability and
416 polymorphism in *V. rupestris* B38 remains to be tested. Given that *V. rupestris* B38 is now
417 known to harbor multiple DM resistance genes (Rpv19 and Rpv28), marker assisted selection
418 will be essential to identifying which resistance alleles are passed on to its progeny.
419 Highly effective MAS, however, will require the development of markers closely-
420 linked to Rpv28.1. The strong synteny (Supplemental Figure 3) and partial conservation of
421 SSR markers between *V. vinifera* and *V. rupestris* indicate that designing SSR primers based
422 on the orthologous *V. vinifera* sequences may be a workable, though potentially ineffective,
423 way to achieve this goal. A genomic library or a genome assembly of *V. rupestris* would
424 make this approach more fruitful. Other marker types, including rhAmpSeq and KASP could
425 also be useful for this purpose and for further mapping work with this population. In
426 addition, it may prove useful for future breeding efforts to survey the extant grapevine
427 germplasm repositories for the markers associated with Rpv28 resistance, and assess allelic

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diversity at this locus. Considering the importance of this species as a resource for grape breeding, the establishment of genomic tools would be a well-justified investment for the grape research community.

Conclusion

The hypothesis that a *V. rupestris* × *V. riparia* F1 progeny can facilitate the mapping of economically relevant loci was supported by the identification of the DM resistance QTL *Rpv28.1 and Rpv28.2* in the *V. rupestris* genome. The novelty of this resistance locus suggests that the biological diversity of North American *Vitis* remains an extensive and still largely unexplored resource for grapevine breeding. In addition, this paper, including the supplementary material, provides a valuable resource for grape breeders and geneticists, as well as for teaching genetic mapping in an outcrossing species.

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Table 1 Distribution of SNP markers and the total genetic length in Kosambi distance across the *V. rupestris* B38 and *V. riparia* HP-1 parental and integrated maps.

Linkage group	Number of markers			Size (cM)			Average distance between markers (cM)		
	V. rupestris map	*V. riparia* map	Integrated map	*V. rupestris* map	*V. riparia* map	Integrated map	*V. rupestris* map	*V. riparia* map	Integrated map
LG1	42	64	125	80.5	94.8	96.0	0.52	0.68	0.77
LG2	50	43	99	64.5	77	75.2	0.78	0.56	0.76
LG3	51	57	115	65.4	75.7	77.3	0.78	0.75	0.67
LG4	61	64	139	71	88.9	87	0.86	0.72	0.63
LG5	63	59	139	71.8	83.5	82.4	0.88	0.71	0.59
LG6	31	53	94	58.6	86.8	85	0.53	0.61	0.90
LG7	98	92	209	95.9	116.7	114.6	1.02	0.79	0.55
LG8	57	50	119	75.7	89.8	88	0.75	0.56	0.74
LG9	66	37	110	63.2	63	67.8	1.04	0.59	0.62
LG10	65	33	107	65.8	80.9	74.8	0.99	0.41	0.70
LG11	42	39	90	65.2	75.2	74.4	0.64	0.52	0.83
LG12	68	71	163	87.6	74.3	90.9	0.78	0.96	0.56
LG13	48	80	159	80	98.3	96.5	0.60	0.81	0.61
LG14	110	86	228	88.4	96.4	93.9	1.24	0.89	0.41
LG15	55	54	117	65.6	95.7	93.3	0.84	0.56	0.80
LG16	78	49	143	66.3	82.1	74.5	1.18	0.60	0.52
LG17	41	53	102	64.4	72.7	71.6	0.64	0.73	0.70
LG18	78	74	172	108.7	125.1	115.3	0.72	0.59	0.67
LG19	73	57	153	62.7	80.5	75.6	1.16	0.71	0.49
Total	**1177**	**1115**	**2583**	**1401.3**	**1657.4**	**1634.1**	**0.84**	**0.67**	**0.63**

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Table 2 Summary of QTL analysis results performed in the F1 hybrid progeny of *V. rupestris* B38 and *V. riparia* HP-1.

Trait	Parent of origin	Peak position (cM)	[a]Interval (cM)	Nearest marker	LOD	[b]LOD threshold	[c]Explained variance (%)	[d]Flanking markers
Sex	*V. riparia*	21.99	21.85-22.2	S2_4599939	60.32	4.8	80.7	S2_3835305, S2_4650244
DM resistance (i*n vitro*)	*V. rupestris*	15.098	1.8-19.8	S10_3024940	5.20	4.8	24.3	S17_17189505, S10_2674703
DM resistance (in greenhouse)	*V. rupestris*	12.456	11.86- 14.85	S10_1285522	32.32	4.8	66.5	S10_2470297, S10_2868961

[a]2-LOD interval on the integrated genetic map.
[b]Genome-wide LOD threshold obtained with 1000 permutations at $p = 0.05$.
[c]Percentage of phenotypic variance explained by QTL.
[d]Markers on each side of the largest LOD pea.

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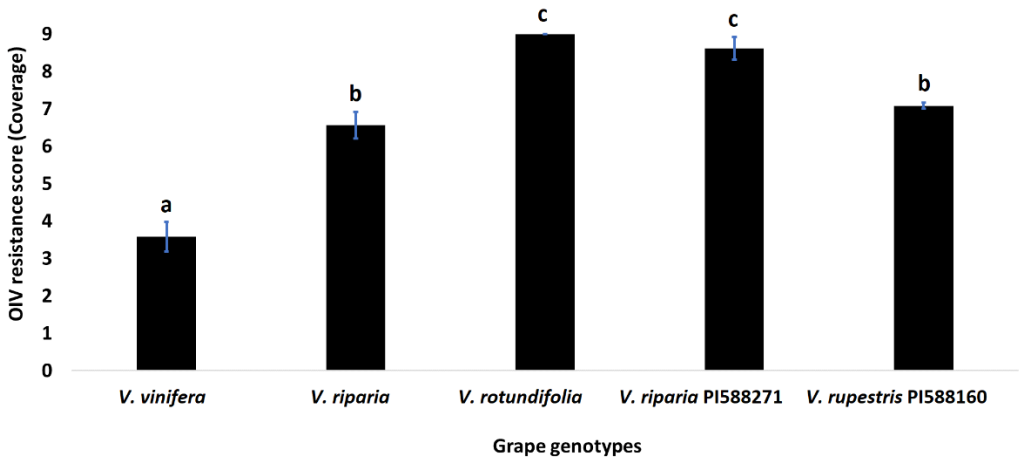

Figure 1 Resistance to *P. viticola* strain MO-1 in five grapevine genotypes. Level of resistance was measured as leaf area covered by sporangiophores following OIV standards in *V. vinifera* F2-35, *V. riparia* Gloire de Montepellier, and *M. rotundifolia* Thomas, and parent genotypes V. riparia HP-1 (PI588271), and *V. rupestris B38* (PI588160).

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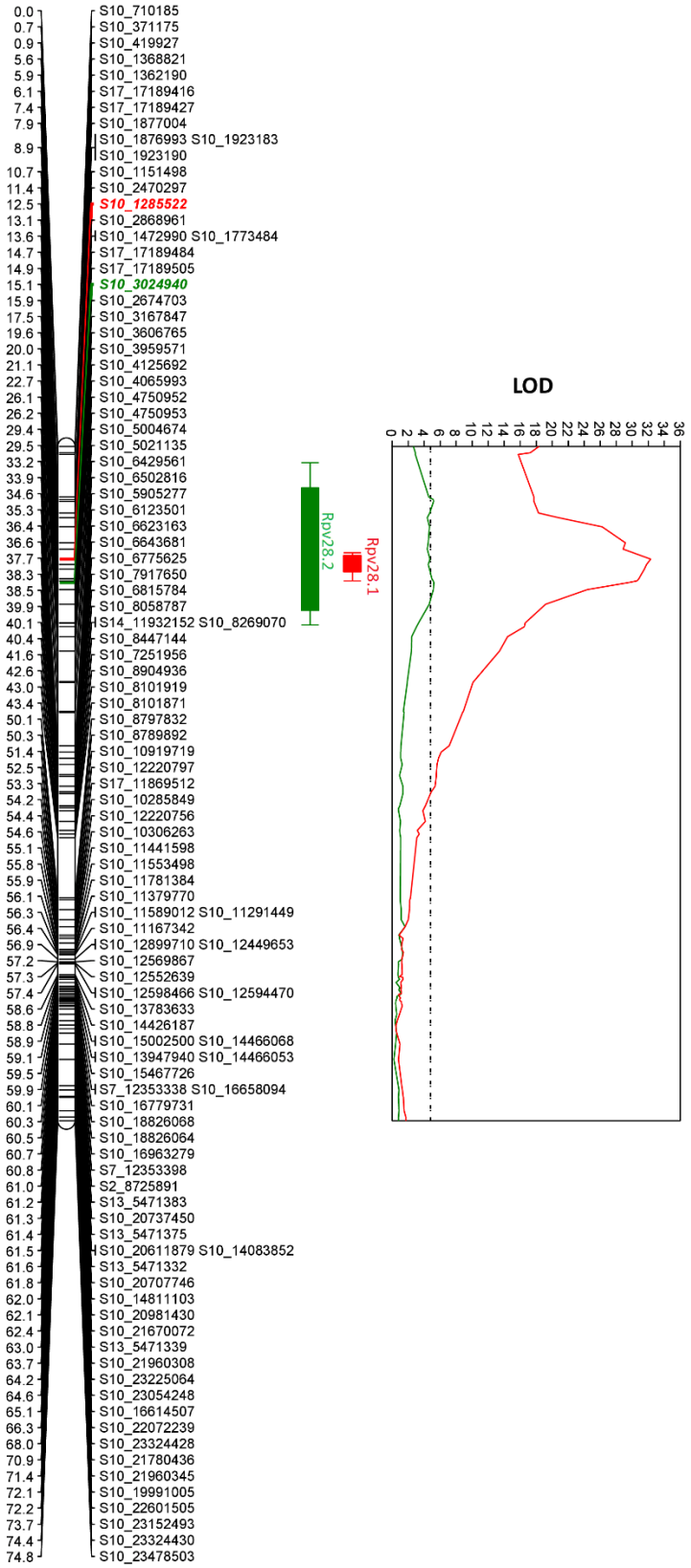

Figure 2 QTL analysis of downy mildew resistance across linkage group 10. The left panel represents the linkage map for LG 10 with marker ID and corresponding genetic position (cM). The right panel shows the LOD scores obtained from interval mapping for downy mildew resistance (red: greenhouse inoculation and green: *in vitro* inoculation) for each marker in the integrated genetic map. The solid boundary of red and green box plots, and extreme boundary represented by their whiskers indicate 1- and 2-LOD intervals for Rpv28.1 and Rpv28.2, respectively. The horizontal black dashed-dotted line represents genome-wide LOD threshold (1000 permutations) at a 5% level of significance. Markers in red and green color on the map represent markers with the largest LOD value.

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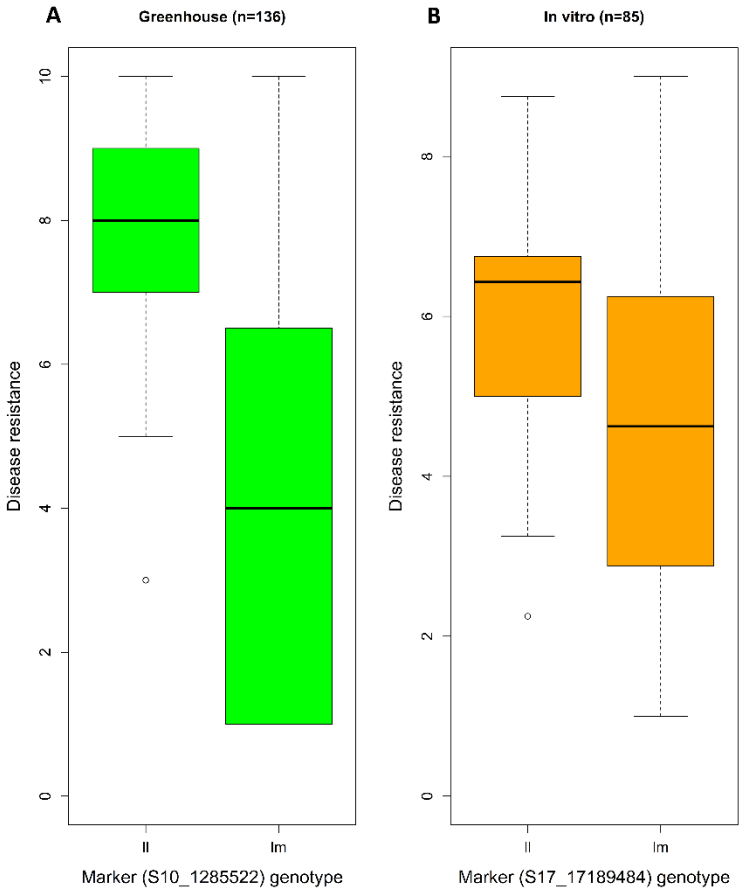

Figure 3 Effect plots showing the relative contribution to downy mildew resistance of QTL Rpv28.1 (S10_1285522) and Rpv28.2 (S17_17189484) in homozygous and heterozygous state.