SZENT ISTVÁN UNIVERSITY
FACULTY OF HORTICULTURAL SCIENCE
DEPARTMENT OF GENETICS AND PLANT BREEDING

IDENTIFICATION OF TWO NOVEL POWDERY MILDEW RESISTANCE LOCI, FROM THE WILD CHINESE GRAPE SPECIES *VITIS PIASEZKII*

DOCTORAL (Ph.D.) THESIS

DÁNIEL PAP

SUPERVISORS:

DR. M. ANDREW WALKER

DR. JÚLIA HALÁSZ

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Ph.D. School Name: Doctoral School of Horticultural Science

Field: Horticultural Biology

Head of the Ph.D. school: Prof. Dr. Éva Zámboriné Németh
Doctor of the Hungarian Academy of Sciences
Department Head, Department of Medicinal and Aromatic Plant
Szent István University, Faculty of Horticultural Science

Supervisor: Dr. Júlia Halász
Department of Genetics and Plant Breeding
Szent István University, Faculty of Horticultural Science

Co-Supervisor: Prof. Dr. Andrew M. Walker
Department Viticulture and Enology
University of California Davis

The applicant met the requirement of the Ph.D. regulations of the Szent István University and the thesis is accepted for the defence process.

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Prof. Dr. Éva Zámboriné Németh
Head of the Ph.D. school

Dr. Júlia Halász
Supervisor

TABLE OF CONTENTS

1. ABBREAVIATIONS

chr	–	chromosome
cM	–	centiMorgan
DNA	–	deoxyribonucleic acid
gDNA	–	genomic deoxyribonucleic acid
IM	–	interval mapping
kb	–	kilobasepare
LOD	–	logarithm of odd
MQM	–	multiple QTL mapping
pBC1	–	pseudo backcross, first generation
PCD	–	programmed cell death
PCR	–	polymerase chain reaction
PM	–	powdery mildew
qPCR	–	quantitative polymerase chain reaction
QTL	–	quantitative trait loci
R	–	resistance
SSR	–	simple sequence repeats, microsatellite markers

2. INTRODUCTION

Numerous old fruit varieties are vegetatively propagated from earliest times of their cultivation, therefore immortalized in the trough centuries (Mullins et al., 1992; Di Gaspero et al., 2012; Myles, 2013). These varieties are remaining the same, while pathogens continue to evolve and are inadvertently moved among continents. The productivity drops because of the onslaught by increasingly virulent and exotic pathogens. Fruits also remain in the same field in monoculture over extended period of time. The only way to protect these crops in the field, is apply large amounts of pesticides periodically. Another, sustainable solution is to shuffle the fruit crop genomes through sexual reproduction, and to introduce disease resistance alleles from their wild relatives (Myles, 2013).

Exploring additional sources for disease resistance and other worthwhile agronomic traits is indispensable for sustainable agriculture. Wild relatives of cultivated grapes, which are potential sources of disease and pest resistance, can be found in North America and Asia. In the past decade, Chinese *Vitis* species have attracted attention from grape breeders because of their strong resistance to powdery mildew and their lack of negative fruit quality attributes that are often present in resistant North American species. Grapevine powdery mildew, *Erysiphe necator* is a major fungal disease in almost all grape growing countries worldwide. Breeding for resistance to this disease is crucial to avoid extensive fungicide applications that are costly, labor intensive and may have detrimental effects on the environment. Combining multiple resistance alleles in the same cultivar is necessary, since a single resistance allele can be easily broken down due to pathogen evolution. Recent genomic techniques make it possible to use genetic resources more effectively and expeditiously.

In this study, we investigated powdery mildew resistance in multiple accessions of the Chinese species *Vitis piasezkii* that were collected during the 1980 Sino-American botanical expedition to the western Hubei province of China. Development of breeding populations and identification of resistance on their genetic maps is a very powerful approach to develop molecular tools for breeders.

While the genetic mapping and the DNA work can be almost semi-automated and generalized among crops, the phenotyping systems are crop and disease specific. Developing a consistent phenotyping system is therefore crucial in order to effectively access the resistance in any breeding population. In this work we compare different phenotyping systems to assess resistance:

1) field evaluations for two successive years under natural and artificial infections;

2) greenhouse evaluations under no-spray conditions;

3) *in vitro* assay with detached leaves visualized under the microscope.

All three systems were measured by categorical scale devised by OIV. The categorical *in vitro* leaf assay was also compared with qPCR by measuring the relative gDNA of the fungal tissue.

3. OBJECTIVES

- Evaluating the *Vitis piasezkii* F1 breeding populations for powdery mildew symptoms in the field to determine the genetic nature of the resistance, whether it's Mendelian or quantitative trait
- Phenotype assessment of the F1 breeding population in controlled environments: evaluate the symptoms in a greenhouse, and investigate visually under a microscope using *in vitro* detached leaf assays
- Utilize a non-visual powdery mildew evaluation technique to confirm visual scores
- Developing a framework map of *V. piasezkii* DVIT2027 with SSR markers
- QTL mapping: identification of potential genetic regions that are responsible for the resistance based on the phenotypic data and genetic framework map
- Challenge the mapped resistance with multiple isolate of powdery mildew to ensure its stability and non-specificity
- Identification of recombinant genotypes in the extended populations, in new generations of crosses. Narrow the genetic region by developing new SSR markers in the flanking genetic regions of the locus
- Explore other available *V. piasezkii* accessions by infecting with grape powdery mildew
- Confirm the co-segregation of the sex linked molecular markers with the flowering type from this Asian *Vitis* species

4. LITERATURE REVIEW

4.1 Significance of the grape and the Vitis genus

The plant family *Vitaceae* includes about 14 genera (Wen et al., 2007). Plants that belong to the *Vitaceae* are mostly lianas. A key morphological characteristic of *Vitaceae* is that a a tendril or an inflorescence always develops opposite to leaf (Mullins et al., 1992). Among these genera, grapevines are classified in the *Vitis* genus, within which they are grouped into one of two subgenera: *Euvitis* and *Muscadinia*. The two subgenera differ in chromosome number, possessing 38 and 40 chromosomes per diploid genome, respectively (Munson, 1909, Patel and Olmo, 1955). Overall there are about 65 species in the genus *Vitis*, all of which are distributed in the northern hemisphere (This et al., 2006). The two centers of diversity within *Vitis* are North America and Eurasia (Aradhaya et al., 2013). In each of these gene centers up to 30 taxa are recognized (Reisch et al., 2012) depending on the author (Munson, 1909; Moore, 1991; Chen et al., 2007; Aradhaya et al., 2013). In addition, there is a single European species which includes two subspecies, the cultivated *Vitis vinifera* L. spp. *sativa* and wild *Vitis vinifera* L. spp. *sylversrtis C.C. Gmel*) (This et al., 2006, Eamunelli et al., 2013).

Wild *Vitis* species are dioecious in contrast to the cultivated vine which is hermaphroditic (This et al., 2006). Hermaphroditism in *V. vinifera* L. spp. *sativa* is probably the result of artificial selection for plants with complete flowers during domestication, to the point of nearly complete fixation among cultivars (Bacilieri et al., 2013).

The delineation of *Vitis* species from one another is less well defined than in other plant genera according to Mullins et al. (1992) since *Vitis* species are interfertile with one another (Emunaelli et al., 2013). “The genus Vitis in North America has long been considered difficult from a systematic standpoint” (Moore, 1991). Species of *Vitis* are considered ecospecies or ecotypes and can be defined as populations of grapevine that are adapted to specific environmental conditions and are distinguishable by morphological characters (Mullins et al., 1992, Zecca et al., 2012). The present taxonomic treatment of *Vitis* species in North America is therefore still based merely on morphological criteria (Aradhaya et al., 2013) and support of other characteristics such as habitat, climate, soil, and geographical distribution (Mullins et al., 1992). Wild relatives of the cultivated grapevine possess resistance to unfavorable environmental and biotic stress factors. Most importantly, today’s grape breeders strive to reduce dependence on chemical disease control and rely instead on the innate disease resistance of grapevines.

The grape is the most economically important of all fruit crops (Myles et al., 2010). In 2011, more than 7 Mha were cultivated on five continents, with a yield of nearly 70 million metric tons. The fruit is consumed as wine, fresh table grapes, raisins, juice, jam, and distilled liquor (pomace brandy). The ten largest producers (measured in production of tons) in the world in order are China, Italy, the United States, France, Spain, Turkey, Chile, Argentina, Iran, Australia, and Brazil. In Hungary, about 70,000 ha are under grape cultivation, producing about ~450,000 t of fresh fruit (FAOSTAT, 2011).

4.2 Cultural history of viticulture

Grapevine is not only a fruit crop, but also an integral part of cultural history. Wine appears in ancient Greek, Roman, and Egyptian cultures as a gift from the gods, and its mystical and cultural attributes remain a part of Christian religions and Western civilization. Domestication of the grapevine began during the Neolithic era, about eight thousand years ago in Transcaucasia (Mullins et al., 1992, This et al., 2006, Myles et al., 2010). It is believed that prehistoric people consumed the berries of *Vitis vinifera* spp. *sylvestris*, the wild progenitor of the cultivated grape, and that wine was probably "invented" accidentally by attempts to store the fruit for winter. The berry skin provided a favorable environment for yeast growth, and the high sugar content of the fruit made it possible to make wine without technical difficulties (Mullins et al., 1992).

The acceleration of transatlantic trade in the 19th century led to the accidental introduction of novel grape pests and pathogens from North American *Vitis* species to the European grapevine, which created major challenges to viticulture.

In the mid-1800s, phylloxera (*Daktulosphaira vitifoliae* Fitch.), an aphid-related insect, grape powdery mildew (*Erysiphe necator*) and grape downy mildew (*Plasmopara viticlola*) were introduced in Europe and caused an epidemic across the continent (Reisch et al., 2012). The solution to the soil-borne phylloxera problem came from the use of grafted plants. The use of North American *V. riparia, V. rupestris, V. berlandieri* and *V. cinerea* as rootstocks to protect valuable cultivars was one of the first successful and widespread applications of grafting as biological pest control. These species were also later used as genetic resources in breeding programs to introgress their resistance and hardiness against various biotic and abiotic stresses into hybrid grapevines and provided reduced the disease susceptibility to the introduced grape powdery mildew (Mullins et al., 1992).

4.3 Genetic tools available for grapevine

The extensive genetic and genomic tools accumulated for grapevine underscore the economic importance of the genus *Vitis*. Several linkage maps and two reference genome sequences have been constructed (Jaillon et al., 2007; Velasco et al., 2007). Moreover, the grape was the first fruit crop for which a reference genome sequence was established. Because the species is highly heterozygous, the first genome sequence was completed using a *V. vinifera* ssp *sativa* individual that resulted from several sequential generations of selfing to obtain a highly homozygous genome for sequencing (Jallion et al., 2007). In the past decade, several studies have focused on the phylogeny and the origin of *Vitis* (Aradhaya et al., 2003, 2013; Wen et al., 2007, Myles et al., 2010, 2011)

4.4 Microsatellite markers

Microsatellite markers or simple sequence repeats (SSRs) are tandem repeats of one to six nucleotides in the genome. The variability in number of these tandem repeats provides co-dominant marker information upon PCR amplification. The variability is believed to be neutral in most cases, and can occur as a result of DNA replication slippage, unequal crossing-over, gene conversion, mismatch/double strand break repair, and retrotransposons (Kalia et al., 2010). The favorable characteristics of microsatellite markers are high levels of polymorphism, reproducibility, and codominance (Emanuelli et al., 2013).

Since 1998, several hundred microsatellite markers have been developed for multiple uses of molecular characterization within the genus *Vitis* (Sefc et al., 1999). Applications of microsatellites include the construction of reference genetic maps (Adam-Blondon et al., 2004; Riaz et al., 2004, 2011, 2012; Doligez et al., 2006; Lamoureux et al., 2006; Welter et al., 2007; Di Gaspero et al., 2007), marker assisted selection (Eibach et al., 2007; Molnar et al., 2007; Katula-Debreceni et al., 2010; Riaz et al., 2011; Li et al., 2013), fingerprinting, pedigree construction, parental analysis (Kiss et al., 2007, Upadhyay et al., 2010, Singh et al., 2013), mapping agronomic traits (Coleman et al., 2009), various evolutionary investigations (Aradhaya et al., 2003, 2013; Myles et al., 2010, 2011; Wen et al., 2007), and several national germplasm collection diversity studies (Pellerone et al., 2001; Aradhya et al., 2003; Leao et al., 2009; Cunha et al., 2013; Rusjan, 2013).

4.5 Grapevine powdery mildew

Grapevine powdery mildew (PM) is caused by the obligate biotrophic fungus *Erysiphe necator* Schwein. (previously *Uncinula necator*). The widely cultivated European grape, *Vitis vinifera* L., is highly susceptible to powdery mildew. All green plant parts suffer from infection resulting in reduced yield due to a decline of leaf photosynthetic capacity and compromised fruit and wine quality (Gadoury et al., 2001, Calonnec et al., 2004, Stummer et al., 2005,). Complete crop loss can occur if infection is not controlled in the early stages of flowering and fruit set. Primary inoculum is the overwintering cleistothecia. During warm season, the conidiophores produce asexual conidia, which spread by air. Both cleistothecia and conidia produce germ tube into primary appressorium to penetrate cuticle layer and epidermal cell wall leading to formation of feeding structure, haustorium. The secondary hyphal structure develops upon establishment of successful parasitic connection (Gadoury et al, 2012). Five to twenty-five days after infection, conidiophores start forming to initiate the mass production of conidia that are spread by air to initiate new infections (Gadoury et al., 2012, Riaz et al., 2013a).

A rigorous regime of synthetic and organic fungicide applications with seasonal rotations (as many as 12 to 20 times in one growing season in California) is required to control the disease and to prevent the pathogen from evolving resistance to fungicides. Excessive application of fungicides leads to increased production costs and adverse impacts on the environment (Gubler et al., 2008, Gadoury et al., 2012, Fuller et al., 2014).

4.6 Resistances against powdery mildew

A number of resistance genes (R-loci) have mapped in *Vitaceae* for various diseases including powdery mildew in several breeding programs around the world. In recent years, wild grapes from Asia have been the focus of several breeding efforts. These species crosses with other *Vitis* varieties with no difficulties, and the fruit quality of the resulting progeny is generally superior to crosses made with North American origin. Asian grape species have a desirable resistance against powdery mildew and downy mildew [*Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni.] (Puchao et al., 1985). Among approximately 35 Asian species (Wan et al., 2008a), R-loci have currently only been identified and mapped in two species. From *V. romentii,* strong resistance against powdery mildew (Riaz et al. 2011; Ramming et al., 2011, Mahanil et al., 2012), and from *V. amurensis* against downy mildew and *Agrobacterium tumafeciens* (Blasi et al., 2011, Schwander et al., 2012, Kuczmog et al., 2012, Venuti et al., 2013).

In North America wild grapes are coevolved with the most viticulturally damaging pests and diseases. *Muscadinia rotundifolia* (Michx.) Small. possesses different cytological and morphological characteristics form others in the *Vitis* genus (Riaz et al. 2012). Several mildew resistance were mapped in *M. rotundifolia* and two R-loci from North American *Vitis* sp. (Dalbó et al., 2001, Welter et al. 2007).

First studied resistant locus was *Run1*, which is a single dominant locus from *M. rotudnifolia* (Michx.) Small., in the chr. 12 (Paquette et al. 2001, Baker et al. 2005). *Run2.1* and *Run2.2* loci were reported by Riaz and collegaues in 2011 from `Magnolia` and `Trayshed` muscandine grapes respectively. Both loci can be found in chr. 18, in the same region, however they can be distinguished by size of the alleles of flanking markers. *Ren5* was a recently observed QTL form the chr. 14 derived from *M. rotundifola* `Regale` (Blanc et al., 2012).

The locus *Ren2* (VIVC 2011, Dalbo et al., 2001) mapped on to the chr. 14 of an intraspecific hybrid, Illinois 547-1 *(V. rupestris* Scheele. × *V. cinerea* Engelm.), possible derived form *V. cinerea.* Similarly, the major QTL, named *Ren3* (Welter et al., 2007) are founded in the chr. 15 of the hybrid cultivar `Regent` with the pedigree of *V. vinifera*, *V. rupestris*, *V. labrusca*, *V. berlandieri*, *V. lincecumii*, and *V. riparia*.

In addition, *Ren1* was found in *V. vinifera* 'Kismish vatkana' (Hoffman et al., 2008, Coleman et al., 2009, Riaz et al. 2013b). In summary powdery mildew resistance loci were identified previously on chromosome 12, 13, 14, 15, 18. The geographic origin and the

genomic location of these loci are presented in Figure 1 and 2. Many of these *R* loci are being used in different breeding programs (Li et al., 2013, Feechan et al., 2015).

Recently a North-American isolate of powdery mildew overcame monogenic resistance conveyed by the *MrRUN1* gene (Feechan et al., 2015). Introgression different resistant loci, derived from various background, into the same cultivar is an effective way to provide a durable, long term resistance in the field of cultivation (Eibach et al. 2007, Peressotti et al. 2010, Ramming et al., 2012, Feechan et al., 2015). To secure these resistances long term durability in competition with the quickly evolving powdery mildew, it is essential explore new effective resistance alleles and combining with exiting advanced breeding lines (Jones et al., 2013). This could potentially led to agrochemical free powdery mildew pest management. Beyond practical advances, that it also could lower the cost of production, it can prevent powdery mildew to accidental adaption to certain fungicides (Délye et al., 1997, Gadoury et al., 2011, Jones et al., 2013, Baudoin et al., 2014) in case of lack of chemical rotation by having multiple layer of "built in" biological defense by default. Powdery mildew resistant grapevine cultivars, with multiple R-loci are on the way to be released in the following years (Fuller et al., 2014).

Breeding grape varieties to resist powdery mildew is a direct strategy to increase farming efficiency and reduce the environmental impact of frequent prophylactic fungicide sprays. Because resistance conferred by a single gene can be overcome by the ongoing evolution of pathogens, multiple resistance genes can be combined into a single genome by the strategy of gene pyramiding (Peresotti et al., 2010).

In the last decade, wild germplasm in Asia gained the attention of grape breeders due to the presence of high levels of resistance to powdery mildew (Puchao & Guoying, 1986, Wang et al., 1998, Wan et al., 2007; Wan et al., 2008a; Wan et al., 2008b; Wang et al., 2008, Li et al., 2008, Blasi et al., 2011, Schwander et al., 2012, Kuczmog et al., 2012, Liu & Li, 2013). In 2007, Wan and colleagues reported several *Vitis* species from China (e.g. *V. romanetii*, *V. piazezkii*, and *V. davidii*) with different resistance level against powdery mildew, however further investigation is needed to explore these loci. Chinese species hybridize easily, and lack the negative fruit properties present in the hybrids of North American species, which make them prime candidates for breeding (Wan et al., 2008b). Among the approximately 35 Asian *Vitis* species, powdery mildew resistance-linked *R* loci have previously been identified and mapped in two full-sib accessions (C166-043 and C166-026) of *V. romanetii* [*Ren4* (Riaz et al., 2011, Ramming et al., 2011, Mahanil et al., 2012].

In addition, in Chinese species resistance to downy mildew and *Agrobacterium tumefaciens* was identified in *V. amurensis* (*Rpv8*, (Blasi et al., 2011); *Rpv10*, (Schwander et

al., 2012); *Rpv12*, (Venuti et al., 2013); Rcg1, (Kuczmog et al., 2012)). Moreover, the *Ren1* locus that provides partial resistance to powdery mildew was also identified in two accessions of cultivated *V. vinifera* from central Asia (Hoffmann et al., 2008, Coleman et al., 2009). In an attempt to explore new potential sources of PM resistance in central Asian accessions, Riaz et al. (2013b) surveyed 380 cultivated and wild accessions of *V. vinifera* and accessions of Chinese species. They identified 10 *V. vinifera* accessions with partial resistance to PM and strong resistance in accessions of the Chinese species, *V. piasezkii*. Powdery mildew resistance has previously been reported in multiple accessions of *V. piasezkii*, a species widely distributed in the wild grape species rich mountain ranges of Northeast and Western China (Wan et al., 2007, Wan et al., 2008a).

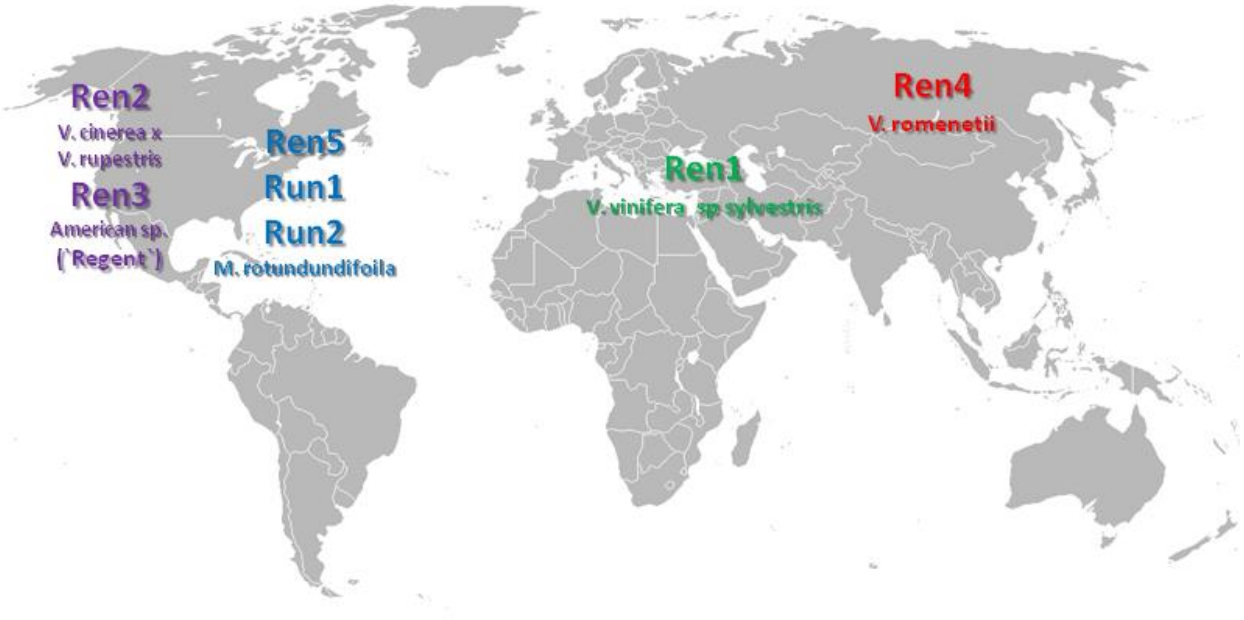


Figure 1. *Geographic origins of known powdery mildew resistance loci*

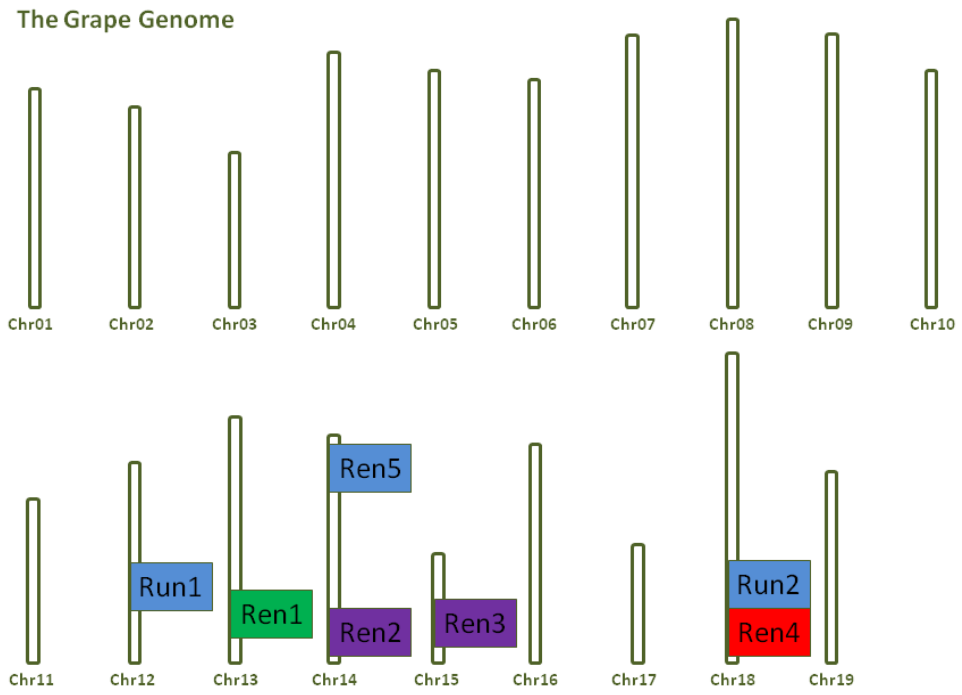


Figure 2. *Genetic locations of the known powdery mildew resistance loci across the genome of the* Vitis

It is a general assumption that PM originated from North America based on the historical records and presence of resistance in many North American species (Brewer and Milgroom, 2010, Gadoury et al., 2012). The presence of strong resistance to powdery mildew in Chinese *Vitis* species is curious. Potentially, these Chinese species could have different mechanisms of resistance at the molecular level. From a breeding perspective, it is critical to combine *R* loci that recognize different molecular patterns into the same genotype to generate durable field resistance that is not overcome by rapidly evolving pathogens (Eibach et al., 2007, Peressotti et al., 2010, Ramming et al., 2012, Feechan et al., 2015). This approach should consider the combination of different host resistance mechanisms and the knowledge of powdery mildew core effectors recognized by different *R* genes. Understanding of the resistance will greatly assist breeders in making decisions about combining different loci to develop breeding lines with durable resistance in the field (Jones et al., 2014). Enhancing genetic resistance of cultivated grapevines would potentially lead to powdery mildew management with reduced or no fungicide applications, lowering costs of production and reducing the impact on the natural environment (Délye et al., 1997, Gadoury et al., 2012, Jones et al., 2014, Kunova et al., 2015).

In this study we investigated powdery mildew resistance in 10 accessions of *V. piasezkii* maintained at the National Clonal Germplasm Repository, Davis, California. Nine of these

accessions were collected during the 1980 Sino-American botanical expedition in the Shennongjia Forestry District, Hubei province of China (Bartholomew et al., 1983, Dosmann and Del Tredici, 2005). Two accessions DVIT2027 and DVIT2032 were identified to be powdery mildew resistant in an earlier study (Riaz et al., 2013b). The DVIT2027 accession was used to develop two F1 breeding populations; a framework genetic map with simple sequence repeat (SSR) markers was developed and two new powdery mildew *R* loci on different chromosomes were identified. These loci have been designated *Ren6* (chromosome 9) and *Ren7* (chromosome 19) in accordance with the guidelines of the International Grape Genome Program (IGGP). The large population size allowed us to study the effect of each locus cumulatively as well as individually. The *Ren6* locus provides complete immunity to the disease by initiating rapid programmed cell death (PCD) at the point of pathogen penetration. The locus *Ren7* provides partial resistance by allowing the pathogen to establish, but limits the amount of hyphal growth and conidiation. The availability of these two new *R* loci will enhance the repertoire of existing *R* loci available for powdery mildew resistance breeding.

5. MATERIALS AND METHODS

5.1. Plant material

The F1 population designated 11-373 was the result of a cross between powdery mildew susceptible and pistillate *V. vinifera* F2-35 ('Carignane' × 'Cabernet Sauvignon') and the resistant Chinese species *V. piasezkii* DVIT2027 (Figure 3 and 4). This accession of *V. piasezkii* was identified to have strong resistance to grape powdery mildew in multi-year field-testing and it hybridizes easily with other *Vitis* species making it an excellent parent for powdery mildew resistance breeding. DVIT2027 is a staminate vine; leaves are simple, unlobed and long cordate with an acute apex. This accession does not have shoots with variable leaf lobing, such irregular lobing is typical of *V. piasezkii*.

Figure 3. Vitis piasezkii *DVIT2027 growing in the greenhouse from hardwood cutting.*

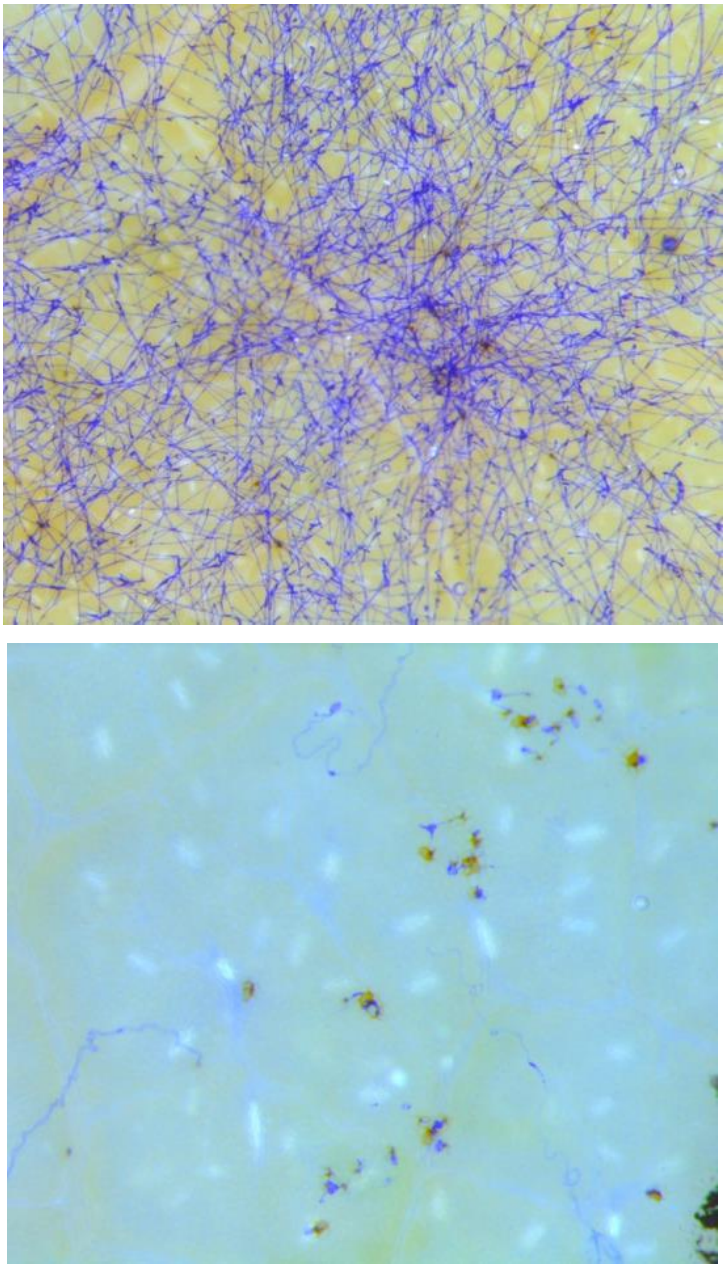


Figure 4. *Susceptible* Vitis vinifera *F2-35 (upper) and the resistant* Vitis piasezkii *DVIT2027 (lower) infected with grape powdery mildew. Fungal structures are stained with Comassie Blue R250.*

The 11-373 breeding population consisted of 536 seedlings that are maintained at the Department of Viticulture and Enology, University of California, Davis, California. The DVIT2027 and eight other accessions of *V. piasezkii* were collected in 1980 during the Sino-American Botanical expedition (Figure 5). The accession DVIT1453 was acquired from

China by H. P. Olmo. All accessions of *V. piasezkii* tested in this study are maintained at the USDA-ARS National Clonal Germplasm Repository, Davis, California.

An additional small F1 mapping population (designated VpF1) was generated by crossing *V. piasezkii* DVIT2027 with a powdery mildew-susceptible *V. vinifera* 'Pinot Meunier' mutant "picovine" line 06C008V0003 (Chaib et al., 2010). The VpF1 population consisting of 31 individuals was maintained in a glasshouse at CSIRO Agriculture, Urrbrae, South Australia. This process was similar to how the *Run1* locus from *M. rotundifolia* was introduced into the same genetic background by crossing the resistant line BC5:3294-R23 with picovine line 06C008V0003 and selecting *Run1* progeny using markers as described previously (Feechan et al., 2013).

Figure 5. *Origin of Vitis piasezkii accessions used in this study (A) Nine accessions including DVIT2027 were collected in the Shennongjia Forest, Hubei Province, China in 1980 (Bartholomew et al 1983).*

5.2. Disease evaluations

The 11-373 seedling population was evaluated for powdery mildew resistance in multiple environments. Severity of the disease symptoms was recorded in two successive years under natural and artificial infections in the field. Disease evaluations were also carried out on four replicates of each seedling plant in a controlled environment in an unsprayed greenhouse, and by detached leaf assay in the laboratory. The powdery mildew mass was quantified on detached leaf samples with a molecular approach using quantitative polymerase chain reaction (qPCR).

Powdery mildew symptoms on canes and leaves were evaluated on all available growing plants in the field. From the base mapping population of 277 seedlings, 253 and 261 seedlings were evaluated in the field in 2013 and 2014, respectively. A total of 258 seedlings from the base population were challenged with powdery mildew and evaluated in the greenhouse. Young uninfected leaves of 258 seedlings were also used in an *in vitro* assay and examined under the microscope (Leica EZ4 D) for severity of mildew infection. To avoid bias, plants in the field were scored three to four times each year and two people independently evaluated the greenhouse and the *in vitro* experiments. Lastly, qPCR was completed on 247 genotypes from the *in vitro* assay leaves to measure the total mass of fungal infection.

5.2.1. Field evaluations

Categorical measurements of phenotypic responses in the field were recorded in August, September and October of 2013 under natural infection conditions. In the spring of 2014 artificial inoculations were carried out at four subsequent times from April to the end of June with three to four week intervals in order to ensure a homogeneously high infection rate. For artificial inoculations, PM conidia were amplified on *in vitro* cultures of *V. vinifera* 'Carignane' leaves and suspended in 0.1% (v/v) Tween-20 solution. Each seedling plant was sprayed with the inoculum suspension using a Perval Sprayer unit (Chicago Aerosol, Coal City, Illinois). The powdery mildew symptoms were evaluated in August and September. A 6 point scoring system was used for both leaf and cane scores in years 2013 and 2014:

0 - no visible symptoms,

1 - one or two spots of infection,

2 - more than two spots of infection but still hard to find,

3 - active PM infection that was easy to observe on the leaves and cane tissue,

4 - PM infection patches on many leaves and cane tissue, and

5 - heavy PM infection on all plant parts.

5.2.2. Greenhouse evaluations

Controlled disease evaluations were performed in an unsprayed shaded greenhouse and on detached leaves in the laboratory. For the greenhouse evaluations, three to four replicates of each genotype were propagated from either green or hardwood cuttings and potted in 10 cm pots. Multiple plants of susceptible control cultivars (*V. vinifera* 'Carignane' and F2-35), and tolerant/resistant controls (*V. vinifera* 'Karadzhandal', *Vitis* hybrids 'Villard Blanc' and e2-9, *V. romanetii* C166-043, and *V. piasezkii* DVIT2027) were used in each round of disease evaluations to monitor the variation in the severity of the screen. The seedling replicates and control cultivars were randomized across the greenhouse and spaced 10 cm apart. The temperature of the greenhouse was set at 23-27°C, lights were used to maintain a minimum 12h daylength if needed, and air humidity was elevated by spraying water on the floor. For inoculum, the C-isolate (Riaz et al., 2013a) was propagated on *in vitro* plated susceptible 'Carignane' leaves. On average approximately 70,000 conidia/ml in 0.1% (v/v) Tween solution were used to infect each plant with a Perval Sprayer unit. Disease evaluations were carried out four weeks post inoculation by two people using a modified OIV-455 scale (IPGRI-UPOV, 1997):

0 - no symptoms,

1 - one or two small patches of PM on the entire plant,

2 - four to five patches of PM,

3 - many leaves have patches of PM,

4 - PM covers entire surface of many leaves on the same plant (Figure 6).

Figure 6. A susceptible and a resistant seedlings from the F1 population after the greenhouse evaluations of powdery mildew. When most of the leaves are covered with the fungi, scored on the scale highest.

5.2.3. In vitro evaluations

Microscopic evaluation of powdery mildew infections were made on *in vitro* cultured detached leaves for all breeding populations and for 10 accessions of *V. piasezkii* along with susceptible and resistant controls. Four fully expanded leaves from the third and fourth position on a shoot were collected, washed and plated as follows: rinse with distilled water, 2-3 min submergence into 0.3% (w/v) sodium hypochlorite solution followed by four to five rinses with sterile distilled water, leaves were dried between sterile paper towels and petioles were trimmed before plating adaxial surface up onto 0.8% agar in 100 x 15mm Petri dishes. Leaves were inoculated using a settling tower procedure modified from Reifschneider and Boiteux (Reifschneider and Boiteux, 1988) to obtain uniform and consistent powdery mildew infections with the C-isolate (Figure 7.) A custom-made settling tower (50 x50 x120 cm) with 25 leaves each in Petris dish placed in it, was attached to a vacuum system for 5 min followed by 10 min of conidia settling after breaking the vacuum.

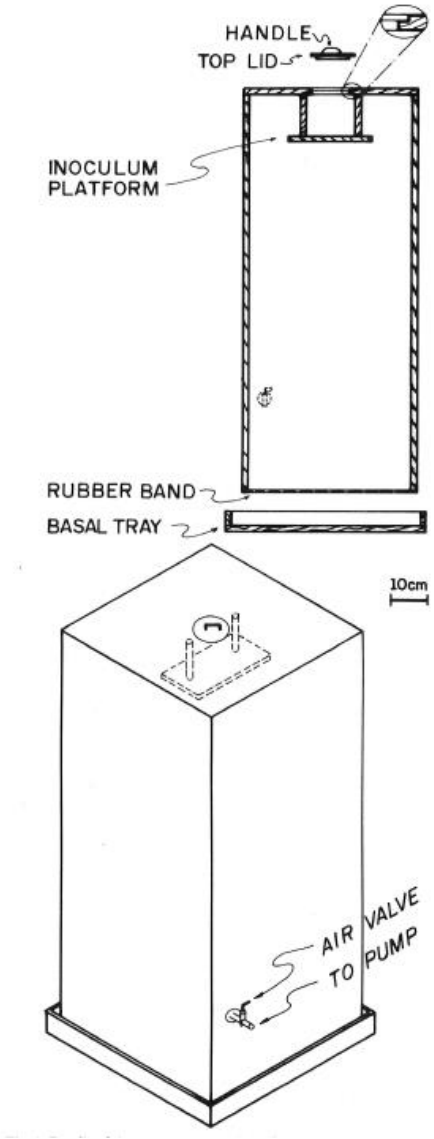


Figure 7. *Schematic figure of the settle tower used during the in vitro powdery mildew assays. (Reifschneider and Boiteux 1988)*

The average infection rate was 2.18 ± 1.5 conidia/mm^2. Two people independently rated powdery mildew growth for all *in vitro* experiments at 14-15 days post inoculation (dpi) using a dissecting microscope (Leica EZ4 D) with the following scale (Figure 8):

0 - no hyphae,

1 - one or two conidia with hyphae,

2 - several conidia with secondary hyphae and establishment of micro colonies,

3 - mycelium on entire leaf surface, limited conidiophore, and

4 - mycelia coverage is extensive, reproduction is prolific, clearly visible with the naked eye.

To obtain better visual observations, staining with Coomassie Brilliant Blue R-250 was carried out on detached leaves as described by Riaz et al (Riaz et al., 2013a).

Figure 8. *Detached, in vitro leaf assay in Petri-dishes after 14 days of the infection. The susceptible leaf is covered with extensive powdery mildew, clearly visible with naked eye, scored four on the scale. The leaf on the right is clear from powdery mildew, appears to be resistant.*

Phenotyping of the VpF1 progeny population was carried out using an Australian powdery mildew isolate (APC1)(Feechan et al., 2013). Inoculum was maintained on detached leaves of *V. vinifera* 'Cabernet Sauvignon' using an 8-10 day rotation and inoculated onto detached leaves of VpF1 progeny as previously described (Donald et al., 2002). Scoring of the frequency of PCD induction in penetrated epidermal cells was carried out 2 dpi using trypan blue as previously described (Feechan et al., 2015).

5.2.4. Molecular disease evaluation

Molecular disease quantifications on 247 genotypes were achieved with qPCR. For each accession, infected leaves from *in vitro* assay were collected after visual examination. The tissue was kept in -20°C until the DNA was extracted from the whole infected leaf with a modified CTAB protocol with the addition of RNase treatment. The accumulated powdery mildew biomass was quantified in qPCR as described in Amrine et al. (2015) with primer

sequences designed for the *E. necator* elongation factor *EnEF1* gene along with *V. vinifera* actin-specific primers:

- *EnEF1* Gene ID: KHJ34692.1;
    - Forward primer: TGGAAAGTCTATTGAGGCAACTCC,
    - Reverse primer: CAACACACATAGGTTTAGATGGAATCA (Jones et al., 2014)
- *Actin* Gene ID: 100232866;
    - Forward primer: CTATGAGCTGCCTGATGGGC,
    - Reverse primer: GCAGCTTCCATCCCAATGAG (Licausi et al., 2010).

The reactions were carried out with SYBR Green Master mix as per the manufacturer's instructions (Applied Biosystems 7500 Real-Time PCR System) using the following temperature profile:

- 2 min at 50°C,
- 10 min at 95°C,
- followed by 40 cycles of
    15s at 95°C and
    60s at 60°C.

DNA samples of three to four biological replicates of each genotype were randomized across reaction plates, and each plate had two replicates of reference *V. vinifera* and *E. necator* DNA samples. Infection coefficients from individual samples were calculated from linearized difference of C_T values with the formula: $2^{-(VvActinCT-EnEF1CT)}$.

A replicated subset of genotypes was also challenged with three additional powdery mildew isolates collected from different locations in California to determine if powdery mildew resistance is race-specific. Two of the tested isolates, Lodi and e1-101 were genetically distinct and grouped in a different clade based on their microsatellite profiles (Jones et al., 2014). The isolate 11-373-J16 was collected from a susceptible seedling from the 11-373 population, which is maintained at UC Davis. All collected phenotypic data was analyzed using R 3.1.3 (R Team, 2015) and the Agricolae package (Mendiburu, 2015).

5.3. Genotyping and genetic map construction

Genomic DNA was extracted from young leaf tissue by a modified CTAB protocol (Riaz et al., 2011). A total of 277 progeny plants of the 11-373 population were used as a base

mapping population to generate a framework genetic map. Five hundred and twenty SSR markers from previously published marker series were tested on a subset of eight samples including parents and progeny. The VMC and VMCNg marker series were developed by the *Vitis* Microsatellite Consortium (Agrogene, Moissy Crameyel, France), VVI series by Merdinoglu et al. (2005), UDV series by Di Gaspero et al. (2005), VChr series by Cipriani et al. (2008), VVMS series by Thomas and Scott (1993), SCU by Scott et al. (2000), VVC by Decroocq et al. (2003), VVMD by Bowers et al. (1999, 1996), and CTG, CF, AF primer sequences were derived from the EST-SSR database (University of California, Davis http://cgf.ucdavis.edu). The sequences of the primer pairs are available from the NCBI database (http://ncbi.nlm.nih.gov) and/or from the aforementioned references. To further saturate and refine the region for chromosome 9 and for chromosome 19, fourteen new SSR markers were developed (PN9 and PN19 series; Table 1) utilizing the 12X genome sequence of PN40024 (Jaillon et al., 2007). The genome sequence was screened with WebSat (Martins et al., 2009) for repetitive sequences and primers were designed with Primer3 software (Untergasser et al., 2012) using the following parameters: 35-60% GC content, 22 bp length, and a calculated T_m of 60°C (Rozen and Skaletsky, 1999).

Polymerase chain reactions of 10µl volume were carried out with fluorescently-labeled forward primers using the following standardized thermocycling profile:

- 5 min at 95°C
- 35 cycles of
  - 45s at 95°C,
  - 45s at 56°C
  - 45s 72°C,
- 10 min at 72°C.

Amplified products of up to five markers were combined depending on the amplicon size and fluorescent labels of the markers and run on an ABI 3500 capillary electrophoresis analyzer with GeneScan-500 Liz Size Standard (Life Technologies, Carlsbad, California, USA). Allele sizes were determined using GeneMapper 4.1 software (Applied Biosystem Co., Ltd., USA).

Table 1 *New SSR makers designed from the 12X genome sequence of PN40024 for chromosome 9 and 19*

Marker Name	Forward Primer Sequence	Reverse Primer Sequence	Amplicon size
PN9-005	tcaattcacctcctcaaggttt	ctagcaatcttttgggcagttt	238
PN9-016	caacgatcacagaaggcaataa	tgtgtatggaagaccaactatgga	251
PN9-042	ctccaccttggtttgcttattc	acaacagcctatgttccagagc	366
PN9-057	gagatgttgtagtgaagatcaagc	agtaggaagagatgtggaaaagag	190
PN9-063	tcctaagacaaagttcccttca	gttacagttgaatccttgcaca	194
PN9-0661	gcatcctctatgtctttattttgaga	gatggtttgcacaagctagaaa	306
PN9-067	gggtagcaacattgaaaaggac	agaattatcttcggccctcatt	303
PN9-0672	gtcaagataaagcctaagtggc	tgagtagatgtgactacaatcctttg	350
PN9-068	ccccaatcttaaatggaaca	tagtgggacgttggacactt	182
PN9-099	tgcaaccataatcaacgtgc	gcatgtgtaaactaagccctaaca	272
PN9-112	tgttagccccatttggatt	ccatttcaacatctctattccc	242
PN9-141	gttggggtcatgcaactatctt	acaatgggctagattaagcgac	383
PN19-018	caaaacaagtccattgcgttta	tgtcccatgacagtttatccag	197
PN19-022	ttttatttccattttctttctttc	aggagagatattttgcattgaag	286

Markers were evaluated for Mendelian segregation ratios using χ^2-tests and the parental and consensus genetic-linkage maps were created using JoinMap 4.1 (Van Ooijen, 2011). Recombination frequencies were set between 0.25 and 0.05 to group the markers. The Kosambi mapping function was used to generate centimorgan (cM) distances (Kosambi, 1944). In the interval regression mapping the independence LOD (logarithm of odd) was set to 5-8 with a one-step interval. Chromosome numbers and their orientation were derived from a consensus grape reference genetic map (Doligez et al., 2006).

Additional *V. piasezkii* accessions were genotyped as described above with the following markers PN9-066.1, PN9-067, PN9-068, VMC4h6, VMC9a2.1, PN19-022 and VMC5h11. The allelic data was analyzed in DARwin6 (Perrier and Jacquemoud-Collet, 2006) to generate a relationship tree with the unweighted neighbor-joining method employing 1,000 bootstrap replications.

5.4. Quantitative trait locus analyses

The quantitative trait locus (QTL) analysis for each trait was carried out using two different approaches with MapQTL 6.0 using both parental and consensus maps (Van Ooijen, 2009 and 2011). First, interval mapping (IM) analysis was carried out with a regression algorithm to detect possible QTLs on both parental maps. Automatic cofactor selection was carried out on five neighboring loci around the potential QTL with the *p* value set at 0.001 with 2,000 iterations. In the next step, multiple QTL mapping (MQM) analysis was carried out for each phenotypic trait using the assigned cofactors from the previous step. To examine the effect of each locus independently, a subset of F1 11-373 progeny were selected based on local haplotypes for either *Ren6* or *Ren7* only and QTL analysis was carried out as described above. The genome wide, and combined significance LOD thresholds, were calculated with 1,000 permutations. The type-I error rate of 0.05 was used to identify significant LOD values.

5.5. Additional pseudo-backcross breeding populations for key recombinant search

Four pseudo-backcross (pBC1) breeding populations were developed using resistant seedlings of 11-373 that inherited either *Ren6* or *Ren7* or both loci. In all four cases, PM resistant seedlings were used as the male parent and the susceptible *V. vinifera* 'Malaga Rosada' was used as the female parent. The populations 13-350, 13-351 (which segregated for *Ren6* only), 13-352 (both *Ren6* and *Ren7*) and 14-353 (*Ren7* only) consisted of 396, 125, 133 and 256 seedlings, respectively (Table 2.). An additional 259 seedlings of the F1 11-

373 population and all pBC1 populations were screened with markers flanking the *Ren6* and *Ren7* loci to identify potential recombinant plants. Disease evaluations were carried out on multiple replicates of all candidate recombinant plants as well as partial subsets of each population in the greenhouse and by the *in vitro* detached leaf assay. Inoculations and scoring was carried out using the experimental procedures described above.

Table 2. *Summary of the utilized breeding population. For creating the framework map and identification of the resistance 277 seedlings of the 11-373 population were genotyped, and phenotyped. The remaining seedling and additional pBCI populations were screened with the flanking markers of the identified resistance loci for potential recombinants. The potential recombinants plants were evaluated for powdery mildew symptoms.*

Population ID	Number of seedling	Female parent	Male parent (*R* loci)
11-373 F1	536	*V. vinifera* F2-35	*V. piasezkii* DVIT2027
13-350 pBC1	396	*V. vinifera* 'Malaga Rosada'	F1 11373-039 (*Ren6*)
13-351 pBC1	125	*V. vinifera* 'Malaga Rosada'	F1 11373-051 (*Ren6*)
13-352 pBC1	133	*V. vinifera* 'Malaga Rosada'	F1 11373-087 (*Ren6*/*Ren7*)
14-353 pBC1	256	*V. vinifera* 'Malaga Rosada'	F1 11373-210 (*Ren7*)

5.6. Gene annotation and identification of transposable elements

Based on the markers linked to the *Ren6* and *Ren7* loci, a 60 kb and 330 kb piece of corresponding genome sequence of PN40024 for each locus was scanned for the presence of transposable elements using CENSOR (Kohany et al., 2006). The gene annotations for the corresponding regions were obtained from *Gramene* (Grimplet et al., 2009) (12.1 assembly, V1 annotation). Both gene and transposable element annotations were overlaid and displayed using the software package Geneious v7.1.7. (Kearse et al., 2012).

6. RESULTS

6.1. Disease evaluations

The F1 11-373 seedling population was evaluated in multiple environments. Field evaluations for leaf and cane powdery mildew symptoms were carried out for two consecutive years (2013 and 2014) in addition to the greenhouse evaluations, *in vitro* assays, and qPCR evaluations. The Table 3 provides the details on the number of seedlings tested in each year, minimum and maximum scores, means, and variances. Lower mean and variance was registered across all progeny for both leaf ($\bar{x}$=0.51, σ^2=0.55) and cane ($\bar{x}$=0.23, σ^2=0.29) evaluation in 2013 compared to 2014 ($\bar{x}$=1.29, σ^2=3.09; $\bar{x}$=0.88, σ^2=2.41 respectively for leaf and cane). The results of all methods used for disease evaluations were significantly correlated to each other (Table 4, p<0.001, Figure 9). The pair wise correlations with the 2013 field scores and any other evaluation were lower (R^2 ranging from 0.25 to 0.63). The highest correlation was observed between the visual scores from the *in vitro* assay in the controlled environment and the greenhouse assay (0.91). Likewise, high correlations were observed between the estimation of accumulated powdery mildew biomass by qPCR and phenotypic evaluations on greenhouse plants (0.77) as well as the *in vitro* assay (0.82).

Table 3. *Descriptive statistics for phenotypic scores of powdery mildew symptoms used for QTL mapping with the 11-373 grapevine mapping population.*

Phenotype Evaluation[a]	Number	Mean	Variance	Min	Median	Max
Leaf 2013	253	0.51	0.55	0	0.33	3.83
Cane 2013	253	0.23	0.29	0	0	3.5
Leaf 2014	261	1.29	3.09	0	0.5	5
Cane 2014	261	0.88	2.41	0	0	5
Greenhouse	258	1.36	1.36	0	0.43	4
in vitro	258	1.55	2.57	0	0.97	4
qPCR	247	6.62	9.63	0.25	6.87	12.67

[a]*Powdery mildew symptoms in the field were evaluated in two subsequent years. Greenhouse, in vitro experiments, and the qPCR-based molecular assay were carried out with three to four biological replicates of each seedling plant in 2014.*

Table 4. *Correlation of average phenotypic scores across different disease evaluation screens developed for mapping resistance in the 11-373 mapping population.*

	Leaf 2013	Cane 2013	Leaf 2014	Cane 2014	Greenhouse	*in vitro*	qPCR
Leaf 2013	1.0	-	-	-	-	-	-
Cane 2013	0.632[a]	1.0	-	-	-	-	-
Leaf 2014	0.591	0.465	1.0	-	-	-	-
Cane 2014	0.633	0.59	0.827	1.0	-	-	-
Greenhouse	0.499	0.374	0.842	0.664	1.0	-	-
in vitro	0.409	0.283	0.786	0.617	0.91	1.0	-
qPCR[b]	-0.365	-0.245	-0.697	-0.546	-0.767	-0.818	1.0

[a]*All R^2 values are significant ($p<0.001$).*

[b]*The qPCR derived infection coefficients normalized with natural logarithm. They correlate inversely with the visual observations.*

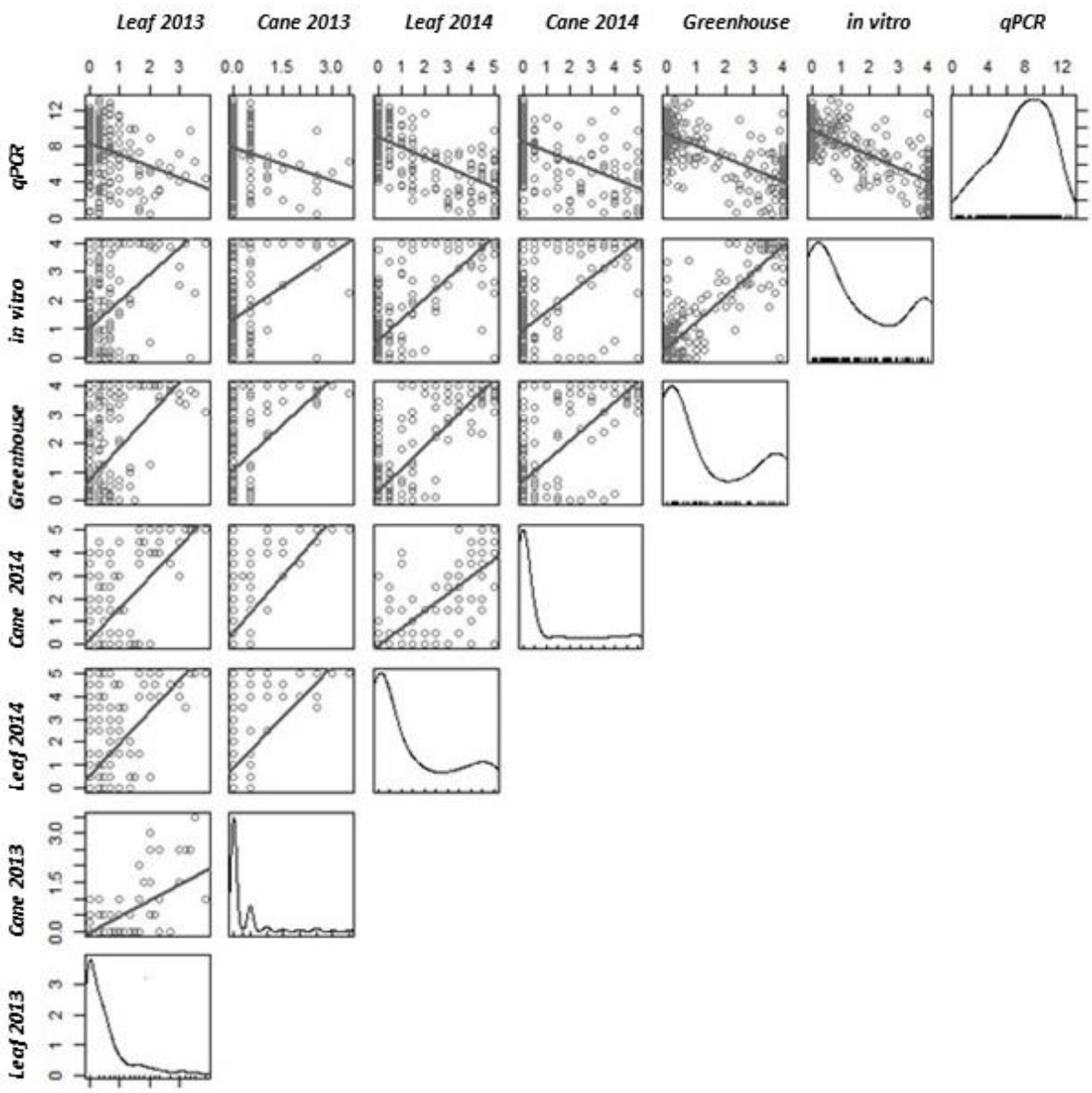


Figure 9. *Pairwise correlation of average phenotypic scores across different disease evaluation screens used for mapping powdery mildew resistance. Respected* R^2 *values are presented in Table 4. The qPCR derived infection coefficients normalized with natural logarithm and its correlate inversely with the visual observations. Diagonal squares represent the distributions of each phenotypic assessment.*

In addition to testing the F1 11-373 population with the powdery mildew C-isolate, a subset of thirty-one F1 genotypes were also challenged with three additional powdery mildew isolates (Lodi, e1-101 and 11-373-J16 (Jones et al., 2014)) in the detached leaf assay using the settle tower infection method. Analysis of variance detected no significant differences among the four powdery mildew isolates (Table 5; p=0.162).

Table 5. *Two-way factorial Analysis of Variance with four powdery mildew isolates that were used to evaluate four genotypic classes of progeny plants with susceptible controls and parental genotypes*

	Df	SS	MS	F	*p* Value
Genotypic Class	3	111.58	37.19	40.835	<2e-16
Strain	3	4.76	1.59	1.743	0.162
Interaction	9	2.01	0.22	0.246	0.987
Residuals	108	98.37	0.91		

6.2. Marker analysis and genetic linkage maps

From a total of 520 markers, 268 and 264 were found to be polymorphic for the female and male parents, respectively. Two hundred and seven markers that were polymorphic for the resistant male parent and one marker for the female parent were applied to the base population of 277 seedlings. A total of 148 markers were fully informative, segregating for parents (ab × cd, ab × ac), and 59 were polymorphic for the male parent DVIT2027 only. The missing allelic information for the complete data set was 3.12%. Of the 208 markers, 34 deviated from the expected Mendelian segregation (p<0.05). All distorted markers are listed with χ^2 values in Supplemental Table 1. Markers with significant deviation from Mendelian ratios were included on all maps if the order of the markers didn't differ from previously reported maps. The flower phenotype was also evaluated for 180 seedlings that bloomed in 2014. Only pistillate and staminate flower phenotypes were observed and they segregated 1:1 (69:74, χ^2 = 0.175 p=0.6759).

Parental and consensus framework genetic maps were constructed with polymorphic marker data. The F2-35 parental map included 144 markers across 19 chromosomes covering 779.61 cM with an average marker distance of 5.41 cM. The DVIT2027 map included 207 markers across 19 chromosomes covering 1002.7 cM with an average marker distance of 5.35 cM. There were only seven gaps that were bigger than 20 cM

(Supplemental Figures 1A-D). The consensus genetic map was 1005.4 cM with an average marker distance of 5.31 cM. Eight newly designed SSR makers (PN9 series) were mapped to their respective location in both parental and consensus genetic maps. In all three maps the order of the markers was consistent and comparable to known reference maps. The parental and consensus framework maps represented complete coverage of the genome based on the markers that are common to other published maps. The summary statistics of both parental and consensus maps are presented in Table 6. Sixteen of thirty-four markers with significant segregation distortion mapped to chromosome 1 (Supplemental Table 1). The flower sex phenotype, as a qualitative marker, mapped to chromosome 2 (Figure 10), the same genomic region as reported in previous studies (Fechter et al., 2012, Riaz et al., 2006, Marguerit et al., 2009).

Table 6. *Summary of the consensus and the two parental genetic framework maps*

Chromosome	Consensus Map			DVIT2027 Map			F2-35 Map		
	No. Markers	cM	Average Distance (cM)	No. Markers	cM	Average Distance (cM)	No. Markers	cM	Average Distance (cM)
1	16	65.89	4.12	16	63.02	3.94	11	74.59	6.78
2	10	35.76	3.58	10	33.15	3.31	3	14.82	4.94
3	6	47.77	7.96	6	47.14	7.86	5	12.81	2.56
4	10	70.82	7.08	10	76.05	7.60	9	46.74	5.19
5	12	28.20	2.35	12	24.10	2.01	9	34.62	3.85
6	13	57.66	4.44	13	58.60	4.51	10	53.75	5.37
7	4	39.97	9.99	4	39.48	9.87	3	12.92	4.31
8	9	48.79	5.42	9	49.51	5.50	5	40.90	8.18
9	18	57.73	3.21	18	53.06	2.95	13	61.64	4.74
10	7	48.13	6.88	7	47.16	6.74	4	26.38	6.59
11	8	49.83	6.23	8	51.21	6.40	5	46.04	9.21
12	8	50.91	6.36	8	46.17	5.77	5	34.63	6.93
13	12	55.94	4.66	12	56.98	4.75	8	42.19	5.27
14	15	63.27	4.22	15	62.16	4.14	9	65.63	7.29
15	4	24.68	6.17	4	19.44	4.86	4	30.10	7.52
16	11	61.56	5.60	11	73.79	6.71	6	15.18	2.53
17	9	50.86	5.65	9	52.71	5.86	6	35.84	5.97
18	14	89.72	6.41	13	92.32	7.10	12	76.19	6.35
19	22	57.94	2.63	22	56.67	2.58	17	54.65	3.21
Total	208	1,005.42	4.83	207	1,002.71	4.84	144	779.61	5.41

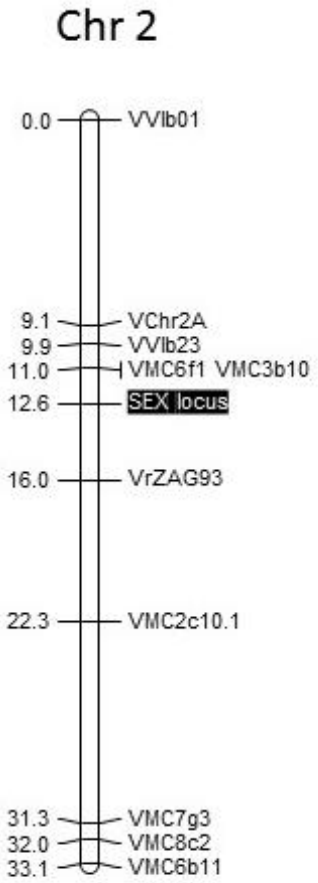


Figure 10. *Localization of Sex locus on the chromosome 2 of the map of* V. piasezkii *DVIT2027 derived from the 11-373 mapping population.*

6.3. QTL-analysis

The QTL analysis was carried out by Interval mapping (IM) and Multiple-QTL Mapping (MQM) using both parental and consensus maps. Significant QTLs were detected on the consensus and DVIT2027 parental maps, but not on the F2-35 map. From hereon we present the QTL results of the male parental map only, since the resistance is derived exclusively from *V. piasezkii* DVIT2027.

The IM analysis identified two resistance loci, the first on chromosome 9 (*Ren6*) and the second on chromosome 19 (*Ren7*). SSR marker PN9-057 and PN9-068 flanked the LOD peak for the *Ren6* locus on chromosome 9. The position of the *Ren6* locus did not change with the method of disease evaluation (Figure 11, Figure 12A; Supplemental Table 2). However, the phenotypic variation explained by the *Ren6* locus varied with the method of

disease evaluation. The maximum variation explained (61.9%) was with the controlled *in vitro* screen method with a LOD 54.3 (Supplemental Table 2). The above-mentioned flanking markers for the *Ren6* locus were used for all subsequent screens for recombinants in additional seedlings of the F1 and pBC1 populations. The IM analysis identified VVIp17.1 and VMC9a2.1 as flanking markers for the *Ren7* locus for the 2013 and 2014 field leaf and cane disease evaluations. However, for the greenhouse, *in vitro* and qPCR assay, the flanking markers were VMC9a2.1 and VMC5h11 (Figure 12B; Supplementary Table 2). The *Ren7* locus explained 19% phenotypic variation with a LOD 11.92 for the cane evaluation from 2014. All three SSR markers (VVIp17.1, VMC9a2.1 and VMC5h11) were used to identify recombinants in additional F1 and pBC1 populations.

Multiple-QTL mapping analysis confirmed the two previously identified loci with the IM approach (Figure 12E). The automatic cofactor selection procedure identified the PN9-068 marker as a cofactor for all disease evaluation approaches except for the 2013 field data for the *Ren6* locus. With the PN9-068 marker as a cofactor, phenotypic variation explained by the *Ren6* locus varied across the method of disease evaluation. A maximum of 62% variation was also observed for *in vitro* analysis (Supplementary Table 2) with LOD 66.28. For the *Ren7* locus on chromosome 19, the VVIu09 marker was selected as a cofactor for the greenhouse and *in vitro* assay and VMC5h11 was used for the qPCR analysis. Both markers are closely linked and are only 0.9 cM apart on the map (Supplemental Figures 1A-D). A maximum of 18.1% variation was observed for the 2014 cane screen with LOD 14.55. The detailed results of IM and MQM are presented in Supplemental Table 2. The alleles of SSR markers that are linked to the *Ren6* and *Ren7* loci are presented in Tables 7A and 7B.

To study the effect of each locus independently, F1 progeny were divided into groups based on the presence of *Ren6* and *Ren7* haplotypes. All genotypes with the *Ren6* linked allele (PN9-068, 174bp) were removed from the datasets, and IM analysis was applied to the remaining genotypes that theoretically only segregated for *Ren7*. The IM analysis in the absence of *Ren6* boosted the impact of the *Ren7* locus to 71.9% explained variation at LOD 35.58 with the greenhouse screen data. The IM analysis was also performed inversely, with genotypes containing the *Ren7* linked allele (VMC9a2.1, 163bp) removed from the genotype file. The *Ren6* locus explained as much as 95.4% of the phenotypic variation (LOD 95.76) in the absence of *Ren7* (Figure 12C-D; Supplemental Table 2C).

To further demonstrate that there were no other genetic factors contributing to powdery mildew resistance, the dataset was reanalyzed following removal of all genotypes with alleles linked to *Ren6* or *Ren7*. Interval mapping on this artificial data set did not reveal any other significant QTLs.

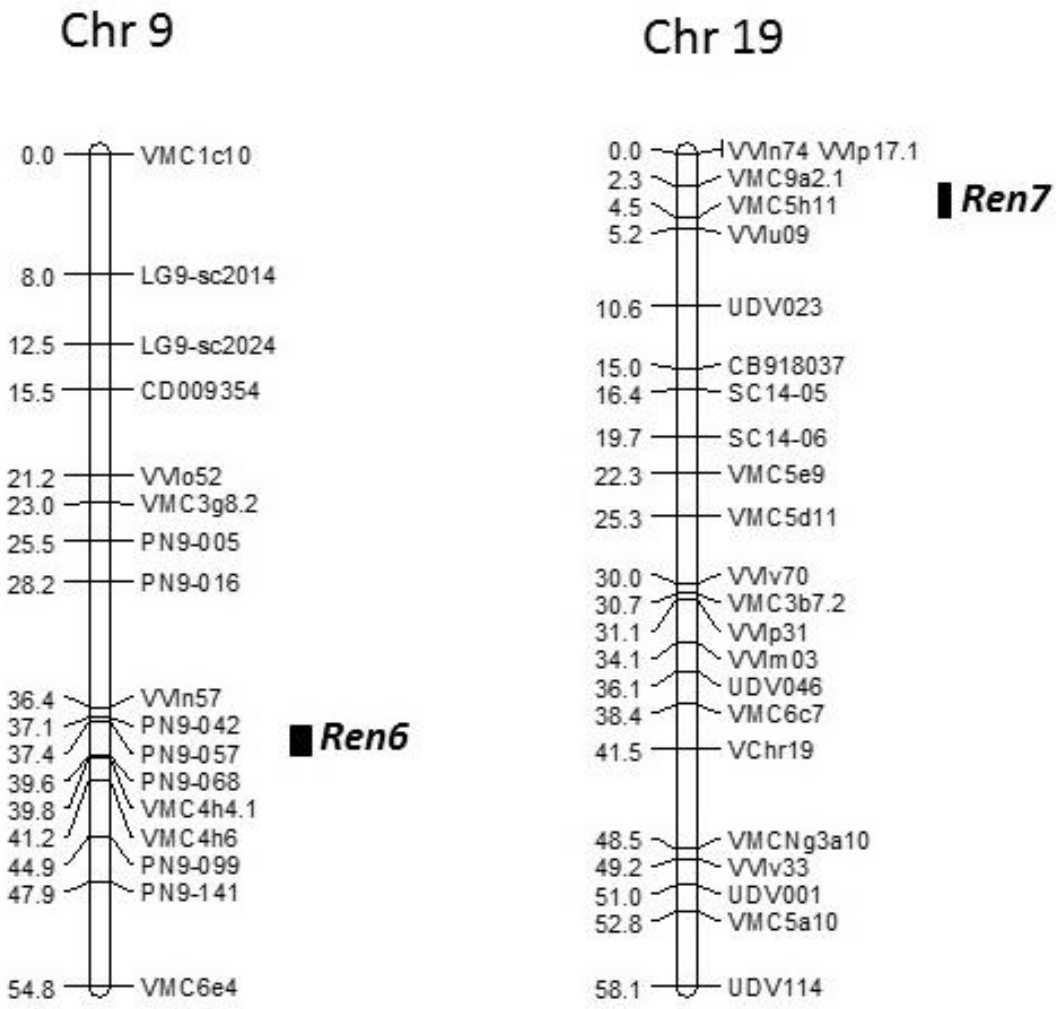


Figure 11. *Genetic location of the identified Ren6 and Ren7 loci on chromosome 9 and chromosome 19 respectively. Ren6 is flanked by makers PN9-057 and PN9-068 spanning 2.2 cM distance. Ren7 is flanked by VMC5h11 and VMC9a2.1 spanning 2.2 cM distance as well.*

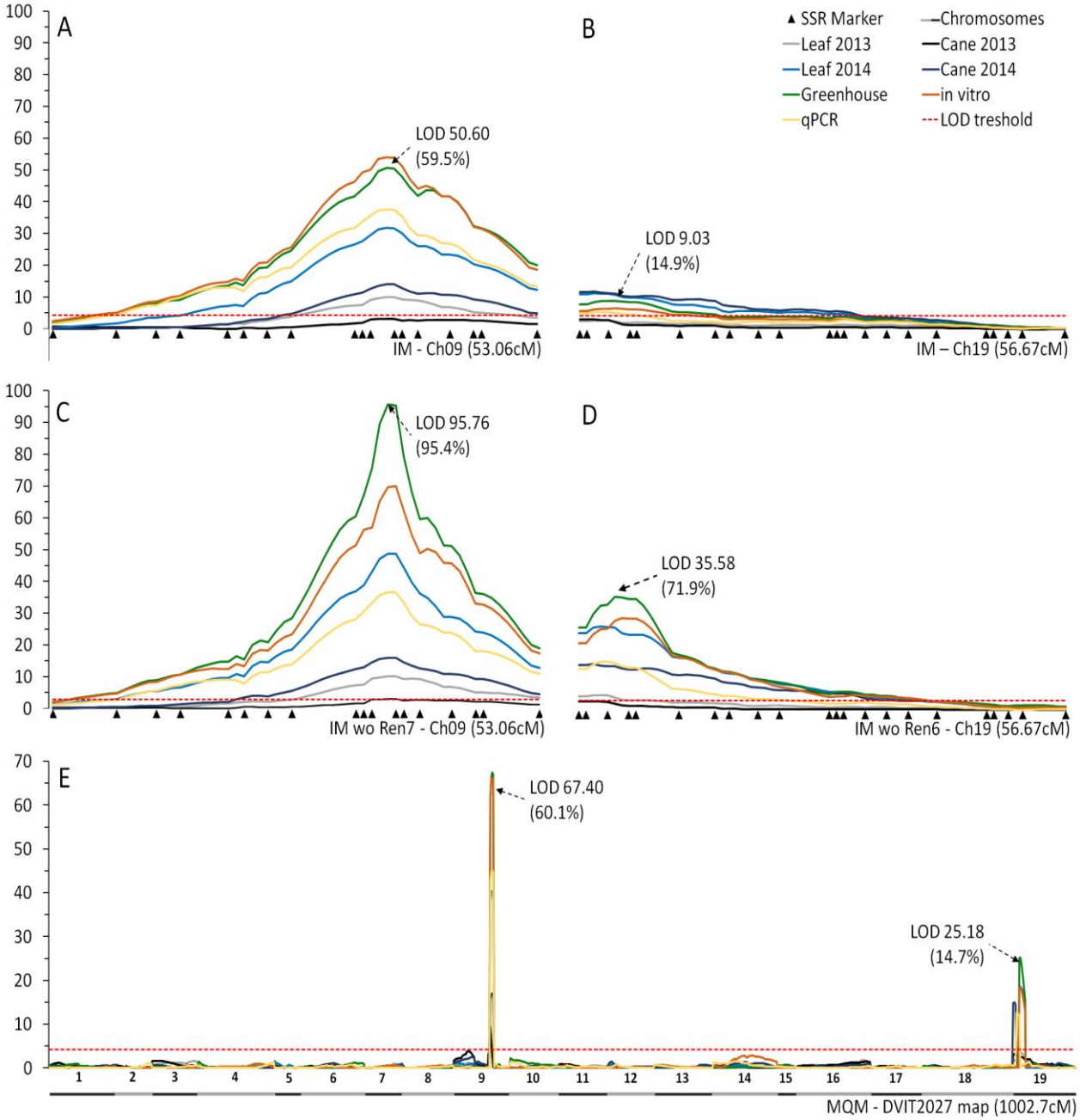


Figure 12. *Identification of Ren6 and Ren7 loci with interval and multiple QTL mapping. (A, B) Results of interval mapping carried out on entire base population for chromosome 9 and 19. (C, D) Interval mapping analysis on subset of genotypes that are selected based on the local haplotype of Ren6 or Ren7 locus, respectively. (E) Results of Multiple QTL mapping on the nineteen chromosomes of DVIT2027. Leaf 2013, Leaf 2014 and Cane 2013, Cane 2014 represent the disease evaluations carried out in the field for the respective year. Greenhouse and in vitro assays were carried out in controlled environments. In all charts the arrow represents the maximum LOD score and the respective percent-explained variation of the greenhouse assay. The red dotted line represents the significance threshold for QTL detection. In all charts the arrow represents the maximum LOD score and the respective percent explained variation of the greenhouse screen for powdery mildew resistance*

6.4. Independent assortment of Ren6 and Ren7 loci in terms of powdery mildew resistance

The two newly identified loci, *Ren6* on chromosome 9 and *Ren7* on chromosome 19, segregate independently of each other and generated four classes of genotypes (*Ren6*$^{+}$/*Ren7*$^{+}$, *Ren6*$^{+}$/*Ren7*$^{-}$, *Ren6*$^{-}$/*Ren7*$^{+}$ and *Ren6*$^{-}$/*Ren7*$^{-}$) (Figure 13). Theoretically, we should expect equal ratios of four phenotypic classes in the F1 progeny since the female parent is susceptible to powdery mildew, lacks both loci and does not contribute any minor genes for resistance. The ratio of the four genotypes was 63:73:61:79 (χ^2=3.245 p=0.3553) confirming that both loci segregate independently of each other and followed Mendel's second law of inheritance. A Tukey's test significantly separated the phenotypic scores of susceptible progeny (*Ren6*$^{-}$/*Ren7*$^{-}$) from the genotypes that have either *Ren6* or *Ren7* or both loci. Significant phenotypic differences were detected between *Ren6*$^{+}$/*Ren7*$^{+}$ or *Ren6*$^{+}$/*Ren7*$^{-}$ and the *Ren6*$^{-}$/*Ren7*$^{+}$ genotypes, in the 2014 field leaf scores, greenhouse, *in vitro,* and qPCR evaluations (Figures 14A-B). These differences were also clearly illustrated by the powdery mildew development observed on leaves from the different genotypes in the *in vitro* assay. Inoculated leaves were harvested 5 dpi and stained with Coomassie Brilliant blue to visualize the development of fungal structures on the leaf surface (Figure 13). Genotypes lacking both *Ren6* and *Ren7* (Figure 13A) showed extensive hyphal growth and conidiophore development after 5 dpi. On *Ren6*$^{-}$/*Ren7*$^{+}$ genotypes (Figure 13B), secondary hyphae were clearly visible on the leaf surface but the density was markedly reduced compared to the fully susceptible *Ren6*$^{-}$/*Ren7*$^{-}$ genotypes. In a very few cases, minor amounts of conidiophore formation was observed on some *Ren6*$^{-}$/*Ren7*$^{+}$ leaves at 14 dpi in the *in vitro* assay represented by the bars on Figure 14B. Powdery mildew development on leaves of genotypes containing *Ren6*$^{+}$/*Ren7*$^{-}$ and *Ren6*$^{+}$/*Ren7*$^{+}$ was very similar (Figure 13C-D , Figure 14B) with little or no secondary hyphae development. The disease symptoms on canes were not significantly different between *Ren6*$^{+}$/*Ren7*$^{+}$ or *Ren6*$^{+}$/*Ren7*$^{-}$ and Ren6^{-}/Ren7^{+} for both years in the field. There was no separation of *Ren6*$^{+}$/*Ren7*$^{+}$ and *Ren6*$^{+}$/*Ren7*$^{-}$ from each other with the 2013 and 2014 leaf scores, greenhouse screen, *in vitro* screen and qPCR results confirming the strong influence of the *Ren6* locus on the phenotype (Figure 14B).

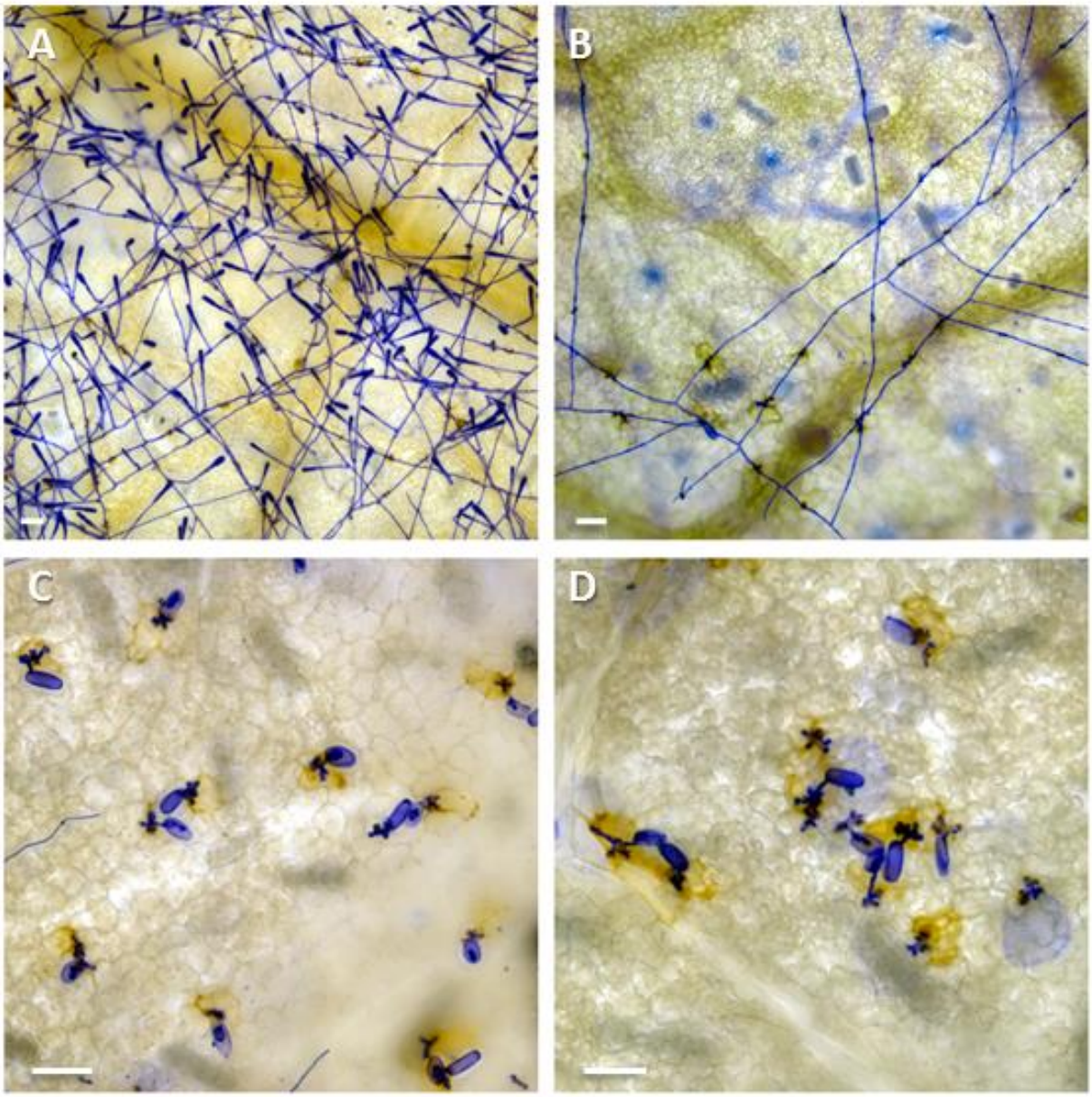


Figure 13. *Comparative development of powdery mildew on in vitro leaves of genotypes containing different combinations of R loci introgressed from* Vitis piasezkii. *Detached leaves were inoculated with powdery mildew using a settling tower, harvested 5 dpi and stained with Coomassie Brilliant blue to visualize the development of fungal structures on the leaf surface. (A) Ren6^{-}/Ren7^{-}, (B) Ren6^{-}/Ren7^{+}, (C) Ren6^{+}/Ren7^{-}, and (D) Ren6^{+}/Ren7^{+} genotypes. The brown cells beneath the appressoria of germinated fungal spores are indicative of the hypersensitive response induced by the R loci. Scale bars represent 50µm.*

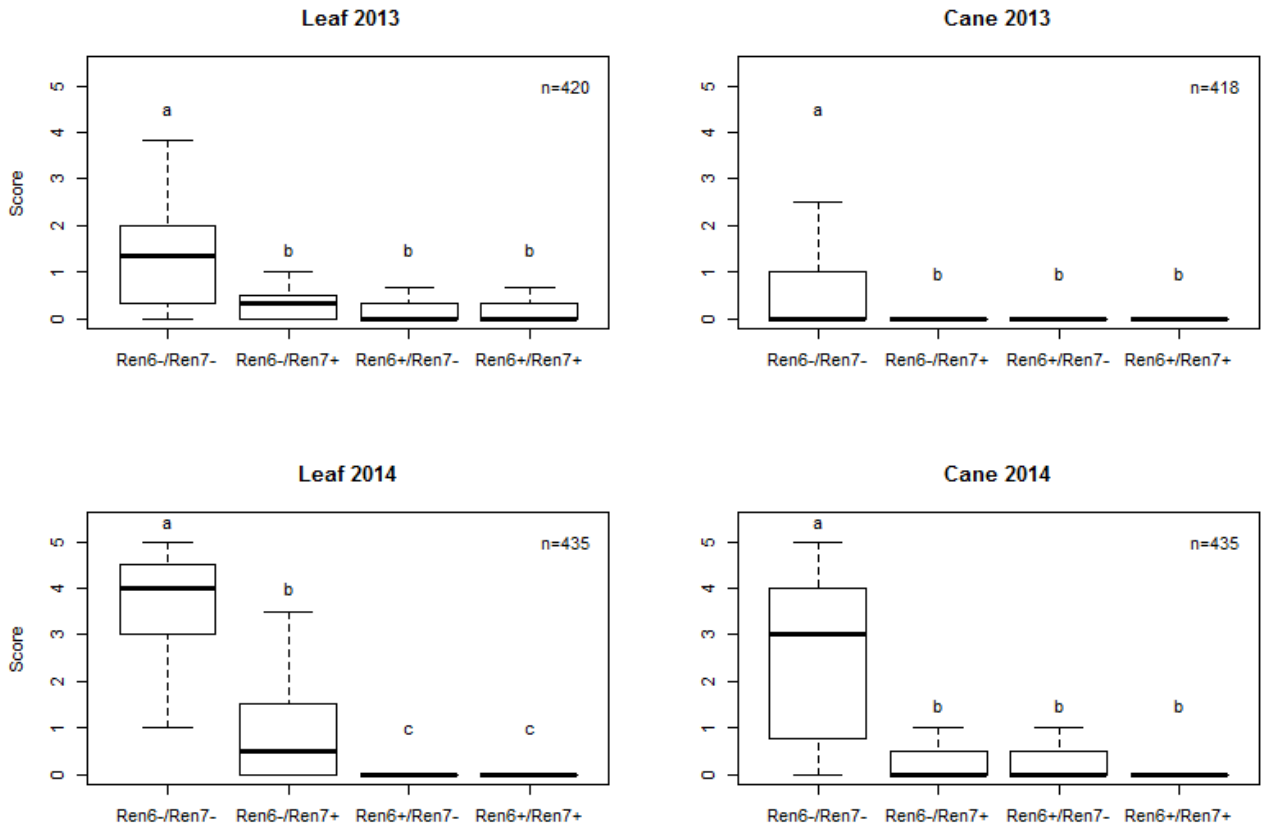


Figure 14A. *Summary of powdery mildew susceptibility of the four genotypic classes within the F1 population. Susceptibility was assessed on leaves and canes of field-grown vines in 2013 using natural powdery mildew inoculations. A 6-point rating scale, 0 (no visible symptoms) to 5 (powdery mildew covers all tissue of interest) was used to determine powdery mildew susceptibility for field evaluations in 2013 and 2014. Significant differences detected with Tukey's test are indicated with different letters. The letter 'n' denotes the number of genotypes used for analysis in each of the disease evaluation method.*

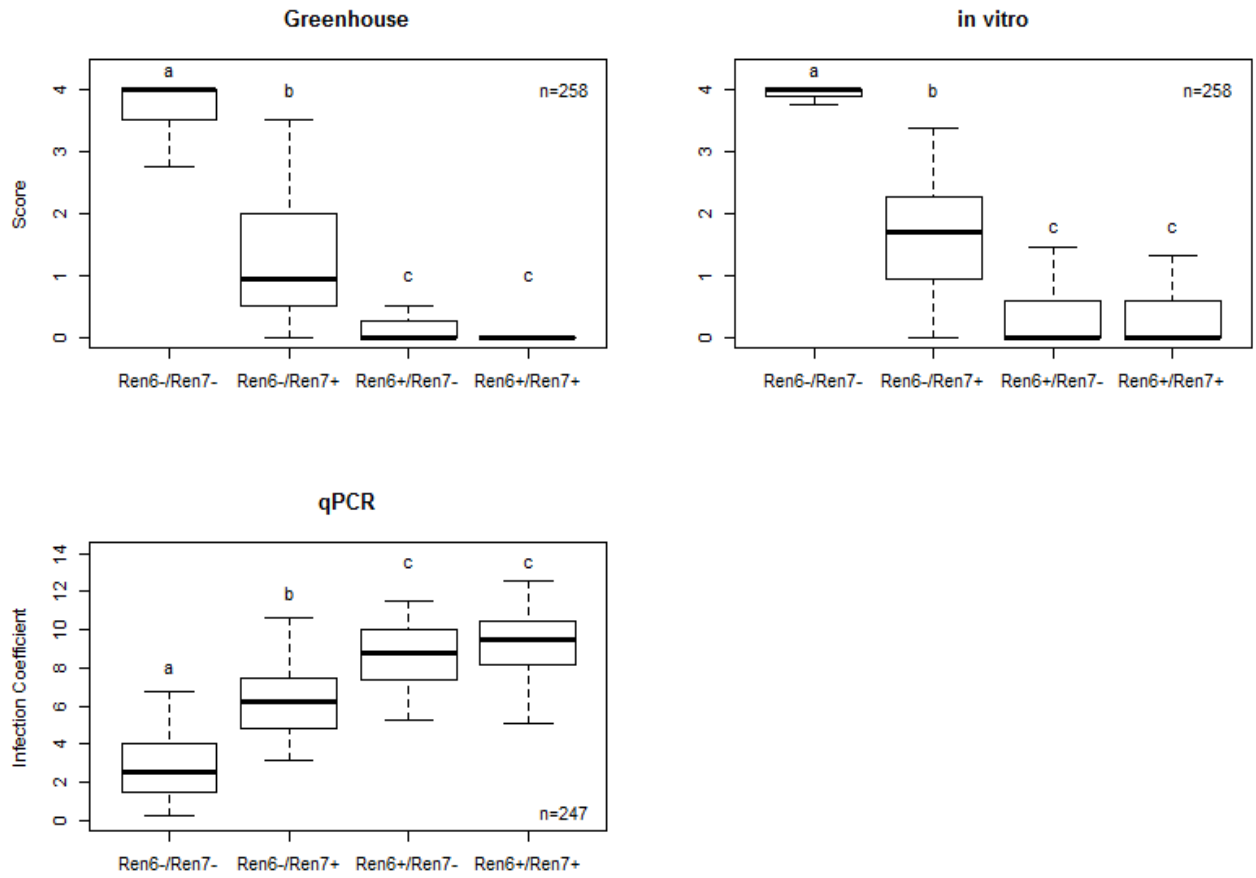


Figure 14A. *Summary of powdery mildew susceptibility of the four genotypic classes within the F1 population. Progeny was evaluated in the greenhouse plants and with detached leaves in vitro assays. Powdery mildew susceptibility was rated using a 5-point scale, 0 (no symptoms) to 4 (powdery mildew covers majority of the leaves) in the greenhouse and in vitro assays. Significant differences detected with Tukey's test are indicated with different letters. The letter 'n' denotes the number of genotypes used for analysis in each of the disease evaluation method. The E. necator biomass was measured by the qPCR. Plotted infection coefficients correspond to natural logarithm-transformed* $2^{-\Delta CT}$ *values. The higher value indicates less biomass accumulation.*

6.5. Characterization of the Ren6 and Ren7 resistance response

There is clear evidence from Figure 13 of a hypersensitive response (HR) to powdery mildew inoculation in genotypes containing either *Ren7* (Figure 13B) or *Ren6* (Figure 13C). In the case of *Ren7* this was mainly associated with epidermal cells penetrated by appressoria on developing secondary hyphae, whereas in *Ren6* genotypes the HR appeared to be more pronounced and was associated with appressoria of germinated spores. This HR is most likely the result of the penetrated epidermal cells undergoing PCD following recognition of specific avirulence effectors secreted by the invading powdery mildew pathogen (Qiu et al., 2015). However, the strength or speed of the PCD response and its effectiveness in restricting hyphal development appears to differ significantly between *Ren6* and *Ren7*.

To further investigate these differences and to enable us to compare the PCD response mediated by the two *R* loci from *V. piasezkii* to the previously characterized *Run1* locus, all three *R* loci were introduced into the same genetic background by crossing with the powdery mildew-susceptible *V. vinifera* 'Pinot Meunier' mutant picovine (Chaib et al., 2010) and disease phenotypes were observed in response to a grapevine powdery mildew isolate from Australia.

A small F1 population (VpF1) of 31 progeny was generated from the cross of DVIT2027 with picovine line 06C008V0003. The *Ren6* marker PN9-067 and the *Ren7* markers VMC9a2.1 and VMC5h11 were found to be informative in this cross and used to genotype the progeny. Percent induction of PCD in penetrated epidermal cells was measured 2 dpi using the vital stain trypan blue that is only taken up by dead plant cells (Feechan et al., 2015). Figure 15A shows that the powdery mildew resistance response mediated by both *Ren6* and *Ren7* involves the induction of PCD in penetrated epidermal cells. This observation confirms that these two *R* loci from *V. piasezkii* are able to recognize powdery mildew isolates from both California and Australia. It also indicates that the PCD-based resistance response mediated by *Ren6* is stronger or more rapid than that mediated by *Ren7*. This is confirmed by the results of a separate study that compared powdery mildew induced PCD induction in selected lines of *Ren6*$^{-}$/*Ren7*$^{+}$, *Ren6*$^{+}$/*Ren7*$^{-}$ and *Run1* F1 progeny in the same genetic background (Figure 15). The results are presented in terms of the percentage of fungal penetrated epidermal cells that have either undergone either effective PCD (no secondary hyphae formation), ineffective PCD (secondary hyphae formation still occurs) or no PCD. The VpF1 progeny containing *Ren6* displayed a very high incidence of effective PCD (i.e. >93%) in penetrated epidermal cells leading to complete suppression of secondary

hyphae formation. In contrast, VpF1 progeny containing *Ren7* displayed much lower levels of effective PCD (<22%) in penetrated cells and much higher levels of ineffective PCD (28-65%), which resulted in much greater levels of secondary hyphal growth. Based on these results, *Ren6* appears to mediate a more rapid/stronger PCD response to powdery mildew infection than *Run1*, while the *Ren7* mediated response is slower/weaker than *Run1*.

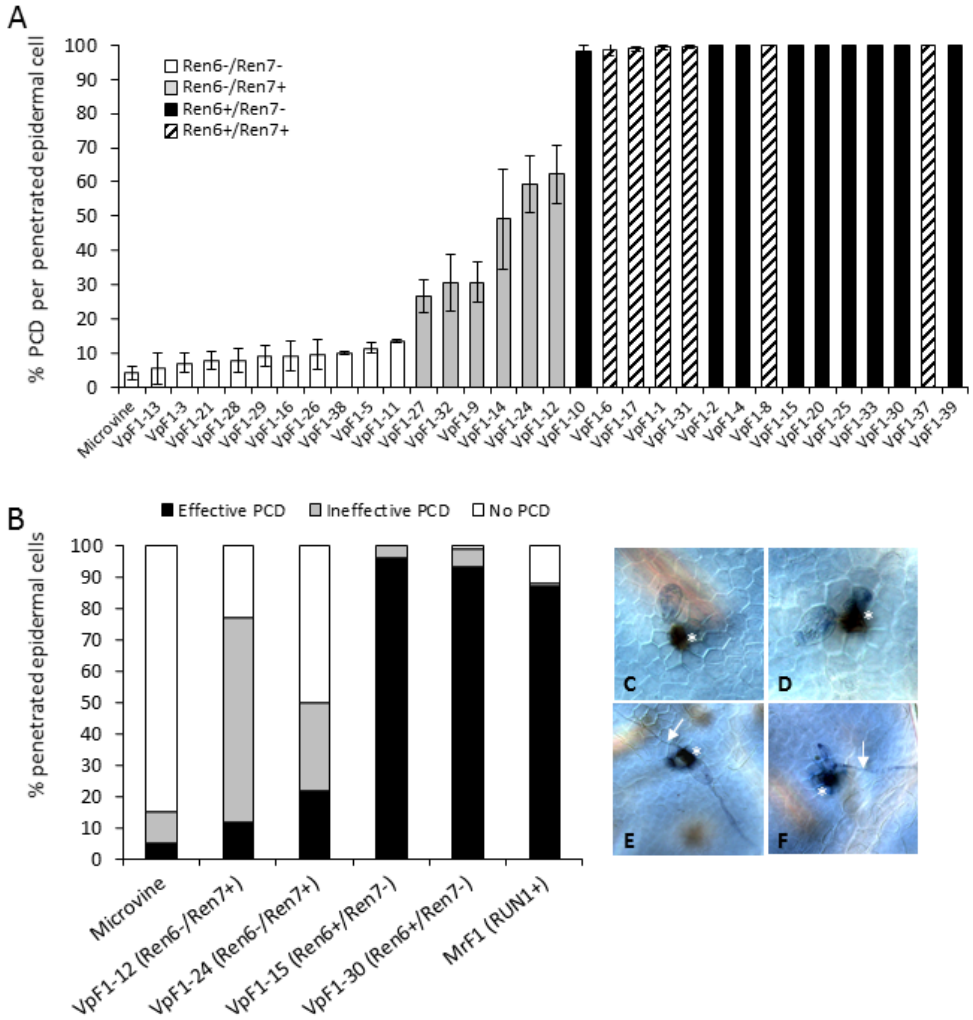


Figure 15. *Comparison of PCD (programmed cell death) induction kinetics in Ren6 and Ren7 genotypes. All data and micrographs were collected 2 dpi and at least 100 germinated spores were scored following trypan blue staining for estimation of PCD. (A) Relative levels of PCD in powdery mildew-penetrated epidermal cells of an F1 microvine population segregating for Ren6 and Ren7. Each data point is the mean ±SE of at least two biological replicates. (B) Proportion of penetrated epidermal cells that show either effective PCD (no secondary hyphae), ineffective PCD (secondary hyphae produced) or no PCD following powdery mildew penetration in four individual VpF1 lines shown in (A). For comparison, two additional microvine lines were included – a susceptible line lacking any R genes and a resistant line containing the Run1 locus. Results are shown from one experiment, but the experiment was repeated twice with the same results. (C-F) Micrographs showing examples*

of effective PCD (C, D) and non-effective PCD (E, F). Epidermal cells that have undergone PCD, as shown by the uptake of trypan blue, are indicated with an asterisk while secondary hyphae are indicated by white arrows.

6.6. Search for additional key recombinants

The screening of additional genotypes of the F1 population 11-373 and the four derived pBC1 populations with markers linked to *Ren6* and *Ren7* loci allowed the identification of additional recombinant genotypes. In the 2.2 cM genetic window of the *Ren6* locus (between PN9-057 and PN9-068) 13 recombination events were identified from 1,169 seedlings. To further refine the 2.3 cM wide genetic window of the *Ren7* locus, 917 seedlings were evaluated with flanking markers. Nine recombinants were found in the F1 population (n=536), five of them lacking the *Ren6* locus. In addition, two pBC1 populations (n=386) were screened within a wider genetic window because of the homozygosity of the VMC9a2.1 marker in the resistant pBC1 parents. Thirteen recombinants were identified; 12 of them did not possess *Ren6*. The haplotype and phenotype of recombinants for both loci are summarized in Tables 7A and 7B.

In the refined genetic map based on the additional recombinant genotypes, the *Ren6* locus resides between markers PN9-066.1 and PN9-067 (Table 7A). The physical distance between these two markers in the PN20024 genome sequence is 22 kb. The refined genetic map of the *Ren7* locus consisted of two new microsatellite markers (PN19 series, Table 1). The *Ren7* locus resides between PN19-022 and VMC5h11 and the corresponding physical distance between these two markers in the PN20024 genome sequence is 330 kb (Table 7B).

Table 7A. *Allelic profiles of Ren6 (A) flanking markers on chromosome 9.*

ID	PN9-057[a]	PN9-063	PN9-066.1	*Ren6*	PN9-067	PN9-067.2	PN9-068	VMC4h4.1	*in vitro* PM	*Ren7*
F2-35	185/187	170/188	390/434	-	284/290	303/303	null/null	247/251	4.00	-
Malaga Rosada	185/185	188/null	387/434	-	284/292	303/303	180/null	211/251	4.00	-
DVIT2027	190/204	166/174	402/408	+	281/288	314/320	171/174	178/229	0.00	+
11373-473	-	+	+	+	+	+	+	+	0.50	-
11373-483	-	+	+	+	+	+	+	+	0.00	-
11373-014	-	-	+	+	+	+	+	+	0.25	-
11373-094	-	-	+	+	+	+	+	+	0.50	+
11373-128	-	-	+	+	+	+	+	+	0.00	-
11373-390	-	-	+	+	+	+	+	+	0.00	-
11373-148	-	-	-	-	-	-	+	+	1.75	+
11373-245	-	-	-	-	-	-	+	+	1.58	+
11373-276	-	-	-	-	-	-	-	+	4.00	-
11373-497	-	-	-	-	-	-	-	-	2.38	+
13350-357	+	+	+	+	+	-	-	-	0.00	-
13351-057	+	+	+	-	-	-	-	-	4.00	-
13350-055	+	+	-	-	-	-	-	-	4.00	-
13351-020	+	+	-	-	-	-	-	-	4.00	-

***Table 7B**. Allelic profiles of Ren7 (B) flanking markers on chromosome 19*

ID	VVin74	VVip17.1	PN19-018	VMC9a2.1	PN19-022	*Ren7*	VMC5H11	VVIu09	*in vitro* PM	*Ren6*
F2-35	278/278	77/77	null/null	163/163	274/null	-	195/198	95/97	4.00	-
Malaga Rosada	278/288	77/87	null/180	163/163	261/274	-	181/195	95/97	4.00	-
DVIT2027	278/280[b]	77/79	null/187	163/165	268/268	+	175/200	99/104	0.00	+
13352-012	-	-	+	ud[c]	+	+	+	+	0.50	+
11373-497	-	-	-	+	ud	+	+	+	2.38	-
14353-026	-	-	-	ud	+	+	+	+	0.81	-
14353-028	-	-	-	ud	+	+	+	+	0.83	-
11373-186	-	-	-	-	ud	+	+	+	2.25	-
11373-415	-	-	-	-	ud	ud	+	+	0.00	+
13352-025	-	-	-	ud	-	-	+	+	4.00	-
11373-471	+	+	+	+	ud	+	-	-	1.88	-
14353-213	+	+	+	ud	+	+	-	-	2.00	-
11373-001	+	+	+	+	ud	ud	-	-	0.00	+
11373-008	+	+	+	+	ud	-	-	-	3.75	-
11373-075	+	+	+	+	ud	ud	-	-	0.13	+
11373-088	+	+	+	+	ud	ud	-	-	0.00	+
11373-150	+	+	+	+	ud	-	-	-	3.50	-
13352-004	+	+	+	ud	-	-	-	-	4.00	-
13352-015	+	+	+	ud	-	-	-	-	4.00	-
14353-126	+	+	+	ud	-	-	-	-	3.66	-
14353-151	+	+	+	ud	-	-	-	-	4.00	-
14353-082	+	+	-	ud	-	-	-	-	4.00	-
14353-086	+	+	-	ud	-	-	-	-	3.25	-
14353-214	+	+	-	ud	-	-	-	-	4.00	-
14353-223	+	+	-	ud	-	-	-	-	4.00	-

[a]Underlined marker names are included in the framework map.

[b]Alleles shown in bold represent the resistant haplotypes.

[c]Undetermined is shown as 'ud'.

6.7. Genotyping and phenotyping of additional **V. piasezkii** *accessions*

The detached leaf *in vitro* assay was carried out on nine additional accessions of *V. piasezkii* maintained at the USDA National Clonal Germplasm Repository, Davis, California. Eight of these accessions were collected from the Shennongjia Forestry District, and one from an undetermined location in China (Figure 5). SSR markers linked to the *Ren6* and *Ren7* loci were used to genotype these accessions to identify other similar haplotypes based on their genotypic and phenotypic profiles. The results of genotyping and phenotyping of these accessions are presented in Table 8. The *in vitro* test was carried out with the C-isolate only and identified seven accessions that exhibited varying levels of resistance to PM. Three accessions (DVIT2026, DVIT2028, and DVIT2032) were resistant to PM in the detached leaf assay and were positive for the SSR marker allele(s) that linked the *Ren6* locus in DVIT2027 (Table 8.). Interestingly, DVIT2026 had both the *Ren6* and *Ren7* linked SSR marker alleles and showed complete immunity to PM in the *in vitro* assay. Five accessions had SSR marker allele(s) that were linked to the *Ren7* locus in DVIT2027 (Table 8.). Unweighted neighbor joining analysis placed DVIT2026 and DVIT2027 in the same clade indicating that they are closely related to each other. Two other *Ren6* like haplotypes (DVIT2028 and DVIT2032) were in different clades; both of them were collected from different sites in the Shennongjia Forestry District (Figure 16). The accessions similar to the *Ren7* haplotype based on the linked markers showed variation in PM infection with symptoms ranging from 1.33 to 2.13 (Table 8.).

Table 8. *Genetic characterization of* Vitis piasezkii *accessions used in this study. Local haplotype of V. piasezkii accessions with SSR markers and results of in vitro powdery mildew resistance evaluations. Collection site # within the Shennongjia Forest as cited in Bartholomew et al (1983). Alleles that are linked with the resistance loci are bold and underlined.*

ID	Site #	*in vitro*	*Ren6*	*Ren7*	PN9-066.1	PN9-067	PN9-068	VMC4h6	VMC9a2.1	PN19-022	VMC5h11
DVIT2027	34	0.00	+	+	**402**/408	281/**288**	171/**174**	**156**/160	**164**/166	**268**/268	175/**200**
DVIT2026	34	0.00	+	+	**402**/402	**288**/297	**174**/190	150/**156**	**164**/166	**268**/268	197/**200**
DVIT2028	29	0.30	+	nd	365/398	**288**/300	174/**174**	154/**156**	166/166	262/**268**	185/197
DVIT2029	29	3.40	-	-	365/405	**288**/292	171/171	160/166	144/169	**268**/286	175/177
DVIT2031	20	1.90	-	+	386/398	286/295	na/na	148/158	166/166	268/286	**200**/210
DVIT2032	20	0.50	+	nd	367/395	288/292	171/**174**	**156**/158	144/166	**268**/274	na/na
DVIT2033	7	1.57	-	+	400/418	287/300	171/171	158/160	152/**164**	262/267	200/**200**
DVIT2034	7	2.13	-	+	412/418	286/300	171/190	154/160	144/**164**	262/270	175/**200**
DVIT2596.01	7	1.33	-	+	394/398	295/303	174/**174**	154/**156**	143/143	**268**/274	175/**200**
DVIT1453	nd	4.00	-	-	379/379	292/292	171/**174**	146/162	144/166	**268**/271	185/202

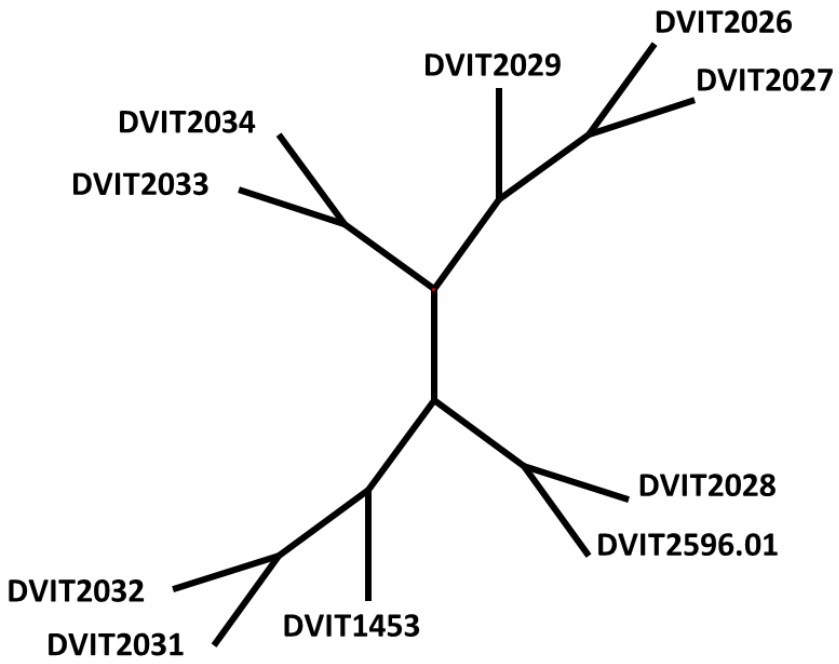


Figure 16. *Unweighted neighbor joining tree derived from local haplotypes of SSR markers (Table 8) of the* Vitis piasezkii *accessions used in this study.*

7. CONCLUSIONS

7.1. Vitis piasezkii *has two unique loci to restrict powdery mildew infection*

In this study we (i) explored powdery mildew resistance in ten accessions of the Chinese species, *V piasezkii*, (ii) developed F1 and pBC1 breeding populations with a single resistance source and (iii) identified two loci *Ren6* and *Ren7* on different chromosomes, chromosome 9 and chromosome 19, respectively. Powdery mildew resistance has not been found to be associated with these chromosomes in previously published studies (Dalbó et al, 2001, Hoffmann et al, 2008, Welter et al, 2007, Coleman et al, 2009, Riaz et al, 2011, Ramming et al, 2012, Blanc et al, 2012, Feechan et al, 2015). The identification of *Ren6* and *Ren7* loci was supported with disease evaluation data obtained from multiple environments. Field evaluations for both leaf and cane symptoms were carried out for two consecutive years without fungicide applications, and this data was confirmed by assays in the greenhouse, *in vitro* on detached leaves, and with qPCR assays. In general, field evaluation results may vary from year to year depending on the inoculum pressure which is strongly influenced by the weather, population biology and strain composition for any given year within a vineyard (Montarry et al., 2008). In agreement with previous reports, we observed that the maturity of the plants plays a role in the variation of disease severity (Barba et al., 2015). We also observed variation in the field evaluation results between the two years of data collection that was reflected in the different values of phenotypic variation explained by both loci in QTL analysis (Figure 12; Supplemental Table 2). Nonetheless, we identified the *Ren6* and *Ren7* loci with significant LOD scores in the same genomic regions, independently of the type of phenotypic data used for the analysis. Identification of two *R* loci for the same pathogen that segregate independently of each other is novel for grape, but has been reported for other crops. For example, two loci were identified for potato virus X (PVX) resistance in potato (Ritter et al., 1991), brown planthopper (*Nilaparvata lugens* Stål.) resistance in rice (Huang et al., 2001), clubroot (*Plasmodiophora brassicae* Woronin.) resistance in *Brassica oleracea* and in *B. rapa* (Voorrips et al., 1997, Suwabe et al., 2003).

We observed four genotypic classes with a 1:1:1:1 segregation in the progeny. However, the phenotypic scores statistically divided into three groups for the leaf data from the field in 2014, the controlled greenhouse assay, *in vitro* scoring, and qPCR results. This was a result of the difficulty in separating the genotypes that carry *Ren6*$^{+}$/*Ren7*$^{+}$ and *Ren6*$^{+}$/*Ren7*$^{-}$ due to the strong effect of the *Ren6* locus on the phenotype. Interestingly, for the cane evaluations

for both years, the *Ren6*$^{-}$/*Ren7*$^{+}$ phenotype was also not distinguishable from the two resistant genotypic classes of *Ren6*$^{+}$/*Ren7*$^{+}$ and *Ren6*$^{+}$/*Ren7*$^{-}$ (Figure 14A). These results indicate that under normal field conditions, the *Ren7* locus could provide effective resistance to cane tissue against the powdery mildew infection. Variation in the level of the powdery mildew resistance between different tissue types was observed in an earlier study (Riaz et al., 2011). However, no information is available about the underlying factors that might contribute to this variation.

7.2. Ren6 and Ren7 confer resistance at the post-penetration phase

There is a significant amount of diversity and variation for powdery mildew disease resistance within the grape genome as demonstrated by the identification of numerous *R* loci from a wide range of wild *Vitis* species. Prior to this work, seven *R* loci had been mapped in different *Vitis* species from North America as well as Central Asia and China (see review by Qiu et al. (2015)). Among all of the loci mapped, the exact position and identity of the gene conferring the resistance at the locus has only been resolved for the *Run1* locus (Qiu et al., 2015, Feechan et al., 2013). The *Run1* locus was found to comprise a family of seven putative Toll/interleukin-1 receptor (TIR)-NB-LRR-type *R* genes, one of which designated *MrRUN1* was found to confer strong resistance in transformed *V. vinifera* cultivars that were otherwise susceptible to the PM infection (Feechan et al., 2013). The *MrRUN1* gene confers resistance via the rapid induction of PCD in penetrated epidermal cells, restricting the availability of nutrients for further growth and development of the fungus. Other powdery mildew *R* loci such as *Run2*, *Ren1* and *Ren2* exhibit a lower frequency of PCD of penetrated cells compared to *Run1* allowing more extensive secondary hyphal development (Feechan et al., 2015). In the case of *Ren1*, the fungus is able to obtain sufficient nutrition to complete its life cycle, although the level of sporulation is approximately 10-fold lower than that observed on susceptible genotypes (Hoffmann et al., 2008).

It is clear that, like *Run1*, both the *Ren6* and *Ren7* loci from *V. piasezkii* confer resistance to powdery mildew through the induction of PCD following fungal penetration (Figure 15). However, the speed and/or strength of PCD induction vary markedly between these two loci. In the presence of *Ren6*, PCD induction is extremely rapid with 92-95% of epidermal cells displaying effective PCD i.e. no development of secondary hyphae, after 2 dpi (Figure 15). The *Ren6* resistance response is even stronger than that mediated by the *Run1* locus in the same genetic background (Figure 15). In contrast, the resistance response of *Ren7* genotypes is much slower than *Ren6* resulting in a high percentage of penetrated epidermal cells in

which either no PCD is observed or the PCD induction can be considered ineffective because the fungus is still able to produce a secondary hyphae (Figure 13-14). What is responsible for the differences in the speed or strength of the post-penetration PCD induction mediated by each of these different R proteins? One possibility is that each of these proteins recognizes different core effectors secreted by *E. necator* and that these effectors are secreted at different stages during the infection process or at markedly different levels. A second possible explanation is that the differences in speed or strength of PCD induction is a reflection of differences in the steady state level of the R protein within the grape leaf epidermal cells. A good demonstration of the influence of R protein levels on the kinetics of the resistance response comes from work on the barley powdery mildew resistance gene MLA12. Shen and collegaues (2003) were able to convert the slow-acting resistance response of MLA12 into a rapid response by over-expression of MLA12 in barley cells with a strong ubiquitin promoter, suggesting that cellular amounts of MLA12, or protein complexes containing MLA12, are rate limiting for the onset or speed of the resistance response.

7.3. Presence of PM resistance in Central Asia and China

Vitis piasezkii is the second Chinese species known to confer strong resistance to powdery mildew for which the *R* locus has been mapped. Powdery mildew resistance was previously mapped to the *Ren4* locus in *V. romanetii* (Riaz et al., 2011, Ramming et al., 2012). Many Central Asian cultivated and wild accessions of *V. vinifera* spp. *sylvestris*, the progenitor of the cultivated *V. vinifera* spp. *sativa*, were also identified to carry partial resistance to the PM (Hoffmann et al., 2008, Coleman et al., 2009, Riaz et al., 2013a, Amrine et al., 2015). The presence of strong resistance to powdery mildew in Asian *Vitis* species appears to be at odds with the current theory regarding the co-evolution of *E. necator* on wild North American grapevines and its subsequent introduction into Europe and to the rest of the world in the mid-nineteenth century (Brewer and Milgroom, 2010). Such a time frame would clearly have been insufficient time, in evolutionary terms, for resistance to develop in the Asian *Vitis* species (Riaz et al., 2013b).

The presence of two different *R* loci to avoid powdery mildew infection is another intriguing aspect that poses more questions. Did these loci evolve independently of each other, or was one derived from the other? The possible answer to this question lies in the comparative sequence analysis of the genomic regions carrying these loci to other sequenced grape genomes. No significant disease resistance-related candidate genes were identified in

22 kb and an expanded 60 kb corresponding genomic region for the *Ren6* locus and a 330 kb region for the *Ren7* locus in the susceptible *V. vinifera* PN40024 (12X.1) reference genome sequence (Supplemental Figure 2). It is most likely that the genetic and physical distances between the Chinese origin *V. piasezkii* and European origin PN40024 does not correlate with each other as we have identified recombinants with the flanking markers for both loci (Tables 7A and 7B). The other hypothesis is that this region is potentially different between two accessions and *V. piasezkii* possesses unique genes that are not present in PN40024 genome sequence. The corresponding regions for both loci in PN40024 genome sequence had large clusters of retro and DNA-transposable elements that are a trademark to be present around and between clusters of disease resistance genes (Coleman et al., 2009). The physical maps for the *Ren6* and *Ren7* loci are the next step forward that would allow direct comparisons to other susceptible genomes and to identify and examine gene structure for these loci.

The other *V. piasezkii* accessions acquired from the Shennongjia Forestry District contained either *Ren6* or *Ren7* or both loci haplotypes further demonstrating that powdery mildew resistance is wide spread (Figure 5, Table 8.). It would be interesting to collect more accessions of *V. piasezkii* from its native habitat in Northeast and Western China to carry out comparative disease evaluations aimed at identifying other accessions with similar or different loci. Such efforts would help to capture the maximum genetic diversity of powdery mildew resistance and potentially help to understand the mode of evolution of the resistance. It is also possible that both loci evolved independently of each other, and later combined into a single line with natural hybridization. In future studies, comparative genome sequence analysis of both the *Ren6* and *Ren7* loci could shed more light on the homology of the resistance genes and potentially explain the evolution of this powdery mildew resistance.

7.4. Implication for breeding durable field resistance

Grape powdery mildew is a rapidly evolving pathogen as a result of its mixed reproductive strategies and strong selection pressure due to the extensive use of synthetic fungicides in all grape growing regions of the world (Jones et al., 2014). Major *R* loci against powdery mildew have been identified in many North American, Central Asian and Chinese species (Hoffmann et al., 2008, Riaz et al., 2013a, Riaz et al., 2011, Ramming et al., 2012, Feechan et al., 2015). In general, major genes confer a strong resistance against specific races of a pathogen and are stable across diverse environmental conditions. However, this monogenic resistance can create high selection pressure on the pathogen that

could lead to the emergence of new virulent isolates (Peressotti et al., 2010, McDonald and Linde, 2002).

Durable disease resistance against pathogens such as powdery mildew is a primary objective of many grape breeding programs worldwide. A common theme among researchers is to adopt strategies to moderate selection pressure by combining or stacking *R* genes from different genetic sources and hence slow the evolution of virulent isolates and achieve durable resistance in the field. The identification of two powdery mildew *R* loci that segregate independently of each other is very important for grape breeders. To date, powdery mildew resistance loci have been identified and mapped on chromosomes 12, 13, 14, 15 and 18 from different native grape species and hybrids from North America, Central Asia and China (Dalbó et al., 2001, Welter et al., 2007, Hoffmann et al., 2008, Coleman et al., 2009, Riaz et al., 2011, Ramming et al., 2011, Blanc et al., 2012, Riaz et al., 2013a, Feechan et al., 2015, Feechan et al., 2013). The presence of *R* gene(s) on different chromosomes makes it easier to stack resistance via marker-assisted selection more effectively (Eibach et al., 2007). Current breeding strategies are also focused on combining *R* genes from different *Vitis* species with the assumption that they will have different recognition specificities (Feechan et al., 2015). This strategy is important to ensure that any mutation in a core effector will not lead to a loss of recognition by both R proteins simultaneously. At present, only limited information is available regarding the race-specificity of the different grapevine powdery mildew *R* loci. A recent study by Feechan and colleagues (2015) demonstrated that the *Run1* and *Run2.1* loci, which originated from different breeding lines of *M. rotundifolia*, show clear differences in recognition of the *Run1*-breaking Musc4 isolate making them good candidates for stacking. Similarly, preliminary studies with *Ren6* and *Ren7* also suggest that the resistance conferred by these two loci is not compromised by the Musc4 isolate (Lance Cadle-Davidson, personal communication). In this regard, the addition of two new *R* loci from *V. piasezkii*, that we showed confer resistance to powdery mildew isolates from North America and Australia, probably evolved to resist isolates in China (Wan et al., 2007, Wang et al., 2008b), making these *R* loci a valuable addition to the repertoire of resistance loci for powdery mildew resistance breeding. *Vitis piasezkii*'s neutral fruit flavor and breeding compatibility with *V. vinifera* cultivars makes it ideal candidate to develop high quality resistant lines in a short interval of time. With the help of tightly linked markers, it will be possible to incorporate these *R* loci into advanced breeding lines that already have powdery mildew *R* loci incorporated from different sources to produce grapevines with durable resistance to this important pathogen.

8. SUMMARY

Grapevine powdery mildew is caused by obligate biotrophic pathogen, ascomycetous fungus *Erysiphe necator*. All cultivated *Vitis vinifera* cultivars are highly susceptible to this pathogen. The fungus infect all living tissue and cause tremendous loss of productivity if not controlled. Up to fifteen organic and inorganic fungicide sprays are carried out to control powdery mildew during the growing season. These chemicals cause tremendous impact on the environment and human health. One way to control powdery mildew is to introgress resistance from the wild grapes.

In recent years wild grapes from Asia have been the focus of several breeding efforts. These species can easily be crossed with other *Vitis* species, and the fruit quality of the resulting progeny is generally superior to crosses made with wild North American species.

This study presents the SSR marker-based framework linkage map of *Vitis piasezkii, a* Chinese origin grape species. A framework genetic map was developed using simple sequence repeat markers in 277 seedlings of an F1 mapping population arising from a cross of the powdery mildew susceptible *Vitis vinifera* selection F2-35 and a resistant accession of *V. piasezkii* DVIT2027.

The seedlings were evaluated against powdery mildew in the field and greenhouse, and in a *in-vitro* assays to quantify the resistance to the disease. In the thesis we present result of reference genetic map and identification of genomic regions that harbor resistance to powdery mildew

Quantitative trait locus analyses identified two major powdery mildew resistance loci on chromosome 9 (*Ren6*) and chromosome 19 (*Ren7*) explaining 74.8% of the cumulative phenotypic variation. The quantitative trait locus analysis for each locus, in the absence of the other, explained 95.4% phenotypic variation for *Ren6,* while *Ren7* accounted for 71.9% of the phenotypic variation. Screening of an additional 259 seedlings of the F1 population and 910 seedlings from four pseudo-backcross populations with SSR markers defined regions of 22 kb and 330 kb for *Ren6* and *Ren7* in the *V. vinifera* PN40024 (12X) genome sequence, respectively.

Both *R* loci operate post-penetration through the induction of programmed cell death, but vary significantly in the speed of response and degree of resistance; *Ren6* confers complete resistance whereas *Ren7* confers partial resistance to the disease with reduced colony size. A comparison of the kinetics of induction of powdery mildew resistance mediated by *Ren6,*

Ren7 and the *Run1* locus from *Muscadinia rotundifolia*, indicated that the speed and strength of resistance conferred by *Ren6* is greater than that of *Run1* which, in turn, is superior to that conferred by *Ren7*.

This is the first report of mapping powdery mildew resistance in the Chinese species *V. piasezkii*. Two distinct powdery mildew *R* loci designated *Ren6* and *Ren7* were found in multiple accessions of this Chinese grape species. Their location on different chromosomes to previously reported powdery mildew resistance *R* loci offers the potential for grape breeders to combine these *R* genes with existing powdery mildew *R* loci to produce grape germplasm with more durable resistance against this rapidly evolving fungal pathogen.

9. NEW SCIENTIFIC ACHIVEMENTS

1. Evaluated the 11-373 mapping populations in multiple environment. Two years of field data, greenhouse test and *in vitro* assays on detached leaves are correlate, validating each other in different environments, infection method, plants development stage.
2. Successfully utilized qPCR as a phenotyping tool for first time for grape powdery mildew resistance mapping. qPCR scores correlate closely with the assessments in controlled environment.
3. Developed framework SSR map for *Vitis piasezkii* DVIT2027, applying 208 markers on 277 seedlings.
4. Discovered two new grape powdery mildew resistance loci (*Ren6*, *Ren7*) from the single accession of *V. piasezkii*. This is the first time that resistance in Chinese species *V. piasezkii* has been tagged and reported. These two genomic regions are on chromosome 9, 19 regions that are not represented before in powdery mildew resistances breeding programs.
5. Confirmed these two loci individually, analyzing subset of the mapping population. Tested a subset of plants with different isolates of powdery mildew, to ensure the durable resistance across isolates.
6. Saturated the genetic framework map in the chromosomal region of interest. Identified and evaluated the recombinants from the F1 and backcross populations for powdery mildew. Developed new SSR markers for in the region of interests to narrow the genetic regions.
7. Confirmed the co-segreagation of the sex linked marker with sex locus from Asian species, *V. piasezkii*.
8. Identified other *V. piasezkii* accession that are potentially carries the *Ren6* and/or *Ren7* loci.

10. LITERATURE

Adam-Blondon, A-F., Roux, C., Claux, D., Butterlin, G., Merdinoglu, D., & This, R. (2004). Mapping 245 SSR markers on the *Vitis vinifera* genome: a tool for grape genetics. *Theor Appl Genet*, **109**, 1017–1027.

Amrine, K. C., Blanco-Ulate, B., Riaz, S., Pap, D., Jones, L., Figueroa-Balderas, R., Walker, M. A. & Cantu, D. (2015). Comparative transcriptomics of Central Asian *Vitis vinifera* accessions reveals distinct defense strategies against powdery mildew. *Horticulture Res*, **2**, 15037

Aradhya, M. K., Dangl, G. S., Prins, B. H., Boursiquot, J. M., Walker, M. A., Meredith, C. P., & Simon, C. J. (2003). Genetic structure and differentiation in cultivated grape, *Vitis vinifera* L. *Genetics Research*, **81**(3), 179-192.

Aradhya, M., Wang, Y., Walker, M. A., Prins, B. H., Koehmstedt, A. M., Velasco, D., Gerrath J. M., Dangl, G. S., & Preece, J. E. (2013). Genetic diversity, structure, and patterns of differentiation in the genus *Vitis*. *Plant Syst Evol*, **299**(2), 317-330.

Bacilieri, R., Lacombe, T., Le Cunff, L., Di Vecchi-Staraz, M., Laucou, V., Genna, B., Péros, J.P., This, P. and Boursiquot, J.M., (2013). Genetic structure in cultivated grapevines is linked to geography and human selection. *BMC Plant Bio*, **13**(1), 1.

Barba, P., Cadle-Davidson, L., Galarneau, E. & Reisch, B. (2015). *Vitis rupestris* B38 confers isolate-specific quantitative resistance to penetration by *Erysiphe necator*. *Phytopathology*, **105**, 1097-103.

Barker, C. L., Donald, T., Pauquet, J., Ratnaparkhe, M., Bouquet, A., Adam-Blondon, A.-F., Thomas, M. & Dry, I. 2005. Genetic and physical mapping of the grapevine powdery mildew resistance gene, Run1, using a bacterial artificial chromosome library. *Theor Appl Genet*, **111**, 370-377.

Bartholomew, B., Boufford, D., Chang, A., Cheng, Z., Dudley, T., He, S., Jin, Y., Li, Q., Luteyn, J., Spongberg, S. & S., Y. T. (1983). The 1980 Sino-American Botanical Expedition to western Hubei Province, People's Republic of China. *J Arnold Arboretum*, **64**, 1-103.

Baudoin, A. (2014). First confirmation of resistance to quinoxyfen in grape powdery mildew in North America. Phytopathology, **104**(11), 160.

Blanc, S., Wiedemann-Merdinoglu, S., Dumas, V., Mestre, P. & Merdinoglu, D. (2012). A reference genetic map of *Muscadinia rotundifolia* and identification of Ren5, a

new major locus for resistance to grapevine powdery mildew. *Theor Appl Genet*, **125**, 1663-1675.

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), pp.43-53.

Bowers, J. E., Dangl, G. S. & Meredith, C. P. (1999). Development and characterization of additional microsatellite DNA markers for grape. *Am J Enol Viticult*, **50**, 243-246.

Bowers, J. E., Dangl, G. S., Vignani, R., & Meredith, C. P. (1996). Isolation and characterization of new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). *Genome*, **39**(4), 628-633.

Brewer, M. & Milgroom, M. (2010). Phylogeography and population structure of the grape powdery mildew fungus, Erysiphe necator, from diverse Vitis species. *BMC Evol Bio*, **10**, 268.

Calonnec, A., Cartolaro, P., Poupot, C., Dubourdieu, D. & Darriet, P. (2004). Effects of *Uncinula necator* on the yield and quality of grapes (*Vitis vinifera*) and wine. *Plant Pathol*, **53**, 434-445.

Chaib, J., Torregrosa, L., Mackenzie, D., Corena, P., Bouquet, A. & Thomas, M. R. (2010). The grape microvine - a model system for rapid forward and reverse genetics of grapevines. *Plant J*, **62**, 1083-1092.

Chen, Z. D., Ren, H., & Wen, J. (2007). Vitaceae. In: Wu Z-Y., Hong D-Y., Raven P. (eds) Flora of China, Vol. 12, Science Press and Missouri Botanical Garden Press, Beijing, China and St. Louis, pp 210–222.

Cipriani, G., Marrazzo, M. T., Di Gaspero, G., Pfeiffer, A., Morgante, M., & Testolin, R. (2008). A set of microsatellite markers with long core repeat optimized for grape (*Vitis* spp.) genotyping. *BMC Plant Biol*, **8**(1), 127.

Coleman, C., Copetti, D., Cipriani, G., Hoffmann, S., Kozma, P., Kovács, L., Morgante M, Testolin R, & Di Gaspero, G. (2009). The powdery mildew resistance gene REN1 co-segregates with an NBS-LRR gene cluster in two Central Asian grapevines. *BMC Genetics*, **10**(1), 89.

Cunha, J., Teixeira-Santos, M., Brazao, J., Fevereiro, P., & Eiras-Dias, J. E. (2013). Portuguese *Vitis vinifera* L. Germplasm: Accessing its diversity and Strategies for Conservation (Chapter 6 In: The Mediteranean genetic code – grapevine and olive), *InTech*.

Dalbó, M., Ye, G., Weeden, N., Wilcox, W. & Reisch, B. (2001). Marker-assisted selection for powdery mildew resistance in grapes. *J Am Soc Hortic Sci*, **126**, 83-89.

Decroocq, V., Fave, M., Hagen, L., Bordenave, L. & Decroocq, S. (2003). Development and transferability of apricot and grape EST microsatellite markers across taxa. *Theor Appl Genet*, 106, 912-922.

Délye, C., Laigret, F. & Corio-Costet, M.-F. (1997). A mutation in the 14 alpha-demethylase gene of Uncinula necator that correlates with resistance to a sterol biosynthesis inhibitor. *Appl Environ Microb*, **63**, 2966-2970.

Di Gaspero, G., Cipriani, G., Adam-Blondon, A. F., & Testolin, R. (2007). Linkage maps of grapevine displaying the chromosomal locations of 420 microsatellite markers and 82 markers for R-gene candidates. *Theor Appl Genet*, **114**(7), 1249-1263.

Di Gaspero, G., Cipriani, G., Marrazzo, M. T., Andreetta, D., Castro, M. J. P., Peterlunger, E. & Testolin, R. (2005). Isolation of (AC)n-microsatellites in *Vitis vinifera* L. and analysis of genetic background in grapevines under marker assisted selection. *Mol Breeding*, **15**, 11-20.

Di Gaspero, G., Copetti, D., Coleman, C., Castellarin, S. D., Eibach, R., Kozma, P., Lacombe, T, Gambetta, G, Zvyagin A, Cindric, P., Kovacs, L., Michele Morgante, M., & Testolin, R. (2012). Selective sweep at the Rpv3 locus during grapevine breeding for downy mildew resistance. *Theor Appl Genet*, **124**(2), 277-286.

Doligez, A., Adam-Blondon, A.-F., Cipriani, G., Di Gaspero, G., Laucou, V., Merdinoglu, D., Meredith, C., Riaz, S., Roux, C. & This, P. (2006). An integrated SSR map of grapevine based on five mapping populations. *Theor Appl Genet*, **113**, 369-382.

Donald, T. M., Pellerone, F., Adam-Blondon, A. F., Bouquet, A., Thomas, M. R. & Dry, I. B. (2002). Identification of resistance gene analogs linked to a powdery mildew resistance locus in grapevine. *Theor Appl Genet*, **104**, 610-618.

Dosmann, M. S. & Del Tredici, P. (2005). The Sino-American Botanical Expedition of 1980: A retrospective analysis of success. *HortScience*, **40**, 302-303.

Eibach, R., Zyprian, E., Welter, L., & Topfer, R. (2007). The use of molecular markers for pyramiding resistance genes in grapevine breeding. *Vitis*, **46**, 120 –124.

Emanuelli, F., Lorenzi, S., Grzeskowiak, L., Catalano, V., Stefanini, M., Troggio, M., & Myles, S. (2013). Genetic diversity and population structure assessed by SSR and SNP markers in a large germplasm collection of grape. *BMC Plant Biology*, **13**(1), 39.

FAOSTAT (2011). Food And Agriculture Organization Of The United Nations: http://faostat.fao.org/site/339/default.aspx

Fechter, I., Hausmann, L., Daum, M., Sorensen, T. R., Viehover, P., Weisshaar, B. & Topfer, R. (2012). Candidate genes within a 143 kb region of the flower sex locus in Vitis. *Mol Genet Genomics*, **287**, 247-259.

Feechan, A., Anderson, C., Torregrosa, L., Jermakow, A., Mestre, P., Wiedemann-Merdinoglu, S., Merdinoglu, D., Walker, A. R., Cadle-Davidson, L., Reisch, B., Aubourg, S., Bentahar, N., Shrestha, B., Bouquet, A., Adam-Blondon, A. F., Thomas, M. R. & Dry, I. B. (2013). Genetic dissection of a TIR-NB-LRR locus from the wild North American grapevine species *Muscadinia rotundifolia* identifies paralogous genes conferring resistance to major fungal and oomycete pathogens in cultivated grapevine. *Plant J*, **76**, 661-74.

Feechan, A., Kocsis, M., Riaz, S., Zhang, W., Gadoury, D., Walker, A. M., Dry, I. B., Reisch, B. I. & Cadle-Davidson, L. (2015). Strategies for RUN1 deployment using RUN2 and REN2 to manage grapevine powdery mildew informed by studies of race-specificity. *Phytopathology*, **105**, 1104-1113.

Fuller, K. B., Alston, J. M. & Sambucci, O. S. (2014). The value of powdery mildew resistance in grapes: Evidence from California. *Wine Econ Poli*, **3**, 90-107.

Gadoury, D. M., Cadle-Davidson, L., Wilcox, W. F., Dry, I. B., Seem, R. C. & Milgroom, M. G. (2012). Grapevine powdery mildew (*Erysiphe necator*): a fascinating system for the study of the biology, ecology and epidemiology of an obligate biotroph. *Mol Plant Pathol*, **13**, 1-16.

Gadoury, D. M., Seem, R. C., Pearson, R. C., Wilcox, W. F. & Dunst, R. M. (2001). Effects of powdery mildew on vine growth, yield, and quality of concord grapes. *Plant Dis*, **85**, 137-140.

Grimplet, J., Cramer, G. R., Dickerson, J. A., Mathiason, K., Van Hemert, J. & Fennell, A. Y. (2009). VitisNet: "Omics" integration through grapevine molecular networks. *PLoS One*, **4** (12), e8365.

Gubler, W. D., R.J. Smith, L.G. Varela, S. Vasquez, J.J. Stapleton, A.H. Purcell 2008. Grape powdery mildew. http://ipm.ucanr.edu/PMG/r302100311.html Accessed 1 July, 2015.

Hoffmann, S., Di Gaspero, G., Kovács, L., Howard, S., Kiss, E., Galbács, Z., Testolin, R. & Kozma, P. (2008). Resistance to *Erysiphe necator* in the grapevine 'Kishmish vatkana' is controlled by a single locus through restriction of hyphal growth. *Theor Appl Genet*, **116**, 427-438.

Huang, Z., He, G., Shu, L., Li, X. & Zhang, Q. (2001). Identification and mapping of two brown planthopper resistance genes in rice. *Theor Appl Genet*, **102**, 929-934.

IGGP International Grape Genome Program. http://www.vitaceae.org/index.php/International_Grape_Genome_Program Accessed 29 September 2015.

IPGRI-UPOV (1997). OIV Descriptors for grapevine (Vitis spp.), Rome, Italy.

Jaillon, O., Aury, J. M., Noel, B., Policriti, A., Clepet, C., Casagrande, A., Choisne, N., Aubourg, S., Vitulo, N., Jubin, C., Vezzi, A., Legeai, F., Hugueney, P., Dasilva, C., Horner, D., Mica, E., Jublot, D., Poulain, J., Bruyere, C., Billault, A., Segurens, B., Gouyvenoux, M., Ugarte, E., Cattonaro, F., Anthouard, V., Vico, V., Del Fabbro, C., Alaux, M., Di Gaspero, G., Dumas, V., Felice, N., Paillard, S., Juman, I., Moroldo, M., Scalabrin, S., Canaguier, A., Le Clainche, I., Malacrida, G., Durand, E., Pesole, G., Laucou, V., Chatelet, P., Merdinoglu, D., Delledonne, M., Pezzotti, M., Lecharny, A., Scarpelli, C., Artiguenave, F., Pe, M.E., Valle, G., Morgante, M., Caboche, M., Adam-Blondon, A-F., Weissenbach, J., Quetier, F., & Wincker, P. (2007). The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature*, **449**, 463–467.

Jones, L., Riaz, S., Morales-Cruz, A., Amrine, K. C., Mcguire, B., Gubler, W. D., Walker, M. A. & Cantu, D. (2014). Adaptive genomic structural variation in the grape powdery mildew pathogen, *Erysiphe necator*. *BMC Genomics*, **15**, 1081.

Kalia, R. K., Rai, M. K., Kalia, S., Singh, R., Dhawan, & A. K. (2011). Microsatellite markers: an overview of a recent progress in plants. *Euphytica*, **177**, 309-334.

Katula-Debreceni, D., Lencses, A. K., Szoke, A., Veres, A., Hoffmann, S., Kozma, P., Kovacs, L. G., Heszky, L., & Kiss, E. (2010). Marker-assisted selection for two dominant PM resistance genes introgressed into a hybrid grape population. *Scientia Horticulturae* **126**, 448-453.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S. & Duran, C. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, **28**, 1647-1649.

Kiss, E., Kozma, P., & Halasz, G., (2007). Hungarian *Vitis* microsatellite database.

Kohany, O., Gentles, A. J., Hankus, L. & Jurka, J. (2006). Annotation, submission and screening of repetitive elements in Repbase: RepbaseSubmitter and Censor. *BMC Bioinformatics*, **7**(1), 1

Kosambi, D. D. (1944). The estimation of map distances from recombination values. *Annals of Eugenics*, **12**, 172-175.

Kuczmog, A., Galambos, A., Horváth, S., Mátai, A., Kozma, P., Szegedi, E. and Putnoky, P., (2012). Mapping of crown gall resistance locus Rcg1 in grapevine. *Theor Appl Genet*, ***125***(7), 1565-1574.

Kunova, A., Pizzatti, C., Bonaldi, M. & Cortesi, P. (2015). Metrafenone resistance in a population of *Erysiphe necator* in northern Italy . *Pest Manag Sci*. **72**(2), 398-404.

Lamoureux, D., Bernole, A., Le Clainche, I., Tual, S., Thareau, V., Paillard, S., Legeai, F., Dossat, C., Wincker, P., Oswald, M., Merdinoglu, D., Vignault, C., Delrot, S., Caboche, M., Chalhoub, B., & Adam-Blondon, A-F. (2006). Anchoring of a large set of markers onto a BAC library for the development of a draft physical map of the grapevine genome. *Theor Appl Genet*,, **113**(2), 344-356.

Leao, P. C. S., Riaz, S., Graziani, R., Dangl, G. S., Motoike, S.Y., Walker, M. A. (2009). Characterization of Brazilian Grape Germplasm Collection using microsatellite markers. *Am J Enol Viticult*, **60**, 4.

Li, C., Erwin, A., Pap, D., Coleman, C., Higgins, A. D., Kiss, E. Hoffmann, S., Kozma, P., & Kovács, L. G. (2013). Selection for Run1-Ren1 Dihybrid Grapevines Using Microsatellite Markers. *Am J Enol Viticult*, **64**(1), 152-155.

Li, D., Wan, Y., Wang, Y., & He, P. (2008). Relatedness of resistance to anthracnose and to white rot in Chinese wild grapes. *Vitis*, **47**(4), 213

Licausi, F., Giorgi, F. M., Zenoni, S., Osti, F., Pezzotti, M. & Perata, P. 2010. Genomic and transcriptomic analysis of the AP2/ERF superfamily in Vitis vinifera. *BMC Genomics*, **11**, 719.

Liu, L. and Li, H., (2013). Review: Research progress in amur grape, *Vitis amurensis* Rupr. *Canadian Journal of Plant Science*, ***93***(4), 565-575

Mahanil, S., Ramming, D., Cadle-Davidson, M., Owens, C., Garris, A., Myles, S. & Cadle-Davidson, L. (2012). Development of marker sets useful in the early selection of Ren4 powdery mildew resistance and seedlessness for table and raisin grape breeding. *Theor Appl Genet*, **124**, 23-33.

Marguerit, E., Boury, C., Manicki, A., Donnart, M., Butterlin, G., Nemorin, A., Wiedemann-Merdinoglu, S., Merdinoglu, D., Ollat, N. & Decroocq, S. 2009. Genetic dissection of sex determinism, inflorescence morphology and downy mildew resistance in grapevine. *Theor Appl Genet*, **118**, 1261-78.

Martins, W. S., Lucas, D. C. S., De Souza Neves, K. F. & Bertioli, D. J. (2009). WebSat-A web software for microsatellite marker development. *Bioinformation*, **3**(6), 282-283.

Mcdonald, B. A. & Linde, C. (2002). Pathogen population genetics, evolutionary potential, and durable resistance. *Annu Rev Phytophatol*, **40**, 349-379.

Mendiburu, F. D. (2015). Package 'agricolae'. Statistical procedures for agricultural research. v1.2-3 Vienna, Austria: Institute for Statistics and Mathematics.

Merdinoglu, D., Butterlin, G., Bevilacqua, L., Chiquet, V., Adam-Blondon, A.-F. & Decroocq, S. (2005). Development and characterization of a large set of microsatellite markers in grapevine (*Vitis vinifera* L.) suitable for multiplex PCR. *Mol Breeding*, **15**, 349-366.

Molnar, S., Galbacs, Z., Halasz, G., Hoffmann, S, Kiss, E., Kozma, P., Veres, A., Galli, Z., Szoke, A., & Heszky, L. (2007). Marker assisted selection (MAS) for PM resistance in a grapevine hybrid family. *Vitis*, **46**, 212-221.

Montarry, J., Cartolaro, P., Richard-Cervera, S. & Delmotte, F. (2008). Spatio-temporal distribution of *Erysiphe necator* genetic groups and their relationship with disease levels in vineyards. *Eur J Plant Pathol*, **123**, 61-70.

Moore, M. O. (1991). Classification and systematics of eastern North American Vitis L. (Vitaceae) north of Mexico. SIDA *Contributions to Botany*, **14**, 339–367.

Mullins, M. G., Bouquet, A., & Williams, L. E. (1992). Biology of the Grapevine. 3rd edn. Cambridge University Press, Cambridge. United Kingdom.

Munson, T.V. (1909). Foundations of American Grape Culture. T. V. Munson and Son. Dension TX.

Myles, S. (2013). Improving fruit and wine: what does genomics have to offer? *Trends in Genetics*, **29**(4), 190-196.

Myles, S., Boyko, A. R., Owens, C. L., Brown, P. J., Grassi, F., Aradhya, M. K., Prins, B., Reynolds, A., Chia, J-M., Ware, D., Bustamente, C. D., & Buckler, E. S. (2011). Genetic structure and domestication history of the grape. *Proceedings of the National Academy of Sciences*, **108**(9), 3530-3535.

Myles, S., Chia, J. M., Hurwitz, B., Simon, C., Zhong, G. Y., Buckler, E., & Ware, D. (2010). Rapid genomic characterization of the genus *Vitis*. *PloS One*, **5**(1), e8219.

NCBI (2013) National Center of Biotechnology Information. http://www.ncbi.nlm.nih.gov/

Patel, G. I., Olmo, H. P. (1955). Cytogenetics of Vitis: I. The hybrid *V. vinifera* × *V. rotundifolia*. *American Journal of Botany*, 141-159.

Pauquet, J., Bouquet, A., This, P. & Adam-Blondon, A. F. (2001). Establishment of a local map of AFLP markers around the powdery mildew resistance gene Run1 in grapevine and assessment of their usefulness for marker assisted selection. *Theor Appl Genet*, **103**, 1201-1210.

Pellerone, F. I., Edwards, K. J., & Thomas, M. R. (2001). Grapevine microsatellite repeats: isolation, characterisation and use for genotyping of grape germplasm from Southern Italy. *Vitis*, **40**(4), 179-186.

Peressotti, E., Wiedemann-Merdinoglu, S., Delmotte, F., Bellin, D., Di Gaspero, G., Testolin, R., Merdinoglu, D. & Mestre, P. (2010). Breakdown of resistance to grapevine downy mildew upon limited deployment of a resistant variety. *BMC Plant Biol*, **10**(1), 147.

Perrier, X. & Jacquemoud-Collet, J. (2006). DARwin software.

Puchao H, Guoying W (1985) Studies on the resistance of wild *Vitis* species native to China to downy mildew, *Plasmopara viticola* (Berk. Et Curtis) Berl. et de Toni

Qiu, W., Feechan, A. & Dry, I. (2015). Current understanding of grapevine defense mechanisms against the biotrophic fungus (*Erysiphe necator*), the causal agent of powdery mildew disease. *Horticulture Res*, **2**, 15020

Ramming, D. W., Gabler, F., Smilanick, J. L., Margosan, D. A., Cadle-Davidson, M., Barba, P., Mahanil, S., Frenkel, O., Milgroom, M. G. & Cadle-Davidson, L. (2012). Identification of race-specific resistance in North American *Vitis* spp. limiting *Erysiphe necator* hyphal growth. *Phytopathology*, **102**, 83-93.

Ramming, D. W., Gabler, F., Smilanick, J., Cadle-Davidson, M., Barba, P., Mahanil, S. & Cadle-Davidson, L. (2011). A single dominant locus, Ren4, confers rapid non-race-specific resistance to grapevine powdery mildew. *Phytopathology*, **101**, 502-508.

Reifschneider, F. J. & Boiteux, L. S. (1988). A vacuum-operated settling tower for inoculation of powdery mildew fungi. *Phytopathology*, **78**, 1463-1465.

Reisch, B. I., Owens, L. C., & Cousins, S. P. (2012). Grape (Chapter 7 in; Badenes ML, Byrne DH (eds.), Fruit Breeding, Handbook of Plant Breeding 8, Springer Science Business Media, LLC 2012).

Riaz, S., Boursiquot, J.-M., Dangl, G. S., Lacombe, T., Laucou, V., Tenscher, A. C. & Walker, M. A. (2013b). Identification of mildew resistance in wild and cultivated Central Asian grape germplasm. *BMC Plant Biol*, **13**, 149.

Riaz, S., Dangl, G. S., Edwards, K. J., & Meredith, C. P. (2004). A microsatellite marker based framework linkage map of Vitis vinifera L. *Theor Appl Genet*, 108, 864–872.

Riaz, S., Hu, R., & Walker M. A. (2012). A framework genetic map of Muscadinia rotundifolia *Theor Appl Genet* **125**(6), 1195-210.

Riaz, S., Krivanek, A. F., Xu, K. & 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**, 1317-29.

Riaz, S., Lejkina, I., Gubler, W. & Walker, M. (2013a). Report of a new grape powdery mildew morphotype with branched conidiophores. *Plant Pathol Quar*, **3**, 19-27.

Riaz, S., Tenscher, A. C., Ramming, D. W. & Walker, M. A. (2011). Using a limited mapping strategy to identify major QTLs for resistance to grapevine powdery mildew (*Erysiphe necator*) and their use in marker-assisted breeding. *Theor Appl Genet*, **122**, 1059-73.

Ritter, E., Debener, T., Barone, A., Salamini, F. & Gebhardt, C. (1991). RFLP mapping on potato chromosomes of two genes controlling extreme resistance to potato virus X (PVX). *Mol Gen Genet*, **227**, 81-85.

Rozen, S. & Skaletsky, H. (1999). Primer3 on the WWW for general users and for biologist programmers. Bioinformatics methods and protocols. Springer.

Rusjan, D. (2013). Genetic and phenotypic diversity and relations between grapevine varieties: Slovenian germplasm (Chapter 7, In: The Mediteranean genetic code – grapevine and olive), *InTech.*

Schwander, F., Eibach, R., Fechter, I., Hausmann, L., Zyprian, E. and Töpfer, R., (2012). Rpv10: a new locus from the Asian *Vitis* gene pool for pyramiding downy mildew resistance loci in grapevine. *Theor Appl Genet*, ***124***(1), 163-176.

Scott, K. D., Eggler, P., Seaton, G., Rossetto, M., Ablett, E. M., Lee, L. S. & Henry, R. J. (2000). Analysis of SSRs derived from grape ESTs. *Theor Appl Genet*, **100**, 723-726.

Sefc, K. M., Regner, F., Turetschek, E., Glossl, J., & Steinkellner, H. (1999). Identification of microsatellite sequences in Vitis riparia and their applicability for genotyping of different Vitis species. *Genome*, **42**, 1-7.

Shen Qh, Z. F., Bieri S, Haizel T, Shirasu K, Schulze-Lefert P (2003). Recognition specificity and RAR1/SGT1 dependence in barley Mla disease resistance genes to the powdery mildew fungus. *The Plant Cell*, **15**, 732-744.

Singh, A., Kumar, K., Gill, M. I. S., Chhuneja, P., Arora, N. K., & Singh, K. (2013). Genotype identification and inference of genetic relatedness among different purpose grape varieties and rootstocks using microsatellite markers. *African Journal of Biotechnology*, **12**(2), 134-141.

Stummer, B. E., Francis, I. L., Zanker, T., Lattey, K. A. & Scott, E. S. (2005). Effects of powdery mildew on the sensory properties and composition of Chardonnay juice

and wine when grape sugar ripeness is standardised. *Aust J Grape Wine Res*, **11**, 66-76.

Suwabe, K., Tsukazaki, H., Iketani, H., Hatakeyama, K., Fujimura, M., Nunome, T., Fukuoka, H., Matsumoto, S. & Hirai, M. (2003). Identification of two loci for resistance to clubroot (Plasmodiophora brassicae Woronin) in Brassica rapa L. *Theor Appl Genet*, **107**, 997-1002.

Team, R. D. C. (2015). A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

This, P., Lacombe, T., & Thomas, M. R. (2006). Historical origins and genetic diversity of wine grapes. *Trends in Genetics*, **22**(9), 511-519.

Thomas, M. R., & Scott, N. S. (1993). Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequence-tagged sites (STSs). *Theor Appl Genet*, **86**, 985-990.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M. & Rozen, S. G. 2012. Primer3: new capabilities and interfaces. *Nucleic Acids Res*, **40**, e115-e115.

Upadhyay, A., Kadam, U. S., Chacko, P., Karibasappa, G. S. (2010). Microsatellite and RAPD analysis of grape (Vitis spp.) accessions and identification of duplicates/misnomers in germplasm collection. *Indian Journal of Horticulture*, **67**(1), 8-15.

Van Ooijen, J. (2009). MapQTL 6.0: Software for the mapping of quantitative trait loci in experimental populations of diploid species. Wageningen, Netherlands: Kyazma B. V.

Van Ooijen, J. (2011). Multipoint maximum likelihood mapping in a full-sib family of an outbreeding species. *Genetics Res*, **93**, 343-349.

Velasco, R., Zharkikh, A., Troggio, M., Cartwright, D.A., Cestaro, A., Pruss, D., Pindo, M., Fitzgerald, L.M., Vezzulli, S.,Reid, J., Malacarne, G., Iliev, D., Coppola, G., Wardell, B., Micheletti, D., Macalma, T., Facci, M., Mitchell, J.T.,Perazzolli, M., Eldredge, G., Gatto, P., Oyzerski, R., Moretto, M., Gutin, N., Stefanini, M., Chen, Y., Segala, C.,Davenport, C., Dematte, L., Mraz, A., Battilana, J., Stormo, K., Costa, F., Tao, Q., Si-Ammour, A., Harkins, T., Lackey, A., Perbost, C., Taillon, B., Stella, A., Solovyev, V., Fawcett, J.A., Sterck, L., Vandepoele, K., Grando, S.M., Toppo, S.,Moser, C., Lanchbury, J., Bogden, R., Skolnick, M., Sgaramella, V., Bhatnagar, S.K., Fontana, P., Gutin, A., Van de Peer, Y., Salamini, F., &

Viola, R. (2007). A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. *PLoS ONE*, **2**(12), 1326-1371.

Venuti S, Copetti D, Foria S, Falginella L, Hoffmann S, Bellin D, Cindric P, Kozma P, Scalabrin S, Morgante M, Testolin R, Di Gaspero g,. (2013) Historical introgression of the downy mildew resistance gene Rpv12 from the asian species *Vitis amurensis* into grapevind varieties, *PLoS ONE* **8**(4): e61228.

Voorrips, R., Jongerius, M. & Kanne, H. (1997). Mapping of two genes for resistance to clubroot (*Plasmodiophora brassicae*) in a population of doubled haploid lines of *Brassica oleracea* by means of RFLP and AFLP markers. *Theor Appl Genet*, **94**, 75-82.

Wan, Y., Schwaninger, H., He, P. & Wang, Y. (2007). Comparison of resistance to powdery mildew and downy mildew in Chinese wild grapes. *Vitis*, **46**, 132.

Wan, Y., Schwaninger, H., Li, D., Simon, C., Wang, Y. & Zhang, C. (2008a). A review of taxonomic research on Chinese wild grapes. *Vitis*, **47**, 81.

Wan, Y., Wang, Y., Li, D. & He, P.(2008b). Evaluation of agronomic traits in Chinese wild grapes and screening superior accessions for use in a breeding program. *Vitis*, **47**, 153.

Wang, X., Wang, Y. & Fei, Z. (2008). Identification and characterisation of resistance gene analogues from wild Chinese *Vitis* species. *J Hortic Sci Biotech*, **83**, 345-350.

Wang, Y. J., Liu, Y. L., He, P. C., Lamikanra, O., & Lu, J. (1998). Resistance of Chinese Vitis species to *Elsinoë ampelina* (de Bary) shear. *HortScience*, **33**(1), 123-126.

Welter, L. J., Göktürk-Baydar, N., Akkurt, M., Maul, E., Eibach, R., Töpfer, R., & Zyprian, E. M. (2007). Genetic mapping and localization of quantitative trait loci affecting fungal disease resistance and leaf morphology in grapevine (*Vitis vinifera* L). *Molecular Breeding*, **20**, 359-374.

Wen, J., Nie, Z. L., Soejima, A., & Meng, Y. (2007). Phylogeny of Vitaceae based on the nuclear GAI1 gene sequences This article is one of a selection of papers presented at the symposium on *Vitis* at the XVII International Botanical Congress held in Vienna, Austria, in 2005. *Botany*, **85**(8), 731-745.

Zecca, G., Abbott, J. R., Sun, W. B., Spada, A., Sala, F., & Grassi, F. (2012). The timing and the mode of evolution of wild grapes *Vitis*. *Mol Phylogenet Evol*, **62**(2), 736-747.

11. SUPPLEMENTAL TABLES AND FIGURES

Supplemental Table 1. *A list of significantly distorted markers with p values*

Chromosome	Locus	Segregation	$\chi 2$	Df	*p* Value[a]
1	VVS29	<abxcd>	8.09	3	**
1	VMC8a7	<abxcd>	7.89	3	**
1	AF378125	<nnxnp>	10.37	1	***
1	VVIq57	<abxcd>	13.54	3	***
1	ctg1010271	<efxeg>	11.97	3	***
1	VMCNg2g7	<nnxnp>	15.36	1	******
1	ctg1008034	<nnxnp>	15.48	1	******
1	VMCNg1h7	<abxcd>	17.53	3	****
1	ctg1025664	<abxcd>	19.91	3	*****
1	ctg1011774	<abxcd>	14.16	3	***
1	VMC7g5	<efxeg>	15.56	3	***
1	VVIs21	<abxcd>	16.36	3	****
1	VMC2b3	<abxcd>	16.66	3	****
1	ctg1026392	<nnxnp>	10.52	1	***
1	VVIf52	<nnxnp>	6.07	1	**
1	VMC9d3	<efxeg>	10.9	3	**
4	VMC2b5	<abxcd>	7.88	3	**
5	SCA20-5	<efxeg>	8.11	3	**
5	VVIv21	<abxcd>	11.24	3	**
5	VMC16d4	<efxeg>	8.04	3	**
6	VMC5c5	<nnxnp>	7.33	1	***
6	VMC2h9	<nnxnp>	7.45	1	***
7	VMC7a4	<efxeg>	8.85	3	**
8	VVIv15.2	<abxcd>	10.91	3	**
8	VMC7h2	<nnxnp>	11.97	1	****
8	UDV126	<nnxnp>	14.65	1	*****
9	LG9-sc2014	<abxcd>	8.95	3	**
9	CD009354	<abxcd>	9.12	3	**
11	VVIm04	<nnxnp>	8.41	1	***
11	VMC3e12	<abxcd>	8.44	3	**
14	VVC62	<abxcd>	8.99	3	**
14	VMCNg1e1	<abxcd>	9.37	3	**
16	UDV086	<abxcd>	49.34	3	******
19	UDV114	<efxeg>	12.13	3	***

[a] *Markers that have significant segregation deviation from the Mendelian ratios are marked with asterisks indicating the significance levels at alpha 0.01=*, 0.05=**, 0.01=***, 0.005=****, 0.001=*****, 0.0005=******, 0.0001=*******.*

Supplemental Table 2A *QTLs detected with interval mapping (IM) in the base mapping population using different disease evaluation assays*

Phenotype	Mapping Method	Chr.	LOD Max	Genome Wide Threshold, 95%	Variance Explained (%)	Phenotypic Variance	Location on the Map (cM)	Flanking Markers	
Leaf 2013	IM	9	9.95	2.70	16.6	0.56	38.613	PN9-057	PN9-068
Cane 2013	IM	9	3.17	2.60	5.6	0.29	38.613	PN9-057	PN9-068
Leaf 2014	IM	9	31.78	2.80	42.9	3.09	38.423	PN9-057	PN9-068
Cane 2014	IM	9	14.05	2.80	22.0	2.41	38.613	PN9-057	PN9-068
Greenhouse	IM	9	50.60	2.80	59.5	2.62	38.423	PN9-057	PN9-068
in vitro	IM	9	54.30	2.80	61.9	2.57	38.423	PN9-057	PN9-068
qPCR	IM	9	37.60	2.80	50.5	9.63	38.423	PN9-057	PN9-068
Leaf 2013	IM	19	2.79	2.70	4.9	0.56	2.006	VVIp17.1	VMC9a2.1
Cane 2013	IM	19	3.05	2.60	5.4	0.29	1.006	VVIp17.1	VMC9a2.1
Leaf 2014	IM	19	11.38	2.80	18.2	3.09	1.006	VVIp17.1	VMC9a2.1
Cane 2014	IM	19	11.92	2.80	19.0	2.41	1.006	VVIp17.1	VMC9a2.1
Greenhouse	IM	19	9.03	2.80	14.9	2.62	3.24	VMC9a2.1	VMC5h11
in vitro	IM	19	6.58	2.80	11.1	2.57	3.24	VMC9a2.1	VMC5h11
qPCR	IM	19	5.44	2.80	9.7	9.63	2.006	VMC9a2.1	VMC5h11

Supplemental Table 2B *QTLs detected with multiple QTL mapping (MQM) in the base mapping population using different disease evaluation assays*

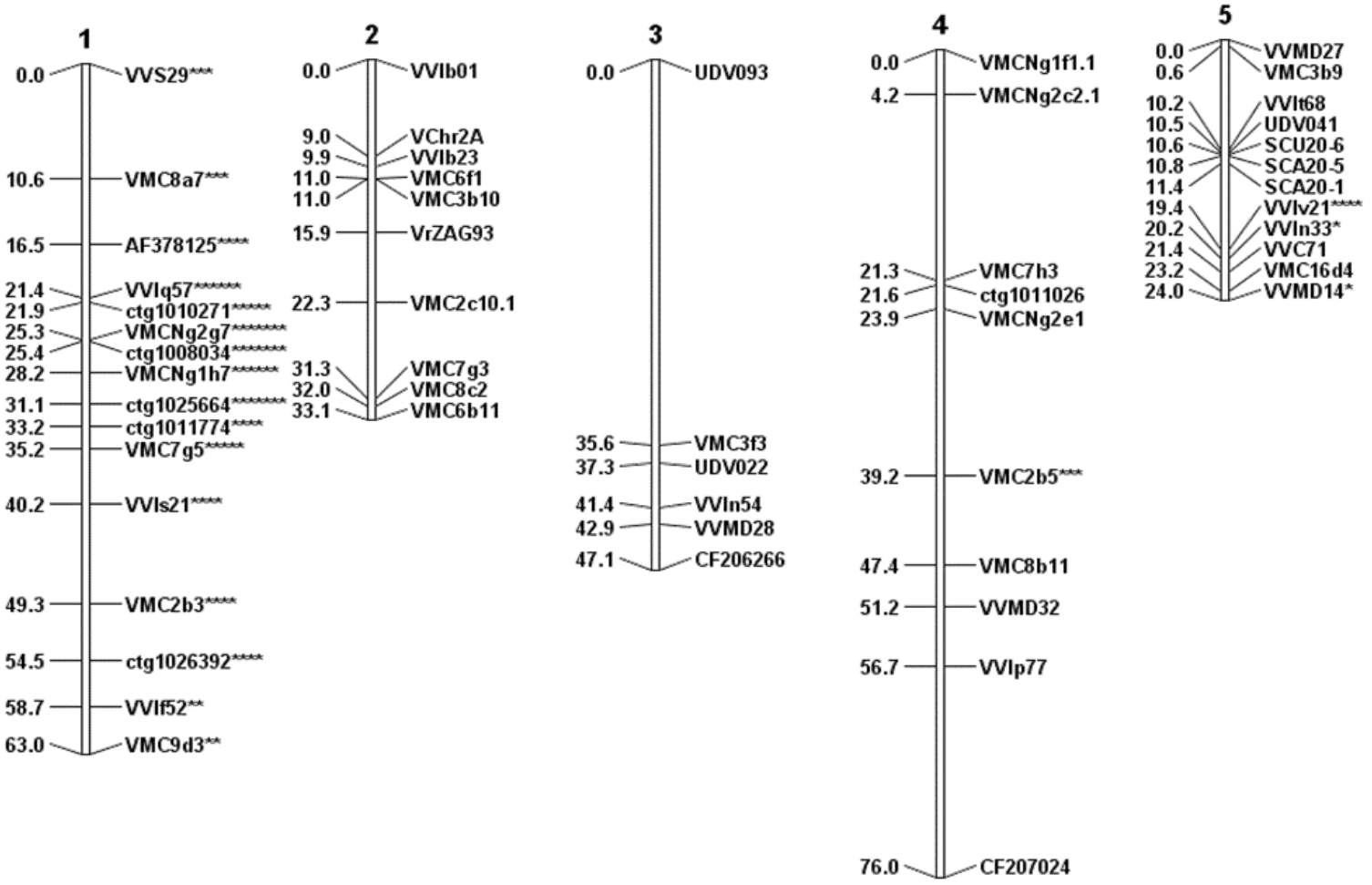
Phenotype	Mapping Method	Chr	LOD Max	Genome Wide Threshold, 95%	Variance Explained (%)	Phenotypic Variance	Location on the Map (cM)	Flanking Markers		Cofactor(s)
Leaf 2013	MQM	9	8.01	2.70	13.0	0.56	36.055	PN9-042	PN9-057	VVIn57, PN9-042
Cane 2013	MQM	9	9.55	2.60	15.4	0.29	36.423	PN9-057	PN9-068	VVIn57, PN9-042
Leaf 2014	MQM	9	40.43	2.80	42.8	3.09	38.423	PN9-057	PN9-068	PN9-068
Cane 2014	MQM	9	17.09	2.80	21.2	2.41	38.423	PN9-057	PN9-068	PN9-068
Greenhouse	MQM	9	67.40	2.80	60.1	2.62	38.423	PN9-057	PN9-068	PN9-068
in vitro	MQM	9	66.28	2.80	62.0	2.57	38.423	PN9-057	PN9-068	PN9-068
qPCR	MQM	9	44.95	2.80	52.0	9.63	38.423	PN9-057	PN9-068	PN9-068
Leaf 2013	MQM	19	3.55	2.70	5.0	0.56	5.357	VVIu09	VMC5h11	VVIu09
Cane 2013	MQM	19	3.18	2.60	4.6	0.29	0.006	VVIp17.1	VMC9a2.1	-
Leaf 2014	MQM	19	18.56	2.80	16.0	3.09	5.357	VVIu09	VMC5h11	VVIu09
Cane 2014	MQM	19	14.99	2.80	18.1	2.41	1.006	VVIp17.1	VMC9a2.1	VVIn74
Greenhouse	MQM	19	25.18	2.80	14.7	2.62	5.357	VVIu09	VMC5h11	VVIu09
in vitro	MQM	19	18.38	2.80	10.7	2.57	5.357	VVIu09	VMC5h11	VVIu09
qPCR	MQM	19	12.71	2.80	10.5	9.63	3.24	VMC9a2.1	VMC5h11	VMC5h11

Supplemental Table 2C*. QTLs detected with interval mapping (IM) in the mapping population using different disease evaluation assays. The IM analysis was carried out with genotypes possessing either Ren6 (IM without Ren7) or Ren7 (IM without Ren6) haplotypes.*

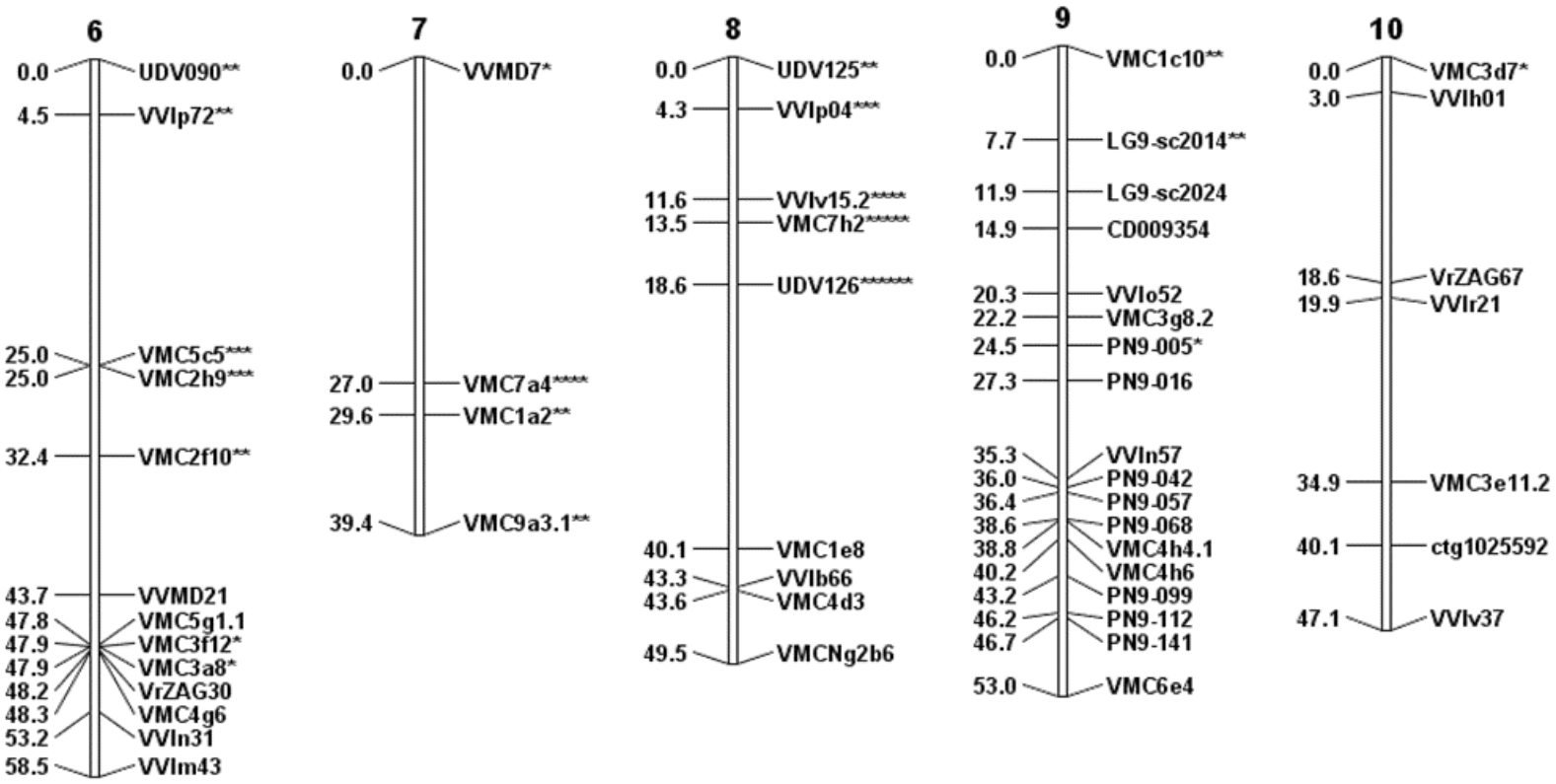
Phenotype	Mapping Method	Chr.	LOD Max	Genome Wide Threshold, 95%	Variance Explained (%)	Phenotypic Variance	Location on the Map (cM)	Flanking Markers	
Leaf 2013	IM wo*Ren7*	9	10.09	2.90	28.6	0.79	38.613	PN9-057	PN9-068
Cane 2013	IM wo*Ren7*	9	2.97	2.90	9.4	0.46	38.613	PN9-057	PN9-068
Leaf 2014	IM wo*Ren7*	9	48.76	2.80	79.2	3.98	38.613	PN9-057	PN9-068
Cane 2014	IM wo*Ren7*	9	16.03	2.90	40.3	3.39	38.613	PN9-057	PN9-068
Greenhouse	IM wo*Ren7*	9	95.76	2.80	95.4	3.31	38.423	PN9-057	PN9-068
in vitro	IM wo*Ren7*	9	69.91	2.90	89.6	3.36	38.613	PN9-057	PN9-068
qPCR	IM wo*Ren7*	9	36.51	2.80	71.2	11.10	38.423	PN9-057	PN9-068
Leaf 2013	IM wo*Ren6*	19	4.66	2.90	15.9	0.85	2.006	VVIp17.1	VMC9a2.1
Cane 2013	IM wo*Ren6*	19	2.76	3.10	9.7	0.53	1.006	VVIp17.1	VMC9a2.1
Leaf 2014	IM wo*Ren6*	19	26.26	2.80	60.8	3.45	2.006	VVIp17.1	VMC9a2.1
Cane 2014	IM wo*Ren6*	19	14.44	2.80	40.3	3.43	1.006	VVIp17.1	VMC9a2.1
Greenhouse	IM wo*Ren6*	19	35.58	2.80	71.9	2.08	3.24	VMC9a2.1	VMC5h11
in vitro	IM wo*Ren6*	19	28.09	2.80	63.3	1.80	3.24	VMC9a2.1	VMC5h11
qPCR	IM wo*Ren6*	19	15.27	2.80	43.3	6.63	2.24	VMC9a2.1	VMC5h11

[a]

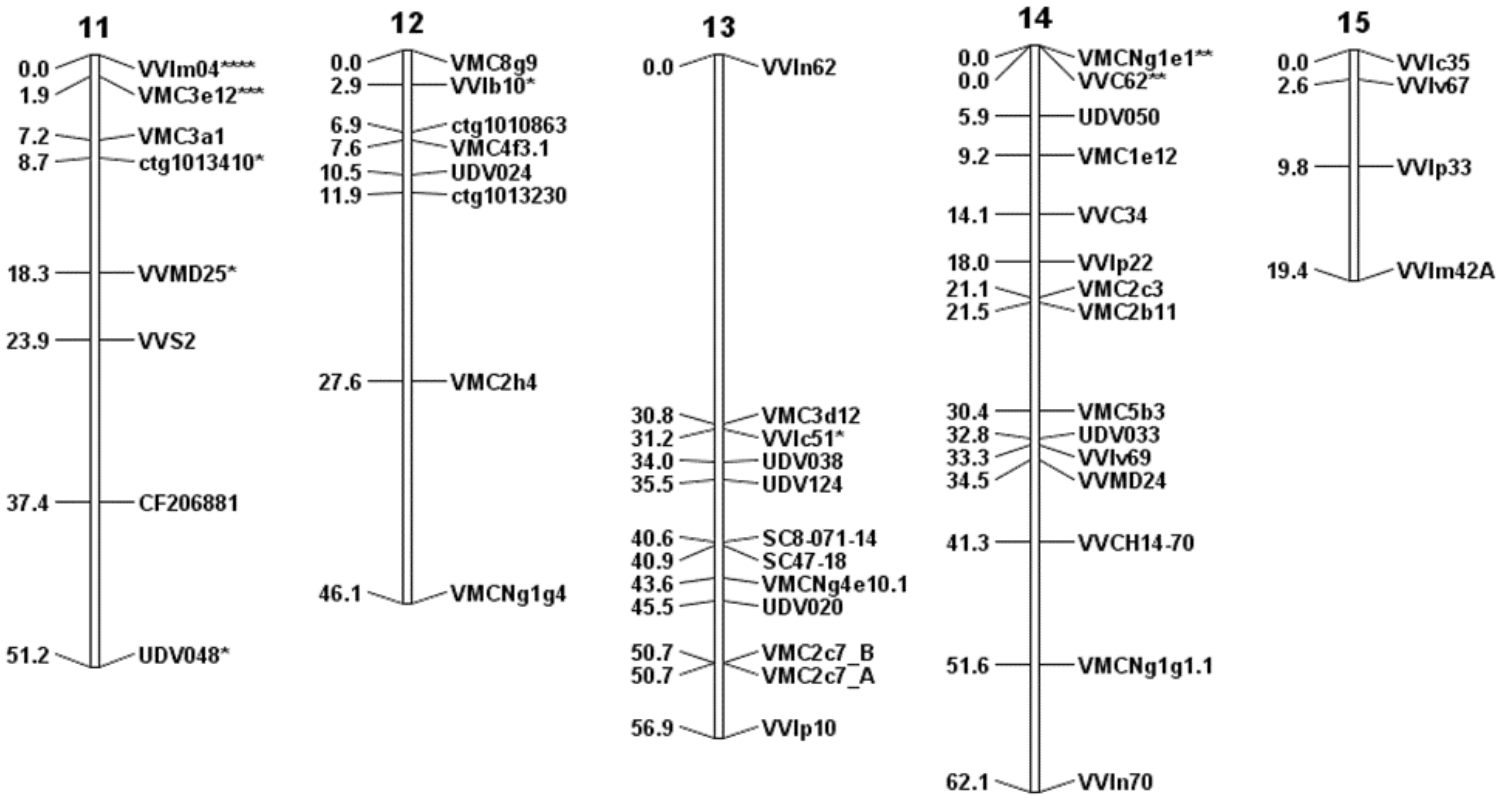
Supplemental Figure 1A. *A framework genetic map of* Vitis piasezkii *DVIT2027 chromosomes 1 to 5. Markers that have significant segregation deviation from Mendelian ratios are marked with asterisks indicating the significance levels at alpha 0.01=*, 0.05=**, 0.01=***, 0.005=****, 0.001=*****, 0.0005=******, and 0.0001=*******.*



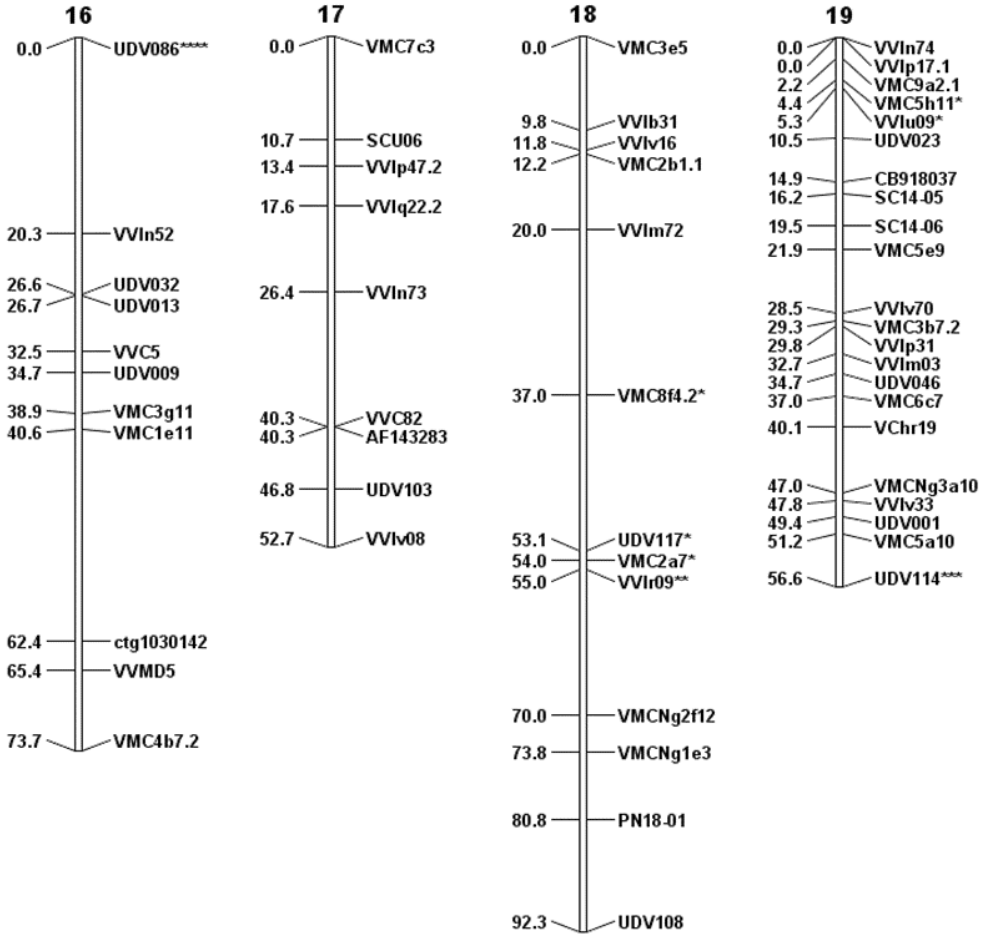
Supplemental Figure 1B. *A framework genetic map of* Vitis piasezkii *DVIT2027 chromosomes 6 to 10. Markers that have significant segregation deviation from Mendelian ratios are marked with asterisks indicating the significance levels at alpha 0.01=*, 0.05=**, 0.01=***, 0.005=****, 0.001=*****, 0.0005=******, and 0.0001=*******.*



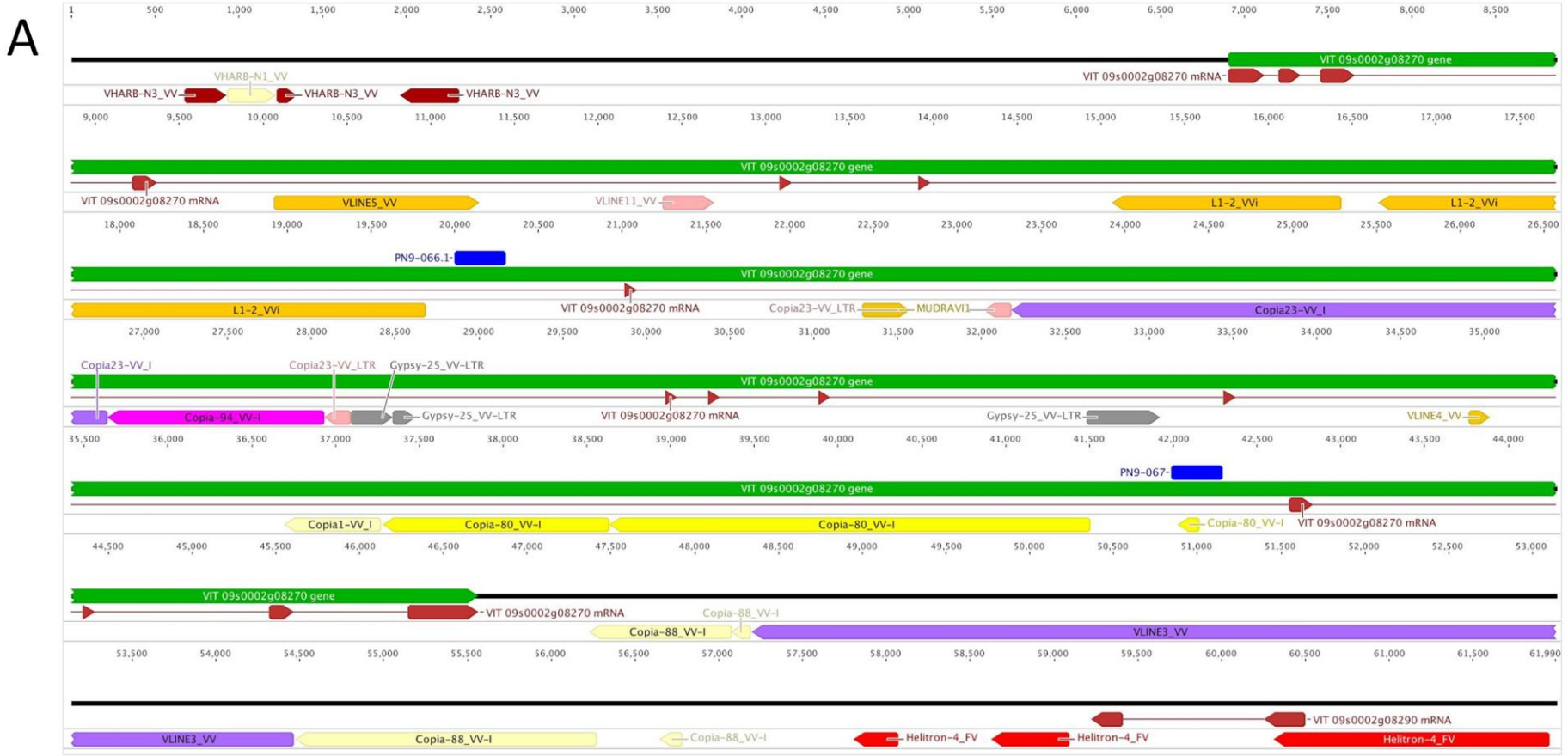
Supplemental Figure 1C. *A framework genetic map of* Vitis piasezkii *DVIT2027 chromosomes 11 to 15. Markers that have significant segregation deviation from Mendelian ratios are marked with asterisks indicating the significance levels at alpha 0.01=*, 0.05=**, 0.01=***, 0.005=****, 0.001=*, 005=**, 001=***, 0005=****, 0001=*****, 00005=******, and 00001=*******.*



Supplemental Figure 1D. *A framework genetic map of* Vitis piasezkii *DVIT2027 chromosomes 16 to 19. Markers that have significant segregation deviation from Mendelian ratios are marked with asterisks indicating the significance levels at alpha 0.01=*, 0.05=**, 0.01=***, 0.005=****, 0.001=*, 005=**, 001=***, 0005=****, 0001=*****, 00005=******, and 00001=*******.*



Supplemental Figure 2A. *Gene and transposon annotation of the corresponding genomic region of Ren6 (A) and Ren7 (B) loci in PN40024 sequence. The green color indicates the annotated genes labeled with gene ID and maroon color is used for the mRNA of corresponding gene. All other colors indicate different type of transposable elements identified with the Repbase database. Transposable elements with size of smaller than 100 bp were not included in the figure.*



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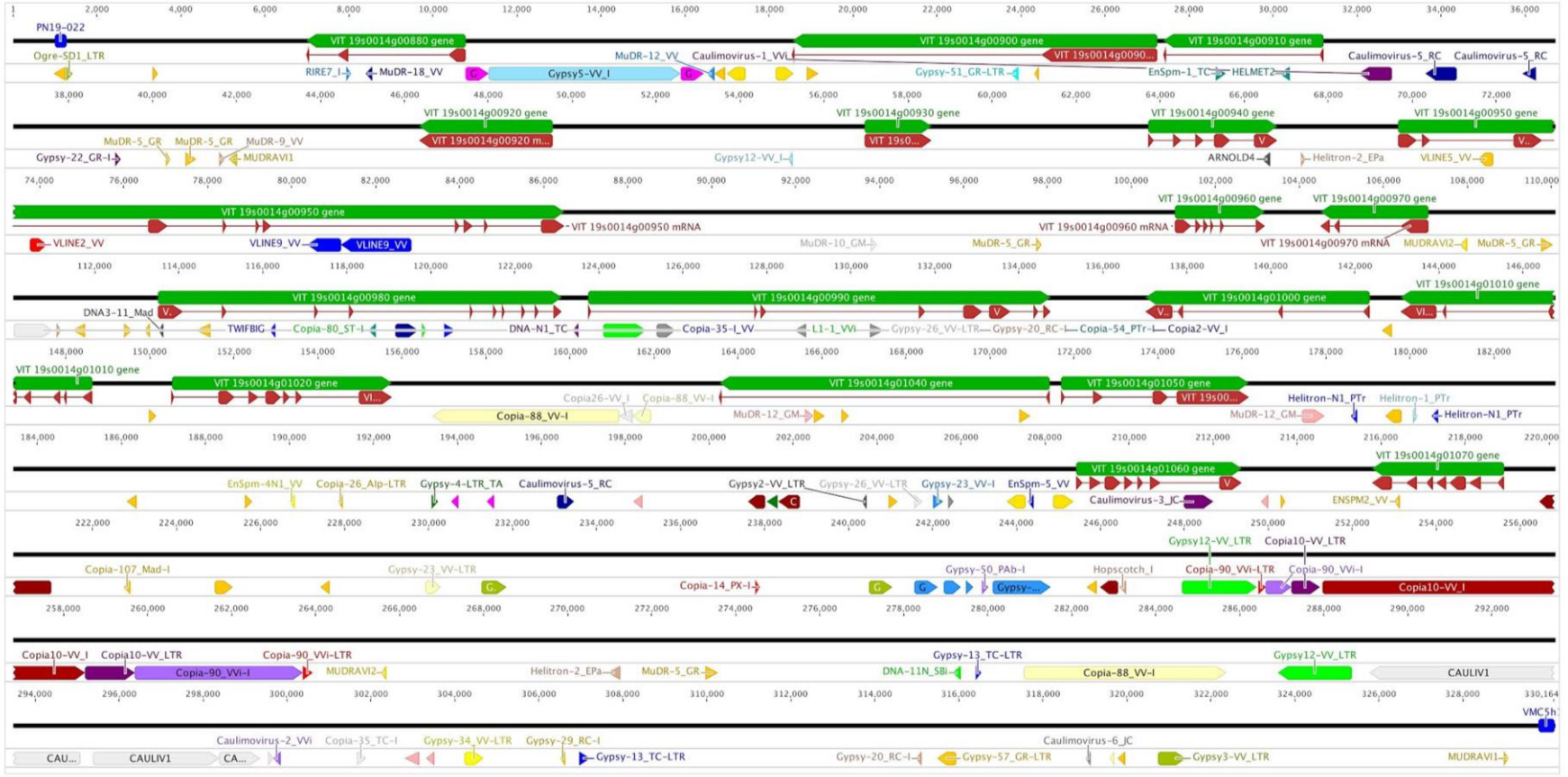
B



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