SZENT ISTVÁN UNIVERSITY

IDENTIFICATION OF

TWO NOVEL POWDERY MILDEW RESISTANCE LOCI,

FROM THE WILD CHINESE GRAPE SPECIES *VITIS PIASEZKII*

PhD THESIS

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1. INTRODUCTION

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. 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 to effectively access the resistance in any breeding population.

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.

2. OBJECTIVES

- Evaluating the *Vitis piasezkii* DVIT2027 derived F1 breeding populations for powdery mildew symptoms in the field to determine the genetic nature of the resistance
- 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 subjective 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

3. MATERIALS AND METHODS

3.1. 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.

3.2. 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.

3.3. 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.

To further saturate and refine the region for chromosome 9 and for chromosome 19, fourteen new SSR markers were developed (PN9 and PN19 series) utilizing the 12X genome sequence of PN40024 (Jaillon et al., 2007).

Amplified products run on an ABI 3500 capillary electrophoresis analyzer. (Life Technologies, Carlsbad, California, USA). Allele sizes were determined using GeneMapper 4.1 software (Applied Biosystem Co., Ltd., USA).

3.4. 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. Then after the identification of cofactors Multiple QTL Mapping (MQM) analysis were carried out.

3.5. Identification of key recombinants

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. 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.

4. RESULTS

4.1. 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 1 provides the details on the number of seedlings tested in each year, minimum and maximum scores, means, and variances.

Table 1. *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 2. *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*

4.2. 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. 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. 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. 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 1E). 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 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. A maximum of 18.1% variation was observed for the 2014 cane screen with LOD 14.55.

To study the effect of each locus independently, F1 progeny was 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 1C-D). 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.

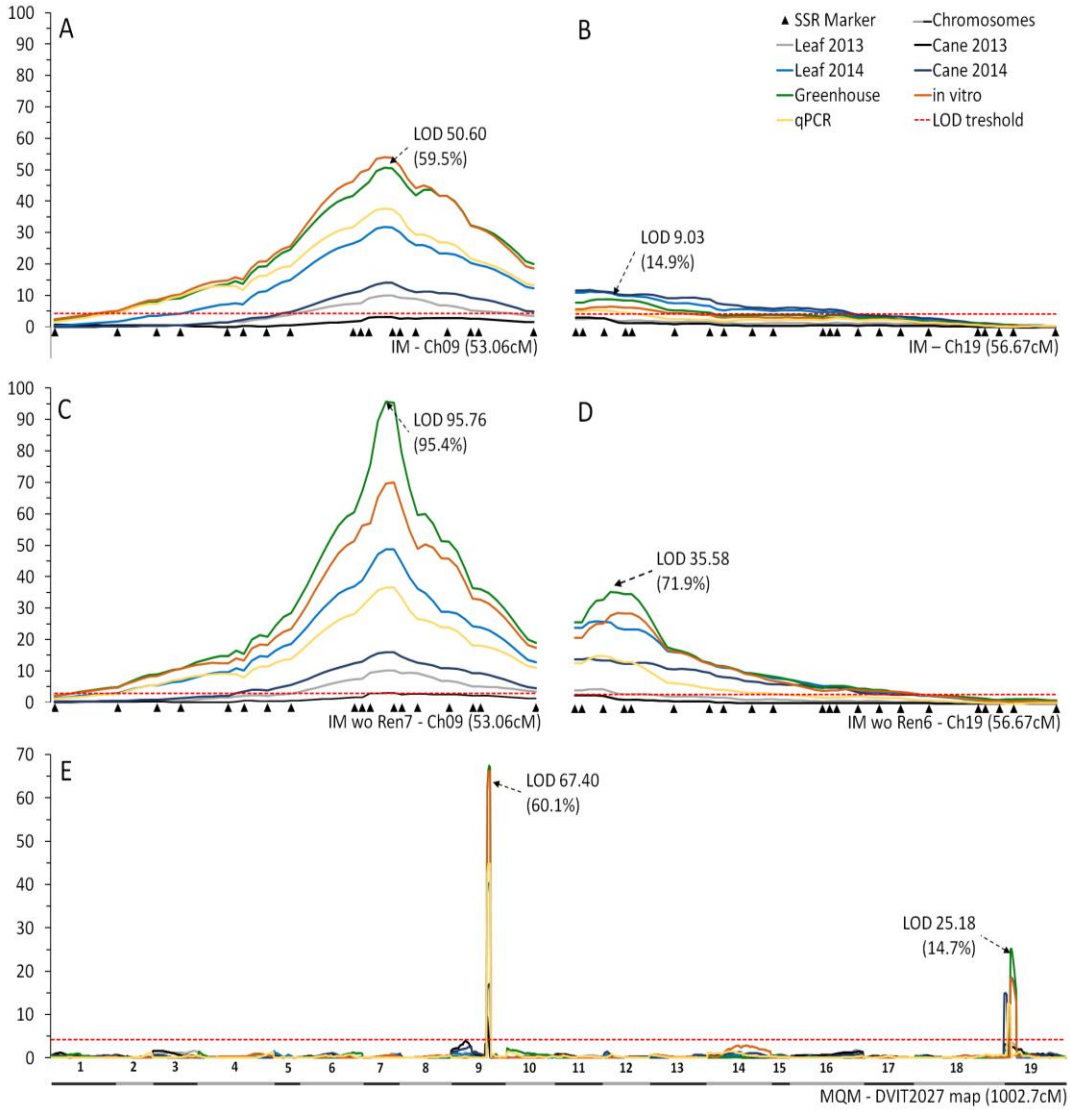

Figure 1. *Identification of Ren6 and Ren7 loci with interval and multiple QTL mapping. (A, B) Results of interval mapping carried out on entire base population for chromosome 9 and 19. (C, D) Interval mapping analysis on subset of genotypes that are selected based on the local haplotype of Ren6 or Ren7 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.*

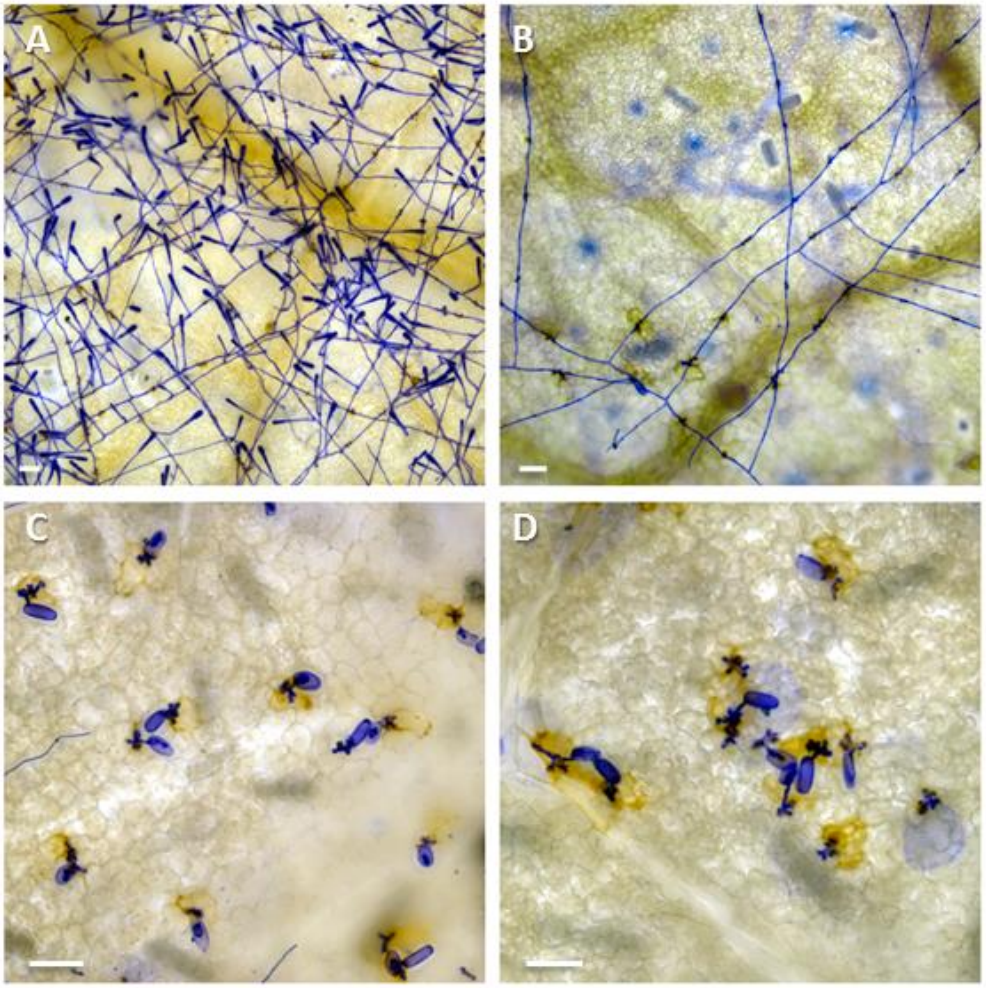

Figure 2. *Comparative development of powdery mildew on in vitro leaves of genotypes containing different combinations of R loci introgressed from* Vitis piasezkii. *Detached leaves were inoculated with powdery mildew using a settling tower, harvested 5 dpi and stained with Coomassie Brilliant blue to 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.*

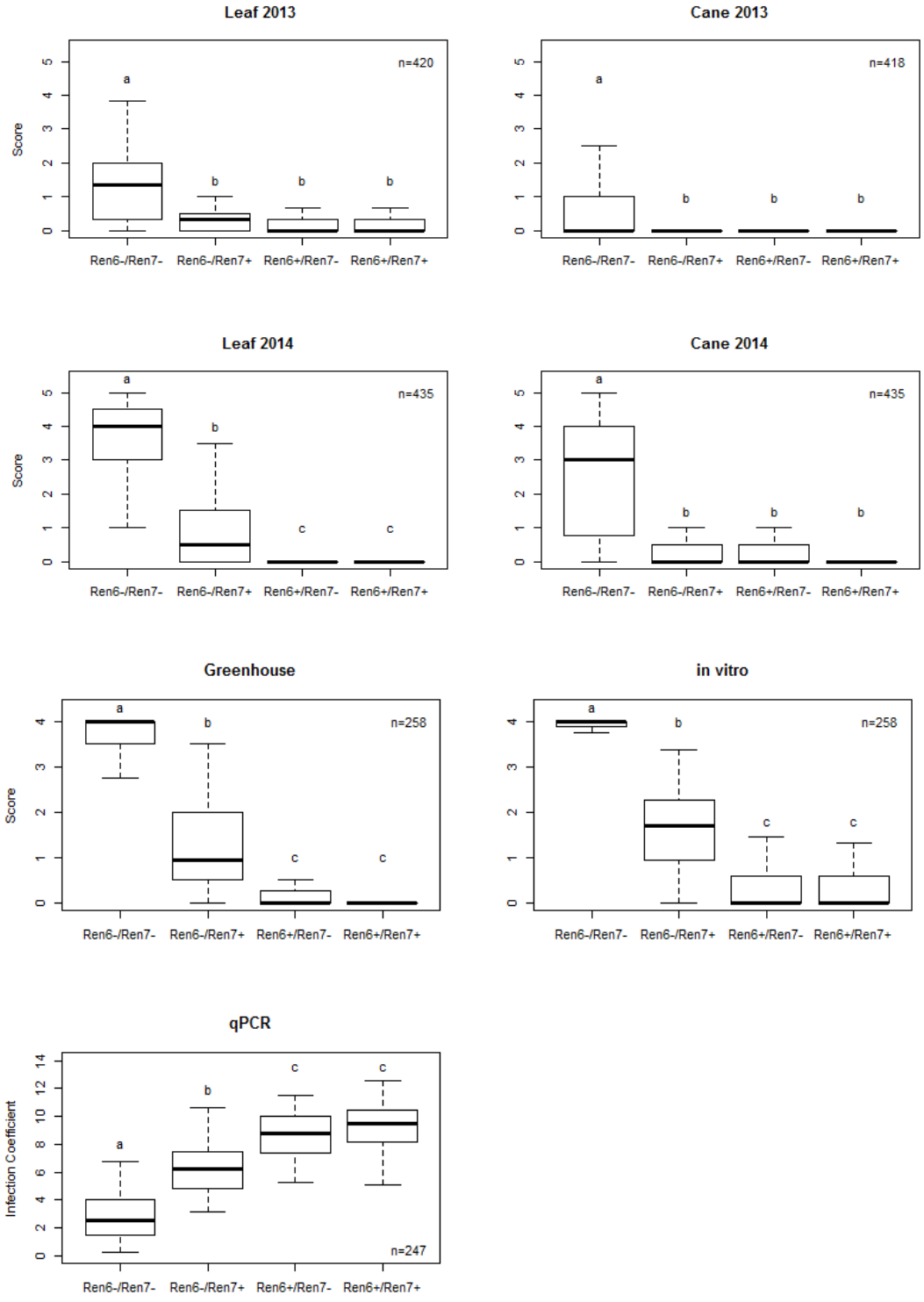

Figure 3. *Summary of powdery mildew susceptibility of the four genotypic classes within the F1 population. Progeny was evaluated in the greenhouse plants and with detached leaves in vitro assays. Significant differences detected with Tukey's test are indicated with different letters. The letter 'n' 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.*

4.3.Characterization of the Ren6 and Ren7 resistance response

There is clear evidence from Figure 2 of a hypersensitive response (HR) to powdery mildew inoculation in genotypes containing either *Ren7* (Figure 2B) or *Ren6* (Figure 2C). 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*.

4.4.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*.

In the refined genetic map based on the additional recombinant genotypes, the *Ren6* locus resides between markers PN9-066.1 and PN9-067. 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). 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.

5. CONCLUSIONS

5.1. 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 1). Nonetheless, we identified the *Ren6* and *Ren*7 loci with significant LOD scores in the same genomic regions, independently of the type of phenotypic data used for the analysis.

5.2. 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.

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. 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.

5.3. 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. 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.

6. 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.

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

7. 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.

8. REFERENCES

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.

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.

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.

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.

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.

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.

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.

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.

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.

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

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.

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.

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., 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.

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.

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.

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., 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.

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.

PUBLICATIONS CONNECTED TO THE DISSERTATION

Papers in impact factored journals:

Pap D., Riaz, S., Dry I.B., Jermakow A., Tencher A.C., Cantu D., Oláh R., Walker, M.A., 2016 Identification of two novel powdery mildew resistance loci, *Ren6* and *Ren7*, from the wild Chinese grape species *Vitis piasezkii*. BMC Plant Biology. **IF 3.813 (2015)**

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 Research, *2*, p.15037. **(IF due in 2017)**

Other papers:

Pap D., Riaz S., Halász J., Tenscher A.C., Walker M.A., 2016 Szőlő lisztharmat (Erysiphe necator Schwein.) fenotipizálási rendszerek alkalmazott összehasonlítása rezisztenciatérképezéshez. Kertgazdaság **(**Kertgazdaság 48 (3): 62-69**)**

Konference papers ("abstract")

Pap D., Riaz S., Tenscher A.C., Walker M.A.: Evaluation of different methods to determine powdery mildew resistance in grape breeding program. 67th American Society for Enology and Viticulture National Conference, Monterey, California USA. June 27-30, 2016

Riaz S., **Pap D.,** Tenscher A.C., Hu R., Walker M.A.: Molecular Strategies to stack powdery mildew from multiple backgrounds in grape breeding program. 67th American Society for Enology and Viticulture National Conference, Monterey, California USA. June 27-30, 2016

Pap D., Riaz S., Tenscher A.C., Hu R., Walker MA.: Genetic mapping of powdery mildew resistance in wild Chinese species, *Vitis piasezkii*. Recent Advances in Viticulture & Enology. UC Davis, Davis, California. May 15 2015 http://wineserver.ucdavis.edu/education/venextention/rave/RAVE_2015_Agenda.pdf

Pap D., Riaz S., Tenscher A.C., Hu R., Walker MA.: Genetic mapping of powdery mildew resistance in wild Chinese species, *Vitis piasezkii*. 65th American Society for Enology and Viticulture National Conference, Austin, Texas. June 23-27, 2014 Book of Conference, page 9. http://www.asev.org/sites/main/files/file-attachments/2014registrationguide.pdf

PUBLICATIONS CONNECTED TO THE DISSERTATION

Papers in impact factored journals

Bearch S., Kovens M., Hubbert L., Honesty S., Guo Q., **Pap D.**, Dai R., Kovacs L. Qiu, W., Genetic and phenotypic characterization of Grapevine vein clearing virus isolates of wild *Vitis rupestris* Scheele. Phytopathology, DOI: 10.1094/PHYTO-04-16-0173-R

Pap, D., Miller, A.J., Londo, J.P. and Kovács, L.G., 2015. Population structure of *Vitis rupestris*, an important resource for viticulture. American Journal of Enology and Viticulture, pp.ajev-2015. **IF 1.388 (2014)**

Konference papers ("abstract")

Campbell C., Conover J., **Pap D.**, Riaz R., Coleman C., Kovács L.G., Walker M.A.: Phenotyping *Vitis piasezkii* X *Vitis vinifera* hybrid population for resistance to *Plasmopara viticola.* Plant Phenomics: From Pixels to Traits - Plant Science Symposium, Lincoln, Nebraska, Nebraska Innovation Campus Conference Center November 15-16 2015, http://www.unl.edu/psi/2015-plant-science-symposium

Conover J., Campbell C., **Pap D.**, Riaz S., Coleman C., Kovács L.G.: Phenotyping a segregant mapping population of *Vitis piasezkii* X *Vitis vinifera* hybrids for quantitative trait koci analysis for resistance to *Plasmopara Viticola.* 4th Annual Grape and Wine Research Symposium, Columbia, Missouri on May 8 2015. http://www.cafnralumni.com/s/1002/gid12_cafnr/index.aspx?sid=1002&pgid=5200&gid=12&cid=9696&ecid=9696&post_id=0

Conover J., Campbell C., **Pap D.**, Riaz S., Coleman C., Kovács L.G.: Phenotyping a segregant mapping population of *Vitis piasezkii X Vitis vinifera* hybrids for quantitative trait loci analysis for resistance to *Plasmopara viticola.* Missouri State University, College of Natural and Applied Sciences, Undergraduate Research Symposium, Springfield, Missouri on April 24 2015. Book of Abstracts, page 4. https://science.missouristate.edu/assets/science/Abstracts2015.pdf

Conover J., **Pap D.**, Riaz S., Coleman C., Kovács L.G.: Differential host series analysis of *Plasmopara viticola* on select varieties of *Vitis vinifera*. Undergraduate Research Symposium, Springfield, Missouri on April. 2014. Book of Abstracts, page 4. https://science.missouristate.edu/assets/science/CNASAbstractsURD2014.pdf

Klein L., **Pap D.**, Kovács L.G., Hyma K., Londo J., Miller A.: Population genetics of native *Vitis riparia* and *V. rupestris* in sympatry and allopatry. 60th annual systematics symposium of the Missouri Botanical Garden, St. Louis, Missouri. October 11-12. 2013 http://www.missouribotanicalgarden.org/plant-science/plant-science/resources/conferences-and-seminars/annual-systematics-symposium.aspx?questionid=833&afmid=9849

Klein L., **Pap D.**, Kovács L.G., Hyma K., Londo J., Miller A.: Population genetics of native *Vitis riparia* and *V. rupestris* in sympatry and allopatry. Third Annual Saint Louis Ecology, Evolution, and Conservation retreat, St. Louis, Missouri. September 28 2013. http://wubio.wustl.edu/events/992

Klein L., **Pap D.**, Kovács L.G., Hyma K., Londo J., Miller A.: Population genetics of native *Vitis riparia* and *V. rupestris* in sympatry and allopatry. Fifth conference of the Grape Research Coordination Network (GRCN), Davis, California. July 11 2013. http://www.vitaceae.org/index.php/Meetings

Pap D., Klein L., Coleman C., Peccoux A., Kovács L. G., Londo J., Miller A.: The genetic structure of rock grape populations on the Ozark plateau. Fifth conference of the Grape Research Coordination Network Davis, California. July 11 2013. http://www.vitaceae.org/index.php/Meetings

Whitehead D., **Pap D.**, Kalapos B., Erwin A., Kovács L.G.: An investigation of the role of Sirtuin proteins in plants. Missouri State University Interdisciplinary Forum Springfield, Missouri. April, 2013. Book of Abstracts page 1. http://science.missouristate.edu/assets/science/CNASAbstractsURD2013.pdf

Pap D., Coleman C., Erwin A., Glasson J., Li C., Kovács L.G.: Exploring rock grapes in Missouri. Missouri State University Interdisciplinary Forum Springfield, Missouri. April 14. 2012. Book of absracts, page 5. http://science.missouristate.edu/assets/science/CNASAbstractsURD2012.pdf

Kovács G.L., Li C., Erwin A., **Pap D.**, Coleman C., Selection for Run1-Ren1 Dihybrid Grapevines Using Microsatellite Markers. 2nd Grape and Wine Research Symposium. University of Missouri, Columbia, Missouri, December 7, 2012. http://www.cafnralumni.com/s/1002/gid12cafnr/index.aspx?sid=1002&pgid=3754&gid=12&cid=6425&ecid=6425&post_id=0