CROP BREEDING AND APPLIED BIOTECHNOLOGY

ARTICLE

Pyramiding of resistance alleles to grape powdery mildew assisted by molecular markers

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Abstract: *Disease resistance gene pyramiding is a widely used strategy to enhance resistance durability. Marker-assisted selection (MAS) was applied to pyramide the alleles Run1 and Ren3, which confer resistance against grape powdery mildew (Erysiphe necator). Two F_1 full-sibs carrying Run1 and Ren3 in heterozygosity were selfed to develop the breeding populations used in the analysis. From the 637 genotyped plants, 313 (50.6%) had the Run1 and Ren3 pyramided. Seven (1.1%) of them exhibited the two resistance alleles in homozygosity. Plants without any resistance alleles had the highest disease severity ($\overline{X}$ = 7.3), while the ones with the Run1 allele both in homozygosity and heterozygosity were highly resistant ($\overline{X}$ = 1.5). Similar level of resistance was observed in the plants containing Run1 and Ren3 pyramided ($\overline{X}$ = 1.3). Plants containing Run1 and Ren3 pyramided in homozygosity are important genetic resources for grape breeding programs in Brazil*.

Keywords: Vitis vinifera*, plant breeding,* Erysiphe necator*, disease resistance, sustainability*

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INTRODUCTION

Powdery mildew is a major fungal grapevine disease worldwide. The disease is caused by *Erysiphe necator* Schwein [syn. *Uncinula necator* (Schwein) Burrill], an obligate biotrophic fungus belonging to ascomycetes and the Erysiphaceae family (Kunova et al. 2021). When not adequately managed, it reduces grape yield and quality and compromises wine quality (Scott 2021). The infection and colonization of susceptible hosts result in abundant production of mycelium, conidiophores and asexual conidia at the adaxial leaf surface, which are initially visualized as roughly circular whitish spots, and later assuming a typical powdery appearance (Kunova et al. 2021).

The pathogen is predominantly found in dry and warm regions (Zendler et al. 2021). Tropical regions in Brazil produce either table and wine grapes. The São Francisco River Valley region is the most prominent of these areas. These regions are frequently characterized by a dry climate throughout the year, and powdery mildew is one of the major challenges for grape production (Camargo et al. 2011). In the Southern region of Brazil, *E. necator* is not epidemic, because the climatic conditions are not conducive to *E. necator* infection and development. However, in this region, some vineyards are grown in protected environments

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(plastic cover). That measure reduces the wetting on the grape tissues and favors the occurrence of powdery mildew (Almança et al. 2017).

The current main strategy for controlling powdery mildew in commercial vineyards is based on preventive fungicide application. This practice increases the production costs, generates health and environmental concerns, and may result on the selection of fungicide-resistant *E. necator* strains (Merdinoglu et al. 2018, Kunova et al. 2021).

Host genetic resistance is considered the most sustainable alternative for grape powdery mildew management (Qiu et al. 2015). Many loci containing alleles associated with resistance to the pathogen have been genetically mapped: *Run1* (Barker et al. 2005); *Run1.2* (Riaz et al. 2011); *Run2.1* and *Run2.2* (Riaz et al. 2011); *Ren1* (Hoffmann et al. 2008); *Ren1.2* (Possamai et al. 2021); *Ren2* (Dalbó et al. 2001); *Ren3* (Welter et al. 2007); *Ren4* (Ramming et al. 2011); *Ren5* (Blanc et al. 2012); *Ren6* and *Ren7* (Pap et al. 2016); *Ren8* (Zyprian et al. 2016); *Ren9* (Zendler 2017); *Ren10* (Teh et al. 2017); and *Ren11* (Karn et al. 2021).

The construction of resistance genes pyramids is considered one of the best strategies to achieve durable resistance against pathogens (Rex Consortium 2016). Resistance allele pyramiding against *E. necator* is being widely used in grape: *Run1* and *Ren3* heterozygous plants (Eibach et al. 2007, Calonnec et al. 2013), *Run1.2* and *Run2* in the Trayshed cultivar (homozygous only for the *Run1.2* locus) and Thomas genotype (heterozygous lines) (Feechan et al. 2015), *Run1* and *Ren1* (Agurto et al. 2017), and *Ren6* and *Ren7* (Pap et al. 2016).

We aimed in the present investigation to apply the MAS to pyramid the resistance alleles associated to the loci *Run1* and *Ren3* and to assess the level of resistance conferred by *Run1* and *Ren3*, isolated or combined, in homozygote and heterozygote state.

MATERIAL AND METHODS

Plant materials

The full-sibs '2000-305-134' and '2000-305-97', selected from the cross 'VHR-3082-1-42' and 'Regent', were selfed to develop two segregating populations UFSC-2013-1 (420 progenies) and UFSC-2013-2 (217 progenies). They carry the resistance alleles *Run1* (Barker et al. 2005) and *Ren3* (Welter et al. 2007), pyramided in heterozygosity.

Genotyping

DNA preparation and quantification followed the methodology described by Sanchez-Mora et al. (2017). For marker-assisted selection, microsatellite markers closely flanking the *Run1* and *Ren3* loci were used. *Run1* is closely linked to *Rpv1*. Therefore, for *Run1* we used the same microsatellite markers (Sc34_8 and Sc35_2) described by Sanchez-Mora et al. (2017). For *Ren3* we used the markers GF15-28 and GF15-30 (Zyprian et al. 2016). The forward primers were labeled with the fluorescent dyes 6-FAM, VIC, and PET. The four microsatellite markers were combined into a single multiplex, amplified with the Kapa2G Fast Multiplex Mix (Kapa Biosystems Inc., Boston, MA, USA) and the fragments separated by capillary electrophoresis in a 3500 XL sequencer (Thermo Fisher Scientific, Waltham, MA, USA), as described by Sanchez-Mora et al. (2017). Gene Mapper Version 4.1 software (Thermo Fisher Scientific, Waltham, MA, USA) was used to call the microsatellite alleles. Based on the genotypic information, the progenies were discriminated into the nine possible allelic combinations of the two independently segregating loci *Run1* and *Ren3* (Figure 1).

Phenotyping

To validate the genotyping data, a subset of plants from both segregating populations representing the nine different genotypes were scored for powdery mildew resistance in greenhouse in two locations and three years. Plants of the two populations were propagated by cuttings and established in a greenhouse at the Videira Experimental Station of Epagri, Videira, SC, and in the Agricultural Experimental Station of the Federal University of Santa Catarina, Curitibanos Campus, Curitibanos, SC. The resistance assays were performed under natural conditions of powdery mildew infection in February in the 2014/15 and 2015/16 crop seasons in Videira and in 2016/17 in Curitibanos.

The level of resistance to powdery mildew was rated according to the OIV-455 scale of the International Organisation

of Vine and Wine (OIV 2009), where each score corresponds to the sporulation intensity of the disease: 1) very low sporulation: small spots or no symptoms, not visible; 3) low sporulation: limited spots < cm in diameter, limited sporulation and mycelium, the presence of *E. necator* is only indicated by a slight ripple in the leaf blade; 5) average sporulation: normally limited spots with diameter of 2-5 cm; 7) high sporulation: vast spots, some limited, abundant mycelium sporulation; and 9) very high sporulation: unlimited stains with entire leaf blade under attack, high fungal sporulation and abundant mycelium.

Statistical analysis

The genotype data were organized in frequency distribution, and the chi-square (χ^2) goodness of fit test was applied to verify if there are allelic or genotypic segregation distortion. The nature of segregation distortion was defined using the methodology described by Lorieux et al. (1995). For the phenotypic data, the frequency of progenies in each resistance class was calculated and plotted on graphs. The Fisher exact test (p value < 0.001) was applied after Bonferroni correction to compare the frequency distribution between the different progenies. When the genotypes had the same resistance score, they were considered dependent, that is, they have the same frequency distribution and therefore have the same level of resistance. In contrast, when the progenies have different scores, they are considered independent and are different. The R statistical program (R Development Core Team 2021) was used for statistical analyses.

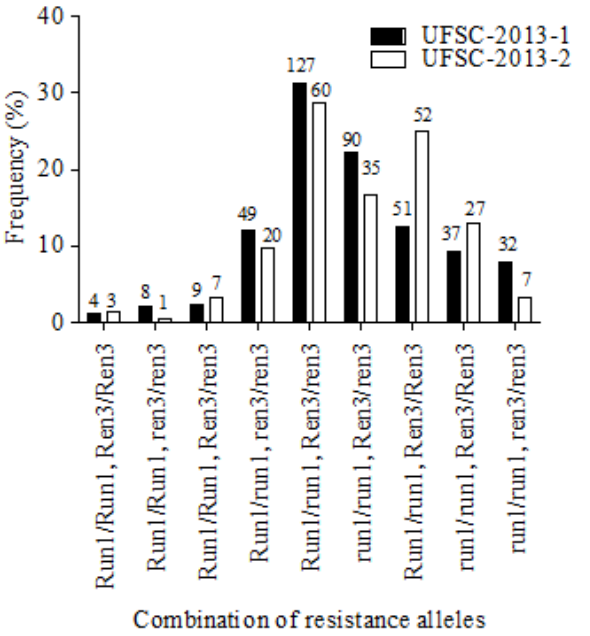

Figure 1. Frequency distribution (%) of individuals segregating for two loci conferring resistance to powdery mildew in 619 plants of the UFSC-2013-1 (χ^2 = 107.86, df = 8, p value < 0.001) and UFSC-2013-2 (χ^2 = 80.604, df = 8, p value < 0.001) selfing populations. Numbers above the bars indicate the number of plants in each genotypic class.

RESULTS AND DISCUSSION

Genotyping

Based on the genotypic data, the 637 plants from the two segregating populations were distributed in nine genotypic classes (Figure 1). Plants were considered to have the resistance allele when no crossing-over was observed between the respective flanking markers. Two plants in the *Run1* locus and 16 plants in the *Ren3* locus showed recombination (2.8%) between the flanking microsatellite markers. From the remaining 619 plants, 313 (50.6%) exhibited the two alleles pyramided, 189 (30.5%) carried only *Ren3*, 78 (12.6%) had only the *Run 1* allele, and 39 (6.3%) did not carry any of the resistance alleles (Figure 1).

Among the plants containing pyramided resistance alleles, 7 (1.1%) exhibited the two resistance alleles in homozygosity (*Run1*/*Run1*, *Ren3*/*Ren3*), 16 (2.6%) carried the *Run1* allele in homozygosity and *Ren3* in heterozygosity (*Run1*/*Run1*, *Ren3*/*ren3*), 103 (16.6%) had the *Run1* allele in heterozygosity and *Ren3* in homozygosity (*Run1*/*run1*, *Ren3*/*Ren3*), and 187 (30.2%) had the two resistance alleles in heterozygosity (*Run1*/*run1*, *Ren3*/*ren3*).

Significant segregation distortion was observed in both segregating populations (Table 1). It was particularly high for the microsatellite markers linked to *Run1* (Table 1; Figure 1). In both populations occurred a suppression of the genotype homozygote for the resistance allele *Run1* (*Run1/Run1*) and, consequently, an excess of heterozygotes (*Run1/run1*) and recessive homozygotes (*run1/run1*) (Table 1). The segregation distortion in this genomic region is due to zygotic selection against the homozygous genotype *Run1*/*Run1*. Segregation distortion in the *Run1/Rpv1* locus was previously reported

Table 1. Frequencies obtained of segregation and chi-square test revealed by SSR markers in the two populations segregating for resistance to powdery mildew

Locus	Populations					
	UFSC-2013-1			UFSC-2013-2		
	n	Genotype frequency	Allele frequency	n	Genotype frequency	Allele frequency
SSR markers: Sc34_8/Sc35_2 (Chr. 12)						
Run1/*Run1*	21	0.05	*Run1*=0.33	11	0.05	*Run1*=0.36
Run1/*run1*	227	0.56	*run1*=0.67	132	0.62	*run1*=0.64
run1/*run1*	159	0.39		69	0.33	
χ^2 (1:2:1)	99.01***			44.49***		
Distortion with one locus[1]			Zygotic		Zygotic	
χ^2 =[*p* (*Run1*) = *q* (*run1*)]			94.10***		33.24***	
$\chi^2 = p^2+2pq+q^2$			27.63***		25.60***	
SSR markers: GF15-28/GF15-30 (Chr. 18)						
Ren3/*Ren3*	92	0.23	*Ren3*=0.51	82	0.39	*Ren3*=0.63
Ren3/*ren3*	226	0.55	*ren3*=0.50	102	0.48	*ren3*=0.37
ren3/*ren3*	89	0.22		28	0.13	
χ^2 (1:2:1)	5.02			27.81***		
Distortion with one locus[1]			Zygotic		Gametic	
χ^2 = [*p* (*Ren3*) = *q* (*ren3*)]			0.08		28.66***	
$\chi^2 = p^2+2pq+q^2$			4.99***		0.19	
Distortion with two loci[1]			Zygotic		Zygotic	
χ^2 = [*p* (*Run1*) = *q* (*run1*), *r* (*Ren3*) = *s* (*ren3*)]			422.47***		223.44***	
$\chi^2 = [p^2+2pq+q^2] \times [r^2+2rs+s^2]$			18031.2***		11560.5***	

[1]According to the methodology employed by Lorieux et al. (1995). *** P<0.001 by chi-square test (χ2).

in other studies (e.g., Barker et al. 2005, Sánchez-Mora et al. 2017). The microsatellite markers linked to *Ren3* showed weak segregation distortion (Table 1).

Segregation distortion is frequently observed in interspecific crosses. In grape, it was reported in interspecific crosses e.g. Horizon x Illinois 547-1 (Dalbó et al. 2001), *Muscadinia rotundifolia* G52 × *Malaga seedling* No.1 (*V. vinifera*) (Pauquet et al. 2001), *V. rupestris × V. arizonica/girdiana* (Riaz et al. 2006), and *M. rotundifolia* cv. Fry × *M. rotundifolia* cv. Trayshed (Riaz et al. 2012).

Phenotyping

To determine the level of resistance to powdery mildew conferred by the loci *Run1* and *Ren3*, individually and in pyramided form, about three-fourth of the genotyped plants were screened in greenhouses under natural infectious conditions of *E. necator* in two environments (Videira, SC, and Curitibanos, SC). Evaluation in greenhouse is a methodology widely used to phenotype resistance to grapevine powdery mildew (Eibach et al. 2007, Gao et al. 2016, Possamai et al. 2021). The greenhouse provides a drier environment that favors infection by the pathogen.

The three phenotyping evaluations (two in Videira and one in Curitibanos, SC) revealed no significant genotype × environment interaction (data not shown), and therefore, the results were analyzed together (Figure 2 and Table 2). The progenies without any resistance allele were susceptible to powdery mildew (average score of 7.0; *p* value < 0.001). *Ren3* conferred partial resistance to the plants, significantly reducing incidence of the disease compared to the susceptible plants (average score of 5.4; *p* value < 0.001). The *Ren3* allele displayed the widest variation in resistance level, ranging from 1 (12.3% of plants) to 9 (14% of plants). The frequencies of the intermediary scores were: 3 (8.8%), 5 (35.1%), and 7 (29.8%). Similar results were observed in previous investigations (e.g., Welter et al. 2007, Eibach et al. 2007, Zendler et al. 2017). The fine mapping of the original *Ren3* genomic region of 'Regent' (Welter et al. 2007) revealed that two distinct loci (*Ren3* and *Ren9*) mediate the partial resistance to *E. necator* (Zendler et al. 2017, Zendler et al. 2021). An impaired recombination frequency between both loci was observed, strongly suggesting that in the present investigation

the two loci are operating together, since both selfing populations inherited *Ren3* from 'Regent'. Therefore, one possible explanation for the phenotypic variation is the recombination between the two loci. However, Zendler et al. (2021) found a recombination frequency of around 2%, which cannot explain the phenotypic variation. The most probable explanation for the variation is associated with the mode of operation of *Ren3/Ren9* resistance alleles. For instance, Zendler et al. (2017) demonstrated that the defense mechanism mediated by *Ren3*/*Ren9* is associated with a post-invasion reaction. The pathogen is able to build a dense mycelial net on plants carrying *Ren3*/*Ren9*, but rarely forms conidia. Mycelial density may also be influenced by genomic background, the presence of other minor QTLs, and/or small environmental variations.

Table 2. Results of Fisher's exact test ($p < 0.01$) comparing the phenotypic response of the allelic combinations of the *Run1* (A) and *Ren3* (B) loci to powdery mildew infection in grape

Loci	Aabb	aabb
AAbb	= *p* value=0.07	≠ (**) *p* value =0.00049
Aabb		≠ (**) *p* value =0.00049
	aaBb	aabb
aaBB	= *p* value =0.11	≠ (**) *p* value =0.00049
aaBb		≠ (**) *p* value =0.00549

DIF: Significant difference indicated by Fisher's exact test at 5% significance level.
** p-value with a significance level lower than 0.01.

The progenies carrying the *Run1* allele were highly resistant (average score of 1.9), differing significantly from *Ren3* (p value < 0.001). Almost 70% percent of the progenies carrying at least one *Run1* allele did not show any symptom of the disease (score 1). This resistance level mediated by *Run1* is already well documented and it is associated with programmed cell death of epidermal cells penetrated by fungi, prohibiting pathogen colonization (Feechan et al. 2013). As *Run1* mostly conferred complete resistance to powdery mildew, no significant difference was observed between progenies carrying *Run1* alone or pyramided with *Ren3* (average score of 1.3), as previously reported (Eibach et al. 2007, Zini et al. 2019, Possamai et al. 2021). Nevertheless, stacking the resistance allele of both loci is an important measure to promote resistance durability since the loci are inherited from different species and the mode of action is different (Feechan et al. 2013, Merdinoglu et al. 2018, Zendler et al. 2021). However, the long-term durability of *Ren3* and *Run1*

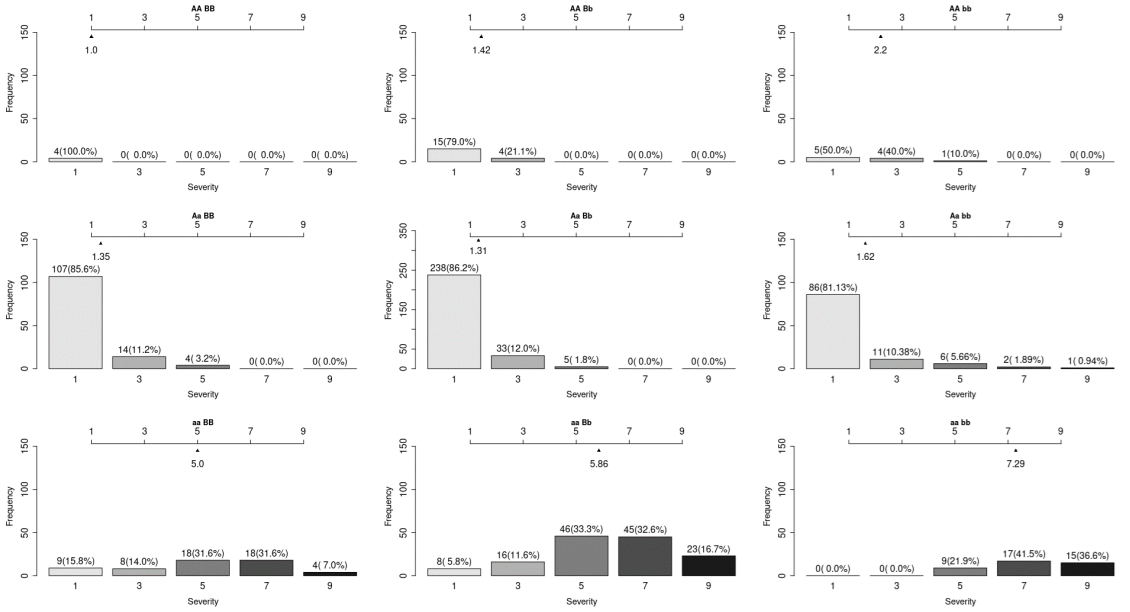

Figure 2. Phenotypic distribution of powdery mildew resistance according to the OIV-455 descriptor ranging from resistant (1) to susceptible (9) for each of the nine genotypic combinations of the loci *Run1* (A) and *Ren3* (B). The upper axis indicates the weighted average of the genetic resistance of the analyzed sample. The inferior axis indicates the distribution of the analyzed allele frequency grouped by the class of severity of powdery mildew infection (OIV-455). Above each column the observed frequency and the respective proportion are indicated.

should be a matter of concern, since isolates that have overcome the resistance of both loci have been reported (Cadle-Davidson et al. 2011, Feechan et al. 2013, Feechan et al. 2015, Teh et al. 2017).

The progenies containing the resistance alleles in homozygosity did not show higher resistance level to powdery mildew compared to those with the alleles in heterozygosity for *Run1*, *Ren3,* and *Run1+Ren3* (Table 2), suggesting a complete dominant allelic interaction in both loci. Although no dosage effect was observed, the development of "breeding lines" homozygous for resistance alleles is an interesting strategy to increase the breeding efficiency, since the use of homozygous lines in crosses would render all progenies with one copy of the resistance alleles, not requiring phenotyping and genotyping evaluations. However, grape commonly undergoes endogamic depression, and obtaining plants with desirable vigor is a challenge. It is noteworthy that the OIV-455 descriptor is more of a quality rating scale; therefore, small differences such as allele dosage effects or the contribution of *Ren3* in increasing the resistance mediated by *Run1* cannot be completely discarded.

In the present study, MAS was effectively used for pyramiding the loci *Run1* and *Ren3*, which confer resistance to grape powdery mildew. The use of MAS, associated to segregating populations obtained by selfing, allowed the selection of plants containing *Run1* and *Ren3* alleles in homozygosity. The use of these plants in new crossing generations permits the obtention of 100% of the progenies containing both resistance alleles in heterozygosity, increasing the breeding efficiency.

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REFERENCES

Agurto M, Schlechter RO, Armijo G, Solano E, Serrano C, Contreras RA, Zúñiga GE and Arce-Johnson P (2017) RUN1 and REN1 Pyramiding in grapevine (*Vitis vinifera* cv. Crimson Seedless) displays an improved defense response leading to enhanced resistance to powdery mildew (*Erysiphe necator*). **Frontiers in Plant Science 8**: 758.

Almança MAK, Frighetto NS, Tonello JC and Lerin S (2017) Diseases incidence and fungicide cost reduction with overhead covered grapes. **Revista Brasileira de Fruticultura 39**: e-020.

Barker CL, Donald T, Pauquet J, Ratnaparkhe A, Bouquet A, Adam-Blondon AF, Thomas MR and Dry I (2005) Genetic and physical mapping of the grapevine powdery mildew resistance gene, *Run1*, using a bacterial artificial chromosome library. **Theoretical and Applied Genetics 111**: 370-377.

Blanc S, Wiedemann-Merdinoglu S, Dumas V, Mestre P and Merdinoglu D (2012) A reference genetic map of *Muscadinia rotundifolia* and identification of Ren5, a new major locus for resistance to grapevine powdery mildew. **Theoretical and Applied Genetics 125**: 1663-1675.

Cadle-Davidson L, Mahanil S, Gadoury DM, Kozma P and Reisch BI (2011) Natural infection of *Run1*-positive vines by naïve genotypes of *Erysiphe necator.* **Vitis 50**: 173-175.

Calonnec A, Wiedemann-Merdinoglu S, Deliere L, Cartolaro P, Schneider C and Delmotte F (2013) The reliability of leaf bioassays for predicting disease resistance on fruit: a case study on grapevine resistance to downy and powdery mildew. **Plant Pathology 62**: 533-544.

Camargo UA, Tonietto J and Hoffmann A (2011) Progressos na viticultura brasileira. **Revista Brasileira de Fruticultura 33**: 144-149.

Dalbó MA, Ye GN, Weeden NF, Wilcox WF and Reisch BI (2001) Marker-assisted selection for powdery mildew resistance in grapes. **Journal of the American Society of Horticultural Science 126**: 83-89.

Eibach R, Zyprian E, Welter L and Töpfer 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 AR, Cadle-Davidson L, Reisch B, Aubourg S, Bentahar N, Shrestha B, Bouquet A, Adam-Blondon AF, Thomas MR and Dry IA (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. **The Plant Journal 76**: 661-674.

Feechan A, Kocsis M, Riaz S, Zhang W, Gadoury DM, Walker MA, Dry IB, Reisch B and 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.

Gao YR, Han YT, Zhao FL, Li YJ, Cheng Y, Ding Q, Wang YJ and Wen YQ (2016) Identification and utilization of a new *Erysiphe necator* isolate NAFU1to quickly evaluate powdery mildew resistance in wild Chinese grapevine species using detached leaves. **Plant Physiology and Biochemistry 98**: 12-24.

Hoffmann S, Di Gaspero G, Kovacs L, Howard S, Kiss E, Galbacs Z, Testolin R and Kozma P (2008) Resistance to *Erysiphe necator* in the grapevine'Kishmish vatkana' is controlled by a single locus through restriction of hyphal growth. **Theoretical and Applied Genetics 116**: 427-438.

Karn, A, Zou C, Brooks S, Fresnedo-Ramírez J, Gabler F, Sun Q, Ramming D, Naegele R, Ledbetter C and Cadle-Davidson L (2021) Discovery of the REN11 locus From *Vitis aestivalis* for stable resistance to grapevine powdery mildew in a family segregating for several unstable and tissue-specific quantitative resistance loci. **Frontiers in Plant Science 12**: 733899.

Kunova A, Pizzatti C, Saracchi M, Pasquali M and Cortesi P (2021) Grapevine powdery mildew: fungicides for its management and advances in molecular detection of markers associated with resistance. **Microorganisms 9**: 1541.

Lorieux M, Perrier X, Goffinet B, Lanaud C and González de León D (1995) Maximum-likelihood models for mapping genetic markers showing segregation distortion. 2. F_2 populations. **Theoretical and Applied Genetics 90**: 81-89.

Merdinoglu D, Schneider C, Prado E, Wiedemann-Merdinoglu S and Mestre P (2018) Breeding for durable resistance to downy and powdery mildew in grapevine. **OENO One 52**: 189-195.

OIV - International Organisation of Vine and Wine (2009) Descriptor list for grape varieties and Vitis species, Paris, France. Available at <http://www.oiv.org>. Accessed on January 07, 2014.

Pap D, Riaz S, Dry IB, Jermakow A, Tenscher AC, Cantu D, Oláh R and Walker MA (2016) Identification of two novel powdery mildew resistance loci, *Ren6* and *Ren7*, from the wild Chinese grape species *Vitis piasezkii*. **BMC Plant Biology 16**: 170.

Pauquet J, Bouquet A, This P and Adam-Blondon AF (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. **Theoretical and Applied Genetics 103**: 1201-1210.

Possamai T, Wiedemann-Merdinoglu S, Merdinoglu D, Migliaro D, De Mori G, Cipriani G, Velasco R and Testolin R (2021) Construction of a high-density genetic map and detection of a major QTL of resistance to powdery mildew (*Erysiphe necator* Sch.) in Caucasian grapes (Vitis vinifera L.). **BMC Plant Biology 21**: 528.

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

R Development Core Team (2021) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available at <http://www.R-project.org/>. Accessed on October 05, 2021.

Ramming DW, Gabler F, Smilanick J, Cadle-Davidson M, Barba P, Mahanil S and Cadle-Davidson L (2011) A single dominant locus, *ren4*, confers rapid non-race- specific resistance to grapevine powdery mildew. **Phytopathology 101**: 502-508.

Rex Consortium (2016) Combining selective pressures to enhance the durability of disease resistance genes. **Frontiers in Plant Science 7**: 1916.

Riaz S, Hu R and Walker MA (2012) A framework genetic map of *Muscadinia rotundifolia.* **Theoretical and Applied Genetics 125**: 1195-1210.

Riaz S, Krivanek AF, Xu K and Walker MA (2006) Refined mapping of the Pierce's disease resistance locus, *PdR1*, and Sex on an extended genetic map of *Vitis rupestris* x *V. arizonica*. **Theoretical and Applied Genetics 113**: 1317-1329.

Riaz S, Tenscher AC, Ramming DW and Walker MA (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. **Theoretical and Applied Genetics 122**: 1059-1073.

Sánchez-Mora FD, Saifert L, Zanghelini J, Assumpcão WT, Guginski-Piva CA, Giacometti R, Novak EI, Klabunde GH, Eibach R, Dal Vesco L, Nodari RO and Welter LJ (2017) Behavior of grape breeding lines with distinct resistance alleles to downy mildew (*Plasmopara viticola*). **Crop Breeding and Applied Biotechnology 17**: 141-149.

Scott ES (2021) 2019 Daniel McAlpine memorial lecture. Grapevine powdery mildew: from fundamental plant pathology to new and future Technologies. **Australasian Plant Pathology 50**: 1-6.

Teh SL, Fresnedo-Ramírez J, Clark MD, Gadoury DM, Sun Q, Cadle-Davidson L and Luby JJ (2017) Genetic dissection of powdery mildew resistance in interspecific half-sib grapevine families using SNP-based maps. **Molecular Breeding 37**: 1.

Welter LJ, Göktürk-Baydar N, Akkurt M, Maul E, Eibach R, Töpfer R and Zyprian EM (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.

Zendler D, Schneider P, Töpfer R and Zyprian E (2017) Fine mapping of Ren3 reveals two loci mediating hypersensitive response against *Erysiphe necator* in grapevine. **Euphytica 213**: 68.

Zendler D, Töpfer R and Zyprian E (2021) Confirmation and fine mapping of the resistance locus *Ren9* from the grapevine cultivar 'Regent'. **Plants 10**: 24.

Zini E, Dolzani C, Stefanini M, Gratl V, Bettinelli P, Nicolini D, Betta G, Dorigatti C, Velasco R, Letschka T and Vezzulli S (2019) R-Loci arrangement versus downy and powdery mildew resistance level: a vitis hybrid survey. **International Journal Molecular Science 20**: 3526.

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