



## Detection of the Presence of Powdery Mildew Resistance -Associated Genes (*Ren1*, *Ren3*, and *Ren9*) in *Vitis labrusca* L. Genotypes

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### ABSTRACT

Powdery mildew disease (*Erysiphe necator* Schwein) is a significant threat to grape cultivation in vineyards. Severe yield and quality losses could occur in vineyards when this pathogen is not managed correctly. Several commercial grape varieties are highly susceptible to powdery mildew. Therefore, large quantities of fungicides are applied throughout the growing season. In addition to yields and quality, new grapevine varieties that are genetically resistant to powdery mildew are required for sustainable viticulture. This study was conducted through molecular screening of powdery mildew resistance genes in nine different *Vitis labrusca* L. genotypes (TEG-VI-1, TEG-VI-2, TEG-VI-3, TEG-VI-4, TEG-VI-5, TEG-VI-6, TEG-VI-7, TEG-VI-8, and TEG-VI-9) grown in the Black Sea Region of Türkiye. After PCR amplifications using *Ren1*, *Ren3* and *Ren9* locus-specific primers, *Ren1*, and *Ren 9* genes were detected in three genotypes (TEG-VI-1, TEG-VI-3, and TEG-VI-4). However, the *Ren3* gene was not detected in any genotypes. It was concluded based on present findings that *Vitis labrusca* L. genotypes with resistance genes could be used as genetic resources in grapevine breeding programs and significant economic benefits can be provided accordingly.

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## *Vitis labrusca* L. Genotiplerinde Küllemeye Dirençle İlişkili Genlerin (*Ren1*, *Ren3* ve *Ren9*) Varlığının Tespiti

### ÖZET

Bağlarda görülen külleme hastalığı (*Erysiphe necator* Schwein) üzüm yetiştiriciliği için büyük bir tehdittir. Bu patojene karşı mücadele edilmediğinde, üzüm verim ve kalitesinde önemli düşüşler meydana gelmektedir. Çoğu ticari üzüm çeşidi, küllemeye karşı oldukça hassastır. Bu nedenle yetiştirme dönemi boyunca fazla miktarlarda fungusit uygulanmaktadır. Sürdürülebilir bir bağcılık için verim ve kalitenin yanında, külleme hastalığına karşı genetik olarak dirençli yeni asma çeşitlerine ihtiyaç duyulmaktadır. Bu çalışmada, Türkiye'nin Karadeniz Bölgesi'nde yetiştiriciliği yapılan dokuz farklı *Vitis labrusca* L. genotipinde (TEG-VI-1, TEG-VI-2, TEG-VI-3, TEG-VI-4, TEG-VI-5, TEG-VI-6, TEG-VI-7, TEG-VI-8 ve TEG-VI-9) külleme hastalığına dirençli genlerin moleküler taraması yapılmıştır. *Ren1*, *Ren3* ve *Ren9* lokuslarına özgü primerler kullanılarak gerçekleştirilen PCR amplifikasyonu sonrasında üç genotipte (TEG-VI-1, TEG-VI-3 ve TEG-VI-4) *Ren1* ve *Ren9* genlerinin bulunduğu tespit edilmiştir. Ancak *Ren3* geni hiçbir örnekte saptanamamıştır. Araştırma sonuçlarında direnç genlerine sahip oldukları belirlenen *Vitis labrusca* L. genotiplerinin, gelecekte dirençli asma çeşitlerinin ıslahında genetik kaynaklar olarak kullanılacakları ve bu sayede önemli ekonomik faydalar sağlanabileceği düşünülmektedir.

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## INTRODUCTION

Cultivation is a process in which humans select and genetically modify organisms for their desired traits (Jiao et al., 2021). This process often leads to a reduction in genetic diversity in the breeding population and the loss of parental genes (Doebley et al., 2006). The occurrence of grapevine species dates to 28 million years ago, and today, there are more than 60 species belonging to the genus *Vitis* (Wan et al., 2013). Of these, *V. vinifera*, native to the Mediterranean and Central Europe, is known to be the most widely cultivated vine species and was cultivated ~8,000 years ago during the Neolithic era (This et al., 2006; Jaillon et al., 2007; Zhou et al., 2017). However, *V. vinifera* is highly susceptible to powdery mildew caused by '*Erysiphe necator* Schw.' (Gadoury et al., 2003; Jiao et al., 2021). *E. necator* infects all green tissues of vines. It is easily recognized through white-grayish powdery mildew symptoms on the surface of shoots, stems, leaves, buds, cluster skeletons, flowers, peduncles, and young berries (Bendek et al., 2002; Calonnec et al., 2004). Moreover, powdery mildew negatively affects cluster weight, ripening, photosynthetic activity, transpiration (Sosa-Zuniga et al., 2022), the sugar/acid ratio, and anthocyanin levels (Calonnec et al., 2004).

Although *V. vinifera* is the most widely cultivated *Vitis* species, the resistance of this species to powdery mildew is lower than that of *Muscadinia* species with germplasms of wild *Vitis* spp. from North America or Central Asia (Riaz et al., 2013; Pap et al., 2016; Mermer Doğu et al., 2022; Sosa-Zuniga et al., 2022). As described in previous studies, the natural powdery mildew resistance of North American and Central Asian genotypes is largely related to the evolutionary history of these genotypes (Hoffmann et al., 2008; Dry et al., 2010; Blanc et al., 2012; Pap et al., 2016). These resistant genotypes can be included in grapevine breeding programs and become valuable germplasm sources (Atak, 2023). In recent years, especially in table grape breeding, this system has increased in demand and is spreading worldwide (Montaigne et al. 2021).

The *Vitaceae* family has two primary gene families responsible for powdery mildew resistance: *Run* (resistance to *Uncinula necator*) and *Ren* (resistance to *Erysiphe necator*) (Sosa-Zuniga et al., 2022). To date, 15 loci belonging to the *Run* and *Ren* gene families associated with the defence response of grapevines against powdery mildew have been described (Maul, 2023). Several of these *Ren* loci are used in different breeding programs to strengthen the defence response of plants and increase plant resistance (Li et al., 2013; Feechan et al., 2015; Agurto et al., 2017). *Ren1* loci have been detected in some genotypes of *V. vinifera*

from Central Asia (e.g., 'Kishmish Vatkana' and 'Dzhandzhal Kara' varieties from Uzbekistan). The *Ren3* locus was discovered in the 'Regent' variety, which has resistant parents such as *V. aestivalis*, *V. berlandieri*, *V. cinerea*, *V. lincedumii* and *V. rupestris* (Eibach & Töpfer, 2003; Welter et al., 2007). Zendler et al. (2017) characterized the *Ren9* locus as a second resistance-coding region during a detailed genetic mapping study of *Ren3* on chromosome 15. However, loci on the same chromosome in different regions can be separated into different genotypes.

Türkiye has reasonably available ecological conditions for various plant species and thus has an important position in world agriculture. It also has genetic potential in terms of plant genetic resources (Ergül & Ağaoglu 2001; Ergül et al., 2011, Dilli et al., 2014). The humid climate of the Black Sea Region of Türkiye, which has an average annual rainfall of more than 1000 mm, limits the viticulture of *Vitis vinifera* L. Therefore, *Vitis labrusca* species or hybrids resistant to fungal diseases can grow in this region (Cangi et al., 2006; Çelik et al., 2008; Tahmaz et al., 2022). These species can be grown without pesticides in the Black Sea Region; thus, the must obtained from these grapes contains resveratrol and antioxidants, which are extremely important for human health and nutrition (Üneş, 2016; Atak & Şen, 2021; Tahmaz et al., 2022). Although it is not known exactly how and when the *Vitis labrusca* L. species, originating from North America, arrived in the region, it was reported that there were several varieties and genotypes of this species in the region, and most of them were resistant to fungal diseases (Cangi et al., 2006; Çelik et al., 2008; Atak, 2017; Tahmaz et al., 2022).

Various studies have revealed that *V. labrusca* genotypes generally exhibit greater mildew resistance than *V. vinifera* varieties. However, the level of resistance may vary from variety to variety (Atak et al., 2017; Sargolzaei et al., 2021). Yıldırım et al. (2019) reported that a genotype derived from hybridization between *V. labrusca* and *V. vinifera* possessed the *Rpv3* gene, which is associated with resistance to mildew. Among these genotypes, '57 Gerze 04' (*V. labrusca* × *V. vinifera*) and 'Mortensen' (*V. labrusca* × *V. vinifera*) demonstrated resistance to mildew, whereas the 'Köfteci Üzüümü' (*V. labrusca* × *V. vinifera*) genotype exhibited a greater level of resistance. Furthermore, this study revealed that resistance to grapevine anthracnose in *V. labrusca* is governed by three independent genes. While *An1* and *An2* are the dominant susceptibility genes, *An3* is the only dominant resistance gene (Mortensen, 1981; Gao et al., 2012).

However, in several studies on the pathogen resistance genes of different *Vitis* spp. Species, there are almost

no studies on *Vitis labrusca* L. Therefore, there is a need for research to elucidate the molecular basis of powdery mildew resistance in genotypes of *V. labrusca* L. species. In this study, molecular analyses were conducted to detect the presence of the *ren1*, *ren3*, and *ren9* genes in nine different *Vitis labrusca* L. genotypes grown in the Black Sea Region of Türkiye.

## MATERIAL and METHOD

### Plant Material

Grapevine genotypes were collected from the Erbaa district of Tokat Province (Türkiye) (Figure 1, Figure 2, Figure 3 and Figure 4). The grapevine genotypes used in this study and their collection sites are

delineated in Table 1. During the winter dormancy period, 20 cuttings were obtained from each of nine different *Vitis labrusca* genotypes. These genotypes were then cultivated in a greenhouse at the Faculty of Agriculture, Tokat Gaziosmanpaşa University, for further study. For molecular analysis, bud samples were collected from all cuttings of the nine genotypes. Three shoot tips and young leaf samples were selected from each genotype to serve as plant material for the study.

Shoot tips taken from plant materials were delivered to the Sivas Cumhuriyet University Advanced Technology Research and Application Center in cooler boxes, frozen at -20°C and stored until analysis.



Figure 1. Naturally-grown *V. labrusca* genotypes  
Şekil 1. Kendiliğinden yetişen *V. labrusca* türüne ait genotipler



Figure 2. Genotype marking process  
Şekil 2. Genotiplerde işaretleme işlemleri



Figure 3. Shoot sampling from marked genotypes after defoliation.  
Şekil 3. Yaprak dökümü sonrası işaretli genotiplerden sürgün örneklerinin alınması

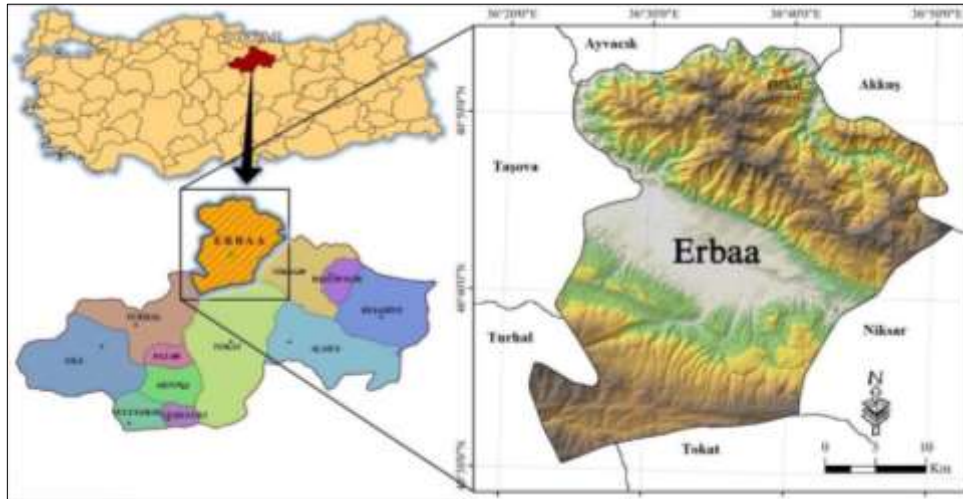


Figure 4. The region from which grapevine genotypes were collected  
Şekil 4. Çalışmada kullanılan asma genotiplerinin toplandığı bölge

### DNA Isolation

Genomic DNA isolation was performed according to Doyle and Doyle (1990) with slight modifications as outlined below:

1. Tissues stored at -80°C were crushed with a pestle and transferred to Eppendorf tubes

(advantageously accelerating this step will provide an advantage in terms of DNA quantity). In cases of low tissue volume, tissue can be crushed inside the Eppendorf tube to prevent DNA loss).

2. Each sample was homogenized by adding 700 µl of preheated 2% CTAB buffer (100 mM Tris-HCl,

- 25 mM EDTA, pH 8.0, and 2.5 M NaCl).
- The tubes were incubated in a water bath at 65°C for 60 minutes with occasional inversion.
  - After incubation, the samples were centrifuged at 7378 ×g for 10 minutes at 4°C, and the supernatant was carefully transferred to a new tube.
  - An equal volume of chloroform:isoamyl alcohol (24:1) was added to the samples and mixed by inversion.
  - The samples were centrifuged at 7378 ×g for 10 minutes at 4°C, and the supernatant was transferred to a new tube.
  - To precipitate the DNA, an equal volume of isopropanol (-20°C) was added to the samples and incubated at -20°C for 30 minutes.
  - The precipitated DNA was centrifuged at 14462 ×g for 10 minutes at 4°C, after which the supernatant was removed.
  - For washing, the pellets in the tubes were centrifuged at 14462 ×g for 5 minutes at 4°C after adding 500 µl of 70% ethanol (-20°C).
  - The resulting pellets were air-dried at room temperature and dissolved in 30/60 µl of TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0).
  - RNase A was added to each sample (1/100 µl DNA sample) and incubated at 37°C for 1 hour.

DNA quality and quantity were evaluated by electrophoresis in a 1% (w/v) agarose gel and using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE), respectively. The isolated DNA was stored at -80°C until PCR was performed.

Table 1. Grapevine genotypes and sampling locations

Çizelge 1. Çalışmada kullanılan asma genotipleri ve toplandıkları lokasyonlar

No	Genotype	Location
1	TEG-VI-1	40°86'10"N 36°65'38"E
2	TEG-VI-2	40°86'17"N 36°68'73"E
3	TEG-VI-3	40°84'49"N 36°66'77"E
4	TEG-VI-4	40°83'03"N 36°66'40"E
5	TEG-VI-5	40°82'93"N 36°66'53"E
6	TEG-VI-6	40°83'94"N 36°67'41"E
7	TEG-VI-7	40°84'09"N 36°67'50"E
8	TEG-VI-8	40°84'50"N 36°66'56"E
9	TEG-VI-9	40°32'40"N 36°44'97"E

### Polymerase Chain Reaction (PCR)

To determine whether the 9 DNA samples used in the study were resistant to powdery mildew, three resistance gene regions, namely, *ren1*, *ren3*, and *ren9* were selected. The sequences of the primers used for these gene regions were obtained from previous studies (Akkurt et al., 2007; van Heerden et al., 2014; Pozharskiy et al., 2020). Information on the primers used for resistance-related marker amplification in *Vitis labrusca* L. genotypes is presented in Table 2.

Table 2. Markers used to describe alleles corresponding to loci associated with resistance to powdery mildew in *Vitis labrusca*

Tablo 2. *Vitis labrusca*'da külemeye dirençle ilişkili lokuslara karşılık gelen alelleri tanımlamak için kullanılan markerler

Locus	Marker	Forward / Reverse primer	Mildew	Fragment Length (bp)	Annealing Temperature (T <sub>A</sub> )	Reference
<i>ren1</i>	GF13-13R	GTGCATCTTCTTCCCAACC/ GCATTTGTCAAAGTCGTGTACTTC	+	214	60	Pozharskiy et al., 2020
<i>ren3</i>	ScORA7-760R	GAAACGGGTGTGAGGCAAAGGTGG/ GGCCATTAGGAAATCAACATTAC	+	760	60	Akkurt et al., 2007
<i>ren9</i>	CenGen6R	TGAATTTTGTCTTTAGGATTTGGA/ CACAAGAACAATTTCTACGCACA	+	287	55	van Heerden et al., 2014

Table 3. PCR amplification conditions for markers associated with powdery mildew resistance in *Vitis labrusca*

Çizelge 3. *Vitis labrusca*'da külemeye dirençle ilişkili markerler için PCR amplifikasyon koşulları

Initial denaturation	5 min at 94°C	
Denaturation	30 s at 94°C	
Annealing	30 s at 55-60°C	30 cycles
Extension	2 min at 72 °C	
Final Extension	10 min at 72°C	

Powdery mildew-related genes and PCR conditions were evaluated with the primers given in Table 3.

All PCRs were prepared in a final volume of 25 µl. The 25 µl reaction volume contained: 0.125 U of Taq DNA polymerase (Fermentas), 2.5 µl of reaction buffer (100 mM Tris-HCl, pH 8.8, 500 mM KCl, and 0.8% Nonidet P-40), 1 µl each of 10 pmol primer, 2.5 µl of 2.5 mM dNTPs (MBI Fermentas), 2.5 µl of 25 mM MgCl<sub>2</sub> and 1 µl of 100-500 ng of template DNA, which was added to a final volume of 25 µl with dH<sub>2</sub>O. PCRs were performed in a Blue-Ray Biotech thermocycler under the following conditions.

The amplification products were subjected to 1.5% agarose gel electrophoresis containing ethidium bromide (2 µg/ml) and imaged under a UV transilluminator.

## RESULT and DISCUSSION

### PCR amplification results for markers associated with the *ren1*, *ren3*, and *ren9* loci in DNA samples

To determine the genotypes associated with powdery mildew resistance, a comprehensive analysis was conducted on nine samples utilizing polymerase chain reaction (PCR) to detect the presence of the *Ren1*, *Ren3*, and *Ren9* loci. Subsequently, the genotypes were classified based on their resistance or sensitivity to powdery mildew, which was determined through visualization of the amplified products via gel electrophoresis.

Analysis via molecular markers identified specific alleles correlated with resistance to powdery mildew within *V. labrusca* genotypes, as detailed in Table 4. Figure 5 shows the results of PCR amplification employing primers tailored for the *Ren1*, *Ren3*, and *Ren9* loci. For the *Ren1* gene, 214 bp PCR products encompassing TEG-VI-1, TEG-VI-2, TEG-VI-3, TEG-VI-4, TEG-VI-5, TEG-VI-6, TEG-VI-7, TEG-VI-8, and TEG-VI-9 were discerned in all examined samples,

constituting a 100% detection rate, as depicted in Figure 6. Conversely, for the *Ren9* gene, a 287 bp PCR product including TEG-VI-1, TEG-VI-3, and TEG-VI-4 was identified in three samples, representing a 33.33% occurrence rate, as highlighted in Figure 7. Notably, the *Ren3* gene was absent in all the samples analyzed.

This investigation corroborates and extends upon the established body of knowledge, highlighting the significant genetic diversity within the *Vitis* genus, especially concerning powdery mildew resistance. The majority of commercial grape cultivars exhibit vulnerability to this pathogen, yet resistance often occurs within wild populations, suggesting substantial potential for breeding. Previous studies have identified *V. labrusca* for its distinctive resistance properties against fungal diseases (Cangi et al., 2006; Çelik et al., 2008; Tahmaz et al., 2022), although the precise molecular mechanisms conferring this resistance remain largely unexplored. In this context, this research aimed to identify the presence of the *Ren1*, *Ren3*, and *Ren9* genes within nine *Vitis labrusca* L. genotypes from the Black Sea region of Türkiye.

Table 4. Distribution of the *ren1*, *ren3* and *ren9* genes in the investigated genotypes

Çizelge 4. İncelenen genotiplerde *ren1*, *ren3* ve *ren9* genlerinin dağılımı

Genotype	Loci		
	<i>ren1</i>	<i>ren3</i>	<i>ren9</i>
TEG-VI-1	+	-	+
TEG-VI-2	+	-	-
TEG-VI-3	+	-	+
TEG-VI-4	+	-	+
TEG-VI-5	+	-	-
TEG-VI-6	+	-	-
TEG-VI-7	+	-	-
TEG-VI-8	+	-	-
TEG-VI-9	+	-	-

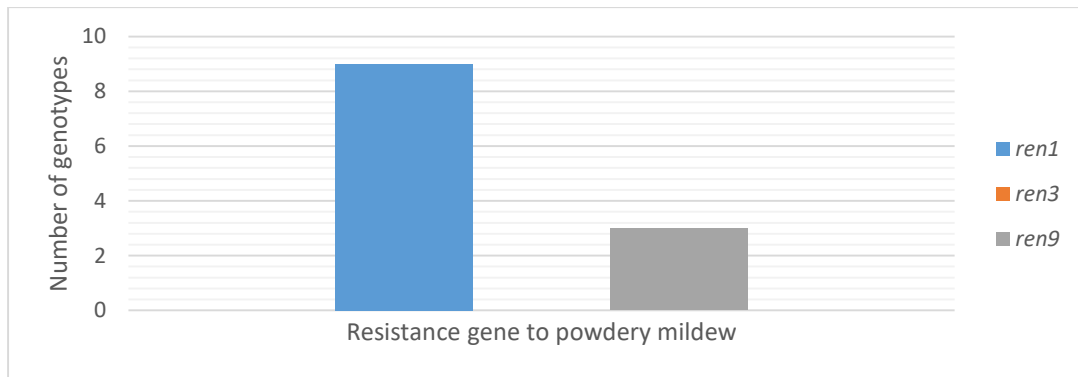


Figure 5. Distribution of the *ren1*, *ren3* and *ren9* genes in the investigated genotypes  
Şekil 5. İncelenen genotiplerde *ren1*, *ren3* ve *ren9* genlerinin dağılımı

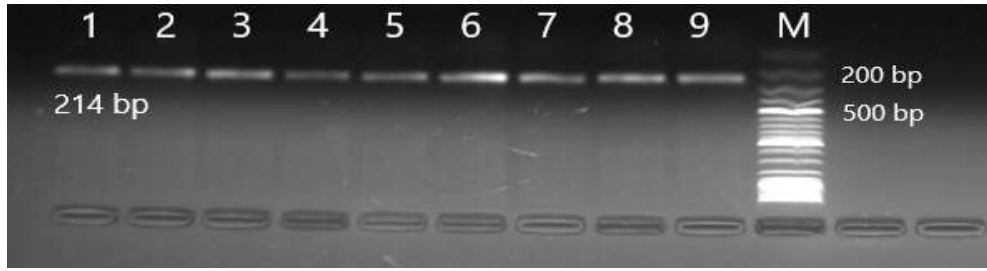


Figure 6. Electrophoretic separation of PCR amplicons of the *Ren1* gene obtained from the GF13-13F / GF13-13R primer pair

Şekil 6. GF13-13F / GF13-13R primer çiftlerinden elde edilen *Ren1* geninin PCR ampliconlarının elektroforetik ayrımı

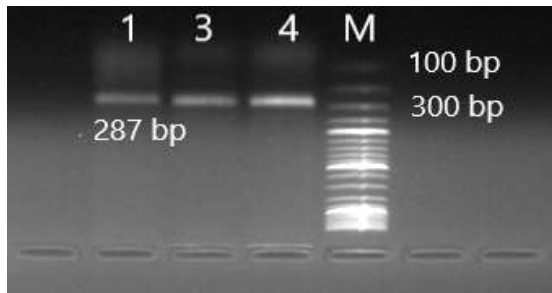


Figure 7. Electrophoretic separation of PCR amplicons of the *Ren9* gene obtained from the CenGen6F / CenGen6R primer pair

Şekil 7. CenGen6F / CenGen6R primer çiftlerinden elde edilen *Ren9* geninin PCR ampliconlarının elektroforetik ayrımı

Molecular analyses in this research revealed that all nine genotypes harbor the *Ren1* gene. The dominant locus *Ren1* (resistance to *Erysiphe necator* 1) belongs to 'Kishmish Vatkana' and 'Dzhandzhal Kara', two Central Asian *V. vinifera* cultivars (Korbuly, 1999; Kozma et al., 2006; Reisch et al., 2014). However, the *Ren3* gene was conspicuously absent in all genotypes examined in this study, consistent with findings from other investigations indicating the gene's sparse distribution among grape varieties (Welter et al., 2007; Pozharskiy et al., 2020). On the other hand, the *Ren9* gene was detected in three out of the nine genotypes analyzed, which suggests a nuanced distribution of resistance genes within *V. labrusca*, indicating the complexity of the mechanisms underlying resistance to powdery mildew.

Interestingly, this study documents the identification of the *Ren1* locus within North American-derived *V. labrusca* genotypes. The detection of the *Ren1* gene in the examined *V. labrusca* genotypes highlights the possibility of natural hybridization between *V. labrusca* and *V. vinifera*, especially in Türkiye, where the cultivation of *V. vinifera* cultivars has historically been widespread. This finding suggests that the examined genotypes may not be pure *V. labrusca* but likely represent hybrids with *V. vinifera*. A similar study was conducted by Cadle-Davidson et al. (2011a)

with different *Vitis* species at two different locations and the results were similar to the results of this study. In this study, two different treatments, natural infection and artificial inoculation with a single isolate, were applied for powdery mildew disease on grapevine leaves and the results revealed significant differences between the species. One of the most susceptible species was *V. vinifera*, while interspecific hybrids were found to be more resistant to powdery mildew. *V. labrusca* was found to be one of the most resistant species. Similar to the results of this study, Reisch et al. (1993) reported that the interspecific hybrid variety Alden was tolerant to powdery mildew and resistant to mildew, while another interspecific hybrid variety, 'Kay Gray', had good resistance to mildew and powdery mildew. Almost all *V. vinifera* cultivars are highly susceptible to powdery mildew; nevertheless, several Vitaceae species have developed resistance mechanisms against this fungus but lack commercial qualities (Riaz et al., 2007; Glawe, 2008; Dry et al., 2010; Gadoury et al., 2012). In this context, resistant genotypes have become valuable germplasm for inclusion in grapevine breeding programs. These natural powdery mildew resistance sources correspond to some North American and Asian genotypes, and the resistance trait is related to their evolutionary history, as described by several works (Riaz et al., 2007; Hoffmann et al., 2008; Coleman et al., 2009; Dry et al., 2010; Feechan et al., 2011; Ramming et al., 2011; Blanc et al., 2012; Gadoury et al., 2012; Qiu et al., 2015; Pap et al., 2016). Resistance to pathogenic microorganisms is a common and important trait to be incorporated into new plant cultivars. Many sources of resistance to grapevine powdery mildew have been identified, including some North American and Chinese species, and even some Asian *V. vinifera* cultivars, which exhibit different levels of resistance but lack commercial qualities (Barker et al., 2005; Welter et al., 2007; Ramming et al., 2011; Blanc et al., 2012; Feechan et al., 2015; Pap et al., 2016). An important issue in the development of new pathogen-resistant cultivars is the emergence of new virulent isolates with the ability to overcome R gene recognition (Peressotti et al., 2010; Cadle-Davidson et al., 2011b).

Hence, pyramiding two or more R genes from different *Vitis* species has become a durable and secure strategy; even if any mutation or loss of an avirulence factor occurs, the pathogen will still be recognized by at least one R gene (Feechan et al., 2015; Armijo et al., 2016; Pap et al., 2016). In a hybridization study, Agurto et al. (2017) used segregating plants from *V. vinifera* 'Dzhandzhal Kara' × *V. vinifera* 'Laszta' and the fifth pseudobackcross of *M. rotundifolia* × *V. vinifera* as two genetically different sources of resistance against the biotrophic fungus *E. necator* carrying *Ren1* and *Run1* loci, respectively, and pyramided them in single grapevine plants until the seventh pseudobackcross with *V. vinifera* 'Crimson Seedless'. Such dual-purpose hybrids can significantly contribute to the diversity of genetic resources in the viticulture industry. By providing valuable genetic material for the development of high-quality grape varieties and offering natural resistance to diseases, these hybrids can support sustainable viticulture practices (Atak & Göksel, 2019). The genotypes examined in the present study have the potential to carry the commercial traits of certain *V. vinifera* high-quality grape varieties, while also harboring the disease resistance characteristics of *V. labrusca*.

Therefore, the discovery and utilization of hybrid genotypes hold considerable promise for transforming and improving the viticulture industry. The outcomes of this study emphasize the strategic significance of amalgamating multiple resistance loci to forge robust and enduring resistance against powdery mildew. The co-occurrence of the *Ren1* and *Ren9* genes within the same genotypes supports the ability of diverse resistance mechanisms to bolster disease resistance. This approach is in harmony with contemporary breeding objectives that aim for sustainable resistance through the incorporation of various modes of action, thereby reducing evolutionary pressures on pathogens and delaying the emergence of virulent fungal strains.

The molecular analysis of the *Vitis labrusca* genotypes TEG-VI-1, TEG-VI-3, and TEG-VI-4 highlighted the co-occurrence of the *Ren1* and *Ren9* genes, marking a noteworthy discovery for grapevine breeding endeavors. The identification of two distinct powdery mildew resistance loci within the same genotype represents a pivotal achievement. This dual locus presence aligns with the broader consensus among researchers advocating for the integration of *Ren* genes from varied genetic backgrounds as a strategy to establish durable resistance in agricultural settings. By pyramiding resistance genes from diverse sources, the intention is to reduce the selection pressure on pathogen populations, thereby decelerating the emergence of virulent fungal strains (Feechan et al., 2015; Pap et al., 2016). Such strategic breeding is deemed crucial for preventing mutations within pathogen effector molecules that might otherwise

elude recognition by the *Ren* proteins, ensuring the continued efficacy of resistance mechanisms (Pap et al., 2016). These findings underscore the strategic importance of considering the geographical origins and genetic diversity of resistance loci. The aim is to compile a mosaic of resistance sources, thereby broadening the genetic base upon which new grapevine cultivars resistant to powdery mildew are developed (Sosa-Zuniga et al., 2022). Zandler et al. (2020) further support this approach by suggesting that resistance traits identified in the *Ren3* and *Ren9* loci from North American grapevine species, when coupled with those exhibiting strong resistance from European origins, can lead to enhanced durability and efficacy of resistance against powdery mildew. This collective body of work advocates for a nuanced and globally informed approach to grapevine breeding. By embracing genetic diversity and the integration of resistance genes from internationally distinct *Vitis* species, breeders can forge new paths toward the cultivation of grapevine varieties endowed with comprehensive and enduring resistance to powdery mildew. This endeavor not only holds promise for safeguarding the viticulture industry against current and future pathogenic challenges but also exemplifies a forward-thinking commitment to the sustainable management of plant health and productivity.

The findings of this study endorse a strategic breeding methodology that capitalizes on genetic diversity and integrates resistance alleles from disparate sources. By prioritizing the combination of resistance genes from geographically and genetically diverse *Vitis* species, breeders can cultivate grapevine varieties with enhanced and lasting resistance to powdery mildew, contributing significantly to the sustainable future of viticulture (Feechan et al., 2015; Pap et al., 2016; Sosa-Zuniga et al., 2022; Zandler et al., 2020). This strategy not only leverages the intrinsic genetic potential within the *Vitis* genus but also serves as a bulwark against the rapid evolution of pathogenic threats, ensuring the longevity and productivity of grapevine populations worldwide.

## CONCLUSION

The findings of the present study may help to elucidate the molecular basis of resistance mechanisms against powdery mildew across various *V. labrusca* genotypes. This research indicated that the distribution of resistance genes differed among the genotypes, suggesting underlying genetic diversity as a potential influencer. These identified grapevine genotypes present an opportunity for registration as new grape varieties that exhibit resistance to *Erysiphe necator*, thereby contributing to the diversification and enhancement of disease-resistant cultivars. The genotypes characterized by these resistance genes are poised to illuminate future research aimed at

augmenting resistance to powdery mildew across different species and varieties. These findings could serve as valuable resources for researchers striving to devise optimal combinations of genes and loci for enhanced disease resistance. This knowledge can significantly accelerate the development of grapevine varieties with improved resilience against powdery mildew, aligning with the broader goals of sustainable viticulture and crop protection. Comprehensive studies involving physiological, biochemical, and transcriptomic analyses are highly recommended to better understand and decipher the intricate effects of these resistance genes. Such in-depth investigations will pave the way for a more nuanced appreciation of the resistance mechanisms at play, potentially leading to the discovery of novel strategies for managing powdery mildew in grapevines. Moreover, it is imperative that future evaluations of these genotypes be conducted under field conditions or controlled environments. Proper experimental setups are essential for validating resistance traits and ensuring the practical applicability of these genotypes in real-world viticultural practices. This approach will not only corroborate the initial findings but also facilitate the integration of these resistant genotypes into breeding programs, thus bolstering global efforts to mitigate the impact of powdery mildew on grape production.

#### Researchers' Contribution Rate Statement Summary

AY prepared the original manuscript. SD, AB, and DSA contributed to supervision, editing, and conceptualization. AY, SD, and AB gathered relevant research articles and reviewed the manuscript. DSA also contributed to reviewing and editing the manuscript. All the authors have read and approved the final manuscript.

#### Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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