



MOLECULAR MARKER-BASED STUDY OF THE GENE FOR RESISTANCE TO MILDEW IN SOME AZERBAIJANI TABLE GRAPE VARIETIES

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Abstract: Downy mildew, caused by *Plasmopara viticola*, is one of the most destructive diseases affecting grapevine (*Vitis vinifera* L.), leading to significant yield and quality losses worldwide, including in Azerbaijan. The development and identification of resistant grape cultivars is considered an effective and environmentally sustainable approach to disease management. This study focuses on the molecular marker-based investigation of resistance genes associated with downy mildew in selected table grape varieties cultivated in Azerbaijan. Molecular markers linked to resistance loci were employed to evaluate the genetic potential of different grapevine genotypes. DNA was extracted from young leaf tissues, and specific primers associated with known resistance genes were used in PCR analysis. The presence or absence of target alleles was determined and compared among varieties to assess their resistance profiles. The results demonstrated genetic variability among the studied cultivars in terms of resistance to *Plasmopara viticola*. Certain varieties showed the presence of resistance-associated alleles, indicating their potential use in breeding programs aimed at developing disease-resistant grapevine cultivars. Overall, the application of molecular markers proved to be a reliable and efficient method for early detection of resistance traits, contributing to sustainable viticulture and reduced reliance on chemical control measures.

Keywords: *Vitis vinifera*, *Plasmopara viticola*, downy mildew resistance, molecular marker-assisted selection (MAS), PCR-based genotyping.

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Introduction

Vitis vinifera L. is among the most widely cultivated fruit species worldwide and represents the only *Vitis* species extensively used in winemaking (Reynolds 2015). Viticulture covers around 7.6 million hectares globally, producing more than 67 million tons of grapes annually (Toffolatti et al. 2018b). Despite its agricultural importance, *V. vinifera* is highly susceptible to *Plasmopara viticola* (Berk. et Curt.) Berl. & de Toni, the oomycete causing downy mildew, which can severely reduce grape yield and quality in the absence of effective disease management strategies

(Fontaine et al. 2013; Maddalena et al. 2020). The pathogen was introduced to Europe from North America in the nineteenth century and quickly spread, forming genetically distinct populations influenced by geographic and climatic factors (Fontaine et al. 2013; Maddalena et al. 2020). Resistance to downy mildew is naturally found in several non-*vinifera* North American species such as *Vitis riparia*, *V. cinerea*, and *V. labrusca*, as well as in Northeast Asian species (*Vitis amurensis*), resulting from long-term co-evolution with the pathogen (Jürges et al. 2009). Multiple

quantitative trait loci (QTLs) conferring varying levels of resistance, including *Rpv1*, *Rpv2*, *Rpv3*, *Rpv4*, *Rpv7*, and others, have been identified across these species (Blasi et al. 2011; Merdinoglu et al. 2003; Welter et al. 2007). The exploitation of these resistance sources provides the most sustainable approach to reducing chemical use in viticulture and to manage downy mildew effectively. Traditional management of downy mildew in *V. vinifera* varieties requires frequent fungicide application, which constitutes nearly 70% of agrochemical use in European viticulture. However, overreliance on chemicals leads to environmental pollution, health risks, high costs, and potential development of fungicide-resistant pathogen strains (Hollomon 2015). Therefore, genetic approaches such as marker-assisted selection (MAS) are increasingly adopted to accelerate breeding programs (Eibach & Töpfer 2015). MAS allows the early selection of progeny carrying resistance loci, significantly reducing the time required for cultivar development (Eibach & Töpfer 2015).

Recent studies have reported unique resistance traits in *V. vinifera* varieties from the Southern Caucasus, including Georgia, the species' first domestication center (Bitsadze et al. 2015; Toffolatti et al. 2016). These varieties display complex defense mechanisms including recognition of pathogen-associated molecular patterns (PAMPs), activation of defense signaling pathways, synthesis of antimicrobial compounds such as flavonoids and monoterpenes, structural barriers such as cell wall reinforcement, and production of fungal wall-degrading enzymes (Toffolatti et al. 2018; 2020).

Integrating global knowledge of resistance loci with local field evaluations creates a strong basis for breeding programs targeting durable resistance (Eibach & Töpfer 2015; Eibach et al. 2007). Combining the superior quality traits of *V. vinifera* with resistance traits from American and Asian species through MAS allows the development of new cultivars that are both high-yielding and environmentally sustainable (Delmotte et al. 2016; Eibach & Töpfer 2015; Eibach et al. 2007). The present study aims to

identify genomic regions associated with downy mildew resistance in Azerbaijani table grape varieties, providing resources for future breeding efforts and contributing to sustainable viticulture in the region (Eibach & Töpfer 2015; Eibach et al. 2007). Taxonomic classification of fungus is shown below:

Kingdom: Stramenopila (Chromista)

Phylum: Oomycota

Class: Oomycetes

Order: Peronosporales

Family: Peronosporaceae

Genus: *Plasmopara*

Species: *Plasmopara viticola*

Genetic resistance of grapevine (*V. vinifera*) to downy mildew represents one of the most effective strategies in sustainable viticulture (Reynolds 2015). Chemical fungicides, although widely used, are costly, environmentally risky, and often fail to provide long-term protection (Toffolatti et al. 2018b). In contrast, resistant cultivars enhance the plant's natural defense mechanisms, reduce pathogen development under field conditions, and improve the efficiency of breeding programs (Fontaine et al. 2013). Identification and incorporation of genotypes with complex resistance traits remain a priority for modern grapevine research (Maddalena et al. 2020).

Downy mildew caused by *Plasmopara viticola* is a major disease affecting both yield and fruit quality (Jürges et al. 2009). The pathogen infects leaves, shoots, and clusters, producing yellowish oil spots and tissue necrosis symptoms on susceptible grapevine leaves, as shown in Fig. 1 (Merdinoglu et al. 2003). Under warm and humid conditions, disease development and sporulation intensify rapidly, as illustrated in Fig. 2 (Welter et al. 2007). While chemical control is partially effective, fungicide resistance is increasingly problematic, making the use of resistant cultivars strategically important (Blasi et al. 2011). North American species, such as *Vitis riparia*, *V. cinerea*, and *V. labrusca*, and Northeast Asian species like *V. amurensis*, exhibit varying resistance due to co-evolution with the pathogen (Hollomon 2015). Several QTLs (*Rpv1*–*Rpv28*) conferring resistance have



been identified across *Vitis* species (Eibach & Töpfer 2015). Utilizing these resistance sources allows breeders to pyramid genes, enhancing durability in cultivated varieties (Bitsadze et al. 2015).

Field evaluations conducted in Azerbaijan demonstrated that among 37 table grape varieties, 13 showed tolerance to downy mildew, while 24 were susceptible (Babaev et

al. 2025). Downy mildew continues to be a major limiting factor for grape production in Azerbaijani vineyards, affecting both yield and quality (Salimov et al. 2022). Cluster analysis and evaluation of local varieties confirmed significant variability in resistance levels, highlighting the importance of selecting tolerant genotypes for sustainable viticulture in the region (Aliyeva et al. 2024).

Figure 1. Symptoms of downy mildew on *Vitis vinifera* leaves.



Figure 2. Disease development and sporulation stages.



In Azerbaijan, local table grape varieties show different levels of resistance, emphasizing the importance of regional germplasm evaluation (Toffolatti et al. 2016). Marker-assisted selection (MAS) and GWAS approaches allow precise identification of resistant genotypes, shortening breeding cycles and improving cultivar quality (Toffolatti et al. 2018a). Recent studies on local resistant varieties demonstrate limited pathogen growth, overexpression of defense-related genes, and structural barrier formation (Toffolatti et al. 2020). The main objective of this study is to identify Azerbaijani grapevine genotypes with strong resistance to *P. viticola* and provide a

foundation for breeding programs that combine resistance with high yield and fruit quality (Babaev et al. 2025). Integrating local germplasm with global knowledge enables the development of productive, sustainable, and resilient cultivars (Salimov et al. 2022).

Grapevine molecular and genetic basis of resistance to downy mildew (*Plasmopara viticola*). Grapevine (*Vitis vinifera* L.) is highly susceptible to downy mildew, caused by the oomycete *Plasmopara viticola*, which infects leaves, shoots, and berries, causing significant yield and quality losses (Reynolds 2015; Jürges et al. 2009). Chemical fungicides are widely used to control this disease, but they are

expensive, environmentally harmful, and often less effective against evolving pathogen strains (Reynolds 2015; Jürges et al. 2009). Genetic resistance offers a sustainable alternative by enhancing the plant's intrinsic defense mechanisms and reducing dependence on chemicals (Jürges et al. 2009). Identifying resistant genotypes is therefore essential for the development of durable and productive grapevine cultivars (Reynolds 2015; Jürges et al. 2009). Resistance in grapevine is mediated by multiple *Rpv* (Resistance to *Plasmopara viticola*) loci, which regulate pathogen recognition, signaling pathways, and defense gene activation. Recent studies have discovered novel *Rpv* loci in Caucasus-derived germplasm, providing new opportunities for marker-assisted selection (MAS) in breeding programs (Reynolds 2015; Jürges et al. 2009). These loci often promote rapid activation of defense responses, including synthesis of antimicrobial compounds, oxidative stress management, and reinforcement of structural barriers in plant tissues (Reynolds 2015; Jürges et al. 2009). Understanding these mechanisms at the molecular level is crucial for improving resistance without compromising yield or fruit quality (Jürges et al. 2009).

Marker-assisted selection, coupled with high-density SNP arrays, accelerates the identification of resistant genotypes and allows pyramiding of multiple *Rpv* loci in elite cultivars (Reynolds 2015; Jürges et al. 2009). Genome-wide association studies (GWAS) have been instrumental in detecting resistance-associated loci, which can then be efficiently integrated into breeding programs (Reynolds 2015; Jürges et al. 2009). Resistant cultivars exhibit limited pathogen growth, reduced sporulation, and enhanced physiological responses that collectively enhance durability of resistance (Jürges et al. 2009). Such strategies contribute to sustainable viticulture while minimizing environmental impact and production costs. The molecular basis of resistance involves coordinated regulation of genes associated with pathogen perception, signal transduction, and secondary metabolite biosynthesis. Resistant genotypes show early

and strong transcriptional responses that prevent pathogen colonization and reduce disease severity. Integrating genetic, molecular, and phenotypic data allows breeders to select high-performing cultivars with durable resistance efficiently. Overall, understanding and utilizing the genetic basis of downy mildew resistance is a cornerstone of modern grapevine breeding and sustainable viticulture practices (Reynolds 2015; Jürges et al. 2009).

Materials and Methods

Characteristics of the research materials

The study used several local Azerbaijani table grapes varieties that differ in their susceptibility to downy mildew. A total of 12 representative varieties were chosen, including early, mid, and late-ripening types, to capture the diversity of fruit characteristics and growth patterns. Healthy cuttings were collected from established vineyards and acclimatized under controlled greenhouse conditions before the experiments. Selection criteria focused on fruit quality, yield potential, and historical observations of disease response.

Isolation of nuclear DNA from grapevine genotypes

Nuclear DNA was extracted from young, healthy leaves of selected Azerbaijani table grape genotypes. Leaf samples were collected in the morning to ensure minimal degradation and immediately stored at -80°C until processing. Prior to DNA extraction, leaves were ground into a fine powder using liquid nitrogen to break cell walls efficiently. The quality and quantity of the samples were maintained to allow reliable downstream molecular analyses. DNA extraction was performed using a modified CTAB (cetyltrimethylammonium bromide) method, which is suitable for grapevine tissues containing high levels of polyphenols and polysaccharides. During the procedure, RNA and proteins were removed using RNase and proteinase K treatments to ensure pure nuclear DNA. Following extraction, DNA was precipitated with cold isopropanol, washed with ethanol, and resuspended in TE buffer for long-term storage. The integrity of extracted DNA was verified by agarose gel electrophoresis, and



concentration was measured spectrophotometrically.

The resulting high-quality DNA was suitable for molecular marker analysis, including SSR, SNP, and QTL mapping for mildew resistance. This method provided consistent yields across all genotypes, ensuring comparability of genetic data. The DNA obtained from resistant and susceptible varieties formed the basis for identifying candidate resistance loci in subsequent genotyping experiments. Proper sample handling and standardized extraction protocols minimized variation and contamination risks. Overall, this approach enabled efficient, reproducible, and high-fidelity nuclear DNA isolation for all grapevine genotypes under study.

Determination of DNA purity and optical density

The purity and concentration of nuclear DNA extracted from table grape genotypes were assessed using a spectrophotometer. Absorbance was measured at 260 nm and 280 nm to calculate the 260/280 ratio, which indicates protein contamination. Additionally, the 260/230 ratio was determined to evaluate the presence of polysaccharides and other impurities. DNA concentrations were adjusted to optimal levels for downstream molecular marker analysis. This procedure ensured that only high-quality DNA was used for subsequent genotyping and resistance studies.

Results and Discussion

Field evaluation of 37 Azerbaijani table grape varieties showed significant variability in susceptibility to downy mildew. Among them, 13 varieties displayed tolerance, exhibiting minimal leaf necrosis and reduced sporangiophore development, while the remaining 24 were clearly susceptible (Babaev et al. 2025). Resistant varieties maintained better overall plant health and higher photosynthetic activity compared to susceptible ones. These findings highlight the importance of identifying genotypes with natural resistance for sustainable viticulture.

Molecular analysis using SSR markers associated with known resistance loci (*Rpv1*–

Rpv10) revealed polymorphisms across the varieties (Eibach & Töpfer 2015). Several resistant accessions carried alleles linked to the *Rpv10* locus, indicating the presence of functional resistance genes. PCR amplification confirmed the expected fragment sizes corresponding to resistant alleles. Varieties lacking these alleles exhibited higher infection severity, confirming the correlation between genotype and phenotype (Eibach & Töpfer 2015).

Some varieties possessed multiple QTLs, suggesting that gene pyramiding can enhance resistance durability. Those combining *Rpv1*, *Rpv3*, and *Rpv10* loci consistently showed the lowest disease severity, supporting the use of multiple loci in breeding programs. Varieties with single loci exhibited moderate susceptibility under high disease pressure. These observations reinforce the importance of integrating molecular markers with field evaluation for selecting superior genotypes (Eibach & Töpfer 2015; Toffolatti et al. 2016).

Environmental conditions influenced pathogen development, particularly warm and humid weather, which increased infection rates in susceptible varieties. Resistant genotypes, however, maintained lower disease indices despite similar environmental pressure, suggesting that genetic factors strongly limit pathogen proliferation. Structural defense mechanisms, such as cell wall reinforcement, and biochemical responses, including antimicrobial compound production, were observed in tolerant varieties. These traits restricted pathogen colonization and sporulation, protecting both leaves and clusters.

Marker-assisted selection enabled early identification of resistant genotypes, allowing for accelerated breeding without reliance on chemical fungicides. Integrating local Azerbaijani germplasm with known resistance loci from global studies provides a strong foundation for developing high-yielding, resistant cultivars (Toffolatti et al. 2016). Overall, combining phenotypic screening and molecular markers ensures efficient selection of grapevine genotypes with durable resistance. These results confirm the potential of local

varieties as valuable genetic resources for sustainable viticulture in Azerbaijan (Babaev et. 2025; Salimov et al. 2022).

Conflict of Interest

Authors declare that there is no conflict of interest.

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