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Research Article

Assessment of Susceptibility to Downy Mildew Disease in Some Grape Varieties and Genotypes Using Marker-Assisted Selection

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Abstract: The leaves of the vine plant, like its fruits, are used in human nutrition and meals in many countries. Grape leaves serve as an important ingredient in traditional foods. In Türkiye, the leaves of Narince, Sultani Çekirdeksiz and Yapıncak grape varieties are preferred for stuffed grape leaf production. However, pesticide residues generate a serious problem for brined vine leaves. Fungicides with different active ingredients are used to combat downy mildew and powdery mildew diseases in viticulture. Improper use of these chemicals results in serious residue problems on product surfaces. Such cases pose serious threats to human health and the environment. This study used the marker-Assisted Selection (MAS) method to identify individuals containing genes resistant to *Plasmopara viticola* in grape genotypes obtained through hybridization. The presence of the Rpv3 gene in hybrid individuals was examined. Total nucleic acids were extracted from fresh leaves of the plants, and the regions related to the Rpv3 gene were amplified on the genomic DNA with GF18-06/GF18-08 primers. PCR products were visualized using an agarose gel electrophoresis system, and allele gene sizes were also determined by fragment analysis. MAS method yielded 27 genotypes with the Rpv3 gene. DNA sizes were also confirmed by fragment analysis. The promising genotypes were selected for future studies.

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1. Introduction

Grapes are utilized in several different ways. Besides grapes, vine leaves are also used as a foodstuff in various countries (Cangi and Yağcı, 2017). Vine leaves are low in calories and rich in dietary fiber, calcium, phosphorus, phenolic compounds, vitamin C and K1 (El Nehir et al., 1997). In Turkey, leaves of Narince, Sultani Cekirdeksiz, and Yapıncak grape cultivars are mainly used for stuffed grape leaves (Cangi and Yağcı, 2017). Vine leaves used as foodstuff are collected from the vineyards where grapes are produced. Leaves are generally collected between May and July. During this period, different contact or systemic fungicides and insecticides are used in vineyards against some pests and diseases (Eutyepa dieback, powdery mildew, downy mildew, leaf scab, etc.) (Yanar et al., 2017; Bakırcı

et al., 2019). When the pesticide application and leaf collection periods are not well adjusted, pesticide residues on vine leaves may generate a serious problem for human health (Cangi et al., 2014).

Hybrid breeding studies in viticulture have a history of about 200 years. The primary objective of hybridisation studies was initially to achieve high yield and quality varieties, but later on, resistance to biotic or abiotic stressors gained greater emphasis (Reynolds, 2015; Atak, 2024). Breeders largely apply the classical hybridisation method. In this method, genetic variation is created with appropriately selected parents and selection is made among the resultant genotypes. Classical breeders determine yield and quality parameters through field observations. In this method, the breeding period usually takes 25-30 years (Eibach and Töpfer, 2015). With the new techniques developed in recent years, new grape varieties, especially seedless, are being developed in a much shorter time (Doyğacı et al., 2024).

Scientists have made great progress in studies on grapevine genetics since the early 20th century (Vivier and Pretorius, 2000). With the introduction of polymerase chain reaction (PCR)-based DNA analyses, the first genetic map was created (Lodhi et al., 1995). Later on, QTL (Quantitative Trait Loci) were defined for some important traits such as powdery mildew and downy mildew (Fischer et al., 2004). Then, breeders went beyond classical methods and applied MAS (Marker Assisted Selection) methods (Zyprian et al., 2003; Eibach et al., 2007) and used molecular markers for identification of genetic resources. In this way, breeders achieved a time savings of 8-10 years in breeding processes (Verma et al., 2019).

Vitis vinifera species are susceptible to downy mildew (Krul and Mowbray, 1984). However, there are also species within the genus *Vitis* that are resistant to downy mildew. For instance, while North American vine species of *V. aestivalis* and *V. labrusca* are moderately susceptible, *V. cardifolia*, *V. rupestris* and *V. rotundifolia* are relatively resistant. Hybridisation studies were carried out between *V. vinifera* and North American species and new cultivars were obtained by combining downy mildew resistance and fruit quality (Wilcox et al., 2015). Among them, Baron, Cabernet blanc, Cabernet Carbon, Cabertin, Piroso, Rondo, Sauvignac and Monarch varieties with Rpv3 locus were published in the Vitis International Variety Catalogue (VIVC) (https://www.vivc.de/). Regent is also among these varieties. QTLs specifically providing downy mildew resistance were identified in Regent variety (Welter et al., 2007).

Several studies were conducted to determine downy mildew resistance through marker-assisted selection in genotypes obtained from Regent variety and hybrid combinations in which this variety was used as parents (Fischer et al., 2004; Akkurt et al., 2022; Polat and Suluhan, 2024). However, there are no studies on the allele sizes associated with the Rpv3 locus in Isabella variety that are tolerant to both powdery mildew and downy mildew (Atak et al., 2017; Yıldırım et al., 2019; Doğu et al., 2023), in Kishmish Vatkana variety that are tolerant to powdery mildew (Kozma et al., 2006; Hoffmann et al., 2008; Coleman et al., 2009; Bozkurt et al., 2023) and in genotypes obtained from hybridisations in which these varieties were used as parents.

The primary objective of this study is to detect downy mildew tolerant genotypes with high leaf quality at an early stage through marker-assisted selection to reduce the negative effects of pesticide use.

2. Material and Methods

2.1. Material

In 2019, classical hybridisation studies were conducted on the combinations of Narince × Isabella (NVL), Narince × Regent (NRG), and Narince × Kishmish Vatkana, resulting in the production of 447 hybrids. In the years 2020 and 2021, these genotypes were evaluated for their suitability in terms of lob number, leaf sinus depth, leaf hair density, and vein thickness for brined vine leaves, and it was generally determined that they were suitable genotypes (Bozkurt, 2023; Bozkurt and Yağcı, 2024).

Narince grape variety is a wine variety originating from Turkey (Anonymous, 2023) and its leaves are also brined (Cangi and Yağcı, 2017) (Figure 1). Regent variety is obtained as a result of interspecies hybridisation. It has Ren3, Ren9 and Rpv3.1 loci. Regent variety is highly tolerant to downy mildew and powdery mildew diseases (Figure 2), (VIVC, 2020). Kishmish Vatkana grape variety is originated from Uzbekistan (VIVC, 2020) and is tolerant to vineyard powdery mildew (Kozma et al., 2006; Hoffmann et al., 2008) (Figure 3). Isabella (*V. labrusca*) grape variety is tolerant to powdery

mildew and downy mildew (Figure 4) (Yıldırım et al., 2019). These three varieties constitute an important genetic resource for resistance breeding studies (Atak et al., 2017).

Figure 1. Narince (VIVC, 2020).

Figure 2. Regent (VIVC, 2020).

Figure 3. Kishmish vatkana (VIVC, 2020).

Figure 4. Isabella (VIVC, 2020).

2.2. Methods

2.2.1. Total genomic DNA isolation

Scions were collected from the genotypes during pruning and subsequently planted in 0.85-liter pots filled with a peat and perlite mixture. The genotypes were cultivated under standard conditions in a climate chamber. Shoots, approximately 2-3 cm in length, were harvested from the plants and used as a DNA source. Molecular analyses were conducted at the Advanced Technology Research and Application Center of Sivas Cumhuriyet University in 2022. About 150 mg of tissue was taken from the shoot tips of F1 plants, transferred to sterile Eppendorf tubes and stored at -80°C until DNA isolation stage. DNA isolation from these tissues was performed in accordance with the protocol of Piccolo et al. (2012).

2.2.2. Polymerase Chain Reaction (PCR)

The Rpv3 gene region was selected to determine whether the DNA samples of 33 genotypes were resistant to downy mildew (Di Gaspero et al., 2012). The 'GF18-06' and 'GF18-08' primers, which were developed from the 12X reference map of grapevine and determined to be related to the Rpv3.1 gene region, were used (Schwander et al., 2012; Zyprian et al., 2016). Information about the primer pairs, fragment length and annealing temperatures is provided in Table 1.

Table 1. Primers and standard characteristics

Gene	Primer	Forward / Reverse primer	Reference	Fragment Length (bp)	Annealing Temperature (T_A)
Rpv3	GF 18-06F	GGTCTCCTAGAAAGCCAAGCAA	Di Gaspero et al., 2012	389	60
	GF 18-06R	TCCCTTTTCCCCTTGTTCTCG			
	GF 18-08F	GACAATAGCGAGAGAGAATGGG		399	
	GF 18-08R	AGTTGGCTAAAACCCTAGAGGC			

All PCR reactions were prepared in 25 µl volume. The 25 µl reaction volume was composed of: 0.125 U Taq DNA polymerase (Fermentas), 2.5 µl reaction buffer (100 mM Tris-HCl, pH 8.8, 500 mM KCl and 0.8% Nonidet P-40), 1 µl of each primer at 10 pmol, 2.5 µl of 2.5 mM dNTP (MBI Fermentas), 2.5 µl of 25 Mm $MgCl_2$ and 1 µl of 100-500 ng template DNA. Final volume was achieved with the use of 25 µl dH_2O. The PCR reactions are enumerated in Table 2 and were performed on a Blue-Ray Biotech thermocycler.

Table 2. PCR reactions

Initial denaturation	at 94 °C for 5 min	
Denaturation	at 94 °C for 30 sec	
Annealing	at 60 °C for 30 sec	30 cycles
Extension	at 72 °C for 2 min	
Final extension	at 72 °C for 10 min	

To determine downy mildew resistant/susceptible genotypes, PCR amplifications of 33 samples were performed with two SSR markers (GF18-06 and GF18-08) specific to the Rpv3.1 gene. Amplification products were run on 1/1.5% agarose gel electrophoresis containing ethidium bromide (2 µg ml^{-1}) and imaged with a UV transilluminator.

Relevant allele sizes of PCR-amplified samples were determined with the use of a Bioanalyzer Qsep100 fragment analyzer. The genotypes for which allele sizes were determined were then assessed as resistant and/or sensitive.

3. Results

Agarose gel electrophoresis revealed that 33 amplified PCR products yielded a PCR product of 389 bp for GF18-06 (Figure 5) and 399 bp for GF18-08 (Figure 6).

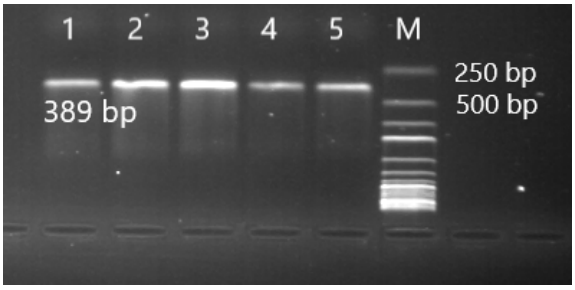

Figure 5. PCR image of Rpv3 gene (GF 18-06).

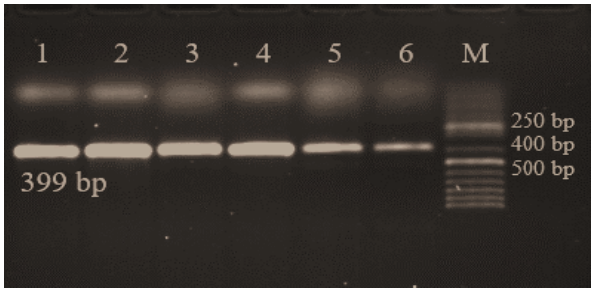

Figure 6. PCR image of Rpv3 gene (GF 18-08).

Allele size images for GF18-06 and GF18-08 primers obtained with a fragment analyzer are shown in Figure 7 and Figure 8.

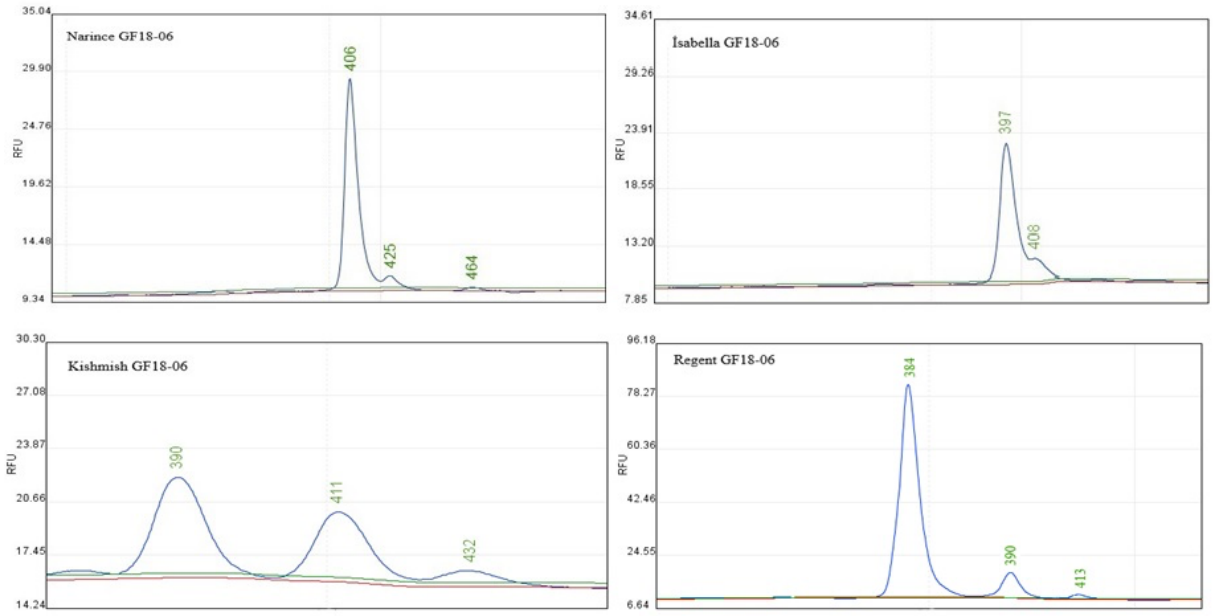

Figure 7. Allele size image for GF18-06 primer to Narince, Isabella, Kishmish, and Regent.

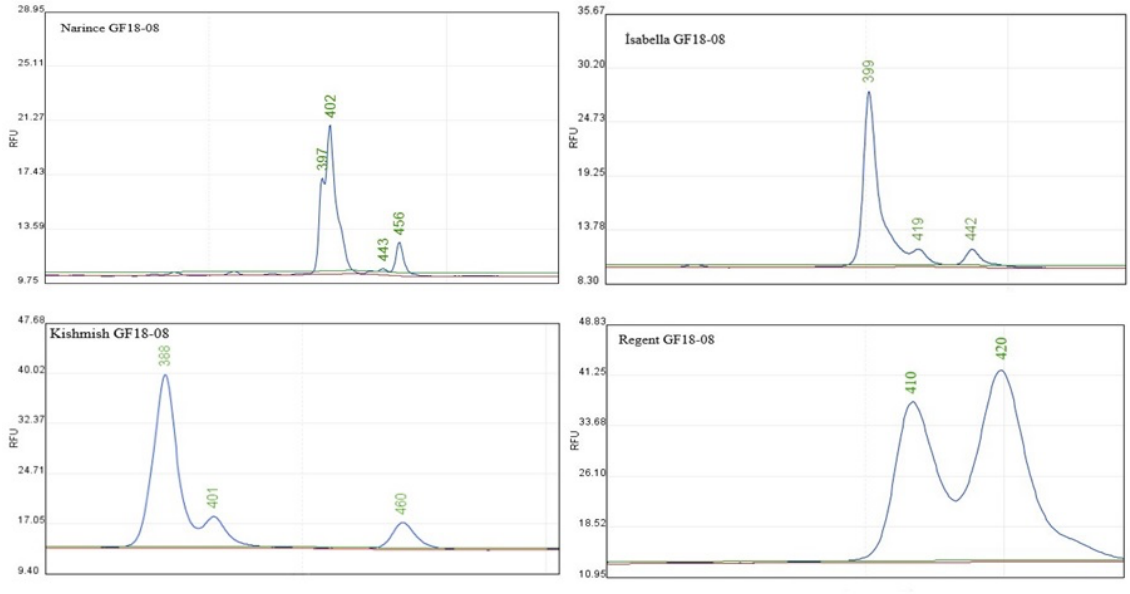

Figure 8. Allele size image for GF18-08 primer to Narince, Isabella, Kishmish, and Regent.

Genetically tested varieties and genotypes all yielded PCR product bands for both primers. With the GF 18-06 primer, allele sizes of 384-390-413 bp were determined in Regent, 390-411-432 bp in Kishmish Vatkana, 406-425-464 bp in Narince and 397-408 bp in Isabella. With the GF 18-06 primer, allele sizes of 410-420 bp were determined in Regent, 388-401-460 bp in Kishmish Vatkana, 397-402-443-456 bp in Narince and 399-419-442 bp in Isabella. Genotypes exhibited bands in the direction of allele sizes derived from the maternal and paternal parents.

In Regent variety with the Rpv3.1 locus, allele sizes of the GF18-06 and GF18-08 primers were taken as reference. Allele sizes of the parents and genotypes are provided in Table 3 and Figure 9, 10, 11, 12, 13, 14 and 15.

Considering the allele sizes of the reference variety Regent, 384-390-413 bp with the GF-18-06 primer and 410-420 bp with the GF 18-08 primer, it was seen that the NRG genotypes carried at least one allele from both primers in the Regent variety. Among them, NRG-147 (2 alleles for the GF18-06 primer, 1 allele for the GF18-08 primer) and NRG-64 (1 allele for the GF18-06 primer and 2 alleles for the GF18-08 primer) were found to be prominent genotypes since they were the same as the Regent variety. The K. Vatkana variety had also 1 allele of the same size (390 bp) in the GF18-06 primer of the Regent variety (Table 3).

The Isabella variety exhibited allele sizes of 397-408 bp with the GF18-06 primer. An allele size of 397 bp was detected in 20 genotypes belonging to the NVL combination; 408 bp in 3 genotypes (NVL-77, NVL-34 and NVL-154). When the allele sizes were examined for the GF18-08 primer, it was determined that 21 genotypes, except for NVL-145, NVL-43 and NVL-186 in the NVL hybrid group and Isabella variety, carried 399 bp allele (Table 3).

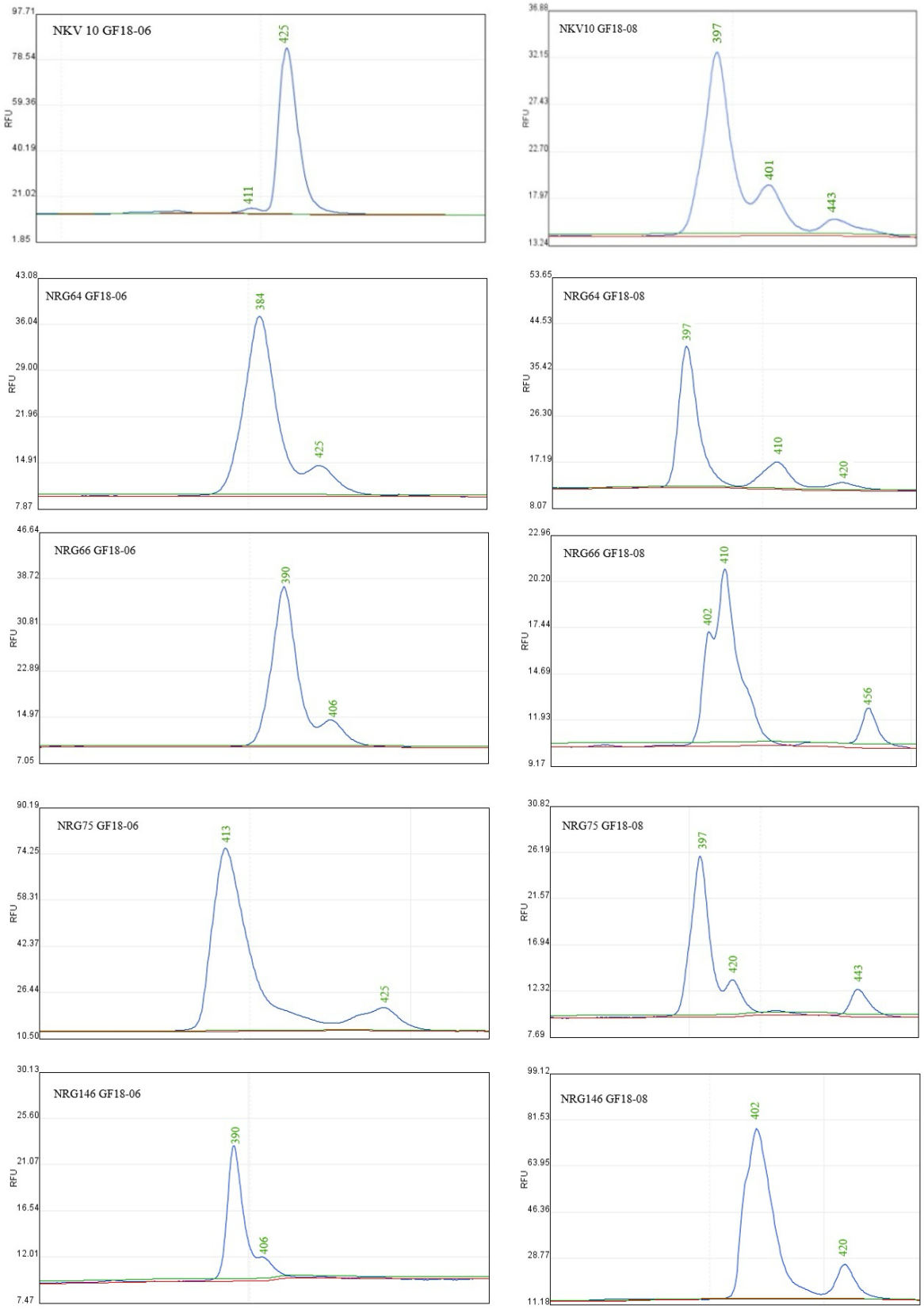

Figure 9. Allele sizes of GF18-06 and GF18-08 primers to NKV-10, NRG-64, NRG-66, NRG-75, and NRG-146.

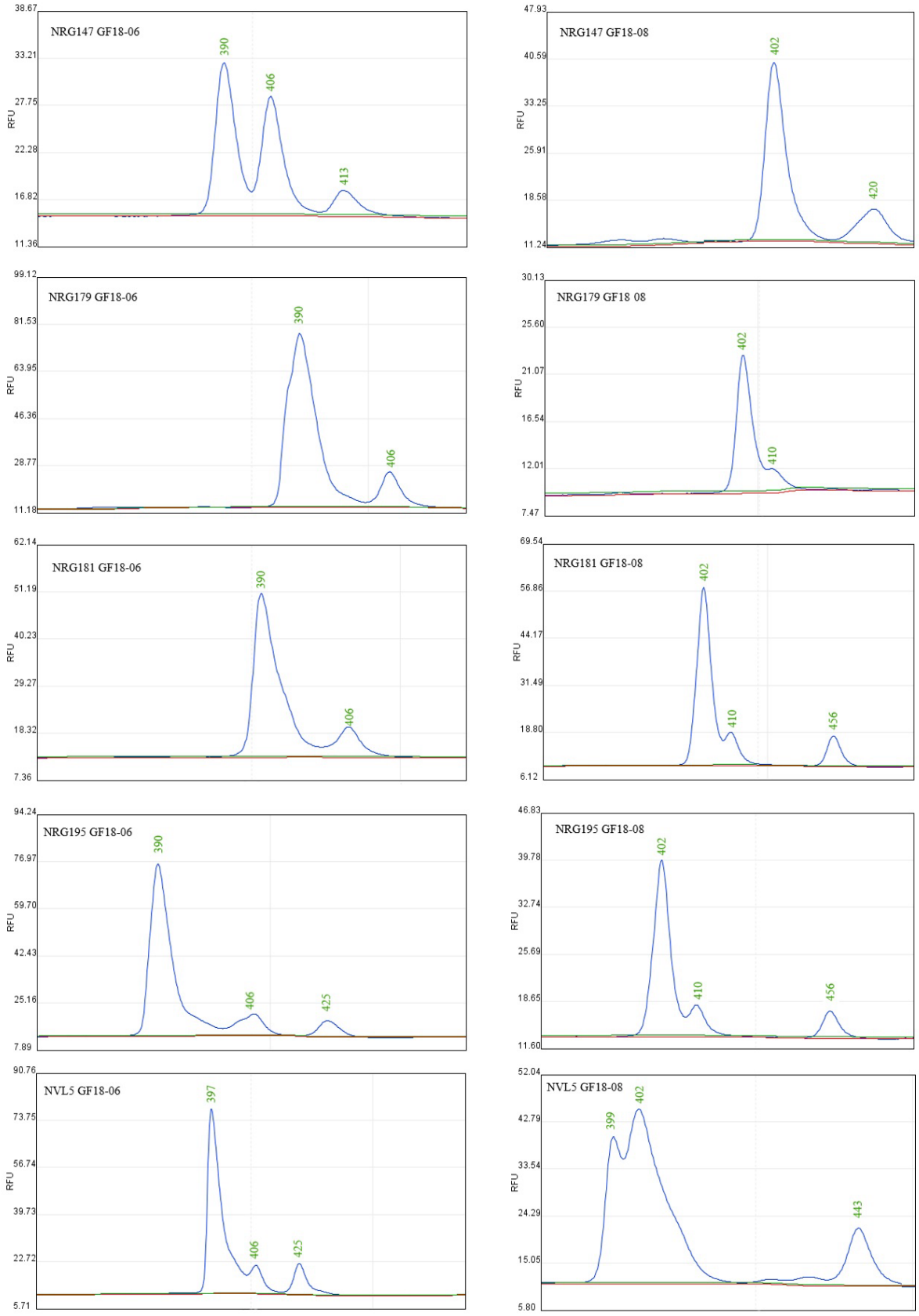

Figure 10. Allele sizes of GF18-06 and GF18-08 primers to NRG-147, NRG-179, NRG-181, NRG195, and NVL-5.

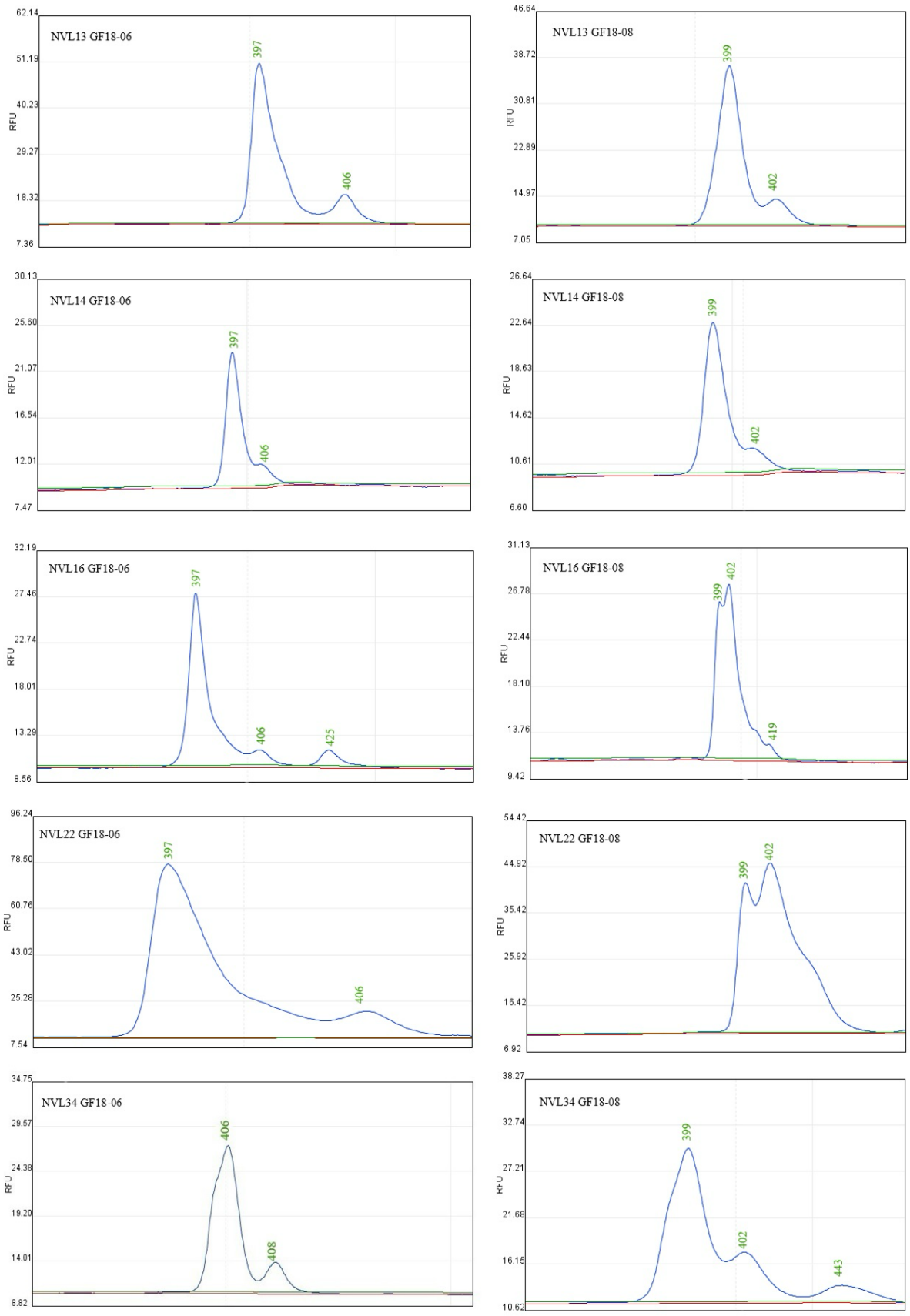

Figure 11. Allele sizes of GF18-06 and GF18-08 primers to NVL-13, NVL-14, NVL-16, NVL-22, and NVL-34.

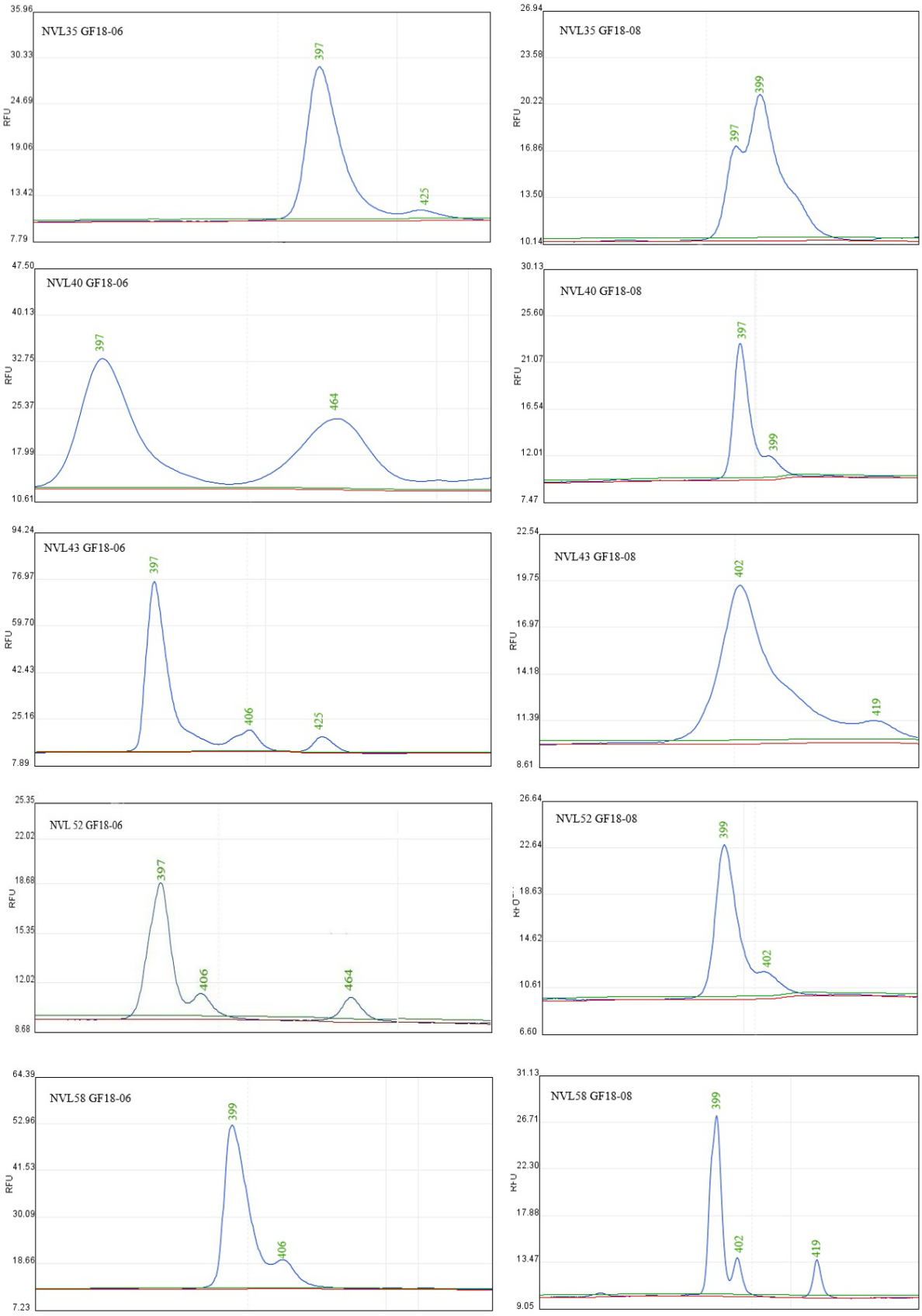

Figure 12. Allele sizes of GF18-06 and GF18-08 primers to NVL-35, NVL-40, NVL-43, NVL-52, and NVL-58.

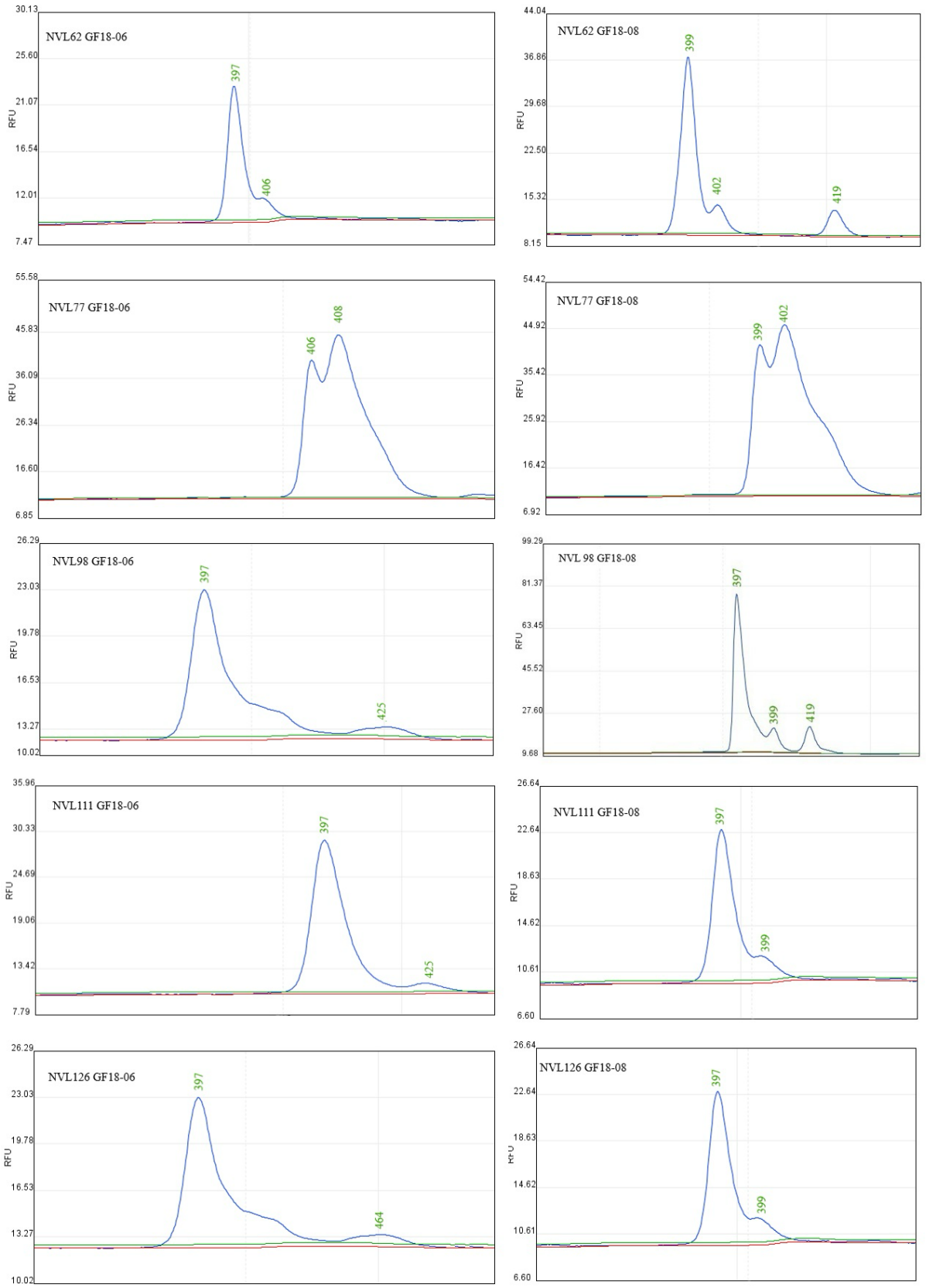

Figure 13. Allele sizes of GF18-06 and GF18-08 primers to NVL-62, NVL-77, NVL-98, NVL-111, and NVL-126.

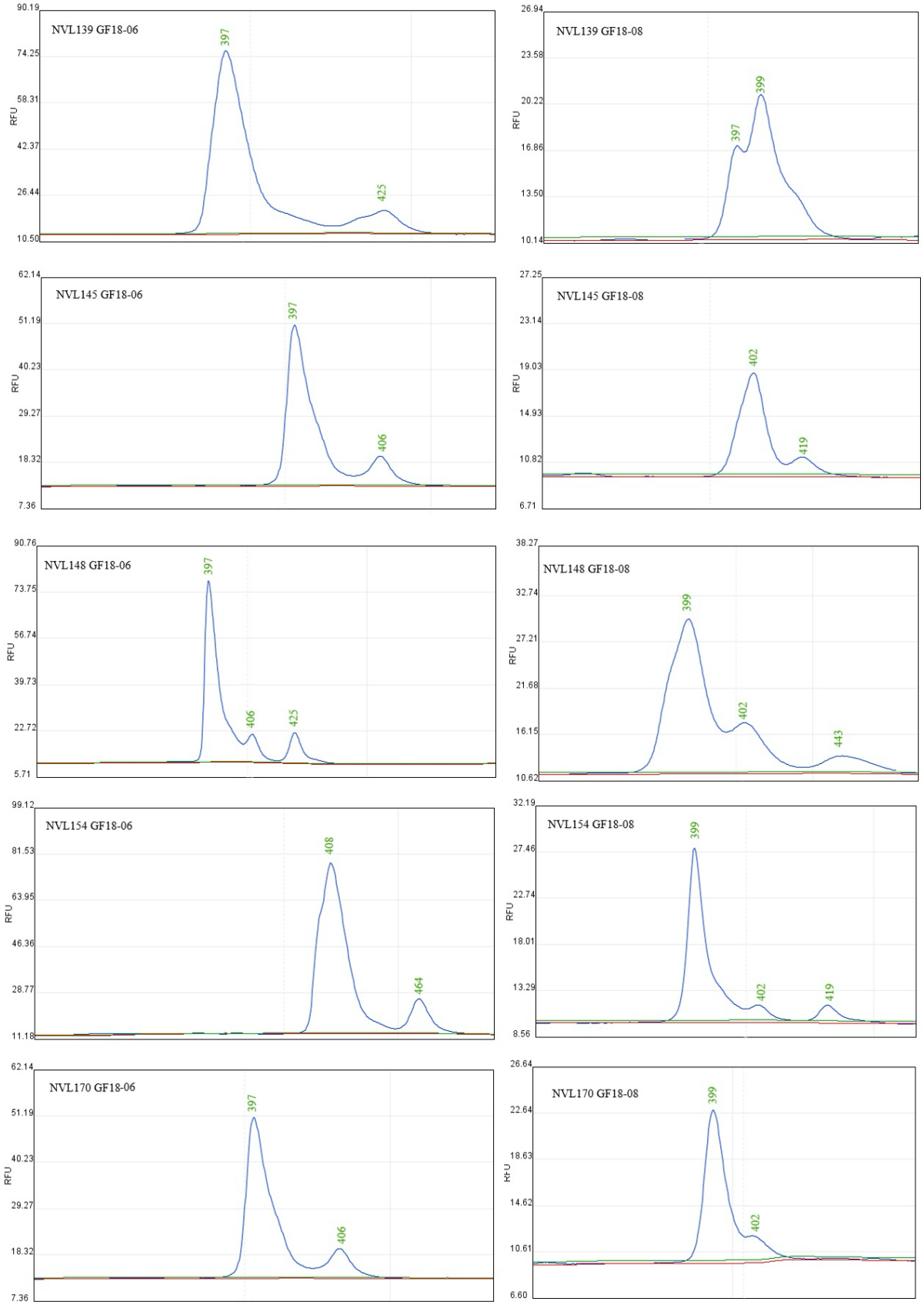

Figure 14. Allele sizes of GF18-06 and GF18-08 primers to NVL-139, NVL-145, NVL-148, NVL-154, and NVL-170.

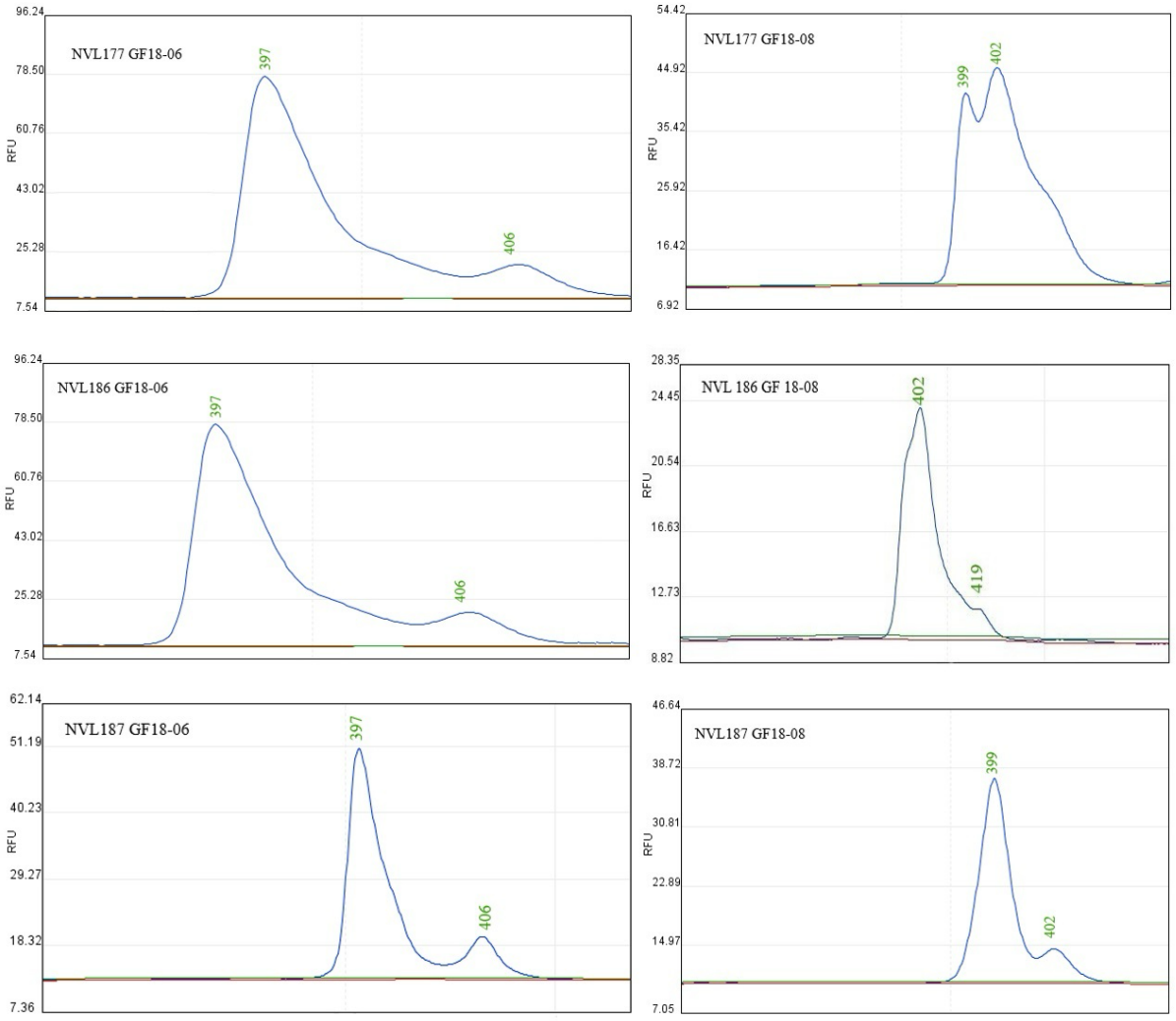

Figure 15. Allele sizes of GF18-06 and GF18-08 primers to NVL-177, NVL-186, and NVL-187.

Table 3. Allele sizes of GF18-06 and GF18-08 primers

Sample	Allele sizes of GF18-06 primer			Allele sizes of GF18-08 primer			
Isabella	397	408		399	419	442	
K. vatakana	390	411	432	388	401	460	
Regent	384	390	413	410	420		
Narince	406	425	464	397	402	443	456
NKV-010	411	425		397	443	401	
NRG-064	384	425		397	410	420	
NRG-066	390	406		402	456	410	
NRG-075	413	425		397	443	420	
NRG-146	390	406		402	420		
NRG-147	390	406	413	402	420		
NRG-179	390	406		402	410		
NRG-181	390	406		402	410	456	
NRG-195	406	425	390	402	410	456	
NVL-005	406	425	397	399	402	443	
NVL-013	397	406		399	402		
NVL-014	397	406		399	402		
NVL-016	406	425	397	399	402	419	
NVL-022	397	406		399	402		
NVL-034	406	408		399	402	443	

Table 3. Allele sizes of GF18-06 and GF18-08 primers (continued)

Sample	Allele sizes of GF18-06 primer			Allele sizes of GF18-08 primer		
NVL-035	397	425		397	399	
NVL-040	397	464		397	399	
NVL-043	397	406	425	402	419	
NVL-052	397	406	464	399	402	
NVL-058	399	406		399	402	419
NVL-062	397	406		399	402	419
NVL-077	406	408		399	402	
NVL-098	397	425		399	397	419
NVL-111	397	425		399	397	
NVL-126	397	464		399	397	
NVL-139	397	425		399	397	
NVL-145	397	406		402	419	
NVL-148	397	406	425	399	402	443
NVL-154	408	464		399	402	419
NVL-170	397	406	425	399	402	
NVL-177	397	406		399	402	
NVL-186	397	406		402	419	
NVL-187	397	406		399	402	

4. Discussion

The results obtained from our study were found to be consistent in many respects when compared with similar studies (Shidfar et al., 2019; Yıldırım et al., 2019; Akkurt et al., 2022). In the present study, the MAS method offered a reliable tool for early selection of resistant genotypes. As it was previously stated in Foria et al. (2018), the present findings confirmed that the response to downy mildew in plants screened for the Rpv3.1 differed based on the genetic background of the plants.

Genotypes with allele sizes of 385-390-407 bp (Akkurt et al., 2022) in relation to Rpv3.1 in the Regent variety with the GF18-06 primer were assessed as resistant candidates. In the present study, an allele size of 390 bp was detected in all genotypes belonging to the NRG hybrid group and these genotypes were assessed as resistant to downy mildew. Detection of an allele size of 390 bp in the Kishmish Vatkana variety suggested that this variety might also be tolerant to downy mildew. The Present findings indicated that the Kishmish Vatkana variety, resistant to powdery mildew (Kozma et al., 2006; Hoffmann et al., 2008; Coleman et al., 2009; Bozkurt et al., 2023), may offer a valuable source also for downy mildew. A similar case is valid for the Isabella variety. The Isabella variety exhibited allele sizes of 397-408 bp with the GF18-06 primer. An allele size of 397 bp in 20 genotypes belonging to the NVL combination and 408 bp in 2 genotypes (NVL-77 and NVL-34) (Table 3). Such a case suggests that the allele size of 397 bp in the Isabela variety might be related to downy mildew resistance. It has been indicated in previous studies where artificial downy mildew tests were conducted on the Isabella variety under both field and laboratory conditions that this variety was resistant to downy mildew (Atak et al., 2017; Yıldırım et al., 2019; Doğu et al., 2023). Unlike the present findings, Zyprian et al. (2016) reported only 387 bp allele associated with downy mildew resistance in the QTL map obtained from the hybrid combination 'GF.GA-47-42' × 'Villard Blanc' with the GF 18-06 marker. In the present study, although 387 bp alleles were not detected, it was thought that 390 bp 'Regent' alleles could provide a source for hybridisation populations for breeding resistance to downy mildew.

Previous studies revealed that downy mildew-resistant Regent variety yielded allele sizes between 399-410-420 bp with the use of GF18-08 primer associated with Rpv3.1 locus and GF18-08 marker had a strong relationship with downy mildew resistance (Uzun et al., 2018; Akkurt et al., 2022). In the present study, NRG-181, NRG-66, NRG-179, NRG-64 and NRG-195 genotypes of Narince x Regent combination yielded an allele size of 410 bp and other genotypes exhibited an allele size of 420 bp. In a similar study, Akkurt et al. (2022) identified 113 of a total of 145 genotypes that gave

amplification products with GF 18-08 marker as resistant. It was particularly indicated that GF 18-08/410 bp 'Regent' allele was strongly associated with downy mildew resistance. In the present study, with GF18-08 primer, an allele size of 399 bp was determined in 21 genotypes belonging to Isabella variety and Narince × Isabella combination. The Present findings suggest that allele size of 399 bp in Isabella variety may be related to downy mildew resistance. Zyprian et al. (2016) used GF18-08 marker in downy mildew resistant varieties Regent, Suberux, Villard Blanc and Seibel 6468 and assessed "399 bp" allele as related to resistance. In a similar study, Possamai et al. (2020) screened 26 different grapevine genotypes, which are Raboso Piave (RP), Kozma 20-3 (K), Solaris (S), Chardonnay and Glera parent grapevine varieties and their hybrids, for specific segregating Rpv loci with the use of SSR markers. It was indicated that 110 different RP x K genotypes carried resistance alleles of Rpv3.1, Rpv12 or both and 255 different RP x S genotypes had resistance alleles of Rpv3.3, Rpv10 or both. It was also indicated that hybrids carrying Rpv3.1 and Rpv12 loci showed the strongest resistance response (low sporulation and necrosis), while those carrying Rpv3.3 locus showed the highest levels of necrosis and the genotypes carrying Rpv10 represented intermediate levels of both sporulation and necrosis. In another study, it was determined that Rpv3.1-mediated resistance identified in Villard Blanc variety was associated with a defense mechanism that resulted in inhibition of pathogen growth and development through triggering the synthesis of stilbenes and HR toxic to fungi (Eisenmann et al., 2019). Additionally, transient co-expression of TIR-NB-LRR gene pairs from Rpv3 locus of *V. vinifera* leaves activated pathogen-induced HR and sporulation was reduced as compared to leaves of non-transformed plants (Foria et al., 2020).

Conclusion

Based on the results obtained in this study, several important conclusions can be drawn regarding the resistance of grapevine genotypes to downy mildew. The molecular analysis indicated that certain genotypes, particularly those from the Narince × Regent (NRG) hybrid group, exhibited allele sizes corresponding to known resistance markers for downy mildew, specifically the 390 bp allele with the GF18-06 primer, which is linked to the Rpv3.1 locus in Regent variety. These genotypes, including NRG-147 and NRG-64, were identified as potential candidates for downy mildew resistance. Additionally, the Kishmish Vatkana variety showed the presence of the 390 bp allele, suggesting a possible tolerance to downy mildew. The Isabella variety also demonstrated promising results, with allele sizes of 397-408 bp with the GF18-06 primer, which were consistent with previously reported downy mildew resistance. The presence of the 399 bp allele in 21 genotypes from the NVL hybrid group further supported this potential resistance. Moreover, the GF18-08 primer revealed strong associations between the 410 bp and 420 bp alleles in NRG genotypes, with the 410 bp allele particularly linked to resistance in previous studies. These findings align with the results of previous research, such as those by Akkurt et al. (2022), and highlight the effectiveness of molecular markers, particularly the GF18-06 and GF18-08 primers, in identifying resistant genotypes. Overall, this study supports the use of molecular marker-assisted selection (MAS) as a reliable tool for early selection of grapevine genotypes resistant to downy mildew. It is recommended that these promising genotypes be further evaluated under field conditions to confirm their resistance profiles and their potential for use in breeding programs aimed at developing downy mildew-resistant grapevine cultivars.

Ethical Statement

This article does not require ethical approval because it does not contain any studies with human or animal subjects.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Author Contributions

The authors of the study contributed equally to all stages of the research.

References

Akkurt, M., Şenses, I., Aktürk, B., Tozlu, I., Özer, N., & Uzun, H. (2022). Marker assisted selection (MAS) for downy mildew resistance in grapevines using Rpv3. 1 associated markers. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *50*(1). DOI:10.15835/nbha50112708

Anonymous, (2023). Kavaklıdere Şarapları. http://www.kavaklidere.com/tr (24.03.2023).

Atak, A. (2024). *Vitis* species for stress tolerance/resistance. *Genetic Resources and Crop Evolution*, 1-20. https://doi.org/10.1007/s10722-024-02106-z

Atak, A., Akkurt, M., Polat, Z., Çelik, H., Kahraman, K., Akgül, D., ... & Eibach, R. (2017). Susceptibility to downy mildew (*Plasmopara viticola*) and powdery mildew (*Erysiphe necator*) of different *Vitis* cultivars and genotypes. *Ciência e Técnica Vitivinícola*, *32*(1). file:///C:/Users/pcx/Downloads/ctv20173201p23.pdf

Bakırcı, G. T., Çınar, E., & Karakaya, S. (2019). Pesticide residues in grape leaves collected from Manisa. *Academic Food Journal*, *17*(1), 55-60. DOI: 10.24323/akademik-gida.544073

Bozkurt, A. (2023). *Improve of new powdery mildew tolerant grape cultivars for stuffed vine leaf* (Doctoral Thesis), Tokat Gaziosmanpaşa University Universitesi, Institute of Science and Technology, Department of Horticulture, Tokat.

Bozkurt, A. , Yağcı, A. & Akgül, D. S. (2023). *Time-Dependent Variation of Susceptibility of Some Grape Cultivars to Powdery Mildew Infections.* Bahçe, 10. Turkey Viticulture and Technologies Symposium, 300-308. https://dergipark.org.tr/en/download/article-file/2979987

Bozkurt, A., & Yağcı, A. (2024). Determination of the possibility of using some hybrid vine genotypes as stuffed vine leaves. *Gaziosmanpasa Journal of Scientific Research, 13(1)*, 31-42. https://dergipark.org.tr/en/download/article-file/3742290

Buonassisi, D., Colombo, M., Migliaro, D., Dolzani, C., Peressotti, E., Mizzotti, C., Velasco, R., Masiero, S., Perazzolli, M., & Vezzulli, S. (2017). Breeding for grapevine downy mildew resistance: a review of ‘‘omics’’ approaches. *Euphytica*, *213*,103.

Burger, P., Bouquet, A., & Striem, M. J. (2009). Grape breeding. Breeding plantation tree crops: Tropical species, *Springer,*Chapter *5,* 161-189. https://doi.org/10.1007/978-0-387-71201-7_5

Cangi, R., & Yağcı, A. (2017). Production of grapevine leaves from vineyard to table. *Nevşehir Journal of Science and Technology, 6*, 137-148. DOI: 10.17100/nevbiltek.288316

Cangi, R., Yanar, Y., Yağcı, A., Topçu, N., & Sucu, S. (2014). The effect of picking period and brining applications on fungicide residue levels in brined vine leaves production. *Journal of Agricultural Faculty of Gaziosmanpasa University, 31*(2), 23-30.

Coleman, C., Copetti, D., Cipriani, G., Hoffmann, S., Kozma, P., Kovács, L., ... & 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), 1-20. doi:10.1186/1471-2156-10-89

Di Gaspero, G., Copetti, D., Coleman, C., Castellarin S.D., Eibach, R., Kozma, P., Lacombe, 5 T., Gambetta, G., Zvyagin, A., Cindrić, P., Kovács, L., Morgante, M., & Testolin, R. (2012). 6 Selective sweep at the Rpv3 locus during grapevine breeding for downy mildew resistance. 7 *Theor Appl. Genet., 124*, 227-286. https://doi.org/10.1007/s00122-011-1703-8

Doğu, D. M., Atak, A., & Özer, N. (2023). An overview of the studies on resistance to downy mildew (*Plasmopara viticola*) on grapevines in Türkiye. *Viticulture Studies*, *3*(1), 41-55. doi.org/10.52001/vis.2023.19.41.55

Doyğacı, Y., Boztepe, Ö., Kandilli, G. G., & Atak, A. (2024). Embryo recovery (rescue) studies in different Vitis species. *BMC Plant Biology, 24(1),* 822. https://doi.org/10.1186/s12870-024-05539-x

Eibach, R., & Töpfer, R. (2015). Traditional grapevine breeding techniques. In Grapevine breeding programs for the wine industry. *Woodhead Publishing,* 3-22. https://doi.org/10.1016/B978-1-78242-075-0.00001-6

Eibach, R., Zyprian, E., Welter, L., & Topfer, R. (2007). The use of molecular markers for pyramiding resistance genes in grapevine breeding. *Vitis-Geilweilerhof*, *46*(3), 120.

Eisenmann, B., Czemmel, S., Ziegler, T., Buchholz, G., Kortekamp, A., Trapp, O., Rausch, T., Dry, I., & Bogs, J. (2019). Rpv3 – 1 mediated resistance to grapevine downy mildew is associated with

specific host transcriptional responses and the accumulation of stilbenes. *BMC Plant Biol*., *19*, 1-17. doi: 10.1186/s12870-019-1935-3

El Nehir S., Kavas A., & Karakaya S. (1997). Nutrient composition of stuffed vine leaves: A mediterranean diatery. *Journal of Food Quality*, *20*, 337-341. https://doi.org/10.1111/j.1745-4557.1997.tb00476.x

Fischer, B. M., Salakhutdinov, I., Akkurt, M., Eibach, R., Edwards, K. J., Toepfer, R., & Zyprian, E. M. (2004). Quantitative trait locus analysis of fungal disease resistance factors on a molecular map of grapevine. *Theoretical and Applied Genetics*, *108*, 501-515. https://doi.org/10.1007/s00122-003-1445-3

Foria, S. (2015). The Rpv3 locus in grapevine: DNA variation and relevance for conventional breeding. Dissertation, Università degli Studi di Udine. https://air.uniud.it/handle/11390/1132809

Foria, S., Copetti, D., Eisenmann, B., Magris, G., Vidotto, M., & Scalabrin, S., (2020). Gene duplication and transposition of mobile elements drive evolution of the Rpv3 resistance locus in grapevine. *Plant J*., *101*(3), 529-542. doi: 10.1111/tpj.14551

Foria, S., Magris, G., Morgante, M., & Di Gaspero, G. (2018). The genetic background modulates the intensity of Rpv3-dependent downy mildew resistance in grapevine. *Plant Breed, 137*, 220-228. https://doi.org/10.1111/pbr.12564

Hoffmann, S., Di Gaspero, G., Kovács, L., Howard, S., Kiss, E., Galbács, Z., ... & 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*(3), 427-438. https://doi.org/10.1007/s00122-007-0680-4 https://www.researchgate.net/publication/331062575

Kozma, P., Kiss, E., Hoffmann, S., Galbacs, Z. S., & Dula, T. (2006, July). *Using the powdery mildew resistant Muscadinia rotundifolia and Vitis vinifera'Kishmish vatkana'for breeding new cultivars*. In IX International Conference on Grape Genetics and Breeding, 827, 559-64. 10.17660/ActaHortic.2009.827.97

Krul, W. R., & Mowbray, G. H. (1984). Grapes. In: Sharp WR, Evans DA, Ammirato PV, Yamady Y (eds) *Handbook of plant cell culture* (pp. 396-434), vol 2. Crop species. MacMillan, New York.

Lodhi, M. A., Ye, G. N., Weeden, N. F., Reisch, B. I., & Daly, M. J. (1995). A molecular marker based linkage map of *Vitis*. *Genome*, *38*(4), 786-794. https://doi.org/10.1139/g95-100

Monteiro, F., Sebastiana, M., Pais, M. S., & Figueiredo, A. (2013). Reference gene selection and validation for the early responses to downy mildew infection in susceptible and resistant *Vitis vinifera* cultivars. *PloS one, 8*(9), e72998. https://doi.org/10.1371/journal.pone.0072998

Piccolo, S. L., Alfonzo, A., Conigliaro, G., Moschetti, G., Burruano, S., & Barone, A. (2012). A simple and rapid DNA extraction method from leaves of grapevine suitable for polymerase chain reaction analysis. *African Journal of Biotechnology, 11*(45), 10305. DOI:10.5897/AJB11.3023

Polat, İ., & Suluhan, E. (2024). Determination of Resistance to Mildew and Powdery Mildew Diseases of Narince x Regent Cross F1 Genotypes by Molecular Markers. *Yuzuncu Yil University Journal of Agricultural Sciences*, *29*(2), 770-779.

Possamai, T., Migliaro, D., Gardiman, M., Velasco, R., & De Nardi, B. (2020). Rpv mediated defense responses in grapevine offspring resistant to *Plasmopara viticola*. *Plants, 9*(6), 781. https://doi.org/10.3390/plants9060781

Reynolds, A. G. (Ed.). (2015). *Grapevine breeding programs for the wine industry*. Elsevier.

Schwander F., Eibach R., Fechter I., Hausmann L., Zyprian E., & Töpfer R. (2021). Rpv10: a new locus from the Asian Vitis gene pool for pyramiding downy mildew resistance loci in grapevine. *Theor. Appl. Genet*., *124*, 163-176. DOI 10.1007/s00122-011-1695-4.

Şenses, İ. (2020). *Marker assisted selection of downy mildew resistance in hybrid grapevine genotipes*. (Master Thesis), Ankara University, Graduate School of Natural and Applied Sciences, Department of Horticulture, Ankara.

Shidfar, M., Akkurt, M., Atak, A., Ergül, A., & Söylemezoğlu, G. (2019). Evaluation of grapevine resistance to downy and powdery mildew in 'Regent'×'Boğazkere' hybrid population segregating for resistance genes. *Acta Scientiarum Polonorum Hortorum Cultus*, *18*(1), 181-188. DOI: https://doi.org/10.24326/asphc.2019.1.18

Töpfer, R., Hausmann, L., & Eibach, R. (2011). Molecular breeding. *Genetics, Genomics, and Breeding Of Grapes*, 160-185.

Uzun, İ., Özer, N., Akkurt, M., Özer, C., & Aydın, S. (2018). *Asmalarda Mildiyö Hastalığına Dayanıklılığın Marköre Dayalı Seleksiyon ve Fenotipleme Yardımıyla Erken Teşhisi*. TÜBİTAK Proje No: 1150176; Antalya.

Verma, M. K., Singh, S. K., Patel, V. B., & Kumar, C. (2019). Grape improvement. Singh SK, Patel VB, Goswami AK, Prakash Jai & Kumar C (eds), *Breeding of perennial horticultural crops*. *Biotech Books*, Chapter 24, 283-314. New Delhi.

VIVC, (2020). Vitis International Variety Catalogue VIVC. https://www.vivc.de/ (Access date: 06.06.2020).

Vivier, M. A., & Pretorius, I. S. (2000). Genetic improvement of grapevine: Tailoring grape varieties for the third millennium-a review. *South African Journal of Enology and Viticulture*, *21(1),* 5-26. DOI: https://doi.org/10.21548/21-1-3556

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*(4), 359-374. https://doi.org/10.1007/s11032-007-9097-7

Wilcox, W. F., Gubler, W. D., & Uyemoto, J. K. (Eds.). (2015). Compendium of grape diseases, disorders, and pests. Tomball, TX: APS Press, *The American Phytopathological Society*, 39-45 https://library.wur.nl/WebQuery/titel/2098746

Yanar, Y., Yanar, D., Erdal, G., Erdal, H., & Yurttaş, F., (2017). Plant Protection Issues Encountered in Vineyards of Manisa Province and Producer Consciousness Level. *Turkish Journal of Weed Science, 20*(1), 18-26.

Yıldırım, Z. (2016). *Determination of powdery mildew and downy mildew resistance in different grapevine genotypes via marker assisted selection cultivated in Black Sea and Marmara Region* (Master Thesis), Ankara University, Graduate School of Natural and Applied Sciences, Department of Horticultural Sciences, Ankara.

Yıldırım, Z., Atak, A., & Akkurt, M. (2019). Determination of downy and powdery mildew resistance of some *Vitis* spp. *Ciencia e Tecnica Vitivinicola*, *34*(1). 10.1051/ctv/20193401015

Zyprian, E., Eibach, R., & T¨opfer, R. (2003) Comparative molecular mapping in segregating populations of grapevine. *Acta Hortic, 603*, 73-77. 10.17660/ActaHortic.2003.603.5

Zyprian, E., Ochßner, I., Schwander, F., Šimon, S., Hausmann, L., Bonow-Rex, M., ... & Töpfer, R. (2016). Quantitative trait loci affecting pathogen resistance and ripening of grapevines. *Molecular Genetics and Genomics*, 291(4), 1573-1594. https://doi.org/10.1007/s00438-016-1200-5