Marker assisted selection (MAS) for downy mildew resistance in grapevines using Rpv3.1 associated markers

Murat AKKURT1*, Irem ŞENSES1, Burak AKTÜRK2, Ilhami TOZLU2, Nuray ÖZER3, Halil Ibrahim UZUN2

1Ankara University, Faculty of Agriculture, Department of Horticulture, Ankara, Turkey; akkurt@agri.ankara.edu.tr
2Akdeniz University, Faculty of Agriculture, Department of Horticulture, Antalya, Turkey; akturkbrk@gmail.com; itozlu@akdeniz.edu.tr; uzun@akdeniz.edu.tr
3Namık Kemal University, Faculty of Agriculture, Department of Plant Protection, Tekirdag, Turkey; nurayozcer62@gmail.com

Abstract

Powdery mildew and downy mildew are primary fungal diseases that cause significant damage in viticulture. Therefore, breeding powdery and/or downy mildew resistance is one of the priority subjects in grapevine breeding programs. This study aims to conduct early-selection by marker assisted selection (MAS) method among 869 genotypes obtained through crossbreeding ‘Alphonse Lavallee’ × ‘Regent’ cultivars using the markers (GF18-06 and GF18-08) associated with downy mildew resistance gene region Rpv3.1 to develop new grapevine cultivars resistant to downy mildew caused by Plasmopara viticola. A total of 869 hybrid plants which were obtained after crossing ‘Alphonse Lavallee’ × ‘Regent’ in a 3-year breeding program were used in the study. The hybrid plants were scored for the resistance level based on their sporulation intensity after artificial inoculation of P. viticola. DNA samples of the hybrid plants were amplified with GF18-06 and GF18-08 markers in Polymerase Chain Reaction (PCR) for MAS. The alleles which were associated to Rpv3.1 resistance locus and the results of resistance scoring were compared, and the applicability of the markers in MAS was verified. It was determined that the GF18-08/410 bp marker can be used successfully for MAS. Gf 18-06 marker 385 bp, 390 bp and 407 bp gave false positive results in our population, respectively 8.86%, 9.02% and 37.94%. Therefore, this may limit its use for MAS.

Keywords: GF 18-08; GF 18-06; grapevine; marker assisted selection; Plasmopora viticola; Rpv3

Introduction

Grapevine (Vitis vinifera L.) is one of the most important perennial crops with high commercial value in the world. Fungal diseases are among the most important concerns of viticulture and cause high losses in vineyard yields. They may also affect the yield of the following year depending on the severity of the disease during the growing period. It may sometimes dry up all vineyard, which poses a great risk for the producer. Today, many fungicides are used quite successfully against fungal diseases. However, a great amount of fungicide is required to reduce the crop loss due to downy mildew disease in grapevine. More than 15 spraying
may be necessary per season in years of highly humid climatic conditions. The excessive pesticide uses increases production costs and, pollutes natural resources. The striking results of research on the amounts of fungicide in viticulture clearly present the significance of the studies for limiting the use of chemicals for sustainable viticulture (Wingerter et al., 2021). Merdinoglu et al. (2018) state that 33% of all the fungicide in Europe are used in vineyards. Data show that approximately 50% of all the fungicide used in European Union countries are actually being applied in vineyards which make up %5 of total farmland in EU (Zendler et al., 2017). On the other hand, EU directive 2009/128 for sustainability management of diseases caused by plant pathogens in the EU recommends a reduction in the number of fungicide treatments in the field (Sargolzaei et al., 2020).

In contrast to rapid increase in world population, the natural areas in cities are gradually decreasing which has led to an increase in people’s need for a healthy life. This is among the reasons why individuals’ eating habits are changing. Crops grown using minimal chemicals have become more popular than conventional farming, and consumers have come to prefer them more. Consumer preferences and the problems faced by growers have changed the direction of breeding studies in viticulture as in many agricultural branches and resulted in demands for products of the plants that are especially resistant or tolerant to diseases.

Although some Vitaceae species have developed resistance mechanisms against diseases caused by fungi, they do not seem to possess the level of quality that most consumers demand (Agurto et al., 2017). Therefore, the crossing is the first among the methods which are used to develop new grapevine cultivars with high quality as well as tolerance to disease and pests. However, classical plant breeding programs are long-term applications and they require years of observation on hybrid plants to see the targeted characteristics.

As a result of the inclusion of biotechnological methods in breeding programs, genetic analyzes related to disease resistance in grapes were carried out by different researchers, resistance-related gene regions have been identified. Molecular markers associated with these resistance-related gene regions have been developed (Akkurt et al., 2007; Welter et al., 2007; Hoffmann et al., 2008; Katula-Debrecheni et al., 2010; Riaz et al., 2011; Di Gaspero et al., 2012; Zyprian et al., 2016). Thanks to the inclusion of the MAS in breeding programs, breeding processes have been shortened, and hybrid plants that do not have the desired character or characteristics can be identified and removed from breeding programs without the need for phenotypic observations. In this way, land, labour, time, and costs in breeding programs have been greatly lowered.

The gene regions that provide resistance to fungal diseases in grapevine are called Rpv (Resistance to Plasmopora viticola) for downy mildew, Run (Resistance to Uncinula necator) and Ren (Resistance to Erysiphe necator) for powdery mildew. 28 gene regions associated with resistance to downy mildew (Plasmopara viticola) and 13 gene regions associated with resistance to powdery mildew (Erysiphe necator) in grapevine have been reported so far (Vitis International Variety Catalogue VIVC, 2022). It is also known that the majority of downy mildew resistant cultivars grown in Europe were developed from a single major resistance locus, Rpv3.1 (Eisenmann et al., 2019).

The cultivar ‘Regent’, which was bred as resistant to fungal diseases, was developed at “Institute for Grapevine Breeding Geilweilerhof” (Siebeldingen/Germany) in Germany in 1996 (Eibach and Töpfer, 2003). Rpv3.1 gene region, which is associated with downy mildew resistance, was first identified in the genetic map of the cultivar ‘Regent’ (Fischer et al., 2004; Welter et al., 2007) and was described in more detail in ‘Bianca’ (V. vinifera cv.) (Di Gaspero et al., 2012). Van Heerden et al., (2014) reported that some Run and Ren genes are located in the same region as Rpv3.1 gene. The resistance mechanism of ‘Regent’ to powdery mildew is based on a ‘post-contamination’ mechanism that restricts pathogen growth and inhibits conidium formation (Zendler et al., 2017). It is known that pathogen that cause fungal diseases in viticulture have different races as well. Therefore, resistance breeding studies aim to combine many gene regions (gene pyramiding) in order to stand against the aforementioned pathogen diversity (Zendler et al., 2017; Zini et al., 2019). It is seen that many recent breeding programs (Eibach et al., 2007; Schwander et al., 2012; Venuti et al., 2013) aim at pyramiding resistance gene regions and transferring these pyramided gene regions to new cultivars (Saifert et al., 2018). Therefore, cultivars such as ‘Regent’, which possess gene regions in their genomes associated with
resistance to different diseases and high-quality characteristics, have high significance and value for breeding studies as well as growers.

This study aims to conduct early-selection via marker assisted selection (MAS) among 869 genotypes obtained through crossing ‘Alphonse Lavalleé’ × ‘Regent’ cultivars using the markers (GF18-06 and GF18-08) associated with resistance gene region \( \text{Rpv3.1} \) to breed new grapevine cultivars resistant to downy mildew. Additionally, hybrid genotypes were scored based on disease severity after being infected with artificial inoculation. By comparing the markers associated with the \( \text{Rpv3.1} \) gene region (GF18-06; GF18-08) with the resistance levels, it was aimed to confirm the usability of these markers for MAS purposes in grape breeding studies for resistance to downy mildew disease.

**Materials and Methods**

**Plant material**

Eight hundred sixty-nine offspring from the crossing population of ‘Alphonse Lavalleé’ (susceptible \( V. \text{vinifera} \) cv) x ‘Regent’ (Interspecific hybrid, complex resistance against downy and powdery mildew) were used in this study.

**DNA isolation and PCR amplification**

Young leaf samples (0.5-1 g) were collected from each plant and kept at -80 °C until use. DNA isolations were performed according to the Plant/Fungi DNA Isolation Kit (Norgen Biotek Corp.) protocol using healthy young leaf samples. Genomic DNAs from the samples were run at 100 V for 1 hour with Agarose Gel Electrophoresis method and visualized by SynGene imaging system. The amount and purity of the DNA were measured with the Nano Drop ND-1000 Spectrophotometer. The final concentration was adjusted to 10 ng μl\(^{-1}\).

Two SSR markers, GF 18-06 and GF 18-08, which were developed from \( \text{Rpv3.1} \) resistant gene region, were used for PCR amplifications (Table 1). PCR was performed in a reaction volume of 10 μl containing 15 ng of DNA, 5 pmol of each forward and reverse primer, 0.5 mM of dNTP, 0.5 unit of GoTaq DNA Polymerase (Promega, Madison, WI) that included 1.5 mM of MgCl\(_2\). PCR amplifications were performed on a Biometra Uno Thermocycler (Biometra, Göttingen, Germany) programmed as follows: initial denaturation step at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 50-60 °C (depending on each primer pair-specific annealing temperature) for 1 min and at 72 °C for 2 min with a final extension at 72 °C for 10 min.

**Determination of fragment sizes**

Fragment sizes of PCR products were determined by Fragment Analyser (Advanced Analytical Technologies, Inc. IS, USA) and allele size of the obtained peak was determined by ProSize software (Data Analysis Software ver. 3.0.1.6).

**Artificial inoculation procedure and determination of resistance of individuals**

Artificial inoculation and disease evaluation were carried out as defined by Özer et al. (2021). An isolate of the pathogen was obtained from a single sporulating (oil spot) lesion on a leaf of cv. ‘Cabernet Sauvignon’, which is highly susceptible to downy mildew. Resistance level of each individual was scored according to Bosso et al. (2014).

**False positive/negative rate**

The resistant phenotype without the relevant allelic variant was considered false negative, and the susceptible phenotype with the relevant allelic variant was considered false positive. False negative and false
Positive detection rates were calculated as the ratio of the number of false positive or false negative samples to the total number of samples from the population.

**Results and Discussion**

In the present study, a total of 1050 hybrid plants were acquired after cross ‘Alphonse Lavallée’ × ‘Regent’ cultivars in the breeding studies to develop new grapevine cultivars resistant to mildew. Some of these plants dried during germination or transition to field and did not develop; therefore, artificial mildew inoculation tests were carried out on 869 hybrid plants which matured vigorously.

Resistance scores of the hybrid plants after inoculation tests were determined as defined by Boso et al., (2014). The resistance scoring defined 61 individuals as extremely resistant (7.01%), 43 as highly resistant (4.9%), 101 as resistant (11.62%), 186 as susceptible (21.40%), 96 as highly susceptible (11.04%), and 382 individuals as extremely susceptible (44.18%) (Özer et al., 2021). Sporulation area after artificial inoculation in ‘Alphonse Lavallée’, ‘Cabernet Sauvignon’ and hybrid genotypes are given in Figure 1. Susceptible cultivars exhibited dense and large sporulation area (Fig 1 A and B) in contrast to resistance genotypes with different levels (example genotypes number 340 and 534). These genotypes were extremely resistant (ER) since the pathogen could not grow on their leaves (Fig 1 C and D). The allele size of these genotypes was GF 18-08/410 bp which is evaluated as a more associated marker for downy mildew resistance (detailed data are given below).

![Figure 1](image)

DNA samples of the hybrid plants were amplified in PCR condition using GF 18-06 and GF 18-08 primers, and allele sizes were determined by ProSize software. The results were compared to confirm the relationship between the resistance scores of the offspring and the allele sizes of the genotypes.
The allele sizes of the genotypes, which were selected as resistant after the pathogen inoculation and the resistance levels of the genotypes (ER: extremely resistant; HR: highly resistant; R: resistant, respectively) are given in Table 1.

Using GF 18-06 marker, allele sizes were obtained as “385-390-407 bp” in the resistant parent ‘Regent’ carrying Rpv 3.1 gene region and as “396-417 bp” in the susceptible ‘Alphonse Lavalleé’. It may be seen that 57 genotypes which have 385 bp allele among ‘Regent’ alleles are defined to be susceptible to mildew at varying levels (8.86% false positive) when resistance levels are evaluated. 390-bp ‘Regent’ allele were defined in 58 susceptible genotypes (9.02% false positive) and 407-bp ‘Regent’ allele in 244 susceptible genotypes (37.94% false positive). No false negative results were detected (Table 1).

Zyprian et al. (2016) reported only paternal 387-bp allele associated to downy mildew resistance in QTL map acquired from ‘GF.GA-47-42’ (maternal genotype) x ‘Villard blanc’ (paternal genotype; Seibel 6468 × Seibel 6905; syn. ‘Subereux’) cross combination with GF 18-06 marker. Although 387 bp alleles were not detected in our research, it was evaluated that 390 bp and 385 bp ‘Regent’ alleles with low false-positive rates could be used in crossing populations for downy mildew resistant breeding.

The results show that the resistant parent ‘Regent’ gave allele sizes between “399-410 bp” and the susceptible parent ‘Alphonse Lavalleé’ between “406-417 bp” by using GF 18-08. Only 32 genotypes, which had 410 bp alleles with GF 18-08 marker, were scored as susceptible on the resistance scale (4.97% false positive) (Table 1). This result was interpreted as that GF 18-08 marker has a strong relationship with downy mildew resistance and would be more appropriate to be used for MAS. A total of 307 individuals with 399 bp alleles were determined as susceptible (47.74% false positive) after scoring and were not associated with downy mildew resistance.

Percentages of resistant genotypes determined by resistance-associated alleles are given in Figure 2. Out of a total of 145 genotypes that gave amplification product with the GF 18-08 marker, 113 were determined resistant as a result of resistance level. 17 of 74 genotypes (22.97%) with GF 18-06/385 bp allele and 68 of 126 genotypes (53.96%) with GF 18-06/390 bp allele were found to be resistant after inoculation. The results show that GF 18-08/410 bp ‘Regent’ allele is the strongly associated marker for downy mildew resistance with a 77.93% percentage. GF 18-06/390 bp allele was found to be the second associated marker with a 53.96% percentage. Other ‘Regent’ alleles were not evaluated to be associated with downy mildew resistance (Figure 2).
GF 18-06 and GF 18-08 SSR primers were developed from 12X reference map of the grapevine in associated with \textit{Rpv3.1} gene region (Schwander \textit{et al.}, 2012; Zyprian \textit{et al.}, 2016). \textit{Rpv3.1} locus in 'Regent' map (Welter \textit{et al.}, 2007) was verified in 'Regent' × 'Red Globe' genetic map developed by Van Heerden \textit{et al.}, (2014) (Zyprian \textit{et al.}, 2016). VMC7F2 SSR marker was mapped in the same region with GF18-08 on the chromosome 18 in the genetic map developed by Van Heerden \textit{et al.} (2014). GF18-08 marker was specifically redesigned for the sequence that encircles the same SSR region as VMC7F2 (Zyprian \textit{et al.}, 2016).

\textbf{Table 1.} Allele sizes for hybrid genotypes selected as resistant after artificial inoculation of \textit{P. viticola} in ‘Alphonse Lavalleé’ × ‘Regent’ population and their resistance levels

<table>
<thead>
<tr>
<th>Genotype No.</th>
<th>Alleles size</th>
<th>Resistance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>GF 18-06</td>
<td>GF 18-08</td>
<td></td>
</tr>
<tr>
<td>Regent</td>
<td>385-390-407</td>
<td>399-410</td>
</tr>
<tr>
<td>A. Lavalleé</td>
<td>396-417</td>
<td>406-417</td>
</tr>
<tr>
<td>1</td>
<td>407-417</td>
<td>406-410</td>
</tr>
<tr>
<td>2</td>
<td>407-417</td>
<td>399-406</td>
</tr>
<tr>
<td>4</td>
<td>390-407</td>
<td>399-410</td>
</tr>
<tr>
<td>7</td>
<td>390-396</td>
<td>399-410</td>
</tr>
<tr>
<td>8</td>
<td>396-407</td>
<td>399-410</td>
</tr>
<tr>
<td>14</td>
<td>396-407</td>
<td>410-410</td>
</tr>
<tr>
<td>18</td>
<td>390-407</td>
<td>406-410</td>
</tr>
<tr>
<td>37</td>
<td>407-417</td>
<td>399-410</td>
</tr>
<tr>
<td>39</td>
<td>407-417</td>
<td>406-410</td>
</tr>
<tr>
<td>52</td>
<td>396-407</td>
<td>399-406</td>
</tr>
<tr>
<td>56</td>
<td>390-410</td>
<td>410-410</td>
</tr>
<tr>
<td>60</td>
<td>396-407</td>
<td>399-410</td>
</tr>
<tr>
<td>64</td>
<td>390-396</td>
<td>406-410</td>
</tr>
<tr>
<td>68</td>
<td>390-396</td>
<td>399-410</td>
</tr>
<tr>
<td>80</td>
<td>396-410</td>
<td>399-406</td>
</tr>
<tr>
<td>81</td>
<td>390-396</td>
<td>406-410</td>
</tr>
<tr>
<td>89</td>
<td>390-407</td>
<td>399-410</td>
</tr>
<tr>
<td>98</td>
<td>396-407</td>
<td>399-410</td>
</tr>
<tr>
<td>100</td>
<td>407-417</td>
<td>399-406</td>
</tr>
<tr>
<td>104</td>
<td>407-417</td>
<td>399-406</td>
</tr>
<tr>
<td>105</td>
<td>390-396</td>
<td>410-396</td>
</tr>
<tr>
<td>120</td>
<td>390-396</td>
<td>399-406</td>
</tr>
<tr>
<td>126</td>
<td>390-396</td>
<td>399-406</td>
</tr>
<tr>
<td>132</td>
<td>390-396</td>
<td>410-396</td>
</tr>
<tr>
<td>140</td>
<td>407-417</td>
<td>399-410</td>
</tr>
<tr>
<td>148</td>
<td>396-407</td>
<td>399-410</td>
</tr>
<tr>
<td>149</td>
<td>407-417</td>
<td>406-410</td>
</tr>
<tr>
<td>158</td>
<td>407-417</td>
<td>399-417</td>
</tr>
<tr>
<td>161</td>
<td>407-417</td>
<td>399-406</td>
</tr>
<tr>
<td>164</td>
<td>385-390</td>
<td>399-406</td>
</tr>
<tr>
<td>168</td>
<td>396-407</td>
<td>399-410</td>
</tr>
<tr>
<td>170</td>
<td>390-407</td>
<td>399-410</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>171</td>
<td>396-417</td>
<td>390-410</td>
</tr>
<tr>
<td>175</td>
<td>407-417</td>
<td>410-410</td>
</tr>
<tr>
<td>178</td>
<td>390-410</td>
<td>399-410</td>
</tr>
<tr>
<td>180</td>
<td>390-396</td>
<td>399-410</td>
</tr>
<tr>
<td>183</td>
<td>390-396</td>
<td>406-410</td>
</tr>
<tr>
<td>187</td>
<td>390-410</td>
<td>399-410</td>
</tr>
<tr>
<td>188</td>
<td>396-407</td>
<td>399-410</td>
</tr>
<tr>
<td>189</td>
<td>396-407</td>
<td>399-410</td>
</tr>
<tr>
<td>199</td>
<td>396-407</td>
<td>406-410</td>
</tr>
<tr>
<td>205</td>
<td>390-396</td>
<td>-</td>
</tr>
<tr>
<td>210</td>
<td>396-407</td>
<td>399-406</td>
</tr>
<tr>
<td>211</td>
<td>390-407</td>
<td>399-410</td>
</tr>
<tr>
<td>215</td>
<td>390-407</td>
<td>399-410</td>
</tr>
<tr>
<td>217</td>
<td>390-407</td>
<td>399-410</td>
</tr>
<tr>
<td>219</td>
<td>390-396</td>
<td>399-410</td>
</tr>
<tr>
<td>223</td>
<td>396-407</td>
<td>406-410</td>
</tr>
<tr>
<td>225</td>
<td>407-417</td>
<td>399-410</td>
</tr>
<tr>
<td>229</td>
<td>396-407</td>
<td>406-410</td>
</tr>
<tr>
<td>234</td>
<td>390-407</td>
<td>399-410</td>
</tr>
<tr>
<td>236</td>
<td>390-407</td>
<td>399-410</td>
</tr>
<tr>
<td>238</td>
<td>390-396</td>
<td>399-410</td>
</tr>
<tr>
<td>241</td>
<td>407-417</td>
<td>399-410</td>
</tr>
<tr>
<td>245</td>
<td>396-407</td>
<td>-</td>
</tr>
<tr>
<td>249</td>
<td>396-407</td>
<td>399-410</td>
</tr>
<tr>
<td>254</td>
<td>396-407</td>
<td>399-406</td>
</tr>
<tr>
<td>258</td>
<td>390-407</td>
<td>399-410</td>
</tr>
<tr>
<td>261</td>
<td>390-396</td>
<td>406-410</td>
</tr>
<tr>
<td>263</td>
<td>390-396</td>
<td>399-410</td>
</tr>
<tr>
<td>264</td>
<td>390-396</td>
<td>406-410</td>
</tr>
<tr>
<td>266</td>
<td>390-396</td>
<td>410-410</td>
</tr>
<tr>
<td>267</td>
<td>396-407</td>
<td>406-410</td>
</tr>
<tr>
<td>269</td>
<td>407-417</td>
<td>399-410</td>
</tr>
<tr>
<td>271</td>
<td>407-417</td>
<td>399-417</td>
</tr>
<tr>
<td>277</td>
<td>407-417</td>
<td>399-410</td>
</tr>
<tr>
<td>281</td>
<td>396-407</td>
<td>399-410</td>
</tr>
<tr>
<td>283</td>
<td>396-407</td>
<td>399-410</td>
</tr>
<tr>
<td>288</td>
<td>396-407</td>
<td>399-406</td>
</tr>
<tr>
<td>289</td>
<td>396-407</td>
<td>399-406</td>
</tr>
<tr>
<td>290</td>
<td>390-396</td>
<td>399-410</td>
</tr>
<tr>
<td>291</td>
<td>390-407</td>
<td>410-410</td>
</tr>
<tr>
<td>301</td>
<td>396-407</td>
<td>399-406</td>
</tr>
<tr>
<td>307</td>
<td>390-396</td>
<td>406-410</td>
</tr>
<tr>
<td>309</td>
<td>385-390</td>
<td>406-410</td>
</tr>
<tr>
<td>315</td>
<td>385-390</td>
<td>410-417</td>
</tr>
<tr>
<td>317</td>
<td>396-407</td>
<td>399-406</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>319</td>
<td>390-396</td>
<td>406-410</td>
</tr>
<tr>
<td>321</td>
<td>385-390</td>
<td>406-410</td>
</tr>
<tr>
<td>322</td>
<td>390-407</td>
<td>399-417</td>
</tr>
<tr>
<td>323</td>
<td>385-390</td>
<td>406-?</td>
</tr>
<tr>
<td>338</td>
<td>407-417</td>
<td>399-406</td>
</tr>
<tr>
<td>340</td>
<td>407-413</td>
<td>399-406</td>
</tr>
<tr>
<td>341</td>
<td>-407</td>
<td>399-406</td>
</tr>
<tr>
<td>342</td>
<td>-407</td>
<td>406-?</td>
</tr>
<tr>
<td>344</td>
<td>407-417</td>
<td>399-410</td>
</tr>
<tr>
<td>345</td>
<td>407-413</td>
<td>399-406</td>
</tr>
<tr>
<td>349</td>
<td>390-407</td>
<td>399-406</td>
</tr>
<tr>
<td>352</td>
<td>396-407</td>
<td>399-406</td>
</tr>
<tr>
<td>353</td>
<td>390-396</td>
<td>399-410</td>
</tr>
<tr>
<td>355</td>
<td>390-396</td>
<td>399-410</td>
</tr>
<tr>
<td>359</td>
<td>396-407</td>
<td>406-410</td>
</tr>
<tr>
<td>370</td>
<td>407-417</td>
<td>399-406</td>
</tr>
<tr>
<td>372</td>
<td>390-390</td>
<td>399-410</td>
</tr>
<tr>
<td>379</td>
<td>407-417</td>
<td>399-410</td>
</tr>
<tr>
<td>387</td>
<td>396-417</td>
<td>399-406</td>
</tr>
<tr>
<td>392</td>
<td>407-417</td>
<td>399-410</td>
</tr>
<tr>
<td>399</td>
<td>396-407</td>
<td>399-417</td>
</tr>
<tr>
<td>401</td>
<td>390-396</td>
<td>399-417</td>
</tr>
<tr>
<td>409</td>
<td>385-390</td>
<td>399-410</td>
</tr>
<tr>
<td>410</td>
<td>396-407</td>
<td>406-410</td>
</tr>
<tr>
<td>412</td>
<td>385-396</td>
<td>399-417</td>
</tr>
<tr>
<td>417</td>
<td>407-417</td>
<td>399-406</td>
</tr>
<tr>
<td>420</td>
<td>407-417</td>
<td>399-399</td>
</tr>
<tr>
<td>423</td>
<td>-407</td>
<td>399-410</td>
</tr>
<tr>
<td>428</td>
<td>407-417</td>
<td>-</td>
</tr>
<tr>
<td>430</td>
<td>396-407</td>
<td>410-</td>
</tr>
<tr>
<td>431</td>
<td>390-396</td>
<td>399-410</td>
</tr>
<tr>
<td>434</td>
<td>407-417</td>
<td>399-399</td>
</tr>
<tr>
<td>435</td>
<td>396-407</td>
<td>399-410</td>
</tr>
<tr>
<td>436</td>
<td>407-417</td>
<td>399-410</td>
</tr>
<tr>
<td>437</td>
<td>396-407</td>
<td>399-410</td>
</tr>
<tr>
<td>439</td>
<td>407-417</td>
<td>410-417</td>
</tr>
<tr>
<td>441</td>
<td>407-417</td>
<td>410-</td>
</tr>
<tr>
<td>442</td>
<td>407-417</td>
<td>399-410</td>
</tr>
<tr>
<td>443</td>
<td>385-396</td>
<td>410-</td>
</tr>
<tr>
<td>444</td>
<td>396-407</td>
<td>406-?</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>446</td>
<td>385-390</td>
<td>399-</td>
</tr>
<tr>
<td>449</td>
<td>407-417</td>
<td>399-410</td>
</tr>
<tr>
<td>452</td>
<td>385-390</td>
<td>399-406</td>
</tr>
<tr>
<td>457</td>
<td>385-396</td>
<td>410-</td>
</tr>
<tr>
<td>459</td>
<td>385-390</td>
<td>406-?</td>
</tr>
<tr>
<td>461</td>
<td>407-417</td>
<td>399-406</td>
</tr>
<tr>
<td>463</td>
<td>396-407</td>
<td>399-410</td>
</tr>
<tr>
<td>469</td>
<td>407-417</td>
<td>399-417</td>
</tr>
<tr>
<td>470</td>
<td>407-413</td>
<td>399-406</td>
</tr>
<tr>
<td>472</td>
<td>385-390</td>
<td>399-410</td>
</tr>
<tr>
<td>473</td>
<td>390-407</td>
<td>410-</td>
</tr>
<tr>
<td>474</td>
<td>390-396</td>
<td>399-410</td>
</tr>
<tr>
<td>475</td>
<td>396-417</td>
<td>399-410</td>
</tr>
<tr>
<td>480</td>
<td>396-413</td>
<td>399-406</td>
</tr>
<tr>
<td>481</td>
<td>390-396</td>
<td>399-406</td>
</tr>
<tr>
<td>483</td>
<td>396-407</td>
<td>399-410</td>
</tr>
<tr>
<td>485</td>
<td>407-417</td>
<td>406-?</td>
</tr>
<tr>
<td>492</td>
<td>407-417</td>
<td>399-410</td>
</tr>
<tr>
<td>495</td>
<td>385-390</td>
<td>399-406</td>
</tr>
<tr>
<td>497</td>
<td>390-396</td>
<td>399-406</td>
</tr>
<tr>
<td>500</td>
<td>407-417</td>
<td>399-410</td>
</tr>
<tr>
<td>505</td>
<td>396-407</td>
<td>399-406</td>
</tr>
<tr>
<td>507</td>
<td>396-407</td>
<td>399-410</td>
</tr>
<tr>
<td>515</td>
<td>396-407</td>
<td>410-</td>
</tr>
<tr>
<td>519</td>
<td>396-407</td>
<td>406-410</td>
</tr>
<tr>
<td>522</td>
<td>396-407</td>
<td>399-</td>
</tr>
<tr>
<td>524</td>
<td>407-417</td>
<td>399-406</td>
</tr>
<tr>
<td>532</td>
<td>396-407</td>
<td>406-410</td>
</tr>
<tr>
<td>534</td>
<td>384-390</td>
<td>410-</td>
</tr>
<tr>
<td>535</td>
<td>390-407</td>
<td>399-417</td>
</tr>
<tr>
<td>538</td>
<td>396-407</td>
<td>399-406</td>
</tr>
<tr>
<td>539</td>
<td>396-407</td>
<td>399-406</td>
</tr>
<tr>
<td>542</td>
<td>396-407</td>
<td>-</td>
</tr>
<tr>
<td>543</td>
<td>396-407</td>
<td>399-410</td>
</tr>
<tr>
<td>547</td>
<td>390-396</td>
<td>399-410</td>
</tr>
<tr>
<td>549</td>
<td>385-390</td>
<td>399-410</td>
</tr>
<tr>
<td>553</td>
<td>390-407</td>
<td>410-</td>
</tr>
<tr>
<td>558</td>
<td>407-417</td>
<td>399-410</td>
</tr>
<tr>
<td>560</td>
<td>390-407</td>
<td>399-410</td>
</tr>
<tr>
<td>568</td>
<td>396-407</td>
<td>-</td>
</tr>
<tr>
<td>570</td>
<td>396-407</td>
<td>399-417</td>
</tr>
<tr>
<td>571</td>
<td>396-407</td>
<td>399-406</td>
</tr>
<tr>
<td>572</td>
<td>396-407</td>
<td>410-417</td>
</tr>
<tr>
<td>573</td>
<td>417</td>
<td>410-</td>
</tr>
<tr>
<td>574</td>
<td>407-417</td>
<td>410-417</td>
</tr>
</tbody>
</table>
Zyprian et al. (2016) found the allele with “399 bp” size as associated with resistance using the GF 18-08 marker. “393-399 bp” allele distribution was acquired in ‘Regent’ with GF 18-08 primer. The alleles for downy mildew resistant/tolerant hybrids are ‘Villard Blanc’ (383-399 bp), Seibel 6468 (387-399 bp), Suberux (383-399 bp), and GF.GA 47-42 (389-392 bp). In the present study, ‘Regent’ gave amplification product with “399-410 bp” size. However, 399 bp ‘Regent’ allele, in contrast to the findings above, was not associated with resistance while 410 bp allele was found to be highly related to downy mildew resistance. It is thought that this difference may be due to the possible differences encountered during the determination of fragment sizes with fragment analyzers. Therefore, it is highly likely that 399 bp allele associated with resistance in the study by Zyprian et al., (2016) corresponds to 410 bp allele in the present study.

The application of MAS in resistance breeding studies provides researchers with useful perspectives from many different aspects. Genotyping genetic resources with molecular markers related to important traits enables the identification of optimized crossover combinations. For instance, MAS may allow target parent selection with the potential to combine different resistance loci in offspring to increase resistance level and sustainability of resistance with regard to resistance to downy mildew (Eibach and Töpfer, 2015). However,
phenotyping remains essential to characterize host-pathogen interaction and to evaluate the effective resistance level of new varieties (Possamai et al., 2020).

On the other hand, it is crucial for early selection with MAS to be effective to select molecular markers that are closely associated with the characteristics examined in the selected parents in hybridization combinations. Kuchel et al. (2007) stated that the frequency of Lr34/Yr18 rust-resistance genes increased from 0.25 to 0.60 with MAS in BC1 wheat population, reported, however, the increase as from 0.25 to only 0.27, and claimed that it resulted from the actually poor relationship between Lr34/Yr18 genes and the markers used in the scanning of BC1 plants (Bernardo, 2008). Similarly, Yıldırım et al. (2019) determined that some grapevine genotypes collected from the Black Sea coast with high humidity and precipitation, which they determined to have resistance-associated alleles as a result of MAS, showed intense mildew symptoms after artificial inoculation. Researchers point out the importance of validating the results of the marker used for MAS with the results of artificial/natural inoculation. Therefore, it is extremely important to validate the selected molecular markers for MAS in different genotypic sources.

Conclusions

The development of new downy mildew resistant cultivars through conventional breeding provides an effective solution for disease management. In such studies, MAS provides rapid and cost-effective genotyping methods. In the present study, it was determined that 385 bp and 390 bp alleles which were acquired with GF18-06 marker associated with Rpv3.1 gene region resulted in 8.86% and 9.02% false positives, respectively, and that 410 bp allele among ‘Regent’ alleles acquired with GF 18-08 marker had high correlation with downy mildew resistance based on resistance scoring results. Therefore, GF 18-08 marker is recommended for MAS in downy mildew resistant grape breeding studies.

Authors’ Contributions

Conceptualization: MA and İŞ; Data curation: MA and BA; Formal analysis: İS and NÖ; Methodology: MA, İŞ, BA, NÖ; Supervision: HİU; Validation: İS and NÖ; Writing – original draft: MA and İŞ; Writing – review & editing: NÖ and HİU.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This work was supported by the National Scientific and Technological Research Council of Turkey (grant number 1150176).

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.
References


Vitis International Variety Catalogue VIVC. Loci for Traits in Grapevine Relevant for Breeding and Genetics. Retrieved 17.01.2022 from: [https://www.vivc.de](https://www.vivc.de)


