Research Article

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Response of hybrid grapes (*Vitis* spp.) to two biotic stress factors and their seedlessness status

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Abstract: Grape varieties belonging to the *Vitis vinifera* species are widely grown worldwide, and many of these are susceptible to powdery mildew and downy mildew. However, wild *Vitis* species are pretty resistant to these diseases. Hybrid genotypes that are resistant or tolerant to diseases can be produced through crossbreeding studies between *V. vinifera* and other *Vitis* species. Today, the demand for new table grape varieties, both seedless and disease-resistant or tolerant, has increased. In our study, we scored the resistance of F1 hybrid grape genotypes developed through crossbreeding with different *Vitis* species to powdery mildew (*Erysiphe necator*) and downy mildew (*Plasmopara viticola*) after natural inoculation. Furthermore, both seedless and disease-resistant genotypes were identified using marker-assisted selection (MAS). Disease scoring of 470 hybrid grape genotypes revealed that 31 were highly resistant to both powdery mildew and downy mildew. To determine seedlessness, 351 genotypes were screened using the 5U_VviAGL11 SSR marker using MAS, resulting in 136 potential seedless genotypes. Among these possible seedless hybrid grape genotypes, 77 genotypes were determined to be very resistant or resistant to both powdery mildew and downy mildew. The fruit characteristics of these 77 hybrid genotypes will be evaluated over the next few years, and they will also be utilized in future breeding studies to develop new varieties suitable for a sustainable viticulture model.

Keywords: MAS; powdery mildew; downy mildew; seedless; resistance; table grape

1 Introduction

Türkiye's location is in a very suitable climate zone for grapevine cultivation, and the history of viticulture dates back to ancient times. In addition, different institutions in Türkiye have conducted breeding studies for many years, and many new grape varieties have been developed as a result of these studies [1]. In particular, recent breeding studies conducted for table purposes have focused on developing new seedless grape varieties that are tolerant or resistant to fungal diseases, in response to consumer demands [2]. Growing table grapes in humid regions is not easy due to the damage caused by fungal diseases, such as powdery mildew and downy mildew. It is generally considered that downy mildew (DM) develops mainly in wet periods [3], whereas powdery mildew (PM) grows in dry periods [4]. Due to these diseases, a large number of fungicides are used on grapes throughout the year. This not only poses a significant threat to human and environmental health but also significantly increases production costs during the growing season [5]. In addition, in parallel with climate change resulting from increasing temperatures, researchers are trying to develop new table grape varieties that are more resistant or tolerant to diseases [6].

While the results of breeding studies in viticulture are typically obtained over many years, the desired outcomes can be achieved in a shorter timeframe with the use of newly developed molecular methods and marker-assisted selection (MAS) [7]. The hybrid population is screened with markers that determine seedlessness, and seedless genotypes are selected; seeded ones are not planted unnecessarily in the experimental plot [8].

Researchers have developed a variety of molecular markers to identify seedlessness in grapes [8–11] random amplified polymorphic DNA (RAPD) markers, sequence

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characterized amplified region (SCAR) markers, and, most recently, simple sequence repeats (SSR) markers. Two SCAR markers (SCC8-1080 and SCF27-2000) were developed through bulk segregant analysis (BSA) by associating the imbalance with a seed development inhibitor (SDI), and they were found to be suitable for populations in which both parents were seedless [12, 13]. Mejia et al. [14] reported that short 'insertions' and 'deletions' (INDELs) suppressed the expression of the VviAGL11 gene, and they reported that the VviAGL11 gene may be responsible for seedlessness.

Hybridization between *V. labrusca* and *Vitis vinifera* increases resistance to fungal pathogens without compromising seedlessness traits. We hypothesized that cross-breeding *Vitis* species with known resistance loci can yield disease-resistant, seedless table grape genotypes, enabling reduced pesticide use and increased sustainability in humid climates. This study assessed the resistance of 470 hybrid genotypes derived from a table grape breeding program to powdery mildew and downy mildew diseases. Additionally, the widely known 5U_VviAGL11 SSR marker was used to determine the seedlessness status of the hybrid genotypes.

2 Materials and methods

2.1 Plant materials and trial area

The trial area is in Yalova Atatürk Horticultural Central Research Institute (YAHCRI) in the centre of Yalova Province. Its coordinates are 40° 39 min 40 s North latitude and 29° 17 min 22 s East longitude; the altitude is 3 m, and the distance to the sea is approximately 150 m. Important climate data for the long-term and trial years of the nursery area where hybrid genotypes are located are given in Figure 1. The hybrid genotypes used in this study were obtained from cross-breeding studies between different *Vitis* species. The origins of the parents used in the cross-breeding studies are briefly as follows;

K-77 (*V. labrusca*): This genotype was taken from a vineyard near Ankara by YAHCRI during a selection study in 2017 and grafted into the genetic resources parcel.

Bronx Seedless (Interspecific Cross) (Goff X Iona) X Sultana): The Bronx Seedless variety was developed in 1925 by the New York State Agricultural Experiment Station and New York Botanical Garden.

Beyaz Çavuş (*V. vinifera*): This variety is grown in different provinces of Turkey. A clone selection study was conducted by YAHCRI in 1997 for this variety, and three clones were selected. Clone No. 13 was used in this study.

Crimson Seedless (*V. vinifera*): This variety was selected in 1983 by David Ramming as a result of breeding work done at the Horticultural Field Station USDA/ARS.

Red Globe (*V. vinifera*): This variety was selected and registered in 1958 as a result of the breeding work done by Olmo et al. at the California Agricultural Experiment Station, University of California.

Yalova Seedless (*V. vinifera*) (Beyrut Hurması × Perlette): This variety was selected and registered as a result of the breeding work carried out in YAHCRI in 1990.

86/1 (*V. vinifera*) (Hafızali X Muscat Reine des Vignes): This hybrid genotype was obtained due to crossbreeding studies conducted by YAHCRI, but it was not registered due to its female flower structure. It was protected in the genetic resources parcel of the same institute and used as a parent in subsequent breeding studies.

Ruby Seedless (*V. vinifera*) (Emperor x Sultana Moscata): This variety was selected and registered in 1950 as a result of the breeding work done by Olmo at the California Agricultural Experiment Station, University of California.

More information and photos of the parents are given in Table 1 and Figure 2. These F1 hybrid grape genotypes constituted the primary material of the study. Following the experiment, specific coding was performed for each F1 hybrid individual obtained from the combinations. While the letter used in the coding represents the year of cross-breeding, the first number on the right side indicates the combination number, and the rightmost number indicates the genotype number. The seeds extracted from the hybrid grapes during harvest were germinated in the greenhouse the following spring, and F1 hybrid grape seedlings were obtained. Cross-breeding, disease scoring, DNA isolation, and PCR were performed in a nursery, an experimental greenhouse, and a biotechnology laboratory located at the Yalova Atatürk Horticulture Central Research Institute. Capillary electrophoresis was performed in the molecular genetic laboratory of the Horticulture Department of Ankara University.

2.2 Scoring the resistance of F1 hybrid grape genotypes to powdery mildew (*Erysiphe necator*) and downy mildew (*Plasmopara viticola*) diseases

This study consists of two main stages. The first part involved scoring the resistance of F1 hybrid grape genotypes to powdery mildew (*E. necator*) and downy mildew (*P. viticola*), and the second part involved determining the resistance of

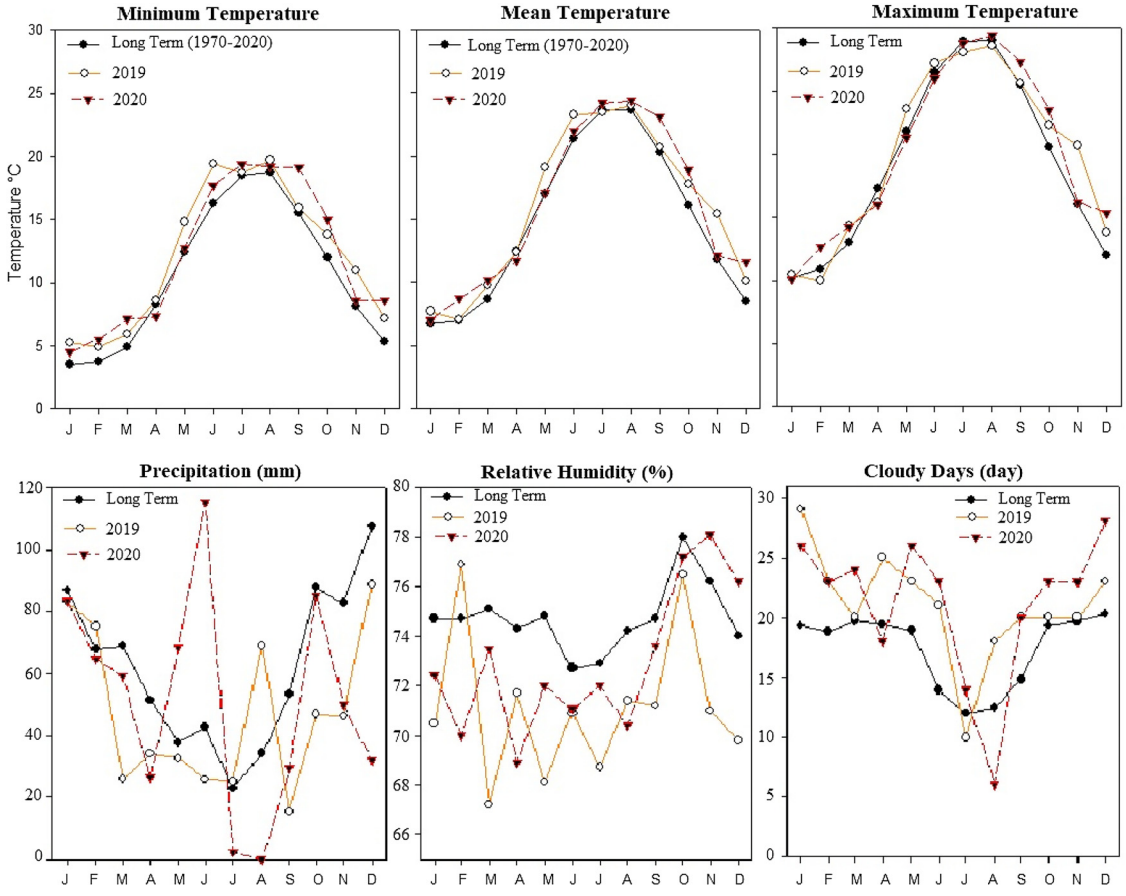


Figure 1: Important climate data for long term and trial years of the nursery area where hybrid genotypes are located.

similar genotypes to seedless genotypes via marker-assisted selection (MAS).

The parents used in the study consist of interspecific hybrids, *V. labrusca* and *V. vinifera*. Among these, the Ren2, Ren3, Ren9 and Ren10 regions of the powdery mildew disease resistance gene loci of interspecific hybrids; *V. vinifera* varieties may contain the Ren1, Ren1.2, Ren 14 and Ren 15 loci. In terms of resistance to Downy mildew disease, interspecific hybrids from parents Rpv4, Rpv7, Rpv11, Rpv17, Rpv18, Rpv20 and Rpv21; *V. vinifera* Rpv28, Rpv29, Rpv30, Rpv31, Rpv36, Rpv37 and finally *V. labrusca* varieties may contain Rpv3.2 loci [1]. F1 hybrid grapevines rooted in pots were assessed to determine the resistant level of the genotypes to powdery mildew and mildew diseases. The disease source grapevine plants were grown in the greenhouse to prevent early transmission of the disease and were placed at equal intervals within the F1 population in the last week of May for downy mildew and in the second week of June for powdery mildew. The distance between diseased plants and F1 hybrid plants varies between 30 cm and 200 cm. In order to ensure equal disease transmission, artificial air movement was created with fans and all hybrid plants were infected equally.

As a result of many years of observations, the resistance status of the parents used in the study against both diseases is as follows; K-77 is resistant to both diseases because it belongs to the *V. labrusca* species. Bronx seedless, an interspecific hybrid, is similarly resistant to both diseases. Other varieties belong to the *V. vinifera* species, of which Red Globe, Beyaz Çavuş, 86/1 and Crimson Seedless are more tolerant to downy mildew than other varieties. In terms of powdery mildew, Red Globe and Crimson Seedless are tolerant, while other *V. vinifera* varieties are sensitive [5, 15].

Following the development of 10 leaves on the shoots, these grapevines were placed in natural environments close to the diseased grapevines on the grape plots of the institute, and they were infected with powdery mildew and downy mildew by the natural inoculation method. At this stage, no fungicide was applied to the grape plants in the main plot or the F1 plants in the pot. Powdery and downy mildew infections on the leaves were examined and scored on the dates when the infection was most intense.

Table 1: Characteristics of the parents of the hybrid genotypes used in the study.

Combination code	Mother (♀) parent	Pollinator (♂) parent
B-5	**K-77 (*V. labrusca*)** This grape genotype was selected by YAHCRI as a result of selection breeding. It has berries that are resistant to fungal diseases, black in colour, and have seeds and an intense foxy flavour.	**Bronx seedless (interspecific cross)** (Goff X iona) X sultana) It has foxy-flavoured, red and seedless fruits.
B-10	**Beyaz Çavuş (*V. vinifera*)** It has a female flower structure, yellowish green colour, slightly oval, and large berries with 1–2 seeds. It has a flavour specific to the variety.	**Crimson seedless (*V. vinifera*)** Its berries are elliptical, red, seedless, crunchy fleshy and large (3.7 g). It harvests in the middle and late period.
B-16	**Red globe (*V. vinifera*)** The berry characteristics are round/slightly elliptical, pinkish red and very large berries (12–14 gr).	**Yalova seedless (*V. vinifera*)** It was registered in Yalova ABKMAE in 1990. The berry is oval, white, large (4–5 gr.), seedless and thin-skinned.
B-20	**86/1 (*V. vinifera*)** (Hafızali X Muscat Reine des Vignes) It has a female flower structure, yellowish green colour, slightly oval, and middle size berries with 1–2 seeds also it has intense muscat flavour	**Bronx seedless (interspecific cross)** (Goff X iona) X sultana)
B-21	**86/1 (*V. vinifera*)** (Hafızali X Muscat Reine des Vignes)	**Ruby seedless (*V. vinifera*)** (Emperor x sultana Moscata) It has red, large and seedless berries.

Variety name of the parents are in bold.

Downy mildew scoring was performed on 21 June, and powdery mildew scoring was performed on 10 August. Powdery and downy mildew infections in the genotypes were detected between the fourth and eighth leaves from the tip of each young hybrid grapevine plant. The coverage area of the infections was determined by calculating the percentage of disease lesions observed in the entire leaf area according to the procedure of OIV-452, 455, and 456 [16]. Tables 2 and 3 show how downy and powdery mildew scoring was performed. Additionally, examples of hybrid genotypes scored for powdery mildew and downy mildew are given in Figure 3.

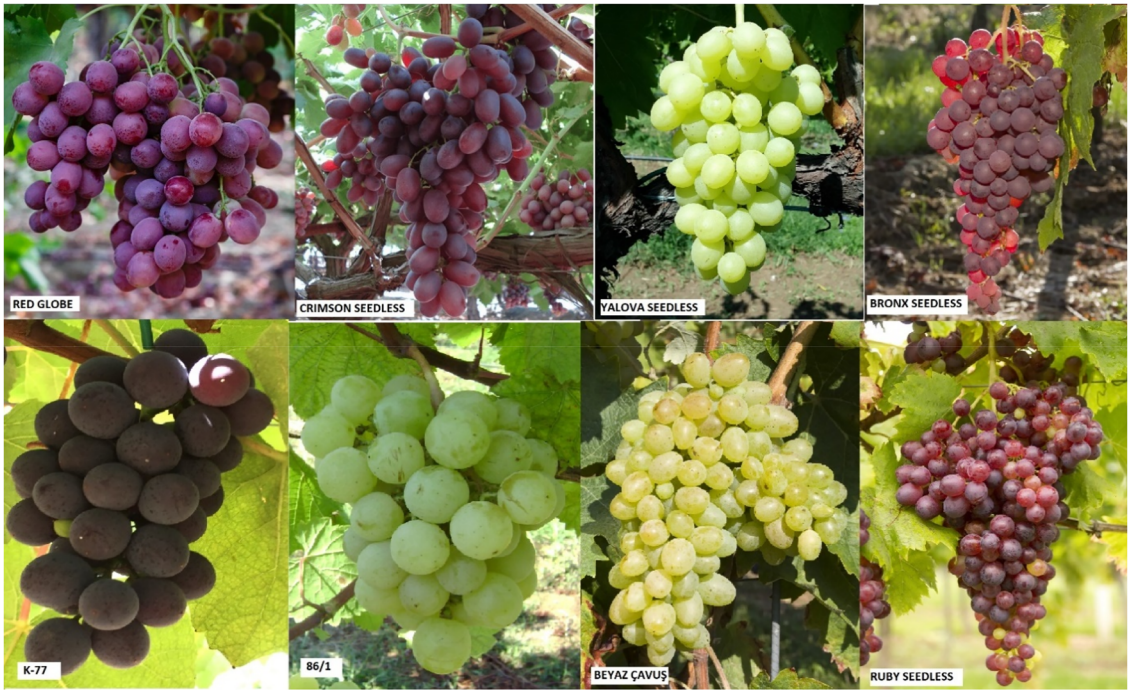


Figure 2: Photos of parents used in cross-breeding studies.

Table 2: Powdery mildew infection scoring and disease resistance definitions of genotypes under the same conditions.

Score	Symptoms	Description
1	Very low (tiny spots or no symptoms; neither visible sporulation nor mycelium)	Very resistant
3	Low (limited patches <2 cm diameter; limited sporulation and mycelium; the presence of *Erysiphe* is only indicated by a slight curling of the blade)	Resistant
5	Medium (patches usually limited to a diameter of 2–5 cm)	Tolerant
7	High (vast patches; some limited; strong sporulation and abundant mycelium)	Susceptible
9	Very high (very vast, unlimited patches or totally attached leaf blades; strong sporulation and abundant mycelium)	Very susceptible

Table 3: Downy mildew infection scoring and disease resistance definitions of genotypes under the same conditions.

Score	Symptoms	Description
1	Very low (tiny necrotic spots or no symptoms; neither sporulation nor mycelium)	Very resistant
3	Low (small patches <1 cm in diameter; little sporulation or mycelium)	Resistant
5	Medium (little patches 1–2 cm in diameter; more or less strong sporulation; irregular formation of mycelium)	Tolerant
7	High (vast patches; strong sporulation and abundant mycelium; leaf drop later than below)	Susceptible
9	Very high (vast patches or totally attached leaf blades; strong sporulation and dense mycelium; very early leaf drop)	Very susceptible

2.3 Determination of seedless genotypes with MAS

For MAS analyses, 100 mg of fresh leaf samples of each genotype were taken, placed in a 2.0 mL high-pressure resistant plastic tube with a metal ball and then immersed in liquid nitrogen for approximately 30 s. After removal from liquid nitrogen, the samples were shredded using a Qiagen Tissuelyser-II shredder until they turned into powder. Using the Qiagen DNeasy Plant Mini Kit, DNA was extracted from the powdered samples.

A Nanodrop spectrophotometer (Implen N-60 Touch) and 1 % agarose gel electrophoresis were used to measure the quantity and quality of the DNA. The DNA isolation processes for genotypes with insufficient DNA quantity and purity were repeated. All DNA was diluted to a final volume of 10 ng/µl. The 5U_VviAGL11 SSR marker was used to determine possible seedless genotypes among the isolated DNA samples. The forward and reverse sequences of this marker are as follows [17].

Forward primer: 5′-CGC CCATTC TCT CTC GCT AT-3′

Reverse primer: 5′-GTG CAA AAA CGC GTA TCC CA-3′

The following mixture was made for the PCR procedure using this primer: 100 ng of DNA, 10 pmol of forward primer, 10 pmol of reverse primer, 12.5 µL of master mix, and a final volume of 25 µl of PCR water. With the mixture prepared in this way, the following program in the Bio-Rad T-100 Thermal Cycler device was used: 3 min at 94 °C, 1 min at 94 °C, 1 min at 55 °C, 2 min at 72 °C, and finally 10 min at 72 °C (35 cycles for steps 2, 3, and 4).

The amplification of PCR products was checked by loading 10 µL of each sample on a 2 % agarose gel. Gel images were taken with a Vilber E-Box imaging system. A 10 µL DNA Ladder (50–1000 bp) was loaded into the first well of each gel, and band sizes were determined with the help of this ladder. For samples that did not work, the PCR process was repeated in the same way. The exact band sizes of the PCR products were determined by capillary electrophoresis in a DNA fragment analysis system.

Peak images were obtained with a capillary electrophoresis (Advanced Analytic Fragment Analyser) system at the Molecular Genetics Laboratory of Ankara University Department of Horticulture. Then, ProSize software was used to determine allele sizes in the peaks.

3 Results

3.1 Scoring of F1 hybrid genotypes for powdery mildew and mildew diseases

Scoring results for powdery mildew (*E. necator*) and downy mildew (*P. viticola*) diseases as a result of natural inoculation of F1 hybrid grape genotypes are given in Tables 4. Additionally, in Figure 4, powdery mildew and downy mildew scores are shown in a circular graph (right) according to the number of genotypes in the combinations (left graph) and the percentage distribution of genotypes.

The study scored 66 hybrid genotypes belonging to the B5 combination (K-77 X Bronx Seedless). Powdery mildew

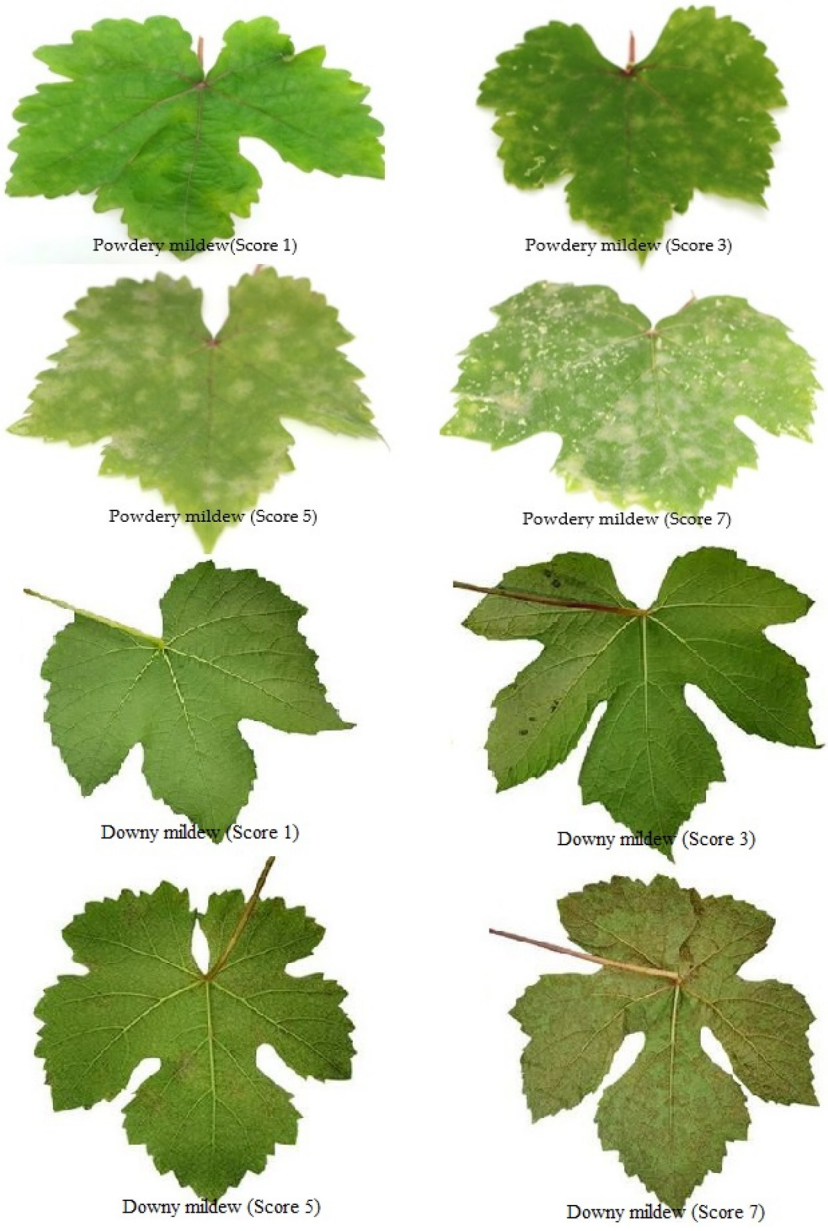


Figure 3: Photos and scores of diseased leaves of some hybrid grapes used in the study.

scoring revealed that 23 genotypes were classified as highly resistant (HR), 32 genotypes as resistant (R), 10 genotypes as tolerant (T), and one genotype as sensitive (S). For downy mildew, 31 genotypes were scored as HR, 28 genotypes as R, and 7 genotypes as T. In the B10 combination (Beyaz Çavuş X Crimson Seedless), a total of 90 genotypes were scored. In these scoring systems, 11 genotypes were defined as HR, 31 as R, 39 as T, and nine as S against powdery mildew disease, while 40 as HR, 38 as R, and 12 as T according to the downy mildew disease scores. In the B16 (Red Globe X Yalova Seedless) combination, 109 genotypes were scored. For powdery mildew, 20 genotypes were scored as HR, 69 as genotype R, 19 as genotype T, and one as genotype S. For downy mildew, 35 genotypes were scored as HR, 60 genotypes as R, and 14 genotypes as T.

For the B20 (86/1X Bronx Seedless) combination, 105 genotypes were scored. For powdery mildew, 31 genotypes were scored as HR, 63 as genotype R, 10 as genotype T, and one as genotype R. For downy mildew, 31 genotypes were identified as HR, 60 as R, and 14 as T. A total of 109 genotypes were scored in the B21 (86/1X Ruby Seedless) combination. According to the powdery mildew disease scores, 29 HR genotypes, 68 R genotypes, and three T genotypes were identified. There were four HR genotypes, 58 R genotypes, 35 T genotypes, and three S genotypes related to downy mildew.

Table 4: Scoring results for powdery mildew and downy mildew diseases as a result of natural inoculation of F1 hybrid grape genotypes. (G.Code: Genotype Code, PM: powdery mildew and DM: downy mildew).

G.Code	PM	DM	G.Code	PM	DM	G.Code	PM	DM	G.Code	PM	DM
B5-2	1	3	**B5-95**	3	3	**B10-167**	5	3	**B10-231**	3	1
B5-3	1	1	**B5-97**	3	3	**B10-168**	3	1	**B10-232**	3	1
B5-4	3	1	**B5-100**	1	1	**B10-169**	3	3	**B10-234**	3	1
B5-5	1	1	**B5-104**	1	1	**B10-170**	7	5	**B10-237**	3	1
B5-6	1	5	**B5-105**	5	3	**B10-171**	5	3	**B10-238**	3	1
B5-7	3	3	**B5-109**	3	3	**B10-172**	5	3	**B10-246**	3	1
B5-8	3	3	**B5-111**	3	3	**B10-173**	5	5	**B10-247**	3	1
B5-9	1	3	**B5-114**	1	3	**B10-174**	3	1	**B10-253**	5	1
B5-10	3	3	**B5-117**	5	1	**B10-175**	3	1	**B10-264**	5	1
B5-11	1	1	**B5-122**	5	5	**B10-178**	5	3	**B10-265**	7	3
B5-15	5	1	**B5-125**	3	1	**B10-180**	5	3	**B10-270**	5	3
B5-16	1	1	**B5-127**	1	5	**B10-181**	1	1	**B10-273**	5	3
B5-17	1	1	**B5-128**	1	3	**B10-182**	5	1	**B10-277**	1	3
B5-18	1	1	**B5-129**	3	1	**B10-183**	5	3	**B10-284**	3	1
B5-19	3	1	**B5-137**	3	3	**B10-185**	5	1	**B10-290**	5	3
B5-20	3	3	**B5-130**	3	1	**B10-186**	5	3	**B10-296**	1	3
B5-21	3	5	**B5-133**	7	3	**B10-187**	1	5	**B10-302**	5	5
B5-22	1	3	**B5-135**	3	3	**B10-190**	7	3	**B10-327**	3	1
B5-24	1	1	**B5-136**	3	3	**B10-193**	3	3	**B10-329**	1	1
B5-25	3	1	**B5-138**	1	3	**B10-194**	1	3	**B10-333**	3	1
B5-26	3	3	**B5-139**	3	1	**B10-195**	1	3	**B10-337**	5	1
B5-27	3	3	**B10-127**	7	5	**B10-196**	1	3	**B16-2**	3	3
B5-29	3	3	**B10-129**	7	5	**B10-198**	3	1	**B16-8**	3	5
B5-31	5	3	**B10-132**	5	3	**B10-199**	3	1	**B16-9**	3	3
B5-32	3	1	**B10-133**	5	3	**B10-200**	5	1	**B16-15**	5	3
B5-33	3	3	**B10-134**	5	5	**B10-203**	5	3	**B16-16**	3	5
B5-35	1	5	**B10-136**	7	3	**B10-204**	5	1	**B16-18**	3	5
B5-37	3	1	**B10-137**	5	5	**B10-205**	3	5	**B16-22**	3	3
B5-39	5	1	**B10-138**	7	3	**B10-207**	1	3	**B16-27**	3	3
B5-41	3	5	**B10-141**	5	1	**B10-209**	7	3	**B16-28**	1	5
B5-46	1	3	**B10-145**	5	3	**B10-213**	3	1	**B16-29**	3	3
B5-49	3	1	**B10-146**	5	3	**B10-214**	3	5	**B16-30**	3	3
B5-52	5	1	**B10-147**	3	5	**B10-215**	3	3	**B16-38**	3	3
B5-58	3	1	**B10-149**	5	1	**B10-216**	3	5	**B16-39**	3	3
B5-59	3	3	**B10-151**	5	1	**B10-217**	1	3	**B16-41**	1	3
B5-61	5	1	**B10-154**	5	1	**B10-218**	5	1	**B16-43**	3	3
B5-62	5	1	**B10-155**	5	1	**B10-220**	3	3	**B16-58**	1	5
B5-67	1	5	**B10-157**	3	1	**B10-221**	5	3	**B16-68**	3	3
B5-69	3	3	**B10-158**	5	1	**B10-222**	7	3	**B16-76**	3	3
B5-70	3	1	**B10-160**	5	1	**B10-223**	1	1	**B16-85**	3	1
B5-71	5	1	**B10-161**	5	1	**B10-224**	5	1	**B16-87**	3	1
B5-81	1	3	**B10-162**	3	3	**B10-226**	3	3	**B16-88**	3	1
B5-82	1	1	**B10-164**	3	3	**B10-227**	3	1	**B16-104**	5	3
B5-93	1	1	**B10-165**	5	3	**B10-229**	3	1	**B16-108**	3	1
B5-84	3	1	**B10-166**	5	3	**B10-230**	3	1	**B16-109**	1	3
B16-126	3	1	**B16-190**	7	3	**B20-68**	1	3	**B20-129**	3	5
B16-128	3	1	**B16-191**	5	1	**B20-69**	1	5	**B20-130**	5	5
B16-134	3	1	**B16-192**	3	3	**B20-70**	3	3	**B20-131**	3	3
B16-135	5	3	**B16-196**	3	3	**B20-71**	5	3	**B20-132**	3	3
B16-140	3	1	**B16-197**	3	3	**B20-72**	1	1	**B20-133**	3	1
B16-142	3	1	**B16-198**	3	3	**B20-73**	1	1	**B20-135**	3	5
B16-143	1	1	**B16-200**	5	3	**B20-74**	1	3	**B20-136**	3	3
B16-144	1	1	**B16-201**	3	5	**B20-76**	3	3	**B20-138**	3	1

Table 4: (continued)

G.Code	PM	DM	G.Code	PM	DM	G.Code	PM	DM	G.Code	PM	DM
B16-145	3	5	**B16-203**	3	3	**B20-77**	3	3	**B20-139**	1	3
B16-146	1	1	**B16-204**	3	5	**B20-80**	5	5	**B20-140**	3	1
B16-147	3	3	**B16-205**	3	3	**B20-81**	3	3	**B20-141**	3	5
B16-148	1	5	**B16-208**	1	5	**B20-82**	3	3	**B20-142**	3	3
B16-149	3	1	**B16-210**	3	3	**B20-83**	3	3	**B20-145**	1	3
B16-150	1	1	**B16-211**	1	3	**B20-84**	1	3	**B20-146**	1	3
B16-151	5	1	**B16-212**	5	3	**B20-85**	1	1	**B20-147**	1	3
B16-154	5	1	**B16-213**	5	3	**B20-87**	3	3	**B20-148**	3	1
B16-155	3	3	**B16-214**	1	3	**B20-88**	3	3	**B20-149**	3	3
B16-156	3	3	**B16-215**	3	3	**B20-90**	3	5	**B20-150**	5	1
B16-157	3	1	**B16-216**	1	3	**B20-93**	1	3	**B20-151**	1	0
B16-159	5	1	**B16-217**	3	3	**B20-95**	5	3	**B20-153**	1	0
B16-160	5	3	**B16-218**	3	3	**B20-96**	3	3	**B20-154**	1	0
B16-162	3	1	**B16-219**	3	3	**B20-97**	3	3	**B20-156**	1	0
B16-163	3	1	**B16-221**	5	3	**B20-98**	3	3	**B20-161**	3	0
B16-164	3	1	**B16-222**	5	3	**B20-99**	3	3	**B20-162**	0	3
B16-165	5	5	**B16-223**	3	3	**B20-101**	3	3	**B20-163**	3	0
B16-167	3	1	**B16-225**	3	1	**B20-105**	3	3	**B20-165**	1	0
B16-168	1	5	**B16-226**	1	3	**B20-107**	3	3	**B20-166**	5	1
B16-169	3	3	**B16-227**	5	3	**B20-110**	3	3	**B20-167**	3	1
B16-170	3	3	**B16-228**	3	1	**B20-111**	3	1	**B20-168**	3	0
B16-171	5	1	**B16-230**	3	1	**B20-113**	1	3	**B20-171**	3	1
B16-172	1	3	**B16-231**	3	0	**B20-114**	1	3	**B20-172**	3	1
B16-173	5	1	**B16-232**	3	3	**B20-115**	3	3	**B20-175**	1	3
B16-174	3	3	**B16-233**	3	3	**B20-116**	3	3	**B20-180**	3	3
B16-176	3	3	**B16-234**	3	1	**B20-117**	1	5	**B20-181**	3	3
B16-177	1	1	**B16-236**	3	1	**B20-118**	1	5	**B20-187**	3	1
B16-178	1	3	**B16-237**	5	3	**B20-119**	5	3	**B20-188**	5	3
B16-182	3	3	**B16-238**	3	3	**B20-120**	3	3	**B20-190**	5	1
B16-183	3	3	**B16-239**	0	1	**B20-121**	5	3	**B20-194**	3	5
B16-184	3	5	**B16-240**	3	1	**B20-122**	3	1	**B20-206**	3	3
B16-185	3	3	**B16-241**	3	3	**B20-124**	3	3	**B20-220**	3	1
B16-186	5	3	**B16-242**	0	1	**B20-125**	3	3	**B20-222**	1	3
B16-187	3	3	**B20-62**	1	3	**B20-126**	3	3	**B20-228**	3	3
B16-188	3	5	**B20-64**	1	1	**B20-127**	3	3	**B20-230**	3	3
B16-189	3	3	**B20-67**	3	3	**B20-128**	3	5	**B20-235**	3	3
B20-259	1	3	**B21-58**	3	3	**B21-96**	1	5	**B21-132**	3	3
B20-286	3	1	**B21-60**	3	3	**B21-97**	1	3	**B21-133**	3	3
B20-296	3	3	**B21-62**	5	5	**B21-99**	3	3	**B21-134**	1	5
B20-323	3	1	**B21-63**	1	3	**B21-100**	3	3	**B21-135**	3	3
B20-355	1	1	**B21-64**	3	5	**B21-101**	1	5	**B21-136**	1	5
B20-379	1	3	**B21-65**	3	5	**B21-102**	3	3	**B21-137**	3	5
B20-399	3	3	**B21-66**	3	3	**B21-103**	1	3	**B21-138**	3	5
B20-422	3	1	**B21-67**	3	5	**B21-104**	3	3	**B21-139**	3	3
B20-423	7	5	**B21-68**	1	3	**B21-105**	3	5	**B21-140**	1	3
B20-425	3	5	**B21-69**	3	3	**B21-106**	5	3	**B21-141**	1	3
B20-427	3	1	**B21-70**	3	3	**B21-107**	3	3	**B21-142**	1	5
B20-435	3	5	**B21-71**	5	3	**B21-109**	3	3	**B21-143**	3	1
B20-437	5	3	**B21-72**	3	3	**B21-110**	3	5	**B21-144**	3	3
B20-451	1	3	**B21-73**	3	5	**B21-111**	1	3	**B21-146**	1	3
B21-4	3	5	**B21-74**	3	3	**B21-113**	1	3	**B21-147**	3	3
B21-7	3	1	**B21-75**	3	5	**B21-114**	3	3	**B21-148**	3	3
B21-11	3	5	**B21-76**	1	3	**B21-116**	3	3	**B21-149**	1	5
B21-20	3	5	**B21-78**	3	5	**B21-117**	3	3	**B21-150**	3	3

Table 4: (continued)

G.Code	PM	DM	G.Code	PM	DM	G.Code	PM	DM	G.Code	PM	DM
B21-28	3	5	**B21-79**	3	5	**B21-119**	1	5	**B21-151**	3	1
B21-31	3	3	**B21-81**	1	7	**B21-120**	3	3	**B21-152**	3	3
B21-35	3	1	**B21-82**	3	3	**B21-123**	3	3	**B21-153**	3	3
B21-37	1	5	**B21-83**	3	5	**B21-124**	3	5	**B21-154**	3	3
B21-40	1	3	**B21-84**	3	5	**B21-125**	3	3	**B21-155**	3	3
B21-42	3	7	**B21-86**	3	5	**B21-126**	3	3	**B21-156**	3	5
B21-44	1	3	**B21-89**	3	3	**B21-127**	3	3	**B21-158**	3	3
B21-47	3	3	**B21-90**	1	5	**B21-128**	3	3	**B21-159**	1	5
B21-51	1	7	**B21-91**	1	5	**B21-129**	1	3	**B21-160**	1	5
B21-56	1	5	**B21-92**	3	3	**B21-130**	1	3			
B21-57	3	5	**B21-95**	3	3	**B21-131**	3	3			

Name of the hybrids (genotypes) are in bold.

Among the hybrid grape genotypes used in the research, 11 genotypes from the B5 combination, three from the B10 combination, seven from the B16 combination, and 10 from the B20 combination were determined to be highly resistant to both diseases. The genotype that is highly resistant to both diseases could not be identified from the B21 combination.

According to the scoring results, the percentages of highly resistant genotypes that received 1 point from both disease scores on a combination basis were as follows: 35.48 % for the B5 (Bronx Seedless X K-77) combination, 32.25 % for the B20 combination (Bronx Seedless X 86/1), 22.58 % for the B16 (Yalova Seedless X Red Globe) combination and 9.67 % for the B10 combination (Crimson Seedless X Beyaz Çavuş). The B5 (Bronx Seedless X K-77) combination had the greatest resistance to various diseases (Figure 4). The main reason for this is that its parents are interspecific hybrids and *V. labrusca* species.

3.2 Determination of seedless genotypes with MAS

In this part of the study, combination B5, which had many problems during the DNA isolation and PCR amplification stages, was excluded from the research. The presence of seedless gene regions was detected in the genotypes of other combinations of PCR products using the 5U_VviAGL11 primer, and their exact band sizes were then determined with the capillary system. The allele sizes of the hybrid grape genotypes were examined with a Fragment Analyser device. The genotypes detected with the 318-bp allele, which is associated with seedlessness, were selected as seedless. As a result of the evaluations made according to the presence of this allele, it was determined that 136 hybrid grape genotypes could be seedless. Tables 5–8 present the MAS results for seedlessness, while Table 9 lists only hybrid genotypes with an allele size of 318-bp that could potentially be seedless.

3.3 Determination of disease resistance and seedless genotypes

Hybrid genotypes that have both the seedlessness-associated gene region and are scored as very resistant or resistant to powdery mildew and downy mildew are shown in Table 10. Additionally, a visual summary of the method used to select disease-resistant and seedless genotypes in the study is presented in Figure 5.

4 Discussion

Consumer demands change annually, and people want to purchase new, healthier seedless varieties that meet their expectations for quality. However, fungal diseases are among the most significant problems preventing these expectations. This study has yielded promising results for developing new varieties that meet consumer expectations. The two most significant fungal diseases that threaten world viticulture are powdery mildew and downy mildew. To control these diseases, numerous pesticides must be applied during the vegetative period [17]. However, because of concerns about residue risk and the health of humans and the environment, consumers are showing more demand for grape varieties that use fewer pesticides, as well as new seedless grape varieties. As a result, breeding studies to develop seedless varieties resistant to these two diseases continue in Türkiye and many other countries.

In a similar study, Wan et al. [18] evaluated the resistance of genotypes belonging to different *Vitis* species to

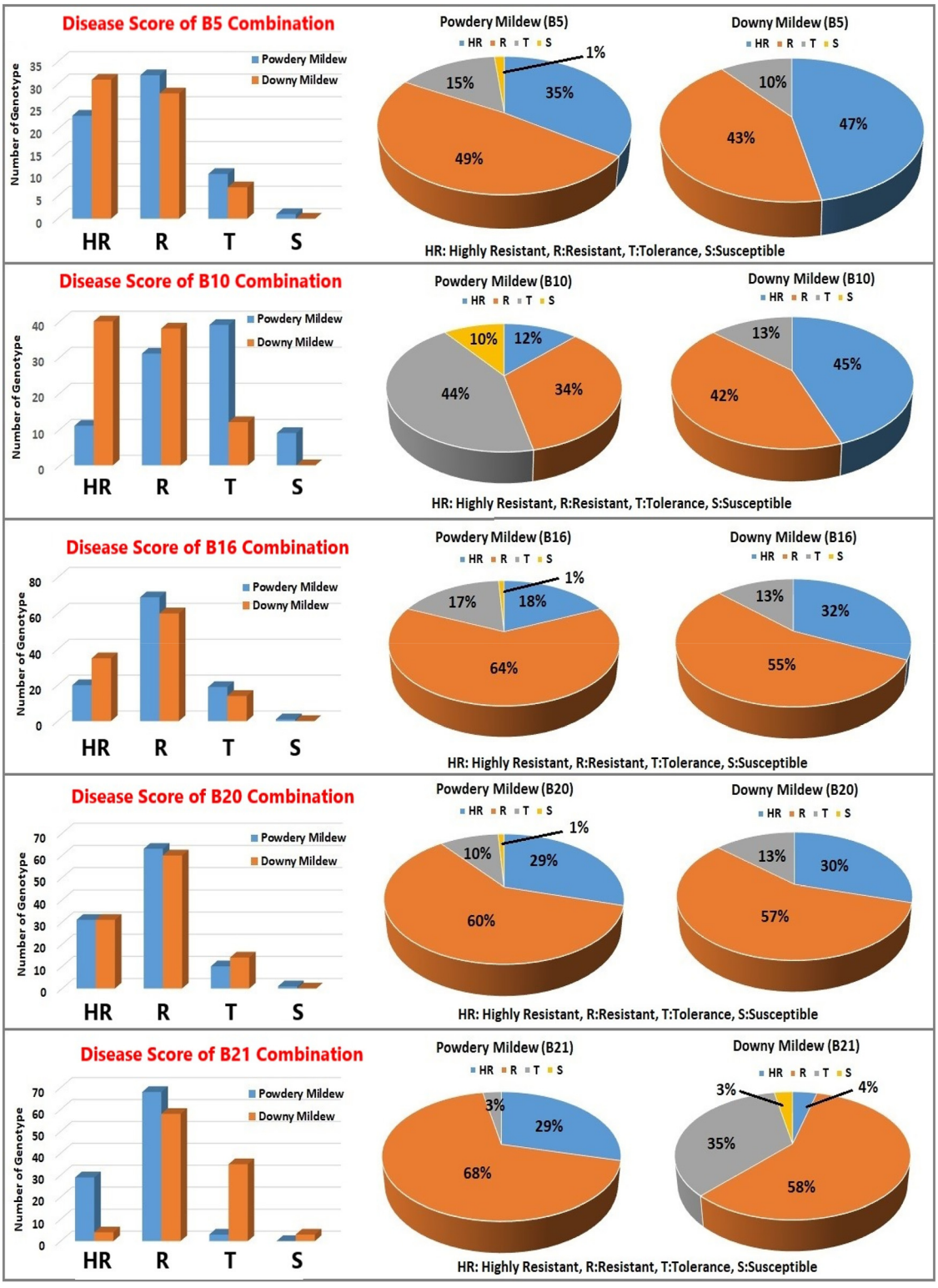


Figure 4: The powdery mildew and downy mildew scores are shown in a circular graph (right) according to the number of genotypes in the combinations (left graph) and the percentage distribution of genotypes.

Table 5: Allele sizes (bp) of genotypes belonging to the B10 combination amplified with the 5U_VviAGL11 primer.

G.Code	Allele size (bp)				G.Code	Allele size (bp)			
B10-127	287	296	327		B10-209	317	325		
B10-129	284	294	315	325	B10-213	313	323		
B10-132	316	326			B10-214	314	324		
B10-133	317	324			B10-215	317	399	463	
B10-136	317	325	398		B10-216	316	324		
B10-137	285	294	306	317	B10-217	293	315		
B10-138	314	324			B10-218	320	331		
B10-145	283	292	323		B10-220	317	324	399	461
B10-147	317				B10-221	311	321		
B10-149	314	324			B10-222	312	322		
B10-155	317	325			B10-223	316	324		
B10-157	317	325	399		B10-224	283	292		
B10-158	284	293	314	324	B10-225	283	293		
B10-160	293				B10-226	284	294	314	324
B10-161	314	324			B10-227	283	291	303	314
B10-162	293	315			B10-229	284	293	305	316
B10-164	293	316			B10-230	283	293	305	315
B10-165	285	294	307	317	B10-231	284	294	306	317
B10-166	284	293	305		B10-232	316			
B10-167	315	324			B10-234	284	293	305	316
B10-168	283	293			B10-237	283	292	313	323
B10-169	284	293	314	324	B10-238	313	322		
B10-170	317	325			B10-240	315	326		
B10-171	284	294	315	325	B10-243	284	293	305	316
B10-172	286	295	316	326	B10-246	316	323		
B10-173	316	324	399	462	B10-247	293			
B10-174	317				B10-252	314	324		
B10-175	284	293	305	316	B10-253	314	324		
B10-178	314	321			B10-256	312	324		
B10-180	314	325			B10-259	286	295	316	326
B10-181	316	324			B10-264	314	325		
B10-182	293				B10-270	313	324		
B10-183	284	293	314	324	B10-273	293	316		
B10-185	313	323			B10-277	318	325		
B10-187	314	325			B10-284	283	292	304	315
B10-190	315	325			B10-289	285	294	317	431
B10-193	285	294	306	317	B10-290	284	293	314	323
B10-194	317	325	399	462	B10-292	284	294		
B10-196	314	325			B10-296	293			
B10-199	284	293			B10-302	316	324		
B10-200	317	325	401	463	B10-323	312	323		
B10-203	293	303	316		B10-327	316	324		
B10-204	317	324	399		B10-333	315	323		
B10-207	284	293	314	324	B10-337	316	324	399	

Table 6: Allele sizes (bp) of genotypes belonging to the B16 combination amplified with the 5U_VviAGL11 primer.

G.Code	Allele size (bp)				G.Code	Allele size (bp)			
B16-9	286	310			B16-169	287	315		
B16-15	284	307			B16-170	314	323		
B16-16	286	314			B16-172	286	310		
B16-18	310	323			B16-173	316	325		
B16-22	316	324			B16-174	314	323		
B16-27	314	322			B16-176	286	314		
B16-28	307				B16-177	292	320		
B16-29	282	291	302	314	B16-178	282	291	302	313
B16-30	285	314			B16-182	314	323		
B16-38	287	314			B16-183	286	314		
B16-39	314	325			B16-184	310	322		
B16-41	315	324			B16-186	315	322		
B16-43	312	320			B16-187	287	315		
B16-58	310	323			B16-188	287			
B16-68	286	314			B16-189	315	323		
B16-76	286	310			B16-191	287	311		
B16-85	286	314			B16-192	311	324		
B16-87	311	323			B16-197	316	324		
B16-88	315	323			B16-200	317	325		
B16-104	315	323			B16-201	189	317		
B16-108	312	325			B16-203	286	315		
B16-109	286	315			B16-204	288	316		
B16-126	286	315			B16-205	290	301	312	
B16-128	279	307			B16-208	286	310		
B16-134	315	323			B16-211	288	316		
B16-135	309	322			B16-212	288	317		
B16-140	287	311			B16-213	281	308		
B16-142	314	322			B16-215	286	314		
B16-143	314	323			B16-216	289	313		
B16-144	285	309			B16-217	311	324		
B16-145	286	314			B16-218	288	317		
B16-146	313	322			B16-219	288	312		
B16-147	316	324			B16-221	317	325		
B16-148	310	323			B16-222	285	313		
B16-149	315	324			B16-225	308			
B16-150	287	311			B16-226	319	327		
B16-151	286	315			B16-227	297			
B16-154	288	312			B16-228	283	304		
B16-155	286	311			B16-230	318	326		
B16-156	310	323			B16-231	291			
B16-157	313	321			B16-232	318	326		
B16-159	285	313			B16-233	291	318		
B16-160	311	324			B16-234	290			
B16-162	287	311			B16-237	319			
B16-163	287	316			B16-238	291			
B16-164	287	315			B16-239	290			
B16-165	316	325			B16-240	291			
B16-167	311	324			B16-241	323			
B16-168	311	323			B16-242	318	328		

powdery mildew and downy mildew diseases using a 0–7 scale after natural infection. According to their scoring, 46 of 66 genotypes were resistant to powdery mildew, 28 to downy mildew, and 19 to both diseases. In another study conducted by Calonnec et al. [19], powdery mildew and downy mildew infections of genotypes were examined using OIV scores, and different resistance levels were determined based on genotype. In this study, according to the literature, 31 hybrid grape genotypes out of 470 were determined to be highly resistant to both diseases (powdery mildew and mildew).

Table 7: Allele sizes (bp) of genotypes belonging to the B20 combination amplified with the 5U_VviAGL11 primer.

G.Code	Allele size (bp)				G.Code	Allele size (bp)			
B20-62	315				B20-133	327			
B20-64	323	330			B20-135	315			
B20-67	312				B20-136	326			
B20-68	312	312			B20-138	326			
B20-69	316	351	382		B20-141	326			
B20-70	303	313	321	331	B20-142	315			
B20-71	317	326			B20-145	324	358	386	
B20-73	325				B20-148	328	339		
B20-74	314	324			B20-149	324			
B20-76	326	392			B20-151	318	326		
B20-77	312	322			B20-152	327			
B20-80	325				B20-156	315			
B20-83	318	326			B20-161	313			
B20-85	325				B20-163	330	336		
B20-87	313	325			B20-165	312	323		
B20-88	313	325			B20-166	313	324		
B20-90	314	326			B20-167	316	326		
B20-93	314				B20-188	319	326		
B20-95	313	324			B20-190	323			
B20-96	312				B20-194	315	326		
B20-99	313	322			B20-206	315	325		
B20-101	312	321	331		B20-220	333			
B20-105	325				B20-222	324			
B20-107	313				B20-259	320	332		
B20-115	314	322			B20-323	320	332		
B20-118	327				B20-355	330	335		
B20-119	313	388			B20-379	327			
B20-121	315	325			B20-399	330	336	365	373
B20-122	325				B20-423	331	337		
B20-124	315	323			B20-425	310	322	339	363
B20-126	326				B20-427	312	323		
B20-128	319	326			B20-435	313	323		
B20-130	309	319			B20-437	312	323		
B20-132	313	323			B20-451	318			

Moreover, according to the results for powdery mildew disease, one genotype from the B5 combination, nine genotypes from the B10 combination, one genotype from the B16 combination, and one genotype from the B20 combination were determined as susceptible. For downy mildew, three genotypes from the B21 combination were detected as susceptible. There was a significant difference in disease resistance between individuals with the same parents. These results indicate that genotypes belonging to different *Vitis* species may have different levels of disease resistance depending on their parents. Researchers have reported that genotypes obtained from breeding programs in which *V. vinifera* varieties, which are generally very sensitive to diseases, are used as parents exhibit the same susceptibility to hybrid genotypes in the F1 stage. However, when different *Vitis* species are included in combinations, more resistance is detected in hybrid genotypes [20]. In this study, hybrid grape genotypes obtained from combinations of *V. vinifera, V. labrusca,* and interspecies hybrids showed similar results for resistance to powdery mildew and downy mildew. Among these genotypes, 16.6 % of the B5 (K-77 X Bronx Seedless) combination genotypes, 3.33 % of the B10 (Beyaz Çavuş X Crimson Seedless) combination genotypes, 6.42 % of the B16 (Red Globe X Yalova Seedless) combination genotypes, and 9.61 % of the B20 (86/1 X Bronx Seedless) combination genotypes are very resistant to both diseases (HR). In other words, a higher resistance/tolerance has been found in combinations using species other than *V. vinifera*. The results are quite similar to the studies conducted by Wu et al. [21] and Atak et al. [15]. Researchers have reported that genotypes belonging to the *V. labrusca* genus exhibit increased resistance. The K-77 genotype, one of the B5 series parents used in this study, belongs to the *V. labrusca* species, and most of the genotypes that are highly resistant to both diseases are composed of the genotypes of this combination.

As in our study, Volynkin et al. [22] scored the hybrid population obtained by crossing *V. vinifera* with wild *Vitis* species and screened them for resistance genes against powdery mildew and downy mildew. They reported that a significant number of hybrid genotypes resistant to both diseases were obtained. This demonstrates the importance of interspecific hybrids in disease resistance and may also guide future studies on other quality criteria. Additionally, Salotti et al. [23] report that knowing the disease resistance of grape varieties will not only influence fungicide selection but also significantly reduce unnecessary use of fungicides. This further increases the importance of understanding the disease resistance of new grape varieties, as demonstrated in our study.

With marker-assisted selection (MAS), the characteristics controlled by more than one gene or locus can be determined quickly. The MAS method is used to determine seedless genotypes at early stages in classical breeding studies, especially in individuals with long juvenile sterility, such as grapevines [24]. Different molecular markers are used to determine seedlessness in grapevine hybrids: two sequence characterized amplified region (SCAR) markers, SCC8 [12], the DNA probes GSLP1 [25] and SCF27 [13], and two simple repeated SSR markers, VMC7F2 [25] and p3_VvAGL11 [14]. Each of these markers has been widely used in grapevine varieties, hybrids, or genotypes with different genetic backgrounds [26–32].

Later, markers linked with the VviAGL11 gene, which is connected to seedlessness, began to be used in this study. Mejía et al. [14] reported that the VviAGL11 gene may be responsible for seedlessness in table grape varieties. Bergamini et al. [28] evaluated VvAGL11 in 475 hybrid F1

Table 8: Allele sizes (bp) of genotypes belonging to the B21 combination amplified with the 5U_VviAGL11 primer.

G.Code	Allele size (bp)				G.Code	Allele size (bp)			
B21-4	326				B21-104	317	325		
B21-7	332				B21-105	316	326		
B21-11	322				B21-106	318	326		
B21-20	318	326			B21-107	315	326		
B21-28	323				B21-109	314	325		
B21-31	318	332			B21-110	316	326		
B21-35	322	335			B21-111	319	327		
B21-37	323				B21-114	318	326		
B21-40	327				B21-116	318	333		
B21-42	326	332			B21-117	317	331		
B21-44	317	328			B21-118	324	329		
B21-47	326				B21-119	314	325		
B21-51	320	334			B21-120	314	325		
B21-56	318				B21-122	317	353		
B21-57	315	326			B21-123	315	392		
B21-58	315	328			B21-124	318	332		
B21-60	322				B21-125	325			
B21-62	319	328			B21-126	325	351	360	387
B21-63	3B21	335			B21-127	326	332		
B21-64	318	326			B21-128	317			
B21-65	318	326			B21-130	322			
B21-66	326				B21-131	318	326		
B21-67	318	326			B21-132	319	326		
B21-68	319				B21-133	319	328		
B21-71	319				B21-134	318	332		
B21-72	316	327			B21-135	318	332		
B21-73	318	332			B21-136	318	327		
B21-74	307				B21-137	327	332		
B21-75	320	332			B21-138	326	332		
B21-76	316	327			B21-139	314	325		
B21-78	326				B21-140	317	325		
B21-79	318	326			B21-141	314	324		
B21-81	326				B21-142	3B21	358		
B21-82	315	326			B21-143	316	324		
B21-83	314	325			B21-144	312	322		
B21-84	329				B21-146	316	324		
B21-88	325	331			B21-148	306			
B21-89	306	317	320	331	B21-149	324	361	390	
B21-90	323	334			B21-150	316	3B21	331	
B21-91	327	333			B21-151	316	325	408	
B21-92	325	332			B21-152	319	320	393	
B21-95	320	333			B21-153	324	359	389	
B21-96	326				B21-154	316	324	352	399
B21-97	316	325			B21-155	324	361	390	
B21-99	327				B21-156	315	325		
B21-100	306	317	3B21	331	B21-158	316	407		
B21-101	318	332			B21-159	325	361	391	
B21-102	316	330			B21-160	316	324	407	466
B21-103	314	326			K-77	282	313		
Sultani Çekirdeksizi	318				Crimson seedless	287	296	318	
Beyaz Çavuş	290	298			Yalova seedless	286	296	318	

Table 9: Hybrid genotypes and their combinations with the gene region associated with seedlessness.

Genotype code	Genotype code	Genotype code	Genotype code	Genotype code	Genotype code	Genotype code	Genotype code
B10-129	B10-181	B10-240	B16-126	B16-204	B21-20	B21-89	B21-128
B10-132	B10-190	B10-243	B16-134	B16-211	B21-31	B21-97	B21-131
B10-133	B10-193	B10-246	B16-147	B16-212	B21-44	B21-100	B21-132
B10-136	B10-194	B10-259	B16-149	B16-218	B21-56	B21-101	B21-133
B10-175	B10-234	B10-273	B16-203	B20-451	B21-82	B21-124	B21-160
B10-137	B10-200	B10-277	B16-151	B16-221	B21-57	B21-102	B21-134
B10-147	B10-204	B10-284	B16-163	B16-226	B21-58	B21-104	B21-135
B10-155	B10-209	B10-289	B16-164	B16-230	B21-62	B21-105	B21-136
B10-157	B10-215	B10-302	B16-165	B16-232	B21-64	B21-106	B21-140
B10-162	B10-216	B10-327	B16-169	B16-233	B21-65	B21-107	B21-143
B10-164	B10-217	B10-333	B16-173	B16-237	B21-67	B21-110	B21-146
B10-167	B10-220	B10-337	B16-186	B16-242	B21-68	B21-111	B21-150
B10-170	B10-223	B16-22	B16-187	B20-71	B21-71	B21-114	B21-151
B10-171	B10-229	B16-41	B16-189	B20-83	B21-72	B21-116	B21-152
B10-172	B10-230	B16-88	B16-197	B20-128	B21-73	B21-117	B21-154
B10-173	B10-231	B16-104	B16-200	B20-151	B21-76	B21-122	B21-156
B10-174	B10-232	B16-109	B16-201	B20-188	B21-79	B21-123	B21-158

Table 10: Hybrid genotypes that have a gene region associated with seedlessness and are also scored as very resistant or resistant to powdery mildew and downy mildew.

G.Code	PM	DM	G.Code	PM	DM	G.Code	PM	DM	G.Code	PM	DM
B10-157	3	1	B10-277	1	3	B16-218	3	3	B21-114	3	3
B10-162	3	3	B10-284	3	1	B16-226	1	3	B21-116	3	3
B10-164	3	3	B10-327	3	1	B16-230	3	1	B21-117	3	3
B10-168	3	1	B10-333	3	0	B16-232	3	3	B21-123	3	3
B10-169	3	3	B16-22	3	3	B16-233	3	3	B21-128	3	3
B10-174	3	1	B16-41	1	3	B16-242	0	1	B21-131	3	3
B10-175	3	0	B16-88	3	1	B20-83	3	3	B21-132	3	3
B10-181	1	1	B16-109	1	3	B20-151	1	0	B21-133	3	3
B10-193	3	3	B16-126	3	1	B20-451	1	3	B21-135	3	3
B10-194	1	3	B16-134	3	1	B21-31	3	3	B21-140	1	3
B10-215	3	3	B16-147	3	3	B21-44	1	3	B21-143	3	1
B10-217	1	3	B16-149	3	1	B21-58	3	3	B21-146	1	3
B10-220	3	3	B16-163	3	1	B21-68	1	3	B21-150	3	3
B10-226	3	3	B16-164	3	1	B21-72	3	3	B21-151	3	1
B10-223	1	1	B16-169	3	3	B21-76	1	3	B21-152	3	3
B10-229	3	1	B16-187	3	3	B21-82	3	3	B21-154	3	3
B10-230	3	1	B16-189	3	3	B21-89	3	3	B21-158	3	3
B10-232	3	1	B16-197	3	3	B21-97	1	3			
B10-234	3	1	B16-203	3	3	B21-100	3	3			
B10-246	3	1	B16-211	1	3	B21-111	1	3			

genotypes for MAS purposes. With their study, they confirmed that the VviAGL11 marker can be used for the early selection of negative stenospermocarpy. In parallel with these results, potential seedless hybrid genotypes were successfully selected by scanning the VviAGL11 gene region for seedlessness in the early stages of our breeding program.

To identify seedless individuals among disease-resistant hybrids, we applied MAS using the 5U_VviAGL11 marker, a widely used tool for early seedlessness screening in grape breeding programs. Ocarez et al. [33] studied the 5U_VviAGL11 marker, which was also used in this study, with hybrid genotypes in which the Sultana parent was used and reported that seedlessness was defined at an allele size of 319 bp. Although Sultana grape was not used as a parent in this study, it has been reported that hybrid genotypes obtained from the Sultana variety carrying a band close to the

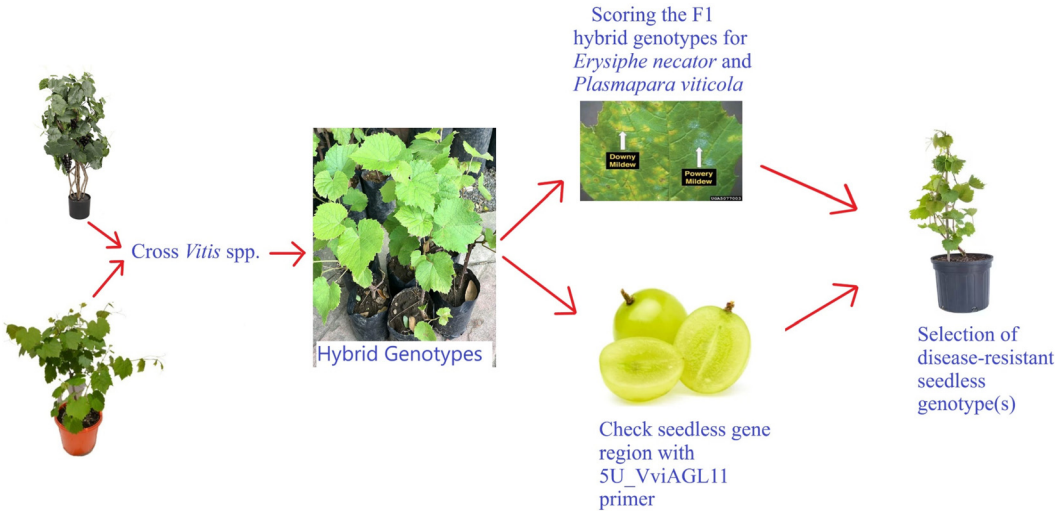


Figure 5: Visual summary of the method followed in the study.

318 bp allele may be potential seedless genotypes. According to different studies, there may be a difference of up to a few bp between the band sizes obtained with the capillary system and those reported in the literature, possibly due to the use of different media and devices [34, 35]. Chen et al. [36] also reported that depending on the seedless parents used in the cross-breeding combination, differences may be seen in the band sizes obtained from the 5U_VviAGL11 primer to determine seedless genotypes. Taken together, the genotypes that differed by only a few bases from the 318 bp allele size were considered to be seedless and were included in the list of potential seedless genotypes, and a total of 136 hybrid genotypes were selected. Wingerter et al., [37] and Li et al., [38] that PCD in grape plants is triggered by resistance (R) loci, R genes, grape regulators, fungal agents, and phytotoxins. Potential PCD loci also play an important role in providing resistance to powdery mildew and downy mildew in our hybrid grapes. Piarulli et al. [39] shared the same objectives as our study, aiming to develop disease-resistant and seedless hybrid genotypes. They identified a significant number of seedless hybrid genotypes resistant to powdery mildew and downy mildew using similar markers. They also explained the effects of parental combinations and other variables on the development of new table grape varieties and the pyramiding of genes of interest.

5 Conclusions

Currently, the demand for seedless grape varieties consumed for table purposes is increasing in Türkiye and the world. This demand has led to an increase in the number of breeding studies aimed at the development of new seedless grapes. In addition, awareness of both human and environmental health is increasing in our country, as well as all over the world, and in parallel with this, new varieties that can be grown organically are in greater demand. Research on the origins and mechanisms of disease resistance or susceptibility in grapevine has been ongoing for many years to fulfil these demands. Almost every year, new gene regions are being discovered in studies on endurance. Different breeding efforts are being initiated to transfer these genes to susceptible varieties. As a result of this study, the resistance levels and seedlessness status of hybrid grape genotypes against powdery mildew and downy mildew diseases, which are very important for viticulture, were determined. The 77 hybrid genotypes that are both disease resistant and seedless were identified. In future studies, the fruit characteristics of these hybrid genotypes will be investigated, and those with higher quality will be registered as a new variety. Those with low potential as new varieties can be used as parents in future breeding studies due to their disease resistance and seedlessness properties.

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