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DETERMINATION OF DOWNY AND POWDERY MILDEW RESISTANCE OF SOME *VITIS* SPP.

DETERMINAÇÃO DA RESISTÊNCIA AO MÍLDIO E OÍDIO DE ALGUMAS *VITIS* SPP.

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SUMMARY

The Black Sea region of Turkey receives an annual rainfall of 2000-2500 mm and viticulture in the region depends on the rainfall limits significantly. In this study, the resistance of eighty different *Vitis* species and cultivars/genotypes to downy and powdery mildew was assessed using Marker Assisted Selection coupled with inoculation observations. Six Simple Sequence Repeats (SSR) and one Sequence Characterized Amplified Region (SCAR) markers were developed for different resistance loci (Run1, Rpv1, Ren1, Rpv3, Ren3) from different *Vitis* genetic resources. Eight of the cultivars/genotypes were characterised as resistant to downy mildew, seven were resistant to powdery mildew, and four were resistant to both diseases. These results, taken together with the disease inoculation observations, revealed 'Köfteci Üzümü' (*Vitis labrusca*) and 'Giresun 3' (*Vitis labrusca*) were the most resistant cultivars/genotypes.

RESUMO

A região do Mar Negro na Turquia apresenta uma precipitação anual de 2000-2500 mm, sendo a viticultura na região muito limitada pela precipitação. Neste estudo, a resistência ao míldio e oídio foi avaliada em oitenta diferentes espécies e cultivares/genótipos de *Vitis* através da Seleção Assistida por marcadores moleculares, juntamente com observações de inoculação no fenótipo. Foram usados seis microssatélites (SSR) e um marcador de Região Amplificada Caracterizada por Seqüência (SCAR) desenvolvidos para diferentes loci de resistência (Run1, Rpv1, Ren1, Rpv3, Ren3), a partir de diferentes recursos genéticos de *Vitis*. Oito cultivares / genótipos foram caracterizadas como resistentes ao oídio, sete ao míldio e quatro a ambas as doenças. Estes resultados avaliados em conjunto com as observações de inoculação da doença, revelaram que os cultivares/genótipos 'Köfteci Üzümü' (*Vitis labrusca*) e 'Giresun 3' (*Vitis labrusca*) foram os mais resistentes.

Key words: Fungal Diseases, Grapevine, Resistant Loci, Inoculation.
Palavras-chave: Doenças fúngicas, Videira, Loci resistentes, Inoculação.

INTRODUCTION

Grapes are one of the most important fruits in the world and are believed to have been cultivated in different regions globally for thousands of years (Santos *et al.*, 2014). It is widely known that Turkey is an important centre of origin both for cultivated *Vitis vinifera* ssp. *sativa* and wild *Vitis vinifera* ssp. *sylvestris* (Arroyo-Garcia *et al.*, 2006; Karataş *et al.*, 2014). Grapes are grown in a large area but over time many pests and diseases have begun to affect yield and quality in the world's vineyards. Powdery [*Erysiphe necator* Schwein. (syn. *Uncinula necator* (Schw.) (Burr.)] and downy mildew [*Plasmopara viticola* (Berk. and Curtis) Berl. and de Toni] are the two most significant diseases that devastate grapes worldwide (Wan *et al*., 2007).

Downy mildew (DM) and powdery mildew (PM) diseases affect grape production and reduce yield in many regions of the world (Jermini *et al*., 2010; Karbalai-Khiavi *et al*., 2012; Figueiredo *et al*., 2017). The incidence of PM has increased in recent years. Climatic conditions and reduced efficacy of fungicides were suggested as possible reasons for this increase (Staudt, 1997; Yıldırım *et al*., 2002; Dean and Gray, 2008). Oliveira and Cunha (2015) reported

that diversity of these pathogens also can affect disease resistance of *Vitis* spp.

These two fungal diseases also cause severe losses in yield and quality in vineyards, particularly in warm and humid climate areas. These two diseases particularly affect leaf, fruit and shoots, leading to significant crop losses. In addition to cultural practices, intensive fungicide applications are used to prevent these diseases. However, according to research in recent years, pesticide residues have negative effects on both human health and the environment (Nicolopoulou-Stamati *et al.*, 2016).

One of the most effective ways to control disease is to develop new cultivars which are resistant to the diseases (Akkurt *et al*., 2007; Yıldırım and Dardeniz, 2010; Özer *et a*l., 2012). Production of grape cultivars with high quality that are simultaneously resistant to fungal diseases is one of the most crucial goals of grape breeding (Töpfer *et al*., 2011; Rex *et al*., 2014).

Therefore, breeding studies have been performed in different world regions since the early 20th century. In recent years, different resistant genomic regions and associated markers were obtained for these diseases (Dalbó *et al*., 2001; Welter *et al*., 2007; Zini *et al.*, 2015).

Grapevines (*Vitis vinifera* L.) in European viticulture is threatened by pathogens like PM and DM, requiring heavy fungicide applications. Resistance traits from wild *Vitis* species are introgressed into the cultivated susceptible *V. vinifera*. Molecular markers linked to traits of interest help to accelerate the process, rationalizing the choice of parental types and replacing part of the phenotypic evaluation with genetic testing in the early stages of plant growth (Rex *et al*., 2014).

Combining resistance gene regions from American wild *Vitis* species, with good wine qualities of *Vitis vinifera* L. has become an important strategy for grapevine breeding. *Muscadinia rotundifolia* was described as totally resistant to PM species. *Muscadine* is seen as a potential source for disease resistance for *V. vinifera*. One gene that has drawn some attention is the Resistance *Uncinula necator* 1 gene (Run1), which is the grapevine PM resistance gene. Three molecular markers were confirmed as good candidates for Run1 marker assisted selection (Pauquet *et al*., 2001; Dry *et al*., 2010). Eibach *et al*. (2007) recommend selection using phenotypic data and molecular markers to identify resistance to PM and DM. Gene pyramiding was used to combine Run1 with Ren1, which is another PM resistant gene found in a *V. vinifera* cultivar, and it was confirmed in a *V. rotundifolia x V. vinifera* BC5 hybrid family (Katula-Debreceni *et al*., 2010).

Grapes of Eurasian origin were believed to be susceptible to American native pathogens like PM and DM. This assumption was explained by the geographical isolation of host and pathogen during their evolution. However, certain East Asian grape species, for example *Vitis piasezkii*, were found to be at least partly resistant to PM (Pap *et al.*, 2016). It was also reported that some *Vitis vinifera* cultivars, such as Kismish vatkana and Dzhandzhal Kara which grow in central Asia, are resistant to PM (Hoffmann *et al.,* 2008; Kozma *et al*., 2014). The resistant loci originating from these varieties is named Ren1 (Resistant to *Erysphe necator* 1).

The regent cultivar, which is resistant to downy and PM obtained from one of these breeding studies, has begun to be widely grown in Germany (Eibach and Töpfer 2003). Regent carries the dominant resistance genes of Ren3 (Resistant to *Erysphe necator* 3) and Rpv3 (Resistant to *Plasmopara viticola* 3). Due to resistance to these diseases, Regent was used as parent in many breeding and QTL studies (Eibach *et al*., 2007; Van Heerden *et al*., 2014).

In this study, the resistance of some grape cultivars or genotypes from different origins to DM and PM was observed over two years and also examined with different markers related to resistance to these diseases.

MATERIAL AND METHODS

Plant Materials

80 cultivars/genotypes considered to be resistant/tolerant to fungal diseases collected from the humid regions of Turkey (mainly Northern part of Turkey) and different part of the world (mainly Northern part of the United States) were used in this study. Some of these cultivars have been obtained as a result of the breeding program in Turkey, USA and Germany. Information from cultivars/genotypes used in the study are given in Table I. Collected grape cultivars/genotypes were grown in Yalova Atatürk Horticultural Central Research Institute greenhouses. Disease infections and inoculations were carried out in a greenhouse using 2-year-old potted vines grown in 5-litre pots and filled with soil mixture (1/3 garden soil, 1/3 peat moss, and 1/3 blended compost). Also optimum climatic conditions (22-25 ºC and 80% Relative humidity) were ensured for the development of diseases in the greenhouse.

TABLE I

The origin of cultivars/genotypes and inoculation results (N: No band associated with resistance allele; ER:Extremely Resistant, HR:Highly Resistant, R:Resistant, S:Susceptible and HS:Highly Susceptible after inoculation).

Origem das cultivares / genótipos e resultados de inoculação (N: Sem banda associada ao alelo de resistência; ER: Extremamente resistente, HR: Altamente resistente, R: Resistente, S: Susceptível e HS: Altamente susceptível após a inoculação)

Code	Cultivar/ Genotype	Species	Origin	Downy Mildew	Powdery Mildew
41	Regent	Interspecies	Ankara/Turkey	HR	R
47	Sugargate	*M.rotundifolia*	USA	ER	HR
121	Razakı	*V. vinifera*	Tekirdağ/Turkey	S	S
1	57 Ayancık 01	*V.labrusca*	Samsun/Turkey	R	R
2	Batum 4	*V.labrusca*	Samsun/Turkey	R	S
3	57 Erfelek 03	*V.labrusca*	Samsun/Turkey	R	S
4	57 Gerze 04	*V.labrusca*	Samsun/Turkey	R	S
5	61 Of 04	*V.labrusca*	Samsun/Turkey	R	R
6	61 Sürmene 01	*V.labrusca*	Samsun/Turkey	R	S
7	61 Sürmene 02	*V.labrusca*	Samsun/Turkey	R	R
8	57 Merkez 02	*V.labrusca*	Samsun/Turkey	R	R
10	53 Merkez 02	*V.labrusca*	Samsun/Turkey	R	S
11	Rizessi	*V.labrusca*	Samsun/Turkey	R	S
12	Rizpem	*V.labrusca*	Samsun/Turkey	HR	S
13	Çeliksu	*V.labrusca*	Samsun/Turkey	R	S
14	Rizellim	*V.labrusca*	Samsun/Turkey	R	S
15	55 Merkez 06	*V.labrusca*	Samsun/Turkey	R	S
16	55 Merkez 12	*V.labrusca*	Samsun/Turkey	R	R
17	55 Merkez 11	*V.labrusca*	Samsun/Turkey	R	R
18	53 Güneysu 01	*V.labrusca*	Samsun/Turkey	HR	S
21	53 Pazar 02	*V.labrusca*	Samsun/Turkey	R	S
22	55 Çarşamba 01	*V.labrusca*	Samsun/Turkey	HR	S
23	28 Tirebolu 02	*V.labrusca*	Samsun/Turkey	R	S
24	28 Görele 01	*V.labrusca*	Samsun/Turkey	ER	S
25	28 Merkez 01	*V.labrusca*	Samsun/Turkey	R	R
26	Concord	*V.labrusca*	Samsun/Turkey	S	S
27	Mars	*V.labrusca X V.vinifera*	Samsun/Turkey	R	S
28	Niagara	*V.labrusca X V.vinifera*	Samsun/Turkey	R	S
31	Valiant	*V.labrusca X V.riparia*	Samsun/Turkey	R	S
32	Kyoho	Interspecies	Yalova/Turkey	R	S
34	Arcadia	*V.vinifera*	Samsun/Turkey	R	S
35	Sunbelt	*V.labrusca X V.vinifera*	Samsun/Turkey	ER	R
37	Boscop Glory	*V.vinifera*	Samsun/Turkey	S	S
38	Orion	Interspecies	Samsun/Turkey	ER	S
40	Staufer	Interspecies	Ankara/Turkey	R	R
42	Sirius	Interspecies	Ankara/Turkey	HR	R
43	Phoenix	Interspecies	Ankara/Turkey	HR	HR
44	Mortensen	*V.labrusca X V.vinifera*	USA	R	S
45	Alden	*V.labrusca X V.vinifera*	USA	R	R
46	Kay Grey	Interspecific hybrid	USA	R	S
48	Muscat Bailey A	Interspecific hybrid	Japan	R	S
49	Isabella	*V.labrusca*	Yalova/Turkey	R	S
50	Rize Geççi	*V.labrusca X V.vinifera*	Yalova/Turkey	S	S
51	Köfteci Üzümü	*V.labrusca*	Yalova/Turkey	H R	R
62	FX1-1	*V.vinifera*	Yalova/Turkey	R	HS
63	BX1-166	*V.vinifera*	Yalova/Turkey	S	S
64	KXP-10	*V.vinifera*	Yalova/Turkey	S	HS
65	Özer Karası	*V.labrusca X V.vinifera*	Yalova/Turkey	S	S
67	86/1	*V.vinifera*	Yalova/Turkey	S	HS
68	85/1	*V.vinifera*	Yalova/Turkey	S	HS

TABLE I (continuation)

The origin of cultivars/genotypes and inoculation results (N: No band associated with resistance allele; ER:Extremely Resistant, HR:Highly Resistant, R:Resistant, S:Susceptible and HS:Highly Susceptible after inoculation).

Origem das cultivares / genótipos e resultados de inoculação (N: Sem banda associada ao alelo de resistência; ER: Extremamente resistente, HR: Altamente resistente, R: Resistente, S: Susceptível e HS: Altamente susceptível após a inoculação)

Code	Cultivar/ Genotype	Species	Origin	Downy Mildew	Powdery Mildew
69	Atak 77	*V.vinifera*	Yalova/Turkey	S	S
70	Pembe 77	*V.vinifera*	Yalova/Turkey	S	HS
71	Arifbey	*V.vinifera*	Yalova/Turkey	R	S
72	FX1-10	*V.vinifera*	Yalova/Turkey	S	HR
73	Gülgönül	*V.vinifera*	Yalova/Turkey	S	S
74	Güzgülü	*V.vinifera*	Yalova/Turkey	S	S
76	Siyah Geççi	*V.vinifera*	Yalova/Turkey	R	HS
81	Yusufeli İri beyaz	*V.vinifera*	Artvin/Turkey	S	HS
85	Giresun 1	*V.labrusca*	Giresun/Turkey	R	R
88	Giresun 2	*V.labrusca*	Giresun/Turkey	R	R
89	Giresun 3	*V.labrusca*	Giresun/Turkey	HR	R
94	Sinop 1	*V.labrusca*	Sinop/Turkey	R	S
95	Sinop 2	*V.labrusca*	Sinop/Turkey	R	HS
97	Sinop 3	*V.labrusca*	Sinop/Turkey	R	HS
98	Sinop 4	*V.labrusca*	Sinop/Turkey	-	-
128	Gürcü	*V.vinifera*	Tekirdağ/Turkey	R	S
131	Favli	*V.vinifera*	Tekirdağ/Turkey	S	HS
132	Mercan	*V.vinifera*	Tekirdağ/Turkey	S	HS
133	Yerli İsabella	*V.labrusca*	Tekirdağ/Turkey	R	S
134	Çınarlı karası	*V.vinifera*	Tekirdağ/Turkey	S	HS
135	Tilki Kuyruk	*V.vinifera*	Zonguldak/Turkey	S	HS
136	Alüzüm	*V.vinifera*	Zonguldak/Turkey	R	S
153	Steuben	Interspecies	Geneva/USA	HR	S
154	Price	Interspecies	Geneva/USA	R	HS
155	Concord Seddless	*V.labrusca X V.vinifera*	Geneva/USA	R	S
156	Edelweiss	Interspecies	Geneva/USA	R	S
157	Canadice	*V.labrusca X V.vinifera*	Geneva/USA	ER	S
184	Çayeli 4	*V.labrusca*	Rize/Turkey	R	R
192	Katıkara 1	*V.labrusca*	Ordu/Turkey	S	H S
193	Katıkara 2	*V.labrusca*	Ordu/Turkey	R	S

DNA isolation, PCR reactions and fragment analyses were conducted at Molecular Genetic Laboratories of Ankara University, Faculty of Agriculture, Department of Horticulture. The present investigation was carried out during the production period of the years 2014-15 and 2015-16.

Inoculation for DM

The method of Rumbolz *et al*. (2002) and Boso *et al*. (2006) were used to propagate sporangia inoculum. *Plasmopara viticola* was obtained from naturally infected plants in the vineyards of the Atatürk Horticulture Central Research Institute Yalova, Turkey. Plants were sprayed with a suspension of sporangia (40,000 sporangia ml^{-1} distilled water) on the abaxial leaf side and whole plants were covered with a polyethylene cover during the night. On following day, the polyethylene covers were removed and incubation lasted 5-6 days at 25-26 ºC. The same inoculation procedure was repeated after 5-6 days in order to provide a better infection.

Inoculation for PM

The modified method of Wang *et al*. (1995) was applied for inoculation of plants with PM. Briefly, fungal conidia were collected from naturally infected leaves from the vineyard, washed with 78% glucose solution, imitating the osmotic pressure of PM conidia and then suspended in sterile water. Vine leaves were inoculated with the conidial suspension at the rate of $2x10^5$ sporangia ml^{-1} by spraying the upper surface of the leaves. Inoculated leaves were immediately covered by thin plastic for 6 hours.

Evaluation of inoculated leaves

For disease evaluation, four young leaves from each genotype or cultivar were arbitrarily selected for resistance evaluation for each disease. The symptoms on each leaf were rated from 0 to 7, based on the estimated percentage of lesion over the whole leaf as follows (Wan *et al*., 2007): Grade 0 = no symptoms, 1 = 0.1-5.0 %, 2 = 5.1-15.0 %, 3 = 15.1-30.0 %, 4 = 30.1- 45.0 %, 5 = 45.1-65.0 %, 6 = 65.1-85.0 %, 7 = 85.1-100.0 %. The grades were then converted into a susceptibility index (SI). Disease severity was evaluated 3 weeks after inoculation.

SI = {[Sum of (Grade value x number of leaves in that grade)] / (Total leaf number x Highest grade value) } x 100.

DM and PM resistance levels of each genotype were rated in five categories based on its SI value: SI = 0 extremely resistant (ER). SI = 0-5 highly resistant (HR). SI = 5.1-25 resistant (R). SI = 25.1-50 susceptible (S). SI = 50.1-100 highly susceptible (HS).

DNA extraction

Young leaves were collected and immediately frozen at deep freeze. DNA extraction was carried out using the method of Norgen Biotek Plant/Fungi Isolation Kit (Norgen Biotek Corporation, Canada). The DNA quality analysed with both methods; gel electrophoresis and spectrophotometer (NanoDrop ND-1000 Spectrohotometer, Wilmington, DE, USA).

PCR Analyses

Six SSR markers; VMC8g9, VMC4f3.1, VMC1g3.2, UDV020a, VMC9h4.2, VMC7f2 and SCAR marker SCORA7 were used for PCR analyses. Markers used in the study and related references are shown on Table II.

TABLE II

Markers used in the study

Marcadores utilizados no estudo

Marker Name	Choromosome	Position on Chromosome (Mb)	Forward primer	Reverse Primer	Reference
VMC8g9	12	20.4	AACATTATCAACAACA TGGTTTTA	ATATTCATCCTTCCCA TCACTA	Barker *et al.* (2005)
VMC4f3.1	12	13.1	AAAGCACTATGGTGG GTGTAAA	TAACCAATACATGCAT CAAGGA	Barker *et al.* (2005)
UDV-020a	13		TGTTGGTGTGTGTTTG TACGTG	TGTTGGCCTGATGTTG AGAG	Hoffmann *et al.* (2008)
VMC9h4.2	13	18.4	GCAGTTGATGCAAAA CAACAGT	CACATCATTCATTGAT GAGGCT	Hoffmann *et al.* (2008)
ScORA7	15	-	GAAACGGGTGTGAGG CAAAGGTGG	GGCCATTAGGAAATCA ACATTAC	Akkurt *et. al.* (2007)
VMC1g3.2	12	10.0	GATAGTTACCATACTT AGTCGGA	ACTTAGCTTCAGAAGA AAATAGA	Merdinoglu *et al.* (2003)
VMC7f2	18	26.9	TCCGACTTTGGGTAAT AAGC	AAGATGACAATAGCG AGAGAGAA	Bellin *et al.* (2009)

PCR reactions for six SSR loci were performed in a reaction volume of 10 µl containing 15 ng of DNA, 5 pmol of each forward and reverse primer, 0.5 mM dNTP, 0.5 unit GoTaq DNA Polymerase (Promega, Madison, WI) that includes 1.5 mM $MgCl_2$. Amplifications were performed on a Biometra Uno Thermocycler (Biometra, Göttingen, Germany) with the following cycling conditions; initial denaturation step 3 min at 94°C, followed by 35 cycles of denaturation at 1 min at 94°C, annealing 1 min at 50-

60°C (depending on each primer pair-specific annealing temperature) and 2 min at 72°C with a final extension 10 min at 72°C. For SCAR marker PCR assays were performed in standard assays in a total volume of 20 µl containing 0.25 mM of each dNTP, 0.25 µM of each primer, 0.5 U Taq DNA polymerase, 1.5 mM $MgCl_2$, and 20-40 ng template DNA. The PCR conditions were as follows: an initial cycle of 3 min at 95°C, followed by 30 cycles of denaturation at 1 min at 95°C in, annealing for 1 min at 60°C, a synthesis step 2 min at 72°C, and final extension 10 min at 72°C.

PCR products from SSR and SCAR markers were checked by gel electrophoresis (1.5-2 % agarose) under UV light. Fragment size of PCR products obtained by SSR technique was determined by Fragment Analyser (Advanced Analytical Technologies, Inc. IS, USA). PCR products were analysed according to the Fragment Analyser protocol. Allele size of the obtained peak images were determined by ProSize software.

In each PCR run, Regent (Interspecies hybrid) and Sugargate (*M. rotundifolia*) were included as the reference cultivars because of the presence of disease resistance loci. Razakı (*V. vinifera* cv.) was used as negative control.

RESULTS AND DISCUSSION

In Turkey, there is a noticeable variety of climates, with considerable differences between the areas, and also with some microclimates due to exposure on slopes and coasts. The north of Turkey is quite rainy, but has relatively warm and humid regions. Turkey is one of the most important countries for vineyard areas and grape production. Due to ideal climatic conditions and suitable growing circumstances, Turkey has very rich *Vitis* germplasm. The northern part of Turkey, especially, has some fungal disease resistant/tolerant genotypes. A large part of the material was collected from this region. These cultivars/genotypes grow in a very humid ecology. They predominantly consist of *V. labrusca* and *V.labrusca x V.vinifera* hybrids.

In this study, fungal disease tolerant/resistant cultivars/genotypes which were collected from humid regions (mainly from Turkey) had disease resistance level determined with molecular markers and also artificial inoculations.

Eighty cultivars/genotypes were analysed using the seven markers considered responsible for DM and PM resistance. Allele sizes of cultivars/genotypes were compared with Regent (interspecies hybrid, complex resistance against DM and PM) and Sugargate (*M. rotundifolia*), both presenting a high degree of tolerance to DM and PM.

Six SSR primers (VMC8g9, VMC4f3.1, VMC1g3.2, UDV20a, VMC9h4, VMC7f2) and one SCAR primer (SCORA7) were used in association with the Run1, Rpv1, Ren1 and Rpv3 resistance genes for marker-assisted selection. VMC8g9, VMC4f3.1, UDV020a, VMC9h4.2 and SCORA7 markers were used for determination of PM resistance. VMC1g3.2 and VMC7f2 markers were used for determination of DM resistance (Table II).

The resistance loci against PM (Run1) is a single dominant gene present in *Muscadinia rotundifolia*, but absent in *Vitis vinifera* species. This resistant locus was introgressed successfully from *Muscadinia rotundifolia* into *V. vinifera* using a pseudo-backcross strategy (Barker *et al*., 2005). Several genetic markers that are linked to the resistance locus were previously identified by researchers. VMC8g9, VMC4f3.1 and GLP1-12 were successfully used to identify PM resistant genotypes (Marguerit *et al*., 2009; Katula-Debreceni *et al*., 2010; Agurto *et al*., 2017).

VMC8g9 and VMC4f3.1 markers were used to determine PM resistance associated with the resistance gene Run1. Molnar *et al*. (2007) used the same markers and they found 186 bp allele size for VMC4f3.1 and 160 bp allele size for VMC8g9 in resistant individuals in the *M.rotundifolia* X *V.vinifera* hybrid population. In our study, no genotype presented a 186 or 160 bp allele size. This result may have occurred because our genotypes did not originate from *M.rotundifolia.* Most of the genotypes used in our study were *V.labrusca* and the natural hybrid of *V.labrusca X V.vinifera* so we could not detect any Run1 positive genotypes. However, Kozma *et al*. (2009) reported that VMC4f3.1 and VMC8g9 markers could be used to identify resistant individuals in their population. They developed new hybrids that are resistant to PM in their project. These hybrids were developed from Kishmish vatkana BC3 and BC4 hybrids and *V. amurensis* X *V. vinifera* hybrids.

VMC1g3.2 marker was described as an Rpv1-associated marker by Wiedemann-Merdinoglu (2003). With this marker, we obtained allele size of between 132-274 bp. A large number of cultivars/genotypes were found to have the same band sizes (140 and/or 153 bp) as the control cultivars (Regent and Sugargate) in our study. Katula-Debreceni *et al.* (2010) identified the allel size of the VMC1g3.2 marker associated with the Rpv1 gene as 122 bp. Cultivars/genotypes used in our study did not show alleles related to resistance.

The resistance gene observed in cultivated plants of *Vitis vinifera* is called Ren1. Ren1 was first determined by Hoffman *et al*. (2008) in 'Kishmish vatkana' and 'Dzhandzhal kara' which are cultivated in Central Asia since the 1920s. As a result of Hoffmann *et al*. (2008), individuals with a size of 161 bp allele for the UDV020a marker and those individuals with VMC9h4.2 marker size of 283 bp allele were associated with Ren1. Ren 1 was detected on chromosome 13, unlike the resistance genes previously found in wild North American grapes and their interspecific hybrids (Coleman *et al.*, 2009). In our study, we tested both markers in relation to Ren 1. When fragment sizes obtained from the VMC9h4.2 marker were examined, in our study, six cultivars/genotypes were identified as having 283 bp alleles (Table III). Contrary to expectations, inoculation results indicate that these cultivars/genotypes were susceptible to PM; however, they were resistant to DM. In case of UDV020 marker, genotype 98 (Sinop 4/*V. labrusca*) showed an allele size of 161bp and was selected as a resistant candidate genotype (Table III).

TABLE III

Markers used in the study and resistant candidate genotypes after MAS

Marcadores utilizados no estudo e genótipos candidatos resistentes após Seleção Assistida por Marcador

Marker	Associated gene	Resistance origin	Resistant-Associated allele size (bp)	Resistant-Candidate Genotypes	Determined allele size (bp)
VMC8g9	Run1	*M. rotundifolia*	160	-	-
VMC4f3.1	Run1	*M. rotundifolia*	186	-	-
VMC1g3.2	Run1	*M. rotundifolia*	122	-	-
UDV020a	Ren1	Kishmish vatkana	161	98 (Sinop 4)	158/161
VMC9h4	Ren1	Kishmish vatkana	283	12 (Rizpem)	283/292
				14 (Rizellim)	283/292
				26 (Concord)	283/292
				27 (Mars)	272/283
				31 (Valiant)	283/283
				32 (Kyoho)	283/290
VMC7F2	Run2.1	*M. rotundifolia*	193	-	-
VMC7F2	Rpv3	Bianca	-	4 (57 Gerze 04)	210/216
				38 (Orion)	210/216
				40 (Staufer)	210/216
				44 (Mortensen)	210/216
				51 (Köfteci Üzümü)	210/216
SCORA7	Ren3	Regent	760	2 (Batum 4)	760
				17 (55 Merkez 11)	760
				37 (Boscop Glory)	760
				38 (Orion)	760
				40 (Staufer)	760
				42 (Sirius)	760
				43 (Phoenix)	760
				47 (Sugargate)	760
				184 (Rize Çayeli 4)	760

Akkurt *et al*. (2007) developed SCAR markers associated with Ren3 resistant loci to *Uncinula necator* from QTL regions of Regent x Lemberger hybrids. The SCORA7 marker was determined to be

useful in marker-assisted selection for resistance to powdery mildew and also later referred to as a Ren3-associated marker in different publications (Eibach *et al.*, 2007; Van Heerden *et al.*, 2014). When the results obtained from the SCORA7 marker were examined, the fragment of 760 bp associated with resistance was obtained in 10 cultivars/genotypes including Regent and Sugargate (Table III). Also, when we evaluated the results from our disease scores, some of these genotypes have this band even though they are susceptible. These are Batum 4 (*V. labrusca*), Orion (interspecies hybrid) and Boscop Glory (*V.vinifera*). The other seven cultivars/genotypes were found to be resistant or very resistant in accordance with the SCORA7 marker (Table III).

The VMC7f2 marker was analysed in our population and we obtained an allele size of between 210 bp and 222 bp. Forty-six cultivars/genotypes showed the same allele sizes for at least one locus of Regent or Sugargate. Also, 5 of these cultivars/genotypes were found to have the same for both alleles (210 bp and 216 bp) compared with Regent (Table III). These 5 genotypes, which have the same allele as Regent, were found to be mildew resistant in inoculation test results. Bellin *et al.* (2009) reported that in the genetic map of 'Bianca' x 'Chardonnay' hybrid population, the VMC7f2 marker is closely related to the Rpv3 gene. 'Bianca' is heterozygous for a dominant gene, located in a 2.9 cM interval at the Rpv3 locus on chromosome 18. 'Villard Blanc' is in the pedigree of 'Bianca' and 'Regent' and it is also a common parent. It is claimed that the downy mildew resistance locus was transferred to 'Bianca' from 'Villard Blanc' (Bellin *et al.*, 2009). This case explains the presence of downy mildew resistant genotypes bearing the VMC7f2 marker and Regent alleles. Riaz *et al.* (2011) studied VMC7f2 marker for *M. rotundifolia* cv. Mognolia and they reported that it was associated with the Run2.1 gene which is responsible for PM resistance on chromosome 18. The researchers detected Run2.1 allele associated with resistance at 193 bp locus. In our study, no cultivar/genotypes which have 193 bp allele related to *Muscadinia* origin resistance locus were found.

Several studies reported that some cultivars or genotypes may be resistant to both PM and DM diseases. Kozma and Dula (2003) studied interspecies hybrids and they reported that some hybrids resistant to PM may also be resistant to DM. They also associated double disease resistance with the Run1 gene. Similarly, Merdinoglu *et al*. (2003) used 208 SSR, 13 ISSR and 151 RAPD markers in order to find markers which are associated with resistance to DM in *Muscadinia* cultivars. They strongly suggested that the identified QTL corresponds to a unique major gene conditioning DM resistance in the *Muscadine* grapes, which was named Rpv1. Moreover, Rpv1 was shown to be tightly linked to the dominant gene conferring resistance to PM, Run1. Similarly, in this study, it was found that especially few *V. labrusca* and interspecific hybrid cultivars/genotypes were resistant to both diseases. It was observed that *V. labrusca* and interspecies cultivars or genotypes are significantly more resistant to both diseases than *V.vinifera* especially in the species-specific evaluation. This was more pronounced for PM disease (Table I).

In this study, eighty cultivars/genotypes were studied with 7 different markers and also PM and DM inoculations were applied over two years. When cultivars/genotypes were evaluated, a total of 204 bands were obtained with at least one locus size the same compared with the control cultivars (Regent and Sugargate). Also 24 cultivars/genotypes have the same locus size for both alleles compared with Regent or Sugargate.

One of the *V. labrusca* genotypes with the code number 94, which gave the same band sizes as Regent for 4 markers, attracted attention. However, according to the results of disease inoculations, this genotype was found to be resistant to DM but sensitive to PM. Nevertheless, when the disease inoculation and molecular analysis results of the study are evaluated together, the results were compatible with each other.

Cultivars/genotypes of *V. labrusca* and interspecies have more gene regions which are associated with disease resistance compared to *V. vinifera* cultivars/genotypes. Especially after evaluation of both diseases, *V. labrusca* and interspecific hybrids were determined to be more resistant. It is clear that it would be appropriate to use *V. labrusca* and interspecific hybrids as parents in resistance breeding programs in the future.

When these results evaluated together with inoculation observations; especially 'Köfteci Üzümü' (a possible *Vitis labrusca* genotype) and 'Giresun 3' (a possible *Vitis labrusca* genotype) were selected as the most resistant cultivars/genotypes (Table I and III). Although no resistance locus could be detected in 'Giresun 3' genotype, inoculation observations indicated high resistance to both diseases.

CONCLUSIONS

In this study, it was determined the resistance of powdery and downy mildew diseases of cultivars/genotypes collected from different humid regions with marker assisted selection and disease

inoculations. The results of the study indicate that the genotypes that belong to *V.labrusca* species are highly has shown to be resistant to diseases. In particular, the resistance to downy mildew disease was higher.

Local genotypes of 'Köfteci üzümü' and 'Gerze 04' were selected as the most resistant genotypes after marker assisted selection and inoculation test. While the genotypes 'Sinop 4', 'Rizpem' and 'Rizellim' revelled resistance to powdery mildew with molecular markers they not presented resistance in inoculation tests. The genotype of 'Giresun 3' was found highly resistant to both diseases as a result of inoculation tests, however as a result of selection by molecular markers, it was not found to be related to resistance to diseases.

According to these results obtained, marker-assisted selection and disease inoculation tests should be applied together in disease-resistant breeding programs for different *Vitis* spp. In addition, cultivars/genotypes belonging to the *V. labrusca* species, which are resistant to fungal diseases, can be used in breeding programs to transfer resistance characteristics to the new high-quality cultivars. Thus, much more environmentally- friendly cultivars can be obtained.

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