Search for donors of powdery mildew resistance genes among seedless and table grape varieties

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Abstract. Powdery mildew (*Erysiphe necator*) is one of the most common and economically significant diseases of grapes. Currently, the main method of controlling the disease is pesticide treatment. Breeding of resistant varieties is necessary to reduce chemical treatments. Currently, a number of grape resistance genes to powdery mildew and DNA markers for identification the allelic status of these genes are known. In a study to determine the presence of resistance loci *Ren3* and *Ren9*, 25 genotypes of table grape varieties were analyzed, including 18 seedless varieties. DNA markers GF15-42, ScORGF15-02 were used to identify *Ren3* gene, and CenGen6 – to identify *Ren9* gene. DNA of cultivars Regent and Seyve Villard 12-375, which have resistance alleles, were used as positive controls. As a result of DNA marker analysis, it was determined that genotypes of table varieties Viking, Kodryanka, Moldova, Nadezhda AZOS, Original and seedless varieties Pamyati Smirnova, Kishmish Zaporozhskiy and Kishmish 342 carry loci of resistance to powdery mildew *Ren3* and *Ren9*.

1 Introduction

Table grapes are beneficial for the people, as they are rich in vitamins of groups A, C, D, E, P, K, PP and B, contain minerals, organic acids, etc. Grapes are one of the products that can be eaten both fresh and dried (raisins) [1]. Most often, the population chooses seedless grapes from table varieties for fresh consumption. Seedless grape varieties also play an influential role in the industry, as almost all dried products are made from them [2].

Fungal diseases pose a great threat to industrial viticulture, as they can lead to the loss of most of the harvest. One of these diseases is powdery mildew of vines. The causative agent of this disease is *Erysiphe necator* Schwein. – biotrophic ascomycete, which colonizes the epidermal cells of photosynthetic tissues and spreads through subsequent asexual cycles [3]. Infected leaves exhibit reduced photosynthesis and often prematurely age and fall off. Early infection of berries causes their cracking, and the overall impact on the harvest includes a decrease in yield, an increase in acidity and a decrease in the content of anthocyanin and sugar in mature berries. Even a low level of powdery mildew

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contamination of berries can lead to spoilage of table grapes and a subsequent deterioration in varietal characteristics. [4].

Fungicidal treatments are currently used to control *Erysiphe necator*. However, for table varieties, this is not always safe, since there is a risk of accumulation of reagent residues in berries that are used for food by people. Therefore, an important task is to create genetically resistant to powdery mildew table grape varieties, which reduces the pesticide load on vineyards. The best quality characteristics of the harvest are possessed by European grapevines (*V. vinifera* L.), among this species there are genotypes with tolerance to powdery mildew, but most varieties are susceptible to this disease. Resistance to *Erysiphe necator* is observed in North American (*V. riparia*, *V. aestivalis*, *V. rupestris*, *V. berlandieri*, etc.), as well as in Asian (*V. romanetii*, *V. piazezkii*, etc.) grape varieties [5, 6]. According to published data, currently more than 10 resistance loci have been identified, which are responsible for resistance to powdery mildew, they have a common symbolism of *Run* and *Ren* [7-12]. It was also possible to map the locus of resistance to *Erysiphe necator* in *V. vinifera* gene pool, namely in the genotypes of varieties Kishmish vatkana (*Ren1*) and Chardonnay (*Sen1*) [13, 14].

Ren3 resistance locus was first described by Fischer et al. (2004) in a cross-population of "Regent" × "Lemberger" and additionally characterized by Welter et al. (2007) [15, 16]. Later, during the mapping of *Ren3* locus in the same hybrid population, Zendler and his colleagues (2017) discovered *Ren9* resistance locus [11]. The *Ren3* and *Ren9* loci were identified on chromosome 15, and DNA markers suitable for detecting the allelic status of these genes were identified [11, 16].

The purpose of this study is to search for donors of the genes of resistance to powdery mildew *Ren3* and *Ren9* among table and seedless grape varieties using recognized DNA markers.

2 Materials and Methods

The study included table and seedless grape varieties growing in the Anapa ampelographic collection, as well as in private farms.

In this work, we used markers GF15-42, ScORGF15-02 to identify *Ren3* gene, and CenGen6 to identify *Ren9* gene. The Regent and Seyve Villard 12-375 varieties were used as positive controls - these genotypes carry alleles of resistance to powdery mildew.

DNA was isolated from the young leaves of the apical part of the shoots of the studied varieties using the CTAB method [17]. PCR was performed using reagents manufactured by OOO «SibEnzim-M» (Russia, Moscow). The volume of the reaction mixture was 20 µl, it contained 50 ng of genomic DNA, 1.5 units of Tag polymerase, 1x buffer for Tag polymerase with ammonium sulfate and magnesium, 2 mM MgCl2, 0.2 mM each dNTP (deoxynucleotide triphosphates). The conditions of the polymerase chain reaction with the marker CenGen6 have been tested in previous studies [18]. For the markers GF15-42 and SCORGF15-02, the annealing temperature of the primers was selected, equal to 60 °C. The optimal protocol was chosen as follows: initial denaturation at +95 ° C – 5 minutes; then 34 cycles of synthesis: denaturation – 10 seconds at +95 ° C, annealing of primers – 30 seconds at +60 °C, elongation – 30 seconds at +72 °C; final elongation – 3 minutes at +72 °C.

The automatic genetic analyzer ABI Prism 3130 was used in the work, on it the fragments obtained by PCR were separated. The size of the amplified fragments was estimated using special software (GeneMapper and PeakScanner). The data obtained were corrected in accordance with the control varieties (Regent and Seyve Villard 12-375), which have a known allelic composition.

3 Results and Discussion

There were analyzed 25 genotypes, including 18 seedless varieties. According to the literature data, the presence of *Ren3* allele of resistance to powdery mildew in grape genotypes correlates with fragments of 199 and 242 base pairs (bp) detected by markers GF15-42 and ScORGF15-02, respectively, determined by PCR analysis. The presence of *Ren9* resistance allele is determined by the detection of the PCR fragment 287 bp when analyzing using the CenGen6 marker. Both resistance loci *Ren3* and *Ren9* were found in the genotypes of varieties Pamyati Smirnova, Viking, Kishmish Zaporozhskiy, Kishmish 342, Kodryanka, Moldova, Nadezhda AZOS, Original (table 1).

Table 1. Identified alleles for the studied loci, bp.

Variety	Pedigree	*Ren3*		*Ren9*
		GF15-42	ScORGF15-02	CenGen6
Regent (control)	Diana x Chambourcin (**Seyve Villard 12-417** x Seibel 7053)	**199**	**242**	277:**287**
Seyve Villard 12-375 (control)	Seibel' 6468 x Seibel' 6905	**199**	**242**	276:**287**
Pamyati Smirnova*	**Seyve Villard 12-375** x Kishmish tairovskiy rozovyi	**199**	**242**	277:**287**
Viking	ZOS-1 x Kodryanka (Moldova (Guzal kara x **Seyve Villard 12-375**) x Marshal'skiy)	**199**	**242**	**287**
Kishmish Zaporozhskiy*	Viktoriya x Rusbol (**Seyve Villard 12-375** x Sverhranniy bessemyannyi)	179:**199**	**242**	277:**287**
Kishmish 342*	**Seyve Villard 12-375** x Perlette	**199**	240:**242**	275:**287**
Kodryanka	Moldova (Guzal kara x **Seyve Villard 12-375**) x Marshal'skiy	**199**	**242**	277:**287**
Moldova	Guzal kara x **Seyve Villard 12-375**	**199**	**242**	276:**287**
Nadezhda AZOS	Moldova (Guzal kara x **Seyve Villard 12-375**) x Cardinal	**199**	**242**	277:**287**
Original	Damasskaya roza x Dattier de St. Vallier (Panse de Provence x **Seyve Villard 12-375**)	185:**199**	**242**	277:**287**
Attika*	Alphonse Lavallee x Kishmish chernyi	179:195	240	276
Bessemyannyi Magaracha*	(Katta-Kurgan x Kirovobadskiy stolovyi) x Sverhranniy bessemyannyi	187:224	240	277
Vanessa seedless*	Seneca x New York 45910	193:**199**	239	277
Vostorg	(Zarya severa x Dolores) x Russkiy ranniy	196	241	277
Kishmish belyi oval'nyi*	Ancient Central Asian variety	185	240	275:277
Kishmish VIRa*	Babara x Kishmish chernyi	187	240	277
Kishmish Moldavskiy*	Pobeda x Kishmish rozovyi	183	240	275:277
Kishmish rozovyi*	Ancient Armenian grape variety	185	240	275:277
Kishmish rozovyi AZOS*	Kriulyanskiy x Yangi Er	185:191	240	272:277
Kishmish Sogdiana*	Pobeda x Kishmish chernyi	**199**	240	272:285

Kishmish chernyi AZOS*	Kriulyanskiy x Yangi Er	183:193	240	272:277
Mars*	Campbell early x Arkanzas 1339	187:**199**	239	275:277
Muskat letniy	Seyve Villard 20-366 x Koenigin der weingaerten	196	240	277
Perlette*	Koroleva vinogradnikov x Sultanina	193:219	240	275:277
Remaily Seedless*	Lady Patricia x NY 33979	179	240	277
Romulus*	Ontario x Kishmish belyi	185	240	277
Ruby Seedless*	Emperor x Sultanina muskatnaya	179	240	275:281

Note: target alleles and genotypes-donors of these alleles are highlighted in bold;
* - seedless grape varieties

Analyzing the pedigree of varieties in which resistance alleles were found, it can be concluded that in all these genotypes, the original donor of *Ren3* and *Ren9* is Seyve Villard 12-375. In varieties Pamyati Smirnova, Moldova, Kishmish 342 genes are inherited directly from Seyve Villard 12-375. The varieties Viking, Kishmish Zaporozhskiy, Kodryanka, Nadezhda AZOS, Original inherited the resistance loci through the descendants of Seyve Villard 12-375 (table 1).

These varieties can be included in further breeding work on the creation of table grape varieties as donors of *Ren3* and *Ren9* genes, since, in addition to presence of these loci of resistance to powdery mildew, varieties have a complex of other economically valuable traits, bunches and berries have an attractive appearance. The genotypes of Pamyati Smirnova, Kishmish Zaporozhskiy and Kishmish 342 can be used in breeding of seedless grape varieties.

4 Conclusion

It was determined by DNA marker analysis that genotypes of table varieties Viking, Kodryanka, Moldova, Nadezhda AZOS, Original and seedless varieties Pamyati Smirnova, Kishmish Zaporozhskiy and Kishmish 342 carry loci of resistance to powdery mildew *Ren3* and *Ren9*.

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