Analysis of the SC8-0071-014 and sc47-18 loci co-segregated with *Ren1* gene in the genotypes of seedless grape varieties

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Abstract. Powdery mildew (*Erysiphe necator*) is one of the most common and economically significant diseases of grapes. The main method of controlling the disease is pesticide treatment. To reduce chemical treatments, it is necessary to select and introduce resistant varieties into production. DNA markers are currently actively used in the study of grape genetic resources. Seedless grape varieties are highly demanded by consumers. *Ren1* is one of the known and mapped vine resistance genes to powdery mildew, inherited from *V. vinifera*; linked DNA markers for this resistance locus are known. A study of 34 seedless grape genotypes was carried out using DNA markers SC8-0071-014 and sc47-18 co-segregated with *Ren1*. In the studied sample of varieties, 12 types of alleles were identified in the sc47-18 locus and 9 types of alleles in the SC8-0071-014 locus. Target fragments, according to linked marker loci, indicating the presence of the *Ren1* resistance gene, were identified in grape variety Lotus (Kriulyanskiy x Yangi Er).

1 Introduction

Powdery mildew disease is caused by the ascomycete *Erysiphe necator* (earlier *Uncinula necator*), which is considered a local pathogen of North America [1]. The pathogen is able to infect all the green tissues of the plant – shoots, leaves, inflorescences, berries. Ascospore colonies are most often localized on the lower surface of the grape leaf, on the upper surface of the leaf they are manifested by a chlorotic spot. Severely affected leaves age quickly, necrotic spots appear on them and they fall off prematurely. On the stems, *Erysiphe necator* initially causes symptoms similar to those on the leaves, but the colonies on the shoots eventually die as the periderm forms, producing a dark, web-like scar on the cane [2]. Since the infection is not limited to specific humidity and temperature conditions, the pathogen poses a global threat to viticulture.

Genetic resistance to powdery mildew is mainly observed in the North American group of grapes, such as *V. aestivalis*, *V. berlandieri*, *V. cinerea* and *V. labrusca*, and Asian – *V. amurensis*, *V. bashinica*, *V. davidii*, *V. liubanensis*, *V. piazezkii* и *V. romanetii* [3, 4]. A

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number of resistance loci under the names *Ren* and *Run* are currently identified and mapped [5-14].

However, in *V. vinifera* grape variety – Kishmish vatkana, locus of resistance to powdery mildew *Ren1*, localized on chromosome 13, was determined [15]. Later *Ren1* was found in another *V. vinifera* variety – Janjal kara, related to Kishmish vatkana [16]. To determine the allelic state of the *Ren1* locus, Coleman et al. (2009) proposed DNA markers sc47-6 and sc47-20, as they were located at a distance of 0.7 cM from the *Ren1* resistance region. Later Li et al. (2013) discovered chromosomal recombination in this region and proposed 4 more markers: SC8-0071-014, sc47-19, sc47-33, and sc47-18 [17]. Further studies showed that SC8-0071-014 and sc47-18 markers co-segregating with the *Ren1* locus are the most suitable for marker selection [18].

Seedless grape varieties are highly demanded by consumers. Growing resistant genotypes of grapes makes it possible to obtain environmentally friendly products. The breeding of resistant varieties is based on the use of existing genetic diversity, the search for donors and sources of valuable traits. Currently, MAS (marker assisted selection) technologies are actively used in grape breeding.

The aim of this work was to study seedless grape genotypes with DNA markers of *Ren1* resistance locus to powdery mildew in order to search for potential donors of the gene.

2 Materials and Methods

DNA marker analysis was carried out on 34 genotypes of seedless varieties and grape hybrids of various genetic origins growing in the Anapa ampelographic collection. Kishmish vatkana was used as a control genotype in DNA analysis.

DNA extraction was carried out by a modified method using CTAB (cytyltrimethylammonium bromide) from tops of young shoots of the studied varieties [19].

The allelic composition of *Ren1* locus was identified using the SC8-0071-014 and sc47-18 markers by polymerase chain reaction (PCR) using an Eppendorf MasterCycler nexus GX2 amplifier (Germany) according to experimentally selected conditions. PCR was performed in 20 µl of a mixture containing 50 ng of DNA, 1.5 units of Taq polymerase, 1x buffer for Taq polymerase with ammonium sulfate and magnesium, 2 mM MgCl2, 0.2 mM of each dNTP (deoxynucleotide triphosphates) (SibEnzym-M, Moscow) and 200 µM of each primer (OOO Sintol, Moscow).

PCR products were separated by capillary electrophoresis on a Nanofor 05 genetic analyzer (Institute of Analytical Instrumentation, Russian Academy of Sciences, St. Petersburg, Russia) with subsequent assessment of their size using special built-in software.

Statistical data processing was carried out using the GenAlEx 6.5 program [20].

Molecular genetic analyzes were performed using the equipment of the Center for Collective Use of the North Caucasus Federal Scientific Center for Horticulture, Viticulture, Winemaking in the direction of "Genomic and post-genomic technologies".

3 Results and Discussion

DNA markers SC8-0071-014 and sc47-18 are used in studies on DNA marker assisted selection and in the determining genotypes with *Ren1* powdery mildew resistance locus [18, 21, 22]. Using these DNA markers, we screened 34 seedless grape varieties genotypes of various origins (*V. vinifera* varieties, interspecific hybrids, varieties of unknown origin). It is known from the literature data that the sc47-18 allele is 249 base pairs (bp) in size and SC8-0071-014 is 143 bp cosegregate with *Ren1* resistance locus. To refine the size of the

PCR fragments, DNA of Kishmish vatkana variety was used in the work, after DNA fingerprinting of this variety was performed, which showed full compliance with the DNA profile of this variety in the international catalog of DNA passports of grape varieties in the VIVC database [23]. In the studied sample of varieties, 12 types of alleles were identified in the sc47-18 locus, the most common alleles were 231 and 236 bp (frequency of occurrence 0,306 and 0,250, respectively). In the locus SC8-0071-014, 9 types of alleles were identified, alleles 160 and 200 bp found in most of the studied genotypes (frequency of occurrence 0,292 and 0,278, respectively). The values of observed heterozygosity (Ho) for both loci exceeded the expected value (He): sc47-18 Ho – 0,889, He – 0,811; SC08-0071-014 Ho – 0,806, He – 0,797.

Target fragments for the sc47-18 marker were identified in 4 samples: Kishmish belyi kruglyi, Kishmish kruglyi, Kishmish chernyi AZOS, Lotus (table). The target allele for the marker SC8-0071-014 was determined only in the Lotus genotype. Thus, Lotus grape variety is a potential donor of *Ren1* powdery mildew resistance gene.

Table 1. Allelic composition of the studied grape varieties for the *Ren1* locus.

Varieties	*Ren1*	
	sc47-18	SC8-0071-014
Kishmish vatkana (control)	231:**249**	**143:**160
Attica seedless	231:231	160:170
Belgradskiy bessemyannyi	236:240	160:200
Bessemyannyi Magaracha	236:240	200:200
Centennial Seedless	231:236	170:200
Vanessa	231:231	170:170
Hybrid Tishchenko	221:240	158:170
Detskiy	221:240	163:163
Kishmish belyi kruglyi	236:**249**	170:200
Kishmish belyi oval'nyi (Sultanina)	231:236	160:200
Kishmish VIR	227:236	200:200
Kishmish chernyi AZOS	231:**249**	160:170
Kishmish dubovskiy	240:240	160:164
Kishmish kruglyi	236:**249**	170:200
Kishmish luchistyi	215:231	160:160
Kishmish melkiy	231:236	160:200
Kishmish moldavskiy	231:236	160:200
Kishmish opushennyi	231:236	160:200

Kishmish OSKHI	236:240	160:200
Kishmish rozovyi	231:236	160:200
Kishmish safed okruglyi	206:242	166:166
Kishmish sieh	231:231	160:160
Kishmish Sogdiana	231:240	158:170
Kishmish suugli (Kishmishi)	231:236	160:200
Kola kishmishnaya	231:236	160:200
Korinka russkaya	221:236	163:200
Lady Patricia	220:237	158:162
Lotus	231:**249**	**143:**160
Pamyati Smirnova	220:236	158:200
Perlette	221:231	160:163
Remaily seedless	220:236	158:200
Rozovyi Bisser	221:236	163:200
Ruby seedless	216:231	160:170
Rusbol	220:227	164:166
Yangi Er	221:231	160:163

Note: target alleles are highlighted in bold.

Variety Lotus obtained at the Anapa zonal experimental station of viticulture and winemaking from crossing the varieties Kriulyanskiy and Yangi Er. In the genotype of Yangi Er variety, we did not find the target alleles for linked DNA markers; thus, it can be assumed that the locus was inherited from Kriulyanskiy variety. According to the VIVC international database, the origin of Kriulyanskiy variety: Angur Kalan x pollen mixture Seyanets 180-2 + Pierrelle + Perle noire. Analyzing the pedigree of the variety, it can be assumed that *Ren1* is inherited from the Angur kalan (Nimrang) genotype – the exact origin of the variety is unknown, presumably Uzbekistan or Tajikistan. Thus, well-known varieties – donors of *Ren1* gene (Kishmish vatkana and Dzhanzhal kara) also belong to the gene pool of Central Asia. According to morphological and biological characteristics, Angur kalan belongs to the eastern ecological and geographical group of grape varieties, characterized by an average degree of powdery mildew damage, depending on the growing conditions [24]. Phytopathological analysis is required to confirm the presence of *Ren1* resistance gene in Lotus variety.

4 Conclusion

Using DNA marker analysis, the genotypes of seedless grape varieties were studied for the presence of *Ren1* powdery mildew resistance locus. Target fragments, according to the

linked marker loci SC8-0071-014 and sc47-18, indicating the presence of *Ren1*, were identified in grape variety Lotus (Kriulyanskiy x Yangi Er).

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