# DNA-marker identification of *Rpv3* and *Rpv12* resistance loci in genotypes of table and seedless grape varieties*

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**Abstract.** DNA markers are widely used in grapevine breeding to create forms with combined resistance genes. Downy mildew is one of the most common fungal diseases of the vine in the world. Growing grapevines with increased resistance allows to reduce the number of chemical treatments. The decrease in the use of pesticides is especially significant for viticulture of table varieties, since berries are directly consumed by humans for food. Currently, more than 20 resistance genes have been identified by molecular methods, and DNA markers for many genes have been developed. The genes *Rpv3* (inherited from North American grape species) and *Rpv12* (derived from *V. amurensis*) are among the most effective and have an additive effect. The study of 14 table grape varieties for the presence of the *Rpv3* gene and 8 varieties for the presence of the *Rpv12* gene was performed by using DNA-marker analysis. The analysis included varieties that could inherit these genes from the parent forms, according to their ancestry. The study was conducted using an automatic genetic analyzer ABI Prism 3130 and special software GeneMapper and PeakScanner, DNA-markers were taken from literature sources. According to the results of DNA-marker analysis, 9 varieties were identified, including 2 seedless varieties, with the $Rpv3_{299\text{-}279}$ allele in the genotypes, which determines resistance to downy mildew, and 3 table varieties with the *Rpv12* gene in the genotypes. One table grape genotype was identified with *Rpv3* and *Rpv12*.

## 1 Introduction

Creating genotypes of grapevines with high quality characteristic and resistance to pathogens is a priority task of most grapevine breeding programs in the world. One of the most common and harmful diseases of grapevines – downy mildew, it is caused by biotrophic oomycetes *Plasmopara viticola* Berl.et de Toni. The pathogen affects only

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grapevines (leaves, shoots, inflorescences, berries). The greatest damage is caused to vineyards in high humidity and warm weather. It is believed that the pathogen downy mildew originally existed on wild vines growing in the forests of South-Eastern North America. The pathogen entered in Europe in the XIX century along with American grape species that were imported to the continent as phylloxera-resistant rootstocks. European variety *Vitis vinifera* L began to be impaired by *P. viticola* and the vineyards were significantly damaged by downy mildew [1]. Creating resistant varieties to downy mildew has become an urgent task for European viticulture. Genotypes that are resistant to downy mildew belong to the grapevines of North America and Asia (*V. aestivalis* Michx., *V. berlandieri* Planch., *V. riparia* Michx., *V. rupestris* Scheele and etc.), also *Muscadinia rotundifolia* [2]. Interspecific hybridization is the basis for breeding of resistant grape varieties.

Creating genotypes with combined resistance genes significantly increases the degree of plant resistance. Methods of DNA marker-assisted selection are actively used for these purposes. Today, more than 20 different loci of downy mildew resistance have been identified in the grapevine genome using molecular genetic analysis [3]. The search for new donors of resistance genes in various germplasm of grapes, their phenotypic assessment and introduction into the breeding process are actively underway [4-11].

Genes *Rpv3* (inherited from North American species) and *Rpv12* (origins from *V. amurensis*) are one of the most effective and have additive effect [10, 11]. For these genes, DNA-markers have been identified that allow identifying the allelic state of the locus.

Cultivation of grape varieties with increased resistance to *P. viticola* allows to restrain the active development of the pathogen, reduce the number of treatments of plantings with fungicides. The reduction in the use of pesticides is particularly significant for viticulture of table varieties, since the berries are directly consumed by humans without any processing. Creating resistant table grape varieties with high consumer properties is an urgent task of modern grapevine breeding. Seedless table varieties are particularly in demand by the population, for this reason, the identification of seedless forms with resistance genes to fungal pathogens is of special interest for breeding. Also, the varieties should have an attractive cluster, a large berry of beautiful color, have transportability and storability.

The purpose of this work is to search for donors of downy mildew resistance genes *Rpv*3 and *Rpv*12 in the gene pool of table and seedless grape varieties and forms using DNA-markers analysis.

## 2 Materials and Methods

The study includes seedless and table grape varieties that are promising for use in breeding as initial forms for a number of economically valuable traits and which, according to their pedigree, could inherit genes *Rpv3* or *Rpv12*.

The study was performed by PCR with analysis of the results on an automatic genetic analyzer ABI Prism 3130. DNA was isolated from the crown of young shoots of plants using the CTAB-method [12]. Microsatellite markers recommended for DNA identification of the studied genes: *Rpv3* – UDV305, UDV737, *Rpv12* – UDV343, UDV360 was used in this work [10, 11]. As controls, we used the DNA of varieties in which these genes were found, according to published data (Seyve Villard 12-375 – for *Rpv3;* Zarya severa – for *Rpv12*).

PCR was performed using reagents produced by «Syntol» (Russia). DNA amplification was performed using the BioRad device (USA) according to the following program: 5 minutes at 95 °C – initial denaturation, followed by 35 cycles: 10 seconds denaturation at 95 °C; 30 seconds annealing of primers; synthesis of PCR fragments lasted 30 seconds at 72 °C; the final cycle-3 minutes at 72 °C. The annealing temperature of the primers was

selected as optimal 55 °C for markers UDV305, UDV737 and 60 ºC for markers UDV343, UDV360. Separation of reaction products by capillary electrophoresis and estimation of the size of amplified fragments was performed using an automatic genetic analyzer ABI Prism 3130 and software GeneMapper и PeakScanner.

## 3 Results and Discussion

We have already started research for searching gene *Rpv3* donors and some of them are published [13-15]. A large work in this direction was done by Di Gaspero and colleagues: DNA-marker analysis of the *Rpv3* locus in 580 genotypes of various origins [10]. This article presents the results of analysis of genotypes of seedless and table grape varieties, which have a number of positive agrobiological and consumer properties and can be used in the breeding process.

The given resistance gene can be found in varieties and forms-interspecific hybrids that have North American grapevine species in their pedigree *V. rupestris, V. lincecumii, V. labruska* or *V. riparia*. It is known that the gene *Rpv3* has seven haplotypes that determine downy mildew resistance [10]. Microsatellite DNA-markers UDV305, UDV737 allow to determine the condition of the locus *Rpv3*. Haplotypes gene *Rpv3*, which form resistance, correspond to the following allelic status of the specified loci: *Rpv3*$^{299\text{-}279}$, *Rpv3*$^{\text{null-}297}$, *Rpv3*$^{321\text{-}312}$, *Rpv3*$^{\text{null-}271}$, *Rpv3*$^{361\text{-}299}$, *Rpv3*$^{299\text{-}314}$, *Rpv3*$^{\text{null-}287}$ (UDV305, UDV737, respectively).

The DNA-marker analysis revealed *Rpv3*$^{299\text{-}279}$ in the genotypes of table grapes Bolgariya ustoychivaya, Il'ya, Muskat letniy, Nadezhda AZOS, Rochefort, Timur, Yubiley Moldavii and seedless variety – Kishmish 342, Lady Patricia (Table 1.). If we analyze the pedigrees of the varieties, it can be seen that the original gene donor is the Save Villard hybrids.

Thus, out of 14 analyzed genotypes, in 9 genotypes was identified the downy mildew resistance gene *Rpv3*, including two seedless grape varieties. The identified genotypes can be used as *Rpv3*$^{299\text{-}279}$ donors in the breeding process, at the same time, they have a number of other breeding-valuable features. Grapevine variety Kishmish 342 and Lady Patricia can be used in breeding as sources of seedless berry traits and simultaneously donors of the downy mildew resistance gene *Rpv3*.

**Table 1.** Results of DNA-marker analysis of grape genotypes

| **Identified alleles of microsatellite loci linked to the *Rpv3* gene** | | | | |
|---|---|---|---|---|
| **Variety (form)** | **Berry color** | **Pedigree** | **UDV305** | **UDV737** |
| **Seyve Villard 12-375 (control)** | Light yellow-green | Seibel' 6468 x Seibel' 6905 | **299**<br>**361** | **279**<br>**299** |
| Bolgariya ustoychivaya | Light amber | Seyve Villard 20-473 x Bulgaria | **299** | **279**<br>295 |
| Valentina | Yellow-green | Demetra x Muskat letniy | 342 | 279<br>293 |
| Vanessa seedless * | Red | Seneca x New York 45910 | 292<br>342 | 279<br>285 |

| | | | | |
|---|---|---|---|---|
| Il'ya | White | Voskovoy (**Seyve Villard 20-374** x Vostorg) x Kishmish luchistyi | **299** | **279** |
| Kishmish 342* | Green-golden | **Seyve Villard 12-375** x Perlette | **299**<br>342 | **279** |
| Kyoho | Dark purple | 62.50% *Vitis vinifera* + 37.50% *Vitis labrusca* | 226<br>313 | 285<br>293 |
| Lady Patricia * | Yellow, with a tan | Seibel 14665 x **Seyve Villard 20-365** | **299** | **279** |
| Muskat letniy | Amber white | **Seyve Villard 20-366** x Koenigin der weingaerten | **299** | **279**<br>285 |
| Nadezhda AZOS | Black | Moldova (Guzal kara x **Seyve Villard 12-375**) x Cardinal | **299** | **279**<br>285 |
| Rochefort | Dark purple | Talisman (**Frumoassa alba** x Vostorg) x Cardinal | **299**<br>342 | **279**<br>285 |
| Seneca | Yellowish green | Luglienga bianca x Ontario | 303<br>343 | 271<br>285 |
| Timur | White, with a tan | **Frumoassa alba** (Guzal kara x **Seyve Villard 20-473**) x Vostorg | **299** | **279** |
| Yubiley Moldavii | Dark pink | (Nimrang x Karmannyi) x **Seyve Villard 20-473** | **299** | **279**<br>295 |
| Yalovenskiy stolovyi | White | Ichkimar x Seyve Villard 20-366 | 299 | 281<br>295 |
| **Identified alleles of microsatellite loci linked to the *Rpv12* gene** | | | | |
| **Variety (form)** | **Berry color** | **Pedigree** | **UDV343** | **UDV360** |
| **Zarya severa (control)** | Black | Seyanets Malengra x *V. amurensis* | **164**<br>200 | 207<br>**213** |
| Vostorg | White, with a tan | (**Zarya severa** x Dolores) x Russkiy ranniy | **164**<br>216 | 207<br>**213** |

| | | | | |
|---|---|---|---|---|
| Vostorg krasnyi | Pink | Vostorg x Original | 200<br>216 | 195<br>207 |
| Vostorg ideal'nyi | White | Seyve Villard 12-375 x **Vostorg** | **164**<br>216 | 207<br>**213** |
| Zolotoy Don | Golden | Biruintsa x Vostorg | 216 | 194<br>209 |
| Korinka russkaya * | Golden, with a pink tint | Zarya severa x Kishmish chernyi | 195<br>200 | 207<br>217 |
| Loza goryanki | Black | Muskat ustoychivyi (Zarya severa x Nairi) x Dzhandzhal kara | 216<br>220 | 198<br>207 |
| Pamyati Dombkovskoy * | Black | Zarya severa x Kishmish unikal'nyi | 200<br>216 | 198<br>207 |
| Rochefort | Dark purple | Talisman (**Frumoassa alba** x Vostorg) x Cardinal | **164**<br>195 | 209<br>**213** |

Note: * - seedless grape varieties

Initially *Rpv12* gene originates from *V. amurensis* Rupr. Thus, varieties with *V. amurensis* in their pedigree may carry the *Rpv12* gene. It is known that Zarya severa variety inherited gene *Rpv12* from *V. amurensis* [11]. In the study, we included table and seedless varieties of Russian selection that have Zarya severa in the pedigree. According to the identified size of the loci UDV343 and UDV360 in genotype Vostorg, Vostorg ideal'nyi and Rochefort, we can draw conclusions about the presence of a functional allele of the *Rpv12* gene in plants of these varieties. Variety Vostorg is present in the pedigree of varieties Vostorg ideal'nyi and Rochefort, thus, it can be considered as a gene donor among the original forms of these genotypes. In Vostorg allele *Rpv12*, which affects the formation of resistance, is inherited from Zarya Severa variety. The *Rpv12* gene was not detected in the analyzed seedless varieties Korinka russkaya and Pamyati Dombkovskoy.

Thus, the genotypes Vostorg, Vostorg ideal'nyi and Rochefort can be involved in the breeding of table grape varieties as donors of *Rpv12* and the harvest of these varieties is characterized by high consumer characteristics.

The variety Rochefort is of interest, since it carries both of the analyzed resistance genes in its genotype according to the data of the DNA-marker assessment.

## 4 Conclusion

22 genotypes of grapevines were analyzed for the presence of *Rpv3* and *Rpv12* downy mildew resistance genes using DNA-markers linked to these genes. 9 varieties were identified, including 2 seedless varieties, which carry the $Rpv3^{299\text{-}279}$ allele in their genotypes, which affects the forming of downy mildew resistance, and 3 table varieties with the *Rpv12* gene in their genotypes, based on the results of DNA-marker analysis. One genotype with both *Rpv3* and *Rpv12* was also identified – Rochefort grape variety. The obtained molecular-genetic data correspond to the information about the pedigree of the studied varieties. The results can be used for the development of breeding programs for creating genotypes of grapevines with combined resistance genes.

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