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DNA-marker based identification of the *RPV3* gene determining downy mildew resistance in grapevines

E.T. Ilnitskaya[1] ✉, M.V. Makarkina[1], S.V. Tokmakov[1], L.G. Naumova[2]

[1] North-Caucasian Federal Scientific Center of Horticulture, Viticulture, Winemaking, Krasnodar, Russia
[2] All-Russian Research Institute of Viticulture and Winemaking named after Ya.I. Potapenko, Novocherkassk, Russia

Downy mildew is one of the most common fungal diseases of the vine, caused by *Plasmopara viticola*. An effective way to control the spread of the pathogen is to cultivate resistant varieties. Cultivars of *Vitis vinifera*, being the basis of high-quality viticulture, practically do not possess genetic resistance to *P. viticola*, so screening for resistance donors is an important stage in breeding. One of the major resistance loci to downy mildew, the *Rpv3* gene, was identified in the genotype of a complex interspecific hybrid of grapes Bianca. Later, it was found that this gene had seven haplotypes of resistance inherited from North American grape species, and that it was possible to identify the allelic status of the gene using DNA-markers UDV305, UDV737. However, only two haplotypes can be combined in one diploid form. To determine the *Rpv3* gene in the grape gene pool we, using these markers, studied 35 different genotypes of grapevines, most of which are interspecies cultivars. Three varieties with known allelic status of the *Rpv3* gene (Dunavski lazur, Noah, Seyve Villard 12-375) were included in the study as reference genotypes. The genotypes were studied through polymerase chain reaction with separation of amplification products by capillary electrophoresis in automatic genetic analyzer ABI Prism 3130. In the studied grape cultivars DNA marker analysis indentified the *Rpv3* gene in sixteen genotypes of interspecific origin, including haplotype $Rpv3^{299\text{-}279}$ found in twelve varieties, $Rpv3^{321\text{-}312}$ – in three, and haplotype $Rpv3^{null\text{-}271}$ – in one variety. Seyve Villard 12-375 turned out to be the donor of resistance gene in the most of the genotypes carrying *Rpv3* in this study. The obtained data can be useful in selection of mildew resistant grape varieties and screening for hybridization pairs.

Key words: grapevine; resistance to downy mildew; gene *Rpv3*; haplotype; DNA-markers; interspecific hybrids.

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✉ e-mail: ilnitskaya79@mail.ru

ДНК-диагностика гена *RPV3*, определяющего устойчивость винограда к возбудителю милдью

Е.Т. Ильницкая[1] ✉, М.В. Макаркина[1], С.В. Токмаков[1], Л.Г. Наумова[2]

[1] Северо-Кавказский федеральный научный центр садоводства, виноградарства, виноделия, Краснодар, Россия
[2] Всероссийский научно-исследовательский институт виноградарства и виноделия им. Я.И. Потапенко, Новочеркасск, Россия

Милдью – одно из наиболее распространенных грибных заболеваний виноградной лозы, вызываемое *Plasmopara viticola*. Эффективным способом контроля распространения патогена является возделывание устойчивых сортов. Сорта *Vitis vinifera*, считаясь основой высококачественного виноградарства, практически не обладают генетической устойчивостью к *P. viticola*. Поиск доноров устойчивости – важный этап в селекции. Один из крупных локусов устойчивости к милдью, ген *Rpv3*, впервые был определен в генотипе сложного межвидового гибрида винограда Бианка. Позже было установлено, что этот ген имеет семь гаплотипов устойчивости, наследуемых от североамериканских видов винограда; идентифицировать аллельное состояние гена можно с помощью ДНК-маркеров UDV305 и UDV737. В одной диплоидной форме могут быть объединены только два гаплотипа. С целью определения гена *Rpv3* в генофонде винограда с использованием указанных маркеров нами проведено изучение 35 генотипов различного происхождения, большинство из которых – межвидовые сорта. Три сорта, аллельное состояние гена *Rpv3* в которых известно, были включены в исследование в качестве референсных генотипов: Дунавски лазур, Ноа, Сейв Виллар 12-375. Работа проведена методом полимеразной цепной реакции с разделением продуктов амплификации методом капиллярного электрофореза при использовании автоматического генетического анализатора ABI Prism 3130. В исследуемой выборке сортов винограда, согласно данным проведенного ДНК-маркерного анализа, ген *Rpv3* определен впервые в 16 генотипах межвидового происхождения, в том числе в ДНК 12 сортов идентифицирован гаплотип $Rpv3^{299\text{-}279}$, в трех – $Rpv3^{321\text{-}312}$, в одном сорте выявлен гаплотип $Rpv3^{null\text{-}271}$. В большинстве идентифицированных нами генотипов, несущих *Rpv3*, донором гена является Сейв Виллар 12-375. Сорта винограда, в которых были идентифицированы гаплотипы *Rpv3*, определяющие устойчивость, характеризуются высоким или повышенным уровнем устойчивости к милдью. Полученные данные могут быть полезны в селекции устойчивых сортов винограда при подборе пар для гибридизации.

Ключевые слова: виноград; устойчивость к милдью; ген *Rpv3*; гаплотип; ДНК-маркеры; межвидовые гибриды.

Downy mildew is one of the most widespread and destructive fungal diseases in the grapevine, caused by oomycete *Plasmopara viticola* Berl. et de Toni. The pathogen affects only the vine developing in its every green organ: leafs, shoots, inflorescences, grapes and tendrils. In favorable conditions such as warm temperature and excessive humidity, mildew may cause harvest failure from 50 to 100 % in different grape varieties (Talash, 2010).

Cultivation of resistant varieties remains one of the most effective methods of disease control that allows one to reduce the amount of pesticide sprayings, and in this way improves the ampelocenosis, food safety and harvest of grapes.

The success of such cultivation is rooted into the genetic diversity of a culture and in many ways is determined by the level of knowledge about an accumulated genetic pool. Identification of the genotypes to serve as resistance donors has been one the topical issues in the science of selection. Being the foundation of high-quality vine growing cultivars *Vitis vinifera* have almost no genetic resistance to *Plasmopara viticola*, while downy mildew – resistant genotypes belong to vine species from North America and Asia (*V. aestivalis*, *V. berlandieri*, *V. cinerea*, *V. riparia*, *V. rupestris*, etc.) as well as to *Muscadinia rotundifolia* (Alleweldt et al., 1988; Wan et al., 2007).

Molecular genetic methods are widely applied these days for identification and mapping of valuable genes, gene- pool diversity analysis, and DNA-marker selection in different breeding programs. The methods have made it possible to determine around 20 mildew-resistant loci in a vine genome (http://www.vivc.de). Many of them have been mapped and given names with their linked DNA-markers identified including those appropriate for DNA marker selection (Eibach et al., 2007; Di Gaspero et al., 2012; Schwander et al., 2012; Venuti et al., 2013; Zini et al., 2014; Ochssner et al., 2016).

The *Rpv3* gene, one of the major loci of resistance, was detected for the first time and mapped at chromosome 18 in the genotype of a complex interspecies hybrid Bianca carrying the geneplasm of *V. vinifera*, *V. labrusca*, *V. rupestris*, *V. berlandieri*, *V. lincecumii* (Bellin et al., 2009). Later, in the course of a large-scale study into the North American species and varieties carrying *Rpv3*, one detected the seven conservative haplotypes of this gene responsible for mildew resistance (Di Gaspero et al., 2012). As the mentioned haplotypes were not found in *V. vinifera*, the authors came to the conclusion that *Rpv3* could be found in the varieties, whose pedigree had several North American species. The valuable haplotypes localize in a single locus, that is why in case of traditional breeding, only two haplotypes can be combined in a single diploid cell.

The performed studied have resulted in determination of tightly linked flanking microsatellite markers to identify such *Rpv3* gene haplotypes as UDV305 and UDV737 (Di Gaspero et al., 2012). The resistant haplotypes of the *Rpv3* gene corresponds to the following allele states of the abovementioned loci (UDV305, UDV737, respectively): $Rpv3^{299\text{-}279}$ (inherited from *V. rupestris*), $Rpv3^{\text{null-}297}$ (*V. rupestris* or *V. lincecumii*), $Rpv3^{321\text{-}312}$ (*V. labrusca* or *V. riparia*), $Rpv3^{\text{null-}271}$ (*V. labrusca* or *V. riparia*), $Rpv3^{361\text{-}299}$ (*V. rupestris*), $Rpv3^{299\text{-}314}$ (*V. rupestris*), $Rpv3^{\text{null-}287}$ (*V. rupestris* or *V. labrusca*). G. Di Gaspero at al. have studied more than 200 grapevine varieties to determine their genotypes and stable *Rpv3*-bearing haplotypes, so their data can be used in screening for hybridization pairs in breeding of downy mildew-resistant cultivars.

The objective of the presented study was using DNA-marker analysis for identification of the allele state of the *Rpv3* gene in different vine cultivars, and comparison of the obtained data against the genotype pedigree.

Materials and methods

The study covered 35 grapevine cultivars from the gene pools of the Anapa ampelographic collection (Anapa) and the collection of Ya.I. Potapenko Research Institute of Viticulture and Winemaking (Novocherkassk). Most of the studied cultivars were interspecies hybrids, whose parents were North American vine varieties and, based on analysis of their pedigree, could carry the resistant haplotypes of the *Rpv3* gene. In the geneplasm of the studied cultivars – potential carriers of the studied gene – were present *V. riparia*, *V. labrusca*, *V. aestivalis*, *V. rupestris*, *V. berlandieri*, *V. lincecumii*. The study also covered a number of genotypes that should have no *Rpv3* gene such as *Vitis vinifera* and its hybrids with *V. amurensis*.

The vines' DNA was extracted from leaves using the CTAB method (Rogers, Bendich, 1985). The genotypes were determined using polymerase chain reaction (PCR) and the DNA-markers recommended for identification of the alleles of the *Rpv3* gene (Di Gaspero et al., 2012). PCR was performed in a finite volume of 25 μl following the standard protocol and using the Sintol reagent kit (Moscow, Russia). DNA amplification was performed in Eppendorf MasterCycler Gradient Thermal Cycler (Germany) with the following protocol for every DNA-marker: 5 minutes at 95 ºC for initial denaturation followed by 35 cycles (10 seconds for denaturation at 95 ºC, 30 seconds for annealing the primers at 55 ºC, 30 seconds for synthesis at 72 ºC, and 3 minutes for the last cycle of synthesis at 72 ºC). The reaction products were separated using capillary electrophoresis, and the size of the amplified fragments was estimated with the ABI Prism 3130 automatic genetic analyzer using software packages GeneMapper and PeakScanner. The DNA of the Dunavski lazur, Seyve Villard 12-375 and Noah varieties with known allele sizes for the studied loci (Di Gaspero et al., 2012) were used as controls to specify the sizes of the amplified fragments.

The molecular genetic study was carried out using the equipment provided by Shared Equipment Center "Genomic and Postgenomic Technologies" of North-Caucasian Federal Scientific Center of Horticulture, Viticulture, Winemaking.

Results and discussion

The performed study determined the allele state of downy mildew-resistant gene *Rpv3* in the genotypes of 35 vine cultivars (Table). The gene's resistant haplotypes were identified in 19 varieties: Dunavsky lazur, Noa, Seyve Villard 12-375, Dekabrskiy, Dunavska gymza, Original, Talisman, Kutuzovskiy, Kodryanka, Rusbol, Storgoziya, R65, Kishmish 342, Srebrostrui, $VIII_2$-2-48, Armalaga, Poliuks, Podarok Magaracha, Melody (Figure).

In the studied sample haplotype $Rpv3^{299\text{-}279}$ occurred more often than others: it was detected in 14 genotypes (see Table).

In 12 cultivars this haplotype was indentified for the first time.

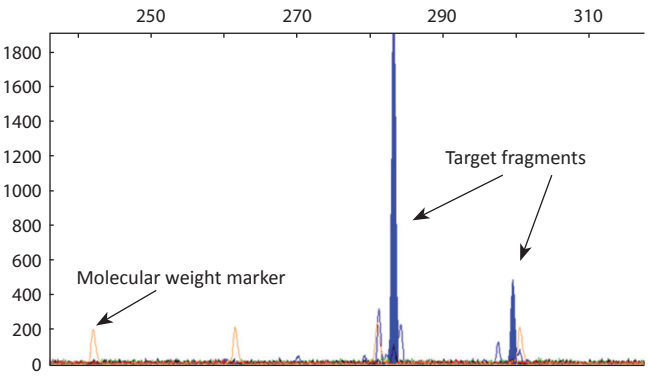
Haplotype *Rpv3*$^{321\text{-}312}$ was for the first time identified in three cultivars: Armalaga, Poliuks and Podarok Magaracha. DNA-marker analysis identified hyplotype *Rpv3*$^{null\text{-}271}$ only in the melody species. The Noah variety that we used as one of the reference genotypes, also carried *Rpv3*$^{321\text{-}312}$and *Rpv3*$^{null\text{-}271}$.

According to the published data, haplotype *Rpv3*$^{299\text{-}279}$ we identified in cultivars Dekabrskiy, Dunavska gymza, Original, Talisman, Kutuzovskiy, Kodryanka, Rusbol, Storgoziya, R65, Kishmish 342, Srebrostruy, VIII$_2$-2-48, is inherited from the geneplasm of *V. rupestris*. In the analyzed sample of grapevine cultivars six genotypes carrying *Rpv3*$^{299\text{-}279}$ out of twelve inherited the resistant allele directly from their parent variety

Results of analysis of vine genotypes of different origin for the SSR loci UDV305 and UDV737 linked with downy mildew-resistant gene *Rpv3**

Species (variety)	Origin	UDV305		UDV737	
		Allele size, bp			
Dunavski lazur	Rkatsitely × Seyve Villard 12-375	299	326	279	295
Noah	*Vitis riparia* × *Vitis labrusca*	321	0	271	312
Seyve Villrd 12-375	Seibel' 6468 × Seibel' 6905	299	361	279	299
Dekabrskiy	Korna niagra × Seyve Villard 12-375	299		279	285
Dunavska gymza	(Mavrud × Pinot noir) × Seyve Villard 12-375	299		279	293
Original	Damasskaya roza × Seyve Villard 20-365	299	322	279	
Talisman	Frumoassa alba × Vostorg	299	326	279	295
Kutuzovskiy	Moldavskiy × Seyve Villard 20-365	299		279	285
Kodryanka	Moldova × Marshalskiy	299		279	285
Rysbol	Seyve Villard 12-375 × Sverkhranniy bessemyanniy	299		279	
Storgoziya	(Mavrud × Pinot noir) × Seyve Villard 12-375	299		279	295
R65	Zala dyoengye × (Gloria × Koroleva vinogradnikov) × Muscat zimniy	299		279	289
Kishmish 342	Seyve Villard 12-375 × Perlette	299	342	279	
Srebrostruy	Rkatsitely × Seyve Villard	299	326	279	295
VIII$_2$-2-48	Moldova × {Pobeda × [Katta-Kurgan × (Kishmish rozoviy × Kishmish beliy)]}	299		279	
Armalaga	(Armlong × Malaga)	321	334	271	312
Poliuks	Oberlen 595 (V. riparia × Game cherniy) × Foster white seedling	229	321	312	
Podarok Magaracha	Rkatsitely × Magarach 2-57-72	321		297	312
Melody	Seyval blanc × Geneva white 5 (Pinot blanc × Ontario)	0		271	
Vesta	(Avgusta × V. amurensis) × (Kentavr magarachskiy × Levokumskiy)	231	285	293	297
B 7-2	*Vitis vinifera* × *Vitis labrusca*	0		295	312
Doyna	Korna niagra × (Cabernet Sauvignon × Seyve Villard 23-657)	290		279	285
Yalovenskiy Stoloviy	Ichkimar × Seyve Villard 20-366	299		281	295
Agadai	Local cultivar of Dagestan *Vitis vinifera*	326		289	295
Alfa	*Vitis vinifera* × *Vitis riparia*	296		285	303
Antaris	Saperavi × Tsimlyanskiy cherniy	326		301	295
Granatoviy	Saperavi × Cabernet Sauvignon	254		283	285
Golubok	Severniy × (Vishnyoviy + Odesskiy ranniy + 1-17-54)	322		285	295
Dmitriy	Varousset × Granatoviy	254		285	295
Dostoyniy	Phillokseroustoychiviy Dzhemete × Muscat Gamburg	320		293	295
Krasnostop AZOS	Phillokseroustoychiviy Dzhemete × Krasnostop anapskiy	320		293	295
Murometc	Severniy × Pobeda	342		285	
Pomoriyski biser	Misket cherven × Seyve Villard 12-375	300		293	301
Cvetochniy	Severniy × pollen of Muscat cultivars	322	333	285	
Fioletoviy ranniy	Severniy × Muscat Gamburg	300		293	301

* Data for the identified alleles are presented in a way chosen by G. Di Gaspero et al. (2012).



Visualization of the results of PCR – product fragment analysis with the UDV737 marker of the Storgoziya cultivar.

Seyve Villard 12-375. These were cultivars Dekabrskiy, Dunavska gymza, Rusbol, Storgoziya, Kishmish 342, and Srebrostruy. The Seyve Villard series are complex interspecies hybrids that are often used in grapevine breeding as resistance donors, and Seyve Villard 12-375 is one of the most known hybrids in the series that carry the geneplasm of *V. vinifera*, *V. labrusca*, *V. rupestris*, *V. berlandieri*, *V. lincecumii*. In the Kodryanka and VIII$_2$-2-48 varieties, haplotype *Rpv3*$^{299\text{-}279}$ is inherited from parent variety Moldova (Guzal' kara × Seyve Villard 12-375), which means in this case Seyve Villard 12-375 has also served as a gene donor. According to the pedigree of R65 (Zala dyoengye (Seyve Villard 12-375 × Jemchug Saba) × (Gloria × Koroleva vinogradnikov) × Muscat zimniy) the resistant allele was inherited from Seyve Villard 12-375. However, Seyve Villard 12-375 is also considered one of the parents of the Pomoriyski biser variety, which carries no *Rpv3* gene haplotype making it resistant to downy mildew.

In the genotype of the Talisman cultivar, the resistant allele was inherited from the Frumoassa alba variety, whose parent is Seyve Villard 20-473. Seyve Villard 20-365 served as a gene donor in varieties Original and Kutuzovskiy.

It is considered that resistant haplotype *Rpv3*$^{null\text{-}271}$ initially originated from either *V. labrusca* or *V. riparia*. This is the haplotype we identified in American variety Melody. In the beginning, when analyzing its pedigree (Seyval blanc × Geneva white 5 (Pinot blanc × Ontario)) for its inclusion as a potential gene donor we assumed Seyval blanc (another name of Seyve Villard 5-276) to be the resistivity donor since its genetic formula contained *V. rupestris* and *V. aestivalis*. However, if we assume that *Rpv3*$^{null\text{-}271}$ is inherited either from *V. labrusca* or from *V. riparia*, the indicated resistance allele in Melody can be inherited from Ontario (25 % *V. vinifera* + 75 % *V. labrusca*).

Haplotype *Rpv3*$^{321\text{-}312}$ was identified in cultivars Armalaga, Poliuks and Podarok Magaracha. The published data indicate the source of this resistant haplotype to be either *V. labrusca* or *V. riparia*. In variety Armalaga, *Rpv3*$^{321\text{-}312}$ was inherited from *V. labrusca*, and in Poliuks – from *V. riparia*, which is confirmed by their pedigrees. Meanwhile, the pedigree of Podarok Magaracha remains an open issue. One of the parent varieties it inherited its mildew resistance from was probably Magarach 2-57-72 (Mtsvane × Sochinskiy cherniy). The Sochinsky cherniy first discovered by P.Ya. Golodriga in the outskirts of Sochi has been lost and its exact genetic origin remains unknown, but its high resistance to fungal pathogens it transfers to its descendants makes it possible to classify it as an interspecies hybrid. The data we collected during the study allow us to assume that the pedigree of this species included either *V. labrusca* or *V. riparia.*

Conclusion

The presented study analyzed 35 vine genotypes of different origin to identify the presence of downy mildew-resistant gene *Rpv3* using DNA markers UDV305 and UDV737. The analysis identified the *Rpv3* gene in 19 grapevine cultivars, including 3 varieties, in which the presence of the gene had been confirmed earlier. The abovementioned markers allow one to identify a certain haplotype of the *Rpv3* gene. That way, for the first time, haplotype *Rpv3*$^{299\text{-}279}$ has been detected in 12 interspecies varieties; *Rpv3*$^{321\text{-}312}$ – in three varieties; and *Rpv3*$^{null\text{-}271}$ – in one variety. For the first time, the presence of the *Rpv3* gene has been confirmed for such grapevine cultivars as Dekabrskiy, Dunavska gymza, Original, Talisman, Kutuzovskiy, Kodryanka, Rusbol, Storgoziya, R65, Kishmish 342, Srebrostruy, VIII$_2$-2-48, Armalaga, Poliuks, Podarok Magaracha and Melody. All the genotypes with *Rpv3* are characterized by high or increased level of downy mildew resistance, which has been confirmed by the results of perennial observations (Petrov, Talash, 2010; Troshin, Radchevsky, 2010). The performed DNA analyses has also allowed us to assume that the parent varieties of Podarok Magaracha, whose pedigree remains unclear, are interspecies varieties carrying the geneplasm of either *V. labrusca* or *V. riparia.*

The results obtained can be used for selection of initial varieties to breed cultivars resistant to downy mildew.

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Conflict of interest

The authors declare no conflict of interest.

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