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DNA marker identification of downy mildew resistance locus *Rpv10* in grapevine genotypes

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Abstract. One of the most common and harmful diseases of grapevine is downy mildew, caused by *Plasmopara viticola*. Cultivars of *Vitis vinifera*, the basis of high-quality viticulture, are mainly not resistant to downy mildew. Varieties with natural resistance to downy mildew belong to the vine species of North America and Asia (*V. aestivalis*, *V. berlandieri*, *V. cinerea*, *V. labrusca*, *V. amurensis*, etc.), as well as *Muscadinia rotundifolia*. The breeding of resistant cultivars is based on interspecific crossing. Currently, molecular genetic methods are increasingly used in pre-selection work and directly in breeding. One of the major loci of downy mildew resistance, *Rpv10*, was first identified in the variety Solaris and was originally inherited from wild *V. amurensis*. DNA markers that allow detecting *Rpv10* in grapevine genotypes are known. We used PCR analysis to search for donors of resistance locus among 30 grape cultivars that, according to their pedigrees, could carry *Rpv10*. The work was performed using an automatic genetic analyzer, which allows obtaining high-precision data. *Rpv10* locus allele, which determines resistance to the downy mildew pathogen, has been detected in 10 genotypes. Fingerprinting of grape cultivars with detected *Rpv10* was performed at 6 reference SSR loci. DNA marker analysis revealed the presence of a resistance allele in the cultivar Korinka russkaya, which, according to publicly available data, is the offspring of the cultivar Zarya Severa and cannot carry *Rpv10*. Using the microsatellite loci polymorphism analysis and the data from VIVC database, it was found that Korinka russkaya is the progeny of the cultivar Severnyi, which is the donor of the resistance locus *Rpv10*. The pedigree of the grapevine cultivar Korinka russkaya was also clarified.
Key words: *Vitis* sp.; target alleles; *Plasmopara viticola*; DNA fingerprinting.

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ДНК-маркерная идентификация локуса устойчивости к милдью *Rpv10* в генотипах винограда

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Аннотация. Милдью – одно из наиболее распространенных и вредоносных заболеваний виноградной лозы, возбудителем которого считают *Plasmopara viticola*. Сорта *Vitis vinifera*, выступая основой высококачественного виноградарства, практически не обладают генетической устойчивостью к милдью. Генотипы, имеющие природную устойчивость к поражению *P. viticola*, принадлежат видам винограда Северной Америки и Азии (*V. aestivalis*, *V. berlandieri*, *V. cinerea*, *V. labrusca*, *V. amurensis* и др.), а также к *Muscadinia rotundifolia*. По этой причине создание сортов винограда с повышенной устойчивостью к патогену основано на межвидовой гибридизации. В настоящее время молекулярно-генетические методы анализа все активнее используют на этапах предселекционной работы и непосредственно в селекции. Один из крупных локусов устойчивости к милдью – ген *Rpv10* – впервые идентифицирован в сорте межвидового происхождения Солярис и изначально происходит от дикого амурского винограда. Известны ДНК-маркеры данного гена, позволяющие детектировать наличие *Rpv10* в генотипах винограда. Методом ПЦР-анализа выполнен поиск доноров гена устойчивости среди генотипов 30 сортов винограда, которые, согласно родословным, могли бы нести ген *Rpv10*. Работа выполнена с использованием автоматического генетического анализатора, что позволяет получать высокоточные данные. По результатам ДНК-маркерного анализа в 10 генотипах винограда выявлено наличие аллели гена *Rpv10*, определяющей устойчивость к возбудителю милдью. Выполнено генотипирование сортов винограда, в которых обнаружен *Rpv10*, с помощью шести стандартных для ДНК-профилирования винограда SSR-маркеров. ДНК-маркерный анализ показал наличие аллели устойчивости у сорта Коринка русская, который, по общедоступным данным, является потомком сорта Заря Севера, не обладающим геном устойчивости *Rpv10*. С использованием анализа полиморфизма микросателлитных локусов и базы данных VIVC уточнена родословная сорта винограда Коринка русская. Установлено, что Коринка русская происходит от сорта Северный – донора локуса устойчивости *Rpv10*.
Ключевые слова: *Vitis* sp.; целевые аллели; *Plasmopara viticola*; ДНК-профилирование.

Introduction

The Eurasian grapevine (*Vitis vinifera* L.) is the most widely cultivated and economically important fruit crop in the world (De Mattia et al., 2008). Grapevines are grown both for direct food consumption and for the production of wine. The issue of creating pathogen-resistant genotypes is relevant in the breeding of table and wine cultivars. Downy mildew is one of the most common and harmful diseases of grapevine, caused by biotrophic oomycete *Plasmopara viticola* Berl. et de Toni. The pathogen has a narrow specialization and affects only grapevines: it develops on all green organs of the plant – leaves, shoots, inflorescences, berries, tendrils. The greatest damage is caused to vineyards in warm periods with high humidity. The creation of new grapevine forms is based on the use of the genetic diversity. The searching and identification of genotypes – donors of resistance, is an important task both for studying the diversity of the existing gene pool and for the purposes of breeding new resistant cultivars.

The *V. vinifera* genotypes, being the basis of high-quality viticulture, are mainly not resistant to *P. viticola*. The breeding of resistant cultivars is based on interspecific crossing. Genotypes with natural resistance to downy mildew belong to the vine species of North America (*V. riparia*, *V. aestivalis*, *V. berlandieri*, *V. cinerea*, *V. labrusca*) and East Asia (*V. amurensis*, *V. piasezkii*), as well as *Muscadinia rotundifolia* (Alleweldt, Possingham, 1988; Wan et al., 2007). It is generally accepted that resistance in American species developed simultaneously with the pathogen, which is endemic to North America. Resistance to *P. viticola* in some forms of *V. amurensis* could have developed through evolution from resistance to *P. cissii* and *P. amurensis,* these microorganisms are endemic to Asia (Riaz et al., 2011).

Molecular genetic analysis methods are successfully used now to identify and map loci of resistance to downy mildew. Both major loci with large influence in phenotypic variation and minor loci with smaller effects were identified (Bellin et al., 2009; Di Gaspero et al., 2012; Schwander et al., 2012; Venuti et al., 2013; Ochssner et al., 2016; Divilov et al., 2018; Lin et al., 2019; Sapkota et al., 2019; Bhattarai et al., 2020; Sargolzaei et al., 2020; Fu et al., 2020). The results of many such studies are successfully used for DNA marker selection to create quality grape cultivars with pyramided resistance genes (Eibach et al., 2007; Zini et al., 2019; Possamai et al., 2020; Ruiz-García et al., 2021).

Thus, a major locus of resistance inherited from wild *V. amurensis* was identified in the genotype of interspecific cultivar Solaris, it was named *Rpv10* (Schwander et al., 2012). The identified locus explained up to 50 % of observed phenotypic variance in the studied mapping hybrid population. Analysis of Solaris cultivar pedigree revealed that the allele that determines resistance to downy mildew was inherited from Severnyi (*V. amurensis* × Seyanets Malengra) cultivar. At the same time, studies have shown that in the genotype of Zarya Severa cultivar, which was selected from the same hybrid population as Severnyi (*V. amurensis* × Seyanets Malengra), the resistance allele is absent (Schwander et al., 2012). In the course of this study, flanking DNA markers of *Rpv10* locus were identified, which make it possible to search for genotypes – donors of the downy mildew resistance locus *Rpv10* in grapevine collections (Marker-Assisted Parental Selection) and in the breeding process to identify hybrid samples carrying the target allele (Marker-Assisted Seedling Selection) according to DNA analysis data.

The aim of the work was to determine *Rpv10* locus in grape cultivar's genotypes using flanking DNA markers.

Material and methods

Grapevine accessions and DNA extraction. We included in the study grape cultivars that could have the resistance locus *Rpv10*, according to analysis of their well-known pedigree: cultivars-descendants of Severnyi cultivar or bred using wild *V. amurensis* (original gene donor). In total, 30 genotypes were analyzed: Amurets, Avgusta, Buytur, Cabernet severnyi, Cvetochnyi, Denisovskiy, Dimatskun, Druzhba, Dushystyi, Fioletovyi ranniy, Golubok, Grushevskiy belyi, Korinka russkaya, Kostyukovskiy, Kristall, Kunleany, Kurchanskiy, Lusakert, Morozko, Murometc, Muscat donskoi, Pamyati Dombkovskoy, Saperavi severnyi, Skromnyi, Stanichnyi, Stepnyak, Sverkhranniy volgodonskiy, Vostorg, Vydvizhenets, Zolotoy Don cultivars. Plant material was collected from the Anapa ampelographic collection (North-Caucasian Federal Scientific Center of Horticulture, Viticulture, Winemaking) and the collection of Ya.I. Potapenko All-Russian Research Institute of Viticulture and Winemaking – branch of Federal Rostov Agricultural Research Center. Genomic DNA samples were isolated from young tops of plant shoots. DNA extraction was carried out by the method based on the use of CTAB (Rogers, Bendich, 1985).

DNA analysis. Three DNA markers were used to identify the allelic status of *Rpv10* locus (GF09-44, GF09-46, GF09-47). The sequence of primer oligonucleotides was synthesized according to information from the literature (Schwander et al., 2012). Polymerase chain reaction (PCR) was carried out in total volume of 25 µl containing about 50 ng of genomic DNA, 1.5 units of Taq-polymerase (SibEnzyme, Russia), 1X Taq-polymerase buffer (SibEnzyme, Russia), 2 µM of $MgCl_2$ (SibEnzyme, Russia), 0.2 µM of each dNTP (SibEnzyme, Russia) and 200 µM of forward and reverse primers (Syntol, Russia). Amplification was carried out on a BioRad Thermo cycler T100 (USA). The following PCR conditions were used: initial denaturation for 5 min at 95 °C, 40 cycles of 30 s denaturation at 95 °C, annealing at 60 °C for 30 s and extension at 72 °C for 40 s, final step – 5 min extension at 72 °C. DNA of Solaris grape cultivar, which carries *Rpv10* resistance allele, was used as a control to identify target alleles and correct the size of the detected PCR fragments.

A standard set of SSR markers for DNA profiling of grapevine genotypes (VVS2, VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, VVMD32, VrZAG62 and VrZAG79) was used for DNA fingerprinting of cultivars (This et al., 2004; This, 2007). Forward primers were labeled as follow: FAM (VVS2, VDMD27, VrZAG62), TAMRA (VVMD5, VVMD25, VVMD28, VVMD32), R6G (VVMD7, VrZAG79). The sequence of primer oligonucleotides was synthesized by Syntol (Russia). The following PCR conditions were applied: initial denaturation for 5 min at 95 °C; 34 cycles of 20 s denaturation at 95 °C, 30 s annealing at Tm (55 °C – VVS2, VVMD5, VVMD7, VVMD27; 58 °C – VrZAG62, VrZAG79; 60 °C – VVMD25, VVMD28, VVMD32) and 40 s extension at 72 °C; final extension of 3 min at 72 °C. To clarify the size

of the detected alleles, we used the DNA of reference cultivars Cabernet Sauvignon and Pinot noir. Fragment analysis was carried out using an ABI Prism 3130 genetic analyzer. Molecular genetic studies were carried out using the instrument park of the center for collective use of technological equipment of the North Caucasian Federal Scientific Center for Horticulture, Viticulture, Winemaking.

Results and discussion

Rpv10 locus detection

At the first stage of the work, 30 grapevine accessions were analyzed using the GF09-46 marker, this microsatellite locus was identified as a closely linked DNA marker, correlating with the presence of *Rpv10* locus, according to the studies of Schwander et al. (2012) (Schwander et al., 2012). The authors found that the PCR product of 416 base pairs size detected by the GF09-46 marker corresponds to the presence of *Rpv10* locus allele which determines downy mildew resistance in the grapevine genotype. The target fragment was identified in ten cultivars on the 30 analyzed accessions: Augusta, Golubok, Denisovskiy, Dimatskun, Korinka russkaya, Morozko, Saperavi severnyi, Stanichnyi, Fioletovyi ranniy, Cvetochnyi (Table 1). Some of the results were published earlier (Ilnitskaya et al., 2019). At the second stage of the study, it was decided to analyze these ten cultivars with DNA markers GF09-44 and GF09-47, flanking the region of the chromosome where *Rpv10* locus is localized, which makes it possible to make sure that there is no crossing-over at this locus in the studied genotypes (Schwander et al., 2012).

Thus, according to the results of DNA marker analysis, target alleles at loci GF09-44 and GF09-47, correlating with the presence of a resistant allele in the *Rpv10* locus, according to published data, were detected in all ten samples (see Table 1).

It has been determined that there was no crossing-over at the analyzed part of the chromosome in the studied genotypes, thus, according to the DNA marker analysis, the presence of the downy mildew resistance locus *Rpv10* in grape cultivars Augusta, Golubok, Denisovskiy, Dimatskun, Korinka russkaya, Morozko, Saperavi severnyi, Stanichnyi, Cvetochnyi and Fioletovyi ranniy is confirmed.

An analysis of the pedigree of these cultivars suggests that the locus is inherited directly from Severnyi cultivar (Saperavi severnyi, Denisovskiy, Golubok, Fioletovyi ranniy, Cvetochnyi) and from the descendants of this cultivar (Avgusta, Morozko, Stanichnyi) (Table 2).

In the genotype of Dimatskun, according to the origin of this cultivar, the *Rpv10* could be inherited from both the paternal and the maternal genotype, since the resistance donor is wild *V. amurensis*, which is present in the pedigrees of both parents of this cultivar. Of interest is the fact that *Rpv10* resistance locus is present in Korinka russkaya cultivar. So, the parents of this genotype are considered to be cultivars Zarya Severa and Kishmish chernyi (http://www.vivc.de). However, Zarya Severa genotype lacks *Rpv10* allele that determines resistance, according to the published data (Schwander et al., 2012) and our research. Thus, the reliability of information about Korinka russkaya cultivar pedigree is questionable.

According to the average long-term data of observations, the greatest field resistance to downy mildew among these cultivars is shown by Stanichnyi cultivar (5–25 % damage). Most likely, Stanichnyi genotype also contains downy mildew resistance genes inherited from North American grape species, this cultivar has a complex interspecific origin (see Table 2).

Fingerprinting

We carried out genotyping of Korinka russkaya and Zarya Severa by nine SSR loci used for DNAfingerprinting and identification of grapevine cultivars (This et al., 2004; This, 2007). The obtained data confirm the assumption that Zarya Severa cannot be the maternal parent of Korinka russkaya cultivar (Table 3).

If Korinka russkaya was bred from Zarya Severa cultivar, then, according to the codominant type of inheritance of SSR loci alleles, one of the alleles of Zarya Severa of each analyzed microsatellite loci would be found in the corresponding lo-

Table 1. The results of grape genotypes analysis with DNA markers linked to downy mildew resistance locus *Rpv10*

Cultivar	The sizes of identified alleles, base pair					
	GF09-44		GF09-46		GF09-47	
Avgusta	**230**	242	**416**	423	296	**299**
Golubok	**230**	–	**416**	–	296	**299**
Denisovskiy	**230**	243	395	**416**	296	**299**
Dimatskun	**230**	242	**416**	423	296	**299**
Korinka russkaya	**230**	242	**416**	423	296	**299**
Morozko	**230**	242	**416**	423	296	**299**
Saperavi severnyi	**230**	243	395	**416**	296	**299**
Stanichnyi	**230**	–	407	**416**	290	**299**
Fioletovyi ranniy	**230**	244	**416**	–	296	**299**
Cvetochnyi	**230**	236	394	**416**	296	**299**

Note. Target fragments that correlated with resistance are shown in bold.

Table 2. Pedigree of the analyzed grape genotypes

Cultivar	Pedigree	Originator (institution, country)
Avgusta	SV 12-309 × Kazachka (Kazachka-1 × Fioletovyi ranniy (**Severnyi** × Muscat Gamburg)	Ya.I. Potapenko All-Russian Research Institute of Viticulture and Winemaking – branch of Federal Rostov Agricultural Research Center, Russia
Golubok	**Severnyi** × a mix of pollen cultivars 40 let Oktyabrya, Odesskiy ranniy and No. 1-17-54 (Alicante Bouschet × Cabernet Sauvignon)	V.Ye. Tairov Institute of Viticulture and Winemaking of the National Academy of Agrarian Sciences of Ukraine, Ukraine
Denisovskiy	**Severnyi** × pollen mix of muscat cultivars (Muscat a petits grains blancs, Muscat fleur d'oranger, Muscat of Alexandria)	Ya.I. Potapenko All-Russian Research Institute of Viticulture and Winemaking – branch of Federal Rostov Agricultural Research Center, Russia
Dimatskun	Karmrayut (Adisi × (***V. amurensis*** × Chernyi sladkiy) × Seedling 1563/1 + 21 (Madeleine Angevine × ***V. amurensis***) × Seyanets Malengra 65/16 (open pollination of Seedling Malengra cultivar)	Armenian Academy of Viticulture, Wine-Making and Fruit-Growing, Armenia
Korinka russkaya	~~**Zarya Severa**~~ **Severnyi** × Kishmish chernyi	I.V. Michurin Federal Scientific Center, Russia
Morozko	Mitsar × Saperavi severnyi (**Severnyi** × Saperavi)	North Caucasian Federal Scientific Center for Horticulture, Viticulture, Winemaking, Russia
Saperavi severnyi	**Severnyi** × Saperavi	Ya.I. Potapenko All-Russian Research Institute of Viticulture and Winemaking – branch of Federal Rostov Agricultural Research Center, Russia
Stanichnyi	Cvetochnyi (**Severnyi** × pollen mix of muscat cultivars) × Zala Gyoengye	Ya.I. Potapenko All-Russian Research Institute of Viticulture and Winemaking – branch of Federal Rostov Agricultural Research Center, Russia
Fioletovyi ranniy	**Severnyi** × Muscat Gamburg	Ya.I. Potapenko All-Russian Research Institute of Viticulture and Winemaking – branch of Federal Rostov Agricultural Research Center, Russia
Cvetochnyi	**Severnyi** × pollen mix of muscat cultivars (Muscat a petits grains blancs, Muscat fleur d'oranger, Muscat of Alexandria)	Ya.I. Potapenko All-Russian Research Institute of Viticulture and Winemaking – branch of Federal Rostov Agricultural Research Center, Russia

Table 3. DNA profiles of grape cultivars Korinka russkaya, Zarya Severa and Severnyi by nine SSRs

Cultivar	Alleles of SSR loci, base pairs								
	VVS2	VVMD5	VVMD7	VVMD25	VVMD27	VVMD28	VVMD32	VrZAG62	VrZAG79
Cabernet Sauvignon	139 151	234 242	239 239	239 249	176 190	234 236	240 240	188 194	247 247
Zarya Severa	135 139	238 240	247 249	255 271	195 195	236 244	242 272	200 204	249 251
Korinka russkaya	**135** 155	236 **238**	**241** 253	245 **255**	182 **184**	218 **244**	**240** 250	**184** 188	247 **255**
Severnyi (VIVC)	129 **135**	238 **238**	**241** 247	237 **255**	182 **184**	**244** 252	237 **240**	**184** 204	**255** 259

Note. The alleles inherited by Korinka russkaya from Severnyi genotype are shown in bold.

cus of Korinka russkaya cultivar. However, in five (VVMD7, VVMD27, VrZAG62, VrZAG79, VVMD32) out of nine studied SSR loci, these cultivars do not have common alleles (see Table 3).

Most likely, *Rpv10* locus in Korinka russkaya is inherited from Severnyi cultivar, according to the analysis of the history of Korinka russkaya genotype origin. In addition, the information that Severnyi cultivar is the parent of Korinka russkaya was found by us in a literary source describing the northern grape cultivars of Russia (Abuzov, 2009). Using data from the DNA profile database of Vitis International Variety Catalogue (http://www.vivc.de), we performed the DNA profiles comparison between Korinka russkaya and Severnyi. The allele from Severnyi cultivar was identified in each analyzed locus of Korinka russkaya, accordingly (see Table 3). So Severnyi is the parent of Korinka russkaya, Zarya Severa is not in the pedigree of Korinka russkaya.

We performed genotyping on VVS2, VVMD5, VVMD7, VVMD27, VrZAG62 and VrZAG79 SSR loci of cultivars, in which *Rpv10* resistance locus was identified (Table 4). The DNA profiles can then be used for the trueness-to-type analysis of accessions. Genotypes Avgusta, Golubok, Denisovskiy,

Table 4. DNA profiles of grape cultivars with detected *Rpv10* locus

Cultivar	Alleles of SSR loci, base pair					
	VVS2	VVMD5	VVMD7	VVMD27	VrZAG62	VrZAG79
Cabernet Sauvignon	139 151	234 242	239 239	176 190	188 194	247 247
Pinot noir	137 151	230 240	239 243	186 190	188 194	239 245
Avgusta	133 133	228 238	237 249	180 190	180 186	255 261
Golubok	129 133	238 248	239 241	182 184	184 188	255 259
Denisovskiy	135 137	236 238	245 247	184 190	202 204	243 255
Dimatskun	133 135	238 240	241 249	182 184	188 196	243 247
Morozko	129 143	226 238	247 249	182 190	202 204	247 255
Saperavi severnyi	129 133	226 238	239 247	182 192	200 204	243 255
Stanichnyi	135 139	238 266	233 243	184 188	188 196	255 259
Fioletovyi ranniy	129 149	234 238	241 249	180 182	184 186	255 259
Cvetochnyi	133 135	230 238	233 247	180 184	196 204	255 255

Dimatskun, Korinka russkaya, Morozko, Saperavi severnyi, Stanichnyi, Cvetochnyi and Fioletovyi ranniy can be used in breeding as donors of *Rpv10.* Also, all these cultivars have increased frost resistance.

Conclusion

Using the DNA markers GF09-44, GF09-46 and GF09-47 linked to downy mildew resistance locus *Rpv10*, we analyzed 30 genotypes of grapes that could inherit this *R*-loci, according to their pedigrees. *Rpv10* locus was detected in the DNA of cultivars Avgusta, Golubok, Denisovskiy, Dimatskun, Korinka russkaya, Morozko, Saperavi severnyi, Stanichnyi, Cvetochnyi and Fioletovyi ranniy. All these cultivars were genetically characterized with the standard set of six SSRs for identification of grape cultivars. It was also shown by the results of SSR analysis of Korinka russkaya and Zarya Severa genotypes that cultivar Zarya Severa is not the parent of Korinka russkaya. The presence of *Rpv10* locus in Korinka russkaya genotype also confirms these data, since Zarya Severa does not carry *Rpv10*. Comparison of Korinka russkaya and Severnyi DNA profiles confirmed the assumption that Severnyi is the parent of Korinka russkaya cultivar. Thus, the pedigree of Korinka russkaya grape cultivar has been clarified.

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