

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

E.T. Ilnitskaya¹✉, M.V. Makarkina¹, S.V. Tokmakov¹, L.G. Naumova²

¹ North-Caucasian Federal Scientific Center of Horticulture, Viticulture, Winemaking, Krasnodar, Russia

² Ya.I. Potapenko All-Russian Research Institute of Viticulture and Winemaking – branch of Federal Rostov Agricultural Research Center, Novocherkassk, Russia
✉ ilnitskaya79@mail.ru

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.

For citation: Ilnitskaya E.T., Makarkina M.V., Tokmakov S.V., Naumova L.G. DNA marker identification of downy mildew resistance locus *Rpv10* in grapevine genotypes. *Vavilovskii Zhurnal Genetiki i Seleksii* = *Vavilov Journal of Genetics and Breeding*. 2023;27(2):129-134. DOI 10.18699/VJGB-23-18

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

Е.Т. Ильницкая¹✉, М.В. Макаркина¹, С.В. Токмаков¹, Л.Г. Наумова²

¹ Северо-Кавказский федеральный научный центр садоводства, виноградарства, виноделия, Краснодар, Россия

² Всероссийский научно-исследовательский институт виноградарства и виноделия имени Я.И. Потапенко – филиал Федерального Ростовского аграрного научного центра, Новочеркасск, Россия

✉ ilnitskaya79@mail.ru

Аннотация. Милдью – одно из наиболее распространенных и вредоносных заболеваний виноградной лозы, возбудителем которого считают *Plasmopara viticola*. Сорты *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, Stanichniy, Stepnyak, Sverkhanni 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 MgCl₂ (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 T_m (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 (Ilitskaya 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 DNA fingerprinting 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 (Severnii × Muscat Gamburg))	Ya.I. Potapenko All-Russian Research Institute of Viticulture and Wine-making – branch of Federal Rostov Agricultural Research Center, Russia
Golubok	Severnii × 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	Severnii × 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 Wine-making – branch of Federal Rostov Agricultural Research Center, Russia
Dimatskun	Karmrayut (Adisi × (<i>V. amurensis</i> × Cherniy sladkiy) × Seedling 1563/1 + 21 (Madeleine Angevine × <i>V. amurensis</i>) × Seyanets Malengra 65/16 (open pollination of Seedling Malengra cultivar))	Armenian Academy of Viticulture, Wine-Making and Fruit-Growing, Armenia
Korinka russkaya	Zarya Severa Severnii × Kishmish cherniy	I.V. Michurin Federal Scientific Center, Russia
Morozko	Mitsar × Saperavi severnii (Severnii × Saperavi)	North Caucasian Federal Scientific Center for Horticulture, Viticulture, Winemaking, Russia
Saperavi severnii	Severnii × Saperavi	Ya.I. Potapenko All-Russian Research Institute of Viticulture and Wine-making – branch of Federal Rostov Agricultural Research Center, Russia
Stanichnyi	Cvetochnyi (Severnii × pollen mix of muscat cultivars) × Zala Gyoengye	Ya.I. Potapenko All-Russian Research Institute of Viticulture and Wine-making – branch of Federal Rostov Agricultural Research Center, Russia
Fioletovyi ranniy	Severnii × Muscat Gamburg	Ya.I. Potapenko All-Russian Research Institute of Viticulture and Wine-making – branch of Federal Rostov Agricultural Research Center, Russia
Cvetochnyi	Severnii × 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 Wine-making – branch of Federal Rostov Agricultural Research Center, Russia

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

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

Note. The alleles inherited by Korinka russkaya from Severnii 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 Severnii cultivar, according to the analysis of the history of Korinka russkaya genotype origin. In addition, the information that Severnii 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 Severnii. The allele from Severnii cultivar was identified in each analyzed locus of Korinka russkaya, accordingly (see Table 3). So Severnii 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	234	239	176	188	247
	151	242	239	190	194	247
Pinot noir	137	230	239	186	188	239
	151	240	243	190	194	245
Avgusta	133	228	237	180	180	255
	133	238	249	190	186	261
Golubok	129	238	239	182	184	255
	133	248	241	184	188	259
Denisovskiy	135	236	245	184	202	243
	137	238	247	190	204	255
Dimatskun	133	238	241	182	188	243
	135	240	249	184	196	247
Morozko	129	226	247	182	202	247
	143	238	249	190	204	255
Saperavi severnyi	129	226	239	182	200	243
	133	238	247	192	204	255
Stanichnyi	135	238	233	184	188	255
	139	266	243	188	196	259
Fioletovyi ranniy	129	234	241	180	184	255
	149	238	249	182	186	259
Cvetochnyi	133	230	233	180	196	255
	135	238	247	184	204	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.

References

Abuzov M. Atlas of Northern Grapes. Smolensk: KFH Pitomnik Publ., 2009. (in Russian)
 Alleweldt G., Possingham J.V. Progress in grapevine breeding. *Theor. Appl. Genet.* 1988;75:669-673. DOI 10.1007/BF00265585.

Bellin D., Peressotti E., Merdinoglu D., Wiedemann-Merdinoglu S., Adam-Blondon A.F., Cipriani G., Morgante M., Testolin R., Di Gaspero G. Resistance to *Plasmopara viticola* in grapevine ‘Bianca’ is controlled by a major dominant gene causing localised necrosis at the infection site. *Theor. Appl. Genet.* 2009;120:163-176. DOI 10.1007/s00122-009-1167-2.
 Bhattarai G., Fennell A., Londo J.P., Coleman C., Kovacs L.G. A novel grape downy mildew resistance locus from *Vitis rupestris*. *Am. J. Enol. Vitic.* 2020;2:12-20. DOI 10.5344/ajev.2020.20030.
 De Mattia F., Imazio S., Grassi F., Baneh H.D., Scienza A., Labra M. Study of genetic relationships between wild and domesticated grapevine distributed from middle east regions to European countries. *Rend. Lincei.* 2008;19:223-240. DOI 10.1007/s12210-008-0016-6.
 Di Gaspero G., Copetti D., Coleman C., Castellarin S.D., Eibach R., Kozma P., Lacombe T., Gambetta G., Zvyagin A., Cindrić P., Kovács L., Morgante M., Testolin R. Selective sweep at the *Rpv3* locus during grapevine breeding for downy mildew resistance. *Theor. Appl. Genet.* 2012;124(2):277-286. DOI 10.1007/s00122-011-1703-8.
 Divilov K., Barba P., Cadle-Davidson L., Reisch B.I. Single and multiple phenotype QTL analyses of downy mildew resistance in interspecific grapevines. *Theor. Appl. Genet.* 2018;131(5):1133-1143. DOI 10.1007/s00122-018-3065-y.
 Eibach R., Zyprian E., Welter L., Töpfer R. The use of molecular markers for pyramiding resistance genes in grapevine breeding. *Vitis.* 2007;46(3):120-124. DOI 10.5073/vitis.2007.46.120-124.
 Fu P., Wu W., Lai G., Li R., Peng Y., Yang B., Wang B., Yin L., Qu J., Song Sh., Lu J. Identifying *Plasmopara viticola* resistance Loci in grapevine (*Vitis amurensis*) via genotyping-by-sequencing-based QTL mapping. *Plant Physiol. Biochem.* 2020;154:75-84. DOI 10.1016/j.plaphy.2020.05.016.
 Ilnitskaya E., Tokmakov S., Makarkina M., Suprun I. Identification of downy mildew resistance genes *Rpv10* and *Rpv3* by DNA-marker

- analysis in a Russian grapevine germplasm collection (Conference Paper). *Acta Hort.* 2019;1248:129-134. DOI 10.17660/ActaHortic.2019.1248.19.
- Lin H., Leng H., Guo Y., Kondo S., Zhao Y., Shi G., Guo X. QTLs and candidate genes for downy mildew resistance conferred by interspecific grape (*V. vinifera* L. × *V. amurensis* Rupr.) crossing. *Sci. Hort.* 2019;244:200-207. DOI 10.1016/j.scienta.2018.09.045.
- Ochssner I., Hausmann L., Töpfer R. *Rpv14*, a new genetic source for *Plasmopara viticola* resistance conferred by *Vitis cinerea*. *Vitis*. 2016;55:79-81. DOI 10.5073/vitis.2016.55.79-81.
- Possamai T., Migliaro D., Gardiman M., Velasco R., De Nardi B. *Rpv* mediated defense responses in grapevine offspring resistant to *Plasmopara viticola*. *Plants*. 2020;9(6):781. DOI 10.3390/plants9060781.
- Riaz S., Tenschler A.C., Ramming D.W., Walker M.A. Using a limited mapping strategy to identify major QTLs for resistance to grapevine powdery mildew (*Erysiphe necator*) and their use in marker-assisted breeding. *Theor. Appl. Genet.* 2011;122:1059-1073. DOI 10.1007/s00122-010-1511-6.
- Rogers S.O., Bendich A.J. Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. *Plant Mol. Biol.* 1985;19:69-76. DOI 10.1007/BF00020088.
- Ruiz-García L., Gago P., Martínez-Mora C., Santiago J.L., Fernández-López D.J., Martínez M.D.C., Boso S. Evaluation and pre-selection of new grapevine genotypes resistant to downy and powdery mildew, obtained by cross-breeding programs in Spain. *Front. Plant Sci.* 2021;12:674510. DOI 10.3389/fpls.2021.674510.
- Sapkota S., Chen L.L., Yang S., Hyma K.E., Cadle-Davidson L., Hwang C.F. Construction of a high-density linkage map and QTL detection of downy mildew resistance in *Vitis aestivalis*-derived "Norton". *Theor. Appl. Genet.* 2019;132:137-147. DOI 10.1007/s00122-018-3203-6.
- Sargolzaei M., Maddalena G., Bitsadze N., Maghradze D., Bianco P.A., Failla O., Toffolatti S.L., De Lorenzis G. *Rpv29*, *Rpv30* and *Rpv31*: three novel genomic loci associated with resistance to *Plasmopara viticola* in *Vitis vinifera*. *Front. Plant Sci.* 2020;11:1537. DOI 10.3389/fpls.2020.562432.
- Schwander F., Eibach R., Fechter I., Hausmann L., Zyprian E., Töpfer R. *Rpv10*: a new locus from the Asian *Vitis* gene pool for pyramiding downy mildew resistance loci in grapevine. *Theor. Appl. Genet.* 2012;124:163-176. DOI 10.1007/s00122-011-1695-4.
- This P., Jung A., Boccacci P., Borrego J., Botta R., Costantini L., Crespan M., Dangl G.S., Eisenheld C., Ferreira-Monteiro F., Grando S., Ibáñez J., Lacombe T., Laucou V., Magalhães R., Meredith C.P., Milani N., Peterlunger E., Regner F., Zulini L., Maul E. Development of a standard set of microsatellite reference alleles for identification of grape cultivars. *Theor. Appl. Genet.* 2004;109:1448-1458. DOI 10.1007/s00122-004-1760-3.
- This P. Microsatellite markers analysis. In: Minutes of the First Grape Gen06 Work-shop March 22nd and 23rd, INRA, Versailles (France). 2007;3-42.
- Venuti S., Copetti D., Foria S., Falginella L., Hoffmann S., Bellin D., Cindrić P., Kozma P., Scalabrin S., Morgante M., Testolin R., Di Gaspero G. Historical introgression of the downy mildew resistance gene *Rpv12* from the Asian species *Vitis amurensis* into grapevine varieties. *PLoS One*. 2013;8:e61228. DOI 10.1371/journal.pone.0061228.
- Wan Y., Schwaninger H., He P., Wang Y. Comparison of resistance to powdery mildew and downy mildew in Chinese wild grapes. *Vitis*. 2007;46:132-136. DOI 10.5073/vitis.2007.46.132-136.
- Zini E., Dolzani C., Stefanini M., Gratl V., Bettinelli P., Nicolini D., Betta G., Dorigatti C., Velasco R., Letschka T., Vezzulli S. R-loci arrangement versus downy and powdery mildew resistance level: a *Vitis* hybrid survey. *Int. J. Mol. Sci.* 2019;20(14):3526. DOI 10.3390/ijms20143526.

ORCID ID

E.T. Ilnitskaya orcid.org/0000-0002-2446-0971
M.V. Makarkina orcid.org/0000-0002-3397-0666
S.V. Tokmakov orcid.org/0000-0002-2092-7757
L.G. Naumova orcid.org/0000-0002-5051-2616

Conflict of interest. The authors declare no conflict of interest.

Received June 17, 2022. Revised August 30, 2022. Accepted August 30, 2022.