

A study of the genetic polymorphism of *Plasmopara viticola* in the vineyards of the Krasnodar Territory

Marina V. Makarkina^{1,*}, Sergey V. Tokmakov¹, and Elena T. Ilnitskaya¹

¹North Caucasian Federal Scientific Center of Horticulture, Viticulture, Wine-making, Functional Research Center “Breeding and Nursery”, 350901 Krasnodar, Russia

Abstract. *Plasmopara viticola* oomycete is a seasonal pathogen that causes one of the most harmful diseases of the grapevine – downy mildew. The study of the biodiversity of *Plasmopara viticola* in various zones of viticulture has fundamental goals, as well as practical ones, as it is important for understanding the epidemiological cycle of *P. viticola* and for refining disease prediction models. The purpose of the work is to study the genetic diversity of *P. viticola* in the vineyards of the Krasnodar Territory, including on the grape varieties with different levels of resistance to downy mildew. The study was conducted on pathogens samples collected on grape plants of varieties with varying degrees of resistance to downy mildew. The collection of material was carried out in May-August 2019 in various zones of the Krasnodar Territory. 48 samples of *P. viticola* were analyzed. The DNA markers BER, ISA, CES, GOB, PV91, PV137, PV143, PV144 recommended for studying the genetic diversity of *P. viticola* were used. The work was performed by PCR. The amplification products were evaluated by the method of fragment analysis. DNA-marker GOB identified 37 alleles of different sizes, PV144 – 20, CES – 10, BER – 3, PV91 – 3, PV137 – 2, ISA – 1, PV143 – 1. It was shown that *P. viticola* populations are variable on different varieties and in different geographical areas. This study was conducted in the Krasnodar Territory for the first time.

1 Introduction

Plasmopara viticola oomycete is a seasonal pathogen that is the causative agent of one of the most harmful diseases of the grapevine – downy mildew. In recent years, the incidence of downy mildew in the world has increased. In some regions, pathogen populations are changing to more aggressive races that can damage previously resistant varieties. Downy mildew causes particular damage in the humid areas of the Black Sea coast of the North Caucasus. The relevance of studying the *P. viticola* biodiversity in different zones of viticulture is determined not only by fundamental goals, but also by the practical significance of such works for a better understanding of the epidemiological cycle of *P. viticola* and for clarifying disease prediction models. The purpose of the work is to study the genetic diversity

* Corresponding author: konec_citatu@mail.ru

of *P. viticola* in the vineyards of the Krasnodar Territory on grape varieties with different levels of resistance to downy mildew.

2 Materials and methods

The study was carried out on pathogen samples collected from plants of various grape varieties that have increased resistance to downy mildew (Augustine, Kishmish 342, Bianka, Kunleany, Vanessa Seedless, TANA19 (Zala Gyoengye x Beisug) and fairly unstable genotypes (Kishmish luchistyi, Kishmish belyi kruglyi, Kishmish rozovyi, Attica, Merlot Noir, Cabernet Sauvignon). The collection was carried out from leaf blades affected by downy mildew in an active phase. Material was collected in May-August 2019 in various zones of the Krasnodar Territory – Anapa, Taman, Krymsk, Krasnodar. 48 samples of *P. viticola* DNA were analyzed (three samples from each cultivar/geographical point). DNA was isolated using the CytoSorb kit (Syntol, Russia). DNA markers BER, ISA, CES, GOB, PV91, PV137, PV143, PV144 recommended for studying the genetic diversity of *P. viticola* were used [1-2]. The work was performed by PCR according to the following scheme: 3 minutes at 95 °C – initial denaturation, then 35 cycles: 20 seconds at 95 °C – denaturation, 30 seconds – annealing of primers GOB, CES, ISA, BER at 60 °C, PV144, PV143, PV137 – at 58 °C, PV91 – at 55 °C; 40 seconds at 72 °C – elongation; then 5 minutes at 72 °C – final elongation. A PCR mixture with a total volume of 25 µl contained: 50-70 ng DNA, 1x PCR buffer for Taq polymerase with ammonium sulfate and magnesium, 0.125 mM/µl dNTP, 0.25 pM/µl of each primer, 0.05 % BSA (bovine serum albumin), 0.125 ea/µl Taq polymerase (SibEnzyme-M, Russia). BSA was added to the PCR mixture to reduce the probability of inhibition of PCR by polyphenols, which accumulate in grape leaves and can affect amplification. The sizes of the amplified fragments were estimated using ABI Prism 3130 automated genetic analyzer (Applied Biosystems, USA) by fragment analysis. The data were processed in the Gene Mapper 4.1 program.

3 Results and discussion

At the first stage of the work, our task was to study the degree of polymorphism of the selected markers in the studied sample (48 samples). The GOB DNA marker was able to identify 37 alleles of different sizes, PV144 – 20, CES – 10, BER – 3, PV91 – 3, PV137 – 2, ISA – 1, PV143 – 1. Thus, the largest polymorphism was detected by markers GOB, PV144, CES, to a lesser extent PV91, BER and PV137. Markers ISA and PV143 did not reveal polymorphism in the studied sample. The results obtained on the level of marker polymorphism correspond to data from published sources [1-2]. So in the work of D. Gobbin and co-authors who studied the diversity of *P. viticola* populations in Italian vineyards, the largest polymorphism was also detected by the GOB marker, which showed 43 different alleles in 190 samples, while CES, ISA, BER – 14, 4 and 3, respectively. [1]. South African *P. viticola* populations, like the European ones, also have a high level of genetic diversity at the GOB and CES loci [3]. In 2012, M. Rouxel and co-authors identified 89 different multilocus genotypes among 96 genotyping *P. viticola* isolates from three European and three North American populations using 35 SSR markers (PV61...PV148) [2]. The greatest polymorphism was revealed by marker PV144 – 11 different types of alleles in the European population and 11 in the North American, by marker PV143 – 3 and 4, respectively, PV127 – 3 and 5, PV91 – 2 and 3 [2].

At the second stage of the study, we were faced with the task of establishing relationships between *P. viticola* populations parasitizing on varieties with different degrees of resistance to downy mildew, as well as on the same varieties in the same and different geographical

locations. The basis of the experiment was the assumption of a possible polymorphism between pathogen populations on grape varieties with a high degree of resistance to downy mildew and susceptible varieties.

Mostly resistant to downy mildew grape genotypes are ones that carry the gene plasm of American and Asian grape varieties, as it is believed that tolerance to the pathogen in grapes developed simultaneously with the evolution of microorganisms - endemic to North America and Asia. Varieties *V. vinifera*, as a rule, are susceptible to downy mildew.

The greatest number of alleles for the GOB marker was found in the analysis of the pathogen collected on the Bianka variety growing in the Taman zone – 6 types of alleles. This genotype is an interspecific hybrid (Villard Blanc (56.19 % *V. vinifera* + 3.13 % *V. labrusca* + 29.16 % *V. rupestris* + 6.25 % *V. berlandieri* + 5.28 % *V. lincecumii*) x Bouvier); American species – resistance donors are present in the pedigree; it can be assumed that the hybrid is populated by a genetically heterogeneous population of *P. viticola* in order to overcome the resistance of the variety. On the hybrid variety Kishmish 342 (Villar blanc x Perlette), which has the same source of resistance in parental forms, 6 types of alleles were also found in the population collected in the zone “Krasnodar”. However, when analyzing a pathogen collected on the same variety (Kishmish 342) in the Anapa zone, only the one allele was determined. It should be noted that the material for the study was selected in the first case in May (in the first generation of the pathogen), and in the second - at the end of June (in the second generation). Thus, it can be assumed that the structure of the pathogen population is affected by the zone of growth of the grape plant and the period of collection of the material (pathogen generation). According to the CES marker, alleles 152, 157 and 164 bp were identified in the pathogen populations collected on varieties growing in the zone “Anapa” (Kishmish 342 and Kishmish rozovyi) and in the zone “Krasnodar” (Kishmish luchisty), respectively, occurring singly. It should be noted that according to preliminary data, the population identified on the variety Kunleany (an interspecific hybrid with *V. amurensis*) showed the most homogeneous genetic structure. The same alleles were identified on all the studied markers on different plants of this variety [Table 1].

Table 1. DNA Analysis Results

Variety/zone and time of collection	Identified alleles, nucleotide pairs (bp)							
	GOB	CES	ISA	BER	PV91	PV137	PV143	PV144
Kunleany, Taman, May	281:285	151:154	134	137	146:154	261:264	139	174:186
	281:285	151:154	134	137	146:154	261:264	139	187:199
	281:285	151:154	134	137	146:154	261:264	139	174:186
Bianka, Taman, May	273:365	122:151	134	137	146:154	261:264	139	178:185
	313:372	122:151	134	137	146:154	261:264	139	199:208
	366:375	122:151	134	137	154	261:264	139	187:199
Augustine, Taman, May	293:366	149	134	137	146	261:264	139	174:186
	366:383	149	134	137	146:154	261:264	139	188:199
	190:293	149	134	137	146	261:264	139	163:185
Kishmish 342, Krasnodar, May	314:375	149:151	134	136	146:154	261	139	179:192
	331:371	149:151	134	136	154	261:264	139	179:192
	272:310	149:151	134	136	146:154	261:264	139	179:192
Kishmish 342, Anapa, July	293	152	134	136	146:149	261:264	139	179
	293	152	134	136	146:149	261:264	139	185
	293	152	134	136	146:149	261:264	139	179:195

Vanessa Seedless, Anapa, July	193:366 280:366 305:366	151:153 153 151	134 134 134	136 136 136	146:149 146:149 146:149	261:264 261:264 261:264	139 139 139	192 192 192
Kishmish luchistyi, Anapa, July	285:302 375:379 285:302	151:156 151 151:156	134 134 134	124 137 137	146:154 146:154 154	261:264 261:264 261:264	139 139 139	158:179 179 179
Kishmish belyi kruglyi, Anapa, July	276:374 289:374 374	151 151 151	134 134 134	136 136 136	146:149 146:149 146:149	261 261:264 261:264	139 139 139	170 170 170
Kishmish rozovyi, Anapa, July	300 300 300	153:157 153:157 153:157	134 134 134	136 136 136	146:149 146:149 146:149	261 261 261	139 139 139	179:186 179:186 179:186
Attica, Anapa, July	293 293 293	149 149 149	134 134 134	136 136 136	146:149 146:149 146:149	261:264 261:264 261:264	139 139 139	179:186 179:186 179:186
TANA19, Krasnodar, August	279:378 279 279:378	149:153 149:153 149	134 134 134	137 137 137	149 146:149 146:149	261 261 261:264	139 139 139	179:192 179:192 179:192
Kishmish luchistyi, Krasnodar, August	280:295 280:295 280:295	164 164 164	134 134 134	137 137 137	146 146:154 146:154	261:264 261:264 261:264	139 139 139	165 165 165
Merlot Noir, Krymsk, August	197:284 357:374 197	148 148 148	134 134 134	137 137 137	146:154 146 146:154	261 261:264 261	139 139 139	163 179:186 179:186
Merlot Noir, Anapa, August	193:290 307:366 193:290	148 148 148	134 134 134	137 137 137	146:154 154 146:154	261:264 261:264 261:264	139 139 139	179:186 195 179:186
Augustine, Krasnodar, August	198:293 302:371 198:293	154:156 154:156 154:156	134 134 134	124 137 137	146:154 146:154 146:154	261 261 261:264	139 139 139	156 162 175
Cabernet Sauvignon, Anapa, August	292:306 361 361	148 148 148	134 134 134	137 137 137	146:154 146:154 146:154	261:264 261 261:264	139 139 139	179:186 189:195 202
Na – number of different alleles	37	10	1	3	3	2	1	20
Ni – number of alleles	83	68	48	48	87	84	48	77

According to the data obtained, in the first year of the study, the most common alleles identified by the most polymorphic marker GOB: 293 nucleotide pairs (bp) (10 alleles of 83 identified) – 12 %, 366 bp (7 alleles out of 83) – 8, 4 % and 285 bp (5 alleles of 83) – 6 %. Rare alleles (occurring once): 190, 272, 273, 276, 284, 289, 292, 305, 306, 307, 310, 313, 314, 331, 357, 365, 372, 379 and 383 bp. The second polymorphic marker PV144 revealed the most common alleles: 179 bp (22 alleles of 77 identified) – 28,6 %, 186 bp (14 alleles of

77) – 18.2 % and 192 bp (9 alleles out of 77) - 11.7 %. Rare alleles: 156, 158, 162, 175, 178, 188, 189, 202 and 208 bp. By CES marker, the most common are: 151 (17 out of 68 identified) – 25 %, 149 bp (12 of 68) - 17.6%, 148 bp (9 out of 68) - 13.2%, 153 bp (7 out of 68) – 10.3 %. Rare alleles (found 3 times each): 122, 152, 157 and 164 bp [Table 1].

The data obtained on the variability of populations of *P. viticola* both within the same variety and on different varieties that differ in the degree of resistance to the pathogen, as well as in different geographical locations, correspond to world research. So in 2006, Rumbou and Gessler, using SSR markers designed by D. Gobbin and co-authors, studied the diversity of *P. viticola* populations collected from three islands in the Ionian Sea west of Greece [4]. It was found that populations of *P. viticola* from previously studied areas of the mainland have high genetic diversity and limited clonality, but populations in the Mediterranean Islands were mostly characterized by limited variations, and epidemics were mainly caused by multiple clonal infections of one or more genotypes. Populations from different islands differed from each other, while genetic divergence was also found among subpopulations of the same site [4]. In addition, a number of studies of populations of *P. viticola* in Europe [4-7], USA [8], South Africa [3], showed a high diversity of the pathogen at different periods of the growing season of a grape plant.

4 Conclusion

According to the results of this work, *P. viticola* populations are variable in different varieties and in different geographical zones. The study of *P. viticola* samples parasitizing on varieties with varying degrees of resistance to downy mildew growing in the same and in different geographical zones did not reveal any patterns. The work continues, it is planned to expand the sample of the pathogen, as well as to attract additional DNA markers to get a more complete information of the genetic structure of *P. viticola* populations. This study was conducted in the Krasnodar Territory for the first time.

The reported study was funded by RFBR and Krasnodar Region according to the research project № 19-416-233038 r_mol_a.

References

1. D. Gobbin, I. Pertot, C. Gessler, European Journal of Plant Pathology **109**, 153 (2003). DOI: 10.1023/A:1022565405974
2. M. Rouxel, D. Papura, M. Nogueira, V. Machefer, D. Dezette, S. Richard-Cervera, S. Carrere, P. Mestre, F. Delmotte, Appl. Environ. Microbiol. **78**, 6337 (2012). DOI: 10.1128/AEM.01255-12
3. T. Koopman, C. C. Linde, P. H. Fourie, A. McLeod, Molecular Plant Pathology **8**, 723 (2007). DOI: 10.1111/j.1364-3703.2007.00429.x
4. A. Rumbou, C. Gessler, Phytopathology **96**, 501 (2006). DOI: 10.1094/PHYTO-96-0501
5. D. Gobbin, M. Jermini, B. Loskill, I. Pertot, M. Raynal, C. Gessler, Plant Pathology **54**, 522 (2005). DOI: 10.1111/j.1365-3059.2005.01208.x
6. A. Rumbou, C. Gessler, Journal of Biological Research **7**, 3 (2007)
7. B. Loskill, D. Gobbin, B. Berkemann-Loehnertz, C. Gessler, IOBC wprs Bulletin **29**, 33 (2006)
8. M. M. Kennelly, D. M. Gadoury, W. F. Wilcox, P. A. Magarey, R. C. Seem, Phytopathology **97**, 512 (2007). DOI: 10.1094/PHYTO-97-4-0512