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

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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

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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 luchisty, 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 ºС – initial denaturation, then 35 cycles: 20 seconds at 95 ºС – denaturation, 30 seconds – annealing of primers GOB, CES, ISA, BER at 60 ºС, PV144, PV143, PV137 – at 58 ºС, PV91 – at 55 ºС; 40 seconds at 72 ºС – elongation; then 5 minutes at 72 ºС – 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 + 31.3 % 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 (Villard 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

<table>
<thead>
<tr>
<th>Variety/zone and time of collection</th>
<th>Identified alleles, nucleotide pairs (bp)</th>
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<tbody>
<tr>
<td></td>
<td>GOB</td>
</tr>
<tr>
<td>Augustine, Taman, May</td>
<td>293:366</td>
</tr>
<tr>
<td></td>
<td>366:383</td>
</tr>
<tr>
<td></td>
<td>190:293</td>
</tr>
<tr>
<td></td>
<td>331:371</td>
</tr>
<tr>
<td></td>
<td>272:310</td>
</tr>
<tr>
<td>Kishmish 342, Anapa, July</td>
<td>293</td>
</tr>
<tr>
<td></td>
<td>293</td>
</tr>
<tr>
<td></td>
<td>293</td>
</tr>
<tr>
<td>Variety</td>
<td>179 bp</td>
</tr>
<tr>
<td>---------</td>
<td>--------</td>
</tr>
<tr>
<td>Seedless, Anapa, July</td>
<td>192</td>
</tr>
<tr>
<td>Kishmish luchisti, Anapa, July</td>
<td>179</td>
</tr>
<tr>
<td>Kishmish belyi kruglyi, Anapa, July</td>
<td>179</td>
</tr>
<tr>
<td>Attica, Anapa, July</td>
<td>179</td>
</tr>
<tr>
<td>TANA19, Krasnodar, August</td>
<td>179</td>
</tr>
<tr>
<td>Kishmish luchisti, Krasnodar, August</td>
<td>179</td>
</tr>
<tr>
<td>Merlot Noir, Krymsk, August</td>
<td>192</td>
</tr>
<tr>
<td>Merlot Noir, Anapa, August</td>
<td>192</td>
</tr>
<tr>
<td>Augustine, Krasnodar, August</td>
<td>192</td>
</tr>
<tr>
<td>Cabernet Sauvignon, Anapa, August</td>
<td>192</td>
</tr>
</tbody>
</table>

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.

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References