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Short Notes

Characterization and genetic diversity of grapevine Pinot gris virus in Serbian vineyards

Sanja ŽIVKOVIĆ[1,*], Bojana VASILIJEVIĆ[2], Tanja VASIĆ[1], Debasis MITRA[3], Darko JEVREMOVIĆ[2]

[1] *University of Niš, Faculty of Agriculture, Kosančićeva 4, 37000 Kruševac, Serbia*
[2] *Fruit Research Institute, Kralja Petra I 9, 32102 Čačak, Serbia*
[3] *Department of Microbiology, Graphic Era (Deemed to be University), 566/6 Bell Road, Clement Town, Dehradun, 248002 Uttarakhand, India*
*Corresponding author. E-mail: zivkovic.sanja@ni.ac.rs

Summary. Sixty-five samples of grapevine from commercial vineyards in the Rasina district of Serbia were tested for the presence of grapevine Pinot gris virus (GPGV), using the reverse transcription-polymerase chain reaction. Fourteen samples of the grapevine varieties 'Red Globe', 'Victoria' and 'Preobraženje' were infected with GPGV. All the infected plants showed symptoms of leaf chlorotic mottling, puckering, and deformation, stunting, and reduced yields. The coding regions of the movement and coat protein (MP/CP) and a region of the RNA-dependent RNA polymerase (RdRP) domains of eight virus isolates were sequenced. Phylogenetic analyses of these genomic regions showed high nucleotide similarity among the Serbian GPGV isolates. This study is the first to describe genetic diversity of GPGV isolates in Serbia.

Keywords: *Vitis vinifera* L., RT-PCR, detection, phylogenetic analyses.

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ORCID:
SŽ: 0000-0002-4451-4957
BV: 0000-0002-9906-6522
TV: 0000-0002-1834-9484
DM: 0000-0003-0525-8812
DJ: 0000-0002-2983-9198

INTRODUCTION

More than 80 viruses naturally infect grapevines (*Vitis vinifera* L.) (Fuchs, 2020). Grapevine fanleaf virus (GFLV), grapevine leafroll-associated viruses (GLRaVs), and grapevine red blotch virus (GRBV) are economically important pathogens that cause severe damage to various plant organs, resulting in low grape yields and reduced berry quality. Most viruses are present in grapevines in mixed infections, although their influence on host plants is minor or unknown.

Grapevine Pinot gris virus was discovered in Trentino, Italy, in 2003, on the cultivar 'Pinot Gris' (Giampetruzzi *et al*., 2012). GPGV is a positive-sense single-stranded RNA virus (*Trichovirus, Betaflexiviridae*) (Tarquini *et al*., 2018). This virus causes chlorotic mottling, puckering, and deformation of 'Pinot Gris' grapevine leaves (Giampetruzzi *et al*., 2012; Martelli, 2014). Stunting, grapevine leaf mottling and deformation (GLMD), discolouration, blistering, and significant yield losses have also been reported on other grapevine cultivars (including 'Traminer,' 'Chardonnay', 'Riesling', and

'Tocai') (Giampetruzzi *et al*., 2012; Malossini *et al*., 2012; Martelli *et al*., 2014; Saldarelli *et al*., 2015; Gentili *et al*., 2017). Latent infections can also be caused by GPGV (Giampetruzzi *et al*., 2012; Saldarelli *et al*., 2013; Eichmeier *et al*., 2016a; 2016b; 2017). GPGV has been frequently identified in mixed infections with other viruses that infect grapevines, making it difficult to determine its influence on vine health. The virus has been reported in numerous countries, including in Europe (Armenia, Belgium, Bosnia and Herzegovina, Croatia, Czech Republic, France, Georgia, Germany, Greece, Hungary, Italy, Moldova, Montenegro, North Macedonia, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Switzerland, Türkiye, Ukraine, and the United Kingdom), in Asia (China, Lebanon, Iran, Japan, Pakistan, and South Korea), Africa (Algeria), and in South America (Argentina) (EPPO, 2024). Hily *et al*. (2020) hypothesized a scenario for GPGV dispersal from an Asian origin to Europe and other world grape producing countries. Movement and exchange of GPGV infected host propagation material (potted vines, cuttings, rootlings, and budwood) is the primary mode for transmission of the virus. Bertazzon *et al*. (2020) reported rapid transmission of GPGV in Italian vineyards, and the grape leaf blister mite (*Colomerus vitis* Pagenstecher) is a putative GPGV vector (Malagnini *et al*., 2016). GPGV does not spread mechanically through pruning or harvesting machinery.

The presence of GPGV in Serbia was confirmed by Bertazzon *et al*. (2016), in one sample from an unknown grapevine cultivar. Since then, no further research has been reported on GPGV presence in this country. The aim of the present study was to investigate presence and genetic diversity of GPGV in Serbia.

MATERIALS AND METHODS

From 2020 to 2022, 65 samples of grapevine leaves were collected from the Rasina district, which is an important grape-producing region in Serbia. Samples were collected from symptomatic and asymptomatic plants. Symptomatic plants had shortened internodes, deformed leaves, and leaf chloroses and mottling. Each sample consisted of five to six leaves picked from different parts of an individual plant. Leaf petiole samples were ground in extraction bags (Flexo), in 2% cetyltrimethylammonium bromide (CTAB) buffer, as described for total nucleic acid (TNA) extraction by Li *et al*. (2008). Reverse transcription (RT) reactions were carried out in two steps with random hexamer $pd(N)_6$ primers and Maxima Reverse Transcriptase (Thermo Scientific). Polymerase chain reactions (PCR) were carried out using two GPGV-specific primer pairs. For the first PCR, the primer pair GPGVRepF/GPGVRepR was used, spanning the RNA-dependent RNA polymerase (RdRp) domain of the replicase gene (525 bp) (Saldarelli *et al*., 2015). The primer pair GPGVDetF/GPGVDetR, spanning the end of the movement protein (MP) and the beginning of the coat protein (CP) gene (585 bp), was used for the second PCR (Saldarelli *et al*., 2015). Polymerase chain reactions were carried out in a TPersonal thermocycler (Biometra), with each reaction containing 1× DreamTaq Green Buffer, 0.2 mM dNTPs, 2.5 mM $MgCl_2$, 0.2 mM each primer, and 0.05U of Green Taq DNA polymerase (ThermoScientific). The thermal cycling conditions were as follows: 5 min at 94°C; 30 cycles each of 30 s at 94°C, 30 s at 55°C, and 60 s at 72°C; and a final step of 10 min at 72°C. Amplicons were visualized using 1.5% agarose gel electrophoresis.

All GPGV-infected samples were additionally tested for the presence of nine other viruses capable of infecting grapevines (GFLV, GRSPaV, GFkV, GLRaV1, GLRaV3, GVA ArMV, GVB, and GlRaV-2), using multiplex RT-PCR as described by Gambino and Gribaudo (2006).

Genetic diversity of the GPGV isolates was assessed by sequencing the amplified products of eight isolates (Table 1) (Macrogen Europe Laboratory, the Netherlands). BioEdit software (Hall, 1999) was used to assemble the raw sequences obtained. Phylogenetic relationships were reconstructed using a Maximum Likelihood (ML) tree, using MEGA11 software (Tamura *et al*., 2021). A phylogenetic tree was generated using the ML method with 1000 bootstrap replications.

RESULTS AND DISCUSSION

The expected 525 bp and 585 bp fragments were obtained in 17% (14 of the 65 tested samples). These results indicate limited distribution of GPGV in the Rasina district of Serbia. GPGV was confirmed in three grapevine cultivars ('Red Globe,' 'Victoria' and 'Preobraženje'), which had symptoms of stunted canes with shortened internodes, chlorotic mottling, and leaf deformation (Figures 1 and 2). GPGV-infected samples were collected from two vineyards located in the village of Božurevac. GPGV-infected samples of 'Victoria' originated from a 15-year-old vineyard, while samples from 'Red Globe' and 'Preobraženje' were taken from a nearby 3-year-old vineyard.

To further characterize the detected virus, fragments of the RdRP and MP/CP genes from eight isolates

Figure 1. Shortened internodes, deformed leaves and stunting of shoots in a GPGV-infected grapevine of 'Red Globe'.

Figure 2. Leaf deformation, chlorosis and mottling in a GPGV-infected grapevine of 'Victoria'.

were selected and partially sequenced (Table 1). Sequences were deposited in NCBI GenBank under accession numbers OP279698 to OP279713. When compared, the detected Serbian GPGV isolates had 97.3 to 100% nucleotide sequence similarities in the RdRP fragments, and 97.6 to 100% similarities in the MP/CP fragments. The sequences from the isolates were compared with those of other GPGV isolates available in the NCBI GenBank database. BLAST analysis of the RdRP sequences showed the greatest (99.62%) nt sequence similarity with isolates from Spain (KY404083), Switzerland (ON237610), and Slovakia (KF134125). Nucleotide sequences of the MP/CP gene showed greatest similarity (98.98 to 99.66%) with isolates from Russia (ON033806) and Hungary (ON360686). In the reconstructed phylogenetic tree with MP/CP sequences, the Serbian isolates clustered into three separate subclusters (Figure 3). In the RdRP sequence phylogenetic tree, the GPGV isolates were clustered into two subclusters (Figure 4). The clustering of the Serbian isolates confirms their close relatedness, and indicates that the samples originated from the same locality. The older vineyard of 'Victoria' grapevines may have been the source of GPGV, that spread to the adjacent younger plants of 'Red Globe' and 'Preobraženje'.

Table 1. Grapevine Pinot gris virus isolates characterized in this study.

Isolate	Year of isolation	Host cultivar	NCBI accession numbers RdRp gene	NCBI accession numbers MP/CP gene
RS-GPGV-1	2021	'Red Globe'	OP279698	OP279706
RS-GPGV-4	2021	'Red Globe'	OP279699	OP279707
RS-GPGV-6	2021	'Red Globe'	OP279700	OP279708
RS-GPGV-8	2021	'Red Globe'	OP279701	OP279709
RS-GPGV-12	2021	'Preobraženje'	OP279702	OP279710
RS-GPGV-13	2021	'Preobraženje'	OP279703	OP279711
RS-GPGV-14	2021	'Victoria'	OP279704	OP279712
RS-GPGV-15	2021	'Victoria'	OP279705	OP279713

In addition to GPGV, other viruses were detected by RT-PCR in the samples tested. GFLV, GFkV, GRSPaV, and GLRaV1 were detected in all the GPGV-infected

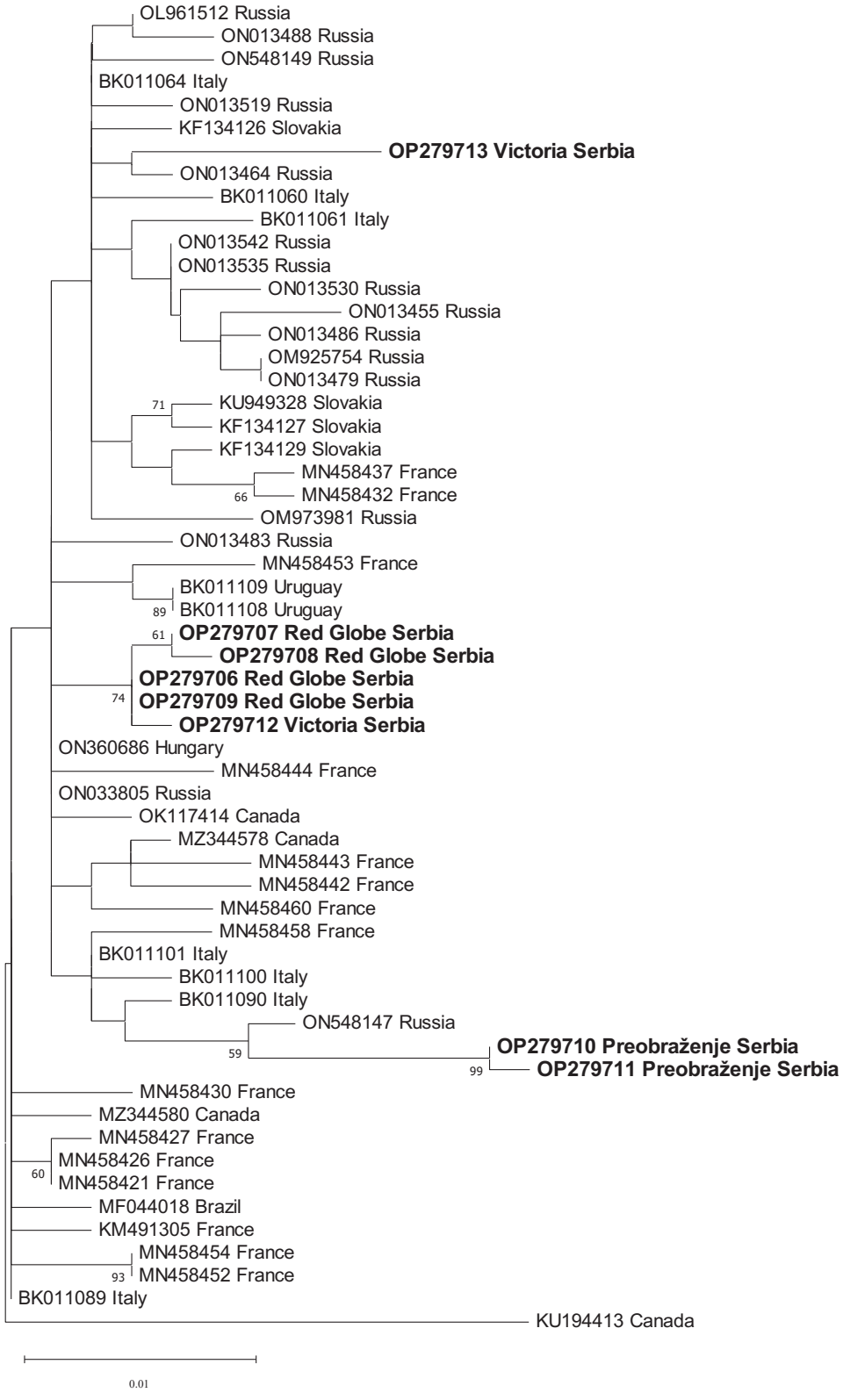

Figure 3. Phylogenetic analysis of partial MP/CP sequences of 58 grapevine Pinot gris virus isolates retrieved from NCBI. Phylogenies were inferred with ML method based on Kimura 3-parameter model with gamma distribution (MEGA11). Bootstrap values (> 50%) are displayed next to respective branches. The NCBI accession numbers are in parentheses. Serbian isolates are in bold font.

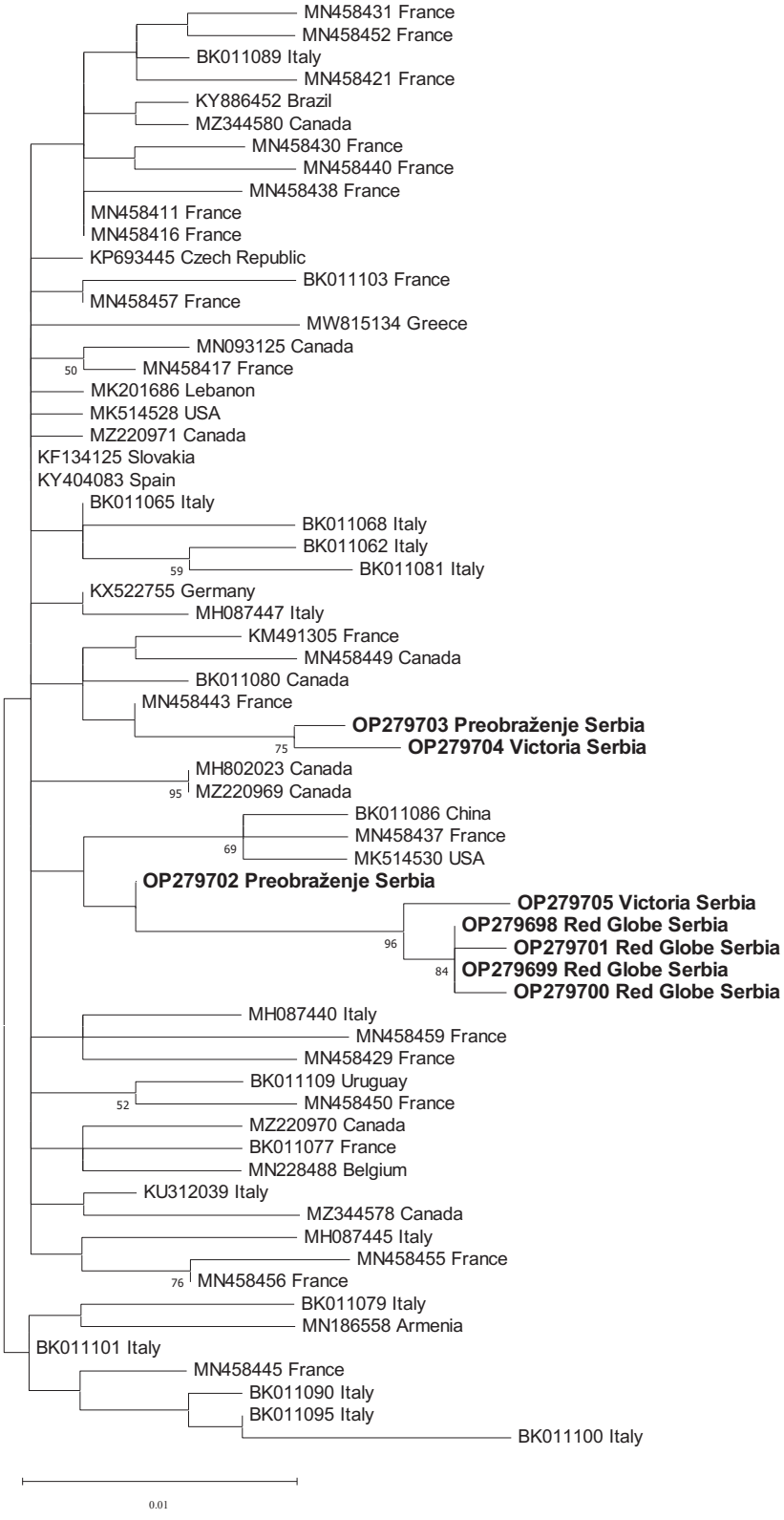

Figure 4. Phylogenetic analysis of partial RdRP sequences of 65 grapevine Pinot gris virus isolates. Phylogenies were inferred with ML method based on Kimura 3-parameter model with gamma distribution (MEGA11). Bootstrap values (> 50%) are displayed next to respective branches. The NCBI accession numbers are in parentheses. Serbian isolates are in bold font.

samples. In varieties 'Preobraženje' and 'Victoria', GFkV and GRSPaV were also confirmed.

All the GPGV-infected samples showed typical symptoms, as described by Giampetruzzi *et al*. (2012). The most significant symptom found in all infected vines was plant stunting, followed by shortened internodes and chlorotic leaves. Saldarelli *et al*. (2015) detected GPGV in asymptomatic samples, but in the present study GPGV was not confirmed in samples with no symptoms. Other viruses that caused similar symptoms (GFLV and GFkV) were detected in all 14 GPGV-positive samples from Serbia. Therefore, it cannot be confirmed which virus was the primary cause of the disease symptoms, or whether the symptoms resulted from mixed virus infections.

The presence of symptoms in infected grapevines depends on the genetic variability of GPGV isolates (Tarquini *et al*., 2018). In addition, the expression of symptoms correlates with different virus variants and/or virus titres. Bertazzon *et al*. (2017) confirmed that symptomatic grapevines have a greater GPGV titres than asymptomatic grapevines. The frequency of manifestation of symptoms has been found to be affected by soil and terrain types (Angelini *et al*., 2015).

The Serbian GPGV isolates showed high nucleotide similarities in both genomic regions examined. Analyses of the nucleotide sequences of partial MP and CP genes showed that the Serbian GPGV isolates were closely related to Russian and Hungarian isolates. Considering the RdRP domain, the Serbian isolates were closely related to Spanish, Swiss, and Slovakian isolates.

This is the first comprehensive study of GPGV in Serbia. Further research should be carried out to characterize the incidence and prevalence of GPGV in vineyards across all districts of the country.

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