INCIDENCE OF GRAPEVINE PINOT GRIS VIRUS IN THE WESTERN BLACK SEA REGION, TURKEY AND PHYLOGENETIC ANALYSIS BASED ON COAT PROTEIN AND MOVEMENT PROTEIN GENES

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Abstract. The aim of this research was to detect the presence of GPGV and to perform phylogenetic analysis of the isolates obtained from different vineyards in Turkey based on the partial coat protein and movement protein gene. A total of 418 grapevine samples were systematically collected from vineyards situated in Amasya, Çorum, and Tokat during the 2017 growing season. These regions hold commercial importance in viticulture in the Western Black Sea Region of Turkey. The collected samples were subjected to comprehensive testing for the presence of Grapevine Pinot gris virus (GPGV). Detection was performed using Reverse Transcription-Polymerase Chain Reaction (RT-PCR) based on the CP and MP gene regions. The infection rate was estimated at 15.87% based on the results of the molecular assays. Seven PCR products were selected and sequenced from both the partial CP and MP gene regions. According to the phylogenetic analysis, all GPGV isolates which belong to the movement protein region obtained in this study, 3 other Turkish isolates and 2 Czech Republic isolates showed the highest similarity. However, the GPGV isolates which belong to the coat protein region were closely grouped with the other Turkish and Slovenian isolates. This study revealed, for the first time, the existence of *Grapevine Pinot gris virus* (GPGV) in vineyards in the Western Black Sea Region of Turkey, and provided additional Turkish isolates which suggest new insights into genetic variability research.
Keywords: *grapevine, GPGV, CP, MP, phylogeny*

Introduction

Vitis vinifera L., commonly known as grapevine, is a significant and widespread fruit species globally. The importance of grapevines goes beyond the production of wine, playing a crucial role in both the food and beverage industries (Viver and Pretioru, 2002). Turkey occupies an important position in the world with a yield of 3,670 million tonnes (FAO, 2021).

To date, more than 86 viruses, from different genera and families, have been documented in association with grapevines (Fuchs, 2020; Al Rwahnih et al., 2021) and *Grapevine Pinot gris virus* is one of them (Giampetruzzi et al., 2012).

After the first determination of GPGV in Turkey (Çağlayan et al., 2015), a comprehensive study has been carried out in our country (Tekirdağ, Manisa, Mardin) on detection, distribution, and molecular characterization of GPGV by Elçi et al. (2018). The current study was the second study related to virus in Turkey and the second for the survey area. Thus, the aim of this research was first to expose the presence of GPGV in the

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Western Black Sea Region of Turkey and to perform the phylogenetic analyzes of the isolates obtained from this study and other GPGV isolates based on partial coat protein (CP) gene and movement protein (MP) sequences.

Review of literature

Furthermore, it is anticipated that additional viral species will be identified and characterized in future studies (Maliogka et al., 2015), such as *Grapevine Pinot gris virus*, which was first described in northern Italy by high-troughput sequencing on a Pinot gris grapevine variety, demonstrating mottling and malformation of the leaves (Giampetruzzi et al., 2012). Subsequently, the virus was detected in Southern Italy (Morelli et al., 2014) and several other countries, including South Korea (Cho et al., 2013), Slovenia (Plesko et al., 2014), Slovakia and Czech Republic (Glasa et al., 2014), Greece (Martelli, 2014), France (Beuve et al., 2015), Uruguay (Jo et al., 2015), Georgia (Casati et al., 2015), Canada (Xiao et al., 2016), China (Fan et al., 2015), USA (Al Rwahnih et al., 2016; Angelini et al., 2016), and Turkey (Gazel et al., 2016). The etiology of the disease is not yet fully understood; indeed, several reports have indicated the presence of *Grapevine Pinot Gris virus* (GPGV) in a significant proportion of symptomatic plants, as well as in a considerable number of asymptomatic grapevines, as demonstrated by Saldarelli et al. (2015). Also, in a recent study by Bertazzon et al. (2017), a notable correlation was observed between the presence of *Grapevine Pinot gris virus* (GPGV) and the manifestation of leaf mottling and deformation in grapevines.

The results of a three-year field study indicated that vines affected by the grapevine Pinot Gris virus (GPGV)-associated disease exhibited a reduction in the number of canes, and a decrease in the quantity and weight of bunches, as reported by Malossini et al. (2012). Recent data on the cultivar *Vitis vinifera* L. cv. Glera have confirmed and corroborated this finding, as documented by Bertazzon et al. (2015). In addition, this emerging disease leads to economic losses due to a decline in the quantity, weight, and quality of berries, as highlighted by Martelli pointed out in 2014.

Grapevine Pinot gris virus (GPGV) is a positive sense single-stranded RNA virus, consist of 7258 nucleotides, excluding the polyA tail. The genomic RNA of GPGV comprises three open reading frames (ORFs). The first ORF, ORF1, is 1865 amino acids (aa) in length, corresponding to a molecular weight of 214 kDa. This ORF encodes, in sequential order, the replicase-associated proteins, including methyl transferase (aa 44–333), helicase (aa 1040–1277), and RNA-dependent RNA polymerase (RdRp, aa 1447–1797). ORF2 encodes for a 376 aa polypeptide 42 kDa in size homologous to movement protein; ORF3 encodes the 195 aa (22 kDa) putative coat protein (CP; Giampetruzzi et al., 2012).

The spread of GPGV can be influenced by the movement and exchange of infected propagation material such as potted vines, bud wood, rootlings and cuttings (Malagnini et al., 2016). There is no evidence to support the transmission of the virus mechanically on pruning or harvesting equipment (Constable et al., 2019). Additionally, it mentions local spread from vine to vine facilitated by vectors. Several studies have documented the apperent absence of spontaneous viral transmission, as determined through the examination of grapevines in close proximity to infected specimens (Al Rwahnih et al., 2016; Wu et al., 2017). Alternatively, an incrise in the prevalence of infected grapevines over a three-year period frame indicated the an active spread of the virus in the vine growing region (Martelli, 2014; Bertazzon et al., 2018). The spread of the disease within vineyards through natural transmission mechanisms can be attributed to the eriophyid

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grape bud mite *Colomerus vitis*, which have been identified as a monophagous vector for the virus under controlled conditions (Malagnini et al., 2016). Moreover, an additional polyphagous vector might play a role in the epidemiology of Grapevine Pinot Gris Virus (GPGV), as shown by the presence of the virus in woody and herbaceous hosts near the vineyards (Gualandri et al., 2017). Subsequent studies are essential to clarify the potential roles of presumed vectors in the epidemiology of *Grapevine Pinot gris virus* (GPGV) and the associated disease.

Methods

Field survey

The material of the study consists of leaf and young shoot samples obtained from grapevines in vineyards in Tokat, Çorum and Amasya provinces (*Figure 1*). Grapevine samples were collected from the Center, Erbaa, Zile, Niksar and Pazar districts of Tokat province, the Center, Alaca, Bayat, Boğazkale, İskilip, Mecitözü, Ortaköy, Osmancık, Sungurlu and Uğurludağ districts of Çorum province and the Center and Taşova districts of Amasya province (*Figure 2*). A total of, 418 grapevine samples with virus-like symptoms and without symptoms were taken in the summer of 2017 (May-June and September) from various vineyards in the Western Black Sea Region of Turkey.

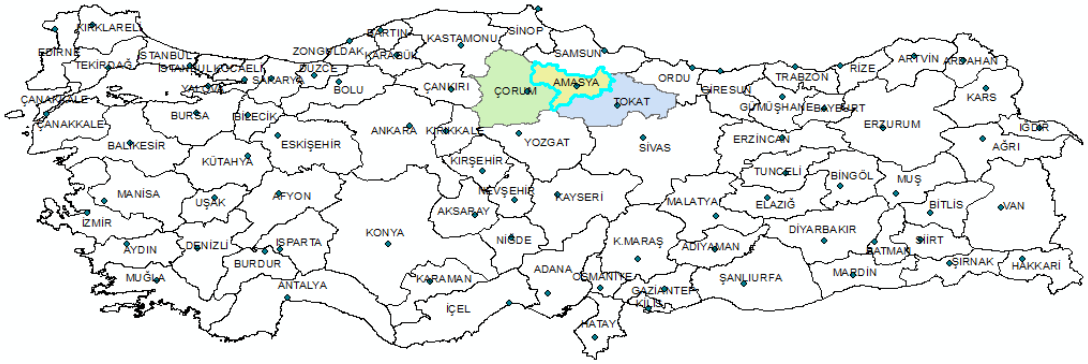

Figure 1. *The provinces where survey studies were conducted*

Figure 2. *The districts where samples were taken*

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In the survey area mostly table grape is grown and local varieties are common, especially Narince which are valued for its leaf besides grape. There are not only self-rooted vines but also grafted ones on rootstocks among the sampled grapevines.

Total RNA extraction, RT-PCR and sequencing

The collected samples were wrapped in paper towels, placed in plastic bags, labeled, brought to the laboratory in ice bags and placed in a deep freezer at – 20 °C.

The extraction of total ribonucleic acids (RNAs) from 100 mg of leaf tissues was performed manually, following the CTAB (cetyltrimethylammonium bromide) method (Li et al., 2008), which is modified during the study to enhance the extraction process. The quantity and quality values of total RNAs were measured by using a NanoDrop spectrophotometer.

The amplification of a partial region of the Coat Protein (CP) and Movement Protein (MP) of *Grapevine Pinot gris virus* (GPGV) was carried out utilizing a pair of universal primers (*Table 1*) as designated by Glasa et al. (2014). cDNA synthesis was practised by two-step RT-PCR protocol: Initially, 500-600 ng of total RNA, 3 µl of Reverse primer and NFh20, adjusted according to the RNA yield, incubated at 95 °C for 5 min then at 0 °C for 5 min. For the next step, 0.2 µl of 200 U/µl Reverse transkriptase, 6 µl of 5X RT buffer, 0.4 µl of 40U/µl RNase inhibitor, 1.5 µl of 10 mM dNTP and 1.9 µl of NFh20 incubated at 42 °C for 1 h (Thermo Fisher Scientific, USA). The PCR was conducted in 25 µl total volume containing 2 µl of cDNA, 2.5 µl of 10X PCR buffer, 1.5 µl of 25 mM $MgCl_2$, 0.4 µl of 10 mM dNTPs, 0.4 µl of 10 µM of forward and reverse primer (*Table 1*) with 0.25 µl of 500 U/µl Taq DNA polymerase (Thermo Fisher Scientific, USA). PCR was performed by the following cycles: primary denaturation at 95 °C for 3 min, 34 cycles of amplification at 95 °C for 45 s, 53 °C for 45 s, 72 °C for 45 s and a final extension at 72 °C for 10 min. The PCR products were analyzed with 1% agarose gels and were monitored with the Gel Imaging System after waiting in ethidium bromide for a while. All of the PCR products were sequenced bidirectionally with the same primers as for PCR.

Table 1. *The primer pair used in RT-PCR assay for detection of grapevine Pinot gris virus*

Primer name	Sequence (5'–3')	Position	Amplicon size (bp)	References
GPG-6609F GPG-7020R	5'-GAGATCAACAGTCAGGAGAG-3' 5'-GACTTCTGGTGCCTTATCAC-3'	CP	411	Glasa et al., 2014
GPG-5637F GPG-5939R	5'-ATTGCGGAGTTGCCTTCAAG-3' 5'-CTGAGAAGCATTGTCCCATC-3'	MP	302	Glasa et al., 2014

Multiple alignments and phylogenetic analysis

Multiple alignments of nucleotide sequences obtained in the present study, alongside pertinent sequences available in the GenBank database, were conducted using ClustalW, which was integrated into the MEGA7 software platform (Kumar et al., 2016). Maximum Likelihood phylogenetic tree was established using the MEGA7 with the best fit model Tamura-3G parameter for coat protein and movement protein gene and 1000 bootstrap. Sequences from this study are given in *Table 2*.

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Table 2. *Grapevine Pinot gris virus isolates analyzed in this study (CP and MP gene)*

Isolate ID	Location	GenBank accession no.	
		Coat Protein	Movement Protein
ATB1-2	Amasya	MN218575	MN175691
TEBp1-2	Tokat	MN218576	MN175692
TEY1-3	Tokat	MN218577	MN175693
TEU1-1	Tokat	MN218578	MN175694
TEY1-2	Tokat	MN218579	MN175695
TED1-2	Tokat	MN218580	MN175696
TEY1-1	Tokat	MN218581	MN175697
TNG1-1	Tokat	MN218582	MN175698

Results

Survey

During the surveys in Amasya, Tokat and Çorum, different types of symptoms such as yellow mosaic and curling, chlorotic mottling and leaf deformations were observed (*Figure 3*).

(a) (b)

Figure 3. *Yellow mosaic and curling of the leaves starting from the tips (a), chlorotic mottling and leaf deformation (b)*

The different types of GPGV-associated symptoms were observed during this research. These types of symptoms were commonly observed in the survey area. The symptom expression could be influenced by host genotype, environmental conditions, virus strain or other viruses infecting the plant at the same time (Martelli, 2017).

Occurrence of GPGV

DNA fragments of 411 bp and 302 bp corresponding to the CP and MP regions of GPGV, respectively were amplified by RT-PCR from only 20 samples of the tested plants. Thus, according to the molecular results, the infection rate for GPGV was 4.78%

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(20/418). A total of 3 samples from Amasya and 17 samples from Tokat provinces were identified as being infected by GPGV, as shown in *Table 3*. Each infected grapevine was detected during the second survey conducted in September. The infection rates for GPGV in the examined samples from Amasya and Tokat were determined to be 8.11% ve 10.55%, respectively. The RT-PCR results of this study proved that GPGV exists (4.78%) in the Western Black Sea Region of Turkey.

Table 3. *Distribution of infected samples determined by RT-PCR tests on province and district basis*

Province	District	Tested samples	Samples infected by GPGV	Tested samples	Samples infected by GPGV	Total infected samples
		May-June		September		
Tokat	Merkez	21	-	20	-	-
	Erbaa	21	-	16	13	13
	Niksar	18	-	10	4	4
	Pazar	8	-	5	-	-
	Zile	24	-	18	-	-
Çorum	Merkez	27	-	21	-	-
	Alaca	11	-	11	-	-
	Bayat	19	-	7	-	-
	Boğazkale	13	-	12	-	-
	İskilip	15	-	4	-	-
	Mecitözü	11	-	4	-	-
	Ortaköy	5	-	4	-	-
	Osmancık	13	-	5	-	-
	Sungurlu	13	-	7	-	-
	Uğurludağ	13	-	5	-	-
Amasya	Merkez	19	-	5	-	-
	Taşova	9	-	4	3	3
Total		**260**	**-**	**158**	**-**	**20**

Phylogenetic analysis

The phylogenetic analysis of the Turkish GPGV isolates was conducted for two genomic regions and compared with international isolates obtained from GenBank. A phylogenetic tree was constructed using the 8 unique coat protein gene sequences from Turkey (MN218575, MN218576, MN218577, MN218578, MN218579, MN218580, MN218581 and MN218582) and 42 sequences from different countries (*Table 4, Figure 4*). The results revealed that the isolates in the study showed a closer grouping with the Tekirdağ and Manisa isolates from our country. Additionally, isolates from Slovakia and Turkey (Manisa, Tekirdağ, Tokat, Amasya) appeared to be a closer group to each other. Although high rates of bootstrap amounts (reliability values) did not appear in the branches of the resulting dendrogram, it was revealed that the isolates from Türkiye and Europe were generally in a closer phylogenetic relationship with each other. In addition, isolates from Far Eastern countries were observed to have a closer phylogenetic relationship to each other. Another phylogenetic tree was constructed using 8 distinct movement protein gene sequences from Turkey (MN175691, MN175692, MN175693, MN175694, MN175695, MN175696, MN175697 and MN175698) and 50 sequences

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from different countries (*Table 5, Figure 5*). All GPGV isolates obtained in the study showed close grouping with 3 other isolates from Türkiye (KY792971, KY792967 and KT267248) and 2 isolates from the Czech Republic (KF356193, KP693445). The obtained dendrogram provides a better knowledge in terms of evolution and geographic distribution of both grapevine and the virus.

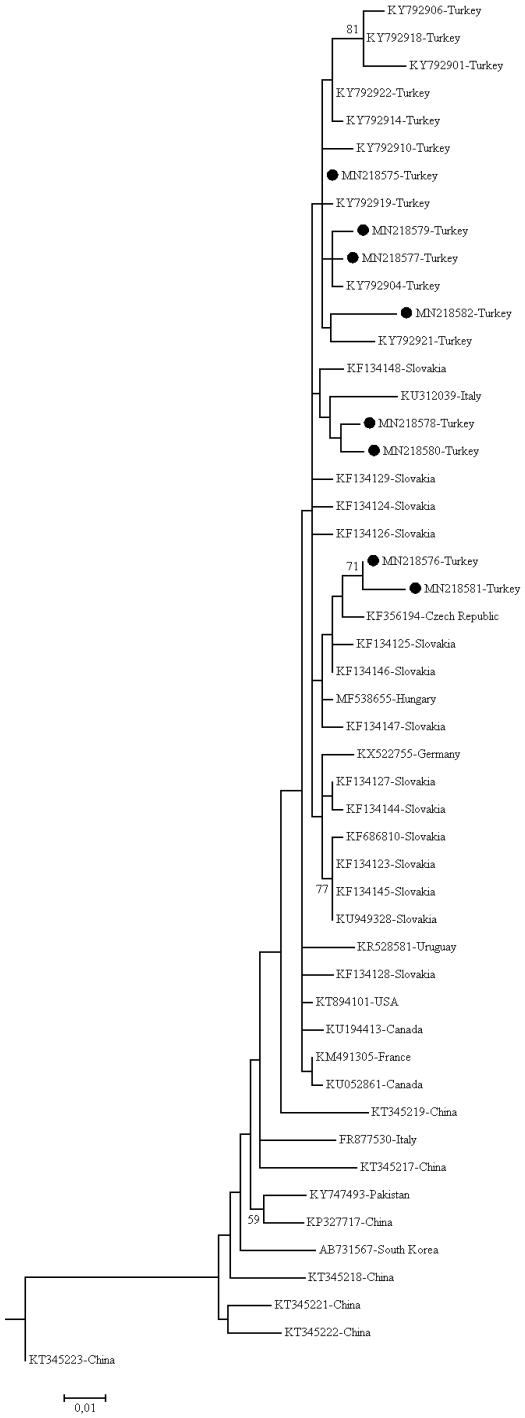

Figure 4. *The global phylogenetic analysis of grapevine Pinot gris virus (GPGV) isolates by Maximum Likelihood method based on the Tamura-3G parameter model, Coat Protein (CP) phylogram*

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Table 4. *The list of isolates used for phylogenetic analysis (CP gene)*

Accession number	Isolate	Host	Origin	Reference
KY792906	17YT	*Vitis vinifera*	Turkey	Elçi et al., 2018
KY792918	429	*Vitis vinifera*	Turkey	Elçi et al., 2018
KY792901	165	*Vitis vinifera*	Turkey	Elçi et al., 2018
KY792922	7	*Vitis vinifera*	Turkey	Elçi et al., 2018
KY792914	2	*Vitis vinifera*	Turkey	Elçi et al., 2018
KY792910	205	*Vitis vinifera*	Turkey	Elçi et al., 2018
KY792919	430	*Vitis vinifera*	Turkey	Elçi et al., 2018
KY792904	17	*Vitis vinifera*	Turkey	Elçi et al., 2018
KY792921	439	*Vitis vinifera*	Turkey	Elçi et al., 2018
KF134148	SK31	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KF134129	SK56	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KF134124	SK01	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KF134126	SK107	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KF134125	SK13	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KF134146	SK03	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KF134147	SK98	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KF134127	SK53	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KF134144	SK77	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KF134123	SK30	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KF134145	SK08	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KF134128	SK312	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KF686810	SK30-1	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KU949328	SK704	*Vitis vinifera*	Slovakia	Glasa et al., 2017
KF356194	TI25_CZ	*Vitis vinifera* cv. Laurot	Czech Republic	Glasa et al., 2014
MF538655	GPGV-HUMK5	*Vitis vinifera* cv. Teleki-Kober 125	Hungary	Czotter et al., 2018
KX522755	Riesling 25-3	*Vitis vinifera* cv. Riesling	Germany	Reynard et al., 2016
KR528581	Tannat-GvPGV	*Vitis vinifera*	Uruguay	Cho et al., 2015
KT894101	GPGV-TN	*Vitis vinifera* cv. Touriga Nacional	USA	Al Rwahnih et al., 2016
KU194413	BC-1	Pinot gris	Canada	Poojari et al., 2016
KU052861	93-9	*Vitis vinifera*	Canada	Xiao et al., 2016
KM491305	Mer	*Vitis vinifera* cv. Merlot	France	Beuve et al., 2015
KT345219	BJ-MGX	*Vitis vinifera* cv. Muscat Hamburg	China	Fan et al., 2016
KT345217	LN-HDQ	*Vitis vinifera* cv. Red globe	China	Fan et al., 2016
KT345218	BJ-MLZ	*Vitis vinifera* cv. Merlot	China	Fan et al., 2016
KT345221	LN-MGX	*Vitis vinifera* cv. Muscat Hamburg	China	Fan et al., 2016
KT345222	LN-PLZ	*Vitis vinifera* cv. Cabernet Franc	China	Fan et al., 2016
KT345223	LN-MRDW	*Vitis vinifera* cv. Moldova	China	Fan et al., 2016
KP327717	Capsid protein gene	*Vitis vinifera* cv. GoldFinger	China	Li et al., 2016
KU312039	GPGV_FEM01	*Vitis vinifera*	Italy	Gualandri et al., 2016
FR877530	complete genome	*Vitis vinifera* cv. Pinot gris	Italy	Giampetruzzi et al., 2012

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Accession number	Isolate	Host	Origin	Reference
KY747493	SL13	*Vitis vinifera* cv. Bai-Ji-Xin	Pakistan	Rasool et al., 2017
AB731567	Cp gene	*Vitis* sp. cv. 'Tamnara'	South Korea	Cho et al., 2013
MN218575	ATB1-2	*Vitis vinifera*	Turkey	In this study
MN218576	TEBp1-2	*Vitis vinifera*	Turkey	In this study
MN218577	TEY1-3	*Vitis vinifera*	Turkey	In this study
MN218578	TEU1-1	*Vitis vinifera*	Turkey	In this study
MN218579	TEY1-2	*Vitis vinifera*	Turkey	In this study
MN218580	TED1-2	*Vitis vinifera*	Turkey	In this study
MN218581	TEY1-1	*Vitis vinifera*	Turkey	In this study
MN218582	TNG1-1	*Vitis vinifera*	Turkey	In this study

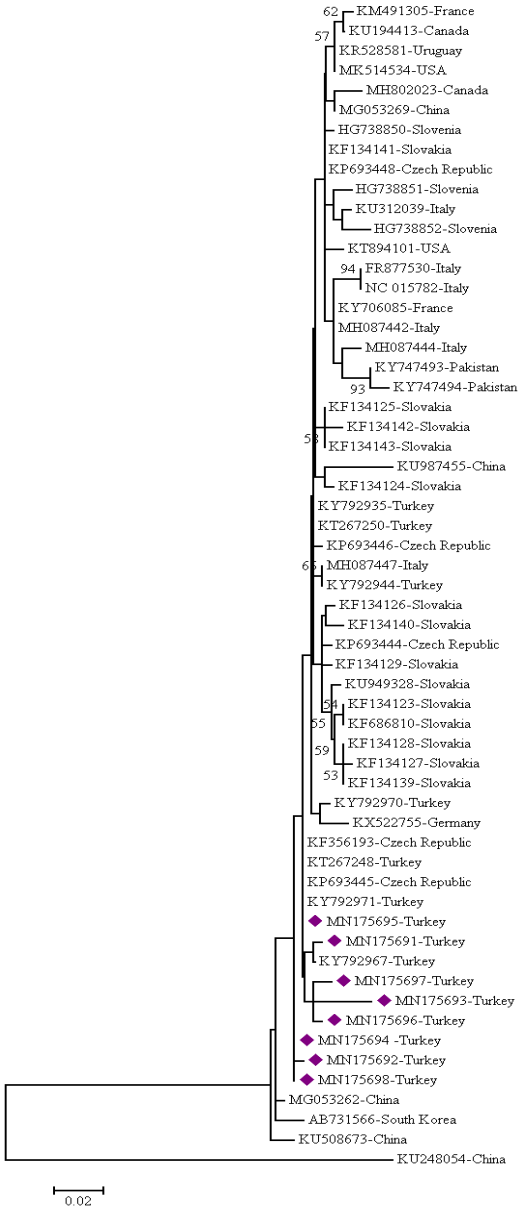

Figure 5. *The global phylogenetic analysis of grapevine Pinot gris virus (GPGV) isolates by Maximum Likelihood method based on the Tamura-3G parameter model, Movement Protein (MP) phylogram*

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Table 5. *The list of isolates used for phylogenetic analysis (MP gene)*

Accession number	Isolate	Host	Origin	Reference
KM491305	Mer	*Vitis vinifera* cv. Merlot	France	Beuve et al., 2015
KY706085	PN	*Vitis vinifera* cv. Pinot Noir	France	Marais et al., 2018
KU194413	BC-1	Pinot gris	Canada	Poojari et al., 2016
MH802023	12G1110	*Vitis vinifera*	Canada	Rott et al., 2019
KR528581	Tannat-GvPGV	*Vitis vinifera*	Uruguay	Cho et al., 2015
KT894101	GPGV-TN	*Vitis vinifera* cv. Touriga Nacional	USA	Al Rwahnih et al., 2016
MK514534	S335	*Vitis vinifera*	USA	Al Rwahnih et al., 2019
MG053269	NX-Mer	*Vitis vinifera* cv. Merlot	China	Fan et al., 2018a
MG053262	BJ-Mer	*Vitis vinifera* cv. Merlot	China	Fan et al., 2018b
KU987455	C75-11	*Vitis vinifera* cv. Shine Muscat	China	Lou et al., 2016
KU508673	Goldfinger	*Vitis vinifera*	China	Li et al., 2018
KU248054	XJ-CaS	*Vitis vinifera* cv. Cabernet Sauvignon	China	Fan et al., 2016
HG738850	088/12	*Vitis vinifera* cv. Pinot Noir	Slovenia	Mavric Plesko et al., 2014
HG738851	100/12	*Vitis vinifera* cv. Pinot gris	Slovenia	Mavric Plesko et al., 2014
HG738852	111/12	*Vitis vinifera*	Slovenia	Mavric Plesko et al., 2014
KF134141	SK77	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KF134125	SK13	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KF134142	SK98	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KF134143	SK03	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KF134124	SK01	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KF134126	SK107	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KF134140	SK31	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KF134129	SK56	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KF134123	SK30	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KF134128	SK312	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KF134127	SK53	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KF134139	SK08	*Vitis vinifera*	Slovakia	Glasa et al., 2014
KU949328	SK704	*Vitis vinifera*	Slovakia	Glasa et al., 2017
KP693448	22_2_3	*Vitis vinifera*	Czech Rebublic	Eichmeier et al., 2016
KP693446	7_2_5	*Vitis vinifera*	Czech Rebublic	Eichmeier et al., 2016
KP693444	TI_21	*Vitis vinifera*	Czech Rebublic	Eichmeier et al., 2016
KP693445	TI_25	*Vitis vinifera*	Czech Rebublic	Eichmeier et al., 2016
KF356193	TI25_CZ	*Vitis vinifera*	Czech Rebublic	Glasa et al., 2014
KU312039	GPGV_FEM01	*Vitis vinifera*	Italy	Gualandri et al., 2016
FR877530	Complete genome	*Vitis vinifera* cv. Pinot gris	Italy	Giampetruzzi et al., 2012
NC_015782	complete genome	*Vitis vinifera* cv. Pinot gris	Italy	Giampetruzzi et al., 2012
MH087442	fvg-Is8	*Vitis vinifera*	Italy	Tarquini et al., 2018
MH087444	fvg-Is13	*Vitis vinifera*	Italy	Tarquini et al., 2018
MH087447	fvg-Is17	*Vitis vinifera*	Italy	Tarquini et al., 2018
KX522755	Riesling 25-3	*Vitis vinifera* cv. Riesling	Germany	Reynard et al., 2016

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Accession number	Isolate	Host	Origin	Reference
AB731566	Mp gene	*Vitis* sp. cv. 'Tamnara'	South Korea	Cho et al., 2013
MN175691	ATB1-2	*Vitis vinifera*	Turkey	In this study
MN175692	TEBp1-2	*Vitis vinifera*	Turkey	In this study
MN175693	TEY1-3	*Vitis vinifera*	Turkey	In this study
MN175694	TEU1-1	*Vitis vinifera*	Turkey	In this study
MN175695	TEY1-2	*Vitis vinifera*	Turkey	In this study
MN175696	TED1-2	*Vitis vinifera*	Turkey	In this study
MN175697	TEY1-1	*Vitis vinifera*	Turkey	In this study
MN175698	TNG1-1	*Vitis vinifera*	Turkey	In this study

Discussion

In recent years, various viruses have emerged in viticulture worldwide and some of which pose significant threat to grapevine production. One such virus, *Grapevine pinot gris virus* (GPGV), was recently discovered in Italy using Next-Generation Sequencing (NGS), and since its identification in 2012, a several studies have focussed on its prevalence and genetic diversity. The aim of this study is to explore the occurrence and phylogeny of GPGV across different grape-growing regions and cultivars. Consequently, GPGV was identified for the first time in Western Black Sea Region and among the three provinces surveyed in this study, Tokat had the highest infectivity rate. However, according the obtained results, GPGV is not a prevalent in survey area compared to the other parts of Turkey especially in Tekirdağ, which is closest to Europe (Elçi et al., 2018). Based on the results of RT-PCR, the total incidence of GPGV was found to be 4.78%. However, the agent was only detected in the September survey, not in September. This situation could be related to climatic conditions, particularly the air temperature. Infected vines were mostly local cultivar, because the cultivated cultivar is generally Narince. We found GPGV not only in samples showing symptoms but also in asymptomatic ones, consistent with the findings of Saldarelli et al. (2015), Bertazzon et al. (2017), and Elçi et al. (2018). The variation in symptoms observed in GPGV-infected plants may be due to co-infection with other pathogens, making it difficult to definitively associate specific symptoms with GPGV alone.

The purpose of conducting the survey in two different time periods was to determine whether the symptoms observed in the green parts of the plants became latent over time, as well as to monitor any symptoms affecting the berries during the harvest season. As a result, no changes were detected in the virus symptoms on the foliage, but smaller grape bunches with shot berries were observed at harvest time.

The grouping of GPGV isolates from this study with other Turkish isolates in the phylogenetic trees suggests that grafts from the vineyard areas of Tekirdağ and Manisa, where another study was conducted, may have been transferred to the research sites in Amasya and Tokat. In a phylogenetic study by Elçi et al. (2018) using the partial CP gene region, Turkish isolates were found to be distinct from other global isolates. However, the new isolates obtained in this study were more closely clustered with those from Slovakia and other Turkish isolates. Their findings indicate that Turkish GPGV isolates exhibit limited genetic diversity among themselves. On the other hand, All GPGV isolates obtained using the MP gene sequences clustered closely with three other isolates from Turkey (KY792971, KY792967, and KT267248) and two isolates from the Czech Republic (KF356193 and KP693445).

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Conclusion

In summary, our study revealed, for the second time, the existence of *Grapevine Pinot gris virus* (GPGV) in vineyards in the Western Black Sea Region of Turkey. In addition, closest countries, Greece and Georgia, have high infection rate (Martelli, 2014; Casati et al., 2015), similar to other research conducted by Elçi et al. (2018) in our country, indicating transmission of the agent via propagation material from border. Research on genetic variability is critical to understand virus emergence and epidemiology, allowing the identification of highly conserved regions of the viral genome and supporting the development of effective, lasting disease management strategies. Consequently, an integrated pest management approach, grounded in the virus's epidemiology and biology, is vital for effective control practices.

To reduce the impact of newly emerging viruses on both current and future grapevine production, optimal strategies will likely involve a comprehensive pest management approach, integrating the most effective control measures based on the virus's biology and its vectors. Understanding the biology and epidemiology of these viruses is, therefore, critical for developing targeted and sustainable management strategies.

Conflict of interest. The authors declare that they have no conflict of interest in the publication.

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REFERENCES

[1] Al Rwahnih, M., Golino, D., Rowhani, A. (2016): First report of grapevine *Pinot gris virus* infecting grapevine in the United States. – Plant Disease 100: 1030.

[2] Al Rwahnih, M., Alabi, O. J., Hwang, M. S., Tian, T., Mollov, D., Golino, D. (2021): Characterization of a new nepovirus infecting grapevine. – Plant Disease 105: 1432-1439. https://doi.org/ 10.1094/PDIS-08-20-1831-RE.

[3] Angelini, E., Bertazzon, N., Montgomery, J., Wang, X., Zinkl, A., Stamp, J., Wei, A. (2016): Occurrence of grapevine *Pinot gris virus* in commercial vineyards in the United States. – Plant Disease 100: 1254.

[4] Bertazzon, N., Filippin, L., Forte, V., Angelini, E. (2015): Grapevine Pinot gris virus seems to have recently been introduced to vineyards in Veneto, Italy. – Archives of Virology 161: 711-714.

[5] Bertazzon, N., Forte, V., Filippin, L., Causin, R., Maixner, M., Angelini, E. (2017): Association between genetic variability and titre of grapevine *Pinot gris virus* with disease symptoms. – Plant Pathology 66: 949-959.

[6] Bertazzon, N., Forte, V., Di GaSpero, M., Angelini, E. (2018): Temporal spread of Grapevine leaf mottling and deformation in the field. – Proc. 19th Congress of ICGV, Santiago, Chile.

[7] Beuve, M., Candresse, T., Tannieres, M., Lemaire, O. (2015): First report of grapevine *Pinot gris virus* (GPGV) in grapevine in France. – Plant Disease 99(2): 293.

[8] Casati, P., Maghradze, D., Quaglino, F., Bianco, P. A. (2015): First report of grapevine *Pinot gris virus* in Georgia. – Journal of Plant Pathology 97: 67-77.

[9] Cho, I. S., Jung, S. M., Cho, J. D., Choi, G. S., Lim, H. S. (2013): First report of grapevine *Pinot gris virus* infecting grapevine in Korea. – New Disease Report 27: 10.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_48774891

[10] Cho, W. K., Jo, Y., Choi, H. (2015): Grapevine *Pinot gris virus* isolate Tannat-GvPGV, complete genome. – National Center for Biotechnology Information, retrieved: January 17, 2020, from https://www.ncbi.nlm.nih.gov/nuccore/KR528581.1?report=genbank.
[11] Constable, F., Tassie, V., Tassie, L., McLoughlin, S. (2019): Grapevine *Pinot gris virus*. – Factsheet, Wine Australia for Australian Wine, Retrieved: January 17, 2023, from https://www.wineaustralia.com/ RD_Factsheets_GrapevinePinotGrisVirus_Jan2019_W2.
[12] Czotter, N., Molnar, J., Szabo, E., Demian, E., Kontra, L., Baksa, I., Szittya, G., Kocsis, L., Deak, T., Tusnady, G. E., Burgyan, J., Varallyay, E. (2018): NGS of Virus-Derived Small RNAs as a Diagnostic Method Used to Determine Viromes of Hungarian Vineyards. – Front Microbiol 9: 122.
[13] Çağlayan, K., Gazel, M., Elçi, E., Öztürk, L. (2015): The situation of the new emerging grapevine viruses in Turkey. – 18th Congress of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine (ICGV). 7-11 Eylül 2015. Ankara, Türkiye, s 83.
[14] Eichmeier, A., Penazova, E., Pavelkova, R., Mynarzova, Z., Saldarelli, P. (2016): Detection of grapevine *Pinot gris virus* in certified grapevine stocks in Moravia, Czech Rebuclic. – Journal of Plant Pathology 98(1): 155-157.
[15] Elçi, E., Gazel, M., Roumi, V., Çağlayan, K. (2018): Incidence, distribution and limited genetic variability among Turkish isolates of Grapevine *Pinot gris virus* from different grapevine cultivars. – Journal of Plant Disease and Protection 125: 469-476. https://doi.org/10.1007/s41348-018-0175-3.
[16] Fajardo, T. V. M., Eiras, M., Nickel, O. (2017): First report of Grapevine *Pinot gris virus* infecting grapevine in Brazil. – Australasian Plant Disease Notes 12: 45. https://doi.org/10.1007/s13314-0170270-5.
[17] Fan, X. D., Dong, Y. F., Zhang, Z. P., Ren, F., Hu, G. H., Li, Z. N., Zhou, J. (2015): First report of grapevine *Pinot gris virus* in grapevines in China. – Plant Disease 100: 540.
[18] Fan, X., Dong, Y., Zhang, Z., Ren, F., Hu, G. (2018a): Detection and sequence analyses of GPGV isolates in China. – National Center for Biotechnology Information, Retrieved: January 17, 2020, from https://www.ncbi.nlm.nih.gov/nuccore/MG053269.
[19] Fan, X., Dong, Y., Zhang, Z., Ren, F., Hu, G. (2018b): Grapevine Pinot gris virus isolate BJ-Mer MP gene, partial cds. – National Center for Biotechnology Information, Retrieved: January 17, 2020, from https://www.ncbi.nlm.nih.gov/nuccore/MG053262.
[20] FAO. (2021): Food and Agricultural Commodities Production, Commodities by Country. – Retrieved: January 17, 2023, from http://www.fao.org/faostat/en/#data/QC.
[21] Fuchs, M. (2020): Grapevine viruses: a multitude of diverse species with simple but overall poorly adopted management solutions in the vineyard. – Journal of Plant Pathology 102: 643-653.
[22] Gazel, M., Caglayan, K., Elci, E., Ozturk, L. (2016): First report of grapevine *Pinot gris virus* in grapevine in Turkey. – Plant Disease 100: 657.
[23] Giampetruzzi, A., Roumi, V., Roberto, R., Malossini, U., Yoshikawa, N., La Notte, P., Terlizzi, F., Credi, R., Saldarelli, P. (2012): A new grapevine virus discovered by deep sequencing of virus- and viroid-derived small RNAs in cv *Pinot Gris*. – Virus Research 163(1): 262-268. https://doi.org/10.1016/j.virusres.2011.10.010.
[24] Glasa, M., Predajna, L., Kominek, P., Nagyova, A., Candresse, T. (2014): Molecular characterization of divergent grapevine Pinot gris virus isolates and their detection in Slovak and Czech grapevines. – Archives of Virology 159: 2103-2107.
[25] Gualandri, V., Asquini, E., Bianchedi, P., Covelli, L., Brilli, M., Malossini, U., Bragagna, P., Saldarelli, P., Si-Ammour, A. (2017): Identification of herbaceous hosts of the Grapevine *Pinot gris virus* (GPGV). – Eur. J. Plant Pathol. 147: 21-25.
[26] Jo, Y., Choi, H., Kyong Cho, J., Yoon, J. Y., Choi, S. K., Kyong Cho, W. (2015): In silico approach to reveal viral populations in grapevine cultivar Tannat using transcriptome data. – Scientific Reports 5: 15841.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_48774891

[27] Kumar, S., Stecher, G., Tamura, K. (2016): MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. – Molecular Biology and Evolution 33: 1870-1874.
[28] Li, R., Mock, R., Huang, Q., Abad, J., Hartung, J., Kinard, G. (2008): A reliable and inexpensive method of nucleic acid extraction for the PCR-based detection of diverse plant pathogens. – Journal of Virological Methods 154: 48-55. https://doi.org/10.1016/j.jviromet.2008.09.008.
[29] Li, Y., Deng, C., Cheng, Y. (2016): First report of Grapevine pinot gris virus infecting grapevine in China. – National Center for Biotechnology Information, Retrieved: January 17, 2020, from https://www.ncbi.nlm.nih.gov/nuccore/KP327717.
[30] Li, Y., Deng, C. (2018): Multiple virus infection in a grapevine from China by small RNA deep sequencing. – National Center for Biotechnology Information, Retrieved: January 17, 2020, from https://www.ncbi.nlm.nih.gov/nuccore/KU508673.
[31] Lou, B. H., Song, Y. Q., Chen, A. J., Bai, X. J., Wang, B., Wang, M. Z., Liu, P., He, J. J. (2016): First Report of Grapevine *Pinot gris virus* in Commercial Grapevines in Southern China. – Journal of Plant Pathology 98(3): 677-697.
[32] Malagnini, V., de Lillo, E., Saldarelli, P., Beber, R., Duso, C., Raiola, A., Zanotelli, L., Valenzano, D., Giampetruzzi, A., Morelli, M., Ratti, C., Causin, R., Gualandri, V. (2015): Preliminary data on the transmission of Grapevine *Pinot Gris virus* by *Colomerus vitis*. – 18th Congress of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine (ICVG). 7-11 Eylül 2015, Ankara, Türkiye, pp. 217-218.
[33] Maliogka, V. I., Martelli, G. P., Fuchs, M., Katis, N. I. (2015): Control of viruses infecting grapevine. – Advances in Virus Research 91: 175-227.
[34] Malossini, U., Moscon, R., Ferrazza, M., Bianchedi, P., Varner, M., Credi, R. (2012): Caratteristichevegeto-produttive div iti pinot grigio e Tramineraromaticoaffette da unanuovavirosisegnalata in Trentino. – IV Convegno Nazionale di Viticoltura Conavi. To Asti, 10-11-12 Iuglio, p37.
[35] Marais, A., Faure, C., Theil, S., Candresse, T. (2018): Full length genome sequence of a French Grapevine Pinot Gris virus symptomatic isolate from France. – National Center for Biotechnology Information, Retrieved: January 17, 2020, from https://www.ncbi.nlm.nih.gov/nuccore/KY706085.
[36] Martelli, G. P. (2014): Directory of virus and virus-like diseases of the grapevine and their agents. – Journal of Plant Pathology 96: 105-120.
[37] Martelli, G. P. (2017): An Overview on Grapevine Viruses, Viroids, and the Diseases They Cause. – In: Meng, B., Martelli, G. P., Golino, D. A., Fuchs, M. (eds.) Grapevine Viruses: Molecular Biology, Diagnostics and Management. Springer International Publishing AG, 32, Switzerland.
[38] Mavrič Pleško, I., Virščeк Marn, M., Seljak, G., Žežlina, I. (2014): First report of Grapevine Pinot gris virus infecting grapevine in Slovenia. – Plant Disease 98: 1014-1014.
[39] Morelli, M., de Moraes, C. A., Susca, L., Saldarelli, P., Gualandri, V., Martelli, G. P. (2014): First report of grapevine *Pinot gris virus* from table grapes in Southern Italy. – Journal of Plant Pathology 96: 439.
[40] Plesko, P. I., Virscek, M. M., Seljak, G., Zezlina, I. (2014): First report of grapevine *Pinot gris virus* infecting grapevine in Slovenia. – Plant Disease 98: 1014.
[41] Poojari, S., Lowery, T., Rott, M., Schmidt, A.-M., Úrbez-Torres, J. R. (2016): First Report of Grapevine *Pinot gris virus* in British Columbia, Canada. – Plant Diseases 100: 1513.
[42] Reynard, J.-S., Schumacher, S., Wenzel, W., Fuchs, J., Bohnert, P., Glasa, M., Wetzel, T., Fuchs, R. (2016): First Report of Grapevine *Pinot gris virus* in German vineyards. – Plant Disease 100(12): 2545-2545.
[43] Salderelli, P., Giampetruzzi, A., Morelli, M., Malossini, C., Pirolo, C., Bianchedi, P., Gualandri, V. (2015): Genetic variability of Grapevine *Pinot gris virus* and its association with grapevine leaf mottling and deformation. – Phytopathology 105: 555-563.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_48774891

[44] Viver, M. A., Pretioru, I. S. (2002): Genetically tailored grapevines for the wine industry. – Trends in Biotechnology 20: 472-478.
[45] Xiao, H., Shabanian, M., McFadden-Smith, W., Meng, B. (2016): First report of grapevine *Pinot gris virus* in commercial grapevines in Canada. – Plant Dis 100: 1030.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2303_48774891