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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Molecular Determination of the Presence of Some Grapevine Viruses and Phylogenetic analyses of Grapevine Virus A in Tokat Province Grapevine Area*

Tokat İli Asma Alanında Bazı Asma Virüslerinin Moleküler Olarak Belirlenmesi ve Grapevine Virus A'nın Filogenetik Analizi

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Abstract

Grapevine (*Vitis* spp.) is one of the major fruit crop with high socioeconomic importance for Turkey. In vineyards, many harmful organism, especially virus infections, weaken the plant and lead to decreases in yield and quality, so it takes the lead in quarantine and certification. This study was carried out to determine some viral agents that cause yield loss in vines produced in Tokat, where viticulture is very important. Samples were collected from young leaves and one-year-old shoots of grapevines showing virus symptoms from some vineyard areas in Tokat Center and its districts. Collected 189 grapevine samples were subjected to the RT-PCR test, which is a molecular method using virus-specific primers, to detect the presence of *Grapevine pinot gris virus* (GPGV), *Grapevine virus A* (GVA), *Strawberry latent ringspot virus* (SLRSV). Out of a total of 189 plant samples, 80 (42.32%) of GVA, 3 (1.58%) of GPGV were detected and SLRSV (0%) was not detected. More than one virus was found in 2 (1.05%) of 189 tested samples. It was determined that the most common virus was GVA, the least detected virus was GPGV in plant samples collected from Tokat Center and its districts. Bidirectional sequence analysis of RT-PCR products of GVA-infected isolates were performed and phylogenetic analyzes were done by comparing them with reference isolates after they were aligned with the MEGAX computer program. Based on phylogenetic analysis studies, GVA showed differential branching with isolates registered in GenBank and isolates obtained in the study. GVA-infected isolates showed similarity with reference isolates at rates of 92-94%. In this study, molecular analysis of Turkish GVA isolates was performed. This molecular information is important as it will shed light on future studies.

Keywords: Phylogenetic analysis, Grapevine virus A, Strawberry latent ringspot virus, Grapevine pinot gris virus, Tokat, *Vitis vinifera*, RT-PCR

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Öz

Türkiye bağcılığı sosyoekonomik anlamda en önemli ürün gruplarının biridir. Bağlarda birçok zararlı organizma grubu özellikle de virüs enfeksiyonları bitkiyi zayıflatıp, verim ve kalitesinde azalmalara yol açtığından karantina ve sertifikasyonda başı çekmektedir. Bu çalışma, bağcılığın çok önemli olduğu Tokat ilinde üretilen asmalarda verim kaybına neden olan bazı viral etmenleri tespit etmek amacıyla yapılmıştır. Tokat Merkez ve ilçelerindeki bazı bağ alanlarından virüs semptomu gösteren asmaların genç yapraklarından ve bir yaşındaki sürgünlerinden örnekler alınmıştır. Toplanan 189 asma örneğine Grapevine pinot gris virus (GPGV), Grapevine virus A (GVA) ve Strawberry latent ringspot virus (SLRSV) varlığını tespit etmek için virüse özgü primerler kullanılarak moleküler bir yöntem olan RT-PCR testine tabi tutulmuştur. Toplam 189 asma örneğinden 80 (%42.32) GVA, 3 (%1.58) GPGV enfeksiyonu tespit edilmiş ve hiçbir örnekte SLRSV (%0) tespit edilmemiştir. Test edilen 189 asma örneğinin 2'sinde (%1.05) birden fazla virüs tespit edilmiştir. Tokat Merkez ve ilçelerinden toplanan bitki örneklerinde en sık görülen virüsün GVA, en az tespit edilen virüsün ise GPGV olduğu belirlenmiştir. GVA ile enfekteli izolatların RT-PCR ürünlerin çift yönlü sekans analizi analizleri yapılmış ve MEGAX bilgisayar programı ile hizalandıktan sonra referans izolatlarla karşılaştırılarak filogenetik analizleri yapılmıştır. Filogenetik analiz çalışmalarına göre, GVA, GenBank'ta kayıtlı izolatlarla ve farklı çalışmada elde edilen izolatlarla farklı dallanma göstermiştir. GVA ile enfekte izolatlar referans izolatlarla %92-94 oranında benzerlik göstermiştir. Bu çalışmada Türk GVA izolatlarının moleküler analizi yapılmıştır. Bu moleküler bilgilerin ileride yapılacak çalışmalara ışık tutacağı öngörülmektedir.

Anahtar Kelimeler: Filogenetik analiz, Grapevine virus A, Strawberry latent ringspot virus, Grapevine pinot gris virus, Tokat, *Vitis vinifera*, RT-PCR

1. Introduction

Grape, which has great importance in human nutrition, is a type of fruit that is consumed both fresh and dry. Our country, is located on the most favorable belt of the world for viticulture; Due to its location in the center of the geography where the gene centers of the vine intersect and where it was first cultivated, it has a very old and deep-rooted viticulture culture and a rich vine gene potential (Durgut and Arın, 2005). It is also an important export product for Turkey's economy (Kaplan and Bayhan, 2017).

Tokat is a province that demonstrates the peculiarities of the transitional climate between the region of Central Anatolia and the Eastern Black Sea region. Viticulture is an important agricultural industry for Tokat and is widely practiced in the districts of Central, Zile, Erbaa, Pazar, Turhal, and Niksar. In Tokat province, 51,762 tons of products are produced from an area of 62,259 hectares (TUIK, 2019). Narince variety, which is grown in the Tokat region and forms a large part of the vineyards, has an important place in commercial ivy leaf production.

The most important factors that reduce the yield and quality of grapes in the vineyard areas are the diseases and harmful factors of the cultivated plant. Among them, virus diseases cause yield losses by affecting crop plants in varying degrees. Pinot gris grapevine virus (GPGV), one of the important viral diseases of vineyards, is a single-stranded RNA virus with positive sensitivity, belonging to the genus *Trichovirus*. It affects the growth of leaves and fruits of grape plants and is known for its resemblance to the internal necrosis virus of grapefruits. It is a new virus, which was first detected in Italy in 2012, and the next-generation sequence analysis method is widely used in the field of plant virology. The entire genome sequence of the Italian isolate GPGV was analyzed and reported to be very closely related to the internal Grapevine necrosis virus (GINV), which belongs to the genus *Trichovirus* (Giampetruzzi et al., 2012). This virus, which has been found mainly in wine grapes in Europe, has so far only been detected in the table grape variety Tamnara, which causes cluster necrosis symptoms in Korea, except in Europe (Cho et al., 2013). It is called by this name because it was first detected in the Pinot gris grape variety in Northern Italy. In the following years, this virus was detected in Korea (Cho et al., 2013), Slovenia (Plesko et al., 2014), Slovakia, Czech Republic (Glasa et al., 2014), France (Beuve et al., 2015), and Turkey (Gazel et al., 2016) and records from different countries are reported every year (Saldarelli et al., 2017). GPGV was detected and recorded in a reverse transcriptase-polymerase chain reaction (RT-PCR) tick assay using specific primers with grape mite samples known as *Colomerus vitis* (=Eriophyes) collected from GPGV-infected vines and symptomatic sites on the leaves. It has been reported to be a possible carrier of this virus (Malagnini et al. 2016).

Grapevine virus A (GVA) is a viral agent occurring worldwide in Vitis species, with filamentous particles 800 nm in length and features showing distinct cross-bands. The virus is a grapevine pathogen that is difficult to transmit to a very narrow range of herbaceous hosts by inoculation of plant sap, transporting infected material over medium and long distances, and between plants by vectors of the cochlea. It is also known for the symptoms seen on the woody part of the vine. Swelling occurs at the graft site, which is seen as rootstock and scion incompatibility. It causes noticeable stunting in vines (Akbaş et al., 2008). GVA has been detected in several countries in the Middle East, including Syria (Mslmanieh et al., 2006), Jordan (Osman and Rowhani, 2008; Osman et al., 2013), Egypt (Fattouh et al., 2014), Afghanistan (Digiaro et al., 1999), Lebanon (Haidar et al., 1996), Palestine (Alkowni et al., 1998; Alkowni et al., 2004), Turkey (Koklu et al., 1998, Balsak and Buzkan, 2021), and other countries such as Italy (Ioannou, 1993), Spain (Zabalgogeazcoa et al., 1997), USA (Goszczynski and Habili, 2012), and Portugal (Digiaro et al., 1999).

Strawberry latent ringspot virus (SLRSV) has a wide host range. It is found among rose, strawberry, peach, nectarine, mint, cucumber, sugar beet, lettuce, tomato, tobacco, vineyard, and many weed hosts (Murant, 1976). Within the geographical range of SLRSV, there are most European countries, Israel, Turkey, Canada, the USA, Australia, and New Zealand (Anonymous, 2020). SLRSV, a *nepovirus*, is transmitted in nature by the nematode *Xiphinema diversicaudatum* (Micol.). The nematode has been determined to carry the virus in both the larval and adult stages. The virus can also be transmitted mechanically, by graft, and by seeds (Lister, 1964; Murant, 1976; Lamberti et al., 1986). The virus is known to cause curling and narrowing of leaves, shrubs, decreased yield, and deformation of fruits and seeds.

Numerous studies have been carried out on grapevines in Turkey; Çığşar (2002) collected and tested a total of 1001 samples to determine the status of viruses and virus-like diseases in the important vineyard areas of the Southeastern Anatolia Region and Nevşehir province between 1999-2002. Biological and serological diagnostic

methods were used on the collected samples. The results showed that the most common virus in the region was GVA (41.2%), followed by Grapevine leafroll-associated virus-1 (GLRaV-1) (38.1%), GFLV (8.1%), and Grapevine fleck virus (GFkV) (5.4%).

In the study conducted by Değer (2015), samples were collected from 11 vineyards in Hatay and 2 vineyards in Gaziantep. According to ELISA results, most common viruses were GLRaV-1 (55.56%), GLRaV 4-9 (43.14%), GLRaV-2 (15.69%), GLRaV-3 (12.42%) and followed by GVA (4.57%) and GLRaV-6 (%0.65). None of the samples, were found infected with GLRaV- 5, GLRaV-7 and Grapevine virus B (GVB). Based on the RT-PCR results, tested samples were infected with GLRaV-1, GLRaV-6, GLRaV-3, GVA, GVB and Grapevine virus D (GVD) at rates 23.94%, 15.38%, 14.77%, 13.64%, %1.26, 1.20%, respectively.

In the vineyard samples collected from Tekirdağ province by Kocabağ et al. (2019), 43.62% GPGV and 1.04% Grapevine Syrah virus-1 (GSyV1) were detected. In the samples collected from Hatay province, only 0.9% GSyV1 was detected, and the tested samples were clear in terms of GPGV, GRBaV, and GRLDaV. In the RT-PCR analyzes of Tekirdağ samples for GPGV, when primers that amplify partial coat protein, movement, and replicase genes were used, PCR products of 411 bp, 302 bp, and 618 bp, respectively, were obtained. As a result of a direct bidirectional sequence analysis of these products, they reported that the nucleotide sequences of all three gene regions showed high homology with different GPGV isolates registered in the GenBank.

This study, it was aimed to define the presence of GVA and SLRSV, which were previously reported serologically in Tokat province, and the presence of GPGV by molecular methods. The presence of the newly reported GPGV in Turkey in the province of Tokat was tested for the first time in this study.

2. Material and Methods

2.1. Land surveys

In the summer of 2019, surveys were carried out in the vineyard areas in Central, Erbaa, Niksar, Zile, and Pazar districts, where viticulture is intensive in the province of Tokat. During the surveys, a total of 189 samples were taken from the young leaves and one-year-old shoots of vines showing virus symptoms in the vineyards. The collected samples were labeled in polyethylene bags and brought to the laboratory in an icebox and stored in the refrigerator at +4°C for a short time and at -20°C for a long time until testing. Guided sampling was done during the surveys, and the number of samples to be taken was determined according to Bora and Karaca (1970), considering the size of each vineyard.

2.2 Total RNA isolation

Isolation of RNA from collected samples grapevine sites was studied according to Foissac et al. (2001) protocol. Accordingly, 100 mg of each plant sample was weighed and 1 ml of extraction buffer (6 M guanidine thiocyanate containing 0.2 M sodium acetate, 25 mM EDTA, 1 M potassium acetate, 2.5% PVP-40, and 1% mercaptoethanol) was added, crushed in mortars and a new aliquot. After transferring to a sterile tube, 100 µl of 10% sodium lauryl sarcosyl solution was added, and then the tubes were incubated at 70 ºC for 10 minutes and then kept on ice for 5 minutes. After centrifugation in eppendorf tubes at 14,000 rpm for 10 minutes, 300 µl of supernatant was transferred to a new eppendorf tube containing 150 µl of ethanol, 25 µl of silica, and 300 µl of 6 M sodium iodide. This mixture was then incubated at room temperature for 10 minutes with intermittent shaking. After centrifuging at 6000 rpm for 1 min, after adding the supernatant, the pellets were washed with 500 µl of wash buffer (10 mM Tris-HCl containing 0.05 mM EDTA, 50 mM NaCl and 50% ethanol). After centrifugation at 6000 rpm, it was washed once again with washing buffer and then again subjected to centrifugation at 6000 rpm, the supernatants were removed and the tubes were dried by inverting on blotting paper. Then, 300 µl of RNase-free distilled water was added to them and incubated at 70 ºC for 4 minutes, then centrifuged at 14,000 rpm for 3 minutes, and the supernatant was transferred into a new eppendorf tube and stored at -20ºC until used in RT-PCR processes.

2.3 RT-PCR method

2.3.1 Complementary DNA (cDNA) synthesis

The RNAs obtained from the RNA isolated samples were removed from -20, and after incubation in a water bath at 65 °C for 5 minutes, they were placed on ice and kept on ice for 3 minutes. Complementary DNA (cDNA) was synthesised using 4 µl of total RNA, 4 µl 5X VS Reaction Buffer, 1 µl VitaScript™ Enzyme Mix, 11 µl nuclease-free dH2O in a 20 µl volume mix and kept at 25ºC for 10 min, followed by 42ºC for one hour and finally 85 ºC for 5 min.

2.3.2 PCR method

PCR processes were performed with virus-specific primers by using the cDNA which is obtained in the first step as a template (*Table 1*).

Table 1. Virus-specific primers, base sequences and binding sites used in the study for GVA, GPGV and SLRSV

TargetVirus/Primer	Primer Sequence	Region	Expected Amplicon Length	Reference
GVA-F	GACAAATGGCACACTACG	CP	429 bp	Minafra et al., 1992
GVA-R	AAGCCTGACCTAGTCATCTTGG			
SLRSV-F	CCTCTCCAACCTGCTAGACT	CP	497 bp	Martin et al., 2004
SLRSV-R	AAGCGCATGAAGGTGTAACT			
GPGV-F	GGAGTTGCCTTCGTTTACGA	CP	770bp	Beuve et al., 2015
GPGV-R	GTACTTGATTCGCCTCGCTCA			

The PCR temperature cycling conditions were as follows for GVA: initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and elongation at 72°C for 1 min. The final cycle was followed by extension at 72°C for 10 min.; for SLRSV; initial denaturation at 95°C for 2 min; 35 cycles of denaturation at 94°C for 30 sec, annealing at 52 °C for 30 sec, and elongation at 72°C for 30 sec. and the final cycle was followed by extension at 72°C for 5 min.; for GPGV, initial denaturation at 94°C for 2 min; 35 cycles of denaturation at 94°C for 1 min, annealing at 59°C for 30 sec, and elongation at 72°C for 1 min. The final cycle was followed by extension at 72°C for 10 min.

2.4 Agarose gel electrophoresis studies

The PCR products obtained as a result of PCR performed with virus-specific primers were subjected to electrophoresis at 100 V for 1 hour in agarose gel prepared at a rate of 1.2% and containing 10 mg/ml ethidium bromide. At the end of the electrophoresis procedure, imaging was performed on the imaging device.

2.5 Phylogenetic Analysis by Maximum Likelihood

For phylogenetic studies, RT-PCR products of 11 isolates of the GVA, which gave positive results at the end of RT-PCR, were sent to the sequence. The obtained sequences were analyzed with the use of Maximum likelihood (ML) method in MEGAX computer program. The data were then compared with the reference isolates registered in the National Center for Biotechnology Information (NCBI) gene bank, and a phylogenetic tree was formed and the degree of relatedness was compared. Phylogenetic analyses were carried out using the maximum likelihood program (with 1000 bootstrap replicates) of MEGAX software (Kumar et al., 2018).

3. Results and Discussion

3.1 Survey results

For the study, a total of 189 vine samples were collected from different districts of Tokat in 2019 (Central n= 43, Erbaa n= 36, Niksar n=32, Pazar n=43 and Zile =35) (*Table 2*).

Symptoms in the samples that were taken; mosaic, yellowing, green veins - yellowing between veins, yellowing in veins - green color formation between veins, discoloration of veins, vein banding, deformities, redness, green

main veins of leaves - reddening between veins, necrotic spots, brown spots, mottling, swelling, inward curling of leaves, fan leaf formation and shrinkage, decrease in fruit set, formation of large and small grains, uneven coloration, growth retardation and general stunting were observed (*Figure1*).

Figure. 1. Some symptoms types observed in field surveys. a) It was tested for the possibility of multiple infections and was found to be infected with Grapevine Virus A. b) Positive band was obtained with reddish and yellowish colors Grapevine Virus A. c) The main veins of the leaves are green-reddening between the veins d) Veins green-yellowing between veins

3.2 RT-PCR Results

As a result of the RT-PCR test performed with GVA-specific primers GVA 1-F and GVA 1-R, bands of the expected size (430 bp) were obtained in a total of 81 samples (*Figure 2*). As a result of the RT-PCR test performed with GPGV-specific primers GPGV 2-F and GPGV 2-R, a band of the expected size (770 bp) was obtained in three samples (N-1, N-2, N-22) belonging to Niksar district (*Figure 3*).

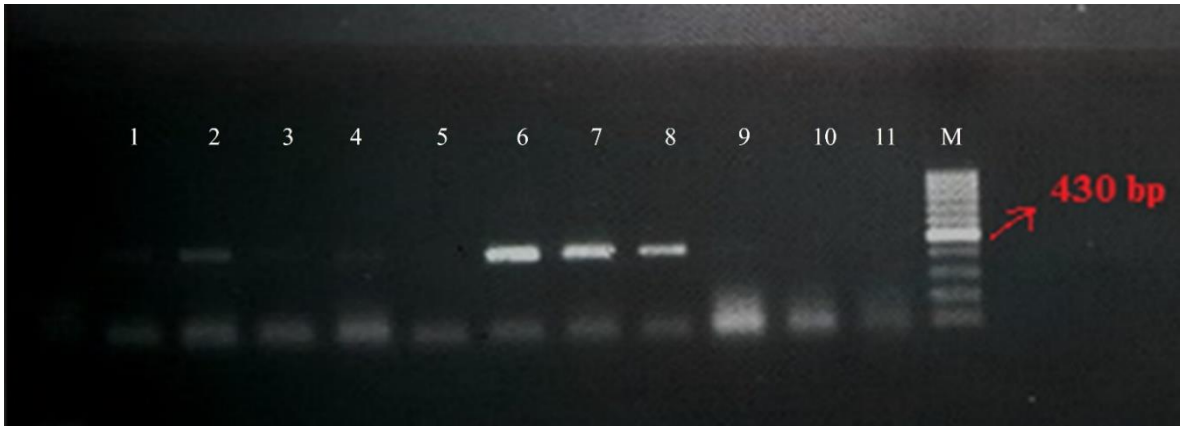

Figure.2. RT-PCR result of some isolates made with a primer specific to Grapevine Virus A. 1, 3, 4, 5, 9, 10: negative samples; 2: N2, 6: N22, 7: T42, 8: U24, 11: negative control, M: 100 bp ladder

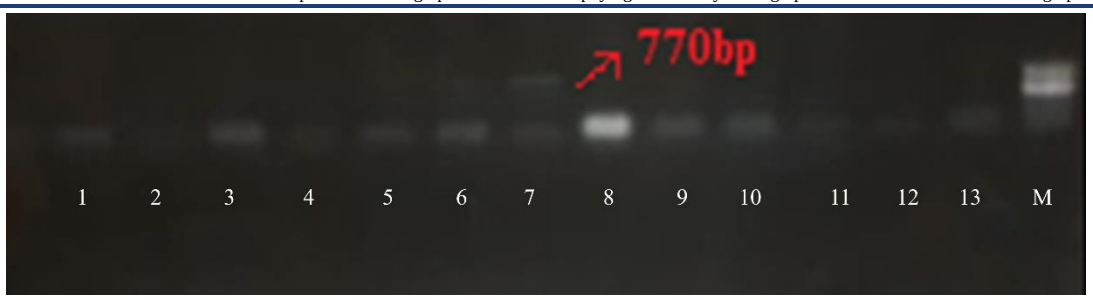

Figure.3. RT-PCR result of N-2 isolate with Grapevine pinot gris virus specific primer. M: 100 bp ladder, 13: Negative control, 7: N1.

A total of 189 samples taken from Central and its districts were tested for the presence of GVA, GPGV, and SLRSV viruses. Positive results were obtained in 81 of the 189 grapevine samples tested. Of the total 189 plant samples tested, 80 (42.32%) were found to be infected with GVA and 3 (1.58%) with GPGV. Mixed infections with GVA and GPGV were detected in two samples. It was determined that the most common virus in plant samples collected from Tokat Center and its districts was GVA, followed by GPGV. No sample infected with SLRSV was detected. The distribution of viruses detected in Tokat vineyard areas by districts is given below (*Table 2*).

Table 2. The number of samples collected from the Center and districts of Tokat province and the distribution of viruses detected in the vineyard areas by districts

DISTRICT	Collected and tested samples	Number of Infected Plants	GVA	GPGV	SLRSV
Center	43	14	14	-	-
Erbaa	36	25	25	-	-
Niksar	32	13	10	3	-
Pazar	43	19	19	-	-
Zile	35	12	12	-	-
Total infected sample		**83**	80	3	-
Total samples	**189**				

3.3 Phylogenetic and BLAST analyses results

In the study, phylogenetic analysis of CP sequences of GVA isolates obtained from this study and reference sequences of isolates available in NCBI databases were constructed.

Sequence information of the GVA CP region from Turkish isolates were submitted to GenBank, with accession numbers: N2 (OP434321), N22 (OP434322), T10 (OP434323), T2 (OP434324), T42 (OP434325), U29 (OP434326), Z14 (OP434327), Z26 (OP434328), D17 (OP434329), D27 (OP434330), and D7 (OP434331). As a result of the phylogenetic analysis performed with the sequence data of the CP region of 11 samples infected with GVA, the D-17 isolate obtained in the study showed similar branching with the USA isolate, while the other isolates showed close similarity to China, Portugal, and South Africa isolate and were grouped with Group I isolates (*Figure 4*).

The sequence analysis of GVA isolates showed that Turkish GVA isolates showed 82-95 nucleotide and 95-99% amino acid similarity among themselves and shared 92-99% sequence and 79-99% amino asit (a.a) similarity with reference isolates from different countries.

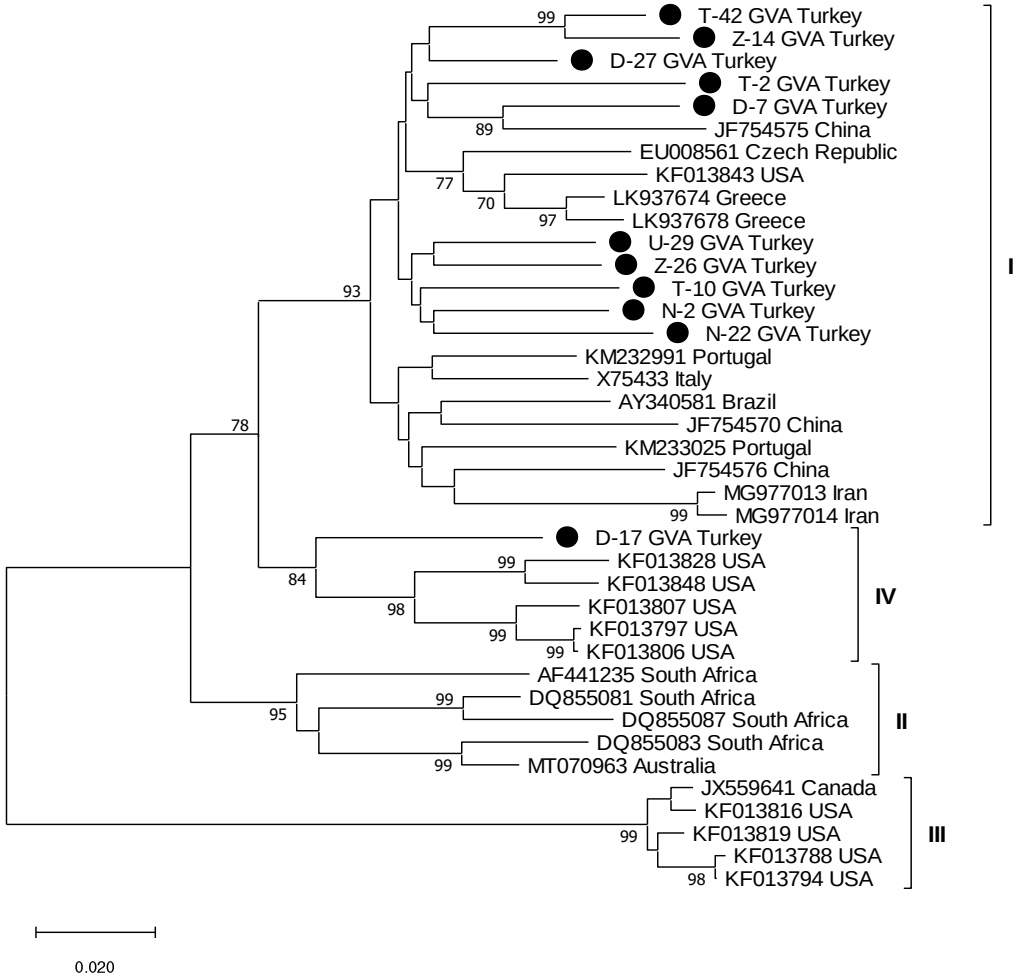

Figure 4. Phylogenetic tree constructed based on the partial nucleotide sequences of the CP gene of new Grapevine Virus A isolates and reference Grapevine Virus A isolates with the use of Maximum likelihood (ML) method. Turkish Grapevine virus A isolates are indicated using a black filled circle.

4. Discussion

Although Anatolia is an important grape producer in the world, it has been observed that viticulture has started to decline in the last 20-25 years. There are various factors among the reasons for this regression. One of them undoubtedly plants protection problems. Among the plant protection factors, virus diseases, which spread rapidly with the production materials and show their visible symptoms 4-5 years after the vineyard establishment, have a separate place and importance. Therefore, it is vital to take the necessary precautions with viruses early. Measures and determinations to be taken for the development of viticulture, which is done intensively in the province of Tokat, gain serious importance. Considering the damage caused by virus diseases in the province of Tokat, where viticulture is common, it is important to determine the presence of virus diseases that will cause damage in the vineyard areas with this study. Because, in the fight against viral diseases, it is primarily the identification of the viral agent.

GVA is a worldwide viral pathogen associated with Kober stem grooving and Shiraz diseases (Minafra et al., 2017). It causes grapevine stem pitting disease and noticeable stunting in grapes (Akbaş et al., 2008), and also is often associated with GLRaV in the field (Sabanadzovic, 2009). In our country where viticulture is common, the damage caused by virus diseases should not be ignored. Detection of its existence and measures to be taken are important to eliminate these adverse conditions.

In a study conducted by Soğukömeroğulları (2017), prevalence of GVA and GVB was investigated in autochthonous grape varieties throughout vineyards of TRC 1 region (Gaziantep, Adıyaman, Kilis) by serological and

biological methods. Total of 66 leaf and shoot samples from Gaziantep (5), Adıyaman (7) and Kilis (54) were tested for GVA and GVB in DAS-ELISA and Double antibody sandwich indirect (DASI-ELISA). Prevalence of GVA was 16 % at Adıyaman, 24 % at Kilis and 20 % at Gaziantep. GVA could not be detected in the samples taken from Adıyaman and Gaziantep, but it was found to be around 11.54% in Kilis.

In previous studies, CP sequence information of GVA has been reported by different researchers (Minafra et al., 1994; Anfoka et al., 2004; Alabi et al., 2014; Goszczynski et al., 2008; Goszczynski and Habili, 2012; Moradi et al., 2018). Firstly, GVA isolates have been grouped into three groups including I, II, and III by Anfoka et al. (2004) and Goszczynski and Habili (2012). After, Alabi et al. (2014) reported that based on phylogenetic analysis of CP sequences of GVA isolates collected from different wine grape cultivars, the GVA isolates were divided into four major clades: groups I, II, III, and IV. Balsac and Buzkan investigated molecularly the prevalence and genetic variability of GVA in autochthonous grapevine cultivars based on the analysis of its CP gene in two important grape regions: Eastern Mediterranean (EM) and Southeast Anatolia (SEA). they reported that RT-PCR revealed a high infection rate of GVA in two major viticultural areas, and high nucleotide and amino acid sequence similarity were seen between the Turkish GVA isolates and the reference isolates in group I and II. In this study, Turkish GVA isolates except for one isolate (D17) were grouped into group I, which include many isolates from different countries such as the USA, Macedonia, Iran, and China. The D17 isolate was grouped into new subgroup IV, which consists of USA, China and Greece isolates. According to the results of the phylogenetic analysis of CP sequences, Balsak and Buzkan (2021) reported that GVA isolates and cultivars in the same location were not phylogenetically related. Similary in this study, one isolate (D17) showed phylogeneticly difference from others.

In another study, Çiftçi et al. (2015) in the spring and autumn of 2014, they conducted field studies in grape growing areas in the Eastern and Southeastern Regions of Turkey. They were collected a total of 87 specimens in the spring and 123 specimens of vines showing signs of the virus in the fall and, were tested by using DAS-ELISA for the presence of GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-4, GLRaV-5, GLRaV-6, GLRaV-7, GLRaV-9, Grapevine fanleaf nepovirus (GFLV), GFkV, GVA, Raspberry ringspot nepovirus (RpRSV), SLRSV, Tomato black ring nepovirus (TBRV), Arabis mosaic virus (ArMV), and also PCR was performed for Grapevine red blotch-associated virus (GRBaV) using the primer pairs designed in the study. The most common virus was GFLV (6.66%), followed by GLRaV 4-9 (3.80%), GLRaV 1+3 (3.81%), GFkV (1.43%) and GVA (0.95%).

Gazel et al. (2016) collected eighteen suspicious samples with leaf deformation and mottling symptoms that reduced fruit yield and quality and applied the RT-PCR test for GPGV. As a result of the test, they obtained the first report of GPGV in the grapevine in Turkey. The presence in the province of Tokat of GPGV, which newly reported in Turkey, was tested molecularly for the first time in this study but sufficient PCR product could not be obtained for the sequence analysis. More detailed studies are planned for the future.

In another study conducted by Turkmen (2020), shoots and leaves were collected from 418 vine plants in 2017 from vineyards in Amasya, Çorum and Tokat, and were investigated for the presence of GFLV and GPGV using RT-PCR methods. At the end of tests, the highest infection rate for GFLV was found in Tokat province, followed by Amasya and Çorum provinces at rates 48.45%, 24.32%, 13.18%, respectively. GPGV was detected only in Amasya and Tokat provinces at a rate of 8.11 % and 10.55 %, respectively. Based on the phylogenetic trees, all the GFLV isolates obtained in the study and 12 Iranian isolates have high similarity. According to sequences of movement protein region of all GPGV isolates obtained in this study, 3 other Turkey isolates and 2 Czech Republic isolates showed the highest similarity. On the other hand, according to coat protein sequences of GPGV isolates were closely grouped with the other Turkish isolates and Slovakian isolates. In the study, sequence results of GPVV isolates, which were positive as a result of RT-PCR, could not be obtained in the study, and it is planned to be done again in the future.

Although no infected samples were detected in the study of SLRSV, the risk of spreading in the region is high. For this reason, it is necessary to take measures to prevent the spread of viral factors and to raise the awareness of producers on this issue. It is also necessary to fight against vector nematodes that play a role in the transmission of this factor. Since other viruses, which are the subject of research, are carried by crustacean lice and mite species, it is necessary to fight vector insects.

5. Conclusions

In conclusion, viruses cause deformations in vine leaves and decrease in leaf quality, as well as damage to fruit. Chemical control against plant virus diseases cannot be eliminated like the fight against insects and fungal diseases.

For this reason, care should be taken to ensure that grafting materials such as scion and rootstock, which play an important role in the spread of GPGV, GVA, and SLRSV agents, are free of viruses, and more importance should be given to the necessary precautions in seedling production. Producers should be made aware of using certified vine samplings. Since GVA and GPGV viruses are transmitted by mechanical means, tools and equipment used in vineyards should be disinfected and protective measures should be taken in the struggle, especially infected vines should be removed. It is important to use molecular methods for the identification of GVA-infected and non-infected vines. In this study, phylogenetic analysis of Turkish GVA isolates was performed for the first time.

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