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Detection of *Grapevine rupestris stem pitting-associated virus* in the vineyards of Southeastern Anatolia and Eastern Mediterranean in Turkey

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ABSTRACT

In this study, it was aimed to survey the vineyards for the presence of the *Grapevine rupestris stem pitting-associated virus* (GRSPaV) in the Southeastern Anatolia (Gaziantep, Adıyaman and Kilis) and Eastern Mediterranean Region (Kahramanmaraş, Hatay, Adana and Mersin) with a newly designed primer pair. A total of 381 samples from 96 vineyards were collected and analysed by RT-PCR technique. Of the 381 samples collected, 40 were found to be infected with GRSPaV. The prevalence rates of GRSPaV were respectively calculated as follows: Kahramanmaras (4.46%), Kilis (2.36%), Adıyaman (1.58%), Gaziantep (1.31%), Adana (0.53%), Mersin (0.26%) and Hatay (0%). As a result, it has been determined that the virus has a significant prevalence in Southeastern Anatolia and Eastern Mediterranean Region.

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KEYWORDS
GRSPaV; vineyards; RT-PCR

Introduction

The grapevine serves as a host to a large number of pathogens such as bacteria, fungi, phytoplasm and viruses. Fungal and bacterial diseases that are harmful to the vineyards can be controlled by chemical methods; however, due to the lack of direct chemical countermeasures against viruses and viroids, the importance of the associated diseases tend to increase [1–4].

Up to now, more than 70 infecting pathogens have been identified, including virus (65), viroid (5), phytoplasm (8) and bacteria (1) restricted by insect-bearing xylem. It is known that viral diseases that are present in the vineyards lead to quality and yield losses [5]. One of the most important diseases is the *Grapevine rupestris stem pitting-associated virus* (GRSPaV). This causes delays in the maturation of the grains of grapes in the cluster, resulting in loss of product and sugar content in the product. In the virus prevalence survey on the selection of the vineyards, the virus was identified in 66% of the 70 selected vineyards in France, 42% of the 53 selected vineyards in Germany and 67% of the 33 selected vineyards in Australia [6]. Due to the variety of the variants of GRSPaV, primer sequences to perform identifications in a broad spectrum are required. With the GRSPaV primers prepared by Meng et al. [7]from the genomic region coding the conserved HEL portion in the viral replicase polyprotein region, further identification has been performed. Polyclonal antiserum of the sheath protein has been used for the serological diagnosis of GRSPaV. Antiserum has been reported to be effective in determining different strains of GRSPaV in ELISA and western blot methods. However, only a limited number of samples are being studied with western blot. In addition, it has been reported that RT-PCR has had numerous advantages such as being much faster, more reliable, and the ability to test multiple samples at the same time, when compared to ELISA and western blot methods [7,8].

This study was designed to make the molecular characterization of GRSPaV with our own designed primer pair in grapevine leaves and shoot samples collected from vineyards in Southeastern Anatolia (Gaziantep, Adıyaman, Kilis) and Eastern Mediterranean Region (Kahramanmaraş, Hatay, Adana and Mersin).

Materials and method

Collection of plant samples

A total of 96 vineyards were investigated at seven locations: 381 samples of leaf, petioles, shoot and phloem tissues were collected from the vineyard areas of Southeast Anatolia (Gaziantep, Adıyaman and Kilis) and Eastern Mediterranean Region (Kahramanmaraş, Hatay, Adana and Mersin) in 2013 and 2014 summer as research materials (Table 1).

Primer design

Within the scope of the research; a pair of primer for diagnosing GRSPaV isolates in Turkey; the new primer pair prepared using regions where the viral genomes of local isolates Tk-21 and Tk345 [9] and ABD GRSPaV-PN1|DQ278635.1| [10] and USA GRSPaV-SG1|

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Table 1. Survey sites and sample numbers from these sites.

Location	Number of vineyards	Number of samples collected
Hatay	10	42
Kahramanmaraş	26	94
Adana	3	20
Mersin	16	64
Gaziantep	17	75
Kilis	10	37
Adıyaman	14	49
Total	**96**	**381**

AY881626.1| [11], which are foreign-referenced isolates, are most varied; are presented in Table 2. A primer pair was obtained using Integrated DNA Technologies Inc. (US) PrimerQuest Tool programme.

Total nucleic acid isolation

Tissues from leaves and phloem were taken at 100 mg and crushed with 1 ml of grinding buffer solution [12]. The whole of the plant extract was taken and 10% Na-LS was added. 30 µl of the supernatant was taken and mixed with an equal volume of NaI, and 150 µl of 90% ethyl alcohol and 25 µl of silica. Discarding the liquid part in the mixture, the remaining precipitate was mixed with 50 µl of distilled water, and it was kept in 70°C for 4 minutes. The nucleic acids in the liquid portion were taken and stored at −20°C for cDNA synthesis.

Viral cDNA synthesis and polymerase chain reaction work

Viral cDNA synthesis was carried out by; mixing 7 µl RNA with 2 µl Random Primer (Thermo Scientific, USA) (0.5 µg/µl) and it was topped up to 13 µl with dH_2O. 0.8 µl of 200 U/µl *Revert Aid Reverse Transcriptase* (Thermo Scientific, USA), 10 µl 5X RT buffer, 2.5 µl 10 mMdNTP was added to the mixture. The reaction was kept at 42°C for 90 minutes and then concluded at 70°C for 10 minutes.

Table 2. RSPYB-R/RSPYB-F primer sequences prepared from the ORF1 helicase region used for PCR detection of Turkish isolates of GRSPaV in grapevine samples.

Primer	Sequence (5′–3′)	Genome location
RSPYB-F	GATGCTTTCATTTCACTTGC	4433–4452
RSPYB-R	CTTGCCAGGAGGAAATCA	4675–4692

Polymerase chain reaction

The PCR reaction was carried out in 25 µl. To the mixture; 3 µl of cDNA, 2.5 µl of 10X PCR thermal reaction buffer, 1.70 µl of 25 mM $MgCl_2$, 2 µl of 10 mM dNTP, 1.5 µl of virus-specific homologous and heterologous primers and 0.15 µl of 5 U/µl *Taq* DNA polymerase (Thermo Scientific, USA) were added. The PCR conditions were used as, starting at 94°C for 5 minutes, 10 seconds at 94°C for each of the 35 cycles and 10 seconds at 52°C and 60 seconds at 72°C. Final extension was determined as 10 minutes at 72°C.

Results and discussion

The symptoms observed were mostly chlorotic spots, yellow mosaic and vein chlorosis. No obvious symptoms of GRSPaV disease were observed on the trunks. This is probably due to the rooted plant density in the vineyards examined.

The designed RSPYB-R/RSPYB-F primer pair the amplified cDNA fragment is 259 bp is 259 bp in size. RT-PCR analyses of 381 samples collected from the survey sites were performed. As a result of the PCR analysis performed, it was determined that 40 samples were infected with GRSPaV. In Figure 1 shown below, gel images of samples determined to be infected with GRSPaV are given.

The % rates of the distribution of the samples that were detected to be infected with GRSPaV according to the locations are given in Table 3, according to the results of the RT-PCR analysis.

In all samples tested, the virus detection rate was found to be 10.50%, as a result of PCR study with RSPYB-F/R primers. Percentage of virus in the Eastern Mediterranean region is 5.25%, while in Southeast

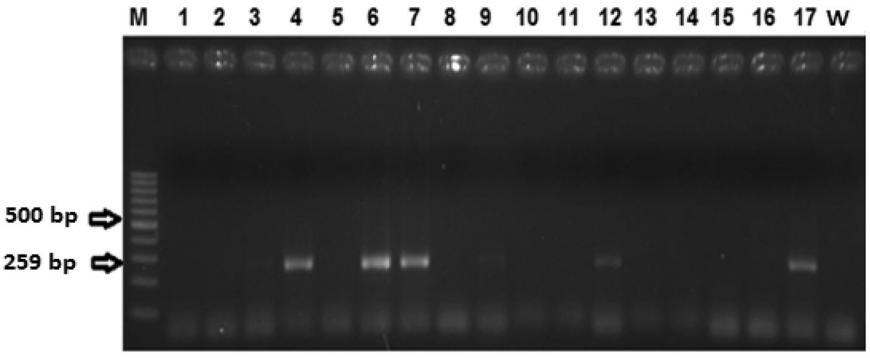

Figure 1. Electrophoresis analysis of the samples collected from Kahramanmaraş, Kilis, Adıyaman and Mersin samples obtained from PCR tests with RSPYB-F/R primer. (6-14-17) Kilis; (1-2-7-8-9-13) Adıyaman; (3-4-10) Kahramanmaraş; (5-15-16-17) (Mersin). W; Water negative control. M; 100 bp DNA marker (Fermentas).

Table 3. Identification of GRSPaV with RSP13/14 and RSPYB-F/R primers in grapevines from in the Eastern Mediterranean and Southeastern Anatolia.

Region	Location	Vineyards	Tested samples	Positive samples RSPYB-F-RSPYB- R	Positive sample rate (%)
Eastern Mediterranean	**Hatay**	10	42	0	0
	Kahramanmaraş	26	94	17	4.46
	Adana	3	20	2	0.53
	Mersin	16	64	1	0.26
Southeast Anatolia	**Gaziantep**	17	75	5	1.31
	Kilis	10	37	9	2.36
	Adıyaman	14	49	6	1.58
	Total	**96**	**381**	**40**	**10.50**

Anatolia this ratio is the same as 5.25%. While virus was identified in all the samples collected in the Southeastern Anatolia Region, the GRSPaV was not found in the samples taken from the location of Hatay in Eastern Mediterranean Region.

In 9 of the 37 samples collected from Kilis province, viruses were identified, and 24.32% of the collected samples were determined to be infected with these viruses. Kilis is the most infected province according to the sample numbers and percentages of GRSPaV collected from the provinces. The follow-up Kilis, Kahramanmaraş is the second province with virus identified in 17 samples among the 94 samples collected. GRSPaV positive samples were 18.1% of the samples collected from Kahramanmaraş. These provinces are followed by Adıyaman (12.4%), Adana (10%), Gaziantep (6.7%), Mersin (1.56%) and Hatay (0%), respectively. Within the 42 samples taken from Hatay, no sample with GRSaV was found.

In a previous study, it was reported that 39 of 106 samples, which were collected from Adana, Mersin, Hatay in Eastern Mediterranean Region, and from Adıyaman, Gaziantep, Kilis and Şanlıurfa in Southeastern Anatolia Region, were contaminated with GRSPaV. In addition, GRSPaV was reported to be found for the first time in Turkey [13]. In a study conducted in Northern Italy, samples from 56 different locations were collected. The collected samples were tested by RT-PCR with a different primer pair (RSP13/RSP14 and RSP21/22). In the tests, 48 samples with RSP13/RSP14 and 37 samples with RSP21/RSP22 were identified to be positive [14].

Conclusions

As a result, GRSPaV is an important viral factor that causes loss of quality and yield in vineyards. Determining its prevalence is extremely important for the countermeasures of this factor. With our study, it was determined that it has a significant prevalence at the result of the scanning with the primer pair that we have recently designed, in the important vineyards of Southeastern Anatolia and Eastern Mediterranean Regions. As the result of the study, according to the number of samples collected, the virus was determined to be most common in Kilis, while the least seen province was Hatay.

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Disclosure statement

No potential conflict of interest was reported by the author.

References

[1] Cramer HH. Plant Protection and World Crop Protection Advisory. Dept. Of Fab. Bayer AG Leverkusen; 1967. p. 524.

[2] Martelli GP, Savino V. Fanleaf degeneration. In: RC Pearson, AC Goheen, editor. Compendium of grape diseases. St. Paul (MI): APS Pres; 1988. p. 48–49.

[3] Szychowski JA, Mckenry MV, Walker MA, et al. The vein-banding disease syndrome: a synergistic reaction between *Grapevine Viroids* and *Fan Leaf* Virus. Vitis. 1995;34:229–232.

[4] Gökçe B. Gaziantep İli Bag Alanlarinda *Bag Sari Benek* (Gysvd-1 ve 2) Hastaliginin Arastirilmasi. Çukurova Üniversitesi Fen Bilimleri Enstitüsü Doktora tezi; 2007.

[5] Martelli GP. Directory of virus and virus-like diseases of the grapevine and their agents. J Plant Pathol. 2014;96 (1 Suppl):1–136.

[6] Goheen AC. Virus diseases and grapevine selection. Am J Enol Vitic. 1989;40:67–72.

[7] Meng B, Johnson R, Peressini S, et al. Rupestris stem pitting-associated virus-1 is consistently detected in rupestris stem pitting-İnfected grapevines. Eur J Plant Pathol. 1999;105:191–199.

[8] Meng B, Gonsalves D. Rupestris stem pitting-associated virus of grapevines: genome structure, genetic diversity, detection, and phylogenetic relationship to other plant viruses. Current Topics in Virology. 2003;3:125–135.

[9] Bayan Y. Bağ Alanlarında *Asma Gövde Çukurlaşması İlişkili Virüs* (*Grapevine Rupestris Stem Pitting-Associated Virus*, Grspav)'Ün Moleküler Karakterizasyonu ve Genomik Çeşitliliğinin Araştırılması [thesis PhD]. Turkey: Kahramanmaras Sutcu Imam University; 2015.

[10] Meng B, Li C, Wang W, et al. The complete genome sequences of two new variants of *Grapevine rupestris* stem pitting-associated virus and comparative analyses. J Gen Virol. 2005;86:1555–1560.

[11] Meng B, Rebelo AR, Fisher H. Genetic diversity analyses of *Grapevine rupestris* stem pitting-associated virus reveal distinct population structures in scion versus rootstock varieties. J Gen Virol. 2006;87:1725–1733.

[12] Foissac X, Svanella-Dumas L, Gentit P, et al. Polyvalent degenerate oligonucleotides reverse transcription-

polymerase chain reaction: A polyvalent detection and characterization tool for *Trichoviruses*, *Capilloviruses*, and *Foveaviruses*. Phytopathology. 2005; 95(6):617–625.

[13] Buzkan N, La Notte P, Karadag S, et al. Detection of *Grapevine Rupestris stem pitting-associated virus* in autochthonous grapevine cultivars in Turkey. J Plant Pathol. 2015;97(2):387–389.

[14] Terlizzi F, Ratti C, Filippini G, et al. Detection and molecular characterization of Italian *Grapevine rupestris* stem pitting-associated virus isolates. Plant Pathol. 2010;59:48–58.