Viticulture Data Journal 5: e133487
doi: 10.3897/vdj.5.e133487

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Research Article

Exploring vineyards of Crete and Thira islands of southern Greece: Incidence of seven major grapevine viruses in indigenous *Vitis vinifera* cultivars

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Academic editor: Laura Rustioni

Received: 30 Jul 2024 | Accepted: 18 Oct 2024 | Published: 24 Oct 2024

Citation: Doupis G, Pitsoli T, Leivadara E, Merkouropoulos G, Taskos D, Grammatikakis S, Grammatikaki M, Pappi P (2024) Exploring vineyards of Crete and Thira islands of southern Greece: Incidence of seven major grapevine viruses in indigenous *Vitis vinifera* cultivars. Viticulture Data Journal 5: e133487. https://doi.org/10.3897/vdj.5.e133487

Abstract

Wine grapes from two of the most well-established viticultural regions in Greece, Crete and Thira islands, were surveyed to determine the prevalence of the seven major viral diseases included in Greek phytosanitary certification requirements. A total of 14 indigenous grapevine cultivars in 30 commercial vineyards were investigated, amounting to a total of 250 samples. PCR assays were performed for the reliable detection of Grapevine virus A (GVA), Grapevine virus B (GVB), Grapevine leafroll associated virus 1 (GLRaV-1), Grapevine leafroll associated virus 3 (GLRaV-3), Grapevine fleck virus (GFkV), Grapevine fanleaf virus (GFLV) and Arabis mosaic virus (ArMV) in asymptomatic vines. The virological problems of each island were differed. In Cretan grafted cultivars, the most prevalent virus was GVA (the virus was detected in 43% of the samples) followed by GLRaV-3 (21%), GFkV (20%), GLRaV-1 (17%) and GFLV (3%). In self-rooted

vines of Thira, 33% of the samples were positive to GVA and none of the other tested viruses was detected. GVB and ArMV were not detected in both islands. Approximately, 40% of the samples were mixed-infected with more than one virus. The production and conservation of virus-free propagation material of the most valuable south Aegean cultivars was accomplished and the evaluation of their agronomical and oenological characteristics of interest is in progress, efforts that could benefit the Greek vineyard.

Keywords

grapevine, vitiviruses, RT-PCR, Crete, Thira, Mediterranean

Introduction

Grapevine hosts a significant number of intracellular viral pathogens responsible for a multitude of degenerative syndromes with the consequent deterioration of viticultural products (Martelli 2017, Fuchs 2020). The presence of viruses, in single or mixed infections, in the vast majority of the viticultural areas and in addition: (i) the widespread use of infected planting material, (ii) the relative contribution of vectors, as well as vegetative propagation and grafting, to the introduction and translocation of these pathogenic agents, (iii) the poor endorsement of virus disease management strategies in the vineyard and (iv) the ineffectiveness of the quarantine programmes ascertain the significant economic threat to the grape industry worldwide (Bisztray et al. 2011, Oliver and Fuchs 2011, Maliogka et al. 2015). In this perspective, several countries have implemented stringent regulations and certification programmes about the production and disposal of targeted viruses-free propagative material in order to limit the actual economic impact of grapevine viral diseases (Messmer et al. 2023).

Greek vineyards, amongst the oldest in the world, are dominated by hundreds of indigenous grapevine cultivars that require extensive research to optimise production (Stavrakaki and Biniari 2016). Clonal selection is considered an effective tool to improve the performance of the vine plant material (Hajdu et al. 2011). Sanitary quality, together with the assessment of genetic diversity and the evaluation of genotype x environment interaction are the main components of the clonal selection programmes (Mannini 2000, Rühl et al. 2004, Loureiro et al. 2011). In Greece, since June 2016, a state decree for clonal selection protocol has been legislated that establishes the implementation of the procedure by the competent research institutes.

Focusing on the sanitary status, clonal selection aims to obtain plant material that does not carry serious viral infections, thus providing the optimum possibility to improve crop yield and grape quality. Nowadays, 96 viral agents belonging to different genera and families are present in the majority of the vine-growing regions worldwide (Fuchs 2020, Javaran et al. 2021). More than 30 of the viruses isolated from grapevines have a great economic impact on the grapevine industry, causing or associated with detrimental diseases such as leafroll, leaf mottling and vein clearing, stem pitting, stem grooving,

corky bark, degeneration and decline (Maliogka et al. 2015). In Greece, according to the existing European Union (EU) directives, the latest law decree imposes that vine propagation material should be free from the Grapevine Viti-viruses A and B (GVA and GVB, family *Betaflexiviridae*, genus *Vitivirus*), the Grapevine leafroll associated closteroviruses 1 and 3 (GLRaV-1 and GLRaV-3, family *Closteroviridae*, genus *Ampelovirus*), the Grapevine fleck virus (GFkV, family *Tymoviridae*, genus *Maculavirus*), the Arabis mosaic virus (ArMV, family *Secoviridae*, genus *Nepovirus*) and the Grapevine fanleaf virus (GFLV, family *Secoviridae*, genus *Nepovirus*). The occurrence of the aforementioned major grapevine diseases represents a significant threat to the growers affecting the quantity and quality of grapes produced.

Viticulture in Greece has a history of over eight thousand years and, in accordance, grape production and wine making have a vital role in the country's cultural heritage and economic growth (Vlachos 2017). Greek vineyards are cultivated in a variety of climatic, topographic and soil conditions at altitudes varying from sea level to often in excess of 1,000 m above sea level. Crete, the largest Greek island and the fifth largest in the Mediterranean is located at the southern limits of the Aegean Sea. Cretan vineyards cover 12.0% of Greece's wine regions and rank third amongst the country's 13 viticultural areas. Indigenous grape varieties grown in Crete include the red-skinned "Kotsifali", "Mandilaria", "Liatiko" and "Romeiko" and the white-skinned "Vilana", "Vidiano", "Dafni" and "Plyto". Thira (also known as Santorini) Island is located about 150 km north of Crete and it is the southernmost member of the Cyclades complex of islands. The phylloxera-free, own-rooted and dry vineyard of Thira grows on volcanic soil covering about one-sixth of the island's total area. Amongst the most prominent Thira varieties are the emblematic "Assyrtiko", "Athiri" and "Aidani" (whites) and the red "Mavrotragano".

In both Crete and Thira islands, the profitability of viticulture has become doubtful since information on the presence of the major grapevine virus diseases is lacking. The present paper reports the results of a survey undertaken from 2018 to 2022 in wine-growing regions of Crete and Thira in order to determine the occurrence and distribution of the most significant grapevine viruses. Incidence of virus infection was evaluated in the widespread local varieties, thus allowing us to apply a sanitary selection process and to introduce the virus-free clones in the certification protocol.

Materials and Methods

From 2018 to 2022, samples were randomly collected from thirty commercial vineyards (twenty-three are located in the Cretan prefectures of Chania, Rethymno, Heraklion and Lassithi whereas the remaining seven vineyards are located in Thira) and subsequently analysed (Fig. 1). To assess the presence of GVA, GVB, GLRaV-1, GLRaV-3, GFkV, GFLV and ArMV, leaves and/or canes, from vines aged between 10 and 30 years, were collected from April to September. The samples were immediately transferred in a cold box and stored for a maximum of 10 hrs. Samples were either processed immediately or stored at 4^{o}C until further processing for the molecular assays. Cultivar selection and number of samples collected for virus tests per sampling area are presented in Table 1.

Table 1.

Cultivar name per sampling area and the respective number of plants collected for virus tests (#). In parenthesis the name of the Prefecture (Ch: Chania, Her: Heraklion, Las: Lassithi, Ret: Rethymno and Thi: Thira).

Location	Cultivar	#	Location	Cultivar	#
Gerani (Ch)	Kotsifali	4	Zaros (Her)	Moschato Spinas	7
Gerani (Ch)	Romeiko	6	Zaros (Her)	Vidiano	10
Plakalona (Ch)	Romeiko	8	Sitia (Las)	Kotsifali	2
Alagni (Her)	Plyto	10	Sitia (Las)	Vilana	2
Archanes (Her)	Assyrtiko	10	Ziros (Las)	Liatiko	4
Archanes (Her)	Athiri	10	Rethymno (Ret)	Vidiano	8
Archanes (Her)	Mavrotragano	10	Gaidouri (Thi)	Assyrtiko	9
Archanes (Her)	Moschato Spinas	11	Gaidouri (Thi)	Mandilaria	3
Dafnes (Her)	Dafni	2	Gaidouri (Thi)	Platani	3
Dafnes (Her)	Kotsifali	3	Imerovigli (Thi)	Assyrtiko	9
Dafnes (Her)	Vilana	3	Marmari (Thi)	Aidani	20
Douli (Her)	Kotsifali	10	Megalochori (Thi)	Aidani	2
Douli (Her)	Mandilaria	10	Megalochori (Thi)	Assyrtiko	2
Douli (Her)	Vilana	10	Megalochori (Thi)	Athiri	1
Asites (Her)	Liatiko	2	Megalochori (Thi)	Mandilaria	1
Kolena (Her)	Kotsifali	5	Megalochori (Thi)	Mavrotragano	1
Panorama (Her)	Mandilaria	7	Potamiotisa (Thi)	Athiri	5
Pyrgou (Her)	Kotsifali	5	Potamiotisa (Thi)	Mavrotragano	5
Voutes (Her)	Liatiko	5	Thirassia1 (Thi)	Assyrtiko	10
Voutes (Her)	Vilana	5	Thirassia2 (Thi)	Assyrtiko	10

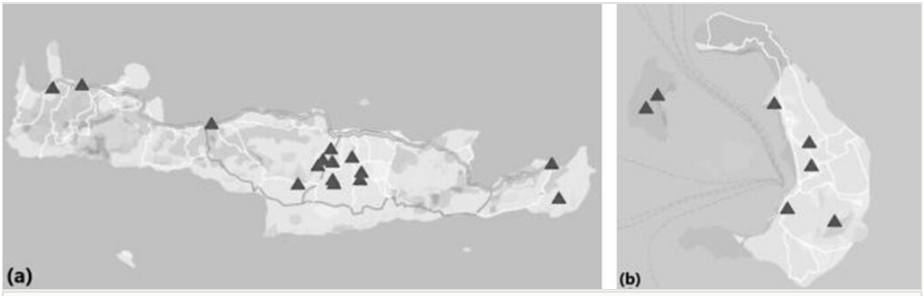

Figure 1. doi

Map of Crete (a) and Thira (b) islands with the locations of the 30 vineyards assessed in this study marked with a triangle.

Total RNA was extracted from phloem scrapings (200 mg) which were placed in a plastic bag and homogenised in 2 ml of grinding buffer according to a slightly modified protocol of Chatzinasiou et al. (2010) as described in Maliogka et al. (2015). The specific primers recommended for the detection of GVA, GVB, GLRaV-1, GLRaV-3, GFkV and GFLV are presented in Table 2. To improve the detection of GVB, new specific primers were designed, based on the amplification of the most conserved regions of the coat protein gene according to a modified protocol of Osman et al. (2013). Primers and TaqMan probe designs were carried out using the highly conserved regions of pathogen's genome, after aligning homologous nucleotide sequences of different isolates deposited in the NCBI databases (Fig. 2).

Table 2.

Primers and probes used for the detections of grapevine viruses in RT-PCR assays.

Target	Primer	Primer sequence (5' – 3')	TaqMan probe sequence (5' – 3')	Authors
GVA	GVA-77 f	CGACCGAAATATGTACCTGAATACTC	CTTCGGGTACATCGCCTTGGTCGG	Osman and Rowhani 2008
	GVA-192 r1	TTTGCTAGCTTTAGGACCTACTATATCTACCT		
	GVA-92 r2	CTTGCTAGCCTTAGGTCCTACTATATCTACCT		
GVB	GVB-Rup	TCTTTCGRACWGAGGGT	AGAACGTCTTCACAGCRCARGC	this study
	GVB-Rdo	AACCACCTATATYTCRACAGA		
GLRaV-1	LR1 HSP70-149 f	ACCTGGTTGAACGAGATCGCTT	ACGAGATATCTGTGGACGGA	Osman et al. 2007
	LR1 HSP70-293 r	GTAAACGGGTGTTCTTCAATTCTC		
GLRaV-3	GLRaV3-56f	AAGTGCTCTAGTTAAGGTCAGGAGTGA	CAGGTAATAGCGGACTGAGACTGGTGGACA	Osman et al. 2008
	GLRaV3-285r	GTATTGGACTACCTTTCGGGAAAAT		
GFkV	GFkV OB f	CGAGAACTCTCTTTTCACCTC	ACCCTCGCCCTCATGCA	Bertolini et al. 2010
	GFkV OB r	CCGGCGTGGATGTAGAG		
GFLV	GFLV-769f	GGGACCACTATGGAYTGGAATGA	AAGTATCCCGGGGTGTATGTGGAAGAGGA	Osman and Rowhani 2008
	GFLV-868r	TTCGGTGATATGGAGAGCGAAT		

For all the above pathogens, quantitative reverse transcription polymerase chain reaction assays (RT-qPCR) were employed and the thermal cycling conditions included incubation at 50°C for 30 min, followed by 95°C for 15 min and 50 cycles of two steps: a) 30 sec at 95°C and b) 60 sec at 60°C. RT-qPCR for each pathogen was performed in a final volume of 25 µl and the reaction mixture consisted of 2 U HotStarTaq, 10x buffer (Qiagen, Hilden, Germany), 4 mM DTT (AppliChem, Darmstadt, Germany), 2 U of Superscript™ II Reverse Transcriptase, (Thermo Fisher Scientific Inc., Waltham, Massachusetts, United States) and DEPC-treated water up to the final volume. All primers

and probes were used in concentration of 0.2 μM and probes were labelled with 6-carboxyfluorescein (6-FAM) at the 5′-end and with Hole Dark Quencher 1 (BHQ-1) at the 3′-end (Integrated DNA Technologies, Leuven, Belgium). Amplifications were performed in a QuantStudio™ 3 Real-Time PCR System (Thermo Fisher Scientific Inc., Waltham, Massachusetts, United States)

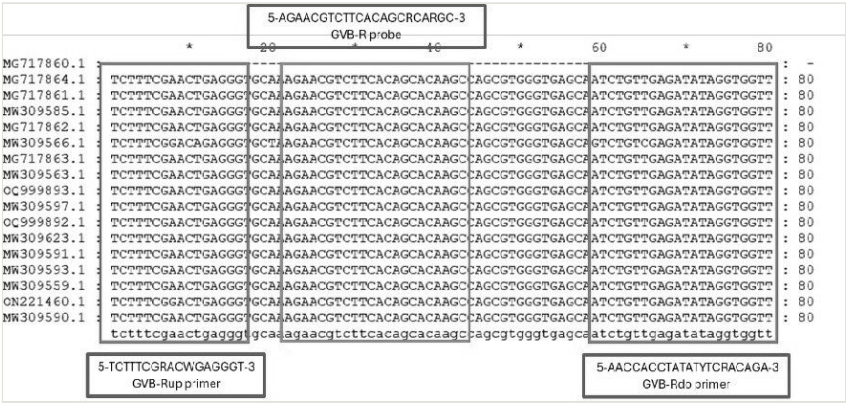

Figure 2. doi

Detection of GVB. Genomic area of the designed primers after aligning homologous nucleotide sequences of different isolates deposited in the NCBI databases.

For the identification of ArMV, new primers were designed, based on aligned sequences of ArMV isolates available in the NCBI databases and a two-step PCR detection system was employed. Primer names, sequence, concentration of each primer, expected size of amplified products and cycling conditions for ArMV two-step detection system are provided in Table 3. Briefly, a first Reverse Transcription-Polymerase Chain Reaction (RT-PCR) assay was conducted in a final volume of 25 μl after the addition of 2 μl of total RNA extract in the reaction mixture along with the specific primer pair ArMV UP-190 and ArMV DO (Integrated DNA Technologies, Leuven, Belgium), 0.1 M DTT (AppliChem, Darmstadt, Germany), 0.2 mM dNTPs, 2 U SuperscriptTM II Reverse Transcriptase (Thermo Fisher Scientific Inc., Waltham, Massachusetts, United States), 10x Taq pol with 15 mM $MgCl_2$ buffer, 2 U DF Taq DNA polymerase (Enzyquest, Heraklion, Crete, Greece) and DEPC-treated water. Subsequently, a nested PCR was performed using 1 μl of the products from the first amplification as a template. The reaction mixture of the nested PCR (final volume of 20 μl) consisted of the 1 U DF Taq DNA polymerase, 10x Taq pol with 15 mM $MgCl_2$ buffer, 0.2 mM dNTPs, the specific primer pair ArMV UP NEST2 and ArMV DO NEST2 and DEPC-treated water up to the final volume of 20 μl. Amplifications were performed in a Techne Prime Thermal Cycler (Cole-Parmer Instrument Co., Europe, UK) and all PCR products were visualized under UV light after analysis by electrophoresis in 1.5% agarose gels in TAE buffer and ethidium bromide staining.

Positive and negative controls used in the experiments aforementioned herein, consisted of lyophilised leaf tissues from infected or non-infected plants provided by the germplasm collection of IOSV (Lykovrisi, Attica and Heraklion, Crete) (data not shown).

Graph Pad Prism 5.0 was used for figure preparation.

Table 3.

Details for the two-step RT-PCR protocol applied for the detection of ArMV virus.

Protocol	Cycling scheme	
RT-PCR	42°C for 1 hr	
ArMV UP-190 0.4 µM	95°C for 15 min	
5' - CGT GGG TTA TGA GYTTTG ATGC - 3'		
ArMV DO 2 1.0 µM	95°C for 30 sec	40 cycles
5' - ARY CCA TGR CAA GCT ATC ATRT - 3'	54°C for 30 sec	
Product size 997 bp	72°C for 1 min	
	72°C for 5 min	
Protocol	Cycling scheme	
Nested PCR	95°C for 15 min	
ArMV UP NEST2 1.0 µM		
5' - TAT GCY GAG TTT GAR GCR GCVAA - 3'	95°C for 30 sec	40 cycles
ArMV DO NEST2 1.0 µM	60°C for 30 sec	
5' - TT GTTG RTT CCA GTT RTT AGT GAC - 3'	72°C for 30 sec	
Product size 336 bp		
	72°C for 5 min	

Results and Discussion

Viral diseases in viticulture cause significant economic losses (Atallah et al. 2011) since they are considered responsible for an array of symptoms leading to qualitative and quantitative yield loss, thus shortening the productive lifespan of the vineyard (Martelli 2017). In the current study, the presence of GVA, GVB, GLRaV-1, GLRaV-3, GFkV, GFLV and ArMV infection was evaluated in a total of 250 asymptomatic samples from 14 cultivars (Table 1), collected from 30 commercial vineyards located in Crete and Thira islands (Fig. 1). Vines identified as visibly non-diseased (absence of virus-like symptoms such as graft union-incompatibility, distortion, discolouration, leaf rolling and mottling, poor fruit setting, irregular ripening and reduced size of the berries) are labelled as asymptomatic. RT-PCR analysis revealed that 124 out of 250 samples showed an infection with one of the tested viruses, representing a percentage of 49.6% (Table 4). Since nearly half of the asymptomatic samples found to be infected, our results together with observations reported by Poojari et al. 2013, Alabi et al. 2016, Moura et al. 2018 and Crnogorac et al. 2021support the finding that grapevine could be infected with viruses without displaying any observable symptoms. The asymptomatic infections could be attributed to: (i) plant tolerance mechanisms that can mitigate the negative effects caused by high-titer virus replication levels, (ii) the presence of mild strains of the detected

viruses in the sampling sites, (iii) a dormant status of the viruses (viruses latency), a time period when no viral replication occurs, referring to the period between viral infection and the elicitation of the visible symptoms and (iv) the influence of host plant x environment interactions on the overall impact of the detected viruses.

Table 4.

Percentage (%) of detected viruses (in single or mixed infections) in tested grapevine cultivars. In parentheses, the grape colour (W: white, R: red). The symbol # represents the number of samples per grapevine cultivar subjected to the virus detection protocols.

Cultivar	#	Virus free	GVA	GVB	GLRaV-1	GLRaV-3	GFkV	GFLV	ArMV
Aidani (W)	22	73%	27%	0%	0%	0%	0%	0%	0%
Assytiko (W)	50	60%	34%	0%	10%	12%	16%	0%	0%
Athiri (W)	16	50%	38%	0%	0%	13%	0%	0%	0%
Dafni (W)	2	50%	0%	0%	0%	50%	0%	0%	0%
Kotsifali (R)	29	55%	31%	0%	7%	3%	10%	3%	0%
Liatiko (R)	11	18%	73%	0%	73%	55%	18%	9%	0%
Mandilaria (R)	21	19%	81%	0%	29%	14%	48%	10%	0%
Mavrotragano (R)	16	25%	69%	0%	19%	13%	6%	0%	0%
Platani (W)	3	67%	33%	0%	0%	0%	0%	0%	0%
Plyto (W)	10	40%	10%	0%	0%	60%	10%	0%	0%
Romeiko (R)	14	0%	100%	0%	29%	50%	21%	0%	0%
Moschato Spinas (W)	18	78%	22%	0%	0%	0%	0%	0%	0%
Vidiano (W)	18	67%	6%	0%	0%	0%	28%	0%	0%
Vilana (W)	20	65%	25%	0%	5%	3%	5%	5%	0%

With the exception of two virus-free plantations in the areas of Rethymno (local cultivar “Vidiano”) and Douli (local cultivar “Kotsifali”), the majority of the Cretan vineyards assessed in this study were found to be infected by various viruses. In particular, for Cretan red-berried cultivars (“Kotsifali”, "Liatiko", "Mandilaria", "Mavrotragano" and “Romeiko”), 73% of the tested samples were infected with at least one virus, while the average infection reported from the white-beried cultivars (“Vilana”, “Vidiano”, “Plyto”, “Moschato Spinas”) was 43% (Table 4). In contrast, in the seven vineyards of Thira, GVB, GVLRaV-1, GLRaV-3, GFkV, GFLV and ArMV were not detected, while the presence of GVA was confirmed in 27 of the 81 vines (33%). Specifically, we report three GVA-positive out of fifteen tested vines in the vineyard “Gaidouri”, four positive out of twenty tested in the vineyard “Marmari”, five positive out of nine in the vineyard “Imerovigli” and six out of ten in the vineyard “Potamiotissa”, whereas in twenty samples from Thirassia (a small island adjacent to Thira, that was once a part of Thira), GVA was detected in only two of the vines. Most GVA infections were detected in the vineyard “Megalochori” (100% positive samples). Based on the severity of viral epidemics in "Megalochori", an effective control strategy against the spread of GVA, starting with clean planting stocks, is more

than mandatory. The reasons why the monitored viruses were more frequently present in Crete compared to Thira and in red compared to white cultivars, could be due to the sanitary quality of the propagating material, as well as the absence of the natural vectors and/or the application of integrated management practices to control the population of the vectors in the vineyard (Maliogka et al. 2015).

Grapevine virus A (GVA) was the most frequent virus in single or mixed infections accounting for an 81% prevalence, whereas GLRaV-3, GFkV, GLRaV-1 and GFLV were detected in 28%, 27%, 23% and 5% of the infected vines, respectively (Table 5). It is worth mentioning that GVB and ArMV were not found in any tested sample collected from Cretan and/or Thira vineyards. A growing number of studies (Kostadinovska et al. 2014, Porotikova et al. 2016, Caruso et al. 2022) indicate the widespread nature of GVA, since the virus has been detected in almost all the grape-growing regions of the world (Goszczynski and Habili 2011, Mostert et al. 2018, Vončina et al. 2019) and, in addition, reports suggest that GVA can naturally spread in wild grapevines (Minafra et al. 2017). GVA, a virus that multiplies in phloem parenchyma cells (Rosciglione et al. 1983) and is closely associated with the Rugose wood (RW) syndrome, is known to be transmitted by grafting and/or different species of mealybugs (family *Pseudococcidae*) and soft-scale insects (family *Coccidae*), species considered primary pests for the vineyards worldwide (Daane et al. 2012). The noteworthy role of mealybugs in GVA transmission, together with the international dissemination of the virus in infected grapevine germplasm, could explain the frequent co-existence of GVA with *V. vinifera* cultivars. In our study, we reported a high prevalence of GVA in Cretan and Thira vineyards. We also documented the complete absence of Grapevine Virus B (another member of the genus *Vitivirus* with a worldwide distribution pattern, transmitted also by mealybugs and soft scale insects) that is not in agreement with the GVB detection ratio in other regions of Greece (Orfanidou et al. 2021), as well as with surveys conducted in other countries (Xiao et al. 2018, Jones and Nita 2019, Rasool et al. 2019). The zero incidence of GVB (Fig. 3) could be attributed to the exchange of GVB-free parental material (rootstocks and scions) within the Cretan and Thira territory. Additionally, the absence of Arabis mosaic virus (ArMV), a *Nepovirus* associated with the disease complex known as fanleaf degeneration, in 250 tested samples confirms the findings of Trudgill et al. (1983) who reported that the virus is transmitted by the nematode *Xiphinema diversicaudatum* and is commonly detected in cooler climates where its vector is present. However, it is important to note that, according to Vončina et al. (2011), when the vector is present, the incidence of ArMV (and consequently the economic impact and the biological damage on grapevine) might increase. Transmission through nematodes is a common feature of numerous grapevine-infecting nepoviruses and of Grapevine fanleaf virus (GFLV) (Digiaro et al. 2017), a major virus characterised as one of the Old World nepoviruses causing discolourations, deformations, progressive decline and decreased yields and quality. GFLV is a soil-borne viral disease transmitted, relatively slowly, by the longidorid nematode *Xiphinema index*. According to Tzortzakakis et al. (2014), the nematode had a high prevalence in grapevines in Crete and thus, in order to prevent the formation of GFLV hotspots, we highlight the importance for the application of preventative measures (implementation of reliable diagnostic techniques for GFLV detection and introduction of GFLV-free planting

material, monitoring of the occurrence of the corresponding vector nematode) against the spread of GFLV in Cretan vineyards.

Table 5.

Percentage (%) of single and multi-infections detected in the grapevines, based on the data obtained from the PCR assays for a total of 124 positive samples.

Single infection	
Virus	Percentage
GVA	44%
GLRaV-3	8%
GFkV	6%
GLRaV-1	2%
GFLV	0%
Multi-infection	
Virus	Percentage
GVA & GFkV	7%
GVA & GLRaV-1	7%
GVA & GLRaV-1 & GLRaV-3	6%
GVA & GLRaV-1 & GLRaV-3 & GFkV	4%
GVA & GLRaV-3	4%
GVA & GLRaV-1 & GFkV	3%
GVA & GLRaV-3 & GFkV	2%
GLRaV-3 & GFkV	2%
GVA & GFLV	2%
GLRaV-3 & GFLV & GFkV	1%
GVA & GFLV & GFkV	1%
GLRaV-1 & GLRaV-3 & GVA & GFLV & GFkV	1%

In the current study, the relatively high rate of PCR detection of GLRaV-3 and GLRaV-1 (Table 5) re-affirms the occurrence of leafroll-associated viruses throughout the Mediterranean vineyards, as reported 25 years ago (Digiaro et al. 1999). As previously mentioned for GVA, the reported efficiency of the most of the common mealybugs (e.g. *Planococcus ficus*, *Pseudococcus maritimus*) and soft scale insects (e.g. *Parthenolecanium corni*, *Ceroplastes rusci*) to act as potential GLRaVs vectors (Almeida et al. 2013), as well as the transmission of GLRaV-1 by the *Eriophyes* mite *Colomerus vitis* (Malagnini et al. 2016) and, additionally, the exchange of infected propagation material have been identified as the major factors responsible for the global spread of GLRaVs. In our survey, similar viral load (27%) was reported for the Grapevine fleck virus (GFkV). Fleck disease is named for the appearance of vein-clearing on young leaves on *Vitis rupestris*, while many grapevine cultivars and rootstocks infected by GFkV alone

could be symptomless. However reports associate GFkV with graft incompatibilities, in case of a co-infection (Sabanadzovic et al. 2017) and, consequently, plant material (rootstocks and scions) must be tested for the virus. GFkV infects only *Vitis* spp. worldwide, is a virus found in the phloem and is non-mechanically transmissible. Natural field transmission of GFkV is questionable since no vector is known and the virus primarily spreads through infected propagating material.

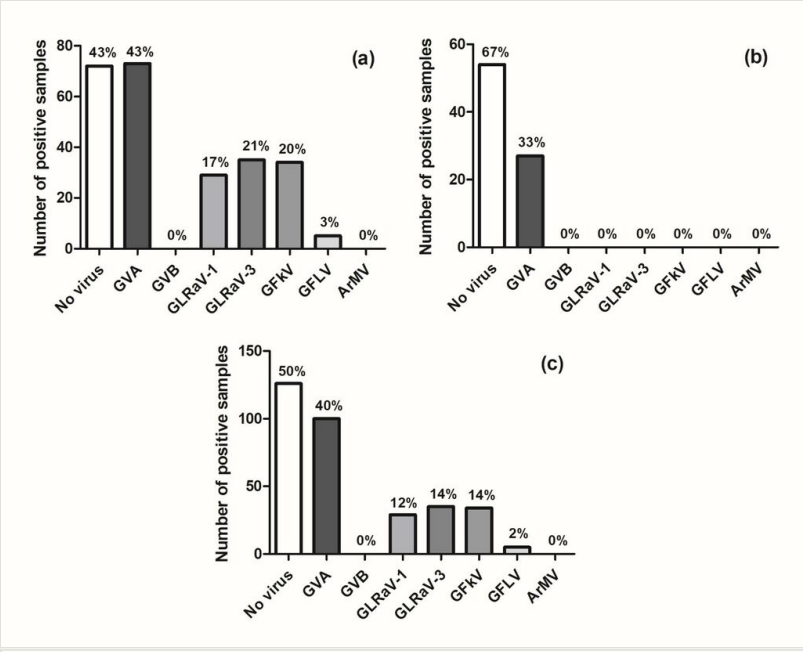

Figure 3. doi

Virus monitoring in the analysed samples from Cretan (A), Thira (B) vineyards and as a total (C) in single or mixed infections. Above each column is the percentage (%) of virus detection in a total of 250 samples (169 samples from Crete and 81 samples from Thira) from 14 cultivars.

Additional analysis revealed that 60% of the detected infections were due to a single infection, whereas two viruses were found in 22% of the infected plants and three viruses in 13% of the tested positive samples (Table 4). Quadruple and quintuple infections were found in five and one (out of 250) vines respectively. The combination of viruses found in mixed infections varied, but was characterised by similarities within the same vineyard. According to Takahashi et al. (2019), transition from the latency period to activation and appearance of obvious disease symptoms sometimes occurs during mixed infections and, in addition, Prosser et al. (2007) suggested that the largest losses in production are caused by complexes of viruses. However, this is not the case in our study, as we report asymptomatic infections even during the presence of different combinations of viruses in individual grapevines. This phenomenon is in accordance with the results of Clingeleffer and Krake (2002) and Komorowska et al. (2013) showing that vines infected with more than one virus are occasionally symptomless.

Overall, this study represents the first extensive survey on the presence of GVA, GVB, GLRaV-1, GLRaV-3, GFkV, GFLV and ArMV in Cretan and Thira vineyards providing useful data for the sanitary status of the most important red- and white-berried indigenous cultivars. In conclusion, our study revealed a significant presence of GVA GLRaV-1, GLRaV-3 and GFkV in Cretan plantations. Conversely, the overall level of viruses in commercial vineyards in Thira is relatively low with the exception of GVA incidence. Based on our findings, we conclude that the use of certified planting material together with the proper control strategies for the different vector species should be essential components for efficient viral disease management. According to the National Regulations and the EU Directives for the production of pathogen-free propagation material, some of the most important Greek varieties are sanitary-certified and are grown in pots in nethouses. In order to maintain a relatively large intravarietal diversity and to produce clones that are potentially of benefit to the grapevine growers, the next step involves the evaluation of the agronomical and oenological characteristics of the certified clones.

Funding program

This study was a part of the project “A collaborative network for the exploitation and clonal selection of Greek vine varieties and valorization of the genetic material” (acronym CloseViva) and has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Programme Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH-CREATE-INNOVATE (project code:T1EDK-04363).

Ethics and security

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflicts of interest

The authors have declared that no competing interests exist.

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