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SHORT COMMUNICATION

Survey of grapevine viruses in Palestine

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Abstract
This study aimed to assess the current status of grapevine virus infections in Palestine, where it is widely cultivated. Almost 500 grapevine samples were collected through several field surveys started in 2019 to 2022 and tested against nine of grapevine-infecting viruses (GVA, GVB, ArMV, GFLV, GFkV, GLRaV-1, GLRaV-2, GLRaV-3, and GLRaV-7) by ELISA. The results were confirmed by RT-PCR. High prevalence of GVA and GLRaV-3 (34.4% and 23.8%, respectively) was revealed, whereas GFkV showed the lowest prevalence among all tested viruses (0.4%). Surprisingly, GFLV reported relatively high incidence (19.8%) where more than one third was detected in Hebron governorate. The most cultivated grapes (Dabouqi and Beiruiti) were found infected with nine tested viruses. This study showed the virus presence in all cultivars, with incidence of 72% of samples infected with at least one virus. These findings strongly support the need of actions to prevent virus spread through certification and sanitation programs.

Keywords Grapevine · Viruses · Survey · Palestine

Grapevines (*Vitis vinifera* L.) represent a critical component of global agriculture, particularly within Mediterranean and Middle Eastern regions. In Palestine, viticulture has both economic and cultural significance, contributing to the livelihoods of farmers and the preservation of traditional agricultural practices. However, the productivity and sustainability of grapevine cultivation are increasingly threatened by the prevalence of grapevine viruses, which can adversely impact yield, fruit quality, and vine longevity, causing severe economic losses (Martelli et al. 2017; Fuchs 2025).

Grapevine viruses belong to diverse families, including *Closteroviridae*, *Betaflexiviridae*, and *Tymoviridae*, among others. These viruses are often transmitted through vegetative propagation, grafting, and insect vectors such as mealybugs and aphids (Al Rwahnih et al. 2009). Viruses commonly infecting grapevines include grapevine leafroll-associated viruses (GLRaVs), grapevine fanleaf virus (GFLV), and grapevine virus A (GVA), each exhibiting distinct symptoms ranging from leaf discoloration to stunted growth and reduced fruit quality (Martelli and Boudon-Padieu 2006).

The epidemiology of grapevine viruses in Palestine remains poorly understood, despite their global significance. Local studies are scarce, and most knowledge relies on research conducted in neighboring regions such as Israel, Jordan, and Lebanon (Abou Ghanem-Sabanadzovic et al. 2018). The last survey on grapevine viruses in Palestine was conducted in 1997 by Alkowni et al. (1998). Given the unique climatic and agronomic conditions in Palestine, it is essential to carry out a comprehensive survey to identify prevalent grapevine viruses, their modes of transmission, and associated economic impacts. Such a survey would provide critical insights to guide management strategies, including the use of virus-free planting materials, vector control, and the implementation of sanitary measures.

The objective of this study is to address the existing knowledge gap by conducting a systematic survey of grapevine viruses in Palestine. This research utilized serological and molecular diagnostic tools (ELISA and RT-PCR) to identify and characterize the viral pathogens present in local vineyards. The findings will contribute to a better

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understanding of the distribution and prevalence of grapevine viruses in the region, and to the development of tailored intervention strategies to safeguard the Palestinian viticulture sector.

Field surveys were conducted through 2019–2022 in the main grapevine growing areas of Palestine (Hebron, Bethlehem, Ramallah, Jericho, Tubas and Jenin) (Fig. 1). During this survey, virus symptoms were frequently observed in most of the vineyards. Virus-infected grapevines often exhibited symptoms such as leaf discoloration (Fig. 2), reduced yield, and compromised fruit quality, leading to substantial economic losses. The most frequent symptoms resembled those induced by leafroll viruses and GVA. Due to the variable symptomatology observed between and within cultivars, which hinders the visual diagnosis, laboratory diagnosis becomes necessary for accurate identification of the etiology of viral diseases.

Almost 500 samples from different local cultivars (Fig. 1) (shoots and mature canes) were randomly collected from the surveyed vineyards.

Considering the possible uneven distribution of viruses within plant samples, at least two different shoots or canes of the same plant were mixed for ELISA (Enzyme-Linked Immunosorbent Assay) and RT-PCR tests. All the samples were stored at 4 °C for laboratory analysis.

DAS-ELISA test was used as serological tests for detection of GVA, grapevine virus B (GVB), Arabis mosaic virus (ArMV), GFLV, grapevine fleck virus (GFkV), GLRaV-1,

Cultivar	*tested*
Beiruti	106
Halawani	65
SPS	63
Beituni	54
Zaini/ Baladi	35
Dabouqi	30
Shami	29
Marrawi	28
Jandali	22
Zaini	22
Unknown	12
Hamadani	6
Perlite Red	6
Ballouti	3
Hamadani/France	3
Red Globe	3
Romi	3
Salti Khdari	3
Shoyoukhi Darawishi	3
Romi/Black	2
Sultanina	2

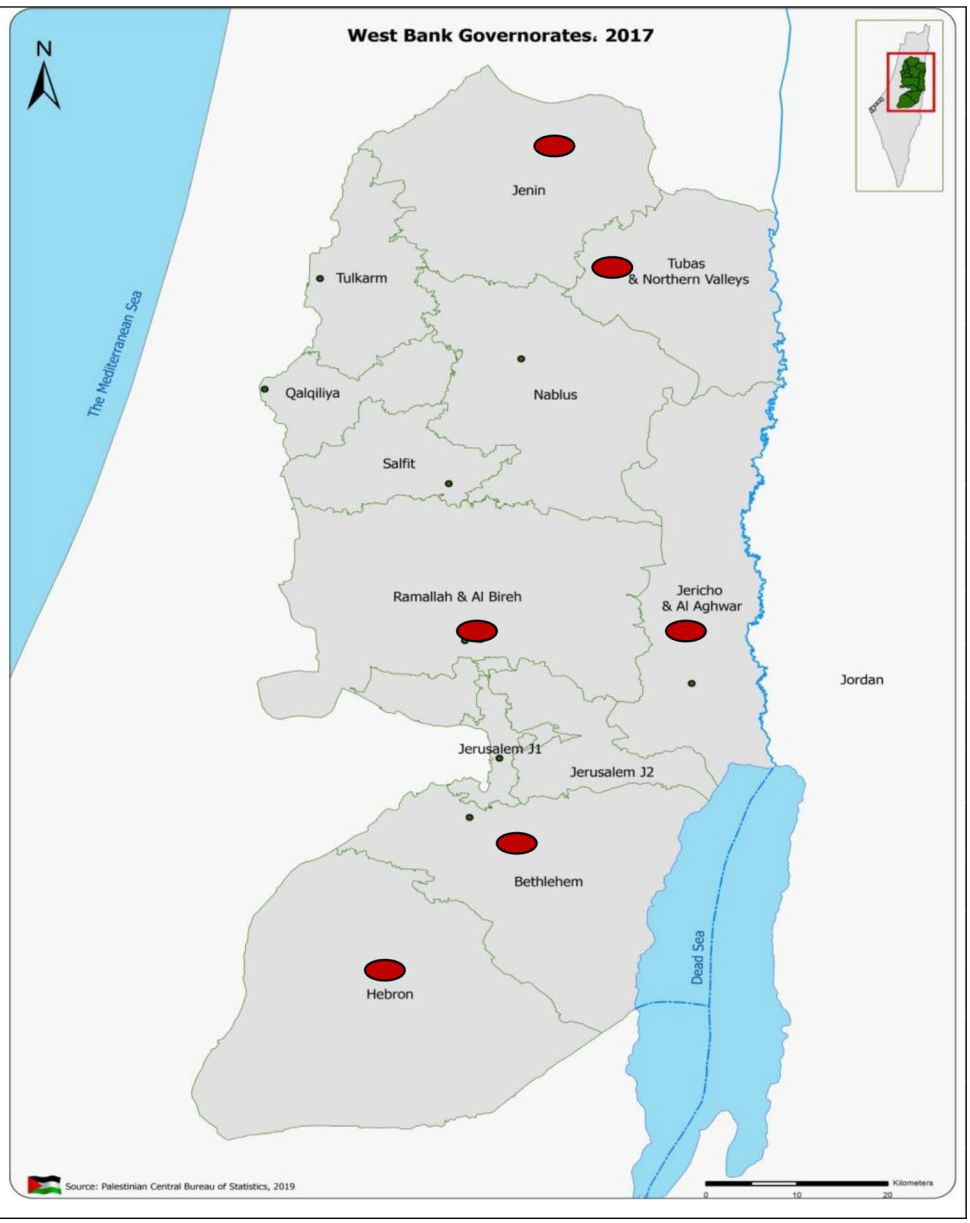

Fig. 1 The number of samples from grapevine cultivars collected from the main grapevine growing areas in West Bank–Palestine

Fig. 2 Leaf discoloration symptoms putatively resembled those induced by grapevine viruses

GLRaV-2, GLRaV-3, GLRaV-7 for all the collected samples. Commercial kits (Agritest, Valenzano, Italy) were used with duplicate wells per sample for each of the tested viruses. Positive and negative controls were also included in each test. Results were analyzed according to the manufacturer's instructions. ELISA positive samples were confirmed by RT-PCR, using the specific primer sets as published in Gambino and Gribaudo (2006) where Vitis 18 S rRNA was used as a PCR positive control. Total RNA was extracted from the collected leaf tissues (petioles, midribs, and basal leaf portions) by the modified Cetyltrimethyl ammonium bromide (CTAB) -based extraction protocol (Doyle and Doyle 1990). Then the air-dried pellet was resuspended in nuclease-free water and subjected to the first-strand cDNA synthesis that was performed by GoScript™ Reverse Transcriptase (Promega). Reaction products were analyzed by electrophoresis on 2% agarose gels in TBE buffer (45 mM Tris-borate, 1 mM EDTA) and visualized by UV light after staining with GelRed.

During this survey several viruses were identified infecting grapevines, including rugose wood associated viruses of the genus *Vitivirus*, grapevine virus A (GVA) and GVB (Xiao and Meng 2023); where GVA recorded the highest prevalence (34.4%) (Fig. 3), particularly in Jericho, Hebron and Bethlehem Governorates (Table 1).

Grapevine leafroll-associated viruses (GLRaV-1; -2; -3; and −7) having a global presence with high prevalence (Xiao et al. 2018) were tested, to mark highest prevalence of GLRaV-3 (23.8%) among all. The highest frequency of GLRaV-3 was reported on grapes tested from Hebron Governorate (51%), followed by GLRaV-2 (26% in Jenin district) (Table 1).

Grapevine leafroll tested viruses were found in all tested cultivars except Sultanina, Red Globe' and Romi/Black. Infectious degeneration and decline are due to infection by multiple viruses of the genus *Nepovirus* (family *Secoviridae*), including GFLV, and ArMV. The soil-borne nematode *Xiphinema index* was linked to the grapevine, and presented a major threat to vineyards worldwide due to its ability to transmit GFLV (Nguyen et al. 2019) through its feeding on the plant. GFLV is known as the most severe viral disease of grapevines. In Palestine, the previous study showed the limited detection of GFLV-infected vines in the fields suggested the scarce presence of *Xiphinema index* (Alkowni 2017). Surprisingly, relatively high incidence was observed for GFLV (19.8%) with the highest prevalence in Hebron governorate (36%). High detection rate of GFLV in this study opens up further study on this virus and the spread of its vector.

Cultivar Sultanina was found infected with only GVA; meanwhile GFLV was the only virus tested positive in Romi black cultivar (Table 2). Surprisingly, almost 50% of Shami grapes was infected by GFLV. Indeed, this virus was reported in very socioeconomic important varieties as Dabouqi, Beiruti, Marrawi, Perlite Red, Zaini/ Baladi, Jandali, Beituni, Halawani, Ballouti, Shoyoukhi/Darawishi, Zaini, Shami, Red Globe and Romi/Black. This was unexpected, since the previous survey reported the very low incidence of this virus in Palestine (Alkowni 2017). ArMV was another nepovirus which still limited to few cultivars, mainly Hamadani, Halawani and Beituni.

Although ELISA testing method approach was reported as sensitive and reliable, but it has certain limitations with detecting of GFkV, due to its inability to identify grapevine

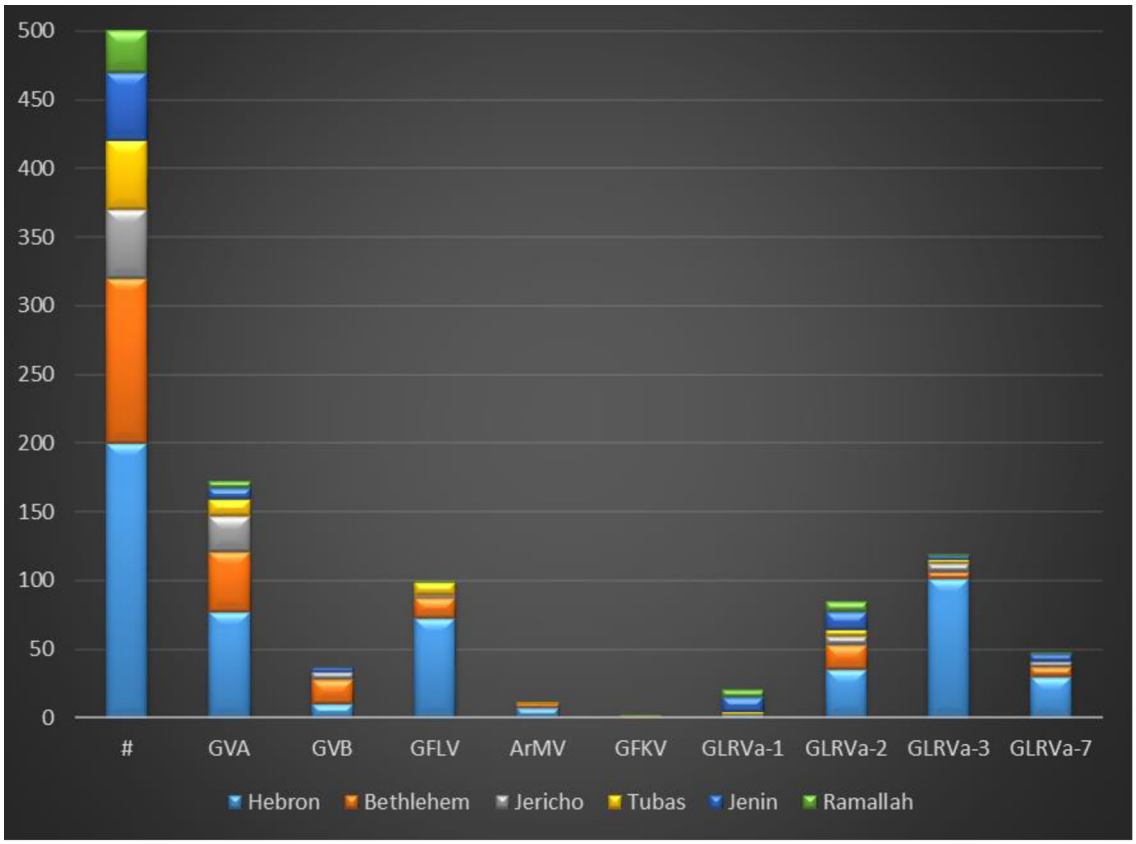

Fig. 3 The prevalence of GVA, GVB, ArMV, GFLV, GFkV, GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-7 infecting grapevine in Palestine. The most prevalent virus was found to be GVA, followed by GLRaV-3

Table 1 The prevalence of tested viruses in each Governorate

Governorate	#	GVA	GVB	GFLV	ArMV	GFKV	GLRVa-1	GLRVa-2	GLRVa-3	GLRVa-7
Hebron	**200**	39%	5%	36%	4%	0%	1%	18%	51%	15%
Bethlehem	**120**	37%	15%	13%	3%	1%	0%	15%	4%	6%
Jericho	**50**	52%	10%	4%	0%	0%	0%	12%	12%	8%
Tubas	**50**	24%	0%	18%	2%	0%	4%	10%	6%	0%
Jenin	**50**	16%	6%	0%	0%	0%	22%	26%	6%	10%
Ramallah	**30**	17%	0%	3%	0%	3%	17%	23%	3%	3%

viruses at low titers (Osman et al. 2008). RT-PCR as well has limitation for GFkV detection due to its genetic variability (de Souza et al. 2024). GFkV was found limited to two cultivars (Dabouqi and Beiruti) with very low incidence (3% and 1%, respectively).

Beiruti and Halawani; the most frequent cultivars in all governorates except Jericho. Meanwhile Beituni; Shami; Marrawi; and Jandali were frequently founded in the southern governorates (Hebron and Bethlehem). The unevenly number of samples collected for each cultivar was challenging to correlate varietal susceptibility or regional virus pressure. GLRaV-2 was the only virus tested in one samples of unknown cultivar which most probably belong to Zaini/Baladi ones from Ramallah governorate.

As a total, the percentage of virus-tested samples was reached 72%; infected with at least one virus. There were not any samples reported infected with all examined viruses.

Mixed infections were found in all governorates. The most common type of infection was single, accounting for 38%, as shown in Table 3. While the majority of grapevines were observed to be of single or double infection (Table 3), The quadruple infection was found in 1.2% of the samples tested, specifically in Hebron.

Beituni; Halawani and Marrawi were reported to have quadruple infections where GVA, GLRaV-1 and GLRaV-3 were in all of them, meanwhile GFLV and GLRaV-7 were alternated in quadruple infection level (Table 4).

Grapevines are a cornerstone of Palestinian agriculture, and the health of vineyards is critical for ensuring

Table 2 Number of cultivar samples that tested positive for each virus

Cultivar	No. tested	GVA	GVB	GFLV	ArMV	GFkV	GLRaV-1	GLRaV-2	GLRaV-3	GLRaV-7
Beiruti	106	35	6	14	1	1	10	20	7	9
Halawani	65	32	3	20	3	0	5	14	24	6
SPS	63	23	5	2	1	0	0	5	7	3
Beituni	54	15	3	14	2	0	0	8	22	10
Zaini/ Baladi	35	11	5	7	1	0	2	10	14	3
Dabouqi	30	6	3	3	1	1	2	2	11	6
Shami	29	18	0	14	1	0	1	5	10	2
Marrawi	28	11	7	4	0	0	0	3	5	3
Jandali	22	9	1	5	0	0	0	3	1	3
Zaini	22	3	2	9	0	0	0	6	5	1
Hamadani	6	1	0	0	0	0	0	0	3	0
Perlite Red	6	3	0	1	0	0	0	1	1	1
Ballouti	3	0	0	1	0	0	0	1	2	0
Hamadani/ France	3	0	0	0	1	0	0	0	3	0
RedGlobe	3	0	1	2	0	0	0	0	0	0
Romi	3	2	0	0	0	0	0	1	0	0
Salti Khdari	3	1	0	0	0	0	0	2	1	0
Shoyoukhi/Darawishi	3	0	0	1	0	0	0	2	3	0
Romi/Black	2	0	0	2	0	0	0	0	0	0
Sultanina	2	2	0	0	0	0	0	0	0	0
Unknown	12	0	0	0	0	0	0	1	0	0
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Table 3 The number of samples with mixed infection that correspond to each governorates

Governorate	Number of tested samples	Negative for tested viruses	Single	Double	Triple	Quadruple
Hebron	**200**	16	79	66	33	6
Bethlehem	**120**	44	46	25	5	0
Jericho	**50**	17	20	10	3	0
Tubas	**50**	24	21	4	1	0
Jenin	**50**	18	23	7	2	0
Ramallah	**30**	21	1	4	4	0

sustainable production and economic stability. This survey provides essential insights into the prevalence and distribution of nine grapevine viruses (Fig. 3), which pose a significant threat to viticulture in the region. This study shows that the tested viruses are spread in all cultivated grapes in Palestine, thus indicating that actions to prevent the dissemination of these viruses are strongly needed. The findings of this study highlight the significance of ELISA as a reliable and effective diagnostic tool for detecting grapevine viruses in Palestine. At least 10% out of the ELISA positive samples were ensured by RT-PCR.

While high-throughput sequencing (HTS) is recognized as the reference method in contemporary plant virology for detecting latent, low-titer, and novel viruses (Fuchs 2025), the effective use of ELISA in our study illustrates its practical value in large-scale surveys. ELISA's reliability, sensitivity, and cost-effectiveness make it an indispensable tool for virus detection in grapevines. Nonetheless, it is crucial to acknowledge the limits of ELISA, including possible cross-reactivity and its incapacity to identify novel or uncharacterized viruses. Integrating ELISA with molecular diagnostic methods such as RT-PCR may improve the precision and thoroughness of future studies. Even though, the prevalence of virus infection by ELISA tests could be underestimated once the ELISA-negative samples were subjected to RT-PCR tests. However, ELISA tests were frequently used for routine diagnosis and laboratory research worldwide especially in virus surveillance programs due to its simplicity and reliability (Zherdev et al. 2018; Aydin 2015).

Early detection through regular monitoring and the use of virus-free planting material are crucial for controlling virus spread. Additionally, implementing vector control measures and improving vineyard sanitation practices can significantly reduce infection rates. Policymakers should prioritize the development of national guidelines and support research initiatives to address the challenges posed by grapevine viruses.

Table 4 Numbers of samples with single and multiple infection for each grapevine variety

Cultivar	Number of tested samples	Negative for tested viruses	Single	Double	Triple	Quadruple
Ballouti	3	1	1	0	1	0
Beiruti	106	33	49	18	6	0
Beituni	54	13	19	13	7	2
Dabouqi	30	9	9	10	2	0
Halawani	65	11	20	18	13	3
Hamadani	6	3	2	1	0	0
Hamadani/ France	3	0	2	1	0	0
Jandali	22	5	12	5	0	0
Marrawi	28	9	9	7	2	1
Perlite Red	6	1	4	0	1	0
RedGlobe	3	0	3	0	0	0
Romi	3	1	1	1	0	0
Romi/Black	2	0	2	0	0	0
Salti Khdari	3	0	2	1	0	0
Shami	29	3	7	13	6	0
Shoyoukhi/Darawishi	3	0	1	1	1	0
SPS	63	31	20	10	2	0
Sultanina	2	0	2	0	0	0
Zaini	22	4	12	4	2	0
Zaini/ Baladi	35	4	13	13	5	0
Unknown	12	11	1	0	0	0

Conclusion

The urgency of comprehensive management strategies in Palestine is underscored by this survey, which also emphasizes the critical role of ELISA in the diagnosis of grapevine viruses. By addressing the challenges identified in this study, stakeholders can act to protect the health of vineyards and ensure the sustainability of grapevine production in the region. The findings serve as a call to action for researchers, policymakers, and farmers to work collaboratively in combating grapevine viral diseases.

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Data availability Adequate and clear descriptions of the applied materials and tools are provided in the materials and method section of manuscript. In addition, the obtained data is clearly justified from the figures and tables in the manuscript.

Declarations

Competing interests The authors declare that they have no competing interests.

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