

## Viruses and virus diseases of grapevine in Palestine

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Surveys were carried out in vineyards in the main grapevine-growing areas of Palestine (Hebron, Bethlehem, Gaza, Jerusalem, Ramallah, Jenin, Jericho and Nablus) to assess the presence and incidence of virus and virus-like diseases. Leafroll symptoms were observed in Bethlehem, Ramallah and Jerusalem in native and imported cultivars, with higher rates in the red-fruited Shami, Beitoni and Smari. Rugose-wood symptoms were also observed in local and foreign cultivars, especially on grafted vines with a high incidence in Bethlehem. Fanleaf symptoms were rarely observed, while phytoplasma-induced symptoms were observed in Jenin, Jericho and Bethlehem on cvs Biadi, Superior Seedless and Beitoni. ELISA tests showed that 463 out of 566 (82%) tested vines were infected by at least one virus. GVA was the prevailing virus (66.1%), followed by GLRaV-1 (45.6%), GLRaV-3 (21.7%), GFkV (15.7%) and GLRaV-2 (8.3%). GVB and GFLV were also detected to a lesser extent, their incidence ranging between 3.7 and 1.2%, whereas GLRaV-7 was detected in a single vine of cv. Sultanina of foreign origin. Vineyards in the Bethlehem area were particularly badly damaged (97.5%), and some local cultivars were totally (Jandali, Marrawi and Shoyoukhi) or heavily infected (Zaini, Biadi and Shami). ELISA testing of 69 young rootstock mother plants showed a relatively high incidence of virus infection (20.3%). Vein necrosis and vein mosaic diseases were also ascertained on graft-inoculated 110R and *Vitis riparia* indicator plants, whereas no viruses other than GFLV were mechanically transmitted from about 200 vines onto inoculated herbaceous hosts.

### Introduction

With an area of cultivation of about 8900 ha, grapevine ranks second among the fruit crops of Palestine, being preceded only by olive. The main grapevine-growing areas are in the southern part of Palestine, Hebron and Bethlehem in particular, with 59% and 16% of the total vineyard area (PCBS, 1996). Table-grape cultivars are by far the best represented comprising both native (e.g. Dabouqi, Zaini, Beitoni, Halawani, Darawishi, Jandali, Marrawi, Romi) and foreign (e.g. Early Superior, Perlette, Flame Seedless, Emperor, Sultanina) cultivars. Although scattered enclaves of self-rooted vines exist, the great majority of commercial vineyards are grafted on American rootstocks (e.g. 161/49, SO4, 1103P, 140Ru, 3309, 41B, *Vitis rupestris*).

The sanitary status of Palestinian viticulture is not totally unknown because of the similarity with the viticultural industries of neighbouring Israel and Jordan, which have been investigated in some detail (Savino *et al.*, 1975; Boscia *et al.*, 1995a; Tanne *et al.*, 1989, 1996). However, since there is no published record of a thorough sanitary survey of Palestinian vineyards, an investigation to this aim was initiated, the preliminary results of which are reported in this paper.

### Materials and methods

#### Field surveys

Field surveys for symptom observation were conducted in autumn 1996 in the main grapevine-growing areas of Palestine (Hebron, Bethlehem, Gaza, Ramallah, Jerusalem, Jenin, Jericho and

Nablus). Samples for laboratory testing (mature canes) were randomly collected in winter from about 5% of the vines in each of 61 commercial vineyards surveyed, and from one young rootstock mother block in Hebron (Beir-Alqos), established with material coming from Israel. The choice of cultivars and rootstocks, and the number of samples to be collected from each of these, was based on their relative economic importance. All the samples were stored at 4°C until used for laboratory analysis.

### *Biological assays*

Leaves from glasshouse-forced cuttings of about 200 selected vines were ground in a mortar in the presence of 0.1 M phosphate buffer pH 7.2 and 2.5% nicotine to inoculate a range of herbaceous hosts. The extracts were rubbed on celite-dusted leaves of *Chenopodium quinoa*, *C. amaranticolor*, *Nicotiana occidentalis*, *N. clevelandii*, *N. benthamiana*, *Petunia hybrida*, *Cucumis sativus* cv. Marketeer and *Vigna unguiculata*. Shoots from about 120 samples, mostly native table-grape cultivars, were green-grafted in spring 1997 onto a range of woody indicator plants including *Vitis riparia*, 110R and LN33, and maintained in a glasshouse (Martelli, 1993).

### *Serological tests*

The presence of grapevine fanleaf nepovirus (GFLV), grapevine fleck virus (GFkV), grapevine A and B vitiviruses (GVA, GVB), and grapevine leafroll-associated 1, 2, 3 and 7 closteroviruses (GLRaV-1, -2, -3, -7) was checked by DAS-ELISA (GFLV, GLRaV-3), protein A DAS-ELISA (GVA), DASI-ELISA (GFkV, GVB), and biotin-streptavidin DASI-ELISA (GLRaV-1, -2, -7) (Boscia et al., 1997a). Extracts of cold-stored mature canes, obtained by macerating cortical scrapings in a mortar in the presence of Tris-HCl (GFLV, GFkV) or PBS-buffer (vitiviruses and closteroviruses), were used for the tests. Polyclonal antisera and monoclonal antibodies raised at the University of Bari (Boscia et al., 1992, 1994, 1995b; Bonavia et al., 1996) were used as reagents.

## **Results**

### *Field surveys*

Due to the late season, the only symptoms observed and identified with reasonable confidence in the field were those typical of leafroll disease (i.e. rolling and reddening of the leaves) in most of the red-fruited cultivars. Fanleaf symptoms were not obvious and rugose wood was only seen in the infrequent cases in which there was a marked corky reaction of the bark at the base of the vines, or when pits and grooves showed on the outer surface of the cortex. Phytoplasma-induced symptoms were observed in Jenin, Jericho and Bethlehem on cvs Biadi, Superior Seedless and Beitoni.

Heavy infestations of unidentified pseudococcid mealybugs, potential clostero- and vitivirus vectors, were observed in many vineyards of the Bethlehem area, where cvs Jandali, Marrawi, Zaini, Halawani and Dabouqi are widely grown.

### *Biological assays*

The preliminary results of testing showed that both vein necrosis and vein mosaic diseases occur in Palestine for the leaves of 110R and *V. riparia* graft-inoculated with some of the tested accessions reacted, 4–6 weeks after grafting, with the typical necrosis of the veinlets (vein necrosis) and chlorotic discoloration and vein banding (vein mosaic).

GFLV was transmitted from a restricted number of vines as confirmed also by ELISA. This virus induced chlorotic and necrotic ringspots, clearing of the veins and deformation of the leaves of

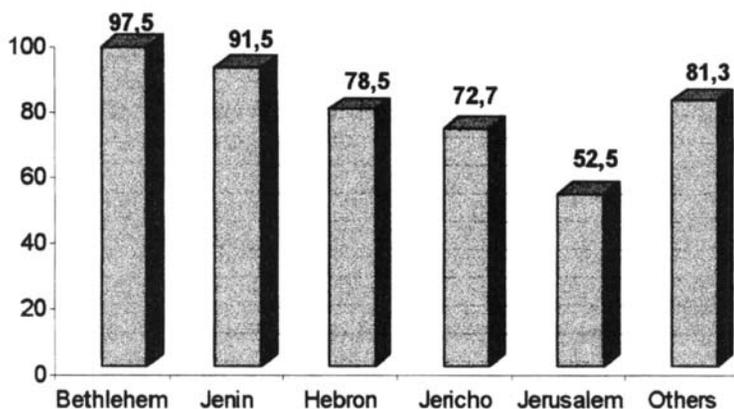


Fig. 1. Incidence of infected grapevines in different regions of Palestine.

*N. occidentalis*, vein clearing of the leaf blade, followed by systemic mottling and deformation in *Chenopodium quinoa* and *C. amaranticolor*, chlorotic leaf mottling and rolling in *N. benthamiana*.

### Serological tests

A total of 635 vines (566 *Vitis vinifera* and 69 rootstocks) were tested by ELISA. About 82% of the European grape samples (463 out of 566) from commercial vineyards contained one or more viruses (Table 1). As to individual viruses, there was a clear-cut prevalence of vitiviruses (GVA and GVB) and closteroviruses (GLRaV-1, -2, -3). GLRaV-7 was found in a single vine of cv. Sultanina. GVA, the most widespread virus in the country, was detected in about two thirds of the samples (66.1%), practically in all Palestinian grape cultivars, especially in the native ones. Besides cv. Shoyoukhi, that was totally infected, high infection rates by GVA were detected in cvs Shami (87.5%), Beiruti (83.3%), Jandali (83.3%), Halawani (76.2%), Marrawi (73.7%) and Zaini (71.1%). GLRaV-1 was the second virus after GVA in term of occurrence, with an average infection level of 45.6%. As to GVA, its incidence was particularly high in local cultivars such as Shami (70%), Jandali (75%), Marrawi (79%) and Shoyoukhi (100%), whereas it was absent in foreign cultivars such as Superior and Perlette. The average infection level of GLRaV-3 was about 22% with peaks of 71.4% in cv. Superior Seedless and 50% in cv. Jandali. The average infection rate was lower for GFkV (about 16%) with peaks of 47.4% in cv. Marrawi and 45.8% in cv. Jandali. These two viruses were detected in all the Palestinian cultivars, with the exception of cv. Biadi for GFkV. Less widespread were the other viruses considered in this study, in particular GFLV (1.4%), GVB (3.7%) and GLRaV-2 (8.3%).

With reference to the regions, the vineyards of Bethlehem (97.5% of infection) and Jenin (91.5% of infection) are particularly badly damaged, while those of Jerusalem appeared less affected since only 52.5% of plants had viruses (Fig. 1). In these areas some local and ancient cultivars were totally (Jandali, Marrawi, and Shoyoukhi) or heavily infected (Zaini, Biadi, and Shami). The situation appeared to be only slightly better for cv. Beitoni (55.8%) and Dabouqi (63.6%), that had infection rates similar to some recently introduced cultivars like Sultanina, Perlette and Superior (Fig. 2).

Of 69 mother plants of American rootstocks tested, 14 (20.3%) were infected by at least one virus (Table 1). Infections prevailed in *V. rupestris* (100%) and in the hybrid 161/49 (100%). In these plants GLRaV-3 was the most represented (10.1%), followed by GFkV (5.8%) and GVA (5.8%) (Table 1). Although the number of rootstock samples considered was low, it is interesting to note that some viruses, such as GLRaV-1, GVB and GLRaV-7 were totally absent, whereas GFLV and GLRaV-2 were detected only in a single sample.

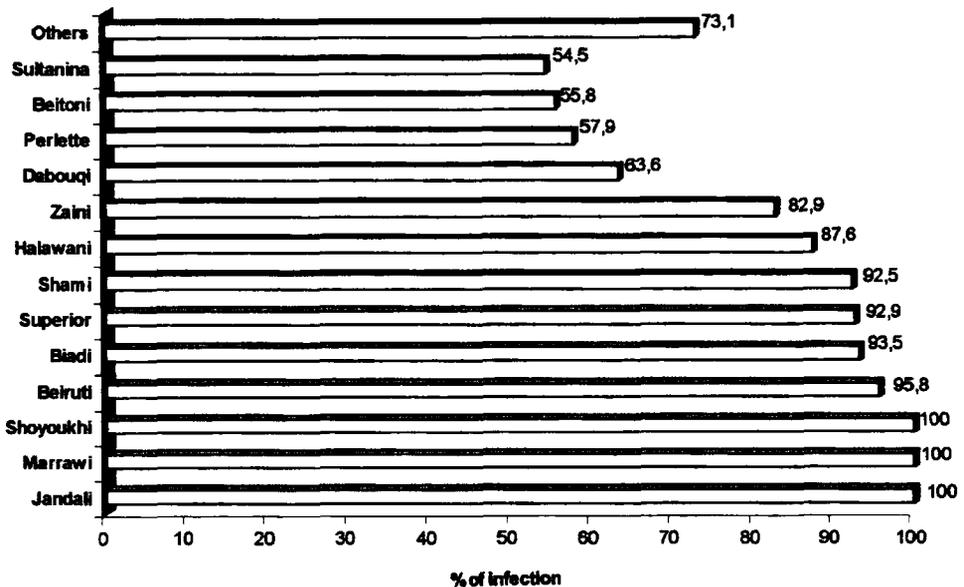
**Table 1.** Incidence of eight different viruses in Palestinian grapevine cultivars and rootstocks

	Total samples	Infected	GVA	GVB	GLRaV-1	GLRaV-2	GLRaV-3	GLRaV-7	GFKV	GFLV
European grapes										
No.	566	463	374	21	258	47	123	1	89	7
%	100	81.8	66.1	3.7	45.6	8.3	21.7	0.2	15.7	1.2
Rootstocks										
No.	69	14	4	0	0	1	7	0	4	1
%	100	20.3	5.8	0	0	1.4	10.1	0	5.8	1.4

## Discussion

From the above data it can be inferred that rugose wood (Kober stem grooving, in particular) and leafroll are by far the most widespread diseases in Palestine. This is not surprising considering that GVA and GLRaV-3, two of the most frequently detected viruses, are agents of these diseases and are both transmitted by mealybug vectors (Boscia *et al.*, 1997b) that are very common in the area.

The very high incidence of GVA infections (66.1%), in particular in local cultivars, seems indicative of the long-lasting existence of this virus in the region. Considering the geographical position of Palestine in the Mediterranean area, the high incidence of GLRaV-1 (45.6%) in comparison with GLRaV-3 (21.7%) seems surprising. This unusual ratio between the two viruses contradicts the notion that GLRaV-1 prevails in the northern and GLRaV-3 in the southern areas of Europe and the Mediterranean (Fortusini *et al.*, 1993; Digiario *et al.*, 1994; Haidar *et al.*, 1996;

**Fig. 2.** Incidence in Palestine of virus infections in ELISA tested grapevines of different cultivars.

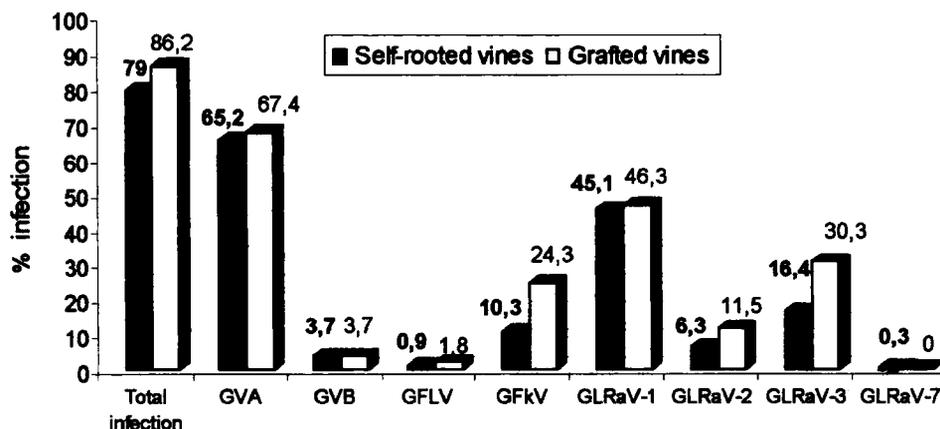


Fig. 3. Influence of grapevine rootstocks on the incidence of viral infection in Palestine.

Mahfoudhi *et al.*, 1998). The incidence of a third grapevine closterovirus, GLRaV-2 (8.3%), is not to be overlooked considering its potential natural spread by mealybugs (Walter & Martelli, 1996) largely present in Palestine. The absence of GLRaV-7, except for one single vine of cv. Sultanina, confirms the restricted area of distribution of the virus (Choueiri *et al.*, 1996). This finding, however, is important and indicative of the potential role that the exchange of propagative material can play in the spread of dangerous pathogens in new areas. Encouraging, if compared with other Mediterranean countries, is the low infection level of GFkV (15.7%) and GFLV (1.2%) (Martelli *et al.*, 1992; Digiario *et al.*, 1994; Mercuri *et al.*, 1994; Haidar *et al.*, 1996; Mahfoudhi *et al.*, 1998). This latter finding in particular, together with the unsuccessful recovery of nepoviruses other than GFLV by mechanical transmission, indicate that nepovirus-induced diseases, including fanleaf, are limited in Palestinian vineyards. The rare findings of GFLV-infected vines in the fields suggest that *Xiphinema index*, its nematode vector, may be equally rare in Palestine, thus confirming the findings from the nearby Lebanon (Haidar *et al.*, 1996).

An initial step in improving the sanitary status of Palestinian vineyards is provided by a rootstock mother-plant plot, constituted with material coming from Israel. Although the infection level ascertained in these plants was moderate, it represents a potential focus for new infections and spread of viral diseases, that will add to the already present viruses. That the rootstocks play an important role in the spread of viral diseases is confirmed by the results obtained in this study. A comparison between grafted and self-rooted vines has clearly shown that grafted plants are constantly more infected than the self-rooted ones (86.2% vs. 79%, respectively) and that these differences are significantly higher for those viruses generally latent in all or in most rootstocks, such as GFkV and GLRaVs (Fig. 3). The same evidence was indirectly obtained by analysing the ELISA results from the different grapevine-growing areas. The lower infection level found in Jerusalem area (52.5%) compared with the other areas of the country finds a possible explanation in the habit of Jerusalem growers to import rootstocks from Israel, where more advanced knowledge and facilities are available for virus diagnosis.

The results of the present survey show that Palestinian viticulture is not in a better sanitary condition than the rest of the Mediterranean countries, therefore the implementation of measures for its improvement should seriously be taken in consideration.

### Acknowledgements

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### **Virus et analogues sur vigne en Palestine**

Des prospections ont été réalisées dans des vignobles des principales régions viticoles en Palestine (Hebron, Bethléem, Gaza, Jérusalem, Ramallah, Jenin, Jéricho et Nablus) afin d'évaluer la présence et l'incidence des maladies à virus et analogues. Des symptômes d'enroulement foliaire ont été observés à Bethléem, Ramallah et Jérusalem sur des cultivars locaux et importés, avec une plus grande incidence chez les cépages à raisin noir (Shami, Beitoni et Smari). Des symptômes de bois strié ont aussi été mis en évidence sur des cultivars locaux et étrangers, en particulier sur des plants greffés, avec une plus grande incidence à Bethléem. Des symptômes de court-noué ont rarement été rapportés, alors que des manifestations imputables à des phytoplasmes ont été observées à Jenin, Jéricho et Bethléem sur les cvs Biadi, Superior Seedless et Beitoni. Les tests ELISA ont révélé que 463 des 566 (82%) vignes examinées étaient infectées par au moins un virus. Le GVA était le virus prédominant (66,1%), suivi du GLRaV-1 (45,6%), du GLRaV-3 (21,7%), du GFkV (15,7%) et du GLRaV-2 (8,3%). Le GVB et le GFLV ont aussi été décelés mais dans une moindre mesure, leur incidence étant comprise entre 3,7 et 1,2%. Parallèlement, le GLRaV-7 a été détecté dans une seule vigne du cv. Sultanina d'origine étrangère. Les vignobles de la région de Bethléem (97,5%) se sont avérés particulièrement endommagés et certains cultivars locaux étaient entièrement (Jandali, Marrawi et Shoyoukhi) ou gravement infectés (Zaini, Biadi et Shami). Le test ELISA exécuté sur 69 jeunes pieds-mères de porte-greffe a montré une incidence relativement élevée d'infection virale (20,3%). La présence de la nécrose des nervures et de la mosaïque des nervures a été mise en évidence sur des plantes indicatrices de 110R et de *Vitis riparia* inoculées par greffe, alors qu'aucun virus, sauf le GFLV, n'a été transmis mécaniquement de 200 vignes environ à des hôtes herbacés.

### **Вирусы и вирусные заболевания виноградинок в Палестине**

Обследования проводились на виноградниках основных регионов производства винограда в Палестине (Хеброн, Вифлеем, Газа, Иерусалим, Рамалла, Иенин, Иерихон и Наблус) с тем, чтобы оценить наличие и распространенность вирусоподобных заболеваний. Симптомы скручивания листьев были обнаружены в Вифлееме, Рамалла и Иерусалиме на местных и импортных селекционных сортах, причем наибольшая заболеваемость была обнаружена на сортах черного винограда (Шама, Бейтони и Смари). Симптомы полосования виноградной лозы были также выявлены на местных и завозных селекционных сортах, в частности, на привитых лозах, причем наибольшая заболеваемость была обнаружена в Вифлееме. Симптомы мозаики были обнаружены редко, в то время как проявления, объясняемые появлением фитоплазмы были обнаружены в Иенине, Иерихоне и Вифлееме на селекционных сортах Биади, Сюперииор Сидлесс и Бейтони. Испытания ELISA показали, что 463 из 566 (82%) обследованных виноградников были заражены по крайней мере одним из вирусов. GVA являлся преобладающим вирусом (66,5%), за которым следовал GLRaV-1 (45,6%), GLRaV-3 (21,7%), GFkV (15,7%) и GLRaV-2 (8,3%). GVB и GFLV были также обнаружены, однако в меньшем количестве, так как заболеваемость ими находилась в пределах от 3,7 до 1,2%. Одновременно GLRaV-7 был обнаружен на единственном винограднике сорта Султанина, имевшего зарубежное происхождение. Виноградинок Вифлеема (97,5%) оказались главным образом подвержены вирусам и некоторые местные сорта оказались подвержены полностью (Яндали, Маррави и Шоюки) или серьезно заражены (Зайни, Биади и Шама). Испытания ELISA, проведенные на 69 молодых подвоях виноградной лозы, показали сравнительно высокую зараженность вирусной инфекцией (20,3%). Некроз прожилок листа и мозаика жилок были также подвергнуты оценке на индикаторных растениях 110R и *Vitis riparia*, инокулированных прививкой, в то время как ни один из вирусов (за исключением GFLV) не был передан механически с почти 200 виноградных лоз на инокулированные травяные хозьява.

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