Detection and Distribution of *Grapevine fanleaf virus* in Some Grapevine Growing Regions in Syria

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Abstract

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Grapevine fanleaf virus is one of the most destructive grapevine viruses in the world. It is transmitted by grafting and the nematode vector *Xiphinema index*. It causes fanleaf, leaf mosaic, shortened internodes and leaf malformation symptoms and affects negatively the yield and fruit quality. The virus has not been studied enough in Syria. Accordingly, this study aimed to assess the distribution of GFLV on different varieties in some grapevine growing regions in Syria. Survey was conducted during 2016/2017- 2017/2018 growing seasons in Lattakia, Tartous, Homs, Hama and Al-Sweida provinces. 360 samples showing different types of symptoms suggestive of virus infection such as mosaic, vein yellowing, chloroses, vein banding, mottling, leaf distortion, and shortened internodes were collected. Out of 360 grape samples with symptoms suggestive of virus infection, 42 samples were found to be infected with GFLV (11.66% of the tested samples), using the enzyme-linked immunosorbent assay (DAS-ELISA). The highest relative infection rate was recorded in Hama and Homs (22.72% and 17.10%, respectively). The highest relative infection rate (22.22% of the tested symptomatic samples) was recorded on the small rose fruit and black Zinni varieties. These results provided new information about the distribution of GFLV in Syria and suggest the possible occurrence of mixed infections with other different viruses affecting grapevine in Syria.

Keywords: GFLV, *Vitis*, Syria, DAS-ELISA.

Introduction

Grapevine is the most widely cultivated and economically important fruit crop in the world (Andret-Link *et al*., 2004a), and it has several nutritional, medical and therapeutic uses, in addition to wine production (Jamal Al-Din, 2010). In 2017, grape production in Syria reached around 40,000 tons (Agricultural Statistical Group, 2017). Grapevine is infected with many viruses and virus-like diseases (Martelli & Boudon-Padieu, 2006), many of which have been recorded in the grapevine producing areas of the Mediterranean basin (Choueiri *et al*., 1997; Katis *et al*., 1990; Padilla, 1992; Pourrahim *et al.*, 2007). The most important reason for the spread of these diseases in grapevine is lack of appropriate sanitary measures (Belin *et al.*, 1999). Several viruses have been recorded earlier on grapevine in Syria (Aldaoud *et al.*, 1991; Al-Shaabi *et al*., 2000; Mslmanieh, 2005; Ghorz El-Din *et al.*, 2008). *Grapevine fanleaf virus* (GFLV) belong to the genus *Nepovirus*., and the family of *Comoviridae* (Mayo & Robinson, 1996). GFLV is among the most severe viral disease of grapevine worldwide (Bovey & Martelli,1992; Martelli, 1993; Raski *et al*., 1983; Zimmermann *et al*., 1988), This virus is present in the majority of vineyards where *Vitis vinifera* grapes and French-American rootstocks are grown. In Syria, the first record of the virus was in 2000, at a rate 4.8% (Al-Shaabi et al., 2000) but without any information about its distribution. The virus causes significant losses in production up to 80-90%, depending on the variety and the virus strain (Legin *et al*., 1993; Martelli & Savino, 1990). It also reduces the fruits yield and quality. The virus could also affect the longevity of grapes (Andret-Link *et al*., 2004a), and reduces plant vigor and the productive lifespan of vineyards. GFLV is naturally transmitted by soil borne nematode vector *Xiphinema index* (Longidoridae: Dorylaimida) (Andret-Link *et al*., 2004b; Cohen *et al*., 1970; Hewitt *et al*., 1958; Zyl *et al*., 2012). The virus is also transmitted by grafting and can survive in the remains of grapevine roots several years after its removal. The symptoms caused by the virus differ according to the virus strain (Legin *et al*., 1993; Martelli, 1993). The most important symptoms are: decline in tree growth, stunting, fanleaf, mosaic, shortened internodes as well as taking an abnormal shape (zikzak), leaf malformation, vein banding, ring and line patterns and flecks, leaf edge yellowing, interveinal chlorosis, leaf shape asymmetry (Martelli, 1993). Many countries have resorted to the production of virus free propagation material to reduce prevent the spread of viral diseases in grapes (Savino, 1992; Walter, 1992).

This study aimed to evaluate occurrence and distribution of GFLV in some Syrian provinces, using DAS-ELISA test.

Materials and Methods

Field survey and sample collection

Field surveys were conducted in several grapevine growing areas of five Syrian provinces (Lattakia. Tartous, Homs, Hama, and Al-Sweida), during 2016/2017- 2017/2018 growing seasons. 360 samples (each sample represented a single vine) showing various degrees of symptoms

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suggestive of virus infection (mosaic, vein yellowing, chlorosis, leaf edge yellowing, vein banding, mottling, leaf distortion, zikzak twig growth, shortened internodes, etc.) were collected.

Serological Tests

Serological tests were carried out in the virology laboratory of the Agricultural Scientific Research Center in Lattakia. Enzyme-linked immunosorbent assay (DAS-ELISA) was used (with polyclonal antibodies produced by the company "AGRITEST"), following the protocol recommended by the company. ELISA plates were read by measuring the light absorbance of each sample at 405 nm wavelength, by the ELISA reader according to the manufacturer's instructions.

Results and Discussion

Symptoms observed

During field survey, different types of symptoms similar to those caused by viral infection were observed, including those caused by GFLV, such as growth decline, stunting, yellowing on one side of the tree, mosaic, mottling, deformation and wrinkling of leaves, leaf shape asymmetry, fanleaf, interveinal chlorosis, shortened internodes with a zikzak shape, vein banding, ring and line patterns. Some of these symptoms were consistent with the results of the DAS-ELISA serological test (Table 1), which confirmed infection with GFLV. Many of these symptoms were identical what was indicated by previous studies on the symptoms caused by GFLV (Martelli, 1993). However, some observed symptoms, such as leaf reddening could be caused by other viruses. The symptoms varied according to the grapevine variety and the surveyed area as shown in Tables 1 and 2.The results of serological tests (DAS-ELISA) indicated an increase in the relative occurrence of GFLV in Syria compared to what it had been recorded previously (Al-Shaabi *et al.,* 2000; Ghorz El-Din *et al.,* 2008). Out of 360 grape samples, 42 samples (11.66%) were found to be infected with GFLV among the tested samples (Table 1). These results were consistent with several previous studies which confirmed the presence of the virus in most areas of the grape cultivation (Al-Shaabi et al., 2000; Andret-Link *et al*., 2004a; Bovey & Martelli, 1992; Martelli, 1993; Raski *et al*., 1983; Zimmermann *et al*., 1988).

The results obtained indicated that the highest relative occurrence of GFLV was recorded on the small rose fruit and black Zinny varieties (22.22%). Although the observed symptoms varied on the same variety at different locations, such differences can be due to the different environmental conditions at the different locations as well as the possibility of infection with different strains of the virus or the presence of other viruses that were not tested for in this study (Legin *et al*., 1993; Martelli & Savino, 1990).

Rate of virus infection according to the sampling sites:

The number of tested samples and the rate of GFLV infection of grape at different sampling sites in each province was calculate (Table 1).

Table 1. Relative GFLV rate of infection of vineyards at different locations in five provinces in Syria during 2016/2017 and 2017/2018 growing seasons.

Province Location	No. of tested samples	No. of infected samples	Relative infection rate (%)
Lattakia			
Al-Hanade	17	1	5.88
Al-Katria	27	3	11.11
Al-Ruemea	6	1	16.66
Slunfe	12	2	16.66
Sheikh Hasamo	25	3	12.00
Al-Bahlolia	6	-	-
Berne	4	-	-
Zgaren	5	-	-
Al-Srskiah	7	-	-
Kassab	15	2	13.33
Lattakia Res. Center	19	-	-
Jableh	8	2	25.00
Sub-Total	**151**	**14**	**9.27**
Homs			
Al-Aawar	8	1	12.50
Al-Thabtea	3	-	-
Zidel	10	2	20.00
Skaraa	2	-	-
Shin	22	4	18.18
Barshin	14	2	14.28
Feroza	8	1	12.50
Special nursery	9	3	33.33
Sub-Total	**76**	**13**	**17.10**
Hama			
Al-Skelbeah	3	1	33.33
Al-Slukiaa	5	1	20.00
Al-Latmaa	9	2	22.22
Deir Shamil	8	1	12.50
Aein Al-Gern	6	1	16.66
Al-Glemaa (nursery)	10	3	30.00
Al-Latmeh (nursery)	3	1	33.33
Sub-Total	**44**	**10**	**22.72**
Tartous			
Mehwarteaa	14	-	-
Hresson	26	3	11.58
Al-Gamasaa	24	2	8.33
Sub-Total	**64**	**5**	**7.80**
Al-Sweida			
Sub-Total	**25**	-	-
Total	**360**	**42**	**11.66**

The results obtained summarized in Table 1 indicated that the relative occurrence of GFLV varied among regions. GFLV was detected in all grape growing sites surveyed except in Al-Sweida province. The average total infection rate in Syria was 11.66%, with the highest in Hama province (22.72%), followed by Homs province (17.10%), then Lattakia (9.27%) and Tartous (7.8%). This could be explained by the random dependence of farmers on the cultivation of desired varieties from unreliable sources. This is more or less similar to the spread of the virus in most areas of grape cultivation worldwide (Andret-Link *et al*., 2004a;

Bovey & Martelli, 1992; Martelli, 1993; Raski *et al*., 1983; Oliver *et al*., 2010; Zimmerman *et al*., 1988). The low infection rates in some provinces, such in Tartous, and their absence in Al- Sweida province does not necessarily mean that vineyards in these provinces are free from the virus, as the number of tested samples was small (25 samples only in Al-Sweida). On the other hand, the wide distribution of the virus on grapevine may be attributed to the expansion of the American hybrid cultivation with good and desirable production characteristics without apparent symptoms, but it is sensitive to infection with many viruses, including GFLV (Cohen *et al.*, 1970), or as a result of the transport of the soil-borne nematodes from *Xiphinema* genus between nurseries and permanent field (Hewitt *et al*., 1958; Zyl *et al*., 2012), as well as the possibility of soil infestation with *Xiphinema* in Syria, as suggested by the results of nematode testing of soil samples collected at the same time of collecting plant samples from the same sites (N. Ali, unpublished data). It is worth noting that several infections with GFLV was found in samples from grapevine nurseries in Hama and Homs (Table 1), which for sure lead to further spread in vineyards using infected grape seedlings.

Relative GFLV infection rate of different grapevine varieties

Results obtained indicated that the relative infection rate with GFLV of different grape varieties was variable (Table 2). The infection rate ranged from 10.71% to 22.22%, the highest (22.22%) was on the small rose fruit and black Zinni varieties, whereas the lowest rate (10.71%) was on the yellow Zinni variety. This indicate that there are most likely other viruses which infect grapevine in Syria.

It is essential to check for the presence of GFLV and other potential viruses in the seedlings stage at the grape nurseries, and make sure that farmers use only virus-free certified plant propagation materials when establishing new vineyards. DAS-ELISA test is a suitable tool and widely available for early detection of the virus. It is also recommended to widen such survey to cover all grape producing regions in Syria. Furthermore, it is essential to conduct surveys based on random sampling of vineyards to evaluate precisely the incidence of GFLV and other viruses that infect grapes in Syria.

Table 2. GFLV relative infection rate (%) of different grapevine varieties surveyed.

Variety	No. of tested samples	No. of infected samples	Relative infection rate (%)
American	20	3	15.00
Small rose fruit (seedless)	9	2	22.22
Small yellow fruit	45	9	20.00
Zinni (yellow) (Bentamoni- Iraq)	17	2	17.64
Sweet (rose)	40	5	12.50
Sweet (Black)	14	3	21.42
Zinni (Black)	18	4	22.22
Zinni Black (Iraq)	5	1	20.00
Zinni (yellow)	28	3	10.71
French	15	3	20.00
Unknown	149	6	4.026

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الملخص

عاقل، إنصاف حسن، نادين علي، نادر أسعد وعماد إسماعيل. 2020. تقصي انتشار فيروس الورقة المروحية للكرمة في بعض مناطق زراعة الكرمة في سورية. مجلة وقاية النبات العربية، 38(4): 339-343.

يُعد فيروس الورقة المروحية (GFLV، جنس: *Nepovirus*، عائلة: *Comoviridae*) من أهم الفيروسات الاقتصادية على نبات الكرمة في مختلف أنحاء العالم. ينتشر الفيروس بالتطعيم وبوساطة النيماتودا الخنجرية (*Xiphinema index*)، ويسبب أعراض الورقة المروحية، موزاييك الأوراق، قصر السلاميات، تشوه الأوراق، كما يؤثر في نوعية الثمار وكمية المحصول. لا توجد دراسات كافية عن الفيروس في سورية، ولذلك هدفت هذه الدراسة إلى تتبّع انتشاره في بعض مناطق زراعة الكرمة في عدد من المحافظات السورية (اللاذقية، طرطوس، حماه، حمص، السويداء) خلال موسمي 2017/2016 و2018/2017. تم جمع 360 عينة فردية من الأوراق والأفرع أبدت أعراضاً شبيهة بأعراض الإصابات الفيروسية كالموزاييك، شفافية العروق، تحزم العروق، البرقشة، الاصفرار، وتشوه الأوراق، تعرج الافرع، وقصر السلاميات. تمّ تشخيص الإصابة بهذا الفيروس باستخدام اختبار الادمصاص المناعي المرتبط بالأنزيم (DAS-ELISA). بيّنت النتائج انتشار فيروس الورقة المروحية على الكرمة في سورية، بنسبة وصلت إلى 11.66% في العينات التي فحصت وظهر عليها أعراض توحي بالإصابة الفيروسية. بلغ عدد العينات المصابة 42 عينة من أصل 360 عينة تم فحصها. أوضحت النتائج تباين في الانتشار النسبي لهذا الفيروس وفقاً لمناطق جمع العينات واختلاف الأصناف، حيث سُجلت أعلى نسبة إصابة في محافظات حماه وحمص بنسب وصلت حتى 22.72%، 17.10%، على التوالي، وكانت أعلى نسبة في صنف الحبة الصغيرة وردية اللون (بدون بذور)، والزيني الأسود بنسبة 22.22%. أظهرت النتائج وجود تباينٍ في أعراض الاصابة الظاهرية على الصنف نفسه المزروع في المنطقة نفسها مما يشير إلى احتمال وجود أكثر من سلالة للفيروس المدروس، أو وجود إصاباتٍ مختلطة مع أنواع فيروسية أخرى.

كلمات مفتاحية: فيروس الورقة المروحية، العنب، سورية، اختبارات مصلية.

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