Detection of Grapevine Fanleaf Virus Using Serological and Biological Assays

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Abstract: The production of grapevine is important agricultural sector in Turkey. But, the production is lower than the other Mediterranean countries. One of the main reasons of low yield is many viruses' diseases. The nepoviruse diseases are responsible for significant losses in vineyards. Grapevine fanleaf virus (GFLV) is the most important deadly virus among them. It is transmitted by graft and vector nematodes. It has not been studied enough about this virus in our country. There is not enough detailed research on this virus in our country. In this study, the maintenance of GFLV in vineyards and their occurrence areas were determined in Adana and Mersin provinces by serological and biological assays. Out of total 384 grapevine samples, 63 plants (16.4%) were found to be infected with GFLV by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). In addition to this, as herbaceous indicator plants; *Nicotiana benthamiana* Domin., *N. tabacum* L., *N. rustia* L., *Chenopodium amaranthicolor* Coste et Reyn., *C. quinoa* Wild., *Cucumis sativus* L., and *Gomprena globosa* L. were used in biological assays. But chlorotic local lesions were observed only on *Nicotiana benthamiana* Domin. among weedy indicator plants by mechanical transmission.

Keywords: GFLV, grapevine, inoculation, *Nicotiana.*

INTRODUCTION

The Eurasian grapevine, *Vitis vinifera* L., is the most widely cultivated and economically important fruit crop in the world [1]. Grape consumed fresh, raisins or as raw in many industries (molasses, wine, vinegar, etc.) is an important food containing sugar, nitrogen minerals and vitamins. Approximately 3.58 million tons of grape production was realized on about 470 thousand hectares of vineyard area in Turkey [2]. Compared with other countries on the Mediterranean coast, the main reason of the low yield is the plants affected by many viruses and virus-like diseases. The most important reason for the occurrence of these diseases in the vineyards is not taking hygienic precautions [3-5]. As a result of some research, the virus diseases in vineyards in some provinces in the Mediterranean, Central Anatolia and Southeast seems to be very common in many grape-growing regions. Among them, Grapevine fanleaf virus (GFLV) is one of the most economically important viral diseases affecting grapevine in many grape-growing regions. This virus is in the first place with a rate of 46.9% infestation in Elazığ province in Turkey [6]. It causes serious economic losses by substantially reducing yield by up to 80% and affecting fruit quality [7]. But the damages induced by the viruses are very variable according to factors as cultivars and clones, locations, age of the plants, rootstocks, crop management, virus or combination of viruses infecting the plants, and environmental conditions [8]. One of the most economically important viruses is GFLV transmitted to plants by vector nematode *Xiphinema index* Thorne et Allen (Nematoda: Longidoridae) [9, 7, 10]. The spread of this deadly viral disease is carried out by infested material or natural soil-dwelling ectoparasitic vector nematode. The studies associated with *Xiphinema* species were carried out in Turkey were given by Kepenekci [11] and Kepenekci *et al*. [12]. In a more recent study, it was shown that GFLV persists in juveniles of *X. index* for over four years [13]. Over the past two years, it was important scientific contributions especially about the biology of nepoviruses and GFLV. [14-15]. The development of molecular tools and the knowledge about the biology of nepoviruses have given new contribution to the study of interactions between nepoviruses and their natural vector nematode.

The objective of this study was to determine the GFLV in vineyards using the serological and biological methods and to identify the regions where the virus has spread in Adana and Mersin provinces in Turkey.

MATERIALS AND METHODS

The study was carried out especially in the elderly and high system vineyards in some regions of the provinces of Adana and Mersin (Figure **1**) from 2002 to 2003. A total of 384 fresh leaves and the bark samples

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Figure 1: Locations of the provinces and districts study was conducted on Turkey's map.

were collected from vineyards showing the virus symptoms during the spring and autumn. All the samples were tested for the presence of GFLV using the double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) tests as described by Clark and Adams [16]. ELISA kits used in the tests were purchased from commercial company (AGDIA) and prepared as recommended according to instructions. A pair of polystyrene ELISA plate wells (two replicates) was used for testing of each sample. The results of absorbance value of each well were read at a wavelength of 405 nanometer (nm) with an ELISA microplate reader. The samples were compared with known negative control wells. In tests, the yellow color formation was observed in wells of infected samples.

In this study, mechanical inoculations were made using 57 plant samples known to be infected with GFLV. *Nicotiana benthamiana* Domin., *N. tabacum* L., *N. rustia* L., *Chenopodium amaranthicolor* Coste et Reyn., *C. quinoa* Wild., *Cucumis sativus* L., and *Gomprena globosa* L. were used as herbaceous indicator plants in trials. The herbaceous indicator plants were inoculated as four replications and one control plant for each sample. Crude sap of leaves that obtained from infected grapevine plants were used to inoculating the healthy host indicator plants. Therefore, fresh vine leaves were crushed in 0.1 M phosphate buffer pH 7.2 containing 2.5% (m/v) nicotine to inoculate mechanically the indicator herbaceous plants. The obtained extract was contaminated to the leaves of plants dusted with carborandum powder. After waiting a few minutes, the plants were washed with tap water without damaging according to the method described by Dijkstra and Jager [17] and Hanna [18]. The herbaceous indicator plants were inoculated as four replications and one control plant for each sample. The inoculated plants were kept at 25 ± 1 °C, 65 ± 10% relative humidity, and under a light : dark cycle of 12 : 12 hours in climate room. Under this condition, they were grown from the seed in pots were used during 4-5 leaves in the experiments. The inoculation studies carried out in the same conditions. Symptoms were monitored every day. Then, leaves of these plants were checked by the DAS-ELISA whether they had been infected by GFLV. The experiments were carried out in the laboratory and climate room of the University of Çukurova, Turkey.

RESULTS AND DISCUSSIONS

In this study, virus infestation rates detected in the tested samples collected from vineyards in the area where the research was conducted is given in Table **1**. A total of 384 plant samples were tested. Of these, 63 samples infected with GFLV were detected by DAS-ELISA. The infection rate of the virus was calculated as 16.4% in the total samples. It can be seen in Table **1** that none of the plant samples taken from Karaisalı and Kozan was infected with GFLV. The virus was detected the most commonly with a rate of 28.6% in Pozantı. The data clearly showed that GFLV is one of the most widespread grapevine viruses in the research area.

As a result of some work done in our country, it is determined that this virus infection rates vary from region to region between 7.7% and 46.9% [19, 3, 4, 20, 6]. Less growth of 6.4-88.9% and the loss of product between 45.0 and 95.1% are reported in infected grapevines according to healthy vines [21-22]. Many

Table 1: Infection Rates of Grapevine Fanleaf Virus According to Regions in the Research Area in 2002 and 2003

Region	Total number of samples	The infected samples	Infection rate (%)
Adana-Balcalı	104	20	19.2
Pozantı	7	2	28.6
Ceyhan	10	2	20.0
Karaisalı	9	---	---
Saimbeyli	20	5	25.0
Kozan	10	---	---
Mersin	114	17	14.9
Tarsus	110	17	15.5
Total	384	63	16.4

studies have reported the use of DAS-ELISA for the detection of GFLV and other grapevine viruses [23-25].

During the surveys in the vineyards symptoms related with GFLV such as growth retardation, low yields, swollen or twin formation of nodes in shoots, the shortening of the intermediate nodes and zigzag development, large and small fruits, mosaic spots, yellowing of leaves, the vein banding, flattening were observed on plants. Hewitt [26-27] stated that the pathogen responsible for the symptoms such as stunting, shortening the shoots, leaf spots, deformities, flowers spills, shortening between the nodes in the early period, zigzag shoots and twin node formation was GFLV. The researcher also reported that the twin node formation and short internodes sometimes can be seen in normal plants but also shortening of especially 8-11 th nodes were the typical symptom of GFLV.

The chlorotic local lesions were observed on leaves of *N. benthamiana*, as shown in (Figure **2**). Similar symptoms were obtained by Martelli [28] and El-Awady *et al*. [25] on the same indicator plant. These symptoms were disappeared spontaneously in about 8-10 days. Positive reactions for virus infection has been determined serologically in 13 samples of *N. benthamiana* inoculated. Optical densities of these plants inoculated with the GFLV were detected using plate reader (Table **2**). The samples gave positive reactions with values ranged between 1.511 and 3.241 compared to values ranged between 0.057 and 0.089 of the negative and healthy samples.

Figure 2: View of the chlorotic local lesions on the leaf of *Nicotiana benthamiana* test plant.

El-Awady *et al*. [25] reported that while non-infected control plants did not show any symptoms, *N. benthamiana* displayed leaf malformation, *Phaseolus vulgaris* L. displayed mottling and ringspots, and *Cucurbita pepo* L. displayed systemic chlorotic mosaic. The studies of indexing on some *Vitis* indicators under greenhouse conditions using the harmful virus diseases in Japan vineyards were done by Tanaka [29] and thus GFLV was determined. He also reported that mechanical inoculation of GFLV on *C. quinoa* and *G. globosa* of the herbaceous indicator is very difficult.

Grapevine fanleaf virus is one of the most severe diseases which affect adversely grape yields and fruit quality all over the world. Those losses caused by GFLV are increasing in Turkey. One of the most serious problems is GFLV transmitted by *X. index*. But the economic significance of the damage caused by this nematode is not well-understood or recognized by growers [12]. The using of nematicides and fungicides fails to control vector for reasons such as the vine roots is a good food for *X. index* and a good inoculum source of the virus in the soil, grapevine roots can live a long time, pesticides are poorly penetrating and vector nematodes live in more depth in the soil [30]. Before planting vineyards, soil analysis should be made whether contaminated with vector nematodes.

Table 2: Optical Densities of Positive and Negative Reactions of *Nicotiana benthamiana* Plants Inoculated with the Grapevine Fanleaf Virus

Number	ELISA value (405 nm)	Mean of negative control (405 nm)	Mean of positive control (405nm)
1	0.388	0.057	3.241
2	0.483	0.057	3.241
3	0.344	0.089	1.511
4	0.454	0.089	1.511
5	0.283	0.089	1.511
6	0.622	0.089	1.511
7	0.244	0.089	1.511
8	0.281	0.089	1.511
9	0.312	0.089	1.511
10	0.355	0.089	1.511
12	0.477	0.089	1.511
13	0.511	0.089	1.511

In conclusion, this study suggests that the grapevine plant materials must be examined for the existence of the virus by serological techniques before using them for production. The DAS - ELISA will be a suitable tool for diagnosing virus in vineyards and for detection the virus-free plants. It is believed that the results from this study are important to change their practices for producing virus free plants in the very near future. The new vineyards should be established using the virus free production materials such as rods, plant cuttings eyes and rootstocks.

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