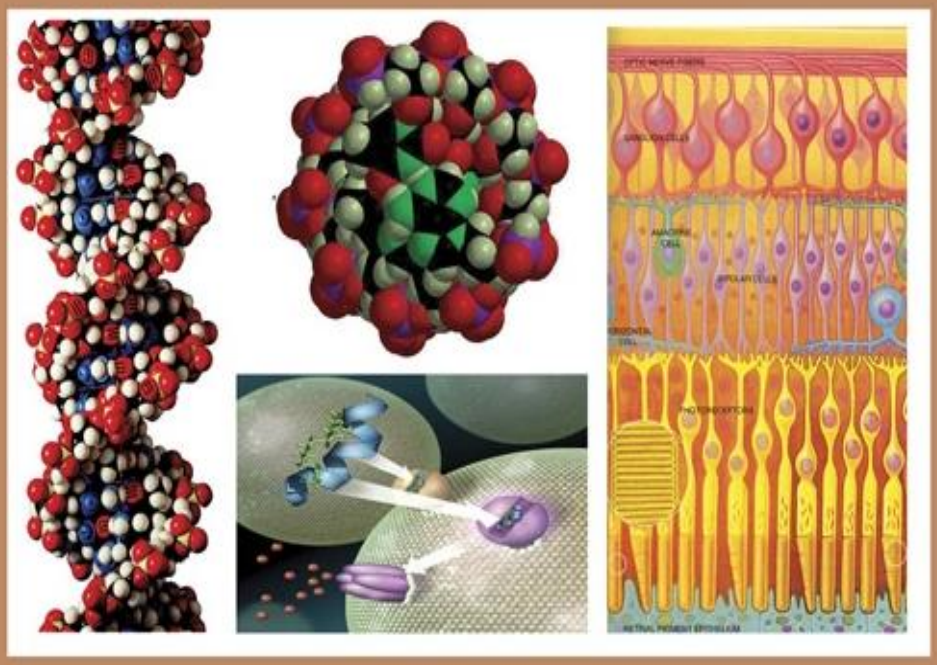
EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
C
PHYSIOLOGY & MOLECULARBIOLOGY



ISSN
2090-0767

WWW.EAJBS.EG.NET

**Vol. 15 No. 2 (2023)**

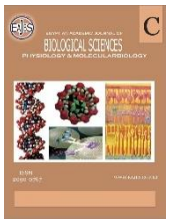
.

---

**Citation**: *Egypt.Acad.J.Biolog.Sci. ( C.Physiology and Molecular biology ) Vol. 15(2) pp101-117 (2023)*
**DOI: 10.21608/EAJBSC.2023.312619**

**Egypt. Acad. J. Biolog. Sci., 15(2):101-117 (2023)**

**Egyptian Academic Journal of Biological Sciences**
**C. Physiology & Molecular Biology**
**ISSN 2090-0767**
**www.eajbsc.journals.ekb.eg**



# Characterization and Elimination of *Grapevine fanleaf virus* Using Thermotherapy in Combination with Meristem Tip Culture

**Tarek A. El Sayed[1], Khaled E. A. Saker[1], Ahmed E. El Shorbagy[1] , Aiman M. Mandour[1], Mahmoud J. Alajouz[2] and Ahmed M. Soliman[1]**

[1]Virus & Phytoplasma Res. Dept., Plant Pathol. Res. Institute, Agricultural Research Center, Egypt.

[2]Plant Production and Protection Dep., Faculty of Agriculture, Alazhar University, Gaza, Palestine.

***E-mail:** drtarekelsayed71@gmail.com ; ahmedsoliman@arc.sci.eg

**ARTICLE INFO**

**Article History**
Received:4/7/2023
Accepted:14/8/2023
Available:18/8/2023

---

***Keywords***:
GFLV; RT-PCR; Thermotherapy; Meristem tip culture.

**ABSTRACT**

*Grapevine fanleaf virus* (GFLV) is considered one of the most prevalent and serious diseases infecting grapevine in the world. Visual observations were conducted in the field concerning the GFLV symptoms in the elder and young trees in Beheira Governorate. Plants with characteristic symptoms of GFLV were collected during the growing season and checked by DAS- ELISA. Leaf samples that reacted positively to GFLV antiserum were used first to inoculate the test plants and then for biological purification of the virus isolate using single local lesions developed on *Chenopodium quinoa*, whereas, *Gomphrena globosa* was used as a source for virus inoculum. Biological indexing was carried out using woody indicator cutting of virus-free rootstocks; freedom and LN33. RT-PCR was done to confirm the ELISA results. Anatomical changes in the infected cells of leaves compared with healthy ones were studied. The infection rate of the virus was 10.29%. There are quantifiable changes in physiological and biochemical markers such as proteins, pigment and carbohydrate content, phenolic compounds, polyphenol oxidase (PPO), peroxidase (POX), and catalase (CAT) activity in grapevine leaves. Infected grapevine leaves had higher levels of phenolic compounds, PPO, POX, and CAT than healthy leaves, although total protein content and pigment levels were in the other direction. Overall, the findings imply that GFLV infection causes considerable changes in enzyme levels that result in the development of symptoms that cannot be reversed. The thermotherapy at 36$^{o}$C for 60 days in combination with meristem tip cultures resulted in 100% virus-free cultures.

## INTRODUCTION

Due to the wine industry and the high demand for both fresh and dried fruit, the grapevine is one of the most economically significant cultivated fruit species in the world (Vivier and Pretorius, 2002). Grape is a significant food source of sugar, nitrogen, minerals, and vitamins that can be consumed fresh, dried, or uncooked in numerous sectors (molasses, wine, vinegar). (Auger *et al*., 1992). In Egypt, Grapevine is the second most important fruit crop after citrus. Nevertheless, the annual production is lower compared to the other Mediterranean countries. The total area of the grapes production reached about 472500.42 hectares yielding about 3.58 million tons (FAO, 2015).

Virus diseases are significantly contributing to the reduced yield of grapevines. The GFLV is one of the most important and widely distributed viruses infecting grapevines at different locations in Egypt (Shalaby *et al*., 2007). It affects productivity and quality and shortens the longevity of grapevines in the vineyard (El-Kady *et al*., 1991; Andret-Link *et al*., 2004). Not taking hygienic precautions is the main factor in the emergence of virus illnesses, particularly GFLV in vineyards. A "fanleaf"-shaped distortion to a yellow mosaic and vein banding are just a few examples of the symptoms of GFLV on leaves (El-Kady *et al*., 1991; Krake *et al*., 1999; Youssef *et al*., 2008). *Xiphinema index*, a nematode, is a vector for the virus (Andret-Link *et al*., 2004). By using contaminated materials for vegetative development or grafting, it is mostly spread across vast distances. As early as three to four weeks following the graft, symptoms start to show up (El-Kady *et al*., 1991; Martelli 1993; Al-Tamimi *et al*., 1998). GFLV is a member of the genius *Nepovirus*, family *Secoviridae*. Its bipartite genome is made up of two positive signal-stranded RNA molecules that are independently encapsidated. (Mayo and Robinson 1996; Jafarpour and Sanfacon 2009; King *et al*., 2011). Both genomic RNAs are polyadenylated at their 3` end and have a tiny virus-encoded protein that is covalently attached to their 5` end. Numerous nepoviruses have identical non-coding regions (NCR) between their two genomic RNAs (Le Gall *et al*., 1995). Studying the interaction between nepoviruses and their worm natural vector (nematode) is made possible by advances in molecular tools and knowledge of the biology of nepoviruses (Sambrook *et al.,* 1989; King *et al*., 2011). Grapevine leafroll-associated virus -1 (GLRa V-1) and GFLV were detected by using DAS-ELISA and RT-PCR (Youssef *et al*., 2008). They used meristem tip culture to produce virus-free plants. Today, several sensitive methods were used for the detection and characterized such viruses including biological indexing, ELISA, PCR and real-time PCR (Vigne *et al.,* 2004; Eichmeier *et al*., 2010; Aseel *et al*., 2019). However, to do so calls for in-depth research and comprehension of the GFLV infection in grapevine's adaptive processes and responses. Our understanding of viruses' interactions with hosts and alterations to the physiology, biochemistry, and molecular biology of the host is limited since viruses cannot be cultivated *in vitro*. Furthermore, a thorough study of the biochemical changes in GFLV-infected grapevine plants is yet lacking. Tissue culture plays an important role in the mass multiplication of crops of economic interest, which are unable to be bulked up to greater quantities to meet the ever-rising market demands of quality plants (Dijkstra and de Jager 1998; Youssef *et al.,* 2008). In this work, GFLV infecting grapevine plants were biologically, serologically and molecularly characterized, *in vitro* produced virus-free grapevine by using thermotherapy in combination with meristem tip culture, and the anatomical abnormalities in infected leaves compared with healthy ones were determined. In order to determine quantitative estimates of physiological and biochemical parameters, such as protein, pigment, and carbohydrate contents, phenolic substances, polyphenol oxidase (PPO), peroxidase (POX), and catalase (CAT) content, the current experiment was carried out.

## MATERIALS AND METHODS

### Field Symptomatology and Virus Isolation:

Visual observations were done in the field concerning the symptoms of GFLV in the elder land and in some regions of the new reclamation lands in Beheira Governorate, Egypt.

A total of 136 samples were collected from seven varieties (Superior, Themson, Early Sweet, Flame, Crimson, King Ruby and Red Globe) in different fields. These samples with typical symptoms of GFLV on infected grapevine including open petiole sinuses, shark-toothed leaf edges, mosaic, vein yellowing, stem fasciation, zigzag stems, leaf

distortion and shortened internodes were collected during the spring and summer of 2021 and 2022 growing season. These samples were serologically tested for the presence of GFLV using DAS-ELISA as described by Clark and Adams (1977). ELISA kits used for the detection of the virus were provided by Agritest, Italy, and prepared according to the manufacturer's instructions. Plates were read at 405 nm with an ELISA reader. Reading twice of healthy plants was considered positive. Grapevine leaf samples that were positive against GFLV antiserum were used to inoculate the tested plants. For biological purification of the virus, isolate using single local lesions onto *C. quinoa* (Kuhn 1964). After three successive single local lesions transfers produced onto *C. quinoa*, the biologically purified virus was propagated mechanically on healthy grapevine seedlings and *Gomphrena globosa*.

**Diagnostic Hosts and Symptomatology:**

Seven plant species and cultivars including *Nicotiana benthamiana*., *N. tabacum* L., *N. rustia* L., *Chenopodium amaranticolor, C. quinoa* Wild., *Cucumis sativus* L., and *G.globosa* L., were mechanically inoculated as described by Noordam (1973) with GFLV-infected sap. The inoculum was prepared by homogenizing the infected leaves with sterilized mortar and pestle in 0.1 M phosphate buffer, pH 7.2 containing 2.5% aqueous nicotine solution**.** The extracted infected sap was rubbed on the carborundum (600 mesh)-dusted leaves of the diagnostic hosts. The inoculated plants were rinsed with tap water immediately after inoculation. Ten seedlings of each healthy host plant were inoculated. An equal number of healthy seedlings of the same cultivar and age were inoculated with tap water and used as controls. Inoculated seedlings were kept under observation in the greenhouse at about 25±1ºC, for 30 days, periodically sprayed with insecticides (Agrothion 57%) to avoid contamination through insect transmission. Symptomless plants were tested for virus infection by DAS-ELISA.

**Biological Indexing Assay:**

Scions taken from bud sticks of naturally infected trees showing GLFV typical symptoms and gave positive results with DAS-ELISA were cleft grafting on woody indicator cuttings (freedom rootstock and LN33) in five replications with three control plants for each sample. The grafted seedlings were embedded in paraffin wax melted at 60-70ºC and dipped immediately in cold water, then in IBA 500 ppm for 10 sec. The grafted cuttings were then grown in plastic bags containing sterilized soil mixture under tunnels (El Sayed 2005) (Fig. 1). The grafted seedlings were kept under observations in the greenhouse at 25±1ºC for 1-2 months and then they were daily inspected for symptoms development. DAS-ELISA was carried out on symptomatic and asymptomatic plants to confirm the graft inoculation success.

**Molecular Detection:**

A reverse transcription-polymerase chain reaction was performed to confirm the presence of GFLV in infected plants. RT-PCR was carried out on RNA preparations with Reverse-iT™ One-Step RT-PCR Kit. This allows RT and amplification to be performed sequentially in the same tube. Infected tissues were extracted using RNeasy Plant Mini Kit (Qiagene, Inc). Primers for GFLV RNA-2 were designed based on highly conserved regions of the sequences available in GenBank, which yielded amplicons for all GFLV isolates. The RT-PCR was performed with total RNA extracted from Plantlets regenerated from meristem tips, using the downstream primer C547 (5` ATTAACTTGACGGATGGCACGC 3`) complementary to nucleotide positions 1064-1083 and H229 (5` ATAAGCATTCGGG ATGGACC 3`) designed by Minafra and Hadidi (1994), In a 50 µL mixture, 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 100 µM each of (dATP, dCTP, dGTP and dTTP), 1.5 mM $MgCl_2$, 1.25 units of *Taq* polymerase and 500 nM of each primer. The mixture was incubated in a thermal cycler. The cycling conditions included a 4 min denaturation step

at 94°C followed by 35 cycles at 94°C for 30 s, 50°C for 30 s and 72°C for 2 min, and a final elongation step at 72°C for 6 min. The RT-PCR products were analyzed by electrophoresis in 1.5% agarose gels and visualized by UV illumination (Bio-Rad) according to Sambrook *et al*. (1989). 100 bp DNA Ladder (Bioneer) was used to determine the expected size About 75 min was required for running agarose gels and staining with ethidium bromide in 10 µg/mL. RNA extraction from healthy plants was used as the negative control.

**Anatomical Changes:**

A comparative anatomical study was carried out on the infected and healthy leaves (controls) to determine the anatomical abnormalities, which may occur in the infected leaves**.** Sections of the infected and healthy leaves were made at 15-17 µm thick using a rotary microtome. Small leaf sections were cut out, killed, and fixed in FAA (10 mL formalin, 5 mL glacial acetic acid, and 85 mL ethyl alcohols, 70%), washed in 50% ethyl alcohol, dehydrated in a series of ethyl alcohols (70, 90, 95, and 100%), infiltrated in xylene, and embedded in paraffin wax with a melting point of 60–63°C. According to Ruzin (1999), sections were mounted on glass slides and stained with aqueous Safranin (1%) and Fast Green (0.1% in 95% ethanol). Microscopically, sections were examined to look for histological signs of notable infection-related reactions.

**Estimation of Chlorophylls:**

According to the non-destructive DMSO method, the contents of chlorophyll 'a', chlorophyll 'b', and total chlorophyll in healthy and infected grapevine leaves were estimated. Test tubes containing the 500 mg of leaf discs were filled with 10 mL of DMSO. The tubes were left in the dark for 1-2 hr. In a spectrophotometer, the absorbance was measured at 663 and 645 nm using the following equations described by Gu *et al*. (2016).

$$Chlorophyll\ a\ (mg/g) = (12.7 * A663) - (2.59 * A645)$$

$$Chlorophyll\ b\ (mg/g) = (22.9 * A645) - (4.7 * A663)$$

$$Chlorophyll\ total\ (mg/g) = (8.2 * A663) + (20.2 * A645)$$

**Estimation of Total Sugars And Starch:**

Total sugar content was estimated using the McCready *et al*., (1950) method and total starch content was determined using the DuBois *et al*., (1956) method. 500 mg of both healthy and sick leaves were taken, properly cleaned with tap water and then distilled water, and then blotted to dry between the folds of filter paper. Leaf samples had their midribs removed, chopped up, and macerated in 5 mL of 80% ethanol. The macerates were put into centrifuge tubes and spun at 5000 rpm for fifteen minutes. Three times, 80% ethanol was used to wash the pellet. The supernatants were combined and filled with 80% ethanol to a predetermined volume. The samples were cooked in a water bath at 85°C until all of the alcohol was gone. The supernatants were gathered and utilized to calculate the sugar content. Starch extraction and quantification were done using the pellet. Twenty milliliters of pooled supernatants from healthy and virus-infected cells were collected separately into test tubes for the measurement of sugar. Each tube received a quick addition of 1 mL of distilled water and 4 mL of cold anthrone reagent. The tubes were then vigorously shaken, incubated for 10 min on an ice bath, and then cooled at room temperature. 4 mL of cold anthrone reagent and 1 mL of distilled water were combined to create the blank. In a spectrophotometer, the samples' absorbance was measured at 625 nm. A D-glucose standard curve was used to measure the amount of total sugars. The pellet that was gathered when making the extract for total sugar was dissolved in 5 mL of 52% perchloric acid (PCA) and heated at 80°C for 10 minutes in order to determine the starch content. Glass wool was used to filter out the solution. With PCA, the filtrate was measured and diluted to 10 mL. Twenty microliters of each type of sample extract healthy and infected were taken individually, mixed with 3 mL of distilled water and 5 mL of anthrone reagent, and then let to sit in an ice bath for 10 min. In a spectrophotometer, the samples' absorbance was measured at 625 nm. The

glucose standard curve was used to determine the amount of starch.

**Estimation of Phenol:**

Folin-Ciocalteu reagent was used to calculate the phenol content. The phenols were extracted using 80% ethanol. Two 5 mL amounts of 80% ethanol were divided between one gramme of pulverized plant material before being centrifuged. The extracts were combined to create a volume of 10 mL. Then, 6 mL of water was added, agitated vigorously, and 0.5 mL of Folin-Ciocalteu reagent was added after 0.1 mL of ethanol extract had been evaporated in a water bath. 2 mL of a 20% sodium carbonate solution was added after 5 min. The absorbance at 660 nm was measured 30 min after the incubation period. According to Folin and Ciocalteu (1927), the phenol concentration of the leaf extract was determined using pyrocatechol as a reference.

**Preparation of Enzyme Extract:**

With a mortar and pestle, 1 g of leaf sample was homogenized in one mL of extraction solution, which contained 50 mM potassium phosphate buffer (pH 7.0), 1% Triton X-100, and 7 mM 2-mercaptoethanol. After centrifuging the homogenate at 12000 rpm for 20 minutes at 4°C, the supernatant was utilized as the crude extract to calculate the activity of POX, PPO, catalase, APX, GPX, and SOD.

**Enzyme Assays:**

Following the oxidation of O-dianisidine, the Malic and Singh (1980) technique was used to measure the peroxidase activity. In a clean, dry cuvette, 3.5 mL of phosphate buffer (pH 6.5), 0.2 mL of enzyme extract, and 0.1 mL of newly made O-dianisidine solution were added for the test. After adding 0.2 mL of 0.2 M $H_2O_2$, the reaction mixture's absorbance was measured at 430 nm every 30 sec for the next three min.The Ngadze *et al.* (2012) method was used to measure the polyphenol oxidase.Polyphenol oxidase activity was determined by measuring the initial rate of quinine formation, as indicated by the increase in absorbance at 420 nm, using a recording spectrophotometer (2401 PC UV-Vis). One unit of enzyme activity was defined as the amount of enzyme that caused a change in absorbance of 0.001/min. PPO activity was assayed in triplicate. The sample cuvette contained 2.95 mL of a 20 nM solution of catechol in 0.1 M phosphate buffer (pH 6.0) and 0.05 mL of enzyme solution. The blank contained only 3 mL of substrate solution. The rate at which $H_2O_2$ dissipated was measured using the method of Maehly and Chance (1959) to assess the catalase activity. The reaction mixture included 50 L of enzyme extract diluted to keep measurements within the analysis's linear range, 2.5 mL of 50 mM phosphate buffer (pH 7.4), and 0.1 mL of 1% $H_2O_2$. A fall in absorbance at 240 nm was seen as the $H_2O_2$ concentration dropped.

**Estimation of Total Protein Content:**

By measuring absorbance at 595 nm and applying the Bradford (1976) method, total protein was calculated calorimetrically. As a benchmark, bovine serum albumin was used. G of protein per gram of leaf tissue was the measurement for the protein content in leaf samples.

**Statistical Analysis:**

The result of every experiment was the same after two replications. Means and standard errors were determined after data were submitted to a variance analysis (Meyers *et al*., 1974).

**GFLV Elimination by Thermotherapy in Combination with Meristem Tip Culture:**

An attempt to eliminate GFLV from infected grapevine trees and produce virus-free healthy propagating materials using thermotherapy in heat chambers according to Mink and Shay (1962). For heat treatment, the infected grapevine seedlings with GFLV under greenhouse conditions were exposed for two months at 36°C in a computerized hot chamber regulated by a thermostat as described by De Sequeira and Posnette (1969). Under this environmental condition, the ability of the viruses to develop is hindered to the point that they are unable to spread to the growing tips of the tree. The budded plants were bent to promote the forcing of the buds. After 8 weeks, plants were removed from the chamber and the buds

were forced. Tissues from these heat-treated shoots were then thoroughly tested by DAS-ELISA for the presence of GFLV. About 0.5 mm of the new apical meristems with primordial from grapevine seedlings treated with thermotherapy was cultivated *in vitro* on tissue culture technique as follows:

**Establishment of Explants in Culture:**

Surface sterilization: The explants were surface sterilized using sodium hypochlorite 15% for 15 min, and then washed with distilled water. The explants were sterilized with 0.1 g of mercuric chloride for 10 min and then washed with distilled water. The explants were sterilized with 70% ethanol for 5 mins and then washed with distilled water.

The medium formulation is often standard, e.g. MS medium Murashige and Skoog (1962). Culture media consists of 30 g of sugar, 1.23 g of woody plant, 1 g of benzylaminopurine (BAP), 0.665 g of indole-3-butyric acid (IBA) and 6 g of agar per liter (Table 1), the pH of the medium was adjusted to 5.8 before autoclaving.

**Controlled Conditions:**

The explants about 0.5 mm long were inserted into the medium and maintained under optimum light (1-10 K-Lux), temperature (25±2°C) and relative humidity (>75%). Under these conditions, the culture is established. Eventually, the culture stabilizes, and then it adapted to the culture and began to grow steadily.

**Table 1:** Tissue culture media composition**.**

| Constituents | Medium | | | |
|---|---|---|---|---|
| | Basal | Starting | Shooting | Rooting |
| MS salt g/L. | 4.4 | 4.4 | 4.4 | 4.4 |
| Sucrose g/L. | 20 | 30 | 30 | 30 |
| Woody plant g/L | 1.23 | 1.23 | 1.23 | 1.23 |
| BAP g/L. | - | 0.5 | 1.5 | - |
| IBA g/L. | - | - | 0.1 | 0.665 |
| Agar g/L. | 6 | 6 | 6 | 6 |
| Charcoal g/L. | - | - | - | 1 |

**Multiplication: Proliferation of Axillary Shoots:**

Repeated parts, where each part of the explants was cultivated in one gar to promote enhanced axillary shoot production encouraged by dividing the explants into two buds where several shoots grow for every shoot when placed in culture. Every month, the shoots were divided and placed on fresh medium to repeat the process of subculture.

**Pre-transplant (Rooting) and Acclimatization:**

Harvested shoots were transferred to a new medium for rooting (30 g of sugar, 1.23 g of woody plant, 0.5 g of BAP, 1 g of IBA and 6 g of agar per liter, pH 5.8). The inoculated explants into the medium were maintained under optimum light (1-10 K-Lux), temperature (25±2°C) and relative humidity (>75%). Under these conditions, the plants were established. It is a process by which physiologically and anatomically plantlets adjust from in *vitro* to *ex-vitro* conditions. Rooted shoots were transferred into a clean potting medium and grown inside a plastic bag to preserve the humidity around the plantlets in the greenhouse. Acclimatization was a relatively slow process and takes 4 to 5 weeks until plantlets possess enough starch reserves for their metabolic activities during acclimatization. Testing the new plants using DAS-ELISA, RT-PCR and biological indexing to be sure that it is virus free.

## RESULTS

**Filed Symptomatology and Sampling:**

A total of 136 samples collected from 6 grapevine varieties in different fields in Beheira Governorate, Egypt were tested for GFLV infection by DAS-ELISA. Data tabulated in Table (2) indicate that the infection rate of the virus was 10.29%. It was also noted that the Flame variety had the highest rate (19.04%), while the Red Globe variety had the lowest one (4.16%).

**Table 2:** Detection of GFLV in collected grapevine cultivars using DAS-ELISA.

| Varieties | | Nº. of healthy samples | Nº. of infected samples | Extinction values (405 nm) | | Infected ratio |
|---|---|---|---|---|---|---|
| | | | | 1 hr. | 2 hr. | |
| **White seedless** | Superior | 22 | 3 | 1.189 | 1. 350 | 13.63 |
| | Thomson | 23 | 0 | 0.232 | 0.285 | 0 |
| | Early sweet | 23 | 2 | 1.100 | 1.464 | 8.96 |
| **Colored seedless** | Flame | 21 | 4 | 1.404 | 1.452 | 19.04 |
| | Crimson King Ruby | 23 | 0 | 0.262 | 0.324 | 0 |
| **Colored seeded** | Red Globe | 24 | 1 | 1.190 | 1.371 | 4.16 |
| | **Total** | 136 | 10 | | | 7.3 |

Positive control: 1.565 and 1.865 while negative control: 0.380 and 0.450.

**Virus Isolation, Propagation and Identification:**

In this study, GFLV was detected by DAS-ELISA. A positive reaction was obtained only between the sap of infected leaves and GFLV-specific antiserum. After biological purification, the virus isolate was propagated on a healthy grapevine mission cultivar and then used in virus identification. Reactions of the diagnostic hosts included seven plants species and cultivars belonging to five families of GFLV infection are summarized in table 3 and Fig. 1. It is obvious that GFLV infection produced chlorotic local lesions on *C. amaranticolor, C. quinoa* and cucumber cv. Balady. Whereas, bean cv. Bountiful reacted with coloristic local lesions followed by systemic mottling, vein clearing and left deformation. Additionally, *N. benthamiana* exhibited faint yellowish lesions followed by systemic mottling and deformation. Moreover, *Pisum sativum* cv. little marvel and faba bean cv. Giza 1 showed systemic mottling and leaf deformation. No symptoms were observed nor could be detected by ELISA for GFLV infection and *Zinnia elegans.*

**Table 3:** Reaction of herbaceous plants inoculated by GFLV.

| Hosts (Family) | Reaction | | |
|---|---|---|---|
| | | Incubation period (day) | Optical density (405 nm.) |
| 1. *Amarantheaceae*<br>*G. globosa* | Chlorosis | 9 days | + 0.735 |
| 2. *Chenopodiaceae*<br>*Ch. amaranticolour*<br>*Ch. Quinoa* | Chlorotic local lesions<br>Chlorotic local lesions | 14 days<br>10 days | + 0.723<br>+ 0.893 |
| 3. *Compositae*<br>*Zinnia elegans* | - | - | - 0.083 |
| 4. *Cucurbitaceae*<br>*Cucumis sativus* cv. Balady | Chlorotic local lesions<br>Mottle leaves | 14 days | + 0.691 |
| 5. *Leguminosae*<br>*Phaseolus vulgaris cv. Bonntifu*<br>*Pisum sativum* cv. Little Marvel<br>*Vicia faba* cv. Giza | Chlorotic ocal lesions, mottle leaves,<br>vein clearing and leaf distortion<br>Systemic mottling and deformation<br>Systemic mottling and deformation | 15 days<br><br>21 days | + 0.593<br><br>+ 0.972 |
| 6. *Solanaceae*<br>*N. benthamiana* | Faint yellowish lesions, systemic mottling and deformation | 17 days | + 0.734 |

Negative control: 0.105, Positive control: 0.525, 10 plants replicates for each host.

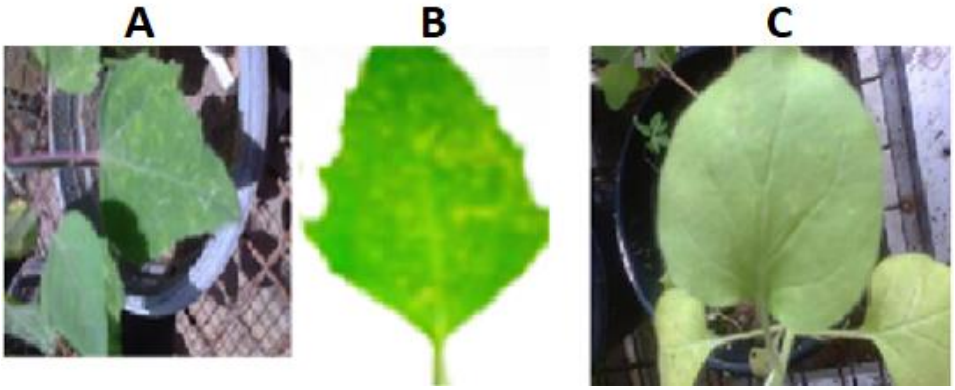



**Fig. 1:** GFLV infection symptoms: chlorotic local lesions on inoculated leaves of *C. amaranticolor* (A) and *C. quinoa* (B) local and systemic symptoms observed on *N. benthamiana* followed by systemic mottling and deformation (C).

**Biological Indexing Assay:**

Scions taken from bud sticks of naturally infected Grapevine trees showing GFLV typical symptoms and gave positive results with DAS-ELISA were cleft grafted on woody indicator cuttings (freedom rootstock and LN33) in five replications and three control plants for each sample. The grafted inoculated cuttings**,** showed in Figure 2. The graft success was relatively high in all indicators. Symptoms could be observed after 3 weeks for GFLV infection. Nevertheless, most of the symptoms developed after 25 days. The first typical GFLV -symptoms observed were leaf yellowing, deformation and after a few days leaf vein clearing and mosaic.

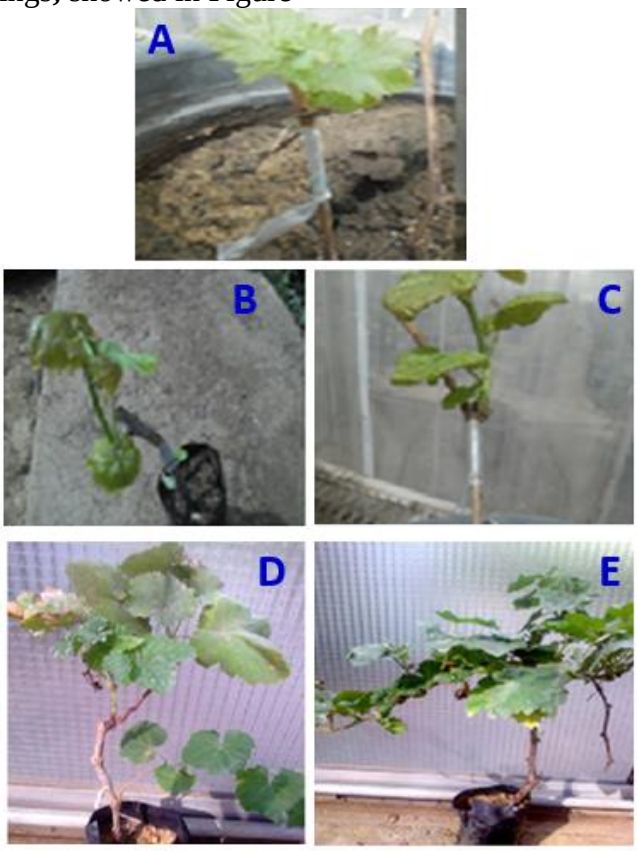



**Fig. 2:** Leaf deformation symptoms in the inoculated cuttings of two grapevine rootstocks genotypes. A: healthy control, (B & C): leaf deformation, after 35 days of GFLV inoculation using the biological indexing assay, D: healthy and E: leaf deformation and zigzag symptoms in the GFLV-inoculated cuttings of grapevine 90 days after inoculation.

**Molecular Detection:**

The coat protein genes of the GFLV isolate were successfully amplified with RT-PCR technique. The expected band size of 321 base pairs was observed. DNA bands of typical GFLV isolate were obtained as shown in Figure 3. The Egyptian isolate of GFLV appears to be recognized by RT-PCR, which is suitable for use on a broad scale. The method utilized in the current study is relatively straightforward, efficient, and accurate for the detection of GFLV infection.

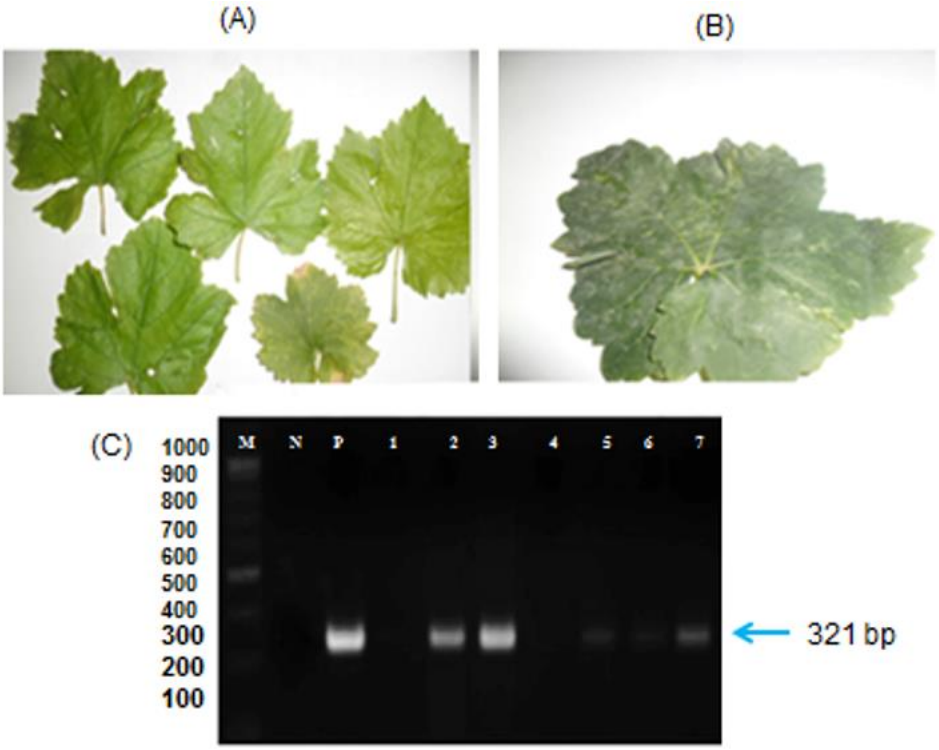



**Fig. 3:** GFLV illustrating mosaic (A), malformation and vein-clearing symptoms (B). C: Agarose gel electrophoresis analysis of RT- PCR showing the amplified fragment of (GFLV) coat protein gene from infected varieties of grapevine. M: 100bp DNA ladder, N: negative control, P: positive control, Lane 1: Crimson cv. Lane 2: Superior cv. Lane 3: Flame cv. Lane 4: Thomson cv. Lane 5: Early sweet cv. Lane 6: Red Globe cv.

**Anatomical Changes:**

The anatomical abnormalities which occurred in the stem of grapevine seedlings infected with GFLV were determined using microtome sections. The obtained results indicated that the cells of pith, xylem, phloem and vascular bundles were compacted and necrosis extended to the vascular bundles in the stem as shown in Figure 4. The number of xylem vessels was clearly reduced. Xylem and phloem cells appear necrotized and blocked with dark stained material. The cells surrounding the vascular bundles and the cambium appear hypertrophic with undulated walls compared with healthy seedlings**.**

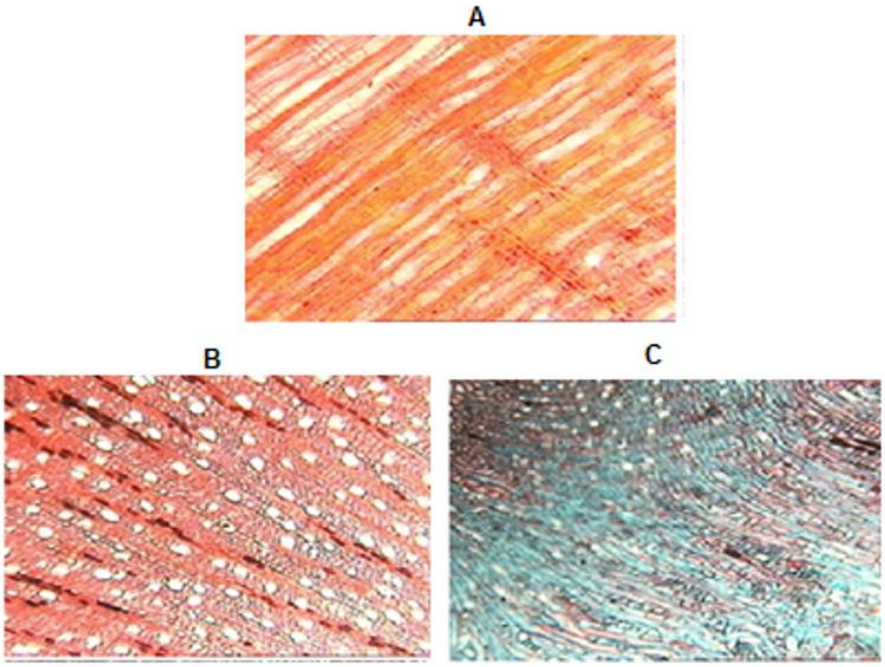



**Fig. 4:** A: healthy xylem, B: infected xylem tissue with necrosis, C: necrosis and death of some xylem vessels. (Magnification X50).

**Biochemical Changes in Infected Grapevine Leaves With GFLV:**

Data in Table 4 showed that;Chlorophyll;The amount of chlorophyll in leaves may be correlated with the physiological effects of stress on growth and yield. The symptoms are brought by changes in leaf's colour brought by virus infection. The relative levels of chlorophyll in healthy and GFLV-infected plants' grapevine leaves were compared. Infected grapevine leaves showed decreased levels of chlorophyll a, b, and total chlorophyll (Fig. 5).

**Carbohydrates:** Regarding the financial harm to the host, the effect of GFLV on the infected host's glucose metabolism is crucial. In the current study, infected plants had higher total sugar and starch levels than healthy plants. Increasing sugar levels during GFLV infection may alter photo-inhibitory mechanisms, which likely cause the symptoms, according to our findings.

**Total protein:** In both cultivars of GFLV-infected plants, a substantial reduction in protein content was observed.

**Total phenol:** Infected leaves have considerably more total phenol than uninfected leaves. Therefore, the increased phenolic content in the diseased grapevine plant may be a factor in the resistance to viral pathogen infection. Increased phenolic levels further imply that the phenol synthesizing pathway accelerated after virus infection.

**Peroxidas** One of the first enzymes to react and offer quick protection against plant diseases is peroxidase (POX). The POXs play a role in lignification, polymerization of glycoproteins rich in hydroxy-proline, control of cell wall elongation, and disease resistance in plants. In comparison to healthy plants, GFLV-infected plants had considerably greater POX activity.

**Polyphenol oxidase** (PPO):In the earliest phase of plant defense, when membrane damage results in the production of phenols such as chromogenic acid, polyphenol oxidase (PPO) plays a crucial role. PPO activity was discovered to be more active in the leaves of GFLV-infected grapevine plants.

**Catalase:** During development, the specialized peroxidative enzyme catalase shields cells from the harmful effects of substrates ($H_2O_2$), which would otherwise be fatal. The leaves of plants that had been infected with GFLV showed an increase in CAT activity.

**Table 4:** Biochemical changes as response of GFLV-infected grapevine plants.

| Parameters | Healthy | Infected | Relative changes% |
|---|---|---|---|
| Chl. a | 2.57 | 1.12 | 56 |
| Chl. b | 1.83 | 0.89 | 51 |
| Chl. ab | 3.73 | 2.17 | 41 |
| Total sugar | 5.90 | 3.21 | 45 |
| Total proteins | 0.94 | 0.83 | 11 |
| Total amino acids | 0.82 | 1.95 | 7 |
| Total phenols | 1.723 | 2.996 | 73 |
| Polyphenol oxidase (PPO) | 0.542 | .986 | 81 |
| Peroxidase (POD) | 0.496 | 1.445 | 95 |
| Catalase (CAT) | 0.647 | 2.653 | 310 |

The percentage of relative changes was measured according to the following equation:

$$\textbf{Relative changes}\ (\%) = \frac{\textbf{Healty} - \textbf{Infected}}{\textbf{Healthy}}\ \textbf{X 100}$$

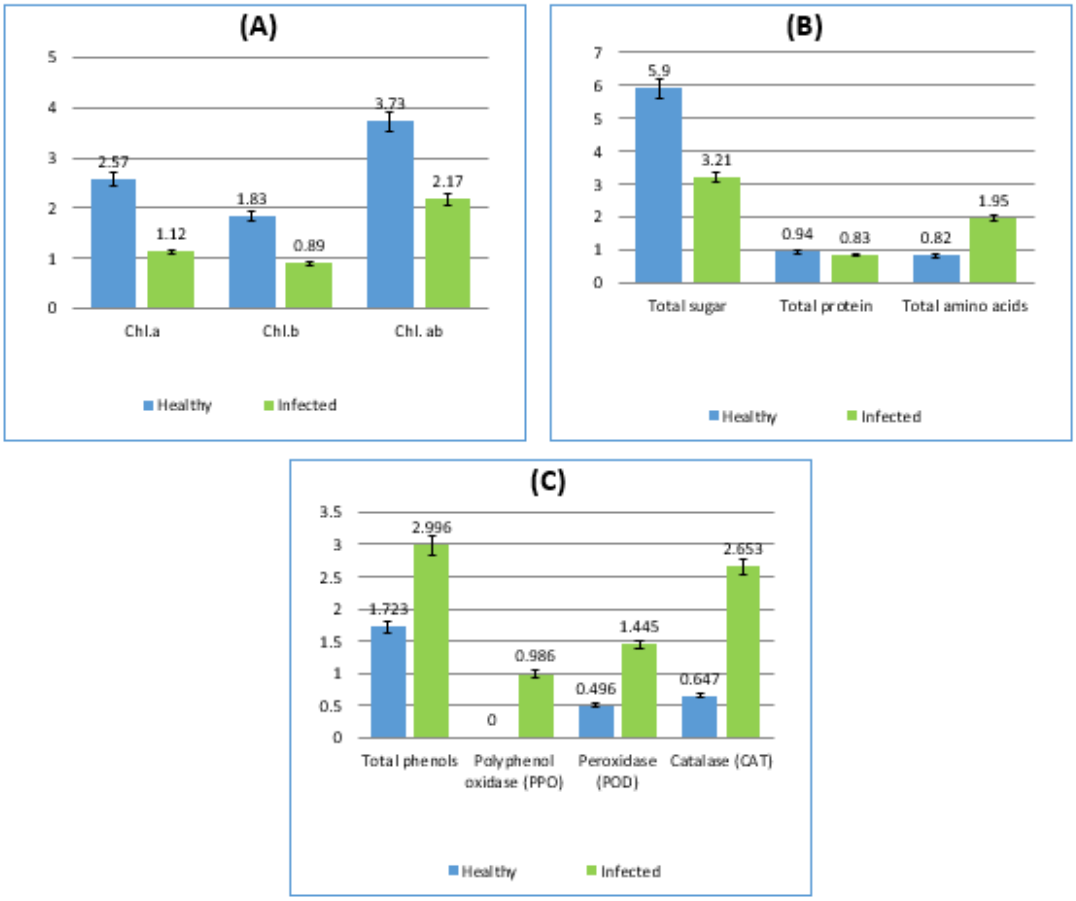



**Fig. 5:** Biochemical changes in GFLV-infected plants

**GFLV Elimination By Thermotherapy in Combination With Meristem Tip Culture:**

Plantlets regenerated from meristem tips treated at 36°C, were tested for GFLV infection visually by DAS-ELISA and RT-PCR. Absorbance values of the treated plants were close to negative control for GFLV. High virus elimination efficiency was achieved by thermotherapy at 36°C for 60 days. All cultures regenerated from meristem tips were found to be 100% virus-free (Fig. 6).

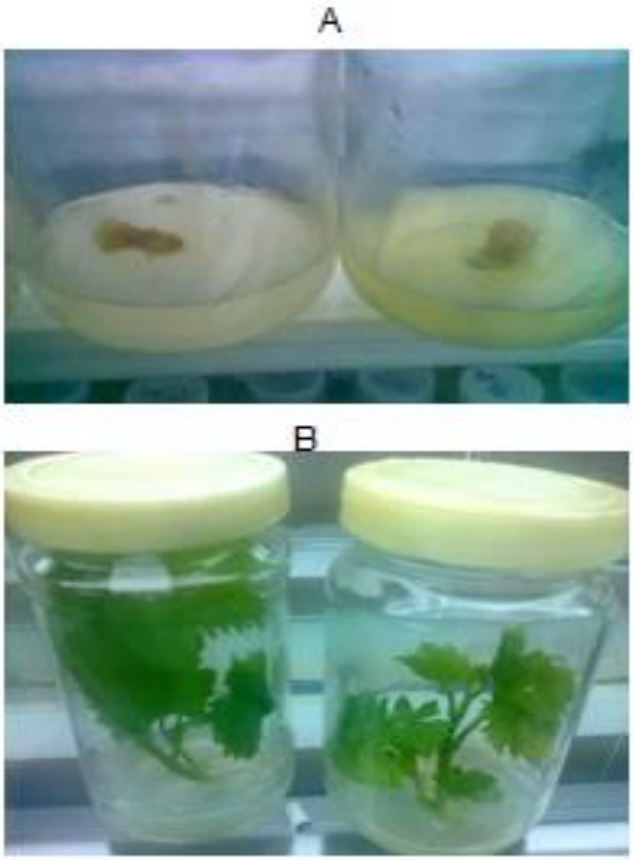



**Fig. 6:** (A): Meristem culture cultivation on Murashige and Skoog media, (B**):** Free virus Grapevine plantlets.

**Molecular Detection of Plantlets Produced By Tissue Culture:**

The coat protein genes of the GFLV isolate were successfully amplified with the RT-PCR technique. The expected band size of approximately 321 base pairs was observed. DNA bands of typical GFLV isolate were obtained as shown in Figure 7. RT-PCR appears to recognize Egyptian isolate of GFLV and would be adaptable for large-scale application. This procedure used in the present study is very simple, quick and reliable for the detection of GFLV infection.

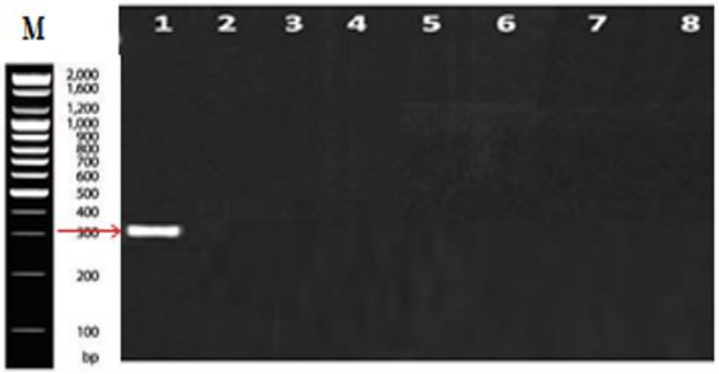



**Fig. 7:** Agarose gel electrophoresis (1%) analysis for tissue culture-produced plantlets. Lane M: 100 bp ladder, Lane 1: virus-specific amplified band corresponding to 321 bp for GFLV, Lanes 2-8: meristematic plantlets negative GFLV.

## DISCUSSION

GFLV is one of the most severe disease-affected grape yields all over the world (Taria and Yilmez 2015). During this study in the vineyards in grapevine–growing areas, symptoms related to GFLV including open petiolar sinuses, shark-toothed leaf edges, mosaic, vein yellowing, stem fascinations, zigzag stems, leaf distortion and shortened internodes were observed (El-Kady *et al.,* 1991; Shalaby *et al*., 2007; Youssef *et al.,* 2008). In diagnostic hosts, the virus isolate produced chlorotic local lesions in *Chenopodium amaranticolor, C. quinoa* and *C. sativus* cv. Balady. Whereas, *Ph. vulgaris* cv. Bountiful reacted with chlorotic local lesions followed by systemic mottling and leaf deformation. In addition, *N. Benthamiana* exhibited faint yellowish lesions followed by systemic mottling. Moreover, *Pisum sativum* cv. Little Marvel and *Vicia faba* cv. Giza 1 showed systemic mottling and deformation. In this work, the virus infection rate reaches 10.29%. Similar results were also obtained by several workers (Martelli 1993; El-Kady *et al*., 1991; El-Awady *et al.,* 2013). In the present work, a positive serological reaction was obtained with GFLV-infected leaves using DAS-ELISA indicating the identity of the virus under study. ELISA provides to be a reliable and sensitive method for detecting and identifying GFLV (Shalaby *et al.,* 2007; Ahmed *et al.,* 2012; El-Awady *et al.,* 2013). In addition, the virus was indexed in healthy grapes by cleft grafting in woody indicator cuttings, which gave the characteristic symptoms. The study of indexing on same *Vities* indicators under greenhouse using harmful virus diseases in Japanese vineyard was done by Tanaka (1988) and thus GFLV infection was confirmed. He also reported that mechanical inoculation by GFLV on *C. quinua and G. globosa* was very difficult, differentiated three isolates of *Citrus psorosis* virus (CPSV) from each other through the symptoms initiated into virus woody indicator plants and different incubation periods (Ahmed *et al.,* 2012). Transmitted GFLV and *Tomato ring spot virus* (TRSV) by side grafting from infected to virus-free grapevine cv. Superior after being tested by ELISA. This study cleared anatomical abnormalities among plants infected with GFLV as compared with healthy plants (controls). The obtained results indicated that the cells of pith, xylem, phloem and vascular bundles were compacted and necrosis extended to the vascular bundles in the steam. The number of xylem vessels was reduced. El-Dougdoug *et al*., (1993) illustrated anatomical changes in virus-infected plants. RT-PCR was found to be a reliable and efficient method for GFLV detection and identification. In the present investigation, the coat protein gene of GFLV isolate was successfully amplified with RT-PCR technique. The expected size of approximately 321 bp was observed in virus-infected plants. This result confirmed the results of biological indexing and ELISA. Whereas, Aseel *et al.,* (2019) used real time-PCR for the detection of GFLV-infected plants in Egypt. Tissue culture has recently become an accepted profitable and established technique for the propagation of many vegetatively propagated plants on a commercial basis (Youssef *et al.,* 2008). They also reported that the usage of meristem and shoot-tip culture for pathogen-free plants is a common practice in the production of virus-free stock. In the present work, the thermotherapy at $36^0$C for 60 days in combination with meristem tip cultures resulted in 100% virus-free cultures. Youssef *et al*., (2008) obtained similar results.

## CONCLUSION

Propagated plant materials should be examined for the existence of viruses biologically, serologically and molecularly before using them for production systems. Thus, a virus-cleaning program should be set up to eliminate GFLV from commercial varieties and rootstock by *in vitro* techniques. This would provide virus-free materials to carry out yield loss and to minimize virus infection and hence produce quality grapevine plants.

**Acknowledgment:**

It is a pleasure to express my deepest gratitude to Prof. Dr. Moustafa El-Kady, professor of Plant Virology, Plant Pathology Research Institute, Agricultural Research Center, for his kind assistance, continuous guidance and valuable suggestions through the course of this study and revision of the manuscript.

**Compliance With Ethical Standards:**

The authors declare that they have no conflict of interest. This article does not contain any studies with human participants or animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study. It also complies with all details of the relevant ethical rules that came under the following headings.

## REFERENCES

Akba, B. and Erdiller, G. (1998). Following Virus Diseases of Connective area Turkey VIII. *Congress of Phytopathology*. Ankara 21-25 Eylül 149-157.

Al-Tamimi, N.; Digiaro, M. and Savion, V. (1998). Viruses of grapevine in Jordan. *Phytopathologia Mediterranea*, 37: 122-126.

Andret-Link, P.; Schmitt-Keichinger, C.; Demangeat, G.; Komar, V. and Fuchs, M. (2004). The specific transmission of grapevine fanleaf virus by its nematode vector *Xiphinema index* is solely determined by the viral coat protein. *Virol*ogy, 320(1): 12- 22.

Aseel, D. G.; Rashad, Y. M. and Hammad, S. M. (2019). Arbuscular Mycorrhizal Fungi Trigge Transcriptional Expression of Flavonoids and Chlorogenic Acid Biosynthetic Pathways Genes in Tomato against Tomato Mosaic Virus. *Scientific reports*, 9: 96

Auger, J.; Aballay, E. E.; Pinto, C. M. and Pastenes, V. C. (1992). Effect of *Grape Fanleaf Virus* (GFV) on growth and productivity of grapevine plants cv. Thompson Seedless. *Fitopatologia*, 27: 85-89.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72: 248-254.

Çigsar, I. (2002). Southeastern Anatolia Region and Nevsehir Biological and Serological Detection with harmful viruses and virus disease in vineyards in the province and are Characterization of Two New nepovirus. Ph. D. Thesis. Zhou. *Institute of Science and Technology 111 s.*

Clark, M. F. and Adams, A. N. (1977). Characteristics of Microplate Method of Enzyme-Linked Immuno Sorbent Assay for the Detection of Plant Viruses. *Journal of General Virology*, 34: 475-483.

Demangeat, G.; Voisin, R.; Minot, J. C.; Bosselut, N.; Fuchs, M. and Esmenjaud, D. (2005). Survival of *Xiphinema index* in Vineyard Soil and Retention of Grapevine fanleaf virus Over Extended Time in the Absence of Host Plants. *Phytopathology*, 95(10): 1151-1156.

Dijkstra, J. and Jager, C. P. (1998). Practical Plant Virology: Protocol and Exercises (Springer Lab. Manuel). *Springer-Verlag* 459s.

DuBois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28: 350-356.

Eichmeier, A.; Baranek, M. and Pidra, M. (2010). Analysis of Genetic Diversity and Phylogeny of Partial Coat Protein Domain in Czech and Italian GFLV Isolates. *Plant Protection Science*, 46(4): 145-148.

El- Kady, M. A. S.; Sadek, A. S.; Gaamal El-Din, A. S. and Tolba, M. A. (1991). Grapevine fanleaf virus in Egypt

proc. 4thNat. Conf. of Pest & Dis. of Veg. & Fruits in Egypt 2: 617- 636.

El Sayed, T. (2005). Setting up of biological indexing of Citrus Tristeza Virus (CTV) and Citrus infectious variegation (CVV) using inoculated indicator cuttings. CIHEAM-IAMB MSc Thesis, Italy, *Collection Master of Science*, 393: 23 p

El-Awady, M. A. M.; El-Den, K. M. E.; Said, M. M.; Ismail, S. E. and El-Tarras, A. A. (2013). Development of the diagnoses tools for *Vitis* spp. grown in Taif- Saudi Arabia infected with *Grapevine fanleaf nepovirus*. *Life Science Journal*, 10(4): 1665-1672.

FAO (2015). FAOSTAT. FAO Statistics Division. Food and Agriculture Organization of the-United Nations, Rome. Available online at http:// www.fao.org/faostat/en/#data

Folin, O. and Ciocalteu, V. (1927). On tyrosine and tryptophane determinations in proteins. *Journal of Biological Chemistry*, 27: 627-650.

Gu, D. -D.; Wang, W. -Z.; Hu, J. -D.; Zhang, X. -M.; Wang, J. -B. and Wang, B. -S. (2016). Nondestructive Determination of Total Chlorophyll Content in Maize Using Three-Wavelength Diffuse Reflectance. *Journal of Applied Spectroscopy*, 83: 541-547.

Hanna, E.; Digiaro, M.; Elbeaino, T.; Choueiri, E.; Jawhar, J.; and Martelli, G.P. (2008). Incidence of viruses and nematode vectors in Lebanese vineyards. *Journal of Phytopathology*, 156: 304-310.

Hefferon, K. and Fuchs, M. (2006). Nepovirus replication. In recent advances in RNA virus replication: Hefferon KL. Eds. Trivandrum, India: *Transworld Research Network* pp. 229-245.

Hewitt, B. W. (1953). Virus Diseases of Grapevine. Plant Diseases. *The Year Book of Agriculture*, 744-753.

Hewitt, B. W. (1954). Some Virus and Virus-Like Diseases of Grapevines. *Bulletin of California Department of Agriculture*, 43: 47-64.

Hewitt, W.B.; Rash, D. J. and Goheen, A. C. (1958). Nematode vector of soilborne fanleaf virus of grapevines. *Phytopathology*, 48: 586-595.

Kepenekci, A. (2014). New Genus *Trıchodorus* Cobb (Stubby Root Nematode) (Triplonchida: Trichodoridae) and Preliminary List of Virus Vector Nematodes Associated In Turkey. *Munis Entomology Zoology*, 9: 227-244.

Kepenekci, İ.; Toktay, H. and Evlice, E. (2014). Plant Parasitic and Virus Vector Nematodes Associated with Vineyards in the Central Anatolia Region of Turkey. *Pakistan Journal of Zoology*, 46(3): 866-870.

King, A. M. Q.; Lefkowitz, E.; Adams, M. J. and Carstens, E. B. (2011). Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses. *Elsevier-Academic Press, Boston, MA. ISBN*-13: 978-0123846846, pp: 811-899.

Köklü, G.; Baloglu, S.; Yilmaz, M. A.; and Ozaslan, M. A. (1983). Study on Determining the Prevalence of *Grapevine Fanleaf Virus* in Vine Varieties in Thrace Region Vineyards. VIII. *Turkey Congress of Phytopathology* Ankara 21-25 September 1998; s. 328-330.

Köklü, G.; Digiaro, M. and Savino, V. A. (2000). Survey of Grapevine Viruses in *Turkish Thrace. Phytopathologia Mediteranea*, 37: 140-142.

Krake, L. R.; Scott, N. S.; Rezaian, M. A. and Taylor, R. H. (1999). Graft-Transmitted Diseases of Grapevines. CSIRO Publishing, *Collingwood, Victoria, ISBN*-13: 9780643063891, Pages:137.

Le Gall, O.; Iwanami, T.; Jones, A. T.; Lehto, K. and Sanfacon, H.; *et al*., (1995). Comoviridae. In: Virus

Taxonomy: VIIIth Report of the International Committee on Taxonomy of Viruses. Fauquet, C.M., M.A. Mayo, J., Maniloff, U. Desselberger and L.A. Ball (Eds.). *Elsevier Academic Press, London, UK* pp: 807-818.

Maehly, A. C. and Chance, B. (1959). The Assay of Catalase and Peroxidase. In: Methods of Biochemical Analysis, Glik, D. (Ed.). *Interscience Publishers, New York*, pp: 357-425

Malic, C. P. and Singh, M. B. (1980). Plant Enzymology and Histoenzymology. Kalyani Publishers, *New Delhi, India* Pages: 286

Martelli, G. P.; Agranovsky, A. A.; Bar-Joseph, M.; Boscia, D.; Candresse, T.; Coutts, R. H.; Dolja, V. V.; Falk, B. W.; Gonsalves, D.; Jelkmann, W.; Karasev, A. V.; Minafra, A.; Namba, S.; Vetten, H. J.; Wisler, G. C. and Yoshikawa, N. (2002). ICTV Study Group on closteroviruses. The family Closteroviridae revised. *Archive of Virology*, 147: 2039-2044.

Martelli, R. E. F. (1993). Diagnosis of plant diseases. *Academic press, New York*; pp. 145- 146.

Mattia, F.; Imazio, S.; Grassi, F.; Doulati, H.; Scienza, A. and Labra, M. (2008). Study of genetic relationships between wild and domesticated grapevine distributed from Middle East regions to European countries. *Rendiconti Lincei*, 19: 223-240.

Mayo, M. A. and Robinson, D. J. (1996). Nepoviruses: Molecular Biology and Replication. In: The plant Viruses: Polyhedral Virions and Bipartite RNA, Harrison, B.D. and A.F. Murant (Eds.). *Springer, New York, USA*. ISBN-13: 978-1-4899-1772-0, pp: 139-185.

McCready, R. M.; Guggolz, J.; Silviera, V. and Owens, H. S. (1950). Determination of starch and amylose in vegetables. *Analytical. Chemistry*, 22: 1156-1158.

Meyers, L.; Grossen, N.; Theory, P.; Design, W. H. and Freeman, S. F. (1974). Analysis of independent group designs. *American Psychological Association*, 237-252.

Minafra, A. and Hadidi, A. (1994). Sensitive detection of grapevine virus A, B, or leafroll-associated virus III from viruliferous mealybugs and infected tissue by cDNA amplification. *Journal of Virological Methods*, 47: 175-188.

Ngadze, E.; Icishahayo, D.; Coutinho, T. A. and van der Waals, J. E. (2012). Role of polyphenol oxidase, peroxidase, phenylalanine ammonia lyase, chlorogenic acid and total soluble phenols in resistance of potatoes to soft rot. *Plant Diseases*, 96: 186-192.

Nogay, A.; Agdaci, M. and Gursoy, Y. Z. A. (1995). Study on Detection of the Virus Diseases and Vectors in Vineyards and American Grapevine Rootstocks in the Marmara Region. VII. *Turkey Congress of Phytopathology. Adana* 26-29; s. 247-251.

Özaslan, M.; Balolu, S. and Yilmaz, M. A. (1991). Virus Diseases of Locally Grown grapevine varieties in Kahramanmaras region. VI. Turkey Congress of Phytopathology. *Proceedings.Turkey Phytopathology Society*, 401-406.

Pesqueira, A. M.; García-Berrios, J. J.; Barrasa, M. and Cabaleiro, C. (2012). Economic Impact of Leafroll Disease in Vineyards of the Cultivar Albariño in Rías Baixas (Spain), *Proceedings of the 17th Congress of ICVG, Davis, California, USA* October 7-14,; p. 55-58.

Rowhani, A.; Maningas, M. A.; Lile, L. S.; Daubert, S. D.; Golino, D. A. (1995). Development of a detection system for viruses of woody plants based on PCR analysis of immobilized

virions. *Phytopathology*, 85: 347-352.

Tanaka, H. (1988). Virus Infection of Grapevine Rootstock Varieties in Japan. *Bulletin of the Fruit Tree Res. Sta. Tsukuba, Ibaraki. Japan* 15: 83-91.

Vigne, E.; Bergdoll, M.; Guyader, S. and Fuchs, M. (2004). Population structure and genetic diversity within *Grapevine fanleaf virus* isolates from a naturally infected vineyards: Evidence for mixed infection and recombinations. *Journal of General Virology*, 85: 2435-2445.

Walker, M. A. (2012). *Xiphinema index* and its Relationship to Grapevines: A review. *South African Journal of Enology and Viticulture*, 33(1): 232.

Youssef, Sahar A.; Al-Dhaher, M. M. A. and Shalaby, A. A. (2009). Elimination of Grapevine fanleaf virus (GFLV) and Grapevine leaf roll-associated virus-1 (GLRaV-1) from Infected Grapevine Plants Using Meristem Tip Culture. *International Journal of Virology*, 5: 89-99.

Yui, Y. D.; Hancheng, H.; Humanting, L.; and Xingqi, G. (1994). Detection Fanleaf Virus by ELISA. *Journal of Shandong Agricultural University*, 25: 82-86.

Zyl, S.; Vivier, M. A.; Raski, D. J. and Goheen, A. C. (1988). Comparison of 1,3- Dichloropropene (Dp) ve Methyl Bromide for Control of *X. index* and *Grapevine Fanleaf* Degeneration Complex. *American Journal of Enology and Viticulture*, 39(4): 334-336.

## ARABIC SUMMARY

### توصيف والتخلص من فيروس الورقة المروحية في العنب باستخدام المعالجة الحرارية بالاشتراك مع زراعة المرستيم القمي

**طارق السيد عبدالباسط[1]، خالد ابراهيم عواد صقر[1]، أحمد النبوي الشوربجي[1]، أيمن محمد مندور[1]، محمود جواد العجوز[2]، أحمد محمد سليمان[1]**

1 قسم بحوث الفيروس والفيتوبلازما - معهد بحوث امراض النباتات - مركز البحوث الزراعية - الجيزة - مصر

2 قسم الأنتاج النباتي والوقاية - كلية الزراعة - جامعة الأزهر – غزة - فلسطين

يعتبر فيروس الورقة المروحية في العنب (GFLV) أحد أكثر الأمراض انتشارًا وخطورة التي تصيب نبات العنب (*Vitis vinifera* L.) في العالم. تم إجراء ملاحظات بصرية في الحقل فيما يتعلق بأعراض GFLV في الأراضي القديمة وفي بعض مناطق أراضي الاستصلاح الجديدة في محافظة البحيرة، مصر. تم جمع النباتات ذات الأعراض المميزة لـ GFLV خلال فصلي الربيع والصيف من موسم النمو 2020 و 2021 وفحصها بواسطة ELISA .تم استخدام عينات الأوراق التي تتفاعل بشكل إيجابي مع المصل المضاد GFLV أولاً لتلقيح نباتات الاختبار (الكاشفة) ثم للتنقية البيولوجية لعزل الفيروس باستخدام البقع الموضعية مفردة على *Chenopodium quinoa* ، بينما تم استخدام *Gomphrena globosa* كمصدر لقاح الفيروس. تم إجراء الأختبارات البيولوجية باستخدام عقل من النباتات الأصل خالية من الفيروسات مثلLN33 .تم إجراء RT-PCR لتأكيد نتائج ELISA. بالإضافة إلى ذلك تم إجراء دراسات تشريحية لدراسة التغيرات التشريحية في الخلايا المصابة لأوراق العنب مقارنة بالخلايا السليمة. وبلغت نسبة الإصابة بالفيروس 10.29٪. لذلك، فإن التغيرات القابلة للقياس الكمي في الصفات الفسيولوجية والكيميائية الحيوية مثل البروتينات ومحتوى الصبغات والكربوهيدرات والمركبات الفينولية والبوليفينول أكسيداز (PPO)والبيروكسيديز (POX) والكتاليز ((CAT) في أوراق العنب. كانت كمية محتويات الكربوهيدرات والمركبات الفينولية و PPO و POX و CAT أعلى بكثير في أوراق العنب المصابة بـ GFLV مقارنة بالسليمة ، بينما أظهر محتوى البروتين الكلي والأصباغ اتجاهًا معاكسًا. بشكل عام ، تشير النتائج إلى أن عدوى GFLV تؤدي إلى تغييرات كبيرة في مستويات الإنزيمات مما يؤدي إلى تطور أعراض. نتج عن المعالجة الحرارية عند 36 درجة مئوية لمدة 60 يومًا بالاقتران مع تقنية زراعة المريستم انتاج نباتات خالية من الفيروسات بنسبة 100٪.