Research Article

# Biological and Molecular Detection of *Fig latent trichovirus* in Naturally Infected Fig Plants

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**Abstract** | Different external symptoms of chlorotic to yellowish mottling, mosaic spots and deformation were observed in leaves of fig plants. To evaluate the presence of Fig latent virus (FLV -1) in fig plants. One hundreds fig trees were collected with virus-like symptoms and symptomless fig trees. The virus was detected by DAS-ELISA. Infected fig leaves were mechanically inoculated on *Chenopodium amaranticolor* L. and reinoculated on *Ch. amaranticolar* L. and *Nicotiana glutinosa* for virus propagation. Fresh wood cuttings (4-6 nodes) were collected from naturally infected fig plants. Healthy fig plants were grafting-inoculated with chip budding of infected fig plants and kept in a greenhouse. Symptoms were confirmed by DAS-ELISA. Strips of infected and healthy *N. glutinosa* leaves were viewed under light microscope. Extracted infectious sap of *N. glutinosa* leaves was examined using Transmission Electron Microscope. Total RNA was isolated from fig leaves showing symptoms and symptomless. Phylogenetic relationships were evaluated. Virus particles were detected by DAS-ELISA in extracts from inoculated host leaves. The virus was transmitted by grafting on fig plants and sap inoculation on a very restricted host range of herbaceous plants where showing latent symptom. The virus with flexible rod particle *ca.* 650 nm long denoted FLV in fig trees in Egypt orchards. The viral Coat protein gene structure resembles that of members of the genus *Trichovirus*in the family *Flexiviridae*. In this study, the virus isolated from fig plants belongs to the genus *Trichovirus* in the family *Flexiviridae*.

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**Keywords** | DAS ELISA, FLV-1, Host range, RT-PCR, TEM, *Trichovirus*



## Introduction

Virus-like diseases of fig trees have been reported to reduce yields significantly. Five distinct virus-like diseases were described in fig plants in Egypt. A "nondescript mosaic" and green-banding were the two predominant symptoms. It was not known if the variations in symptoms were induced by more than one virus or by a variation in response among the several species. During a survey for viruses in a fig germplasm collection of the Faculty of Agriculture of the University of Bari (Southern Italy), filamentous virus-like particles were very frequently observed in negatively stained leaf dips from a number of fig

accessions, regardless of whether they showed mosaic symptoms or not. Similar particles were also found in 1-year-old symptomless seedlings from seeds collected from mosaic diseased figs (Castellano *et al.*, 2009; Gattoni *et al.*, 2009). The present study aims to evaluate the presence of Fig latent trichovirus (FLV-1) the most important RNA viral agents causing the disease by applied biological, DAS- ELISA and RT-PCR methods in different fig orchards of Egypt.

## Materials and Methods

*Plant samples*

One hundred fig trees were collected at autumn season 2018/2021; with naturally virus-like symptoms and symptomless fig trees (*Ficus carica*, cv. sultani, family, Moraceae) from farms of Barshom village, Agriculture Research Station in El-Qanater El Khiria and Department of Horticulture, Faculty of Agriculture, Ain Shams University, Qalubia governorate. Healthy host range plants were obtained from Virology greenhouse and fig plants (*Ficus carica*, cv. Sultani) obtained from Department of Horticulture, Faculty of Agriculture, Ain Shams University.

*Detection of the virus*

The virus was detected based on distinct viral symptoms. DAS-ELISA using specific FLV-1 polyclonal antibody (Pab) kit was kindly provided from (Institute Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), German Collection of Microorganisms and Cell cultures, Inoffenstrasse 7B, 38124 Branuschwieg, Germany) according to Clark and Adams (1977).

*Isolation of the virus*

Infected fig leaves which gave positive result with (Pab) kit FLV- ELISA were grinded in 0.1 M phosphate buffer pH 7.2 (2/1w/v) in sterilized mortar and pestle. The filtrated crude sap was centrifuged at 6000 rpm for 15 min. Virus inoculum was mechanically inoculated on indicator *Chenopodium amaranticolor* L. plants. The morphologically identical local lesions were crushed in 0.1M phosphate buffer and re-inoculated on *Ch. amaranticolar* L. and *Nicotiana glutinosa* for virus propagation. The inoculated plants were kept under insect-proof cages under greenhouse conditions at 26 ±1˚C and 16 h daylight to 21 days.

*Host range*

According to Gattoni *et al.* (2009), seventeen herbaceous plant species (five seedlings of each host) belonging to four families (Table 1) were mechanically inoculated with infectious crude sap and observed daily for symptoms development under observation in insect-proof greenhouse at 26±1˚C for three to five weeks after inoculation and confirmed by DAS-ELISA.

**Table 1:** *Reaction of host plants inoculated with isolated virus.*

| ELISA (OD at 405 nm) | Symptoms | Host | Family |
|---|---|---|---|
| 0.135 | NS | *Beta vulgaris* | *Chenopodiacaea* |
| 0.095 | NS | *Ch. Album* | |
| 0.297 | Ch L L | *Ch. Amaranticolor* | |
| 0.283 | Ch L L | *Ch. Quinua* | |
| 0.365 | M, | *Cucumus sativus* cv Beta-, Alpha | Cucrubiacaeae |
| 0.175 | NS | *Cucumber pepo* cv skandrani | |
| 0.158 | NS | *V. faba L. Giza 2* | *Fabiaceae* |
| 0.276 | Vc, mM | *P. vulgaris* cv.contender. | |
| 0.312 | mM | *Datura metel* L | Solanicaeae |
| 0.078 | NS | *D. stramonium* L. | |
| 0.325 | mM | *N. glutinosa* L. | |
| 0.085 | NS | *N.rustica* L. | |
| 0.273 | mM | *N. tabaccum* cv.Samson L. | |
| 0.192 | M, | *N. tabacum* cv. white burly | |
| 0.123 | mM , C | *C. annum.* cv. californiawonder | |
| 0.285 | mM | *P. hyprida* | |
| 0.372 | M | *S. esculantum* L. cv. Castle rock | |

*Five replicates for each plant species. NS: no symptoms; Ch LL: chlrotic local lesions; VC: vein clearing; M: mosaic; mM: mild mosaic; C: crinkling. Optical density at 405 nm Negative control= 0.114, Positive control= 0.475.*

*Grafting transmission*

A fresh, semi-hard wood cutting with 4-6 nodes were collected from naturally infected fig plants which gave positive result with (Pab) FLV kit ELISA. The healthy fig plants cv. Sultani cultivated in the peat moss and perlite media inside a plastic bag were grafting inoculated with chip budding (blind eye) of infected fig plants and control was done at the same pot according to Roistacher (1991). Grafting inoculated fig trees were kept in a greenhouse conditions at temperatures 24 ºC to 27 ºC maximum during the daytime and 18 ºC to 21 ºC minimum in the night). Symptom observations were routinely carried out since shoots flushing and confirmed by DAS-ELISA.

*Inclusion bodies*

The strips of infected and healthy *N. glutinosa* leaves were separated using a forceps in distilled water and other strips, treated with 5% Triton-XI00 for 5 minutes, then immersed directly in mercuric bromophenol blue stain for 15 minutes, then transferred to 0.5% acetic acid for 15 minutes according to the method (Jordan and Baker, 1955) then washed in distilled water for 10 minutes and mounted on glass slide. The strips were viewed under light microscope at magnification of 400X.

*Electron microscopy*

The extracted infectious sap of *N. glutinosa* leaves was clarified by n-butanol and chloroform (1:1V:V) at room temperature and centrifuged at 6000 rpm at 4°C for 15 min. Few drops of clarified sap were placed on carbon coated grids for one min and stained with 2 % uranyl acetate according to Milne (1993). The dried grids were examined using Transmission Electron Microscope (JEOL-JEM-1010 Electron microscope) in the Regional Center for Mycology, Al-Azhar Univeresity.

*Extraction of total RNA*

It was isolated from four naturally fig leaves showing symptoms and symptomless according to the instruction manual of High Pure RNA tissue kit (Version 1, 2000) from Roche diagnostics GmbH, Germany.

*Reverse transcription polymerase chain reaction (RT-PCR)*

Two oligonucleotide primers, CPtr15′ CCATCTTCACCACACAAATGTC3′ 5040-5060nt position and CPtr2, 5′ CAATCTTCTTGGCCTCCATAAG3′ 5398-5419nt position were synthesized for FLV-1 coat protein CP gene according to Gattoni *et al.* (2009). The oligonucleotide primers were synthesized in ThennoHybaid GmbH, Germany. cDNA was synthesized and amplified as the following procedure according to Latinović *et al.* (2019). cDNA was performed at 50°C for 30 min (reverse transcription). For each sample, 1 µg of total RNA, 3 µl of the primer (CPtr1 5′ CCATCTTCACCACACAAATGTC3′), 20µl of reaction solution 4 µl of 5X first strand cDNA buffer (250 mM Tris-HC1, pH 8.3; 500 mM KC1; 15 mM MgCl2), 5 µl of 0.3 M 2-ß mercaptoethanol, 2.5 µl of 10 mM each deoxynucleotide triphosphate (dNTPs), 1 µl of RNasin (40 units/ µl), 2 µl of 0.1 M dithiothreitol (DTT), 4.5 µl deionized water, and 1 µl (10.000 units/ µl) of Moloney Murine Leukamia. Virus reverse transcriptase (MMLV-RT), (Promega, Co) were mixed with annealing reaction mixture, and incubated for 1 hour at 42°C. Amplification was performed in thin walled PCR tubes. Each tubes containing the following reaction mixture: 5 µl of 10xPCR buffer (1 x= 10mM Tris-HC1, pH 8.3; 50 mMKCl and 0.001 % gelatin), 3 µl of 25 mM $MgCl_2$, 1 µl of 10 mM dNTPs, 5µl of 10 pmol each (CPtr1) and (CPtr2) primers for FLV-1-CP, 2.5 units of Taq DNA polymerase, and sterile water to a volume of 50 µl, in a programmable thermocycler. Five µl of the cDNA was added to the PCR reaction and amplified with the following cycling parameters: 95°C for 15 min (initial PCR activation step) and followed by 40 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 1 min, and a final extension step at 72°C for 10 min. Aliquots 10 µl of RT-PCR amplified DNA product were mixed with 2 µl of gel loading buffer and separated on a 1% agarose gel in 1 X TBE buffer. DNA was stained with 0.5 µg/ml ethidium bromide added to the gel at a concentration of 0.5 µg/ml. DNA was visualized on a UV transilluminator (wavelength=254 nm) and photographed using Gel Documentation System (GELDOC 2000, BioRad, USA). pGEM DNA Ladder (Promega) was used to determine the size of RT- PCR amplified cDNA products.

Multiple alignments of sequences were performed using DNAMAN software (Wisconsin, Madison, USA) and clustal w (Ver.1.74) program (Thompson *et al.*, 1994). The nucleotide distances were estimated considering alignment gaps and using the Jukes and Cantor's method (Jukes and Cantor, 1969) for correction of superimposed substitutions with the Molecular Evolutionary Genetics Analysis (MEGA) software (Ver. 4.0) (Tamura *et al.*, 2007). Phylogenetic relationships were evaluated using un-weight Pair Group Method with Arithmetic Mean (UPGMA) through DNAMAN software and Neighbour Joining (NJ) implemented through MEGA 4.0 software, and bootstrap analysis (1000 replicates) was performed to assess the reliability of the constructed phylogenetic tree.

## Results and Discussion

Fig latent virus (FLV) (genus Trichovirus and family Betaflexiviridae) was isolated from naturally infected fig trees cv. Sultani. It was identified biologically serologically and molecularly.

The virus incidence in farms, Qalubia governorate was 7.5% out of 200 fig trees. Viral symptoms on leaves included crinkling, mild mosaic, veinal necrotic and leave deformation (Figure 1) and confirmed by FLV DAS-ELISA.

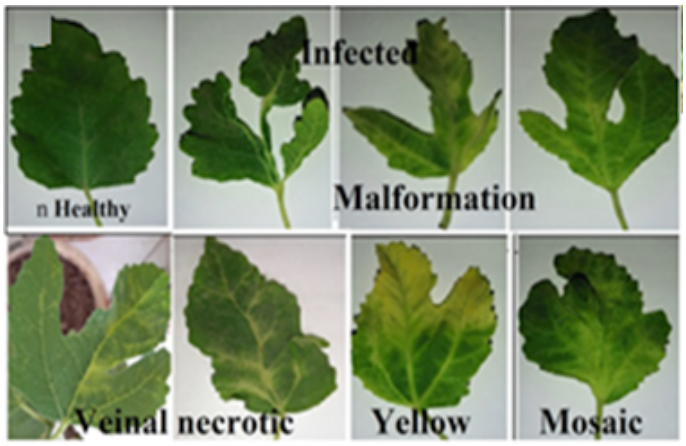



**Figure 1:** *Field fig trees showing viral symptom on leaves.*

*Virus isolation*

The virus was isolated on *Ch. amaranticolor* and showed homologous small round local chlorotic lesions at 12 to 15 days post inoculation under greenhouse condition. The virus was propagated on *N. glutinosa* L. and exhibited systemic infection (vein clearing and mild mosaic) on leaves (Figure 2) and confirmed by DAS-ELISA. The isolated virus was identified based on host range, mode of transmission, inclusion bodies, virus morphology and coat protein gene.

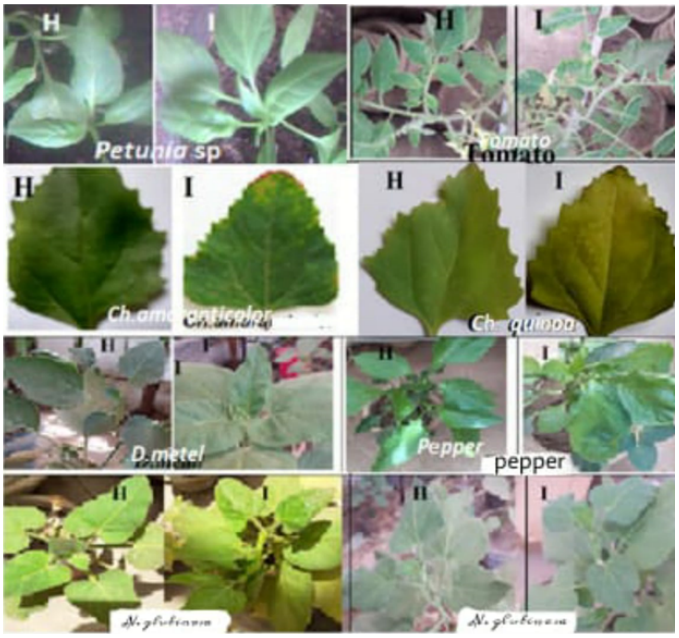



**Figure 2:** *Reaction of host plants inoculated with isolated virus showing none visible symptoms, slightly systemic symptoms on N glutinosa L., N. tabacum cv. white burly, Pepper (Capsucum annum), P. hyprida and tomato, slightly local symptoms on Ch. amaranticolor L. and Ch. quinua.*

*Host range*

The host plants inoculated with isolated virus at the 4-5 leaves old stage showed different reactions (Figure 2). The reaction was divided into four categories React and none visible symptoms (after 40 days inoculation) like, *Datura metel* L., *Cucumis sativus* cv Beta-, Alpha, *Nicotina tabaccum* cv. samson and *Phasolus vulgaris* cv. Contender, Slightly systemic symptoms (after 40 days inoculation) like, *Nicotiana glutinosa* L., *N. tabacum* cv. *white burly*, Pepper *Capsucum annum*, *Petunia hyprida* and Tomato *Solanium Esculantum* cv. Castle rock, slightly local symptoms (after 15 to 20 days inoculation) like, *Ch. amaranticolor* L. and *Ch. quinua*, *gave* local chlorotic *symptom* and no react like, *Ch. album,* Squash *Cucumber pepo* cv. skandrani, *Datura stramonium* L, *Nicotina rustica* L and *Viccia faba* Giza2 (Table 1 and Figure 2). Virus particles were detected by DAS-ELISA in extracts from inoculated host leaves. These results suggest that virus can be transmitted by sap inoculation to herbaceous hosts, in which it does not multiply efficiently under our experimental conditions.

*Virus transmission*

The isolated virus was mechanically transmitted by infectious sap to the host range plants. Grafting transmission as well as the virus was easily grafting transmitted by blind eye to healthy fig plants and showed slightly symptoms (latent) symptoms (Figure 3) at 24 to 32 days post grafting and confirmed by DAS-ELISA.

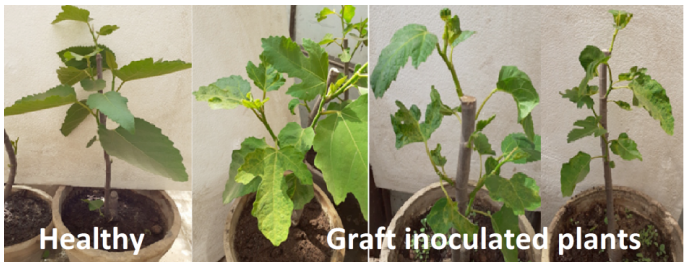



**Figure 3:** *Reaction of fig plants grafting inoculated with isolated virus showing latent systemic symptoms none visible symptoms compared with healthy ones.*

*Morphological of virus particles*

The partial purified virus particles prepared from infected *N. glutinosa* leaves showed flexible rod shape of the most frequent 50 particles by TEM and measured 650 nm and wide 12 nm (Figure 4).

*Inclusion bodies*

The cytoplasmic crystalline and amorphous viral inclusions were detected in epidermal strips of

systemically infected *N. glutinosa* leaves (Figure 5).

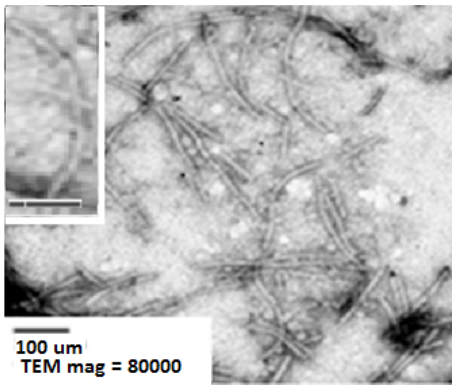



**Figure 4:** *The flexible rod viral particles of FLV-1 detected by negative stain TEM.*

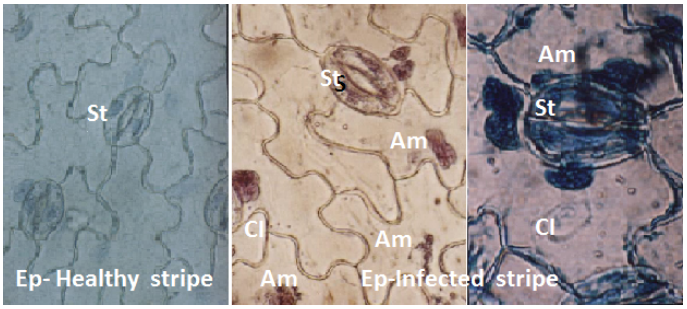



**Figure 5:** *Photograph showing the crystal (Cl) and amorphous (Am) inclusion bodies in cells of epidermal (Ep) strips and stomata (St) of infected and healthy N. glutinosa leaves.*

*Serological characters*

The infectious sap of naturally infected fig trees and herbaceous hosts was reacted by serological precipitation with specific polyclonal antibodies of FLV using DAS-ELISA.

*Molecular characters of FLV*

Extracted total RNA from infected fig leaves was confirmed integrity and quantity by gel electrophoresis and UV spectrophotometer. The purity of yield RNAs was measured by an A260/280 absorbance ratio (1.6) indicating high yield and purity of the extracted RNAs. The concentration of yield RNA was 0.12 mg of infected tissues.

cDNA of coat protein gene of infected fig leaves were reverse transcribed using sense primer coat protein, CPtr-s (5′CCATCTTCACCACACAAATGTC3′). cDNA of coat protein gene nucleotides was amplified using (1 µl of cDNA) mixed with PCR reaction mixture, taq DNA polymerase and two primers (sense CPtr-s and antisense CPtr-s) directly. Electrophoresis analysis of RT-PCR-product was done using 1.5% agarose gel. The fragment of RT-PCR-product expected 400 bp corresponding to the C-terminal region of CP gene. The size of the RT-PCR amplicon for FLV-CP was estimated by comparing its electrophoretic mobility with DNA Ladder as shown in (Figure 6).

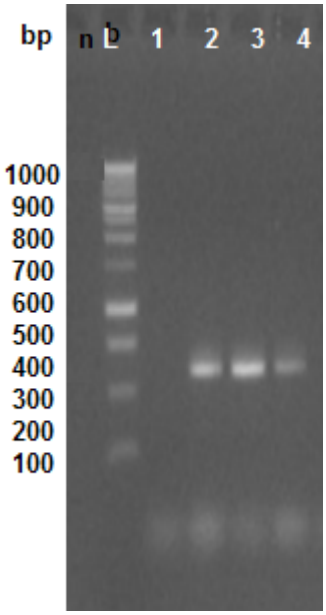



**Figure 6:** *Electrograph of 1.5% agarose gel electrophoresis showing RT-PCR product amplified FLV-CP from extracted RNA from infected fig leaves (1, 2, 3, 4) using (sense CPtr-s and antisense CPtr-s primers . L: DNA ladder weight marker (100 bp ladder).*

*Nucleotide sequence analysis*

The partial nucleotide sequence of the amplified C-terminal region of CP gene of isolate was done from the forward direction at Macrogen3730XL6-1518-009, Korea to determine the relationship with other recommended FLV strains registered in GenBanklt2456301 (Figure 7). Nucleotides were found to be 302 bp mRNA linear corresponding to the C-terminal region of CP gene (Figure 7).

*Fig latent virus MZ076515*

```
  1 atgaaagaag acgccttcaa gggagtaaaa gaagaggaca ggtcaagagt ctggagaaac
 61 atatttggga atatagccat acttggatcc tgtgaggaga cagagttccc acccctagag
121 ttggaggtgg aggatctcaa aggtcagaag atcgcgtctt acactctctc agatatcatt
181 gagctgataa gggattaccg tgacacccac aagagccagt cgatcaagat gatgacactc
241 agaaatatat gtgaggcatt tgcccacgag gctcgactct tccttatgga ggccaagaag
301 at
```

**Figure 7:** *The nucleotide sequence of partial CP gene (302 bp) of FLV strain (MZ076515).*

*Nucleotide sequence analysis*

The partial nucleotide sequence was aligned with four registered FLV isolates in gene bank (Table 2) using the clustal W program with minor manual adjustments. The alignment showed 302bp positions including the gaps.

**Table 2:** *The partial nucleotide sequences producing significant alignments of FLV (MZ076515) CP gene with four FLV isolates recorded in gene bank.*


*Phylogenetic trees*

The genetic distance between the isolated virus and Nahav (KM5167521), I5o5 (FN3775731), Loestanr-1(MG4075531) and Mazandaran, (KM5167521) of FLV-1 isolates recorded in gene bank was 0.008 genetic distance value (Table 2 and Figure 8). The higher genetic distance value was recorded for FLV-1 isolate Nahav (accession no.KM5167521) with 98.3% similarity. The lower genetic distance value was recorded for FLV isolate Mazandaran (accession no.KM5167521) with 100% similarity (Table 2 and Figure 8). Phylogenetic trees showing the relationship of isolated FLV with other members of the family *Flexiviridae* in the CP gene. The neighbour-joining tree was produced and boots trapped 1000 times using CLUSTAL X. Branch lengths are proportional to sequence distances. The scale represents a relative genetic distance of 0.008

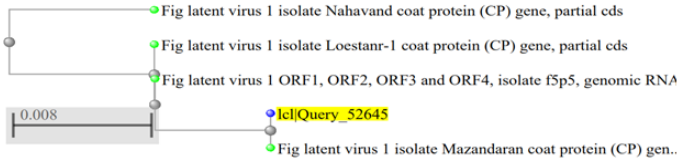



**Figure 8:** *Phylogenetic trees showing the relationship of isolated FLV with Nahavand (KM5167521), I5o5 (FN3775731), Loestanr-1(MG4075531) and Mazandaran, (KM5167521) of fig latent virus isolates recorded in gene bank.*

*Amino acids translation*

The partial nucleotide sequence of partial (CP) gene was translated to amino acids sequence**.** The predict number of amino acids produced were 100 starting with Tyrosine (Figure 9).

*Fig latent virus-1 MZ076515*
/translation="MKEDAFKGVKEEDRSRVWRNIFGNIAILGSCEETEFPPLELEVE
DLKGQKIASYTLSDIIELIRDYRDTHKSQSIKMMTLRNICEAFAHEARLFLMEAKK

**Figure 9:** *Translation of partial nucleotide sequence of CP gene for FLV strain (MZ076515) produced 100 amino acids.*

Amino acids sequencing analysis of CP gene: The amino acids sequence was aligned with eleven FLV isolates registered in gene bank (Table 3 and Figure 10) using the clustal W program with minor manual adjustments. All of these sequences were multiple-aligned using the clustal w program with minor manual adjustments (Figure 10). The alignment showed 100 amino acids positions.

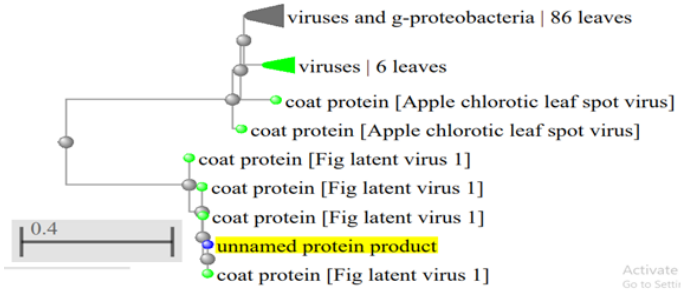
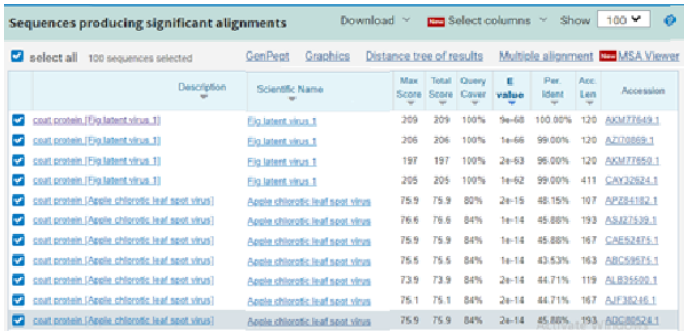



**Figure 10:** *Neighbour joining tree of coat protein gene of FLV-1 CP isolate and 10 isolates published in GenBank based on the amino acid sequence of the CP gene. Numbers represent bootstrap percentage values based on 1000 replicates.*

**Table 3:** *Nucleotide distances and standard error between coat protein gene of FLV isolate and isolates published in GenBank.*

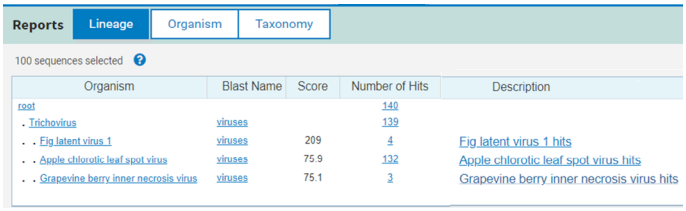



*Phylogenetic tree of CP gene*

The relationship of isolated FLV with other members of the family *Flexiviridae* (Table 3) produced the neighbour-joining tree and boots trapped 100 times using clustal X. Branch lengths are proportional to sequence distances. The genetic distance of amino acid sequences was limited 0.4 genetic distance value (Table 3) between the isolated virus and four FLV strains published in GenBank with accession number AKW77649-1, AZT10869-1, AKW77650-1, CAY32624-1 and seven of Apple chlorotic leaf spot virus with accession number, APZ84182-1, ASJ27539-1, CAE52475-1, ABC59575-1, ALB35600-1, AJF38246-1and ADC80524-1. The higher genetic distance value was recorded for FLV-1 isolate (accession no. AKW77649-1, AZT10869-1, AKW77650-1 and CAY32624-1) with 100% similarity. The lower genetic distance value was recorded for FLV-1 isolate and Apple chlorotic leaf

spot virus with accession number APZ84182-1 with 80% similarity and Apple chlorotic leaf spot virus with accession number ASJ27539-1, CAE52475-1, ABC59575-1, ALB35600-1, AJF38246-1and ADC80524-1with 84% similarity (Table 2 and Figure 8).

**Table 4:** *Classification and characteristics of Fig latent virus.*




*Virus taxonomy*

According to the nucleotide and amino acid sequences of CP gene related to the strains recorded in gene bank (Table 4). The isolated virus classified and characteristic belongs to (genus Trichovirus with number of Hits 140 and family Betaflexiviridae). Fig latent virus 1 (FLV-1) (genus Trichovirus with number of Hits 140 and family Betaflexiviridae ) according to the nucleotide and amino acid sequences of CP gene related to the strain recorded FLV 1 with Score 209 and number if Hits 128 on gene bank (Table 4).

A filamentous virus FLV-1 was detected and isolated in naturally infected fig trees showing latent symptoms by biological, serological and molecular methods. The virus infection was higher by grafting transmission than most of the other grafting transmitted plant viruses. However, the availability of sensitive and specific diagnostic tools allows a careful detection of FLV in certification programs to be run in future for the crop.

FLV (genus Trichovirus and family Betaflexiviridae) (Adams *et al*., 2005; Elbeaino *et al*., 2009; Walia *et al*., 2009) was isolated biologically from naturally infected fig trees cv. sultani, and identified biologically, serologically and molecularly (Gattoni *et al*., 2009; Shahmirzaie *et al*., 2010; Latinović *et al*., 2019).

The incidence of virus found was 7.5% out of 200 natural virus symptom and symptomless fig trees cultivated at some farms in Qalubia governorate based on distinct viral symptoms (lead narrow, mild mosaic, necrotic vein) as mentioned by Gattoni *et al*. (2009) and gave positive result with FLV kit specific polyclonal antibody by DAS-ELISA, Gattoni *et al*. (2009). A survey for the preliminary assessment of the incidence of FLV-1 infections and the association of this virus with symptoms, showed that FLV-1 infects a high percentage (68%) of 40 different cultivars tested from the fig germplasm collection of the University of Bari (Gattoni *et al*., 2009, 2010). A virus with filamentous particles *ca.* 700 nm long, denoted Fig latent virus is widespread in Apulian (Southern Italy) fig orchards, in trees with or without mosaic symptoms and in symptomless seedlings (Gattoni *et al*., 2009, 2010).

The infectious sap gave local chlorotic lesions on *Ch. amaranticolor* after 12 to 15 days post inoculation. The homologous local lesions (small round chlorotic lesions) re-inoculated on *N. glutinosa* L exhibited systemic infection (as vein clearing, and mild mosaic) on leaves and confirmed by DAS-ELISA. These results were in agreement with Saldarelli *et al*. (1999) and Gattoni *et al*. (2009, 2010) who detected the virus particles after 20 days in inoculated leaves of *Ch. quinoa, Ch. amaranticolor*. The reaction of isolated virus and host plants was divided into four categories, none visible symptoms, lightly systemic symptoms and no react. These results suggest that virus can be transmitted by sap inoculation to herbaceous hosts, in which it does not multiply efficiently under our experimental conditions. This virus was transmitted by sap inoculation to a very restricted range of herbaceous hosts without inducing apparent symptoms (Gattoni *et al*., 2009, 2010; Shahmirzaie *et al*., 2010).

The isolated virus was easily grafting transmitted by blind eye by 85 %. The inoculated fig plants showed variable symptoms. This virus was transmitted by sap inoculation to a very restricted range of herbaceous hosts without inducing (Gattoni *et al*., 2009, 2010; Shahmirzaie *et al*., 2010) apparent symptoms.

Virus particles were detected after 24 to 32 days in inoculated leaves. The most frequent size of isolated virus particles was *ca.* 650 nm long and *ca.*12 nm wide with model length more 50 particles were detected in partial purified preparation of infected *N. glutinosa* leaves. Gattoni *et al*. (2009, 2010) found virus particles showing a distinct cross banding were plentiful. Most of the particles were fragmented, so their size was determined from leaf dip preparations. The most frequent length of some 60 particles measured was *ca.* 700 nm. Shahmirzaie *et al*. (2010) reported that a virus with filamentous particle *ca.* 700 nm long. The crystal and amorphous inclusion bodies were detected

in cells of epidermal strips obtained from infected *N. glutinosa* leaves.

The isolated virus have antigenicity characteristic with which the infectious sap of naturally infected fig trees and herbaceous hosts reacted by serological precipitation with specific polyclonal antibodies of FLV particles using DAS ELISA. Gattoni *et al*. (2009, 2010) found the antiserum had a titre of 1:160. It clearly decorated homologous virions. These, however, were not decorated by antisera to ACLSV, GINV, ChMLV and GVA. The serological assay, DIBA, was also performed by using specific polyclonal antiserum Shahmirzaie *et al*. (2010). The purity of yield extracted from symptomatic leaves RNAs was confirmed by gel electrophoresis and quantity an A260/280 absorbance ratio by UV spectrophotometer. RT-PCR was performed by using specific primers (CPtr1/ CPtr2) dsRNAs. Latinović1 *et al*. (2019) detected four viruses detected: Fig leaf mottle-associated virus 1 (FLMaV-1), Fig mosaic virus (FMV), Fig mild mottle associated- virus (FMMaV) and Fig badna virus 1 (FBV- 1). Most of the viruses were present in mixed infections.

cDNA-FLV-*CP* from inoculated *N. glutinosa* was amplified using PCR reaction mixture with product expected 400 bp corresponding to the C-terminal region of CP gene and confirmed by electrophoresis on 1.5% agarose gel (Gattoni *et al*., 2009, 2010). RT-PCR on silica-extracted TNAs template from tissue of a number of different fig trees from the above mentioned collection (Foissac *et al*., 2001; Gattoni *et al*., 2009). A fragment DNA band with the size of 389 bp was amplified using specific primers (CPtr1/ CPtr2) by RT-PCR method for the CP gene of virus Shahmirzaie *et al*. (2010).

The partial nucleotide sequence of FLV strain CP gene found to be 302 bp mRNA linear done from the forward direction at Macrogen 3730XL6-1518-009, Korea and recorded in gene bank with accession number (MZ076515). The alignment with four FLV-1 isolates registered in gene bank by clustal W program with minor manual adjustments, resulting 302 bp positions including the gaps. The genetic distance between FLV strain and FLV-1 isolates, Nahav and (KM5167521) with 98.30% and Mazandaran (accession no.KM5167521) with 100% similarity. Phylogenetic trees showed the relationship of isolated FLV-1 with other members of the family *Flexiviridae* in the CP gene. The neighbour-joining tree was produced and bootstrapped 1000 times using clustal X. Branch lengths are proportional to sequence distances. The scale represents a relative genetic distance 0.008 (Boscia *et al*., 1993; Gattoni *et al*., 2009).

The predicted 100 amino acids were produced from translation of partial (CP) gene nucleotide sequence starting with tyrosine. The alignment of predicted 100 amino acids with the clustal W program with minor manual adjustments, with genetic distance was limited 0.4 between the isolated virus and four FLV-1 strains published in GenBank with 100% similarity according to Caglayan *et al*. (2012).

The isolated virus was named FLV-1 and classified to genus Trichovirus and family *Flexiviridae* according to the nucleotide and amino acid sequences of *CP gene* related to the strains recorded in gene bank. The phylogenetic trees showed the relationship of isolated FLV with other members of the family *Flexiviridae*in the CP gene. Branch lengths are proportional to sequence distances. The scale represents a relative genetic distance of 0.4. The viral genome structure and organization resembles that of members of the genus *Trichovirus*, family *Flexiviridae* and, indeed, FLV clusters with trichoviruses in phylogenetic trees constructed with coat protein sequences (Gattoni *et al*., 2009, 2010).

## Novelty Statement

For the first time, FLV-1 is isolated in Egypt, and it is one of the latent viruses. It is transmitted through vegetative propagation, so it must be detected by PCR and serology. It reduces the horticultural properties of the fig trees in terms of quantity and quality.

## Author's Contribution

Conceptualization: Hanaa H.A. Gomaa, Mona A. Ismail and Khalid A. El-Dougdoug; Methodology: Hanaa H.A. Gomaa, Mona A. Ismail and Khalid A. El-Dougdoug; Formal analysis and investigation: Dalia Y.Z. Amin and Khalid A. El-Dougdoug; Writing-original draft preparation: Dalia Y.Z. Amin; Writing-review and editing: Hanaa H.A. Gomaa, Mona A. Ismail and Khalid A. El-Dougdoug; Supervision: Hanaa H.A. Gomaa, Mona A. Ismail and Khalid A. El-Dougdoug.

*Conflict of interest*

The authors have declared no conflict of interest.

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