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Cytological and molecular studies of an Egyptian isolate of *Carnation vein mottle Potyvirus*

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Abstract

Carnation vein mottle virus CarVMV was isolated from infected carnation plants and identified by host-range, serological detection and maintained on Carnation (*Dianthus caryophyllus* L.) and / *or Gompherena globosa*. Morphological studies of CarVMV were conducted by light and electron microscopy. Light and electron microscopy revealed amorphous cytoplasmic inclusions in infected leaf cells. In some cases, however, inclusions have a characteristic shape, spindle, circular or sledge-like. Pinwheel inclusions characteristic of *Potyvirus* which include CarVMV were also observed. The nuclei were variable in size and shape, sometimes complete destruction of the nucleolus could be observed. The virus infection can cause many cytological changes in the chloroplasts and mitochondria.

A combined assay of reverse transcription and polymerase chain reaction utilizing degenerate primers derived from conserved regions in the genome of potyviruses was designed to amplify a 335 bp cDNA fragment from infected plant using degenerate oligonucleotide primers specific for detection of *Potyvirus* group. Amplification of total RNA extracted from infected carnation suggesting the presence of one *Potyvirus* in the tested carnation plant. Nucleic acid spot hybridization assay also was used to confirm identification of the present virus as one of the *Potyvirus* group using DIG labeled general cDNA probe for detection of potyviruses group. It showed high levels of hybridization signal, whereas no RT-PCR and hybridization were observed with uninfected carnation tissues.

Key words: *Potyvirus* group- amorphous cytoplasmic inclusion- hybridization- RT-PCR

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Introduction

Carnation (*Dianthus caryophyllus* L.) is one of the most important cut-flower crops grown worldwide on commercial scale, and it ranks among the top five cut-flowers. Cut-flower is economically important as an export ornamental crop in Egypt.

Carnation is susceptible to infection by several viruses that cause significant losses (**Lisa, 1995**). These viruses are *Carnation mottle virus* (CarMV), *Carnation vein mottle virus* (CarVMV), *Carnation etched ring virus* (CERV), *Carnation necrotic fleck virus* (CNFV), *Carnation latent virus* (CLV) and *Carnation ring spot virus* (CarRSV). Among them, CarVMV is the most important and widespread virus

CarVMV causes significant economic losses to farmers. Although its infection leads to mild symptoms, it causes sometimes severe infection in all types of carnations. This virus is responsible for the poor quality of cut-flower in terms of size, split calyces and reduced vigour, in addition to lesser yields in terms of lateral shoots, total number of flowers and fresh weight (**Lovisolo and Lisa, 1978** and **Lisa, 1995**).

Thus, this investigation was designed for: Isolation and diagnosis of the *Carnation vein mottle virus*, study the cytological effects of virus infected plants using light and electron microscopy and molecular detection of CarVMV by PCR and dot- blot hybridization technique.

Materials and Methods

Isolation:

Samples from naturally infected carnation (*Dianthus caryophyllus* L.) plants exhibiting characteristic *Carnation vein mottle Potyvirus* (CarVMV) symptoms (diffuse chlorotic spotting and mottling of leaves flower breaking and malformation) were collected from different gardens and greenhouses in Giza Governorate, Egypt. Twenty carnation seedlings grown in clay pots (18 cm dim.) contains sterilized soil ,kept in insect –proof greenhouse were inoculated with sap extracted from infected plants. The inoculated plants were observed for symptoms appearance. For biological purification, single local lesion technique was carried out (**Jiang *et al.*1992**) using *Chenopodium quinoa* as a local lesion host. Then the virus was cultured in *Gompherena globosa* and assayed in *Ch. quinoa*. Whereas *D. caryophyllus* L. was used as propagative host for the following experiments.

Modes of transmission and indicator host plants:

Different plant species belonging to 5 different families were mechanically inoculated with infectious sap prepared by homogenized infected tissues in sodium phosphate buffer, pH 7(1:1w/v). Four seedlings of each host were inoculated and observed daily from symptoms development. The same number of healthy seedlings of the same species and age were left without inoculation to serve as a control. 2-3 weeks post inoculation; plants were visually examined for

symptoms appearance and assayed using serological method.

The green peach, nonvinulleferous aphid (*Myzus persicae* Sulz), was used to study the virus transmission; that had been kept on cabbage seedlings under insect-proof cages. Aphids were starved for one hour, before being allowed to feed on infected carnation leaves for 5 min and then transferred to 10 healthy seedlings (five aphids / seedling). After 24 hours, aphids were killed by spraying with a systemic insecticide (Actra) with rate of 1ml/l. The same procedure was used except that the aphids were fed on virus free seedlings. Plants were visually examined daily up to three weeks and the percentage of transmission was recorded.

Serological diagnosis:

The identity of the virus isolate was confirmed by specific double-antibody sandwich enzyme-linked immuno-sorbent assay (DAS-ELISA). CarVMV IgG and conjugated IgG were purchased from Sanofi, Sante Animal, Paris, France.

Cytological studies:

a- Light microscopic examination:

To study the chemical nature of the inclusion bodies, epidermal strips taken from the lower surface of systematically infected *Nicotiana clevelandii* leaves were treated with 5% triton X-100 for 10 minutes to disrupt the plastids and facilitate the observation of the inclusions. Then the strips were stained by immersing them in a stain containing 100 mg bromophenol blue and 10 g mercuric chloride in 100 ml distilled water for 15 min. The treated strips were then placed in 0.5% acetic acid for 15 min ,washed in tap water for 15 min and then mounted in water (**Christie and Edwardson,1986**). After staining, the strips were viewed and photographed with a Zeiss Photomicroscope II, to detect the protein contents of inclusion bodies.

b-Electron microscopic examination:

Dianthus caryophyllusL., leaves inoculated 15 days previously with CarVMV, and healthy ones were collected, the cytological changes were investigated with electron microscope based on the method given by **Spurr (1969), Weintraub and Ragetli (1970)** , with recorded modifications by **Allam *et al*(2000)**. The samples were cut into small pieces 2-3mm² and fixed in cold 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 for 2-3 hours. They were rinsed with distilled water (once before) washing three times, ten minutes each with 0.1M sodium phosphate buffer, pH 7.2. The specimens were post fixed in 1% Osmium tetra-Oxide (OsO_2) for 60-90 min. and then dehydrated in ascending grades of ethanol 30,40,50 and 60%for 30 min each for 2 times. The blocks were sectioned using the ultramicrotome and the gold and / or silver sections which were stained with uranylacetate and acetone 1:1 (V/V)for 10 minutes, followed by staining with Reynold's lead citrate for 20 minutes and washed with double distilled water several times and dried on a filter paper. The ultrathin sections were followed by electron microscopy using JOEL-100 CX electron microscope, Faculty of

Science, Zagazig University, Zagazig Egypt.

Molecular studies:

Preparation of total RNA

Total RNAs were prepared from young infected and healthy carnation leaf tissues according to the instruction manual of High Pure RNA tissue kit (Version 1, 2000) from Roche diagnostics GmbH, Germany. 50 mg leaf tissues were homogenized in 400 µl lysis /Binding buffer (4.5M guanidine-HCl, 100 mM sodium phosphate, pH 6.6). The lysate was centrifuged for 2 min at maximum speed in a microcentrifuge and 200 µl of absolute ethanol was added to the lysate supernatant. The high pure filter tube and the collection tube were combined and the sample was pipetted in the upper reservoir and centrifuged for 30 s at maximal speed (13,000 xg). The flow through was discarded and 500 µl of wash buffer I (5 M guanidine-HCl, 20 mM Tris-HCl, pH 6.6, in ethanol) was added to the upper reservoir, and centrifuged for 15 s at 10,000 rpm. The flow through was discarded again and 500 µl wash buffer II (20 mM NaCl, 2 mM Tris-HCl, pH, 7.5 in ethanol) was added to the upper reservoir of the filter tube and centrifuged 15 sec at 8000 xg. After the flow through was discarded, 100 µl of elution buffer (nuclease–free, sterile, double dist. Water) was added to the upper reservoir of the filter tube and centrifuged at 8000 xg. The eluted RNA was stored at -80 ºC for later analysis.

Reverse transcription- polymerase chain reaction:

The used oligonucleotide primers designed according to **Langeveld *et al* (1991)** were follows, the upstream primer U335 [5`- gaa ttc atg RtNt ggt gYa tHg aNaa Ygg –`3] and downstream primer D335 [5`- gag ctc gcN gYY ttc atY tgN RHD WKN gc-3`]. (R= a and g ; N=a, c, g and t ; Y=c and t ; H= a,c and t ; D= a, g and t; W=a and t ; K= g and t)were used. For each sample 1 µg TNA, 1. µg of downstream primer, 6 µl of 5x first strand cDNA buffer (250 mM Tris-HCl; pH 8.3; 375 mM KCl, 15 mM MgCl2), 3 µl of 0.1 M dithiothreitol (DTT), were added to a final volume of 30µl using deionized water. The mixture was heated for 5 min at 100°C, and directly chilled on ice for 2 min, then incubated at room temperature for 1 h to allow primer annealing to the RNA template. Twenty µl of reaction solution (4 µl of 5x first strand cDNA buffer (Promega corp, Madison, WI), 5 µl of 0.3 M 2-β mercaptoethanol, 2.5 µl of 10 mM each deoxynucleotide triphosphate (dNTPs), 1µl of RNasin (40 units /µl , Promega corp , Madison, WI), 2 µl of 0.1 M DTT ,4.5 µl deionized water, and 1 µl of cloned Moloney murine LeuKemia virus reverse transcriptase (MMLV-RT)(200 units /µl , Promega Co.) were mixed with annealing reaction mixture, and incubated at 42°C for 1h **(Hadidi *et al.*1993).**

RT-PCR amplification was amplified in thermoblock cycler PCR, (UNOII, Biometra), 35 reaction cycles were performed with 3 min at 94°C and 94°C 30 sec for denaturation, 5 min at 60°C for primer annealing, 1 min at 72°C for synthesis with 7 min at 72°C

for final extension **(Langeveld *et al.* 1991).**

Aliquots of 5 ul from each of PCR amplified DNA products were mixed with gel loading buffer. Separation was done on a 1% agarose gel in 1xTBE buffer pH 8.3 (1x = 89 mM Tris , 89 mM borate , and 2 mM EDTA). DNA was stained with ethidium bromide added to the gel at a concentration of 0.5 ug / ml. DNA was visualized on a UV transilluminator and photographed using DNA documentation gel analysis. PCR DNA marker (Promega) was used to determine the size of RT- PCR amplified cDNA products.

Nnucleic acid spot hybridization:

Prope preparation:

The amplified 335 bp fragment from the virus was eluted and purified from agarose gel electrophoresis and labeled with DIG labeling system according to the manufacturer directions (Boehringer Mannheim GmbH, Mannheim, Germany). Normally for DNA fragments the random primed labeling method is preferred to enzymatically synthesize DIG labeled DNA probes **(Feinberg and Vogelstein, 1983; and Holtke and Kessler, 1990).**

For optimal results, the template DNA should be purified as follows: An equal volume of phenol / chloroform (1:1 V/V) was added, vortexed and centrifuged in a microcentrifuge for 2 min at 12,000 rpm at 4°C. The aqueous phase was transferred to a fresh Eppendorf tubes 1.5 ml and 2.5 volumes of ice cold absolute ethanol were added, mixed by inversion, and incubated at -70 °C for 30 min. The samples were centrifuged at 4 °C for 5 min, the supernatants were discarded and the pellets were dried under vacuum. The pellets were dissolved in 50 µl distilled water. Template DNA (PCR products) was diluted to 0.5 -3 µg to a total volume of 15 µl and denature by heating for 10 min in a boiling water bath and quickly chilling on ice, 2 µl of hexanucleotide mixture, 2 µl DTP labeling mixture, 1µl Klenow enzyme, mixed and centrifuged briefly and incubated for at least 60 min at 37°C (prolonged incubation at 37°C up to 20 h increased the yield of DIG labeled DNA, 2 µl of 0.2 M of EDTA was added to stop the reaction. The labeled DNA was precipitated by adding 2.5 µl of 4 M LiCl, and 75µl of prechiled (-20°C) ethanol. The mixture was mixed very well, then left for 30 min at -70°C or for 2 h at -20°C, then centrifuged for 15 min. The pellets were washed with 50 µl cold ethanol 70 % then dried briefly under vacuum and dissolved in 50 µl TE buffer.

Molecular hybridization for detection of *carnation vein mottle Potyvirus* :

Sap extractions were prepared by grinding 50 mg of fresh plant tissue in 100 ul of AMES buffer (0.5 M sodium acetate, 10 mM MgCl2, 20 % ethanol, 3 % SDS and 1 M NaCl) **(Laulhere and Rozier, 1976 and Podleckis *et al.*, 1993).** The homogenates were incubated 5 min at 37°C before extraction with an equal volume of chloroform. The aqueous phase were collected and reserved in a fresh microcentrifuge tubes. Five ul aliquots were spotted onto a nitrocellulose membrane. The membrane was air

dried and irradiated with UV cross linker and kept at room temperature until hybridized.

Membranes were prehybridized (for blocking the) in hybridization tube at 68 °C for at least 1 h. The membranes were hybridized with 20 ml per 100 cm^2 of hybridized solution containing 5-25 ng of freshly heat DIG labeled cDNA probe denatured (Boiling water bath). The membranes were incubated for at least 6 h to overnight at 68 °C. The membranes were washed 2x 5 min at room temperature with at least 50 ml of 2xSSC, 0.1 % SDS (w/v) per 100 cm^2 membranes and 2x15 min at 68 c with 0.1 x SSC, 0.1 % SDS (w/v). Membranes were equilibrated in Genius buffer 1(100 mM Tris-HCl , 150 mM NaCl , pH 7.5) for 1 min with at least 20 ml of prehybridizated solution per 100 cm2 of membranes. The buffer was discarded , then 100 ml of Genius buffer 2 (2 % blocking reagent dissolved in Genus buffer 1 and diluted 1 : 10) was added and membranes were incubated for 30 min at minimum. Anti digoxigenin alkaline phosphatase was diluted 1: 5000 in Genius buffer 2, and incubated in this buffer for 30 min. The membranes were equilibrated in Genius buffer 3 (100 mM Tris –HCl, 100 mM NaCl , 50 mM MgCl2, pH 9.5) for 2 min, then immunologically detected. The membranes were incubated for 5 min to 16 h in 10 ml of freshly prepared color solution (45 ul Nitro blue tetrazolume and 35 ul X-Phosphate solution were added to a 10 ml of Genius buffer 3) in box in the dark (did not shake during color development) when the spot or band intensities were achieved, the reaction was stopped by washing the membranes for 5 min with 50 ml of water .The results were documented by photocopying the wet filter or photography

Resultis and Discussion

Modes of transmission and indicator host plants:

The virus was easily transmitted mechanically to several plants species belonging to 5 families. The results reported in a combined form in Table (1) and Fig (1) showing symptoms of different hosts. The following hosts did not become infected upon mechanical inoculation: *Datura stramonium, Petunia hybrida, Pisum sativum cv.* Linkolin , *Phaseolus vulgaris* cv.Giza3, *Physalis floridana* and *Nicotiana rustica, N.tabacum var.White Burley and N. glutinosa.* These results were confirmed using back assay inoculation into *Ch. quinoa* using three plants /tested host. The obtained results of inoculated plants are in agreement with those reported for CarVMV before **(Weintraub and Ragetli, 1970) and Hollings and Stone ,1977).**

Myzus persicae Sulz was able to transmit CarVMV from carnation plants to carnation (100 %). This result also agree with those of **Kassanis(1955) and Jiang *et al.* (1992).**

Serological diagnosis:

Positive reaction obtained between infected tissue extract and CarVMV specific antiserum indicated that the virus under study is CarVMV.

Amorphous cytoplasmic inclusions induced by CarVMV was observed by light microscopy in infected epidermal strips of *Nicotiana clevelandii*, 21 days after inculcation (Fig2).

Cytopathological effects of virus infection have been well illustrated by **Rubio (1959)** and **Francki *et al*.(1985).** They stated that, many viruses have no detectable cytological effects on nuclei, other give rise to intranuclear inclusion of various sorts and might affect the nucleolus or the size and shape of the nucleus, even though they appear not to replicate in this organelle.

b- Electron microscopic examination:

Electron microscopic examination of infected leaves revealed various cytological abnormalities which have been absent in healthy tissues. The most prominent structural alteration seen in infected cells are inclusion bodies that are in fact aggregates of virus particle (Matthews, 1991). Examination of ultrathin section of healthy carnation showed healthy mesophyll cells which are nearly round chloenchyma with uniformly thin cell wall and contained nucleus, chloroplasts and mitochondria (**Fig.3a**).

Examination of ultrathin sections of infected carnation leaves with CarVMV revealed the presence of completely destroyed cells and the chloroplasts became swollen, disorganized, reduced and filled with vacuoles and clumped (**Fig3b**). While the hypertrophied nuclear membrane was irregular and chromatin stained dark.These results are similar to those obtained by **Moghal and Francki (1981) and Omar *et al* (1995).**

In particularly, a larg number of cylindrical inclusions in the cytoplasm were appeared as pinwheels and bundles inclusions (**Fig 4**)and (**Fig.5a and b**).Also, paramural bodies were observed similar with those reported on *Datura stramonium* infected with *Potyvirus (Matthews*,1991; **Omar *et al.*,1995** and **Kashiwazaki *et al*.,1998).**

Molecular studies:

RT-PCR has been utilized successfully to detect *Carnation vein mottle* as a *Potyvirus* from infected plants tissues. RT-PCR amplification of viral RNA was carried out on the total RNA isolated from infected and uninfected plant materials using specific primers for detection of potyviruses designed to amplify 335 bp. Electrophoresis analysis of RT-PCR product showed a single amplified fragment of 335 bp presumably from the part of coat protein gene of CarVMV (**Fig.6**). To develop a similar identification method for the new virus belonging to the *Potyvirus* group, local conserved regions in the core domain of the potyvirus coat protein and in the Nib replicase protein were selected to provide nucleotide sequences of the construction of degenerate primers for application in a Potyvirus group specific combined assay of RT-PCR. The available *potyvirus* sequence data made possible the development of a method for the identification of potyviruses based upon the RT-PCR. Indeed, the positive identification was obtained for the virus isolated from carnation plants by this technique. The sequence between the downstream and upstream primers used in this study

represents a highly conserved region of the coat protein gene which should be identical in size in every potyviruses (**Langeveld *et al.*1991**). The primers used in this study complementary to conserved genomic sequences shared all known members of a virus group have been shown to enable the identification of a new related member of the animal *Hepadnavirus* group (**Mack & Sninskey ,1988**), and recently of a group of plant DNA and RNA viruses (**Rybicki & Hughes , 1990 and Langeveld *et al.*, 1991**).

Chen *et al.* (2001) reported that an universal primer (Sprimer: 5'-GGX AAY AAY AGY GGX CAZ CC-3', X=A, G, C or T; Y=T or C; Z=A or G), designed from the consensus sequences that code for the conserved sequence GNNSGQP in the NIb region of members of the family *Potyviridae*, was used to amplify by RT-PCR the 3'-terminal genome regions from infected plant samples representing 21 different viruses in the family. Sequencing of some of the fragments (c. 1.7 kb) showed that the type strain (ATTC PV-107) of *Oat necrotic mottle virus* is not a distinct species in the genus *Rymovirus*, but is synonymous with *Brome streak mosaic virus* (genus *Tritimovirus*) and that *Celery mosaic virus* is a distinct member of the genus *Potyvirus* not closely related to any other sequenced species. Potyviruses infecting crops in China were also investigated, such that viruses infecting cowpea and maize in Hangzhou, Zhejiang province were respectively *Bean common mosaic virus* and *Sugarcane mosaic virus* and that one on garlic in Nanjing, Jiangsu province namely *Onion yellow dwarf virus* were also identified as *Potyvirus*. Fragments were also sequenced from Chinese isolates of *Lettuce mosaic virus* and *Soybean mosaic virus* (from Hangzhou), *Turnip mosaic virus* (2 different isolates from Zhejiang province) and RNA1 of *Wheat yellow mosaic virus* (from Rongcheng, Shandong province).

Nucleic acid spot hybridization with degenarat cDNA probe for potyviruses detection was also used to detect the virus in infected plant materials which gave a positive reaction while no signal was observed with uninfected plant materials. Five µl of extraction from infected and uninfected were spotted onto a nylon membrane. After hybridization, samples containing viral RNA were easily detected. **Fig.(7)** showed dot blot hybridization of Dig - labeled cDNA probe with nucleic acid extracts from plant tissues infected with CarVMV. Reaction of blue signal was resulted from 4 infected carnation leaves samples Lane (1, 2, 3: A,B &C). No hybridization was observed between the cDNA probe and uninfected carnation leaves (Lane4: A, B&C) respectively. These results are similar to those obtained by **White and Bancroft (1982)** and **Podlecis *et al.* (1993)**

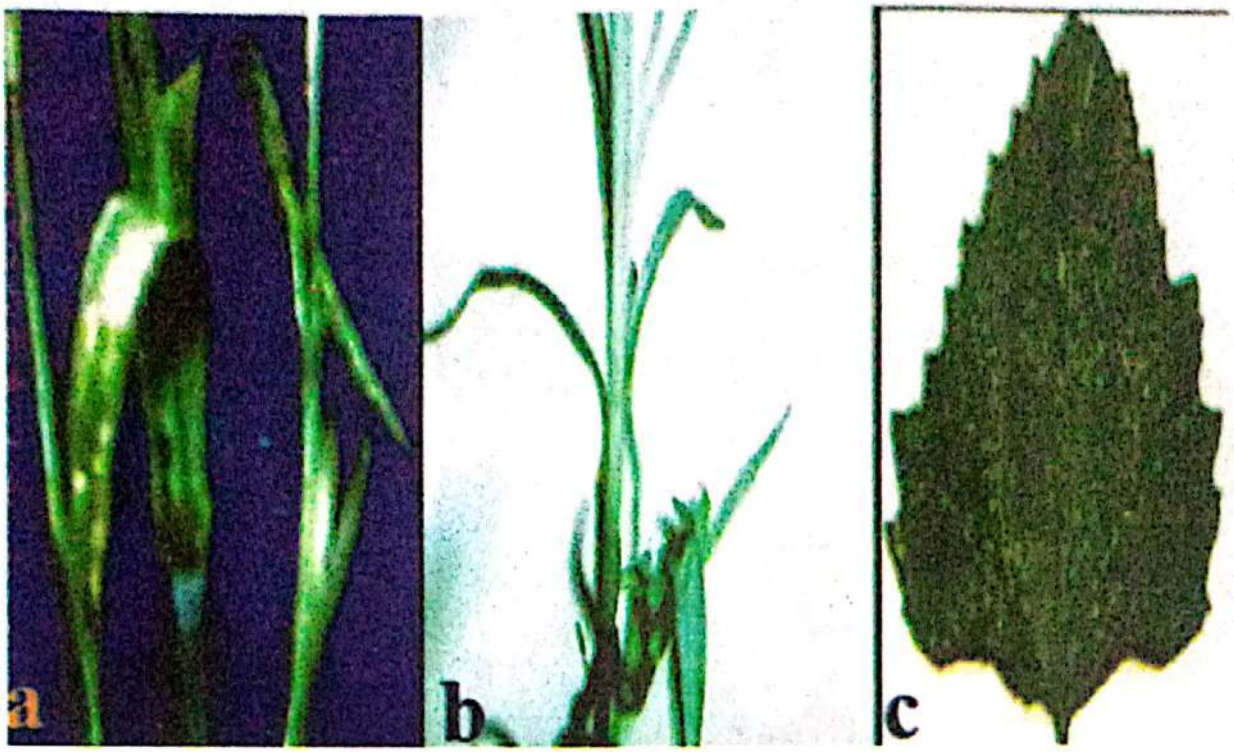

Fig. (1) : Symptoms of Car **VMV** on artificially infected host plants

a- *Dianthus barbatus* show systemic vein-clearing in the youngest leaves developing into a conspicuous leaf mottling.

b- *Dianthus caryophyllus* appeared leaf mottling .

c- C. *quinoa* show local chlorotic spots observed, 7-10 days after inoculation, followed by systemic yellowish veinal flecks and spots with some puckering, buckling and distortion.

Fig. (2) : Light microscopy of Car **VMV** inclusions, amorphous inclusions (IB arrow) near the nucleus (Nu) in *Nicotiana clevelandii,* 21 days after inculcation, staining with bromophenol blue ,(NU) (X 1200).

Table (1). Symptomatological responses of host range to CarVMV isolated from infected carnation plants.

Host plant	Inoculation(days)	Symptoms	Back inoculation
Family: Amaranthaceae			
Gompherena globosa	21	M	+
Family:Chenopodiaceae			
Ch .amaranticolor Cost & Reyn	6-10	LL	+
Ch. quinoa Willd.	7-10	CS	+
Family :Charyophllaceae			
Dianthus caryophyllus L.	15-21	M&CS	+
Dianthus barbatus	15-21	Mo&CS	+
Family: Fabaceae			
Pisum sativum cv. Linkolin	30	-	-
Phaseolus vulgarisL. Cv.Giza 3	30	-	-
Family: Solanaceae			
Datura stramonium	30	-	-
Lycopersicon esculentum Mill. cv. Ice	15	M	+
Nicotiana. clevelandii	21	MM	+
N. glutinosa	30	-	-
N. rustica	30	-	-
Nicotiana. tabacum var. White Burley	30	-	-
Petunia hybrida	30	-	-
Physalis floridana	30	-	-

CS= Chlorotic Spots; LL.= Local Lesion; MM = Moderate Mosaic; Mo=Mottle.M=Mosaic

Back assay was done by inoculation of *Ch. quinoa* Cost & Rey

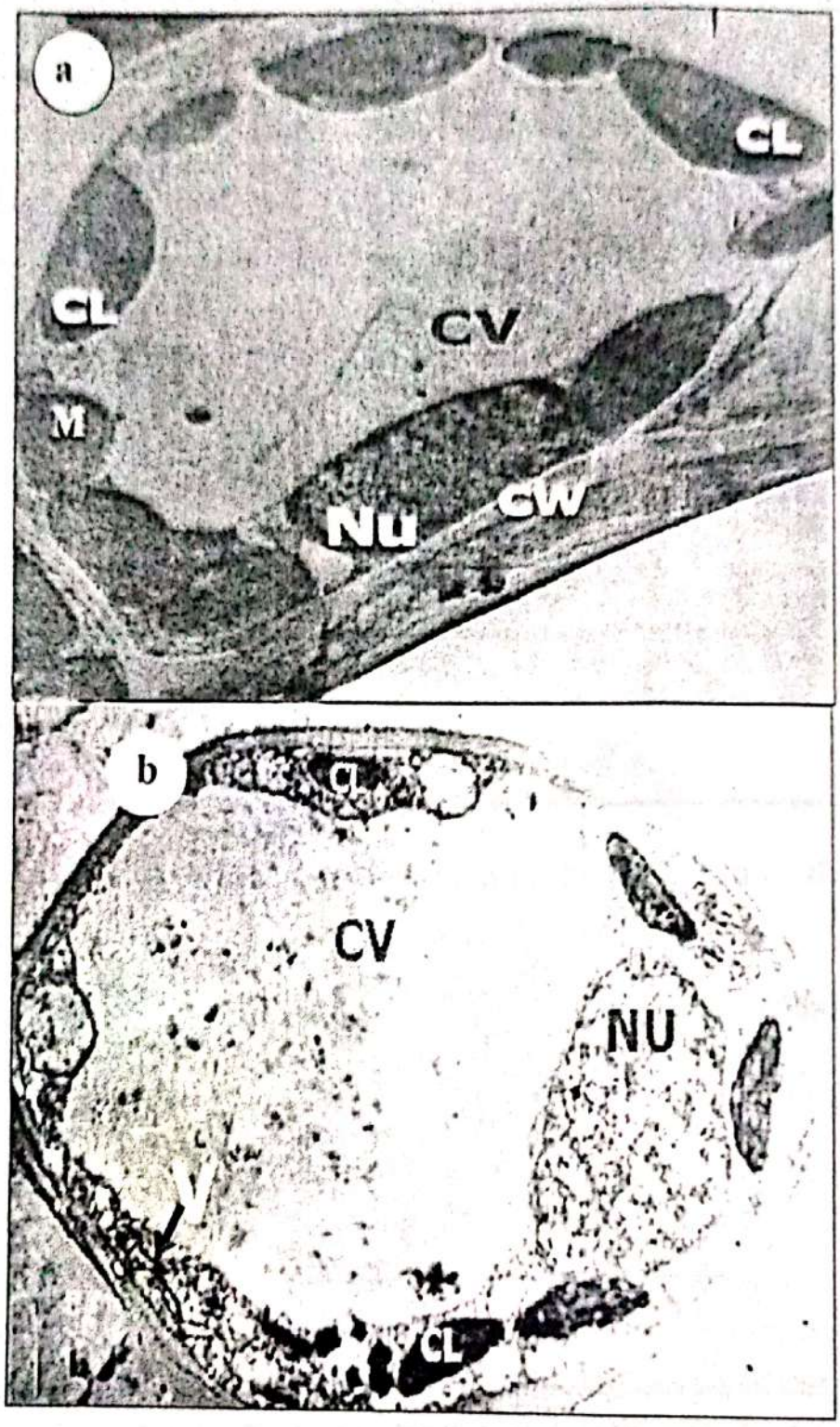

Fig. (3):a- Electron micrograph of ultathin-section of mesophyll cell of healthy carnation leaf showing the cell wall(CW),nucleus(NU), chloroplast(CL),Mitochondria (M),(X-5000).

b- Electron micrograph of ultathin-section of mesophyll cell of infected carnation leaf with CarVMV, the chloroplast (CL) was slightly affected and reduced in size also see the abnormal electron density of the chromatin potions virus like particles (V). (X-5000)

Fig.(4): Inclusions in the form of pinwheel(PW) , bundles inclusions(BU) that may represent a loose pinwheel arrangement(arrow) (10000X).

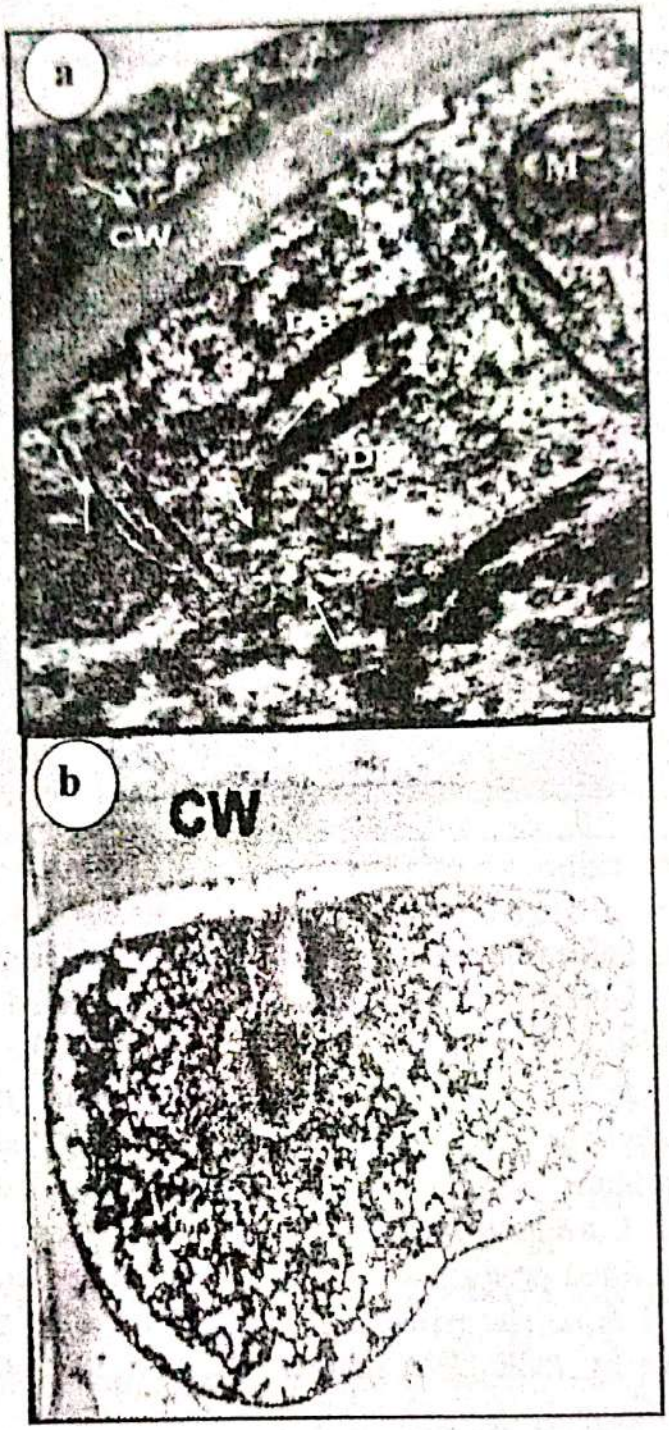

Fig.(5):a- Portion of mesophyll cell of *Dianthus caryophyllus* infected with CarVMV. Note the dense bands (DB), singly and bundles (arrows) in cytoplasm near cell wall vacuole (CV).Arrow points to the more dispositions of particles as a sheet and degenerated mitochondria(M)(28000X).

b- Electron micrographs of thin section prepared from a carnation leaf cells, 15 days from inoculation with CarVMV, Note. the cell wall (CW)and paramural bodies (10000X).

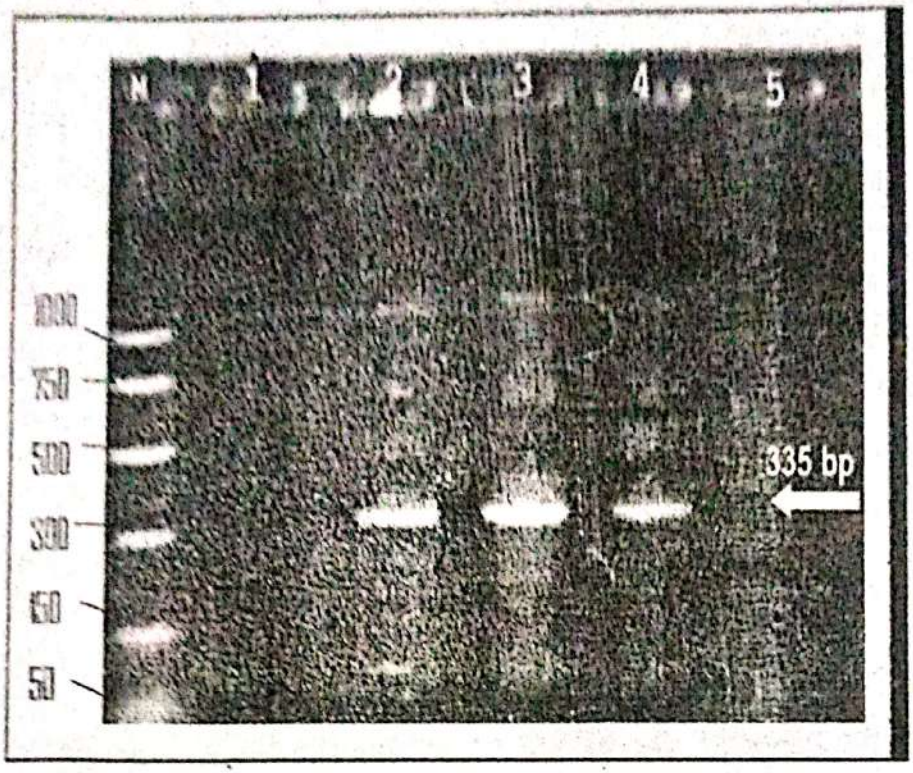

Fig.(6): Agarose gel electrophoretic stained by ethedium bromide analysis of RT-PCR amplification of the total RNA obtained using High Pure RNA tissue kit (Version 1, 2000) from Roche diagnostics GmbH, Germany, from infected and uninfected plant materials with CarVMV showing amplification of a 335 bp fragment with fully degenerate primers U335/ D335 on three carnation leaves samples infected with *Carnation vein mottle Potyvirus*.(Lane 2, 3 and 4). RT-PCR amplified product was observed with uninfected carnation leaves Lane 1 &5). The arrow indicated a 335 bp for the RT-PCR products, lane M: PCR DNA marker (50, 150, 300, 500, 750, and 1000 bp (Promega).

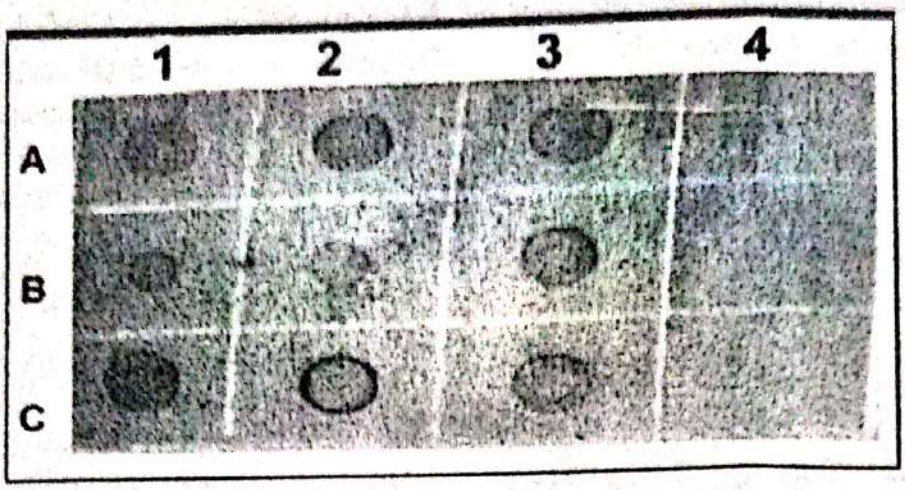

Fig.(7): Dot-blot hybridization assay showing the colored spots of hybridized nucleic acid extracted from nine samples of Carnation plants infected with *Carnation vein mottle Potyvirus* (Lane 1, 2, 3 :A, B &C) , using degenerate cDNA probe labeled with dig-11-dUTP. The extracted nucleic acids diluted 10 fold in 8 X SSC containing 10% formaldehyde and 5 µl of diluted extract were spotted on nitrocellulose membrane. No hybridization signals were observed with healthy plant (Lane 4 A, B &C).

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الملخص العربى

دراسات تشريحية وجزيئية على العزلة المصرية لفيروس تبرقش العروق فى القرنفل

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تم عزل فيروس تبرقش العروق فى القرنفل وتعريفة بأستخدام العوائل المشخصة والتشخيص السيرولوجى وإكثار الفيروس فى نباتات القرنفل والعائل المشخص جمفرينا جلوبوز *Gomphrena globosa* .

وأظهرت الدراسه أنه نتيجة الأصابة تحدث تغيرات داخلية للتركيب التشريحى بأستخدام الميكروسكوب الضوئى والميكروسكوب الألكترونى وجود الأجسام المحتواه بأشكال مختلفة فى سيتوبلازم خلايا الأوراق المصابة ، ولقد شوهدت الأجسام المغزلية والدائرية بالأضافة الى الأجسام المحتواه pinweel وهى المميزة للمجموعة الفيروسية التى يتبعها الفيروس *Potyvirus* . وكذلك فلقد أدت الأصابة الى ظهور تغيرات فى شكل وحجم النواة والى تغيرات فى الكلوروبلاست والميتوكوندريا.

وبينت الدراسة الجزيئية التى تمت على الجينوم الفيروسى بأستخدام إختبار الأستنساخ العكسى •تفاعل البلمرة المتسلسل أن البادئ المتخصص لمجموعة *Potyvirus* له القدرة على التعرف على جزء cDNA فى المنطقة 335 فى نباتات القرنفل المصابة مما يؤكد أن الفيروس أحد أفراد *Potyvirus* وبأستخدام *DIG المعلم للتعرف على مجموعة Potyvirus* أظهر نسبة عالية من التهجين مع النباتات المصابة فى حين لم تظهر النباتات السليمة اى تفاعل.