Biological, cytopathological and molecular studies of Potato virus Y isolated from pepper grown under greenhouse conditions in Egypt

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Abstract — *Potato virus Y* (PVY) was isolated from naturally infected pepper leaves collected from greenhouses in Egypt. Host range and symptomology of pepper isolate of PVY mechanically inoculated into the local lesions host *Chenopodium amaranticolor*. The virus isolate reacted with mild mosaic symptoms and vein clearing on *Nicotiana tabacum* L. cv. White Burely, Dark green mosaic, curl and deformation on *Capsicum annum* L. cv. California Wonder, on *Lycopersicum esculentum* L. cv. Castle Rock. No symptoms were observed on *Solanum tuberosum* L. cv. Diamond. Electron microscopy of the virus dip preparation showed flexuous rod shaped particles. Pinwheel and laminated inclusion bodies by electron microscopy of ultrathin sections prepared in pepper infected plants but not in those of healthy pepper plants. induced by PVY were observed and there were change in the different tissues and cells organelles under electron microscope in infected epidermal strips of pepper leaves. PVY could be effectively detected using RT-PCR. The primers used in this study amplified of full length of the viral coat protein gene (800bp). PCR positive samples were sequenced. The coat protein gene was deposited in the Gen Bank by accession no. (LC060904.1) and PVY isolate was compared with those of other PVY isolates available in the NCBI database with the program BLAST.

Key words :Pepper, PVY^{NP}, ELISA, Inclusion bodies, Electron microscopy, RT-PCR, Sequencing.

1 INTRODUCTION

There are more than 200 cultivars in use for *Capsicum* species. Most of these cultivars belong to the species *Capsicum annuum* (Family: *Solanace*ae). Pepper is very important vegetable crops worldwide. It provides spice and color to foods while providing essential vitamins and minerals in many poor households. Pepper, *Capsicum annuum* is commonly divided into two groups (hot and sweet pepper) (Zhigila *et al.,* 2014 and Green and Kim, 1991).

The greenhouse cultivated pepper in Egypt was about 1,703,775 m2 (2,493 greenhouses) yielding 16,049 tons according to statistics of ministry of agriculture and land reclamation (2012).

Pepper is subjected to many diseases and disorders including fungal, bacterial, phytoplasma and viral caused diseases, Viruses were found to be the most serious disease causing problem in both hot and sweet pepper. Forty-nine virus species infect pepper among which about 20, belonging to 15 different taxonomic groups, have been reported to cause damages in pepper crops (Green and Kim, 1991 and Moury and Verdin, 2012).

Potato virus Y (PVY) which is the model member of *Potyviruses* is a flexuous particles 680–900 nm long and 11–13 nm wide (Moury and Verdin, 2012). PVY have a genome of single-stranded, positive-sense RNA~10kb long. PVY have a monopartite genome that contains only one RNA molecule. The genomic RNA contains a major single encoding a large polyprotein that is co-translationally processed into ten functional proteins (Ha *et al*., 2008). The virus is the most common *Potyvirus* infecting pepper. PVY is common in open field or plastic tunnel pepper cultivation in warm climates. Its prevalence is quite high all around the Mediterranean basin (Moury and Verdin, 2012). It occurs worldwide although it appears to be more important in warmer areas. Disease incidence may be as high as 100% in some areas, resulting in considerable crop loss (Green and Kim, 1991).

The most common symptom induced by PVY in pepper is systemic vein clearing progressing into a mosaic or mottle and generally dark green vein banding in leaves. Vein and petiole necroses often develop, depending on the pepper genotype and, possibly, on the PVY isolate. In some pepper genotypes, systemic necrosis upon PVY infection was shown to depend on the presence of one major dominant gene. In some extreme cases, stem and apical bud necrosis can lead to plant death. Necrotic spots, mosaic patterns, and distortions may develop on fruits of some cultivars. However, fruit symptoms do not always occur in PVY infected pepper plants (Moury and Verdin, 2012, Mascia *et al.,* 2010 and Mostafae *et al*., 2012)

Although mosaic, mottle, dark green veinbanding, veinclearing and yellowing are typical symptoms of infection of pepper by PVY, other symptoms such as leaf crinkling, leaf distortion and stunted growth are also common, depending on the virulence of the strain and the host-pathogen interaction. Vein necrosis, followed by top necrosis and death of the plant can also occur. When plants are infected early, fruit set is reduced and fruits show

pronounced mosaic patterns, making them unmarketable (Green and Kim, 1991). (Romero *et al*., 2001) mentioned that local lesions were formed on inoculated leaves of *Chenopodium amaranticolor*. mild mosaic on *Nicotiana tabacum*, severe mosaic on *Capsicum annum* and no infection on *Solanum tuberosum*.

The virus induces typical type IV inclusions (pinwheels, scrolls and short curved laminated aggregates (Edwardson *et al.,* 1984 and Green and Kim, 1991). Kogovsek *et al.,* (2011) described the ultrastructural changes, such as changes in chloroplast structure and optically dense thickenings of outer cell wall, in the cells with well- developed pinwheels, nuclei with unusual invaginations were found. Tubular aggregates, cytoplasmic inclusion bodies, accumulation of peroxisomes, and nuclei with invaginations were not found in any healthy tissues. In contrast to the healthy leaf tissues, in infected leaves an increased number and size of plasmodesmata between cells in vascular tissue.

The pepper strains of PVY have been grouped into three pathotypes, PVY-0, PVY-I and PVY-1-2, by reactions on *C. annuum* Bastidon, Yolo Wonder, Yolo Y, Florida VR-2 and Serrano VC. Pathotype PVY-0 is the most common. Pathotypes PVY-1 and PVY-1-2 appear predominantly in tropical and subtropical countries (Green and Kim, 1991). There are four major clades among PVY isolates named N, O, C, and Chile. Among them, only members of clades C and Chile can infect pepper crops efficiently and only clade C isolates are prevalent in the Mediterranean basin. Members of the other PVY clades are mostly prevalent on potato or other *Solanaceous* species and poorly infectious in pepper after inoculation in laboratory conditions (Moury and Verdin, 2012).The genetic variability of pepper-infecting PVY isolates is much little than the one found within PVY isolates from potato. Thus, all pepper-infecting isolates analyzed belong to the same genetic strain PVY^{NP}, even the PVY^{C1} group, a potato isolate group able to infect pepper. These results indicate that the specificity of the different isolates of PVY with respect to their host is stricter in the case of pepper isolates, since they do not infect potato (Romero *et al*., 2001). The RT-PCR assays were used for the detection of this virus by using specific primer pairs designed to amplify the full-length of the coat protein gene (810 bp) Shalaby *et al.*(2002).

The present study, biological, Cytopathological and Molecular Studies were carried out for the specific detection of PVY in pepper samples under greenhouse condition in Egypt.

2 MATERIALS AND METHODS

Survey of infected pepper plants under greenhouse condition:

Visual inspection of symptomatic pepper plants under greenhouse conditions was carried out in five Governorates during 2013-2014. The symptomatic pepper plants (131) were collected. The collected samples were screened for PVY incidence using DAS-ELISA with PVY specific antiserum according to (Clark and Adams, 1977).

Isolation and identification of the virus:

To obtain a pure isolate of PVY, biological purification was carried out on *Chenopodium amaranticolor* plants by local lesion technique according to (Kuhn, 1964). The purified isolate was propagated on *Capsicum annum* cv. California wonder and used as source of PVY for the further experiments. The virus was identified as PVY on the basis of host range, transmission experiments, electron microscopy and molecular biological studies.

Mechanical transmission:

The collected leaf samples were homogenized with 0.1M phosphate buffer- solution pH 7.2 (1:2 w/v) in a sterilized mortar. The infectious sap was passed through double layers of cheesecloth and used immediately for mechanical inoculation into healthy Pepper. Seedlings of pepper dusted with carborunndum (600 mech) were inoculated with the sap. The plants were grown in the greenhouse (20-25 °C). The inoculated seedlings were examined daily up to 20 days for symptoms development. The developed symptoms were recorded and tested by indirect ELISA for the presence of PVY using the specific antiserum.

Host rang studies:

Leaves of infected pepper cv. California wander were ground in a mortar and pestle with 0.01 M Phosphate buffer, pH 7.2. Seven of plant species belonging to two families i.e. *Solanaceae* and *Chenopodiaceae* were inoculated mechanically, and kept in the glasshouse at 20-25°C for three weeks. Symptomatic and a Symptomatic plant were tested by indirect ELISA for the presence of PVY using the specific antiserum.

Electron microscopy:

Morphology of PVY particles:

Applying dip preparation technique descripted by *Lin et al.* (1977).carbon coated cupper grid (400mish) were dipped in sap expressed from PVY infected leaves, then negatively stained by 20% Phosphotungestic Acid (PTA) for 2min, then air dried and examined using transmission electron microscope JEOL(JEM-1400TEM, Japan) at The Electron Microscope Unit, Faculty of Agriculture, Cairo University. Electron micrograph was taken the candidate magnification electron micrographs were captured using camera.

Cytopathological studies:

The preparation of Pepper leaf samples infected by PVY were prepared for examination by electron microscopy. The method descripted by Rocchetta *et al.* (2007) and El-banna *et al.* (2007), was followed ultrathin sections (90-100µm) were prepared, mouted on copper grids (400mish) were stained with uranyl acetate and lead citrate. Stained sections were examined by transmission electron microscope JEOL (JEM-1400TEM, Japan) at the candidate magnification. Images were captured using CCD camera model AMT. this work was done in the Research Park (FARP), TEM lab. Faculty of Agriculture, Cairo University.

Molecular biological studies:

Source of samples:

Three of pepper samples showing viral infection collected from the experimental station of faculty of

agriculture in Giza Governorate, which checked by indirect ELISA were used in the study.

RNA extraction:

Total RNA extraction was done using extraction kit (GF-1 Total RNA Extraction Kit (50 preps) cat #GF-TR-050).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR):

The following specific primers pairs designed to amplify the full-length of the coat protein gene of PVY (810 bp): CPf (TCAAGGATCCGCAAATGACACAATTGATGCAGG) CPr (AGAGAGAATTCATCACATGTTCTTGACTCC) by adapted by Shalaby *et al.* (2002). Reverse transcription and PCR were carried out according to the manufacturer's recommendations using One Step RT-PCR Kit (Thermo scientific). The RT-PCR conditions were 06 min at 42°C, 1min at 95°C, 40 cycles of 1 min at 95°C, 1 min at 58°C, 1 min at 72°C and 10 min at 72°C. the amplified product was resolved by electrophoresis in 1% agarose gel.

Sequence analysis:

The amplified PCR product of sample corresponds to portion of CP gene and 3' UTR was purified using QIAquik PCR purification kit. DNA sequencing was carried out with the Bigdye terminator V3.1 cycle sequencing kit (Applied Biosystems) and an Applied Biosystems 3500 sequencer. DNA sequencing was carried out in one direction using the PCR product specific primer PVY. The sequence of PVY isolate was identified in Gene Bank by accession no.: (LC060904.1). PVY isolate was compared with those of other PVY isolates available in the NCBI database with the program BLAST i.e. PVYNP- Tu12.3, PVY-GWB5, PVY-LYE84.2 and PVY- Foggia.

3 RESULTS

Survey PVY infected pepper plants under Greenhouse condition:

Total of Pepper samples were obtained during the 2013-2014 growing seasons. Leaves of pepper showing symptoms suspected to be of viral nature were collected. These samples were collected from greenhouses in five Governorates, i.e. Giza, Dakahlia, Qalubia, Alexandria and Ismailia. the leaves samples were tested by indirect ELISA using the antisera from PVY as showed in Table (1).

Table(1): Survey PVY on pepper plants under greenhouse condition in five Governorates:

ELISA PVY	No. Sample	Cultivars	Greenhouse location	Gov.	
Giza	Experimental station Section 4	Balady 700	8	N	
		Helpino	8	N	
		Omega	8	N	
		Topstare	8	P	67%
		Ananeen	4	P	50%
	Ayat center (Village Tema)	Shunghii	8	N	
		Olymbes	4	N	
		Bunjii	4	N	
	Blue Nile	Canerade	16	N	
		Frezno	10	P	20%
Alexandria	El-Alamiaa	Streck	12	P	17%
Ismailia	Technogreen	85	10	N	
Qalubia	Bahteem	unknown	1	N	
Dakahlia	Kaha Research center	Sonar	15	N	
		Lisar	15	P	06%
Total			131		

P (positive), N (negative), % infection= (No. samples gave positive reaction / No. of tested samples) x100.

Isolation and identification of the virus:

Host range and symptomatology of infecting by PVY:

Seven plants species belonging to two families were mechanically inoculated with the sap extracted from infected plants. Inoculated seedlings were observed for symptoms expression. The data in Table (4) show that *Potato virus Y* reacted with local lesions on the inoculated leaves of *Ch. amaranticolor* L. 6 days after inoculation, on *Nicotiana tabacum* L. cv. White Burely the virus reacted systemically with mild mosaic symptoms and vein clearing 23 days after inoculation, on *Ch. album*., *Datura stramonium* L. and *Solanum tuberosum* L. Diamond No symptoms were observed after inoculation, , on *Lycopersicon esculentum* L. Castle Rock mild mosaic symptoms were observed after inoculation on newly formed leaves and *Capsicum annum* L. California Wonder as dark green mosaic, crinkling and malformation (Fig. 1).

Table (2): Host range and symptoms reaction tested of PVY (pepper isolate)

Tested plant species	Symptoms
Chenopodiaceae	
Ch. amaranticolor L.	Local Lesions
Ch. album	_____
Solanaceae	
Datura stramonium L.	_____
Solanum tuberosum L. Diamond	_____
Nicotiana tabacum L. cv. White Burely	Mild Mosaic, Vein Clearing
Lycopersicon esculentum L. Castle Rock	Mild Mosaic
Capsicum annum L. California wondar	Dark Green Mosaic, Curl, deformation

Fig. 1. Host range and symptoms reaction of PVY an inoculated plant species, (A): local lesions in *Ch. amaranticolor* L., (B): Mild mosaic symptoms on *Lycopersicon esculentum* L. Castle Rock were observed after inoculation with PVY of newly formed leaves. (C,D): *Nicotiana tabacum* L. cv. White Burely plants showing mild mosaic symptoms and vein clearing symptoms, (E): Vein clearing , mosaic and deformation of PVY infected leaves on *Capsicum annum* L. California wondar, (F): Healthy Pepper leaves.

Electron microscopy:

Morphology of PVY particles:

Electron microscopy of the virus dip preparation, inspection of negatively stained dip preparation of PVY by transmission electron microscope revealed the presence of nonenveloped flexuous virus particles measuring 849 nm (Fig. 2).

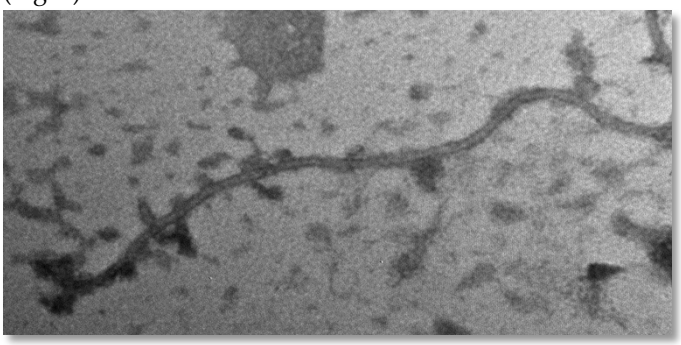

Fig. 2. An electron micrograph of PVY particles negatively stained with 2% phosphotungestic acid (100,000 x).

Cytopathological Studies:

Typical pinwheels inclusion bodies (Edwardson *et al.*, 1984) were found in ultrathin sections of leaves of PVY infected peppers (Figs 3A and B). Pinwheels and laminated aggregates were observed in the cytoplasm of infected cells (Fig. 4). Pinwheels or aggregates were not found in ultrathin sections prepared from leaf tissue of healthy pepper plants. Regarding The change in the different tissues and cells organelles of pepper plants as a result of PVY infection were observed. The chloroplast was destroyed leaving the thilakoids and grana plates free in the cytoplasm (Figs 5A and B), full laysis of the chloroplast was also observed (Fig. 6). The nucleolus disappeared from the nucleus, the nuclear membrane was disappeared and the chromatin was segmented (Figs 7A and B if compared with that of healthy tissues (Fig. 7C). The phloem tissue in infected pepper leaf was dramatically affected Cell wall showed degradation, irregular shape, thickening, invagenation and dissociation (Figs 8A and B). The phloem tissue was showing intracellular and intercellular nicrotic area (Fig. 8B), there were abnormal structure and uncapsidated virus particles in the sieve elements, the cell membrane was dissociation and companion cell was plasmolyses(Fig. 9).

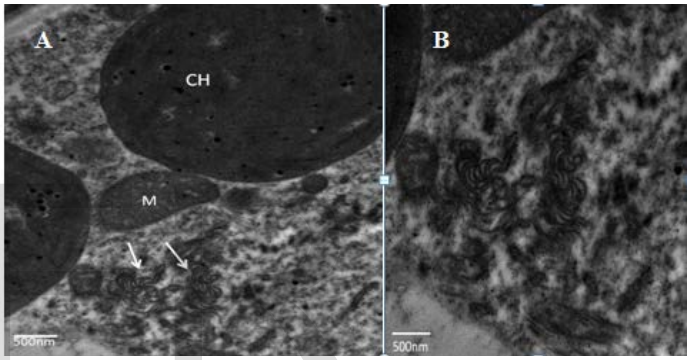

Fig. 3. (A): An electron micrograph showing pinwheel inclusion bodies (arrows) inside the cytoplasm of parenchyma cells infected with PVY (CH=chloroplast, M= mitochondria), (B): Magnified part of (A) Representing pinwheel inclusions at high magnification.

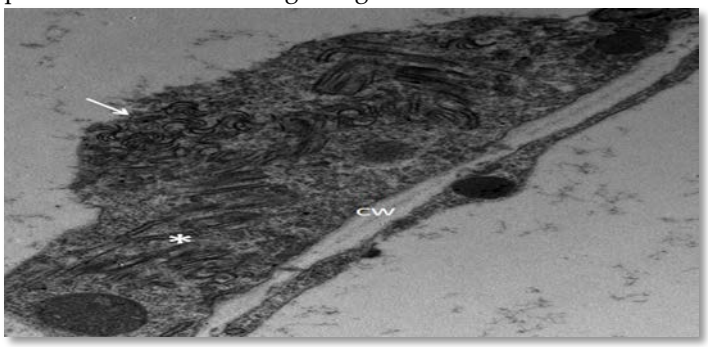

Fig. 4. Transmission electron micrograph of ultra thin section of pepper leaf parenchyma tissue as there are two types of inclusion bodies, pinwheel (arrow) and laminated (*). Cell wall (CW) lyses and disorganization is obvious in the fully destroyed cell.

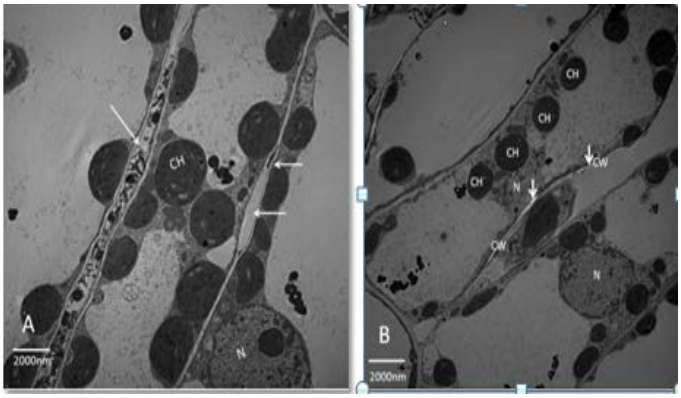

Fig. 5. (A): An electron micrograph of ultrathin section in the mesophyll layer of PVY infected leaf. The numerous chloroplasts are misshapen, the nucleus (N) in not affected and intracellular space is enlarged (arrow), containing electron dance materials and cell debris (CH= chloroplast), (B): Palisade cell containing multiple chloroplasts (CH) which seem spherical in shape. The nucleus (N) is degenerated and contains less chromatin matrix and disappearance of the nucleoli. Dissociation of the cell wall (CW) (arrows) is also remarkable.

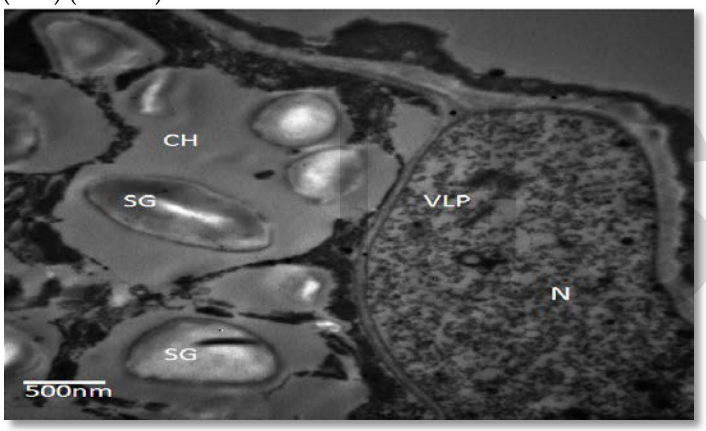

Fig. 6. Lyses of the chloroplast (CH), leaving empty structure containing only starch granules (SG). The nucleus (N) is contained virus like particles (VLP).

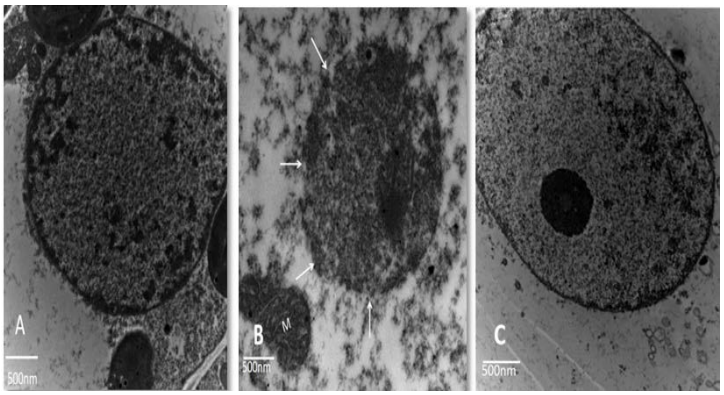

Fig. 7. (A): The nucleus of phloem parenchyma cell, the nucleoli disappeared, the chromatin is segmented, (B): the nucleus of infected parenchyma cell. The nuclear membrane disappeared in several sites (arrows) and mitochondrion (M) is degenerated, (C): nucleus fully organized in healthy plant tissue.

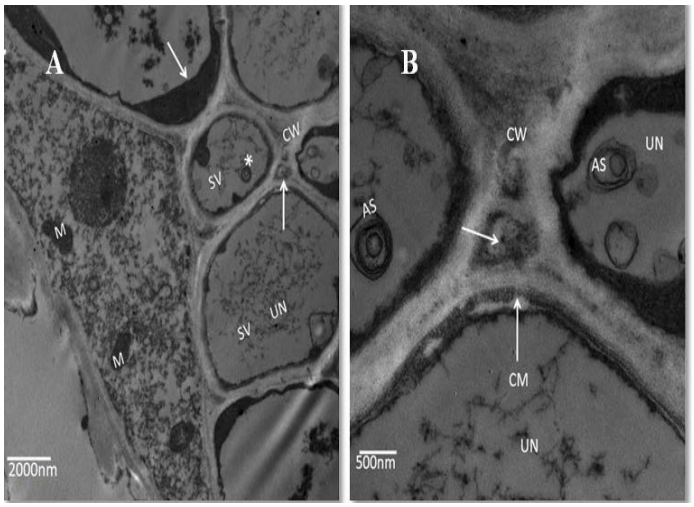

Fig. 8 (A): An overall view of phloem tissue of pepper leaf infected with PVY, showing cell wall (CW) thickening, (B): magnified port of (Fig. 11A) showing intracellular and intercellular necrotic area (arrow). Abnormal structure in the sieve elements (SV) dissociation of the cell membrane (CM) is also obvious (*). Uncapsidated virus particles (UN) are also observed in sieve elements. Loss number mitochondria (M) and degenerated.

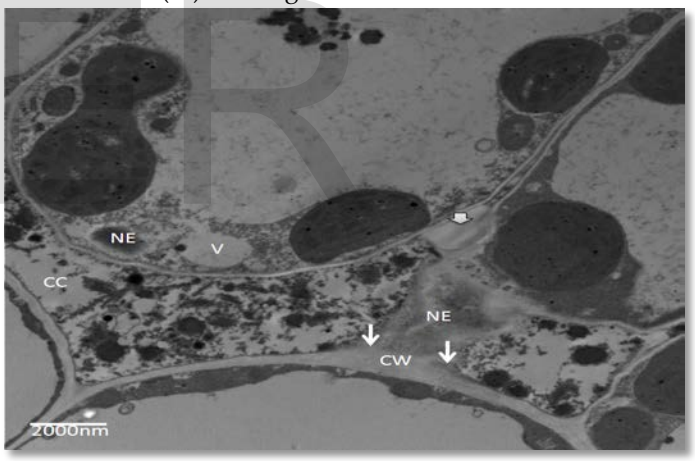

Fig. 9. Part the phloem tissue of pepper leaf infected with PVY. Necrosis (NE) of and lyses of the sieve element is clear, plasmolyses of the companion cell (CC) cell wall (CW) degradation (arrow). Cell wall invagination (head arrow), V= Value.

Molecular biological studies:

(RT-PCR):

The pepper isolate of PVY was effectively detected using RT-PCR. The primers used in this study amplified of full length of the viral coat protein gene (800 bp) of PVY isolate from pepper leaves successfully as showing in Fig. (10).

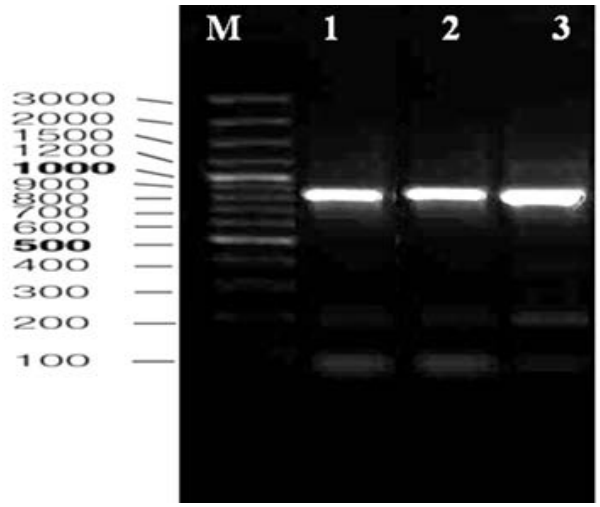

Fig. 10. Agarose gel electrophoresis showing the migration of CP gene of PVY isolate obtained by RT-PCR. M: 100bp ladder,1,2,3: pepper infected samples .

DNA sequencing and phylogenetic analysis

One of PCR positive sample was sequenced. The nucleotide sequence for the completed CDNA, of the coat protein gene was deposited in the GenBank at The DNA Data Bank of Japan (DDBJ, National Institute of Genetics, Japan) by accession no.: (LC060904.1) as the Egyptian isolate of the PVY. The sequence of PVY isolate together with the predicted partial coat protein amino acid sequence was as shown in Fig. (11). PVY isolate was compared with those of other PVY isolates available in the NCBI database with the program BLAST. The nucleotide sequences alignment in Fig. (12). showed 99% similarity with (PVYNP-Tu12.3/ AJ303095.1) isolate from Turkey, 98% similarity with (PVY-GWB5/ AY615284.2) isolate from Europe and 97% similarity with (PVY-LYE84.2/ AJ439545.1 and PVY-Foggia/ EU482153.1) respectively isolates from France and Italy.

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282 atgccaactgtgatgaatgggcttatggtttggtgcattgaaaat
    M  P  T  V  M  N  G  L  M  V  W  C  I  E  N
327 ggaacctcgccaaatgtcaacggagtttgggttatgatggatgga
    G  T  S  P  N  V  N  G  V  W  V  M  M  D  G
372 agcgaacaagttgaatatccgttgaaaccaattgttgagaatgca
    S  E  Q  V  E  Y  P  L  K  P  I  V  E  N  A
417 aaaccgacccttaggcaaatcatggcacatttctcagatgttgca
    K  P  T  L  R  Q  I  M  A  H  F  S  D  V  A
462 gaagcgtacatagaaatgcgcaataaaaaggaaccatatatgcca
    E  A  Y  I  E  M  R  N  K  K  E  P  Y  M  P
507 cgatatggtttaattcgaaatctgcgggatataagtttagcgcgc
    R  Y  G  L  I  R  N  L  R  D  I  S  L  A  R
552 tatgcctttgacttttatgaagttacatcacgaacgccagtgagg
    Y  A  F  D  F  Y  E  V  T  S  R  T  P  V  R
597 gctagggaagcgcacatacaaatgaaggccgcagcattaaaatca
    A  R  E  A  H  I  Q  M  K  A  A  A  L  K  S
642 gcccaacctcgacttttcgggttggatggtggcatcagtacacaa
    A  Q  P  R  L  F  G  L  D  G  G  I  S  T  Q
687 gaggagaacacagagaggcacaccaccgaggatgtttctccaagt
    E  E  N  T  E  R  H  T  T  E  D  V  S  P  S
732 atgcatactctacttggagtcaagaacatgtga 764
    M  H  T  L  L  G  V  K  N  M  *
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Fig. 11. The single nucleotide sequence derived of the PCR product of CP gene of PVY pepper isolate. The predicted amino acid sequence of part of the coat protein is shown below the nucleotide sequence.

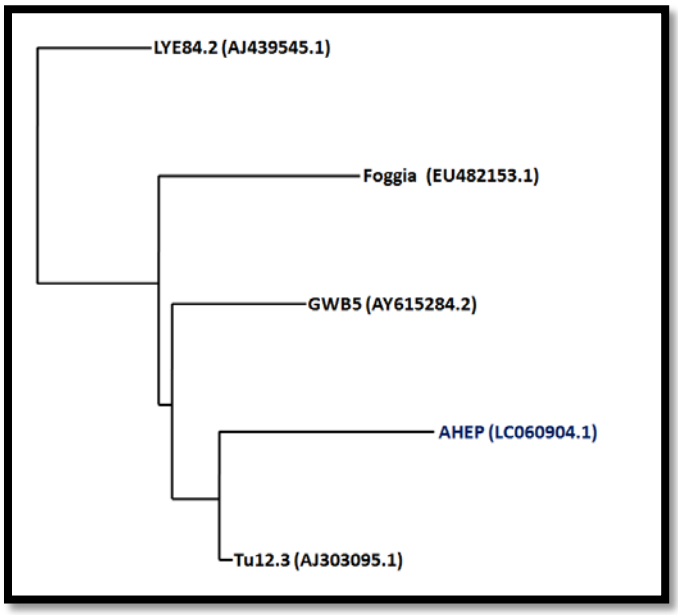

Fig. 12. Phylogenetic tree to PVY pepper isolate with those of other PVY isolates available in the NCBI database with the program BLAST. by neighbor joining method.

4 Discussion

The ongoing study is describing the presence of Potato virus Y on pepper in Egypt. This drown conclusion was built on results of comparatively extensive experiments dealing with biological, serological, molecular biological on the virus isolate under study.

Viral diseases constitute the major limiting factor in pepper cultivation throughout the world. . Forty-nine virus species have been shown to infect pepper among which about 20, belonging to 15 different taxonomic groups, have been reported to cause damages in pepper crops (Moury and Verdin, 2012).

Virus diseases are an important factor contributing to low yields and reduced fruit quality. One hundred percent losses of marketable fruit have been reported arid in some areas infection with viruses has rendered the growing of peppers uneconomical, causing whole fields to be abandoned prior to harvest. (PVY) is the most common *Potyvirus* infecting pepper. It occurs worldwide although it appears to be more important in warmer areas. Disease incidence may be as high as 100% in some areas, resulting in considerable crop loss (Green and Kim, 1991).

In the present study (PVY) was isolated from greenhouse growing pepper plants in different locations repesenting five Governorates in Egypt (Giza, Dakahlia, Qalubia, Alexandria and Ismailia). As virus can be mechanically transmitted with plant sap as it was mentioned by (kotzampigikis *et al.,* 2009 and Mehle *et al.,* 2014), this incidence might be attributed to transplants handling and closed spaces between plants.

Symptoms of PVY on pepper are mosaic, curl and malformation on the newly formed leaves symptoms as it was distribution by (Romero *et al.,* 2001; El-Araby *et al.,* 2009; Moury and Verdin, 2012 and Mostafae *et al.,* 2012).

host plants including *N. tabacum* cv. White Burley was tested in this investigation to check the mechanical transmissibility of the virus and study the different hosts reaction as a result of *Chenopodium amaranticolor* plants were used as a local lesion host for pepper isolate of PVY as well it was used for biological purification of the virus isolate. On the other hand including *N. tabacum* cv. White Burley was used as propagative host for the pepper isolate of PVY. The isolate under study didn't infect the tested potato cv. Diamond tested, so it was considered non potato isolate is confirmed by the findings of (Romero *et al.*, 2001)

By electron microscopy of dip preparation of PVY isolated of pepper is flexuous rod-shaped particles (849 nm) as distribution by (Kitajima *et al*., 1962; Mcdonald and Bancroft, 1977; Mcdonald and Kristjansson, 1993 and Moury and Verdin, 2012). The virus was found to induce cytoplasmic cylindrical inclusions consisting of pinwheels and laminated aggregates through investigation of ultrathin sections prepared from infected leaves. These findings were mentioned by (Edwardson *et al.*; 1984; Green and Kim,1991 and Kogovsek *et al.*, 2011). In the parts of inoculated leaves by PVY without spot necrosis, a decrease in size of chloroplasts From the edge to the middle of a spot necrosis, swelling of chloroplasts, loosening of thylakoid structure and changes density of chloroplasts were followed. In the middle of spot necrosis the cytoplasm of the cells was dense, no vacuoles were present in the cells, cells were shrunk, the cell wall was wrinkled and the intracellular spaces were enlarged. An increase in the number of chloroplasts per cell was observed in the green parts of yellow-green leaves (Pompe-Novak *et al.*, 2001). Changes in chloroplast structure and optically dense thickenings of outer cell wall. in the cells with well- developed pinwheels, nuclei with unusual invaginations were found. The histopathological changes in viral infected tissues depended upon the virus type, host and the time of infection. The change in the different tissues and cells organelles of PVY infected were observed as compared to healthy controls (Kogovsek *et al.*, 2011).

Numerous efforts have been initiated to characterize PVY isolates using an RT-PCR assay targeting specific areas of the genome (Piche *et al.*, 2004). There is a continuum of variants or strains that cannot be distinguished into strains and species. Recently, nucleotide sequence of the genome and amino acid sequence of the CP seem to provide suitable information for identifying strains and species in the Potyvirus group. the similarity percentage of CP amino acid sequence ranges from 90 to 99% among strains of the same species. (D'Aquino *et al.*, 1995). All pepper-infecting isolates analyzed belong to the same genetic strain (PVY^{NP}), even the PVYC1 group, a potato isolate group able to infect pepper. Pepper-PVY isolates are a single genetic strain, containing several pathotypes (Romero *et al.*, 2001). phylogenetic analysis of PVY isolates shows that pepper-PVY isolates from pepper fields has highest percentage of similarity with the non potato isolates (Mostafae *et al.*, 2012). After phylogenetic analysis it was noticed that pepper isolate has high similarity with Tu12.3 strain that was isolated from Turkey.. For this reason pepper infecting isolates could be classified within the PVY^{NP} group.

5 REFERENCES

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