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Identification and Bioinformatics Analysis of Coat Protein Gene of a Strain of Zucchini Yellow Mosaic Virus

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ABSTRACT

In this study, the focus was on the bioinformatics analysis of the coat protein (*cp*) gene of a specific Egyptian isolate of *Zucchini yellow mosaic virus* (ZYMV), which was identified based on both biological and molecular characteristics. The presence of ZYMV was confirmed in 27 squash samples using DAS-ELISA and RT-PCR techniques employing ZYU-F and ZYD1186-R primers that flank the *cp* gene of ZYMV. Transmission experiments involving mechanical and aphid transmission, specifically through *Aphis gossypii*, were carried out on various hosts, including *Chenopodium amaranticolor*, which served as an indicator necrotic local lesion host. Using light and electron microscopies, amorphous and cylindrical inclusions (pinwheels and scrolls) were observed, respectively. A partially purified virus preparation of ZYMV was obtained from infected squash leaves using a protocol involving polyethylene glycol purification and ultracentrifugation, revealing the presence of filamentous viral particles that were negatively stained with 2% uranyl acetate. Subsequently, the ZYMV-*cp* gene was amplified *via* RT-PCR for sequencing following the purification of PCR products from the agarose gel. The nucleotide sequences obtained, consisting of 831 base pairs, and encoding a 236 amino acid open reading frame, were deposited under the ID: LC778450.1 as the ZYMV-Shrouk-23 strain in GenBank. Bioinformatics analyses were conducted, comparing the Egyptian isolate to 25 overseas isolates of ZYMV. The resulting phylogenetic analysis dendrogram revealed a distinct clade with identities ranging from 91.94% to 99.16% based on nucleotide sequences and from 96.19% to 100.00% based on deduced amino acid sequences.

INTRODUCTION

Zucchini yellow mosaic virus (ZYMV) is recognized as one of the most detrimental viruses affecting cucurbit crops, significantly impacting their productivity. Various studies (Provvidenti *et al.*, 1984, Desbiez *et al.*, 2002, Tobias and Palkovics 2003, Radwan *et al.*, 2007, Khalifa *et al.*, 2015, Kheder *et al.*, 2017, Ahsan *et al.*, 2023; Ali *et al.*, 2023, Farg *et al.*, 2024) have highlighted the severe consequences of ZYMV infection on cucurbits.

The virus has been identified in key cucurbit-growing regions by researchers (Lovisolo 1979, Lisa *et al.,* 1981, Lecoq *et al.,* 1981, Desbiez and Lecoq 1997, Ahsan *et al.,* 2023, Farg *et al.,* 2024) and has been reported in various African countries including Egypt, Sudan, Tunisia, Algeria, Morocco, Madagascar, Mayotte, Mauritius, Nigeria, Reunion, South Africa, and Swaziland (Tsai *et al.,* 2010).

The impact of ZYMV on cucurbit crops can lead to substantial losses and hinder the growth of these plants in affected areas (Mostafa and Abou-Ela, 2011, Radwan *et al.,* 2007, Ahsan *et al.,* 2023, Farg *et al.,* 2024). Foliar symptoms induced by ZYMV include systemic chlorosis, mosaic patterns, severe leaf deformation, vein banding, reduced leaf laminae, and stunted plant growth (Verma *et al.,* 2004).

Transmission studies have shown that ZYMV can be mechanically transmitted using infectious sap, both in controlled settings and naturally in field conditions (Dodds *et al.,* 1984, Wong *et al.,* 1994, Pospieszny *et al.,* 2003, Khalifa *et al.,* 2015, Spadotti *et al.,* 2015, Hammad *et al.,* 2022). Furthermore, ZYMV can be efficiently transmitted in a non-persistent manner by various aphid species such as *Aphis citricola*, *A. gossypii*, *A. middletonii*, *A. craccivora*, *Acyrthosiphon p*isum, *Lipaphis eryimi*, and *Myzus persicae* (Vega *et al.,* 1995, Desbiez *et al.* ,1999). The transmission of ZYMV by aphids requires the presence of two viral proteins, namely the helper component-protease (HC-Pro) and coat protein (CP), acting through specific motifs like CP DAG motif, HC-Pro KLSC, and PTK motifs (Shabanian *et al.,* 2007, Simmons *et al.,* 2011, Zhe *et al.,* 2017).

In Egypt, several studies have been carried out on ZYMV, revealing its detrimental effects on squash (*Cucurbita pepo L.*) plants in field conditions. Researchers have observed severe symptoms such as mosaic patterns, yellowing, malformations, and stunted growth in squash plants infected with ZYMV (Abdel-Ghaffar *et al.,* 1998, Tsai *et al.,* 2010, Khalifa *et al.,* 2015, Kheder *et al.,* 2017, Mahfouzea *et al.,* 2018, Hammad *et al.,* 2022, Ali *et al.,* 2023). The presence of potyviruses, including ZYMV, has been associated with the formation of cylindrical inclusions, which serve as diagnostic markers for group-level infections (Lisa *et al.,* 1981, Shukla and Ward 1989, Vega *et al.,* 1995, Abdel-Ghaffar *et al.,* 1998). Additionally, electron microscopy studies have identified filamentous flexuous particles in partially purified ZYMV preparations (Lin *et al.,* 2002, Pospieszny *et al.,* 2003).

The ZYMV-*cp* gene was amplified by reverse transcription-polymerase chain reaction (RT-PCR), sequenced and analyzed compared to those strains or isolates documented in GenBank (Usher *et al*., 2012, Romay *et al*., 2014, Khalifa *et al*., 2015, Spadotti *et al*., 2015, Nasr-Eldin *et al*., 2016, Ali *et al*., 2023) in which identities ranging from 82 to 99% were obtained. The molecular weight of ZYMV-CP among different strains ranged from 34 to 36 kDa (Lisa *et al*., 1981, Lin *et al*., 2002).

This study aimed to conduct a bioinformatics analysis of the coat protein (*cp*) gene of a ZYMV isolate from Egypt, identified through assessments of both biological and molecular characteristics.

MATERIALS AND METHODS

1. Collection of Squash Samples:

Leaves as well as fruits of squash plants exhibiting ZYMV-like symptoms were collected from the greenhouse of Qaha Experimental Station, Agricultural Research Center (ARC), Qalyubia Governorate and from open fields of 10th of Ramadan city, Sharkia Governorate and Faculty of Agriculture, Ain Shams University (Qalyubia Governorate). Samples were separately placed into sterile paper pages and kept at 4^{o}C till use.

2.ELISA Detection of ZYMV:

The presence of ZYMV in the collected squash samples was detected by

DAS-ELISA using ZYMV-specific antiserum (Sanofi Company) according to the protocol of Abdel-Ghaffar *et al*. (1998).

3. Molecular Confirmation of ZYMV:

Molecular detection of ZYMV in the squash samples was conducted using RT-PCR. The total RNA was extracted from squash samples using a Total RNA Mini Kit (Plant)-Geneaid according to the manufacturer's instructions. Two primers specific to ZYMV-*cp* gene (ZYU-F: 5'-GCT CCA TAC ATA GCT GAG ACA GC-3' and ZYD1186-R: 5'-TAG GCT TGC AAA CGG AGT CTA ATC-3') (Choi *et al*. 2002) were used for the RT-PCR detection of ZYMV. The reaction was achieved using the Thermo Scientific™ Verso 1-Step RT-PCR and the following cycling program, 50°C for 15 min (One cycle), 95°C for 3 min (one cycle), 35 cycles each of 94°C/30 sec, 65°C/30 sec, 72°C/1 min, and a final extension cycle at 72°C for 5 min. The PCR products were electrophoresed in 1.0 % agarose gel at 60 V in Tris-acetate-EDTA (TAE) buffer containing ethidium bromide and visualized under a UV transilluminator and photographed using a mobile camera.

4. Mechanical Transmission and Differential Hosts:

The highest ELISA-positive sample which showed PCR product specific to ZYMV-*cp* gene *via* RT-PCR was selected for biological characterization of the ZYMV isolate. The infectious viral sap was prepared in 0.01M phosphate buffer, pH 7.0 containing carborundum as an abrasive material, and then some hosts including squash cv. Eskandarani, cantaloupe, watermelon, cucumber, luffa and *Chenopodium amaranticolor* grown in 10 cm-plastic pots containing sterile soils (Three replicates for each) were mechanically inoculated according to the method of Spadotti *et al*. (2015), and some plants were left without any treatments to serve as a control. All plants were kept under greenhouse conditions at 28±2°C for a maximum of 21 days for symptoms development.

5. Aphid Transmission:

According to the method of Abdel-Ghaffar *et al*. (1998), the virus was transmitted using a set of viruliferous cotton aphid (*Aphis gossypii*) to squash plants (Two plants per pot with 3-4 leaves) in insect-proof-cages under greenhouse conditions.

6. Inclusions of ZYMV:

The presence of amorphous and/or cylindrical crystalline inclusion bodies in the cytoplasm of ZYMV-infected squash leaf cells, was detected by light microscopy of stripes prepared from squash leaves infected with ZYMV and stained with 1% Trypan blue dye as described by Allam *et al*. (2000). The ultrathin sections of ZYMV-infected squash leaves were prepared as described by Abdel-Ghaffar *et al*. (1998), stained with 2% uranyl acetate and Reynold's lead citrate and followed by an examination in the transmission electron microscope (TEM) at the Electron Microscopy Unit, Department of Physics, Faculty of Science, Al-Azhar University using Jeol-JEM-2100 Plus microscope.

7. Purification and Morphology of ZYMV:

Partially purified ZYMV preparations were prepared from *Cucurbita pepo* cv. Eskandarani using polyethylene glycol (PEG) and ultracentrifugation protocol as previously reported by Abdel-Halim *et al*. (2000). The final viral pellets were resuspended in 1.0 mL of 0.01M sodium phosphate buffer, pH 7.2. Partially purified virus preparation was negatively stained with 2% uranyl acetate according to the protocol of Milne and Lesemann (1984). The grids were examined *via* TEM as abovementioned. The concentration of ZYMV particles in the partially virus purified preparation was determined based

on NanoDrop assay (Desjardins and Conklin 2010).

8. Sequencing of ZYMV-*cp* Gene:

PCR product of ZYMV-*cp* gene, amplified *via* RT-PCR using ZYU-F and ZYD1186-R primers, were cut and purified from the agarose gel according to the manufacturer's protocol of GeneDirex PCR Clean-Up & Gel Extraction Kit (NA006-0100). Using the Genetic Analyzer (ABI Prism 310, version 3.4, Semi Adaptive, version 3.2), the nucleotide sequences of ZYMV-*cp* gene were determined.

9. Bioinformatics analysis of ZYMV-*cp* gene:

The DNA sequence was translated to amino acids using the Sequence Manipulation Suite (SMS) program (https://www.bioinformatics.org/sms2/orf_find.html). Nucleotide sequences of ZYMV-*cp* gene and its deduced amino acids were aligned and compared to the most similar isolates or strains of ZYMV documented in GenBank using Clustal Omega Multiple Sequence Alignment (MSA) (https://www.ebi.ac.uk/jdispatcher/msa/clustalo). The comparison was based on Jotun Hein's algorithms (Hein 1990) and the method given by Higgins and Sharp (1989). The nucleotide sequences were documented in GenBank among the DDBJ Annotated/Assembled Sequences (https://www.ddbj.nig.ac.jp/ddbj/submission.html).

RESULTS AND DISCUSSION

1. Detection of ZYMV in Collected Squash Samples Using ELISA and RT-PCR Techniques:

A number of 27 squash samples (22 leaves and 5 fruits) exhibited virus-like symptoms were collected from two different environments, *i.e*., greenhouse (16 samples) and open field (11 samples) conditions (Table 1) (Fig. 1 A, B, C). Collected leaf samples showed different symptoms including mosaic, severe mosaic, vein banding, yellows, leaf deformation, blisters, rolling, and filiform shapes. On the other hand, fruits showed mottling, severe mottling and blisters. Only four-leaf samples exhibited single symptom (QaL-01, QaL-02, QaL-07 and FAL-02). Results in Fig. (1 D, E) showed that the squash fruits exhibited mottling and blisters were economically infeasible fruits. These results were in harmony with those reported by different investigations in which ZYMV causes destructive diseases to zucchini squash (*Cucurbita pepo*) in addition to a large number of cucurbitaceous plants (Lisa *et al*. 1981, Greber *et al*. 1988, Abdel-Ghaffar *et al*. 1998, Dukić *et al*. 2002, Radwan *et al*. 2007, Mostafa and Abou-Ela 2011, Khalifa *et al*. 2015, Spadotti *et al*. 2015, Ahsan *et al*. 2023, Ali *et al*. 2023, Farg *et al*. 2024).

The presence of ZYMV in the 27 collected squash samples was detected *via* DAS-ELISA using a specific antiserum (Table 1). At the level of fruit samples, four out of the five samples representing 80% were produced from ZYMV-infected squash plants. It was noted that 12 (54.55%) out of 22 leaf samples showed ELISA positive values indicating its infection with ZYMV, and variation in ELISA values could reflect the virus concentrations in leaf samples.

Table 1: Symptomatology of squash samples collected from greenhouse and open field environments exhibiting ZYMV-like symptoms and the presence of ZYMV in each sample as confirmed *via* DAS-ELISA and RT-PCR.

Qalyubia Governorate					
Greenhouse (Leaves)					
Codes	**Sources**	**Symptoms**	**ELISA Detection**		**RT-PCR detection**
			Values	**Results**	
S01	QaL-01	Blisters	0.341	-	-
S02	QaL-02	Mosaic	0.361	-	-
S03	QaL-03	Yellows	0.236	-	-
S04	QaL-04	Mosaic, Blisters	0.652	+	+
S05	QaL-05	Mosaic, Blisters	0.717	+	+
S06	QaL-06	Mosaic, Rolling	0.233	-	-
S07	QaL-07	Mosaic, Leaf deformation	0.523	+	+
S08	QaL-08	Mosaic, Leaf deformation	0.460	-	+
S09	QaL-09	Mosaic, Blisters, Vein banding	0.983	+	+
S10	QaL-10	Mosaic, Blisters, Leaf deformation	0.789	+	+
S11	QaL-11	Mosaic, Blisters, Leaf deformation	0.663	+	+
S12	QaL-12	Mosaic, Severe Blisters	0.834	+	+
S13	QaL-13	Severe Mosaic, Severe Blisters	0.955	+	+
Greenhouse (Fruits)					
S14	QaF-14	Mottling, Blisters	0.533	+	+
S15	QaF-15	Mottling, Blisters	0.692	+	+
S16	QaF-16	Mottling, Blisters, Crinkling	0.788	+	+
Open fields (Leaves)					
S17	FAL-01	Mosaic	0.459	-	+
S18	FAL-02	Mosaic, Blisters	0.462	-	+
S19	FAL-03	Yellows, Rolling	0.273	-	-
Sharkia Governorate					
Open field (Leaves)					
S20	10^{th}RL-01	Blisters, Filiform shape	1.109	+	+
S21	10^{th}RL-02	Mosaic, Blisters	0.412	-	-
S22	10^{th}RL-03	Mosaic, Blisters	0.288	-	-
S23	10^{th}RL-04	Mosaic, Blisters	0.887	+	+
S24	10^{th}RL-05	Mosaic, Leaf deformation	0.563	+	+
S25	10^{th}RL-06	Mosaic, Blisters, Filiform shape	0.989	+	+
Open field (Fruits)					
S26	10^{th}RF-07	Mottling	0.356	-	-
S27	10^{th}RF-08	Severe Mottling, Blisters	1.076	+	+
Negative control		Healthy	0.232	-	-

QaL: Qalyubia leaf. QaF: Qalyubia fruit. FAL: Faculty of Agriculture leaf. 10^{th}RL: 10^{th} of Ramadan leaf. 10^{th}RF: 10^{th} of Ramadan fruit.

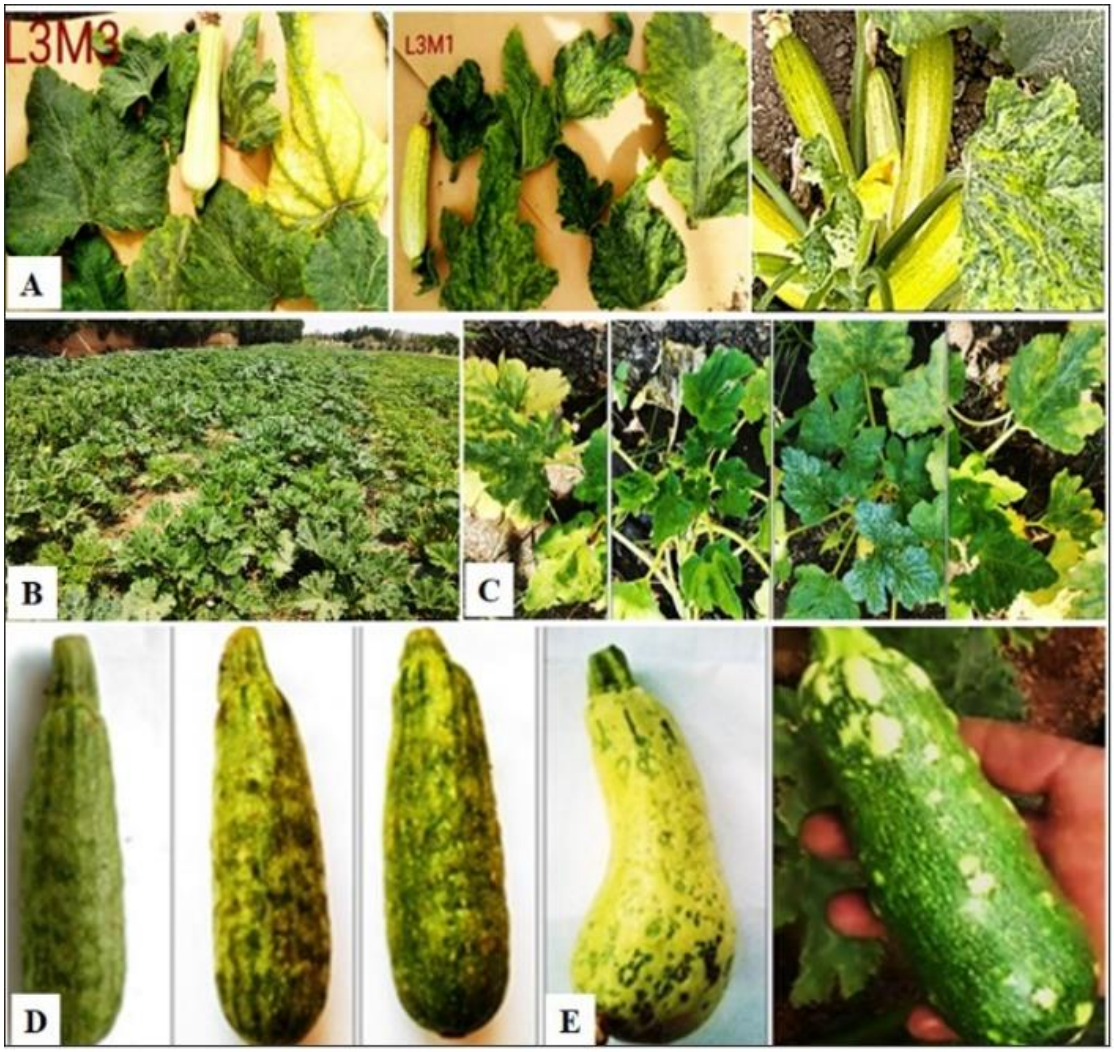

Fig. 1: Samples of squash leaves and fruits collected from greenhouse of Qaha (A), 10th of Ramadan land (B), open field at Faculty of Agriculture (C), squash fruits from greenhouse of Qaha (D) and squash fruits from 10th of Ramadan land (E) exhibiting ZYMV-like symptoms.

Regarding RT-PCR detection of ZYMV in the squash samples, results in Table 1 and supported by Fig. (2) showed that all positive-ELISA samples appeared positive when subjected to RT-PCR detection. Interestingly, three negative-ELISA samples (QaL-12 FAL-01 and FAL-02) with ELISA values of 0.460, 0.462 and 0.459, respectively, showed RT-PCR positive results. Similar observations were reported by Bhardwaj and Kulshrestha (2020) who showed that PCR and its various types, *i.e.*, RT-PCR and immunocapture RT-PCR have been proven to be more sensitive than ELISA technique in detecting plant viruses.

ZYMV was successfully detected *via* ELISA in different cucurbits including squash (Zhe *et al*. 2017), zucchini (Ali *et al*. 2023), melon (Al-Musa, 1989), watermelon (Dietzgen and Herrington, 1991), cucumber (Asad *et al*. 2019) and in different infected plants (Sako *et al*. 1989, Tsai *et al*. 2010, Usher *et al*. 2012). RT-PCR was also applied for detecting the presence of ZYMV in different plant samples based on primers flanking the *NIb* and *cp* genes (Thomson and Dietzgen, 1995, Lin *et al*. 1998, Hosseini *et al*. 2007, Shabanian *et al*. 2007, Romay *et al*. 2014) or *cp* and part of *CI* protein genes of ZYMV (Pfosser and Baumann 2002) or part of 3'-untranslated region (3′-UTR) and the entire *cp* gene (Nasr-Eldin *et al*. 2016) and *P1* gene (Ali *et al*. 2023).

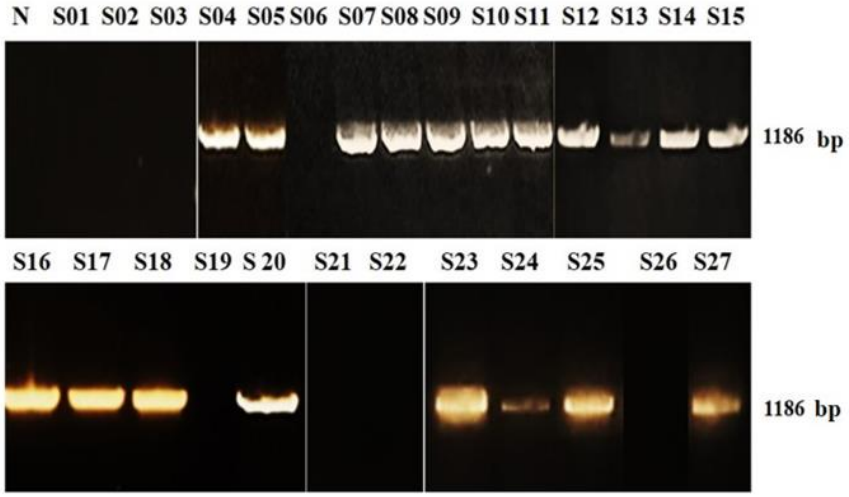

Fig. 2: RT-PCR detection of ZYMV in ELISA tested squash samples exhibiting ZYMV-like symptoms collected from greenhouse and open fields. N: Negative control (PCR mixture without any template). (S01-S27, Squash samples).

2. Symptomatology and Differential Host:

Results in Table 2 showed the reactions of different tested hosts to ZYMV infection. Symptoms included severe systemic symptoms (Mosaic, vein banding, blisters, leaf deformation, yellows, filiform shape) on cantaloupe (Fig. 3A), watermelon (Fig. 3B), cucumber (Fig. 3C) and luffa (Fig. 3D). Whereas local symptoms in the form of necrotic local lesions appeared on *Chenopodium amaranticolor* (Fig. 3E). Results in Fig. (4) Showed the stages of symptoms development on squash plant cv. Eskandarani 7, 10-, 13-, 16- and 21-days post inoculation with ZYMV-infectious sap under greenhouse conditions. These symptoms gradually developed from mosaic up to filiform shape. These results are in agreement with those reported by several investigations biologically identified ZYMV isolates affecting different cucurbits and differential hosts (Hseu *et al*. 1985, Vega *et al*. 1995, Tsai *et al*. 2010, Usher *et al*. 2012, Romay *et al*. 2014, Khalifa *et al*. 2015, Nasr-Eldin *et al*. 2016, Mahfouzea *et al*. 2018, Hammad *et al*. 2022, Ali *et al*. 2023). They also revealed that ZYMV induced chlorotic local lesions on *Gomphrena globose*, *Ch. quinoa*, *Ch. amaranticolor*. While, Huang *et al*. (1986), Vega *et al*. (1995), Hosseini *et al*. (2007) and Nasr-Eldin *et al*. (2016) showed that necrotic local lesions were induced on the leaves of *Ch. amaranticolor*.

Table 2: Reaction of different hosts to ZYMV infection under greenhouse conditions.

Hosts	Reactions	Descriptions
Squash cv. Eskandarani	Severe systemic symptoms	Mosaic, vein banding, blisters, leaf deformation, yellows, filiform shape
Cantaloupe	Severe systemic symptoms	Mosaic, vein banding, blisters, leaf deformation
Watermelon	Severe systemic symptoms	Mosaic, vein banding, blisters,
Cucumber	Severe systemic symptoms	Mosaic, blisters, leaf deformation
Luffa	Severe systemic symptoms	Mosaic, vein banding, blisters, leaf deformation
Chenopodium amaranticolor	Local symptoms	Necrotic local lesions

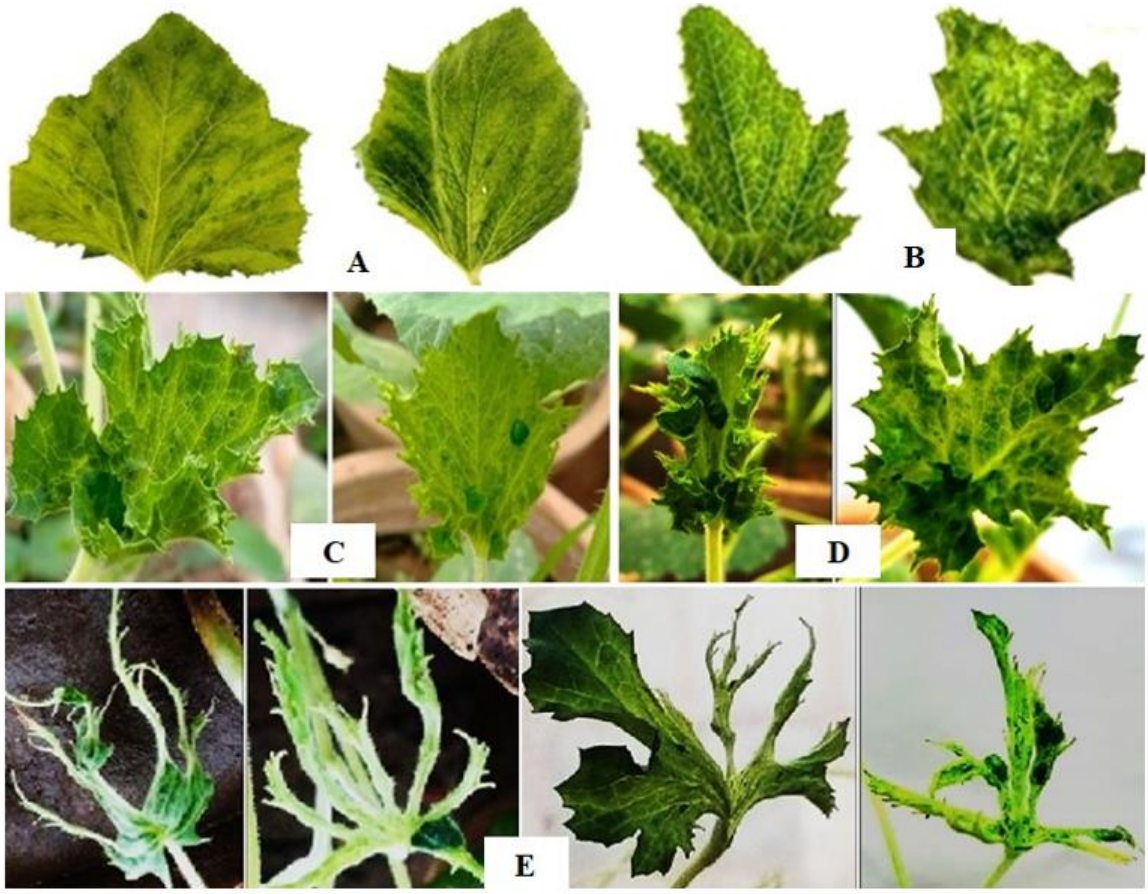

Fig. 3: Leaves of Cantaloupe (A), Watermelon (B), Cucumber (C), Luffa (D) and *Chenopodium amaranticolor* 15,10, 15, 15 and 10 days post mechanical inoculation with ZYMV infectious sap, respectively, under greenhouse conditions.

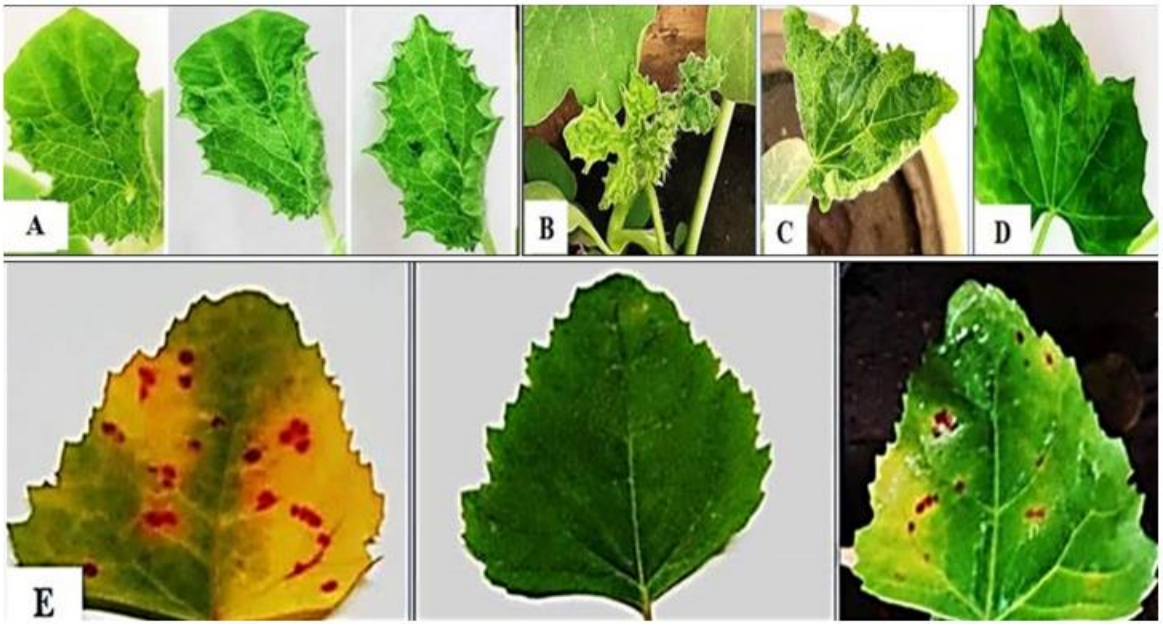

Fig. 4: Squash leaves cv. Eskandarani mechanically inoculated with ZYMV infectious sap 7 (A), 10 (B), 13 (C), 16 (D) and 21 (E) days post inoculation under greenhouse conditions. Note the development of symptoms starting from mosaic up to filiform shape.

3. Insect Transmission:

In this study, ZYMV was successfully transmitted in a nonpersistent manner to squash (Fig. 5) *via* cotton aphid (*Aphis gossypii*). Symptoms developed on plants 7-10 days post insect ZYMV-feeding. The experimental results are in agreement with those reported by several studies (Greber *et al*. 1988, Abdel-Ghaffar *et al*. 1998, Mahgoub *et al*. 1998, Shabanian *et al*. 2007).

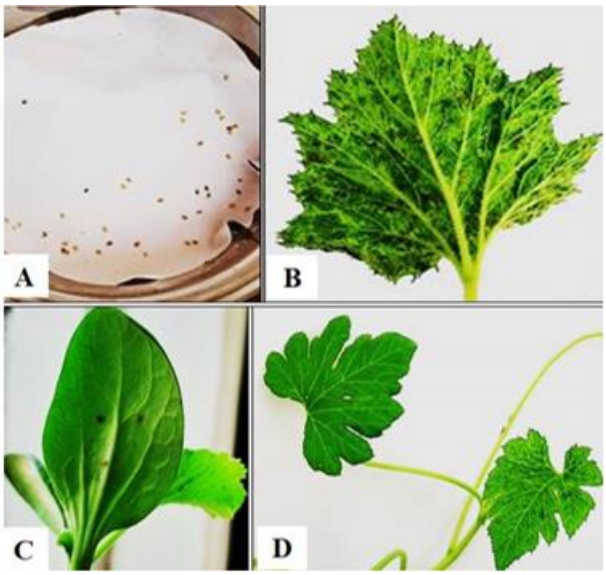

Fig. 5: Stages of insect transmission of ZYMV on squash using cotton aphid (*Aphis gossypii*). A): Starving stage, B): Feeding on ZYMV-infected leaf, C): Feeding on cotyledon of healthy plant and D) mosaic symptoms 10 days post insect inoculation of ZYMV.

4. Inclusion Bodies of ZYMV:

Results in Fig. (6) showed that light microscopy of ZYMV-infected squash stripes revealed that the virus-induced amorphous and proteinous crystalline inclusions in the cytoplasm of infected squash leaves stained with 1% Trypan blue dye 10 days post inoculation with virus-containing infectious sap. Transmission electron microscopy of ultrathin sections of ZYMV-infected squash leaves showed the presence of cylindrical inclusions belonging to subdivision I (pinwheels (Fig. 7A) and scroll (Fig. 7B)) in the infected cells. Interestingly, filamentous virus-like particles also occurred close to the scroll inclusions (Fig. 8) in the cytoplasm near the mitochondria.

These results agree with that reported by Suzuki *et al*. (1990) in which ZYMV produced amorphous inclusion and cylindrical inclusion proteins in *Cucurbita maxima* leaf tissues. Wong *et al*. (1994), Vega *et al*. (1995), Abdel-Ghaffar *et al*. (1998), Usher *et al*. (2012), and Zellnig *et al*. (2014) found that ZYMV-induced cytoplasmic pinwheels and scrolls in plant infected tissues as appeared by transmission electron microscopy. ZYMV induces CIs of subdivision I according to the classification of Edwardson and Christie (1978). Some isolates were found to induce CIs of types III and IV (Edwardson and Christie 1996).

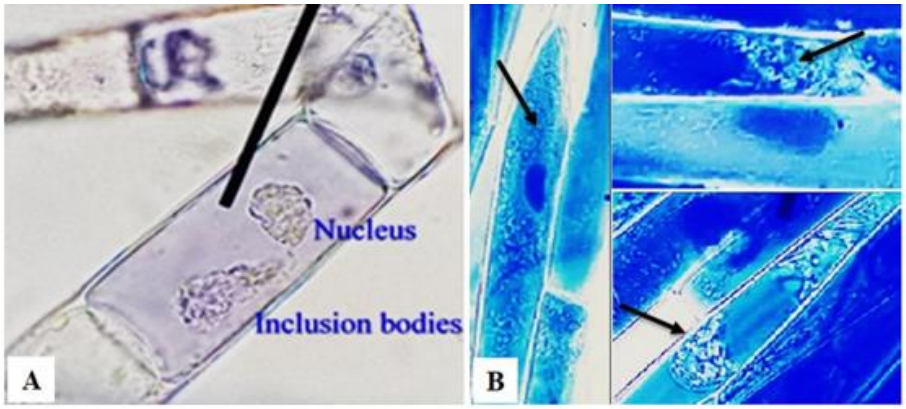

Fig. 6: (A) Amorphous inclusion bodies and (B) cylindrical crystalline inclusions (**Arrow**) induced in the cytoplasm of ZYMV-infected squash leaf cells, stained with 1% Trypan blue dye. Nucleus appeared with dark blue color.

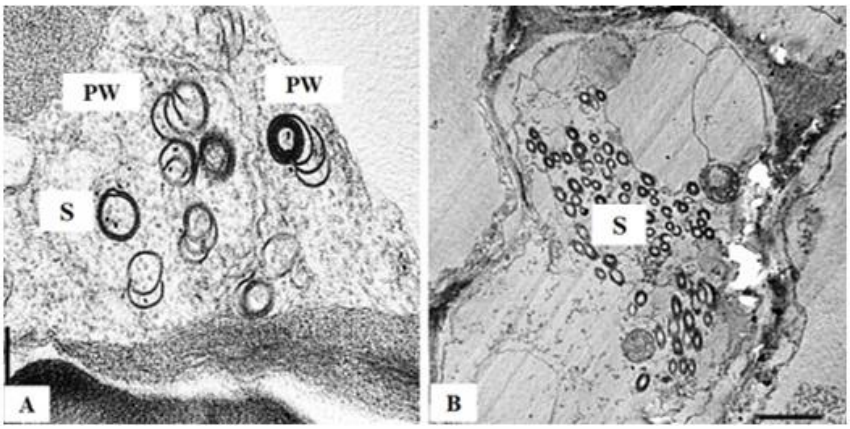

Fig. 7: Electron micrograph shows ultrastructure of ZYMV-infected squash plant leaves. Note presence of pinwheels (PW) (A) and scroll (S) (A&B) cylindrical inclusions characteristic to subdivision I of potyviruses.

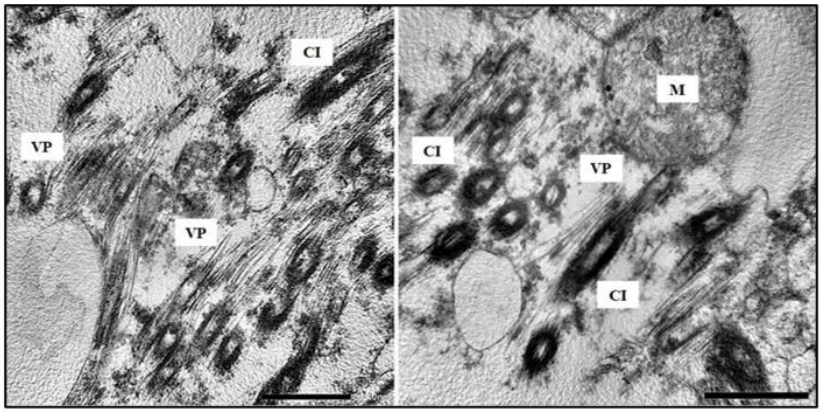

Fig. 8: Electron micrograph shows ultrastructure of ZYMV-infected squash plant leaves. Note presence of virus particles (VP) associated with cylindrical inclusions (CI) which appeared as scroll close to mitochondria (M).

5. Purification and Morphology of ZYMV:

Flexuous filamentous virus particles of length ranged from 725 to 750 nm and width from 11 to 13 nm appeared in aggregates when negatively stained with 2% uranyl acetate (Fig. 9). Based on the NanoDrop spectrophotometer

measurement (Fig. 10) the yield of purified virus ranged from 11-12 mg/100 g of ZYMV-infected squash leaves. Similar results were also recorded by Abdel Ghaffar *et al*. (1998) who stated that potyviruses including ZYMV had filamentous particles ranging from 700 to 900 nm in length. Also, Nasr-Eldin *et al.* (2016) used transmission electron microscopy to determine the morphology of ZYMV in purified virus preparation obtained from ZYMV-infected squash plants and filamentous virus-like particles measuring 750x13 nm were present.

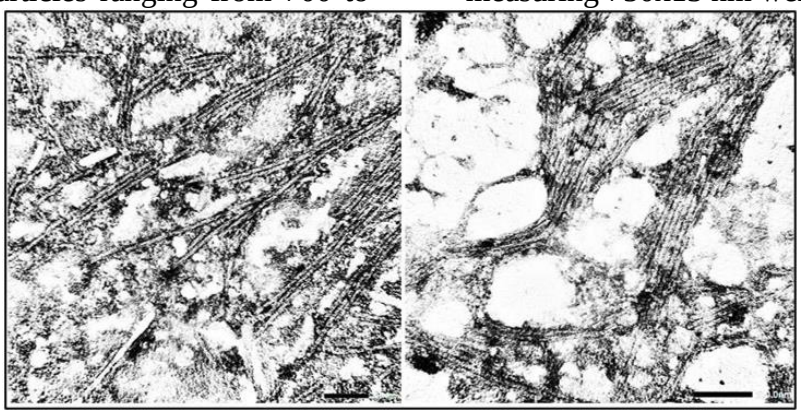

Fig. 9: Electron micrographs show filamentous viral particles negatively stained with 2% uranyl acetate in the partially purified virus preparation.

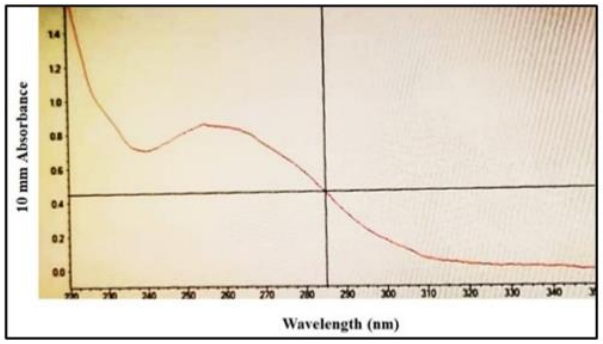

Fig. 10: Nanodrop assay of purified virus preparation.

6. Sequencing of ZYMV-*cp* Gene and Its Bioinformatics Analysis:

The PCR product of ZYMV-*cp* gene was cleaned (eluted) from agarose gel (Fig. 11) to determine its nucleotide sequences. A DNA fragment of 831 nucleotides was sequenced as shown in Fig. (12) and found to contain an open reading frame in sense orientation (Document in GenBank, ID: LC778450.1) which was deduced to 236 amino acids of ZYMV *cp* gene of Shrouk-23 strain plus a stop codon (Fig. 13).

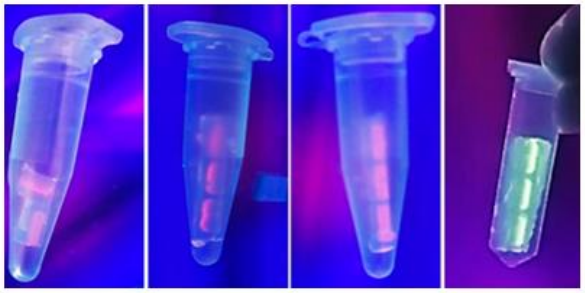

Fig. 11: Extraction of PCR product of ZYMV-*cp* gene for determination of its nucleotide sequences.

```
  1 CCATACTTAC CTGGGACAGC ACTGCGTAGG TGATGCACTG ACAAGGGAGC AGATACAAGT
 61 GAACTGGCAC GCTACCTACA AGCCCTCCAT CAAGACATCT TCTTTGAACA AGGAGACACT
121 GTGATGCTCC AATCAGGCAC TCAGCCAACT GTGGCAGATG CTGGAGCTAC AAAGAAAGAC
181 AAAGAAGATG ACAAAGGGAA AAACAAGGAC GTTACAGGCT CTGGCTCAGG TGAGAAAACA
241 GTAGCAGCTG TCACGAAGGA CAAGGATGTG AATGCTGGTT CTCATGGGAA AATTGTGCCG
301 CGTCTTTCGA AGATCACAAA GAAAATGTCA TTGCCACGCG TGAAAGGAAA TGTGATACTC
361 GATATTGATC ATTTGCTGGA ATATAAACCG GATCAAATTG AGTTATATAA CACACGAGCG
421 TCTCATCAGC AGTTCGCCTC TTGGTTCAAC CAGGTTAAGA CGGAATATGA TTTGAACGAG
481 CAACAGATGG GAGTTGTAAT GAATGGTTTC ATGGTTTGGT GTATTGAAAA TGGCACTTCA
541 CCCGACATTA ATGGAGTGTG GGTTATGATG GACGGAAATG AGCAAGTTGA GTATCCCTTG
601 AAACCAATAG TTGAAAATGC AAAGCCAACG CTGCGGCAAA TAATGCATCA TTTTTCAGAT
661 GCAGCGGAGG CATATATAGA GATGAGAAAT GCAGAGGCAC CATACATGCC GAGGTATGGT
721 TTGCTTCGAA ACCTACGGGA TAGGAGTTTA GCACGATATG CTTTCGATTT CTATGAAGTC
781 AATTCTAAAA CTCCTGAAAG AGCCCGCGAA GCTGTTGCGC AGATGAAAGC A
```

Fig. 12: Partial nucleotide sequence of Zucchini yellow mosaic virus Shrouk-23 *cp* gene, documented in GenBank as ID: LC778450.1, Length: 831 nts.

```
  1 ATGCTCCAATCAGGCACTCAGCCAACTGTGGCAGATGCTGGAGCT
     M  L  Q  S  G  T  Q  P  T  V  A  D  A  G  A
 46 ACAAAGAAAGACAAAGAAGATGACAAAGGGAAAAACAAGGACGTT
     T  K  K  D  K  E  D  D  K  G  K  N  K  D  V
 91 ACAGGCTCTGGCTCAGGTGAGAAAACAGTAGCAGCTGTCACGAAG
     T  G  S  G  S  G  E  K  T  V  A  A  V  T  K
136 GACAAGGATGTGAATGCTGGTTCTCATGGGAAAATTGTGCCGCGT
     D  K  D  V  N  A  G  S  H  G  K  I  V  P  R
181 CTTTCGAAGATCACAAAGAAAATGTCATTGCCACGCGTGAAAGGA
     L  S  K  I  T  K  K  M  S  L  P  R  V  K  G
226 AATGTGATACTCGATATTGATCATTTGCTGGAATATAAACCGGAT
     N  V  I  L  D  I  D  H  L  L  E  Y  K  P  D
271 CAAATTGAGTTATATAACACACGAGCGTCTCATCAGCAGTTCGCC
     Q  I  E  L  Y  N  T  R  A  S  H  Q  Q  F  A
316 TCTTGGTTCAACCAGGTTAAGACGGAATATGATTTGAACGAGCAA
     S  W  F  N  Q  V  K  T  E  Y  D  L  N  E  Q
361 CAGATGGGAGTTGTAATGAATGGTTTCATGGTTTGGTGTATTGAA
     Q  M  G  V  V  M  N  G  F  M  V  W  C  I  E
406 AATGGCACTTCACCCGACATTAATGGAGTGTGGGTTATGATGGAC
     N  G  T  S  P  D  I  N  G  V  W  V  M  M  D
451 GGAAATGAGCAAGTTGAGTATCCCTTGAAACCAATAGTTGAAAAT
     G  N  E  Q  V  E  Y  P  L  K  P  I  V  E  N
496 GCAAAGCCAACGCTGCGGCAAATAATGCATCATTTTTCAGATGCA
     A  K  P  T  L  R  Q  I  M  H  H  F  S  D  A
541 GCGGAGGCATATATAGAGATGAGAAATGCAGAGGCACCATACATG
     A  E  A  Y  I  E  M  R  N  A  E  A  P  Y  M
586 CCGAGGTATGGTTTGCTTCGAAACCTACGGGATAGGAGTTTAGCA
     P  R  Y  G  L  L  R  N  L  R  D  R  S  L  A
631 CGATATGCTTTCGATTTCTATGAAGTCAATTCTAAAACTCCTGAA
     R  Y  A  F  D  F  Y  E  V  N  S  K  T  P  E
676 AGAGCCCGCGAAGCTGTTGCGCAGATGAAAGCA
    R  A  R  E  A  V  A  Q  M  K  A  *
```

Fig. 13: ORF1 CDS translation of ZYMV *cp* gene of Shrouk-23 strain.

Data in Table 3 shows the identities percent of ZYMV *cp* gene partial nucleotide sequence and deduced amino acids of Shrouk-23 strain (Acc. LC778450.1) compared to 25 isolates or strains isolated from different hosts of several countries including Egypt as documented in GenBank. Identities based on nucleotide and deduced amino acid sequences ranged from 91.94 to 99.16% and from 96.19 to 100.00%, respectively. ZYMV-Shrouk-23 strain showed the highest identities of 99.16 and 100.00% at the level of nucleotides and amino acid, respectively, when compared to the DSMZ PV-1383 strain of France (OQ847410.1) isolated from *Cucurbita pepo*. When three Egyptian strains named Qalyubia-EG (MG021246.1 & AUI80735.1), ZYMV-Sohag-Eg-2 (MK457355.1&QEE84036.1), Giza-Almansouria (OM925548.1 & WAW38338.1) isolated from *Cucurbita pepo*, and Egz4 strain (MT383107.1 & QNS28121.1) obtained from zucchini compared to ZYMV-Shrouk-23 strain (Acc. LC778450.1) isolated from *Cucurbita pepo* L., identities ranging from 94.39 to 98.28 and from 97.00 to 100.00% based on

nucleotides and amino acids, respectively, were recorded. Dendrograms in Fig. (14) and Fig. (15) show the genetic relationship between the partial nucleotide sequence of ZYMV *cp* gene and its deduced amino acids of Shrouk-23 strain (Acc. LC778450.1) and 25 different isolates or strains from different hosts of some countries including Egypt.

Table 3. Identities percent of ZYMV *cp* gene partial nucleotide sequence and deduced amino acids of Shrouk-23 strain (Acc. LC778450.1) compared to 25 different isolates or strains isolated from different hosts and locations including Egypt as documented in GenBank.

Isolates or Strains	Countries	Hosts	Nucleic acid		Amino acids	
			Accession	Identities (%)	Accession	Identities (%)
Nt-5	Australia	*Cucumis melo*	MN598576.1	97.95	QID92252.1	99.15
R-111	Canada	Zucchini	OK558794.1	97.59	UZP17463.1	98.73
CH99/116	China	*Cucurbita maxima*	AY611021.1	96.27	AAT47153.1	98.73
Cha-Zuc	China	*Cucurbita pepo*	KU366270.1	97.95	ANN89692.1	99.15
Qalyubia-EG	Egypt	*Cucurbita pepo*	MG021246.1	94.56	AUI80735.1	99.57
ZYMV-Sohag-Eg-2	Egypt	*Cucurbita pepo*	MK457355.1	94.39	QEE84036.1	99.49
Egz4	Egypt	Zucchini	MT383107.1	98.28	QNS28121.1	97.00
Giza-Almansouria	Egypt	*Cucurbita pepo*	OM925548.1	94.42	WAW38338.1	100.0
124L11	France	Zucchini	JN861009.1	92.45	AFB82651.1	97.01
DSMZ PV-1383	France	*Cucurbita pepo*	OQ847410.1	99.16	WIW79928.1	100.0
10	Hungary	*Cucumis sativus*	AJ251527.1	93.02	CAB63753.1	97.88
ZYMV-Iq	Iraq	Zucchini	MT882336.1	97.95	QWL14830.1	100.0
DSMZ PV-0416	Italy	*Cucurbita pepo*	OQ335839.1	97.71	WIW79771.1	100.0
11spno3	Mali	*Cucumis sativus*	HM005309.1	97.11	ADG01846.1	100.0
14spno1-5	Mali	Watermelon	HM005312.1	97.23	ADG01849.1	100.0
---	New Zealand	Zucchini	AY995216.1	91.94	AAX89507.1	96.19
TV1	South Africa	*Cucurbita moschata*	KJ789918.1	98.29	AII82115.1	98.73
Sud.C.20	Sudan	*Cucurbita maxima*	KC695817.1	93.98	AGS48096.1	98.91
SYZY-3	Syria	*Cucurbita pepo*	AB458596.1	93.50	BAH97118.1	100.0
23-8ZYMV	Syria	Zucchini	MK606175.1	93.62	QJB23284.1	100.0
E-7	Turkey	*Cucurbita pepo*	KP872578.1	93.99	AKZ42412.1	100.0
S5	Turkey	*Cucumis melo*	KP872581.1	94.42	AKZ42415.1	100.0
DSMZ PV-1261	UK	*Cucurbita pepo*	OM471983.1	98.44	UOF93247.1	99.58
G72	USA	*Cucurbita pepo*	JN192417.1	97.59	AFK23486.1	97.46
1st	USA	*Cucurbita pepo*	JN192428.1	98.07	AFK23497.1	99.15

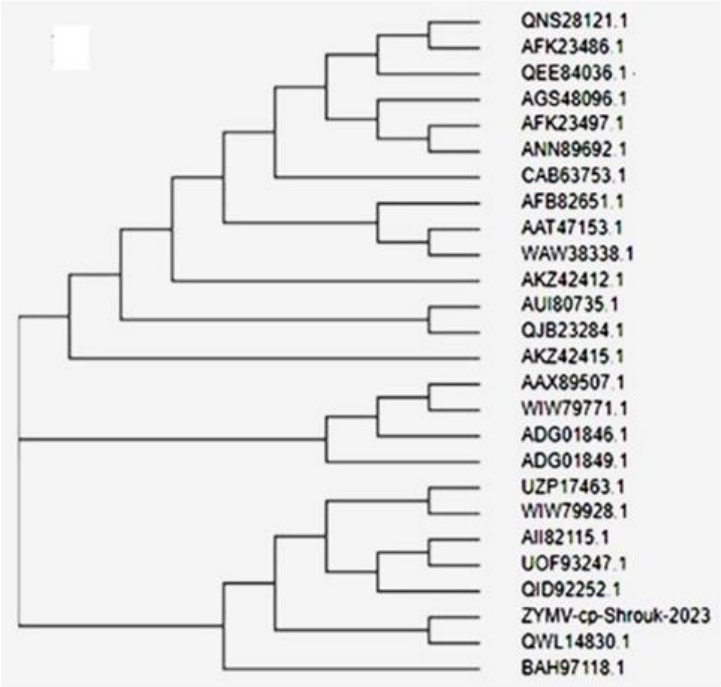

Fig. 14: A dendrogram shows the genetic relationship between the partial sequence of ZYMV *cp* gene of ZYMV-*cp*-Shrouk-2023 strain and different isolates or strains isolated from different hosts of several countries including Egypt.

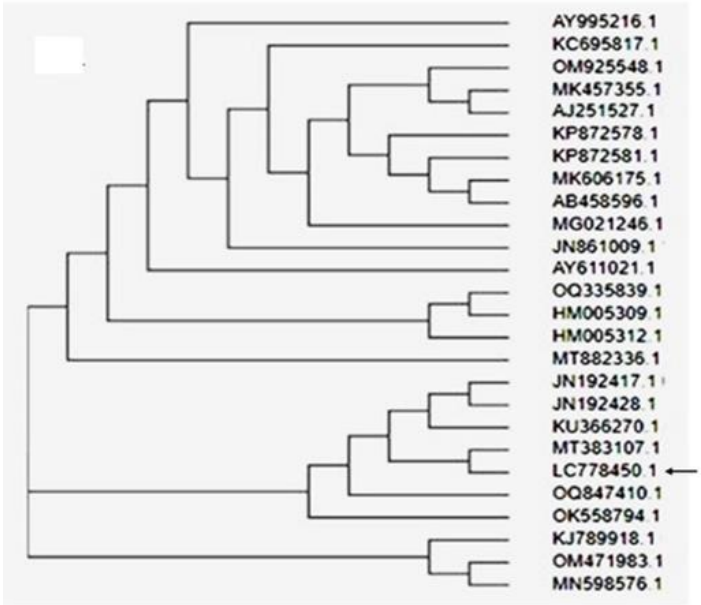

Fig. 15: A dendrogram shows the genetic relationship between the deduced amino acids from the partial sequence of ZYMV *cp* gene Shrouk-23 strain (LC778450.1, **Arrow**) and different isolates or strains isolated from different hosts and countries.

Results of bioinformatics of the ZYMV-*cp* gene in this study are in agreement with that of Kwon *et al.* (2005) who reported that the nucleotide sequences of ZYMV-PE, ZYMV-PA and ZYMV-PS showed identities of more than 96.0% and 98.1% at the levels of nucleotide sequences and deduced amino acid. Also, Coutts *et al*. (2011) determined the nucleotide sequences of *cp* gene in 42 Australian isolates. When the nucleotide sequences were compared to 101 ZYMV sequences documented in GenBank, three distinct groups with four and two subgroups

according to collection location were found. Identities ranging from 94.6 to 99.0% were found between the three sequences from the Northern Territory and isolates from the United States, China, Japan and Iran. In addition, Usher *et al*. (2012) sequenced the partial coat protein gene of the ZYMV-KZN isolate which revealed 95–98% sequence identity when compared to isolates of central Europe and the Indian subcontinent. While 90–93% sequence identity was obtained when compared to isolates from Taiwan and Singapore. Spadotti *et al*. (2015) showed that variability of the nucleotide sequence of *cp* gene ranged from 82 to 99 % when compared to different ZYMV isolates from different geographical locations.

Conclusion

ZYMV was detected in various squash samples, including leaves and fruits, using DAS-ELISA and RT-PCR. RT-PCR has proven higher sensitivity compared to ELISA. An isolate was selected and underwent comprehensive biological identification. Within the cytoplasm of infected cells, amorphous and cylindrical inclusions characteristic of subdivision I of potyviruses were observed. Filamentous virus particles were partially purified with a satisfactory yield. The cp gene was isolated via RT-PCR, purified, and sequenced. The sequenced gene comprised 831 nucleotides, encoding 236 amino acids, and was deposited in GenBank as the Shrouk-23 strain (Acc. LC778450.1). Bioinformatics analyses were conducted on both the nucleotide and amino acid sequences, comparing them to 25 strains from overseas documented in GenBank, and their identities were determined.

Declarations:

Ethical Approval: It is not applicable.

Conflicts of Interest: The author declares no conflicts of interest.

Authors Contributions: All authors were responsible for the study design, experiment execution, data analysis, and manuscript drafting.

Funding: No funding was received.

Availability of Data and Materials: All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

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