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Molecular Identification and Analysis of Coat Protein Gene of *Cucucmber mosaic cucumovirus* Sugar Beet Egyptian Isolate

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ABSTRACT

Cucumber mosaic cucumovirus beet Egyptian isolate (CMV-Beet-EG) was detected serologically by DAS-ELISA using polyclonal antibodies in naturally infected sugar beet plants. The virus was isolated by single local lesion method on *Chenopodium amaranticolor* and then, propagated on sugar beet plants. The virus isolate was confirmed serologically by using dot and tissue printing immunoassay. The coat protein gene of CMV-Beet-EG was successfully amplified using specific primer by RT-PCR and the expected size was 820 bp. Sequence analysis cp gene revealed 97.3 and 96.4% identified similarity with CMV strains sequences published in GeneBank. The highest content of nitrogen bases was for Thymine (T) 164 (26.5%) followed by Cytosine (C) 163 (26.3%), then Adenine (A) 148 (23.9%) and Guanine (G) 144 (23.3%). The ratio of A/T and G/C were 0.902 and 0.883, the percentage of A+T and G+C was found 50.4 and 49.6. The deduced amino acids sequence of CMV-Beet-EG/CP gene was 206 amino acids with a molecular weight of 22.983 kDa and isolelectric point of 9.95. There are total 20 amino acids, the amino acid Serine (S) has the highest content and ferquency of all amino acids 25 and 12.1% while the lowest content and frequency of amino acid was 1 and 0.5% to the each of amino acid Histidine (H) and Trpyptophan (W). The CMV-Beet-EG/CP gene sequence was recorded in GeneBank with Accession No. JX826591.

Key words: CMV, *Beta vulgaris*, RT-PCR, cp-sequence, bioinformatic

INTRODUCTION

Sugar beets (*Beta vulgaris* L.); family, Chenopodiaceae account for 25% of the world supply of raw sugar but the majority of production comes from sugar cane. Sugar beet was naturally infected with 16 plant viruses all over of the world (Fauquet *et al*., 2005). In Egypt, sugar beet found to be naturally infected with 7 viruses (El-Kady *et al*., 1985; Abdel-Ghaffar *et al*., 2003).

Cucumber mosaic cucumovirus (CMV) belongs to genus, *Cucumovirus* and family, *Bromoviridae* it consists of isometric particles with a diameter of about 28 nm (Rybicki, 1995), has

an extraordinary wide host range (Roossinck, 2002). The CMV is responsible for yield losses in many crops (Hsu, 2002). Its may be transmitted by large number of aphid species, through seeds and by vegetative propagation (Gallitelli, 2000).

Bromoviridae (CMV) is a single-stranded positive-sense tripartite genome RNA virus, the genomic RNAs are designed as RNA 1 of 3,360 nucleotides (nt), RNA 2 of 3,050 nt and RNA 3 of 2,200 nt. The RNA 1 and 2 are encapsidated separately while RNA 3 is encapsidated with another RNA (RNA 4) having a subgenomic function (Palukaitis *et al*., 1992).

RNAs 1 and 2 codes for proteins those are associated with the replication of the viral genome (Hayes and Buck, 1990). RNA 2 also encodes an additional protein that is a suppressor of post transcriptional gene silencing and affects multiple functions including long distance movement and host range (Brigneti *et al*., 1998). The RNA 3 is dicistronic, coding for both the 3a movement protein (Kaplan *et al*., 1995) and the Capsid Protein (CP), the latter being translated from a subgenomic messenger (RNA 4) (Schwinghamer and Symons, 1977).

The present study aims to characterize the CMV-Beet-EG isolate molecularly upon coat protein gene of CMV by RT-PCR; identify the similarity level of gene sequence with other sequences published in GeneBank by bioinformatics analyses.

MATERIALS AND METHODS

Source of virus isolate: Samples showing distinctive viral symptoms of *Beta vulgaris* L. cv. Gazella plants naturally infected were collected randomly from Kafr El-Sheikh Governorate.

The CMV was detected serologically by DAS-ELISA according to Clark and Adams (1977), using polyclonal antibody specific against CMV, kindly provided from Prof. Dr. Stephan Winter, Head of Plant Virus Department Leibniz-Institut Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), German collection of microorganisms and cell cultures, Inhoffenstrasse 7B, 38124 Braunschweig, Germany.

The collected infected samples which gave positive serological reaction using IgG-CMV by DAS-ELISA, were used for virus isolation and propagation. The virus was isolated biologically by single local lesion method on *C. amaranticolor* (Noordam, 1973; Megahed *et al*., 2012). For biological purification, one single local lesion was separated and grinding in 1 mL of 1:2 (w/v) of 0.1 M phosphate buffer, pH 7.2, containing 1% Na_2SO_3, 0.02 M EDTA, 0.1% β-mercaptoethanol and 1.5% Triton X-100. The extracted sap was mechanically inoculated on middle leaves of indicator plants. The extraction of resulted local lesions was inoculated on healthy *Beta vulgaris* cv. Gazella plants as a CMV propagative host. The inoculated plants were kept in an insect proof under greenhouse conditions (25-28°C) for 21 days until symptoms appeared.

Dot and tissue printing immunoassay (DBIA and TPIA) were used for confirmation of CMV-Beet-EG isolate using specific CMV-polyclonal antibodies as described by Lin *et al*. (1990).

Molecular identification: Total RNA was extracted from infected sugar beet leaves by using a method described by Gibbs and Mackenzie (1997), the extracted RNA was evaluated by electrophoresis and spectrophotometer and then, keept at -80°C.

Amplification of CMV-Beet-EG/cp gene: The cDNA was synthesis using (200 U μL^{-1}) reverse transcriptase (Promega). The amplification was carried out by the UNOII thermocycler system (Biometra, Germany) and using 0.2 mL micro amplification PCR tubes using each complementary reverse primer 5'-AAC ACG GAA TCA GAC TGG GAG-3' and forward primer

5'-TTG AGT CGA GTC ATG GAC AAA TC-3' (Lin *et al*., 2004; Sofy and Soliman, 2011). Hard denaturation of the DNA was performed at 94°C for 3 min, followed by 35 cycles of amplification with denaturation at 94°C for 1 min annealing at 45°C for 2 min and extension 72°C for 1.5 min. A single trailing cycle of long extension at 72°C for 7 min ended the run. The gel was visualized with UV light after staining for 10 min with 10 mg mL^{-1} of ethidium bromide using Gel Documentation System (Gel Doc 2000 BIO RAD 1000/115 V~50/60 Hz-150 VA).

DNA sequencing of CMV-Beet-EG coat protein gene: DNA fragments were purified from agarose gel using the gel slicing and melting method described by Wieslander (1979). The nucleotide sequence was determined using an automated DNA sequencer (ABI Prism 3730XL DNA analyzer) and analyzed by FinchTV™ version 1.4.0 software.

Sequence analysis: The sequence data of multiple nucleotides, deduced amino acids and multiple alignment of the cp gene for CMV-Beet-EG were translated, analyzed and has been submitted to GenBank Database (accession No. JX826591) by using DNAMAN program (DNAMAN V 5.2.9 package, Madison, Wisconsin, USA). Phytogenetic relationship and antigenic index of with the available sequences were carried out using ClustalW (Ver.1.74) program (Thompson *et al*., 1994).

RESULTS

Virus isolation: The CMV-Beet-EG isolate was detected in the collected naturally infected sugar beet plants showing vein clearing, mottling, mosaic and blister, where gave positive results by DAS-ELISA using specific CMV-IgG. The virus isolate was biologically isolated by single local lesion from *C. amaranticolor* produce necrotic L.L. surrounded with little halo-edge without brown color. It was propagated in healthy sugar beet plants and exhibited vein clearing, narrow leaf and deformation (Fig. 1). This result was confirmed by DAS-ELISA.

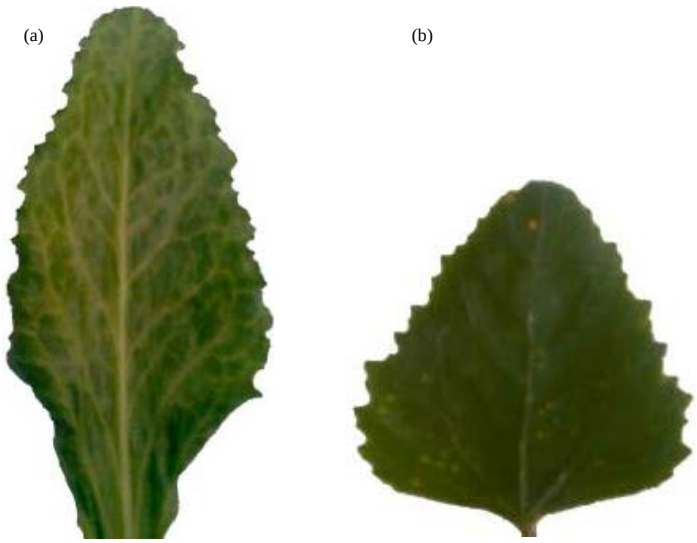

Fig. 1(a-b): (a) Infected sugar beet and (b) *C. amaranticolor* leaves inoculated with CMV-Beet-EG, showing external systematic and local symptoms

Fig. 2: Dot and tissue printing immunoassay for CMV-Beet-EG precipitation against specific IgG-CMV polyclonal, -ve: Negative samples +ve: Positive samples

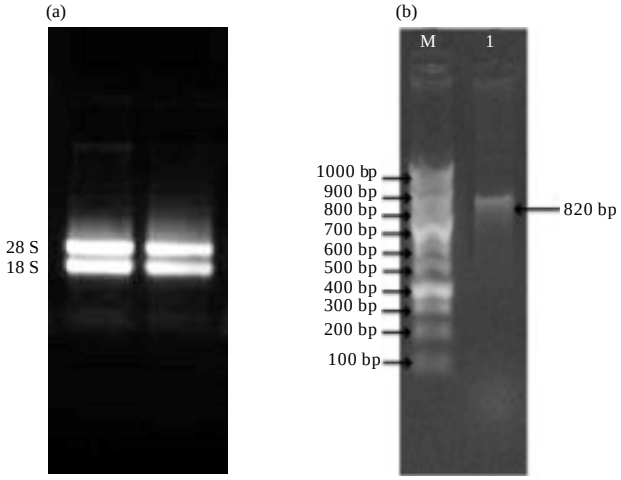

Fig. 3(a-b): The 1% agarose gel electrophoresis showing, (a) Total RNA and (b) Amplified PCR product of CMV-Beet-EG/cp gene of the correct size 820 bp, M: Molecular weight of DNA Marker, Lane 1: amplified CMV-Beet-EG/cp gene isolate

CMV confirmation: The sap extract of inoculated sugar beet was serologically precipitated reaction against specific polyclonal IgG-CMV by immunoblotting and printing and revealed a purplish blue color developed, whereas other extract from healthy plants remain green in the negative reactions (Fig. 2).

Molecular identification: Total RNA was extracted from CMV-Beet-EG infected sugar beet isolate. The integrity and quantity of the purified RNA were confirmed by gel electrophoresis (Fig. 3a) and UV spectrophotometer. The concentration of RNA was 95 μg/0.2 g of infected tissues and the purity was measured by $A_{260/280}$ absorbance ratio which it was 1.5.

The total RNAs prepared from infected sugar beet were reverse transcribed by MMLV reverse transcriptase. The reverse transcription was primed for RNA using Oligo (dt) as minus sense primer (5'-AAC ACG GAA TCA GAC TGG GAG-3') for CMV. The resulting complementary DNA (cDNA) of cp gene/CMV-Beet-EG was amplified by PCR after adding one set of primer CMV-Beet-EG, for

cp-gene. The efficiency of DNA amplification from CMV-Beet-EG infected leaf tissue was detected by analysis of PCR product using 1.0% agarose gel electrophoresis. The size of amplified CMV-DNA fragments was as expected 820 bp (Lane 1) (Fig. 3b).

Partial nucleotide sequence of CMV-Beet-EG/cp gene: The partial nucleotide sequence (619 nucleotides) of the CMV-Beet-EG/cp gene was aligned with other coat protein sequences of CMV isolates published in GeneBank as shown in Fig. 4 using DNAMAN program

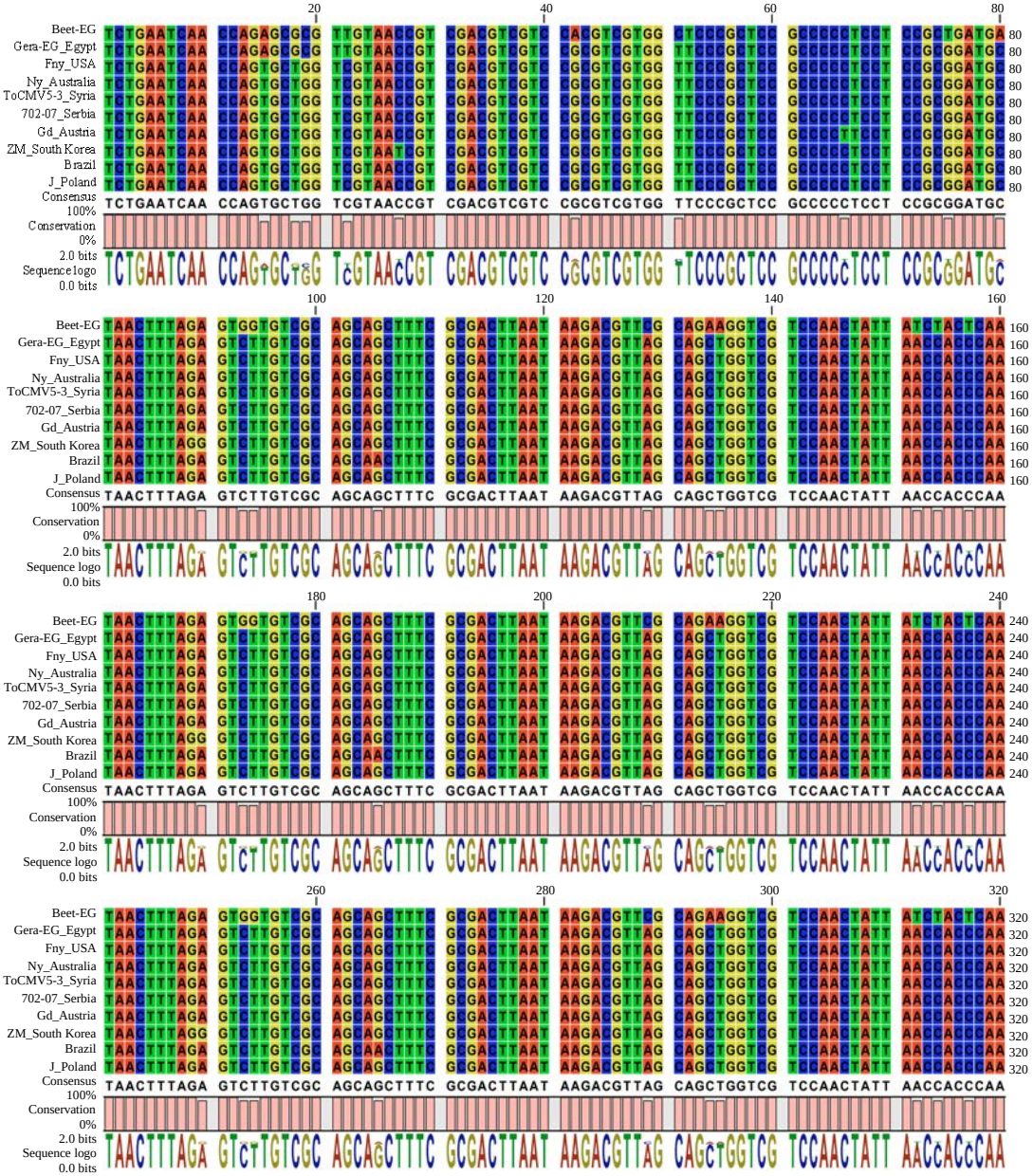

Fig. 4: Continue

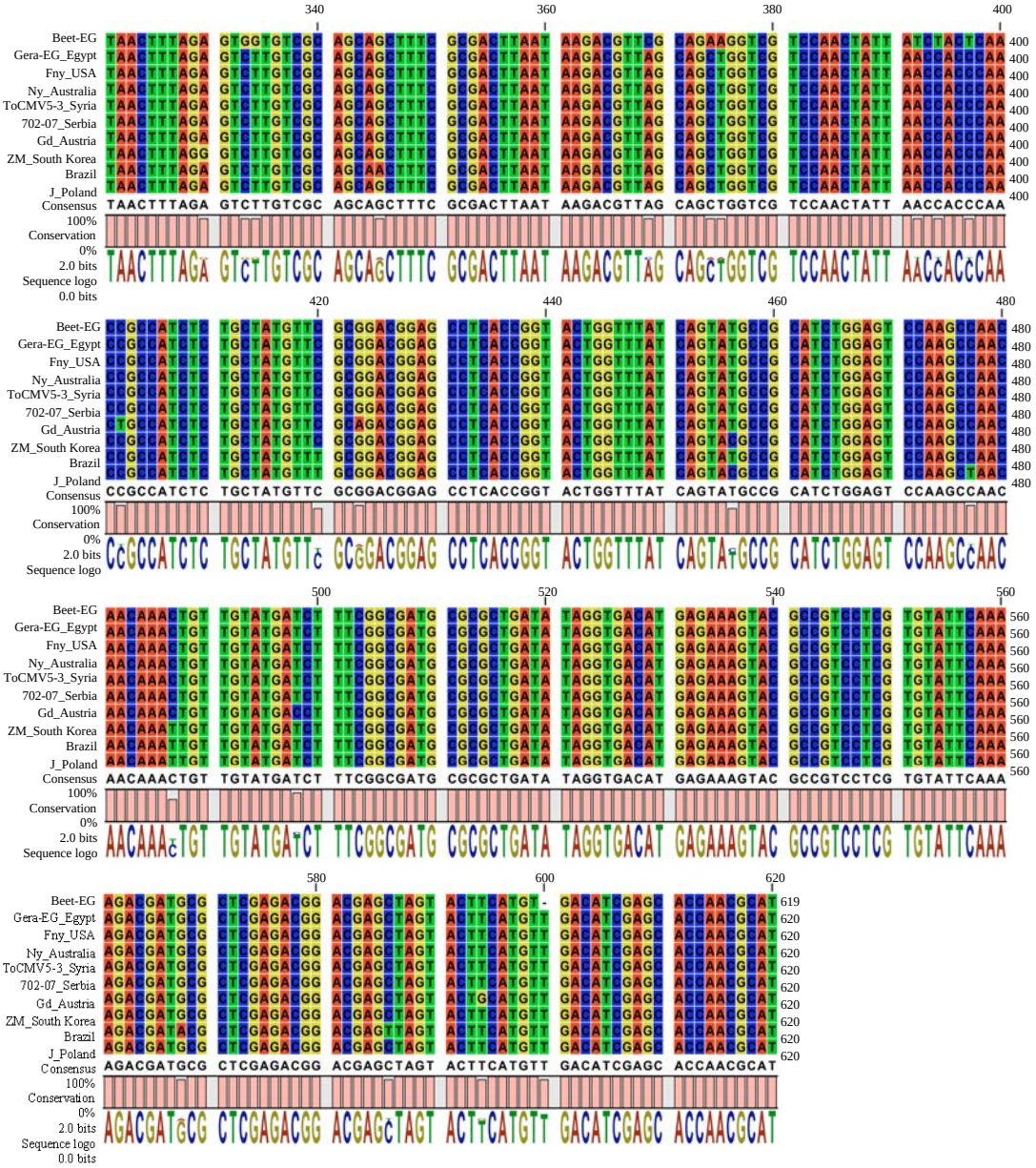

Fig. 4: Partial nucleotide sequence alignment of the cp-gene for CMV-Beet-EG using DNAMAN program

(DNAMAN V 5.2.9 package, Madison, Wisconsin, USA). It was noticed the variation in nucleotide sequence of CMV-Beet-EG/cp gene sequence and other sequences due to replacement of either pyrimidines with purines or opposite revealed there are 31 position of replacement.

The changes of nucleotide at position 18 and 19 of CMV-Beet-EG and Gera-EG replaced T and G by G and C, respectively. At position 42 G replaced by A and at 51 T replaced by C.

The Gd-Austria isolate C replaced by T at 66, the ZM-South Korea at position 90 A replaced by G, the CMV-Beet-EG at positions 93 and 94 (C and T) replaced by G while, Brazil isolate the G

(a)
Homology matrix of 10 sequences

Beet-EG	=	100%
Gera-EG-Egypt	=	97.3% 100%
Fny-USA	=	96.4% 99.2% 100%
Ny-Australia	=	96.4% 99.2% 100.0%
ToCMV5-3-Syria	=	96.4% 99.2% 100.0%
702-07-Serbia	=	96.4% 99.2% 100.0%
Gd-Austria	=	95.6% 98.4% 99.2% 99.2% 99.2% 99.2% 100%
ZM-South Korea	=	95.6% 98.4% 99.2% 99.2% 99.2% 99.2% 98.4% 100%
Brazil	=	95.5% 98.2% 99.0% 99.0% 99.0% 99.0% 98.2% 98.5% 100%
J-Poland	=	95.5% 98.2% 99.0% 99.0% 99.0% 99.0% 98.2% 99.2% 98.7% 100%

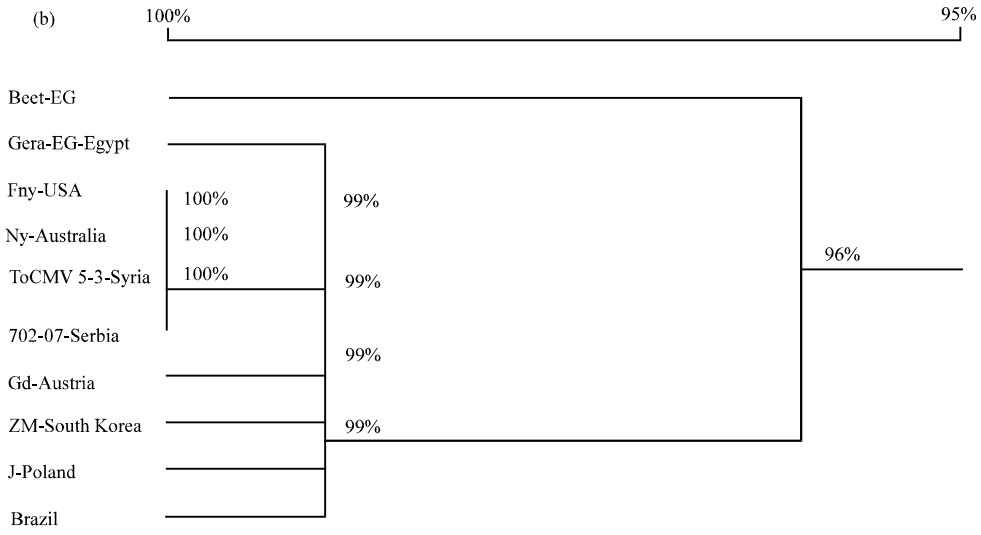

Fig. 5(a-b): (a) Similarity index and (b) Consensus phylogenic tree of nucleic acid sequence constructed from the multiple alignment of the CP/CMV-Beet-EG (under study) and nine CMV isolates

replaced by A at 105. CMV-Beet-EG at positions 152, 154, 157 and 168; the A, C, C and A replaced by T in all previous position. J-Poland isolate G replaced by A at 185 position while CMV-Beet-EG A replaced by T at 211 and Brazil isolate C replaced by T at position 249. At position 262, T replaced by C for both CMV-Beet-EG and Gera-EG isolates, where at 265 C replaced by T for each ZM-South Korea and J-Poland. CMV-Beet-EG at 347 position C replaced by T while in Gd-Austria isolate sequence C replaced by T at 402, on the other position 420, C replaced by T of Brazil and J-Poland. At position 487, C replaced by T of ZM-South Korea, Brazil and J-Poland, where at 498 T replaced by C of Gd-Austria. The Brazil replaced G and C by A and T at 268 and 286, respectively while T replaced by G at 594 of Gd-Austria isolate and finally the CMV-Beet-EG at position 600, the nucleotide was deleted.

The similarity index of CMV-Beet-EG presented in Fig. 5a, revealed the nucleotide sequence of CMV-Beet-EG under study which has 97.3% identified to Gera-EG (Accession No. JQ013954) isolate and 96.4% to Fny-USA (Accession no. MCVCP3A1), Ny-Australia (Accession No. CMU22821), ToCMV5-3-Syria (Accession No. AB448693) and 702-07-Serbia

Table 1: Bases composition of partial CP sequence CMV-Beet-EG and different three CMV isolates published in GeneBank

					Nitrogen base													
					A		T		A+T		G		C		G+C			
CMV isolates	Main host	Accession No.	Total base pairs (bp)	M.wt. (kDa)	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	A/T ratio	G/C ratio
Beet-EG	Sugar beet	JX826591	619	188.179	148	23.9	164	26.5	312	50.4	144	23.3	163	26.3	307	49.6	0.902	0.883
Gera-EG	Geranium	JQ013954	320	188.486	147	23.7	162	26.1	309	49.8	145	23.4	166	26.8	311	50.0	0.896	0.873
Fny-USA	Cucumber	MCVCP3A1	620	188.46	146	23.5	164	26.5	310	50.0	145	23.4	165	26.0	310	50.0	0.890	0.878
Brazil	Amazon Lily	AY380812	620	188.424	148	23.9	168	27.1	316	51.0	143	23.1	161	26.0	304	49.8	0.880	0.888

(Accession No. GQ340670) CMV isolates. The results in Fig. 5b, represented the phylogenetic tree of CMV-Beet-EG and other nine CMV isolates revealed that, CMV-Beet-EG has identified 96% with sub-group consist of Gera-EG (99%); Fny-USA, Ny-Australia, ToCMV5-3-Syria and 702-07-Serbia (99%) and Gd-Austria, ZM-South Korea, J-Poland and Brazil (99%).

The nitrogen base composition variation of cp gene for CMV-Beet-EG Table 1 revealed that, the highest content for Thymine (T) was 164 (26.5%) followed by Cytosine (C) 163 (26.3%), then Adenine (A) 148 (23.9%) and Guanine (G) 144 (23.3%). Comparison between nitrogen base composition of partial cp gene sequence for CMV-Beet-EG (Accession no. JX826591) and three CMV isolates published in GeneBank, Gera-EG (Accession No. JQ013954), Fny-USA (Accession No. MCVCP3A1) and Brazil (Accession No. AY380812) was done to determine the ratio of A/T and G/C and percentage of A+T and G+C Table 1. The ratio of A/T, G/C were 0.902 and 0.883 of CMV-Beet-EG (Accession no. JX826591); 0.896 and 0.873 of Gera-EG (Accession No. JQ013954); 0.890 and 0.878 of Fny-USA (Accession No. MCVCP3A1) and 0.880 and 0.888 of Brazil (Accession no. AY380812). The percentage of A+T and G+C in Table 1 were found to be 50.4 and 49.6%; 49.8 and 50.2%; 50.0 and 50.0% and 51.0 and 49.0% for CMV-Beet-EG (Accession No. JX826591), Gera-EG (Accession No. JQ013954), Fny-USA (Accession No. MCVCP3A1) and Brazil (Accession No. AY380812), respectively.

Deduced amino acids sequence of CMV-Beet-EG/cp gene: The predicted numbers of amino acids produced from translation of partial cp gene nucleotide sequence of CMV-Beet-EG were 206 amino acids starting with Serine (S) and ended with Alanine (A) (Fig. 6).

The deduced amino acids sequence of CMV-Beet-EG/cp gene, 206 amino acids was aligned with other deduced amino acids sequence of CP/CMV isolates published in GeneBank as shown in Fig. 7, CMV-Beet-EG has 6 different amino acids present at position from 200-206 which absent in all other aligned isolates. The presented data in Fig. 7 revealed that, there are 17 differences relative to replacement position of amino acid as following.

The CMV-Beet-EG at positions 5, 7, 27, 32, 43, 45, 51, 52, 53, 71 and 116, the amino acids Serine, Glycine, Alanine, Leucine, Leucine, Alanine, Asparagine, Histidine, Proline, Threonine and Serine replaced with Arginine, Arginine, Aspartic acid, Valine, Phenylalanine, Glutamic, Isoleucine, Tyrosine, Serine, Serine and Phenylalanine, respectively. AAQ89596-Brazil at 190 Alanine replaced by Threonine, where at position 2 Glutamic replaced by Glycine while in isolate Lucknow-India Aspartic acid replaced by Threonine at 194. At positions 134, Alanine replaced by Threonine for Mf-South Korea, where at position 16 and 190, the amino acids Arginine and Alanine replaced by Cysteine and Threonine, respectively for Pf-USA isolate.

The similarity index of deduced amino acids sequence constructed from the multiple alignment of the CP/CMV-Beet-EG (under study) and nine CP/CMV isolates (Fig. 8a) revealed the deduced

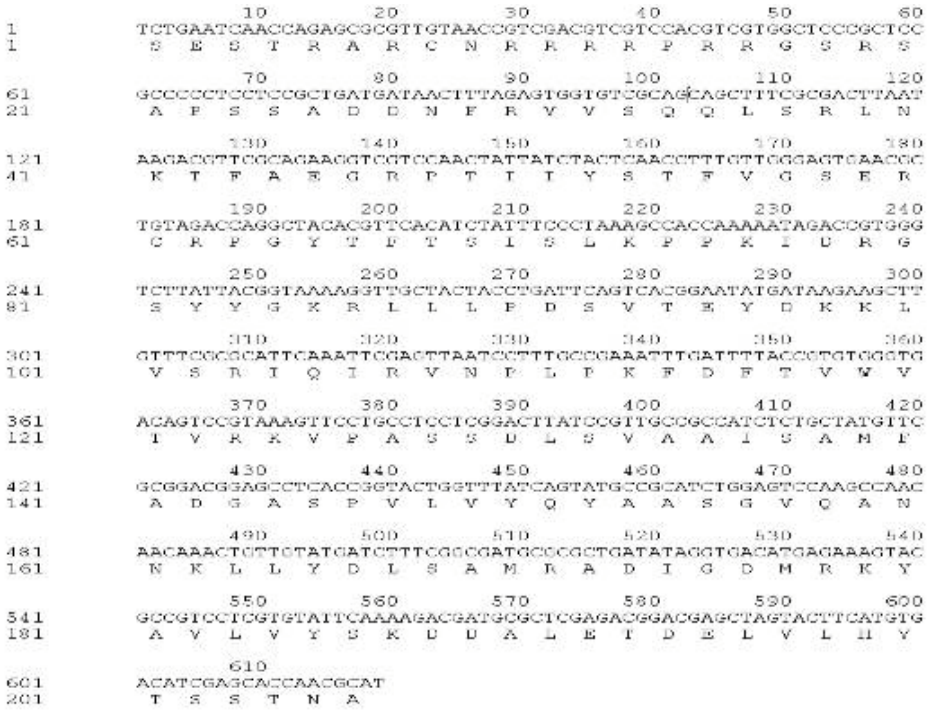

Fig. 6: Predicted amino acid of the cp-gene of the isolated CMV-Beet-EG

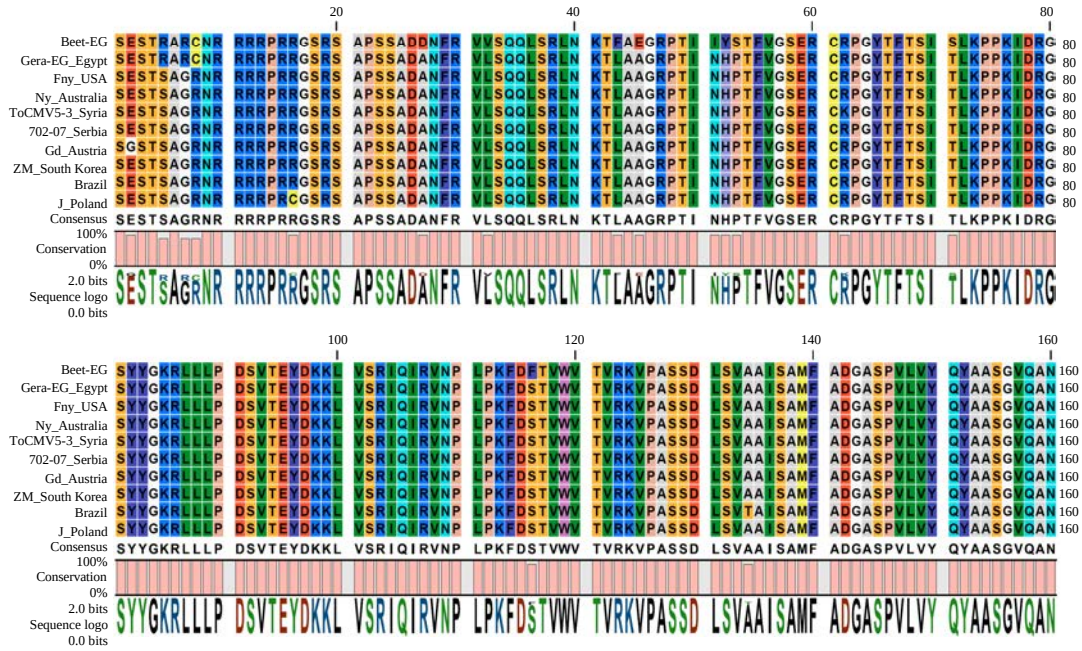

Fig. 7: Continue

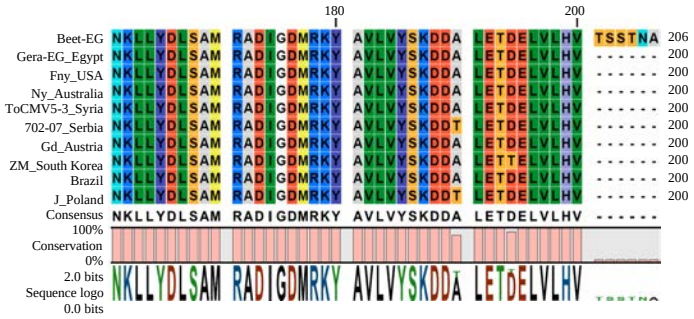

Fig. 7: Deduced amino acids sequence alignment of the CP for CMV-Beet-EG using DNAMAN program

(a)

Homology matrix of 10 sequences

Beet-EG	=	100%
Gera-EG-Egypt	=	95.5% 100%
AAQ89571-Brazil	=	94.0% 98.5% 100%
Fny-US	=	94.0% 98.5% 100.0% 100%
Woj-Poland	=	93.5% 98.0% 99.5% 99.5% 100%
AAQ89596-Brazil	=	93.5% 98.0% 99.5% 99.5% 99.0% 100%
RT88-Spain	=	93.5% 98.0% 99.5% 99.5% 99.0% 99.0% 100%
Lucknow-India	=	93.5% 98.0% 99.5% 99.5% 99.0% 99.0% 99.0% 100%
Mf-South Korea	=	93.5% 98.0% 99.5% 99.5% 99.0% 99.0% 99.0% 99.0% 100%
Pf-USA	=	93.0% 97.5% 99.0% 99.0% 98.5% 99.5% 98.5% 98.5% 98.5% 100%

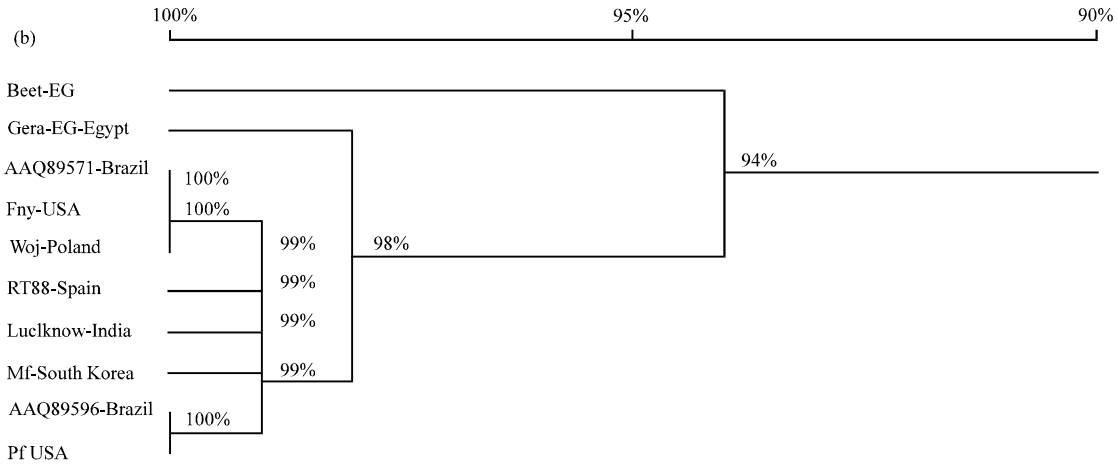

Fig. 8(a-b): (a) Similarity index and (b) Consensus phylogenic tree of deduced amino acids sequence constructed from the multiple alignment of the CP/CMV-Beet-EG (under study) and nine CP /CMV isolates

amino acids sequence of CMV-Beet-EG has 95.5% identified to Gera-EG (Accession No. AEZ03836) isolate; 94.0% to AAQ89571-Brazil (Accession No. AAQ89571) and Fny-USA (Accession No. NP-040777) and 93.5% to Woj-Poland (Accession No. ACM89097), AAQ89596-Brazil (Accession No.

Table 2: The predicted amino acids of partial cp gene sequence for CMV-Beet-EG isolate and different three CP/CMV isolates published in GeneBank

	CMV isolates							
	Beet-EG		Gera-EG		Fny-USA		AAQ89596-Brazil	
Amino acid	No.	%	No.	%	No.	%	No.	%
Alanine (A)	18	8.7	19	9.5	19	9.5	18	9.0
Cysteine (C)	2	1.0	2	1	1	0.5	1	0.5
Aspartic acid (D)	14	6.8	13	6.5	13	6.5	13	6.5
Glutamic (E)	6	2.9	5	2.5	5	2.5	5	2.5
Phenylalanine (F)	7	3.4	5	2.5	5	2.5	5	2.5
Glycine (G)	9	4.4	9	4.5	10	5.0	10	5.0
Histidine (H)	1	0.5	2	1.0	2	1.0	2	1.0
isoleucine. (I)	8	3.9	7	3.5	7	3.5	7	3.5
Lysine (K)	11	5.3	11	5.5	11	5.5	11	5.5
Leucine (L)	17	8.3	19	9.5	19	9.5	19	9.5
Methionine (M)	3	1.5	3	1.5	3	1.5	3	1.5
Asparagine (N)	7	3.4	7	3.5	7	3.5	7	3.5
Proline (P)	11	5.3	12	6.0	12	6.0	12	6.0
Glutamine (Q)	5	2.4	5	2.5	5	2.5	5	2.5
Arginine (R)	21	10.2	21	10.5	20	10.0	20	10.0
Serine (S)	25	12.1	22	11.0	23	11.5	23	11.5
Threonine (T)	12	5.8	11	5.5	11	5.5	12	6.0
Valine (V)	18	8.7	17	8.5	17	8.5	17	8.5
Trpyptophan (W)	1	0.5	1	0.5	1	0.5	1	0.5
Tyrosine (Y)	10	4.9	9	4.5	9	4.5	9	4.5
Total no. of amino acid	206		200		200		200	
M.wt. (kDa) of total amino acid	22.983		22.238		22.123		22.153	
Isoelectric point	9.95		10.19		10.18		10.18	
Accession no. of CMV isolates	JX826591		AEZ03836		NP-040777		AAQ8959	
CMV main host	Sugar beet		Geranium		Cucumber		Amazon Lily	

AAQ89596), RT88-Spain (Accession No. CAH17692), Lucknow-India (Accession No. ABC18318), Mf-South Korea (Accession No. CAB77390) and Pf-USA (Accession No. CAB41491) CP/CMV isolates.

A phylogenetic tree in Fig. 8b, revealed that, CMV-Beet-EG has identified 94% and with 5 sub-group for other nine CMV isolates, sub group 1 has 100% consist of 6 minor groups for Gera-EG, sub-group, (AAQ89571-Brazil, Fny-USA and Woj-Poland), RT88-Spain, Lucknow-India, Mf-South Korea and (AAQ89596-Brazil and Pf-USA). Sub-group 2 has 99% and sub-group 3 has 98%. Comparison between the predicted amino acids of partial cp gene sequence of CMV-Beet-EG (Accession No. JX826591) and three CP/CMV isolates published in GeneBank, Gera-EG (Accession No. AEZ03836), Fny-USA (Accession No. NP-040777) and AAQ89596-Brazil (Accession No. AAQ89596) was done in Table 2.

The amino acid Serine (S) has the highest content No. of all amino acids 25 (12.1%) for CMV-Beet-EG (Accession No. JX826591), 22 (11.0%) for Gera-EG (Accession No. AEZ03836), 23 (11.5%) for each Fny-USA (Accession No. NP-040777) and AAQ89596-Brazil (Accession No. AAQ89596). The deduced amino acids sequence of cp gene for CMV-Beet-EG isolated from sugar

beet, Table 2 have a M.wt 22.983 kDa, isolelectric point 9.95 of CP and the type of amino acids consists of 20 amino acids; the lowest content of amino acid was 1 (0.5%) to the each two amino acid Histidine (H) and Trpyptophan (W). The M.Wt and isolelectric point of deduced CP/CMV-Gera-EG isolated from Geranium (Accession No. AEZ03836) were 22.238 kDa and 10.19, respectively and Trpyptophan (W) has the lowest content of amino acid 1 (0.5%) of 200 total no of amino acid (Table 2). The CP of deduced amino acids consist of 200 amino acid specific to cp gene of Fny-USA (Accession No. NP-040777) isolated from cucumber and AAQ89596-Brazil (Accession No. AAQ89596) isolated from Amazon Lily have a M.wt 22.123 and 22.153 kDa, respectively and the same isolelectric point 10.18. Also the two amino acid Cysteine (C) and Trpyptophan (W) have the same lowest amino acid content 1 (0.5%) of two each CP/CMV isolates (Table 2).

DISCUSSION

In the present study, the CMV was detected in naturally infected *B. vulgaris* L. cv. Gazella which showed distinctive viral symptoms in the form of vein clearing, mottling, mosaic and blister. The detected samples gave $^{+}$ve reaction against specific antiserum by using DAS-ELISA technique, as consistent with Abdelkader *et al.* (2006). CMV-Beet-EG was biologically purified by single local lesion assay on *C. amaranticolor* and sugar beet was used as a propagative host which reacted as vein clearing, narrow leaf and deformation symptoms as reported by many investigators (El-Afifi *et al.*, 2007; Sofy and Soliman, 2011; Megahed *et al.*, 2012).

The results of DBIA and TPIA were found to be sensitive techniques for CMV immunogene detection in all infected sugar beet plants, when the antigenic determinants of CMV-Beet-EG isolate were identified by using polyclonal antibodies specific CMV which give serologically precipitation reacted in DAS-ELISA and in DBIA and TPIA where the purple color appeared clearly, the obtained results in accordance with many authors (Hu and Chang, 2006; Megahed *et al.*, 2012).

Total RNA of CMV-coat protein gene beet Egyption has 95 μg/0.2 g of infected tissues and the purity was measured by $A_{260/280}$ absorbance ratio 1.5 indicated haigh yield and purity of the extracted RNA. CMV-coat protein gene beet Egyption isolate was successfully amplified using RT-PCR from infected sugar beet in fragment of about 820 bp using specific synthesized primers according to Lin *et al.* (2004). Other expected size were obtained by authors; Abdelkader *et al.* (2006) detected the size of amplified cDNA fragment of CMV-EG isolated from sugar beet as 650 bp; Yu *et al.* (2005) the size of DNA fragment was 600 bp for CMV/cp of cucumber isolate while the result of the CMV-cp amplified from *Capsicum annuum* showed the band of the fragment with 740 bp (Wu *et al.*, 2007). As well as, Sofy and Soliman (2011) found the amplified fragment of CMV/cp Gera-EG was 657 bp.

The nucleotide sequence of coat protein gene of CMV-Beet-EG isolate under study revealed 619 nucleotides of cp-sequence has similarity CMV isolates belonging to subgroup IA in accordance with Abdelkader *et al.* (2006) and Sofy and Soliman (2011), in a different percentages; 97.3% identified similarity to Gera-EG-Egypt (Accession No. JQ013954) isolate and 96.4% to Fny-USA (Accession No. MCVCP3A1), Ny-Australia (Accession No. CMU22821), ToCMV5-3-Syria (Accession No. AB448693) and 702-07-Serbia (Accession No. GQ340670) CMV isolates.

The nucleotide sequence of coat protein gene of CMV-Beet-EG showed the highest content for Thymine (T) 164 (26.5%) followed by Cytosine (C) 163 (26.3%), then Adenine (A) 148 (23.9%) and Guanine (G) 144 (23.3%). The similarity index of deduced amino acids sequence constructed from the multiple alignment of the CP/CMV-Beet-EG and nine CP/CMV isolates revealed the deduced amino acids sequence of CMV-Beet-EG is 95.5% identity to Gera-EG-Egypt (Accession No.

AEZ03836), 94.0% identity to AAQ89571-Brazil (Accession no. AAQ89571) and Fny-USA (Accession No. NP-040777) and 93.5% identity to other isolates. The deduced amino acids sequence of cp gene for CMV-Beet-EG isolated from sugar beet have a M.Wt 22.983 kDa, isolelectric point 9.95 of 206 amino acids consists of 20 amino acids.

CONCLUSION

It was concluded that, based on the results obtained by the RT-PCR, sequence and bioinformatics analyses, the CMV-Beet-EG isolated from sugar beet cultivated areas in Egypt is a new CMV isolate belongs to subgroup IA and recorded in GeneBank with Accession No. JX826591.

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REFERENCES

Abdel-Ghaffar, M.H., M.I. Salama and S.Y.M. Mahmoud, 2003. Electron microscopy, serological and molecular studies on an Egyptian isolate of beet mosaic potyvirus. Arab Univ. J. Agric. Sci., 11: 469-484.

Abdelkader, H.S., E.H. Khatab and A.I.A. El-Fattah, 2006. Detection and identification of an Egyptian isolate of Cucumber Mosaic Virus (CMV) affecting sugar beet using non-radioactive RNA Probe. Pak. J. Biotech., 3: 1-10.

Brigneti, G., O. Voinnet, W.X. Li, L.H. Ji, S.W. Ding and D.C. Baulcombe, 1998. Viral pathogenicity determinants are suppressors of transgene silencing in *Nicotiana benthamiana*. EMBO J., 17: 6739-6746.

Clark, M.F. and A.N. Adams, 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol., 34: 475-483.

El-Afifi, S.I., A.M. El-Borollosy and S.Y.M. Mahmoud, 2007. Tobacco callus culture as a propagating medium for cucumber mosaic *Cucumovirus*. Int. J. Virol., 3: 73-79.

El-Kady, M.A., A.S.G. El-Din, A.M. Nakhla and A.M. Abdel-Salam, 1985. A strain of cucumber mosaic virus isolated from sugar beet in Egypt. Proceedings of the 1st National Conference of Pests and Diseases of Vegetables and Field Crops in Egypt, October 21-23, 1985, Ismailia, Egypt.

Fauquet, C.M., M.A. Mayo, J. Maniloff, U. Desselberger and L.A. Ball, 2005. Virus Taxonomy: VIII Report of the International Committee on Taxonomy of Viruses. 2nd Edn., Elsevier Academic Press, San Diego, California, USA., ISBN-13: 978-0122499517, Pages: 1162.

Gallitelli, D., 2000. The ecology of *Cucumber mosaic virus* and sustainable agriculture. Virus Res., 71: 9-21.

Gibbs, A. and A. Mackenzie, 1997. A primer pair for amplifying part of the genome of all potyvirids by RT-PCR. J. Virol. Methods, 63: 9-16.

Hayes, R.J. and K.W. Buck, 1990. Complete replication of a eukaryotic virus RNA *in vitro* by a purified RNA-dependent RNA polymerase. Cell, 63: 363-368.

Hsu, H.T., 2002. Biological Control of Plant Pathogens (Viruses). In: Encyclopedia of Pest Management, Pimentel, D. (Ed.). Chapter 28, CRC Press, New York, USA., ISBN: 9780824706326.

Hu, W.C. and Y.C. Chang, 2006. *Cucumber mosaic virus* in New Guinea impatiens in Taiwan. Acta Hortic., 722: 241-246.

Kaplan, I.B., M.H. Shintaku, Q. Li, L. Zhang, L.E. Marsh and P. Palukaitis, 1995. Complementation of virus movement in transgenic tobacco expressing the cucumber mosaic virus 3a gene. Virology, 209: 188-199.

Lin, H.X., L. Rubio, A.B. Smythe and B.W. Falk, 2004. Molecular population genetics of *Cucumber mosaic virus* in California: Evidence for founder effects and reassortment. J. Virol., 78: 6666-6675.

Lin, N.S., Y.H. Hsu and H.T. Hsu, 1990. Immunological detection of plant viruses and a mycoplasmlike organism by direct tissue blotting on nitrocellulose membranes. Phytopathology, 80: 824-828.

Megahed, A.A., K.A. El-Dougdoug, B.A. Othman, S.M. Lashin, M.A. Ibrahim and A.R. Sofy, 2012. A new Egyptian satellite strain of cucumber mosaic cucumovirus. Int. J. Virol., 8: 240-257.

Noordam, D.D., 1973. Identification of Plant Viruses: Methods and Experiments. Center for Agricultural Publishing and Documentation, Netherlands, ISBN-13: 9789022004647, Pages: 207.

Palukaitis, P., M.J. Roossinck, R.G. Dietzgen and R.I. Francki, 1992. Cucumber mosaic virus. Adv. Virus Res., 41: 281-348.

Roossinck, M.J., 2002. Evolutionary history of *Cucumber mosaic virus* deduced by phylogenetic analyses. J. Virol., 76: 3382-3387.

Rybicki, E.P., 1995. The Bromoviridae. In: Virus Taxonomy: Classification and Nomenclature of Viruses, Murphy, F.A., C.M. Fauquet, D.H.L. Bishop, S.A. Ghabrial and A.W. Jarvis *et al.* (Eds.). Springer-Verlag, Vienna, Austria, ISBN-13: 9783211825945, pp: 450-457.

Schwinghamer, M.W. and R.H. Symons, 1977. Translation of the four major RNA species of cucumber mosaic virus in plant and animal cell-free systems and in toad oocytes. Virology, 79: 88-108.

Sofy, A.R. and A.M. Soliman, 2011. Molecular identification of a Cucumber mosaic virus subgroup I Egyptian isolate from geranium based on bioinformatics analysis of CP gene sequence. Egypt. J. Virol., 8: 178-194.

Thompson, J.D., D.G. Higgins and T.J. Gibson, 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res., 22: 4673-4680.

Wieslander, L., 1979. A simple method to recover intact high molecular weight RNA and DNA after electrophoretic separation in low gelling temperature agarose gels. Anal. Biochem., 98: 305-309.

Wu, L.J., K.L. Hu and Z.M. Wu, 2007. Transformation of *Capsicum annuum* with coat protein gene of *Cucumber mosaic virus*. J. South China Agric. Univ., 28: 69-72.

Yu, C., J. Wu and X. Zhou, 2005. Detection and subgrouping of *Cucumber mosaic virus* isolates by AS-ELISA and immunocapture RT-PCR. J. Virol. Methods, 123: 155-161.