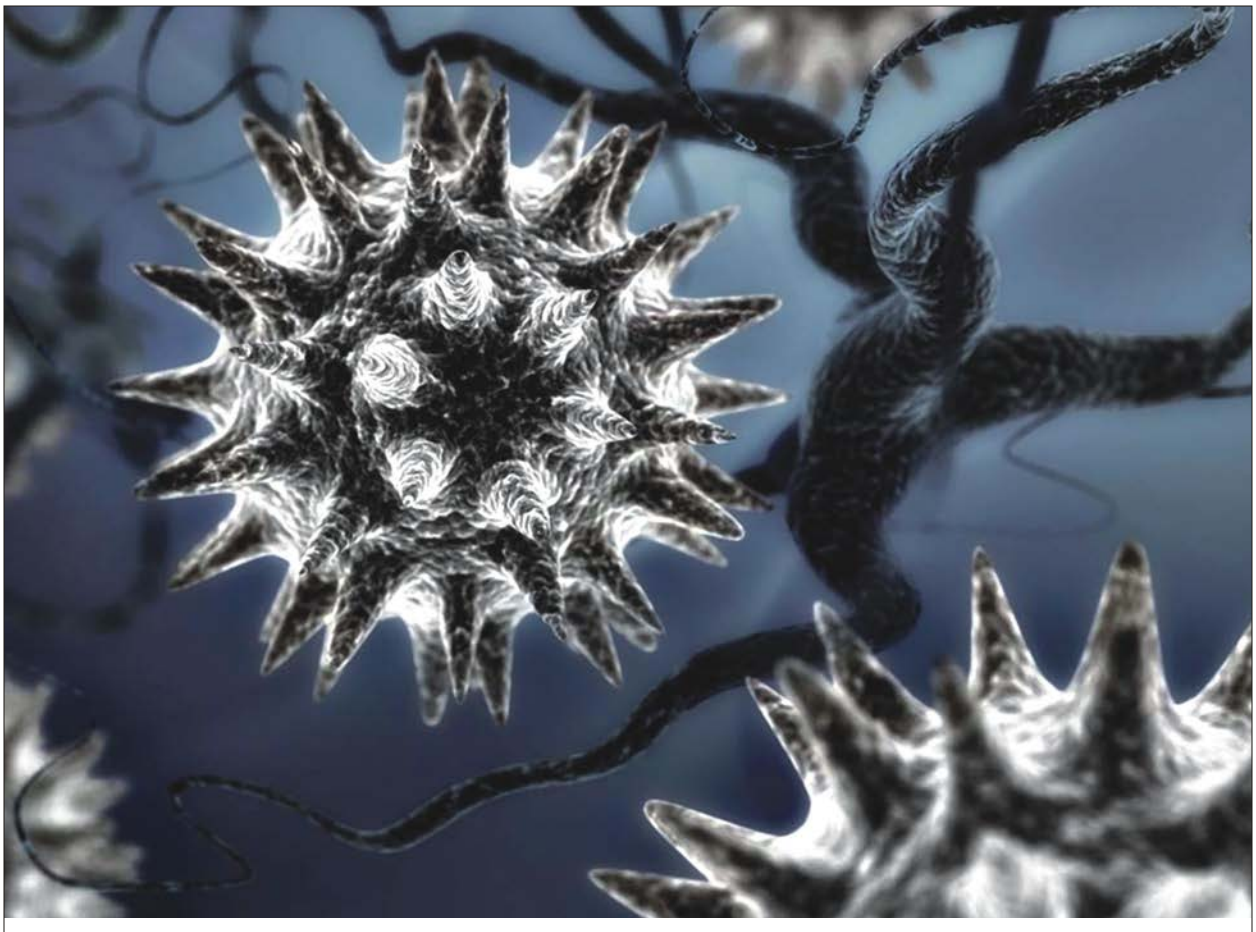

International Journal of Virology

ISSN 1816-4900

Academic Journals Inc.

www.academicjournals.com

International Journal of Virology 8 (3): 240-257, 2012
ISSN 1816-4900 / DOI: 10.3923/ijv.2012.240.257

A New Egyptian Satellite Strain of Cucumber Mosaic Cucumovirus

[1]A.A. Megahed, [2]Kh. A. El-Dougdoug, [2]B.A. Othman, [1]S.M. Lashin, [3]M.A. Ibrahim and [4]A.R. Sofy

[1]Department of Plant Pathology, National Research Centre, Dokki, Cairo, Egypt
[2]Department of Agriculture Microbiology, Faculty of Agriculture, Ain Shams University, Shoubra EL-Kheima, Cairo, Egypt
[3]Department of Molecular Biology, National Research Centre, Dokki, Cairo, Egypt
[4]Departmnet of Botany and Microbiology, Faculty of Science, Al-Azhar University, 11884 Nasr City, Cairo, Egypt

Corresponding Author: A.A. Megahed, Department of Plant pathology, National Research Centre, Dokki, Cairo, Egypt

ABSTRACT

Cucumber mosaic cucumovirus satellite strain Egyption isolate (st-CMV-EG) was detected using polyclonal antibodies by DAS-ELISA form naturally infected cucumber leaves collected from protected agriculture. The samples which give positive reaction were used to virus isolation. st-CMV-EG isolate has a wide host-range belonging to 6 families. The virus isolate was inactivated at 70°C, it was infected at dilution 10^{-4} and longevity was 4 days at room temperature. Amorphous and crystalline inclusion bodies were detected in hair cells of epidermal strips of CMV-inoculated cucumber leaves. The U.V absorption ratio, A_{260}/A_{280} and $A_{max.}/A_{min.}$ were 1.204 and 1.101, respectively, as well as virus yield was 1.43 mg/100 g fresh leaves in purified virus preparation. Electron micrograph of the purified virus isolate showed spherical particles (28 nm.). The virus isolate was detected serologically by using dot blot immunoassay. CMV-RNA was successfully detected in infected cucumber leaves using specific primer of *cp* gene by RT-PCR and the expected size was about 582 bp. Sequence analysis of CMV-*cp* gene of cucumber Egyption isolate was indicated 38% similarity to that of AB024493 and D00542.

Key words: st-CMV-EG, *Cucumis sativus*, symptomatology, virus stability, inclusion bodies, DBIA, RT-PCR, CP-sequence

INTRODUCTION

Cucumber mosaic virus (CMV), a member of the family Bromoviridae (genus Cucumovirus), has an extremely wide host range, including more than 1200 plant species in 500 genera of 100 families (Tobias *et al*., 1982). It causes a broad spectrum of symptoms from mild to severe mosaic, stunting, chlorosis, necrosis and filiformism depending on virus strain and the host (Carrere *et al*., 1999).

The satellite virus had no serological relationship with helper virus or other satellite viruses. These experiments are a good example of the need for care when attempeting to delineate a newly isolated satellite RNA (Valverde and Dodds, 1987). Cucumber mosaic virus supports satellite RNAs consisting of single-strand, linear RNA molecules of 330 to 390 nucleotide. The satellite RNAs of often attenuated the symptoms induced by CMV in certain host plants (Hidaka *et al*., 1988), but in some cases quite dramatic increases in symptom severity may occur (Kaperi *et al*., 1988).

Sharma *et al.* (2005) reported that, CMV was detected and characterized by bioassay, double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA), electron microscopy, immunosorbent electron microscopy, cytopathology, reverse transcription polymerase chain reaction (RT-PCR) and sequencing.

RNAs 1 and 2 code for proteins that are associated with the replication of 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). 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).

This present study aims to isolation and identification of st-CMV isolate biologically, serologically and molecular characters from infected cucumber plants under protected greenhouse conditions in Egypt.

MATERIALS AND METHODS

Source of virus isolate: Ten samples of infected cucumber plants (*Cucumis sativus* cv. Barakoda) showing distincted viral symptoms were collected from protected greenhouses and used for st-CMV isolation.

The virus was detected using polyclonal antibody specific CMV by DAS-ELISA (kits provided by Ssnofi, Sante, Animal, Paris, Farance) according to Clark and Adams (1977).

Isolation and propagation of virus isolate: The infected cucumber plants which gave $^{+}$ve reaction against IgG-CMV by DAS-ELISA were used for virus isolation. The inoculum of infectious sap was mechanically inoculated on *Chenopodium amaranticolor* as indicator host. The single local lesion assay was used for biological purification of isolate and propagated on healthy *Nicotiana glutinosa* plants as CMV propagative host.

Host range and symptomatology: Eighteen plant species belonging to 6 families (Cucurbitaceae, Solanaceae, Chenopodiaceae, Leguminoseae, Compositae and Amaranthaceae). Table 1 were mechanically inoculated with virus inoculum using five plants of each host. The results were confirmed by DAS-ELISA test against IgG-CMV.

Virus stability: Thermal Inactivation Point (TIP), Dilution End Paint (DEP) and Longevity *in vitro* (LIV) was performed according to Noordam (1973), using *C. amaranticolor* as local lesion host to CMV.

Inclusions bodies: Crystalline Inclusion Bodies (CIB) were examined in the epidermal strips from the lower surface leaves of CMV-infected cucumber plant (15 days after inoculation). As well as the amorphous inclusion bodies using bromophenol blue and mercuric chloride. The strips were examined under light microscope, magnification of 400-X (Mazia *et al.*, 1953).

Virus purification: The particles of virus isolate were purified by the method described by Murant (1965). The purified isolate was diluted in extraction buffer 10^{-1} and measured at range 230-300 nm by spectrophotometer (Shimadzu UV-2401 PC UV-Vis) at Molecular Biology Lab., National Research Centre (NRC). The virus yield determined using an extinction coefficient of 5 for CMV (Noordam, 1973).

Negative staining method: Morphology particles of the virus isolate was examined using negative stain method according to Noordam (1973) by JOEL-TEM-1010 electron microscope unite, electron microscope Department, NRC.

Dot blot immunoassay (DBIA): it was used for identification of virus isolate from infected cucumber as described by Lin *et al.* (1990) using polyclonal antibodies (CMV-IgG).

Molecular studies

Extraction of total RNA (T-RNA): Total RNA was extracted from infected cucumber leaves by using a method described by Gibbs and Mackenzie (1997). The extracted RNA was stored at -80°C.

cDNA synthesis: Ten microliters of total RNA were added to mixture contained 6 μL of 5X first strand buffer (250 mM Tris-HCl pH 8.3, 375 mM KCl and 15 mM $MgCl_2$), 3 μL of 0.1 M dithiothreitol (DTT), 1 μg of complementary specific primer CP/CMV and sterile H_2O to a final volume of 30 μL. The primer annealed at 65°C for 30-45 min. The annealed reaction containing 20 μL of cDNA: 4 μL of 5X first strand buffer, 2 μL of 0.1 M DTT, 1 μL of RNAsin (40 units, Promega Corp., Madison, US), 5 μL of 0.3 M β-mercaptoethanol, 2.5 μL of 10 mM dNTPs and 1 μL of *Moloney murine leukemia virus* (MMLV) (200 U μL^{-1}) reverse transcriptase (Promega, Corp.). Reaction was mixed briefly and incubated for 1-1.5 h at 42°C (Hsu *et al.*, 1995).

Amplificaion of CMV/CP coding sequence: Amplification was performed in thin-walled PCR tubes contained the reaction mixture as following: 5 μL of cDNA reaction, 45 μL of the amplification mixture containing 5 μL of 10X Taq DNA polymerase buffer (20 mM Tris-HC1, pH 8.2, 10 mM KCl, 6 mM Gelatin, 2 mM $MgCl_2$, 0.1% Triton X-100 and 10 μg mL^{-1} of nuclease-free BSA), 1 mM each dNTP, 50 ng of each complementary reverse primer 5'-TTGGATCCTCAGACTGGGAG-3' and forward primer 5'-AACCATGGACAAATCAGAA-3' (Abdelkader *et al.*, 2006), 2.5 unites of Taq polymerase (5 unites μL^{-1}) (Promega) and completed with sterile distilled water. The amplification was carried out using the UNOII thermocycler system (Biometra, Germany) and using 0.2 mL micro. Amp. PCR tubes. 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 PCR products were analyzed by electrophoresis onto 1% agarose gel in 1X TAE buffer (40 mM Tris-acetate, 1 mL acetic acid, 2 mM Na-EDTA and ethidium bromide [10 mg mL^{-1}]).

DNA sequencing of CMV coat protein gene: DNA fragments were purified from agarose gel using the gel slicing and melting method described by Wieslander (1979). The nucleotide sequence of the cp for CMV was carried out at Macrogen 3730XL6-1518-009, Korea by ABI 1.6.0 sequencer. The sequence data was translated and analyzed by DNAMAN program.

RESULTS

Virus isolation: The virus isolate was detected in the naturally infected cucumber plants showing mosaic, net yellow and malformation while gave positive results by DAS-ELISA using specific CMV-IgG. It was biologically isolated by single local lesion from *C. amaranticolor* gave chlorotic local lesions surrounded by yellowing hallo and propagated in healthy *N. glutinosa* and cucumber plants as well as confirmed by DAS-ELISA.

Table 1: Reactions of host range plants inoculated with CMV*

	Tested plants			
Virus reaction	Family	Scientific name	Incubation period (day)	External symptoms
Local symptoms	Chenopodiaceae	*C. amaranticolor*	4	CLL
		C. metel	4	CLL
		C. murale	4	NLL
	Amaranthaceae	*Gomphrena globosa*	4	CLL
Systemic symptoms	Cucurbitaceae	*Cucumis sativus* cv. Beith Alpha	15	SM, B, Mf
		Cucumis sativus cv. Atlas	15	SM, B, Mf
		Cucurbita pepo cv. Eskandrani	10	Vc, SM,B, GVB, Mf
	Solanaceae	*N. glutinosa*	14	SM, FL, Mf
		N. tabacum cv. White Burly	20	SM, B
		D. metel	10	M, B
		Petunia hybrida	12	SM, Mf, D
	Compositae	*Helicrysum bracteatum*	17	M
Symptomless	Cucurbitaceae	*Cucurbita maxima*	37	NS
	Solanaceae	*N. rustica*	30	NS
		L. esculantum	30	NS
		Capsicum annum	34	NS
	Leguminoseae	*Vigna sinensis*	30	NS

NS: No symptoms; S: Severe; CLL: Chlorotic local lesions; M: Mosaic; B: Blisters; N: Necrosis; Mf: Malformation; VB: Vein banding; Vc: Vein clearing; FL: Fern leaf and D: Discoloration, NLL: Necrotic local lesions, SM: Severe mosaic, Y: Yellowing. *All CMV infected hosts were detected by DAS-ELISA and local lesion host

Virus identification: The virus isolate was identified as CMV on the basis of biological properties (external symptoms, host range, mode of transmission, virus stability, inclusion bodies and virus morphology), serological reactions (DAS-ELISA and DBIA) and molecular characters (RT-PCR and CP-sequence).

Host range: The reaction of eighteen plant species belonged to 6 families to the virus are summarized into three symptom types in Table 1. Local symptoms *C. amaranticolor, C. metel* and *Gomphrena globosa* produced chlorotic local lesions while *C. murale* produced necrotic local lesions on inoculated leaves after 4 days post inoculation. (Fig. 1; 4, 9, 10). Systemic symptoms, cucumber plants showed severe mosaic, blisters and malformation (Fig. 1; 1). While squash plants gave severe mosaic, green vein banding, blisters and malformation (Fig. 1; 2). *N. glutinosa* appeared severe mosaic, fern leaf and malformation; *N. tabacum* cv. White Burly produced severe mosaic; *Datura metel* showing severe mosaic and malformation, *Helicrysum bracteatum* showed yellowing symptoms and *Petunia hybrida* gave severe mosaic, malformation and discoloration (Fig. 1; 3, 5, 6, 7, 8). Symptomless, the following species did not react with CMV; *Cucurbita maxima, N. rustica, Lycopersicon esculantum, Capsicum annum, Vigna sinensis* and *Vicia faba* (Table 1).

Virus stability in crude sap: The stability of CMV isolate in infectious crude sap extracted from infected *Cucumis sativus*. It was determined by local lesions on leaves of *C. amaranticolor* as an indicator host. The virus isolate was inactivated at 70°C for 10 min., DEP was 10^{-4} and CMV kept its infectivity for 4 days at room temperature 25-28°C.

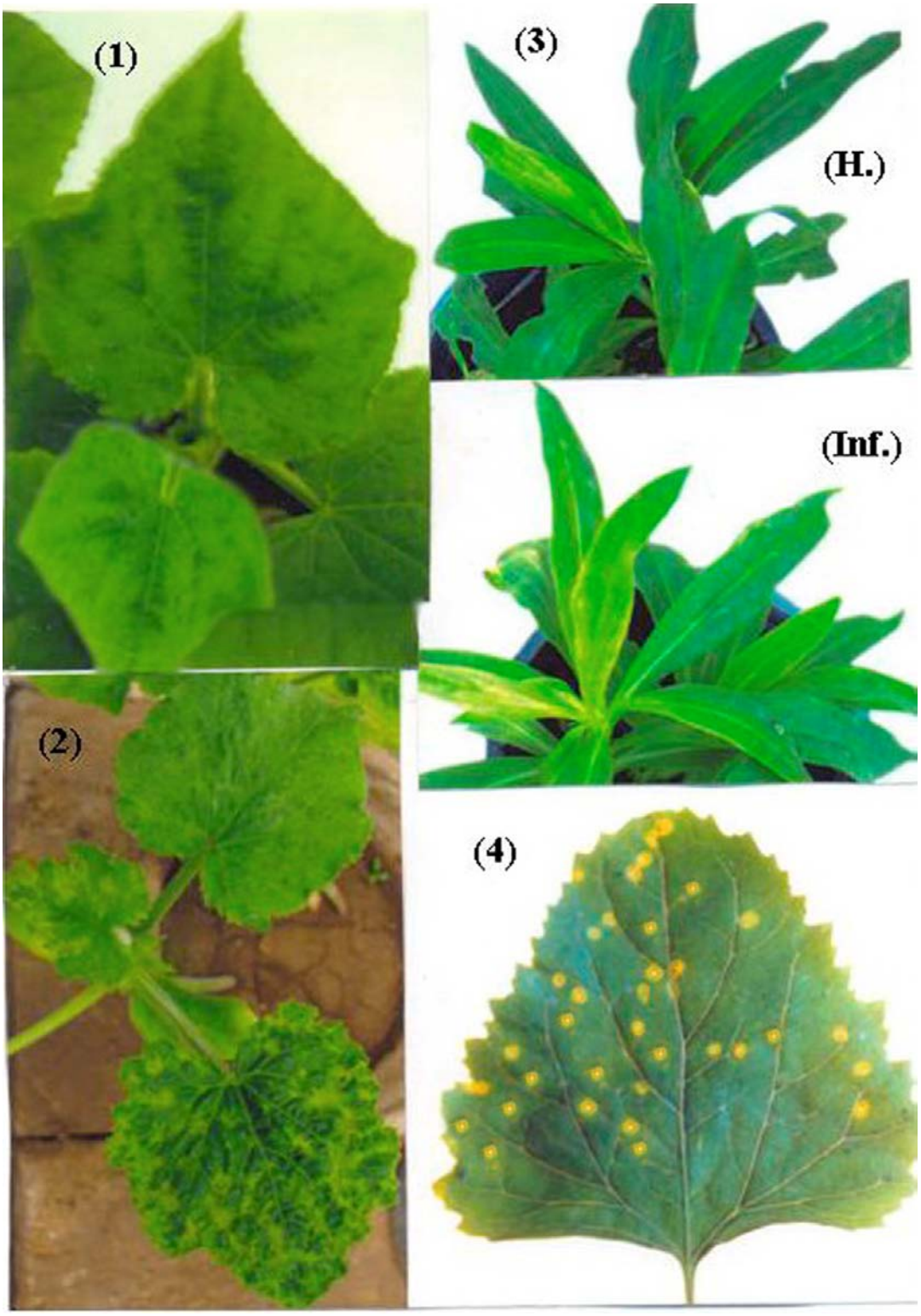

Fig. 1: Continued

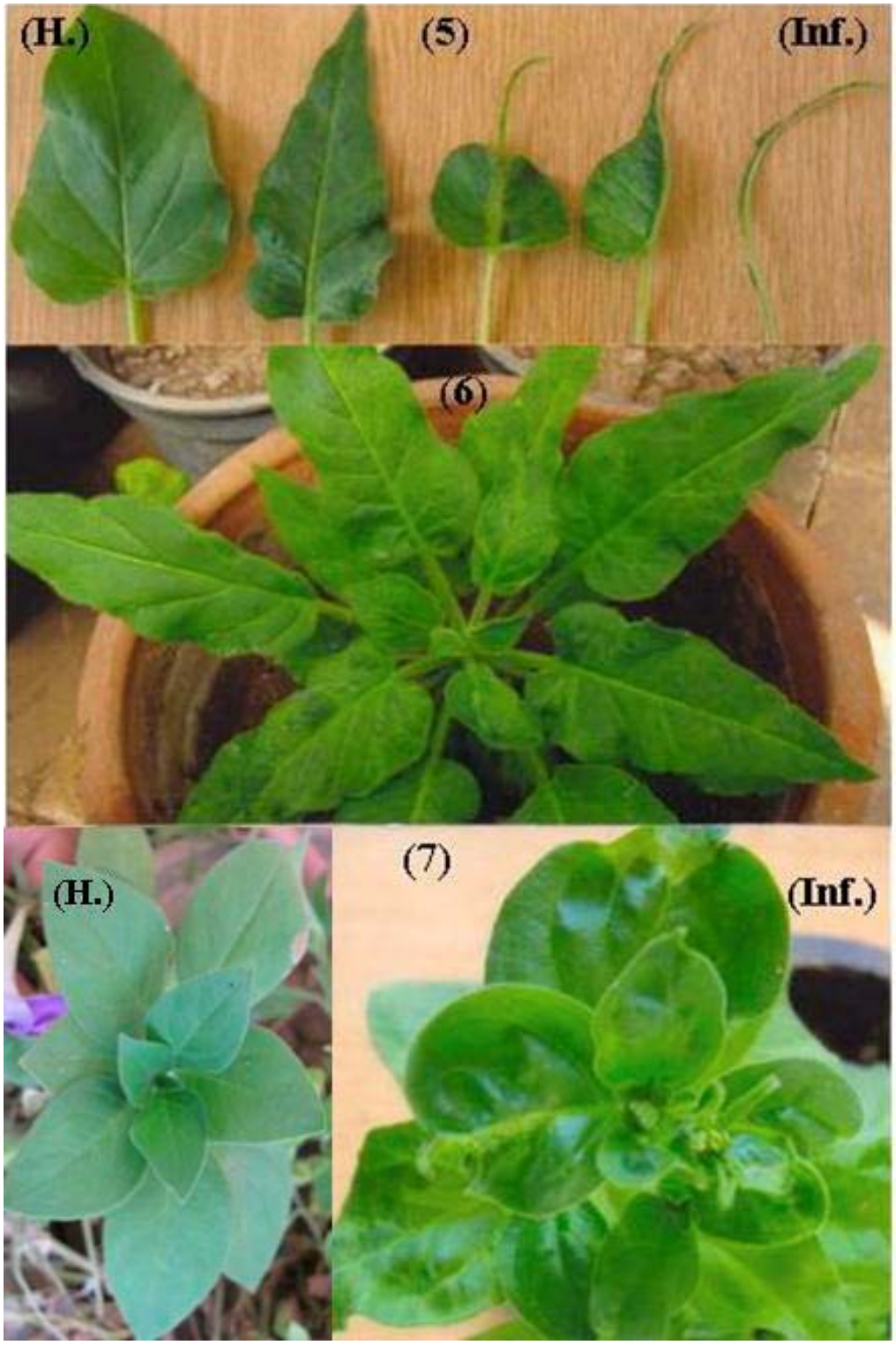

Fig. 1: Continued

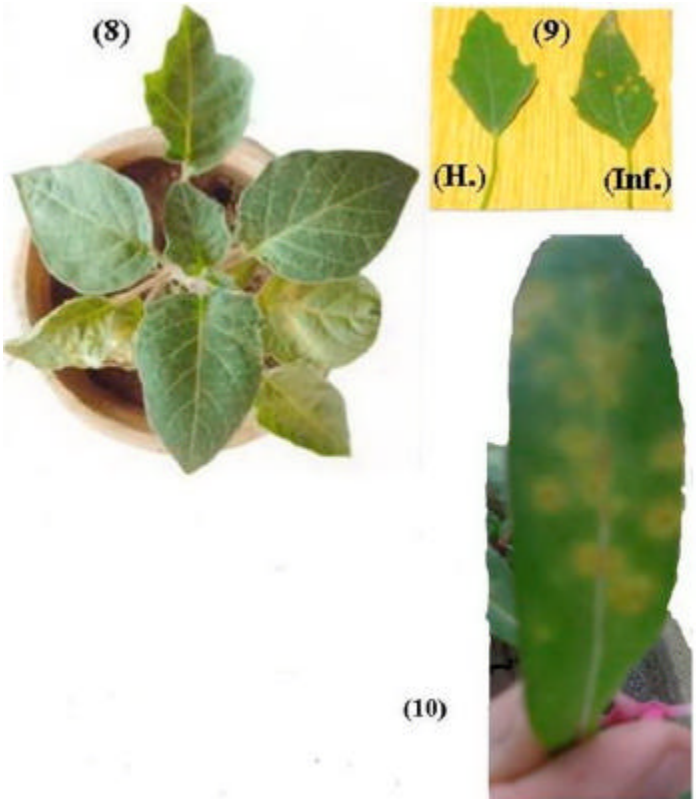

Fig. 1(1-10): Host plants mechanically inoculated with CMV isolate showing different types of symptoms on leaves: (1) *Cucumis sativus*, (2) *Cucurbita pepo*, (3) *Helicrysum bracteatum*, Healthy (H.) and infected (Inf.), (4) *C. amaranticolor*, (5-6) Development of symptoms on *N. glutinosa*, Healthy (H.) and infected (Inf.), (7) *Petunia hybrida*, Healthy (H.) and infected (Inf.), (8) Infected *D. metel*, (9) *C. murale*, Healthy (H.) and infected (Inf.) and (10) Infected *G. globosa*.

Crystalline and amorphous inclusions: The crystalline inclusions induced by CMV were observed in epidermal and hair cells, as well as amorphous inclusions stained by bromophenol blue and mercuric chloride in leaves, 15 days post virus inoculation, Fig. 2.

The purified virus: The purification method of CMV isolate gave high yield of virus particles (1.43 mg/100 g fresh weight leaves). The UV spectrum of CMV particles was showed that, the absorption ratio of min., max. nm, $A_{max.}/A_{min.}$, A_{260}/A_{280} and A_{280}/A_{260} were 248, 261, 1.101, 1.204 and 0.83, respectively.

Electron microscopy of purified CMV isolate was negatively stained with uranyl actate (2%), showed isometric particles with 30 nm in diameter.

Serological reaction: The virus antigen was serologically precipitated reaction against specific polyclonal IgG-CMV by immunoblotting, Fig. 3. A purplish blue color was developed with infected cucumber in the positive reaction, whereas extracts from healthy plants remain green in the negative reactions.

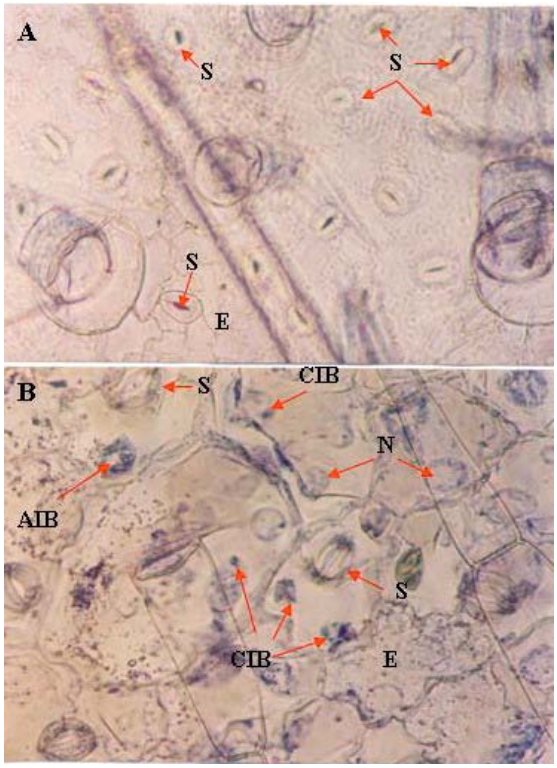

Fig. 2(A-B): Light micrograph of epidermal strips and hairs (A) healthy and (B) CMV infected cucumber leaves (15 days post inoculation) showing cytoplasmic inclusion bodies. Magnification (X-400). N: Nucleus, S: Stomatic, E: Epiderms, CIB: Crystalline Inclusion Bodies and AIB: Amorphous Inclusion Bodies

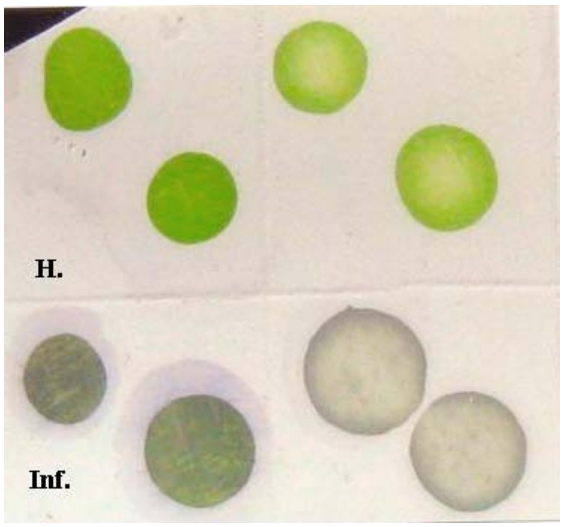

Fig. 3: Dot Blot Immunoassay for CMV precipitation against specific IgG-CMV polyclonal. H: Healthy samples, Inf.: Infected samples

Molecular characters of CMV isolate: The concentration of the RNA was 95 µg/0.2 g of infected tissues and the purity was measured by $A_{260/280}$ absorbance ratio 1.5.

cDNA of CP-CMV was synthesized by MMLV reverse transcriptase. The resulting complementary DNA (cDNAs) was amplified by PCR after adding one set of primer CMV for *cp* gene. The efficiency of DNA amplification from CMV infected leaf tissues was detected by analysis PCR product using 1.0% agarose gel electrophoresis. The size of amplified CMV-DNA fragment was as expected 582 bp. (Lane 2) in Fig. 4.

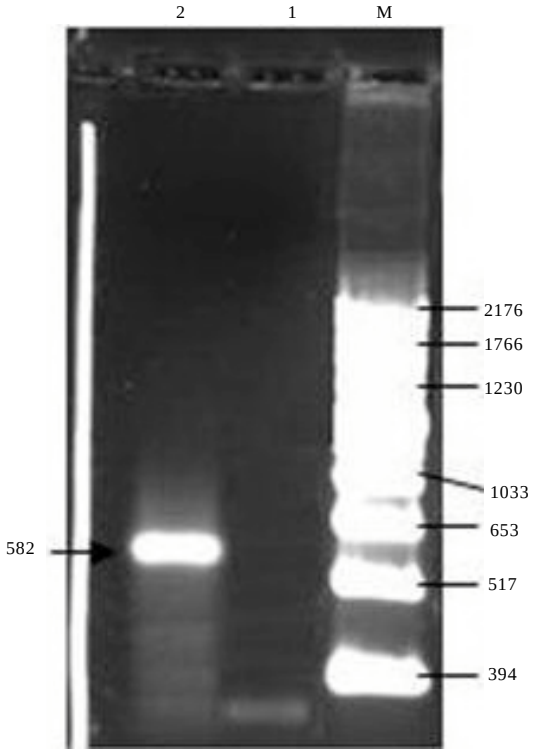

Fig. 4: One % agarose gel electrophoresis showing the amplified PCR product of CMV/CP of the correct size (582 bp). Lane 1: Healthy *N. glutinosa*, Lane 2: amplified CMV/CP isolate, M: DNA marker

Partial nucleotide sequence of CMV-*cp* gene: The partial nucleotide sequence (582 nucleotide) of the CMV-EG-CP was aligned with other coat protein sequences of CMV satellite RNA published in GeneBank as shown in Fig. 5 by using DNAMAN program (DNAMAN V 5.2.9 package, Madison, Wisconsin, USA).

A Phylogenetic tree of CMV-EG presented in Fig. 6 revealed the nucleotide sequence of CMV under study has 41% identified to AB024493 and D00542 satellite RNA CMV isolates.

The nucleotide sequence of *cp* gene for CMV-EG (Table 2) revealed the highest content for Thymine (T) 154 (26.5%) followed by Guanine (G) 153 (26.3%), then Adenine (A) 138 (23.7%) and Cytosine 137 (23.5%).

Comparison between nitrogen bases composition of partial *cp* gene sequence for CMV-EG (Accession no. EU365893) and two CMV satellite RNA isolates published in GeneBank, AB024493

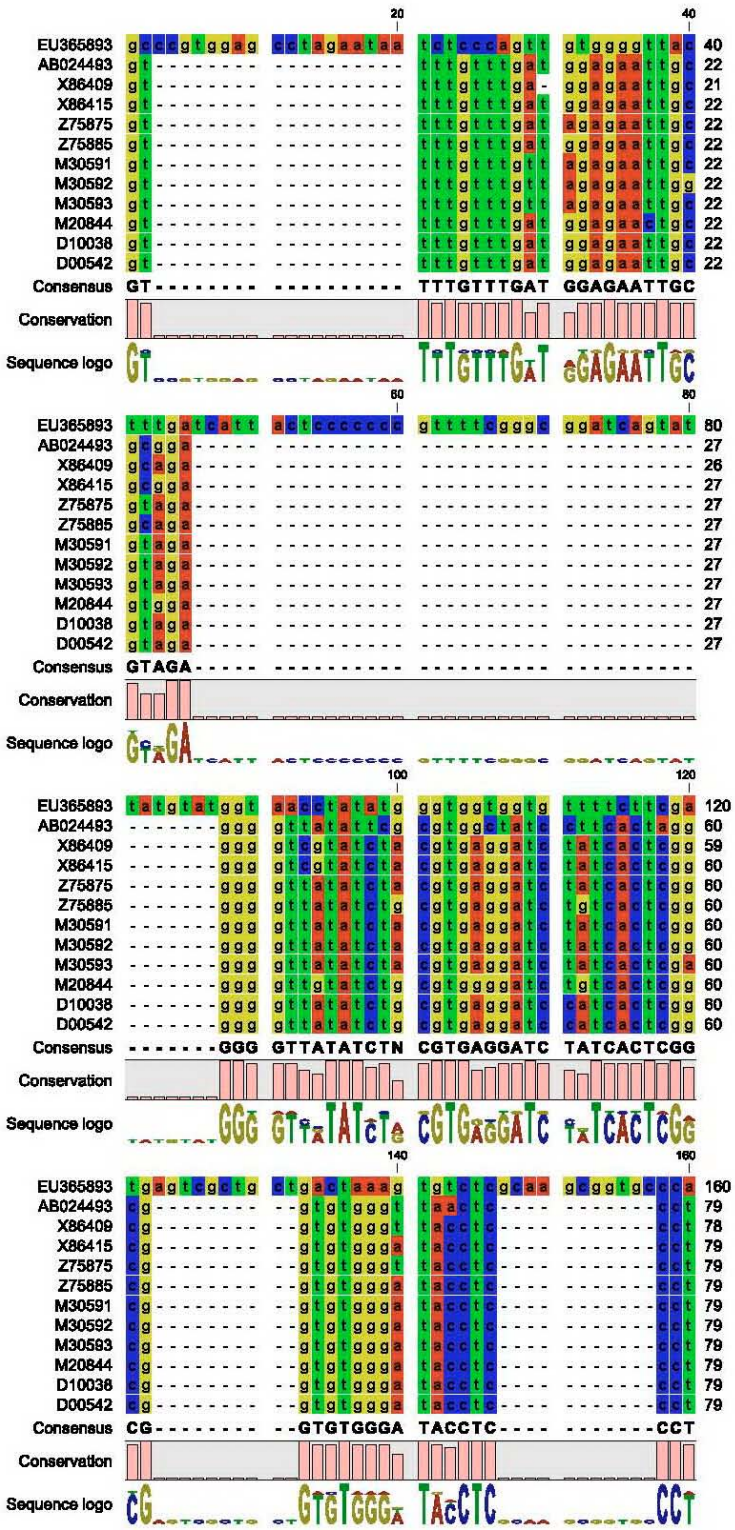

Fig. 5: Continue

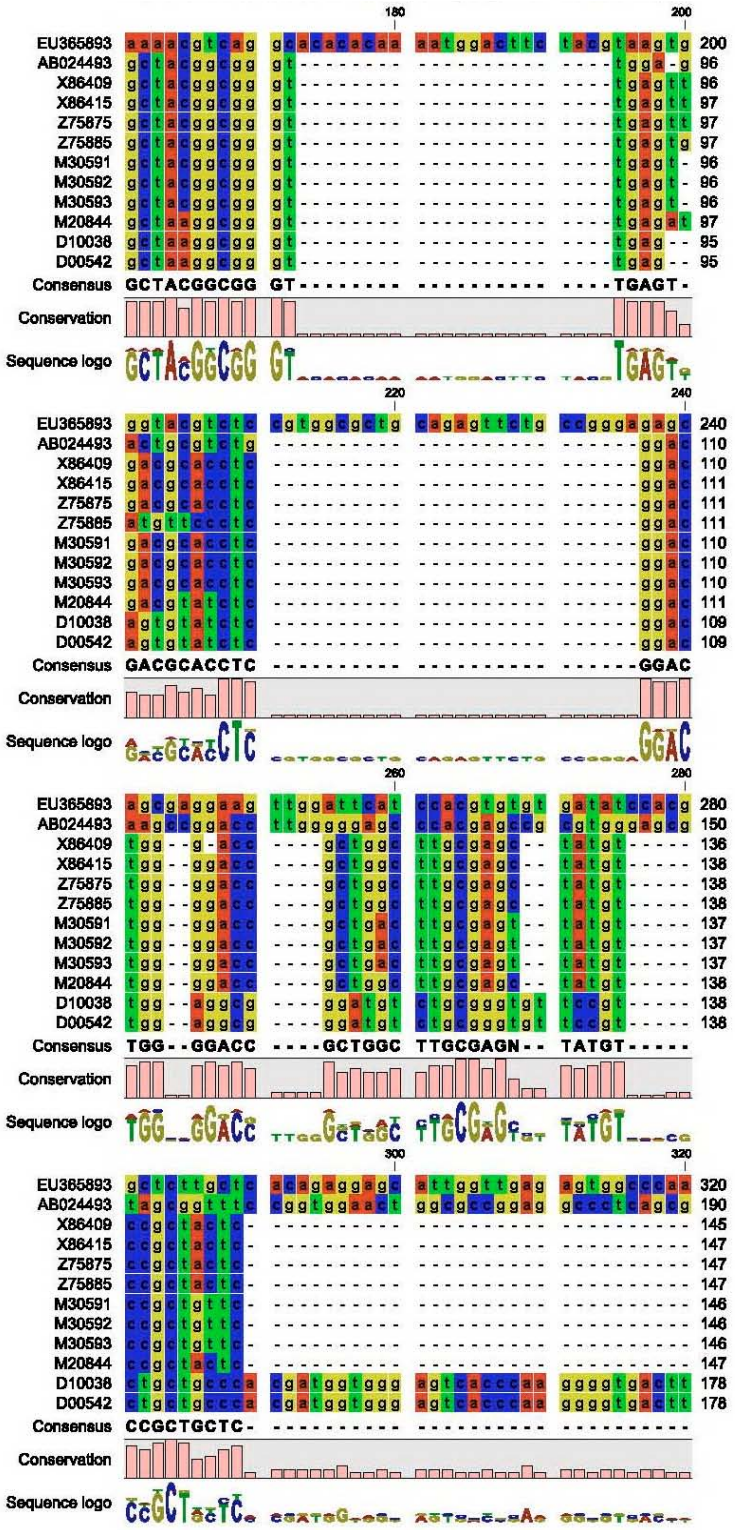

Fig. 5: Continue

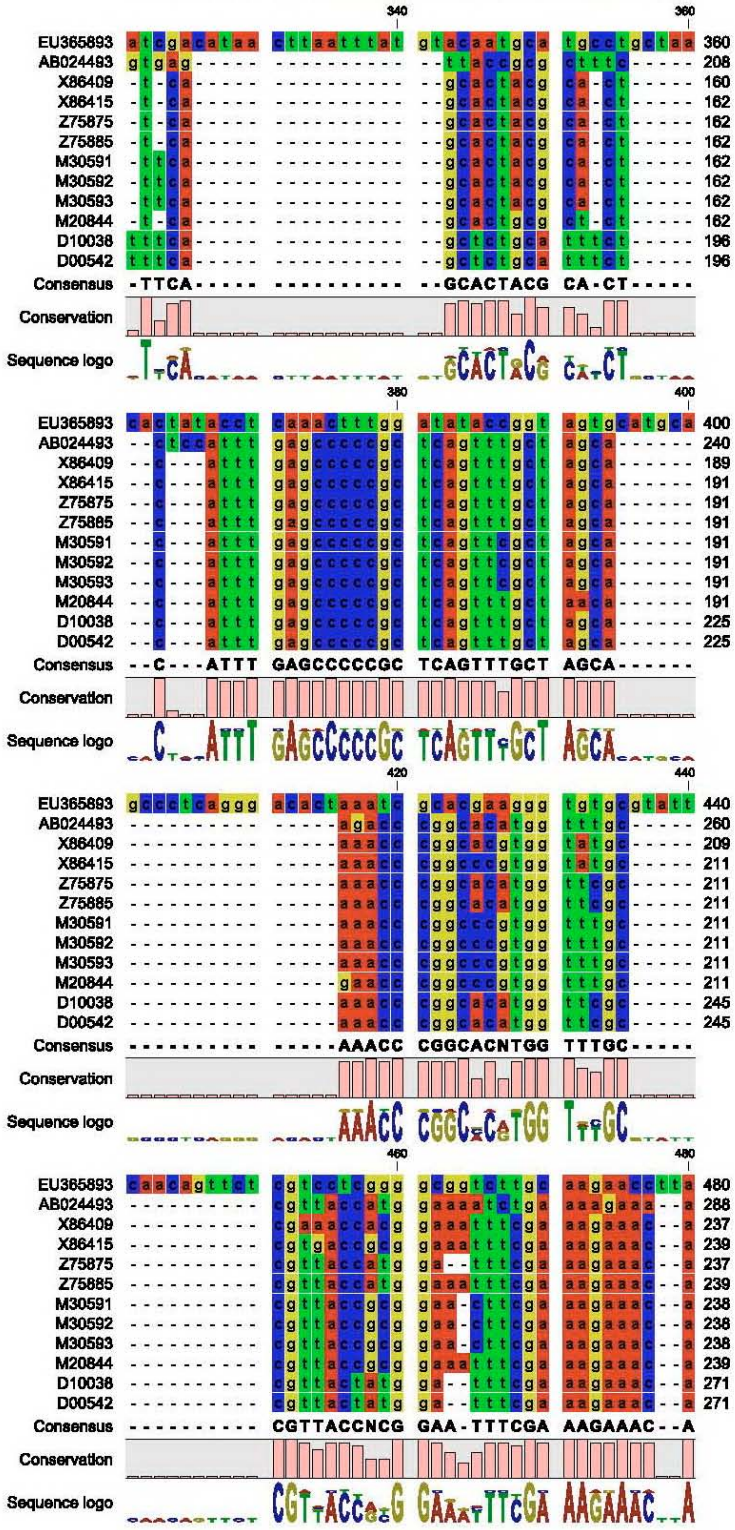

Fig. 5: Continue

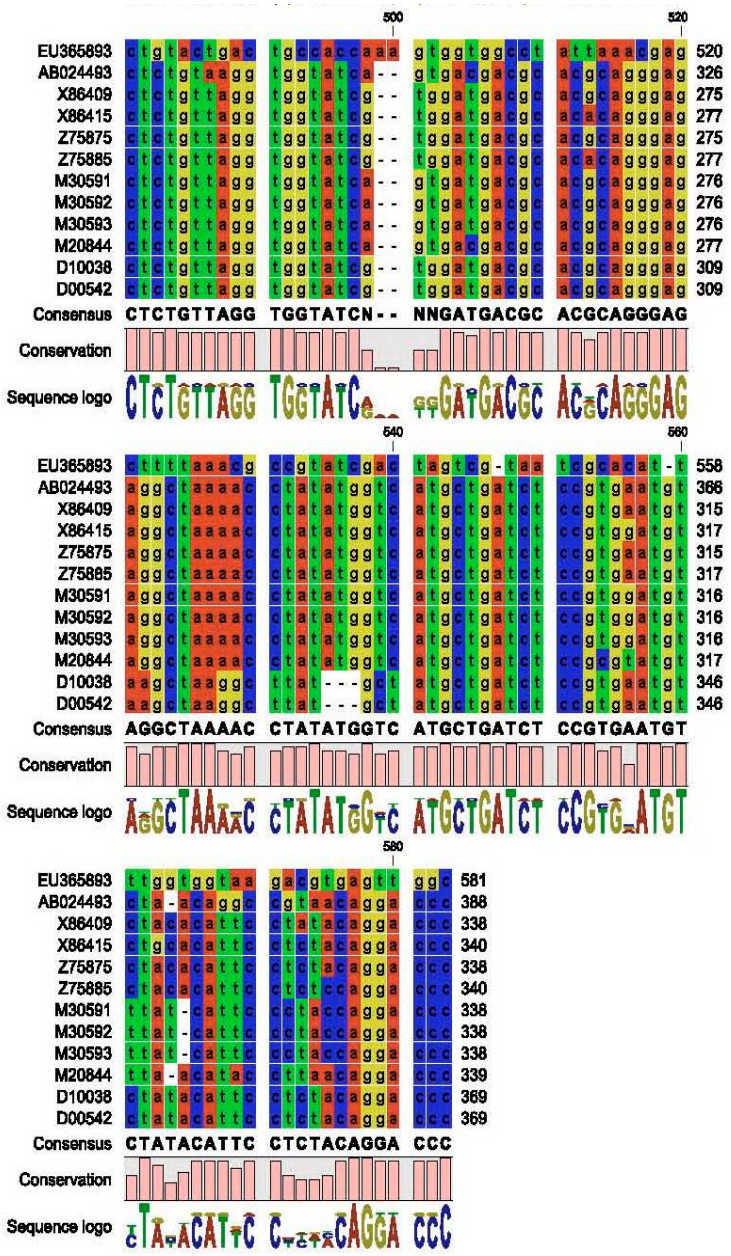

Fig. 5: Partial nucleotide sequence alignment of the CP for CMV-EG using DNAMAN program

and D00542 (Hidaka *et al*., 1984) was done to determine A/T, G/C ratio as well as A+T and G+C percentage was 0.896, 1.116, 50.2 and 49.8% of CMV-EG, 0.909, 1.291, 43.3 and 56.7% of AB024493 and 0.715, 1.309, 47.4 and 52.6% to D00542, respectively.

Deduced amino acids sequence of CMV*cp* gene: The predicted numbers of amino acids produced from translation of partial *cp* gene nucleotide sequence of CMV-EG were 193 amino acids starting with proline (P) and ended with leucine (L), in Fig. 7.

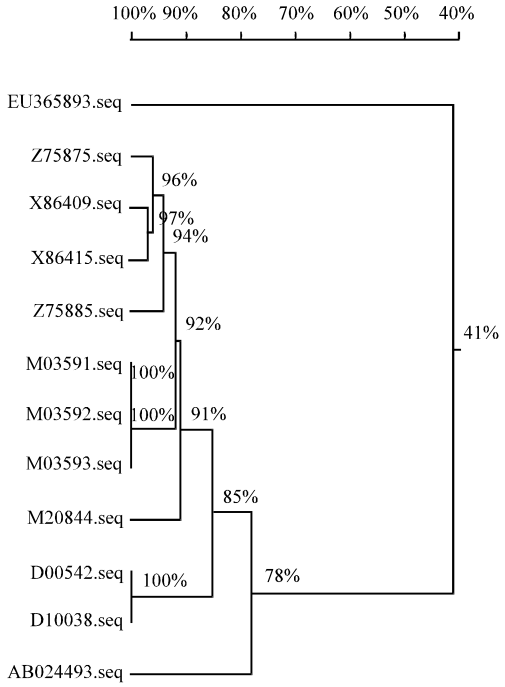

Fig. 6: Consensus phylogenic tree constructed from the multiple alignment of the CP/CMV-EG (under study) and eleven satellite CMV isolates

```
(Translation of DNAMAN1(1-582 Universal code
Total amino acid number: 193, MW=20996
Max ORF starts at AA pos 1(may be DNA pos 2) for 193 AA(579
bases), MW=20996
   GCCCGTGGAGCCTAGAATAATCTCCCAGTTGTGGGGTTACTTTGATCATTACTCCCCCCC 1
   P  V  E  P  R  I  I  S  Q  L  W  G  Y  F  D  H  Y  S  P  P   1

  GTTTTCGGGCGGATCAGTATTATGTATGGTAACCTATATGGGTGGTGGTGTTTTCTTCGA 61
  F  S  G  G  S  V  L  C  M  V  T  Y  M  G  G  G  V  F  F  D   21

 TGAGTCGCTGCTGACTAAAGTGTCTCGCAAGCGGTGCCCAAAAACGTCAGGCACACACAA 121
 E  S  L  L  T  K  V  S  R  K  R  C  P  K  T  S  G  T  H  K    41

AATGGACTTCTACGTAAGTGGGTACGTCTCCGTGGCGCTGCAGAGTTCTGCCGGGAGAGC  181
M  D  F  Y  V  S  G  Y  V  S  V  A  L  Q  S  S  A  G  R  A    61

AGCGAGGAAGTTGGATTCATCCACGTGTGTGATATCCACGGCTCTTGCTCACAGAGGAGC  241
A  R  K  L  D  S  S  T  C  V  I  S  T  A  L  A  H  R  G  A    81

ATTGGTTGAGAGTGGCCCAAATCGACATAACTTAATTTATGTACAATGCATGCCTGCTAA  301
L  V  E  S  G  P  N  R  H  N  L  I  Y  V  Q  C  M  P  A  N    101

 CACTATACCTCAAACTTTGGATATACCGGTAGTGCATGCAGCCCTCAGGGACACTAAATC 361
 T  I  P  Q  T  L  D  I  P  V  V  H  A  A  L  R  D  T  K  S    121

 GCACGAAGGGTGTGCGTATTCAACAGTTCTCGTCCTCGGGGCGGTCTTGCAAGAACCTTA 421
 H  E  G  C  A  Y  S  T  V  L  V  L  G  A  V  L  Q  E  P  Y    141

CTGTACTGACTGCCACCAAAGTGGTGGCCTATTAAACGAGCTTTTAAACGCCGTATCGAC  481
C  T  D  C  H  Q  S  G  G  L  L  N  E  L  L  N  A  V  S  T    161

            TAGTCGTAATCGCACATTCTTGGTGGTAAGACGTGAGTTGGC         541
              S  R  N  R  T  F  L  V  V  R  R  E  L           181
```

Fig. 7: Predicted amino acid of the *cp* gene of the isolated CMV-EG

Table 2: Comparison between bases composition of partial CP sequence CMV and different two CMV satellite isolates published in GeneBank

CMV strains	Total base pairs (bp)	Molecular weight (Kda)	Nitrogen bases A No.	A (%)	T No.	T (%)	A+T No.	A+T (%)	G No.	G (%)	C No.	C (%)	G+C No.	G+C (%)	A/T ratio	G/C ratio
CMV-EG	582	177.609	138	23.7	154	26.5	292	50.2	153	26.3	137	23.5	290	49.8	0.896	1.116
AB024493	388	119.019	80	20.6	88	22.7	168	43.3	124	32.0	96	24.7	220	56.7	0.909	1.291
D00542	369	112.782	73	19.8	102	27.6	175	47.4	110	29.8	84	22.8	194	52.6	0.715	1.309

Comparison between the predicted amino acids of partial *cp* gene sequence of CMV-EG showed that, the type of amino acids consists of 20 amino acids. Leucine and serin has a high frequency and percentage 20 and 10.36%, respectively while tryptophan has a low frequency and percentage 1 and 0.51%.

DISCUSSION

In the present study, the CMV was detected in naturally infected cucumber plants cv. Barakoda which showed distincted viral symptoms in the form of mosaic, mottle, blisters, crinkle, net yellow and malformation. The detected samples gave $^{+}$ve reaction against specific antiserum by using DAS-ELISA technique (Cardin and Moury, 2007).

CMV was isolated by single local lesion assay on *C. amaranticolor* as reported by many investigators (Osman *et al*., 2005; Awasthi *et al*., 2005; El-Afifi *et al*., 2007). *N. glutinosa* was used as a propagative host, where as revealed severe systemic symptoms in the form of severe mosaic, fern leaf and malformation. These symptoms were differed than common CMV which showing mosaic only. These results in agreement with Kim *et al*. (2011).

The CMV isolate under study was identified based on biological, serological and molecular characters. It infected 12 plant species and cultivars, 8 out of 12 plants showed systemic symptoms while only 4 species showed local lesions, Table 1. These results were in accordance with that of many investigators (Roossinck 2001; Osman *et al*., 2005; Hu and Chang, 2006).

Duffus (1973) stated that, disease symptoms on plants in the field are almost always inadequate on their own to give a positive identification. This is particularly so when several viruses cause similar symptoms. Matthews (1991) reported that, since, early days of plant virology, searches have been made for suitable species or varieties of host plant that will give clear, characteristic and consistent symptoms for the virus or viruses being studied usually under greenhouse conditions, such as indicator hosts provide one of the most basic tools for routine diagnosis. Many good indicator species have been found in the genera, *Nicotiana, Solanum, Chenopodium, Cucumis, Phaseolus, Vicia* and *Brassica*. In this investigation *C. amaranticolor* and *C. murale* were used as an indicator plants.

The virus isolate is sap transmissible. Its thermal inactivation point is 70°C, dilution end point 10^{-4} and the virus completely inactivated after 4 days at room temperature. These results were differed than CMV common which have 65°C, 10^{-3} and 4 days of TEP, DEP and LIV, respectively. These results were compatible with that found by Kiranmai *et al*. (1997) and Osman *et al*. (2005).

Matthews (1991) reported that, stability of the virus (thermal inactivation point, dilution end point and ageing at room temperature) as measured by infectivity (often in crude extracts) was an important criterion in attempting to establish groups of viruses.

The CMV-EG isolate formed cytoplasmic inclusions in infected epidermic cell leaves.

Virus purification was applied using different centrifugation which give 1.43 mg/100 g fresh weight leaves. The absorption ratio A_{260}/A_{280} was 1.204. These results agreed with those reported by Hancheng *et al.* (1992).

Electron microscopy of purified CMV showed isometric particles shape with 30 nm in diameter, the same result was mentioned by El-Afifi *et al.* (2007) and Cardin and Moury (2007).

The results of the dot blot immunoassay technique was found to be sensitive to detect CMV in all infected plants in accordance with that obtained by Awasthi *et al.* (2005) and Zein *et al.* (2007). The antigenic determinants of CMV-EG isolate were identified by using polyclonal antibodies specific CMV which give serologically precipitation reacted in DAS-ELISA and in DBIA where the purple color appeared clearly.

RT-PCR of the CMV/CP-Egyptian isolate was used to amplify a fragment of about (582 bp) using primers according to Yu *et al.* (2005) and Abdelkader *et al.* (2006). Yu *et al.* (2005) mention that, the size of DNA fragment was 600 bp for CMV/CP, the results were expected as the same range of CMV/CP with the obtained results while (Wu *et al.*, 2007) the result of the CMV-CP amplified from *Capsicum annuum* showed the band of the fragment with 740 bp. As well as, Akhtar *et al.* (2008) found that, amplified PCR product was 850 bp from the CMV infected tomato leaves.

The nucleotide sequence of *coat protein* gene of CMV was 890 nucleotides which revealed more than 90% similarity with the CMV subgroup I strains (Haq *et al.*, 1996) while CMV-EG isolate under study revealed 582 nucleotides of CP-sequence with 41% identified to AB024493 and D00542 satellite RNA CMV isolates.

The nucleotide sequence of *cp* gene for CMV-EG revealed that the highest content for Thymine (T) 154 (26.5%) followed by Guanine (G) 153 (26.3%), then Adenine (A) 138 (23.7%) and Cytosine 137 (23.5%).

Comparison between nitrogen bases composition of partial *cp* gene sequence for CMV-EG (Accession no. EU365893) and two CMV satellite RNA isolates published in GeneBank, AB024493 and D00542 (Hidaka *et al.*, 1984) was done to determine A/T, G/C ratio as well as A+T and G+C percentage were 0.896, 1.116, 50.2 and 49.8% of CMV-EG., 0.909, 1.291, 43.3 and 56.7% of AB024493 and 0.715, 1.309, 47.4 and 52.6% to D00542, respectively.

Comparison between the predicted amino acids of partial *cp* gene sequence of CMV-EG has showed that, the type of amino acids consists of 20 amino acids. Leucine and serin have a high frequency and percentage 20 and 10.36%, respectively while tryptophan has a low frequency and percentage 1 and 0.51%.

As the previous results, it was found that, the obtained CMV-EG isolate under study was differed from other common CMV isolates on biological characters symptomatology on cucumber, *N. glutinosa* and *C. amaranticolor*. On other hands, the obtained nucleotide sequence of CMV-EG had a similarity (41%) with different eleven satellite RNA Cucumber mosaic virus which published in GeneBank, so we suggest that, the obtained isolate is a satellite CMV and its nucleotide sequence was published in GeneBank with Accession no. EU365893 as a new Egyptian satellite strain of Cucumber mosaic cucumovirus.

REFERENCES

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.

Akhtar, K.P., K.H. Ryu, M.Y. Saleem, M. Asghar, F.F. Jamil, M.A. Haq and I.A. Khan, 2008. Occurrence of *Cucumber mosaic virus* subgroup IA in tomato in Pakistan. J. Plant Dis. Prot., 115: 2-3.

Awasthi, L.P., K. Pardeep and K.M. Nehal, 2005. Management of Cucumber mosaic virus disease in cucumber through root extract of *Boerhaavia diffusa*. Ann. Plant Prot. Sci., 13: 256-257.

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.

Cardin, L. and B. Moury, 2007. First report of cucumber mosaic virus in echium candicans in France. Plant Dis., 91: 1516-1516.

Carrere, I., M. Tepfer and M. Jacquemond, 1999. Recombinants of cucumber mosaic virus (CMV): Determinants of host range and symptomatology. Arch. Virol., 144: 365-379.

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.

Duffus, G.E., 1973. The yellowing virus disease of beet ADV. Virus Res., 18: 347-386.

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.

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.

Hancheng, Z., L. Huanting and Y. Dunyu, 1992. Identification of cucumber mosaic virus from ambary plant. J. Shandong Agric. Univ., 23: 165-169.

Haq, Q.M.R., B.P. Singh and K.M. Srivastava, 1996. Biological, serological and molecular characterization of a cucumber mosaic virus isolate from India. Plant Pathol., 45: 823-828.

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.

Hidaka, S., K. Hanada, K. Ishikawa and K. Miura, 1988. Complete nucleotide sequence of two new satellite RNAs associated with cucumber mosaic virus. Virology, 164: 326-333.

Hidaka, S., K. Ishikawa, Y. Takanami, S. Kubo and K. Miura, 1984. Complete nucleotide sequence of RNA 5 from cucumber mosaic virus (strain Y). FEBS Lett., 174: 38-42.

Hsu, Y.H., C.W. Wu, B.Y. Lin, H.Y. Chen, M.F. Lee and C.H. Tsai, 1995. Complete genomic RNA sequences of cucumber mosaic virus strain NT9 from Taiwan. Arch. Virol., 140: 1841-1847.

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

Kaperi, J.M., M.E. Tousignant and M.T. Steen, 1988. Cucumber mosaic virus-associated RNA 5 XI. Comparison of 14 CARNA 5 variants relates ability to induce tomato necrosis to a conserved nucleotide sequence. Virology, 163: 284-292.

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.

Kim, M.K., H.R. Kwak, S.H. Lee, J.S. Kim, K.H. Kim, B.J. Cha and H.S. Choi, 2011. Characteristics of *Cucumber mosaic virus* isolated from *Zea mays* in Korea. Plant Pathol. J., 27: 372-377.

Kiranmai, G., P. Sreenivasulu and M.V. Nayudu, 1997. Characterization of Cucumber mosaic cucumovirus isolates naturally infecting three solanaceous vegatible crops in Andhra Prodesh. Indian Phytopathol., 50: 416-425.

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

Matthews, R.E.F., 1991. Plant Virology. 3rd Edn., Academic Press, Inc., London, pp: 402.

Mazia, D., P.A. Brewer and M. Alfert, 1953. The cytochemical staining and measurement of protein with mercuric bromphenol blue. Biol. Bull., 104: 57-67.

Murant, A.F., 1965. The morphology of cucumber mosaic virus. Virology, 26: 538-544.

Noordam, D.D., 1973. Identification of Plant Viruses. Methods and experiments. Center for Agricultural Publishing and Documentation, Wageningen, Netherlands, Pages: 207.

Osman, M., K. El-Dougdoug, E.T. Abd El-Salam, R.M. Taha and R.M. El-Hamid, 2005. Histo-cytopathological effects of cucumber mosaic cucumovirus on squash leaves. Egyptian J. Virol., 1: 343-353.

Roossinck, M.J., 2001. Cucumber mosaic virus, a model for RNA virus evolution. Mol. Plant Pathol., 2: 59-63.

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.

Sharma, A., B.K. Mahinghara, A.K. Singh, S. Kulshrestha and G. Raikhy *et al*., 2005. Identification, detection and frequency of lily viruses in Northern India. Sci. Horticult., 106: 213-227.

Tobias, I., D.Z. Maat and H. Huttinga, 1982. Two Hungarian isolates of cucumber mosaic virus from sweet pepper (*Capsicum annuum*) and melon (*Cucumis melo*): Identification and antiserum preparation. Eur. J. Plant Pathol., 88: 171-183.

Valverde, R.A. and J.A. Dodds, 1987. Some properties of isometric virus particles which contain the satellite RNA of tobacco mosaic virus. J. Gen. Virol., 68: 965-972.

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 Agricult. Univ., 28: 69-72.

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

Zein, H.S., M. Nakazawa, M. Ueda and K. Miyatake, 2007. Development of serological procedures for rapid and reliable detection of Cucumber mosaic virus with dot-immunobinding assay. World J. Agric. Sci., 3: 430-439.