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# Biological and Molecular Characterization of Cucumber Mosaic Virus Isolate Causing Severe Mosaic in *Gherkin* (*Cucumis anguria* L.) in India

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***Gherkin* (*Cucumis anguria* L.) is an important cucurbitaceous vegetable crop grown in southern states of India *viz.*, Andhra Pradesh, Karnataka and Tamil Nadu for slicing and pickling. The crop infected by Cucumber mosaic virus (CMV) is a most destructive disease on gherkin growth and yield. During 2013-14 survey revealed that, disease incidence was ranging from16.00 to 59.65 per cent with highest incidence was recorded in Kolar (51.27%) and least was in Bangalore rural (17.15%). The virus can readily be transmitted by sap and aphids on cultivar green long which showed systemic mosaic with dark and light green or with yellow patches with slight puckering. Crop like Ash gourd, Bottle gourd, Bush squash, Cucumber, Muskmelon, Pumpkin, Ridge gourd, Round melon and Chilli also showed mosaic symptoms, Tomato showed shoestring symptom, *Chenopodium* and Cowpea showed necrotic local lesions upon inoculation with CMV. Virus reacted strongly to cucumovirus specific antisera through DAS-ELISA. PCR product of CMV infected sample with CP-primers indicated~700bp-DNA and highest nucleotide homology with the strains of CMV subgroup-IB (95-99%) and nucleotide sequence conservation (99%) with AN-strain (subgroup-IB).**

**Keywords:** Cucumber mosaic virus, Gherkin, Coat protein, DAS-ELISA, PCR.

*Cucumber mosaic virus* (CMV, genus: *Cucumovirus*, family: Bromoviridae) is one of the most widespread plant viruses in the world with extensive host range infecting about 1000 species including cereals, fruits, vegetables and ornamentals and its economic impact, CMV has been considered as one of the most important viruses (Roossinck, 1999; Palukaitis and Arenal, 2003). CMV is readily transmitted in a non-persistent manner by more than 75 species of aphids (Palukaitis *et al.*, 1992). Weed hosts function as a reservoir for the virus and serve as sources of inoculum for the development of disease epidemics. Transmission through planting materials is also a significant reason in some crop and weed hosts (Hsu *et al.*, 2000). CMV is a multicomponent virus with a single stranded positive sense RNA. RNA 1 and 2 are associated with viral genome replication while RNA 3 encodes for movement protein and coat protein. Numerous strains of CMV have been classified into two major subgroups (subgroups I and II) on the basis of serological properties and nucleotide sequence homology (Palukaitis *et al.*, 1992). The subgroup I has been further divided into two groups (IA and IB) by phylogenetic analysis (Roossinck *et al.*, 1999). The subgroup IB is suggested to contain the 'Asian strains' whereas other members of subgroup I have been kept under subgroup IA. The nucleotide sequence identity between CMV subgroup II and I strains ranges from 69 to 77%, while above 90% within subgroup (Palukaitis and Arenal, 2003).

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Gherkin (*Cucumis anguria* L.) is an important cucurbitaceous vegetable crop grown in southern states of India like Andhra Pradesh, Karnataka and Thamil Nadu for slicing and pickling. Not only the gherkins are consumed in India, but also exported to other countries like Russia, followed by USA, Canada and Europe valued upto Rs.502 crores. Bottled gherkins pickled in vinegar contribute nearly 50% of the exports (Sukumaran, 2007). They are usually picked when 4 to 8 cm in length and pickled in jars or cans with vinegar or brine to resemble a pickled cucumber. The term can also be used to refer to the West Indian Burr Gherkin, a related species, originally from West Africa and introduced to the West Indies, probably by the Portuguese (Mugadur and Nittur, 2011). Viruses are the most common causes of diseases affecting cucurbits. These diseases result in losses through reduction in growth and yield and are responsible for distortion and mottling of fruit, making the product unmarketable. More than 25 viruses belonging to genera *Cucumo, Como, Tobamo, Poty* and *Ilarvirus* are known to infect cucurbits world wide (Lovisolo, 1980). The mosaic disease in cucurbits was reported to cause by several viruses including members of genera *Cucumo, Como, tobamo* and *potyvirus* (Mukhopadhyay, 1985). The genus *Cucumovirus* is the major virus group infecting cucurbits, of which *Cucumber Mosaic virus* (CMV) is one of the most wide spread virus in the World infecting over 1000 plant species belonging to more than 85 families (Rossinck, 2002) causing yield losses as high as 40-60% (Varma and Giri, 1998). Although the occurrence of CMV on gherkin have been reported (Rashmi, 2005), but exact identification of the causal virus was remained unaddressed. In this article, we report the occurrence of CMV on the basis of biological and coat protein (CP) gene sequence-based properties, a new record on *Cucumis anguria* L. in India.

## MATERIALS AND METHODS

### Virus isolate

A roving survey was conducted in the districts of Southern Karnataka *viz*., Bangalore urban, Bangalore rural, Chikkaballapura, Kolar, Tumkur and also in Chittoor district of Andhra Pradesh to study the incidence of cucumber mosaic virus disease in Gherkin crop. Among the different districts surveyed, the maximum disease incidence was recorded in Kolar (51.27%) followed by Chittoor (40.27%), Tumkur (35.62%), Chikkaballapura (31.45%), Bangalore urban (23.34%) and Bangalore rural (17.15%). Among the varieties grown in the surveyed area, cv. Green long was found highly susceptible to CMV followed by Sparta and Azax and the results were tabulated in Table 1.

The infected plants exhibiting symptoms like mosaic pattern of light and dark green or yellow and green on the leaves, vein banding, malformation of leaves, yellow spotting and stunting were collected and subjected to Double Antibody Sandwich enzyme linked immunosorbent assay (DAS-ELISA) using antisera specific to genus CMV (Fig. 2), a few of them reacted positively with CMV antiserum. Such samples were maintained through mechanical inoculation by extracting sap in chilled 0.1 M phosphate buffer (pH 7) containing 0×1% 2-mercaptoethanol in a mortar kept in an ice tray. The extracted sap was rubbed on the leaves of test plants dusted with celite, which were then washed off with tap water after 2–3 min.

### Host range

The Study was undertaken to know the infectivity of CMV to other host plants. Healthy gherkins and other plant species *i.e.,* Cucumber (*Cucumis sativus* L.), Ash gourd (*Benincasa hispida* Thumb.), Ridge gourd (*Luffa acutangula* L.), Bush squash (*Cucurbita pepo* var. *Clypeata*), Muskmelon (*Cucumis melo* L.), Pumpkin (*Cucurbita maxima* Duchesne), Round melon (*Praecitrullus fistulosus* ), Watermelon (*Citrullus lanatus* Thumb.) and bottle gourd (*Lagenaria siceraria* Standl.) belongs to family Cucurbitaceae, Tobacco (*Nicotiana tobaccum* L. and *Nicotiana glutinosa* L.), Tomato (*Solanum lycopersicum* L.), Chilli (*Capsicum annuum* L.) and Brinjal (*Solanum melongena* L.) belongs to Solanaceae, Papaya (*Carica papaya*) belongs to Caricaceae, Bhendi (*Abelmoschus esculentus* (L.)) and Cotton (*Gossypium hirsutum*) belongs to Malvaceae, Castor (*Ricinus communis*) belongs to Euphorbiaceaae, Lima bean (*Phaseolus lunatus*) belongs to Fabaceae and Radish (*Raphanus sativus*) belongs to the family Cruciferecae were raised in polyethylene bags in insect proof

glasshouse. Seedlings at two leaf stage were inoculated with standard extract of virus by mechanical sap inoculation as described earlier. In each plant species, ten plants were inoculated and one set of un-inoculated plants were maintained as control. The inoculated plants were kept in the insect proof glass house and examined periodically for symptom expression. The standard extract was also inoculated to local lesion hosts *viz*., *Chenopodium amaranticolor* L. belongs to Chenopodiaceae and cowpea (*Vigna unguiculata* L. Walp.) belongs to Legminosae to confirm the presence of virus. The symptoms expressed by the different plant species were recorded.

**Double antibody sandwich ELISA**

Double antibody sandwich (DAS) ELISA was done on polystyrene plates using the protocol described by Clark *et al.* (1986). Wells were initially coated with CMV IgG at 1 ¼g per ml of coating buffer. Antigen was prepared by grinding leaf tissues in 5 vol of PBS-T containing 2% polyvinyl pyrrolidone (PVP) and 0×2% BSA followed by centrifugation at 8000 rpm for 1 min. Supernatant obtained was used to load onto ELISA plates. CMV specific alkaline phosphatase conjugate was used at 1 : 200 dilution. One hour after the addition of substrate (*p*-nitrophenyl phosphate, Genei, Bangalore), the ELISA reactions were read at 405 nm by using an ELISA reader.

**RNA isolation and reverse transcriptase polymerase chain reaction (RT-PCR)**

RNA was extracted using RNA extraction kit (Shrimpex Biotech services Pvt. Ltd.). RT-PCR was performed in the same tube without any buffer changes in between as described by Pappu *et al* (1993). The primers designed for the CP gene sequences of CMV (based on multiple sequence alignments of CP sequences available in GenBank) were used to prime the amplification. Genome sense primer 5'-ATGGACAAATCTGAATCAAC-3' was derived from the beginning of the first 20 bases of the coding region while antisense primer, 5'-TCAAACTGGGAGCACCC-3' represented last 17 bases of the coding region of the CP gene. The PCR reaction (25.0¼l) contained 2.5¼l of 10X PCR buffer (Supplied with the enzyme), 2.0¼l 25mM $MgCl_2$, 2.0¼l 2mM dNTPs mixture, 0.5¼l Forward primer (20pmol/µl), 0.5¼l Reverse primer (20pmol/µl), 0.5¼l Taq Polymerase(3U/µl), 5.0¼l c-DNA and 12.0¼l deionised nuclease free water. The PCR amplification was carried out in a thermal cycler (Eppendorf) with the following conditions; initial denaturation at 94°C for 3 min. followed by 35 cycles having the following parameters 1 min. of denaturation at 94°C, 1 min. of annealing at 53°C and extension for 2 min. at $72^0$C followed by a final extension for 10 min. at $72^0$C. Amplified DNA fragments were electrophoresed in 0.8 per cent agarose gel.

**Sequencing of the CMV CP gene**

After successful confirmation for the presence of expected coat protein gene, the DNA was isolated in large scale using RNA extraction kit (Shrimpex Biotech services Pvt. Ltd.) and sequenced using the automated sequencing facility at Chromous Biotech Pvt. Ltd., Bangalore. Sequencing was done in both directions using M 13 forward and reverse primers.

**Sequence analysis**

The CP gene sequence obtained was subjected to Basic Local Alignment Search Tool (BLAST) in National Centre for Biotechnological Information (NCBI) Database and was compared with various CMV strains belonging to subgroup I (A and B) and II and also with the CP gene sequences of CMV Indian isolates (Table 2) and were edited by using DNASTAR programme.

Sequence data were compiled using MEGA 6.06 version programme. Multiple sequence alignments were made using Clustal W. The aligned files created by ClustalW were bootstrapped 1000 times for generating neighbour-joining phylogenetic tree using Tree Explorer. Tomato aspermy virus (TAV) CP gene (accession No. AJ550020) was used as outsource (Verma *et al.*, 2006).

## RESULTS

**Symptomatology**

In CMV inoculated gherkin cv. Green long, characteristic symptoms of mosaic was developed within 8 to 10 days after inoculation. Initially, alternate green and yellow patches and reduction in leaf size was observed. Later, the infected plants developed symptoms such as vein-banding, severe mosaic and slight puckering with downward curling of the leaves. Often stunting, leaf distortion and wrinkled foliage and mottling were also observed (Fig. 1).

**Host Range studies**

Among the test plant species inoculated with the crude sap, Ash gourd, bottle gourd, Bush squash, Cucumber, Muskmelon, Pumpkin, Ridge gourd, Round melon and Chilli showed systemic symptoms like mosaic mottling and vein banding and Tomato showed shoestring symptoms upon inoculation. Localized chlorotic followed necrotic lesions were observed on leaves of *Chenopodium amaranticolor and Vigna unguiculata L. Walp.*, four to five days after inoculation (Table 3 & Fig. 3).

**Detection of Cucumber Mosaic Virus in Gherkin through DAS -ELISA**

DAS-ELISA technique was used for the detection of cucumber mosaic virus of gherkin. Infected samples showed strong positive reaction to Alkaline phosphatase labelled CMV specific antisera. Absorbance values of duplicate wells were recorded at 30 minutes after adding substrate at 405nm. O.D. values of infected samples were 2 to 3 times higher than that of healthy/buffer control samples, which is indicated by high contrast yellow colour (yellowish green) developed by the CMV infected gherkin plants in ELISA (Fig. 2).

**Amplification of coat protein gene of CMV**

The total RNA was isolated from CMV infected gherkin leaf sample and cDNA was synthesized using reverse transcriptase enzyme. The cDNA obtained in RT-PCR step was used as template for converting double stranded DNA by using gene specific forward and reverse primers in the presence of taq DNA polymerase. The PCR successfully amplified the CP gene of ~700 bp from infected gherkin leaf samples confirmed with positive control (Banana). The amplicon of CMV Guntur isolate CP genes were confirmed by electrophoresis and ~700 bp band was found confirming the presence of CP gene (Fig. 4) and no amplification obtained in healthy leaf samples.

**Sequence analysis of coat protein gene of CMV**

PCR products were purified and

**Table 1.** Incidence of Cucumber mosaic virus on gherkin in different districts of Southern Karnataka

| Sl. No. | Districts & Taluk | Name of the Location | Variety/ Line* | Disease Incidence (%)* | PDI (mean) |
|---|---|---|---|---|---|
| 1 | Bangalore urbanBengalore north | ZARS, GKVK | Green long | 23.34 | 23.34 |
| 2 | Bangalore rural Doddaballapur | Challahalli | Azax | 16.00 | 17.15 |
| | | Konenahalli | Azax | 17.33 | |
| | | Linganahalli | Sparta | 18.12 | |
| 3 | ChikkaballapuraChikkaballapura | Poshottahalli | Azax | 33.59 | 31.63 |
| | | Rayamakalahalli | Green long | 44.32 | |
| | | Doddamarali | Azax | 16.98 | |
| | ChikkaballapuraBaagepalli | Gorthapalli | Green long | 44.32 | 32.44 |
| | | Bagepalli rural | Green long | 34.52 | |
| | | Gollapalli | Azax | 18.48 | |
| | ChikkaballapuraGowribidanuru | Bommashettihalli | Sparta | 36.57 | 30.28 |
| | | Machenahalli | Sparta | 21.63 | |
| | | Kudumalakunte | Azax | 32.65 | |
| 4 | KolarKolar rural | Gadadasanahalli | Green long | 56.35 | 51.27 |
| | | Sugatur | Green long | 68.79 | |
| | | Doddabommanahalli | Azax | 28.67 | |
| 5 | TumkurSira | Baladevarahatti | Green long | 59.65 | 35.62 |
| | | Dasegowdanahatti | Azax | 27.64 | |
| | | Agrahara | Azax | 19.58 | |
| 6 | AdditionalChittoor (Andhra Pradesh) | Venkatagirikota | Green long | 50.73 | 40.27 |
| | | Santhipuram | Green long | 43.65 | |
| | | Nagalapuram | Sparta | 26.42 | |

*Rabi* 2013-14; *Stage of the crop: 30-45 days

sequenced. The size of the product obtained was 631 bp. The coat protein nucleotide sequence of cucumber mosaic virus infecting gherkin was compared with sequences of other CMVs obtained from the NCBI database. Sequence analysis of CP of CMV-Gherkin revealed 79-80 per cent nucleotide homology with CMV strains of subgroup II. However with subgroup IA it showed 89-92 per cent nucleotide homology. CMV-Gherkin showed a high nucleotide homology with the strains of CMV subgroup IB (95-99%). It clearly indicated that test virus (CMV-Gherkin isolate) belong to CMV subgroup IB. Additionally, test virus showed a very high homology and sequence conservation (99%) in terms of nucleotide with the AN strain (subgroup IB) as mentioned in Table 2.

A phylogenetic tree (Fig. 5) constructed using CP gene nucleotide alignment of various strains of CMV and TAV as an out-group also favours the results of sequence similarity of CMV-Gherkin with CMV subgroup IB (AJ810260.1|AN isolate). Clear clusters of CMV subgroups IA, IB and II formed in the dendrogram.

A: Mosaic of dark green and yellow patches

B: Blistering and puckering of leaves

C: Stunting of plant

**Fig. 1.** Mechanically inoculated gherkin plants showing CMV symptoms

## DISCUSSION

The results presented reveal the occurrence and identification of CMV on gherkin on the basis of biological and CP sequence similarities. The virus was identified as a member of subgroup IB. DAS-ELISA method standardized in the present study could be used to detect CMV infection in gherkin as well as other hosts. This would help in identifying and certifying planting material to check spread of the virus.

In host range studies, Cucumber (*Cucumis sativus* L.), Ash gourd (*Benincasa hispida* Thumb.), Ridge gourd (*Luffa acutangula* L.), Bush squash (*Cucurbita pepo* var. *Clypeata*), Muskmelon (*C. melo* L.), Pumpkin (*C. maxima* Duchesne), Round melon (*Praecitrullus fistulosus* ), Watermelon (*Citrullus lanatus* Thumb.) and bottle gourd (*Lagenaria siceraria* Standl.) belongs to family Cucurbitaceae, *Chenopodium amaranticolor* L. belongs to Chenopodiaceae, Tobacco (*Nicotiana tobaccum* L. and *N. glutinosa* L.), Tomato (*Solanum lycopersicum* L.), Chilli (*Capsicum annuum* L.) and Brinjal (*Solanum melongena* L.) belongs to Solanaceae, Cowpea (*Vigna unguiculata* L. Walp.) belongs to Legminosae, Papaya (*Carica papaya*) belongs to Caricaceae, Bhendi (*Abelmoschus esculentus* (L.)) and Cotton (*Gossypium hirsutum*) belongs to Malvaceae, Castor (*Ricinus communis*) belongs to Euphorbiaceae, Lima bean (*Phaseolus lunatus*) belongs to Fabaceae and Radish (*Raphanus sativus*) belongs to the family Crucifereceae were inoculated by sap to study the infectivity of CMV to a range of host plants.

**Fig. 2.** Detection of CMV from symptomatic gherkin plants through DAS-ELISA. Yellow colour - positive; No colour development – negative

The virus produced systemic symptoms *viz*., chlorotic lesions, severe mosaic mottling and leaf distortion on all cucurbitaceous crops except watermelon at seven to ten days after inoculation. Localized chlorotic lesions followed by necrotic were observed on leaves of *C. amaranticolor* and Cowpea (*V. unguiculata* L. Walp.) at four to five days after inoculation. However, virus failed to infect solanaceae family *species N. tabaccum* L., *N. glutinosa* L., Brinjal, Bhendi and other crops belonging to families Caricaceae, Malvaceae, Euphorbiaceae, Fabaceae and Crucifereceae. The results obtained on host range of CMV are in conformity with the findings of Chandankar *et al*. (2013), host range of CMV on thirty one crops of different families; eleven crops belonging to Cucurbitaceae, Compositae, Chenopodiaceae, Amaranthaceae, Leguminosae and Solanaceae family showed viral disease expression. However, inoculum failed to induce symptoms on crops belonging to Crucifereae, Caricaceae and Malvaceae.

Use of Alkaline-phosphatase labelled cucumovirus specific antisera was greatly facilitated for identification of the virus. The Double Antibody Sandwich enzyme linked immunosorbent assay (DAS-ELISA) resulted in positive reaction to cucumovirus specific antisera. Several researchers used ELISA for identification of cucumber mosaic virus *viz.*, Korbin and Kaminska (1998), Krstic *et al.* (2002), Dukic *et al.* (2002), Sevik *et al.* (2003), Gholamalizadeh *et al*. (2008) and Shomaila Iqbal *et al*. (2012).

Attempts have been made to characterize the CMV at molecular level by amplifying the coat protein gene by using specific primers. The total RNA was isolated from leaf sample of gherkin infected with CMV and cDNA was synthesized using reverse transcriptase enzyme. The cDNA obtained was used as template for converting double stranded DNA using gene specific forward and reverse primers in presence of taq DNA polymerase. The PCR successfully amplified the CP-gene, the amplicons of CP gene of test virus

**Table 2.** Coat protein gene sequences of various Cucumber mosaic virus strains used for comparison

| Subgroup | Strain | Accession number | Origin | % Identity |
|---|---|---|---|---|
| IA | Kor | L36251 | Korea | 89 |
| | FT | D28487 | Japan | 90 |
| | C | D00462 | USA (NY) | 91 |
| | KM | AB004780 | Japan | 91 |
| | Sny | U66094 | Israel | 92 |
| | Ny | U22821 | Australia | 92 |
| | Fny | D10538 | USA (NY) | 92 |
| | FC | D10544 | USA | 92 |
| IB | D | AF281864 | India | 95 |
| | Phym | X89652 | India | 95 |
| | H | AF350450 | India | 95 |
| | CWL2 | JN054635 | Malaysia | 96 |
| | Oxalis | JQ779842 | India | 96 |
| | KS44 | AJ810259 | Thailand | 96 |
| | Rauvolfia | DQ914877 | India | 96 |
| | Kerala | AY754359 | India | 97 |
| | Jasmine | KF129062 | India | 98 |
| | AN | AJ810260 | India | 99 |
| II | Trk7 | L15336 | Hungary | 79 |
| | S | AF063610 | USA | 79 |
| | SP103 | U10923 | USA | 79 |
| | Q | M21464 | Australia | 80 |
| | LS | AF127976 | USA | 80 |
| | m2 | AB006813 | Japan | 80 |

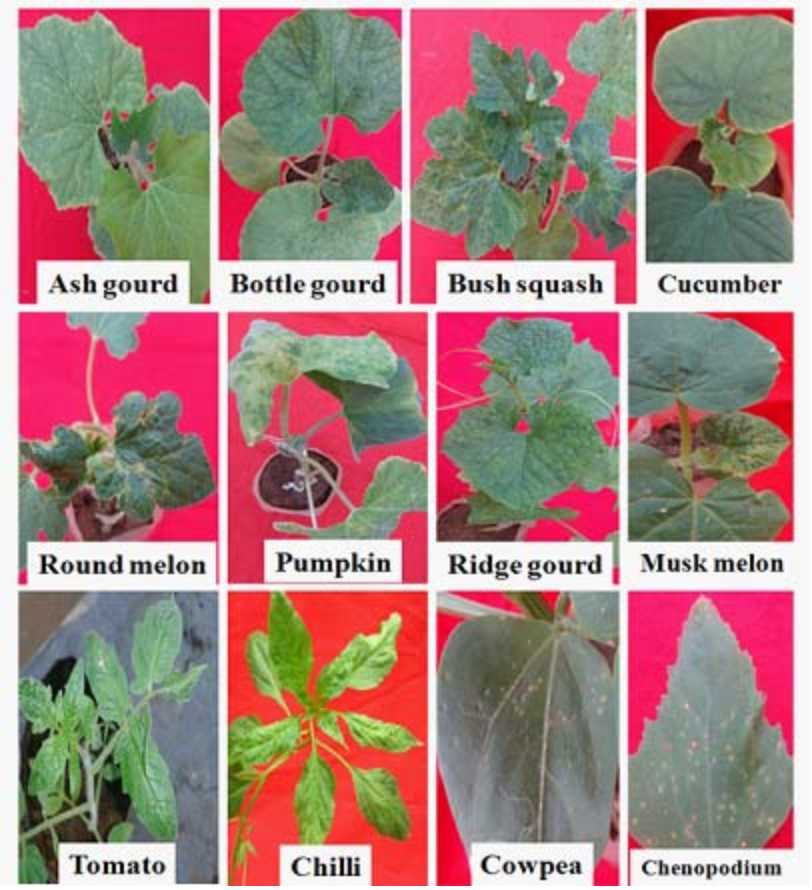
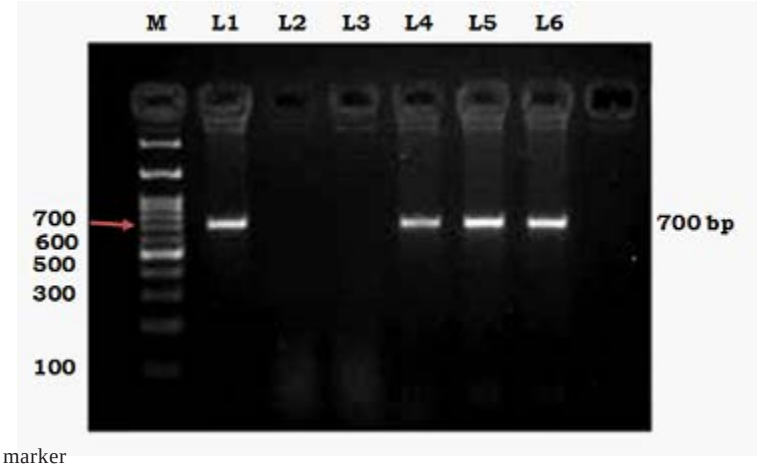



**Fig. 3.** Plants exhibiting different kinds of symptoms upon mechanical inoculation with Gherkin isolate of CMV under green house condition




Lane M: 100 bp marker
Lane 1: Positive control (CMV infected Banana leaf)
Lane 2: Healthy gherkin leaf sample
Lane 3: Water control
Lane 4, 5 & 6: Symptomatic gherkin leaf sample

**Fig. 4.** Amplification of CP gene from CMV infected gherkin leaf samples

(CMV-gherkin isolate) was estimated electrophoretically as ~700 bp confirming the presence of CP gene. PCR products were purified and sequenced with specific primer to a size of 631 bp.

The gherkin CMV showed sharing 99% homology with CMV isolate AN infecting chilli. Phylogenetic analysis of CP genes of CMV indicated that CMV isolates fall into three sub-groups *viz*., Subgroup IA, IB and II. In the present study, the test virus shares 95-99 per cent nucleotide sequence homology with the subgroup IB, and clustered with the AN isolate belongs to subgroup IB. It clearly indicated that test virus (CMV-Gherkin isolate) belong to CMV subgroup IB.

Similarly, Madhubala *et al*. (2005) characterized CMV causing mosaic, leaf distortion and stunting in vanilla (*Vanilla planifolia* Andrews) on the basis of biological and coat protein (CP) nucleotide sequence properties. CP gene of the virus was amplified using RT-PCR and were cloned and sequenced. The sequenced region contained a single open reading frame of 657 nucleotides potentially coding for 218 amino acids. Sequence analyses with other CMV isolates revealed the greatest identity with black pepper isolate of CMV (99%) and the phylogram clearly showed that CMV infecting vanilla belongs to subgroup IB.

Hareesh *et al.* (2006) reported the natural infection of CMV in Indian long Pepper (*Piper longum* L.) and Betel vine (*Piper beetle* L.) was detected by RT-PCR. The amplicons of CP gene sequences were cloned and sequenced using the genome sense primer 5' ATGGACAAAT CTGAATCAAC 3' derived from beginning of the first 20 bases of the coding region and the antisense primer, 5' TCAAACTGGGAGCACCC 3' represented last 17 bases of the coding region of the CP gene. Zitikaite and Staniulis (2006) identified the 400bp DNA fragment of CMV infecting cucumber based on cDNA amplified product through PCR using virus-specific oligonucliotides.

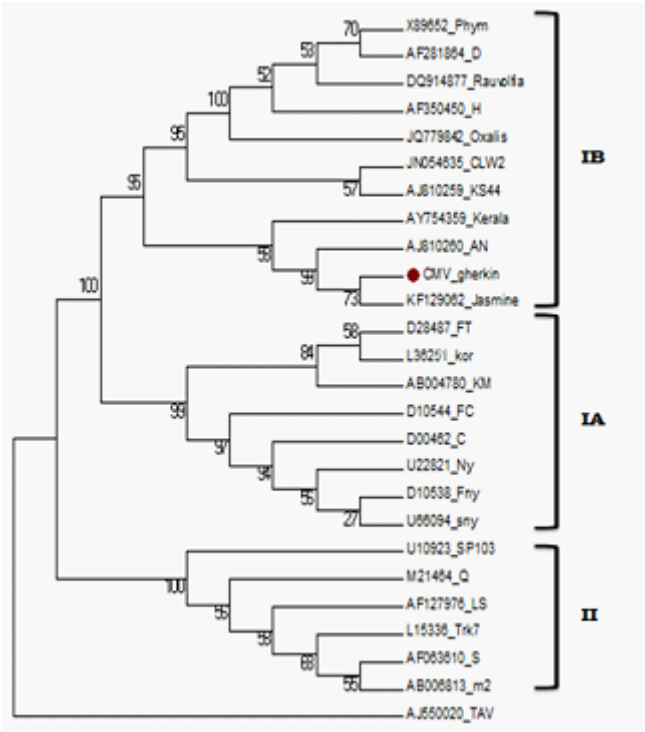



**Fig. 5.** Phylogenetic relationship of CMV-Gherkin with the strains of CMV subgroups I (A and B), II and Indian strains based on the amino acid alignment using ClustalW through TreeExplorer. Tomato aspermy virus (TAV) (Acc. No. AJ550020) was used as an outgroup. The numbers below the joining lines are bootstrapping values

The Complete coat protein (CP) gene of CMV infecting banana was sequenced by Shahanavaj Khan *et al.* (2011). The sequenced regions were found to contain single open reading frame of 657 nucleotides, potentially coding 219 amino acids. Phylogenetic analysis of nucleotide and amino acid sequence of CP gene revealed that CMV infecting banana belonging to IB subgroup.

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**Table 3.** Host range of cucumber mosaic virus disease of gherkin*

| Sl. No. | Name of the host | Scientific name | Transmission (%) | Symptoms observed | Confirmation by DAS-ELISA |
|---|---|---|---|---|---|
| Cucurbitaceae | | | | | |
| 1 | Cucumber | *Cucumis sativus* L. | 70.0 | GYP, BP, SG, RF | + |
| 2 | Ash gourd | *Benincasa hispida* Thumb. | 30.0 | CS, GYP, SG, RF | + |
| 3 | Ridge gourd | *Luffa acutangula* L. | 80.0 | GYP, BP, SG, RF | + |
| 4 | Bush squash | *Cucurbita pepo* var. *Clypeata* | 90.0 | GYP, BP, SG, RF | + |
| 5 | Muskmelon | *Cucumis melo* L. | 80.0 | GYP, BP, SG, RF | + |
| 6 | Pumpkin | *Cucurbita maxima* Duchesne | 40.0 | CS, GYP, SG, RF | + |
| 7 | Round melon | *Praecitrullus fistulosus* | 50.0 | GYP, BP, SG, RF | + |
| 8 | Watermelon | *Citrullus lanatus* Thumb. | — | No symptoms | - |
| 9 | Bottle gourd | *Lagenaria siceraria* Standl. | 60.0 | GYP, BP, SG, RF | + |
| Solanaceae | | | | | |
| 10 | Tobacco | *Nicotiana tabaccum* L. | — | No symptoms | - |
| 11 | Tobacco | *Nicotiana glutinosa* L. | — | No symptoms | - |
| 12 | Tomato | *Solanum lycopersicum* L. | 70.0 | GYP, fern leaf | + |
| 13 | Brinjal | *Solanum melongena* L. | — | No symptoms | - |
| 14 | Chilli | *Capsicum annuum* L. | 50 | Mosaic with downward curling | + |
| Chenopodiaceae | | | | | |
| 15 | Chenopodium | *Chenopodium amaranticolor* L. | 100.0 | Chlorotic local lesions followed by necrosis | + |
| Leguminosae | | | | | |
| 16 | Cowpea | *Vigna unguiculata* L. Walp. | 100.0 | Chlorotic local lesions followed by necrosis | + |
| Caricaceae | | | | | |
| 17 | Papaya | *Carica papaya* | — | No symptoms | - |
| Malvaceae | | | | | |
| 18 | Bhendi | *Abelmoschus esculentus* (L.) | — | No symptoms | - |
| 19 | Cotton | *Gossypium hirsutum* | — | No symptoms | - |
| Euphorbiaceae | | | | | |
| 20 | Castor | *Ricinus communis* | — | No symptoms | - |
| Fabaceae | | | | | |
| 21 | Lima bean | *Phaseolus lunatus* | — | No symptoms | - |
| Cruciferaceae | | | | | |
| 22 | Radish | *Raphanus sativus* | — | No symptoms | - |

* Experiment conducted twice
** No. of plants inoculated: 10
GYP- Green yellow patches
BP- Blistering and Puckering
SG – Stunted growth
RF- Reduced flowering
CS- Chloratic spots

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