TÜBİTAK

**Turkish Journal of Agriculture and Forestry**

**http://journals.tubitak.gov.tr/agriculture/**

**Research Article**

Turk J Agric For
(2015) 39: 421-428

doi:10.3906/tar-1405-143

# Partial characterization and development of sensitive and reliable diagnostic for the detection of cucumber mosaic virus

**Shahanavaj KHAN**[1,2,3,*]

[1]Department of Biosciences, Jamia Millia Islamia, New Delhi, India
[2]Department of Bioscience, Shri Ram Group of College (SRGC) Muzaffarnagar, UP, India
[3]Nanomedicine & Biotechnology Research Unit, Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

**Received:** 30.05.2014 • **Accepted:** 21.04.2015 • **Published Online:** 12.06.2015 • **Printed:** 30.06.2015

**Abstract:** A survey on *Cucumber mosaic* virus (CMV) was conducted on 21 banana orchards in three bananas producing states: Karnataka, Maharashtra, and Uttar Pradesh. Sampling was done during winter and autumn and all samples were initially examined for the presence of CMV using DAS-ELISA. Out of 300 tested samples, the presence of CMV was observed in 13. The lowest rate of infection was determined in Uttar Pradesh followed by Maharashtra, while high infections were observed in samples from Karnataka. Three CMV infected samples represented in each state were amplified through RT-PCR using the movement protein (MP) gene specific primers and cloned in the pGEMT essay vector. The RT-PCR products of amplified samples were sequenced. Gene sequences of CMV-KAR, CMV-MR, and CMV-UP isolates under study coding for the movement protein revealed 99.76%–99.88% and 99.28%–99.64% sequence identity at the nucleotide and amino-acid level. These isolates were compared with 29 CMV isolates reported from various plants around the world, which formed three distinct subclades: -IA, -IB, and -II. These isolates were most closely related to subgroup-I isolates, sharing up to 95.81% and 96.84% sequence identity at nucleotide and amino acid, respectively. However, these isolates shared 81.79%–81.90% and 82.80%–83.15% nucleotide and amino acid identity with subgroup II isolates. The isolates under study clustered with the CMV subgroup-IB as confirmed by phylogenetic analysis. Further, we standardized the minimum limit of RNA transcript for the sensitivity of RT-PCR with respect to the coat protein (CP) gene and movement protein (MP) gene. The results of RT-PCR showed that the CP gene was more sensitive than the MP gene for the detection of CMV as it amplified the gene product up to 0.02 ng RNA. The CP gene based RT-PCR assay may be a more sensitive, reliable, and convenient molecular tool for detection of the CMV pathogen, and can be used in quarantine, eradication, and tissue culture certification programs.

**Key words:** CMV, DAS-ELISA, infection rate, RNA, RT-PCR, detection limit

## 1. Introduction

Banana is the premier fruit of Asia and the Pacific region. It is the fourth most important food crop after rice, wheat, and maize, with numerous medicinal properties (Simmonds, 1966). It is the largest fruit crop in India, accounting 33% of the total fruit production, and has great socioeconomic significance. The crop is reported to be infected by a number of pests and diseases including a number of viruses such as *Banana bunch top virus* (BBTV) (Banerjee et al., 2014), *Banana streak virus* (BSV) (Baranwal et al., 2014), *Cucumber mosaic virus* (CMV) (Khan et al., 2011), *Banana bract mosaic virus* (BBrMV) (Balasubramanian and Selvarajan, 2014), *Banana mild mosaic virus* (BanMMV) (Teycheney et al., 2007), and *Banana dieback virus* (BDBV) (Hughes et al., 1998). Vegetative propagation of the banana crop results in continuation of viruses from one generation to the next generation, decreasing the yield of the products and the health of the plants. CMV, a member of the family Bromoviridae, is one of the most important widespread viruses in the world, infecting more than 1200 species of 100 plant families, including both monocot and dicot plants with severe damage in several of the most economically important fruit, pulse, and vegetable crops (Srivastava et al., 2004; Madhubala et al., 2005; Bashir et al., 2006; Verma et al., 2006; Dubey et al., 2010; Koundal et al., 2011). RNA viruses do not have proofreading ability of the RNA replicase and as a result CMV also has potential for high genetic variation in the same/different plant as well in the same/diverse location in a different part of the world.

The genome of the CMV consists of three plus-sense single stranded RNAs (RNA 1, RNA 2, and RNA 3) and a

* Correspondence: khan.shahanavaj@gmail.com

subgenomic RNA (RNA 4) encoded by the 3′-half of RNA 3, which is involved in encapsidation (Suzuki et al., 1991; Palukaitis and Garcia-Arenal, 2003; Jacquemond, 2012). Many strains of CMV described from different parts of the world have been put into two subgroups, I and II, based on nucleic acid hybridization (Owen and Palukaitis, 1988), gene sequences (Owen et al., 1990; Szilassy et al., 1999), restriction fragment length polymorphism (RFLP) (Rizos et al., 1992), and serology (Hu et al., 1995). The diverse strains of CMV are divided into two main groups, I and II, according to their genomic sequences. Furthermore, group I is divided into two subgroups, IA and IB, on the basis of phylogenetic analysis (Palukaitis and Garcia-Arenal, 2003; Moury, 2004; Liu et al., 2009). However, limited reports are available on molecular characterizations and phylogenetic analysis of CMV based on the movement protein gene (Srivastava et al., 2004). This is the first report of a complete MP gene of the CMV from Indian field samples of subgroup-IB. Moreover, the aim of the present study was to develop a sensitive and reliable RT-PCR assay for the detection of the CMV pathogen from the lower limit of RNA extracted from plant samples. The RT-PCR based diagnostic assay may be used effectively for quarantine and indexing of mother plants in the tissue culture industry to increase yield through crop improvement.

## 2. Materials and methods

### 2.1. Sample collection, screening, and maintenance of virus culture

The survey was conducted in 21 banana orchards in three main growing banana states: Karnataka, Maharashtra, and Uttar Pradesh. Naturally affected samples of banana showing mosaic and yellow stripes on leaves (Karnataka), chlorotic and leaf distortion (Maharashtra), and chlorotic with stunting of plant (Uttar Pradesh) and symptomless samples were collected from banana growing regions of India. The samples were initially subjected to direct antigen coating-enzyme-linked immunosorbent assay (DAC-ELISA) using antiserum against CMV followed by RT-PCR. Collected leaf samples were properly labelled, sealed in plastic bags, and stored at –80 °C until experiments for testing, which were generally performed within 3 months of sample collection. Natural symptomatic plant samples were also maintained under insect-proof glasshouse conditions.

### 2.2. RNA isolation and RT-PCR

Total RNA was extracted from 100 mg of infected and healthy (negative control) banana leaf tissue using the RNeasy Plant Mini Kit according to the manufacturer's instructions (Qiagen Inc., Chatsworth, Los Angeles, CA, USA). The purity of RNA was checked by electrophoresis on 2% agarose gel and the concentration quantified using a NanoDrop ND-1000 UV–vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The cDNA synthesis was carried out using AMV RTase (10 U) at 42 °C for 90 min, in a total volume of 20 mL containing RNAase inhibitor (25 U) and 1 mM each of dNTP and movement protein (MP) gene specific downstream primer (15 pmol). The primer pair used for amplification of MP genes was derived from the previously reported MP gene sequence of CMV (Srivastava et al., 2004). The upstream primer 5'-ATGGCTTTCCAAGGTACCAGT-3' and downstream primer 5'-CTAAAGACCGTTAACCACCTG -3' represented the first and the last 21 bases of the coding region of the MP gene, respectively. Prior to amplification, the template was incubated at 72 °C for 5 min and snap-cooled on wet ice for 2 min. Reverse transcription-PCR was performed in an automated thermal cycler (Biometra) using the following parameters: one cycle at 42 °C for 45 min for complementary DNA synthesis, then 35 cycles at 94 °C for 30 s, 57 °C for 1 min, and 72 °C for 1 min followed by one cycle at 72 °C for 60 min. Products and 100 bp DNA ladder (Fermentas, Canada) were resolved following electrophoresis through a 1% agarose gel containing ethidium bromide.

### 2.3. Cloning and sequencing of MP gene

The product amplified from each infected sample was purified after electrophoresis using a DNA extraction kit as per the manufacturer's instructions (Fermentas, Canada). Purified DNA fragments were ligated into a pGEM-T Easy vector (Promega, Madison, WI, USA) and competent *Escherichia coli* cells (XL1-Blue strain) were transformed by standard molecular biology procedures (Sambrook and Russell, 2001). Clones were designated as CMV-KAR, CMV-MH, and CMV-UP for Karnataka, Maharashtra, and Uttar Pradesh samples, respectively. Two clones of each isolate were sequenced in both directions using universal T7 and SP6 primers.

### 2.4. Sequence analysis and phylogenetic study

MP gene sequences of three CMV isolates infecting banana with Accession No. DQ642018 (CMV-KAR), DQ642019 (CMV-MH), and DQ642017 (CMV-UP) were submitted to the EMBL database. The MP gene of CMV isolates under study was compared with that of 29 isolates of CMV obtained from GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html). The sequence data, accession number, strains/isolates, and country of origin are given in Table 1. Multiple sequence alignment and identity matrix of the sequences were calculated using ClustalW software. Phylogenetic analysis of the MP gene of CMV isolates was performed in MEGA4 with the sequence of *Peanut stunt virus* (PSV) as out-group added to the alignment profile (Tamura et al., 2007). The maximum parsimony method was used to infer the evolutionary history. A phylogenetic tree was generated (with 1000 replicates) using the close-neighbor-interchange algorithm (Nei and Kumar, 2000).

**Table 1.** List of various Indian banana orchards that are supposed to be autochthonous, sites of sampling, and number of initially tested samples by DAS-ELISA followed by RT-PCR with total percentage of CMV infection.

| Samples collection and location of orchards | No. of tested samples | No. of infected samples | | Infection rate in % (RT-PCR) |
|---|---|---|---|---|
| | | DAS-ELISA | RT-PCR | |
| Karnataka orchard 1 | 14 | 02 | 02 | 14.285 |
| Karnataka orchard 2 | 14 | 01 | 01 | 7.142 |
| Karnataka orchard 3 | 15 | 01 | 01 | 6.666 |
| Karnataka orchard 4 | 14 | 00 | 00 | 0.0 |
| Karnataka orchard 5 | 14 | 01 | 01 | 7.142 |
| Karnataka orchard 6 | 15 | 01 | 01 | 6.666 |
| Karnataka orchard 7 | 14 | 00 | 01 | 7.142 |
| (Symptomless) | | | | Total = 7% |
| Maharastra orchard 1 | 15 | 01 | 01 | 6.666 |
| Maharastra orchard 2 | 12 | 00 | 00 | 0.0 |
| Maharastra orchard 3 | 16 | 01 | 01 | 6.25 |
| Maharastra orchard 4 | 15 | 00 | 00 | 0.0 |
| Maharastra orchard 5 | 14 | 00 | 00 | 0.0 |
| Maharastra orchard 6 | 16 | 01 | 02 | 12.50 |
| Maharastra orchard 7 | 12 | 00 | 00 | 0.0 |
| (Symptomless) | | | | Total = 4% |
| Uttar Pardesh orchard 1 | 20 | 01 | 01 | 5.0 |
| Uttar Pardesh orchard 2 | 15 | 00 | 00 | 0.0 |
| Uttar Pardesh orchard 3 | 10 | 00 | 00 | 0.0 |
| Uttar Pardesh orchard 4 | 14 | 00 | 00 | 0.0 |
| Uttar Pardesh orchard 5 | 11 | 00 | 00 | 0.0 |
| Uttar Pardesh orchard 6 | 16 | 00 | 00 | 0.0 |
| Uttar Pardesh orchard 7 | 14 | 00 | 01 | 7.142 |
| (Symptomless) | | | | Total = 2% |

### 2.5. Development of sensitivity RT-PCR assay for the detection of CMV through CP gene and MP gene

In order to evaluate the analytical sensitivity of the assay, RT-PCR experiments were performed on ten-fold dilution series of RNA with a 200 ng known quantity of RNA (6 tubes with a range of 200, 20, 2.0, 0.2, 0.02, 0.001 ng) by CP and MP gene specific primers. The dilutions were made in nuclease-free water to determine the detection limit of RNA concentration for the detection of virus in infected samples as per the protocol by Pérez and Díaz de Arce (2009). The PCR thermal cycling parameter was used as described above for MP gene and CP gene amplification.

## 3. Results

### 3.1. Survey and sample collection

A total 300 samples were collected from Karnataka (86 symptomatic and 14 symptomless samples), Maharashtra (88 symptomatic and 12 symptomless samples), and Uttar Pradesh (86 symptomatic and 14 symptomless samples) and analyzed initially by DAS-ELISA assay and further confirmed through a CP specific primer pair through RT-PCR as discussed previously by Khan et al. (2011). The incidence of CMV infection was more prevalent in Karnataka, with a rate of 7% (07 samples out of 100), followed by Maharashtra (4%; 4 samples out of 100) and Uttar Pradesh (2%; 2 samples out of 100) (Table 1).

**3.2. RT-PCR and cloning of amplified product in pGEMT**

RT-PCR followed by agarose gel electrophoresis of the PCR products revealed the expected size fragment of an MP gene fragment of about 840 bp in CMV infected samples of each state, Karnataka, Maharashtra, and Uttar Pradesh, except for the negative control (tissue culture based healthy sample). The PCR products were successfully cloned in pGEM-T Easy vector. Furthermore, restriction digestion of plasmid DNA isolated from each clone showed positive conformation of several clones having the insert of expected size.

**3.3. Sequence analysis and phylogenetic study**

The complete nucleotide sequences of three clones of CMV-KAR, CMV-MH, and CMV-UP isolates were determined by sequencing in both reverse and forward directions using T7 and SP6 promoter primers. The sequences have been deposited in the EMBL database with the accession numbers DQ642018 (CMV-KAR), DQ642019 (CMV-MH), and DQ642017 (CMV-UP) as indicated in Table 2. The sequenced region in all three isolates had a complete open reading frame (ORF) of 840 bases, which potentially

**Table 2.** Source of CMV movement protein gene sequences used in multiple alignment studies. Asterisk (*) indicates banana isolates under study (NA: not available).

| S.No. | Accession Number | Strain/isolate | Origin/Country |
|---|---|---|---|
| 1. | DQ642018* | CMV Banana /KAR | India |
| 2. | DQ642019* | CMV Banana /MH | India |
| 3. | DQ642017* | CMV Banana /UP | India |
| 4. | DQ885889 | CMV Banana / Lucknow | India |
| 5. | EF178298 | CMV Banana / Lucknow-Gmt | India |
| 6. | AF268598 | CMV Banana/Xb | China |
| 7. | AM117572 | CMV Tagetus erectus | India |
| 8. | AF414078 | CMV Amaranthus | India |
| 9. | DQ914878 | CMV Rauvolfia | India |
| 10. | AM396982 | CMV Tagetes erecta | India |
| 11. | DQ873411 | CMV Datura | India |
| 12. | DQ885887 | CMV Tom-shoestring | India |
| 13. | DQ656120 | CMV Chrysanthemum | India |
| 14. | D10538 | CMV Fny | USA |
| 15. | D16405 | CMV Legume | Japan |
| 16. | AJ304398 | CMV ALS | Netherlands |
| 17. | AJ304399 | CMV ALS | Netherlands |
| 18. | U20219 | CMV Ixora | USA |
| 19. | U66094 | CMV Sny | Israel |
| 20. | U37227 | CMV S | USA |
| 21. | D83958 | CMV Y | Japan |
| 22. | AY429437 | CMV CS | China |
| 23. | AY429432 | CMV Ca | China |
| 24. | AB042294 | CMV | Japan |
| 25. | AF198103 | CMV LY | Australia |
| 26. | AJ276481 | CMV Mf | South Korea |
| 27. | L15336 | CMV Trk7 | NA |
| 28. | D28780 | CMV NT9 | Taiwan |
| 29. | Y16926 | CMV | Italy |
| 30. | D00385 | CMV O | Japan |
| 31. | M21464 | CMV Q | NA |
| 32. | D12499 | CMV Y | Japan |
| 33. | U15730 | *Peanut stunt virus* (PSV) | USA |

coded for a protein of 279 amino acids. Sequence alignment of the MP gene of the CMV isolates under study showed 99.76%–99.88% and 99.28%–99.64% identities at nucleotide and amino acid level, respectively. Sequence alignment between the MP gene of the CMV isolates under study and other reported Indian isolates of CMV showed 98.69%–98.81% and 99.64%–100% sequence identity at nucleotide and amino acid level, respectively. Moreover, the results of sequence alignment of the MP gene of the CMV isolates under study and 29 diverse isolates of CMV reported from different parts of the world revealed that the isolates under study were more closely related to subgroup-IB isolates, sharing 81.64%–95.81% and 83.64%–96.84% sequence identity with reference to nucleotide and amino acid level, respectively. However, the isolates under study were distantly related to the members under subgroup-II, sharing only 81.79%–81.900% sequence identity at the nucleotide level and 82.80%–83.15% sequence identity at the amino acid level (Table 3). Moreover, phylogenetic analysis of the amino acid sequences of the MP gene under study showed that CMV isolates formed one cluster along with IB subgroup isolates. The remaining isolates formed two distinct clusters with IA and II subgroup isolates (Figure 1).

### 3.4 Comparison of RT-PCR sensitivity for the detection of CMV through CP gene and MP gene

RT-PCR using primer pairs of the CP gene was successful amplified about 650 bp fragments in RNA concentration ranging from 200 to 0.02 ng (Figure 2a). However, primer pairs of the MP gene showed amplification of about 850 bp fragment in RNA concentration ranges from 200 to 0.2 ng (Figure 2b).

## 4. Discussion and conclusion

CMV is a very common plant virus that infects many plant families and can cause significant economic losses in many fruit, vegetable, ornamental, and horticultural crops. Banana, cucurbit, tomato, and pepper are the crops most commonly affected by CMV (Palukaitis and Garcia-Arenal, 2003; Bashir et al., 2006; Dubey et al., 2010). A total of 300 samples were collected from three banana growing states in different geographical areas in India. CMV causing yellow mosaic and stripes on the leaves of banana was specifically observed in winter. The presence of virus was initially tested by DAS-ELISA assay. The samples were further confirmed through RT-PCR for CMV infection. Two asymptomatic samples were also CMV positive in RT-PCR. This disparity in results may show the existence of CMV isolates in the form of latent infection that are not easily detected by indexing on ELISA although detectable by RT-PCR analysis. Moreover, as a limited number of samples were analyzed, it was impossible to find a possible correlation between the observed symptoms and the presence of virus. An improved balanced strategy for plant health management might be required for virus free mother plants. The RT-PCR assays, due to the fact that they are specific, reliable, and sensitive, and can be performed rapidly and at a reasonable cost, have been confirmed to be valuable techniques in the detection of virus mediated diseases in plant science. The most commonly used molecular technique is RT-PCR for the detection of RNA viruses. The RT-PCR method was more sensitive than direct action coating ELISA (DAC-ELISA) to detect CMV infection in banana as previously confirmed in many reports (Raj et al., 2003; Reddy et al., 2011).

CMV infection was determined in 13 banana samples (4.33%) out of the total 300 samples. The highest CMV infection rate was determined in Karnataka (7%) followed

**Table 3.** Percent identity in movement protein gene at nucleotide and amino acid level among CMV banana isolates from India and different parts of the world using ClustalW program (% nucleotide identity top right and % amino acid identity bottom left).

| | DQ642018 | DQ642019 | DQ642017 | DQ885889 | EF178298 | AF268598 |
|---|---|---|---|---|---|---|
| DQ642018 | X | 99.76 | 99.88 | 98.69 | 98.69 | 81.79 |
| DQ642019 | 99.28 | X | 99.88 | 98.81 | 98.81 | 8.79 |
| DQ642017 | 99.64 | 99.64 | X | 98.81 | 98.81 | 81.9 |
| DQ885889 | 99.64 | 99.64 | 100 | X | 100 | 81.67 |
| EF178298 | 99.64 | 99.64 | 100 | 100 | X | 81.67 |
| AF268598 | 82.80 | 82.80 | 83.15 | 83.15 | 83.15 | X |

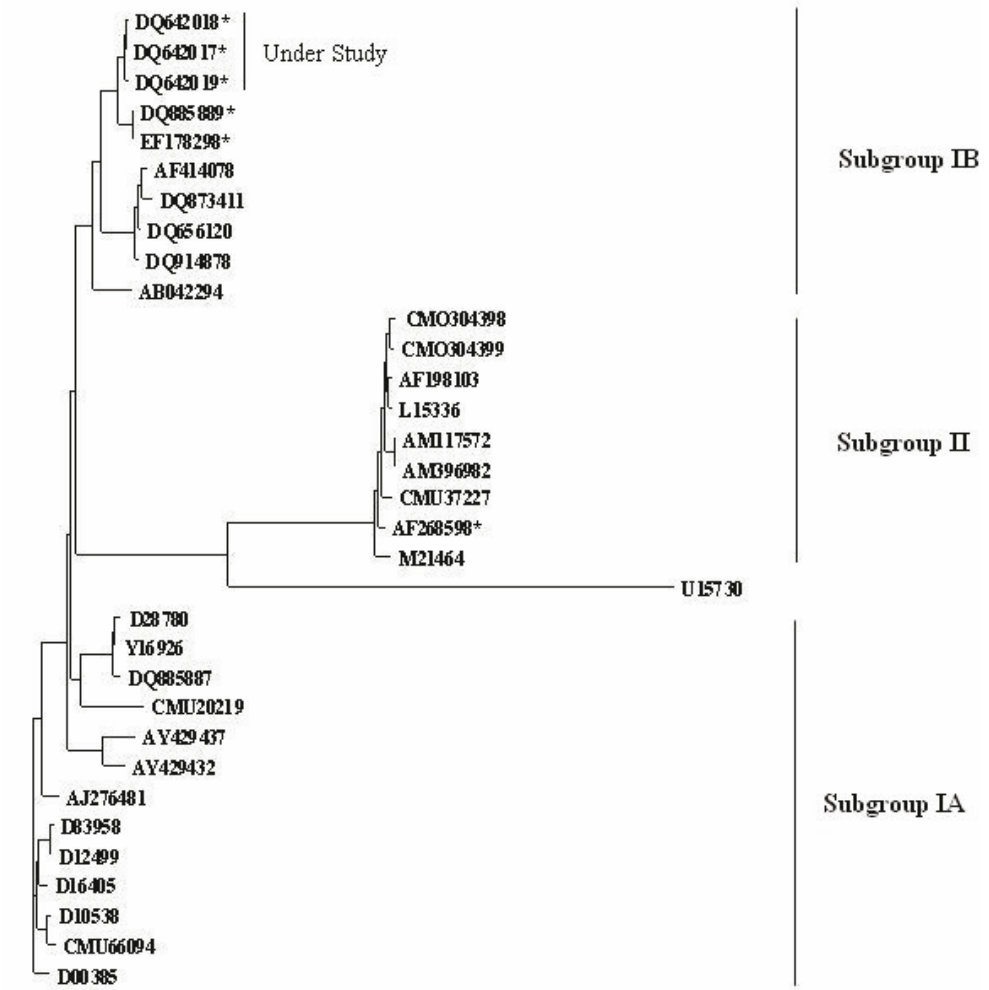
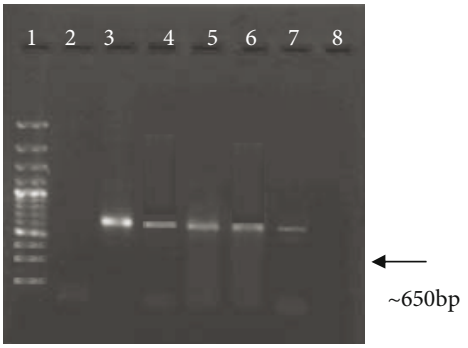



**Figure 1.** Phylogram, drawn by neighborhood joining bootstrap method (bootstrap analysis with 1000 replicates) in Clustal X (version 1.83), illustrating phylogenetic relationship based on multiple alignments of the movement protein gene sequences of 26 distinct CMV and 6 banana isolates of CMV MP along with PSV as out-group. The sequences used in the analyses were obtained from GenBank/EMBL (see Table 1). Asterisk (*) indicates CMV isolates infecting banana.

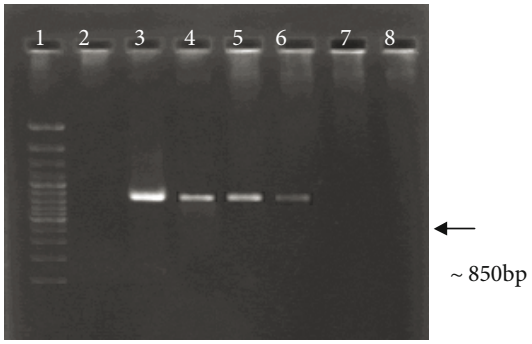
by Maharashtra (4%) out of 100 samples collected from each location (Table 1). Such high infection in Karnataka may be due to the existence of other leguminous family crops such as cowpea, fava bean, and tepary bean together with banana orchards, which may be infected with CMV and may act as a source of transmission of virus. The three isolates of CMV representing each banana growing state were studied for their molecular characterization. These CMV isolates (CMV-KAR, CMV-MH, and CMV-UP) under study were characterized based on complete MP gene analysis. The sequence of CMV isolates under study was significantly identical at both nucleotide and amino acid level (>99% similarity). However, a previous report on the CP gene of CMV isolates showed 90%–98% sequence identity with the CMV subgroup I isolates (Srivastava et al., 2004). The data of MP sequence analysis showed that the 38 nucleotides from positions 596–633 (38 nt) are identical among all CMV strains (data not shown). Furthermore, alignment of its inferred amino acid sequence showed the 67 amino acid from position 88–154 are conserved among all the strains of subgroup I. Therefore, the MP gene may be better suited than the CP gene for the development of transgenic plants due to their more conserved nature among CMV subgroup I isolates. The complete MP amino acid sequence of isolates under study was compared with that of 29 CMV isolates reported from 10 different countries and various hosts over the last 20 years (Table 1).

Although there are some reports on serological and CP based molecular characterization of many Indian strains of CMV, their isolation and molecular characterization




a

**Figure 2a.** Agarose gel electrophoresis of RT-PCR products (CMV CP gene specific primers) with different dilutions of RNA. Lane 1: 100 bp Ladder; Lane 2: Negative control; Lane 3: 200 ng RNA; Lane 4: 20 ng RNA; Lane 5: 2 ng RNA; Lane 6: 0.2 ng RNA; Lane 7: 0.02 ng RNA; Lane 8: 0.002 ng RNA.




b

**Figure 2b.** Agarose gel electrophoresis of RT- PCR products (CMV MP gene specific primers) with different dilutions of RNA. Lane 1: 100 bp Ladder; Lane 2: Negative control; Lane 3: 200 ng RNA; Lane 4: 20 ng RNA; Lane 5: 2 ng RNA; Lane 6: 0.2 ng RNA; Lane 7: 0.02 ng RNA; Lane 8: 0.002 ng RNA.

based on complete MP sequence data have not yet been done except for some preliminary findings (Srivastava et al., 2004; Khan et al., 2011). Therefore, this study was planned to obtain a better understanding of the right taxonomic position of CMV isolates infecting banana and the phylogenetic relationship with other CMV strains reported from India and different parts of the world. The phylogenetic analysis of these isolates based on complete MP sequence illustrated two major groups: I and II. The I-group was further divided into two subgroups: IA and IB (Owen and Palukaitis, 1988; Liu et al., 2009). The phylogenetic analysis revealed that the isolates under study were more closely related to the IB-subgroup along with other isolates reported from India and Japan (Srivastava et al., 2004). The majority of CMV isolates in India along with the isolates under study are grouped in subgroup-IB.

Additionally, the present study developed a molecular diagnostic tool for the detection of CMV population based on the requirement of minimum detection limit of RNA with CP and MP gene specific primers by RT-PCR. The sensitivity of an RT-PCR assay is important for efficient detection of CMV in tissue culture based banana plant samples as well as field samples. The coat protein gene specific RT-PCR assay is more efficient, reliable, and convenient as it may detect the infection of CMV in up to 10-fold lower (0.02 ng) concentration of RNA. It is reported that a fourth RNA, RNA 4, which is subgenomic to RNA 3, serves as a monocistronic messenger and encoded the coat protein in addition to RNA 1, 2, and 3. Therefore, the CP gene based RT-PCR assay might be a more sensitive and reliable molecular tool for the detection of the CMV pathogen in mother plants. The outcome of detection limit describes a simple, rapid, sensitive, specific, reliable, and significant RT-PCR assay for CMV detection in plant samples. The RT-PCR assay is more cost-effective than real time PCR, but real time PCR has tremendous potential for quantitative applications.

This finding might be helpful in determining the evolution of Indian isolates of CMV and the design of strategies for efficient control of CMV. The outcome of the study provides information on the ways in which CMV infections can impair the sustainability of modern farming systems of banana. The timely identification and characterization of the virus seems to be the most urgent requirement for tissue culture based mother plants. Genetic engineering for the development of transgenic plants and quarantine inspection of plant propagules are needed for well characterized genes and powerful diagnostics to help achieve sustainability. Although many detection techniques such as ELISA, dot blot hybridization/ slot blot hybridization, and restriction fragment length polymorphism (RFLP) are available, RT-PCR-based assays are much more reliable and sensitive. The established diagnostic technique could be helpful to ensure the production of virus free tissue culture based plants and can be used in quarantine, eradication, and certification programs. Although the presence of CMV is well known in many regions and many countries worldwide, to the best of our knowledge this is the first report of CMV based on the MP gene in India.

**Acknowledgment**

The author is grateful to the Deanship of Scientific Research and Research Center, College of Pharmacy, King Saud University.

## References


Balasubramanian V, Selvarajan R (2014). Genetic diversity and recombination analysis in the coat protein gene of Banana bract mosaic virus. Virus Genes 48: 509–517.

Banerjee A, Roy S, Behere GT, Roy SS, Dutta SK, Ngachan SV (2014). Identification and characterization of a distinct banana bunchy top virus isolate of Pacific-Indian Oceans group from North-East India. Virus Res 183: 41–49.

Baranwal VK, Sharma SK, Khurana D, Verma R (2014). Sequence analysis of shorter than genome length episomal Banana streak OL virus like sequences isolated from banana in India. Virus Genes 48: 120–127.

Bashir NS, Kalhor MR, Zarghani SN (2006). Detection, differentiation and phylogenetic analysis of cucumber mosaic virus isolates from cucurbits in the northwest region of Iran. Virus Genes 32: 277–288.

Dubey VK, Aminuddin, Singh VP (2010). Molecular characterization of Cucumber mosaic virus infecting Gladiolus, revealing its phylogeny distinct from the Indian isolate and alike the Fny strain of CMV. Virus Genes 41: 126–134.

Hu JS, Li HP, Barry K, Wang M, Jordan R (1995). Comparison of dot blot ELISA and RT-PCR assays for detection of two Cucumber mosaic virus isolates infecting banana in Hawaii. Plant Dis 79: 902–906.

Hughes Jd'A, Speijer PR, Olatunde O (1998). Banana die-back virus - a new virus infecting banana in Nigeria. Plant Disease 82: 129.

Jacquemond M (2012). Cucumber mosaic virus. Adv Virus Res 84: 439–504.

Khan S, Jan AT, Aquil B, Haq QMR (2011). Coat protein gene based characterization of Cucumber mosaic virus isolates infecting banana in India. Journal of Phytology 3: 94–101.

Koundal V, Haq QM, Praveen S (2011). Characterization, genetic diversity, and evolutionary link of Cucumber mosaic virus strain New Delhi from India. Biochem Genet 49: 25–38.

Liu YY, Yu SL, Lan YF, Zhang CL, Hou SS, Li XD, Chen XZ, Zhu XP (2009). Molecular variability of five Cucumber mosaic virus isolates from China. Acta Virol 53: 89–97.

Madhubala R, Bhadramurthy V, Bhat AI, Hareesh PS, Retheesh ST, Bhai RS (2005). Occurrence of Cucumber mosaic virus on vanilla (*Vanilla planifolia* Andrews) in India. J Biosci 30: 339–350.

Moury B (2004). Differential selection of genes of cucumber mosaic virus subgroups. Mol Biol Evol 21: 1602–1611.

Nei M, Kumar S (2000). Molecular Evolution and Phylogenetics. New York, NY, USA: Oxford University Press.

Owen J, Palukaitis P (1988). Characterization of cucumber mosaic virus. I. Molecular heterogeneity mapping of RNA 3 in eight CMV strains. Virology 166: 495–502.

Owen J, Shintaku M, Aeschleman P, Ben Tahar S, Palukaitis P (1990). Nucleotide sequence and evolutionary relationships of cucumber mosaic virus (CMV) strains: CMV RNA 3. J Gen Virol 71 (Pt 10): 2243–2249.

Palukaitis P, Garcia-Arenal F (2003). Cucumoviruses. Adv Virus Res 62: 241–323.

Pérez LJ, Díaz de Arce H (2009). A RT-PCR assay for the detection of encephalomycarditis virus infections in pigs. Brazilian Journal of Microbiology 40: 988–993.

Raj SK, Srivastava A, Chowdhury MR, Johri JK (2003). Association of cucumovirus and potyvirus with betelvine (*Piper betle* L.) as evidenced by ELISA and RT-PCR. Indian J Exp Biol 41: 273–276.

Reddy CV, Sreenivasulu P, Sekhar G (2011). Duplex-immunocapture-RT-PCR for detection and discrimination of two distinct potyviruses naturally infecting sugarcane (*Saccharum* spp. hybrid). Indian J Exp Biol 49: 68–73.

Rizos H, Gunn LV, Pares RD, Gillings MR (1992). Differentiation of cucumber mosaic virus isolates using the polymerase chain reaction. J Gen Virol 73 (Pt 8): 2099–2103.

Sambrook J, Russell DW (2001). Molecular Cloning, a Laboratory Manual. 3rd ed. Cold Spring Harbor, NY, USA: Cold Spring Harbor Laboratory Press.

Srivastava A, Chandra G, Raj SK (2004). Molecular characterization of a strain of cucumber mosaic virus based on coat protein and movement protein genes. Acta Virol 48: 229–239.

Suzuki M, Kuwata S, Kataoka J, Masuta C, Nitta N, Takanami Y (1991). Functional analysis of deletion mutants of cucumber mosaic virus RNA3 using an in vitro transcription system. Virology 183: 106–113.

Szilassy D, Salanki K, Balazs E (1999). Molecular evidence for the existence of two distinct subgroups in cucumber mosaic cucumovirus. Virus Genes 18: 221–227.

Tamura K, Dudley J, Nei M, Kumar S (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 24: 1596–1599.

Teycheney PY, Acina I, Lockhart BE, Candresse T (2007). Detection of Banana mild mosaic virus and Banana virus X by polyvalent degenerate oligonucleotide RT-PCR (PDO-RT-PCR). J Virol Methods 142: 41–49.

Verma N, Mahalingam BK, Ram R, Zaidi AA (2006). Coat protein sequence shows that Cucumber mosaic virus isolate from geraniums (*Pelargonium* spp.) belongs to subgroup II. J Biosci 31: 47–54.