Molecular Characterization of Coat Protein Gene of Blackgram Yellow Mosaic Virus (BGYMV) from Karnataka, India

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ABSTRACT

Yellow mosaic virus is the most destructive disease of urdbean causing 5-100 per cent yield loss. BGYMV belongs to genus *Begomovirus* of the family Geminiviridae transmitted by white fly (*Bemisia tabaci* Gennadius). Polymerase chain reaction of yellow mosaic virus infecting blackgram samples using MYMV-CP-F/MYMV-CP-R primers amplified the expected product of size 1000 bp from blackgram infected samples. Expected PCR products of size 1000 bp obtained were cloned, sequenced and assembled. The total number of sequences obtained from blackgram yellow mosaic was 880 bp with 106 bp of pre-coat protein region and 774 bp of coat protein region of blackgram yellow mosaic virus. 257 amino acid lengths were predicted after translation of the nucleotide sequences. The cluster phylogram based on pairwise and multiple sequence alignment of the nucleotide sequence of the CP gene of 8 isolates of MYMV and 13 isolates of MYMIV indicated that the present isolate causing blackgram yellow mosaic virus formed cluster with other known isolates of MYMV. Sequence comparisons indicated that BGYMV has the highest nucleotide sequence identity of about 98.7 per cent, 98.4 per cent and 98.3 per cent and with MYMV-Namakkal:MoB [DQ865201.1]; MYMV-Madurai:SB [AJ421642.1], MYMV-Tamil Nadu:MB [AJ132575.1] and MYMV-Maharashtra:SB [AF314530.1] isolates, respectively. The nucleotide sequence identity of BGYMV with MYMV ranged from 94.4-98.7 per cent. The nucleotide sequence identity of BGYMV with MYMIV ranged between 79-80.7 per cent. The deduced amino acid sequence of individual proteins were compared with those of other begomoviruses, the maximum homology of 99.2 per cent and 98.8 per cent was noticed with MYMV-Tamil Nadu:MB [AJ132575.1]; MYMV-Maharashtra:SB [AF314530.1] and MYMV-Namakkal:MoB [DQ865201.1]; MYMV-Madurai:SB [AJ421642.1] isolates, respectively. The deduced amino acid identities of BGYMV with Mungbean yellow mosaic virus revealed identities between 99.2-95.7 per cent. Deduced amino acid sequence comparison revealed that BGYMV revealed identities ranged from 84-85.9 per cent with Mungbean yellow mosaic India virus at amino acid level. The results of the present study revealed that coat protein gene of yellow mosaic virus infecting blackgram (BGYMV-Hebbal-Bangalore) is a Mungbean yellow mosaic virus (MYMV) but not Mungbean yellow mosaic India (MYMIV) virus and it is a variant of mungbean yellow mosaic virus since it showed 94.4-98.7 per cent identity at nucleotide level with other MYMV isolates.

Keywords

Blackgram yellow mosaic virus (BGYMV), Coat protein, Characterization, Phylogenetic analysis, Nucleotide sequence identity, Amino acid identity

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Introduction

Black gram or Urdbean (Vigna mungo L. Heper), is one of the important pulse crops grown throughout India. It is consumed in the form of dal (whole or split, husked and unhusked). This pulse legume is used for green manuring after picking the pods because of its characteristics to fix the atmospheric nitrogen. The plant with deep tap roots binds soil particles and helps in conservation of soil. Urdbean is widely cultivated in India, Myanmar, Sri Lanka, Thailand, the Philippines and Pakistan. In India, it is grown in states like Madhya Pradesh, Rajasthan, Uttar Pradesh, Orissa, Maharashtra, Andhra Pradesh, Tamil Nadu and Karnataka. In India, it is cultivated in an area of 32.15 lakh ha, with a production of 17.66 lakh tonnes and productivity of 549 kg/ha. The area under cultivation in Karnataka is 0.93 lakh ha, with a production of 0.35 lakh tones and productivity of 376 kg/ha (Anon., 2012).

Several viruses infecting blackgram are yellow mosaic, leaf curl and leaf crinkle which are considered to be economically important resulting in crop losses (Biswas et al., 2012; Malathi and John, 2008; Qazi et al., 2007). Amongst viral diseases, yellow mosaic virus is the most destructive disease of urdbean causing 5-100 per cent yield loss (Nene, 1972; Singh et al., 1980; Rathi, 2002). The symptoms of blackgram yellow mosaic virus (BGYMV) firstly appear on young leaves in the form of yellow, diffused, round spots scattered on the leaf lamina. The infected leaves turn necrotic. The diseased plants usually mature later and bear relatively few flowers and pods. The pods are stunted and mostly remain immature but whenever seeds are formed they are small in size (Nene, 1972; Singh et al., 2002). BGYMV is not seed-transmitted, but numerous alternate hosts and the whitefly vector provides primary source of inoculum. This disease is transmitted by whitefly (Bemisia tabaci Gennadius) and not by mechanical inoculation or by seed (Shad et al., 2005). BGYMV belongs to genus Begomovirus of the family Geminiviridae (Bos, 1999). The virus has geminate particles of size 20 x 30 nm and single stranded DNA genome of 2.8 Kb (Roger Hull, 2004).

Two distinct begomoviruses, Mungbean yellow mosaic virus (MYMV) and Mungbean yellow mosaic India virus (MYMIV) were known to cause yellow mosaic disease on urdbean (Islam et al., 2012; Malathi and John, 2008; Ilyas et al., 2010; Islam et al., 2012; Shahid et al., 2012; Tsai et al., 2013). MYMV is confined to Thailand, Vietnam, and Peninsular region of India, whereas MYMIV occurs in Northern India, Pakistan, Nepal, Bangladesh and Indonesia. In this regard, an attempt has been made to characterize coat protein gene of YMV infecting urdbean and to confirm that Blackgram yellow mosaic virus (BGYMV) from Karnataka is an isolate or variant of Mungbean yellow mosaic virus (MYMV) rather than Mungbean Yellow Mosaic India Virus (MYMIV).

Materials and Methods

DNA extraction

Blackgram plants showing severe yellow mosaic and mottling symptoms were collected from field at the MRS, Hebbal, Bangalore, Karnataka (south India) during the 2012 (Plate 1). Samples from healthy plants were collected as controls.

The total genomic DNA was extracted from leaf tissues of healthy blackgram plants and YMV infected blackgram plants based on the method of Rouhibakhsh et al. (2008). One hundred and fifty milligrams of fresh YMV infected leaf tissues were ground with liquid nitrogen using sterile pestle and mortar. The whole ground sample was transferred into a
fresh 1.5-ml eppendorf tube. 1500 µl of pre-
warmed (65°C) DNA extraction buffer was
added to ground sample taken in 1.5-ml
eppendorf tube (added in situ just before DNA
extraction). The whole crude sap was
incubated for 30 min at 60°C in a water bath
with occasional mixing. The supernatant (750
µl) was transferred into a fresh 1.5-ml
eppendorf tube and mixed with equal amount
(750 µl) of Phenol: chloroform: isoamyl
alcohol (25: 24:1) by vertexing. The samples
were then centrifuged at 13,000 rpm for 10
min using micro centrifuge. The aqueous
supernatant was collected in to a fresh 1.5-ml
eppendorf tube. The DNA was precipitated by
mixing with 300 µl of chilled isopropanol +
30 µl of 7.5 M Ammonium acetate by
inversion. The tubes were centrifuged at
13,000 rpm for 10 min. The resulted pellet
was washed with 70 per cent ethanol, dried in
a vacuum drier for 10 min and re-suspended
with 40 µl of T_{10}E_{0.1} buffer (10 mM Tris-HCl
of pH 8.0 and 0.1 mM EDTA of pH 8.0) and
stored at -20°C. All the DNA extracts were
further diluted from 1:10 to 1:40 in single
distilled water (SDW) before using for PCR
amplifications. The quality and quantity of
DNA was assessed at 260 nm and 280 nm
using UV spectrophotometer.

**PCR amplification and gel electrophoresis**

In order to determine the nucleotide sequence
of coat protein of blackgram yellow mosaics
virus, specific primers available in the
literature were tried to amplify coat protein
region of yellow mosaic viruses of nearly
1000 bp. Primers specific to MYMV
(MYMV-CP-F-ATG GG (T/G) TCC GTT
GTA TGC TTG / MYMV-CP-R-GGC GTC
ATT AGC ATA GGC AAT) were used for
amplification of coat protein gene of
Blackgram yellow mosaics virus (BGYMV).
Primers were designed to get the complete
coop protein gene of yellow mosaic viruses of
legume hosts by taking 100 extra nucleotides
on both the sides of the gene (Naimuddin and
Mohd. Akram, 2010).

PCR was performed in Thermocycler
(Eppendorf Mastercycler gradient, Hamburg,
Germany) programmed for one step of initial
denaturation at 94°C for 2 min and 35 cycles of
denaturation at 94°C for 1 min, annealing at
55°C for 2 min for primers MYMV-CP-F/
MYMV-CP-R and extension at 72°C for 3
min, followed by one step of final extension at
72°C for 10 min. PCR was conducted with
Dream Taq Master mix (Fermentas) in total
reaction mixture volume of 25 µl that
contained Dream Taq Master mix- 13 µl; 
dH2O - 4 µl; forward and reverse primers (20
pmole/ µl)- 2 µl each; DNA template (total
nucleic acid-100ng/µl)- 4 µl, and. PCR
products were subjected to electrophoresis in 1
% agarose at 50 V for 45 minutes in
Electrophoresis system - SCOTLAB
(Anachem Ltd.) in Tris-acetate- EDTA buffer
containing ethidium bromide @ 0.1 %. The
gel was observed under Gel Documentation
System (IMAGO Compact Imaging System, B
& L Systems, Isogen Lifescience, The
Netherlands).

**Cloning and sequencing of coat protein
gene of YMV infecting mungbean**

The PCR products were purified from agarose
gel using Qiagen Gel Extraction kit (Qiagen,
Hilder, Germany). All amplicons were cloned
into the plasmid vector pTZ57R/T using
InsTAClone™ PCR Cloning Kit following the
manufacturer’s instructions. Transformed
colonies were screened and selected on LB
agar medium amended with ampicillin, X-gal
and IPTG. Isolated plasmids from transformed
positive clones were confirmed for the
presence of insert using the respective CP
specific primers. The resultant positive clones
were fully sequenced in both directions using
universal M13 forward and reverse primers.
Full length sequence of coat protein of YMV
was obtained by aligning of forward and reverse reaction sequences.

**Phylogenetic analysis, nucleotide sequence and amino acid sequence comparison of coat protein gene of yellow mosaic virus of urdbean with other geminiviruses**

Pairwise and multiple sequence alignment of the full length of coat protein sequence of various YMV was done using MEGA 5.1 multiple alignment tool. The phylogenetic neighbor-joining trees and evolutionary analysis were conducted using MEGA 5.1 software package (Tamura et al., 2007) based on coat protein gene sequences of MYMV with 21 other geminivirus sequences downloaded from NCBI Genbank (Table 1). Robustness of trees was determined by bootstrap sampling of multiple sequence alignment with 1000 replications. Comparision of the nucleotide and amino acid sequences of YMV was analysed by using sequence identity matrix tool of Bio-Edit software (Version 7.9.1).

**Results and Discussion**

Young leaves showing characteristic yellow mosaic symptoms were collected from field infected urdbean plants and used total DNA was isolated according to Rouhibakhsh et al. (2008). DNA from healthy plants was also isolated. Total DNA was used as template in PCR reactions. A set of degenerate primers specific to coat protein region of MYMIV (NM1/NM2), MYMV (MYMV-CPF/MYMV-CP-R) available in the literature were synthesized.

Polymerase chain reaction of yellow mosaic virus infecting blackgram samples using MYMV-CP-F/MYMV-CP-R primers amplified the expected product of size 1000 bp from blackgram infected samples. PCR products from the yellow mosaic affected samples of blackgram when analysed on gel, yielded an amplicon of expected size of nearly 1000 bp (Plate 2). But no amplicon was observed in PCR products from healthy plants indicating no infection by MYMV in plants that were free from yellow mosaic symptoms. No amplification of PCR products was observed with NM1/NM2 primers which were highly specific to MYMIV, suggesting that yellow mosaic virus infecting blackgram in Bangalore is an isolate of MYMV but not MYMIV. Expected PCR products of size 1000 bp obtained were cloned, sequenced and assembled. The total number of sequences obtained from blackgram yellow mosaic was 880 bp with 106 bp of pre-coat protein region and 774 bp of coat protein region of blackgram yellow mosaic virus. 257 amino acid lengths were predicted after translation of the nucleotide sequences. The complete nucleotide sequence of the CP gene of BGYMV, Hebbal, Bangalore isolate had single open reading frame (ORF) of 774 base pairs and 257 amino acids.

The cluster phylogram based on pairwise and multiple sequence alignment of the nucleotide sequence of the CP gene of 8 isolates of MYMV and 13 isolates of MYMIV indicated that the present isolate causing blackgram yellow mosaic virus formed cluster with other known isolates of MYMV. The present isolate clustered with MYMV-Maharashtra, MYMV-Tamil Nadu, MYVMadurai and MYVM-Nammakal isolates infecting soybean, mungbean, soybean and mothbean respectively (Fig. 1).

Sequence comparisons indicated that BGYMV has the highest nucleotide sequence identity of about 98.7 per cent, 98.4 per cent and 98.3 per cent and with MYMV-Namakkal:MoB [DQ865201.1]; MYMV-Madurai:SB [AJ421642.1], MYMV-Tamil Nadu:MB [AJ132575.1] and MYMV-Maharashtra:SB [AF314530.1] isolates, respectively (Table 2).
The nucleotide sequence identity of BGYMV with MYMV ranged from 94.4-98.7 per cent. The nucleotide sequence identity of BGYMV with MYMIV ranged between 79-80.7 per cent. BGYMV had 80.7 per cent, 79.9 per cent and 79.8 per cent identity with MYMIV-India:SB [AY049772.1], MYMIV-Pakistan:MB [AY269992.1] and MYMIV-Pakistan:BG [FM208845.1]; MYMIV-Indonesia:YLB [JN368437.1]; MYMIV-Bangladesh:MB [AF314145.1] isolates, respectively.

When the deduced amino acid sequence of individual proteins were compared with those of other begomoviruses, the maximum homology of 99.2 per cent and 98.8 per cent was noticed with MYMV-Tamil Nadu:MB [AJ132575.1]; MYMV-Maharashtra:SB [AF314530.1] and MYMV-Namakkal:MoB [DQ865201.1]; MYMV-Madurai:SB [AJ421642.1] isolates, respectively (Table 2). The deduced amino acid identities of BGYMV with Mungbean yellow mosaic virus revealed identities between 99.2-95.7 per cent. BGYMV shared 99.2 per cent homology with MYMV-Tamil Nadu:MB [AJ132575.1], MYMV-Maharashtra:SB [AF314530.1] and 98.8 per cent with MYMV-Namakkal:MoB [DQ865201.1]; MYMV-Madurai:SB [AJ421642.1] isolates. Deduced amino acid sequence comparison revealed that BGYMV revealed identities ranged from 84-85.9 per cent with Mungbean yellow mosaic India virus at amino acid level. BGYMV showed 85.9 per cent homology with MYMV-Palampur:FB [FN794200.1], MYMV-Jabalpur:SB [AJ416349.1] and MYMV-Pakistan:MB [AY269992.1]. 85.6 per cent homology was obtained with MYMV-Varanasi:Do [AY547317.1], MYMIV-India:SB [AY049772.1] and MYMIV-Pakistan:BG [FM208845.1] isolates. The results from the phylogenetic analysis, nucleotide sequence identity and amino acid identity confirmed that blackgram yellow mosaic virus from Bangalore is an isolate of MYMV.

Phylogenetic tree based on full length of coat protein gene sequences of four isolates of blackgram yellow mosaic virus with other isolates formed two major clusters of MYMIV and MYMV. The four clusters formed unique cluster with MYMIV group that cause yellow mosaic disease symptoms MYMIV group that cause yellow mosaic disease symptoms in blackgram (AF126406), mungbean (AY271893), soybean (DQ389146) and cowpea (DQ389153). The nucleotide sequence analysis of the four blackgram isolates infected by yellow mosaic virus with other geminivirus sequences showed that YMV isolate had >92 per cent homology with MYMIV and less than 80 per cent homology with MYMV, MSV, BGYMV, ToLCV, DoYMV and HgYMV. A comparison of predicted amino acid sequence of coat protein gene of four isolates blackgram yellow mosaic virus from Andhra Pradesh with other amino acid sequences indicated that YMV had >92 per cent homology with MYMIV and less than 66 per cent homology with all other geminiviruses (Obaiah, 2011). The results obtained are in conformity with earlier investigations carried out by Naimuddin and Mohd. Akram (2010), Kamaal Naimuddin et al. (2011), Mohammad Nurul Islam et al. (2012), Naimuddin and Akram (2012) and Sachan Mansi et al. (2010).

Coat protein genes have traditionally proven useful for plant virus identification and classification. Because of its high degree of conservation, the coat protein ORF (CP or AV1) is the only begomovirus sequence approved by the International Committee on Taxonomy of Viruses for ascertaining the identity of a begomovirus (Mayo and Pringle, 1998), and the sequence comparison has been used to identify and classify geminiviruses (Malla Padidam et al., 1995; Brown et al.,
The CP gene is the sole structural protein of geminiviruses and has been shown to play a determinative role in the transmission of the viruses (Pradeep Sharma et al., 2005). The CP gene is the most highly conserved gene in the family Geminiviridae. These sequences which effectively predicts discrete strains, species and taxonomic lineage of begomoviruses, has been accepted by ICTV as desirable marker for virus identity when full length genomic sequences are not available (Rybicki et al., 1998). The utility of the CP gene sequences for these purposes is likely possible because the CP sequences are optimally average variable and conserved regions to arrive at a prediction more in line with extent of sequence variation and conservation across the entire genome (Brown et al., 2001).

As per the latest guidelines if nucleotide identity at coat protein sequence is >90%, it will be considered as variant, strain or isolate of the same virus and <90% will be considered as distinct species in begomovirus classification (Fauquet et al., 2008). The International Committee on Taxonomy of Viruses (ICTV) accepts the classification of begomoviruses based on CP gene sequences, when full length sequences are not available (Rybicki et al., 1998). Member of the genus Begomovirus are known to form clusters according to geographical origin with distinct branches for viruses from America, Africa and Asia. The results of the phylogenetic analysis, nucleotide sequence comparison and amino acid sequence comparison of the present study revealed that coat protein gene of yellow mosaic virus infecting blackgram (BGYMV-Hebbal-Bangalore) is a Mungbean yellow mosaic virus (MYMV) but not Mungbean yellow mosaic India (YMIV) virus and it is a variant of mungbean yellow mosaic virus since it showed 94.4-98.7 per cent identity at nucleotide level with other MYMV isolates.

**Plate.1** Urdbean plants showing typical symptoms of yellow mosaic virus
| Sl. No. | Virus species                  | Abbreviation          | Geographical origin | Host species | Accession number |
|--------|-------------------------------|-----------------------|---------------------|-------------|-----------------|
| 1.     | Mungbean yellow mosaic virus  | MYMV-Haryana:MB       | Haryana             | Mungbean (MB) | AY271896.1      |
| 2.     | Mungbean yellow mosaic virus  | MYMV-Namakkal:MoB     | Namakkal            | Mothbean (MoB) | DQ865201.1      |
| 3.     | Mungbean yellow mosaic virus  | MYMV:Combodia:MB      | Combodia            | Mungbean (MB) | AY271892.1      |
| 4.     | Mungbean yellow mosaic virus  | MYMV-Madurai:SB       | Madurai             | Soybean (SB)  | AJ421642.1      |
| 5.     | Mungbean yellow mosaic virus  | MYMV-Pakistan:SB      | Pakistan            | Soybean (SB)  | AY269991.1      |
| 6.     | Mungbean yellow mosaic virus  | MYMV-Maharashtra:SB   | Maharashtra         | Soybean (SB)  | AF314530.1      |
| 7.     | Mungbean yellow mosaic virus  | MYMV-Thailand:MB      | Thailand            | Mungbean (MB) | AB017341.1      |
| 8.     | Mungbean yellow mosaic virus  | MYMV-Tamil Nadu:MB    | Tamil Nadu          | Mungbean (MB) | AJ132575.1      |
| 9.     | Mungbean yellow mosaic India virus | MYMIV-Indonesia:SB | Indonesia          | Soybean (SB)  | JN368438.1      |
| 10.    | Mungbean yellow mosaic India virus | MYMIV-Akola:MB | Akola              | Mungbean (MB) | AY271893.1      |
| 11.    | Mungbean yellow mosaic India virus | MYMIV-India:SB | India              | Soybean (SB)  | AY049772.1      |
| 12.    | Mungbean yellow mosaic India virus | MYMIV-Indonesia:YLB | Indonesia          | Yard long bean (YLB) | JN368437.1  |
| 13.    | Mungbean yellow mosaic India virus | MYMIV-Pakistan:BG | Pakistan           | Blackgram (BG) | FM208845.1      |
| 14.    | Mungbean yellow mosaic India virus | MYMIV-Pakistan:MB | Pakistan           | Mungbean (MB) | AY269992.1      |
| 15.    | Mungbean yellow mosaic India virus | MYMIV-Indonesia:YLB | Indonesia          | Yard long bean (YLB) | JN368434.1  |
| 16.    | Mungbean yellow mosaic India virus | MYMIV-Indonesia:YLB | Indonesia          | Yard long bean (YLB) | JN368432.1  |
| 17.    | Mungbean yellow mosaic India virus | MYMIV-Nepal:MB | Nepal              | Mungbean (MB) | AY271895.1      |
| 18.    | Mungbean yellow mosaic India virus | MYMIV-Varanasi:Do | Varanasi           | Fieldbean (Do) | AY547317.1      |
| 19.    | Mungbean yellow mosaic India virus | MYMIV-Bangladesh:MB | Bangladesh        | Mungbean (MB) | AF314145.1      |
| 20.    | Mungbean yellow mosaic India virus | MYMIV-Jabalpur:SB | Jabalpur           | Soybean (SB)  | AJ416349.1      |
| 21.    | Mungbean yellow mosaic India virus | MYMIV-Palampur:FB | Palampur           | Frenchbean (FB) | FN794200.1      |
**Table 2** Nucleotide and amino acid sequence identities of coat protein gene of yellow mosaic virus infecting blackgram with other geminiviruses

| Sl. No. | Accession number | Sequences               | Nucleotide sequence identity | Amino acid sequence identity |
|---------|------------------|-------------------------|------------------------------|------------------------------|
| 1.      | AY271896.1       | MYMV-Haryana:MB         | 94.4                         | 95.7                         |
| 2.      | DQ865201.1       | MYMV-Namakkal:MoB       | 98.7                         | 98.8                         |
| 3.      | AY271892.1       | MYMV:Combodia:MB        | 96.3                         | 97.2                         |
| 4.      | AJ421642.1       | MYMV-Madurai:SB         | 98.7                         | 98.8                         |
| 5.      | AY269991.1       | MYMV-Pakistan:SB        | 94.8                         | 96.4                         |
| 6.      | AF314530.1       | MYMV-Maharashtra:SB     | 98.3                         | 99.2                         |
| 7.      | AB017341.1       | MYMV-Thailand:MB        | 96.6                         | 98.0                         |
| 8.      | AJ132575.1       | MYMV-Tamilnadu:MB       | 98.4                         | 99.2                         |
| 9.      | JN368438.1       | MYMIV-Indonesia:SB      | 79.7                         | 84.0                         |
| 10.     | AY271893.1       | MYMIV-Akola:MB          | 79.0                         | 84.4                         |
| 11.     | AY049772.1       | MYMIV-India:SB          | 80.7                         | 85.6                         |
| 12.     | JN368437.1       | MYMIV-Indonesia:YLB     | 79.8                         | 85.2                         |
| 13.     | FM208845.1       | MYMIV-Pakistan:BG       | 79.8                         | 85.6                         |
| 14.     | AY269992.1       | MYMIV-Pakistan:MB       | 79.9                         | 85.9                         |
| 15.     | JN368434.1       | MYMIV-Indonesia:YLB     | 79.7                         | 84.4                         |
| 16.     | JN368432.1       | MYMIV-Indonesia:YLB     | 79.7                         | 84.8                         |
| 17.     | AY271895.1       | MYMIV-Nepal:MB          | 79.3                         | 85.2                         |
| 18.     | AY547317.1       | MYMIV-Varanasi:Do       | 79.3                         | 85.6                         |
| 19.     | AF314145.1       | MYMIV-Bangladesh:MB     | 79.8                         | 84.0                         |
| 20.     | AJ416349.1       | MYMIV-Jabalpur:SB       | 79.5                         | 85.9                         |
| 21.     | FN794200.1       | MYMIV-Palampur:FB       | 79.5                         | 85.9                         |
Plate 2 Amplification of coat protein gene of YMV infecting urdbean using MYMV-CP-F/MYMV-CP-R primer pair

Lane:
M- 1Kb Marker (NEB 1 kb DNA ladder)
Lane 1 – Healthy urdbean plant DNA
Lane 2 – Water control
Lane 3, 4, 5 - Specific PCR product of 1000 bp from from BGYMV infected sample

Figure 1 Phylogenetic tree obtained from comparison of complete nucleotide sequence of coat protein gene of BGYMV with other geminiviruses from database. The dendrograms are calculated using neighbor-joining algorithm of MEGA 5.1 version. Numbers at nodes indicate percentage bootstrap confidence scores (1,000 replications)
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