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Molecular Detection and Partial Characterization of Coat Protein Gene of Moth Bean Yellow Mosaic Virus (MBYMV) from Northern Karnataka

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ABSTRACT

Background: Moth bean [*Vigna aconitifolia* (Jaq.) Marechal] is characterized as one of the most drought hardy, short duration, annual legume crop. It is mainly grown in Northern districts of Karnataka. Moth bean crop suffers from many diseases *viz*., yellow mosaic, bacterial blight, root rot, anthracnose and powdery mildew. Moth bean is targeted by YMV which causes severe damage to grain and fodder yields. Since not much work has been carried out on characterization of moth bean yellow mosaic virus in Northern Karnataka, an attempt was made to partially characterize coat protein gene of Moth Bean Yellow Mosaic Virus (MBYMV).

Methods: The total genomic DNA was extracted from leaf tissues of healthy moth bean plants and yellow mosaic virus infected plants utilizing by modified CTAB method. Specific primers for yellow mosaic viruses were tried to amplify coat protein region of MBYMV.

Result: Moth bean leaf samples showing yellow mosaic symptoms gave positive results with MYMV specific primer pairs (MYMV-CP-F/MYMV-CP-R) and yielded amplicons of ~1000 bp. The 1000 bp PCR products were directly sequenced and assembled. Phylogenetic tree based on full length coat protein gene sequence of MBYMV with other geminiviruses sequences downloaded from NCBI Genbank formed three major clusters of MYMV, HgYMV and MYMIV. The present MBYMV isolate formed unique cluster with MYMV group.

Key words: Coat protein, Geminivirus, Moth bean, Yellow mosaic.

INTRODUCTION

Moth bean [*Vigna aconitifolia* (Jacq.) Marechal] belongs to genus *Vigna* sub family Papilionaceae and family Fabaceae (Marechal *et al*., 1978). Moth bean is known as dew gram, aconite bean and kidney gram along with vernacular names in various parts of nation *viz*., moth, tangle, matki (Hindi), kheri (Bengali), kumkuma pesalu (Telugu), tulkapayir (Tamil) and madike (Kannada).

Moth bean is cultivated as food crop in India and different parts of Asia and Africa. The grains have protein (22-24%) and vitamins. In India and South-Western United States, plants were used to improve soil fertility, as green manure, pasture or forage crop. Moth bean helps in symbiotic nitrogen fixation and thus enhance soil fertility (Yogeesh *et al*., 2012). The seeds are utilized therapeutically in diet during illness. In India, significant food items produced are dhal, kheech, papad, bhujia, mangori and sprouts (Sharma and Joshi, 1993).

Moth bean is a local crop of India, Pakistan and Myanmar. It is the most significant pulse crop of semi-arid and arid region of North-western states. In India, moth bean is cultivated in an area of 993.08 thousand ha with a production and productivity of 346 thousand ton and 226 kg per ha, respectively during 2018-19 (Anonymous, 2021). In Karnataka, moth bean is mostly cultivated in northern region during *kharif* season, with a yearly precipitation of 594 mm from June to October.

Moth bean crop suffers from yellow mosaic, bacterial blight, root rot, seedling blight, anthracnose, *Alternaria* leaf spot, powdery mildew and root knot diseases (Prema, 2021). Among various diseases, yellow mosaic, a viral disease causes detrimental effect on pulse crops by reducing their yield (Kang *et al*., 2005). Yellow mosaic disease poses constraints to agriculture and breeding programme to develop resistant sources (Kumar and Khan, 2019). Yellow mosaic disease (YMD) transmitted by *Bemisia tabaci* is the most serious disease of moth bean as it unfavourably affects the seed and fodder yield (Ahamed and Harwood, 1973 and Vir *et al*., 1984). In India, first time reporting of the yellow mosaic disease was recorded during seventies in Uttar Pradesh and Rajasthan (Tyagi and Mathur, 1978 and Nene, 1972). In pulses, the characteristics symptoms of YMD show up as

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golden yellow colour mosaic patches on leaves which might be incompletely or totally yellow. Infected plants scarcely bear flowers and pods with some immature and deformed seeds. The disease causes decrease in number of seeds per pods, number of pods per plant and yield loss of about 75.30 per cent (Lodha *et al*., 1986). The disease may occur at any phase of plant development. If the incidence occurs at initial stage, plant may not blossom and the yield reduction might be as high as 90 per cent (Bhati *et al*., 1987).

Legumes infecting begomoviruses have a place within family Geminiviridae (Fauqet *et al*., 2003). These begomoviruses are transmitted by means of white fly, *Bemisia tabaci* and cause YMD that severely affect pulses (Varma and Malathi, 2003). It is not yet evident that the yellow mosaic in various pulses is due to various strains of same or by different infectious virus species (Qazi *et al*., 2007). The yellow mosaic disease across tropical and subtropical regions of Asia are recognized as four bipartite begomoviruses *viz*., Mung Bean Yellow Mosaic Virus (MYMV), Mung Bean Yellow Mosaic India Virus (MYMIV), Horse Gram Yellow Mosaic Virus (HgYMV) and Dolichos Yellow Mosaic Virus (DoYMV). These begomoviruses are altogether referred to as Legume yellow mosaic viruses (Fauquet and Stanley, 2003; Qazi *et al*., 2007 and Briddon *et al*., 2010).

Geminiviruses measure about 15-19 nm × 30 nm in size and are geminate molecules comprising of two incomplete icosahedron particles which are bipartite or monopartite with circular single stranded (ss) DNA of around 2600 nucleotides (Harrison *et al*., 1977; Francki *et al*., 1980). The Geminiviruses have been assembled into different genera: *Begomovirus, Mastrevirus*, *Curtovirus* and *Topocuvirus* relying upon their genome structure, host range and vector specificity (Fauquet and Stanley, 2003 and Fauquet *et al*., 2003).

Coat protein is predominantly conserved among the Begomoviruses originating from the similar geographical regions (McGrath and Harrison, 1995 and Maruthi *et al*., 2002). Coat protein is a multifunctional protein which is associated with symptom expression, viral DNA replication and vector transmission. Therefore, coat protein was utilized to describe and find the relationship of several Begomoviruses. Hence, the core region of the coat protein sequence is helpful for virus diversity identification and grouping purposes (Harrison *et al*., 2002).

Since not much work has been carried out on molecular characterization of Moth bean yellow mosaic virus in Karnataka and the literature available was sparse, the current research was carried out with an intention to detect and partially characterize coat protein gene of moth bean yellow mosaic virus at molecular level.

MATERIALS AND METHODS

The present investigations on Moth bean yellow mosaic virus (MBYMV) was carried at College of Agriculture, Vijayapur, University of Agricultural Sciences, Dharwad, Karnataka, India during December 2021.

Collection of yellow mosaic virus (YMV) affected moth bean samples

Moth bean leaves infected by yellow mosaic virus were collected from fields in polythene covers and stored at -80°C for molecular analyses.

Extraction of total DNA using modified Cetyl Trimethyl Ammonium Bromide (CTAB) method

Severe yellow mosaic symptoms showing moth bean plants were collected from field (Fig 1). The total genomic DNA was extracted from leaf tissues of healthy moth bean plants and yellow mosaic virus infected plants by modified CTAB method of Rouhibakhsh *et al*. (2008) and Prema (2013). All the DNA extracts were further diluted from 1:10 to 1:40 in sterile distilled water (SDW) before using for PCR amplifications. Quality and quantity of DNA was checked by using agarose gel electrophoresis and spectrophotometer.

Amplification of the coat protein gene of moth bean yellow mosaic virus (MBYMV)

In order to amplify the coat protein of moth bean yellow mosaic virus, specific primers available in the literature for yellow mosaic viruses were tried to amplify coat protein region of nearly 1,000 bp (Naimuddin and Akram, 2010). 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) and HgYMV (HgYMV-CP-F- ATG CTT GCA ATT AAG TAC TTG CA/HgYMV-CP-R- TAG GCG TCA TTA GCA TAG GCA) were used for amplification of coat protein gene of moth bean yellow mosaic virus (MBYMV). Samples were taken for polymerase chain reaction (PCR) along with positive control (C-TAB extracted MYMV DNA) and negative control (healthy DNA). After preparing PCR mixture, the DNA template was added and tubes were spun quickly and embedded into the wells of a thermal cycler (Eppendorf thermocycler).



Fig 1: Moth bean plants showing typical symptoms of yellow mosaic disease.

PCR was performed in Thermocycler 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, HgYMV-CP-F/HgYMV-CP-R and extension at 72°C for 3 min, followed by one step of final extension at 72°C for 10 min. PCR mixture was prepared with Dream Taq Master mix (Fermentas) in total reaction mixture volume of 25 µl that contained Dream *Taq* Master mix- 13 µl; dH_2O - 4 µl; forward and reverse primers (20 pmole/µl)- 2 µl each; DNA template (total nucleic acid-100 ng/µl)- 4 µl. After completion of the reaction, the products were kept at 4°C prior to gel analysis. 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 and L Systems, Isogen Lifescience, The Netherlands).

Sequence analysis and phylogenetic tree construction

The 1000 bp amplicon amplified from MBYMV infected moth bean samples were sent to direct sequencing. The sequences obtained from both MYMV-CP-F forward and MYMV-CP-R reverse reactions were aligned and joined together to get full length sequence using 'nucleotide blast' at basic blast programmes and 'align two (or more) sequences' at specialized blast programmes freely assessing in 'Basic Local Alignment Search Tool' (BLAST) at the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov). The phylogenetic neighbour-joining trees and evolutionary analysis were built using MEGA 10.1.8 software package (Tamura *et al*., 2007). Robustness of trees was determined by bootstrap sampling of multiple sequence alignment with 1000 replications. Per cent identity of nucleotide alignments were done with Bio-Edit software (Version 7.2.5).

RESULTS AND DISCUSSION

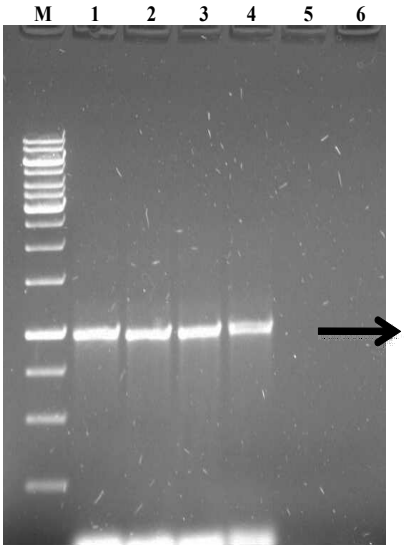
The modified C-TAB method contained some important chemicals like 100 mM (0.1M) Tris HCl (pH 8.0), 20 mM (0.02 M) EDTA (pH 8.0), 1M NaCl, C-TAB (2%) (Cetyl Trimethyl Ammonium Bromide), SDS (1%), PVP 40 (2%) and sodium sulphate (0.65%) β-mercaptoethanol (0.2%) and 1.4-2.0 M concentration of sodium chloride which played a major role in isolation of good quality DNA. So, the modified C-TAB method was used for isolation of viral DNA from infected and healthy moth bean plants (Rouhibakhsh *et al*., 2008 and Prema, 2013). A good quality DNA of MBYMV infected leaf of moth bean was obtained from modified CTAB with RNase treatment and viral DNA extracted from common CTAB method without RNase treatment produced some contamination. Yellow mosaic virus infected mung bean samples were also used for DNA extraction to serve as positive control. Obtained viral DNA was subjected to spectrophotometer readings in order to measure the quality of isolated viral DNA. The DNA concentration varying from 120 ng/µl to 2500 ng/µl and reading of A 260/280 ration ranged between 1.9-2.20 were obtained. This isolated viral DNA produced a greater amplification of coat protein gene in polymerase chain reaction.

Rouhibakhsh *et al*. (2008) who found that modified CTAB method containing chemicals like β-mercaptoethanol and sodium chloride resulted in successful amplification of PCR product from the leaves of legumes rich in polyphenols, tannins and polysaccharides. A good quality DNA of MBYMV infected leaf was obtained from modified CTAB with RNase treatment and viral DNA extracted from conventional CTAB method without RNase treatment produced some contamination. Prema (2013) illustrated that CTAB method utilized for viral DNA extraction from leguminous plants appeared to inhibit amplification of PCR product due to the presence of large number of phenolic compounds and glycoproteins in leaves.

The good quality DNA extracted by using modified C-TAB method was subjected to Polymerase chain reaction (PCR). PCR was mainly conducted to establish a close association of begomoviruses through the amplification of geminiviruses coat protein specific PCR product. To know exact amplification and to get quality PCR product, various dilutions of DNA or template were exposed to PCR *i.e*., 1:10 to 1:40 dilution. In order to know, which is the exact group of yellow mosaic virus affecting moth bean, MYMV CP and HgYMV CP specific primers were used for amplification of MBYMV CP gene. Successful amplification of full length of coat protein gene of approximately 1000 bp from the MBYMV infected moth bean and mung bean PCR samples was obtained at an annealing temperature of 55°C for 2 min. Among different dilutions, a clearly visibly band and specific PCR product of 1000 bp (1 kb ladder) was obtained with 1:40 dilution from MBYMV infected moth bean plant. The dilution of 1:10, 1:20 and 1:30 product of PCR were also amplified at approximately 1000 bp but visibility of band was comparatively low may be due to presence of some phenols and polysaccharides. The MBYMV specific coat protein gene of approximately about 1000 bp was obtained only from the MBYMV infected moth bean and mung bean PCR sample. But none of healthy moth bean plant, healthy mung bean plant samples and water control produced any kind of amplification. The amplification of full length of coat protein gene of MBYMV was obtained only with MYMV-CP-F and MYMV-CP-R primer pairs but not with HgYMV-CP-F and HgYMV-CP-R primer pair (Fig 2).

The above investigations are in conformity with the findings of several workers. The coat protein gene (CP) of yellow mosaic virus infecting various legumes showing specific yellow mosaic symptoms was successfully amplified using specific primers. Nucleotide sequences of 885 bp, 867 bp, 893 bp, 886 bp, 889 bp, 889 bp and 880 bp were obtained from yellow mosaic virus infecting horsegram, frenchbean, soybean, limabean, polebean, mungbean and urdbean, respectively (Prema and Rangaswamy, 2018a).

Comparison of nucleotide sequence of coat protein gene of MBYMV infecting moth bean was done with those of selected begomoviruses which were obtained from NCBI database (Table 1). Nearest homology sequence were identified with respect to nucleotide sequence data by using BLAST programme. Pairwise and multiple sequence alignment with respective reference strain sequence were made by using MEGA 10.1.8 version software. The total length of nucleotide sequence of MBYMV infecting moth bean consisted of 972 nucleotides.



Lane: M- 1Kb Marker (Fermentas 1 kb DNA ruler).
Lane 1, 2- Specific PCR product of 1000 bp from MBYMV infected sample.
Lane 3, 4- Specific PCR product of 1000 bp from MYMV infected sample.
Lane 5- Healthy moth bean plant DNA.
Lane 6- Water control.

Fig 2: Amplification of coat protein gene of YMV infecting moth bean using MYMV-CP-F/MYMV-CP-R primer pair.

Per cent nucleotide sequence identity value of MBYMV infecting moth bean was represented with many other begomoviruses (Table 2). Comparison of nucleotide sequence of MBYMV-AC-Vijayapur with many other begomoviruses revealed 84-85 per cent identity with isolate of Horse gram yellow mosaic virus (HgYMV), 79-85 per cent identity with Mung bean yellow mosaic India virus (MYMIV) and 70-75 per cent identity with Dolichos yellow mosaic virus (DoYMV). The MBYMV-AC-Vijayapur isolate had 97-99 per cent nearer identity with seven other MYMV sequences such as, MYMV-Belagavi:MoB (99.12%), MYMV-Dharwad:MB (98.83%), MYMV-Maharashtra:SB (98.68%), MYMV-Madurai-MB (98.10%), MYMV-Tirunelveli:BG (97.95%), MYMV-Thailand:MB (97.61%) and MYMV-Vietnam:MB (97.36%).

Prema and Rangaswamy (2018b) indicated that SBYMV has the highest nucleotide sequence identity of about 98.3 per cent and 95.4 per cent with HgYMV-Tamil Nadu:HG [AJ627904.1] and HgYMV-Srilanka:FB [GU323321.1], respectively. The nucleotide sequence identity of SBYMV with MYMV ranged from 85.1-86.9 per cent. The nucleotide sequence identity of SBYMV with MYMIV ranged between 81.6-79.9 per cent. 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

Table 1: List of geminiviruses used for comparison of coat protein gene, their origin, host species and NCBI accession numbers.

Virus species	Abbreviation	Accession number	Geographical origin	Host
Mung bean yellow mosaic virus	MYMV-Belagavi:MoB	MN698295.1	Belagavi	Moth bean
Mung bean yellow mosaic virus	MYMV-Dharwad:MB	MN602425.1	Dharwad	Mung bean
Mung bean yellow mosaic virus	MYMV-Maharashtra:SB	AF314530.1	Maharashtra	Soybean
Mung bean yellow mosaic virus	MYMV-Madurai:MB	AJ132575.1	Madurai	Mung bean
Mung bean yellow mosaic virus	MYMV-Tirunelveli:BG	KC911717.1	Tirunelveli	Black gram
Mung bean yellow mosaic virus	MYMV-Thailand:MB	AB017341.1	Thailand	Mung bean
Mung bean yellow mosaic virus	MYMV-Vietnam:MB	JX244173.1	Vietnam	Mung bean
Horse gram yellow mosaic virus	HgYMV-Bengaluru:HG	AM932427.1	Bengaluru	Horse gram
Horse gram yellow mosaic virus	HgYMV-Bengaluru:SB	MK391940.1	Bengaluru	Soybean
Mung bean yellow mosaic India virus	MYMIV-Belagavi:MoB	MN698289.1	Belagavi	Moth bean
Horse gram yellow mosaic virus	HgYMV-Belagavi:MoB	MN698287.1	Belagavi	Moth bean
Horse gram yellow mosaic virus	HgYMV-Bengaluru:FB	AM932425.1	Bengaluru	French bean
Horse gram yellow mosaic virus	HgYMV-Belagavi:CP	MN698285.1	Belagavi	Cow pea
Horse gram yellow mosaic virus	HgYMV-Chamarajnagar:MB	MK391941.1	Chamarajnagara	Mung bean
Mung bean yellow mosaic India virus	MYMIV-Hyderabad:SB	FM208834.1	Hyderabad	Soybean
Mung bean yellow mosaic India virus	MYMIV-India:MB	MH255791.1	India	Mung bean
Mung bean yellow mosaic India virus	MYMIV-Delhi:RG	KX363947.1	Delhi	Red gram
Mung bean yellow mosaic India virus	MYMIV-Bengal:SB	HF922628.1	Bengal	Soybean
Dolichos yellow mosaic virus	DoYMV-India:HB	NC005338.2	India	Hyacinth bean
Dolichos yellow mosaic virus	DoYMV-India:HB	NC024779.1	India	Hyacinth bean

[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 (Prema and Rangaswamy, 2018c).

Phylogenetic tree constructed was based on full length coat protein gene sequence of MBYMV with 20 other geminivirus sequences. The phylogenetic neighbor-joining trees formed three major clusters in which, MBYMV-AC-Vijayapur appeared in cluster I, Mung bean yellow mosaic India virus (MYMIV) appeared in cluster II and Horse gram yellow mosaic viruses (HgYMV) appeared in cluster III (Fig 3). The MBYMV-AC-Vijayapur isolate formed cluster with MYMV infecting moth bean, mung bean, black gram and soybean. In order know the association of Dolichos yellow mosaic virus (DoYMV) with isolated MBYMV- AC-Vijayapur strain,

Table 2: Nucleotide per cent identity of coat protein gene of MBYMV-AC-Vijayapur with other begomoviruses.

Virus species	Abbreviation	Accession number	Per cent identity (%)
Mung bean yellow mosaic virus	MYMV-Belagavi:MoB	MN698295.1	99.12
Mung bean yellow mosaic virus	MYMV-Dharwad:MB	MN602425.1	98.83
Mung bean yellow mosaic virus	MYMV-Maharashtra:SB	AF314530.1	98.68
Mung bean yellow mosaic virus	MYMV-Madurai:MB	AJ132575.1	98.10
Mung bean yellow mosaic virus	MYMV-Tirunelveli:BG	KC911717.1	97.95
Mung bean yellow mosaic virus	MYMV-Thailand:MB	AB017341.1	97.61
Mung bean yellow mosaic virus	MYMV-Vietnam:MB	JX244173.1	97.36
Horse gram yellow mosaic virus	HgYMV-Bengaluru:HG	AM932427.1	85.44
Horse gram yellow mosaic virus	HgYMV-Bengaluru:SB	MK391940.1	85.32
Mung bean yellow mosaic India virus	MYMIV-Belagavi:MoB	MN698289.1	85.19
Horse gram yellow mosaic virus	HgYMV-Belagavi:MoB	MN698287.1	85.02
Horse gram yellow mosaic virus	HgYMV-Bengaluru:FB	AM932425.1	84.88
Horse gram yellow mosaic virus	HgYMV-Belagavi:CP	MN698285.1	84.73
Horse gram yellow mosaic virus	HgYMV-Chamarajnagar:MB	MK391941.1	84.58
Mung bean yellow mosaic India virus	MYMIV-Hyderabad:SB	FM208834.1	80.06
Mung bean yellow mosaic India virus	MYMIV-India:MB	MH255791.1	79.71
Mung bean yellow mosaic India virus	MYMIV-Delhi:RG	KX363947.1	79.41
Mung bean yellow mosaic India virus	MYMIV-Bengal:SB	HF922628.1	79.30
Dolichos yellow mosaic virus	DoYMV-India:HB	NC005338.2	75.00
Dolichos yellow mosaic virus	DoYMV-India:HB	NC024779.1	70.00

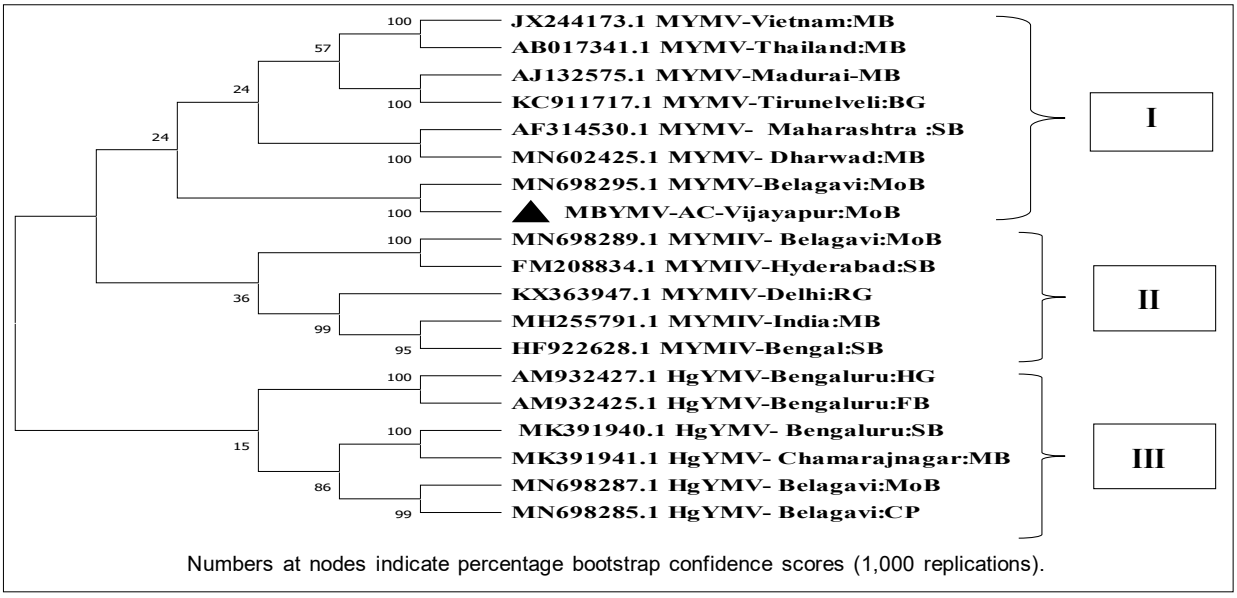


Fig 3: Phylogenetic tree obtained from comparison of complete nucleotide sequence of coat protein gene of Moth bean yellow mosaic virus (MBYMV) infecting moth bean with other geminiviruses like HgYMV, MYMV and MYMIV.

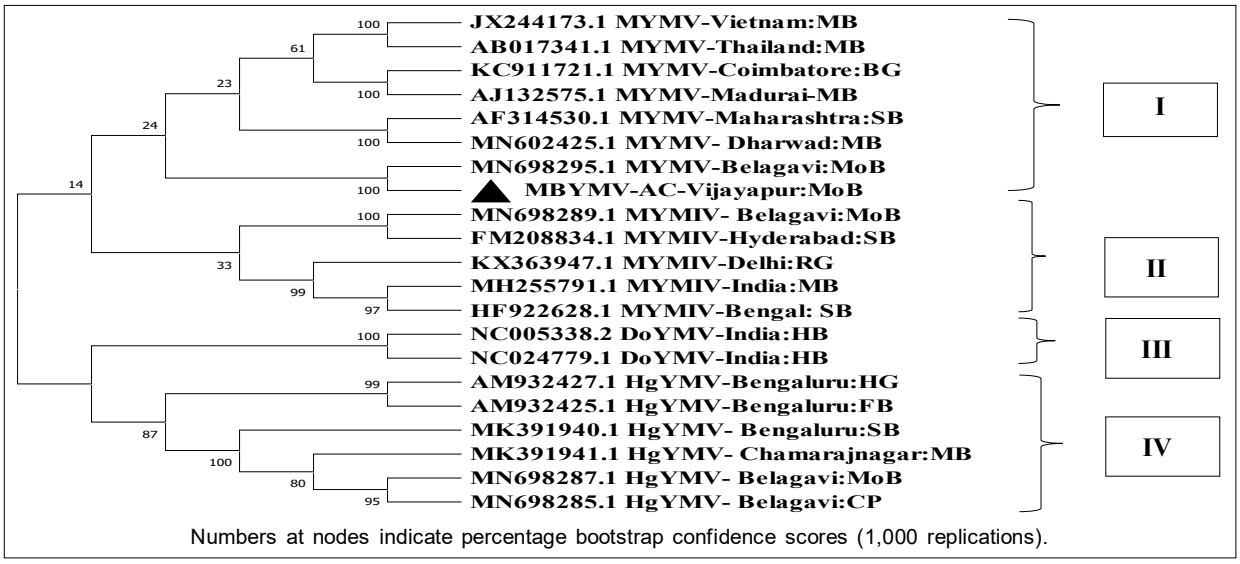


Fig 4: Phylogenetic tree obtained from comparison of complete nucleotide sequence of coat protein gene of Moth bean yellow mosaic virus (MBYMV) infecting moth bean with other geminiviruses like HgYMV, MYMV, MYMIV and DoYMV.

another phylogenetic tree was constructed which formed four clusters in which MBYMV-AC-Vijayapur appeared in cluster I and Mung bean yellow mosaic India virus appeared in cluster II, Dolichos yellow mosaic virus in cluster III and Horse gram yellow mosaic viruses appeared in cluster IV (Fig 4).

Prema and Rangaswamy (2018d) revealed that yellow mosaic virus infecting mungbean is a Mungbean yellow mosaic virus (MYMV) but not Mungbean yellow mosaic India virus (MYMIV) and is a variant of Mungbean yellow mosaic virus (MYMV). Prema and Rangaswamy (2020) analysed that phylogenetic tree based on coat protein gene sequences of HgYMV with 23 other geminivirus sequences formed three major subgroups consisting of of MYMIV, HgYMV and MYMV. HgYMV-GKVK-Bangalore isolate fell within the HgYMV subgroup and deviated from MYMV and MYMIV isolates. Qiu *et al*. (2019) who showed that phylogenetic tree constructed with 44 BYMV-SB1 isolates were closely associated with MYMV-5W9 isolate.

CONCLUSION

The outcome of the investigation from coat protein sequence similarity at nucleotide level and phylogenetic relationship confirmed the existence of MYMV strain on moth bean in northern parts of Karnataka. It is evident that moth bean in AC-Vijayapur infected with yellow mosaic disease is more closely related to other MYMV isolates rather than MYMIV, HgYMV and DoYMV isolates.

Conflict of interest

The authors declare no conflict of interest.

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