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Coat-protein Gene Based Identification and Characterization of Yellow Mosaic Virus Infecting Blackgram in Northern Karnataka, India

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Authors' contributions

This work was carried out in collaboration between both authors. Author SBV performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author PGU designed the study, managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: Coat-protein (CP) based identification and characterization of yellow mosaic virus infecting blackgram.
Place and Duration of Study: The study was carried out during *Kharif* 2023 at College of Agriculture, Vijayapur.
Methodology: The whole genomic DNA was isolated from the leaf tissues of both healthy blackgram plants and plants infected with the yellow mosaic virus by using a modified CTAB method. Specific primers for YMV were tried to amplify coat protein region of blackgram YMV.

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Results: Sequence analysis revealed that the *CP* gene of the begomovirus under study shared 99.8 per cent similarity with MYMV Shivamogga isolate (OM106035.1) at the nucleotide level. When comparing the deduced amino acid sequences of individual proteins from YMV infecting blackgram at Vijayapur with those of other begomoviruses, the highest identity was found with the MYMV Shivamogga isolate (OM106035.1), showing 84.59 per cent similarity. A phylogenetic tree constructed based on the full-length coat protein gene sequence of MYMV, along with 37 other *Geminivirus* sequences, formed two major clusters in which MYMV-Vijayapur isolate appeared in Cluster I.

Conclusion: The results of the current study, based on similarities in coat protein sequence at both the nucleotide and amino acid levels, as well as phylogenetic analysis, confirmed the prevalence of MYMV strain rather than MYMIV, HgYMV and DoYMV in blackgram in northern parts of Karnataka.

Keywords: Coat protein; geminivirus; blackgram; yellow mosaic virus.

1. INTRODUCTION

"Blackgram (*Vigna mungo* L.), commonly known as urdbean in India, belongs to the *Leguminosae* family. It is a significant short-duration, self-pollinating legume. Renowned for its rich nutritional profile, blackgram contains proteins (25-26%), carbohydrates (60%), fats (1.5%), minerals, amino acids and vitamins. Consequently, it serves as an economical source of protein for vegetarians. It also aids in the process of atmospheric nitrogen fixation into the soil. This highly valued pulse crop is cultivated across various regions of India" (Archana et al., 2018). "The optimal climate for blackgram cultivation ranges between 27-30°C, with moderate rainfall and loamy soil that has high water-holding capacity. Its maturity typically occurs within 90-100 days" (Swaminathan et al., 2023). "This crop is grown during both *Kharif* and summer seasons, particularly in central Indian conditions" (Marabi et al., 2017).

"Blackgram originates from the Indo-Burma region and extensively cultivated in major tropical and subtropical nations worldwide" (Biswas et al., 2008). "In India, it ranks as the third most significant pulse crop following chickpea and pigeon pea" (Salam et al., 2009). "India holds the title of the world's largest producer of blackgram, with cultivation spanning approximately 40.02 lakh hectares, resulting in a production of 26.31 lakh tonnes and a productivity rate of 657 kg/ha. The primary blackgram cultivating states include Andhra Pradesh, Bihar, Karnataka, Maharashtra, Madhya Pradesh, Orissa, Rajasthan, Tamil Nadu, Uttar Pradesh and West Bengal. Andhra Pradesh leads the chart as the top blackgram producing state. In Karnataka, it is cultivated across roughly 0.71 lakh hectares, with a production of 0.33. lakh tonnes and productivity rate of 466 kg/ha. Notable blackgram cultivating districts in Karnataka include Kalburgi, Bidar, Mysuru, Belagavi, Dharwad, Bagalkot and Vijayapur" (Anonymous, 2023).

"The low yield of blackgram is attributed to various factors, primarily due to attacks by numerous insects (such as pod borers and sucking pests) and diseases (including Alternaria leaf spot, Cercospora leaf spot, Powdery mildew and viral diseases), as well as other abiotic factors from seedling to maturity stages. Among these constraints, Yellow Mosaic Disease (YMD) caused by Mungbean Yellow Mosaic Virus (MYMV) stands out as the most serious disease and a significant bottleneck for blackgram cultivation and production" (Biswas et al., 2012).

"In the realm of pulses, yellow mosaic disease is attributed to four distinct begomoviruses based on viral nucleotide sequence identity. These include Mungbean Yellow Mosaic Virus (MYMV), Mungbean Yellow Mosaic India Virus (MYMIV), Horsegram Yellow Mosaic Virus (HgYMV) and Dolichos Yellow Mosaic Virus (DoYMV)" (Mishra et al., 2020). "YMV is a single-stranded DNA virus belonging to the genus Begomovirus within the family *Geminiviridae"* (Malathi and John, 2008). In India, Nariani (1960) initially documented MYMV in mungbean fields at the Indian Agricultural Research Institute (IARI) in New Delhi during the 1950s.

"YMVs belong to the genus Begomovirus, are transmitted by the whitefly (*Bemisia tabaci*), and possess bipartite genomes. DNA-A encodes proteins essential for encapsidation, replication and transcription regulation, while DNA-B encodes proteins responsible for intra and intercellular movement. The DNA-A and DNA-B components of begomoviruses share a highly conserved intergenic common region (CR), which contains a stem-loop structure. Within this

loop lies the invariant nanonucleotide motif (TAATATTAC), serving as the origin of viral strand replication" (Bhaskara et al., 2015).

"The insect vector can acquire and transmit the virus within a minimum acquisition and inoculation access period of 10-15 minutes each. The optimal acquisition access period (AAP) ranges between 4-6 hours, while the inoculation access period (IAP) is typically 4 hours. Yellow Mosaic Virus (YMV) is highly destructive and primarily spreads through whiteflies. This virus is not transmitted through seeds, soil or sap" (Nair and Nene, 1973).

"The symptoms of the disease manifest as small yellow flecks in the veinlets of leaves, developing in to more prominent and irregular yellow and green patches, alternating with each other. However, in susceptible genotypes, affected plants produce only a few pods that are smaller in size and deformed" (Verma et al., 1992). "The degree of yield loss largely depends on the age of the plant at the time of infection and the severity of the disease" (Naimuddin et al., 2011). The YMD causes 85-100 per cent yield loss in the plants that are infected at the seedling stage (Nene, 1972).

The goal of the current study was to identify and characterize the coat protein gene of the blackgram yellow mosaic virus at the molecular level. Since, not much work has been done on the molecular characterization of the virus and to determine whether a single strain or mixed strains of YMV are prevalent in northern Karnataka, the present study was initiated.

2. MATERIALS AND METHODS

The present investigation on blackgram yellow mosaic virus was carried at College of Agriculture, Vijayapur, University of Agricultural Sciences, Dharwad, Karnataka, India during July-October months of *Kharif*, 2023.

2.1 Extraction of Total DNA using Modified Cetyl Trimethyl Ammonium Bromide (CTAB) Method

Leaves of blackgram plants (DBGV-5) exhibiting severe yellow mosaic symptoms were collected from the field (Fig. 1). Total genomic DNA was extracted from leaf tissues of both healthy plants and those infected with yellow mosaic virus disease, using the modified CTAB method developed by Rouhibakhsh et al. (2008). The quality and quantity of the DNA were assessed through agarose gel electrophoresis and spectrophotometry.

2.2 Amplification of the Coat Protein Gene of Blackgram Yellow Mosaic Virus

To amplify the coat protein gene of the blackgram yellow mosaic virus, specific primers documented in the literature for yellow mosaic viruses were used to target the coat protein region, approximately 1,000 bp in size (Naimuddin and Akram, 2010). Primers specific to MYMIV (NM1/AV1-F: GTATTTGCAKCAWGTTCAAGA and NM2/AV1-R: AGGDGTCATTAGCTTAGC), MYMV (MYMV-CPF: ATGGG (T/G) TCCGTTGTATGCTTG and MYMV-CPR: GGCGTCATTAGCATAGGCAAT) and HgYMV (HgYMV-CPF: ATGCTTGCAATTAAGTACTTGCA and HgYMV-CPR: TAGGCGTCATTAGC ATAGGCA) were employed to amplify the coat protein gene of blackgram yellow mosaic virus. PCR was performed using these primers with samples taken along with a positive control (CTAB-extracted MYMV DNA) and a negative control (healthy DNA). After preparing the PCR mixture, the DNA template was added, the tubes were briefly spun, and placed into the wells of a thermal cycler (Eppendorf thermocycler) for amplification.

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 NM1/AV1-F/ NM2/AV1-R, 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 reaction mixture volume of 25 µl that contained PCR buffer - 12.5 µl; SDW - 4 µl; forward and reverse primers (20 pmole/µl) - 2 µl each; Taq Polymerase (3 U/µl) - 0.5 µl; DNA - 2 µl; DNTPs - 2 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 60 V for 1 hr in Electrophoresis system.

2.3 Sequence Analysis of Coat Protein Gene and Phylogenetic Tree Construction

The 1000 bp amplicon amplified from YMV infected blackgram samples using MYMV

Fig. 1. Blackgram plants showing typical yellow mosaic disease symptoms

specific primers were sent for sequencing directly. The sequence obtained was searched in NCBI- BLAST to know the sequence similarity with other begomoviruses. Sequence identity matrix tool, Bio Edit software were used to assemble and analyze the data. Multiple alignments were made and sequence was obtained by ClustralW programme. The phylogenetic tree was constructed based on the available sequences using MEGA 11 Software (Valli et al., 2022).

3. RESULTS AND DISCUSSION

The modified CTAB method incorporated several key chemicals, including 100 mM (0.1 M) Tris-HCl (pH 8.0), 20 mM (0.02 M) EDTA (pH 8.0), 1 M NaCl, CTAB (Cetyl Trimethyl Ammonium Bromide) (2%), SDS (1%), PVP 40 (2%), sodium sulfate (0.65%), β-mercaptoethanol (0.2%) and 1.4-2.0 M sodium chloride. These components were crucial for isolating high quality DNA. This modified CTAB method was utilized to isolate viral DNA from both infected and healthy blackgram plants (Rouhibakhsh et al., 2008). High quality DNA was successfully extracted from YMV infected blackgram leaves using this technique.

The DNA extracted through the modified CTAB method was then subjected to Polymerase Chain Reaction (PCR). The PCR effectively amplified the *CP*-gene from YMV infected blackgram leaf samples. Amplification of the coat protein gene of YMV in blackgram resulted in a DNA fragment of approximately 1000 bp (Fig. 2), while no PCR product was obtained from DNA extracted from healthy samples or water control.

Sequencing of PCR products containing the *CP*-gene of YMV using specific primers was performed at Eurofins Genomics India Pvt. Ltd., Bengaluru. The nucleotide sequence of MYMV-Vijayapur isolate was deposited in NCBI database and the accession number obtained was PP470806.1. The results of a BLAST search (http://www.ncbi.nih.gov/BLAST) were used to identify sequence homology. The nucleotide sequence of the coat protein gene of the yellow mosaic virus infecting blackgram was compared with selected begomovirus sequences obtained from the NCBI database (Table 1). The sequence data revealed that the *CP* gene of the begomovirus under study shared 99.80 per cent similarity with MYMV Shivamogga isolate (OM106035.1) at the nucleotide level. When the deduced amino acid sequence of individual proteins of YMV infecting the blackgram at Vijayapur was compared with those of other begomoviruses, the highest identity was found with the MYMV Shivamogga isolate (OM106035.1), showing 84.59 per cent similarity. Based on this sequence comparison, the test isolate was confirmed as mungbean yellow mosaic virus. The nucleotide sequence identity and deduced amino acid sequence identity of MYMV with other begomoviruses ranged from 92.62-99.8 per cent and 70.03-84.59 per cent, respectively.

A phylogenetic tree was constructed based on the full-length coat protein gene sequence of MYMV of blackgram and 37 other *Geminivirus* sequences downloaded from the NCBI GenBank. The phylogenetic neighbor-joining trees and evolutionary analysis were conducted using MEGA 11 with 1,000 bootstrap values and it

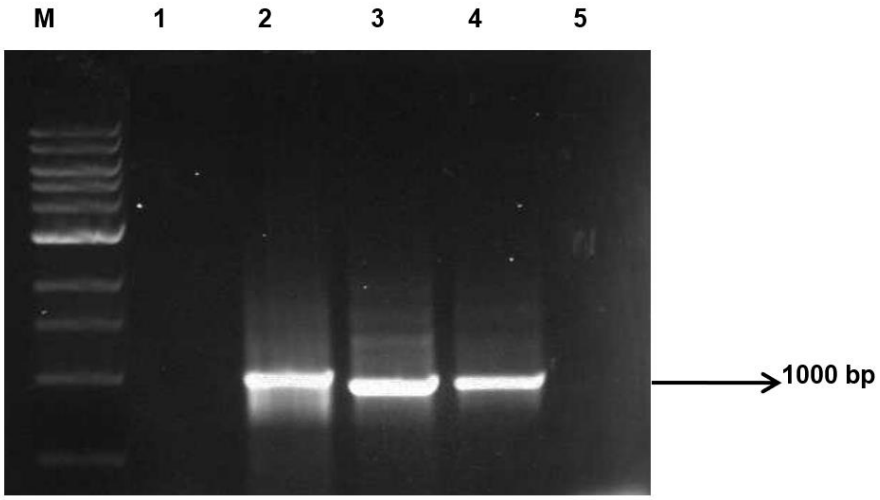
formed two major clusters. The MYMV-Vijayapur isolate appeared in Cluster I and showed similarity with MYMV-Shivamogga isolate (Fig. 3). Pairwise per cent identities of coat protein nucleotide sequences of MYMV and MYMIV isolates was done by using Sequence Demarcation Tool (SDT) and the analysis result showed that highest identity was with MYMV-Shivamogga isolate (Fig. 4). Based on these sequence comparisions, the Vijayapur test isolate was confirmed as mungbean yellow mosaic virus.

These results are consistent with those of several other researchers. Archana et al. (2018) demonstrated that MYMV infecting blackgram from Mandya (Karnataka) was a strain of the MYMV group, with 95 per cent similarity, rather than MYMIV and HgYMV.

Phylogenetic analysis showed that the blackgram yellow mosaic virus clustered with MYMV isolates, having a nucleotide sequence identity of 94.4 to 98.7 per cent with MYMV and 79 to 80.7 per cent with MYMIV. Amino acid sequence comparisons showed high homology with MYMV isolates. This indicated that the virus was a variant of the Mungbean yellow mosaic virus (MYMV), and not of Mungbean yellow mosaic India virus (MYMIV) (Prema and Rangaswamy, 2018).

The results of phylogenetic analysis, nucleotide sequence comparison, and amino acid sequence comparison of the coat protein gene indicated that HgYMV, FBYMV, SBYMV, LBYMV and PBYMV were grouped together as one group of yellow mosaic viruses, while MYMV and BGYMV formed another distinct group of begomoviruses. The findings of this experiment strongly confirmed that yellow mosaic disease affecting various leguminous crops in Bengaluru was caused by two different groups of yellow mosaic viruses (Prema and Rangaswamy, 2018a).

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 isolates. Prema and Rangaswamy (2018c) 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.



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

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

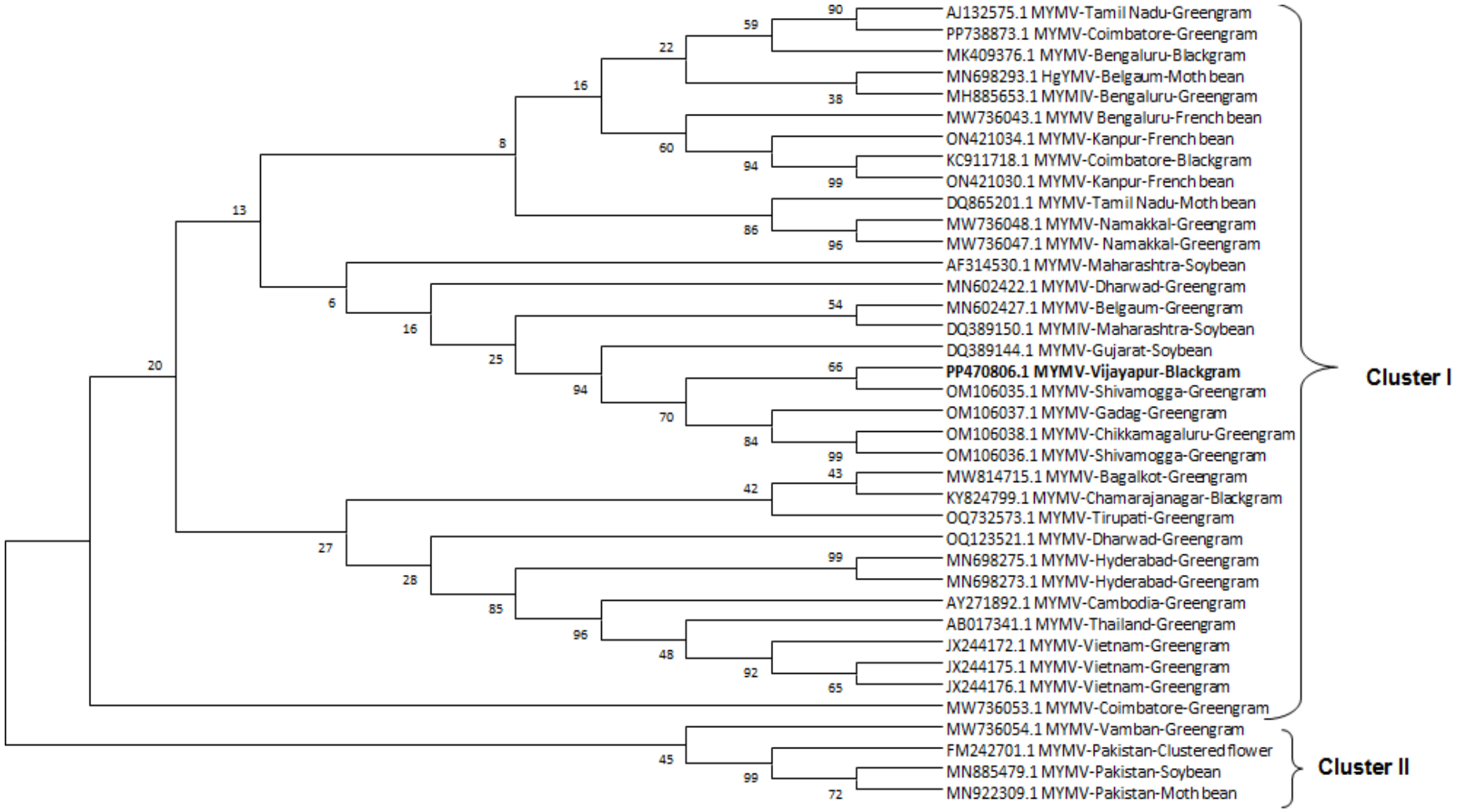


Fig. 3. Phylogenetic relationship of *CP* gene of Vijayapur strain of YMV infecting blackgram with other reference strains

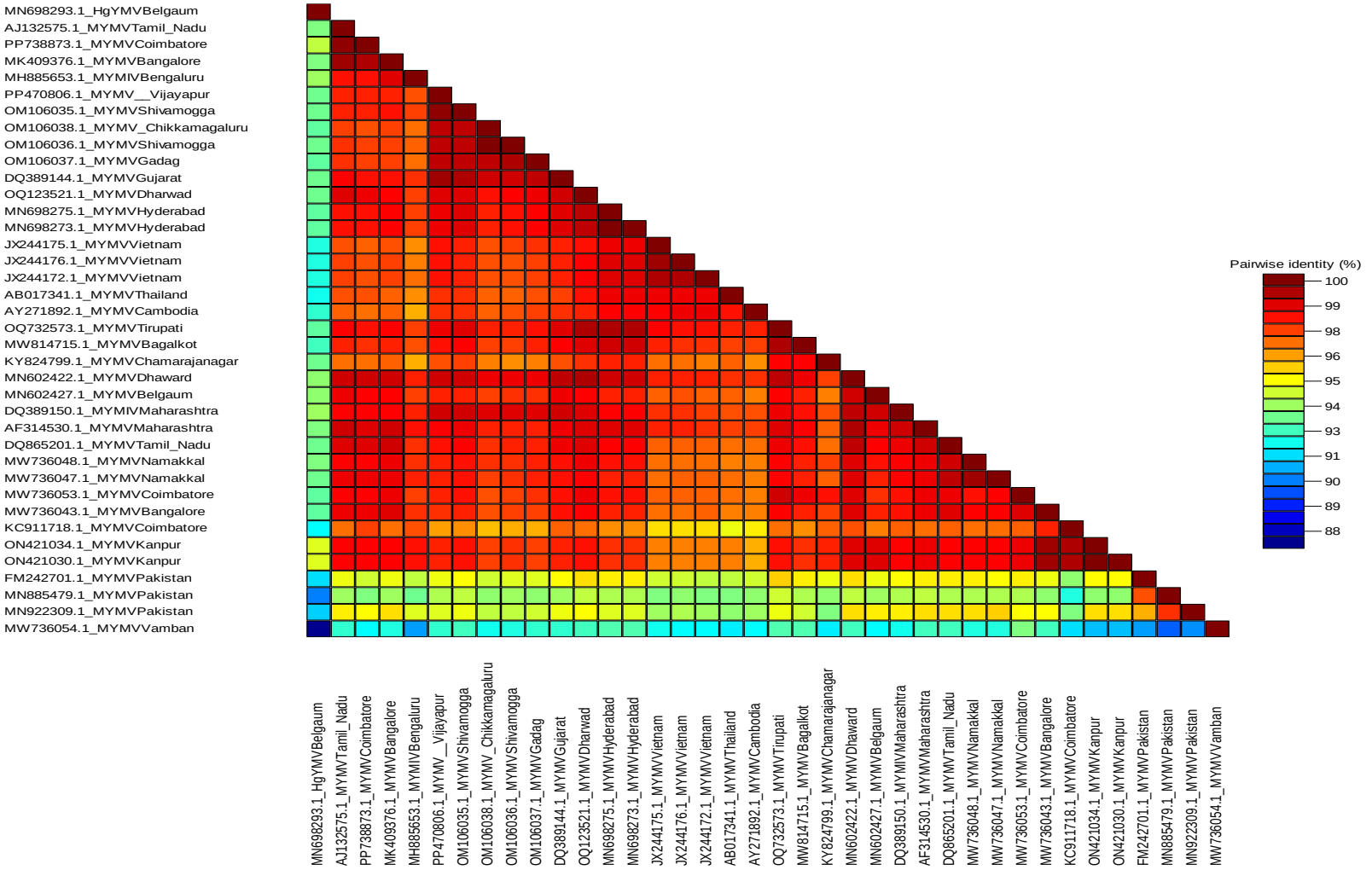


Fig. 4. Pairwise per cent identities of coat protein nucleotide sequences of MYMV and MYMIV isolates along with MYMV Vijayapur isolate

Table 1. Nucleotide and amino acid sequence identities of coat protein gene of YMV infecting blackgram with other *Geminiviruses*

Sl. No.	Accession number	Virus species	Geographical origin	Nucleotide sequence identity (%)	Amino acid sequence identity (%)	Host crop
1	OM106035.1	Mungbean yellow mosaic virus	MYMV-Shivamogga	99.80	84.59	*Vigna radiata*
2	OM106038.1	Mungbean yellow mosaic virus	MYMV- Chikkamagaluru	99.20	83.08	*Vigna radiata*
3	OM106037.1	Mungbean yellow mosaic virus	MYMV-Gadag	99.20	83.08	*Vigna radiata*
4	OM106036.1	Mungbean yellow mosaic virus	MYMV-Shivamogga	99.10	82.78	*Vigna radiata*
5	MN602422.1	Mungbean yellow mosaic virus	MYMV- Dharwad	98.90	82.87	*Vigna radiata*
6	OQ123521.1	Mungbean yellow mosaic virus	MYMV-Dharwad	98.80	82.87	*Vigna radiata*
7	MN698275.1	Mungbean yellow mosaic virus	MYMV-Hyderabad	98.60	81.87	*Vigna radiata*
8	MN698273.1	Mungbean yellow mosaic virus	MYMV-Hyderabad	98.60	81.87	*Vigna radiata*
9	OQ732573.1	Mungbean yellow mosaic virus	MYMV-Tirupati	98.50	81.96	*Vigna radiata*
10	AF314530.1	Mungbean yellow mosaic virus	MYMV-Maharashtra	98.30	80.66	*Glycine max*
11	JX244175.1	Mungbean yellow mosaic virus	MYMV-Vietnam	98.20	80.97	*Vigna radiata*
12	MW814715.1	Mungbean yellow mosaic virus	MYMV-Bagalkot	98.10	80.36	*Vigna radiata*
13	JX244176.1	Mungbean yellow mosaic virus	MYMV-Vietnam	98.10	81.27	*Vigna radiata*
14	JX244172.1	Mungbean yellow mosaic virus	MYMV-Vietnam	98.10	81.57	*Vigna radiata*
15	DQ865201.1	Mungbean yellow mosaic virus	MYMV-Tamil Nadu	98.20	81.27	*Vigna aconitifolia*
16	MN602427.1	Mungbean yellow mosaic virus	MYMV-Belgaum	98.00	81.27	*Vigna radiata*
17	MW736053.1	Mungbean yellow mosaic virus	MYMV-Coimbatore	98.00	80.73	*Vigna radiata*
18	AJ132575.1	Mungbean yellow mosaic virus	MYMV-Tamil Nadu	98.00	80.97	*Vigna radiata*
19	DQ389144.1	Mungbean yellow mosaic virus	MYMV-Gujarat	99.58	83.44	*Glycine max*
20	MK409376.1	Mungbean yellow mosaic virus	MYMV-Bangalore	97.90	80.66	*Vigna mungo*
21	MW736048.1	Mungbean yellow mosaic virus	MYMV-Namakkal	97.90	81.57	*Vigna radiata*
22	MW736047.1	Mungbean yellow mosaic virus	MYMV-Namakkal	98.00	81.27	*Vigna radiata*
23	MW736043.1	Mungbean yellow mosaic virus	MYMV- Bengaluru	97.80	80.36	*Phaseolus vulgaris*
24	AY271892.1	Mungbean yellow mosaic virus	MYMV-Cambodia	97.70	78.62	*Vigna radiata*
25	AB017341.1	Mungbean yellow mosaic virus	MYMV-Thailand	97.41	74.20	*Vigna radiata*
26	KC911718.1	Mungbean yellow mosaic virus	MYMV-Coimbatore	95.92	72.78	*Vigna mungo*
27	PP738873.1	Mungbean yellow mosaic virus	MYMV-Coimbatore	97.85	79.15	*Vigna radiata*
28	FM242701.1	Mungbean yellow mosaic virus	MYMV-Pakistan	94.91	75.23	*Rhynchosia capitata*
29	MN885479.1	Mungbean yellow mosaic virus	MYMV-Pakistan	94.11	74.32	*Glycine max*
30	MN922309.1	Mungbean yellow mosaic virus	MYMV-Pakistan	94.19	76.23	*Vigna aconitifolia*

Sl. No.	Accession number	Virus species	Geographical origin	Nucleotide sequence identity (%)	Amino acid sequence identity (%)	Host crop
31	MN698293.1	Horsegram yellow mosaic virus	HgYMV-Belgaum	93.11	74.02	*Vigna aconitifolia*
32	KY824799.1	Mungbean yellow mosaic virus	MYMV-Chamarajanagar	96.02	73.79	*Vigna mungo*
33	MW736054.1	Mungbean yellow mosaic virus	MYMV-Vamban	92.62	70.03	*Vigna radiata*
34	ON421034.1	Mungbean yellow mosaic virus	MYMV-Kanpur	97.93	81.78	*Phaseolus vulgaris*
35	ON421030.1	Mungbean yellow mosaic virus	MYMV-Kanpur	97.93	81.78	*Phaseolus vulgaris*
36	DQ389150.1	Mungbean yellow mosaic India virus	MYMIV-Maharashtra	99.05	81.90	*Glycine max*
37	MH885653.1	Mungbean yellow mosaic India virus	MYMIV-Bengaluru	97.26	77.06	*Vigna radiata*

Moth bean leaf samples exhibiting yellow mosaic symptoms tested positive with MYMV-specific primer pairs (MYMV-CP-F/MYMV-CP-R), producing amplicons of approximately 1000 bp. These 1000 bp PCR products were directly sequenced and assembled. The phylogenetic tree, based on the full-length coat protein gene sequence of MBYMV and other geminivirus sequences from the NCBI GenBank, revealed three major clusters: MYMV, HgYMV, and MYMIV. The current MBYMV isolate formed a unique cluster within the MYMV group (Appu and Prema, 2024).

4. CONCLUSION

The results of the current study, based on similarities in coat protein gene sequence at both the nucleotide and amino acid levels, as well as phylogenetic analysis, confirmed the prevalence of MYMV strain rather than MYMIV, HgYMV and DoYMV in blackgram in northern parts of Karnataka.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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