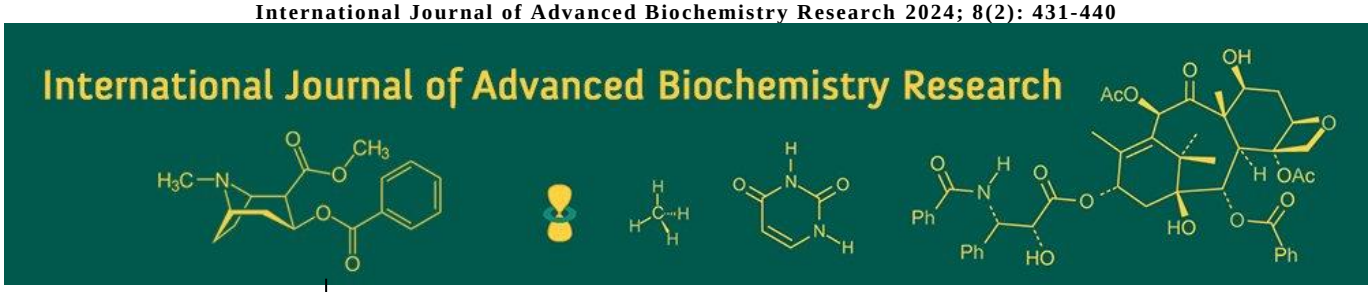

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Bharsakale RD
PG Students, Department of Plant Biotechnology, Vilasrao Deshmukh College of Agricultural Biotechnology, VNMKV, Parbhani, Maharashtra, India

Wankhade DJ
Senior Research Assistant, Department of Plant Biotechnology, Vilasrao Deshmukh College of Agricultural Biotechnology, VNMKV, Parbhani, Maharashtra, India

Jadhav PK
PG Students, Department of Plant Biotechnology, Vilasrao Deshmukh College of Agricultural Biotechnology, VNMKV, Parbhani, Maharashtra, India

Gholave KK
Project Assistant, Department of Plant Biotechnology, Vilasrao Deshmukh College of Agricultural Biotechnology, VNMKV, Parbhani, Maharashtra, India

Deshmukh AS
Senior Research Assistant, Department of Plant Biotechnology, Vilasrao Deshmukh College of Agricultural Biotechnology, VNMKV, Parbhani, Maharashtra, India

Chavan RL
Assistant Professor, Department of Plant Biotechnology, Vilasrao Deshmukh College of Agricultural Biotechnology, VNMKV, Parbhani, Maharashtra, India

VR Hinge
Assistant Professor, Department of Plant Biotechnology, Vilasrao Deshmukh College of Agricultural Biotechnology, VNMKV, Parbhani, Maharashtra, India

Corresponding Author:
Bharsakale RD
PG Students, Department of Plant Biotechnology, Vilasrao Deshmukh College of Agricultural Biotechnology, VNMKV, Parbhani, Maharashtra, India

Coat protein-based molecular characterization of the mungbean yellow mosaic virus (MYMV) infected cowpea (*V. unguiculata* (L.) Walp.) in Maharashtra

Bharsakale RD, Wankhade DJ, Jadhav PK, Gholave KK, Deshmukh AS, Chavan RL and VR Hinge

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Abstract
The Begomoviruses genus' Mungbean Yellow Mosaic Virus (MYMV) causes considerable annual yield losses in crops like soybean, mungbean, cowpea, and black gram, amounting to about $300 million. This study provides proof that symptomatic samples taken from different parts of Maharashtra include MYMV infection. The gene-specific amplification of the CP gene (719 bp and 387 bp) served as the basis for the confirmation. Its identity as MYMV was confirmed by the astonishing 96.52% similarity between the MYMV coat protein (AV1) gene sequence (Accession No. JQ004982.1) and the CP gene sequence found in cowpea samples from the Pune region. A BLASTp investigation of the coat protein of the Mungbean Yellow Mosaic Virus in Vigna (Accession No. AAW50913.1) indicated a 93.41% similarity. With an identity of 82.99%, the MYMV strain that infected cowpea in the Pune area showed the most genetic divergence from Indian strains (MYMIV and HYMV) and a close genetic relationship (96.52%) with MYMV. The 70 different CP gene sequences of MYMV that were analyzed phylogenetically clustered into three groups based on nucleotide and protein sequences. Four sequences (45%), including the MYMV Pune isolate, showed the highest pairwise identity (96-98%) out of the 70 sequences analyzed, whereas about 25% of the total sequences showed lower pairwise identities (84-87%). Significant differences in restriction sites were found among the selected CP gene sequences under examination after an *in silico* restriction analysis was conducted by using the CisSERS tool. The MYMV strain's broad distribution in Maharashtra, its ability to infect other legume crops, and its ongoing evolution with the Mungbean Yellow Mosaic India Virus (MYMIV) strain are all highlighted by this study.

Keywords: Mungbean yellow mosaic virus (MYMV), gene-specific amplification, coat protein (CP) gene

Introduction
Production of mungbeans suffers greatly by Yellow Mosaic Disease (YMD), which has a ripple effect throughout the sector. The symptoms of this illness include slowed growth, little yellow spots or specks on certain leaves, and, in extreme cases, a total yellowing and necrosis of all foliage. YMD causes delayed maturation, reduced flower and pod production, and frequently results in mottling in growing pods, all of which lead to a lower seed output and lower-quality seed. According to John *et al*. (2008) [4], this disease wreaks damage on legume crops in India, especially mungbean (*Vigna radiata* (L.) Wilczek), urdbean (*Vigna mungo* (L.) Hepper), soybean (*Glycine max* (L.) Merr.), and cowpea (*Vigna unguiculata* (L.) Walp. The effects of YMD are severe, resulting in late crop maturity and the production of few flowers and pods, frequently characterized by mottling and poor quality. This disease can occasionally result in yield losses of up to 100% and can result in the death of plants that are affected during the early vegetative stage. Cowpea, a vital legume, is in a particularly bad predicament since it has been documented to be infected by over 140 viruses, 20 of which, like the mungbean yellow mosaic India virus, are known to spread widely and cause significant output losses. When considering soybeans, mungbeans, cowpeas, and black gram as a whole, the economic cost of viral infections on legume crops in India is enormous, totaling to almost $300 million per year (Kumar *et al*., 2017) [8].

The Mungbean Yellow Mosaic Virus (MYMV) can infect mungbean crops at all growth stages and is transmitted by whiteflies in a persistent circulative manner. The four distinct Begomovirus species known as Mungbean Yellow Mosaic Virus (MYMV), and Mungbean Yellow Mosaic India Virus (MYMIV) jointly cause Yellow Mosaic Disease (YMD). In mungbean, soybean, and urdbean, these viruses have been known to cause YMD. Both MYMV and MYMIV are members of the 'Legumoviruses' subgroup of bipartite begomoviruses, which are evolutionarily distinct from one another (Ilyas *et al*., 2009). Begomoviruses have a bipartite genome and are made up of two closed, tiny, circular single-strand DNA viruses (Kumar *et al*., 2017) [8]. DNA-A and DNA-B, the two genetic components, are packed independently into viral particles. The genome is about 2.7 kb in size and has a common region (CR) that is about 220 bp long. According to Akram *et al*. (2015) [9], more than 10 different species of legume are reported to be infected with yellow mosaic disease from these eight distinct bipartite begomovirus species.

The majority of plant viruses lack envelopes. Critical proteins involved in insect transmission, DNA replication, and replication start are encoded by DNA-A in the case of the Mungbean Yellow Mosaic Virus (MYMV). The coat protein (AV1) is one of these proteins that is necessary for encapsidation. DNA-A also codes for the proteins replication enhancer protein (Ren), DNA helicase, rolling circle replication-initiation protein, and replication-associated protein (Rep). Within the replication complex, the coat protein (AV1) is essential for making contact with host cells and transferring the viral genome, which suppresses the plant's innate immune response. The coat protein (CP) and viral genome exit the infected plant at the end of the infection phase. Contrarily, the two important genes nuclear shuttle protein (NSP) and movement protein (MP) are encoded by DNA-B. These proteins have a major impact on symptom onset and host range and are essential for viral cell-to-cell and long-distance movement (Choudhury *et al*., 2006) [18]. It's crucial to remember that cowpea lacks a known natural source of MYMIV resistance, making the process of resistance breeding difficult (Kumar *et al*., 2017) [8].

Materials and Methods

Sample Collection and Genomic DNA Extraction

In 2020, leaf samples were systematically collected from a variety of regions in Maharashtra, including Pune, Latur, Parbhani, and Amaravati, at various times and field locations, showing symptoms typical of MYMV infection, such as yellow specks on young leaves, emergence of trifoliate leaves from the apex, and stunted plant growth leading to complete yellowing. Using an adapted version of Shahakar and Peter's (2015) [22] CTAB (Cetyltrimethylammonium Bromide) technique, genomic DNA was extracted from sixteen different MYMV-infected plant samples. The isolated DNA was then subjected to spectrometric evaluation and agarose gel electrophoresis for analysis.

Designing CP gene-specific primers and PCR amplification

Two primer sets have been designed using the primer 3 program (https://primer3.ut.ee/) to target conserved genomic regions in order to identify MYMV infection and amplify its coat protein gene. Sequences for the MYMV coat protein gene were obtained from the NCBI nucleotide database. The BioEdit sequence alignment program (Version 7.0) was then used to align these sequences. Primers were carefully created based on conserved regions within these sequences after the alignment procedure. Using developed gene-specific primers, DNA samples that had previously been extracted were used as templates for the amplification of the coat protein gene AV1. To guarantee reliable and precise amplification, the PCR reaction's parameters and its components were carefully adjusted.

Phylogenetic Analysis of the Sequence in Comparison to Other Begomoviruses

A range of begomovirus species, strains, and variations representing different crops and geographical areas were chosen for the phylogenetic analysis. The neighbor-joining method was used to create the phylogenetic tree, and 1000 bootstrap replications were carried out using the online program http://www.phylogeny.fr/simple phylogeny.cgi.

Sequence Analysis and Structural Model Validation

Pairwise percent identities were calculated using the SDTv1.2 software to assess sequence similarities. Additionally, analysis via Expasy's ProtParam server and the SOPMA server. To evaluate the quality of the structural model, homology modeling was conducted, with a specific focus on the amino acid region. This analysis included the examination of a Ramachandran plot using the Structural Analysis and Verification Server (SAVS).

Results

Survey for the identification of MYMV disease in different regions of Maharashtra

Disease indications were observed in crops at different development stages, from early stages to develop plants, showing characteristic indications such as leaf curling, twisting, yellowing, crinkling, and hindering (see Figure 1 and 2 for visual representation). A total of 16 tests collected from diverse territories were examined, counting tests from mungbeans, soybeans, and cowpea, all of which shown symptoms of yellow mosaic or brilliant mosaic. Outstandingly, cowpea tests from the Amravati and Latur local showed yellowing with slight leaf twisting consistently.

Amplification of Viral coat protein Region

Using specially created primer pairs, RB-MYMVF1 + RB-MYMVR1 and RB-MYMVF2 + RB-MYMVR2, the AV-1 gene within the DNA-A molecule was successfully amplified by PCR. Using a reference ladder of 100 base pairs, the amplification of the coat protein (CP) gene produced a 719-bp fragment with primer pair 1 and a 387-bp fragment with primer pair 2. (see Figure 4 and 5).

Nine of the sixteen samples that were analyzed showed evidence of successful amplification with primers specific to the CP gene. Specifically, using the indicated gene-specific primer pair (Figure 4 and 5), the CP gene was amplified in infected samples of Cowpea (samples 1 and 2), Mungbean (samples 3, 4, and 5), and Soybean (samples 6, 7, 8, and 9).

Sequence analysis using the BLAST search tool

Sequence analysis was conducted employing the BLAST search tool, and the results of the sequence similarity using BLASTn are presented in Table 2. The CP gene isolated

from infected mungbean samples exhibited a remarkable 96.52% similarity to the MYMV isolate coat protein (AV) gene, specifically the Navsari sequence (Accession No JQ004982.1). This result unequivocally confirms the identity of MYMV, establishing that the yellow mosaic virus infecting mungbean in the Latur region indeed belongs to the MYMV strain.

BLASTp analysis was performed using the translated sequence of the same CP gene, and the results of this analysis indicated that the yellow mosaic virus infecting mungbean in the Latur region displayed a substantial 93.41% similarity to the coat protein [MYMV-Vigna] with Accession No. AAW50913.1 (see Table 2 and Figures 1 and 2). This finding further reinforces the identity of the mungbean yellow mosaic virus infecting mungbean in the Latur region. The percentage of similarity among the MYMV isolates, based on CP gene sequence, ranged from 96.52% to 83.33% (see Table 2), while the similarity percentages among the MYMV isolates, based on CP gene protein sequences, ranged from 93.16% to 49.74 (see Table 3).

Notably, the MYMV strain infecting soybean exhibited a high degree of similarity, with a nucleotide sequence identity of 95.47%, and a protein sequence identity when compared to the MYMV VDCOAB Latur isolate.

Phylogenetic Analysis of MYMV Coat Protein Gene Isolates

A total of 70 distinct viruses, including our own MYMV VDCOAB Latur isolates, were aligned with MYMV sequences isolated from various host crops. Comparisons of the CP gene sequence of MYMV isolate VDCOAB-Latur with other bipartite begomoviruses revealed the closest homology to MYMV coat protein isolate (AV1) gene [Accession no. JQ004982.1] at 96.52% identity, followed by MYMV-NAV coat protein gene [Accession no. DQ389144.1] at 96.17% identity. Notably, MYMV isolate VDCOAB-Latur exhibited distinct differences from Indian strains (MYMIV and HYMV) and MYMIV isolate Mo1-Belgaum clone [Accession no. MN698287.1] with 87.97% identity. These findings highlight that MYMV CP isolates VDCOAB Latur sequence shared a sequence similarity ranging from 96.52% to 83.33% with other begomoviruses (see Table 2).

A phylogenetic tree was constructed based on the genetic distance among the 70 isolates of begomoviruses, which clustered all strains into four groups (see Figure 2). The MYMV VDCOAB Latur isolate was placed in the first group and exhibited high similarity to Accession No. JQ004982.1 (96.52%). The other strains of begomoviruses were clustered into groups II, III, and IV. In group II, MYMV VDCOAB isolate was notably distinct from Cajanus cajan yellow mosaic virus isolates Accession No. MF35965.1, exhibiting 87.73% similarity. Group IV included seven isolates of MYMIV (see Figure 2).

Additionally, a phylogenetic tree was constructed based on the translated protein sequences of the 70 distinct MYMV strains, revealing the formation of four distinct clades (see Figure 4). The MYMV VDCOAB Latur isolate was positioned in the first clade and exhibited high similarity to MYMV (AAW50913.1) at the protein sequence level. Clade II included the HYMV strain, which showed slight distinctions from the Latur isolate. The third cluster encompassed all distinct groups of YMV, while in the fourth clade, the MYMIV strain was grouped with the cowpea golden mosaic virus (Accession No. ANS81423.1).

Percent Identity Study for Nucleotide Sequence among MYMV CP Gene Isolates

Pairwise percent identities were determined using the SDTv1.2 software, and the results are illustrated in Figure 8, providing a comprehensive estimate of sequence identities. Among the 70 sequences analyzed, approximately 51% of them, including the MYMV VDCOAB Latur isolate, exhibited the highest pairwise identity levels, ranging from 96% to 99%. Furthermore, around 22% of the sequences demonstrated identity levels falling within the range of 91% to 95%. A similar proportion, approximately 22% of the total sequences, displayed the lowest pairwise identities, ranging from 82% to 86%. Notably, the range of pairwise identity from 82% to 99% was observed in the smallest proportion of sequences, constituting less than 1%.

Secondary Structure Prediction

In our study, we employed SOPMA to calculate the secondary structural features of the protein sequences. The results unveiled that the predominant secondary structure element across all sequences was the Random coil, followed by alpha helix, extended strand, and beta turns. Notably, this finding aligns with the observations made by Prajapati and Bhagat in 2012, where alpha-helix also dominated among secondary structure elements, followed by extended strand, random coil, and beta turns in their sequences.

Homology Modeling of MYMV Coat Protein Sequence

Upon subjecting the MYMV coat protein sequence to BLASTP analysis against the PDB database, we found a substantial 82.79% similarity with Aqueratum yellow vein virus (6f2s.1 J). Subsequently, we utilized the Swiss model web server for structure prediction and homology modeling of the MYMV coat protein sequence (see Figure 6). While the QMEAN score was determined to be -2.52 with a positive sign (Table 2), it's important to note that the stability of the structure relies significantly on the Ramachandran plot of residues. A similar approach was undertaken by Patel and Kalaria in 2018 [11] for homology modeling of the Papaya Leaf curl virus coat protein, utilizing Aqueratum yellow vein virus (6f2s.1 J) as a reference, which displayed a similarity of over 30 percent.

Validation of Homology Modeling

After constructing the model, it becomes crucial to validate it to ensure the stability and reliability of the model's stereochemistry, aligning it with typical values observed in crystal structures. To assess the quality of the modelled structure, we employed the PROCHECK tool to calculate the Ramachandran plot, an established method for analyzing residue regions.

The Ramachandran plot depicts the phi-psi torsion angles for all residues within the structure. The darkest areas within the plot represent the 'core' regions, indicating the most favorable combinations of phi-psi values. The percentage of residues residing within these 'core' regions serves as an excellent indicator of stereochemical quality (Patel and Kalaria, 2018) [11]. In the case of our predicted MYMV coat protein model, the Ramachandran plot revealed that 89.1% of amino acids resided within the most favorable region (>90%), accounting for 172 amino acids (see Fig 7).

Table 1: List of samples collected for studying MYMV infection in mungbean and other crop spp. in Maharashtra region

Sr. No.	Region/Locality		Host	Symptoms	Season
1.	Pune	Shikhrapur	*Vigna unguiculata*	Yellow mosaic	Rabi
2.		Shikhrapur	*Glycine max*	Yellow mosaic	Rabi
3.	Amaravati	Paratwada	*Vigna unguiculata*	Golden Mosaic	Rabi
4.		Rajna Purna	*Vigna unguiculata*	Yellow Flecks	Rabi
5.		Dhamangaon Gadi	*Vigna unguiculata*	Golden Mosaic	Kharif
6.		Anjangaon Surji	*Vigna unguiculata*	Yellow mosaic	Kharif
7.		Rajna Purna	*Glycine max*	Yellow Flecks	Kharif
8.	Latur	College farm	*Vigna radiata L.*	Yellow mosaic	Kharif
9.		Ring road	*Vigna radiata L.*	Golden Mosaic	Kharif
10.		Kalgaon Wadi	*Vigna radiata* L.	Yellow Flecks	Kharif
11.		Renapur Naka	*Glycine max*	Leaf curl with yellow flecks	Kharif
12.		College farm	*Glycine max*	Leaf curl with yellow flecks	Kharif
13.	Parbhani	University field	*Glycine max*	Leaf curl with yellow flecks	Kharif
14.		University field	*Vigna radiata* L.	Leaf Curl with yellow mosaic	Kharif
15.		Tadkadas	*Vigna radiata* L.	Golden mosaic	Kharif
16.	Dharwad	Hubli	*Vigna radiata* L.	Golden mosaic	Kharif

Symptoms observed in field samples: VF-Vein flecking; Mo-Mosaic pattern; CR- Citrus ringspot; YV-Yellow veins; BM-Blochy mottle

Table 2: List of primers used in PCR reaction for amplification of coat protein

Mol. Marker	Pair of Primers	Sequence 5` to 3`	Length	GC Content %	Tm	Amplicon size (bp)
DNA-A (CP)	RB-MYMV-F1	ATGCCAAAGCGGAATTACGATAC	23	43.48	53	719
	RB-MYMV-R1	GGATTTGATGCATGAGTACAT	21	38.10	49	
	RB-MYMV-F2	TTGTGAAGGACCGTGTAAGG	20	50.00	52	387
	RB-MYMV-R2	GCTCCTTGCTCGCATATTGGC	21	57.14	56	

Tm: Melting Temperature; GC: G or C content; nt: Nucleotide; bp: Base pair.

Table 3: Description of MYMV begomoviruses used for phylogenetic analysis with MYMV VDCOAB, Latur isolate

Sr. No.	Description	Scientific Name	Per. Ident.	Acc. Len	Accession No.
1.	Mungbean yellow mosaic virus coat protein (AV1) gene, Navsari	MYMV	96.52	1285	JQ004982.1
2.	Mungbean yellow mosaic virus isolate Green AV1-like gene, Chamarajanagar	MYMV	95.12	898	KY824800.1
3.	Mungbean yellow mosaic virus isolate Mu1-Dharward clone	MYMV	95.47	2728	MN602422.1
4.	Mungbean yellow mosaic virus isolate Mu1-Belgaum clone	MYMV	95.12	2729	MN602427.1
5.	Mungbean yellow mosaic virus isolate Mo2-Belgaum clone	MYMV	95.47	2729	MN698295.1
6.	Mungbean yellow mosaic virus isolate Chikkaballapura 2 CP gene	MYMV	95.47	989	MK409378.1
7.	Soybean yellow mosaic virus partial av1 gene for coat protein	MYMV-Madurai	95.47	571	AJ315963.1
8.	Mungbean yellow mosaic virus-Thailand DNA A	MYMV-Thailand	95.12	2726	AB017341.1
9.	Mungbean yellow mosaic virus clone	MYMV	94.77	2724	KC911718.1
10.	MYMV DNA-A	MYMV	94.08	2729	AY271892.1
11.	Mungbean yellow mosaic virus AV1-like gene	MYMV	92.23	870	KY824803.1
12.	Mungbean yellow mosaic virus clone SR-77 pre-coat protein (AV2), coat protein (AV1)	MYMV	91.30	2738	MN885479.1
13.	Mungbean yellow mosaic India virus isolate Mo1-Belgaum clone	MYMIV	87.97	2746	MN698289.1
14.	Horsegram yellow mosaic virus isolate Tumakuru 1 coat protein gene	HYMV	87.59	983	MK391938.1
15.	Mungbean yellow mosaic India virus clone SR-13coat protein (AV1)	MYMIV	84.81	2746	MN885468.1
16.	Mungbean yellow mosaic India virus clone SR-13	MYMIV	84.81	2746	MN885468.1
17.	Indian mungbean yellow mosaic virus	IMYMV	84.07	774	AF361434.1
18.	Mungbean yellow mosaic India virus clone pBdAk05	MYMIV	83.76	2747	KP779635.1
19.	Mungbean yellow mosaic India virus isolate MYMIV-CKTD	MYMIV	82.99	2741	MF683072.1

Table 4: Description of translated protein MYMV begomoviruses used for phylogenetic analysis with MYMV VDCOAB, Latur isolate

Sr. No.	Description	Scientific Name	Per. Ident.	Acc. Len	Accession No.
1.	coat protein [Mungbean yellow mosaic virus-Vigna]	MYMV-Vigna	93.41	139	AAW50913.1
2.	coat protein [MYMV]	MYMV	74.78	257	QBQ95430.1
3.	coat protein [HYMV]	HYMV	73.04	257	QBQ18380.1
4.	coat protein [Mungbean yellow mosaic India virus]	MYMIV	68.70	169	ASV64937.1
5.	AV1 [MYMV]	MYMV	57.59	257	AGS77255.1
6.	Alt Name: Full=Coat protein; Short=CP [MYMV-Vigna]	MYMV-Vigna	57.07	257	Q9YPS5.1
7.	AV2 [MYMV]	MYMV	57.07	257	AKM97294.1
8.	coat protein [MYMV]	MYMV	57.07	257	QUP79384.1
9.	coat protein [MYMV]	MYMV	56.54	257	ABI21815.1
10.	coat protein [MYMV]	MYMV	56.54	257	QHD57743.1
11.	coat protein [MYMV-Soybean [Madurai]]	MYMV Soybean [Madurai]	56.54	257	CAD18840.1
12.	coat protein [MYMV - Soybean [Pakistan]]	MYMV Soybean [Pakistan]	54.45	257	AAP34718.1
13.	coat protein [Horsegram yellow mosaic virus]	HYMV	51.83	257	QHD57735.1
14.	coat protein [MYMIV]	MYMIV	50.26	257	AGF33892.1
15.	coat protein [MYMV [Urdbean: New Delhi:2011]]	MYMV [Urdbean: New Delhi]	49.74	257	AFI25066.1
16.	coat protein [MYMIV - [Cowpea Pakistan]]	MYMIV [Cowpea Pakistan]	49.74	257	AAP34712.1
17.	coat protein AV1 [CGMV]	CGMV	49.74	257	AAM33132.1
18.	coat protein [HYMV]	HYMV	49.21	257	QHD57841.1

Fig 1: Symptomatic leaf samples of similar to Mung bean Yellow Mosaic Virus (MYMV) disease on Soybean (*Glycine max*) (a and b) and Cowpea (Vigna unguiculata) (c) in Latur region

Fig 2: Symptomatic leaf samples of Mung bean Yellow Mosaic Virus (MYMV) like disease incidence on Soybean (*Glycine max*) in Latur (d and e) and Parbhani (f) region

Fig 3: MYMV disease infected leaf samples of mungbean at first trifoliate stage (a), flowering stage (b), and harvesting stage (c) growth stages of MYMV disease on Mungbean (*Vigna Radiata* L. Wilczek)

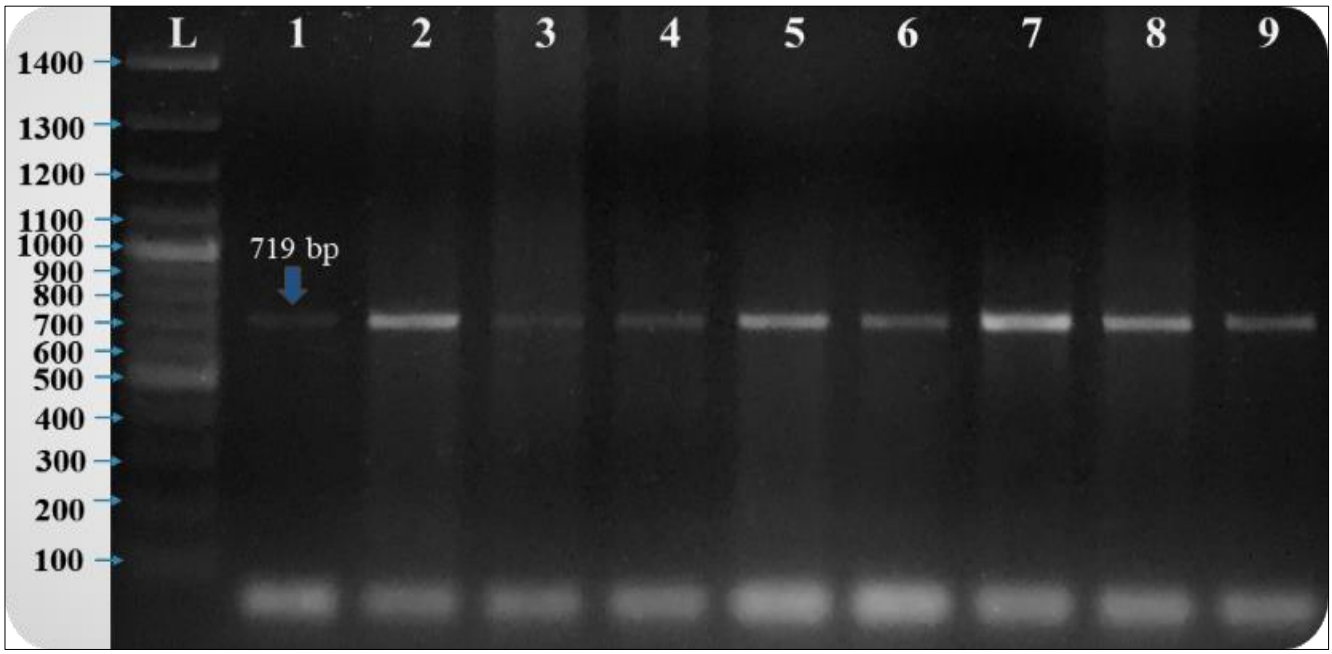

Fig 4: PCR amplification of MYMV CP gene with primer pair 1 (719 bp) in infected samples of cowpea (1 to 2), Mungbean (3, 4 and 5) and soybean (6 to 9); L: 100 bp DNA ladder

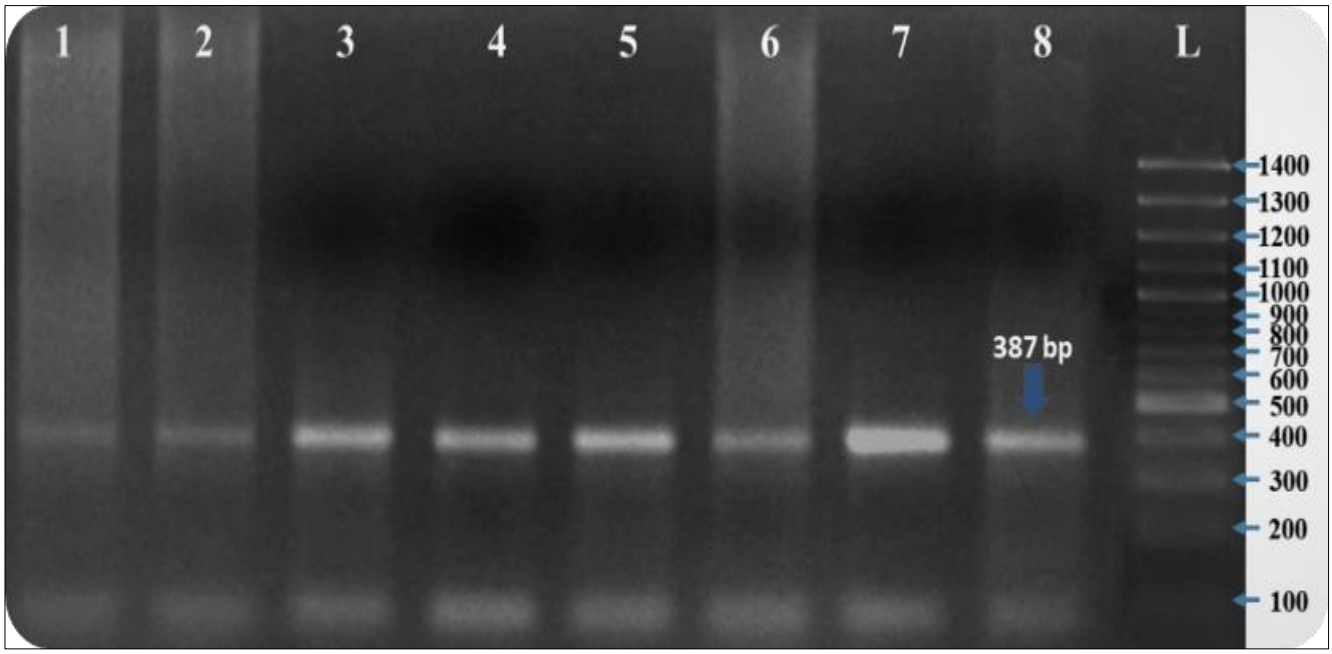

Fig 5: PCR amplification of MYMV CP gene with primer pair 2 (387 bp) in infected samples of cowpea (1 to 2), Mungeban (3, 4 and 5) and soybean (6 to 8); L: 100 bp DNA ladder

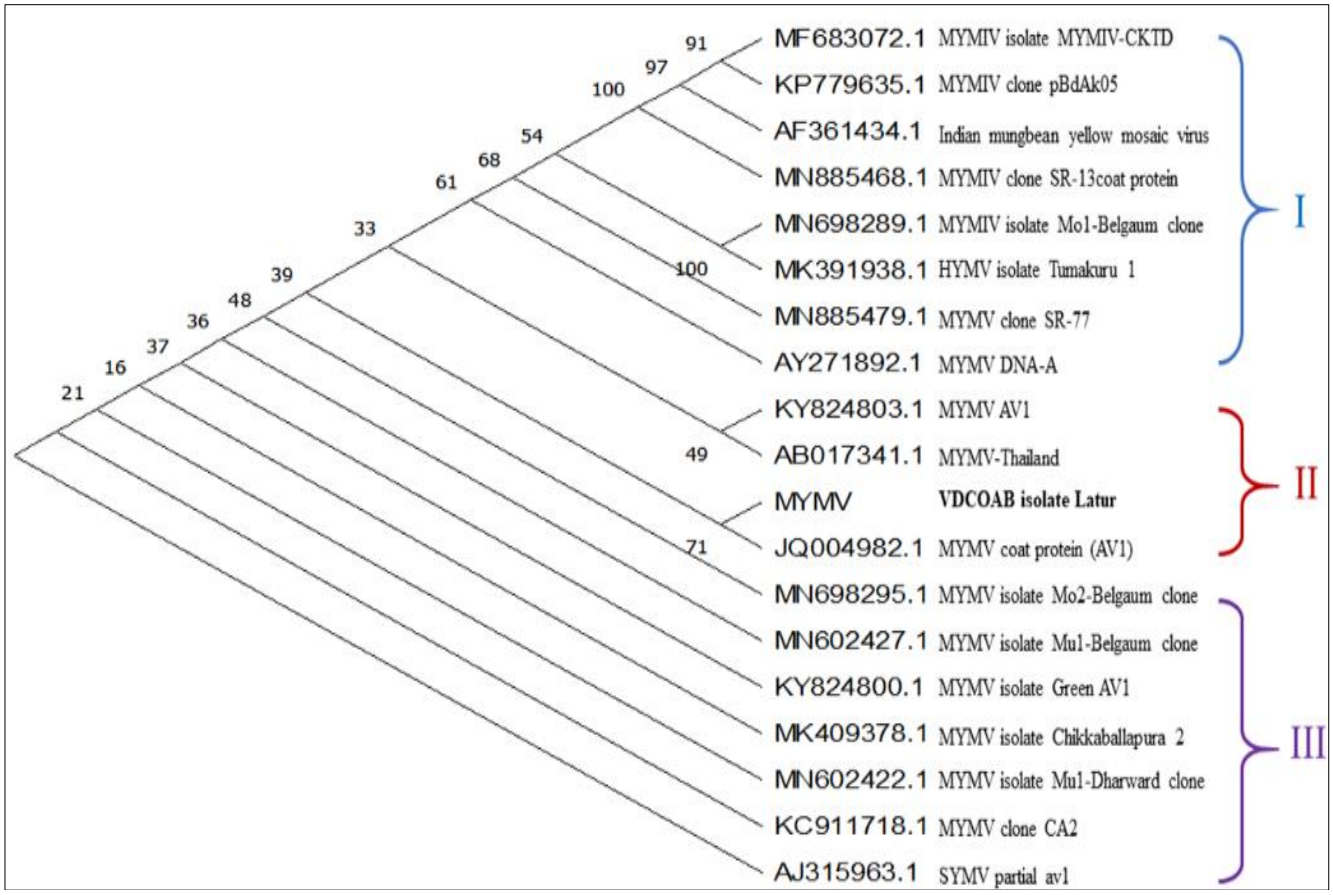

Fig 6: Phylogenetic tree constructed based on nucleotide sequence of MYMV's CP gene to other begomoviruses using PHYLIP. The numbers at the nodes represent % bootstrap confidence scores (1000 replications)

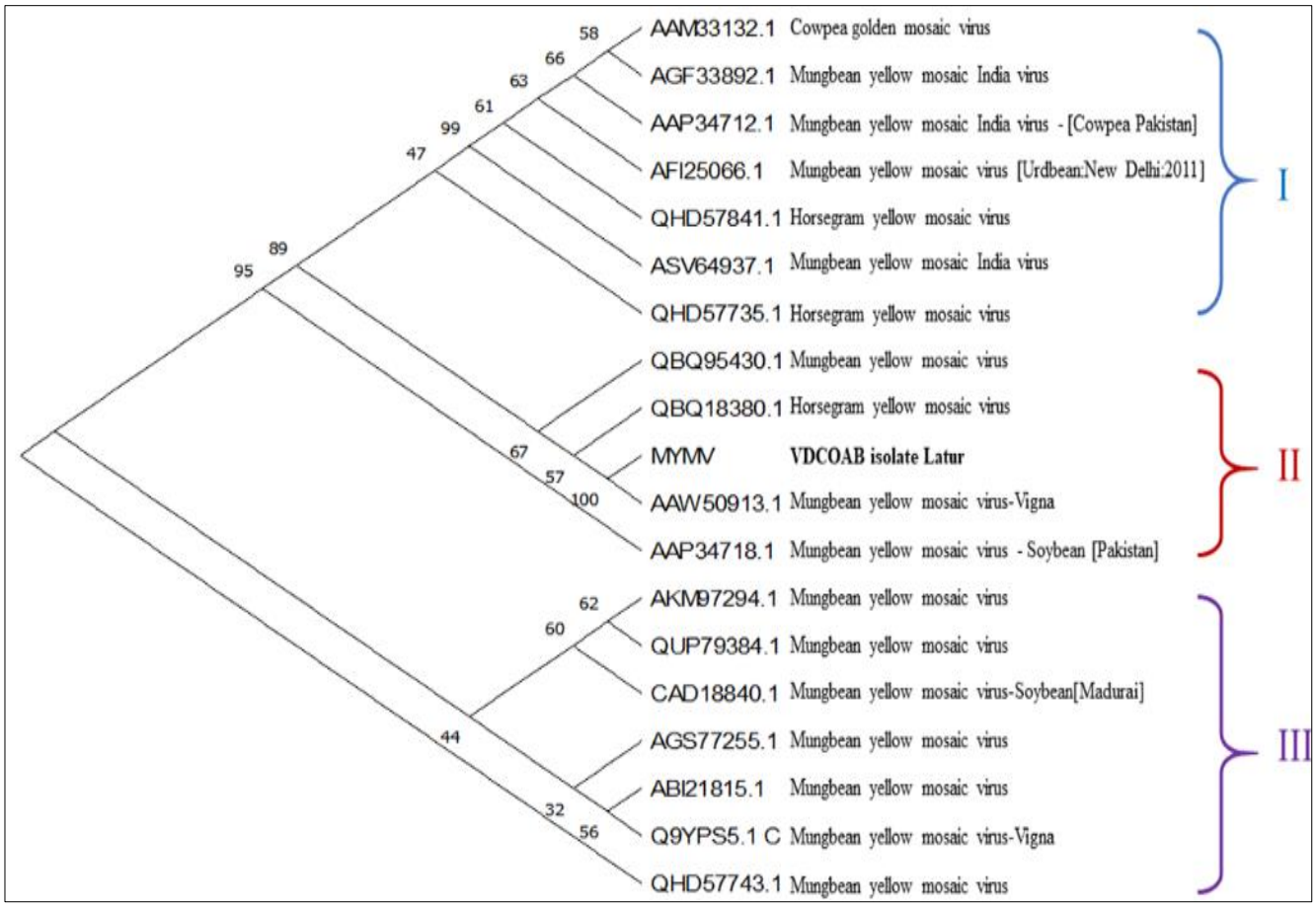

Fig 7: Phylogenetic tree constructed based on protein sequence of MYMV's CP gene to other begomoviruses using PHYLIP package. The numbers at the nodes represent % bootstrap confidence scores (1000 replications)

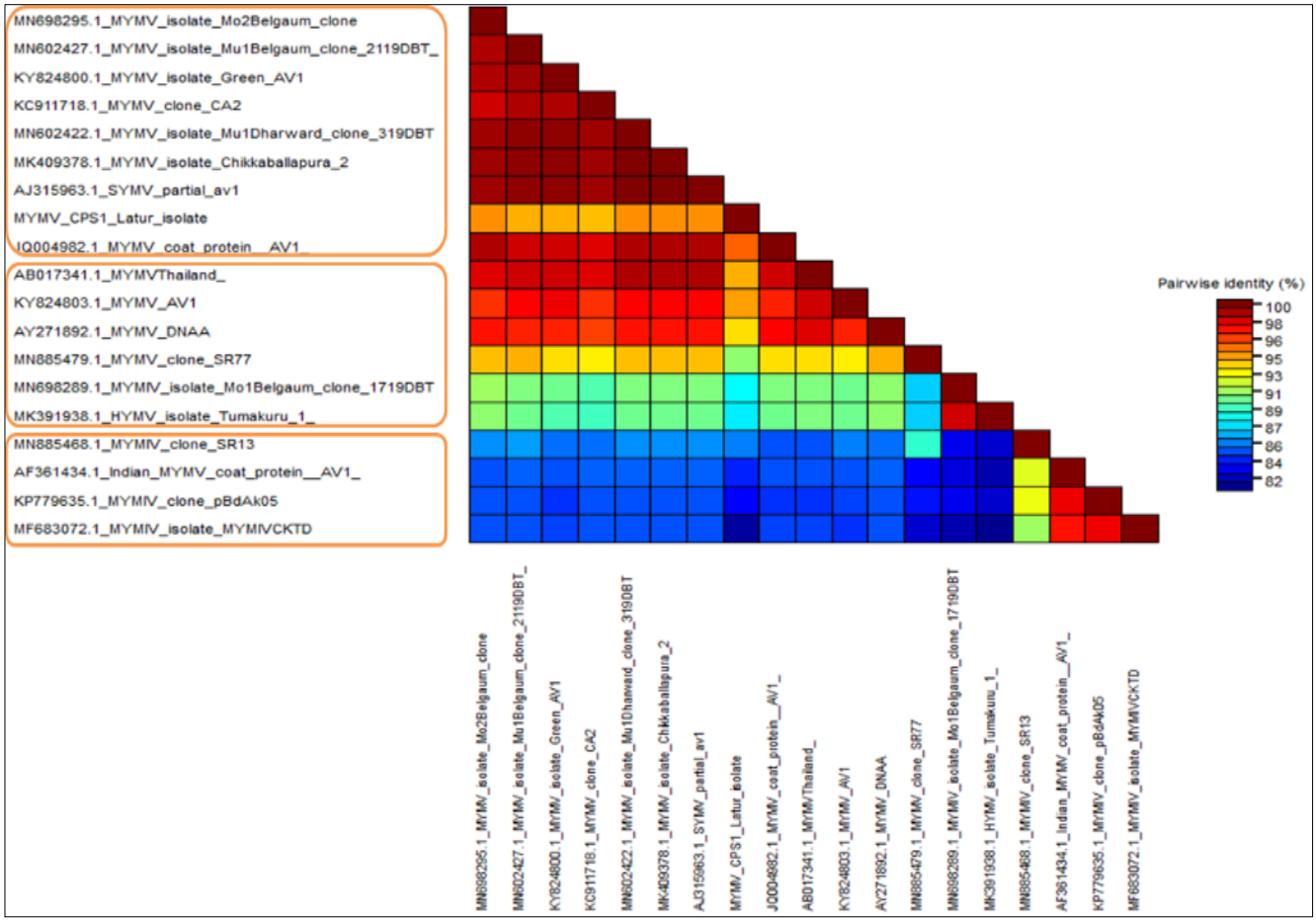

Fig 8: Showing pairwise percent identities of CP nucleotide sequences among MYMV isolates by using SDT (Version 1.2)

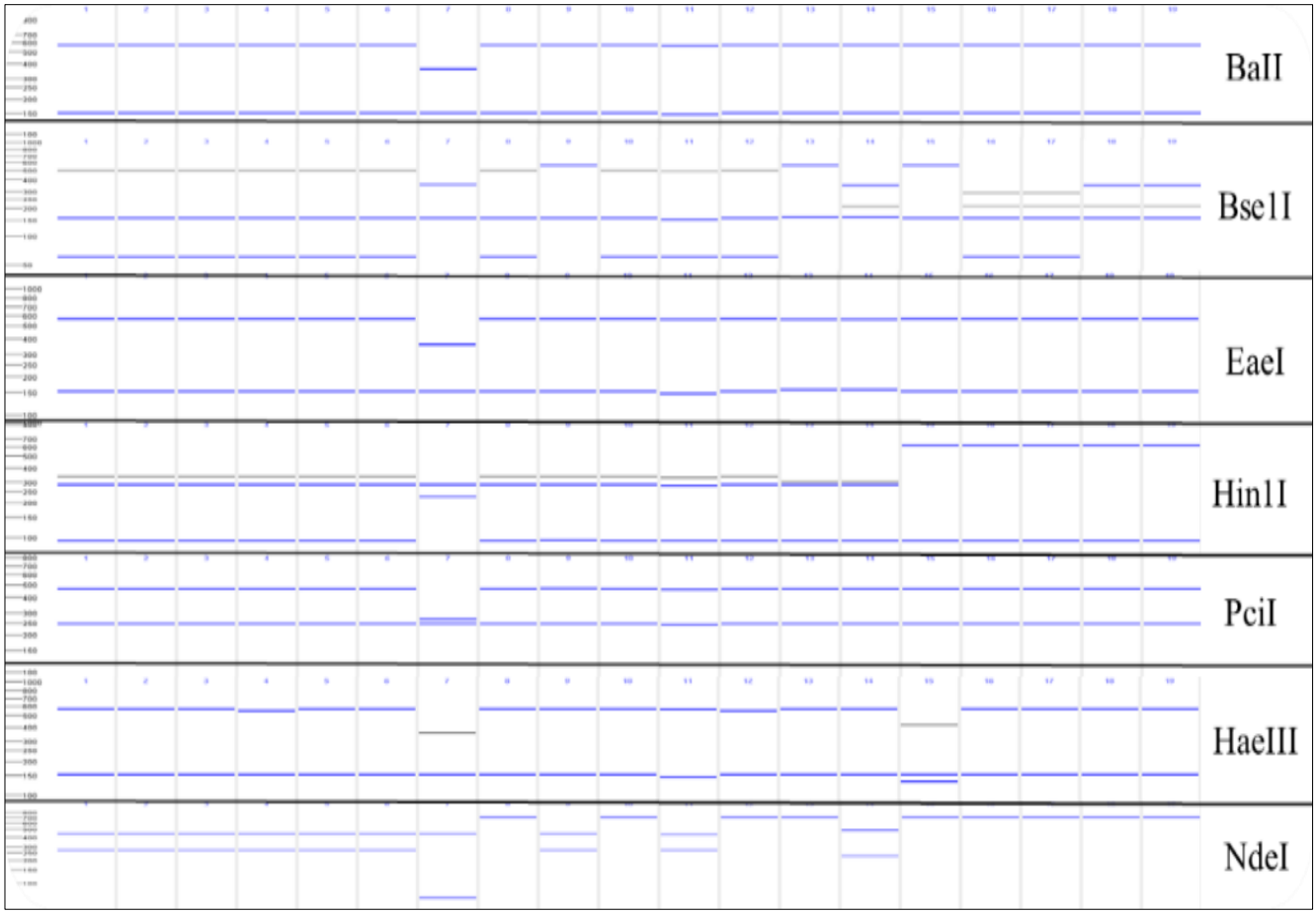

Fig 9: *In-Silico* anlyzed predicted gel images of aligned primer-1 with different Restriction enzymes

Discussion

The findings of this study shed light on the significant impact of Mungbean Yellow Mosaic Virus (MYMV) on legume crops, particularly mungbean (*V. radiata* (L.) Wilczek), urdbean (*V. mungo* (L.) Hepper), soybean (*Glycine max* (L.) Merr.), and cowpea (*V. unguiculata* (L.) Walp.) in the Maharashtra region. Yellow mosaic disease (YMD) caused by MYMV poses a major challenge to legume production, leading to severe yield losses, late maturation, reduced flowering, pod mottling, and ultimately, poor seed quality. The economic impact of this disease is substantial, with estimated annual losses of up to $300 million, affecting soybeans, mungbeans, cowpeas, and black gram. The transmission of MYMV by the whitefly in a persistent circulative manner further exacerbates the disease's prevalence, allowing it to infect legume crops at all growth stages. Moreover, MYMV is part of a group of begomoviruses collectively known as "Legumoviruses," alongside Mungbean Yellow Mosaic India Virus (MYMIV). These viruses, characterized by bipartite genomes (DNA-A and DNA-B), play a pivotal role in the development of symptoms and host range. Notably, MYMV infection leads to the expression of various proteins, including coat protein (AV1), nuclear shuttle protein (NSP), and movement protein (MP), which contribute to viral cell-to-cell and long-distance movement. Our study extends beyond the identification of MYMV's presence in different regions of Maharashtra; it delves into the genetic diversity of the virus, offering insights into its evolution and relationship with other begomoviruses. Through sequence analysis and phylogenetic studies, we discovered that the MYMV strain in Maharashtra exhibits variations compared to Indian strains (MYMIV and HYMV), emphasizing the ongoing evolution of these viruses.

The phylogenetic analysis revealed the clustering of MYMV isolates into distinct groups based on nucleotide and protein sequences. Notably, the MYMV VDCOAB Latur isolate showed a close genetic relationship with MYMV coat protein isolate (AV1) from Navsari, Gujarat. These findings underscore the complexity of MYMV and its ability to adapt to different environments and host crops. Further, the pairwise percent identity analysis highlighted the diverse sequence similarities among MYMV isolates, with some displaying remarkably high pairwise identities, while others exhibited more significant variations. This diversity in sequence identity is a testament to the genetic plasticity of MYMV. In our study, we also explored the prediction of the secondary structure of the coat protein highlighted the prevalence of random coils, followed by alpha helices, extended strands, and beta turns. This insight into the protein's secondary structure aids in comprehending its functionality within the viral lifecycle. The validation of our homology model through the Ramachandran plot demonstrated the stability and reliability of our structural predictions, with a substantial proportion of amino acids residing in the most favorable region.

Overall, our study not only confirms the prevalence of MYMV in Maharashtra but also provides valuable insights into its genetic diversity, phylogenetics, and structural characteristics. These findings contribute to the growing body of knowledge surrounding begomoviruses and their impact on legume crops, ultimately paving the way for improved disease management strategies and crop protection measures. Further research into MYMV and related viruses is warranted to fully grasp their complexity and devise effective mitigation strategies.

Conclusion

In conclusion, this study sheds light on the prevalence, genetic diversity, and evolutionary dynamics of Mungbean Yellow Mosaic Virus (MYMV) in Maharashtra, focusing on soybean, mungbean, cowpea, and black gram crops. The confirmation of MYMV infection in symptomatic samples from various regions through gene-specific amplification of the coat protein (CP) gene establishes a robust foundation for understanding the molecular characteristics of the virus. The high genetic similarity (96.52%) between the MYMV coat protein gene sequence in cowpea samples from Pune region and a known MYMV sequence (Accession No. JQ004982.1) indicates the presence of a closely related strain in the region. The genetic divergence of the Pune isolate from other Indian strains (MYMIV and HYMV) underscores the complexity and diversity within the MYMV population. Phylogenetic analysis revealed the clustering of 70 MYMV CP gene sequences into three groups based on nucleotide and protein sequences. Notably, the MYMV Pune isolate, along with three other sequences, displayed the highest pairwise identity (96-98%) among the analyzed sequences, suggesting a distinct genetic lineage within the MYMV population. *In silico* restriction analysis using the CisSERS tool unveiled significant differences in restriction sites among the examined CP gene sequences, providing additional insights into the genetic variations and potential implications for MYMV evolution. The study's findings highlight the broad distribution of the MYMV strain in Maharashtra, emphasizing its ability to infect various legume crops. Furthermore, the ongoing evolution of MYMV with the Mungbean Yellow Mosaic India Virus (MYMIV) strain indicates a dynamic and adaptive nature, posing challenges for crop management strategies. In summary, this research contributes valuable information to the field of plant virology, providing a detailed characterization of MYMV in Maharashtra and paving the way for further studies aimed at developing effective strategies for the management and control of this economically significant plant virus.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors declare there are no competing interests.

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