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Molecular Detection of Mungbean Yellow Mosaic India Virus (MYMIV) Infecting Soyabean from Karnataka

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Abstract

Yellow mosaic disease (YMD) is a major limiting factor in soybean production and known to be caused by mungbean yellow mosaic India virus (MYMIV), a member of the genus, *Begomovirus*, family, *Geminiviridae*. In the present study, one each symptomatic and asymptomatic soybean leaf samples were collected from Eastern Dry Zone (Bengaluru) and Northern Transitional Zone (Dharwad) of Karnataka, for molecular detection and coat protein gene sequence analysis. Total DNA was extracted and PCR detection was carried out using designed MYMIV coat protein (AV1) gene-specific primers, which resulted in expected amplicon of 670 bp. Sequencing and comparative analysis of the AV1 gene revealed 96.55-99.82% nucleotide identity with other MYMIV isolates reported from India. Phylogenetic analysis showed that the Bengaluru isolate clustered closely with MYMIV Indore-3 (LC271566.1) while the Dharwad isolate grouped with MYMIV Jabalpur (LC271564.1). Further, Sequence Demarcation Tool (SDT) and recombination breakpoint analyses confirmed species identity and revealed no evidence of recombination events in the coat protein region. Based on coat protein sequence, these findings indicate that the MYMIV isolates from Bengaluru and Dharwad represent potentially virulent variants, posing a considerable threat to soyabean cultivation due to their adaptability, broad host range and potential for rapid dissemination.

Keywords : Molecular characterization, YMD, MYMIV, Soyabean, Phylogenetic analysis

Grain legume crops rank third behind cereals and oilseeds with respect to area and major legume crops including mungbean (*Vigna radiata*), blackgram (*Vigna mungo*), pigeonpea (*Cajanus cajan*), soyabean (*Glycine max*), mothbean (*Vigna aconitifolia*) and common bean (*Phaseolus vulgaris*). Yellow mosaic disease (YMD) caused by begomo viruses is one of the major constraints for legume productivity in South-East Asia and serious threat in India (Varma & Malathi, 2003 and Akram *et al*., 2024) and causing substantial economic losses in legume cultivation, with estimated annual yield losses of approximately 300 million USD in mungbean, soyabean and blackgram. In India, the disease poses a particularly severe threat, leading to yield reductions of up to 85 per cent (Karthikeyan *et al*., 2014).

Based on sequence identity analysis, yellow mosaic virus isolates of grain legumes are differentiated into four species. These species are: *Begomovirus vignaradiatae* (mungbean yellow mosaic virus or MYMV), *Begomovirus vigna radiata indiaense* (mungbean yellow mosaic India virus or MYMIV), *Begomovirus lonicera musivi* (horsegram yellow mosaic virus or HgYMV) and *Begomovirus dolichoris* (dolichos yellow mosaic virus or DYMV) and they are collectively named as legume yellow mosaic viruses (LYMVs) or 'legumoviruses' (Qazi *et al.*, 2007). Among these, MYMIV and MYMV are the most important. MYMIV is predominantly distributed in the northern, central and eastern regions of India (Usharani *et al.*, 2004), whereas MYMV is more prevalent in the southern (Karthikeyan *et al.*, 2004) and western regions. These species are known to be transmitted by whiteflies (*Bemisia tabaci* Genn.) (Mandal *et al.*, 1997). The characteristic symptoms of yellow mosaic are scattered small chlorotic specks or yellow spots on the leaf lamina which coalesce to form irregular yellow and green patches at maturity (Malathi, 2007; Varma *et al.*, 2011 and Purushotham *et al.*, 2025)

Based on genome composition, begomoviruses are categorised into monopartite (single genomic component) and bipartite begmoviruses (two single stranded DNA designated as DNA A and DNA B, each of size 2.7kb). They have particle morphology of characteristic twinned (geminate) para icosahedral structure (18×30 nm). In bipartite begomoviruses, DNA A component contains six open reading frames (ORFs), two in the viral sense strand and four in the complementary sense strand and encodes for coat protein (CP, V1/AV1) and Pre-coat protein (AV2/V2) in viral strand and replication initiation protein (Rep, C1/AC1), transcription activator protein (TrAP, C2/AC2), replication enhancer protein (REn, C3/ AC3) and PTGS suppressor (C4/AC4) on complementary strand (Seal *et al.*, 2006), In addition to these, another complementary strand gene (AC5) is uniquely conserved in legume infecting Yellow Mosaic Viruses (YMVs), has been shown to have a possible role in viral genome replication (Raghavan *et al.*, 2004). DNA B has two ORFs encoding movement proteins, BV1 (nuclear shuttle protein, NSP) on the virus-sense strand and BC1 (movement protein, MP) on the complementary-sense strand (Rojas *et al.*, 2005). The DNA A and DNA B components of a begomovirus have a highly conserved non-coding intergenic region referred to as common region (CR), which contains a stem-loop structure with the loop containing the invariant nano nucleotide sequence TAATATTAC that represent the origin of viral strand replication (Hanley-Bowdoin *et al.*, 1999).

Soyabean (*Glycine max* [L.] Merr.), a native of China, is an economically important legume crop and a major source of edible oil, protein and animal feed. India ranks among the leading soyabean producing countries globally, with major cultivation concentrated in Madhya Pradesh, Maharashtra and Rajasthan (Singh and Pathak, 2025). Major constraint in soyabean production and yield loss is due to yellow mosaic disease, caused either by MYMV or by MYMIV (Gupta *et al.*, 2013). YMD of soyabean was first reported in 1935 from the USA (Pierce, 1935) and in 1960 (Nariani, 1960) from India and since then, it is of regular occurrence at alarming levels because of its rapid spread through the vector, *B. tabaci*. Due to their high recombination rates and error-prone replication strategies, several sequence variants of begomovirus species have been reported from different legume crops across the Indian subcontinent. Therefore, the characterization of sequence variants occurring in a particular region is a crucial prerequisite for their management (Mishra *et al.*, 2017; Kumari *et al.*, 2024 and Khatib *et al.*, 2025).

Previous studies have reported that soyabean is susceptible to both MYMV and MYMIV. In view of this, the present investigation was under taken to determine whether the yellow mosaic virus infecting soyabean in Karnataka is an isolate of *MYMIV* rather than *MYMV*. Notably, genomic information on *MYMIV* isolates from Karnataka is currently lacking. Therefore, this study was aimed at characterizing the AV1 (coat protein) gene of *MYMIV* infecting soyabean in Karnataka to better understand its genetic identity and evolutionary relationship with other YMV isolates.

MATERIAL AND METHODS

Sample Collection

Two infected leaf samples from soyabean fields showing symptoms like typical yellow mosaic were collected from two different districts of Karnataka

(University of Agricultural Sciences, Dharwad campus and University of Agricultural Sciences, GKVK campus, Bengaluru) representing and Transitional Zone and Eastern Dry Zone, respectively during the year 2024 (Plate 1 and Fig.1). These samples were named as Dharwad isolate and Bengaluru isolate, respectively. Samples from healthy plants were collected for using as controls.

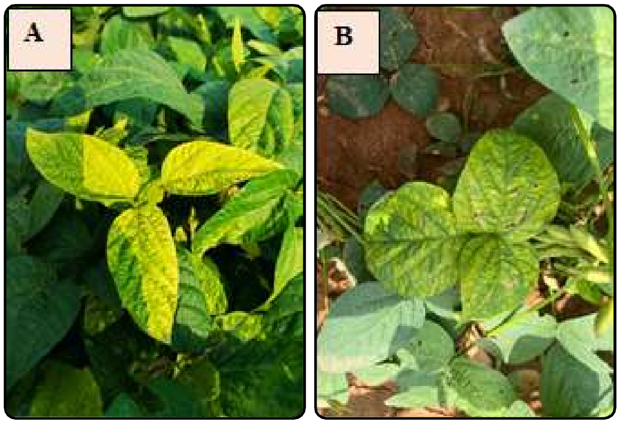

Plate 1: Soyabean plants showing typical yellow mosaic disease symptoms collected from: A) Dharwad and B) Bengaluru

PCR Amplification of MYMIV AV1 gene

To amplify the AV1 gene of MYMIV, specific primers targeting AV1 gene was designed using Primer 3 software (Untergasser *et al.*, 2012) to yield approximately 670bp in size. The details of the primers designed for MYMIV are present in Table 1. PCR was performed by utilizing designed primers using extracted DNA from both symptomatic and asymptomatic leaf samples as template.

PCR reaction mixture (25 µl) contained PCR buffer - 12.5 µl; sterile distilled water - 4 µl; forward and reverse primers (20 pmol/µl) - 2 µl each; Taq Polymerase (3 U/µl) - 0.5 µl; Template DNA - 2 µl; DNTPs - 2 µl and subjected to the short spin to ensure proper mixing and PCR was performed in Proflex thermal cycler (Carlsbad, California, United States). After completion of the reaction, PCR products were subjected to agarose gel electrophoresis on 1 per cent agarose gel at 70 V for 1 hr. The amplified PCR product specific to MYMIV AV1 gene was purified and sequenced by Sanger sequencing method at Eurofins Genomics India Pvt. Ltd., Bengaluru, India.

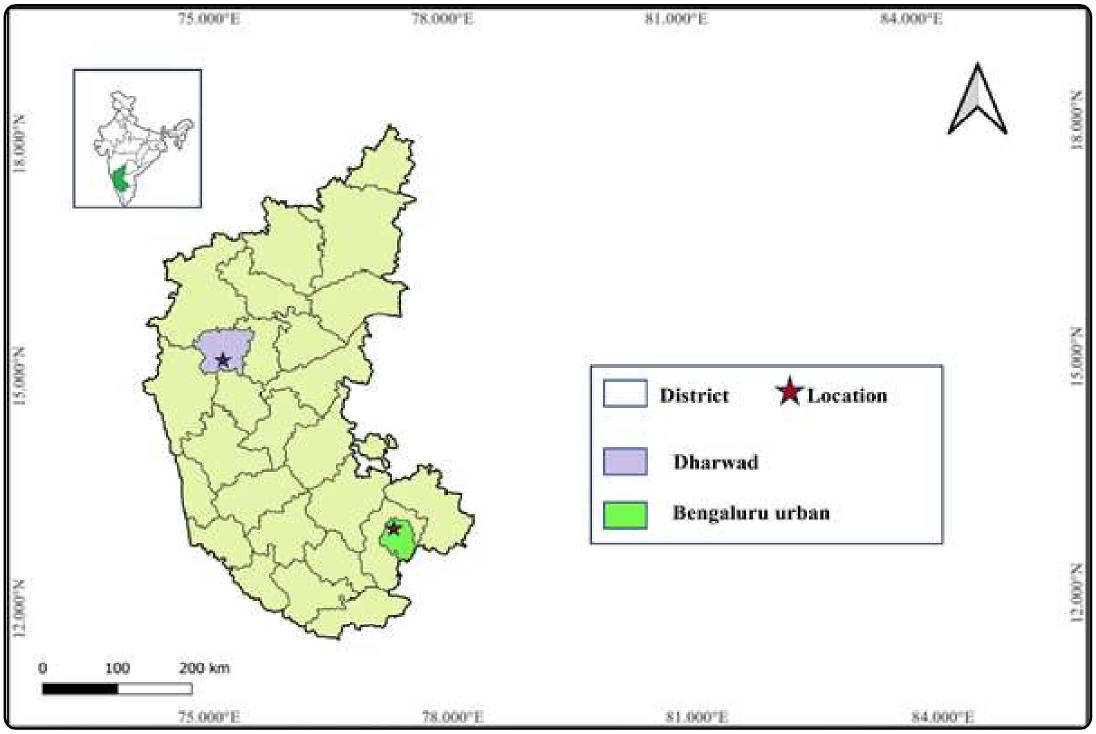

Fig. 1 : Map showing the locations from which soyabean leaf samples were collected for the study

TABLE 1

Details of MYMIV AV1 gene specific primers and PCR conditions used in the current study

Primer sequences (5' to 3')	PCR conditions	Expected Amplicon size
F-GCCTTCTCTACCCCGATGTC R-GG TTTTCATACTTCGCTGCCT	Initial denaturation: 94 °C for 5 min Denaturation: 94 °C for 1min Annealing: 55 °C for 2 min Extension: 72 °C for 3 min Final extension: 72 °C for 7 min Number of cycles: 35	670 bp

Sequence Comparison and Phylogenetic Tree Construction for Coat Protein gene of MYMIV Isolates

The 670 bp amplicon amplified from YMV infected soyabean samples using MYMIV specific primers were excised from agarose gel, eluted, purified, and sequenced by Sanger's dideoxy chain-termination method (ABI 3730 (48 capillaries) electrophoresis). The sequences obtained were queried at NCBI database using to BLASTn tool to know the sequence similarity with other begomoviruses. BioEdit software was used to assemble and analyze the data. Pairwise sequence identity was estimated by aligning the assembled viral genome sequences with their respective reference sequences using ClustalW programme (Larkin *et al*., 2007). Phylogenetic tree was generated in MEGA version 11 (Tamura *et al*., 2021) using the Neighbor-Joining (NJ) method based on the Kimura 2-Parameter (K2P) model (Kimura, 1980) with 1000 bootstrap replicates to assess branch support and per cent identity of the MYMIV Bengaluru and Dharwad isolates was calculated utilizing the Sequence Demarcation Tool (SDT) version 1.2 (Muhire *et al*., 2014).

Recombination Analysis

In order to know the possible recombination events in coat protein gene of MYMIV Bengaluru and Dharwad isolates, neighbor-net analysis was performed with 1000 bootstrap replicates using Splits Tree version 5.3.0 (Huson and Bryant, 2006). Recombination break point analysis was performed using RDP5 (Martin *et al*., 2020) package version 5.101 employing six different algorithms Recombination Detection Programme (RDP), GENECONV, Max Chi, Chimaera, Si Scan and 3Seq) using the begomovirus sequences used for phylogenetic analyses. Recombination events detected by at least three algorithms in the reconstructed viral genomes were considered as significant.

RESULTS AND DISCUSSION

The YMD caused by begomoviruses is the major constraint for the production of soyabean in the world. In Karnataka, YMD in soybean is known to be caused by two closely related begomoviruses, MYMIV and MYMV both of which are responsible for significant yield reduction (Qazi *et al*., 2007). To assess whether there exists any regional variation among the MYMIV strains, infected leaf samples were collected from two different districts of Karnataka, which are geographically apart by distance. These samples were subjected to molecular detection using the coat protein specific gene followed byphylogenetic analysis of its sequence to characterize the virus isolates and to understand their genetic relationships and evolutionary divergence within the coat protein.

The DNA was extracted from the symptomatic and asymptomatic leaf samples collected from soybean fields and PCR was carried out using MYMIV specific primers designed targeting AV1 gene (coat protein). The PCR resulted in expected amplicon of 670bp size from both Dharwad and Bengaluru symptomatic samples, no PCR amplicon was obtained from asymptomatic samples, indicating the association of MYMIV in the symptomatic samples. The Coat protein (CP) gene is the highly conserved in the family, *Geminiviridae* and coat protein gene sequence of begomoviruses effectively predicts discrete strains, species and taxonomic lineage. In an earlier report, Prema and Rangaswamy (2018) used the primers specific to MYMV targeting coat protein gene with an expected band size of 1000 bp for the detection of virus. Similarly, several other researchers have also designed primer sets targeting the coat protein region for the detection of legume yellow mosaic viruses (Obaiah, 2011; Akram *et al.*, 2011; Naimuddin *et al.*, 2011).

Plate 2 : Agarose gel showing PCR amplification of MYMIV coat protein (AV1) gene from the leaf samples of soyabean. Lane L-Ladder (100bp), H-Healthy sample, 1- Bengaluru isolate, 2-Dharwad isolate.

Sequencing of PCR products was performed at Eurofins Genomics India Pvt. Ltd., Bengaluru. The obtained nucleotide sequences of AV1 region of MYMIV isolates in the current study were having 670 nucleotide length and compared with other selected coat protein gene sequences of 28 begomovirus isolates retrieved from NCBI database.

Phylogenetic analysis helps to trace the evolutionary origins and relationships between different plant viruses, offering a deeper understanding of how viruses adapt to different plant species and environments (Gorbalenya and Lauber, 2017 Phylogenetic analysis of the coat protein gene sequence of MYMIV Dharwad and Bengaluru isolates with the corresponding coat protein gene sequence of selected reference isolates sequences retrieved from the NCBI GenBank provided further evidence of a shared evolutionary origin or geographic linkage, as indicated by close clustering of Bengaluru isolate with MYMIV isolate Indore 3 (LC271566.1) infecting soyabean and Dharwad isolate with MYMIV isolate Jabalpur (LC271564.1) infecting pigeonpea (Table 2 and Fig. 2). In a similar study by Arvind *et al.* (2024), characterized begomoviruses infecting soybean and reported that the virus responsible for yellow mosaic disease in the Tarai region shared a close relation ship with MYMIV.

TABLE 2

MYMIV and other selected *begomovirus* isolates sequences available in the NCBI GenBank used in study for analysis

Accession No.	Virus	Strain/isolate	Host	Origin
LC271564.1	MYMIV	Jabalpur 2	Pigeonpea	India
LC271566.1	MYMIV	Indore 3	Soyabean	India
KC243784.1	MYMIV	Hyderabad	Mungbean	India
PQ272939.1	MYMIV	Kanpur	Mungbean	India
OQ107488.1	MYMIV	Hoshangabad	Mungbean	India
OQ107477.1	MYMIV	Bhopal	Mungbean	India
OQ107505.1	MYMIV	Dewas	Mungbean	India
OQ107485.1	MYMIV	Ubaidullaganj	Mungbean	India
MW717940.1	MYMIV	Ludhiana	Mungbean	India

Continued....

TABLE 2 Continued....

Accession No.	Virus	Strain/isolate	Host	Origin
MW717953.1	MYMIV	New Delhi	Mungbean	India
MW717961.1	MYMIV	Coimbatore	Mungbean	India
MW717946.1	MYMIV	Hisar	Urdbean	India
PQ215582.1	MYMIV	Sehore	Pigeonpea	India
OQ107501.1	MYMIV	Indore	Mungbean	India
OQ107484.1	MYMIV	Phanda	Urdbean	India
AY269992.1	MYMIV	Pakistan	Mungbean	Pakistan
MW717956.1	MYMIV	Namakkal	Mungbean	India
OQ107478.1	MYMIV	Bhopal	Mungbean	India
AF314530.1	MYMV	Maharastra	Soyabean	India
MK409378.1	MYMV	Chikkaballapura	Sissoo spinach	India
MN698295.1	MYMV	Belagum	Moth bean	India
MW736043.1	MYMV	Bengaluru	French bean	India
MW736044.1	MYMV	Dharwad	Mungbean	India
AB017341.1	MYMV	Thailand	Mungbean	Thailand
MH885653.1	MYMV	GKVK	Mungbean	India
AJ421642.1	MYMV	Madurai	Soyabean	India
OR621379.1	DYMV	Tamilnadu	Lablab bean	India
MW731691.1	DYMV	Washington	Country bean	Bangladesh

Further, shared nucleotide identity of coat protein gene of MYMIV Dharwad and MYMIV Bengaluru isolates with other MYMIV isolates infecting different crops from various regions ranged from 96.55 per cent to 99.82 per cent. The results of the phylogenetic analysis and nucleotide sequence comparison coat protein gene of YMV isolates infecting soyabean (MYMIV Dharwad and Bengaluru) revealed that, these isolates might be strains of MYMIV. However, complete genome sequencing of these isolates will reveal their exact taxonomic status.

In a similar kind of work, Swapna and Prema (2024) showed that blackgram YMV 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. Similarly, molecular characterization of samples from three agroclimatic zones of central India using coat protein gene primers also confirmed MYMIV infection, with the Jabalpur isolate (OM643233) showing nucleotide similarity up to 97.9 per cent nucleotide with other MYMIV isolates from India and abroad (Amrate *et al.*, 2023). However, complete genome sequencing and analysis involving more sample size will provide much more insights into the geographical variations in these isolates.

For begomoviruses, as per the ICTV, complete DNA A sequence comparison is essential for species demarcation (Brown *et al.*, 2015). However, in the present study we analysed the coat protein (AV1) region of MYMIV, which is highly conserved and used for initial level species identification based of specific primers within begomoviruses. The pair wise identity values obtained for the coat protein region were further validated using the Sequence

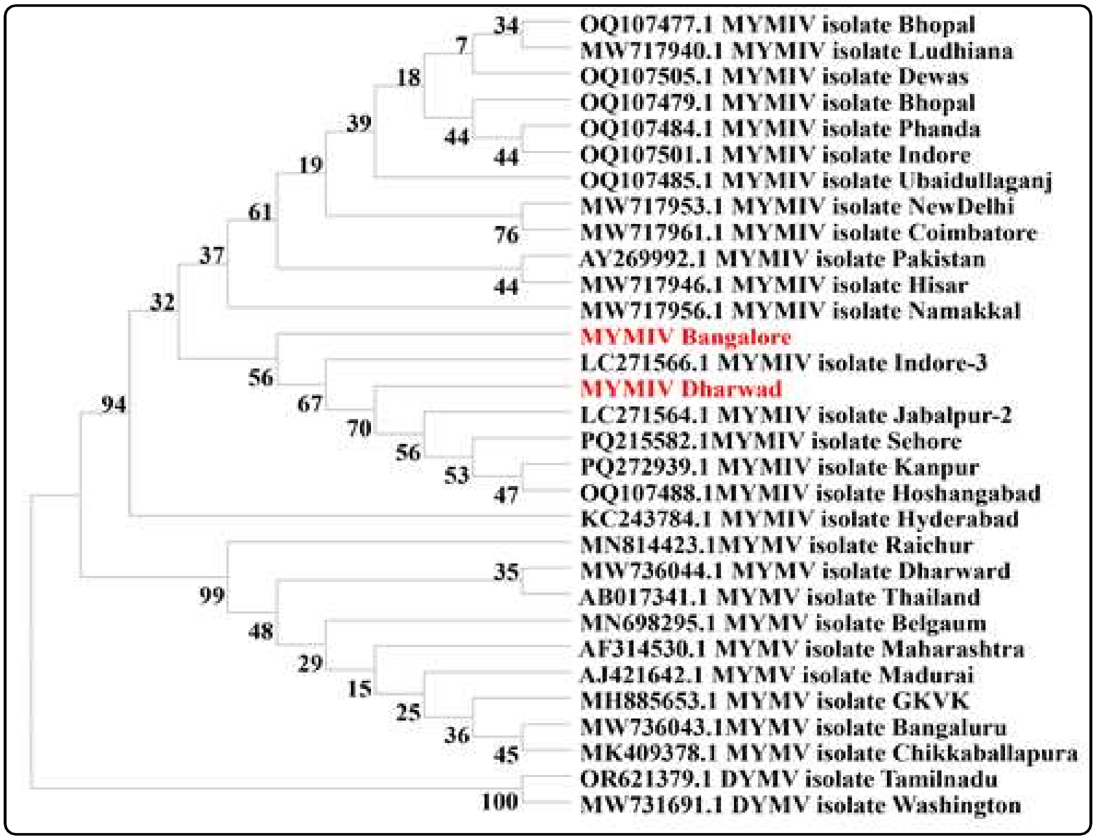

Fig. 2 : Phylogenetic tree constructed for coat protein gene sequence of MYMIV Bengaluru and Dharwad isolate along with other begomoviruses sequences retrieved from NCBI GenBank using Neighbor joining algorithm with 1000 bootstrap replicates. Horizontal distances are proportional to sequence distances; vertical distances are arbitrary.

Demarcation Tool (SDT), which generates a colour-coded matrix of identity scores (Fig. 3). These results clearly confirm that the present isolates cluster within the MYMIV species, indicating them as possible strains of MYMIV.

A neighbor-net network analysis (using Splits Tree version 4.11.3) was conducted for the coat protein region of MYMIV Bengaluru and Dharwad isolates, along with other retrieved sequences from NCBI. The analysis revealed a tree-like structure with straight branches, indicating the absence of potential recombination events in coat protein region (Fig. 4). This result was further validated by recombination break point analysis using RDP4 software, which showed no detectable recombination events. Variations or conserved nature within each individual gene will contribute to the extent of variability in the particular virus population. Supporting this, Akram *et al.* (2024) reported that tests of neutral evolution revealed MYMV and MYMIV are predominantly under purifying selection and exhibit signs of population expansion. Similarly, Shahid and Al-Sadi (2024) observed no evidence of recombination events in MYMIV isolates from Oman infecting mungbean, further confirming the stability of the viral genome.

Interestingly, even though the present isolates (Bengaluru and Dharwad) under study, were collected from locations separated by more than 400 km, no recombination events in their coat protein region were detected. This indicates that despite the considerable geographical distance, the isolates have maintained a high degree of genetic stability. The absence of recombination in the AV1 gene of these isolates

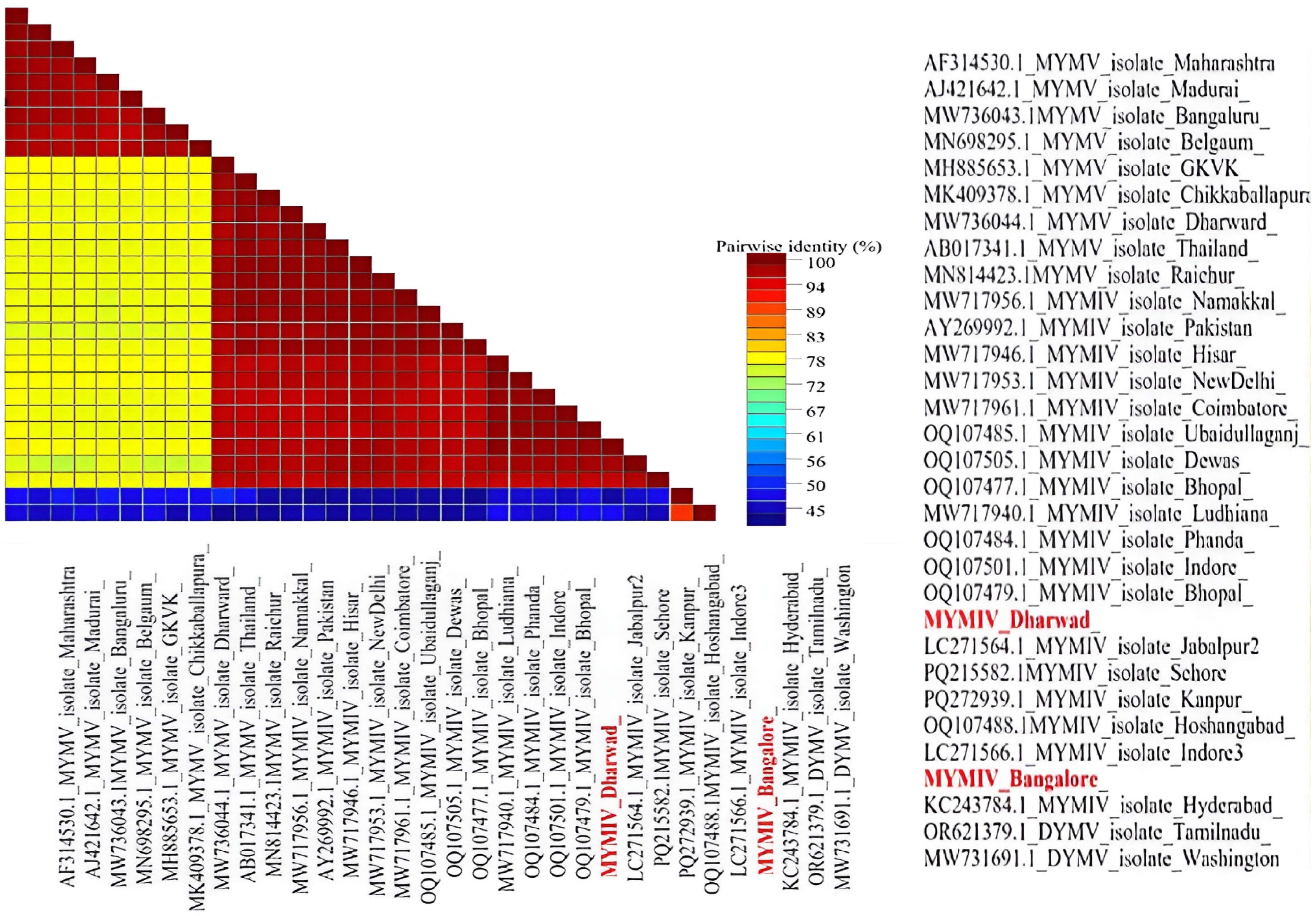

Fig. 3 : Graphical representation of percentage pairwise genomic scores and nucleotide identity plot for coat protein region of MYMIV Bengaluru and MYMIV Dharwad isolates with reference sequences using Sequence Demarcation Tool (SDTv1.0)

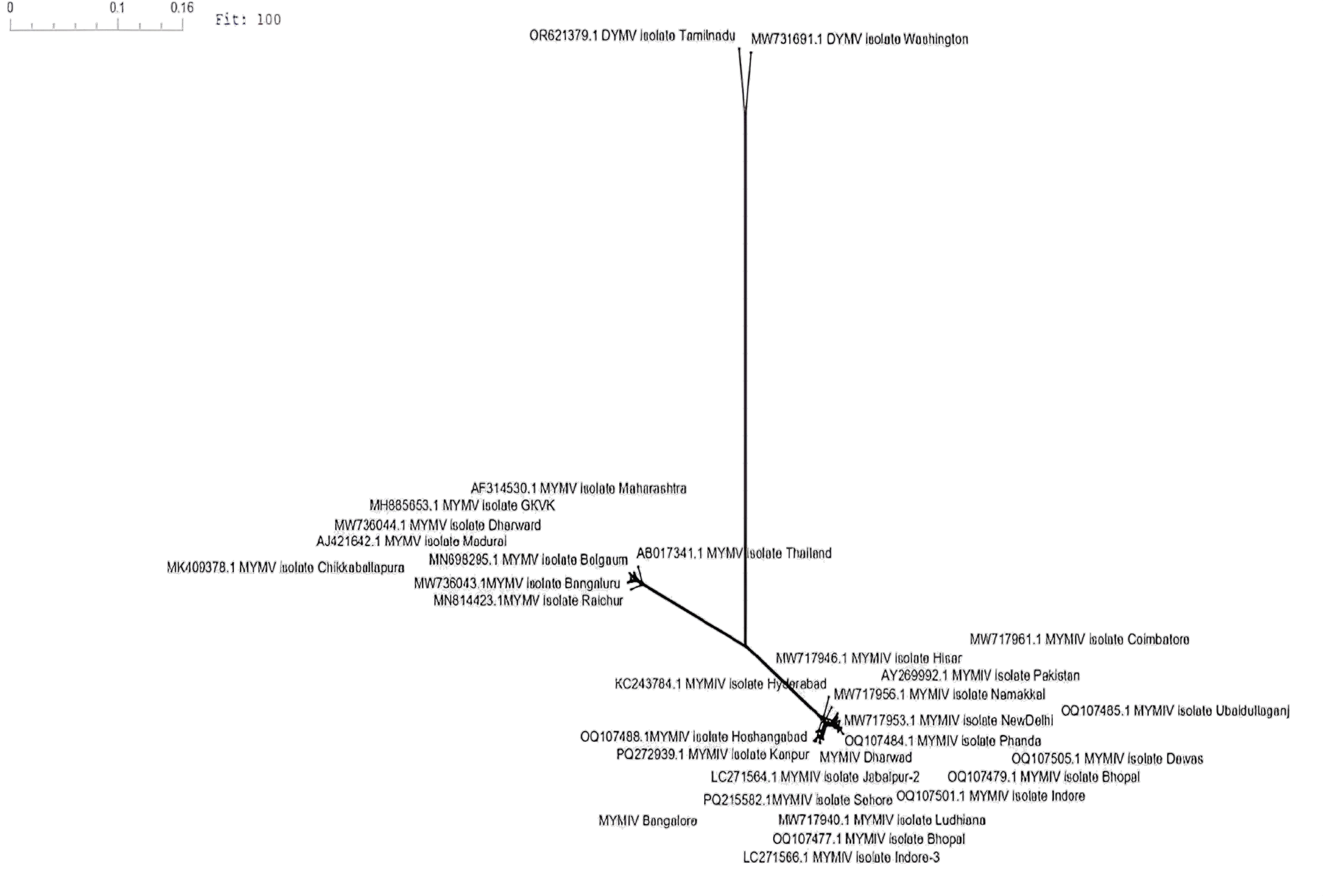

Fig. 4 : A neighbor-net constructed for coat protein region of MYMIV Bengaluru and Dharwad isolate with other selected Begomoviruses from NCBI database by using Split-Tree v5.

suggests that MYMIV primarily evolves through purifying selection acting on existing genetic variation rather than through genetic exchange with other viral strains, shows its conserved evolutionary pattern across different regions.

This study provides valuable insights into the molecular and phylogenetic characteristics of MYMIV isolates infecting soybean in Karnataka based on coat protein gene sequencing, highlighting their high genetic similarity and absence of recombination despite wide geographic separation. The findings emphasize the evolutionary stability and limited genetic diversification of MYMIV, suggesting its adaptation under purifying selection pressure. The findings contribute to a deeper understanding of begomovirus evolution and support the development of sustainable disease management and breeding strategies for resistant soyabean cultivars.

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