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SHORT COMMUNICATION

# Full Genome Characterization of the Causal Virus of Yellow Mosaic Disease in Mungbean Minicore Accessions

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## Abstract

The mungbean (*Vigna radiata*) minicore collection represents global genetic diversity in a compact germplasm panel and serves as an important resource for trait discovery and resistance breeding. However, the widespread occurrence of yellow mosaic disease (YMD) threatens its effective utilization in northern India. During Kharif 2024 at Kanpur (India), YMD-affected accessions from the minicore collection were sampled to identify and characterize the associated causal agent. PCR assays using species-specific primers confirmed mungbean yellow mosaic India virus (MYMIV) infection in all examined samples. Complete genome sequencing of a representative isolate yielded DNA-A (2746 nt) and DNA-B (2672 nt) components that shared high nucleotide identity with MYMIV isolates reported previously. The DNA-A and DNA-B components of the identified isolate, designated MYMIV-[Mu3-Kanpur], were cognate and exhibited 90% sequence similarity within the common region. Recombination analysis revealed a recombination event in the DNA-A component, indicating ongoing viral evolution. This study provides the first complete molecular characterization of MYMIV associated with YMD in the mungbean minicore collection and highlights the need to integrate molecular diagnostics with phenotypic evaluations for the effective identification and deployment of durable resistance sources.

**Keywords** Begomovirus · Genome · Minicore · Mungbean · Mungbean yellow mosaic India virus · *Vigna radiata*

**Significance Statement:** The mungbean minicore collection is a globally important germplasm resource for crop improvement, yet its utilization is constrained by yellow mosaic disease (YMD). Through complete genome characterization, this study identified mungbean yellow mosaic India virus (MYMIV) as the causal agent of YMD in susceptible minicore accessions and detected evidence of viral recombination. These findings provide a molecular framework for resistance screening and the development of durable YMD-resistant mungbean varieties.

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## Introduction

Mungbean [*Vigna radiata* (L.) R. Wilczek] is an important grain legume cultivated widely across Asia, with India being the largest producer [1–3]. Improving resistance to major biotic stresses remains a key breeding objective for enhancing crop productivity. The efficient utilization of genetic diversity conserved in germplasm collections is central to this effort. To facilitate germplasm-based crop improvement, a globally representative mungbean minicore collection comprising 296 accessions was developed from a larger germplasm set, capturing substantial species diversity in a manageable panel for trait discovery and breeding [4, 5]. This minicore collection has been widely used for the identification of accessions with superior agronomic performance and resistance to several abiotic and biotic stresses [6–12].

Yellow mosaic disease (YMD) is among the most destructive diseases limiting mungbean production in South Asia and is caused by a group of 'whitefly-transmitted begomoviruses' collectively referred to as legume yellow mosaic viruses (LYMVs) [13–19]. As it leads to plant malformation, reduced photosynthetic efficiency and restricted plant growth [20, 21], resulting in yield losses ranging from 32–78% following late infection and reaching 100% when infection occurs at early crop stages [19, 22]. In mungbean, YMD has been associated with mungbean yellow mosaic India virus (MYMIV), mungbean yellow mosaic virus (MYMV), dolichos yellow mosaic virus (DoYMV), and horsegram yellow mosaic virus (HgYMV) [16, 19, 23, 24]. Among these, MYMIV and MYMV are the predominant YMD-associated viruses in India, exhibiting distinct geographical distributions and host ranges [19, 24–27]. Because multiple YMD-causing viruses may coexist within the same geographical region and mixed infections have been reported, symptom-based diagnosis alone is insufficient for reliable identification of the causal virus. Accurate molecular identification is therefore essential for dependable resistance screening and deployment of resistant germplasm [19, 28, 29].

Although the mungbean minicore collection has been evaluated for YMD response, the molecular identity of the virus associated with YMD in this globally important germplasm resource remains unknown. During field evaluation of the minicore collection under north Indian conditions, several accessions exhibited characteristic YMD symptoms. Therefore, the present study aimed to identify and characterize the associated causal virus using PCR-based diagnostics and complete genome sequencing. To our knowledge, this is the first report providing complete molecular characterization of a YMD-associated virus infecting the mungbean minicore collection, thereby establishing a molecular basis for future resistance screening and breeding efforts.

## Materials and Methods

A mungbean minicore collection comprising 296 accessions and three check varieties (Virat, IPM 2-14 and Shikha) was evaluated during Kharif 2024 at ICAR-Indian Institute of Pulses Research (ICAR-IIPR), Kanpur, in two replications [5]. Meteorological data corresponding to the crop growing period were obtained from the meteorological observatory of ICAR-IIPR, Kanpur. Yellow mosaic disease (YMD) severity was assessed at 45 days after sowing using a modified 0–9 disease rating scale [30]. Leaf samples from ten highly susceptible accessions exhibiting typical YMD symptoms (disease score 6–9) were collected and designated M1–M10. Total genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Germany), and then subjected to rolling circle amplification (RCA) using the REPLI-g Mini Kit (Qiagen, Germany). RCA products were screened for mungbean yellow mosaic India virus (MYMIV), mungbean yellow mosaic virus (MYMV), dolichos yellow mosaic virus (DoYMV), and horsegram yellow mosaic virus (HgYMV) using previously reported species-specific coat protein gene primers and PCR conditions [31]. Virus-positive samples were subjected to complete genome amplification of DNA-A and DNA-B components using overlapping primer pairs and PCR conditions described previously [28, 29]. PCR products were resolved on 1% agarose gel, and representative amplicons from samples M1 and M6 were subjected to Sanger sequencing.

Sequence reads were assembled and edited using BioEdit v7 [32], followed by BLASTn, ORF Finder, and BLASTp analyses. Phylogenetic relationships were inferred using the Maximum Likelihood method with the Tamura–Nei model and 1,000 bootstrap replicates in MEGA v11 [33, 34] using a dataset of 49 representative begomovirus species, with maize streak virus (EU628631) as the outgroup (Online Resource 1). The phylogenetic trees were visualized using the Interactive Tree of Life (iTOL) online tool (https://itol.embl.de). Additional phylogenetic analysis was performed using complete MYMIV and MYMV genome sequences available at NCBI. Recombination events were identified using RDP4 [35], while pairwise nucleotide identity comparisons were conducted in SDT v1.3 using reference sequences of 13 legumoviruses and cowpea golden mosaic virus (CpGMV) [15, 19, 36]. Characterized sequences were deposited in GenBank database.

## Results

During the crop growth period (July–August 2024), daily maximum temperatures ranged from approximately 34–36 °C and minimum temperatures from 24 to 25 °C, accompanied by high relative humidity (84–91%), frequent rainfall (265 mm in July and 358.6 mm in August), and moderate sunshine (3.6–3.7 h per day), providing favourable conditions for whitefly multiplication and yellow mosaic disease development. Approximately 7% of the mungbean minicore accessions exhibited high susceptibility to yellow mosaic disease (YMD) (disease score 6–9) under natural field conditions. Symptoms initially appeared as small yellow flecks that progressively developed into a characteristic yellow mosaic pattern, followed by extensive chlorosis and plant decline, whereas unaffected accessions remained symptomless (Fig. 1a, b). PCR-based screening of ten symptomatic samples (M1-M10) using species-specific primers detected MYMIV in all samples, as evidenced by the expected ~900 bp amplicon (Fig. 1c). No amplification

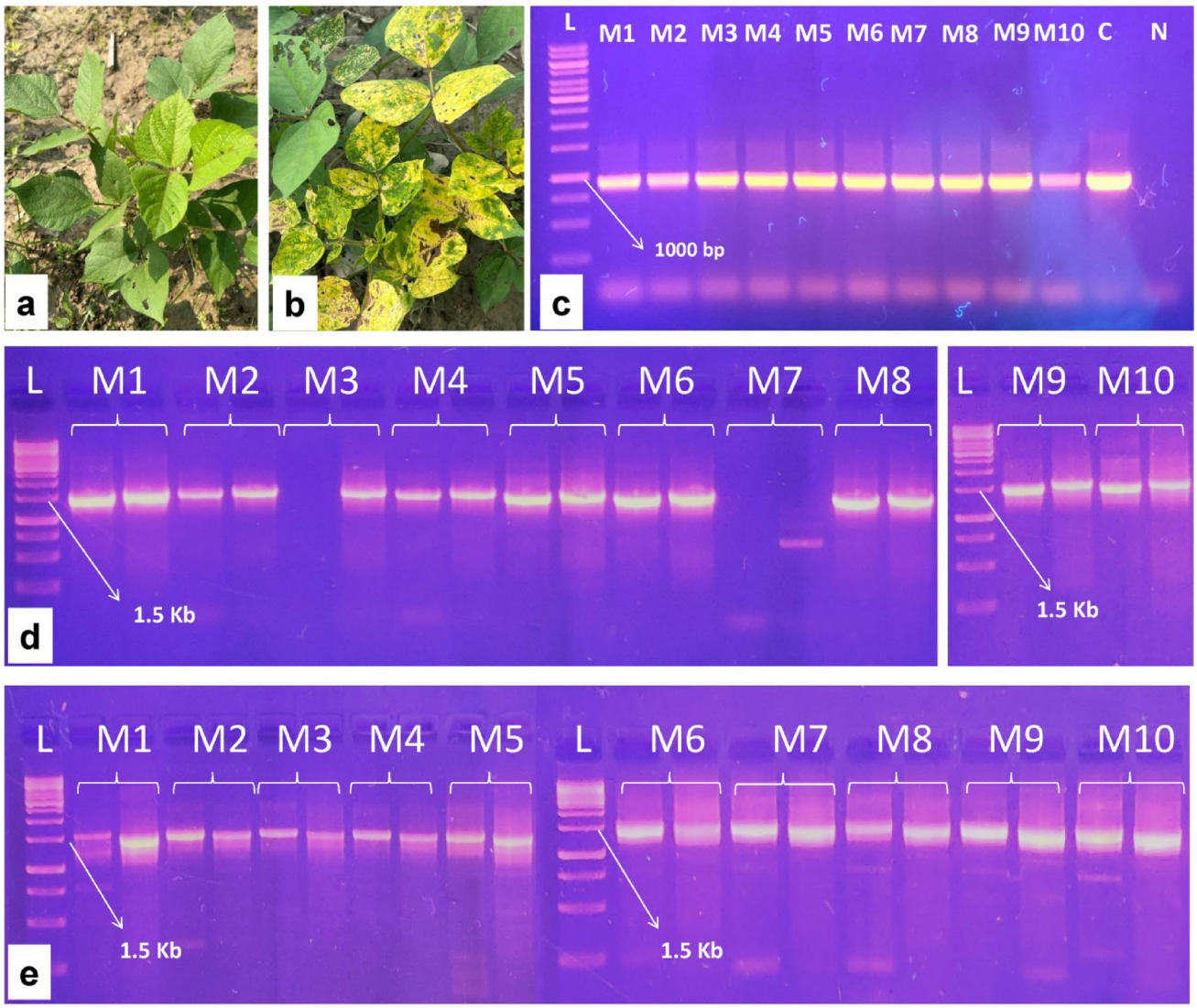


**Fig. 1** Detection and molecular characterization of mungbean yellow mosaic India virus (MYMIV) infecting mungbean minicore accessions.**a** Healthy mungbean plant. **b** Mungbean (*Vigna radiata*) plant exhibiting typical symptoms of yellow mosaic disease (YMD). **c** PCR-based amplification of the MYMIV coat protein (CP) gene (~900 bp) using MYMIV-specific primers, from ten (M1 to M10) symptomatic samples. L = 1 kb DNA ladder, C = Control positive for MYMIV, N = Negative control. **d** PCR based amplification of the complete DNA-A component of MYMIV using two overlapping primers pairs: MIA-F1/R1 and MIA-F2/F3 shown in the first (left) and second (right) lanes, respectively for each sample, generating ~1.5 kb amplicons that together span the full-length DNA-A genome. **e** PCR based amplification of the complete DNA-B component of MYMIV using two overlapping primers pairs: MIB-F1/R1 and MIB-F2/R3 shown in the first (left) and second (right) lanes, respectively for each sample, producing ~1.5 kb amplicons that together span the full-length DNA-B genome. L = 1 kb DNA ladder

was obtained with primers specific to MYMV, DoYMV, or HgYMV, confirming MYMIV as the causal agent of YMD in the tested accessions. Consistent symptom expression across infected plants and identical PCR banding patterns among all MYMIV-positive samples further indicated low viral diversity under the prevailing field conditions.

Complete genome amplification of MYMIV was performed using overlapping primer pairs targeting DNA-A and DNA-B components and yielded the expected ~1.5 kb amplicons. Samples M3 and M7 failed to amplify for DNA-A (Fig. 1d, e). Therefore, the complete genome was successfully amplified for 8 out of 10 samples in this study. Representative samples M1 and M6 were selected for Sanger sequencing. The assembled DNA-A sequences were 2746 nt in length and shared 100% nucleotide identity. BLASTn analysis revealed 99.96% identity with a previously reported MYMIV DNA-A sequence from mungbean (MW814711). The DNA-A component contained seven predicted ORFs, comprising AV1 and AV2 in the virion-sense orientation and AC1-AC5 in the complementary-sense orientation. Similarly, the DNA-B sequences from M1 and M6 were 2672 nt long and shared 100% nucleotide identity. The highest nucleotide identity (99.89%) was observed with a reported MYMIV DNA-B sequence from mungbean (MW814722). The DNA-B component contained two ORFs, BV1 and BC1, encoding the nuclear shuttle and movement proteins, respectively. The common region of DNA-A and DNA-B shared 90% nucleotide similarity and contained the conserved origin of replication features characteristic of bipartite begomoviruses, confirming the cognate nature of the two genomic components. Based on genome organization, nucleotide identity, and cognate DNA-A/DNA-B relationship, the causal agent was identified as an isolate of MYMIV and designated as MYMIV-[Mu3-Kanpur]. The DNA-A and DNA-B sequences were deposited in GenBank under accession numbers PX215754 and PX215755, respectively.

Phylogenetic analysis of both DNA-A and DNA-B components placed MYMIV-[Mu3-Kanpur] within the legumovirus clade, clearly separated from other Old World begomoviruses and clustering with established MYMIV isolates (Fig. 2). Another group comprising of New World begomoviruses was observed to be distinct from Old World begomoviruses. Inclusion of additional MYMIV and MYMV genome sequences in an elaborated phylogenetic analysis did not alter the overall clustering pattern, and the studied isolate, MYMIV-[Mu3-Kanpur] consistently grouped within the MYMIV clade. Based on these results, pairwise full-length sequence comparison of the identified isolate with the RefSeq accessions of legumoviruses

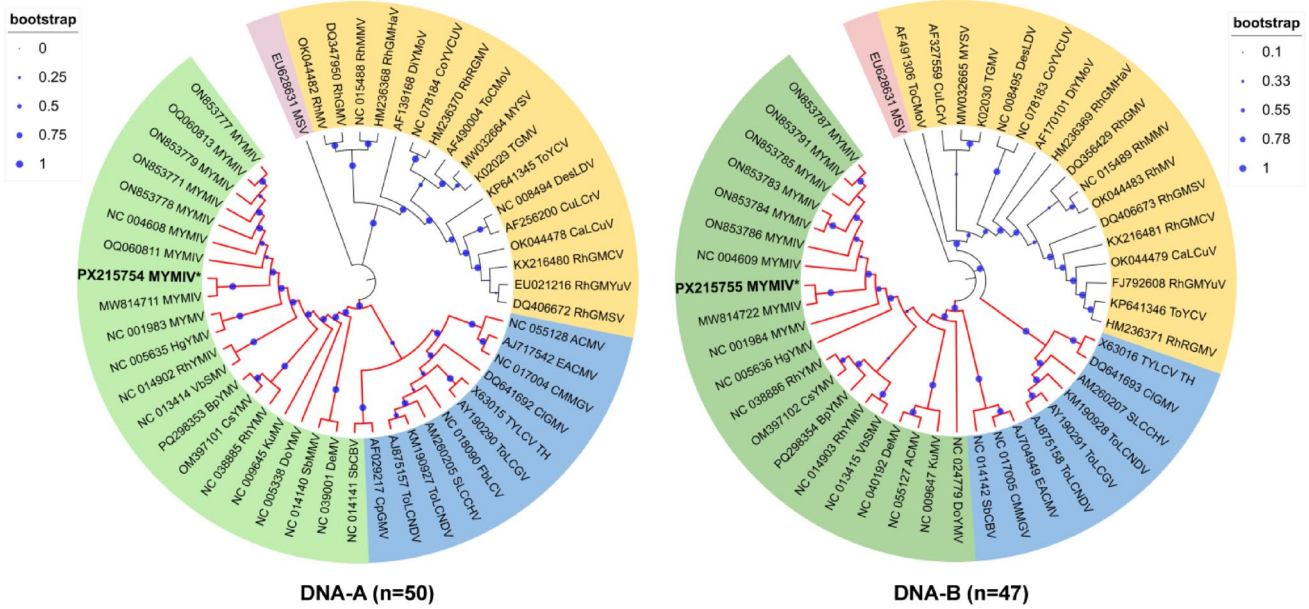


**Fig. 2** Phylogenetic analysis of the complete DNA-A (PX215754) and DNA-B (PX215755) genome sequences of the MYMIV isolate identified in the present study (shown in bold*) with 49 DNA-A and 46 DNA-B sequences retrieved from NCBI. The evolutionary history was inferred using the Maximum Likelihood method implemented in MEGA11 with 1000 bootstrap replicates. Bootstrap values (%) based on 1,000 replicates are indicated at the corresponding nodes, and branch lengths are proportional to the number of nucleotide substitutions per site. The red-coloured branches represent the Old World begomovirus clade, comprising the green sector representing legumoviruses, including the MYMIV isolate identified in the present study, and the blue sector representing the remaining Old World begomoviruses. The yellow sector represents New World begomoviruses, whereas the pink sector represents the outgroup sequence of maize streak virus (MSV). Each taxon is represented with NCBI accession number followed by abbreviated name of corresponding virus species. Details of sequences retrieved from NCBI are provided in Online Resource 1

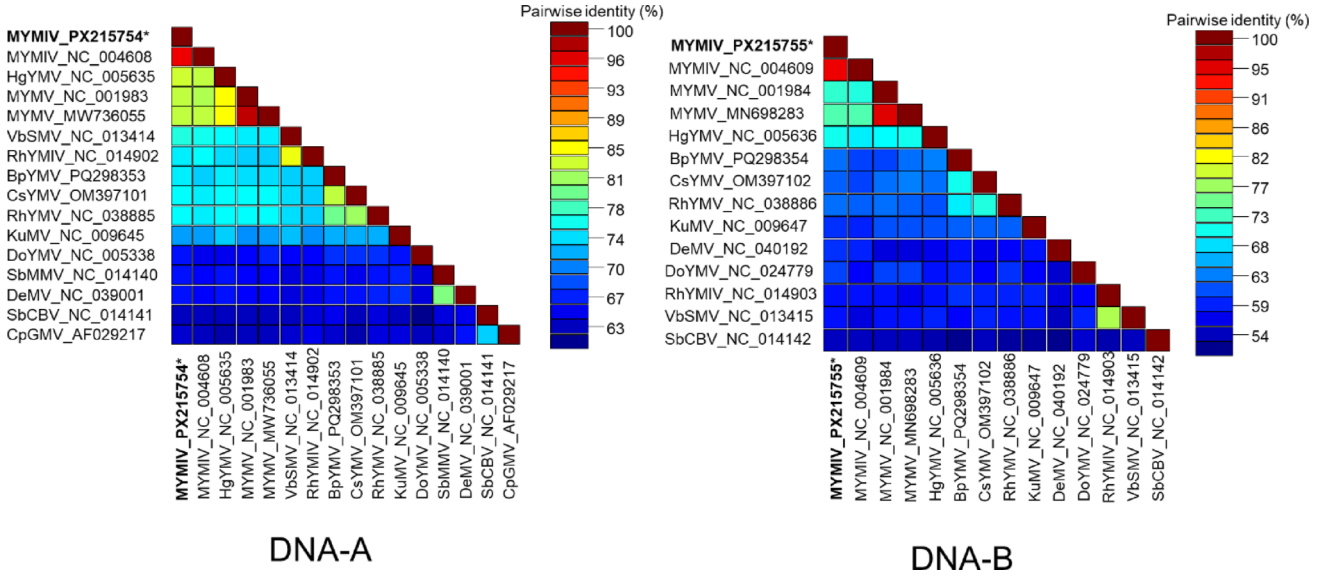


**Fig. 3** Heatmap showing pairwise nucleotide sequence identity of the complete DNA-A and DNA-B genomes of the MYMIV isolate identified in the present study (shown in bold) compared with representative legumoviruses. Pairwise nucleotide identities were calculated using the Sequence Demarcation Tool (SDT v1.2), with colour intensity representing the degree of sequence similarity

revealed that the isolate MYMIV-[Mu3-Kanpur] was highly identical with MYMIV. Similar results were observed in SDT analysis also when compared to full-length sequences of legumoviruses (Fig. 3).

Evidence of a recombination event was observed only in the DNA-A component of the isolate MYMIV-[Mu3-Kanpur]. Seven out of nine detection methods namely, RDP ($p$ value= $1.163 \times 10^{-06}$), GENECONV ($p$ value = $1.023 \times 10^{-08}$), BootScan ($p$ value = $8.172 \times 10^{-12}$), MaxChi ($p$ value = $2.891 \times 10^{-09}$), Chimaera ($p$ value = $1.136 \times 10^{-07}$), SiScan ($p$ value = $1.347 \times 10^{-15}$) and 3Seq ($p$ value = $1.198 \times 10^{-05}$) supported the presence of recombination events significantly. The beginning break point was identified at position 2082 and the ending break point at position 8 of the sequence without gaps. The major and minor parents were identified as MYMIV DNA-A (OQ060811) identified from mungbean and MYMIV DNA-A (ON853779) identified from cowpea, respectively.

## Discussion

The mungbean minicore collection was developed to capture maximum genetic diversity for trait discovery and breeding [5]. The occurrence of highly susceptible accessions within this globally important germplasm resource highlights the need for precise characterization of the virus associated with YMD, as resistance to one LYMV species may not necessarily confer resistance to another, and breeding decisions based solely on symptom expression may therefore be misleading. The predominance of MYMIV observed in the present study agrees with previous reports from northern and central India, where MYMIV has been identified as the principal causal agent of YMD in mungbean, whereas MYMV predominates in southern and western regions [19]. Complete genome analysis revealed a typical bipartite MYMIV genome with cognate DNA-A and DNA-B components sharing 90% identity within the common region, consistent with previous reports on legumoviruses [37–39]. The recovery of cognate genome components, rather than mixed genomic components, further strengthens the molecular identification of MYMIV as the causal virus associated with YMD in the evaluated minicore accessions.

Phylogenetic and pairwise sequence analyses confirmed that the identified isolate, designated MYMIV-[Mu3-Kanpur], belongs to MYMIV. Notably, recombination analysis revealed a statistically significant recombination event in the DNA-A component. Since DNA-A encodes proteins involved in replication, pathogenicity, and host-virus interactions, recombination within this component may contribute to the emergence of variants with altered fitness, host adaptation, and epidemiological success [40–43]. The observed recombination event is consistent with recombination being one of the principal evolutionary mechanisms driving begomovirus diversification and adaptation. The identification of mungbean- and cowpea-derived MYMIV isolates as the parental sequences suggests ongoing genetic exchange among virus populations infecting different pulse hosts. Such events may facilitate cross-host adaptation and potentially influence the durability of host resistance, particularly in regions where multiple legumes are cultivated and exposed to high whitefly pressure [44, 45]. Overall,

this study provides the first molecular characterization of a YMD-associated virus infecting the mungbean minicore collection and identifies a recombinant MYMIV isolate circulating under north Indian field conditions. These findings highlight the importance of integrating molecular diagnostics into germplasm evaluation to ensure accurate resistance phenotyping and emphasize the need for continued surveillance of MYMIV evolution to support the development of durable YMD-resistant mungbean cultivars.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s40009-026-02416-z.

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**Author Contributions** IS and DK wrote the original draft. UC, MAk, and AP conceived the idea. AP acquired the fund for the current study. RMN provided the seeds. AP, MAk, PG helped in conducting field experiments. IS and MAg conducted field evaluation. IS, DK, and MAk conducted lab studies. DK prepared the illustrations and performed the data analysis. All authors read and approved the final manuscript.

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**Data Availability** All genomic data generated in this study have been deposited in the GenBank database under accession numbers PX215754 and PX215755.

## Declarations

**Competing interests** The authors declare that they have no competing interests.

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